ANTIDIABETIC AND HEPATO-RENAL PROTECTIVE EFFECTS OF ETHYL ACETATE FRACTION OF METHANOL LEAF EXTRACT OF *Solanum macrocarpon* Linn IN STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR RATS

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A THESIS IN THE DEPARTMENT OF BIOCHEMISTRY SUBMITTED TO THE FACULTY OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

DECEMBER, 2023

CERTIFICATION

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DEDICATION

God Almighty, the creator of heaven and earth and the giver of life for sustenance.

All my teachers, past and present, for their influence.

My parents of blessed memory, for persistence and perseverance.

My wife of blessed memory and my children for their understanding and support.

ACKNOWLEDGEMENTS

I will like to honour the Almighty God for His faithfulness, divine protection and provision during the period of this project. May His name be praised for ever more. Amen.

Words are not sufficient to express my heartfelt gratitude to my learned, versatile, amiable and God fearing supervisor, Professor C.O.O. Olaiya for selfless and corrective contributions. I will forever remember your concern, encouragement, understanding and support during the dark and challenging period of this program. God will reward and bless you and family abundantly in Jesus name.

I appreciate Professor O.A. Adaramoye, Head of Biochemistry Department, University of Ibadan, for his motivation and for putting at my disposal all needed materials and equipment used for this project. God bless you sir, for being compassionate, thoughtful and kind.

I seize this opportunity to thank in a special way Professor Oyeronke A. Odunola for her contributions towards this project and to my entire endeavors over the year. I will never forget your selflessness, understanding, encouragement and all round support; God will continue to bless you and your family in Jesus name.

I thank Professor O.O. Olorunsogo, for his fatherly care and for the big role he played in this project. Remain blessed sir.

I cannot thank enough Professor E.O. Farombi for his brotherly care, support and encouragement. I am grateful for the privilege and opportunity to work in his laboratories during the course of this project. God bless you sir

I specially recognize the contributions of my teachers, Professor. M.A. Gbadegesin, Dr. Sarah O. Nwozo, Dr. O.A. Abolaji, Dr. Oluwatoyin Adeyemo Salami, Dr. S.O. Owumi, Dr. Olugbami, Dr. Adegoke, Dr. A.O. Olowofolahan. I value immensely the contributions of Drs. I.A. Adedara, J.O. Olanlokun, I.O. Awogbindin, M.A. Esan, J. Achem and Kate E. Nwokocha.

I am grateful to all the non-teaching staff in the department most especially Rev. S.O. Okewuyi, Mr. Eric Sabo, Mrs. Abiodun Bakare, Mrs. Omolara Ogunbiyi, Mr. O.M. Bassir, Mr. Adebiyi Isiaka, and Mrs. Juliana Ogunmokun.

My gratitude goes to all the janitorial staff of the department.

I would like to appreciate the following for the technical support received:

Professor K.O. Soetan, Department of Veterinary Physiology and Biochemistry, University of Ibadan.

Dr. A.O. Aina, Department of Veterinary Anatomy, University of Ibadan.

Dr. Babatunde Osho, Macpherson University.

Dr. Kayode Afolabi, I.A.R. & T., Apata, Ibadan.

Mr. A.O. Adebisi, Oyo State Hospital, Jericho, Ibadan.

Mrs. Okunola, Physiology Department, U.I Ibadan.

Mr. Ejiro, Veterinary Department, University of Ibadan.

Mr. Otegbade Olabode Peter, Histopathology section, U.C.H. Ibadan.

Mr. Tosin Ale, Pharmacy Dept., University of Ibadan.

Mr. Adio, Central Research Laboratory, Polytechnic Ibadan.

Miss Amaka, Drug Metabolism and Molecular Toxicology Research Laboratories, Dept., of Biochemistry, University of Ibadan.

Staff of the Central Laboratory, University of Ibadan,

I value the cooperation of all members of staff of Kenneth Dike Library, University of Ibadan.

I enjoy the wonderful company and support of my course mates, most especially Mr. Akinbode Akinlolu Otitooluwa, Cancer and Molecular Biology Laboratories. God bless you.

I cannot thank enough Dr. Kayode Olayele Karigidi for his outstanding contributions to the accomplishment of this research work. You are just wonderful and God bless you and family in Jesus name.

I appreciate Dr. Ogunyemi Micheal, Omolekan Tolulope, Mrs. Bukola Ailenokhuoria, Miss. Olufolake Sowobi, Dimeji Macauly, Taiwo, Olatinwo Mercy, Saviour Usin, Davies Kanyinsola and all I.T. students for their wonderful contributions towards the accomplishment of this research work. I value greatly the technical support and assistance received from Mrs. Wuraola Familusi, the laboratory technologist at the Nutritional and Industrial Biochemistry Laboratories.

Very many thanks to my siblings: Oluremi Anjorin, Akinyemi Anjorin, Grace Modupe Adesina, and Francis Ige Anjorin for their financial, moral and emotional support throughout the programme. Remain blessed.

My wife, Ellen Adeyinka Anjorin of blessed memory and my children, Ayobami Anjorin, Morenike Anjorin, Olatubosun Anjorin, and Oluwatobilola Anjorin. I appreciate your understanding, support, and concern. God bless you all.

My deep appreciation goes to Mrs. Esther Oluwakemi Asooto – Mama Tosin, and her team for typing and computer work.

I express my profound gratitude to all those who have contributed directly or indirectly towards the success of this work. God bless you all.

ABSTRACT

Diabetes mellitus (DM), a metabolic disease, is becoming more prevalent globally. *Solanum macrocarpon* (Linn.) is traditionally used in the treatment of DM but there is paucity of information on the scientific evaluation of its diabetic activity. The aim of this study was to evaluate the antidiabetic activity of the methanol leaf extract of *Solanum macrocarpon* in streptozotocin-induced diabetic male Wistar rats.

Solanum macrocarpon leaves were air-dried, pulverised, extracted with methanol (SME) and then fractionated into *n*-hexane, chloroform, ethyl acetate (EA), *n*-butanol and methanol. Phytochemical screening and antioxidant [Total flavonoids content, 2,2 diphenyl-1-picryhydrazl reduction (DPPH), Total Antioxidant Capacity (TAC), and Reducing Power (RP)] activity were evaluated in SME and its fractions while α-amylase inhibition (α-AI), α-glucosidase inhibition (α-GI), Metal Chelating (MC) and Nitric Oxide (NO) scavenging activities were evaluated in SME only by spectrophotometry. Diabetes mellitus was induced using streptozotocin (50mg/kg i.p.) and animals with fasting blood glucose (FBG) ≥ 250mg/dL were considered as being diabetic. Twenty-four rats (139-146g) were grouped (n=6): Groups 1 (control), 2 (diabetic control), 3 (diabetic rats + EA fraction, 300mg/kg) and 4 (diabetic rats + Glibenclamide, 1mg/kg) and treated orally for 28 days. The animals were sacrificed and FBG, Oral Glucose Tolerance Test (OGGT), glycated haemoglobin, urea, creatinine, Alkaline Phosphatase (ALP), Glutathione Peroxidase (GPx), Aspartate Transaminase (AST), Superoxide Dismutase (SOD), and levels of GSH and malondialdehyde were assessed by spectrophotometry. Histological examinations of the liver and kidney were performed using standard procedures. Chemical profiling of EA fraction was determined using Fourier Transformed Infrared (FT-IR) spectroscopy and GC-MS. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

The presence of alkaloids, saponins, phenols, flavonoids, steroids, and terpenoids were detected in SME and its fractions. Antidiabetic activity analysis showed that SME exhibited α-AI (59.9%) and α-GI (41.7%) activities. The SME also exhibited NO scavenging (77.4%) and MC (48.8%) activities as well as TAC (0.021mg/gAAE). The EA fraction possessed the highest total flavonoids (0.23mg/gQUE), TAC (0.05 mg AAE/g) and DPPH (92.6%) scavenging activity. In diabetic rats+EA fraction, body weight (36.5%) increased, while decrease in FBG (60%), OGTT and glycated haemoglobin decreased relative to control was observed. Creatinine (28.2%), urea (18.5%), AST (16.3%), ALT (20%), and ALP (31.1%) were reduced in diabetic rats+EA fraction relative to diabetic controls. Activities of SOD (0.104 vs 0.128 units/mg protein), CAT (0.121vs 0.155units/mg protein), and GPx (8.59 vs 10.17 units/mg protein) as well as GSH level (2.66 vs 3.64 μmol/L) elevated in the diabetic rats+EA fraction compared with diabetic. Malondialdheyde levels (26.7%) reduced relative to diabetic control. The diabetic rats+EA fraction showed reversal of hepatic and renal periportal inflammatory infiltration observed in the diabetic controls. Presence of hydroxy groups in EA fraction (3500 cm^{-1}) was revealed, and squalene intermediates as the most abundant compounds.

The ethyl acetate fraction of *Solanum macrocarpon* ameliorated hyperglycaemia and protected renal and hepatic tissues through antioxidant mediated mechanisms in streptozotocin-induced diabetic rats.

Keywords: *Solanum macrocarpon*, Hyperglycaemia, Hepatoprotection, Nephroprotection.

Word count: 476

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LIST OF ABREVIATIONS USED

AAS	Atomic Absorption Spectrophotometer
ADA	American Diabetes Association
ADH	Antidiuretic Hormone
AGE_s	Advanced Glycation end-products
AIDS	Acquired Immunodeficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AMA	American Medical Association
AMD	Age-related macular degeneration
ANOVA	Analysis of Variance
AODM	Adult onset diabetes mellitus
ARIS	Atherosclerosis Risk in Communities Study
AST	Aspartate Transaminase
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydrotoluene
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea nitrogen
BUT	Butanol Fraction of *Solanum macrocarpon* extract
BW	Body Weight
CAT	Catalase
CDK	Cyclin Dependant Kinase
CHL	Chloroform fraction of *Solanum macrocarpon* extract
CHO	Carbohydrate
CNS	Central Nervous System
CRD	Chronic Respiratory Disease
CRF	Chronic Renal Failure
CVA	Cerebro Vascular Accident
CVD	Cardiovascular Disease
DAN	Diabetes Association of Nigeria
DB	Direct bilirubin
DCCT	Diabetes Control and Complication Trial

DKA	Diabetic Ketoacidosis
DKD	Diabetes Kidney disease
DM	Diabetes mellitus
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid
DPPH	2, 2, diphenyl-1-picrylhydrazil
EA	Ethyl acetate fraction of *Solanum macrocarpon*
ED	Erectile Dysfunction or Erectile Disorder
EDTA	Ethylenediaminetetraacetic Acid
ESRD	End Stage Renal Disease
FAO	Food and Agriculture organisation
FBG	Fasting Blood Glucose
FBS	Fasting Blood Sugar
FDA	Food and Drug Administration
FPG	Fasting Plasma Glucose
FT- IR	Fourier Transform Infra-Red
GC–MS	Gas chromatography – Mass Spectroscopy
GDM	Gestational Diabetes Mellitus
GHB	Glycated Heamoglobin
GLIB	Glibenclamide
GP_X	Glutathione peroxidase
GSH	Glutathione
GST	Glutathione–s– transferase
GTT	Glucose Tolerance Test (OGTT).
H & E	Heamatoxylin and Eosin
Hb	Hemoglobin
HbAIC	Glycated Hemoglobin
HBP	Hexosamine Biosynthetic Pathway
HCT	Hematocrit
HDL	High Density Lipoprotein
HEX	Hexane fraction of *Solanum macrocarpon*
HIV	Human Immunodeficiency Virus
Ib	Pound
IDDM	Insulin-dependent diabetes mellitus

IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired Glucose Tolerance
IHD	Ischemic Heart Disease
IR	Insulin Resistance
IR	Insulin Receptor
Kg	Kilogram
LADA	Latent Autoimmune Diabetes in Adults
LDL	Low Density Lipoprotein
LDSO	Median Lethal dose
LPO	Lipid Peroxidation
MCH	Mean Corpuscular hemoglobin
MCHC	Mean Corpuscular hemoglobin concentration
MDA	Malondialdehyde
MET	Methanol fraction of *Solanum macrocarpon*
MI	Myocardial Infarction
MODY	Maturity Onset Diabetes of the Young
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NCD	Non-Communicable Disease
NCD-RISC	NCD Risk Factor Collaboration
NGT	Normal Glucose Tolerance
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NO	Nitric Oxide
OECD	Organisation of Economic Cooperation and Development
OGTT	Oral Glucose Tolerance Test
PBS	Phosphate-buffered Saline
PG	Plasma Glucose
PH	Hydrogen ion Concentration; Negative logarithm of Hydrogen ion activity
PHGPx	Phospholipid Hydropreoxide Glutathione Peroxidase
PLT	Platelet Count
PP	Postprandial (After a meal)
PUFA	Polyunsaturated Fatty Acids

RBC	Red Blood cell
RBG	Random Blood Glucose
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SDG	Sustainable Development Goals
SDS	Sodium Dodecyl Sulphate
SIGT	Screening for Impaired Glucose Tolerance
SM	*Solanum macrocarpon*
SME	Crude extract of *Solanum macrocarpon*
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Science
STZ	Streptozotocin
T2DM	T2 Diabetes Mellitus
TAC	Total Antioxidant Capacity
TB	Total bilirubin
TB	Tuberculosis
TBA	Thiobarbituric Acid
TBAR	Thiobarbituric Acid Reactive Species
TBHQ	Tertiary Butylhydroquinone
TC	Total cholesterol
TFC	Total flavonoid content
TPC	Total phenolic content
Tris	Tris (hydroxymethyl) aminomethane
UKPDS	UK Prospective Diabetes Study
UN	United Nations
UNSDGs	United Nations Sustainable Development goals
WBC	White blood cell
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Today, globally, human life is being threatened by those non-infectious diseases that develop gradually but progress over an extended period of time, otherwise known as non-communicable diseases (NCDs). Characteristically they are caused through interplay of factors between genetics, physiology, environment, life style, and usually affect people for a long time (WHO, 2021). Prominent instances encompass coronary disorders such as cerebrovascular accidents, heart attacks, Chronic Respiratory Diseases (CRDs) including asthma and chronic bronchitis, malignancies, and DM (WHO, 2021). One of the first human diseases to be reported is diabetes mellitus (DM), as evidenced in a codex from Egypt dating to around 1500 BC that mentions "excessive discharge of urine" and the earliest cases listed were thought to be type 1 diabetic cases (WHO, 2014). Diabetes mellitus is among the main traumatic and problematic epidemiological issues in twenty-first century and it has a significant impact on both socio- economic development and public health worldwide (Lin *et al.,* 2020).

Diabetes is that constantly recurring situation that commences whenever either pancreas could not produce adequate insulin or incapability of our body in properly utilising insulin that is synthesised (WHO, 2022; WHO, 2008). Diabetes mellitus is an endocrine disease with multiple etiologies that is characterised by inconsistencies in lipid, protein, and carbohydrate metabolism. These inconsistencies are caused by problems with insulin synthesis by the pancreas, insulin resistance/action, or a partnership between the two, which raises blood glucose levels (Karigidi and Olaiya, 2020; Karaiede and Gnudi, 2016).

The two main kinds of DM are Non insulin-dependent diabetes mellitus (NIDDM), and Insulin-dependent diabetes mellitus (IDDM). Consequent to absolute destruction of pancreatic β-cells, production of insulin decreases and this happens to be the

characteristic feature of IDDM. This poses difficulties in maintaining the levels of blood glucose at normal optimum standards. Type 1 DM has been observed to be present in both the adult as well as the children where elevated glucose levels were recorded in fasting and post prandial blood (Mishra and Garg, 2011). Sole hormone which controls how much sugar enters most of the body's cells, particularly the muscles, the liver, and the adipose tissue is insulin. Thus, all forms of DM can emanate from lack or shortage of insulin or by its receptors insensitivity (David and Doleres, 2011). Reduced glucose levels lead to decrease in insulin released from β cells and subsequent promotion of glycogenolysis. Primary regulator of this process is glucagon that operates in reverse direction to insulin (Röder *et al.,* 2016). The importance of glucose homeostasis in DM cannot be over emphasized and all organs involved in glucose homeostasis, including the brain, the pancreas, the kidneys, the liver, and the gastrointestinal system, are negatively impacted by DM. The standing hallmarks of DM include polyuria, polydipsia and polyphagia. Generally, diabetes is being conceived as a controllable but not treatable disease.

There is a steep increase in the population of people being affected by DM now worldwide and WHO has reported a global shoot up in population of diabetics in comparison to 108 million which was the figure recorded in 1980 (WHO, 2016). From statistics, about six hundred and ninety-three million grown ups globally are predicted to be diabetic by the year 2045 if there are no operative preventive measures in place, up from the predicted 451 million adults who had it in 2017 (Cho *et al.,* 2018; IDF, 2017). Around five hundred and thirty-seven million grown ups between twenty to seventy-nine years of age globally have DM and it has been reported that the figure will rise to 783 million by 2045, with 24 million individuals anticipated to live with the disease in Africa and roughly 3.6 million in Nigeria (IDF, 2023). Since 1990, DM has significantly increased in prevalence around the globe. Due to the complex character or multifold targets of this disease, the conventional medications (insulin and hypoglycemic agents) employed in treating diabetes have their limitations (Marles and Farnsworth, 1995). These synthetic drugs are also costly. Consequently, search for antidiabetic medications has been redirected in emphasis towards botanicals which have little or no negative impact (Horie *et al.,* 1997).

It should be noted that DM has historically been treated using a variety of natural therapies, and many of the pharmaceuticals that are presently in the market are either

extracted from or transformed from the lead compound that was isolated from natural sources. Discovery of metformin from *Galega officinalis* is an attestation to that fact. Many publications based on valid experimental screening procedures have confirmed that well above 800 plants exhibit anti-diabetic activity (Patil *et al.,* 2011). Phytochemicals which are present in plants have been reported to elicit beneficial and positive biological activities, leaning on their efficacy and safety (Dey *et al.,* 2012). By examining the biochemical and histological characteristics of the kidneys, liver, and pancreas, several reports on the reno-hepatic and anti-diabetic actions of medicinal plants have been documented (Zangeneh *et al.,* 2018). Prior research works have suggested that medicinal plants and herbs may be involved in regulating excessive blood sugar, enhancing kidney biomarkers, and restoring liver functioning (Abdallah *et al.,* 2020). Contributions or part played by oxidative stress in the origin and progression of DM complication have inspired application of antioxidants for both prophylaxis and curative measure in this disease.

Natural botanicals especially vegetables have been shown as veritable and reliable sources of antioxidants. Antioxidants are substances that considerably block or slow down the decomposition of the substrates when present in quantities that are less than those of the oxidizable substrates. Antioxidants have been shown to be effective in enhancing glucose disposal; decreasing risk of onset of DM as well as decreasing the impact of associated complications of DM. Antioxidant are endowed with free radical scavenging power. To inhibit or delay the process of oxidation, artificial antioxidants including butylated hydroxytoluene, propyl gallate, and butylated hydroxyanisole have been employed but these antioxidants are found to be hazardous to humans' health and due to this reason emphasis has been shifted to focusing on identifying prospective potential antioxidants from plants origin. According to reports, good providers of antioxidants which are natural are fresh fruits, and vegetables therefore including these botanicals in our normal diets will bring various health benefits because of the significant effects of the antioxidant secondary metabolites resident in them which will eventually protect the human body from oxidative assaults and injuries (Rahaman *et al.*, 2005). The modus operandi of antioxidants may be via removal of or decrease in local oxygen levels, or by removal of catalytic metal ions or scavenging the initiating free radicals or by interrupting the initiated sequence of oxidation process (Pryor and Porter, 1990).

Though advancements in diabetes treatment and management are on-going, it is necessary to be acquainted with the latest developments in this direction which include continuous glucose monitoring (CGM) systems, insulin pump technology, targeted therapies, personalised medicine, digital health solutions, genomics, proteomics, and artificial intelligence (AI) and machine learning algorithms.

Solanum macrocarpon Linn. (SM) is a vegetable that is commonly known as "Igbagba" or "Igbo" in the South Western Nigeria; "Gorongo" in some parts of Northern Nigeria, "Gboma" in the local language (Fon) in Benin, as well as other parts of Africa. It is also known as African eggplant in many areas around the globe. SM belongs to the family solanaceae just like tomato, pepper, and eggplants (Cerpadec-Ong, 2008). The Solanaceae family exhibits the traits of ethno-botany, meaning that humans use them widely. It is a valuable and effective origin and bank of food, spices, and medicine. Eight (8) solanaceae species have been reported in literature to be endowed with antidiabetic properties (Kandimalla *et al.,* 2015). Despite the lack of scientific backing, traditionally, people, particularly those living in tropical regions, use the leaves of SM for DM therapy as well as prophylactic and curative of many other illnesses and diseases. Its young leaves and fruits are prepared as vegetables and eaten with foods like pounded yam, yam flour meal and other solid foods. The leaves are bitter in taste when prepared and can be made into different traditional sauce in various parts of Africa (Grubben and Denton, 2004). Its aqueous extract has been reported as a good reservoir of secondary metabolites of biological significance (Oyesola *et al.,* 2022). Additionally, it has been demonstrated that SM lowers cholesterol and reduce or mitigate redox imbalance in experimental Wistar rats, by Okesola *et al.,* 2020 and Dougnon *et al.,* 2014 respectively. Despite widespread utilisation of SM as prophylactics and curative in DM in folkloric medicine, there is a dearth of scientific evidence to validate this claim. In particular, there are no studies that examine the effects of SM methanol leaf extract's ethyl acetate fraction on hepato-renal damage and diabetes brought on by the streptozotocin treatment of male Wistar rats. The intention of this research study was to examine hepato-renal protective influence and antidiabetic potential of the fraction on male Wistar rats having diabetes induced by streptozotocin. This was done since hyperglycemia is linked to complications of the kidney and liver.

1.2 Statement of Problem

Diabetes mellitus, one of the numerous chronic lethal diseases that have developed during this modern period is potentially deadly and now threatens global health. Both the social and economic growth of the world's population as well as public health are severely hampered by its threat. Several drugs have been developed to combat DM, but these are not without side effects. Therefore, traditional, complementary and alternative therapy is emerging as a promising preventive and therapeutic option in DM management. Several studies have suggested antidiabetic activity of *Solanum macrocarpon* and other antidiabetic plants. However, the biological cum therapeutical activity of this plant in DM is poorly explored and not fully understood. Even with the medicinal potential of *Solanum macrocarpon* Linn. in treating diseases especially DM, scientific evidence to reach this notion of treating diabetes mellitus with a traditionally approach is poorly documented and thus leaving one of the neglected vegetable relatively unexplored.

1.3 Justification of the Study

Solanum macrocarpon Linn. one of the forgotten vegetables has historically been used to cure and manage a number of illnesses, such as constipation, heart issues, and diabetes, traditionally. This crucial medicinal plant's biological function is not currently known. Exploration-cum- in-depth understanding of the role of this plant in diabetes may present the natural key to unlock DM complication mystery.

1.4 Aim and Objectives

1.4.1 Aim

The present study's aim was to assess the anti-diabetic effect of the methanol leaf extract of *Solanum macrocarpon* Linn in streptozotocin-induced diabetic male Wistar rats.

1.4.2 Specific Objectives

Precise objectives of this research are to:

1. determine the phytochemical constituents, elemental analysis and preliminary characterization of *Solanum macrocarpon* Linn.
2. evaluate the antioxidant, metal chelating and anti-radical potential of methanol crude extract *in vitro.*
3. determine the inhibitory effects of the methanol crude extract from SM on starch hydrolyzing enzymes (α - amylase and α – glucosidase) *in vitro.*

4. test the effects of SM crude extract on male Wistar rats from a toxicological perspective.
5. examine how SM crude extract and its solvent fractions affect diabetic, metabolic, haematological, and histopathological indicators in male Wistar rats that have been induced to develop diabetes through the use of streptozotocin.
6. use FT-IR and GC-MS to, respectively, pinpoint the functional groups and active components contained in the SM extract and its solvent fractions.

CHAPTER TWO
LITERATURE REVIEW

2.1 Definition and Description of Diabetes Mellitus

One of the most crippling and fatal chronic diseases of the twenty-first century, diabetes mellitus is life-threatening and has emerged as a global health issue (WHO, 2014). Various peoples' health is being negatively impacted in various countries, including Nigeria. It was appropriately described as a pandemic metabolic illness by Kotha *et al.,* in 2017. Diabetes mellitus, together with its consequences, can damage a number of body organs, causing high number of illnesses and death (ADA, 2018; WHO, 2016). Diabetes mellitus, an endocrine disorder that is characterizes by high blood glucose which is brought on by a flaw in the body's pancrea's ability to synthesis insulin, loss of power by appropriate insulin receptor to profitably use the hormone produced, or combination. Prolonged high blood sugar or long term hyperglyceamia causes cardiac, renal, and hepatic failure; dysfunction and damages to the nerves, eyes and the blood vessels according to the American Diabetes Association (ADA). Persistent hyperglycemia can cause microvascular and macrovascular problems to appear and worsen, according to Basha and Sankaranarayanan, 2014.

2.2 Holistic History of Diabetes Mellitus and Development of Therapies

Vecchio *et al.,* 2018 claimed that DM, one of the most studied illnesses in medical history, is also one of the earliest ailments recognised to affect people. Its symptoms were first reported in antiquity. A number of symptoms associated with diabetes mellitus as a disease were first recorded historically some 3,500 years ago in ancient Egypt, Greece, Arabia, and Asia, but its pathophysiology was never mentioned. Hesy-Ra, an Egyptian medical practitioner of the third papyrus dynasty, is credited to be the pioneer in recognising polyuria as a symptom of diabetes around 1552 BC, according to Ebers papyrus, which has been sufficiently documented (Harikumar *et al.,* 2015; Ebers, 1937). Appollonius of Memphis, a Greek, is thought to have used or coined the words "diabetes," "to pass through," or "syphon", approximately 250 BC (Poretsky, 2009). The first mention of the link between polyuria and a sweet-tasting material in

urine is in Sanskrit literature from the 5th to 6th century BC, during the time of Sushruta, Charaka, and Vaghbata (Algaonker, 1972). In his book "Samhita" written about the fifth century BC, Sushruta used the term "Madhumela," which translates to "honey-like urine," to describe diabetes. He also noted that in addition to having a sweet taste, urine can also be sticky to the touch and can draw ants. According to him, excessive consumption of foods like rice, cereal, and sweets is a contributing factor to diabetes, which disproportionately affects the wealthy. Sushruta recommended physical activity circa 600 BC, in order to keep the humors in balance and lessen the effects of obesity and diabetes (Tipton *et al*., 2008). Charaka and Sushruta charted the morphological variations among patients passing high volumes of "sweet urine" between 600 and 400 BC (Ramachandran and Snehalata, 2009).

They were able to differentiate amongst type 1 and type 2 DM for the first time, with juvenile onset DM being linked with young people and adult onst DM being associated with obesity. With strong thirst, excessive drinking, and enormous amounts of urine that tasted sweet as defining symptoms, Chen Chuan baptized the illness "Hsiano Kho Ping" in the seventh century and noted the sweet urine in DM. Weight loss, polydipsia, and polyuria were all identified by Chang Chung-Ching (Ca. 160–Ca. 219) as signs of a distinct illness. Since the eighth century, doctors have noticed that diabetics are more prone to getting skin illnesses including furuncles, rat ulcers, and vision issues. In his "Canon of Medicine" book, written in the 11th century AD, Avicena recorded and noted gangrene and sexual dysfunction as side effects of diabetes. Moses Maimonides (1138–1204) provided a thorough description of diabetes, which included acidosis symptoms.

Aretaeus of Cappadocia (120–200 AD) was the first to fully describe diabetes clinically and empathically. He described it as a terrible illness that was uncommon in men and likened it to the “transformation or breaking down of muscle and appendages” into urine. He provided the first description of diabetics' "intense thirst" in addition to reporting on the large amount of urine that is excreted through the kidneys (Laios *et al.,* 2012; Eknoyan, 2006). In his writings, Jalinoos (131–201 AD) used the terms "Diarrhoe Urinosa (Diarrhoea of urine)" and "dipsakos (the thirsty disease)" to describe the illness (Pickup and Williams, 1997). Abu Bakr Muhammad Ibn (865–925 AD) offered remedies list for the management of obesity and polyuria. In the 11th century, "water tasters" who tested the urine of patients who were thought to

have diabetes made the diagnosis. Since diabetics' urine contains salt, Phillip Paracelsus (1493–1541) reported that this is the cause of their polyuria and kidney thirst, in the 16th century (Harikumar *et al.,* 2015; Pickup and Williams, 1997). He was the first to recognize diabetes as a systemic disease. Thomas Willis (Britain) coined the name "Mellitus" or from "Honey", a Latin derivative (which means "Honeylike"), in 1675 after rediscovering the sweetness of patients' urine and blood—a condition that had previously been noted by the ancient Indians. This is where the modern history of diabetes begins. He accurately stated that the urine was "Wondrously or beautifully sweet as if enliven with sugar or honey" to characterize the urine that was high in glucose. In addition to being the first to describe the sugary quality of the urine, he used this word in differentiating between DM and diabetes insipidus that is similarly characterized by frequent urination (Poretsky, 2009).

In the modern era, the development of experimental medicine and the history of diabetes coexist. While Johann Conrada Brunner (1653 - 1727 AD) came dangerously near to discovering pancreatic diabetes in 1682 after incompletely removing a dog's pancreas, Thomas Sydenham (1624 - 1689 AD) conjectured that diabetes was a system disease occurring in the blood (Pickup and Williams, 1997). The first person to confirm the existence of excess brown sugar capable of fermentation in the urine and blood as the reason of their sweetness was Mathew Dobson (Britain; 1732–1784) in 1775 (Ahmed, 2002; Dobson, 1776). Thomas Crawley was the first to propose or indicate a connection between diabetes and the pancreas in 1788 (Vecchio *et al.,* 2018). British author John Rollo made it clear that diabetes is a stomach ailment, that cataract is caused by diabetes, and that some diabetics have an acetone-like odor to their breath (Kim and Kim, 2006).

The relationship between the food ingested by diabetics and the amount of sugar found in their urine was confirmed and verified by Rollo (Bernard, 1877). William Hyde Wollaston, an Englishman, noted the presence of sugar in diabetics' blood in 1806. The first person to determine and confirm that the sugar previously described in this condition is glucose was the French chemist Michael Eugene Chevreul in 1815, opening the door and possibility for its quantification for both therapeutic and diagnostic purposes (Fournier, 2011). The theory that diabetes is caused by an excess of glucose production and the involvement of the liver in glycogenesis were both established by Claude Bernard (France, 1813-1873), who also discovered the

glycogenic action of the liver and isolated glycogen in 1875 AD. Additionally, he described how the Central Nervous System (CNS) influences blood sugar levels, outlined the process of gluconeogenesis, and was the first to link diabetes with glycogen metabolism (Karamanou *et al.*, 2016; Bernard, 1850). The word "Coma" was used in the beginning to classify a diabetes complication by William Prout (1785–1859) in 1848. In 1862, Frederick Pavy, who lived from 1829 to 1911, used Fehling's test to establish and validate a measurable relationship between the severity of hyperglycemia and glucosuria (Rubin, 2016). Noyes, H. D. found "Retinitis" formation in glucosuric patients in 1809 (Pickup and Williams, 1997). The pancreatic islet cells and the tiny clusters of ductless cells were discovered by Paul Langerhans (1847–1888) in 1869 and in 1893 AD, Edouard Laguesse (1861 - 1927 AD) named the cluster of cells discovered by Langerhans as Islets of Langerhans to honour him and speculated that they might constitute the endocrine tissue (Pickup and Williams, 1997).

Dickinson W.H. described diabetes as "a disease of the nervous system" that manifests as the production of urine that contains sugar in 1815 AD. It was established that the pancreas was implicated in the development of the disease when Mering J.V. (1849–1980 AD) and Minkowski O. (1858–1921) discovered that pancreatectomized (pancreas removed) dogs developed deadly diabetes in addition to digestive disturbances. They then assumed DM as hormonal deficiency syndrome (Harikumar *et al.,* 2015). In 1901, a connection was established between the non-performance of the islet of Langerhans and subsequent development of diabetes, by Eugene L. Opie (1873–1962 AD) (Opie, 1901). The hormone that lowers blood sugar and is produced by islet tissue was given the name "Insulin" in 1909 by Jean De Meyer (Belgian, 1878–1934), which is derived from the Latin word "Insula" (Meyer, 1909).

The association between the islet of Langerhans and diabetes was confirmed by Moses Barron (1883–1974) in 1920. According to Barron, the islet secretes a hormone directly into the lymph or bloodstreams that regulates and controls carbohydrate metabolism Best C.H. (1899-1978 AD) and Sir. Banting F.G. (1891-1941 AD) demonstrated that insulin can effectively treat diabetic hyperglycemia. As a result of Sir Harold Harry Himsworth's discovery that there are two distinct forms of diabetes based on “sensitivity of insulin" (Himsworth, 1936a), new therapeutic options became available. In 1959, researchers distinguished between juvenile onset DM and adult

onset DM for the first time, identifying type 1 as being IDDM and adult onset DM as being NIDD.

Since January 1922, injections of insulin have been used to treat diabetes; the first recipient was 14-year-old Leonard Thomson, a "charity patient" at Toronto General Hospital. Lispro, novel fast-acting insulin for the treatment of DM, was introduced in August 1996 under the trade name Humalog. Although it was created in 1922, the FDA only authorized the use of metformin in the USA in 1995. Sulfonylureas, an oral drug, were created in the 1950s to treat type 2 diabetes and also Precose, in September 1995, an oral medication was also licensed for use by those with type 2 DM.

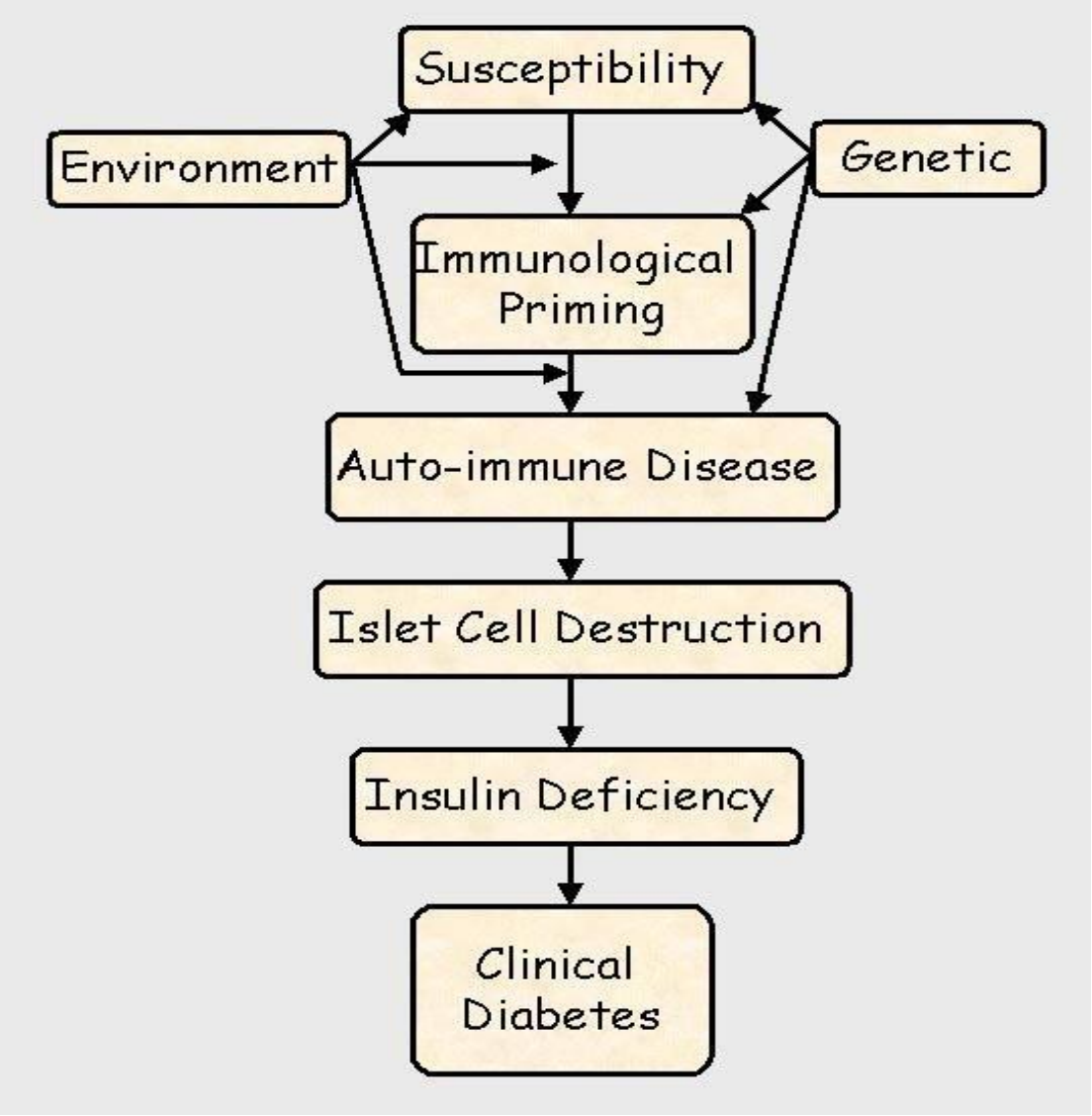


Figure 2.1: Possible Mechanism for Development of Type 1 Diabetes Source – ADA, 2018.

2.3 Classification of Diabetes Mellitus

While different forms of DM exist and illnesses that can cause diabetes, diabetes mellitus can be broadly categorised into two classes etiopathogenetically.

2.3.1 Type 1 Diabetes Mellitus

Previously, immune-mediated DM, juvenile-onset DM, or IDDM were the names this type of diabetes was known as. Absolute insulin insufficiency brought on by the complete loss of pancreatic cells characterizes this kind of diabetes. According to Goldenberg and Punthakee (2013), the cause or origin is due to auto-immune disruption or injury to pancreatic cells. In between five and ten percent of DM patients, are of type 1. Although the reason is connected to a total lack of insulin production, type 1 diabetes mellitus is now incurable even in persons with confirmed autoantibodies (ADA, 2018). This condition is typically identified in children and teenagers.

2.3.2 Type 2 Diabetes Mellitus

This kind of DM was formerly regarded as NIDDM or adult onst DM. It is responsible for approximately 90-95% of entire population of diabetics. Numerous interplay of factors including genetic predisposition, sedentary lifestyle, environmental influences, excessive body fat and obesity, are responsible for inducing this type of diabetes. Exact causes or etiologies cannot be pin-pointed but many people with this kind of diabetes are noticed to be obese, and obesity on its own promotes some degree of insulin resistance (ADA, 2010). Type 2 diabetics do not require insulin therapy to survive either in the early stages or throughout the course of their life. Mak and De Fronzo (1992) noted that type 2 DM is distinguished due to considerable impaired insulin sensitivity, as well as irregular or impaired insulin secretion patterns that lead to long term high blood sugar. To clarify and comprehend the various factors that lend to the occurrence of type 2 diabetes, numerous postulations have been put up.

Postulation 1

This suggests that the first step in glucose disposal may be constrained, which frequently are observed in obese people. Notion claiming that a great number of people with this type of DM (type 2) have excessive weight may partly provide reason for ineffective uptake of glucose (ADA, 2009). It is observed that reduction in sensitivity of insulin receptors as well as in their functionality has been connected with the presence of excessive weight. According to Muoio (2010), insulin insensitivity,

hyperinsulinemia, other disease states such as heart and circulatory disease; degenerative and metabolic problems, have all been linked to type 2 diabetes' abdominal obesity, also referred to as central body obesity.

Postulation 2

Relationship exists between phosphorylation and adult onset DM and it is a complicated one which contributes critically in pathophysiology of DM. Numerous cellular activities including gene expression, signal transduction, and metabolism are regulated supremely by phosphorylation. In type 2 DM, phosphorylation has to do with insulin signaling pathway and glucose metabolism.

This concept states that the reduction or failure of the glucose transport mechanism results from the process of intracellular phosphorylation, which appears to be disturbed and may lend to the occurrence of type 2 DM (Kelley *et al.,* 1996). The probable consequence of this is a reduction in the amount of glucose that is transported inward and an escalation in the concentration of sugar in the bloodstream for kidney elimination. Blood sugar concentration increases due to reduced sugar transport to cell surface. Resulting hyperglycemia may cause glucose poisoning due to the fact that hyperglycemia affects synthesis of insulin thus exacerbating insulin resistance. By serially impairing the β-cells' capacity to release insulin, glucose toxicity "incapacitates" them (Consoli, 1992). Treatment for glycemic control may be able to change the weakening process for the better but may not be able to undo the harm already done (Kelley *et al.,* 1996).

According to Gibney *et al.,* 2004, increased waist circumference and the ratio of the waist to the hips, as well as increased hip skinfold thickness, are ideal markers for abdominal obesity. These researchers went on to say that there is an elevated risk of metabolic problems in people with waist circumferences over 41 inches for men and 35 inches for women.

According to Nydahl *et al.,* 1993, research on the "Syndrome X", which is associated with a number of issues relating to insulin resistance and/or hyperinsulinemia and obesity, a high central fat distribution, hypertension, and dyslipidemia, has opened up the fact that there is a connection between abdominal fat, hypertension, and insensitivity of insulin. Loss of power by β-cells of pancreas, insulin resistance as well

as uncontrolled hepatic glucose/glycogen synthesis, make up the trio of this kind of DM.

2.3.2.1 Common History of Type 2 Diabetes

90–95% of diagnosed instances of diabetes are people suffering from adult onset DM. Main problem of type 2 diadetics is insulin resistance (Rewers and Hamman, 1995). The quantity of insulin secreted by the pancreatic cells to make up for a loss in the body's insulin sensitivity is increased in a proportional manner. The compensation may initially be effective and maintain normal glucose levels for many years, but as the cell capacity fails, its effectiveness decreases. Clinically, this failure can be identified by a combination of mild postprandial blood glucose increase and reduced glucose tolerance. The function of pancreatic cells declines as insulin resistance worsens, impairing their ability to control the generation of hepatic glucose. The blood glucose level rises as a result of all these abnormalities. Hyperglycemia can be brought on by β-cell malfunction and insulin resistance, and this can progress to overt type 2 DM. The first asymptomatic stage with normal blood glucose can progress to high blood glucose necessitating medication intervention.

Understanding the pathological variations between the early (insulin resistance) and late (insulinopenic) stages of the disease's development requires knowledge of the common progression of DM. Awareness and understanding of various stages involved in DM are essential for choosing treatment goals and altering treatment strategies. It may take a very long time for DM to advance and proceed from the asymptomatic stage through a lot of phases to visible diabetes. Numerous lifestyle changes, such as preserving a healthy body weight is good and advocated in this kind of DM. It is currently believed or assumed that drug therapies aimed at correcting chronic low-grade inflammation may also be a realistic or practicable treatment objective.

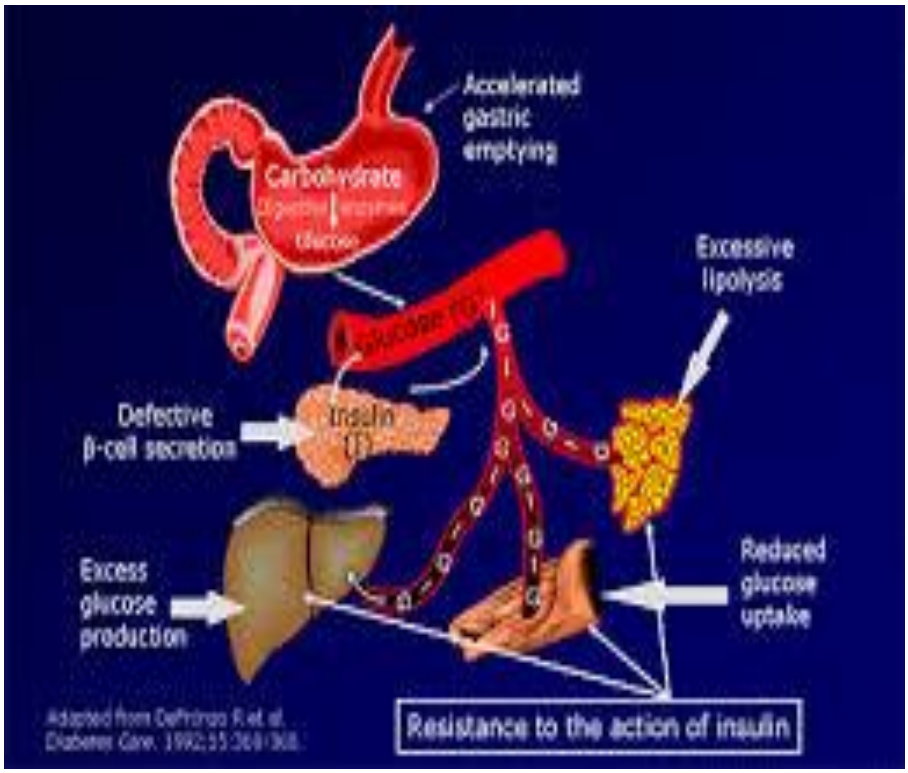


Figure 2.2: Pathophysiology of Type 2 Diabetes
Source: Adapted from Defronzo *et al.*, 1992.

2.3.2.2 Type 2 Diabetes Mellitus and Insulin Resistance

Connection exists between adult onset DM and insulin resistance and for well over 50 years this is known. In addition to being the most accurate forecaster of subsequent occurrence of this type of disease insulin resistance is not only essential in the development of DM but also a therapeutic target when hyperglycemia is present (Galicia-Garcia *et al*., 2020). Muscle insulin resistance has a hereditary component that can be observed in the general population. Exercise can improve mitochondrial dysfunction and lessen resistance to insulin (Sangwung *et al*., 2020; Kim *et al.,* 2008).

2.3.3 Other Forms of Diabetes Mellitus

About 5% in all instances of DM that have been diagnosed fall into the category of type 3 diabetes.

Type 3 diabetes mellitus is the general term employed when Alzheimer's disease is triggered by insulin resistance in the brain. The World Health Organisation (WHO) has characterised and categorised the types of diabetes that make up Type 3. Genetic defect in β-cells is associated with type 3A DM (examples are maternally inherited DM, MODY, and deafness). Type 3B DM is linked to a genetic form of insulin resistance (example is leprechaunism, commonly reffered to as Donohue syndrome), and Type 3C is linked to pancreatic conditions and injuries (including pancreatitis, pancreatic cancer, hemochromatosis, cystic fibrosis, and pancreatectomy) (Kim *et al.,* 2008).

This is the result of pancreatitis, a pancreatic tumor, cystic fibrosis, and trauma injuring a pancreas that was previously healthy. As a result, the pancreas is unable to make sufficient amounts of insulin, digestive enzymes, and other hormones. When pancreatic inflammation, neoplasia, or resection causes β-cell malfunction that affects insulin secretion, diabetes of the exocrine pancreas results. Type 3D, Type 3E, Type 3F, and Type 3G are caused, in that order, by infections (Congenital rubella), chemicals and pharmaceuticals (Glucocorticoids, β-Blockers), hormonal abnormalities (such as Cushing's sickness and syndrome), and a rare kind of diabetes mellitus occurring due to activity of the immune system (Stiff-Person syndrome).

2.4 Type 4 Diabetes Mellitus – Gestational Diabetes Mellitus

Landon and Gabe (2011) and the American Diabetes Association (2021) both states that gestational diabetes mellitus (GDM), which affects two to five percent of pregnancies, is the root cause of 90-95% of all cases of pregnancy-related DM.

Diabetes is a health issue that is alarmingly spreading over the world (Bener *et al.,* 2011; Ferrara, 2007). The term "gestational DM" refers to glucose intolerance that emerges prior to, throughout, and after pregnancy (ADA, 2021; Bhattacharya *et al*., 2022; Landon *et al.,* 2009). According to this definition, gestational DM is the absence of diabetes before pregnancy in pregnant women. When you experience hyperglycemia (high blood glucose levels) while pregnant, you develop gestational diabetes. It normally begins to occur in the third trimester (between 24 and 28 weeks) and usually goes away after delivery. Type 2 DM is likely to progress in women who develop gestational diabetes during pregnancy. To transport blood glucose into the cells, where it may be utilised for generation of energy insulin is necessary if the body is unable to synthesis enough insulin, sugar from the meal taken will linger in the bloodstream and cause high blood glucose concentration.

Insulin controls or maintains maternal metabolism, and due to hormonal changes during pregnancy, its physiology is changed (Lowe and Karban, 2014). Due to hormonal changes, a pregnant woman needs two to three times more insulin from the week 13 of the pregnancy to the end of pregnancy than a non-pregnant woman (hormones released by the placenta that resist insulin) and the demands of the fetus's growth. Cortisol, estrogen, progesterone, and growth hormones prevent insulin from working by inhibiting it, which makes it resistant to insulin (Feig *et al.,* 2008). In the body, β-cells store and release insulin, but when insulin resistance arises, β-cells are unable to overcome their resistance (Cerf*,* 2013), this causes gestational diabetes mellitus (Kapustin, 2008). According to Feig *et al.,* 2008, in pregnancy, insulin resistance is highest from the week 13 of the pregnancy to the end of pregnancy. Gestational diabetes affects 2.98 out of every 1000 pregnancies in Nigeria, according to the research of Wokoma *et al.,* in 2001.

However, Ewenighi *et al.,* 2013 found that the prevalence increased with maternal age, with rates of 3.3% in the 15–24 year age range, 4.2% in the 25–34 year range, and a peak of 17.6% in the 34–44 year range. The estimated prevalence is 4.2% on average.

2.5 Pathogenesis of Insulin Resistance

Pancreatic β-cells secret insulin which is a peptide hormone. Major purpose of insulin is to keep blood sugar levels balanced. According to Kahn *et al.,* 2014, insulin impacts

the metabolism of glucose, amino acids, and fats and primarily acts on the hepatic, muscle, and fat tissues, where it fixes to the cell surface insulin receptors. This initiates a signaling cascade that turns on anabolic metabolism and the Krebs cycle's intake of glucose for cellular energy. By blocking gluconeogenesis and glycogenolysis, insulin reduces the amount of glucose that the liver produces, favouring the storage of glycogen instead. The anabolic effects of insulin cause protein synthesis to maintain muscle mass and energy storage in adipose tissue (Hers, 1990). Poor or insufficient response to a specific concentration of insulin is a hallmark of insulin resistance (Himsworth, 1936b), one of the acknowledged factors in the pathophysiology of diabetes.

Higher insulin to sugar ratios imply greater insulin resistance when comparing fasting blood sugar and insulin levels, which is a method for detecting insulin resistance indirectly. Insulin resistance at the hepatic and muscular level is the distinctive feature of glucose intolerance in persons with DM type 2. To get glucose to the muscle and fat tissue, insulin is necessary. In absence of insulin signaling in muscle, production of glucose transporters and absorption of insulin from the blood stream are inhibited (Wheatcroft *et al.,* 2003). Even if fasting insulin and glucose levels are high, the primary source of insulin resistance is the liver, which occurs in the baseline state, and the absence of insulin signaling to inhibit gluconeogenesis results in irregular overproduction of glucose.

Therefore, the primary factor causing a high fasting plasma glucose concentration is the liver's increased rate of glucose synthesis. Insulin resistance results from both reduced regulation of production of glucose by the liver and decreased absorption of glucose by the muscles after consumption of glucose (Cersosimo *et al.,* 2018).

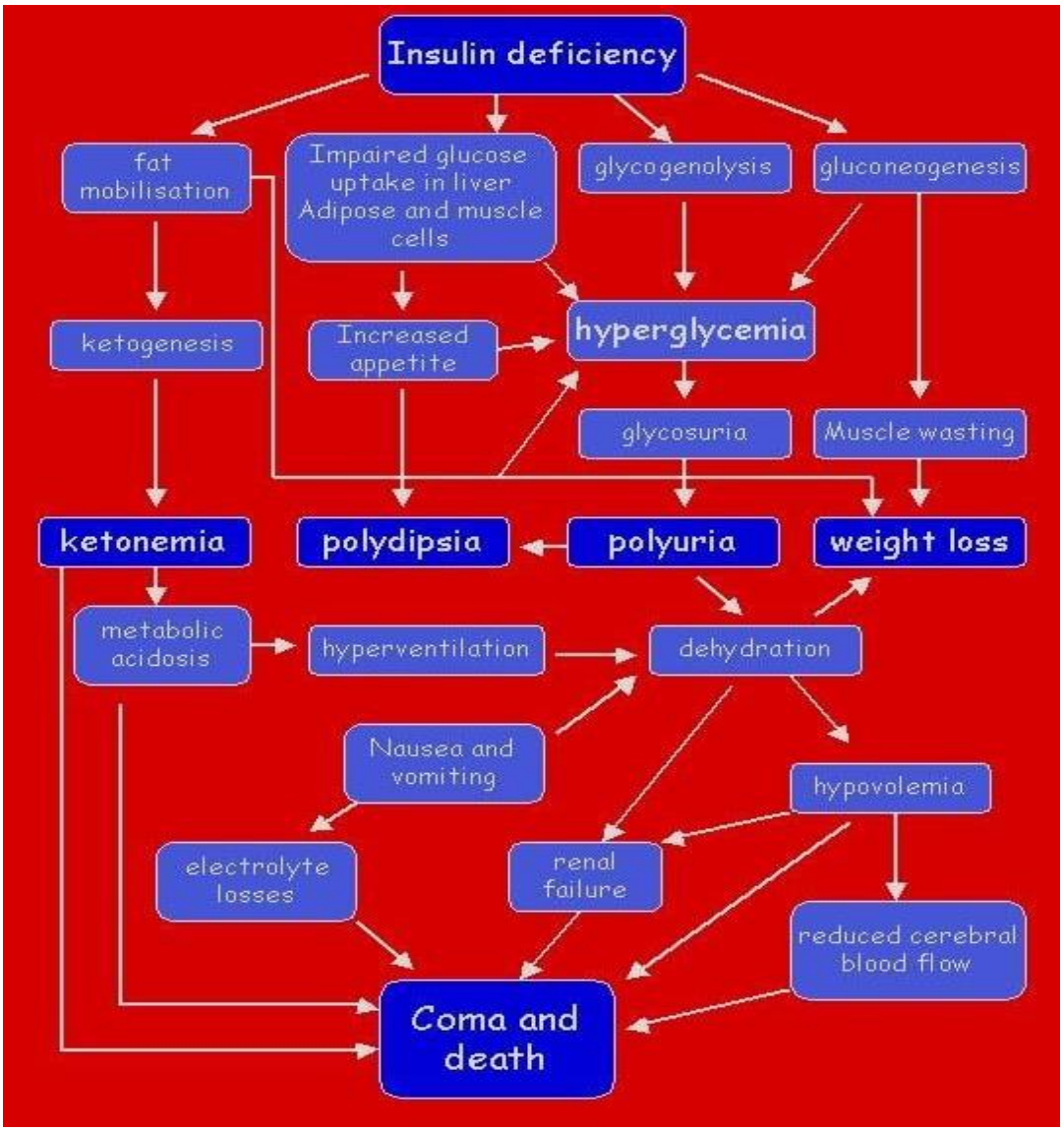


Figure 2.3: Effects of Insulin Deficiency
Source – ADA, 2018.

2.6 Etiology of Insulin Resistance

There are numerous known insulin resistance causes, and many of them are treatable. Alterations in lifestyle and faulty genes that are essential for insulin function are among the causes of insulin resistance. One or more specific hormonal or metabolic factors, such as an excess of glucocorticoids (both endogenous and exogenous), an excess of growth hormones, such as acromegaly, an excess of catecholamine, or an excess of glucagon, can also be the reason. Reversible factors connected with inflammation, including sepsis, fever and infections, are also linked to insulin resistance.

Environmental factors are now being identified as insulin resistance contributors. With the movement of ethnic groups from a developing portion of the world to a more developed and westernized region, it has been noted that the prevalence of insulin resistance rises. Japanese Americans in Seattle, USA, have been found to have a four times higher frequency of DM type 2 than Japanese citizens in Tokyo (Fujimoto *et al*., 1994). There are various other factors that lend to insulin resistance in addition to BM1 and ethnicity (Fujimoto *et al.,* 2000; Fujimoto *et al.,* 1991; Fujimoto *et al.,* 1989; Fujimoto *et al.,* 1987). Insulin resistance has several causes, with inflammation appearing as one of them. Insulin resistance is correlated with age, sex, and race.

2.7 Signs and Symptoms of Diabetes Mellitus

Both types of diabetes have warning indicators just like for any other condition or disease. It is important to recognize or differentiate between a sign and a symptom. Both signs and symptoms are indicators that something is wrong with the body, but there is a distinction between the two in terms of medicine (Ramachandran, 2014). The only people who can identify and recognize symptoms are those who are really experiencing them. Examples include experiencing pain, nausea, dizziness, and problems with eyesight or sight.

Inner symptoms are felt. Symptoms are arbitrary signs of illness. Only when they are notified by the affected person can other people become aware of the indicators (Abdul-Ghani and DeFronzo, 2009). Externally visible indicators are called signs. Moisture, colour, temperature, heart rate, and other parameters must be seen and interpreted by another person. Family members or medical staff can view or read these. They serve as warning signs. Signs are observable indications of a disease, such as a cough or skin rash (Abdul-Ghani and DeFronzo, 2009). If there is overlap, there can be

concurrent symptoms and signs. Although many people frequently ignore them, there are warning signs and symptoms that can assist to suspect or discover diabetes. This chronic disease progression is made possible by disregarding the symptoms and warning indications or signs. Because of the late development of the condition and the lack of knowledge about the effects or consequences of hyperglycemia, many people do not take these signs and symptoms seriously. Most of the time, people are unaware that damage may have already occurred years before symptoms appear. Early diagnosis of the disease's signs and symptoms would help to control it and avert vascular consequences. The best defense against diabetes and its complications is early detection and attention to warning symptoms (Ramachandran, 2014).

These signs include chronic fatigue syndrome, unexplained weight loss, irritability, oral cavity and dry mouth, impaired visual acuity, sexual incompetence, *Acanthosis nigricans*, evidence of obesity, burning pain, not forgetting numbness on the feet. The typical signs of undiagnosed diabetes include weariness, restlessness, unexplained weight loss, and physical pain (Ramachandran, 2014). Polyuria (frequent urine), polydipsia (severe thirst), and polyphagia (extreme hunger) are unique hallmark of DM. The afore-discussed warning indicators of type 1 DM might occur spontaneously or quickly, whereas the above-discussed warning indicators of type 2 diabetes can appear slowly. Years may go by while a person has type 2 DM without being aware of it or knowing it. About 50% of those with type 2 DM have no symptoms and are unaware that they are sick.

Usually, gestational diabetes has no noticeable signs or symptoms. The majority of pregnant women do not exhibit or display signs or symptoms of gestational diabetes, so doctors and other medical professionals always want to examine pregnant women for the condition. However, some expectant women do experience a few modest gestational diabetes signs or symptoms that are quite similar to those of the other types of diabetes. These warning signs and symptoms include:

(i) Feeling thirsty and drinking more than normal.
(ii) Feeling worn out, especially in the morning, which is different from the usual pregnancy weariness.
(iii) Frequent and excessive urinating
(iv) Urine sample showing sugar presence

(v) Dry mouth goes hand in hand with increased thirst. More water is being sought after than before.

2.8 Etiology of Diabetes Mellitus

This review of literature discusses the prevalence and consequences of diabetes, type 2 diabetes complications, prevention of DM and its complications, management through insulin and pharmaceutical interventions, herbs, exercise, and lifestyle change, as well as factors that are related to causes of diabetes mellitus like reactive nitrogen species (RNS), reactive oxygen species (ROS), and redox imbalance. According to Alberti and Zimmet (1998), diabetes mellitus can result from either inadequate insulin production, insulin resistance, or a combination of both. In addition to being partly inherited, stress, illnesses, and environmental factors like exposure to a causal agent can also cause diabetes mellitus.

The susceptibility of individuals to some of the triggers is genetically determined. HLA class 11 genes (HLA-DR, DQ, and DP) have been directly linked to the genetic component causing this vulnerability. IDDM is the name given to the HLA class 11 genes (Redondo *et al.,* 2018). According to research by Kooti *et al.,* 2016, free radicals, which are exceedingly harmful to health, are believed to play a role in the emergence of diabetes mellitus. According to Monnier and Colette, 2008, the key factors causing peripheral and cadiovacular diseases; retinopathy, neuropathy, and nephropathy in DM, especially in type 2 diabetes, are excessive protein glycation and redox imbalance.

2.8.1 Genetic Vulnerability and Hereditary Predisposition

Genetics is important since genetics heavily influences who will likely acquire type 1 DM. Usually, true parents frequently pass on their genes to their offspring. The chance of developing type 1 DM is correlated with presence of certain gene types that encode for the synthesis of cell-surface proteins called Human Leukocyte Antigens (HLAs), which are resident on white blood cells (Harikumar *et al.,* 2015; Zimmet*,* 1995; Japan and Pittsburgh Childhood Diabetes Research group, 1985). The likelihood of getting type-1 DM increases since the specific HLA-DQA1, HLA-DQB1 and HLA–DRBI gene variances are present. These genes encode information for the synthesis of macromolecules that are indispensable in immune system. White blood cells known as "t cells" target and kill β-cells in Type 1 diabetes, a condition known as auto-immune destruction of β- cells. Although it has been hypothesized that environmental variables,

such as meals, viruses, and toxins, are instrumental to acquiring type 1 DM but their definite roles are yet to be elucidated. Although viruses cannot directly cause DM by themselves, but a correlation between viruses and type 1 diabetes is evident given the high number of people who are diagnosed with the condition during or after a viral illness. Coxsackievirus B, Cytomegalovirus, Adenovirus, Rubella, and Mumps are some examples of such viruses. Dietary conditions may also raise or lower the chance of developing type 1 diabetes mellitus. For example, infants who breastfed and those who are given dietary booster like vitamin D may have a lower chance of developing the disease, whereas infants exposed to cow's milk and cereal proteins at birth may have a higher chance of doing so (Harikumar *et al.,* 2015; Hother-Nielson *et al.,* 1988).

According to numerous studies, offspring of type 1 DM fathers are more vulnerable to acquire autoimmune DM than offspring of type 1 DM mothers. Type 1DM patients share the fever tendencies of other family members who have the disease. Numerous additional loci linked with type 2 diabetes mellitus have been found thanks to the development of genome-wide association screening (Majithia and Florez, 2009). The clinical assessment of type 2 DM employed genetic statistics from the family history (Majithia and Florez, 2009). Researchers have discovered that type 2 DM has a powerful hereditary part including monogenic forms of non-ketogenic DM for example Maturity onset diabetes of the young (MODY) 1-6 under 25 years of age and polygenic diseases like common type 2 DM (Majithia and Florez, 2009; Kota *et al*, 2012).

According to studies by Majithia and Florez, 2009; Groop and Tuomi, 1997, the overall community has a lifelong risk of type 2 DM of about 7%, children with one diabetic parent have a 40% risk and children with both parents having the condition have a 70% likelihood. According to Lyssenko *et al.,* 2008, there is a two-fold greater chance of developing type 2 DM in first-degree families with the background of the condition. 20 well-known genetic variations that are frequently linked to type 2 DM have been discovered through genetic research such as loci with common allele detection, assessing genetic types utilising candidate gene techniques, and comprehensive genome-wide association studies (GWAS) on type 2 DM and obesity (Riddeerstrale and Groop, 2009).

Despite the fact that just only one gene has a definite connection to insulin resistance; numerous genes have been implicated in playing significant roles in the etiologies of

type 2 DM. Eight genes namely CDKAL1, CDKN2A/2B, IGF2BP2, KCNQ, KCNJII, TCF7L2, SLC30A8, and HHEX, are among the loci that appear to influence β-cells' capacity to secrete more insulin while responding to a physiological change in the system milieu. PPARG is associated with insulin sensitivity, whereas CAPNIO gene functions in facilitated diffusion. While fat mass, melanocortin 4 receptor and obesity-associated genes are connected to obesity, 8 other loci lack any discernible activity in type 2 DM (Ridderstrale and Groop, 2009). It is noteworthy that 38% of siblings and 33.33% of kids of type 2 diabetics would eventually have the disease or impaired glucose metabolism. Identical twin studies have revealed that when diabetes strikes one, it strikes the other 90–100% of the time, as opposed to 50% of type 1 diabetes mellitus participants, as claimed by numerous researchers.

2.9 Diagnosis of Diabetes Mellitus

Early diagnosis is beneficial because it will enhance the patient's state of health. The following techniques may be employed in diagnosis of DM and pre-diabetes.

2.9.1 Fasting Blood Glucose Test – After a fast of at least eight hours, this measures or determines blood sugar. This test is helpful in identifying pre-DM and DM problems and is most accurate when performed in the morning (ADA, 2022).

2.9.2 Oral Glucose Tolerance Test – This is also known as plasma sugar test. This checks blood sugar levels after a minimum of eight hours of fasting and two hours after consuming a drink that contains glucose (beverage or water). This approach may be employed in detecting DM or pre-DM condition. (It’s considered a more sensitive procedure than the fasting blood glucose test). Another method for diagnosing gestational diabetes is to use the plasma glucose value obtained during the OGTT. In this instance, the blood glucose concentrations are observed roughly on 4 occasions during the period of investigation and gestational DM is present if the blood sugar levels are above normal at least twice (ADA, 2022).

2.9.3 Random Plasma Glucose Test – This is accomplished by randomly monitoring blood sugar levels not taking into cognizance the time of the last food. Procedure is not engaged in determining pre-DM; rather, it is only used to confirm DM when it is present alone or in combination with other symptoms (ADA, 2022).

2.9.4 Glycated Hemoglobin Test – A glycated hemoglobin reading of greater than 6.5% can be used to diagnose diabetes using the HPLC assay. Zinc transporter and autoantibody (ZnT8Ab) blood testing is advised when a person is first diagnosed with

DM as to ascertain if it is type 1 DM or any other form of DM. ZnT8Ab testing is beneficial and required to enable accurate diagnosis and prompt therapy (ADA, 2022).

2.10 Global Diagnostic Criteria

Diabetes mellitus investigation depends on exhibition of chronic high glucose concentration according to the definition of the World Health Organisation (WHO) criteria (Alberti and Zimmet, 1998), and the testing can be accomplished by any one of the procedures previously described above.

2.10.1 Fasting Plasma Glucose Test

The American Diabetes Association (ADA) and World Health Organisation (WHO) committee consented that the diagnostic level of the fasting blood glucose should be fixed at >7.0 mmoll/L (>126 mg/dL) venous plasma or >6.1 mmol/L (110 mg/dL) venous whole blood after observing eight to ten hours of fasting overnight (IEC, 2009).

Impaired fasting glucose (IFG), a type of pre-DM, is present when the fasting blood sugar level is between 100 and 125 mg/dL (5.6 and 6.9 mmol/L), indicating a high likelihood of developing type 2DM. FPG readings under 100mg/dL are considered normal.

Table 2.1 – Fasting Plasma Glucose Test

Reading (mg/dL)	Status
≤ 99	Normal
100 – 125	Pre-DM (IFG)
≥ 126	DM

***Source*: American Diabetes Association, 2014. WebMD LLC**

The procedure should be conducted a second time the following day for confirmation if the result was as indicated (i.e. > 126 mg/dL).

2.10.2. Oral Glucose Tolerance Test (OGTT)

Prior to undertaking the OGTT test, it is demanded that one fasts for a minimum of 8-10 hrs. Plasma glucose concentration is estimated immediately preceding and 2 hrs. Post consumption of a liquid containing 75 g of dissolved glucose.

Blood glucose concentration between 140 and 190 mg/dL two hours after consuming the liquid indicates pre-DM, also referred to as impaired glucose tolerance (IGT), but not yet evident. It demonstrates the likelihood of developing type 2 DM.

If a further test confirms the same outcome subsequently, then a blood sugar level of 200 mg/dL or higher indicates that a person has diabetes.

Plasma glucose readings obtained during the OGTT are used to diagnose gestational DM based on the same premise.

Table 2.2 – Oral Glucose Tolerance Test

2Hrs- Reading (mg/dL)	Status
≤ 139	Normal
140 – 199	Pre-DM (IGT)
≥ 200	DM

Source: American Diabetes Association, 2014 WebMD LLC

A repeat test at a subsequent date with same outcome of 200mg/dL or higher suggests that the person has DM.

Table 2.3 – Gestational Diabetes

Period	Plasma Glucose- Result (mg/dL)
Fasting	≥ 95
At 60 minutes	≥ 180
At 120 minutes	≥ 155
At 180 minutes	≥140

Source: American Diabetes Association, 2014 WebMD LLC

2.10.3 Random Plasma Glucose Test

A single random measurement of circulating glucose, typically 200 mg/dL, is normally diagnostic when the patient exhibits the typical symptoms of polyuria, polydipsia, unexplained weight loss (despite nutrient intake), tiredness, impaired visual acuity, famine, drowsiness, and unhealing wound. A second or third blood glucose reading should be used to confirm the diagnosis when the symptoms are missing. A plasma venous glucose level exceeding 200 mg/dL (or 11 mmol/L) or a whole blood venous glucose level of 10 mmol/L (or 180 mg/dL) or greater is regarded as diagnostic two hours after a 75 g glucose load. If the outcome shows that a person has DM, then FPG or OGTT is repeated or done once more for confirmation on a different day (ADA, 2009).

2.10.4 Glycated Heamoglobin

Glycated heamoglobin, HbA1C referred to as is a type of hemoglobin that measures the average blood glucose concentration over a long time. The hemoglobin is the glycosylated form of it and represents a major type of adult haemoglobbins. The HbA1c concentration in the blood correlates directly with the average level of glucose in a patient's blood over sixty-to ninety days and makes it an excellent measuring standard for long term controlof diabetics. Glycated heamoglobin at any point in time represents the mean blood glucose level for the past 2-3 months since an average lifespan of a red cell is approximately 4 months. The analysis of the glycated heamoglobin can provide a 90 days' time-averaged picture for a clinician on how much glucose is present in blood amongst patients (Sidorenkov *et al.,* 2011).

Heamoglobin is a protein present in erythrocytes carrying oxygen from the lungs to other destinations in the body. When glucose is found in the bloodstream, it can get attached to the hemoglobin molecule then forming glycated heamoglobin. There is interaction between glucose and the amino group of the heamoglobin. The production of a ketoamine is caused by a non-enzymatic binding of the glucose molecule to haemoglobin. As the blood glucose level increases or rises the quantity of heamoglobin that is attached also increases. The synthesis rate is directly linked to the levels of plasma glucose.

Steps involved in determining HbA1C include collection of blood from the patient, estimation of the concentration of the HbA1C in the laboratory employing specialized

approaches, reporting the result as percentage. HbA1C for normal person is between four and half percent to five and half percent. In diabetics, the level is a function of numerous factors such as the overall health status, attendant complications, and age but it is reasonable to keep the level below seven percent so as to reduce the risk of protracted complication. Higher levels of HbA1C show inappropriate blood sugar control over the past ninety days.

HbA1C is only one of the tools used to keep an eye on DM, although it has been recommended as a dependable replacement to fasting plasma glucose for detecting high blood glucose and keeping an eye on glycemic control, especially in type 2 DM. Due to the multifactorial nature of DM, other recommended beneficial tests include renal function testing, cholesterol assessment, protein analysis, eye testing, and neuron and nephron assessment. Although OGTT is the gold standard for diagnosing DM, FPG and RPG are useful, inexpensive, and user-friendly techniques, particularly in situations where OGTT cannot be used (ADA, 2009).

2.11 Glycated Heamoglobin-Historical Development and Significance

In the early 1900s, researchers observed that the blood of diabetics showed propensity to become darker and sticky and that the stickiness is associated with the presence of sugar in the blood.

In 1931, two German Biochemists namely Leonor Michaelis and Maud Menten discovered that glucose has the ability to combine with heamoglobin and form a stable compound while in 1958, two Dutch researchers namely Francis John de Bruijn Meyering and Samuel Rahbar Huisaman did pioneering work of identifying and characterizing the first glycated heamoglobin variant denoted as HbA1a in diabetics. In 1968, Samuel Rahbar an Iranian-American Biochemist discovered multiple forms of glycated heamoglobin and showed that their levels rise in diabetics. He suggested that estimation of glycated heamoglobin may be a standard for blood sugar control whereas in the younger part of 1970s, the term HbA1C was conceived by a group of researchers and measuring HbA1C to obtain data on individual's blood glucose level over a period of time becomes useful (Gebel, 2012). Between 1983 and 1993, the Diabetes Control and Complication Trial (DCCT) clinical study finally revealed the association between HbA1C levels and the risk of DM-related complications. DCCT research finding confirmed HbA1C as a vital marker in monitoring diabetes management and in setting of glycemic targets (Nathan and DCCT/EDIC Research Group, 2014). This noble

discovery has transformed diabetes management, making available a detailed understanding of blood glucose control.

An international specialist group with members from the International Diabetes Federation (IDF), American Diabetes Association (ADA), and European Association for the Study of Diabetes not long ago suggested measuring HbA1C with a ceiling of > 6.5% to diagnose DM (Pani *et al.,* 2020; American Diabetes Association, 2010). The ADA accepted and adopted this approach in 2010 (American Diabetes Association, 2010).

People with various renal disorders or prolonged infections will typically have aberrant RBCs survival and reduced HbA1C, which will make it difficult to utilise HbA1C to gauge their glucose consumption. The same is also available to those who have hemoglobinopathies and iron deficient anemia, especially in Africa (American Diabetes Association, 2010). Epidemiological data suggests a link between high HbA1C levels and the risk of developing coronary and ischemic heart disease (Gao *et al.,* 2008).

2.12 Effect of Diabetes Mellitus

Global

Around the world, the occurrence of DM—particularly type 2 DM—is increasing at a worrisome rate. In 1985, about thirty million (30) people were suffering from diabetes and the figure rose to two hundred and thirty million (230) people which is roughly six percent (6%) of global population, by the end of year 2006. 184,000,000 people representing eighty percent (80%) of this figure are domiciled in the developing world (Oguejiofor *et al.,* 2014; Roglic *et al.,* 2005; King *et al.,* 1995). In 2011, in accordance with IDF's guesstimate, three hundred and thirty-six (336) million adults between the age of 20 – 79 year, out of the global population of seven billion people were suffering from diabetes mellitus (IDF, 2015).

Worldwide, over ninety percent (90%) of cases of DM, the number of sufferer showing classical symptoms of polyphagia, polydipsia, weight loss and polyuria is negligible in contrast to the frequency of asymptomatic or unidentified DM. It has been projected by the World Health Organisation, that DM is responsible for 3.8 million deaths yearly, a number that is the same size as the mortality recorded for HIV/AIDS (WHO, 2013).

In developed and poor countries around the world, there are differences in the causes of mortality in DM. While coronary disease is the principal origin of mortality in the diabetic community in advanced countries (Cusick *et al.,* 2005; Winer and Sowers, 2004), in the developing nations such as African countries, the leading causes of mortality in diabetic populations are infections and severe metabolic disorders, not coronary or kidney issues (Azevedo and Alla, 2008). The leading causes of mortality in Nigeria include diabetic complications such as ketoacidosis, hyperglycemic hyperosmolar, and hypoglycemia. Globally, the cost of diabetes care is very high and about USD 465 billion is expended on that annually

Africa

Before now, Africa was thought to be free from the so called "diseases of affluence" ravaging the developed nations, particularly DM. Medical records from nineteen fifty-nine until the middle of the 1980s indicated that less than 2% of Africans had DM, the estimate went up to 3.6 % in South Africa in 2011, although (Motala *et al.,* 2003). In the year 2000, about 7.1 million Africans were reported to be diabetic with a projection of 18.6 million people by 2030 (Wild *et al.,* 2004). In 2011, 14.7 million Africans ranging in age from 20-79 are diabetic and this is projected to 28 million in 2030. In Africa between 70 % - 90 % of diabetes cases are type 2 (Stephani *et al*., 2018; Azevedo and Alla, 2008; Levitt, 2008) and it's more common among the rich people, thus tagged "illness of the rich". Diabetics are likely to be found in the cities because here people tend not to exercise yet they eat food laden with saturated fat and refined sugar making them obese. Although there has been a global warning, the situation with DM on the continent of Africa and in other places where Africans reside is getting worse every day.

Nigeria

The seventh most populated country in the world is Nigeria, which has the highest population in Africa. The current population of Nigeria is very much over 170 million people (185, 989, 640 according to 2017 revision of the world population prospects) and counting with about seventy-six (76) million adult and roughly 3.1 million people with DM (Adeloye *et al*., 2021). According to IDF report 2010, the estimated prevalence for Nigeria was 3.9 % and number grew to 4.9 %, more than doubling the prior national frequency of 2.2 % (Akinkugbe, 1997). However, according to Bashir *et al.,* 2021, currently the pooled prevalence of pre-diabetes in Nigeria is 13.2 % while

World Bank collection of development indicators reported diabetes prevalence of 3.6 % (ages 20-79) for Nigeria in 2021.

The risk factors for DM in Nigeria include poor family background, city living, eating unhealthy food, smoking cigarettes regularly, becoming older, being overweight, and not exercising. A metropolitan lifestyle, eating bad food, and advancing age are the leading risk factors for DM in Nigeria. Bad food which is primarily composed of bad cholesterol and energy laden foods lends to the induction of obesity and ultimately DM. Poor eating nature is the most common of the risk factors for DM in Nigeria and this can be attributed to the innumerable fast food outlets in a lot of cities within Nigeria. Urbanisation has been linked to a reduction in activity, which can solely result in metabolic syndrome (Assah *et al.,* 2011). In Nigeria, age-related physical activity and decreased response to insulin are recognised as major risk factors for type 2 diabetes.

Recommendation – It is highly advised that there be national diabetes preventive and care policies.

2.13 Risk Factors for Diabetes Mellitus

2.13.1 Risk Factors for Type 1 Diabetes

Though the definite cause or causes of type 1 DM are not known for certain, but the factors that can cause increased risk include the followings:

(a) Family History – The likelihood that someone may develop type 1 DM increases if their biological father, mother, brother, sister, or other family members have the disease (Vehik *et al*., 2007).

(b) The existence of immunodestructive cells (Autoantibodies). An individual is more likely to develop type 1 DM if they have autoantibodies. Though it is not everyone with autoantibodies that develops diabetes mellitus.

(c) Environmental Factors – Situations whereby someone is exposed to viral diseases or illness can activate or spark-off type 1 diabetes (Ilonen *et al*., 2013).

(d) Geography – Location seems to matter. The prevalence of type 1 DM has been found to be greater in Finland and Sweden (Samuelsson *et al*., 2019).

2.13.2 Risk Factors for Type 2 Diabetes and Pre-Diabetes

Factors that can lead to type 2 DM or to pre-DM include;

(a) Family History – If the biological mother, father, brother, or sister has type 2 DM, the risk is increased (Amuta *et al*., 2017).

(b) Inactivity – Lack of physical activity or exercise, or inadequate physical activity. Physical activity helps in controlling our body weight. The body enhances insulin responsiveness by metabolizing glucose for energy and improving cellular insulin sensitivity (Venkatasamy *et al*., 2013).

(c) Weight – Excess fatty tissue in the body makes the body cells resistant to insulin (Hardy *et al*., 2012).

(d) Age – Risk of getting diabetes types 2 increases as one gets older. Attributable reasons may include less of frequent exercise, loss of muscle mass and weight gain as one gets older. It is crucial to highlight that type 2 DM is also becoming more prevalent in young people, including kids, teenagers, and adolescents (Al-Sofiani *et al*., 2019).

(e) Race or Ethnicity – The cause of the greater risk among Blacks, Hispanics, American Indians, and Asian Americans is still unknown (Spanakis and Golden, 2013).

(f) Gestational DM –The likelihood of developing type 2 DM and pre-DM increases if GDM develops during pregnancy. Mothers who give birth to children whose weight are greater than 916 lbs. run the risk of developing type 2 DM (Ornoy *et al*., 2021).

(g) Unusual Amounts of Triglycerides and cholesterol - It should be emphasized that the likelihood of acquiring type 2 DM is extremely greater when the body has low levels of "good" cholesterol. People who have high levels of circulating triglycerides in their blood have an increased risk of acquiring type 2 DM (Martín-Timón *et al*., 2014).

(h) Polycystic Ovary Disease – Women with polycystic ovarian syndrome, which is identified by an abnormal menstrual cycle, prodigious hair growth, and excessive body fat, have an improved chance of emerging type 2 DM (Dennett and Simon, 2015).

(i) High Blood Pressure – Having blood pressure ≥ 140/90 (mmHg) increases the chance of type 2 DM (Kim and Kim, 2022).

2.13.3 Risk Factors for Gestational Diabetes

Some women are at risk than others

Risk factors include:

(a) Ancestral history/ self-history – The risk increases with people who have pre-DM. There is a very high likelihood that others may have diabetes if an immediate family member, such as a brother or sister, does. Risk is also high if one has gestational diabetes in previous pregnancy or pregnancies and particularly with baby weighing over and above 4kg. Unexplained still-birth is also a risk factor (Lewandowska, 2021).

(b) Age – Women above twenty-five (25) years of age are at a higher risk (Mazumder *et al*., 2022).

(c) Race or Ethnicity – Asian-American women, American Indian women, Black women, and Hispanic women have all been found to be at increased risk, albeit the reasons for this are yet unknown (Hedderson *et al*., 2010).

(d) Weight – Over weight before pregnancies increased the risk (Hedderson *et al*., 2008).

2.14 Complication of Diabetes

Complication of diabetes mellitus could be very severe, debilitating, disabling, fatal or even life threatening. With a protracted period of illness, there is a high probability of damage to organs such as kidneys, eyes, and others. Several studies have shown that communities of African descent had a high incidence of microvascular complications and a low frequency of macrovascular complications, in part because of the existence of hypertension, ineffective diabetes management, and restricted access to healthcare. According to these researches, between twenty-one to twenty-five percent of those with type 2 DM exhibit retinopathy at diagnosis. Retinopathy typically affects fifteen to fifty-five percent of people with a high frequency of proliferative retinopathy and macular edema (Mbanya *et al.,* 2010; Tumosa, 2008). The main cause of adult vision loss is diabetic retinopathy, which also puts diabetics at 6 time's higher risk for cataracts and less than two times higher risk for open angle glaucoma (Mash *et al.,* 2007). Five to eight percent of type 2 diabetics have coronary heart disease, and up to fifty percent of them have heart muscle damage. About fifteen percent of people who suffer a cerebrovascular accident have DM, while five percent of those with DM initially present with a stroke. Peripheral vascular disease is responsible for about

twenty percent of the lesions in diabetic feet, and the prevalence of blood circulation disorders varies between sites from 4% to 28% (Kengne *et al.,* 2005). It should be noted that DM requires careful monitoring and control. In the absence of adequate management, it can result into a terribly high blood sugar levels thereby leading to complications.

2.14.1 Long-term Complication of Diabetes

The long-term complications of DM begin slowly. Complications are more likely to occur the longer someone has DM and poorly manages their blood sugar.

Possible complications include kidney damage, nerve damage, and coronary disease. Also, injury to the feet, and to the eyes. According to research, two to thirty-nine percent of persons with type 2 DM have retinopathy, while eight to eighteen percent have nephropathy, five to thirteen percent have neuropathy, and eight percent have coronary disease (Engelgau *et al.,* 2000). Long-term complications of diabetes mellitus can be broadly categorized into microvascular and macrovascular types.

Microvascular complications involve the following areas: Diabetic nephropathy involves damage to the kidney and can result in renal failure; diabetic retinopathy involves damage to the eyes and can rapidly cause loss of vision and even blindness; Damage to the nerve in diabetic neuropathy can cause loss of sensitivity or sensations, and impotency. Macrovascular complications have to do with cardiovascular disease (this affects the heart and blood vessels) including strokes, heart attacks, insufficient flow of blood to the lower limb. In the case of macrovascular complications in type 2 DM, the chance of myocardial infarction and stroke is about 2 – 5 times greater than that in the overall community (Marshall and Flyvbjerg, 2006)

2.14.2 Complication in Baby

Complication in baby can take place due to gestational diabetes, as explained:

Excess growth- As a result of excessive glucose crossing the placenta and activating the foetus' pancreas to synthesis more insulin, the baby may develop macrosomia (grow overly big) and require a Caesarean section when it is born.

Low blood sugar- Because their own insulin production is high, children of moms with gestational DM frequently experience low blood sugar, occasionally right after being put to bed;

Type 2 diabetes later in life- Children born to moms with gestational diabetes are more likely to become obese as children and develop type 2 DM as adults.

Death - Neglected or untreated gestational DM might result in the infant's mortality either before or right after birth.

2.14.3 Complication in Mother

Complications developed in mother include: Preeclampsia, headaches, as well as blurred vision and this is dangerous to baby and mother, subsequent gestational DM (one can continuously have gestational DM).

2.15 Prevention of Diabetes Mellitus

It should be noted that type 1 DM cannot be prevented. However, the same beneficial lifestyle modifications that aid in the treatment of type 2 DM, gestational DM, and pre-DM can also help in the prevention of type 1 DM.

Eat healthy food- Food higher in fibre and lower in fat and calories should be chosen. Embrace fruits, vegetables and whole grains. Fight boredom.

Physical exercise- Engage in more physical exercise. Go as much as thirty (30) minutes of moderate aerobic activity regularly on a weekly basis; one hundred and fifty (150) minutes of aerobic exercise per week is recommended and good

Weight loss- Endeavour or try as much as possible to shed excess weight. People weighing over 200LB (90.72kg) should try to reduce their weight thereby reducing the risk of diabetes.

It is advised that women should not endeavour to lose weight during pregnancy.

Medication- Metformin is one example of an oral antidiabetic medication that may be effective in lowering or reducing the risk of type 2 diabetes.

The best course of action for healthy living is a beneficial lifestyle adjustment, and you should routinely evaluate your sugar level at least once a year.

2.15.1 Prevention of Diabetes Complications

Diabetes complications can be prevented if proper steps have been taken at the right time. Clinical studies have shown that maintaining a firm management of blood sugar, blood pressure, and cholesterol levels lowers the risk of developing diabetes-related macrovascular complications, such as myocardial infarction and stroke, as well as

microvascular complications, such as nephropathy, neuropathy, diabetic foot, retinopathy, and coronary mortality (Van- Bruggen *et al.,* 2009)

Numerous investigations such as the United Kingdom Prospective Diabetes Study in juvenile onset and Diabetes Control and Complications Trail (DCCT) in adult onset, have demonstrated that the risk of microvascular complications decreases with decreasing levels of glycosylated haemoglobin (Marshal and Flyvbjerg, 2006).

Early drug therapy initiation by the doctor, patient adherence to the treatment schedule, and the will to change one's lifestyle are all necessary for preventing DM complications. There should be a follow-up of diabetes prevention, to carry out foot examination, blood and urine tests, eye examination, counseling of the patients, concerning the dangers involved in diabetes mellitus and the importance of life-style change (Goderis *et al.,* 2009). There should be appropriate treatment goals for cholesterol, blood pressure, HbA1C, and blood sugar levels (Van –Brirggen *et al.,* 2009).

2.16 Treatment and Management of Diabetes Mellitus

The ultimate goal or aim in DM treatment or management is to restore carbohydrate metabolism to normal state as much as possible. Medically DM has no known cure for now but all types of diabetes mellitus are treatable or manageable.

Basically, managing or possibly preventing both short-term and long-term complication of DM should be the focus of the treatment. Treatment must be able to effectively manage associated comorbidities including hypertension and dyslipidemia in addition to preventing both macro and micro consequences (Amanda and Siulva, 2011). In order to reduce both insulin resistance and β-cell insufficiency, effectuate glycemic control, and avoid further complications, type 2 DM necessitates a composite strategy to treatment (Rosenbloom, 2009). The conceived approach combines drug therapy with behavioural lifestyle interventions with the goals of promoting weight loss or preventing further weight gain, normalising glycemia, and controlling comorbidities like hypertension, dyslipidemia, nephropathy, and hepatic steatosis (Rosenbloom *et al.,* 2009).

2.16.1 Drug Treatment

Functionally, anti-diabetic drugs cure DM by bringing down blood sugar levels. Numerous anti-diabetic medications are available on the market, but which one should

be used is a function of the patient's age, diabetes, physiological condition, and other criteria. The medications that can be used include glp-1 receptor agonists, sglz2 inhibitors, thiazolidinediones, insulin, sulfonylureas, α-glycosidase inhibitors, incretin mimetics, DPP-4 inhibitors, amylin analogue, etc.

2.16.2 Procedures for Choosing Antidiabetic Drug Treatment

For emphasis, choosing the appropriate antidiabetic agent is not just by free-will approach but a function of consideration of a myriad of factors including the diabetic's metabolic situation, co-existing comorbidity, preferred life style and regulatory authority restrictions. After examining the various probable side effects of drugs as enumerated in Table 2.4, then selection is done according to the apparent metabolic phenotype as listed in the guidelines in Table 2.5. Usually, treatment commences on low dose with succeeding gradual adjustment to metabolic demand. If good success is not achieved due to side effects, or inadequate glycemic control then one can change therapy to another option. Early combination of therapy is recommended as the best option (Standl *et al.,* 2003).

Table 2.4 Guide on Choosing a Diabetic Treatment (Probable side effects of pharmacological treatment alternatives in type 2 diabetes)

S/N	Probable side-effect	Stay-away or reconsider
1.	Impaired kidney function	Sulfonylureas, Biguanides
2.	Impaired liver function	Glitazones, α-glucosidase inhibitors, Glinides, Biguanides.
3.	Unwanted weight gain	Glinides, insulin, sulfonylureas, Glitazones
4.	Hypoglycemia	Glinides, sulfonylureas, insulin.
5.	Impaired cardio – pulmonary function	Glitazones, Biguanides
6.	Gastrointestinal Symptoms	α-glucosidase inhibitors, biguanides

Ref: Standl *et al.,* 2003
The main factors affecting treatment selection are BMI, hypoglycemia risk, renal function, and cardiopulmonary function, especially edema.

Table 2.5: Guidelines for Choosing the Appropriate Oral Antidiabetic Drug According to the Metabolic Condition.

S/N	Metabolic condition	Antidiabetic agent (drug)
1.	Fasting hyperglycemia	Glitazone, Long acting Sulfonylureas, Biguanides.
2.	Insulin deficiency	Sulfonylureas, Glinides (Insulin)
3.	Postprandial hyperglycemia	Short acting sulfonylureas, α-glucosidase inhibitors, Glinides.
4.	Insulin resistance	Glitazones, α-glucosidase inhibitors, Biguanides.

Ref: Standl *et al.,* 2003

The decision on the choice of the therapy is a function of the stage of the disease with its metabolic phenotype. Regulatory issues also help in determining what choice to make (Restrictions on drugs from the authority). Availability of the drug in the market is another determining factor

2.17 Developmental History of the Major Classes of Antidiabetic Drugs

Development of antidiabetic drugs has evolved over the years and it started with the discovery of insulin in Canada by Sir Frederic Banting and Charles Best in 1921. In the 1950s, the first generation Sulfonylureas, the first oral antidiabetic drugs were developed. Metformin the first Biguanide was first synthesized in 1922 but its antidiabetic properties were not known until in the late 1950s and it became well known in the 1970s. Development of Amylin commenced in the 1980s. In the 1990s Alpha-glucosidase inhibitors were introduced while Thiazolidinediones were developed. DPP-4 inhibitors were approved in the mid-2000s while GLP-1 receptor agonists were developed in 2005. SGLT2 inhibitors were approved in the beginning of the 2010s. Over the years especially between 1990-2000 insulin analogues were developed to improve insulin therapy.

2.17.1 Amylin Analogues

Amylin, commonly known as islet amyloid polypeptide (IAPP), is a peptide hormone that is important for controlling hunger and glucose homeostasis. Through the 1980s work of Susan Bonner-Weir and collaborators, Amylin was first developed as a unique hormone.

Historically, Amylin was first discovered specifically in 1987 and is co-secreted with insulin by pancreatic β-cells in response to food stimulation. It is a member of the pancreatic islet amyloid deposit, a pancreatic β-cell hormone. It was identified as the unique islet amyloid component that is only present in type 2 diabetic pancreatic islets. It lends to the gradual β-cell destruction observed in type 2 diabetics and as well responsible for circulating hormones with a variety of metabolic tasks such as preventing eating, inhibiting pancreatic glucagon synthesis, and blocking gastric emptying. This informative idea about amylin's function and mechanism of action prompted the development of amylin analogues now used in the treatment of DM in obese people (Lutz, 2022). Pramlintide looks like or resembles amylin and it's employed in curing both type 1 and type 2 diabetics.

2.17.2 Biguanides

In 1879, Bernhard Rathke was the first person to synthesize Biguanide (Rathke, 1879) and this can be produced from the reaction between dicyandiamide and ammonia via the Pinner-type process:

$$C_2H_4N_4 + NH_3 \rightarrow C_2H_7N_5$$

Biguanidine particularly refers to a group of medicaments which acts as oral antihyperglycemia and employed in curing DM and pre- DM (Rang *et al.,* 2003)

In 1920s, guanidine compounds observed to lower blood glucose level in animal study were discovered in Galega extract, from *Galega officinalis* (French lilac) and these have been employed in the treatment of DM for ages (Witters, 2001).

In 1950s, biguanides, colourless organic solids which dissolve in water to produce highly basic solutions were reintroduced into the market for type 2 DM treatment and Metformin is the most reputable and dominant drug in this group presently (Rathke, 1879)

2.17.3 Bile Acid Sequestrants

Bile Acid Sequestrants are also referred to as bile acid-binding resins and are employed to lower cholesterol levels in the blood. They are powerful metabolic regulators, and effective hormones, which work as signaling molecules on both nuclear and G protein-coupled receptors. They are found to prompt numerous signaling pathways along multiple target organs. They are medicaments that assist in lowering the LDL (bad cholesterol) and they operate by occluding or obstructing the absorption of bile acid in the stomach. They enhance the excretion of bile in the feces. This mechanism progresses to increased cholesterol catabolism and reduced cholesterol concentration in the bloodstream. In 1990s, it was observed that BAS improved glycemic control in type 2 diabetics (Sonne *et al.,* 2014). BAS have been found to lower blood sugar for type 2 diabetics via modulation of the TGR5-dependent synthesis of the GIT-derived GLP-1 and not through reduced gluconeogenesis or glucose absorption.

2.17.4 Dopamine Agonist

Dopamine is a neurotransmitter produced in the brain and its functions include movement, memory, attention, mood and others while dopamine agonists, act directly on the dopamine receptor and mimic its effect by binding to and activating dopamine

receptors in the brain and other parts of the body. Dopamine agonists are known to affect glucose homeostasis. Bromocriptine, discovered by Arthur Stoll along with his research scientists at Sandoz in 1965, is a dopamine D2 agonist used in treating type 2 DM and very effective in reducing glycemic levels, and has minimal side effects (Kabir *et al.,* 2022). The first dopamine agonist to be introduced into clinical practise was bromocriptine (2-bromo-ergocryptine mesylate), an ergot derivative with D_2R agonist and D_1R antagonist characteristics (Gillam and Molitch, 2011).

2.17.5 DPP4 – Inhibitors

The term "incretin," which refers to a hormone of the gastrointestinal system that triggers the pancreas' internal synthesis, was coined by Ernest H. Starling in the early 1900s (Moore *et al.,* 1906). The evolution of the DPP-4 inhibition concept for glucose-lowering therapy in type 2 DM was based on incretin properties. In 1930s, La Barre and Still (La Barre and Still, 1930) and Heller (Heller, 1935) demonstrated how treating laboratory animals with gut extract led to the discovery that the circulating blood glucose level had been lowered. This advanced and led to the identification of GIP and GLP-1as major incretin hormones. DPP – 4 inhibitors were developed to circumvent the inactivation of the internally originated GLP-1. They are considerably new being approved in the mid-2000s and work by inhibiting the enzymes DPP-4 which breaks down incretin hormones that stimulate insulin release and reduce glucagon synthesis. DPP-4 inhibitors aid in the regulation of blood sugar levels by maintaining or preserving incretin function. They seem to be more beneficial to the overweight diabetics. Relatively, they are devoid of serious side effects when compared with other antidiabetic medicaments (Ahrén, 2019).

2.17.6 Glinides

Research on Glinides formulations began in the 1970s but not until in the 1990s that the first generation glinides, nateglinide and repaglinide were developed. Glinides are also known as meglitinides and are employed to lower blood glucose levels in type 2 diabetics. They function by stimulating insulin release from the β-cells of the pancreas in response to meals.

Glinides are secretagogue characterized and identified by expeditious and shortened duration of action (Holstein and Egberts, 2003). They are prandial glucose regulators and a group of oral medicaments developed for treatment of type 2 DM. Moving towards the end of the 1970s, a compound identified as HB699 but subsequently

referred to as meglitinide was made by adding COOH to the monosulfonylurea moiety of glibenclamide, and it was found to block insulin production and ATP-sensitive potassium channels, hence decreasing blood sugar levels (Henquin, 1990). Common examples are nateglinide and repaglinide.

2.17.7 α-Glucosidase Inhibitors

Alpha-gluccosidase inhibitors were introduced in the 1990s and they function by inhibiting intestinal enzymes that break down carbohydrates causing gradual absorption of glucose and thus lowering of post food sugar spikes.

Alpha glucosidase inhibitors area special class of antidiabetic drugs derived from bacterial cultures or their derivatives.

In 1977, Schmidt isolated acarbose from the cultures of *Actinoplanes* and subsequently Pul *et al.,* synthesized AGIs and showed their hypoglycemic action and in 1990 Bayer pharmaceutical Ltd introduced them to the market in Germany.

In 1979, Schmidt began the development of miglitot from microbial cultures of *Bacillus* and *Streptomyces*. Miglitol was semi-synthetically produced from 1 – deoxynojirimycin and was made popular by Pfizer with the brand name Glyset.

In 1994, Takeda pharmaceutical Ltd in Japan developed voglibose which was derived from antibiotic validamycin A that is obtained from *Streptomyces hygroscopicus var. limoneous.* Voglibose has been in the market in India since 2005 but not in USA.

Alpha amylase and other alpha glucosidases can both be blocked by acarbose, which disallows the body from absorbing starch and other carbohydrates from the brush border of the enterocytes of the jejenum in the intestine. In reality, they are not endowed with pancreato-centred modus operandi. The two principal medicaments in this family are acarbose (precose) and miglitol (glyset) (Alpha Glucosidase Inhibitors, 2021).

2.17.8 SGLT – 2 Inhibitors

Sodium-glucose cotransporter2 inhibitors were approved in the 2010s and function by inhibiting sglt2 in the kidneys causing increased excretion of glucose in the urine and subsequently lowering of blood sugar levels.

SGLT-2 inhibitors facilitate the kidneys' disposal of excessive blood glucose in the urine. The protein, SGLT-2 is a very high capacity glucose transporter residing on the cell surface of the tubule cells located earlier in the tubule, at the apical membrane of

the S_1 and S_2 sector of proximal tubule. As urine is produced, SGLT-2 directly accounts or is responsible for ninety percent (90 %) of the total glucose absorption. As a result of its role in glucose reabsorption, SGLT-2 is a promising drug target to alter blood glucose level. The glucose-lowering apparatus of SGLT-2 is a function of perfectly functioning kidneys, thus individuals with impaired kidney function are advised not to use it. The pioneer or first SGLT-2 inhibitor was canagliflozin (Kaushal *et al*., 2014).

2.17.9 Sulfonylureas

Jabon and his collaborators discovered in 1942 that a number of sulfonamides exhibited blood sugar lowering properties in laboratory animals. In the 1950s scientists discovered that a couple of sulfa drugs employed in treating bacterial infections coincidentally possess a surprising side-effect of reducing blood sugar levels. Consequent upon these discoveries carbutamide (1-butyl-3-sulfonylurea) was produced as the pioneer sulfonyl drug for use as antidiabetic. It was not long after its discovery that this drug was withdrawn from the market for safety reasons as it became harmful to bone marrow. In 1960s avalanche of sulfonylureas were produced as antidiabetic agents and they were classified into two major groups. The first generation sulfonylureas, chlorpropamide and tolbutamide which function by activating the release of insulin from the pancreas thus assisting in the control of blood glucose levels in type 2 DM have gone into extinction and no longer in use. Second generation group members like gliclacide, glibenclamide, glipizide and glimepiride are in use currently for the treatment and management of DM, sulfonylureas should be considered only for diabetics of regular weight or those who are not overweight (Sola *et al.,* 2015).

2.17.10 Thiazolidinediones (TZDs)

Thiazolidinediones, also referred to as glitazones were developed and introduced in the 1990s, with the objective of improving insulin sensitivity in peripheral tissues prompting them to respond actively to the effect of insulin. They are sulfur containing pentacyclic compounds widely found in nature in different forms. As insulin sensitizers that operate on within- the- cell metabolic pathway, propelling activity of insulin in order to increase its sensitivity in major organs or tissues. Thiazolidinediones are medicaments utilised for treating and managing type 2 DM and were discovered while screening compounds for hypoglycemic effect or activity in ob/ob mouse (Fujiwara *et al.,* 1988), and subsequently it was revealed that they improve the activity

of insulin in different strains of insulin resistant diabetic obese animal model (Olefsky, 2000). Thiazolidiinediones are insulin sensititising glucose –lowering medications that function principally by activating PPARγ which in turns regulates glucose, lipid, and protein metabolism. They include three compounds namely troglitazone, rosiglitazone, and pioglitazone but rosiglitazone and pioglitazone are presently available in the market in USA and a couple of other places (Della-Morte *et al.,* 2014).

2.17.11 Insulin

In 1921, Sir. Frederic G. Banting, together with members of his team namely Charles H. Best and J.J.R. Maclead developed insulin at the University of Toronto. The experimental procedures started in May 17, 1921 and by September, these researchers have shown that a dog with its pancreas removed developed diabetes, and that when isletin from pancreas extract was intravenously administered into the dog, blood glucose levels were decreased. The isolated canine pancreatic insulin demonstrated its potency in lowering blood sugar levels in diabetic dogs. James B. Collip later carried out the purification of isletin. The first synthetic human insulin manufactured through genetic engineering was created in 1978 utilising the bacteria *E. coli*. Insulin is the best medication for type 1 diabetes. It can complement or replace the body's natural insulin in an effort to achieve normal or nearly normal blood sugar levels and avoid or lessen complications (Vecchio *et al.,* 2018).

2.18 Allopathic Drugs used for Diabetes Mellitus Treatment and their Adverse Side Effects

There are different classes of synthetic antidiabetic drugs and each class has peculiar or distinct deleterious effects and each person's response to these drugs varies. It is advisable and relevant to know or understand the probable negative effects of each class in order to make authentic decision on diabetes management.

Adverse effects include hypoglycemia which is the familiar one, weight gain, nausea, diarrhea, stomach upset, vitamin B_{12} deficiency and anaemia, neurological issues, fluid retention and edema, upper respiratory tract infection, headaches, pancreatitis, increase urination and urinary tract infections, and gastrointestinal disturbances (Mathew and Thoppil, 2022).

Allopathic was coined in early 19th Century by a German physician recognized as Christian Friedrich Samuel Hahnenmann and it is modern medicine adopting or embracing evidence – based approach when treating sicknesses and diseases. It relies

on literature guide and diagnostic tests prior to making decision on what will be the best course of treatment. Allopathy is a structure in which clinical professionals and other allied health care professional (such as pharmacists, nurses, therapists) focus on treating symptoms and ailments using active pharmaceutical medicaments, rays, vaccination, theather operation and other related medical means. This approach of modern medicine has been adopted in the treatment of many health challenges including chronic diseases such as DM. Main grounds of displeasure or bitterness against this modern or orthodox medicine are the associated multiple side effects, ineffectiveness and absence of curative value in a number of chronic disorders (Ameade *et al.,* 2018).

2.18.1 Pharmacological Treatment of DM

In pharmacological treatment of DM, it is very crucial to know the physiological status of the patient's especially with respect to insulin. Is it deficiency of insulin or resistance or the two?

Treatment plans are separated into two, namely:

(a) Non-Insulin Therapies

(b) Insulin Therapies

2.18.1.1 Non-insulin Therapies

2.18.1.1.1 Insulin Sensitizers

Insulin sensitizers are drugs that enhance the body's response to insulin and prompting the cells to be more sensitive to insulin action. As a result of sensitivity enhancement they bring about improvement in the glucose uptake from the bloodstream into the cell thus resulting to lowering of the blood glucose. They are medicaments designed to lower blood sugar levels by enhancing the activity of the hormone insulin in peripheral tissues, e.g. Biguanide and Thiazolidinediones. Biguanides work by decreasing insulin resistance, increasing glucose uptake, and decreasing hepatic gluconeogenesis, while Thiazolidinediones work peripherally by binding to the PPAR-γ nuclear receptor in adipocytes and myocytes to increase glucose uptake and reduce insulin resistance (Di Magno *et al.,* 2022; Quinn *et al.,* 2008). By enhancing the action of insulin on insulin receptors and resulting in a decrease in the concentration of hepatic, muscle, and adipose glucose, metformin works to combat or reverse insulin resistance.

Gastrointestinal problems including constipation; vitamin B deficiency, anaemia, fluid retention, edoema, and weight gain are some of the side effects that are related to these medications.

2.18.1.1.2 Insulin Secretagogues

Insulin secretagogues are drugs which activate the pancreas to produce and release insulin and they are useful for type 2 diabetics.

A chemical known as a secretgogue is one that induces the secretion of another substance. It is known that insulin secretagogues stimulate the release of insulin from the pancreas, which promotes facilitation of the absorption of glucose by muscle and adipose tissue, hence lowering the formation of liver glucose e.g. sulfonylureas and glinides (Hemmingsen *et al.,* 2016). These substances lower blood glucose via stimulation of insulin secretion in the body thus increasing insulin levels in the blood. Sulfonylureas like Glyburide and Glipizide work by directly stimulating the ATP-sensitive K-channel in the pancreatic -cells, which in turn causes the production of insulin. They generate increase in insulin secretion from pancreas. Hypoglycemia, weight gain, and neurological issues are associated adverse effects (Galicia-Garcia *et al*., 2020).

2.18.1.1.3 Alpha-glucosidase Inhibitors

Alpha-glucosidase inhibitors work by preventing the breakdown of carbohydrates by intestinal enzymes (Gong *et al*., 2020). The alpha glucosidase which are present in the brush border of the small intestine is inhibited; and thereby delay carbohydrate absorption. Acarbose carries out its function by decreasing glucose absorption at the intestine. These drugs primarily target postprandial hyperglycemia. Associated adverse effects include gastrointestinal disturbances- bloating, flatulence, and diarrhea (Telagari and Hullatti, 2015).

2.18.1.1.4 Incretins

These medications come in solid form, like DPP-4, or in injectable form, like GLP-1. These drugs are not recommended for those with background of medullary cancer or endocrine cancer. Associated adverse effects include acute pancreatitis (Lotfy *et al*., 2011).

2.18.1.1.5 GLP-1 Receptor Agonists

They are produced to mimic the actions of GLP-1. They work by boosting insulin production, reducing glucagon release, delaying stomach emptying, and enhancing satiety. These medications are injected, and they work by increasing the production of insulin while decreasing the production of glucagon after a meal in a glucose-dependent manner (Zhao *et al*., 2021).

2.18.1.1.6 DPP – 4 Inhibitors

DPP-4 inhibitors function by inhibiting the enzyme DPP-4 which breaks down incretin hormones that activate insulin release and reduce glucagon synthesis (Godinho *et al*., 2015).

The protein DPP-4, which is found in cell membranes, breaks down GLP-1 and gluco-dependent insulinotropic polypeptide quickly. The suppression of DPP-4 will increase insulin secretion and reduce glucagon secretion in a glucose-dependent manner. By delaying the inactivation of the incretin hormones GLP-1 (glucagon-like peptide) and GIP (glucose-dependent insulinotropic peptide), DPP-4 inhibitors address insulin insufficiency (McIntosh, 2008). DPP-4 inhibitors typically work and function on post-meal blood glucose levels, while a drop in fasting glycemia is also seen.

Associated adverse effects include headaches, pancreatitis, and upper respiratory tract infections.

2.18.1.1.7 Pramlintide

Pramlintide, a synthetic version of amylin, works by inhibiting the release of glucagon, slowing down gastric emptying, and reducing appetite via central pathways. It works by primarily affecting postprandial sugar levels.

Associated side effects include hypoglycemia and various stomach problems.

2.18.1.1.8 Bromocriptine

When administered two hours after waking up, bromocriptine ameliorates glucose control in type 2 diabetics. It has no known mechanism for now.

2.18.1.1.9 SGLT-2 Inhibitors

SGLT-2 inhibitors are drugs that are specifically prescribed for type 2 diabetics. They work by blocking SGLT-2 in the kidneys, which results in glucose excretion in the urine and lowers blood sugar levels (Hsia *et al*., 2017). The primary function of SGLT-2, a protein acting as a sodium glucose co-transporter in the proximal tubules of the

kidney, is to reabsorb the glucose that has been filtered out of the urine and put it back into circulation (Srinivas *et al.*, 2021). When this protein is inhibited, glucose is excreted in the urine at blood glucose levels that are lower than usual (approximately 120 mg/dl as opposed to 180 mg/dl). Urinary tract infections and excessive urination are some of the side effects. There could also be Infections with yeast in the genitalia or vagina. Polyuria is a possibility as well.

2.18.2 Insulin Therapies

The mainstay of diabetes management is insulin therapy, particularly for type 1 diabetics and those with type 2 diabetes who cannot maintain adequate glycemic control by oral medications or lifestyle changes alone. The therapy depends on administering exogenous insulin to control hyperglycemia (McFarlane, 2009).

The first treatment for DM was insulin, which was developed in 1921 but whose human clinical trials began in 1922. This treatment is effective at regulating glucose metabolism and bringing down blood sugar levels that are too high. It can be used to lower any elevated glycated haemoglobin. The treatment aids in lowering triglycerides and raising levels of healthy cholesterol. Insulin's actions can be rapid, short-acting, basal, or intermediate, among others. Hypoglycemia and problems with the injection site are two related adverse effects (Wilcox, 2005).

2.18.2.1 Insulin Pump Therapy

Insulin pump therapy, also referred to as continuous subcutaneous insulin infusion (CSII) uses portable electronic insulin pump for delivery of materials using different approaches. The procedure permits admitting different insulin basal rate anytime. Insulin pump therapy allows administration of different basal insulin rates at any time (Berget *et al*., 2019; Edelman *et al*., 2010; Krentz and Bailey, 2005).

2.19 Medicinal Plants and Herbs: Historical Perspective

It is in God’s agenda that plants play prominent role in the cycle of nature because life on earth fundamentally relies on them. Plants cater for all the need of man with respect to food, clothing, shelter; fragrance, flavour and medicine. In God’s blue-print for the survival of man and animal as revealed through the sacred scripture is that both shall depend on plants and/or herbs for food and healing.

“He caused the grass to grow for cattle, and herb for the service of man; that he may bring forth food out of the earth” (Bible – Psalm 104:14 KJV)

And by the river upon the bank thereof, on this side and on that side, shall grow all trees for meat, whose leaf shall not fade, neither shall the fruit thereof be consumed; it shall bring forth new fruit according to his months, because their waters they issued out of the sanctuary; and the fruit thereof shall be for meat, and the leaf thereof for medicine or healing (Bible – Ezekiel 47:12 KJV).

In the midst of the street of it, and on either side of the river, was there the tree of life which bare twelve manner of fruits, and yielded her fruit every month; and the leaves of the tree were for healing of the nations (Bible – Revelation 22:2 KJV).

Botanists and Zoologists, through empirical observations have been able to prove that lower animals share survival trait as they use plants for healing. Dogs have instinctive herbal knowledge like man, they chew grass whenever they are sick while chicken eat plants for healing and good health. Hence we have dog grass and chick-weed. Dogs chew dog-grass (*Agrospyron canina)* as a form of laxative and worm expellant. So also, domestic cats use dog-grass for same purpose especially for purging. Chicken also eat chick-weed (*Stellaria media)* which is very nutrient dense to improve, boost, and sustain their health; and for inflammation of the digestive, renal, respiratory and reproductive tract (Oladeji and Oyebamiji, 2020).

Chimpanzees have been observed to chew the leaves of bitter leaf (*Vernonia amygdalina*) whenever they have stomach upset (Huffman, 2003). Some animals chew special kinds of leaves during labour e.g. pregnant elephants have been observed to eat the bark of the red sering tree (*Burkea spp*) to induce labour (Shuker, 2001). Through intuition, man commenced using herbs and plants to prevent, cure or manage disease right from the dawn of creation. As early as the period of the Neanderthal man (400,000 – 40,000 years ago) herbs and plants have been used, medicinally (Hardy, 2021) and according as substantiated by Karen. Hardy and Stephen Buckey in "Archeology", that *Achillea millefolium* (Yarrow) was used as a form of laxative to solve and clear gut problems and other ailments while *Matricaria chamomilla* L. (Chamomile) was used in treating hay fever, inflammation, muscle spasms, menstrual disorder, gastrointestinal problems, haemoroid, and rheumatic pain (Najafi Mollabashi *et al*., 2021; Miraj and Alesaeidi, 2016).

Use of the plants as sources of relief for different ailments is as old as mankind itself with recorded practice dating back to at least 4000 years ago (Hutchinson and Dalziel, 1963). Plants or materials from plant origin have been utilised by man for therapeutic

or curative, and as well as for prophylactic purposes as far back as to the Sumerian and Akkadian civilization about 3rd Millennium BC (C. 3000 BC). Hundreds of therapeutic plants, including opium, were enumerated on clay tablets during the Sumerian era, which is when the first recorded evidence of herbal treatments first appeared (Petrovska, 2012; Kamboj, 2000). Facts exist of spices, herbs, plants that have been used to treat diseases and for revitalizing the body systems, in almost all ancient civilizations – the Egyptians, the Chinese, the Indians, the Greeks and the Romans.

The Ebers Papyrus from ancient Egypt, C.1500 BC, describes over 800 prescriptions from about 700 plants, for therapy including garlic, onion, pomegranate, and aloe. Between 14 and 15000 BC, herbalists, mostly Europeans and Chinese described and identified many medicinal plants. The first illustration of herbal medicine can be seen on the French cave walls at Lascaux, and it is radiocarbon dated to the period between 13,000– 25,000 BC.

In India, "RigVeda, written by Vyasa, between 3,500 – 1,800 BC provides records of herbs particularly spices used in treating ailments while there are records of compilation by different authors of about 1000 herbs used in China. Shen – nung Pen Ts'ao Chung, also known as the "Canon of Herbs" and composed by Emperor Shen Nung, the divine husbandman, is a priceless ancient Chinese text that dates back to 2500 BC. It lists about three hundred and sixty-five medicaments (obtained from minerals, plants, and animals) and provides instructions on how to prepare and use them. Between 1518 and 1593, Li Shih Chen also penned Pen Ts'ao Kang Mu, also known as "the great herbal," which provides a thorough explanation of more than 1,800 plant and animal components, minerals, and metals, as well as their therapeutic properties and applications.

Hippocrates (460 BCE – 375 BCE), the Greek physician who is being described as the father of modern medicine listed several hundred herbal remedies (Yapijakis, 2009). In the rich rain forest regions of West Africa, most especially in Nigeria, there exists a cultural consumption of plants materials as dietary staples as well as application in ethno-medical practice (Waterman, 1986). Every human has developed its own peculiar kind of food and ethno-medicine from various vegetal resources in its environment and catchment areas.

Up to the time (16th and 17th centuries) when Paracelsus gave birth to a new school of chemistry known as iatrochemistry, which emphasized application of chemistry to

medicine, plants remain the only source of treatment and prophylaxis (Kelly, 2009). However, with the problems associated with synthetic drugs, including toxicity, inefficacy and high cost, emphasis is now back to development and usage of medicinal plants as it were in the beginning.

2.20 Definition and Characteristic of Medicinal Plant

The term "medicinal plants" refers to plants with therapeutic benefits and one or more of their organs or portions that contain components that can be used as medicines or as the basis for chemotherapeutic semi-synthesis (WHO, 2008).

According to WHO technical report, this information has been expanded to add the followings:

(a) Plants or its leaves, roots, barks, stems utilised for standard medical preparation e.g. concoction, and admixture e.g. Cascara bark;

(b) Herbs employed in extracting substances that are pure e.g. yams;

(c) Multipurpose plants used for flavours, perfume, and seasoning e.g. garlic, and ginger;

(d) Invisible and tiny plants e.g. fungi used in producing antibiotics;

(e) Plants used in clothe making e.g. cotton, and flax (Sofowora, 2008; Sofowora *et al.,* 2013).

Trees, herbs, vines, epiphytes, ferns and aquatic plants establish the medicinal flora. About 70–90% of the world's population depends on Herbal medicine, which encompasses all treatment modalities that are unique to individual cultures. A significant portion of traditional therapies utilise plant extract or their active ingredients (Rungsung *et al*., 2015). It means that approximately 3/4 of the global population relies on alternative and complementary healthcare for primary care. These estimable herbal practices resident in developing countries have been taken as an integral part of the customary inheritance of the world (Farnsworth, 1998; Farnsworth, 1988). Medicinal plants are regarded as a repository or bank of very many types of bioactive compounds possessing myriad therapeutic properties. Herbal medications are a pain reliever antibiotic, antiviral, cancer fighting, anti-malarial, anti-inflammatory, and anti-diabetic. A lot of the plants consumed either as food or as vegetable, as drug in concoctions, decoction, infusion, and tisane or for maceration actually contain many compounds with medicinal or therapeutic properties. These naturally occurring compounds being described as secondary plant metabolites include the saponins, the

flavonoids, the alkaloids, the tannins, the hemagglutinins, the protease inhibitors, and the polyphenols. These phytochemicals are available in different concentrations in plants and they elicit various physiological actions. The medicinal potency of medicinal plants resides in these active substances that are present in them. The most important of these active ingredients include tannins, phenolic compounds, flavonoids, and alkaloids (Akindele and Adeyemi, 2007). The pharmacologically active phytochemicals of the herbal plants, notably tannins, flavonoids, phenolics, alkaloids, steroids, cardiac glycosides, and fatty oils, are typically conserved in their particular portions including foliage, blossoms, seeds, fruits, rhytidome, and roots etc (Sharma *et al.,* 2021). With nearly 8,000 phenolic structures now known, phenolic compounds, also known as polyphenols, are one of the many and widely distributed classes of phytochemicals in the flora (Harbone, 1980). Roughly fifty percent of the over eight thousand naturally occurring plant phenolics are found to be flavonoids (Harbone and Herbert, 1993). Antioxidant flavonoids protect against diabetes, cardiovascular disease, certain malignancies, and age-related cell component degenerations. Flavonoids are antioxidants due to their molecular structure. Flavonoids' antioxidant and free radical scavenging activities are due to their hydroxyl group location and other factors. Plants with polyphenolic components, such as flavonoids, have been found to have antioxidant properties (Cook and Samman, 1996). Many plants have been found to exhibit antidiabetic, antimicrobial, antimalarial activities.

2.21 Medicinal Plant as an Alternative Source of Antidiabetic Agent

Since the beginning of existence, man has used plants for therapeutic purposes, and now, people all over the world are aware of the value of using plants as a source for improving their health and curing disease (Vinatoru, 2001). All across the world, medicinal herbs are utilised to cure and prevent various illnesses, particularly in developing nations where infectious diseases are prevalent or endemic and where modern healthcare facilities and services are shamefully and gravely inadequate (Zaidan *et al.,* 2005). Natural products, especially extracts from plant sources, offer unlimited possibilities for therapeutic discovery due to their unmatched chemical variety (Dzobo*,* 2022; Thomford *et al.,* 2018). Dr. Fereydon Batmanghelidj argued that because the human body is natural, it can only be healed by natural substances rather than by inorganic chemical drugs, in one of his books on health titled "Water cures:

Drug kills" (Batmanghelidj, 2003) Traditional medicines are cheap, accessible, and have few side effects, therefore 80% of the world uses them for primary care (Ekor, 2014; Robinson and Zhang, 2011). Natural remedies with plant origins have been used both prophylactically and therapeutically in Nigeria for a very long time. According to Duraipandiyan *et al.,* 2006, avalanche of compounds presents in plants that are used to make herbal treatments can be utilised to treat bacterial, viral, and chronic illnesses. Alkaloids, saponins, flavonoids, tannins, and other phytochemicals found in natural products are risk-free substitutes that work well and have negligible to no negative side effects. There are numerous beneficial biological properties of medicinal plants that have been documented, including antidiabetic, antibacterial, anticancer, antioxidant, analgesic, antidiarrheal, anti-inflammation, and potential healing effects (Sasidharan *et al.,* 2011).

2.22 Herbs used in Treating Diabetes Mellitus

Because they have no adverse effects, herbal medications and derivatives may be excellent treatments for type 2 diabetes, its complications, and related comorbidities. Numerous potent herbal plants with their unique active principles have been demonstrated to be therapeutically helpful against DM, and large-scale medicinal plants have long been used in treating, preventing, and controlling the disease and its complications globally (Gothai *et al.,* 2016). Numerous bioactive compounds derived from plants that have hypoglycemia-inducing properties exhibit antidiabetic effectiveness that is on par with or even superior to those of the most widely used oral hypoglycemic medications on the market today (Patel *et al.,* 2012). Given the great frequency of DM, its multifactorial nature, and the toxicity of current anti-diabetic drugs, the pharmaceutical industry struggles to produce a safe and effective treatment. Complementary and alternative medicine uses more than 1,200 therapeutic natural products because of their effective blood sugar-lowering abilities according to ethno-pharmacological surveys (Kesari *et al.,* 2007; Marles and Farnsworth, 1995). It has been reported that about 800 plants have antidiabetic properties. The ability of medicinal herbs and other therapeutic agents to restore the glycemic balance or homeostasis of hyperglycemic situations is evidence of their antidiabetic action (Ighodaro *et al.,* 2017). There is a need for novel chemicals to treat DM because there is now no safe and effective medication for the condition or any of its consequences. Herbal therapy is safer and more effective than synthetic because phytochemicals in

plant extracts spontaneously target metabolic pathways (Zaidan *et al.,* 2005). Due to their natural origin and lower toxicity, herbal medicines are currently gaining popularity for the treatment and management of DM in both developed and developing countries. Natural products will continue to hold their position as a dependable arsenal of pharmaceuticals, particularly now that nutraceuticals are receiving so much attention. According to Ćorković *et al*. (2022), plant diets high in polyphenols block key enzymes including α-amylase and α-glucosidase, which are linked to type 2 DM and tissue lipid peroxidation and have similar effects to insulin in glucose consumption.

Table 2.6: Plants Used in Treating Diabetes Mellitus Traditionally

S/N	Plant Name	Family	Extract of the Plant part used	Antidiabetic Assay	Mechanism of Action	References
1.	*Adansonia digitata*	Bombacaceae	Stem, Bark	STZ	Antidiabetic	Sundarambal *et al.,* 2015
2.	*Alangium lamarckii*	Alangiaceae	Leaves	STZ-Nicotinamide	Antidiabetic	Kumar *et al.,* 2011
3.	*Albizia odoratissima*	Mimosaceae	Bark	Alloxan	Antidiabetic	Kumar *et al.,* 2011
4.	*Allium Sativum bulbs*	Liliaceae		STZ	Antidiabetic	Eidi *et al.,* 2006
5.	*Aloe vera*	Asphodelaceae	Leaves	STZ	Antidiabetic	Tanaka *et al.,*2006
6.	*Annona Squamosa*	Annonaceae	Leaves	STZ	Antidiabetic	Kaleem *et al.,* 2006
7.	*Anona muricata*	Annonaceae	Leaves	STZ	Antidiabetic	Florence *et al.,* 2014
8.	*Artemisia afra (Jack) Ex wild*	Asteraceae	Leaves and Roots	STZ	Antidiabetic	Afolayan and Sunmonu, 2012
9.	*Aspalathus linearis*	Fabaceae	Shoots	Insulin resistant cell line	Hypoglycemic	Muller *et al.,* 2012
10.	*Axonopus compressus*	Poaceae	Leaves	Alloxan	Antidiabetic	Ibeh *et al.,* 2011
11.	*Berberis vulgaris*	Berberidaceae		STZ	Hypoglycemic	Meliani *et al.,* 2011
12.	*Brassica juncea*	Cruciferae	Seed		Hypoglycemia	Thirumalai *et al.,* 2011
13.	*Carica papaya*	Caricacea	Seed	STZ	Antidiabetic	Ugwu *et al*., 2023
14.	*Caesalpinia digyna*	Fabaceae	Root	STZ	Antidiabetic	Kumar *et al.,* 2012
15.	*Cassia auriculata*	Cesalpiniaceae	Leaves	STZ	Antihyperglycemia	Gupta *et al.,* 2011
16.	*Centaurium erythrea*	Gentianaceae	Leaves	STZ	Antidiabetic	Sefi *et al.,* 2011
17.	*Chaenomeles sineusis*	Resaceae	Fruit	STZ	Antidiabetic	Sancheti *et al.,* 2013
18.	*Clausena anisata (wild)*	Rutaceae	Roots	STZ	Hypoglycemic	Ojewole, 2002
19.	*Cocos nucifera*	Arecaceae	Leaves	STZ	Antihyperglycemic	Naskar *et al.,* 2011

20.	*Costus speciosus*	Costaceae	Rhizome	STZ	Antidiabetic	Eliza *et al.,* 2009
21.	*Cyclorarya paliurus*	Cyclocaryaceae	Bark	Glucosidase	Hypoglycemic	Li *et al.,* 2011
22.	*Cyclopia intermedia*	Fabaceae	Shoot	STZ	Antidiabetic	Muller *et al.,* 2011
23.	*Dillenia indica*	Dilleniaceae	Leaves	Alloxan	Antidiabetic	Kumar *et al.,* 2011
24.	*Enicostemma Littorale*	Gentianaceae	Whole plant	STZ	Antidiabetic	Sonawane *et al.,* 2010
25.	*Garcinia kola*	Guttiferae	Seed	STZ	Hypoglycemic	Adaramoye, 2012
26.	*Hybanthus enneaspermus*	Violaceae	Whole plant	STZ	Antidiabetic	Patel *et al.,* 2011
27.	*Hypoxis hermerocallidea Fisch*	Hypoxidaceae	Corms	STZ	Antidiabetic	Ojewole, 2006
28.	*Leonotis leonurus*	Lamiaceae	Leaves and flower	STZ	Lowering blood glucose	Mazimba, 2015
29.	*Lippa nodiflora*	Verbenaceae	Whole plant	STZ	Antidiabetic	Balamurugan and Ignacimuthu, 2011
30.	*Lithocarpus polystchus*	Fagaceae	Leaves		Hypoglycemic	Hou et eal., 2011
31.	*Momordica charantia*	Cucurbitaceae	Whole plant	STZ	Hypoglycemic	Sathishsekar and Subramanian, 2005
32.	*Musa sp. Var.*	Musaceae	Flower	α-glucosidase and Pancreatic amylase	Antidiabetic	Ramu *et al.,* 2015
33.	*Ocimum sanctum*	Lamiaceae	Aerial part		Antidiabetic	Patil *et al.,* 2011
34.	*Ophipogon japonicas*	Asperagaceae	Root		Hypoglycemic	Chen *et al.,* 2011
35.	*Opuntia streptacantha*	Cactaceae	Leaves	STZ	Antihyperglycemic	Andrade-Cetto and Wiedenfeld, 2011
36.	*Prosopis glandulosa*	Fabaceae	Whole plant		Antidiabetic	George *et al.,* 2011
37.	*Psidium guajava*	Myrtaceae	Fruit	STZ	Antihyperglycemic	Huang *et al.,* 2011
38.	*Sclerocarya birrea*	Anacardiaceae	Stem, Bark, Root	STZ	Antidiabetic	Gondwe *et al.,* 2008
39.	*Solanum torvum*	Solanaceae	Fruit	STZ	Antihyperglycemic	Gandhi *et al.,* 2011
40.	*Solanum xanthocarpum*	Solanaceae	Leaves	Alloxan	Antihyperglycemic	Peongothai *et al.,* 2011
41.	*Strychnos henningsii*	Longaniaceae	Stem, Bark	STZ	Antihyperglycemic	Oyedemi *et al.,* 2013

42.	*Symplocos cochinchinensis*	Symplocaceae	Bark	STZ	Antidiabetic	Sunil *et al.,* 2011
43.	*Telfairia occidentalis*	Cucurbitaceae	Leaves	Alloxan	Antidiabetic /Hyperglycemic	James *et al.,* 2016
44.	*Terminalia sericea*	Combretaceae	Stem, Bark	α-glucosidase / α-amylse	Antidiabetic	Nkobole *et al.,* 2011
45.	*Tulbaghia violacea*	Alliaceae	Whole plant	Cell line	Antidiabetic	van Huyssteen *et al.,* 2011
46.	*Vaccinium Arctostaphylos*	Ericaceae	Fruit	Alloxan	Antidiabetic	Feshani *et al.,* 2011
47.	*Viscum schimperi*	Viscaceae	Aerial parts		Antihyperglycemic	Abdel-Sattar *et al.,* 2011
49	*Vitex negundo*	Lamiaceae	Leaves	STZ	Antihyperglycemic	Sundaram, *et al.,* 2012
50.	*Zygophyllum album*	Zygophyllaceae	Whole plant	STZ	Antidiabetic	Ghoul *et al.,* 2012

2.23 Essential Trace Elements, Vitamins and Carotenoids from Plants and Herbs with Antidiabetic Activity

Plants and herbs possess trace elements, vitamins and carotenoids that are beneficial for disease control such as in DM.

2.23.1 Essential Trace Elements from Herbs with Antidiabetic Activity

Natural products, including herbs, are used as abundant, dependable, and well-balanced sources of crucial trace elements in the prevention and treatment of DM. The structure and function of the enzymes involved in glucose or carbohydrate metabolism depend heavily on trace elements. For healthy health, they are necessary and needed (Nashiru, 2019). The essential physiological operations that trace elements and a few metals support include the Krebs cycle, glycolysis, protein, and lipid metabolism required for maintaining energy production and survival. They act as co-enzymes and co-factors in these reactions. The physiological processes occurring in the human body, such as glucose balance and metabolism, are actively participated in by trace elements. Trace elements causes type 2 DM, insulin resistance, obesity, and other metabolic disorders, according to Wiernsperger and Rapin (2010). There is evidence that several trace elements are present in low quantities in diabetic patients, and it has been documented that deficits of a number of trace elements can lead to or triggers DM complications (Pasula and Sameera, 2013). Numerous studies have linked DM to a few trace components. Trace elements have been looked at as potential preventative and therapeutic agents for type 1 and type 2 DM as well as their associated comorbidities (Mooradian, 1994). It has been found that the trace elements zinc, chromium, vanadium, and selenium (Se) are all extremely closely associated to DM. Additionally; several other trace elements have value.

Zinc acts vitally in balancing the amount of insulin hexamer and insulin storage in the pancreas and improves insulin concentrations in type 1 and 2 DM. It relates to the production of insulin by pancreatic β-cells and also its release. Additionally, it works as a strong antioxidant and protects bodily cells from free radicals (Wiersperger and Rapin, 2010). According to Norouzi et *al*., (2018) Zn regulates the insulin receptor-initiated signal transduction process and the production of insulin receptors. It encourages the use of glucose by adipose and muscle cells (Ranasinghe *et al*., 2015). Diabetics have reduced plasma Zn levels and intestinal Zn absorption rates (Farooq *et al*., 2020).

Chromium actively contributes to both glucose metabolism and homeostasis. Chromium is important for lipid metabolism, and a dietary shortage can cause lipid diseases, hyperglycemia, and decreased glucose tolerance (Staniek, 2011).

Chromium supplementation taken orally has been found to help DM patients with their problems. Trivalent chromium (Cr^{3+}) is necessary to maintain stable and appropriately controlled normal glucose metabolism.

Vanadium has been shown to have anti-diabetic characteristics and is described as an element that mimics the effects of insulin (Pasula and Sameera, 2013). It serves as a co-factor of enzymes that are vital to energy metabolism and is critical in the control of intracellular signaling. Additionally, it speeds up glycogen synthesis while slowing gluconeogenesis (Cohen, 1995). It has been discovered that increasing insulin sensitivity with a daily dose of 10 mg vanadyl sulfate is beneficial (Cohen, 1995).

Seleniun-In diabetes mellitus, there has been evidence of selenium decrease (Wiernsperger and Rapin, 2010). It lowers the risk of cardiovascular disease, reduces cellular damage, improves antioxidant defenses, raises immune system activity, manages typhoid function, and guards against heart disease.

Magnesium- According to some reports, magnesium is a co-factor in numerous enzymes involved in carbohydrate oxidation as well as the cell membrane's mechanism for transferring glucose. It may significantly affect insulin release. Magnesium deficiency is more common in type 2 diabetics than the general population and has been linked to DM complications, notably retinopathy (Mooradian, 1994). It reinforces cell membranes and strengthens the immune system**.**

Molybdate- Molybdate lowers blood sugar in severely insulin-resistant diabetics. It is linked to an improvement in pancreatic insulin reserves and a considerable reduction in hyperinsulinemia (Ozcelikay *et al*., 1996).

Manganese - Manganese serves as a co-factor in the activation of manganese superoxide dismutase, an antioxidant enzyme critical to cellular metabolic processes. This antioxidant enzyme helps the body break down carbohydrates, lipids, proteins, and other substances for the production of energy. It protects cell membranes and tissues from damage and degeneration (Li and Yang, 2018).

Minerals known as trace elements can be present in minute amounts in biological tissues. About 0.02 % of the body's weight is made up of essential trace elements, which serve a critical function as the active sites of enzymes or as minute amounts of bioactive compounds. When ingested in quantities greater than those necessary for nutritional value, they produce pharmacological effects (Gholamhoseinian *et al.,* 2020).

2.23.2 Essential Vitamins from Herbs with Antidiabetic Activity

Vitamins aid in maintaining the body's healthy operation. They support healthy blood coagulation, maintain healthy nerves, help the body fight infections, and produce energy from food. They aid in healthy development and growth. Vitamins in the right amounts safeguard and boost the efficacy of diabetes-treating herbs and natural remedies. Retinol, alpha-tocopherol, ascorbic acid, and carotenoids including β-carotene, β-cryptoxanthin, and lycopene are notable vitamins.

Retinol, commonly known as vitamin A, aids in the modulation of immune system processes and lowers the morbidity associated with infectious disorders (Campa *et al*., 2017).

Alpha-tocopherol – The antioxidant properties of alpha-tocopherol, or vitamin E, are acknowledged. It can then provide protection against redox imbalance, which causes DM. DM causing free radicals are, stopped (Rizvi *et al*., 2014).

Thiamine- The two most crucial activities carried out by thiamine, generally known as vitamin B1, are alpha-keto acid decarboxylation and transketolation. While decarboxylation processes are associated with carbohydrate metabolism, transketolation is connected to the activity of the pentose phosphate pathways (Fattal-Valevski, 2011).

Nicotinic Acid/Nicotinamide- Although its usage in diabetics has been reduced because of its negative effects on glycemic control, nicotinic acid therapy for dyslipidemia has been shown to be effective at doses of 1-3 g daily (Goldberg and Jacobson, 2008).

Nicotinamide prevents auto-immune pancreatic cell death by preserving intracellular NAD levels and preventing the DNA repair enzyme poly (ADP-ribose) polymerase (PARP). Nitric oxide radicals respond to nicotinamide as a weak antioxidant (Hummel *et al.,* 2006). In general, it can help with both type 1DM and type 2DM management.

Ascorbic acid – Eight human enzymes employ ascorbic acid (vitamin C) as an electron donor, according to Gulfraz *et al*. (2011). It prevents DM-causing oxidative damage.

2.23.3 Carotenoids from Herbs with Antidiabetic Activity

Carotenoids are present throughout nature and can be found in plants, algae, and bacteria. They defend against redox imbalance and free radical damage. In the world of plants, they are very common. Animals must consume plants to get the carotenoids they need because they cannot produce them on their own (Goodwin, 1980). Examples include lycopene, β-carotene, and xantophylls. Numerous epidemiological studies have connected a high consumption of carotenoids to a decrease in the prevalence of chronic diseases like DM.

B-Carotene-Antioxidants like β-carotene combat free radical oxidations, which include the peroxidation of lipids that is characteristic of many degenerative illnesses.

Lycopene-Antioxidant qualities of lycopene, particularly those present in tomatoes, can provide relief from the oxidative stress that leads to DM.

Herbs are rich in bioactive phytochemicals like carotenoids, which are known to have a variety of beneficial biological benefits. Fruits and vegetables contain the majority of the 650 known naturally occurring carotenoids (Britton, 1995).

2.24 Modus Operandi of Active Substances in Plants Used in Treating Diabetes Mellitus

Due to the multifactorial nature of diabetes mellitus, numerous pathways will be necessary to treat the condition; with the hyperglycemia process associated with type 2 DM being of particular importance. Several pathways of the hyperglycemic process can be attacked by natural products, such as plants, herbs, or their active ingredients. The human body's metabolic and absorption processes for carbohydrates, insulin synthesis and release, aldose reductase pathway, gluconeogenesis, glycolysis, Krebs cycle, cholesterol synthesis, and free radical scavenging action are all affected by extracts of medicinal plants (Prabhakar, 2016). Phytochemical components can influence numerous biological processes and operate on a range of targets to treat both acute and chronic complications of DM (Arokiyaraj *et al.,* 2011; Kar *et al.,* 2003). According to El-Abhar and Schaalan (2014), plants and herbs have a variety of activities that enable them to out-perform or to be more effective than traditional

synthetic diabetic medications. The following methods enable herbs with a hypoglycemic impact to be effective:

By blocking α-glucosidase, a digestive enzyme produced by the small intestine's brush border, glucose absorption from the intestine is prevented,

Inhibiting α-amylase secreted from the salivary gland,

Increasing insulin synthesis from the β-cells of the pancreas,

Reducing hepatocytes' capacity to produce glucose or boosting GLUT's ability to transport glucose into peripheral tissues,

Stimulating the action of incretin peptide analogue,

Increasing insulin sensitivity and improving muscle and adipose tissue's ability to absorb glucose

Reduction of glycated plasma protein content and HbA1C

Reduced fasting, post-meal, and insulin requirements,

Inhibition of DPP-4 enzymes,

Nourishment of pancreatic β- cells,

Enhancement of GLP-1,

Regulation of GLUT-4 (Vinodhini *et al.,* 2022; El-Abhar and Sachaalam, 2014).

Plant's activity categorization according to mode of action:

2.24.1 Agents Stimulating Pancreatic B-Cell Regeneration and Insulin Releasing Activity

These agents primarily work to regenerate residual β-cells in insulin-dependent diabetes, as well as to repair or renew partially destroyed β-cells and regenerate pancreatic cells, both of which enhance plasma insulin concentration and insulin activity. They promote the regeneration of β-cells, prevent the necrosis of β-cells, and control pancreatic processes that mimic the release of insulin. They help to reduce pancreatic lesions, reverse β-cell damage, and regenerate and replenish or repopulate pancreatic β-cells (Wang *et al*., 2021; Zhong and Jiang, 2019).

2.24.2 Agents Acting as Antioxidants

Aerobic metabolism produces oxygen free radicals, but cells have a defence mechanism to eliminate them (Chandra *et al.,* 2000). According to certain studies (Vona *et al*., 2021; Sharifi-Rad *et al*., 2020; Unuofin and Lebelo, 2020), plants with antioxidant properties have a good impact on experimental DM while others inhibit

the production of lipid peroxide, reactivate antioxidant enzymes, and increase GSH levels.

2.24.3 Agents Stimulating Glucose Receptor

Multiple pathways, each divided into distinct domains, are involved in the action of insulin. GLUT-4 translocation to plasma membrane, which occurs as a result of a cascade of processes triggered by insulin activation, leads to glucose absorption in muscle and adipocytes. These agents increase glucose receptor stimulation, which improves glucose absorption at the tissue and cellular levels and helps to lessen and alleviate DM complications. They can lead to an increase in liver and glycogen content and work by boosting insulin receptor activity at the tissue and cellular level (Petersen and Shulman, 2018).

2.24.4 Agents Modulating Glucose Absorption

The prevalence of type 2 DM is significantly influenced by postprandial hyperglycemia (PPHG), which can cause non-enzymatic glycation of a variety of proteins and lead to chronic complications. By inhibiting the actions of the enzymes α -glycosidase and α-amylase, the level of PPHG can be controlled. This is a crucial tactic for treating people who have type 2 diabetes and those who are borderline (Abd Rashed and Rathi, 2021; Brewer *et al*., 2016; Oboh *et al*., 2016 Priya and Bhaskara Rao, 2016).

2.24.5 Agents Inhibiting Aldose Reductase Pathway

One of the pathways in the human body that converts too much glucose into other safe chemicals is the polyol pathway, which is catalized by the enzyme aldose reductase. According to Prabhakar (2016), the final product of this pathway comprises toxic compounds that result in neuropathy, nephropathy, retinopathy, and cataract. Aldose reductase enzyme is connected to the aetiopathology of diabetes complication such nephropathy, cataract, retinopathy, and neuropathy. Aldose reductase pathway inhibitors have been shown to inhibit this enzyme. Numerous plants and herbs, as well as the active ingredients in them, have been discovered to have the capacity to inhibit the aldose reductase pathway (Dodda and Ciddi, 2014; Kanchan *et al*., 2016).

2.25 Reasons for Increased Patronage and Self-Medication with Herbal Medicine

Numerous components found in plant extracts are thought to work in concert or synergistically with one another.

Herbal products are inexpensive and cost-effective, whereas synthetic pharmaceuticals are expensive and toxic.

Products made from herbs are widely accessible, including vitamins, tea, extracts, and essential oils.

It is said that herbal medications are more effective. Herbal remedies are said to strengthen immunity without interfering with the body's regular physiological functions.

Customers favour natural treatments and put more faith in complementary and alternative therapies.

False belief, that herbal medications are superior to synthetic drugs, in terms of quality, and effectiveness.

Herbal remedies are thought to be more effective in treating some ailments where conventional therapy or medications have failed, due to unsatisfactory results from mainstream medicine...

Patients feel that herbal remedies are more effective alternatives because they believe that modern medical professionals cannot detect their ailments effectively...

With the recent advancements in science and technology, herbal medicine has improved in terms of quality, efficacy, and safety.

Migration in the direction of self-treatment (Bandaranayake, 2006). Confidentiality is important since many people are reluctant to talk about their health problems with strangers, especially with medical professionals.

Fear of incompetence, as shown by the potential for incorrect diagnosis and unsuitable treatment.

Lack of time, to routinely visit a doctor, especially, in cases that call for follow-up (Studdert *et al.,* 1998).

Inability to choose a preferred physician or doctor

Religion and spirituality have more of an impact than scientific principles (Parle and Bansa, 2006; Zeil, 1999; Astin, 1998).

Advanced marketing strategies, and tenacity of the sales staff, used by manufacturers of natural medications; radio, television, and road-side advertisements of various kinds (Parle and Bansa, 2006; Brevort, 1998)

2.26 Non Drug Treatment of Diabetes Mellitus

Non Drug treatment involves lifestyle change, exercise, and diet.

2.26.1 Life Style Change Used in Controlling Diabetes

Changes to one's way of life are referred to as lifestyle changes.

Seven life-style principles of effective diabetes care are listed below.

Knowledge of diabetes,

Receive regular diabetic care.

Find out how to manage your diabetes,

Observe the ABC's for diabetics.

Keep an eye on your diabetic ABCs,

Prevent persistent diabetic issues,

Check on persistent problems and address them.

2.26.2 Exercise

Regular, high-quality exercise is essential for preventing or treating diabetes mellitus.

Regular exercise benefits include:

Shedding weight,

Lowering blood sugar levels and maintaining them there for several hours following,

Lowering blood pressure and cholesterol,

Lowering of stress,

Increasing tissue insulin sensitivity- insulin lowers blood glucose by transporting it to cells.

2.26.3 Diet

Prioritise high-fiber, low-fat foods. The body's sensitivity to insulin will rise just from this.

Dietary weight loss makes a diabetic's body more responsive to insulin.

2.26.3.1 Types 1 Diabetes Treatments

There is currently no recognized medical treatment for Type 1 DM which is a lifelong condition. It is brought on by a lack of insulin, and Type 1 diabetes requires regular

insulin use. It can be breathed in or injected. A special diet, especially one high in fibre and good consistent exercise will help.

2.26.3.2 Type 2 Diabetes Treatments

Although it may also last a lifetime, this may be more amenable to therapy. This condition is caused by the cells' resistance to insulin. Treatment options include drugs that boost pancreatic insulin production, improve target organ insulin sensitivity, and slow down the pace at which the body absorbs glucose from the gastrointestinal system. Some diabetics have had success treating their symptoms without taking medication by combining a customized diet, consistent exercise, and body weight management.

2.26.3.2.1 Healthy Eating – Fruits, vegetables, whole grains, legumes, etc. are high-fiber, low-fat foods.

2.26.3.2.2 Regular Exercise – Aerobic activity for 30–45 minutes a day, five days a week, is good.

2.26.3.2.3. Blood Sugar – regularly checking blood sugar levels.

2.26.3.2.4 Medication – rigorous adherence to insulin therapy or diabetic medicines.

2.27 Data Analysis for a Healthy Living

Carbohydrates	- Between 45 and 65 % of daily calorie intake
Fat	-25–35 % of daily calories
Protein	- 15–25 % of daily calories (Frank, 2011).

2.28 Current Advances in the Modes of Treatment and Management of Diabetes Mellitus

2.28.1 Continuous Glucose Monitoring Systems.

These devises can make available real-time glucose measurements, enabling diabetics to continuously check their own blood sugar levels. They can help people make wise decisions regarding their food, activity, and insulin dosage, which will result in better DM management.

2.28.2 Insulin Pump Technology

This has to do with systems that automatically deliver insulin. These so-called artificial pancreas systems use CGM and insulin pumps to automatically change the amount of insulin delivered based on blood sugar levels. This helps people keep their blood sugar levels more consistently constant and lowers their risk of hypoglycemia.

2.28.3 Targeted Therapies

This entails the creation of brand-new medications that specifically address the causes of DM. For instance, SGLT-2 inhibitors and GLP-1RAS have been successful in decreasing blood sugar levels, encouraging weight loss, and lowering cardiovascular risks in people with type 2 DM.

2.28.4 Personalised Medicine

Progress in modern genetic and molecular research has opened the door to personalised medicine techniques for the treatment of DM. This makes it possible to identify those who will respond better to particular treatment approaches.

2.28.5 Digital Health Solution

Management of DM has altered as a result of the development of digital health technologies. People can use mobile applications and wearable technology to track their blood sugar levels, medication compliance, exercise, and food consumption. This results in enhanced patient-provider communication and access to care.

2.28.6 Behavioral Interventions

Behavioural therapies have drawn attention in the management of DM as a result of the importance of lifestyle adjustment. People can develop healthier habits and improve their glycemic control with the use of techniques like peer support programmes, motivational interviewing, and cognitive-behavioural therapy.

2.28.7 Artificial Intelligence and Machine Learning

Large datasets are being analysed and predictive models for diabetes treatment are being created using artificial intelligence and machine learning algorithms. These technologies can help with early diagnosis of complications, customised treatment planning, and optimising insulin dosing algorithms.

2.29 Distinction between Diabetes Mellitus and Diabetes Insipidus

Diabetes mellitus and diabetes insipidus are the primary types. Diabetes insipidus is a complex multifaceted clinical syndrome that affects water balance. It produces significant amounts of urine despite no blood glucose spike. Blood pressure regulation, renal function, salt homeostasis, and ADH hyposecretion (osmotic balance management) are some of the causes. Hyperglycemia is caused by insulin hyposecretion or absence.

The symptoms of excessive urination and excessive thirst do not distinguish the two disorders. Glucosuria (glucose in the urine), polyuria (increased urine volume due to glucose's osmotic effect), polydipsia (extreme thirst), and polyphagia (excessive appetite) are also symptoms of DM. Kidneys attempt to remove the extra glucose by excreting it in urine. The person with diabetes insipidus can produce up to 19 litres of pee each day, which results in a commensurate rise in the amount of water lost from bodily fluid while the average person produces 1-2.8 litres of urine per day. These cause extreme thirst, dehydration, an increase in food cravings, strength loss, and emaciation. Ketone bodies do not develop in the blood or urine. Despite acceptable glucose levels, the kidneys cannot properly concentrate urine.

2.30 Experimental Induction of Diabetes Mellitus

According to Hunt and Roughead (2003), animals can be experimentally induced to develop DM by either pancreatectomy or the injection of cell poisons such as alloxan or streptozotocin. Alloxan, and STZ two frequently used diabetogenic agents, are toxic glucose analogues that exhibit cytotoxic action on the pancreas by harming or damaging the β-cells in the islet of Langerhans, impairing their ability to produce enough insulin (Lenzen, 2008; Szkudelski *et al.,* 2001). Due to the structural similarities between the two compounds and glucose, glucose can compete with them, making fasting animals more vulnerable or susceptible. Both substances have a fair amount of instability (King, 2012).

2.30.1 Streptozotocin

Streptozotocin, also known as streptozocin, isostazin, or sonostar, is sold under the brand name Zonosar and is referred to by the abbreviation STZ.

2.30.2 History

Scientists at Up John, a pharmaceutical firm that is now a part of Pfizer, in Kalamazoo, Michigan, USA, originally identified, extracted, and the first to isolate the naturally occurring chemical, streptozotocin from a soil bacterium called *Streptomyces achromogenes* (Eleazu *et al*., 2013). The antibiotic streptozotocin was first discovered in the 1950s to have broad-spectrum activity. According to Abdollahi and Hosseini (2014), in mammals, streptozotocin is particularly toxic to the β-cells that make insulin in the pancreas. The FDA approved STZ for pancreatic islet cancer treatment in July 1982. Streptozotocin, a naturally occurring molecule, causes type 1 DM in animal models and type 2 DM with repeated low doses. It also treats metastatic Langerhans

islet cancer (Brentjens and Saltz, 2001). Since 1963, streptozotocin has been used in animal models to study the pathogenesis of DM, its consequences, and prospective treatments. According to Bolzán and Bianchi, (2002), streptozotocin is a monofunctional nitrosourea derivative. Deoxyglucose enhances cell membrane transport while nitrosourea promotes β-cell toxicity in streptozotocin. Streptozotocin destroys β-cells, especially pancreatic ones, when it causes hyperglycemia. STZ, like alloxan, produces free radicals, which alters the body's defences against reactive species.

2.30.3 Chemistry

2.30.3.1 Chemical and Physical Properties

Streptozotocin, a nitrosourea analogue, has an N-Methyl-N-Nitrosourea (MNU) moiety with a methyl group linked to one end and a glucose molecule linked to other end (Gushiken *et al.,* 2016). Streptozotocin, or 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose, is a cytotoxic glycosamine-nitrosourea compound.

2.30.3.2 Chemical Structure

The chloroethyl side chains that are found in other nitrosourea are absent from streptozotocin.

2.30.3.3 Chemical Formula

The molecular formula is $C_8H_{15}N_3O_7$

2.30.3.4 Physical Properties

The crystalline powder of streptozotocin, which has a melting point of 115 ^{0}C, is a combination of the stereoisomers and is either light yellow or off-white (ivory) in colour. Refrigeration and light protection are required for the lyophilized, light-yellow injectable powder.

2.30.3.5 Molecular Weight

The molar mass is 265.221 g/mol.

2.30.3.6 Solubility

In contrast to streptozotocin great solubility in water, ketone, and lower alcohols, streptozotocin has a relatively limited solubility in polar organic solvents (NTP, 2005). While the reconstituted solution is stable for two (2) days at room temperature, it should be discarded after eight hours but STZ is stable for three years when refrigerated. It is a hydrophilic substance that can be used as an alkylating agent and is

comparatively stable for at least an hour at a pH of 7.4 and a temperature of 37 ^{0}C (Povoski *et al.,* 1993; Lee *et al.,* 1993).

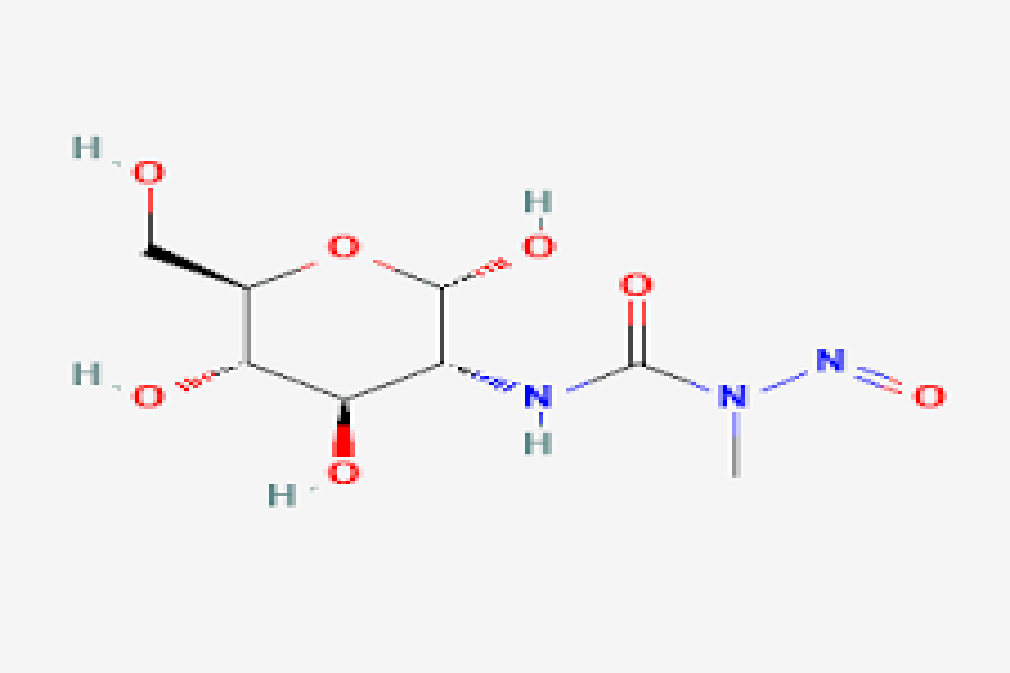


Figure 2.4: Structure of Streptozotocin

Source: Adapted from Mawlieh *et al*., 2020

2.30.3.7 Uses

Streptozotocin is used as a chemotherapeutic medication to treat some Islet of Langerhans cancers and as an animal model for type 1 diabetes. It is FDA-approved to treat metastatic pancreatic islet cell cancer (Brentjens and Salts, 2001), but due to its pancreatic-cell-specific toxicity, it has become an investigational drug used to produce insulitis and diabetes mellitus in animal models (NTP, 2005). In conclusion, streptozotocin preferentially kills pancreatic cells, causes hyperglycemia, prevents DNA synthesis in mammalian cells and microorganisms by cross-linking DNA strands and alkylation, and also affects all phases of the mammalian cell cycle. In humans, streptozotocin is likely tetratogenic, mutagenic, and carcinogenic. The antibiotic streptozotocin also works against Gram-negative bacteria.

2.31 Mechanism of Action of Streptozotocin

In both bacterial and human cells, streptozotocin works as an inhibitor of deoxyribonucleic acid (DNA) synthesis (Bolzan and Bianchi, 2002). The bacterial DNA is degraded and destroyed as a result of a unique interaction STZ has with cytosine groups in the bacterial cell (Reusser, 1971). The mechanism that involves the formation of free radicals during STZ metabolism causes DNA and chromosomal damage in mammalian cells (Bolzan and Bianchi; 2002). This biochemical process results in the death of mammalian cells, and STZ also inhibits or blocks the entry of cells into the mitotic process. Streptozotocin is diabetogenic because it has the ability to selectively cause necrosis to kill beta-cells that produce insulin. STZ's glucose moiety allows it to cross pass the plasma membrane's low affinity glucose transporter GLUT 2 and damage DNA by alkylating it, is thought to be responsible for its specific toxicity to β-cells (Szkudelski, 2001). It is widely acknowledged that DNA alkylation contributes to STZ's cytotoxicity, and that this activation of poly adenosine diphosphate-poly (ADP-ribose) synthase results in a rapid and lethal depletion of NAD in pancreatic beta cells of islet of Langerhans thus causing death (the depletion of NAD in the pancreas results into cessation of NAD- dependent energy formation and finally death of β-cell) and release of Nitric Oxide (NO). Numerous studies have demonstrated that free radicals can increase oxygen free radicals such as hydroxyl radicals and nitric oxide, which may contribute to β-cell damage and STZ's diabetogenic action. Highly reactive carbonium radicals from STZ molecule breakdown have this effect (Mythili *et al.,* 2004). Typically, STZ spontaneously breaks

down to carbonium ions (CH^{3+}), which alkylate purine and pyrimidine bases to form DNA adduct (Rosol *et al.,* 2013). Given that STZ and glucose are similar, it is easily transported into cells by the glucose protein GLUT 2, but is not recognised by other glucose transporters. Since β-cells have a relatively high concentration of GLUT 2, this explains why STZ is relatively toxic to β-cells (Wang *et al.,* 1998). GLUT 2 transports streptozotocin into beta cells; hence its toxic effect requires its expression. According to Sviglerova *et al.,* (2017), stimulation of inducible NO-synthase, an increase in NO concentration, and enhanced H_20_2 generation are necessary for the signal pathways of STZ's toxic effects. The toxicity of streptozotocin may also be explained by its function as an agent for protein alkylation and as a source of NO. Because STZ enters β-cells via GLUT 2, its toxic effects are not limited to β-cells alone and can harm other organs as well, including the liver and kidneys. STZ breaks into its glucose and methylnitrosourea moiety after being taken in by β-cells. Methylnitrosourea alters biological macromolecules, breaks up DNA, and kills β-cells due to its alkylating characteristics, leading to an insulin-dependent state of diabetes. The ability of STZ to suppress glucose-induced insulin release may be due to its targeting of mitochondrial DNA and resulting interference with the signaling function of the β-cell mitochondrial metabolism.

The glucose moiety in STZ is what causes it to selectively accumulate in pancreatic cells. The likelihood that STZ could be used as an animal model for diabetes mellitus was increased by the discovery that the drug preferentially destroys pancreatic islet cells, which generally regulate blood glucose levels by releasing the hormone insulin (Rerup, 1970).

Streptozotocin causes hyperglycemic non-ketotic diabetes mellitus in a variety of animal species (Weir *et al.,* 1981). Because STZ-induced diabetes resembles hyperglycemic non-ketotic DM in humans and is frequently accompanied by kidney hypertrophy, which increases hepatotoxicity danger, oxidative stress, and hypercholesterolemia and also end-stage renal damage, its use in inducing diabetes in experimental animals is now generally accepted (Maric-Bilkan *et al.,* 2012; Gezginci-Oktayoglu *et al.,* 2009).

STZ stimulates the immune system, alkylates DNA, and generates nitric oxide, which results in the fast necrosis of pancreatic beta-cells (Szkudelski, 2001; Kwon *et al.,* 1994). Additionally, required for the onset of diabetes is nuclear DNA fragmentation in

pancreatic cells (Yamamoto *et al.,* 1981). DNA strand breakage and damage caused by STZ can be repaired by NAD-dependent enzymes like Poly (ADP-ribose) synthetase.

2.32 Pharmacological Data

2.32.1 Dosage and Effects

A single 50 mg/kg rat dosage causes cell necrosis. β-cell loss, and islet atrophy. According to Deeds *et al*. (2011), a number of variables, including the animals being studied, their age, gender, and the degree of induction needed, influence the dosage to be utilised for inducing diabetes.

Intraperitoneally administered high dosages of STZ (45 to 75 mg/kg) are toxic to cells that produce or secrete insulin causing the emergence of experimental Type 1 diabetes. Low dosage of STZ (20–60 mg/kg) given intraperitoneally to neonatal rats destroy IR, disrupt IR signaling, and result in Type 2 diabetes (Akbarzadeh *et al*., 2007).

2.32.2 Route of Administration

Effective routes of administration that have been observed are:

(i) Intraperitoneal (ii) Intravenous

The route of administration is also influenced by a variety of parameters (Deeds *et al.,* 2011). When injected intravenously, STZ rapidly drops in plasma concentration within fifteen minutes and concentrates in the liver and kidneys (Eleazu *et al.*, 2013).

2.32.3 Bio-Availability

Bioavailability is 17-25% (100% if intravenous)

Metabolism

The liver and kidneys are metabolic centres. The kidneys metabolise and excrete 20 % of the medicine (or its N-nitrosourea-containing metabolites) (Eleazu *et al.*, 2013).

2.32.4 Biological Half Life

Streptozotocin's biological half-life is estimated to be between 35 and 40 minutes (35-40 min.).

2.32.5 Factors Affecting the Sensitivity of Streptozotocin

The interaction of various elements, such as the animal species or strains being used, gender, nutrition, circadian rhythm, dosage, etc., impacts the sensitivity of STZ (Deeds *et al.,* 2011).

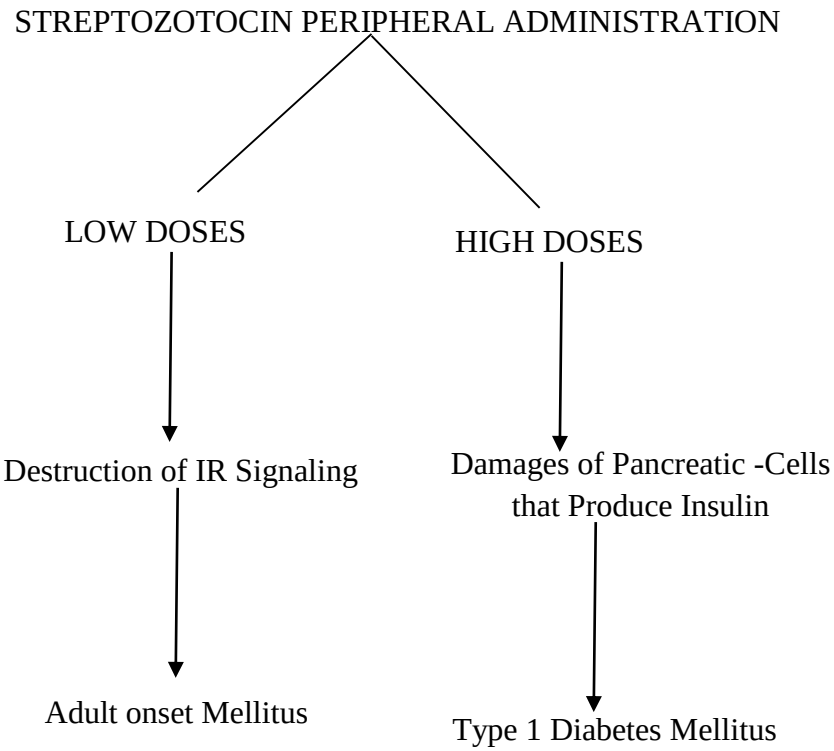


Figure 2:5 Peripheral Mechanisms of STZ.

2.32.6 Safety

The fact that streptozotocin interacts with DNA and can cause cytotoxic effects in animals suggest that exposure to STZ poses a risk to human health and safety.

2.33 Other Experimental Models to Induce Diabetes Mellitus

Animal models have historically been important for researching, characterizing, and comprehending disease pathogenesis as well as for determining targets, evaluating novel therapeutic practitioners, and *in vivo* therapy. Understanding the inciting chemical's role in type 1 and type 2 DM is crucial. Animal models are still the most efficient way to comprehend the complex aetiology and multi-systemic interactions present in DM, regardless of *in vitro* and *in silico* research (Kottaisamy *et al.,* 2021). According to Frode and Medeiros (2008), diabetes can be induced in a variety of animal species through pharmacological, surgical, or genetic manipulation. Rats, mice, hamsters, guinea pigs, and rabbits are just a few of the rodent species that provide good research subjects for DM. Research on DM makes extensive use of pharmacological drugs and animal models to understand the pathophysiology, comorbidities, genetic, and environmental influences (Vineeta and Janeshwar, 2014). Appropriate experimental models are necessary to understand pathogenesis, complications, and testing of various treatment medicines (Patel *et al.,* 2012). Methods for studying diabetes in animals include, but are not limited to, those that are genetically or spontaneously derived, chemically induced, diet induced, surgically induced, and transgenically or knocked out derived (Srinivasan and Ramarao, 2007; Rees and Alcolado, 2005; Chen and Wang, 2005; Cheta, 1998).

Recently produced genetically altered animals, pharmaceutical treatments, surgical interventions, viruses, and diabetogenic hormones have been used to research DM. Several genetically altered animal models, including transgenic, generalised knock-out, and tissue-specific knock-out mice, have been used to test prospective anti-diabetic drugs (Frode and Medeiros, 2008). Currently, using drugs such as Alloxan, Streptozotocin, and Dithizone; Monosodium Glutamate, viruses, and genetically modified rats to induce DM is the best and fastest method. The five most powerful diabetogenic agents include chemicals, peptides, biological agents, potentiators, and steroids, however the two most commonly used chemical agents are alloxan and streptozotocin (Fajarwati *et al*., 2023; Ighodaro *et al*., 2017).

2.33.1 Alloxan

Alloxan is a well-known chemical used in diabetogenic studies. It was first prepared in 1818 by Luigi Valentino Brugnatelli (1761-1818), but Friedrich Wohler and Justus Von Liebig gave it its name in 1838. Alloxan has been used to induce experimental DM in rats, mice, and dogs at various doses since Duns et al.'s discovery of alloxan-induced β- cell necrosis in rabbits in 1943 (Patel *et al.,* 2012). The pancreatic islet's β-cell is selectively necrosed by the urea derivative alloxan (Etuk, 2010).

Alloxan – Formula-OC [N (H) CO2C (OH) 2]

2.33.2 Streptozotocin

As discussed under history in 2.29.2.

2.33.3 Dithizone

Dithizone induces DM in cats, rabbits, golden hamsters, and rodents (Goldberg *et al.,* 1991; Halim *et al.,* 1977)

2.33.4 Gold Thioglucose

According to Heydrick *et al.,* 1995 and Stauffacher *et al.,* 1967, the chemical, gold thioglucose is a diabetogenic agent that causes type 2 DM and induces hyperphagia. It is a derivative of glucose that undergoes precipitation with methanol, followed by water-methanol crystallisation. In a group of mice with normal genetics, gold thioglucose-induced obesity causes diabetes.

2.33.5 Monosodium Glutamate

Without polyphagia, monosodium glutamate induces type 2 DM (Sartin *et al.,* 1985). It is a naturally occurring, freely soluble non-essential amino acid (Nagata *et al.,* 2006). Following consumption, it produces a significant insulin response. For this model, you can use mice, cats, rabbits, or golden hamsters.

2.33.6 Virus

The occurrence of juvenile-onset diabetes mellitus (DM) can be attributed to viral infections and autoimmunity specifically targeting β-cells (Craighead, 1978). The newly discovered juvenile-onset diabetes (type 1) caused by viral infections was described by Gamble *et al*. (1969). According to Gamble *et al.,* 1969, two viruses—the Coxsackie and D-Variant of Encephalomyocarditis—were implicated and described. Coxsackie virus can infect and kill pancreatic acinar cells without harming the neighbouring islets of Langerhans, unlike EMC-D virus, which kills pancreatic beta-

cells in some inbred mice, causing insulin-dependent hyperglycemia (Vineeta and Janeshwer, 2014; Yoon *et al.,* 1980; Lansdown and Brown, 1976).

2.33.7 Hormone Induced Diabetes

Growth hormone has been linked to renal problems in diabetes mellitus (Thirone *et al.,* 2002). While prolonged growth hormone administration causes permanent DM with loss of pancreatic islets tissues and β-cells, sequential or recurrent growth hormone treatment of adult cats and dogs develops DM with all of its symptoms, including severe ketonuria and ketonemia (Kumar *et al.,* 2012).

2.33.8 Corticosteroid Induced Diabetes

Müller (2016) reported that Ingle and co-researchers in 1941 provided an explanation for the hyperglycemia and glucosuria observed in cortisone-treated, force-fed rats. When normal rats are administered high doses of adrenal cortical steroid, DM is induced. The term "steroid diabetes" was first coined and used by Ingle in 1940 to describe this condition (Simmons *et al*., 2012). Steroid-induced diabetes mellitus is attributed to the administration of dexamethasone and prednisone. Glucocorticoids block insulin, causing insulin resistance, hyperglycemia, and hyperlipidemia. Liver gluconeogenesis increases hepatic glucose synthesis (Ferris and Kahn, 2012).

2.33.9 Others

Ighodaro *et al*. (2017) identified anti-insulin serum, high fructose load, and high glucose load as additional diabetogenic agents.

2.34 Differences between Streptozotocin and Alloxan

Streptozotocin and alloxan are the most notable diabetogenic chemical compounds in experimental diabetic study, and both of them are cytotoxic glucose analogues. The mechanisms of the selectivity of the β-cell action in both compounds are similar, however the two drugs' cytotoxic processes differ (Lenzen, 2008).

Streptozotocin has a higher inductive capacity than alloxan, and it is less toxic to pancreatic β-cells than alloxan, which produces hydroxyl radicals that lead to β-cells' death since they have weaker antioxidant defenses.

Streptozotocin is also more specific to pancreatic β-cells because it is taken up by the β-cell-specific GLUT 2 transporter but not by other glucose transporters, whereas alloxan is not only specific to the pancreas but also appears toxic to some other organs,

such as the kidneys, where it induces diabetic renal changes and nephrotoxic alteration (Evans *et al.,* 1984).

Alloxan results in a higher mortality rate for animals than STZ due to a significant drop in body weight, which also harms other organs. The anti-diabetic effects of streptozocin at lower doses are equivalent to those of alloxan at larger levels. Additionally, according to Lenzen *et al*. (1987), streptozotocin does not inhibit or block glucokinase and does not cause early hypoglycemia following injection. Alloxan causes severe diabetes, yet even before medication intervention is used or applied the diabetes it causes can be reversed. At pH 7.4 and 37 ^{0}C, streptozotocin is comparatively stable for at least an hour (Lenzen, 2008). According to a study and sub-meta-analysis conducted (2000-2016) by Ighodaro *et al*. in 2017, Alloxan was utilised in 30.3 % of diabetes research, while STZ was applied in 57.9 %. The remaining 11.8 % consisted of different approaches such as glucose, fructose, and genetic mice.

2.35 Glucose Homeostasis

The precise regulation of blood glucose level is crucial for maintaining normal human body function. This regulation is achieved through a complex network of various hormones and neuropeptides, primarily secreted from the pancreas, brain, liver, intestines, adipose tissue, and muscle tissue (Röder *et al*., 2016). Insulin, the hormone that lowers blood sugar, together with its antagonistic candidate, glucagon, are secreted by the pancreas, which makes it stand out as a significant actor in the hormonal and peptide network. Nevertheless, the disruption of the intricate interplay between hormones and peptides can result in metabolic disorders, specifically Adult onset Diabetes, which is high-prevalence, comorbid, and costly (Röder *et al*., 2016). Hence, it is imperative to pursue progress in the development of innovative therapeutic strategies, alongside the existing anti-diabetic drugs and pharmaceuticals.

Glucose homeostasis is the complex interaction of organs, hormones, metabolic systems, and brain regulatory processes that maintain blood glucose levels between 70 and 110 mg/dL.

Glucose powers all human cells, sometimes known as sugar. Blood glucose levels are kept within a safe range by the glucose homeostasis cycle, and when this cycle is disturbed, manifold metabolic disorders including DM, manifest. In addition, numerous chemicals and other important components, such as glucose, exhibit a lack

of regulation. The process of regulating blood glucose involves several organs, including the pancreas, liver, kidneys, gastrointestinal tract, and brain. Since carbohydrate digestion and absorption as glucose into the bloodstream take place here, the gastrointestinal tract, first, the stomach and small intestine regulate the system. The pancreas produces, stores, and secretes insulin and glucagon, opposing chemicals that regulate glucose metabolism. Subsequently, these hormones facilitate the transportation of glucose in its simple form into the liver, where it undergoes storage as glycogen. The kidneys play a supportive role to the liver by aiding in gluconeogenesis during the post-absorptive phase and facilitating insulin clearance in the postprandial phase (Dimitriadis *et al.,* 2021).

Fasting lowers blood glucose levels, which in turn inhibits the pancreas' ability to produce insulin through the action of hormones known as catecholamines (Fernandez-Tresguerres *et al.,* 2005). Glucagon activates hepatocyte receptors to activate the phosphorylase enzyme and start glycogenolysis. Glycogenolysis is the metabolic process that converts glycogen into glucose, raising blood sugar levels, managing hypoglycemia, and maintaining blood glucose homeostasis.

Diabetes mellitus is characterised by the inability to maintain normal plasma glucose levels, resulting from the disruption or breakdown of glucose homeostasis. This usually results from pancreatic β-cells producing less insulin or developing insulin resistance. Diabetes can cause acute complications such diabetic ketoacidosis, retinopathy, nephropathy, neuropathy, and cardio-cerebrovascular disease. In addition to cutaneous infections, potential adverse consequences encompass coma, profound dehydration, and hyperglycemic hyperosmolar syndrome.

2.35.1 Organs Involved In Glucose Homeostasis

The maintenance of glucose homeostasis involves the following organs:

Pancreas

Both endocrine and exocrine functions are performed by the pancreas. Exocrine functions are concerned with producing and secreting digestive enzymes, whereas endocrine functions are responsible in the synthesis, and secretion of hormones.

Liver

The liver controls glucose absorption from the portal system and manufactures glucose from glycogen and non-carbohydrate precursors.

Kidneys

The kidneys work by filtering the blood and removing waste materials.

GIT

The mouth, oesophagus, stomach, and intestines make up the gastrointestinal tract (GIT), which helps people digest food, absorb energy and nutrients, and excrete waste.

Brain

Neurons use the most glucose, which is the brain's principal energy source (Mergenthaler *et al*., 2013). The brain uses 120 g of glucose each day, 60–70 % of the body's glucose metabolism. When the blood glucose level falls below 400 mg/dL, the brain will function abnormally; this could result in death or irreparable harm or damage. Ketone bodies can penetrate the neuronal blood-brain barrier and be used for energy in hypoglycemia, whereas fatty acids cannot. In a state of starvation, the brain is limited to using glucose or ketone bodies, specifically acetoacetate and hydroxybutyrate, as its primary sources of energy.

Diabetes mellitus adversely affects every organ involved in maintaining glucose homeostasis.

Summary

Glucose powers muscle and other cells. Glucose is mostly from meals and the liver. Once in the bloodstream, insulin helps sugar enter cells. When one hasn't eaten, the liver breaks down glycogen to glucose to maintain glucose levels.

2.36 GLUT 2 (Glucose Transporter 2)

In mammalian cells, glucose serves as the primary fuel, and members of the glut protein family facilitate its diffusion or transport (Mueckler, 1994). Hepatocytes are the main cells that express GLUT 2, a high-K_m isoform of the glut protein family and a bidirectional transporter that allows glucose to move in both directions. It is also expressed by pancreatic β-cells, basolateral membranes of intestine absorptive cells, and renal proximal convoluted tubule cells. To enable an unrestricted (non-rate-limiting) transit of glucose into and out of various cell types, GLUT 2 functions as a high capacity transport mechanism. It contributes significantly to the maintenance of glucose homeostasis in living things by enhancing the facilitative diffusion of glucose through the plasma membrane more quickly (Evans *et al.,* 2003). It is associated with the initiation of the glucose-mediated insulin secretion cascade, as well as the detection of glucose in pancreatic β-cells, the liver, and the hypothalamus. GLUT2

exhibits the least pronounced apparent affinity for glucose among all glucose transporters. GLUT 2 may be involved in diabetic mellitus (DM) aetiology. In animal models of diabetes, pancreatic β-cells express less GLUT 2, while liver expression increases (Narasimhan *et al.,* 2015). Bidirectionality is required in the hepatic cells for the uptake of glucose for glycolysis and the release of glucose during the process of gluconeogenesis. To improve precise measurement of the blood glucose levels in the intracellular milieu of these cells, free flowing glucose is required in the β-cells of the pancreas. Three monosaccharides—fructose, glucose, and galactose—are all transported by GLUT 2 from intestinal mucosal cells into the portal circulation. Hepatocyte plasma membranes' major glucose transporter is the same. GLUT 2 is also involved in the second stage of trans-epithelial glucose transport in the small intestine and kidney proximal convoluted tubule (Thorens, 2015).

Intestinal cells transport cytosolic GLUT 2-containing vesicles to the apical membrane following sugar-rich meals. In reaction to high plasma sugar levels, pancreatic β-cells generate insulin, which internalises GLUT 2 and slows sugar uptake in the intestine during digestion (Tobin *et al.,* 2008). Controlling GLUT 2 level affects how sugar is transported. Hepatocytes' sinusoidal membranes express GLUT 2, which insulin then internalises in endosomal fractions. This is important because insulin inhibits hepatic glucose synthesis (Girad, 2006). The liver up-regulates GLUT 2 gene transcription in postprandial hyperglycemic states or Adult Onset (Im *et al.,* 2005).

DM is characterised by hyperglycemia and protein alteration by monosaccharides. Glycosylation alters protein structure and function by producing advanced glycation end products and ROS (Gillery, 2006). Excess glucose-induced oxidative stress causes diabetes complications and tissue damage. Hyperglycemia affects liver cells, which regulate and metabolise glucose. Human hepatocytes, particularly Chang's liver cells, have been employed in several investigations.

2.37 How Hormone Insulin Works

Pancreatic insulin regulates blood sugar levels. It helps cells use glucose. When eaten, carbohydrates are converted to glucose during digestion. After absorption, glucose raises blood sugar. Insulin is released as blood sugar rises. Insulin opens cells to glucose. It interacts with insulin receptors on muscle and fat cells. This binding triggers biochemical mechanisms that cause cells to take glucose from the blood (Röder *et al*., 2016).

Following entry into the cells, glucose can be utilised as a source of energy. It can be converted into the body's main energy currency, ATP (Adenosine Triphosphate), alternatively, it can be stored as glycogen in the muscle and liver for later use.

Other nutrients in the body are also affected by insulin's effects on metabolism. It promotes the absorption of amino acids into cells, which are necessary for synthesis of protein. Additionally, insulin inhibits the liver's stored glycogen from being broken down, thus limiting the amount of glucose that is released into the bloodstream (Fu *et al*., 2013).

Insulin aids glucose uptake and storage, keeping blood sugar levels stable. During fasting or between meals, insulin secretion decreases, allowing glucose from glycogen to be released into the bloodstream.

Diabetes affects insulin synthesis and efficacy, causing high blood sugar. In Juvelline diabetes, the pancreas doesn't make insulin, but Adult onset DM reduces cell sensitivity to insulin. To compensate for insulin insufficiency or resistance, blood sugar management often requires insulin injections or other drugs (ADA, 2009).

Figure 2.6: Structure of Insulin
Source: Vivo.colostate.edu

2.38 Pancreas

The pancreas is located above the stomach on the left side of the abdomen. The pancreas is near the liver, spleen, and small intestine. The pancreas is 6–10 inches long and shaped like a flat pear or a fish stretched horizontally across the abdomen (Slack, 1995).

The pancreas' widest part, the head, is towards the belly. The stomach and first intestine meet there. The pancreas releases digestive enzymes into partially digested stomach food in the intestine. The pancreas has a neck and a tail on the left (Kulenović and Sarac-Hadzihalilović, 2010).

The celiac axis, portal vein, superior mesenteric artery, and vein feed the pancreas and other abdominal organs. These large arteries surround the pancreas. 95% of the pancreas is exocrine tissue, which produces digestive enzymes, and 5% is islets of Langerhans, endocrine cells. The grape-like Islets of Langerhans produce hormones that regulate pancreas output and blood sugar (Karpińska and Czauderna, 2022).

2.38.1 Functions of the Pancreas

The right chemicals are produced by a healthy, functioning pancreas in the appropriate amounts and at the proper times to break down or digest the food we consume. Exocrine and endocrine functions are the two main categories into which the pancreas's functions fall. Endocrine function pertains to the production of hormones that control blood sugar levels, whereas exocrine activity deals with the production of enzymes involved in digestion (Karpińska and Czauderna, 2022).

2.38.1.1 Exocrine Function

The following digestive enzymes, trypsin and chymotrypsin, amylase, and lipase, are produced by the exocrine pancreatic glands. Trypsin and chymotrypsin break down proteins, lipase break down lipids, while amylase breaks down carbohydrates. The pancreatic duct releases these fluids when food enters the stomach (Pierzynowski *et al*., 2012).

The pancreatic and common bile ducts combine in the duodenum to form the Ampulla of Vater. Bile is produced by the liver's common bile duct and gall bladder. The duodenum releases pancreatic secretions and bile to digest carbs, lipids, and proteins (Karpińska and Czauderna, 2022).

2.38.1.2 Endocrine Functions

The pancreas' endocrine islet cells (Islets of Langerhans) release vital hormones into the circulation. Two major pancreatic hormones are insulin and glucagon. The kidneys, liver, and brain require proper blood sugar levels (Kulenović and Sarac-Hadzihalilović, 2010).

2.38.1.3 Pancreatic Disorders

Diseases that modify the function of the pancreas can influence it.

Pancreatitis, Mucinous Cystic Neoplasm (MCN), and Intraductal Papillary Mucinous Neoplasm (IPMN) are pancreas illnesses. MCN is more common in women 40–50. These diseases have distinct symptoms and treatment methods (Pierzynowski *et al*., 2012).

2.38.1.4 Pancreatitis

Pancreatitis occurs when pancreatic enzymes destroy organs and produce inflammation. This could manifest as a sudden, intensely painful attack or as a chronic illness that worsens over time.

2.38.1.5 Precursors to Pancreatic Cancer

Pancreatic cancer's cause is unknown; however, risk factors enhance its likelihood. Smoking, chronic pancreatitis, and pancreatic cancer syndromes are risk factors. IPMNS and PanIN are pancreatic cancer precursors (Kulenović and Sarac-Hadzihalilović, 2010).

2.38.1.6 Pancreatic Cancer

Pancreatic adenocarcinoma, an exocrine tumour of pancreatic duct cells, is the most prevalent type of pancreatic cancer. Only 5 % of pancreatic cancers are neuroendocrine or islet cell tumours (Karpinska and Czaudern, 2022).

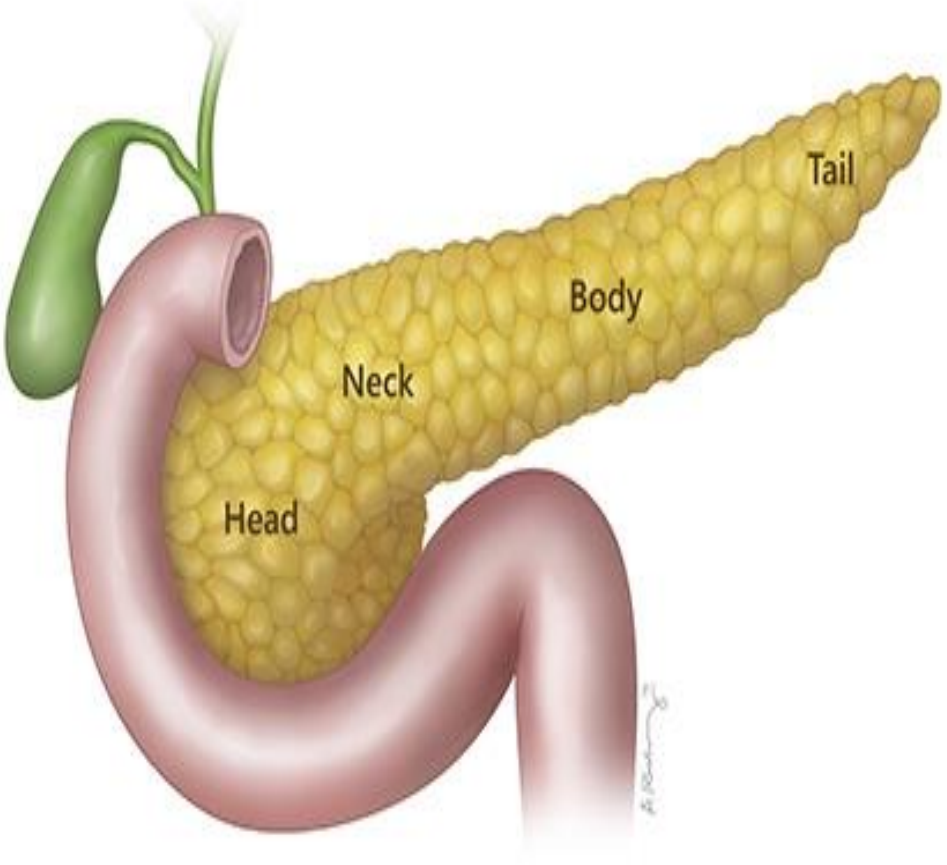


Figure 2.7: Structure of Pancreas
Source: Karpinska and Czaudern, 2022

2.39 The role of Fatty Acids in Type 2 Diabetes Mellitus

Fatty acids are the basic components of fat in both the human body and consumed food. For simple absorption into the circulation, fats are converted to fatty acids during digestion. The molecule known as triglyceride is typically formed when three fatty acid molecules are linked together. Fatty acids are a type of carboxylic acids characterised by the presence of an aliphatic chain, which can exhibit either saturated or unsaturated properties. Any fat with fatty acid chains that only contain single bonds is said to be saturated. Since fatty acids (FAs) control the movement of glucose transporters, the binding and signaling of insulin receptors, as well as the fluidity and permeability of cell membranes, the development of insulin resistance (IR) and adult onset diabetes mellitus may be significantly influenced by FAs. Fatty acid combinations in phospholipids and triglycerides are most associated with type 2 diabetes risk (Shetty and Kumari, 2021).

2.39.1 Odd- Chain Saturated Fatty Acids

Pentadecanoic and heptadecanoic acids are odd-chain saturated fatty acids that lessen type 2 diabetes risk. Even-chain saturated fatty acids including myristic, palmitic, and stearic acids increase type 2 diabetes. SFAs are not a homogenous group; distinct SFAs are associated to type 2 DM in opposite directions (Forouhi *et al.,* 2014). Refined sugars and carbs enhance de-novo lipogenesis (DNL), hepatic steatosis, even-chain SFAs, and type 2 diabetes (Forouhi *et al.,* 2004). Pentadecanoic and heptadecanoic acids are odd-numbered fatty acids (ONFA) which are a minor group of fatty acids prevalent in the body and reduced in type 2 diabetes (Pfeuffer and Jaudszus, 2016).

2.40 Enzymes Involved in Regulation and Absorption of Carbohydrates

2.40.1 α - Amylase

Known for its presence in pancreatic juice and saliva, the alpha-amylase enzyme, breaks down the α-bonds of big α-linked polysaccharides, including insoluble starch molecules and glycogen, into shorter chain absorbable molecules, like dextrins and maltose (Peyrot des Gachons and Breslin, 2016).

2.40.2 α - Glucosidase

Mammalian α-glucosidase, an exo-enzyme, is responsible for catalysing the final stages of the digestion of starch and disaccharides, which are prevalent in the human diet. It is located in the intestinal villi of the small intestine's mucosa brush border cells

of enterocytes. Glucose is created by this enzyme by hydrolyzing carbohydrates (Dhital *et al.,* 2013).

2.40.3 β - Glucosidase

The main enzyme presents in cellulase, β-glucosidase, completes the process by changing cellobiose into glucose, which completes the breakdown of cellulose (Singhania *et al.,* 2013). B-glucosidase is a universal enzyme found in bacteria, fungi, plants, and animals. It may be found in organs such the liver, small intestine, kidneys, and spleen where cytosolic β-glucosidase is responsible for breaking down beta-d-glucoside.

The only carbohydrates that can be quickly absorbed from the intestine are monosaccharides; all other carbohydrates require enzymatic breakdown before they can be absorbed. The main digestive enzymes for dietary carbohydrates digestion are pancreatic and intestine glucosidases; inhibitors of these enzymes may be useful in delaying the absorption of glucose. Kazeem *et al*. (2013) claim that amylase and glucosidase inhibitors slow down the small intestine's process of breaking down carbohydrate and reduce levels of postprandial blood glucose. Inhibitors of α-glucosidase, competitively inhibit the enzymes that transform non-absorbable carbs into simple absorbable carbs, blocking small intestine carbohydrate absorption. Glucoamylase, sucrase, maltase, and isomaltase are enzymes. They reduce the probability of a 3 mmoL/L increase in post-prandial glucose concentration by slowing the absorption of carbohydrates (Akmal and Wadhwa, 2022). Alpha-amylase, maltase, sucrase, and dextranase are all inhibited by acarbose, but it is particularly efficient against glucoamylase and has little effect on lactase, which is a β-glucosidase (Miura *et al.,* 1998).

2.41 Free Radicals

Free radicals are molecules with unpaired electrons in atomic orbitals (Lobo *et al*., 2010). Free radicals are unpaired electron atoms or molecules. Radical organisms usually have unpaired electrons. It can also be an atom, molecule, or compound with a very unstable atomic or molecular structure (i.e., electron distribution). To become stable, a free radical may bond to another molecule or interact with other free radicals.

2.41.1. Characteristics of Free Radicals

Most radicals are extremely unstable and highly reactive.

Radicals can receive or give electrons to other molecules, according to Cheeseman and Slater (1993).

2.41.2 Structure Action of Free Radicals

Several clinical illnesses depend on free radicals including superoxide anion, nitric oxide, OH^-, H_2O_2, hypochlorite, O^{2-}, and peroxynitrite radicals. Biologically important molecules including DNA, proteins, fluid, and carbohydrates can be damaged by these highly reactive species when they come into contact with the cell nucleus and membranes (Young and Woodside, 2001). Attacks by free radicals on crucial macromolecules can result in cellular damage and homeostasis disruption or disorder. Free radicals typically affect DNA, proteins, lipids, and other molecules negatively or adversely, which leads to the development of many human diseases.

2.41.3 Types of Free Radicals

ROS and RNS are two types of damaging free radicals.

2.41.3.1 Reactive Oxygen Species

Oxygen radicals and radicals that can easily become radicals are reactive oxygen species.

Reactive oxygen species components are:

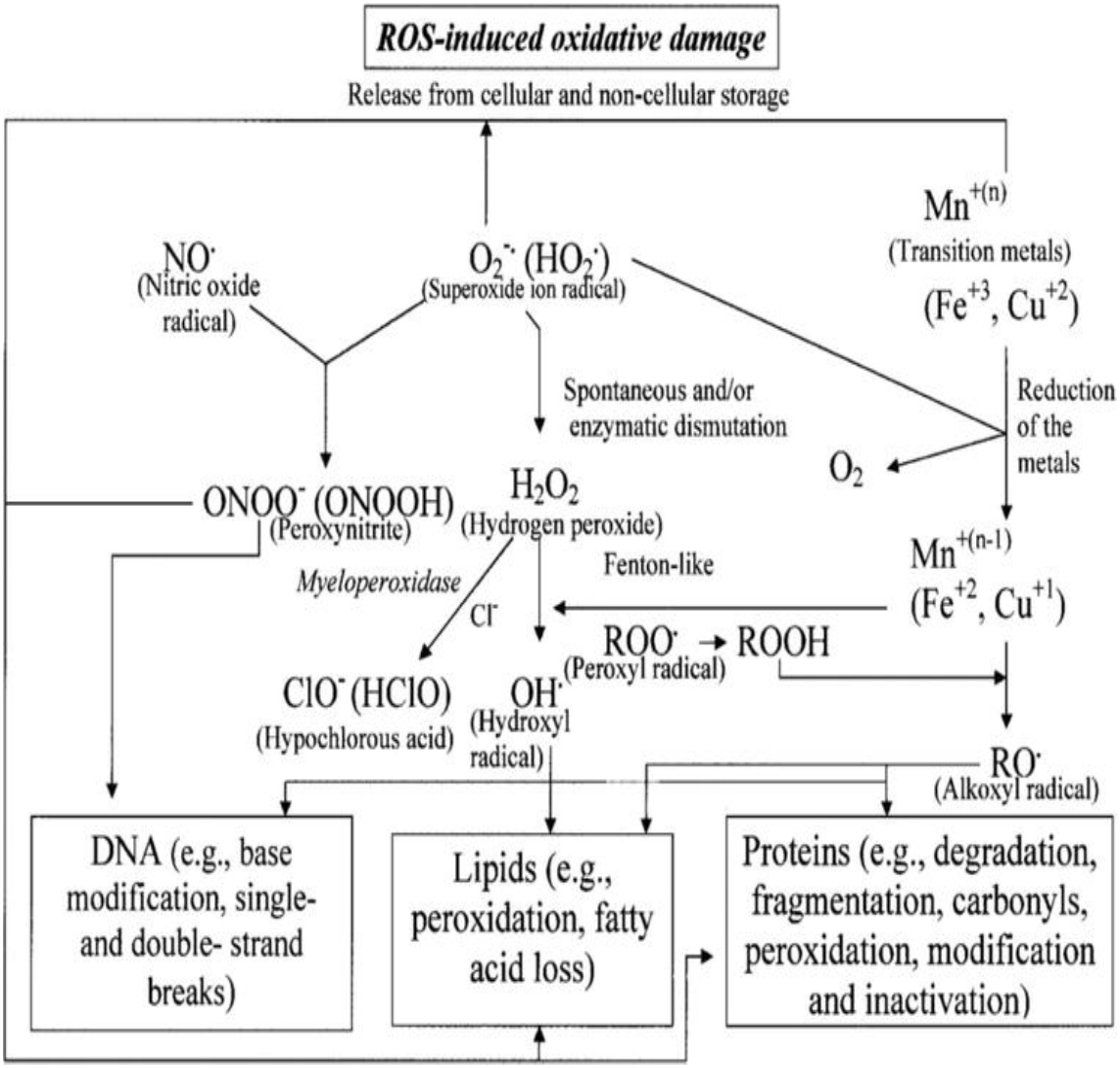


Figure 2.8: ROS Induced Oxidative Damage
Source – Adapted from Kohen and Nyska, 2020.

Super Oxide Anion

This oxygen molecule has an additional electron, and as a result, it has the potential to damage molecules like DNA and mitochondria. It is possible for super oxide to dismutate into hydrogen peroxide, or H_2O_2, in various ways, including diet and the body (Wang *et al.*, 2018).

$$O2^{0-} + O2^{0-} + 2H^{+} \longrightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow \text{Intermediate species} \longrightarrow OH + OH^{-} + Fe^{3+}$$

Hydroxyl Radical (OH•)

Nearly any surrounding organic molecule, including DNA, proteins, lipids, and carbohydrates, can be damaged by extremely reactive molecule, which is created when an oxygen molecule is reduced. Enzymatic reactions are unable to remove the hydroxyl radical OH (Phaniendra *et al.*, 2015).

Hydroperoxyl Radical

Is the protonated form of superoxide, known as "perhydroxyl radical", which also goes by that name. Its chemical formula is HO_2. It is a reactive oxygen species with crucial atmospheric functions (Collin, 2019). In an aqueous solution, the superoxide anion, O_2^- and hydroperoxyl radical are in equilibrium.

Peroxyl Radical (ROO•)

Lipid peroxidation and protein oxidation start with peroxyl radicals (RO_2) (Ayala *et al.*, 2014). Lipid and protein peroxides degrade into peroxyl and alkoxyl radicals (RO) when heated or transition metal ions are added. O2 and carbon-centreed radicals can easily form peroxyl radicals.

$$>C. + O_2 \longrightarrow >C - OO^{0}$$

Alkoxyl Radical (RO•)

Lipid peroxidation, or oxidative deterioration, produces alkoxyl radicals via non-enzymatic methods such the Fenton reaction, one-electron reduction, or the mixing of two peroxyl radicals. Alkoxyl radicals can oxidise DNA and cause death. Numerous age-related degenerative diseases, including diabetes mellitus, cancer, asthma, arthritis, atherosclerosis, stroke, cataractogenesis, and hepatitis, are caused by reactive oxygen species (Phaniendra *et al.*, 2015).

2.41.3.2 Reactive Nitrogen Species

This includes radical and non-radical species such nitrous acids (HNO_2) and the nitrite ion (NO_2^-).

Food contains nitrogen as nitrates, amines, nitrites, peptides, proteins, and amino acids. *In vivo*, it metabolises nitric oxide, higher nitrogen oxides, and peroxynitrite (Davies and Dean, 1997; Beckman *et al.,* 1994). The main reactive nitrogen species are HNO_2, NO_2^-, N_2O_3, and NO.

Peroxynitrite Anion

Although peroxynitrite is stable at a strongly alkaline pH, it reacts with CO_2 and subsequently undergoes protonation, isomerization, and disintegration under conditions of physiological pH to produce harmful substances such as NO_2^0, NO^{2+} and $OH^{\bullet}$. Noxious compounds can alter enzyme activity, reduce antioxidants, oxidise nitrates, lipids, proteins, and DNA, disturb cytoskeletal organisation, and disrupt cell signal transmission (Beckman *et al*., 1994). Adding super-oxide and nitric oxide radicals quickly creates peroxynitrite, a toxic chemical (Davies and Dean, 1997).

$$^{\bullet}NO + O_2^{0-} \longrightarrow ONOO^-$$

Peroxynitrite (ONOO-), Nitrous acid (HNO_2), and Di Nitrogen Trioxide (N_2O_3) can cause DNA nitration and deamination**.**

In hepatitis or other chronic inflammatory conditions, these reactive nitrogen species may increase the cancer risk formation (Beckman *et al.,* 1994; Ohshima and Bartsch, 1994).

2.41.3.3 Non Free Radical

Non-free radical oxygen intermediates. Singlet oxygen ($^1O_2$), ozone (O_3), H_2O_2, and HOCl are examples.

Hydrogen Peroxide

This isn't a free radical in and of itself, but it may be easily changed into one like OH, which then causes the harm. An enzymatic antioxidant called peroxidase neutralises hydrogen peroxide (Lobo *et al.,* 2010).

Singlet Oxygen

Our immune system produces singlet oxygen, and this can cause low density lipoprotein (LDL) to oxidise (Lobo *et al.,* 2010).

2.42 Production of Free Radical in the Human Body

ROS and RNS free radicals can form within and outside the body. The body's pro-oxidative enzyme system and glucoxidation manufacture them. X-rays, UV rays, cigarette smoke, certain medications, environmental contaminants, and industrial poisons can create free radicals (Bagchi and Puri, 1998).

2.42.1 Sources of Internal Production of Free Radicals

These include arachidonate pathways, exercise, inflammation, phagocytosis, peroxisomes, mitochondria, xanthine oxidase, and ischemia/reperfusion injury.

Cells produce free radicals through enzymatic and non-enzymatic mechanisms. Prostaglandin production, the respiratory chain, phagocytosis, and the cytochrome p-450 system generate free radicals (Liu *et al.,* 1999). Ionising reactions and non-enzymatic oxygen-organic molecule interactions generate free radicals (Lobo *et al.,* 2010).

2.42.2 Sources of External Generation of Free Radicals

External sources of free radical formation include radiation, tobacco smoke, and other forms of smoke, environmental contaminants, certain medications, industrial solvents, and pesticides (Ebadi 2001; Bagchi and Puri, 1998).

Free radicals are atoms with unpaired electrons. ROS are oxygen-containing free radicals. Reactive nitrogen species (RNS) are molecules derived from nitric oxide (NO) and superoxide anion ($O_2^{\cdot -}$) and formed by enzymes such NOS, NADPH oxidase, XO, lipoxygenase, and cyclooxygenase. Free radicals can damage DNA by disrupting replication, maintenance, and other processes. They can break or alter DNA bases by interacting with them. Free radical attack is linked to cancer, atherosclerosis, diabetes, Parkinson's, Alzheimer's, and cataracts. Oncogene activation and chromosomal abnormalities cause cancer (Lea, 1966). Dietary lipids in the artery wall and serum may undergo free radical reactions that produce peroxides and other compounds that damage endothelial cells and alter the arterial wall, causing atherosclerosis (Harman, 1992). Free radicals' tendency to concentrate in cell membranes (called "Lipid peroxidation") makes cell membrane lipids extremely sensitive to oxidative damage. ROS oxidation causes cell membrane brittleness and increased permeability, causing cell death (Mercola, 2011)

2.43 The Involvement of Reactive Oxygen Species in the Etiology of Diabetes Mellitus

2.43.1 What are Reactive Oxygen Species?

ROS, also called oxygen radicals, are free radicals.

These unstable oxygen-containing compounds interact easily with cell molecules. Superoxide (O^{-}_{2}), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^{\cdot}$) are cell byproducts of aerobic metabolism. Reactive oxygen species damage cell DNA, RNA, and proteins, causing cell death (Checa and Aran, 2020).

2.43.2 What is Etiology?

Etiology refers to the scholarly investigation of the underlying causes, origins, or rationales that contribute to the current state or functioning of phenomena. The "role of reactive oxygen species in the etiology of diabetes mellitus" refers to the mechanism through which reactive oxygen species cause diabetes. Numerous investigations have indicated that β-cell dysfunction can result from prolonged exposure to high levels of free fatty acids, hyperglycemia, or both (Evans *et al.*, 2003). β-cells are vulnerable to reactive oxygen species (ROS) because they cannot quench free radicals or produce antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxide. Rat β-cells are peroxide-sensitive and lack GPx activity (Malaisse *et al.,* 1982). Therefore, it is not surprising that oxidative stress can kill mitochondria and impair insulin production. Grankvist *et al*. (1981) found low quantities of antioxidant enzymes CuZn-SoD, Mn-SOD, catalase, and GPx in pancreatic islets. These and other findings support the concept that islets are vulnerable to ROS damage because of poor antioxidant activity.

Oxidative stress caused by short-term or transient exposure of β-cell preparations to hydrogen peroxide (H_2O_2) increases CDK production, decreases insulin mRNA, cytosolic ATP, and calcium flux in the cytosol and mitochondria, and causes apoptosis (Evans *et al.,* 2003; Kaneto *et al.,* 1999; Maechler *et al.,* 1999). Reactive oxygen species (ROS) have a major role in diabetes complications. Hyperglycemia increases oxidative stress and reactive oxygen species (ROS) in diabetes. Vascular cells, such as renal cells, can create ROS under hyperglycemic situations (Hunjoo *et al.,* 2008). Baynes and Thorpe, (1999) shows that diabetes tissues produce ROS. The mitochondrial electron transport chain (ETC) (Brownlee, 2001), membrane-bound

NADPH oxidase (Mohazzab *et al.,* 1994), and non-enzymatic glycosylation are ROS producers in cells.

ETC activation in diabetics produces a lot of ROS. Type 2 diabetes, most common metabolic disorder, is deadly. Insulin resistance and pancreatic beta-cell dysfunction differentiate. Type 2 DM is worsened by chronic hyperglycemia and increased reactive oxygen species ROS, which decrease beta-cell function and increase insulin resistance. Chronic hyperglycemia and ROS cause atherosclerosis in diabetics (Kaneto *et al.,* 2010).

2.44 Oxidative Stress

Oxidative stress occurs when free radicals and antioxidants are imbalanced. Thus, oxidative stress occurs when free radicals overwhelm our antioxidant defences. Oxidation, a large-scale chemical reaction, occurs when free radicals interact with other molecules in the body due to their odd number of electrons.

Lipids, proteins, and nucleic acids are damaged by oxidative stress. Oxidative stress shortens telomeres on chromosomes, the body's most accurate biological timekeeping mechanism. DNA double strand breaks can cause oxidative damage and telomere shortening. Oxidative stress causes most telomere shortening due to DNA replication-induced single-strand DNA damage (Reichert and Stier, 2017). Oxidative stress may be detected by 8-hydroxy-2-deoxyguanosine (Valavanidis *et al.,* 2009; Toyokuni *et al.,* 1997).

2.44.1 Short Term Oxidative Stress

Tissue injuries cause short-term oxidative stress. Toxins, hypertoxia, trauma, infections, heat, and excessive activity harm tissue. According to Lobo *et al.* 2010, damaged tissues are more likely to produce free radical-producing enzymes like xanthine oxidase, lipogenase, and cyclooxygenase, activate phagocytes, release free iron and copper ions, and disrupt the electron transport chains of oxidative phosphorylation, which increases reactive oxygen species (ROS).

2.44.2 The Body's Response to Oxidative Stress

When free radicals outnumber antioxidants, they can damage fatty tissues, proteins, and DNA. Diabetes, atherosclerosis, inflammation, high blood pressure, heart disease, and neurological diseases like Parkinson's and Alzheimer's may result from this injury (Lobo *et al.,* 2010; Stefanis *et al.,* 1997).

2.44.3 Human Diseases and Oxidative Stress

Oxidative stress causes several clinical disorders.

2.44.3.1 Diabetes Mellitus and Others

ROS cause age-related eye diseases, diabetes and its complications, and neurological problems like Parkinson's disease (Nita and Grzybowski, 2016; Lobo *et al.,* 2010; Rao *et al.,* 2006).

2.44.3.2 Cardiovascular Diseases

Polyunsaturated fatty acids make up most of blood LDL (low density lipoprotein), and their oxidation causes atherosclerosis (Stefanis *et al.,* 1997). Lipid oxidation can cause atherosclerosis-related foam cells and plaque. Oxidised low-density lipoprotein (LDL) is cytotoxic, atherogenic, and can damage endothelial cells, according to Lobo *et al*. (2010).

2.44.3.3 Cancer and Carcinogenesis

Cancer can be caused by an imbalance between reactive oxygen species (ROS) and the antioxidant defence system (Lobo *et al.,* 2010; Rao *et al*., 2006). ROS, RNS, and their biological metabolites affect carcinogenesis, mutation, and transformation. ROS damage DNA, causing strand breakage, nucleotide changes, and protein cross-links. Lobo *et al*. (2010) say radiation disrupts DNA with hydroxyl radicals (HO), causing mutagenesis.

2.44.3.4 Aging

Free radical cell damage causes age-related pathology (Ashok and Ali, 1999). Reducing free radicals slows ageing.

2.44.3.5 Oxidative Damage to Protein

Proteins can undergo the following oxidative modifications:

Free radicals cleave peptides by oxidising amino acids.

Protein cross-linking caused by interactions with lipid peroxidation. Methionine, cystein, arginine, and histidine, among other amino acids, are particularly susceptible to oxidation, according to Freeman and Crapo, 1982. An enzyme becomes more vulnerable to proteolysis as a result of free radical-induced protein changes. Oxidised proteins may disrupt enzymes, receptors, and membrane transport processes. Peroxyl radicals oxidise proteins. Lobo *et al*. (2010) found that protein oxidation alters signal transmission, enzyme activity, heat stability, and proteolysis vulnerability, accelerating ageing.

2.44.3.6 Lipid Peroxidation

Lipid peroxidation generates free radicals from a secondary free radical. This secondary free radical can operate as a second messenger or directly interact with biomolecules, causing biochemical lesions. The hydroxyl radical initiates ROS by removing hydrogen atoms to form the lipid radical and diene conjugate. Oxygen creates a peroxyl radical that attacks extra fatty acids, forming lipid hydroperoxide (LOOH) and another radical. This promotes lipid peroxidation. Assays for lipid peroxidation use alkanes, malonaldehyde, and isoprotanes. Diabetes, neurological disorders, and ischemia reperfusion injury are linked to these substances (Lobo *et al.,* 2010; Lovell *et al.,* 1995).

2.44.3.7 DNA Oxidative Damage

Oxidative degradation of DNA and RNA is widely accepted. Woo *et al*. (1998) say cancer and ageing target DNA. Oxidative damage to mitochondrial DNA causes diabetes and cancer (Toyokuni *et al*., 1997).

2.45 Risk Factors for Oxidative Stress

Free radicals, a consequence of metabolism, are essential to the body's complex health system. Man is prone to free radicals due to environmental variables such as radiation, ozone, pesticides, cleansers, cigarette smoke, pollution, and secondary smoke. High-fat, sugar, and alcohol diets are also unhealthy (Lobo *et al*., 2010; Pham-Huy *et al*., 2008).

2.46 Prevention and Management of Oxidative Stress

The logical course of action is to increase your antioxidant levels while lowering the generation of free radicals. Making sure your diet contains enough antioxidants is a key preventative approach. Every day, consume a variety of fruits, vegetables, and other nutritional items, such as citrus fruits, carrots, tomatoes, fish, almonds, vitamins C and E, turmeric, onions, ginger, melatonin, and green tea. Regular moderate exercise is beneficial because it raises natural antioxidant levels and reduces oxidative stress-related damage. Exercise lengthens life, slows the effects of ageing, and lowers the risk of DM, cancer, and other disorders (Dhalaria *et al*., 2020; Lobo *et al*., 2010).

Avoid secondary smoke and don't smoke. Be mindful of the environment and use chemicals like cleansers and insecticides carefully. Wear sunscreen to protect your skin, and drink less alcohol. Sleep for lengthy periods of time because it affects a

variety of functions, including hormone production, the balance of antioxidants and free radicals, and brain cum cognitive function. Avoid overeating because both excessive and regular eating generates oxidative stress in the body (Dhalaria *et al.,* 2020).

2.47 The Role of Fruits and Vegetables in Preventing Cellular Oxidative Damage

Vitamins, minerals, phytochemicals, and fibre are abundant in fruits and vegetables. These phytochemicals modulate hormone metabolism and cholesterol synthesis, detoxification enzymes, platelet aggregation, the immune system, blood pressure, and antioxidant, antibacterial, and antiviral effects in animal and cell-culture models. Fruit and vegetables' ability to regulate various prospective diseases and their contents—a preventive mechanism—has also been established in human dietary experiments. In plant-based foods such as grains, nuts, legumes, vegetables, and fruit, a variety of phytochemicals that are biologically active have been discovered. The fact that fruits and vegetables have the widest number of botanical diversity of all the plant diets is significant (Liu, 2013).

Flavonoid molecules from fruits and vegetables have been proven to be efficient in preventing cellular oxidative damage even in tiny amounts *in vitro,* and research is also pointing to the fact that an appreciable amount of this flavonoid would likely be just as helpful *in vivo* (Panche *et al.,* 2016).

Numerous scientific studies have shown how flavonoid chemicals from fruits and vegetables affect a number of *in vitro* indicators of oxidative cellular damage. The crucial elements in this case are absorption and metabolism. The ability to see effects on *in vivo* assessments of oxidative cellular damage depends on whether the antioxidant components in fruits and vegetables are absorbed in adequate amounts and in a manner that will allow for this (Kurutas, 2016).

Taking into account, the anthocyanins and flavonols, two forms of flavonoids, anthocyanins appear to be absorbed around 10 % (1/10) less efficiently than flavonol quercetin. To demonstrate antioxidant benefits *in vivo*, a dietary intake of anthocyanins that is rather substantial appears to be required. The antioxidant activity will decrease as cyaniding 3-glucoside and quercetin are metabolised through methylation or conjugation with glucuronide or sulphate. However, quercetin metabolites seem to

retain some of their antioxidant function in living things (Mattioli *et al*., 2020; Ullah *et al*., 2020).

Diabetes, cancer, rheumatoid arthritis, coronary artery disease, and stroke may be caused by reactive oxygen species from exogenous and endogenous causes. Vitamins C, E, and β-carotene provide this protection. Fruit and vegetable-rich diets minimise disease risk (Forma and Zhang, 2021; Pizzino *et al*., 2017).

Vegetable-rich diets may prevent chronic diseases like diabetes, cancer, heart disease, and many common cancers. Vegetables and fruits affect human biology. High fruit and vegetable intake reduces chronic disease risk, according to epidemiological studies (Aune *et al*., 2017).

Polyphenolic flavonoids are found in fruits, vegetables, and plants. They are mostly flavonols, flavones, flavanones, and anthocyanins. Flavonoids and other plant phenolics offer several biological benefits, including antioxidant, antidiabetic, anti-inflammatory, platelet aggregation inhibition, antibacterial, and anticancer properties (Panche *et al*., 2016; Pandey and Rizvi, 2009). It has been established that compounds derived from anthocyanins and flavonols, especially quercetin, are crucial for dietary intake and wellness (Samtiya *et al*., 2021).

In fruits and vegetables, hydrophilic antioxidants make up more than 85 % of the total antioxidant content. Increased antioxidant capacity should result from more effective fruits and vegetables. Metabolic/absorption pathways may have an impact on how effective they are (Forma and Zhang, 2021).

Fruits and vegetables are full of flavonols, especially quercetins, which contribute to their antioxidant properties. Nevertheless, more than one hundred (100) compounds can contribute to the available antioxidant ability in diverse fruits and veggies' (Samtiya *et al*., 2021).

The secondary plant metabolites known as anthocyanins give many plant tissues their blue, purple, or red colour. They primarily exist as glycosides of the corresponding antocyanidin chromophores. Antioxidant capabilities are present in anthocyanins and anthocyanidin (the aglycone form). Anthocyanins' phenolic structure transmits significant antioxidant activity in a model system either by giving free radicals hydrogen atoms from the hydroxyl moieties or by donating electrons (Forma and Zhang, 2021; Pizzino *et al*., 2017).

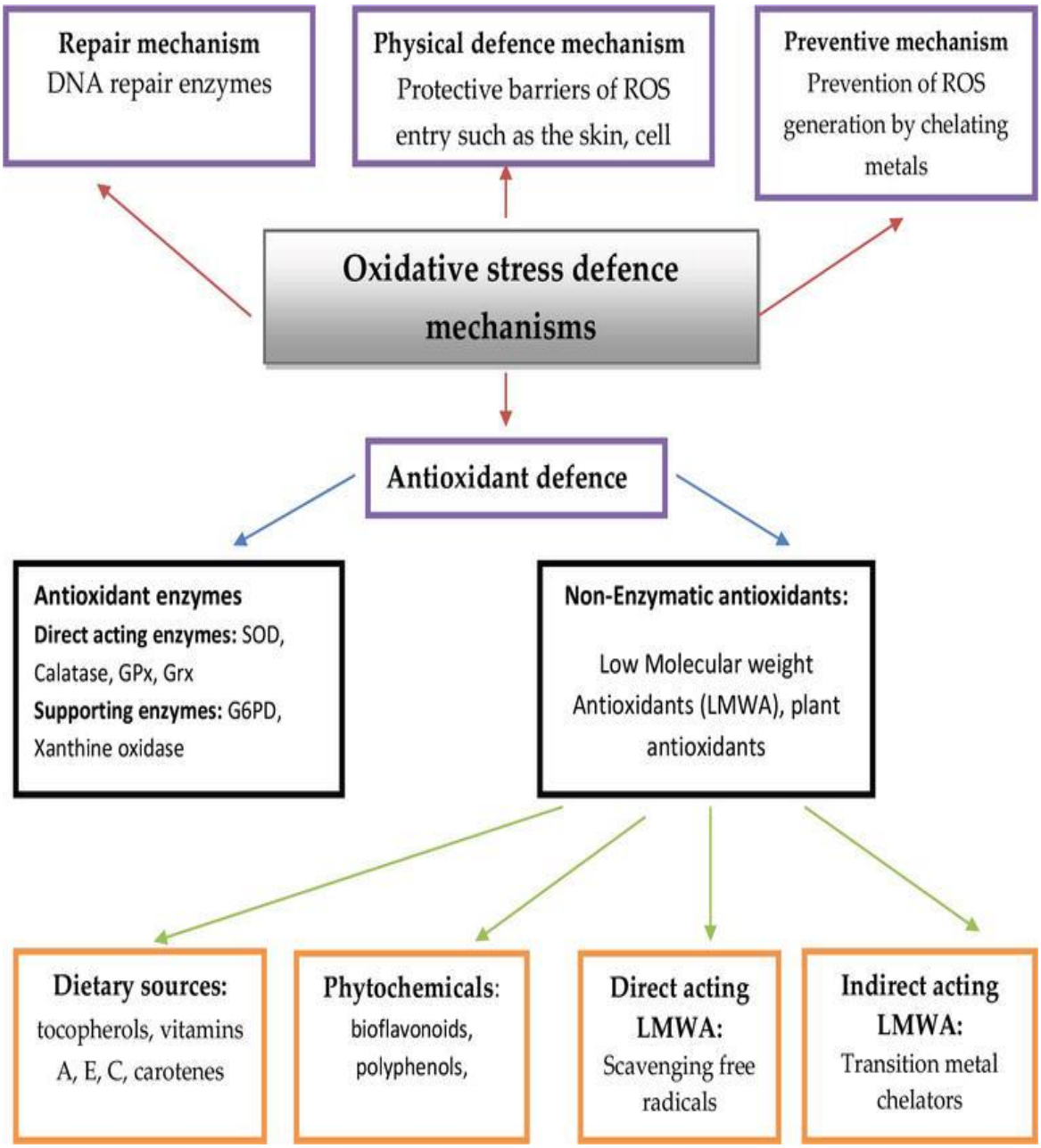


Figure 2.9: Oxidative Stress Defense Mechanism
Source – Adapted from Engwa 2018.

Common anthocyanidin aglycones include cyaniding, malvidin, delphinidin, peonidin, petudin, and pelargonidin. The 3', 4', and 5' positions are where chemical structures differ from one another. 90 % of fruit contains cyaniding, the most common anthocyanidin (Forma and Zhang, 2021). It is clear that both anthocyanins and flavonols can act as antioxidants when used in *in vitro* assay scenarios. Whether anthocyanins enter cells or the relevant subcellular compartments in a concentration high enough to influence metabolic processes *in vivo* is unknown (Pizzino *et al*., 2017).

Dietary anthocyanins have been observed to exhibit protective effects against oxidative stress in animal models at concentrations as high as 1-2 mg/kg diet. Although it is unclear if anthocyanins' vasoprotective effects on humans are due to an antioxidant mechanism, they do appear to have some effects (Ockermann *et al*., 2021).

The antioxidant power of the metabolite will be reduced by methylation at the 3' position of quercetin and cyanidin 3-glucoside. Antioxidant capacity may also be impacted by further conjugation with glucuronide or sulphate, depending on the conjugated position (Wang *et al.,* 1997; Keli *et al.,* 1996; Cao *et al.,* 1996; Steinmetz and Potter, 1991).

2.48 Phytochemical and Phytochemistry

The word "phyto," which means "plant," is derived from the Greek word "Phyton". The scientific study of these chemicals produced by plants is known as phytochemistry. Phytochemicals are physiologically active substances of plant origin. The interaction between organic chemistry and natural products like oil, carbohydrates, alkaloids, flavonoids, saponins, and terpenoids, among others, is the subject of phytochemistry, according to (Abe, 2013). It also considers metabolites' structural makeup, biosynthetic processes, roles, and modes of action in living systems, as well as potential medical, industrial, and commercial uses. To develop new drugs and treatment agents for important diseases, phytochemical expertise is crucial.

Secondary metabolites known as phytochemicals are produced by plants as a kind of defense against diseases, herbivores, UV radiation, and other environmental dangers. but current research has shown that these bioactive chemicals can also protect human beings against diseases (Usin *et al*., 2023; Divekar *et al*., 2022). Plant compounds known as phytochemicals, which are unnutritive and related to plant pigments, have

anti-inflammatory and anti-disease qualities. Since the human body does not require them to maintain life, they are non-essential nutrients. Several noteworthy phytochemicals may be found in various food sources. For instance, tomatoes are rich in lycopene, while soy has isoflavones. Fruits are known to include flavonoids, while onions, leeks, and garlic are notable sources of allysulfides. Carotenoids can be found in fruits as well as carrots, while tea and grapes are known for their polyphenol content (Engelmann *et al*., 2011).

2.48.1 Functions of Phytochemicals

Over 1,000 phytochemicals produced by plants are known, with each working differently. Phytochemicals have been reported to have synergistic and overlapping modes of action in the body system, Abe (2013). Important functions of the phytochemicals include antioxidant activity, regulation of detoxification enzymes, modulation of hormones metabolism, induction of immune system, regulation of DNA replication, antibacterial activity and infection control (Zhang *et al*., 2015).

2.48.1.1 Antioxidant Activity

Antioxidant phytochemicals protect cells from damage caused by oxidative damage and lower cancer and diabetes risks. Allylsulfides, found in foods like onions, leeks, and garlic; carotenoids, flavonoids, and polyphenols, found in foods like tea and grapes, are phytochemicals having antioxidant activity (Zhang *et al*., 2015).

2.48.1.2 Hormones Modulation

Some phytochemicals exhibit hormonal activity regulation by mimicking human estrogen and thus help in reducing menopausal symptoms, and osteoporosis e.g. Isoflavones found in Soy (Miadoková, 2009).

2.48.1.3 Enzyme Stimulation

Cabbage indoles activate enzymes that reduce oestrogen's effectiveness, lowering breast cancer risk. Terpenes (found in citrus fruits and cherries) and protease inhibitors (found in soy and beans) are two other phytochemical that control enzymes (Licznerska *et al*., 2013; Pasqualini and Chetrite, 2005).

2.48.1.4 DNA Replication Interference

A number of phytochemicals interfere with DNA replication e.g. the presence of saponins in beans has been discovered to impede cell DNA replication, hence limiting or halting the growth of cancer cells (Usin *et al*., 2023). Hot peppers include a

compound called capsaicin that protects DNA from carcinogenesis (Adetunji *et al*., 2022).

2.48.1.5 Antibacterial Activity

Allicin in garlic acid has antibacterial properties (Ankri and Mirelman, 1999).

2.48.1.6 Binding on Cell Wall

Phytochemicals that adhere to cell walls prevent infections from attaching. Cranberries' protoanthocyanidins prevent adhesion. Cranberries prevent UTIs and promote tooth health (Jepson *et al*., 2012).

By working with spices, fruits, vegetables and phytochemicals may slow down ageing and lower diseases risk, including diabetes mellitus, heart disease, cancer, urinary tract infections, cataracts, high blood pressure, osteoporosis, and stroke (Boeing *et al*., 2012).

To get adequate phytochemicals, eat whole grains, legumes, fruits, vegetables, and other phytochemical-rich foods. Fruits and vegetables are rich in vitamins, minerals, fibre, and low in saturated fat (Cena and Calder, 2020).

2.49 Phytochemicals

Tannins

The ability to give brown leather gave tannins—which are abundantly found in plants—their name (Falcão and Araújo, 2018). There are two classes of tannins:

(a) Hydrolysable tannins, which are gallic acid esters, and also glycosides of these esters.
(b) Polymers called condensed tannins that come from different flavonoids.

Tannins are non-crystalline, colourless substances, form astringent colloidal solutions with water.

Plant tissues that contain tannins include those in their leaves, buds, seeds, roots, and stems.

The tannins have therapeutic effects. Additionally, to stopping bleeding, they assist in burn recovery. While inwardly healing the wound, they prevent infection. They have reno-protective properties since they safeguard the kidneys and also provide treatment from ailments such as weariness, skin ulcers, haemorrhage, dysentery, diarrhoea, and so on (Adase *et al*., 2022; De Jesus *et al*., 2012). They contain antiviral properties and

are applied to get rid of the venom from bee stings or poison from oak, providing relief (Kaczmarek, 2020). They assist in eliminating all irritants from the skin by having an astringent quality (Usin *et al*., 2023).

Saponins

Many plants contain saponins, which foam. Soap plants are organic surfactants. They function as immune system stimulators and are utilised as natural antibiotics, cough suppressants, and diuretics (Shi *et al*., 2004). They bind cholesterol and prevent cell growth and division as a result (Elekofehinti *et al*., 2021). It has been discovered that they are hazardous to several creatures, including fish (Sharma *et al*., 2023).

Steroids

A low polarity organic solvent, such as ether or chloroform, can be used to extract steroid-containing lipids from cells as they are insoluble in water. Most plant steroid substances are glycosides, and they have the ability to stimulate or activate cardiac muscles (El-Mallakh *et al*., 2019). Steroidal glycosides are referred to as saponins because of their capacity to produce foam in water (much like soap solution) (Wiesner *et al*., 2017). One type of steroid is cholesterol. For the separation and identification of natural steroids, respectively, paper chromatography and thin layer chromatography (TLC) are typically employed.

Glycosides

The majority of the plant's components include glycosides, which are phenolic compounds. Simple glycosides are optically active, colourless, and soluble in water, but they do not reduce Fehling's solution. When glycosides are hydrolyzed by inorganic acids, a sugar and a hydroxylic molecule termed an aglycone are created, which can be either an alcohol or a phenol (Kotik *et al*., 2023). Every glycoside is often connected with an enzyme that exists in various plant cells, in its natural state.

Yan *et al*. (2008) isolated phenylpropanoid glycosides, which have been used to treat liver, lung, and laryngeal carcinoma. Ning *et al*. (2006) isolated cycloartane glycoside; and Kuang *et al*. (2008) isolated phenolic glycoside, which has been used to treat skin infections like psoriasis, rash, and dermatitis.

Alkaloids

Different plant components contain alkaloids. Alkaloids (alkali-like compounds) were the collective name for all organic bases that could be derived from plants. Alkaloids

are described as naturally occurring bases with pyridine rings (Dey *et al*., 2020), they are also basic plant components with at least one nitrogen atom in a heterocyclic ring, according to Kurek, (2019). Due to their high risk, alkaloids are only used medicinally in very small amounts.

Alkaloids are typically crystalline, non-volatile solids that are colourless and soluble in substances other than water, such as ether, chloroform, and ethanol. Many alkaloids taste bitter and have optical activity (laevorotatory) (Dey *et al*., 2020). Most alkaloids have basic characteristics, intricate structures, and physiological effects. Eight groups can be formed from them, as follows:

1. Phenyl ethylamine group e.g. tyramine
2. Pyrrolidine group e.g. hygrine
3. Piperidine and pyridine groups, such as ricinine
4. Pyrrolidine – pyridine group e.g. tropine
5. Quinoline group e.g. galipine
6. Isoquinoline group e.g. papaverine
7. Thebaine, codeine, and phenanthrene group of drugs, such as morphine
8. Indole group e.g. gramine

Awang *et al*. (2008) reported two novel quinazolinedione alkaloids, whereas Jin *et al.,* 2008, recovered two novel phenantrene alkaloids. Headache, stomach discomfort, dysentery, postpartum haemorrhage, and amenorrhea may all be treated with these substances.

Flavonoids

All phyto components whose structures are based on the flavone skeleton are referred to as flavonoids, and they can be divided into six primary classes as follows:

1) Chalcones
2) Flavones, which are typically found in herbaceous families including the Labiatae, Umbelliferae, and Compositae
3) Apigenin (petroselinumcrispum, apiumgraveolens), and luteolin (equisetum arvense)
4) Quercitol (rutagraveolens, fagopyrumesculentum, sambucusinigra), Flavonol (usually in woody angiosperms), kaempferol, and myricetin
5) Flavanone
6) Anthocyanins

7) Isoflavonoids

Flavonoids have anti-inflammatory, antibacterial, anti-cancer, and anti-allergic properties. Flavonoids exhibit potent antioxidant properties. According to Ricardo *et al.,* 2004, flavanol contains both antifungal and anti-inflammatory properties while according to Lin *et al.,* 2000, the following isolated flavone glycosides have galloyl substitutions:

They demonstrated considerable antioxidative actions, specifically

Apigenin 6 - C - (2" - 0 - galloyl) - - D - glycopyronoside and

Apigenin 8 - C - (2" - 0 - galloyl) - - D – glycopyranoside. Fang *et al.,* 2006, have isolated 6 new flavanone glycosides that have antibacterial activities.

2.50 Antioxidants

Historical

The term "antioxidant" originally referred only to chemicals that protect the organism from oxygen oxidation. Antioxidants were first used in the 19th and 20th centuries to vulcanize rubber, polymerize fuels to prevent engine fouling, and prevent metal corrosion (Xu *et al*., 2022).

Definition of Antioxidant

Antioxidant definitions are as varied as the writers who write them or the fields in which they are studied, even though they are all simply different ways of describing the same thing.

Antioxidants are chemicals that delay or stop substrate oxidation when present in low levels, according to Valko *et al.*, 2007. Lobo *et al.,* (2010) define an antioxidant as a stable chemical or substance that may neutralise an agitated free radical by supplying an electron.

Biochemical/Medical Definition

Antioxidants including enzymes, vitamin E, and beta-carotene protect animal tissues against oxidation (Lobo *et al.,* 2010).

Biological Definition

Products are treated with antioxidants to postpone or prevent oxygen-induced degradation. Either it is natural or synthetic antioxidant (Amarachukwu Uzombah, 2022).

Chemical Industry Definition

Antioxidants prevent rubber and plastic from spontaneously oxidizing (Xu *et al*., 2022). Oxygen-substrate radical chain reactions start autooxidation. Radical scavengers like sterically hindered phenols and amines interrupt radical chain processes (Lucarini and Pedulli, 2010).

Food industry Definition

Antioxidants prevent lipid oxidation and reduce reactive oxygen species' negative effects on physiology (Pizzino *et al*., 2017). ROS can cause DNA mutation, membrane protein damage, and cell membrane disintegration. Arteriosclerosis, cancer, diabetes mellitus, liver damage, inflammation, skin damage, coronary heart disease, arthritis, and many other pathological conditions can accelerate ageing and other pathological conditions. Antioxidants neutralise free radicals or reactive oxygen species (ROS) before they damage cells (Lobo *et al*., 2010).

Modes of Action of Antioxidants

Antioxidants can inhibit or delay cell damage, primarily by scavenging free radicals (Halliwell, 1995). Chain reactions are stopped by antioxidants before crucial components are harmed. Chain breaking mechanism and removal of reactive species initiators are the two main ways by which antioxidants work, according to Rice-Evans and Diplock, 1993.

1. Mechanism for Chains Breaking

By employing this method, the primary antioxidant stops a free radical that is already active in the system by giving it an electron**.**

2. Removal of Reactive Species Initiators

Secondary antioxidants quench catalysts that start reactions, eliminating ROS and RNS initiators.

Antioxidants can perform their duties in biological systems by a variety of processes, such as electron donation, metal ion chelation, control of gene expression, and co-antioxidants (Krinsky, 1992).

Production of Antioxidants

(a) The body creates several antioxidants during regular metabolic processes, including glutathione, ubiquinol, and uric acid (Shi *et al.,* 1999).

(b) Dietary antioxidants and bioactive ingridients are weaker. Tocopherol, vitamin C (ascorbic acid), and beta-carotene must be eaten because the body cannot generate them (Levine *et al.,* 1999). Dietary antioxidants help to scavenge reactive oxygen/nitrogen species (ROS/RNS) to block radical chain reactions or prevent reactive oxidants from being produced.

Classifications of Antioxidants

Antioxidants have two categories. Both enzymatic and non-enzymatic antioxidants. Antioxidants come from two sources: natural and manufactured. Antioxidants can either be water- or lipid-soluble.

Natural and Synthetic Antioxidants

Phenolic artificial antioxidants predominate. According to Madhavi *et al*. (1995), chemical structures affect these compounds' antioxidant activity, volatility, solubility, and thermal stability. Examples include TBHQ, BHA, and BHT.

Natural Antioxidants

Plants contain antioxidant flavonoids. Fruits, vegetables, and dietary supplements contain antioxidants like ascorbic acid, hydroxybenzoic acid, hydroxycinnamic acid, α-tocopherol, phenolic acid (salicyclic acid), flavonoids, trans cinnamic acid, isoflavonoids, and phenolic polymers (tannins) (Craft *et al.,* 2012).

Water Soluble (Hydrophillic) Antioxidants

Blood and cytosol (cytoplasmic matrix) contain water-soluble antioxidants-glutathione, vitamin C, and polyphenols.

Lipid Soluble (Lipophillic) Antioxidants

Vitamin E, carotenoids, vitamin A, and lipoic acid are lipid-soluble antioxidants found in cell membranes.

The cell must safeguard its fatty membranes and watery contents from free radicals. Lipid-soluble antioxidants inhibit cell membrane peroxidation (Dean and Davies, 1997).

Enzymatic Antioxidants

Enzymatic antioxidants eliminate free radicals. They use copper, zinc, manganese, and iron trace metal co-factors to transform harmful oxidative products into hydrogen peroxide and water. Enzyme-produced antioxidants cannot be supplemented. Antioxidant enzyme networks protect cells from oxidative injury (Sies, 1997).

Oxidative phosphorylation converts super oxide to hydrogen before reducing it to water. Superoxide dismutases remove hydrogen peroxide, followed by catalases and other peroxidases (Ho *et al.,* 1998).

Super Oxide Dismutase

The super oxide anion is converted into hydrogen peroxide and oxygen by super oxide dismutases (SODs) (Zelko *et al.,* 2002). These enzymes are found in extracellular fluids and most aerobic cells, according to Johnson and Giulivi (2005). These enzymes are categorised into three main categories by the metal co-factor:

(a) Copper and zinc are bound together in Cu/Zn,

(b) iron or manganese are bound together in Fe/Mn.

(c) Ni – This binds nickel, according to Wuerges *et al*. (2004).

The localization of SOD isozymes in higher plants has been found to be limited to specific cell compartments. Mn-SOD has been identified in mitochondria and peroxisomes, while Fe-SOD was initially observed in chloroplasts but has now also been discorved in peroxisomes. Cu-ZN-SOD, on the other hand, is present in cytosol, peroxisomes, chloroplasts, and apoplast (Corpas *et al.,* 2006; Wuerges *et al.,* 2004).

Super oxide dismutase exists in mammals in three different forms, including humans, other mammals, and chordates. SOD1 is a dimer (2 units) and is found in the cytoplasm. SOD2 is a tetramer (4 units) and is found in the mitochondria. SOD3 is also a tetramer and is found in the extracellular environment. SOD2 has manganese in its reactive core, whereas SOD1 and SOD3 have copper and zinc (Cao *et al.,* 2008).

Catalase

Catalase, found in most living things exposed to oxygen, catalyses the breakdown of hydrogen peroxide into water and oxygen (Chelikani *et al.,* 2004). Hydrogen peroxide, a damaging byproduct of many metabolic processes, must be quickly converted into less harmful molecules to prevent damage. Cells use catalase to do this because it reduces the reactivity of hydrogen peroxide into gaseous molecules (Gaetani *et al.,* 1966).

Every known animal employs catalase, which is notably abundant in the liver (Eisner and Aneshansley, 1999) and used in every organ. Iron and manganese serve as co-factors in catalase.

Glutathione System

Glutathione, peroxidases, reductases, and s-transferases form glutathione systems in animals, plants, and microbes.

Glutathione peroxidase, which has four selenium co-factors and breaks down organic hydroperoxide and hydrogen peroxide, uses at least four isozymes in animals (Brigelius-Flohe, 1991).

The most common glutathione peroxidase 1, is good at scavenging hydrogen peroxide, while 4 is best at lipid hydroperoxides. According to Papp *et al*., 2007, the liver possesses a high concentration of glutathione peroxidase and reductase enzymes, which convert organic and hydrogen peroxides into alcohols.

Glutathione-s-transferases have high lipid peroxide activity. These liver-concentrated enzymes are engaged in detoxifying metabolism (Hayes *et al.,* 2005).

Non-enzymatic Antioxidants

Vitamin E stops free radical chain reactions after five reactions. Vitamin C, plant polyphenols, Vitamin E, carotenoids, selenium, melatonin, uric acid, and glutathione are examples of non-enzymatic antioxidants.

Ascorbic Acid

Vitamin C—ascorbic acid—is a monosaccharide antioxidant found in plants and mammals. Humans cannot synthesise vitamin C, thus they must get it from their diet (Smirnoff, 2001). Glutathione, glutaredoxins, and protein disulfide isomerase diminish it in the cell (Meister, 1994). It reduces and neutralises reactive oxygen species (ROS), especially hydrogen peroxide (Padayatty *et al.,* 2003). It can also be a substrate for plant stress-resistant ascorbate peroxidase (Shigeoka *et al.,* 2002).

Melatonin

Animals and algae produce melatonin, also known as N-acetyl-s-methoxy tryptamine (Caniato *et al.,* 2003). It rapidly passes the blood-brain barrier and can permeate cell membranes, but it does not engage in redox cycling, which prevents molecules from repeatedly reducing and oxidising. Melatonin is called a terminal (or suicide) antioxidant because it reacts with free radicals to form stable end products that cannot be returned to their original state (Tan *et al.,* 2000).

Tocotrienols and Tocopherols

Tocopherols and tocotrienols, eight fat-soluble antioxidant vitamins, make up vitamin E (Herrera and Barbas, 2001). α-Tocopherol has the highest bioactivity, thus the body

absorbs and processes it. According to Traber and Atkinson (2007), α-tocopherol interacts with lipid radicals from the lipid peroxidation chain reaction to inhibit membrane oxidation. It prevents membrane oxidation. It eliminates the propagation reaction's free radical intermediates. Ascorbate, retinol, and ubiquinol are antioxidants that reduce oxidised α-tocopherol radicals to the active reduced state (Wang and Quinn, 1999).

Uric acid

Plasma antioxidant capacity is roughly half uric acid. Like ascorbate, uric acid can produce active oxygen species.

Glutathione

The body can synthesise the cystein-containing peptide glutathione from its amino acids (Meister and Anderson, 1983). Due to its thiol group, cystein is an antioxidant and can be oxidised and reduced. Glutathione reductase reduces glutathione in cells. Glutathione directly reduces oxidants and other metabolites (Meister, 1988). Glutathione is one of the most essential cellular antioxidants due to its high concentration and significance in cell redox state (Mattill, 1947). Kinetoplastids use trypanothione and actinomycetes use mycothiol (Fairlamb and Cerami, 1992). Every cell in the body includes glutathione, the "master antioxidant" that boosts the effectiveness of other antioxidants. Pompella *et al.* (2003) describe glutathione as a tri-peptide with a gamma peptide linkage between the glutamate side chain's carboxyl group and the cystein amine group, which is connected to a glycine via a normal peptide linkage. Body glutathione is reduced (GSH) and oxidised (GSSG). Cysteine's thiol group can produce unstable chemicals including reactive oxygen species (ROS) and H^+ + e when reduced. After receiving an electron, glutathione becomes reactive and quickly forms glutathione disulfide (GSSG) with another reactive glutathione. Cells have high glutathione levels (up to 5mM in the liver), which may cause this reaction. From GSSG, glutathione reductase (GSR) produces GSH (Couto *et al.,* 2013). Healthy cells and tissues contain over 90% GSH and fewer than 10% GSSG. Oxidative stress is indicated by a higher GSSG/GSH ratio.

Antioxidants Defense System

Antioxidants are electron donors, peroxide decomposers, enzyme inhibitors, synergists, radical scavengers, hydrogen donors, metal chelators, and singlet oxygen quenchers. Antioxidants in intracellular and extracellular settings can detoxify ROS,

according to Frei *et al.* (1988). Antioxidant defence system action includes de novo, repair, radical scavenging, prevention and adaptability.

First Line of Defense

This technique prevents free radical synthesis by using antioxidants. Antioxidants initially convert hydrogen peroxide into alcohols and water to prevent free radicals. Glutathione peroxidase, glutathione-s-transferase, PHGPX, and peroxidase convert lipid hydroperoxides into alcohols. PHGPX can diminish biomembrane-bound phospholipid hydroperoxides, making it unique. Catalase and glutathione peroxidase convert hydrogen peroxide to water (Lobo *et al*., 2010).

Second Line of Defense

Radical scavenging uses antioxidants to eliminate active radicals from the environment to prevent chain initiation and propagation. Hydrophilic and lipophilic antioxidants scavenge free radicals naturally. Hydrophilic free radical scavengers include thiols, albumin, bilirubin, uric acid, and vitamin C (Lobo *et al*., 2010). Vitamin E and ubiquinol, lipophilic antioxidants, scavenge free radicals.

Third Line of Defense

Repair antioxidants are needed for this de novo and repair process. Proteinases, proteases, and peptides are found in mammalian cell cytosols and mitochondria as repair and de novo antioxidants. These enzymes detect, extract, and eliminate oxidised proteins to prevent buildup. The DNA repair system, which protects against oxidative damage, relies on glycosylases and nucleases (Lobo *et al*., 2010).

Fourth line of defense

Adaptation requires signalling. Free radical production and response signals create and transport the right antioxidant (Niki, 1993). In conclusion, antioxidants do protect tissue from free radical-induced damage by reducing, scavenging, or decreasing the production of free radicals. Because synthetic antioxidants are bad for people, it's important to find effective, non-toxic chemicals or molecules having antioxidant properties. Plants contain a large number of natural antioxidants (Lobo *et al*., 2010).

2.51 *Solanum macrocarpon* Linn.

The Solanaceae, one of the most important ethno-botanical families of angiosperms, contains plants and herbs used by herbalist or tradationalist to cure a variety of

diseases. *Solanum*, a diverse family genus, is important. More than 2,000 *Solanum* species exist worldwide, with most in regions characterised by a warm climate and located near or within the tropics and few in temperate regions (Kandimalla *et al.,* 2015). Hu *et al*. (1999) list twenty-one species and one variant of this genus as herbal remedies. This study used *Solanum macrocarpon* Linn, a perennial indigenous vegetable (UIV) that has been ignored and underused. The cultivated form is called "Igbagba" or "Igbo" in the South West of Nigeria, "Gorongo" in the North, and "Gboma" elsewhere in West Africa. Most call it African aubergine. The bitter leaves and fruits are used in soups and other dishes like vegetables (Gbite and Adesina, 1998). This plant has dark green, alternating leaves that are 4–15 cm wide and 10–30 cm tall. It grows 1–5 metres tall. Wavy, lobed, oval leaves. Leaf sides have hairy stellate or simple hairs, star-shaped blooms on short-stalked inflorescences with 2 to 7 blossoms. These purple or pale purple blossoms can appear white. This plant is popular for its edible fruit, which looks like an eggplant but has a smoother, glossier surface. The huge, oblong berry is green when immature and golden or orange when mature. Smoothing the fruit's top and bottom creates a 5-7 cm long, 7-8 cm wide groove. West and Central African cuisine uses the fruit as a vegetable. Fruit provides vitamins, minerals, and antioxidants, making it a healthy food. Because of its nutritional and therapeutic properties, several African countries grow it. Fruits have many seeds and partially veiled calyx lobes. Oblong seeds are 2-3.5 mm wide and 3-4.5 mm long. This crop is sometimes decorative and given to livestock as leaves (Han *et al*., 2021; Oboh *et al*., 2005).

Traditional medicine uses SM extensively, in addition to its edible applications, to treat a wide range of ailments, such as heart disease, digestive issues, lung infections, constipation, and worm infestation. Scientific evidence strongly supports its antibacterial, antioxidant, and anti-inflammatory effects, which may assist to explain some of its medicinal effects. Its fruits, leaves, and root all have medicinal properties (Ilodibia *et al.,* 2016). The leaves offer a number of medical benefits, according to Komlaga *et al.* (2014).

Research indicates that SM has antibacterial properties, which implies it may be able to inhibit the development of microorganisms like bacteria, viruses, and fungi. In addition, Ilodibia *et al*. (2016) reported that SM leaf extract demonstrated significant

inhibitory activity against *Aspergilus niger, S. aureus, Candida albican*, and *Eschericia coli.* Mustapha *et al*. 2022 reported that SM extract has high antimicrobial activities against oral pathogens, particularly *Streptococus mutans*. It has the ability to shield the body from free radicals, which can hasten the development of chronic ailments like as cancer, diabetes, and cardiovascular disorders. SM leaves' antioxidant activity was reported by Ajiboye *et al*. (2018), Komlaga *et al*. (2014), and Salawi *et al*. (2013). It's anti-inflammatory. Inflammation is a normal response to injury or illness, but chronic inflammation can accelerate the development of diabetes, arthritis, and heart disease.

In hypercholesterolemic rabbit models, SM was found to have anti-inflammatory and hypolipidemic potential by Odetola *et al.* in 2004. Adewale *et al*. (2015) demonstrated that SM leaf extract has hepato-protective benefits against $CC1_4$ (Carbon tetrachloride), a toxin that can harm the liver, kidneys, and nervous system if consumed or inhaled.

The antioxidant activity, nutrient profile, and non-toxic nature of SM extract and powdered leaves were described by Doughnon *et al.* (2012). Hepato-protective effects of SM fruit extract on liver functions were reported by Gupta *et al.* (2011) *Solanum macrocarpon*'s usage in folk medicine is supported by these factors.

Solanum macrocarpon- scientific classification.

Kingdom	-	Plantae
Clade	-	Tracheophytes
Clade	-	Angiosperm
Clade	-	Eudicots
Clade	-	Asterids
Order	-	Solanales
Family	-	Solamaceae
Genus	-	Solanum
Species	-	*Solanum macrocarpon*
Binomial Name	-	*Solanum macrocarpon*

(Oboh *et al.,* 2005; Alsherbiny *et al.,* 2018; Sánchez-Mata *et al.,* 2010).

Figure 2.10: *Solanum macrocarpon* Linn
Source - Oboh *et al.*, 2005.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials, Reagents, and Chemicals

Gallic acid, Quercetin, Folin – Ciocalteu's Phenol reagent, Ascorbic Acid, Aluminum Chloride, Ferric Chloride ($FeCI_3$), 1,1 – Diphenyl-2 – P_1crylhydrazl (DPPH), Trichloroacetic Acid (TCA), Sulphuric Acid, Sodium Carbonate (Na_2CO_3), Sodium nitrite ($NaNO_2$), Potassium Ferricyanide [$K_3Fe(CN)_6$], Disodium Hydrogen Phosphate (Na_2HPO_4), Sodium hydroxide (NaOH), Sodium Dihydrogen Phosphate (NaH_2PO_4), Sodium Azide, Dipotassium hydrogen Orthophosphate (K_2HPO_4), Dipotassium hydrogen Phosphate trihydrate ($H_7K_2O_7P$), Potassium dihydrogen Phosphate (KH_2PO_4) n-Hexane, Chloroform, Ethyl Acetate, Acid, Sulfanilic Acid, Naphthyl Ethylenediamine dihydrochloride, SDS, Tris- HCI, Ferrous Sulphate, Ferrozine Solution, Thiobarbituric Acid (TBA), Ammonium Molybdate, Starch, Alpha-Amylase Solution, Alpha glucosidase solution, Glutathione,), $5,5^1$- dithio-bis-2 nitrobenzoic acid (DTNB), hydrogen peroxide (H_2O_2) and Epinephrine were obtained from sigma chemical Co. (St. Louis, Mo, USA). Distilled water used was prepared at University of Ibadan, Biochemistry Department Laboratory. Analytical-grade, high-purity chemicals and reagents were used in the investigation.

3.2 Equipment

SpectraMax plate reader (Molecular Devices, CA, USA) and ultraviolet (UV) visible spectrophotometer (model spectrum lab 7525) were used to detect optical absorbance, and 1 cm quartz cells measured absorption. Thermo scientific Sorval WX series and Centurion Scientific C2 series ultra-model centrifuges were used. Agilent Technologies' 7890 GC system and Agilent Technologies' 5979 MSD were used to determine the GC-MS, and Buck Scientific model 530 infrared spectrophotometer was employed to determine the Fourier Transform Infra-Red (FT-IR). Gallenkamp top loading balance and a Mettler balance (Model Toledo - PL 203) were used for all weighings. Plant extract was concentrated using rotary evaporator (model – Heidolph Laborata 4000). The water bath utilised was a DK 420 electrical thermostatic water boiler (Model YLD-2000). Whatman filter paper – No. 1 was used.

3.3 Collection of Plant Materials and Identification

The plant materials utilised in this investigation consisted of freshly developed leaves of *Solanum macrocarpon* Linn. The leaves were bought during the month of October in the year 2016 at Bodija Market, an open-air market place located in Bodija, a region within the Ibadan North Local Government area of Oyo State. Geographical coordinates of location in Nigeria are $7^0 24' 7.0632"$ North Latitude and $3^0 55' 2.3268"$ East Longitude. Taxonomist expertly identified and authenticated plant samples at the Herbarium, Department of Botany, University of Ibadan, Ibadan, Nigeria. **A voucher specimen with the reference UIH – 22543, dated 3/11/2016, was deposited at that exact Herbarium.**

Plate 3.1 *Solanum macrocarpon* Leaves

3.4 Preparation of and Fractionation of Extract

Fresh *Solanum macrocarpon* Linn. leaves were cleaned with distilled water to remove dirts, before air drying at room temperature for twelve days, at Nutrition and Industrial Biochemistry laboratories, University of Ibadan. When properly dried, leaves were milled to a fine powder. About 1000 g of fine powder was extracted using 6.5 L of methanol by cold maceration for 6 days with periodic shaking. Methanol content was filtered first employing cloth muslin and when the solvent was concentrated, it was filtered using filter paper (Whatman paper no.1). Resultant filterate was concentrated under reduced pressure in vacuum with water bath set at 40 ^{o}C for I hr. employing rotary evaporator-Heidolph Laborota 4000.

Highly concentrated crude methanol extract (120.20 g) obtained was fractionated further employing vacuum liquid chromatographic method, with these chemicals in rating of increasing polarity: n-hexane, chloroform, ethyl acetate, n-butanol and methanol. Yield obtained: n-hexane (HF: 2.834 g; 4.498 % w/w), chloroform (CF: 1.973 g; 3.131 % w/w), ethyl acetate (EAF: 5.838 g; 9.266 % w/w), n-butanol (BF: 6.797 g; 10.78 % w/w); methanol (MF: 16.109 g; 25.569 % w/w), crude (CRE: 120.20 g; 11.26 % w/w). Crude and fractions were screened phytochemically using standard procedures (Meng *et al.,* 2020; Tiwari *et al*., 2011; Ayoola *et al*., 2006).

3.4.1 Fractionation Procedure- Vacuum Liquid Chromatography

Principle

The extract sample was first extracted with n-hexane to remove any non-polar or lipophilic substances or compounds. The obtained residue was then extracted with chloroform to remove any pigments, including chlorophyll, and the extract from chloroform was further partitioned in separating funnel using ethyl acetate, where all biologically active substances, such as flavonoids, anthraquinone, anthocyanine, and benzo pyrans, were excepted (Extracted) then n – butanol was used to remove other hydrocarbons and finally methanol, according to the standard procedure.

Organic solvents (n-hexane, chloroform, ethyl acetate, n-butanol, and methanol) were used to fractionate the crude extract in an order of increasing polarity. A combination consisting of 150 g of silica gel (for TLC) and about 90 g of SM crude extract dissolved in 100 ml of methanol after which the entire mixture was air dried.

The dried extract and silica gel were introduced into a sintered glass apparatus, which had been pre-loaded with 100 g of silica gel and coupled to a vacuum pump. The

experimental procedure involved passing n-hexane through the apparatus, followed by doing the identical procedure for chloroform, ethyl acetate, n-butanol, and methanol in order to acquire their respective solvent fractions.

Utilising rotary evaporator with a temperature set up at 30 ^{0}C for n-hexane and chloroform fractions and at 40 ^{0}C for methanol, ethyl acetate, and n-butanol respectively.

The concentrates were further concentrated using a vacuum set at 40 ^{0}C with a pressure of 700 mmHg. The different concentrates were weighed and percentage yield calculated.

Using DPPH, Total Antioxidant Capacity (TAC), and reducing tests, the potential antioxidant ability of all resultant fractions were tested *in vitro*.

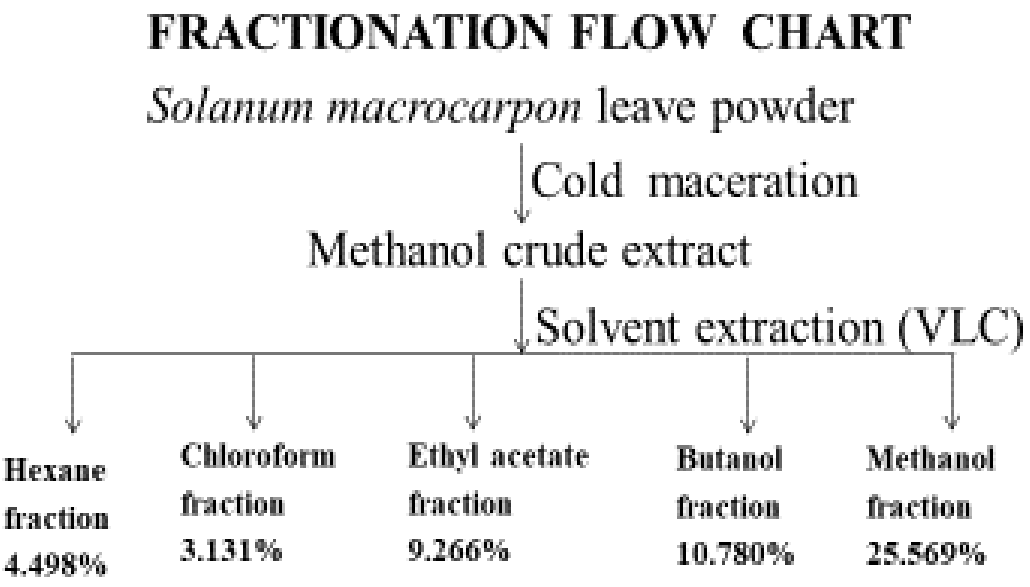


Figure 3.1: Fractionation Flow Chart (Researcher)

3.5 Phytochemical Screening of the Crude Extract and the Solvent Fractions of *Solanum Macrocarpon* Linn

Qualitative evaluations of crude extract and fractions were carried out to confirm the presence or not of certain bioactive constituents, including saponins, proteins, phenols, flavonoids, alkaloids, steroids, cardiac glycosides or cardenolides, phytosterols, diterpens, carbohydrates, tannins, terpenoids and anthraquinone using different standard procedures (Tiwari *et al.,* 2011; Ayoola *et al.,* 2008; Sofowora, 1993; Trease and Evans, 1989).

3.5.1 Test for Saponin (Sofowora, 1993)

0.5 g of the extract was introduced into test tube containig 5 ml distilled water. The solution was strongly stirred and checked for steady foam. Saponins were indicated by 10 minutes foaming.

3.5.2 Test for Phenol (Tiwari *et al.,* 2011)

0.5 g of extract and 3–4 drops of ferric chloride solution were used in the ferric chloride test. Produced colour was bluish-black, which meant phenol.

3.5.3 Test for Flavonoids (Tiwari *et al.,* 2011)

(a) **Lead acetate test**- A small amount of lead acetate solution was applied to the extract. The appearance of a yellow precipitate was a sign of flavonoids.

(b) Ammonia test – A 5 mL volume of ammonium hydroxide was first added to the extract's aqueous filterate. A 1 mL of concentrated sulphuric acid was then added. The observation of a yellow colour that disappeared upon standing was taken as proof that flavonoids existed.

3.5.4 Test for Alkaloids (Trease and Evans, 1989)

(a) Wagner's test—In 5 milliliters of mild hydrochloric acid, 0.5 grams of the extract were dissolved, then filtered. The filtrate was treated using Wagner's reagent (iodine in potassium iodide). Alkaloids were recognized by a brown, reddish precipitate.

(b) Dragendroff test – Acid alcohol was used to dilute 0.5 grams of extract to 10 mL which was then boiled and filtered. Five milliliters of filtrate received two milliliters of ammonium hydroxide. Using 5 ml of chloroform, the alkaloid base was extracted by shaking. Chloroform part was extracted using 10 mL of acetic acid. An aliquot of this had the regent Dragendroff added to it. Based on

the presence of reddish brown precipitate, alkaloids were assumed to be present.

3.5.5 Test for Steroids (Phytosterol)

Liberman Burchard's test- Extract passed through a chloroform treatment and filtration according to Liberman Burchard's test. After being treated with a few drops of acetic anhydride, the filtrate was heated and chilled. There was addition of concentrated sulfuric acid. Brown rings forming at the intersection were interpreted as phytosterol presence evidence.

3.5.6 Test for Terpenoids

Salkowski's Test- Before filtering, 2 mL of chloroform and 0.5 g of the extract were combined. Carefully adding 3 mL of strong sulfuric acid produced a coating. Because of the reddish-brown color of the interface, terpenoids were thought to be present.

3.5.7 Test for Diterpenes

Copper acetate test- Three to four drops of copper acetate were added after the extract was dissolved in water. Diterpenes was confirmed by emergence emerald green colouring.

3.5.8 Test for Tannins- 0.5 g of the extract was boiled in 10 mL of water in a test tube before being filtered. After adding a few drops of 0.1% ferric chloride, the solution's color was examined. A brownish green or blue-black coloring indicates the presence of tannin**.**

3.5.9 Test for Anthraquinones

In 10 mL of H_2S0_4, 0.5 g of the extract was boiled before being filtered while still hot.. Filterate was shaken with 5 mL chloroform. It was then added 1 mL of the ammonium hydroxide solution into chloroform layer already pipetted into another test tube. The solution colour was checked. Anthraquinone was confirmed by a colour change.

3.5.10 Test for cardiac glycosides/cardenolides:

Keller- Killiani test- To 0.5 g of extract that has been diluted to 5 mL in water, glacial acetic acid and one drop of ferric chloride solution were added. Underneath was a pure 1 mL of sulfuric acid. A deoxysugar, which is unique to cardenolides, was indicated by a brown ring at the contact.

3.6 Determination of Proximate Content

According to analysis techniques from the Association of Official Chemists (18th edition, 2005), the proximate composition was established.

3.6.1 Estimation of Crude Protein

Semi-micro Kjeldahl method was employed.

Reagents

1. Concentrated H_2SO_4 as supplied
2. 0.01 N HCL
   0.818 mL of 37.5 % HCI was diluted to 1000 mL of distilled water
3. 40 % (W/V) NaOH
   40 g of NaOH was dissolved in distilled water (100 mL)
4. 2 % Boric Acid solution (BA) (H_2BO_3)
   2 g of BA was dissolved in distilled water (100 mL)
5. Methyl- Red Bromocresol Green Mixed Indicator
6. Kjeldahl catalyst pill, as directed.

Procedure

Digestion, distillation, and titration are explained as due procedure.

One catalyst tablet and 10 mL of concentrated sulphuric acid were put to a Kjeldahl digestion tube that contained 0.5 g of finely powdered dried extract. They were digested for four hours in a fume cabinet by placing them in the proper digestion block heater hole, which produced a clear, colorless solution in the tube. 5 mL of the digest and 5 mL of 40 % (w/v) NaOH were combined in the distillation device. The mixture was blended with indicator solution after being steam-distilled for 2 minutes into a 50 mL conical flask containing 10 mL of 2% boric acid. The indicator solution and boric acid will turn green from red once all of the released ammonia has been accounted for. The green color of the distillation's green solution was titrated against 0.01 N HCI until the point at which all nitrogen trapped as ammonium borate [$(NH_4)_2BO_3$] was released as NH_4CI. At that point, the green color changed to ruby.

$$.\%\ N = \text{Titre value} \times \text{Nitrogen atomic mass} \times \text{Normality of HCI utilised} \times 4 \qquad 3.1$$

$$\text{Crude Protein (\%)} = \%N \times 6.25 \qquad 3.2$$

3.6.2 Determination of Crude Fat

Reagent

Petroleum Spirit or Ether.

Procedure

Weighing 1 g of the dried material into a fat-free extraction thimble, which was then put in the extractor attached to the Soxhlet flask, was the first step in the extraction process. Petroleum ether was poured into the Soxhlet until it was 3/4 full, and the apparatus was then heated for 6 hours while water ran continuously to condense the ether vapour. The sample thimble was removed and dried. The oil or fat flask was taken out and dried to a constant weight in the oven.

$$\% \text{ Crude Fat} = \frac{W_1 - W_0}{Sample\ wt} \times 100 \qquad 3.3$$

W_0 = Initial weight of a dry Soxhlet flask

W_1 = Final weight of oven dried flask + fat

$W_1 - W_0$ = Weight of the Fat.

3.6.3 Determination of Crude Fibre

Reagents

1. 0.255 N H_2SO4

   25 mL of 1 N H_2SO_4 was diluted with distilled water to 100 mL

2. 0.313 N NaOH

   13.16 g of NaOH pellets were dissolved in distilled water (1000 mL)

3. Acetone as supplied.

Procedure

2 g of dry material and 100 mL of sulphuric acid (0.255 N) were vigorously stirred in a fibre flask. Filtering followed, one hour of reflux heating. After the filtrate had been decanted, the residue was added back to the fiber flask along with 100 mL of NaOH (0.313 N) and heated for an additional hour of reflux. 19 mL of acetone were added after filtration to dissolve any remaining organic materials. Before adding the residue to the crucible, 50 mL of hot water was poured over it. To remove the water, the residue was subsequently oven-dried over the following day at 105 °C. Residue was weighed in the desicator before ashing at 550 ^{0}C for 4 hours in the muffle furnace. Weighing of crucible and grey followed. Fibre content was determined as below:

$$\% \text{ Fibre} = \frac{W_1 - W_2}{Sample\ wt} \times 100 \qquad 3.4$$

W_1 = wt of crucible + residue

W_2 = wt of crucible + grey ash

$W_1 - W_2$ = wt of fibre

Nutrient (Unit)	Amount	Nutrient (Unit)	Amount
Proximates		Vitamins	
Sugars, total (g)	3.53	Vitamin K(Phylloquinone) (µg)	3.5
Fibre, total dietary (g)	3	Vitamin E,(α-tocopherol)(mg)	0.3
Carbohydrate (g)	5.88	Vitamin A, RAE (µg)	23
Total lipid (fat) (g)	0.18	Vitamin A, RAE (µg)	1
Protein (g)	0.98	Fola, DFE (µg)	22
Energy (Kcal)	25	Vitamin B6 (mg)	0.084
Water (g)	92.3	Niacin (mg)	0.649
Minerals		Riboflavin (mg)	0.037
Zinc, Zn (mg)	0.16	Thiamin (mg)	0.039
Sodium, Na (mg)	2	Vitamin C (mg)	2.2
Potassium, K (mg	229	Lipids	
Phosphorus	24	Cholesterol (mg)	0
Magnesium, Mg (mg)	14	Fatty acids, total polysaturated (g)	0.076
Iron, Fe (mg)	0.23	Fatty acids, total monosaturated (g)	0.016
Calcium, Ca (mg)	9	Fatty acids, total saturated (g)	0.034

Nutritional value of eggplant per 100g (USDA report 11209)

Figure 3.2: Nutritional Composition of Eggplant
Source: Adapted from Oladosu *et al*., 2021

3.6.4 Ash Determination

Procedure

Two grams of dried sample were weighed and put in a porcelain crucible. Then, for almost four hours, this container was heated at 550 ^{0}C in a muffle furnace, during which time the material would have been transformed into white ash. The crucible and its contents were then chilled in an air-cooled desiccator to a temperature of roughly 100 ^{0}C, and then brought to room temperature, where they were weighed.

$$\text{Ash Content} = \frac{\text{Wt of Ash}}{\text{wt of sample}} \times 100 \qquad 3.5$$

3.6.5 Determination of Dry Matter and Moisture Content

Reagents:

(i) Silica gel

(ii) Grease

Procedure

2 g of the dry sample was weighed, transferred to a crucible that had already been weighed, and placed in an oven set at 100 ^{o}C to dry to a consistent weight over a period of 24 hours. After 24 hours, the crucible and its contents were weighed.

Calculation

$$\% \text{ Dry Matter} = \frac{W_3 - W_0}{W_1 - W_0} \times 100 \qquad 3.6$$

W_0 = Weight of empty crucible

W_1 = Weight of crucible and sample

W_3 = Weight of crucible and oven – dried sample

$$\% \text{ Moisture Content} = \frac{W_1 - W_3}{W_1 - W_0} \times 100 \qquad 3.7$$

3.6.6 Carbohydrate Determination

The following calculation was used to determine the amount of carbohydrates-

% carbohydrate = 100% - [%Ash + % crude fat + % crude fibre + % crude protein + % Moisture] 3.8

3.7 Elemental Analysis

3.7.1 Micronutrient

According to the WHO, micronutrients are minerals and vitamins that the body needs in very minute amounts but that have a significant impact on health. A dangerous and potentially fatal condition can result from a lack of any one of them. They perform different tasks, such as production of enzymes, hormones, including other compounds necessary for healthy growth and development (WHO www.who.int). Iron, manganese, zinc, copper, cobalt, iodine, chromium, molybdenum, selenium, fluoride, nickel chlorine, and boron are a few examples. Vitamins are separated into water-soluble and fat-soluble categories. Numerous phytochemicals, also known as phytonutrients, are micronutrients found in plants.

3.7.2 Macronutrient

According to the WHO, macronutrients are nutrients that produce energy or calories and are consumed in substantial amounts to support bodily processes and activities. Protein, lipids, and carbohydrates are the three basic categories of macronutrients. A few examples of macrominerals are magnesium, potassium, calcium, phosphorus, sodium, sulphur, chloride, carbon, and hydrogen.

3.7.3 Determination of Mineral Elements

3.7.3.1 Macrominerals – calcium, potassium, sodium

Reagents

1. 2 M HCL

   Add 167 mL of conc. HCI to 1000 mL of distilled water

Procedure

1 g of the sample's ash was digested in 5000 µl of 2 M HCI, boiled to dryness, added 5000 µl of 2 M HCI again, heated to boiling, and filtered through Whatman No. 1 filter paper. A Jenway Digital Flame Photometer (PFP 7 model) with filters matching calcium, potassium, and sodium was used to read the concentration of the prepared filtrate in a 100,000 µl volumetric flask made up with distilled water. AAS was used to assess various minerals besides phosphorus.

Calculation

$$\% \text{ Ca, K, Na} = \frac{Meter\ \text{Re}ading\ (MR) \times Slope \times Dilution\ Factor}{10000} \qquad 3.9$$

MR X Slope × Dilusion Factor = Concentration in part per million (PPM or mg/kg). Concentration is obtained in percent when divided by 10000.

3.7.3.1.1 Determination of Phosphorus

Determination by Vanado-Molybdate Colorimetric or Spectrophotometric Method

Reagent:

1. 2 M HCI

   Add 167 mL of conc. HCI to 1000 mL of distilled water. Yellow vanadate-molybdate solution.

Procedure

1 g of the sample's ash after being exposed to 2 M HCI in the manner described above for determining calcium, vanadate-molybdate yellow solution (10 ml) was applied to the filterate (10 mL) as stated above, and the solution was made up with distilled water to 50 mL, then left to stand for 600 seconds to accomplish the yellow development. The phosphorus concentration was determined using a phosphorus calibration curve after the spectrophotometric reading at 470 nm has been taken.

Calculation:

$$\% \text{ Phosphorus} = \frac{Absorbance \times Slope \times Dilution\ Factor}{10{,}000} \qquad 3.10$$

3.7.3.2 Determination of Mineral Element

Microminerals – Fe, Zn, Cu, Mn, Se.

Reagent:

2M HCL

Procedure

Following the same steps used to assess potassium and calcium levels above, 1 g of the sample's ash was digested and added to a 100,000 µl volumetric flask filled with distilled water. In the Buck 200 Atomic Absorption Spectrophotometer (AAS), the diluent was then aspirated through the suction tube. Using proper fuel and oxidant combinations, appropriate cathode lamp was used to read each trace element at its corresponding wavelength.

3.8 Determination of *in Vitro* Antioxidant Activity

Principle

The extract sample was dissolved in water to produce a concentration of 1 mg/ml. This concentration was then diluted to create a range of concentrations for antioxidant tests. Reference compounds were utilised in all of the experiments to make comparisons.

3.8.1 DPPH Radical Scavenging Assay (Gyamfi *et al.*, 1999)

Using the DPPH free radical scavenging assay, the ability of the investigated extract to scavenge free radicals was evaluated. Free radicals that are stable are present in the chemical DPPH.

The 2, 2-Diphenyl-1-picrylhydrazl (DPPH) assay is the most popular antioxidant test for plant extract. In this experiment, a chemical or antioxidant with a tenous A-H bond would interact with the stable free radical DPPH (2, 2-Diphenyl-1-picrylhyrazl, max 517 nm), discolouring it.

Principle

Antioxidants scavenge the DPPH radical by giving it a proton, which causes the production of the reduced DPPH. A reduction in absorbance at 517 nm can be used to assess change in colour from purple to yellow that occurs when reduction is taking place. A linear relationship exists between antioxidant content and absorbance. The scavenging activity of free radicals increased with a higher percentage of free radical inhibition. The amount of the colour change, which is caused by the antioxidant or sample's (extract) ability to donate hydrogen, is a reflection of how well that substance can scavenge free radicals. Depending on how many electrons are taken up, the electrons couple off, and stoichiometrically the solution loses colour (Kedare and Singh, 2011; Sannigrahi *et al*., 2009).

The free radical scavenging ability of the crude extract and fractions was evaluated *in vitro* using the 2, 2 -Diphenyl-1- picrylhydrazl (DPPH) assay in accordance with the procedure established by Blois (1958) as modified by Manzocco *et al.,* 1998, and Gyamfi *et al.,* 1999.

Method

2 ml of the DPPH solution (0.025 g/L) and (1000µl, 1mg/ml) of the methanol extract were combined. Thirty minutes of incubation at 25 ^{0}C in a dark environment with vigorous shaking of the reactants followed. At 517 nm against a blank, the absorbance was measured. Without using any sample, the control was created as before. The following equation was used to calculate the radical scavenging activity based on the percentage of DPPH radical scavenging.

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \qquad 3.11$$

The effective concentration (EC_{50}) (mg/ml) at which DPPH radicals were 50 % scavenged was calculated by interpolating from a linear regression analysis.

3.8.2 Phosphomolybdate assay

The total antioxidant capacity of the extract and fractions was assessed using a phosphomolybdate test with ascorbic acid as the reference. According to approach of (Prieto *et al.,* 1999), the green phosphomolybdenum complex production was utilised in assessing antioxidant activity of the sample.

Principle

This process reduces phosphomolybdic acid to phosphomolybdenum blue complex sodium. Nitrite oxidises the phosphomolybdenum blue complex, weakening its blue colour. Spectroscopic total antioxidant capacity assays quantify antioxidant capacity by generating phosphomolybdenum complex. In acidic pH, the sample analyte's conversion of MO (VI) to MO (V) forms a green phosphate MO (V) complex thus, confirming the test. The modified Prieto *et al.* method is used in calculating the total antioxidant capacity.

Method

A mixture of 0.3 ml of the sample solution and 3 ml of the phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was used. The mixes were placed in test tubes, sealed, and incubated at 95 °C in boiling water bath for 90 minutes. A uv-vis spectrophotometer was used to gauge the aqueous solution's absorbance at 695 nm in comparison to a blank after cooling to room temperature. 1 ml of the reagent solution and the proper volume of sample solvent utilised as standard blank were incubated. Phosphomolybdenum Reduction Potential (PRP) of the sample was calculated using the following equation.

$$\text{Antioxidant effect (\%)} = \frac{Control\ Abs - Sample\ Abs}{Control\ Abs} \times 100 \qquad 3.12$$

Ascorbic acid equivalents are used to express total antioxidant capability. This test is helpful for determining the crude extract's overall or total antioxidant capability.

3.8.3 Nitric Oxide Scavenging Activity

The nitric oxide radical scavenging activity assay was used to determine the percentage of nitric oxide radical scavenging activity of the studied extract, and EC_{50} value.

Principle

The approach of Garrat (Garrat, 1964) was employed to examine the scavenging ability of nitric oxide radicals. At physiological P^H, the breakdown of aqueous sodium nitroprusside solution produces nitric oxide spontaneously. In an aerobic environment, this nitric oxide reacts with oxygen to produce stable products (nitrate and nitrite), which may be found via the Griess-Illovoy reaction (Marcocci *et al.*, 1994; Green *et al.,* 1982; Garrat, 1964).

Citrulline is produced from arginine by certain nitric oxide synthases by a five electron oxidative process, culminating in the production of NO, and these enzymes are in charge of creating NO in biological tissues (Haynes *et al.,* 2003; Bredt, 1999; Moncada *et al.,* 1989). Smooth muscle relaxation, platelet aggregation inhibition, neuronal signalling, and cell-mediated toxicity modulation are just a few of the physiological activities that nitric oxide (NO), a powerful pleiotropic mediator, mediates. A diffusible free radical called nitric oxide serves as an effector molecule in numerous biological processes, such as neuronal communication, vasodilatation, antimicrobial, and anticancer actions (Sarwar *et al.,* 2015). In pathological circumstances, nitric oxide and superoxide anion also interact to generate the extremely deadly compound-peroxynitrite. Nitric oxide inhibitors have been shown to improve various areas of inflammation in tissue damage caused by inflammatory disorders. Nitric oxide production is decreased as a result of nitric oxide scavengers' competition with oxygen.

Method

Procedures as modified by (Garrat, 1964).

The following ingredients were added to 0.5 ml of extract at various concentrations (0.2-0.8 mg/ml), along with 0.5 ml of phosphate buffer saline (0.2 M, p^H 7.4) and 1 mL of 0.10M (10 mM) of sodium nitroprusside that had been prepared right away. 30 minutes were spent incubating the mixture at 27 ^{0}C. Following incubation, 0.5 ml of the incubated solution was removed and combined with 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid), before being incubated for 5 minutes at room temperature with 1 ml of napthylethylenediamine dichloride (0.1% w/v). Allow the mixture to remain for an additional 30 minutes. The generated yellow chromophore was read against a blank at 546 nm. The following equation can be empolyed to evaluate the percentage of nitric oxide radical scavenging activity:

$$\text{Scavenging activity (\%)} = \frac{Absorbance\ of\ \ control - Absorbance\ of\ \ sample}{Absorbance\ of\ \ control} \times 100 \qquad 3.13$$

By extrapolating from linear regression analysis, the extract concentration that provides 50% inhibition (EC_{50}) was determined.

3.8.4. Reducing Power Assay

According to a modified version of Oyaisu's (1986) approach, the extract's reducing power assay was estimated.

Principle

Electron donors stabilise free radicals, stopping the chain process. The reducing assay relies on reaction mixture absorbance rising. Absorbance increases antioxidant activity. In this instance, the antioxidant combines with ferric chloride, potassium ferricyanide, and trichloroacetic acid to generate a colored complex at 700 nm. With an antioxidant, potassium ferricyanide reacts with ferric chloride to create potassium ferrocyanide and ferrous chloride. Gupta *et al*. (2012) and Jayanthi and Lalitha (2011) found that the reducer turns the Fe^{3+}/ferricyanidide complex into ferrous form. Antioxidants are reductants but it does not mean that reductants are automatically antioxidants (Gülcin, 2012; Prior and Cao, 1999). Jayaprakasha *et al*. (2001) found that increase in absorbance during reaction demonstrates the reducing ability of extract. Reducing ability of compounds is a testament to their antioxidant properties (Jung *et al.,* 2008).

Method

In this process, 1 ml of the extract (250 µg/ml to 1000 µg/ml) was added to 500 µl of 1 % potassium ferricyanide (K_3Fe (CN) 6) and 1000 µl of 0.2 M phosphate buffer (pH 6.6), and the mixture was then incubated at 50 ^{0}C for 30 minutes before receiving 0.5 ml of 10 % trichloroacetic acid (TCA). The mixture was then centrifuged at 3000 rpm for 30 minutes. 1 ml of the solution's supernatant, or upper layer, was blended with 1 ml of freshly produced ferric chloride, 0.1 % (w/v) and I ml of distilled water. Absorbance was calculated at 700 nm and compared to a control. A higher reducing power is indicated by the reaction mixture's increased absorbance (Oyaisu, 1986).

3.8.5 Metal chelating activity

Procedure of Dinis *et al*. (1994) as modified was used in assessing chelation of ferrous ion by the extract.

Principle

Ferrozine has the ability to quantitatively chelate Fe^{2+}, resulting in the creation of a complex red molecule. The complex's red color is diminished because this complex formation process is restricted or interrupted in the presence of additional chelating

agents. According to Soler-Rivas *et al.* (2000), reaction measurement calculates the chelating activity competition between ferrozine and ferrous ions. Ferrozine and divalent iron combine to form a stable, crimson or purple complex species that is extremely soluble in water. The transition metal ions Fe^{2+} have the ability to transport a single electron, allowing them to promote the formation of numerous radical reactions, even when radical reactions start with relatively inactive radicals. One of the processes underlying the antioxidative effect is the chelation of transition metals, which inhibits the catalysis of Fenton-type reactions and the breakdown of hydroperoxide (Decker and Welch, 1990; Gordon, 1990). Plant extract antioxidants have chelating activity, which prevents the transport of electrons by forming a coordinated complex with metal ions. As a result, the oxidation reaction is halted and no free radicals are produced.

Method

50 µl of newly manufactured ferrous sulphate (0.01 %) was combined with 0.5 ml of extract. 100 µl of 3 mM Ferrozine initiated the process. The mixture was shaken ferociously and incubated for 10 minutes at room temperature before the absorbance was measured at 562 nm. The amount of Ferrozine-Fe^{2+} compound formation that the extract suppressed was used to determine its Fe^{2+} chelating activity.

$$\text{Chelating effect (\%)} = \frac{Ao - As}{Ao} \times 100 \qquad 3.14$$

Ao= control Absorbance

As= sample Absorbance

3.8.6 Estimation of Total Phenolic Content of Extract

Employing the spectrophotometric technique described by Singleton and Rossi, 1965, as updated by Kim *et al*., 2003a; Kim *et al.,* 2003b. The extract's and the fractions' total phenolic content was calculated.

Principle

This is a sensitive and quantitative method because Folin-Ciocalteu's reagent, which gives a blue colour complex with a broad range of light absorption and a maximum at 765 nm, is sensitive to reducing compounds like polyphenols. The phosphomolybdic/phosphotungistic complexes in the alkaline medium are what the Folin-Ciocalteu assay relies on to transfer reducing equivalent (electrons) from phenolic substances. When the absorbance at 765 nm is monitored on an ultraviolet-visible spectrophotometer, the blue colour complex [$(PMOW_{11}O_{40})$] that is produced as

a result of this reaction can be detected (Anderson and Francis, 2004; Singleton and Rossi, 1965). The hydroxy group-containing aromatic chemicals known as phenolics are highly prevalent in the plant kingdom. Their assessment is based on the phenols that were reduced by the phosphomolybdic-phosphotungistic elements of the Folin-Ciocalteu's reagent. Phenolic molecules undergo oxidation in a basic medium, producing the super-oxide ion, which then interacts with molybdate to produce molybdenum oxide. Because most phenolic compounds are in dissociated forms (as conjugate bases or phenolate anions) at the assay's working p^H of 10, they can be more easily oxidized using Folin-Ciocalteu's reagent. The compound that turned out to be blue in colour as a result, had a very high absorption at 765 mm.

3.8.6.1 Standard Solution Preparation

Carefully measured 10 mg of gallic acid was put into a 10 mL volumetric flask, which was then filled to the mark with distilled water to make 1 mg/ml solution. This laid the groundwork for gallic acid calibration curve construction.

3.8.6.2 Procedure

0.5 mL of extract (1 mg/ml) was combined with approximately 0.05 mL of Folin-Ciocalteu phenol reagent. After waiting for five minutes, the mixture was well mixed by adding 2000 µl of 7% Na_2CO_3 solution and 5000 µl of distilled water. Absorbance was measured at 750 nm after keeping for 90 minutes at 25 ^{0}C in a dark room. The gallic acid solution calibration curve was extrapolated to measure total phenolic content. The amount of total phenolics was given in mg/ml GAE because gallic acid served as the reference.

3.8.7. Estimation of Total Flavonoid Content

Total flavonoids of extract and fractions were determined spectrophotometrically using approach of Zhishen *et al*. (1999) with modifications of Park *et al.,* (2008).

Principle

Aluminium chloride-flavonoids complex formation. Aluminium chloride forms acid-labile compounds with flavonoids' A or B rings' ortho-dihydroxy groups. It gives compouds which are acid-stable with flavonols and flavones C-3 or C-5 hydroxy group and C-4 keto group. When flavonol mixes with $Alcl_3$ and sodium acetate, green colour is produced (Kalita *et al.,* 2013; Chang *et al.,* 2002; and Mabry *et al*., 1970). The ability of flavonoids to act as antioxidants depends on their molecular structure. Their antioxidant and free radical scavenging capacities are influenced by the positioning of

functional hydroxy groups and other chemical characteristics. The most prevalent dietary flavonoid, quercetin, is a potent antioxidant because it has the structural characteristics needed to scavenge free radicals. Flavonoids content is reported as Quercetin equivalents.

3.8.7.1 Preparation of the Standard Solution

To create a solution containing 1 mg of quercetin per milliliter (1 mg/mL) 10 mg of quercetin was accurately weighed into a 10 ml volumetric flask, dissolved with water, and then the volume was increased to 10 ml using distilled water. The calibration curve for quercetin was made using this solution.

3.8.7.2. Procedure

A 10 ml test tube was used in mixing the sample (300 µl), 30% methanol (3,444 µl), 150 µl $NaNO_2$ (0.5 M), and 150 µl $AlCl_3$. $6H_2O$ (0.3 M). After 5 minutes, 1000 µl NaOH (1M) was added. Following mixing, absorbance was measured at 506 nm against blank. The total flavonoid contents were extrapolated in milligrams of quercetin equivalent per gram of dry material using a calibration curve.

3.8.8. Inhibitory Effect of Extract and Solvent Fractions on Lipid Peroxidation in Rat Pancreas

To assess the extract and fractions' lipid peroxidation protection, the amount of MDA [Malondialdehyde] formed after Fe^{2+}-induced lipid peroxidation, was determined spectrophotometrically (Ohkawa *et al.,* 1979).

Lipid peroxidation occurs when free radicals or non-radical species damage carbon-carbon double-bonded lipids. This mechanism removes hydrogen from a carbon, introduces oxygen, and produces hydrogen peroxide and lipid peroxyl radicals (Yin *et al.,* 2011).

3.8.8.1 Preparation of Pancreas Homogenates

The rats used in the experiment were rapidly dissected to remove the pancreas after being sacrificed by cervical decapitation. This organ was weighed after being placed on ice, then it was homogenised with a mortar and pestle in normal, ice-cold saline water (1/10 w/v). The resulting homogenates passed throught ten minutes of centrifugation at a speed of 3000 rpm. The supernatant for the lipid peroxidation experiment was taken out and divided into individual tubes (Oboh *et al.,* 2007; Bellé *et al.,* 2004).

3.8.8.2 Lipid Peroxidation and Thiobarbituric Acid Reaction

Principle

Malonaldehyde (MDA), a consequence of lipid peroxidation, interacts with thiobarbituric acid (TBA) at high temperature and acidity to form a red adduct. Spectrophotometric or colorimetric method is utilised in identifying this adduct.

Lipid peroxidation, in which ROS break down polyunsaturated fatty acids (PUFA), produces malonaldehyde (MDA). Lipid peroxidation produces MDA, a reactive aldehyde and reactive electrophile that causes cell stress. AGEs are made through MDA. MDA production is employed as a biomarker for oxidative stress in humans (Del-Rio *et al*., 2005).

3.8.8.3 Procedure/Assay

Lipid peroxidation assay was performed using a modified version of the method published by Ohkawa *et al*. (1979). 300 microliters of distilled water, 100 microliters of pancreas supernatant fraction, 30 microliters of 0.1 M pH 7.4 Tris-HCl buffer, 30 microliters of freshly made 250 mM ferrous sulphite (pro-oxidant), and 0–100 microliters of sample extract/fraction made up the reaction mixture. Then, for an additional two hours, same reaction mixture was incubated at 37 degrees Celsius. A colour reaction started to appear after 300 microliters of 8.1 % SDS (Sodium Dodecyl Sulphate) were added to this same reaction mixture comprising of supernatant. The mixture was then treated with 500 microliters of 0.8 % TBA (Thiobarbituric Acid) and 500 microliters of acetic acid/HCl with pH 3.4. At a temperature of 100 ^{0}C, this mixture was incubated for an hour. TBAR (Thiobarbituric Acid Reactive Species) generated was measured at 532 nm in a UV-visible spectrophotometer. Absorbance was compared to the absorbance of standard curve using MDA [malonaldehyde]. Calculated and displayed as a percent control, was the MDA generated (Oboh *et al.,* 2007).

3.8.9. Inhibitory Effect of Extract on Starch Hydrolyzing Enzymes –Alpha-Amylase and Alpha-Glucosidase

The extract's *in vitro* antidiabetic activity was evaluated using enzyme inhibition assay models for alpha-amylase and alpha-glucosidase. Alpha-glucosidase and alpha-amylase contribute in the breakdown of polysaccharides. Pancreatic alpha-amylase breaks down long chain carbohydrates or large polysaccharides into disaccharide or oligosaccharide before the activity of alpha-glucosidase, which turns disaccharide into

monosaccharides (glucose), which is easily absorbed into the blood stream. By converting dietary carbohydrates into glucose, these enzymes' bodily functions are to blame for postprandial hyperglycemia. Because this extract can block these enzymes, diabetic patients may suffer less postprandial hyperglycemia.

3.8.9.1 α- Amylase Inhibition Assay

Principle

Amylase activity can be evaluated *in vitro* by hydrolyzing starch in the presence of enzyme alpha-amylase. This process is estimated by utilizing iodine which produces blue colour with starch. If the blue colour is more intense, the extract (substrate) may have the ability to block the enzyme α-amylase. Conversely, if the blue colour is less intense, starch is being hydrolyzed into monosaccharide as a result of enzyme-induced hydrolysis. Sheikh *et al*. (2008) found a direct correlation between the degree of blue color intensity in the test sample and alpha-amylase inhibitory action.

3.8.9.2 Procedure

According to the procedure outlined by Worthington (1993), an assessment of the extract's ability to inhibit α -amylase was performed. 500 µl of SM extract and 500 µl of 0.2 M sodium phosphate buffer with pH 6.9 and 0.006 M sodium chloride, with alpha-amylase solution (0.5 mg/ml) were incubated at 25 ^{0}C for ten minutes. Then each tube received 500 µl of a 1 % starch solution in a 0.02 M sodium phosphate buffer solution with a p^{H} of 6.9 and 0.006 M sodium chloride. The subsequent ten minutes of incubation of reaction solutions at 25 ^{0}C was followed by the stopping of the reaction with 1.0 ml of the Diniotrosalicyclic Acid Colour Reagent. After a third incubation, which lasted for five minutes, the test tubes were cooled to room temperature. The absorbance was measured at 540 nm following dilution of the reaction mixture with 10,000 µl of distilled water. For positive control, the widely used anti-diabetic drug called acarbose was employed. Utilizing the following formula, the extract's percentage of enzyme inhibitory activity was calculated:

$$\% \text{ Inhibition} = \frac{Ac - As}{Ac} \times 100$$

3.15

Ac= Control Absorbance

As= Sample Absorbance

3.8.9.3 α- Glucosidase Inhibition Assay

Extract's alpha-glucosidase inhibition experiment was carried out using a modified version of approach from Apostolidis *et al*., 2007.

Procedure

After being pre-incubated at 25 °C for ten minutes, 500 µl of the extract was then suspended in 1000 µl of alpha-glucosidase solution (1.0 U/L) that was made in 0.1 M phosphate buffer (pH 6.9). 500 µl of 0.005 M nitrophenyl glycopyranoside solution prepared in 0.1 M phosphate buffer (pH 6.9) was added after the pre-incubation time. The reaction mixtures were incubated at 25 ^{0}C for five minutes. The reaction mixture's absorbance was measured at 405 nm. According to Apostolidis's equation, the extract's percentage enzyme inhibitory activity was estimated as shown below.

$$\% \text{ Inhibition} = \frac{Abs\,control - Abs.sample}{Abs.of\ control} \times 100 \qquad 3.16$$

Abs. control = Control Absorbance

Abs. sample = Sample Absorbance

3.9 Experimental Animals and Diet

Male Wistar rats in good health, weighing between 114 and 164 g, were obtained from animal house, Department of Physiology, College of Medicine, University of Ibadan, Ibadan. The animals were housed in controlled environment with 12-hour light/12-hour dark cycle, a temperature of 24 ± 2 °C, and relative humidity of 50 ± 10 %. They were kept in cages in disease causing agent-free animal facility. The rats' food consisted of standard Ladokun pellets and unlimited amounts of water (*ad libitum*) throughout the experiment. The University of Ibadan's Animal Care and Use Research Ethical Committee (UI-ACUREC) granted consent for research to be conducted in compliance with ethical standards. (Approval number: UI-ACUREC/024-0219/21)

For diabetes induction, healthy male Wistar rats weighing (139 - 146 g) were obtained and used.

3.9.1 Acute Toxicity Evaluation

The protocols for acute toxicity studies were carried out in accordance with the OECD's 425, acute toxic class (three animals were employed), criteria (OECD, 2008). Rats weighing 114–164 g were randomly divided into seven groups of three rats each.

To enable individual identification, the rats were marked. The test animals were fasted overnight and treatment with various doses of the methanol extract of *Solanum macrocarpon* leaf corresponding to 100, 300, 500, 1,000, 2,000, 5,000 mg/kg administered orally to six groups (Table 3.1), the 7^{th} group serving as control received only clean water. The animals were placed under observation immediately for four hours, for any gross behaviour, neurological change and then morbidity and mortality for the fourteen days of the experiment.

Table 3.1: Experimental Design for Acute Toxicity

Group	Treatment
Group 1	Control – water
Group 2	SM Plant extract 100 mg/kg body weight
Group 3	SM Plant extract 300 mg/kg body weight
Group 4	SM Plant extract 500 mg/kg body weight
Group 5	SM Plant extract 1000 mg/kg body
Group 6	weight
Group 7	SM Plant extract 2000 mg/kg body weight
	SM Plant extract 5000 mg/kg body weight

3.9.2 Collection of Blood and Tissue from the Animals

Overnight fasted rats were sacrificed by cervical dislocation while receiving only a little anaesthetia, following the fourteen-day experimental activity. Blood obtained via decapitation was placed into sample bottles for preparation of serum and plasma, which was used to estimate various biochemical parameters. After being harvested, histopathological examinations were carried out on pancreas.

3.9.3 Body Weight Changes

Prior to administering the extract on the first day of the experiment, the initial body weights of each animal in this study were recorded. After the experimental fourteen-day period had passed, the weights of each animal were reported.

3.9.4 Preparation of Solution and Reagent

3.9.4.1 Preparation of 0.1 M citrate buffer pH 4.5

Combine 50 mL of 0.1 M sodium citrate and 50 mL of 0.1 M citric acid to make 0.1 M citrate buffer. For adjustments, use NaOH.

Dissolve 1.47 g of sodium citrate dehydrate {(MW 294.10 g/moL) – ($C_6H_9Na_3O_9$)} in 50 mL of distilled water.

Dissolve 0.960 g of citric acid {(MW 192.12 g/moL) – ($C_6H_8O_7$)}

3.9.4.2 Preparation of STZ (50 mg/kg)

Streptozotocin powder {(MW 265.221 g/m) – ($C_8H_{15}N_3O_7$)}

Dissolve 270 mg of STZ in 0.1 M citrate buffer.

3.9.5 Induction of diabetes mellitus

After an overnight fast, the rats received a single intraperitoneal injection of STZ (50 mg/kg) in a cold, freshly made citrate buffer (0.1 M, p^H 4.5) for induction of DM (Adedara *et al*., 2014). Rats were given unlimited access to water and food (*ad libitum*) following administration of STZ. Three days after receiving STZ injections, blood samples through the tail vein were collected, and the FBG concentration was assessed using an Accu-check glucometer. Rats were declared diabetic and chosen for the study if their FBG levels were higher than or equal to 250 mg/dL.

3.9.6 Experimental Design for the *in Vivo* Antidiabetic Study

After DM induction, thirty-six rats were divided into six groups, each with six animals. Throughout the course of the investigation, the groupings persisted. Solvent fractions and crude SM extract were dissolved in corn oil prior to administration.

Group 1: Normal control rats fed rat pellets and distilled water

Group 2: Diabetic control rats (DC) fed rat pellets and distilled water

Group 3: diabetic rats given 300 mg/kg SME

Group 4: Diabetic rats given 300 mg/kg EA

Group 5: Diabetic rats given 300 mg/kg BUT

Group 6: Diabetic rats given 1 mg/kg GLIB

Throughout the course of the treatment, weekly weight checks and FBG tests were performed. Every day, animals received doses via an oral intragastric tube. The animals were fed SME, EA, BUT, and GLIB orally for 28 days. The experiment with overnight-starved rats came to an end on day 28.

3.9.7 Evaluating the Animals for Diabetic Symptoms

Each animal spent the final week of the experimental period in a metabolic cage. To evaluate the animal's diabetic symptoms and examine the efficacy of the medication, measurements of food intake, water intake, and excreted urine volume were taken. Centrifuge tubes were used to collect urine (Shim *et al.,* 2007).

Table 3.2: Experimental Design for *in Vivo* Antidiabetic Study

Group	Treatment
1	Normal control
2	Diabetic control (1 dose of STZ (50 mg/kg) administered
3	Diabetic rats administered 300 mg/kg SME
4	Diabetic rats administered 300 mg/kg EA
5	Diabetic rats administered 300 mg/kg BUT
6	Diabetic rats administered 1 mg/kg GLIB

3.9.8 Oral Glucose Tolerance Test

On the 28th day, overnight-fasted rats' fasting blood glucose levels were assessed prior to the animals being sacrificed via the tail vein. Following glucose administration (1000 mg/kg), the animals' postprandial blood sugar levels were assessed from a vein in the tail at 0, 15, 30, 60, and 120 minutes after consumption. ACCU-Check glucometer was used to measure the glucose levels (Du Vigneaud and Karr, 1925). The glycemic index can be determined using this measured glucose concentration level.

The glycemic index was determined by taking record of the initial and final blood glucose levels, using the formula below:

$$\text{G. I. (\%)} = \frac{Initial\ (diabetic\ control) - Final\ (Treatment\ group)}{Final\ (Treatment\ group)} \times 100 \qquad 3.17$$

G. I. = Glycemic Index

3.9.9 The Survival Rates

Prior to the animals being sacrificed, survival rate at the end of the trial was computed applying ratio of the number of rats that survived to that point to the total number of rats used in the trial (Shim *et al.,* 2007).

3.9.10 Animal Sacrifice

Employing cervical dislocation, rats were sacrificed on day 28 while being given a mild anaesthetia (sodium pentobarbitone). Blood was drawn after the abdomen was ripped open. Samples were frozen before being utilised for various biochemical analyses after being centrifuged at 3000 × g for ten minutes in order to separate the plasma from blood cells. Organs such as kidneys, pancreas, and liver were harvested, rinsed with cold saline three times, and blotting dry, weighing, storing in plain tubes, fixing in formalin, and storing in the freezer until they were needed.

3.9.11 Serum Preparation

Blood obtained in plain bottle was allowed to clot by being left at temperature of the room unattended to for roughly 20 min. Then there was elimination of the clot via centrifugation at 1,000–3000 × g for ten minutes inside refrigerated centrifuge. Serum was the resultant supernatant, which was then retained in the freezer.

3.9.12 Preparation of Tissue Homogenate

0.1 M phosphate buffer (pH 7.4) was used in producing 10 % homogenate of washed excised tissues at 40 °C using a homogenizer equipped with a Teflon plunger. Then, using a cold centrifuge, the homogenates were spun for ten minutes at 10,000 × g. Using Bradford method (1976), the supernatants collected were utilised to determine the protein concentrations, as well as enzymes activities and other biochemical parameters. For histological analysis, 10 % formalin-preserved slices of the pancreas, liver, and kidneys were employed.

3.9.13 Body Weight Changes

At the commencement of the trial on day one, body weights of the numerous rats were recorded prior to medication delivery. The weights were recorded after the 28th day, when the experiment came to an end.

3.10 Biochemical Evaluations in Plasma and Serum Samples i*n Vivo* Assays

3.10.1 Determination of Alanine Transaminase

According to instructions in Randox Diagnostic Kit, the assay was evaluated by employing Reitman and Frankel (1957) colorimetric method.

Principle

This assay relies on the reaction between Ketoglutarate and alanine in the presence of ALT to form pyruvate and glutamate.

Materials and Reagents

1. R_1: 100 mmol. Phosphate buffer (pH 7.4), 200 mmol, alanine and 2 mmol. L-oxoglutarate, as supplied
2. R_2: 2 mmol. 2, 4 dinitrophenyl hydrazine as supplied.
3. Sodium Hydroxide 0.4 M (MW – 39.997 g/mol).
   1.6g of NaOH pellets were dissolved in distilled water (100,000 µl)

Procedure

Water bath was used to heat 0.1 mL of reagent (R1) for 1800 seconds at 37 ^{0}C after 100 µl of sample had been added. After 0.5 mL of reagent (R2) was added, the mixture was allowed to remain at temperature of the room for 1200 seconds. 5.0 mL of NaOH was then added, and after 300 seconds, a spectrophotometric reading at 540 nm was taken.

3.10.2 Determination of Aspartate Transaminase

According to the instructions in the Randox Diagnostic Kit, this was assessed using the Reitman and Frankel (1957) colorimetric approach.

Principle

This assay is a function of chemical interaction between aspartate and ketoglutarate to produce Oxalo-acetate and glutamate in the presence of AST.

Materials and Reagents

1. Reagent R_1:
   R_1: L – aspartate (100 mmol.), 100 mmol phosphate buffer (pH 7.4), 2 mmol L-Oxoglutarate, as supplied.
2. Reagent R_2:
   2, 4 dinitrophenyhydrazine (2 mmol.) as supplied.
3. 0.4 M NaOH (MW-39.997 g/mol.)
   100 ml of distilled water was used to dissolve 1.6 g of NaOH.

Procedure

100 µL of sample was mixed with 100 µL of reagent (R_1) and placed over water-bath for 1800 seconds at 37 ^{0}C. Subsequently, 500 µL of reagent (R_2) was introduced and allowed to stay at ambient temperature for 1200 seconds. After that, 50,000 µl of NaOH was added, and after 300 seconds, absorbance reading was taken at 505 nm.

3.10.3 Determination of Alkaline Phosphatase

Randox (Colorimetric) methods of Rec (1972) was employed

Principle

The ability of the sample's alkaline phosphatase to catalyse the hydrolysis of the colourless substrate P-nitrophenylphosphate in the presence of magnesium ions into the bright yellow-coloured stable product P-nitrophenol, which can be read at 405 nm, is what allows this reaction to take place. Diethanolamine was the buffer utilised.

P-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ Phosphate + P-nitrophenol.

The amount of alkaline phosphatase present in the sample has a direct relationship to the colour intensity that results.

Reagents

R1a: Buffer

Diethanolamine buffer	l mol/l, pH 9.8	
$MgCI_2$	0.5 mmol/l	as supplied

R1b: Substrate

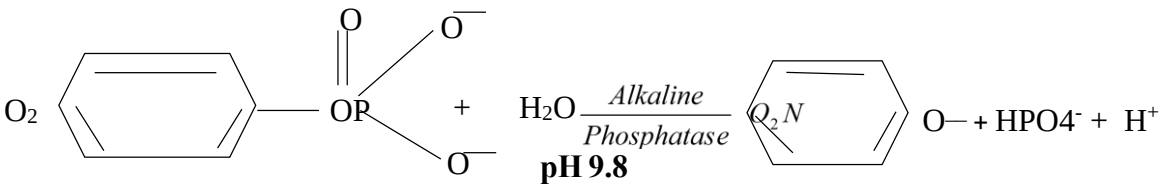
P-nitrophenylphosphate 10 mmol/1 as supplied

Reagent reconstitution: 50,000 µl of R1a was mixed 50,000 µl of R1b and ready for use

Procedure

The sample (10 µl) and reconstituted reagent (1000 µl) were mixed and shaken. 180 seconds were allowed for the reaction before spectrophotometric measurement at 405 nm was effected. The difference in absorbance was multiplied by 3300 to get the ALP activity (U/I).

$$U/I = 3300 \times \Delta A \text{ 405 nm/min}$$



3.10.4 Determination of Urea

This was determined colorimetrically using Weatherburn's (1967) technique, following Randox instruction.

Principle

Urea in serum is degraded to ammonia in the presence of enzyme urase. Following its interaction with phenol when hypochlorite is present (Berthelot reaction), ammonia produced is evaluated photometrically, to form indophenol (Blue complex), which absorbs most strongly at 546 nm.

$$\text{Urea} + H_2O \xrightarrow{Urease} 2NH_3 + CO2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \rightarrow \text{indophenol (Blue Compound)}$$

Reagents

R1a: EDTA (116 mmo1/1) as supplied

Urease (1 g/1) as supplied

R1b: Sodium nitropruside (6 mmo1/1)

R2: Phenol (diluted) (120 mmo1/1) as supplied

R3: Sodium hypochlorite (diluted) 27 mmo1/1 as supplied

Sodium hydroxide (NaOH) 0.14 N as supplied

Reagent Reconstitution

Reagent R1a (1000 µL) was added to R1b (3700 µL) to make

R_1 and mix gently, then ready for use.

Reagent 2 (11000 µl) was diluted with 6000 microlitres of distilled water and mix. Reagent 3 (2200 µL) was diluted with 75000 µL of distilled water, mix thoroughly

Procedure

10 µL of sample and 100 µL of R_1 were thoroughly mixed then incubated at 37 ^{0}C for 600 seconds. Following addition of 2.5 mL of R3 and 2.5 mL of R2 in rapid succession, once again at 37 ^{0}C the reaction was incubated for 2700 seconds. Then spectrophotometric reading was recorded at 546 nm.

3.10.5 Determination of Creatinine

This was evaluated colorimerically using the Bartels and Bohmer (1972) approach, following Randox instruction.

Creatinine-picrate complex, also known as Janovski complex (creatinine alkaline picrate), is created when creatinine interacts with picric acid in an alkaline medium (Jaffe's reaction). There is direct proportionallty between the intensity of the complex produced and the content of creatinine.

Reagents

R1a: Picric Acid 35 mmo1/1 as supplied

R1b: Sodium Hydroxide 0.32 mo1/1 as supplied

Reagent Reconstitution

Reagent (2000 µL) of R_1a and (2,000 µL) of R_1b were thoroughly mixed together in readiness for use.

Procedure

The sample (0.1 mL) and 1000 L of the reconstituted reagent were added and properly mixed together. Every 30 seconds for 120 seconds, spectrophotometric readings, at 492 nm were taken. Following, is the equation for determination of creatinine concentration:

$$\frac{\Delta \text{A Sample}}{\Delta \text{A standard}} \times \text{Standard Concentration (mg/dl)} = \text{Creatiine(mg/dl)} \qquad 3.18$$

3.10.6 Determination of Cholesterol

Allain *et al.,* 1974 Randox (Enzymatic) approach was applied.

To determine cholesterol, the procedure involves enzymatic hydrolysis and oxidation. As a result of reaction between 4-aminoantipyrine and hydrogen peroxide, indicator quinoneimine is produced in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow[esterase]{Cholesterol} Cholesterol + Fatty\,acids$$

$$\text{Cholesterol} + O_2 \xrightarrow[Oxidase]{Cholesterol} Cholestene-3-one - H_2O_2$$

$$\text{4-Aminoantipyrine } 2H_2O_2 + \text{Phenol} \xrightarrow{Peroxidae} quinonei\min e + 4H_2O$$

Reagents

R_1: Pipes Buffer 80 mmo1/1, p^H 6.8

4-Aminoantipyrine 0.25 mmo1/1

Phenol 6 mmo1/1

Peroxidase ≥ 0.5 u/ml

Cholesterol esterase ≥ 0.15 u/ml

Cholesterol oxidase ≥ 0.10 u/ml

All reagents were supplied

Procedure

Reagent R1 (1000 µl) was added to the sample (10 µl) and thoroughly mixed. After incubating at 37 °C, for five minutes, spectrophotometric reading was obtained within an hour, at 546 nm

Calculation

Cholesterol was calculated as below:

$$\text{Cholesterol concentration in sample} = \frac{\Delta\,\text{A Sample}}{\Delta\,\text{AStandard}} \times Conc.of\ Std \qquad 3.19$$

3.10.7 Determination of High Density Lipoprotein (HDL- CH)

This was determined by employing precipitation approach of Friedewald *et al*., (1972), following Randox instruction.

Principle

Precipitation procedures utilising various reagents are typically used to reevaluate plasma or serum HDL-C content. Chylomicron fractions and Low density lipoproteins (LDL and VLDL) precipitate quantitatively when phosphotungstic acid is applied in

the presence of magnesium ions. High Density Lipoprotein, or HDL, is identified by centrifuging the cholesterol fraction that remains in the clear supernatant.

Reagents

R_1: Phosphotungstic Acid 0.55 mmo1/1

Magnesium Chloride 25 mmo1/1

Procedure

Addition of 400 µl of the sample to 1000 µl precipitan (R1) (phosphotungstic acid and magnesium chloride) was effected then properly mixed, stirred, then kept at temperature of the roon for ten minutes. Following that, there was centrifugation of the reaction at 4000 rpm for five minutes. There was separation of clear supernatant and amount of cholesterol in it was calculated. Absorbances of test sample and that of standard were measured at 546 nm respectively.

Calculation

(1) Concentration of HDL Cholesterol in supernatant =

$$\frac{\Delta \text{ A Sample}}{\Delta \text{ AStandard}} \times \text{Conc. of Standard} \qquad 3.20$$

(2) Concentration of LDL

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{2.2} - \text{HDL Cholesterol} \quad \text{in mmo1/1.} \qquad 3.21$$

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL Cholesterol} \quad \text{in mg/dL} \qquad 3.22$$

3.10.8 Determination of Albumin

Grant *et al*. (1987) was employed following Randox technique.

Principle

The anionic dye-indicator Bromocresol green BCG (3, 3', 5, 5' - tetrabromo-m cresol sulphonephthalein) changes colour from yellow-green and green-blue at acidic P^H and its quantitative or specific binding to serum albumin determines the quantity of albumin that is available in the sample. Maximum absorption by albumin-BCG complex occurs at 578 nm. Amount of albumin present in sample is directly related to how intense the colour that results.

$$\text{BCG + Albumin} \xrightarrow{PH\,4.2} BCG - Albu\,\text{min}\ Complex$$

Reagents

R_1: BCG Concentrate

Succinate Buffer 75 mmo1/1; p^H 4.2

Bromocresol green 1.7 mmo1/1

Reagent Reconstitution

1 bottle of R1 can be used after being diluted with 87 mL of distilled water.

3000 µl of diluted of R1 (the BCG reagent) was added to 10 µl of sample, and then mixed thoroughly. Five minutes of the reaction were spent incubating it at 25 ^{0}C. At 578 nm maximum absorption of albumin-BCG complex occurred. . In comparison to blank, absorbances of sample and that of standard were measured. At room temperature, colour is stable for an hour.

Calculation

$$\text{Albumin Conc. (g/dL)} = \frac{\text{A Sample}}{\text{A Standard}} \times \text{conc. of standard} \qquad 3.23$$

$$= x \text{g/dL}$$

3.10.9 Determination of Total Bilirubin

Randox (colorimetric) method, developed by Jendrassik and Grof in 1938, was used to determine this.

Principle

The principle of this approach is based on the interaction of direct (conjugated) bilirubin with diazotized sulphanilic acid to produce blue-colored complex in an alkaline medium. Total bilirubin is measured by the reaction of diazotized sulphanilic acid in the presence of caffeine, which releases albumin-bound bilirubin.

Reagents

R_1:	Sulphanilic acid	29 mmol/l
	Hydrochloric acid	0.17 N
R_2:	Sodium Nitrite	38.5 mmol/l
R_3:	Caffeine	0.26 mol/l
	Sodium Benzoate	0.52 mol/l
R_4:	Tartrate	0.93 mol/l
	Sodium Hydroxide	1.9 N

All reagents are ready for use.

Procedure

Total bilirubin

After adding 200 µL of R1, 50 µL of R2, and 1000 µL of R3 to 200 µL of sample, it was appropriately mixed. After the reaction had stood for ten minutes at 25 ^{0}C, R4 was added, mixed, and given twenty minutes to stand at 25 ^{0}C before absorbance at 578 nm was measured.

Calculation

Total Bilirubin (mg/dL) = 10.8 × A_{TB} (578 nm)

Procedure: Direct Bilirubin

Sample was mixed after adding R1, R2, and 0.9 % NaCL then, after standing for ten minutes at 25 ^{0}C, absorbance was measured at 546 nm.

Calculation:

Direct Bilirubin (mg/dL) = 14.4 × A_{DB}

3.10.10 Total protein

Randox (Bi-uret technique) method developed by Weichselbaum, 1946 was used to determine this.

Principle

In an alkaline solution, the interaction of cupric ions with the protein peptide will result in the formation of a colourful complex.

Reagents

R_1:	Biuret Reagent	
	Sodium hydroxide	100 mmol/l
	Na-K tartrate	16 mmol/l
	Potassium Iodide	15 mmol/l
	Cupric sulphate	6 mmol/l
R_2:	Blank reagent	
	Sodium Hydroxide	100 mmol/l
	Na-K tartrate	16 mmol/l

Reagent Reconstitution

Contents of bottle R1 were diluted with 400 mL of distilled water while 400 mL of distilled water was utilised in diluting the content of bottle R2.

Procedure

Diluted R1 (1.0 mL) was added to the sample (0.02 mL), which was then properly mixed before being incubated for 30 minutes at 25 ^{0}C. Reagent's absorbance was read then used to compare the samples (A sample) and standards (A standard) absorbances. At 546 nm, coloured complex absorbed maximally.

Calculation

$$\text{Total Protein} = \frac{\text{A Sample}}{\text{A standard}} \times \text{Standard Concentration} \qquad 3.24$$

3.10.11 Glucose Determination

Barham and Trinder (1972), Randox (colorimetric) approach was used to estimate this.

Principle

After enzymatic oxidation and with glucose oxidase being present, glucose is measured. When phenol and 4-aminophenazone react with hydrogen peroxide under the catalysis of peroxidase, a red-violet quinoneimine dye is produced that can be used as an indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4 - \text{aminophenazone} + \text{phenol} \xrightarrow{GOD} \text{quinoneimine} + 4H_2O$$

Reagents

R_1a:	Buffer	
	Phosphate Buffer	0.1 mol/l, p^H 7.0
	Phenol	11 mmol/l as supplied.
R_1b:	GOD PAP Reagent	
	4-aminophenazone	0.77 mmol/l
	Glucose oxidase	≥ 1.5 K u/l
	Peroxidase	≥1.5 Ku/l

Reconstitution of Reagents

A portion of R1a was used to rebuild one through of R1b, and the entire contents were then transferred to R1a.

Principle

To the sample (10 µL) was added 1000 µL of R_1, mixed together and incubated at 37 ^{0}C for ten minutes. The absorbance was measured at 546 nm, within 1 hr.

Calculation

$$\text{Glucose concentration (mg/dL)} = \frac{\text{A Sample}}{\text{A Standard}} \times \text{Standard Conc (mg/dL)} \quad ..3.25$$

3.10.12 Determination of Glycated Heamoglobin

This was evaluated using Diatek (immunoturbidimetric) method according to Trivelli *et al.,* 1971.

Principle

Direct estimation of the HbA1C in whole EDTA blood is accomplished using a technique that depends on the interaction of antibody and antigen. As HbA1C from the test samples is absorbed on the latex particles and reacts with Anti-HbA1C (antigen-antibody reaction), agglutination results. In hemolyzed blood, total haemoglobin and HbA1C bind to the latex particles with equal affinity. Mouse antihuman HbA1C monoclonal antibody causes the formation of a latex-Hb A1C-mouse antihuman HbA1C antibody complex (R2). Monoclonal antibodies are used to identify HbA1C. Polyclonal antibodies can agglutinate the particles when they are directed against monoclonal antibodies, and the turbidity that results is measured spectrophotometrically. Absorbance is a measurement of agglutination. A calibration curve yields the HbA1C value.

Reagents

Reagent 1 (R_1) latex reagent
Latex 0.13 %
Buffer
Stabilizer Sodium azide 0.95 g/L

Reagent 2 (R_2) HbA1C Antibodies
Buffer.
Mouse anti-human HbA1C monoclonal antibody 0.05 mg/mL
Goat anti-mouse IgG polyclonal antibody 0.08 mg/dL
Stabilizers

Reagent 3 (R_3) Hemolysing reagent
Water and stabilizers

All Reagents are Ready to Use

Sample collection: Use fresh EDTA blood

Preparation of Heamolysate.

Test tubes containing samples, calibrators, and controls received 0.5 ml of hemolysate reagent. Well-mixed 10 µl of whole blood was added to this. After thoroughly blending it, it was left to stand for 5 minutes to complete the lysis.

Procedure

Reagent Blank: Reagent 1 (375 µl) and reagent 2 (125 µl) were mixed together.

1. A cuvette was filled with reagent 1 (375 µl). After adding 10 µl of hemolysate (consisting of samples, calibrators, and controls), carefully shaking it, and incubating it for five minutes.
2. Following that, reagent 2 (125 µl) was added, thoroughly mixed, and incubated for five minutes at 37 °C.
3. The measurement of absorbance was recorded at wavelength 600 mm (590 - 670 nm) against reagent blank.
4. Calibration curve was plotted and concentration of samples calculated thereof. Hitachi 9.7 autoanalyser was used.

3.10.13 Determination of Potassium

The evaluation was conducted using the Randox (colorimetric) approach as described by Berry *et al*. (1988).

Principle

Potassium is assessed enzymatically through the utilisation of potassium-dependent pyruvate activity, with phosphoenolpyruvate as the substrate. When lactate dehydrogenase (LDH) is present, the pyruvate molecule produced will undergo a reaction with nicotinamide adenine dinucleotide (NADH), resulting in the production of lactate and the regeneration of nicotinamide adenine dinucleotide (NAD+). Proportionate relationship exists between reduction in absorbance at 340 nm and concentration of potassium.

$$PEP + ADP \xrightarrow[PK]{K^t} Pyruvate + ATP$$

$$Pyruvate + {}^{LDH}NaDH + H^+ \rightarrow Lactate + NAD^+$$

Reagents:

R_1: Buffer/Enzymes/Substrate

Tris Buffer 250 mmol/l; pH 8.2

Cryptand 12 mmol/l

PEP ≥ 3.3 mmol/l

ADP ≥ 3.15 mmol/l

α - Oxogluterate ≥ 1.2 mmol/l

NADH ≥ 0.35 mmol/l

GLDH ≥ 11 u/ml

PK ≥ 1.2 u/ml

R_2: Enzyme/Diluent

LDH ≥ 65 u/ml

Reconstitution of Reagents

R_1**:** Contents of 1 via of enzyme/substrate R1b was in a part of buffer R1a, then the whole contents were transferred to buffer R1a.

R_2**:** Content of 1 via enzyme R2b was dissolved in a part of diluent R2a, then the whole contents were transferred to diluent R2a.

Procedure

All reagents were mixed together as explained in the principle. The absorbance was then measured at 340 nm and the value obtained is directly proportioned to the concentration of potassium.

3.10.14 Determination of Sodium

The evaluation of this was conducted using the Randox (enzyme colorimetric) approach as described by Berry *et al.*, 1988.

Principle

The evaluation of sodium is conducted enzymatically through the measurement of sodium-dependent β-galactosidase activity using ONPG as the substrate. The absorbance of the compound 0-nitrophenyl at a wavelength of 405 nm exhibits a direct relationship with the quantity of sodium.

$$\text{ONPG} \xrightarrow[\text{galactosidase}]{Na^+} \textit{galactose}$$

ONPG = O – nitrophenyl β-D-galactopyranose

Reagents

R_1: Buffer/Enzymes

Tris buffer 450 mmol/l; pH 9.0

Cryptand 5.4 mmol/l

β - Galactosidase ≥ 0.8 u/ml

R_2: Diluent/Substrate

Tris buffer 10.0 mmol/l

O-nitrophenyl galactoside 5.5 mmol/l

Reconstitution of Reagents

R_1: One bottle of buffer R1a was transferred to one bottle of enzyme R1b and dissolved completely by swirling gently. The entire contents were transferred to diluent R2a.

Procedure

All the reagents were mixed together as explained in the principle and the absorbance was measured at 405 nm. The value obtained corresponds to the concentration of sodium.

3.11 Haematological Parameters in Blood

The haematological parameters were evaluated using Mindray BC – 5300 hematology auto analyser.

Principle of Detection

The analyzer employs the coulter principle to ascertain the quantity and volume distribution of white blood cells, basophils, red blood cells, and platelets. Colorimetry is employed to quantify the concentration of haemoglobin. The device integrates three widely used technologies, specifically laser scatter, flow cytometry, and chemical dye, in order to provide a dependable and precise differentiation of white blood cells into five distinct parts.

Reagents

(1) Diluent (2) LEO I Lyse (3) LEO 11 Lyse (4) LH Lyse (5) 20 μL of blood sample.

3.12 Biochemical Parameters in Tissues

3.12.1 Determination of Protein Concentration

According to Gornal *et al*. (1949) description, with a small modification, the Bradford technique (1976) was used to measure the protein concentration of liver, kidney, and pancreatic homogenates using Bovine Serum Albumin (BSA) as standard.

Principle

The Brilliant Blue G dye (Coomassie blue) and proteins in solution must form a complex in order for this reaction to happen. In an acidic environment, the dye has the

capacity to attach to proteins. A shift in the dye's maximum absorption wavelength from 465 to 495 nm is brought about by the protein-dye combination. A standard curve with known protein concentrations is created, and the protein concentration is calculated by extrapolating the absorbance values of the samples. The amount of protein present is proportional to the absortion value.

Reagents

1. Bradford reagent.
2. In one litre of distilled water, 100 mg of Coomassie Blue, 50 mL of 85 % ethanol, and 100 mL of 85 % phosphoric acid were combined.
3. Stock of Bovine Serum Albumin (BSA).

A stock solution of 1 mg/mL was created by dissolving 0.01 g of BSA in 10 mL of distilled water.

Standard Bovine Serum Albumin Curve for Bradford Assay

After making serial dilutions of the stock solution, which contains 0.1671 mg – 1 mg of protein per millilitre, 1.4 mL of the Bradford reagent was added to 30 µl of each protein standard solution in the test tubes. After 300 seconds of the mixture remaining at room temperature, the absorbance of the resultant solution was measured with a spectrophotometer at 595 nm. The optical densities were plotted against the concentration of the BSA protein.

Table 3.3: Protocol for Protein Standard Curve

Test Tube No	1	2	3	4	5	6
Stock BSA (µL)	5	10	15	20	25	30
Phosphate Buffer (µL)	25	20	15	10	5	0
Bradford Reagent (mL)	5	5	5	5	5	5
BSA Conc. (mg/mL)	0.167	0.333	0.500	0.667	0.833	1.00
Absorbance at 595 nm	0.163	0.329	0.496	0.663	0.829	0.996

Procedure for the Determination ff Protein in The Sample

In a cuvette, 1.4 mL of the reagent and 30 µl of the homogenate were mixed. A blank was created using 1.4 mL of the homogenate and 30 µl of distilled water. The samples were read at 595 nm by spectrophotometer. Concentration of protein was calculated using BSA standard calibration graph constructed when optical densities were plotted against the protein concentrations.

Estimation of the amount of protein present in homogenate:

To lower the protein content in the supernatant fractions to a level within the detection range of Bradford reagent, homogenates of the tissues from kidneys, liver, and pancreas were diluted with distilled water. The protein concentration of each sample was calculated by extrapolating from a standard curve made using BSA, and actual protein concentration in the samples was then calculated by multiplying the result by the dilution factor.

3.12.2 Assessment of Lipid Peroxidation Generation

The method employed to assess lipid peroxidation was that described by Farombi *et al.,* 2000. On the test samples, quantification of thiobarbituric acid reactive compounds formed was carried out.

Principle

It has been discovered that malondialdehyde (MDA), a byproduct of lipid peroxidation, interact with thiobarbituric acid to generate a pink chromophore that absorbs at 532 nm. The method depends on the interaction of MDA, a result of lipid peroxidation, and 2-thiobarbituric acid (TBA). When 2-thiobarbituric acid (TBA), a chromogenic reagent, is combined with MDA formed by peroxidation of fatty acid membranes and food products, an acidic complex that is pink in color and absorbs most strongly at 532 nm is created on heating. This pink chromophore is easily extracted into butanol and other organic solvents. Since MDA is frequently used to calibrate this test, the results are presented as the amount of free MDA generated.

Reagents

1. 30 % Trichloroacetic Acid (TCA) – (mol. wt.- 163.39 g/mol)
   Small quantity of distilled water was used in dissolving 15 g of TCA; then 50 mL of distilled water was then introduced.
2. 0.75 % Thiobarbituric Acid (TBA) – (mol. wt. 144.15 g/mol.)

0.375 g of TBA was dissolved in 0.1 M of HCL and the solution was made up to 50 mL by employing same. Stirring in a hot water bath (50 ^{0}C) helped the substance to dissolve.

3. 0.15 M Tris-KCL (p^H 7.4) – (mol. wt. Tris 121.1 g/moL; mol. wt. KCL 74.6 g/mol.)

   Distilled water was used to dissolve 0.559 g of KCL and 0.909 g of Tris Base before being diluted to make 50 mL. The p^H was changed to 7.4 after that.

4. 0.1 M Hydrochloric acid – (mol. wt. 36.458 g/mol.).

   Distilled water was mixed with 13 µl of concentrated HCI (36.5–38 %) before the volume was increased to 15 mL.

Procedure

100 microliters sample aliquot was combined with 400 microliters of Tris-KCI buffer, 125 microliters of 30 % TCA, 125 microliters of 0.75 % TBA, and for 45 minutes at 80 degrees Celsius was placed inside water bath. This solution was then centrifuged at 3,000 rpm for ten minutes after being chilled in ice to room temperature. Using a spectra-max plate reader, absorbance of the collected clear supernatant was measured at 532 nm against a reference blank of distilled water.

Calculation

According to Adam-Vizi and Seregi (1982), MDA level was estimated applying a extinction coefficient of 0.156 $\mu M^{-1\text{-}cm\text{-}1.}$

Lipid peroxidation (nmole MDA/mg protein) =

$$\frac{Absorbance \times Volume\ of\ \ mixture}{^{E}532nm \times Volume\ of\ \ sample \times mg\ \ protein/ml} = \text{mmol/mg protein} \qquad 3.26$$

3.12.3 Determination of Nitric Oxide Generation Level

Using the approach developed by Green *et al*. (1982), the level of NO activity was determined. As a measure of nitric oxide (NO) generation, tissue nitrite (NO_2-) was estimated. According to Schulz *et al*. (1999), quantification was based on the Griess reaction.

Principle

The Griess reagent's ability to react with nitrite and produce a coloured azo-dye product that absorbs most effectively at 550 nm is essential to the technique.

A brief or transient diazonium salt will be produced when the nitrite in the tissue homogenate reacts with a diazotizing agent, such as sulfanilamide, in an acidic medium. The formation of a stable azo compound will subsequently be accomplished by allowing this salt interreacts with the coupling agent N-naphthyl-ethylenediamine.

Sulfanilamide and N-naphthyl-ethylenediamine can be combined in an acidic medium first, preceeding reaction with nitrite. This product's bright pink-purple tint will give way to a nitrite test with great sensitivity that may be utilised in measuring nitrite concentration as low as 0.5 μM level. The sample's nitrite content has a linear relationship with adduct's absorbance at 550 nm. Serum concentrations of NO^{3-} and NO^{2-}, which quickly recombine in aqueous solution to generate their stable oxidative metabolites (NO^{3}- and NO^{2}-) (Palmer *et al.,* 1987), are thought to be a measure of NO generation.

Preparation of Reagents

1. 0.1% N-(1-napthyl) ethylenediamine dihydrochloride-(mol. wt. 259.17 g/mol). A modest amount of distilled water was used to dissolve 0.1 g of 0.1% N-(1-napthyl) ethylenediamine dihydrochloride and then made up to 100 mL.
2. 5% Phosphoric acid – (mol. wt. 97.994 g/mol)
   95 mL of distilled water was mixed with 5 mL of concentrated phosphoric acid.
3. 1 % sulphanilamide – (mol. wt. 172.2 g/mol).
   100 mL of 5 % phosphoric acid was used to dissolve 1 g of sulphanilamide. By combining equal quantities of solutions 1 and 3 (1:1), Griess reagent was produced.
4. 20 mmol/L sodium nitrite – (mol. wt. 68.9953 g/mol.)
   In a little amount of distilled water, 0.138 g of sodium nitrite ($NaNO_2$) was dissolved and then made to 100 mL.

Table 3.4: Calibration of Sodium Nitrite Standard Curve

$NaNO_2$ (μmoL)	0.0	0.2	0.4	0.6	0.8	1.0
$NaNO_2$ (μmoL)	0.0	0.1	0.2	0.3	0.4	0.5
Distilled water (mL)	1.0	0.9	0.8	0.7	0.6	0.5
Griess reagent	1.0	1.0	1.0	1.0	1.0	1.0
Absorbance (540 nm)	0.00	0.077	0.112	0.166	0.255	1.215

Procedure

In accordance with Griess protocol (Green *et al.,* 1982), nitrite amount in serum or supernatant was determined by incubating 50 microlitres of the homogenate with 50 microlitres of Griess reagent [0.1% N-(1-naphtyl) ethylenediamine dihydrochloride, 1 % sulfanilamide in 5 % phosphoric acid; 1:1] kept at temperature of the room for twenty minutes. Utilising a spectra - max plate reader, absorbance at 550 nm was read. The sample's OD 550 was compared to the known sodium nitrite content in a standard solution to estimate the nitrite concentration. Distilled water was used to dilute stock solution of 20 mmol/L $NaNO_2$ in order to create calibration curve at various concentrations.

3.12.4 Determination of Hydrogen Peroxide Concentration

Hydrogen peroxide level was measured by using the protocol of Wolff, (1994).

Principle

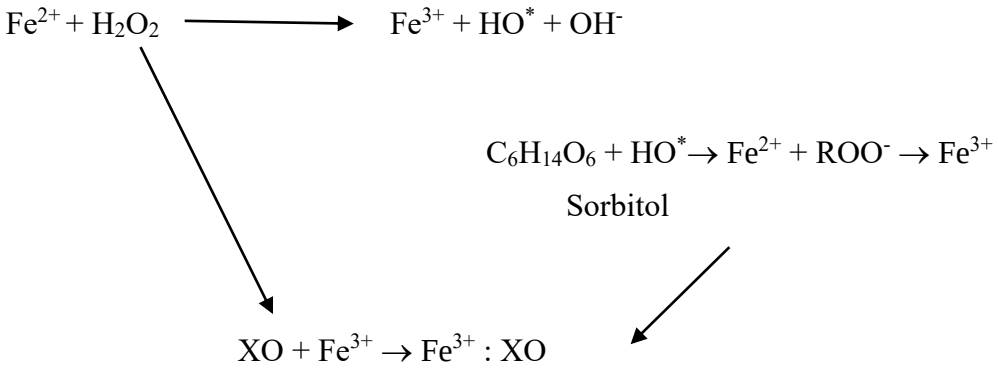
This technique is a function of peroxides (ROOH, where R= H in the case of hydrogen peroxides) oxidaising ferrous ions (Fe^{2+}) to give ferric ions (Fe^{3+}). This assay depends majorly on the capability of hydrogen peroxide oxidaising ferrous ions to ferric ions. Hydrogen peroxide will oxidise iron (Fe II) in an acidic solution to iron (Fe III), and the latter iron (Fe III) will react with the indicator xylenol orange to generate a complex that is blue-purple and absorbs most efficiently at 560 nm.

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + HO^0 + OH^2$$

$$Fe^{3+} + XO \longrightarrow Fe^{3+} : XO$$

Sorbitol addition starts a chain reaction with the hydroxyl radical that is produced thereby increasing Fe (III) yield. This increases the response per H_2O_2 molecule, making the technique more sensitive.

Hydroxyl radicals interact with sorbitol to generate extra Fe^{3+} for the color-producing reaction with xylenol orange, enhancing colour intensity depending on sample homogenate content.



Preparation of Reagents

1. 100 µmol/L xylenol orange – (mol. wt. 760.6 g/mol.)
   A little amount of distilled water was used to dissolve 0.0152 g of xylenol orange, which was then increased to 200,000 µl
2. 250 µmol/L Ammonium Ferrous Sulphate (mol. wt. 392.14 g/mol.)
   196 mg of Ammonium ferrous sulphate was dissolved in a little amount of distilled water and later increased to 200,000 µl.
3. 100 mm/L sorbitol (mol. wt. 182.2 g/mol)
   In a little amount of distilled water, 3.64 g of sorbitol was dissolved, and 200 mL of the mixture was then created.
4. 250 mmol/L H_2SO_4
   Using distilled water, 1000 µl of 1 M H_2SO_4 was diluted and then increased to 40,000 µl.
5. 0.1 M Phosphate Buffer ($_P{}^H$ 7.4)
   9.73 g of potassium dihydrogen phosphate and 4.96 g of dipotassium hydrogen phosphate were dissolved in a little amount of distilled water and then increased to mark in one litre flask and P^H was appropriated to 7.4.
6. FOXl reagent (100,000 µl) = 10 mM xylenol orange + 10,000 µl sorbitol + 50,000 µl AFS + 30,000 µl distilled water

Procedure

Samples followed norms like standard. 30 µl sample homogenate was combined with 170 µl FOXl reagent then properly mixed and kept at the temperature of the room for 30 minutes incubation. Then; spectra-max plate reader was employed to measure the absorbance at 560 nm. H_2O_2 calibration graph was employed in determining peroxide levels.

3.12.5 Estimation of Glutathione S-transferase Activity

Activity of glutathione s-transferase was determined using Habig *et al*. (1974) technique. The GST test measures total GST activity in plasma and crude cell or tissue extracts. The process works by conjugating 1-chloro, 2,4-dinitrobenzene (CDNB) to glutathione's thiol groups, which causes increase in absorbance at 340 nm.

Principle

Through the reduced glutathione's thiol group, GST catalyses the conjugation of L-glutathione to CDNB. The assay is based on the idea that all glutathione s-transferase isozymes are known to display noticeably high levels of activity when given 1-chloro-2-4-dinitrobenzene (CDNB) as the second substrate.

$$GSH + CDNB \xrightarrow{GST} GS - DNB \text{ Conjugages} + HCl$$

When thiol group of the reduced glutathione is conjugated to CDNB substrate, the rate of increase in absorbance at 340 nm, which is directly connected with the GST activity in the sample, increases.

Reagents

1. 0.2 M GSH
   Dissolve 12 mg L-glutathione reduce (GSH) in 0.1 mL ice cold distilled water.
2. 0.1 M phosphate buffer, p^H 6.5
3. 20 mM CDNB

Procedure

The table below displays how the estimating medium was created. Reaction was allowed to proceed for 180 seconds, and microplat reader was employed in collecting readings every 30 seconds against a blank at 340 nm.

Table 3.5 Estimation of Glutathione S-Transferase Activity Preparation

Reagent	Blank	Sample
CDNB (20 mM)	10 µL	10 µL
0.1 M phosphate buffer pH 6.5 (20,000 µl)	170 µL	170 µL
500 µl GSH		
10,500 µl Distilled water		
Sample		20 µL

Calculations

The extinction co-efficient of CDNB conjugates at 340 nm = 9.6 mM^{-1} cm^{-1}

Calculating change in absorbance

$$\Delta A_{340} / \min = \frac{A_{340}(\text{final reading}) - A_{340}(\text{Initial Reading})}{\text{Reduction time (min)}} \qquad 3.27$$

Subtract the ΔA_{340}/min. of blank from ΔA_{340}/min. sample. Use the rates to calculate the GST specific activity as:

$$GST\ activity = \frac{\Delta A_{340} / \min \times \text{reaction volume} \times \text{dilution factor}}{9.6 \times \text{sample volume} \times \text{mg protein/mL}} \qquad 3.28$$

= μmole/min/mg protein

Path length for 96 well plate = 0.6 cm

3.12.6 Estimation of Reduced Glutathione (GSH) Level

Method outlined by Beutler *et al.* (1963) was being used in measuring reduced glutathione level.

Principle

Most non-protein sulfhydryl groups are reduced glutathione.

In the microplate reader test method, glutathione (GSH) was oxidaised by 5, 5^1-dithio-bis (2-nitrobenzoic acid) (DTNB) sulfhydryl reagent to produce TNB, the yellow derivative detectable at 412 nm. Ellman's reagent, 5', 5^1-dithios - (2 nitrobenzoic acid) - DNTB, can form a relatively stable yellow complex (chromophoric product) with molar absorption at 412 nm on interaction with reduced glutathione. GSH concentration in sample is directly proportional to absorbance of complex at 412 nm.

$$R - S - S - R + GSH \rightarrow R - S^- + GSS^-$$

$R - S^-$ = yellow complex

Reagents

1. Glutathione working standard

   100 mL of 100 mM phosphate buffer with a pH of 7.4 was used to dissolve 40 g of GSH.

2. 100 mM phosphate buffer (pH 7.4)

   (a) 7.1628 g of $Na_2HPO_4.12H_2O$ were dissolved in 200,000 μl of distilled water to create 0.1 M $Na_2HPO_4.12H_2O$ (mol. wt. 358.22 g/mol).

   (b) 1, 5603 mg of $NaH_2PO_4.2H_2O$ was dissolved in 100,000 μl of distilled water to create 0.1 M $NaH_2PO_4.2H_2O$ (mol. wt. 156.03 g/mol). After combining

solutions (a) and (b), 0.1M phosphate buffer was prepared and pH was then set to 7.4.

2. DTNB, Ellman's reagent.

   40 g of DTNB in 100 mM phosphate buffer was increased to 100,000 µl to yield this reagent.

3. Precipitating solution

   4,000 mg of sulphosalicyclic acid was dissolved in 100,000 µl of distilled water to create 4% sulphosalicyclic acid ($C_7H_6O_5S.2H_20$; mol. wt. 254.22 g/mol.).

Procedure

The table shows how GSH functional standards were serially diluted.

Table 3.6: Protocol for GSH Standard Curve

Stock (mL)	Phosphate Buffer (mL)	Ellman's Reagent (mL)	Absorbance (412 nm)	GSH conc. (μg/mL)
0.02	0.48	4.50	0.040	8
0.05	0.45	4.50	0.101	20
0.10	0.40	4.50	0.194	40
0.20	0.30	4.50	0.380	80
0.30	0.20	4.50	0.572	120
0.40	0.10	4.50	0.749	160

To each part, 4.5 mL of DTNB was added. GSH correlated with absorbance at 412 nm. Because the colour developed cannot be stable after that time, the readings were recorded within 10 to 15 minutes of administering Ellman's reagent. Each sample was made in three copies. The optical density was plotted against concentration in a graph.

Determination of GSH Concentration in Samples

80 microlitres of precipitating solution were added to 80 microlitres of the homogenate sample in an eppendorf tube, which was later vortexed and centrifuged at 4,000 rpm for five minutes. In a 96-well microplate, 150 microlitres of Ellman's reagent was mixed with 50 microlitres of the supernatant. Spectramax plate reader was employed in measuring reaction mixture's absorbance at 412 nm in comparison to reagent blank.

3.12.7 Glutathione Peroxidase Activity

Method developed by Rotruct *et al*. (1973) was utilised in measuring glutathione peroxidase (GPx) activity.

By monitoring GSSG formation by coupling to the glutathione reductase-catalyzed process, or by measuring ROOH or GSH consumption at regular intervals, one can assess the glutathione peroxidase activity in biological tissues.

Principle

Glutathione perioxidase (GPx), as part of the mechanism in which it lowers cumene hydroperoxide, oxidises GSH to create GSSG, which forms the basis of this reaction. The specialised mechanism exhausts NADPH after glutathione reductase (GR) converts the GSSG to GSH. When NADPH is detected at 340 nm, GPx activity decreases proportionally. Hydrogen peroxide (H_2O_2) and a range of organic hydro peroxides are both substrates for Se-dependent GSH peroxidase. A set amount of time is given to glutathione peroxidase to conjugate hydrogen peroxide to glutathione before the process is halted. The outstanding GSH will interact with DTNB and GSH consumed indicates enzyme activity.

$$2\ GSH + H_2O_2 \rightarrow GS - GS + 2\ H_2O$$

Reagents

1. Sodium Azide (mol. wt. 65.009 g/mol.)
   10 mL of pure water was used to dissolve 0.65 milligrammes of sodium azide.
2. 20 mL of 100 mM phosphate buffer (pH 7.4) was used to make up the final volume after 0.0245 g of GSH (mol. wt. 307. 33 g/mol.) was dissolved in it.

3. Hydrogen peroxide (0.0025 M)

   2.8 µL of 30% hydrogen peroxide was added to distilled water and volume increased to 10 mL using distilled water.

4. Trichloroacetic acid (10%)

   By dissolving 3.0 g of TCA in distilled water, a volume of 30 mL was created.

5. Dipotassium hydrogen orthophosphate (mol. wt. 174.2 g/mol.)

   A small amount of distilled water was utilised to dissolve 3.666 g of K_2HPO_4 and then made to a 55 mL solution.

6. Ellman's reagent (DTNB)

   11.088 mg of DTNB reagent was dissolved into 100 mM phosphate buffer with a P^H of 7.4, and then made up to 28 mL.

7. Phosphate buffer (100 mM, pH 7.4)

   In 90,000 µl of distilled water was added 1.36 g of potassium dihydrogen phosphate (mol. wt. 136.086 g/mol.) and 2.28 g of dipotassium phosphate trihydrate (mol.wt. 228.2 g/mol.) and volume made to 100 mL with same.

Procedure

In a test tube containing 50 µl of phosphate buffer, 30 µl of sodium azide, 60 µl of GSH, 30 µl of hydrogen peroxide was added 50 µl of glutathione peroxidase.

$$\text{Activity}\ (\text{u/mg}) = \frac{(\Delta E_{340\text{nm}}/\text{min})\,(\text{Total vol})\,(\text{Enzy.dil})\,(2)}{(G.22)\,(\text{Enz. vol})(\text{mg enz/mL})} \qquad 3.29$$

3.12.8 Determination of Superoxide Dismutase Activity

Activity of SOD was evaluated by employing Misra and Fridovich (1972) technique.

Principle

SOD's ability to halt adrenaline autoxidation drives the reaction. At p^H 10.2, SOD suppresses auto-oxidation, simplifying the enzyme assay. Superoxide radical (O^{2-}) oxidises epinephrine, forming adrenochrome. pH and epinephrine concentration increase adrenochrome synthesis per superoxide radical.

Reagents

1. A carbonate buffer solution with a concentration of 0.05 M and a p^H of 10.2.

   A total of 14.3 grammes of sodium carbonate decahydrate ($Na_2CO_3.10H_2O$) with a molar mass of 286.144 grammes per mole, and 4.2 grammes of sodium bicarbonate ($NaHCO_3$) with a molar mass of 84.007 grammes per mole, were dissolved in a small volume of distilled water and subsequently diluted to a

final volume of 1000 mL. The solution was modified to a pH of 10.2 using sodium hydroxide (NaOH).

2. 0.3 mM Epinephrine {(mol. wt. 183.204 g/mol.) ($C_9H_{13}NO_3$)}
   11mg of Epinephrine was dissolved in distilled water (200 mL)

Procedure

0.3 mL of 0.3 mM epinephrine and 2.5 mL of 0.05 M carbonate buffer (p^H 10.2) were added to 50 µl of tissue homogenate, mixed by inversion, and monitored for change in absorbance every 30 seconds for a total of 150 seconds at 480 nm. The sample and the water that replaced it were both placed in the same reference cuvette.

Calculation

$$\%\,\text{inhibition} = \frac{100 - (100 \times \text{increase in absorbance per min. for sample})}{\text{increase in absorbance per min. for blank}} \qquad 3.30$$

The amount of SOD required to achieve a 50% inhibition of the auto-oxidation of epinephrine was provided as 1 unit of SOD activity.

3.12.9 Determination of Catalase Activity

The Claiborne *et al*. (1985) technique was used to evaluate catalase activity. The catalase activity is measured by the H_2O_2 breakdown at 240 nm.

Principle

Hydrogen peroxide rapidly breaks down into water (H_2O) and oxygen (O_2).

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

As catalase breaks down hydrogen peroxide into water and oxygen, loss of absorbance is noticed at 240 nm. Despite not having an absorbance maximum at this wavelength, hydrogen peroxide's absorbance has a strong enough correlation with concentration to be used in a quantitative experiment. In Noble and Gibson's 1970 study, they employed extinction co-efficient of 0.0436 mM^{-1} cm^{-1}.

Reagents

1. 50 mM phosphate buffer (p^H 7.0)
   1.42 g of Na_2HPO_4 (mol. wt. 141.958 g/mol.) and 1.56 g of $NaH_2PO_4.2H_2O$ (mol. wt. 156.008 g/mol.) were dissolved in distilled water (200 mL) and standardized with HCL/water.

2. 19 mM H_2O_2

Procedure

2,950 µl of 19 mM hydrogen peroxide and 50 µl of the tissue homogenate were pipetted into 1 cm quartz cuvette. The mixture was placed inside a spectrophotometer, following quick invertion of the liquid to mix it. For 150 seconds, absorbance change was read at 240 nm every 30 seconds.

Calculation

$$\text{Catalase activity} = \frac{\Delta A_{240} / \text{min.} \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{mg protein/mL}} \qquad 3.31$$

$$= \mu\text{mole } H_2O_2/\text{min/mg protein}$$

Catalase activity was measured in moles of H_2O_2 degraded per minute per milligramme of protein.

3.13 Histopathological examinations were carried out according to the procedures described by Avwioro, (2002).

3.13.1 Gross Processing

Gross processing, or "grossing," is the process of preparing pathology specimens for forthcoming microscopic analysis through cutting and tissue sample as well as through plain eye examination to get diagnostic data. Inspecting, describing, measuring, inkling, and sectioning specimens for diagnostic processing are the activities.In this study, tissues were evaluated, sliced into 4 mm pieces, placed in pre-labeled cassettes, and fixed in 100% formal saline for 24 hours.

3.13.2 Tissue Processing

Tissue preparation involves four steps to prepare tissues for paraffin embedding. This is accomplished in order of fixation, dehydration, clearing, and infiltration.

3.13.4 Fixation – This has to do with stabilizing tissue protein in order to prevent changes such as decay.

3.13.5 Dehydration- This is about removal of water from the tissue.

3.13.6 Clearing- This concerns removal of dehydrants from the tissue in readiness for infiltration.

3.13.7 Paraffin Infiltration

This study processed tissues automatically using the Moderl Leica TP 1020. To dehydrate the tissues, 10% formal saline at stations 1 and 2 and alcohol at stations 3 to 7 in varied concentrations (absolute I and absolute II) were used. After two xylene

cleanings at stations 8 and 9, the tissues were infiltrated and impregnated in three wax baths. The machine's 12-hour programming lets tissues stay in each station for an hour. This study processed tissues automatically using the Moderl Leica TP 1020. To dehydrate the tissues, 10% formal saline at stations 1 and 2 and alcohol at stations 3 to 7 in varied concentrations (absolute I and absolute II) were used. After two xylene cleanings at stations 8 and 9, the tissues were infiltrated and impregnated in three wax baths. The machine's 12-hour programming lets tissues stay in each station for an hour.

3.13.8 Embedding

This method employs a mould to implant tissues or specimens. Because of their thinness, tissue blocks need a supporting medium.

A semi-automatic tissue embedding centre applied paraffin wax as solid support medium to every treated tissue in this study. After burying and orienting the tissue in molten paraffin wax, it was dispensed into a metal mould, filled with a pre-labeled cassette, and transported to a cold plate to harden. Removed from the moulds was the tissue block.

3.13.9 Microtomy

Cutting tissue into thin slices bridges the gap between sample collection and microscopic analysis (www.labce.com).

This study's embedding blocks were sliced with a 6 μm rotary microtome to reveal the tissue surface. Before sectioning, surfaces can chill on the ice. There was division of tissues at 4 micrometres (ribbon section).

3.13.10 Floating

This is about an organ or one of its pieces being moved out of place or being abnormally mobile.

This investigation floated sections on a Raymond lamb water bath at 600 c for one hour.

3.13.11 Drying and Staining

This approach enhances microscopic sample contrast.

This study dyed slides with Haematoxylin and Eosin then dried them on a Raymond Lamb hot plate at 600 c for an hour. A microscope inspected the specimens.

3.14 Chromatography

Laboratory chromatography separates mixtures into their components by passing them through a medium in which the components are suspended or in solution and flow at different speeds. The fluid solvent, or mobile phase, transports the mixture through a stationary phase system. How the mixture separates depend on the percentage of each solute spread between the mobile phase and stationary phase. Mobile and stationary phases partitioning cause the separation. The gas and liquid chromatography are utilised when mobile phase is gas or liquid, respectively.

3.14.1 Types of Chromatography

Gas chromatography

Gel permeation chromatography

Column chromatography

Thin layer chromatography (TLC)

Paper chromatography

Affinity chromatography

High pressure liquid chromatography (HPLC)

Ion exchange chromatography

Vacuum liquid chromatography

(Hardwood and Mody, 1989; Encyclopedia Britannica)

3.14.2 Gas Chromatography-Mass Spectrometry

This analysis identifies test sample compounds. It analyses plant extracts and solvent fractions.

Separation techniques are the GC's guiding principle.

Compound identification is based on molecular mass, molecular structure, and computed fragments.

Principle

Separation techniques have both stationary and mobile stages. Mobile phase is the carrier gas- Helium 99.99 Purity, while stationary phase is the column. Extract and solvent fractions that have previously been packaged in via bottles are placed in the sample compartment before being injected into the column that performs the separation, where they are separated into various components at various retention times. The column is filled with extract and solvents at a temperature of 250 ^{0}C. MS applies molar mass and structure to the MZ (Mass to Charge Ratio) spectrum.

GC-MS mass spectra are evaluated using the NIST database of over 62,000 patterns.

100 % credit for highest peak

All values over 3 % have a value assigned.

Agilent Technologies 7890 and an MS Agilent Technologies 5975 USA are the GC-MS models used in this study.

Identification of samples is normally carried out.

3.15 Spectroscopy

Spectroscopy examines and measures electromagnetic radiation-produced spectra. It deals with how matter affects how light and other radiation are absorbed and emitted. It involves dividing electromagnetic (light) radiation into its individual or constituent wavelengths (spectrum), much as how a prism divides light into a rainbow of colours.

3.15.1 Types of Spectroscopy

Infrared (IR) spectroscopy

Raman spectroscopy

Nuclear magnetic resonance spectroscopy

X-Ray spectroscopy

Ultraviolet – Visible (UV/VIS) spectroscopy

3.15.2 Broad Classification

Atomic absorption spectroscopy – AAS

Atomic fluorescene spectroscopy- AFS

Atomic emission spectroscopy – AES

AAS includes methods such as infrared (IR) spectroscopy and ultraviolet-visible (UV/VIS) Spectroscopy.

3.15.3 FT – IR Spectroscopy

Fourier Transform Infrared spectroscopy is a crucial analytical technique for researchers and has been developed as a tool for the simultaneous determination of organic constituents such as chemical bond, as well as organic content (including protein, carbohydrate and lipid). It is used for both organic and non-organic materials without causing damages to the sample.

It is a platform to identify functional groups.

Infrared (IR) spectrum is divided into 3 wave-number regions namely:

Far IR (< 400 cm^{-1})

Mid IR (400 – 4000 cm^{-1})

Near –IR spectrum, most widely utised in analysis of sample is separated into four min regions.

The four regions are as follows:

The single bond region (2500 – 4000 cm^{-1}) → O-H; N-H, CH

The triple bond region (2000 – 2500 cm^{-1}) → CΞC; CΞN

The double bond region (1,500 – 2000 cm^{-1}) → C = C; C=O, C = N

Principle

Samples are IR-exposed and the sample absorbs and transmits infrared light.

Procedure – A small amount of the sample is required. Take a little of the sample (extract) and put it inside the mortar and add potassium bromide to it and then blend them together properly. Then take a small portion of the blended material and put it inside sample holder and tighten it with both hands. Then lose it and it will give you a glass-like pellet. Hang the sample holder inside the infra-red spectrophotometer for scanning to give the spectrum. Substances that are not known can be identified by comparing the spectrum with reference from the library database. From there you obtain the functional groups.

The machine used for this work is Buck scientific model 530 infrared spectrophotometer.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Screening, Evaluation of Antioxidant and Nutritional Properties of *Solanum aacrocarpon*

4.1.1 Phytochemical Screening of Methanol Leaf Extract of *Solanum macrocarpon*

Table 4.1 reveals the phytochemicals that were present in the extract.

Table 4.1: Qualitative Phytochemical Screening of Methanol Extract of *Solanum macrocarpon* Linn.

TEST	CONTENT
SAPONINS	+++
PROTEIN	+
PHENOL	+
FLAVONOIDS	+++
ALKALOIDS	+++
STEROIDS	++
PHYTOSTEROLS	+
DITERPENES	+++
TANNINS	+++
ANTHRAQUINONE	++
TERPENOIDS	+

-Absent; + present; ++ moderately present; +++ highly present

4.1.2 Evaluation of the Nutritional Properties of *Solanum macrocarpon*

Table 4.2 reveals the proximate composition of the leaves of SM while tables 4.3 and 4.4 respectively indicate the micromineral and macromineral profiles. According to Kouame-Osnou *et al*., 2020, the leaves of SM provide nutrients useful for human well-being.

Table 4.2: Proximate Composition of *Solanum macrocarpon* Linn

Nutrients	**g/100 g**
Crude protein	21.73 ± 0.01
Ash	8.74 ± 0.00
Crude fat	3.87 ± 0.00
Crude fibre	16.77 ±0.00
Moisture content	10.61 ±1.35
Carbohydrate	38.28 ±0.05

Data are mean±SD

Table 4.3: Macromineral Profile of *Solanum macrocarpon* Linn

Macromineral	g/100 g
Ca	0.23±0.00
Mg	0.25±0.00
K	0.91±0.00
Na	0.30±0.00
P	0.37±0.01

Data are mean±SD

Table 4.4: Micromineral Profile of *Solanum macrocarpon* Linn

Micromineral	Ppm
Fe	173.66±0.03
Zn	46.73±1.75
Cu	10.13 ±0.29
Mn	25.79±0.20
Se	0.02±0.00

Data are mean±SD

4.1.3 Evaluation of the Antioxidant Properties of *Solanum macrocarpon*

Tables 4.5, 4.6, 4.7, and 4.8 reveal the activity of the extract via DPPH, metal chelating, α-glucosidase and α-amylase inhibition respectively. These assays verified that SM has the ability to act as an antioxidant and an anti-diabetic agent. It can be used to treat postprandial hyperglycemia because it inhibits the enzymes α-amylase and α-glucosidase.

Table 4.5: DPPH Scavenging Activity of *Solanum macrocarpon* Linn.

Concentration µg/ml	**(% inhibition)**
200	36.27
400	42.78
600	46.04
800	49.70
1000	53.53

Data are mean of three determinations

Table 4.6: Scavenging Activity of Nitric Oxide

Concentration µg/ml	% Inhibition
100	41.93
200	53.22
300	62.90
400	67.74
500	77.42

Data are mean±SD

Table 4.7: Metal Chelating Activity of *Solanum macrocarpon* Linn.

µg/ml	Citric Acid % inhibition	SM % inhibition
200	34.82±0.39	33.46±1.16
400	51.16±0.77	35.01±0.00
600	58.85±0.29	42.41±3.11
800	60.60±0.48	47.66±1.75
1000	66.14±1.75	48.83±0.00

Data are mean±SD

Table 4.8: Percentage Inhibition of Alpha-Glucosidase in *Solanum macrocarpon* Linn.

µg/mL	% inhibition
10	22.25± 5.11
20	23.97± 4.15
50	34.59± 5.43
100	37.63± 3.20
150	41.73± 5.25

Data are mean±SD

Table 4.9a: Percentage Inhibition of Alpha-Amylase in *Solanum macrocarpon* Linn.

µg/mL	% inhibition
10	33.11±1.33
20	34.98±2.12
50	49.58±2.56
100	52. 27±2.45
150	59.93±2.34

Data are mean±SD

Table 4.9b: Percentage Inhibition of α-Amylase and α-Glucosidase of Acarbose

S/N	Concentration µg/ml	% inhibition of α-amylase	% inhibition of α-glucosidase
1	10	35.50	24.25
2	20	40.20	31.97
3	50	63.92	51.72
4	100	105.20	77.50
5	150	120.62	85.41
6	IC_{50}	234.50	104.00

4.1.4 Phytochemical Screening of Solvent Fractions of *Solanum macrocarpon*

Tables 4.10, 4.11, 4.12, 4.13 reveal the phytochemical, DPPH, and bioactive compounds reports respectively while Figures 4.1, 4.2, 4.3, 4.4, and 4.5 show TPC, TFC, TAC, reducing power, and lipid peroxidation reports respectively.

Table 4.10: Phytochemical Profile of Crude Extract and Fractions

S/N	Constituent	Crude Extract	Hexane Fraction	Chloroform Fraction	Ethyl Acetate Fraction	Butanol Fraction	Methanol Fraction
1	Terpenoids	+	-	-	+	+	++
2	Steroids	+	++	++	+	+	-
3	Saponins	+++	-	-	+	+	++
4	Tannins	+++	-	-	+	+	++
5	Flavonoids	+++	+	-	+	+	-
6	Cardiac Glycosides	-	-	-	-	-	-
7	Anthraquinone	++	-	+	-	-	-
8	Alkaloids	+++	-	+	+	+	+
9	Phenols	-					
10	Proteins	+					
11	Phytosterols	+					
12	Diterpenes	+++					
13	Carbohydrate	-					

---: Absent; +: present; ++: present in high concentration; +++: present in abundance.

Table: 4.11 DPPH (%) Inhibition of Crude Extract and Solvent Fractions of *Solanum macrocarpon* Linn.

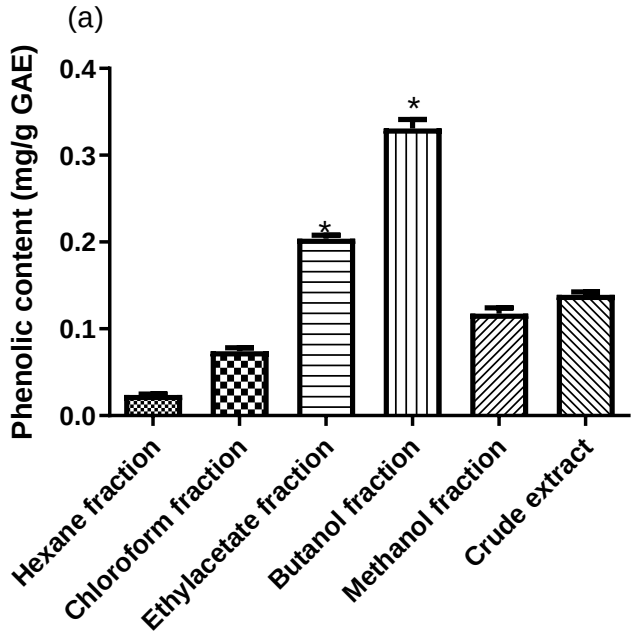
mg/mL	Ascorbic acid	Crude extract	Hexane fraction	Chloroform fraction	Ethyl acetatete fraction	Butanol fraction	Methanol fraction
0.2	94.97± 8.15	62.08±7.90*	53.28±9.78*	59.32±5.90*	60.73±9.84*	98.89±8.54	49.81±12.00*
0.4	95.05±10.98	80.91±9.76	54.63±5.32*	62.21±7.60*	81.62±8.90	94.09±10.00	70.63± 9.80*
0.6	95.18±10.31	94.28±11.29	61.25±9.65*	69.92±9.51*	90.23±8.69	94.47±12.00	94.15± 11.79
0.8	95.31±12.12	93.34±11.34	67.48±8.45*	84.89±9.76	92.54±13.00	94.05±3.54	95.57±12.60
1.0	95.37± 9.87	94.54±8.56	74.43±9.79*	87.79±9.76	92.61±9.85	95.82±10.00	96.14± 12.98

Data are mean±SD

*Significantly different ($p \leq 0.05$) when compared with the standard

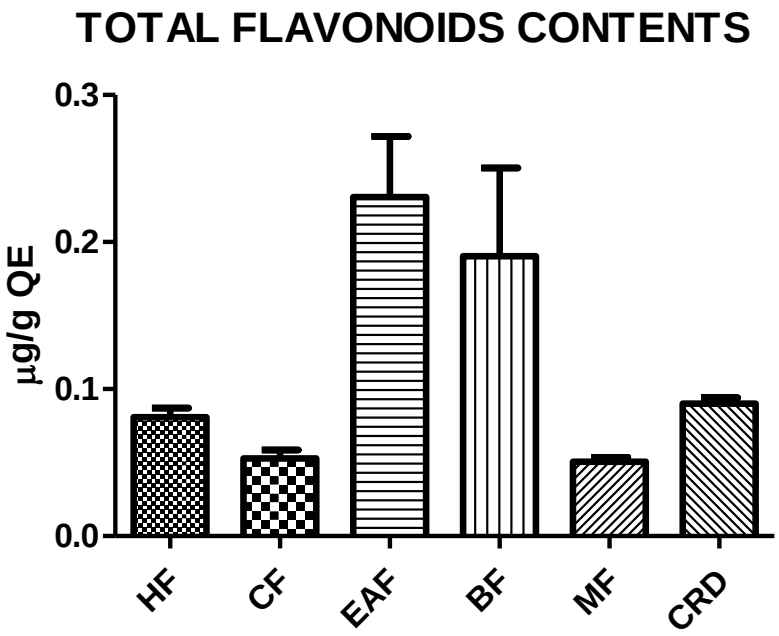
Table 4.12: IC_{50} Values

Product	IC_{50} Value (µg/ml)
Ascorbic Acid	428.2
Crude Extract	7.432
Hexane Fraction	6.651
Chloroform Fraction	3.225
Ethyl Acetate Fraction	0.04046
Butanol Fraction	1.033
Methanol Fraction	143.1



Solanum macrocapon **extract and fractions**

Figure 4.1: Total Phenolic Content of Crude and Solvent Fractions of *Solanum macrocarpon*
*Significantly different ($p \leq 0.05$) when compared with other groups

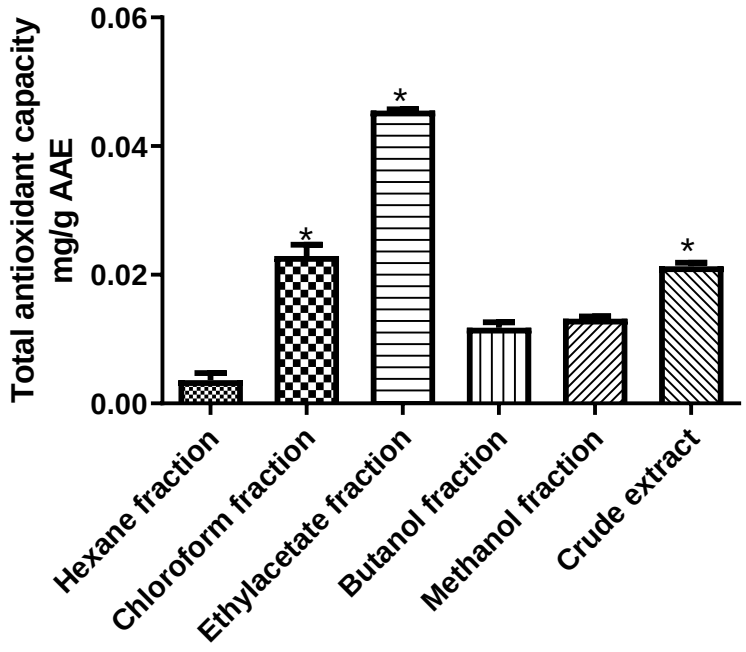


***Solanum macrocapon* extract and fractions**

Figure 4.2: Total Flavonoid Content of Crude and Solvent Fractions of *Solanum macrocarpon*

*Significantly different ($p \leq 0.05$) when compared with other groups

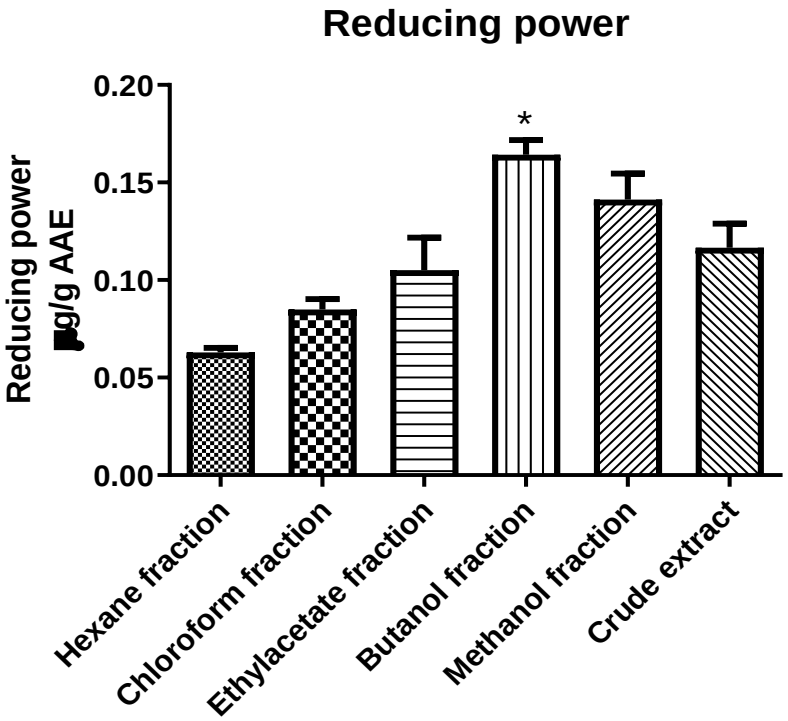
Key: HF: Hexane fraction; CF: Chloroform fraction; EAF: Ethyl actate fraction; BF: Butanol fraction; MF: Methanol fraction; and CRD: Crude extract.



Solanum macrocapon **extract and fractions**

Figure 4.3: Total Antioxidant Capacity of Crude and Solvent Fractions of *Solanum macrocarpon*

***** Significantly different ($p \leq 0.05$) when compared with other groups.



Solanum macrocapon **extract and fractions**

Figure 4.4: Reducing Power of Crude Extract and Solvent Fractions of *Solanum macrocarpon*

*****Significantly different ($p \leq 0.05$) when compared with other groups.

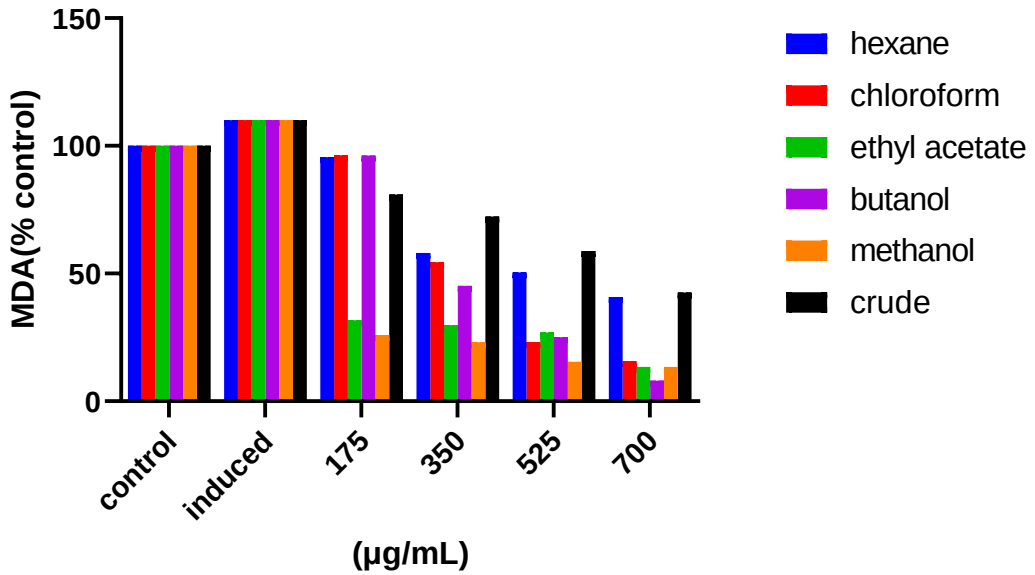


Figure 4.5: Lipid Peroxidation Inhibition of Methanol Extract of *Solanum macrocarpon* and Solvent Fractions

Table 4.13a: Bioactive Components of Methanol Extract of *Solanum macrocarpon* Linn.

Parameters	**Value (s)**
Phenolics (mg GAE/g)	0.042± 0.000
Flavonoids (mg QUE/g)	0.393±0.009
Total antioxidant capacity (mg AAE/g)	0.103± 0.000
Reducing power (mg AAE/g)	0.261± 0.001

Data are mean±SD

Table 4.13b: Extraction Yield of Different Fractions of Extract Including Crude

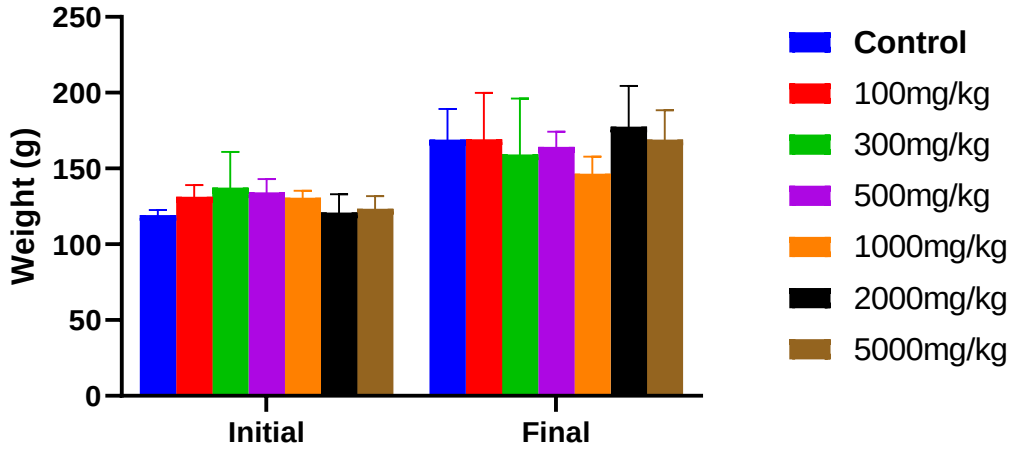
Plant Extract Fraction	Extraction Value%
Hexane	4.498
Chloroform	3.131
Ethyl Acetate	9.266
Butanol	10.78
Methanol	25.569
Crude	11.262

4.1.5 Acute Toxicity Evaluation of methanol extract of *solanum macrocarpon* Linn on rats

Table 4.12 reveals that there was no mortality, no morbidity or any strange behaviour from the animals during the experimental period while Tables 4.13, 4.14, and 4.15 indicate the normalcy of the haematological parameters, electrolytes status and lesion profile respectively. Figures 4.6, 4.7, 4.8, 4.9 and 4.10 reveal weight indifference, normal liver function test, bilirubin status, total protein level and photomicrograph of the pancreas respectively.

Table: 4.14: Acute Toxicity of *Solanum macrocarpon* Linn. Extract on Rats

Grouping	**Dose (mg/kg bw)** **Crude SM extract**	**Mortality/morbidity after 14 days**
Control	Distilled water	0/3
Group 1	100	0/3
Group 2	300	0/3
Group 3	500	0/3
Group 4	1000	0/3
Group 5	2000	0/3
Group 6	5000	0/3



***Solanum macrocapon* extract administration**

Figure 4.6: Body Weight (g) of *Solanum macrocarpon* Linn. Treated Rats
*Significantly different ($p \leq 0.05$) when compared with other groups.

Table 4.15: Hematological Parameters of Acute Toxicity Test

	PCV (%)	Hb (g/L)	RBC (10^6)	WBC (10^3)	PLT (10^5)	N (%)	L(%)	M(%)	Eos (%)	Bas (%)
Control	44.50±0.50	15.25±0.35	8.20±0.10	7.05±0.07	5.75±0.35	66.50±6.50	33.00±6.00	0.50	0.00	0.00
100 mg/kg	47.00±1.00	15.80±0.4	9.95±0.35*	8.88±0.38*	4.45±0.45*	59.50±1.50	39.50±0.50	1.00	0.00	0.00
300 mg/kg	44.00±1.00	14.65±0.25	8.33±0.13	7.60±0.6	4.85±1.15*	69.00±2.00	31.00±2.00	0.00	0.00	0.00
500 mg/kg	49.50±1.50*	16.35±0.45*	10.86±0.26*	9.03±0.13*	4.58±0.48*	61.00±2.00	39.00±2.00	0.00	0.00	0.00
1000 mg/kg	41.50±1.50*	14.10±0.40*	8.06±0.07	7.33±0.22	5.04±1.87*	66.50±2.50	33.00±2.00	0.00	0.50	0.00
2000 mg/kg	45.00±2.00	15.35±0.55	8.63±0.78	7.21±0.91	4.99±0.92	70.00±2.00	30.00±2.00	0.00	0.00	0.00
5000 mg/kg	36.50±1.50*	12.40±1.50*	6.96±0.76*	6.55±0.45	3.99±0.14*	63.50±1.50	35.50± 1.00	0.50	0.50	0.00

Data are mean±SD
*Significantly different ($p \leq 0.05$) when compared with the control

Table: 4.16: Electrolytes Status and Kidney Function Test of The Acute Toxicity Test

	Na^+(mmol/L)	K^+(mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)	Creatinine (g/dL)
Control	138.50±1.5	3.95±0.15	102.5±2.50	22.00±2.00	28.00±1.00	0.65±0.05
100 mg/kg	141.00±1.0	4.00±0.10	107.5±2.50*	21.00±1.00	28.50±2.50	0.65±0.65
300 mg/kg	138.50±2.5	3.85±0.15	102.5±2.50	24.50±0.50*	28.50±1.50	0.70±0.10
500 mg/kg	137.50±1.5	3.95±0.15	102.5±2.00	24.00±1.00	27.00±2.00	0.65±0.05
1000 mg/kg	141.50±1.5	4.00±0.20	107.5±1.50*	22.50±1.50	36.00±4.00*	0.85±0.05
2000 mg/kg	137.00±1.0	3.80±0.20	102.5±1.80	23.00±1.00	25.50±2.50	0.65±0.05
5000 mg/kg	136.00±1.0	3.65±0.25	102.5±1.70	25.00±1.00	20.00±1.00	0.50±0.05

Data are mean±SD
*Significantly different ($p \leq 0.05$) when compared with the control

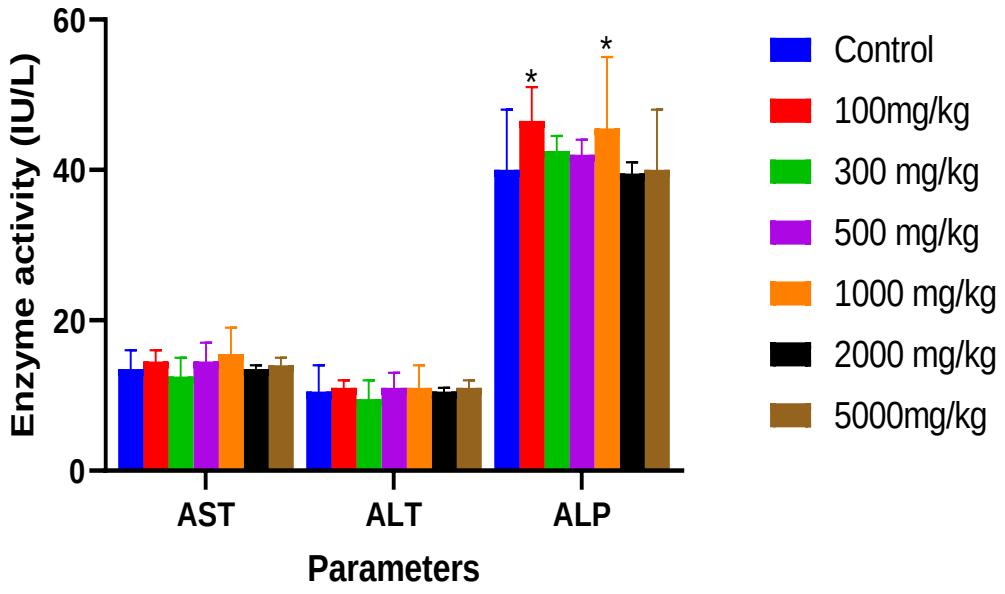


Figure: 4.7: Effect of *Solanum macrocarpon* Linn. on Hepatic Enzymes of Healthy Rats

*Significantly different ($p \leq 0.05$) when compared with the control

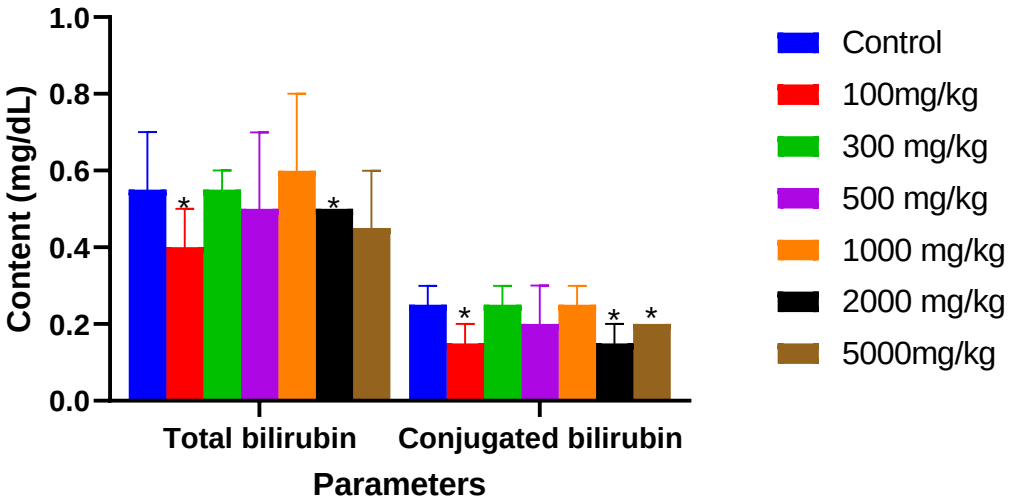


Figure: 4.8: Effect of *Solanum macrocarpon* Linn. on Total and Conjugated Bilirubin of Healthy Rats

*Significantly different ($p \leq 0.05$) when compared with the control

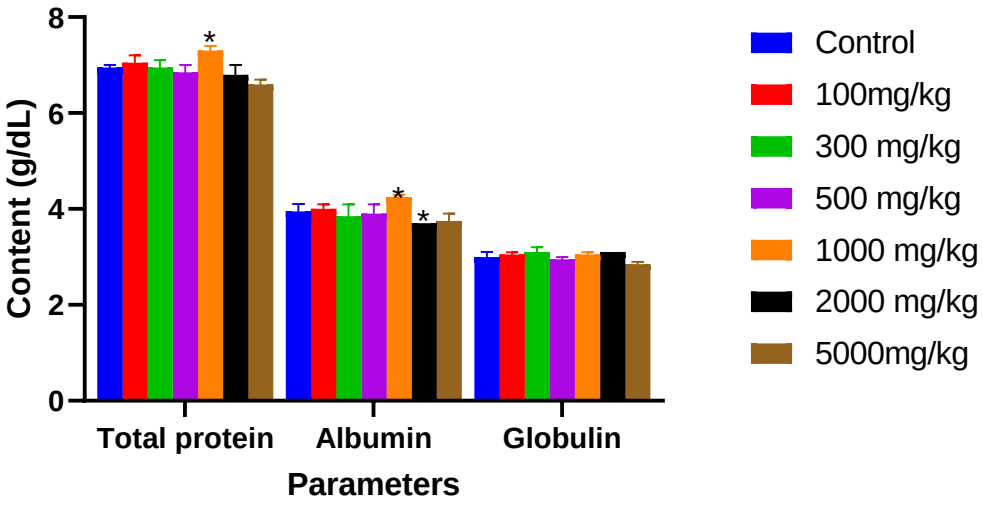


Figure: 4.9: Effect of *Solanum macrocarpon* Linn. on Total Protein, Albumin and Globulin of Healthy Rats

*Significantly different ($p \leq 0.05$) when compared with the control

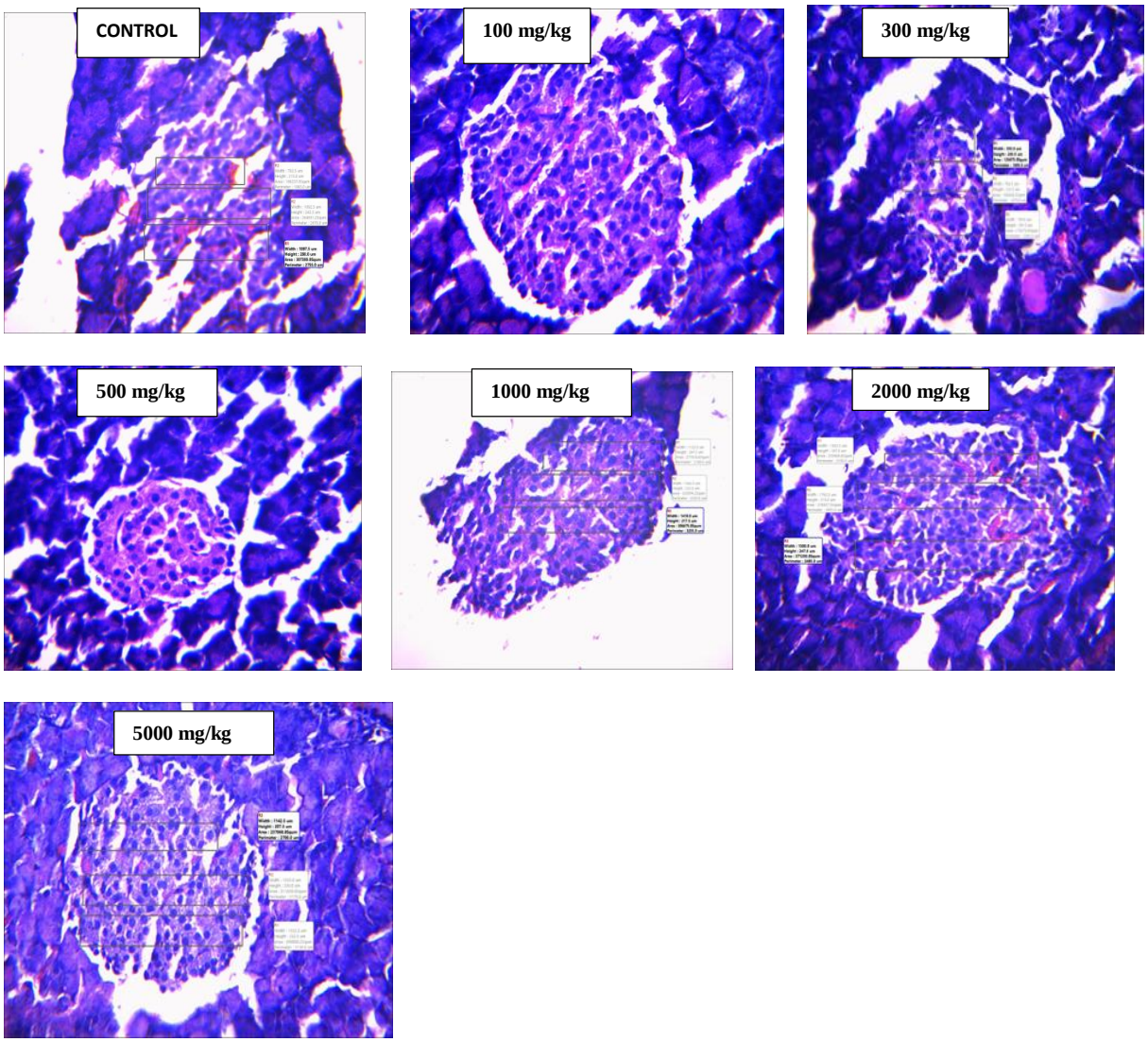


Figure 4.10: Photomicrograph of Pancreas of Healthy Rats Treated with *Solanum macrocarpon* Extract.

Table: 4.17: Lesion Profile of *Solanum macrocarpon* Linn. Extract Treated Rats

Grouping	Lesion	Others
Control	No visible lesion	
100 mg/kg bw	No visible lesion	Congestion of parenchyma
300 mg/kg bw	No visible lesion	Interstitial congestion
500 mg/kg bw	No visible lesion	Interstitial congestion
1000 mg/kg bw	No visible lesion	
2000 mg/kg bw	No visible lesion	
5000 mg/kg bw	No visible lesion	Congestion and oedema parenchyma

4.1.6 Antidiabetic Activity of *Solanum macrocarpon* Linn. and Solvent Fractions on Streptozotocin-induced Diabetic Rats

Table 4.18 shows that, when compared to diabetic control rats, EA considerably reduced blood glucose levels, and Table 4.19 demonstrates that, when compared to diabetic control rats, EA greatly improved body weight reduction. Figures 4.11, 4.12, and 4.13 show that when compared to the diabetic control rats, EA dramatically decreased feed intake, urine output, and water consumption. When compared to diabetic rats but not significantly different from other groups, Figure 4.14 of the OGTT test demonstrates that BUT considerably lowered the blood glucose level after 2 hours. Figure 4.15 shows that, when compared to the diabetic control, EA considerably lowered the level of glycated haemoglobin.

Table 4.18: Effect of *Solanum macrocarpon* Extract and Solvent Fractions on Blood Glucose (mg/dL) of Streptozotocin-Induced Diabetic Rats

Days	0	7	14	21	28
Control	90.67±12.42	89.50±7.89	90.07±8.06	89.00±6.00	88.33±6.88
DC	285.00±16.37[a]	385.85±23.50[a]	468.00±49.92[a]	454.00±36.40[a]	495.33±24.74[a]
SME	300.67±10.69[a]	270.30±18.50[ab]	250.07±23.69[ab]	203.33±35.56[ab]	179.00±10.00[ab]
EA	279.33±28.57[a]	240.15±15.50[ab]	205.33±16.50[ab]	160.00±10.00[ab]	114.00±8.18[ab]
BUT	274.33±15.01[a]	256.62±20.56[ab]	190.33±23.86[ab]	145.22±16.74[ab]	139.33±8.08[ab]
GLIB	268.67±8.02[a]	201.15±11.25[ab]	137.67±11.59[ab]	120.00±8.88[ab]	105.33±4.51[ab]

Data are mean±SD

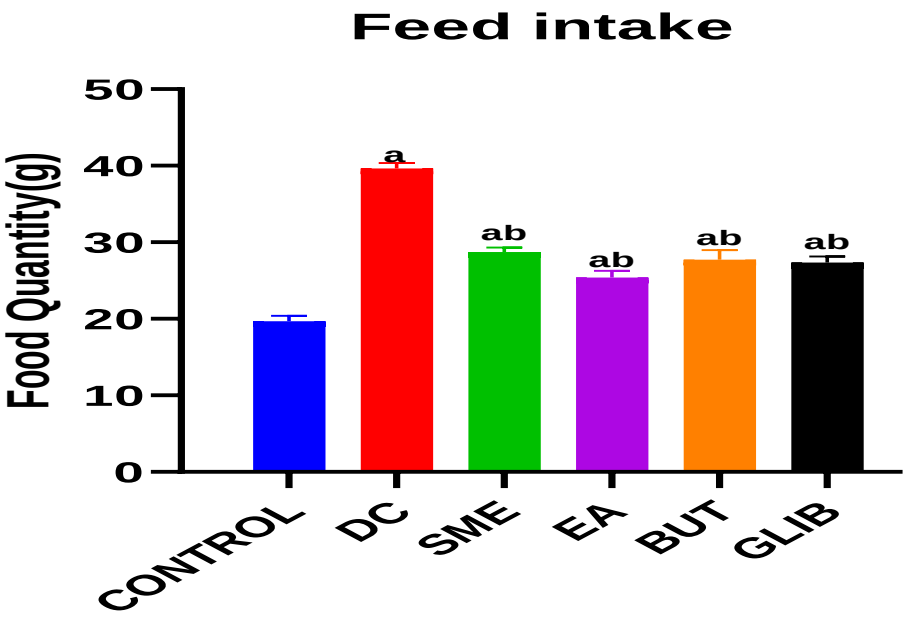
[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups

Table 4.19: Effect of *Solanum macrocarpon* Extract and Solvent Fractions on Weight (g) of Streptozotocin-Induced Diabetic Rats

	0	7	14	21	28	Survival Rate%
Control	140.56±3.45	153.00±6.25	168.67±2.51	188.00±8.71	203.33±8.79	100
DC	142.70±2.18	148.67±6.11[a]	151.00±6.24[a]	162.67±3.51[a]	171.67±2.08[a]	50.00
SME	145.15±3.10	154.67±2.30[b]	162.67±3.05[b]	176.00±3.00[ab]	187.00±5.00[ab]	83.30
EA	142.56±2.88	158.00±2.51[b]	166.00±3.60[b]	180.00±2.65[b]	194.00±3.01[ab]	83.30
BUT	140.12±3.10	153.67±3.51[b]	163.00±3.00[b]	172.67±3.15[b]	182.00±7.94[ab]	66.60
GLIB	139.80±3.99	154.67±7.57[b]	163.67±7.57[b]	173.33±5.51[b]	190.67±4.72[ab]	83.33

Data are mean±SD

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups

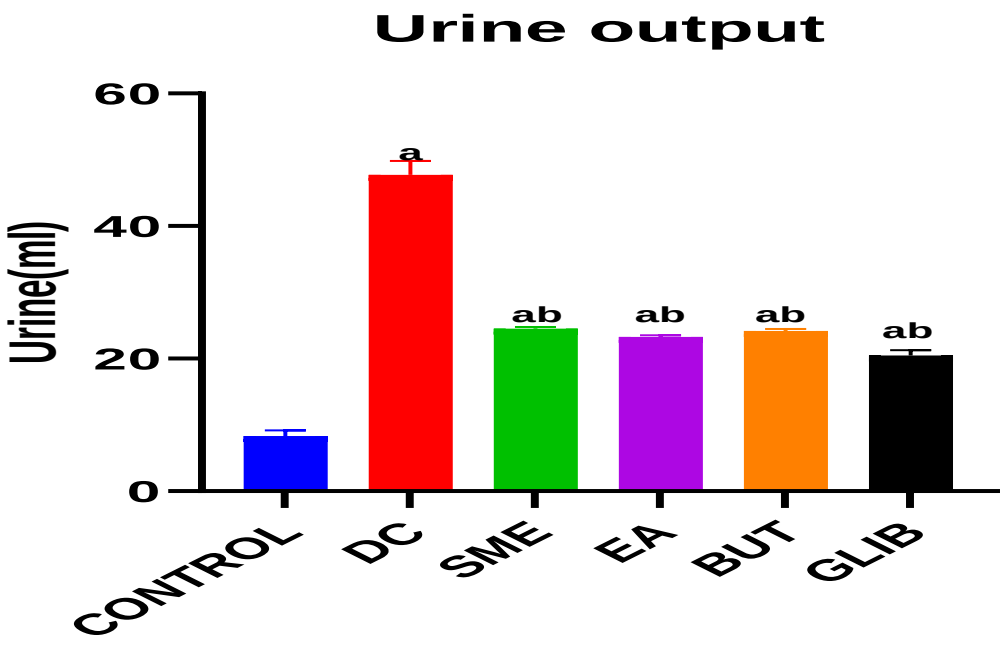


***Solanum macrocarpon* extract and fractions**

Figure 4.11: Effect of *Solanum macrocarpon* Linn Extract and Solvent Fractions on Feed Intake of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

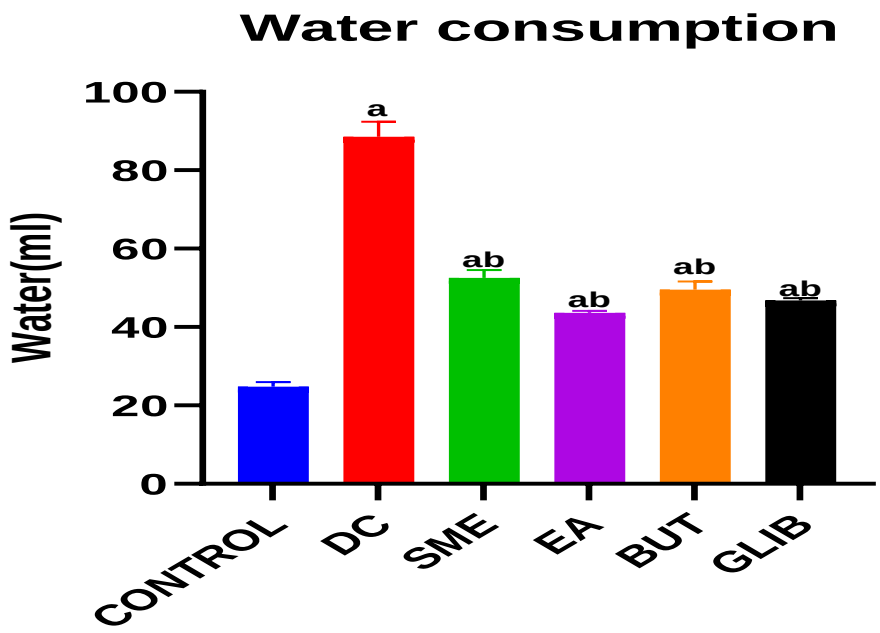


***Solanum macrocarpon* extract and fractions**

Figure 4.12: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Urine Output of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

Figure 4.13: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Water Consumption of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

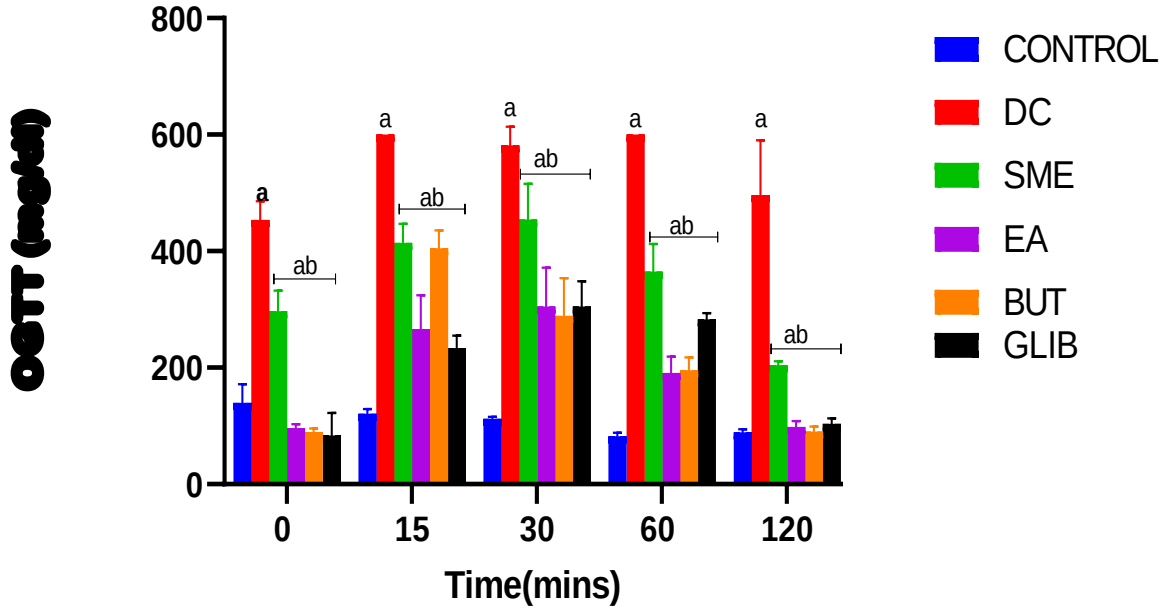
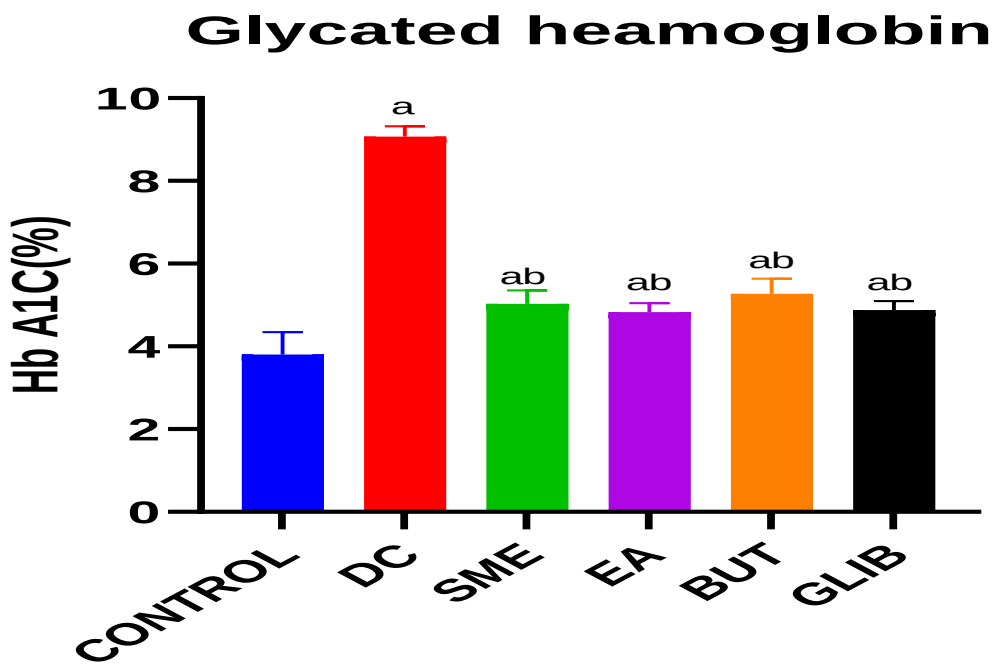


Figure 4.14: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on OGTT of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

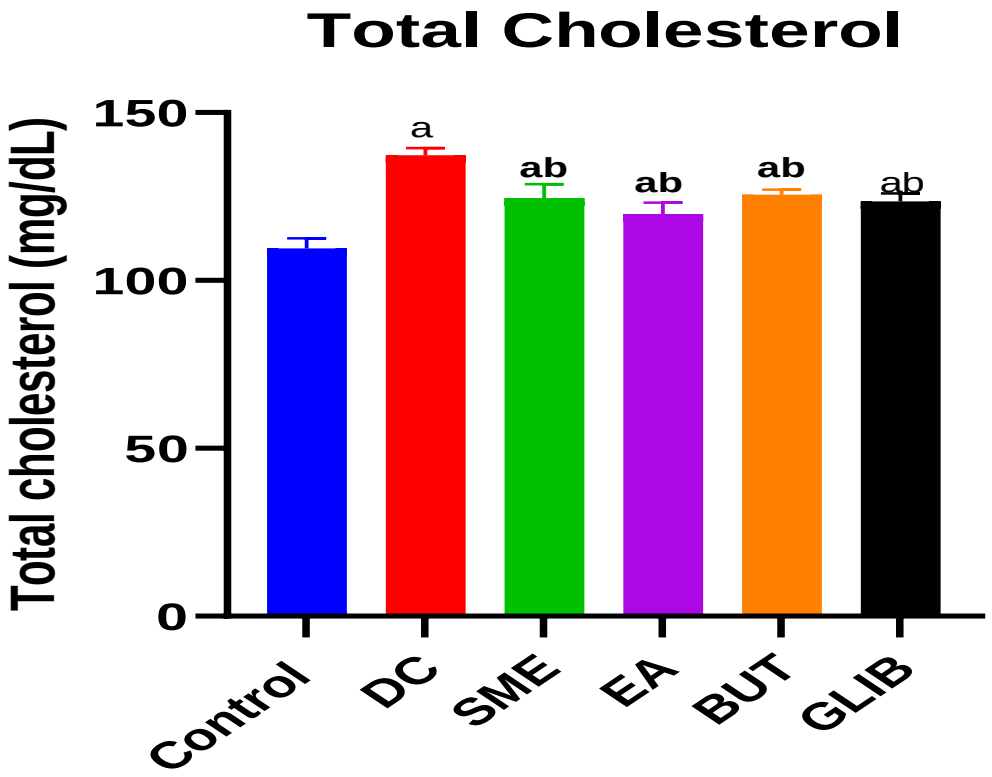
Figure 4.15: Effect of *Solanum macrocarpon* Extract and Solvent Fractions on Glycated Heamoglobin of Streptozotocin Induced Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

4.1.7 Effect of *Solanum macrocarpon* Linn Extract and Solvent Fractions on Lipid Profile on Streptozotocin Induced Diabetic Rats

Figures 4.16, 4.17, 4.18, 4.19 and 4.20 revealed that administration on EA modulated the aberrations in the lipid profiles of diabetic rats.

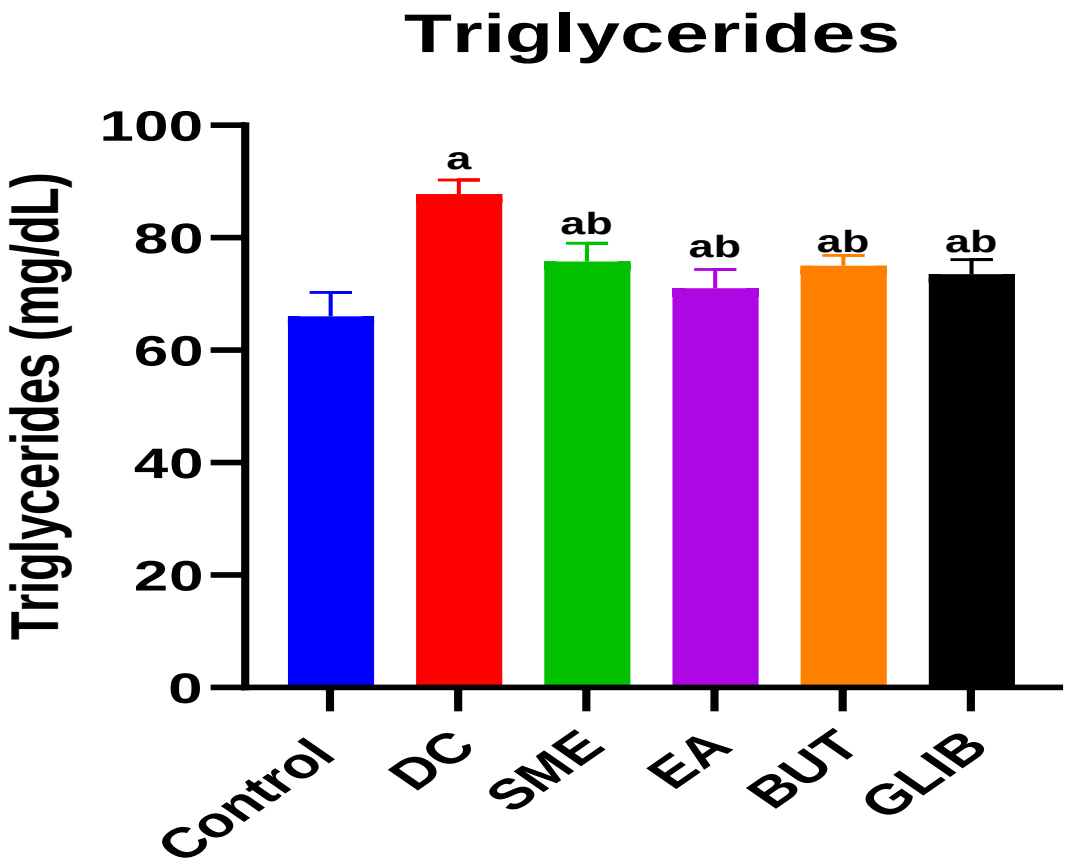


***Solanum macrocarpon* extract and fractions**

Figure 4.16: Effect of *Solanum macrocarpon* Linn Extract and its Solvent Fractions on Total Cholesterol of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

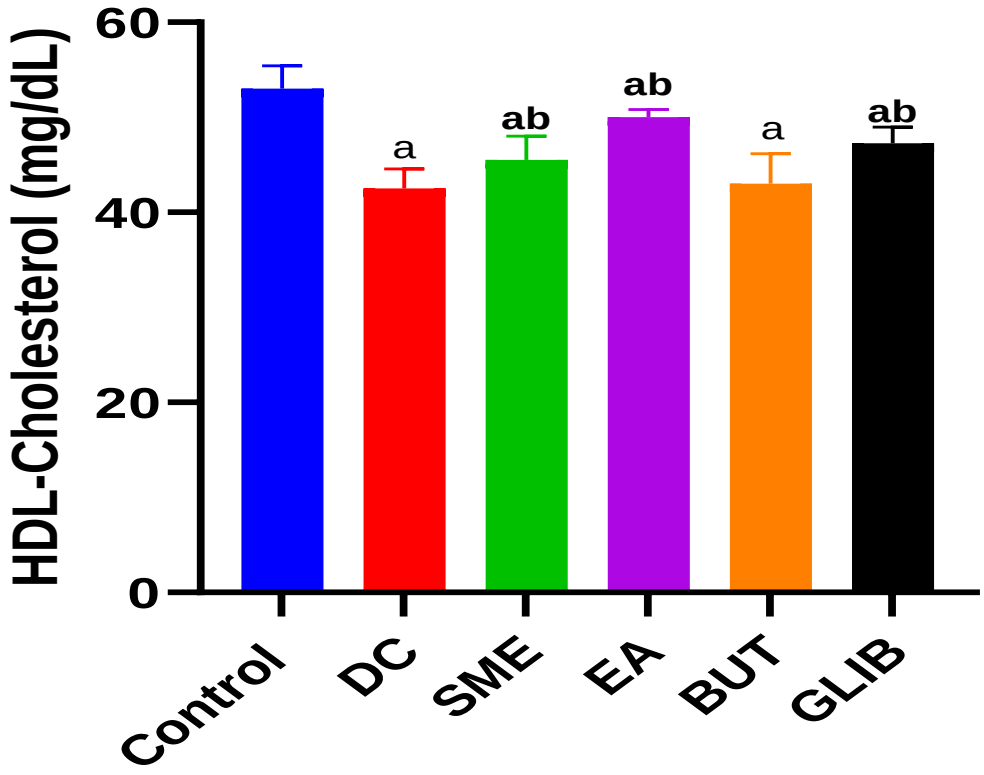


***Solanum macrocarpon* extract and fractions**

Figure: 4.17: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Triglycerides of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

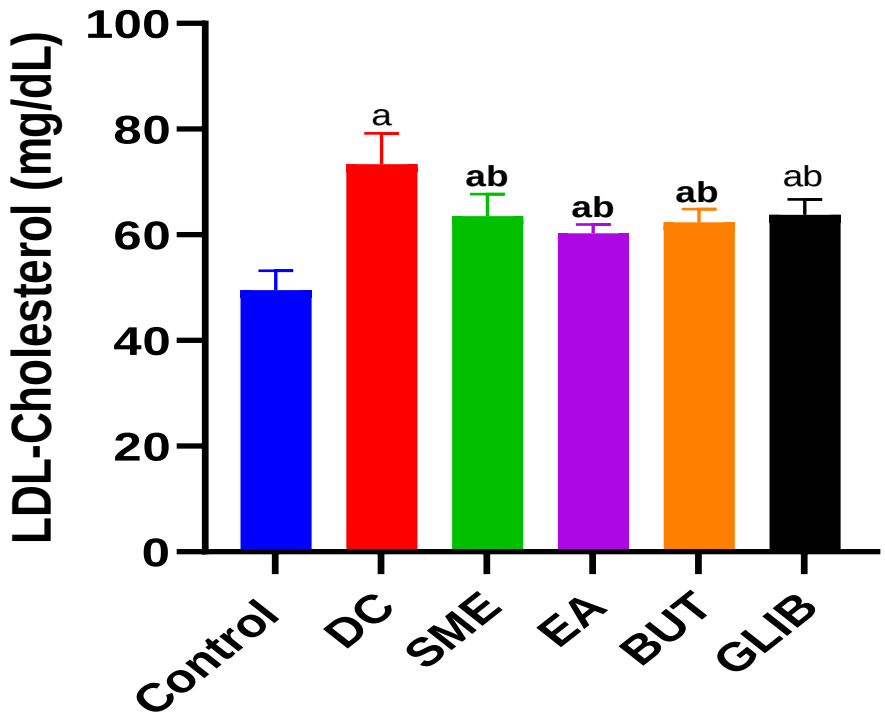


***Solanum macrocarpon* extract and fractions**

Figure 4.18: Effect of *Solanum macrocarpon* Linn. extract and Solvent Fractions on HDL-Cholesterol of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

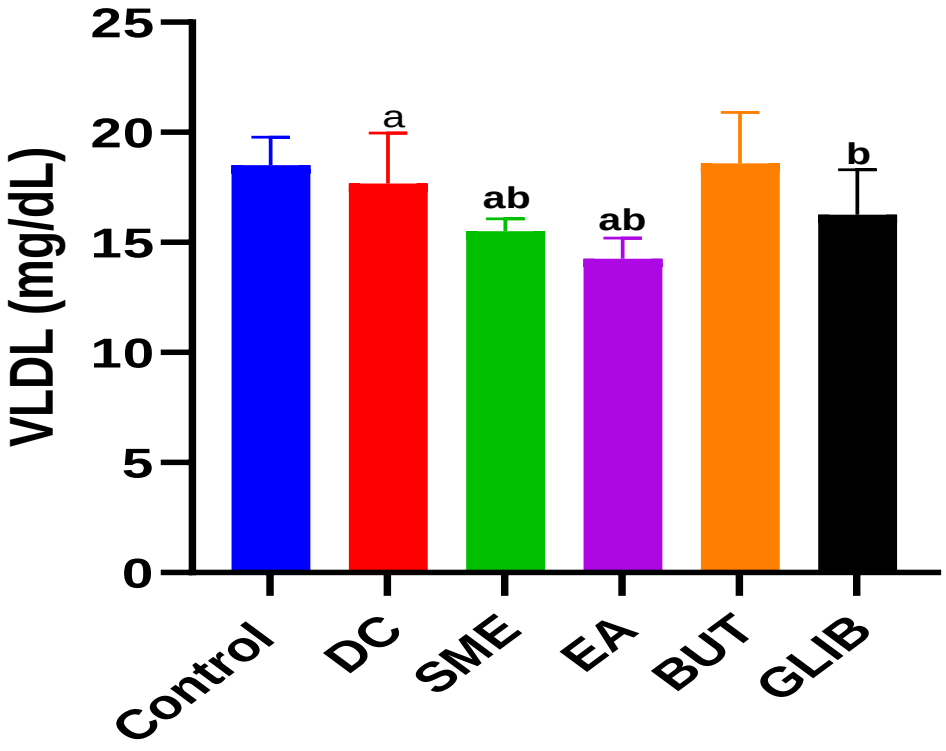


***Solanum macrocarpon* extract and fractions**

Figure 4.19: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on LDL-Cholesterol of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

Figure 4.20: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on VLDL-Cholesterol of Diabetic Rats

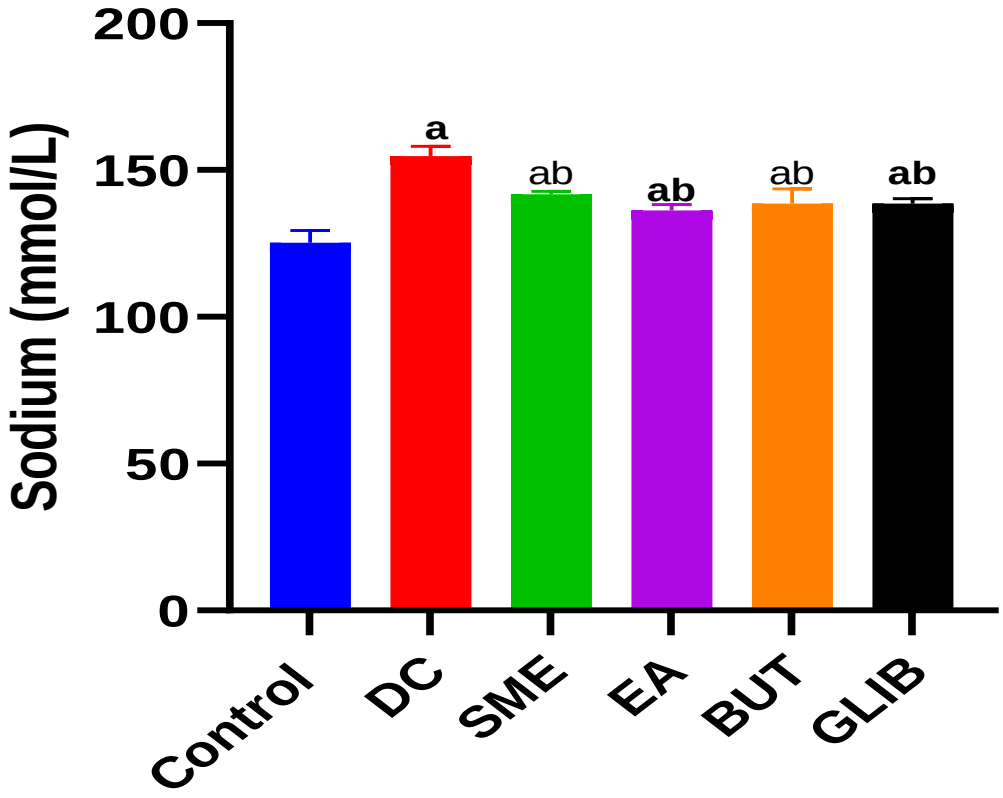
[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

Table 4.20: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Heamatogical Indices of Diabetic Rats

	Control	DC	SME	EA	BUT	GLIB
PVC (%)	43.00±1.02	34.33±0.60[a]	37.67±0.58[ab]	38.33±0.58[ab]	37.67±0.53[ab]	40.67±1.15[ab]
RBC (10^6) cell/mm^3	12.02±0.34	7.23±0.27[a]	7.65±0.39[b]	9.25±0.96[ab]	8.85±0.22[ab]	9.93±0.06[ab]
WBC (10^3) cell/mm^3	6.68±0.30	10.62±0.57[a]	8.10±0.30[ab]	8.08±0.63[ab]	7.29±0.74[b]	8.50±0.35[ab]
PLT (10^5) Cell/mm^3	6.18±0.85	2.96±0.12[a]	4.33±0.44[ab]	4.30±0.92[ab]	3.97±0.42[ab]	5.54±1.25[ab]
HB (g/L)	15.2±0.89	9.93±0.46[a]	11.60±0.70[ab]	12.03±0.25[ab]	12.73±0.47[ab]	14.00±0.10[ab]
N (%)	63.33±6.11	59.00±7.00	66.67±3.21	63.00±1.00	63.33±6.66	65.33±7.51
L (%)	36.67±6.10	40.67±7.02[a]	32.33±3.21	36.33±1.15	35.67±6.65	34.67±7.50
M (%)	00	00	0.67	0.33	0.67	00
E (%)	00	0.33	0.33	0.33	0.33	00
B (%)	00	00	00	00	00	00

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups
DC=Diabetic control, SME=Methanol extract of *Solanum macrocarpon*, EA= Ethyl acetate fraction of methanol extract of *Solanum macrocarpon* BUT = Butanol fraction of methanol extract of *Solanum macrocarpon* GLIB=Glibenclamide

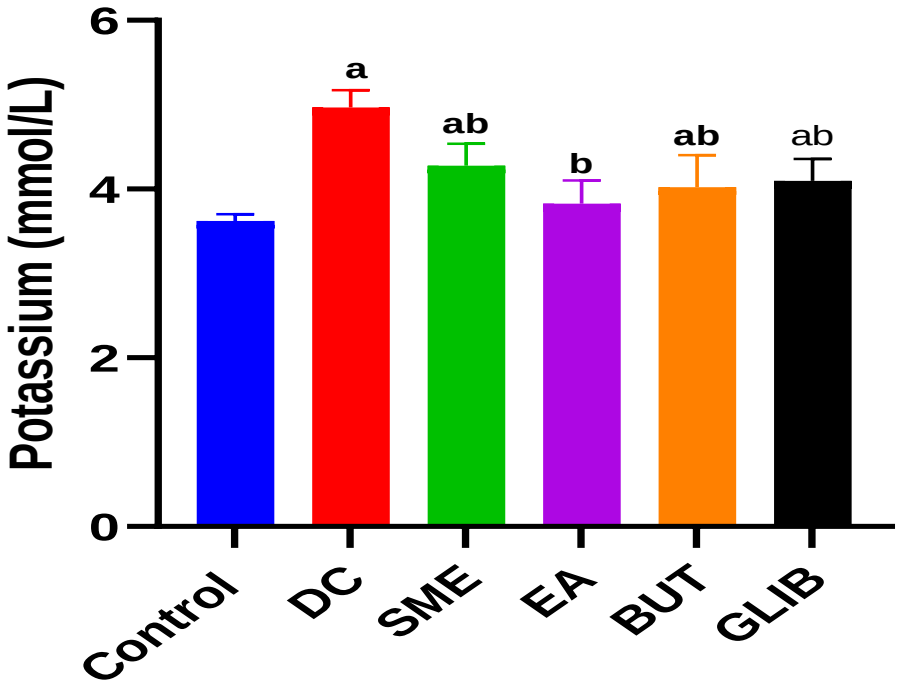


***Solanum macrocarpon* extract and fractions**

Figure 4.21: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Sodium of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

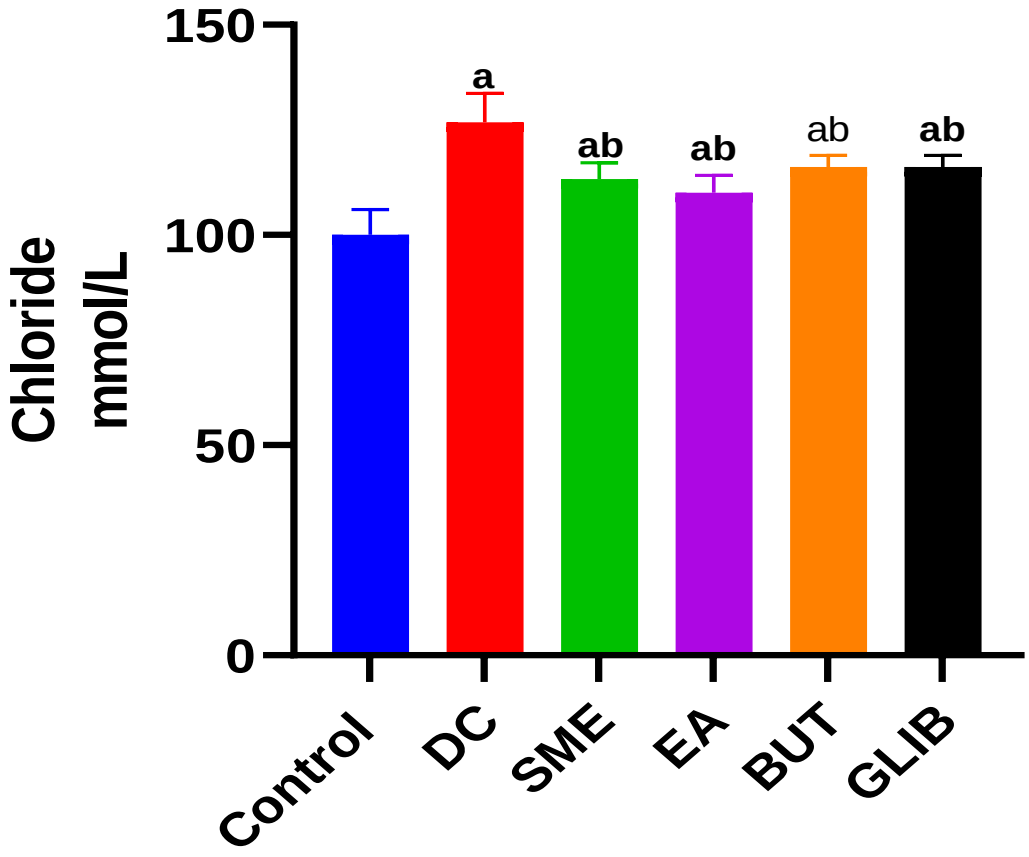


***Solanum macrocarpon* extract and fractions**

Figure 4.22: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Potassium of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

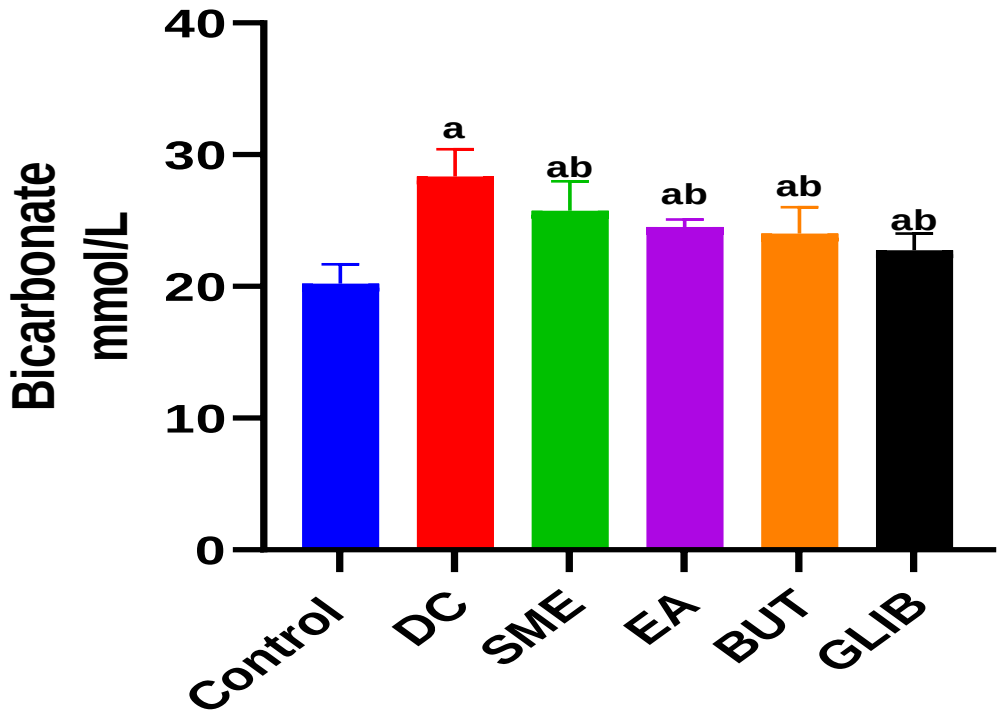


***Solanum macrocarpon* extract and fractions**

Figure 4.23: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Chloride of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

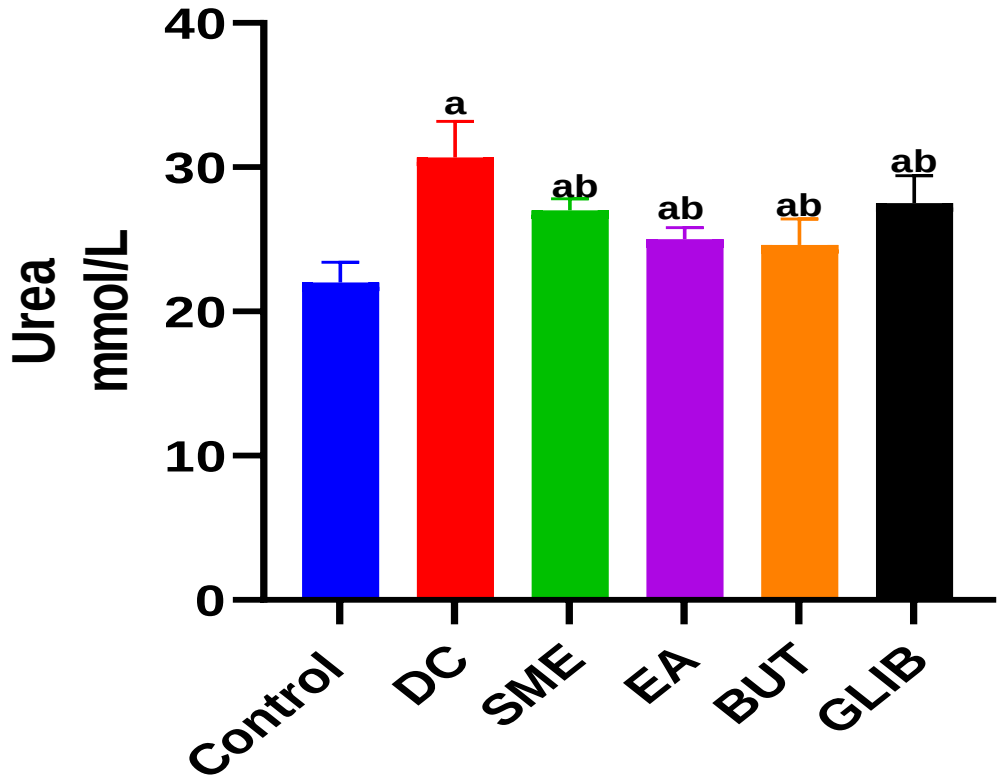


***Solanum macrocarpon* extract and fractions**

Figure 4.24: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Bicarbonate of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

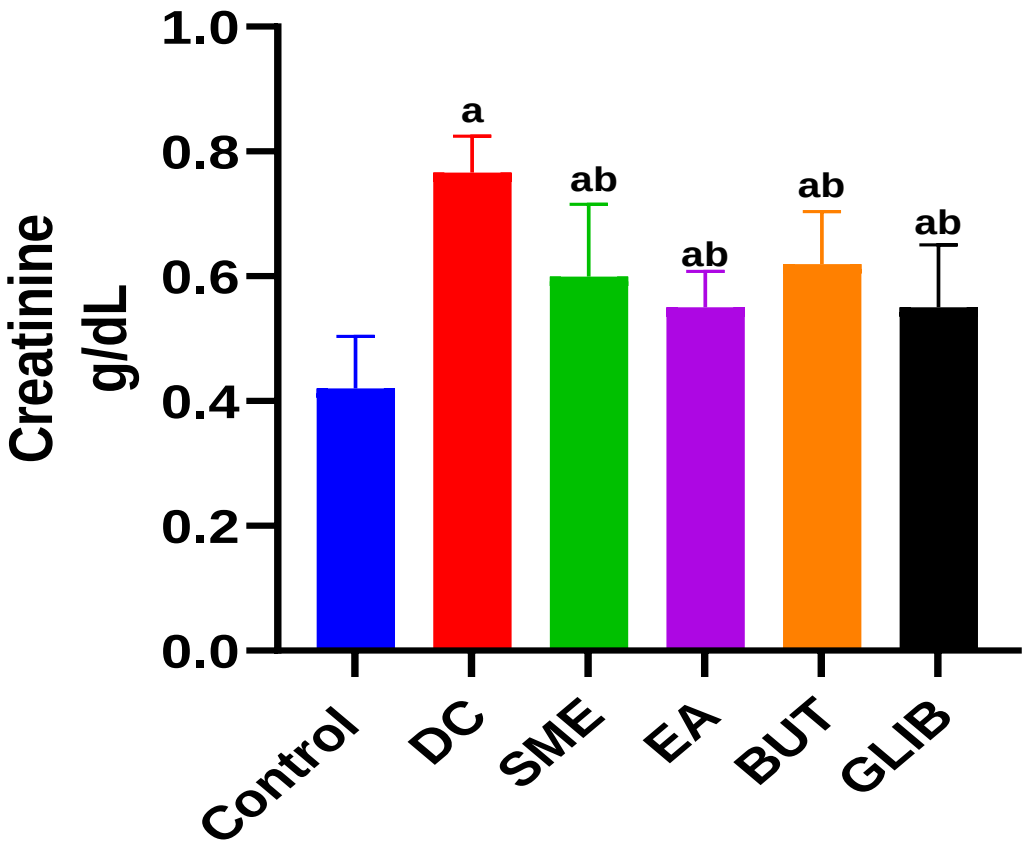


***Solanum macrocarpon* extract and fractions**

Figure 4:25: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Urea of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

Figure 4.26: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Creatinine of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

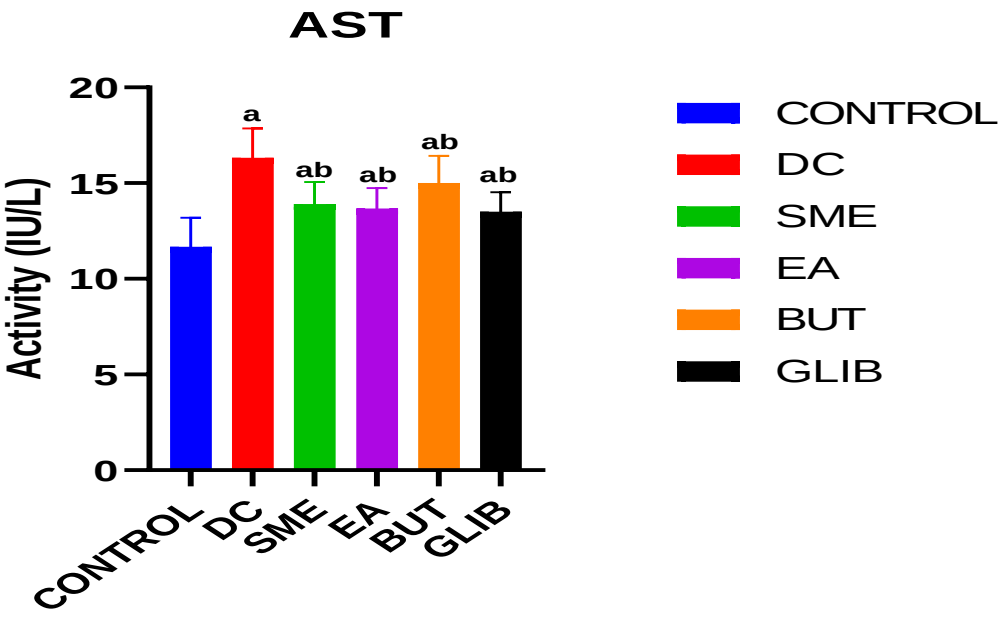
Table 4.21: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Some Liver and Kidney Function Biomarkers in Streptozotocin Induced Diabetic Male Rats

	Control	DC	SME	EA	BUT	GLIB
TP	7.73±0.21	5.90±0.26^{ab}	6.60±0.20^{ab}	6.87±0.15^{ab}	6.53±0.06^{ab}	6.80±0.10^{ab}
ALB	4.87±0.12	4.03±0.11^{a}	4.33±0.21^{ab}	4.73±0.06^{b}	4.50±0.20ab	4.77±0.12^{b}
GLB	2.87±0.15	2.06±0.32^{a}	2.27±0.18^{a}	2.13±0.12^{a}	2.23±0.15^{a}	2.03±0.06^{a}
TB	0.58±0.10	0.33±0.06^{a}	0.41±0.12^{ab}	0.45±0.08^{ab}	0.40±0.10^{ab}	0.47±0.15^{ab}
AST (IU/L)	11.67±1.52	16.33±1.53^{a}	13.90±1.15^{at}	13.67±1.07^{b}	15.00±1.43^{at}	13.50±1.02^{at}
ALT (IU/L)	13.00±0.95	17.33±1.53^{a}	14.33±0.58^{at}	13.87±0.48^{b}	14.50±0.65^{at}	13.33±1.53^{b}
ALP (IU/L)	35.67±1.15	65.33±4.73^{at}	48.67±2.08^{at}	45.00±2.05^{at}	46.50±2.65^{at}	46.33±1.73^{at}

Data are mean±SD

^{a} $p < 0.05$ when control group was compared with experimental groups, ^{b} $p< 0.05$ when diabetic group was compared with treated groups

DC=Diabetic control, SME=Methanol extract of *Solanum macrocarpon*, EA= Ethyl acetate fraction of methanol extract of *Solanum macrocarpon* BUT = Butanol fraction of methanol extract of *Solanum macrocarpon* GLIB=Glibenclamide

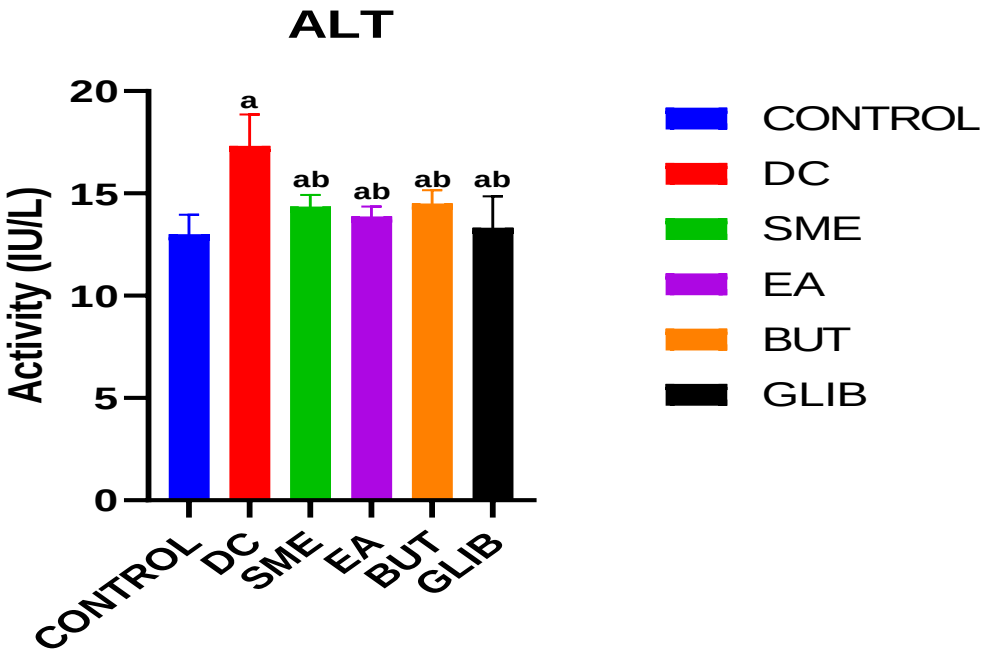


***Solanum macrocarpon* extract and fractions**

Figure 4.27: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Aspartate Transaminase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

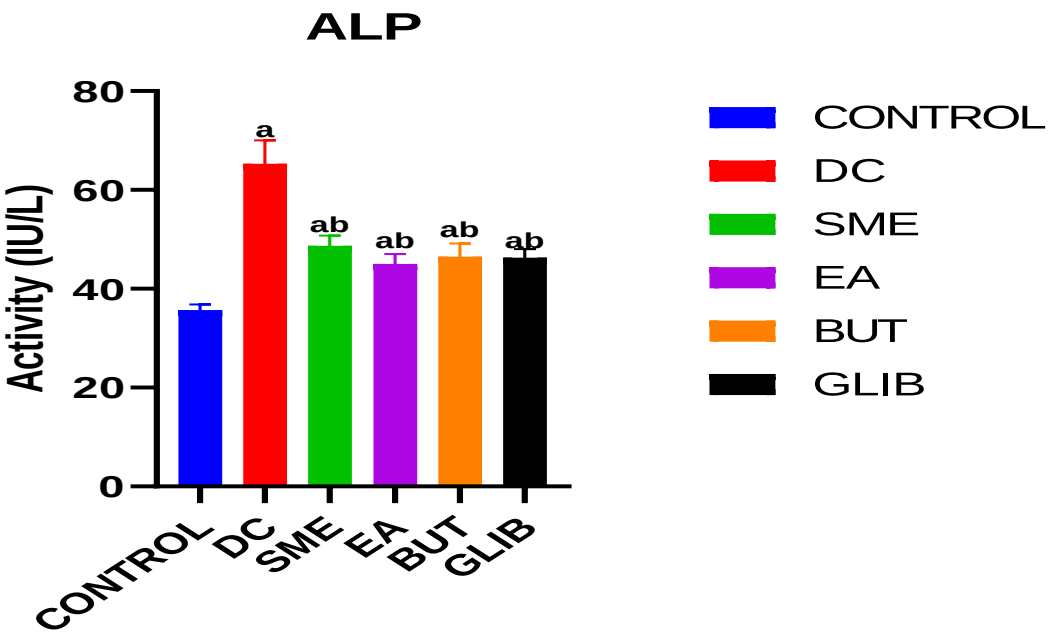


***Solanum macrocarpon* extract and fractions**

Figure 4.28: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Alanine Transaminase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

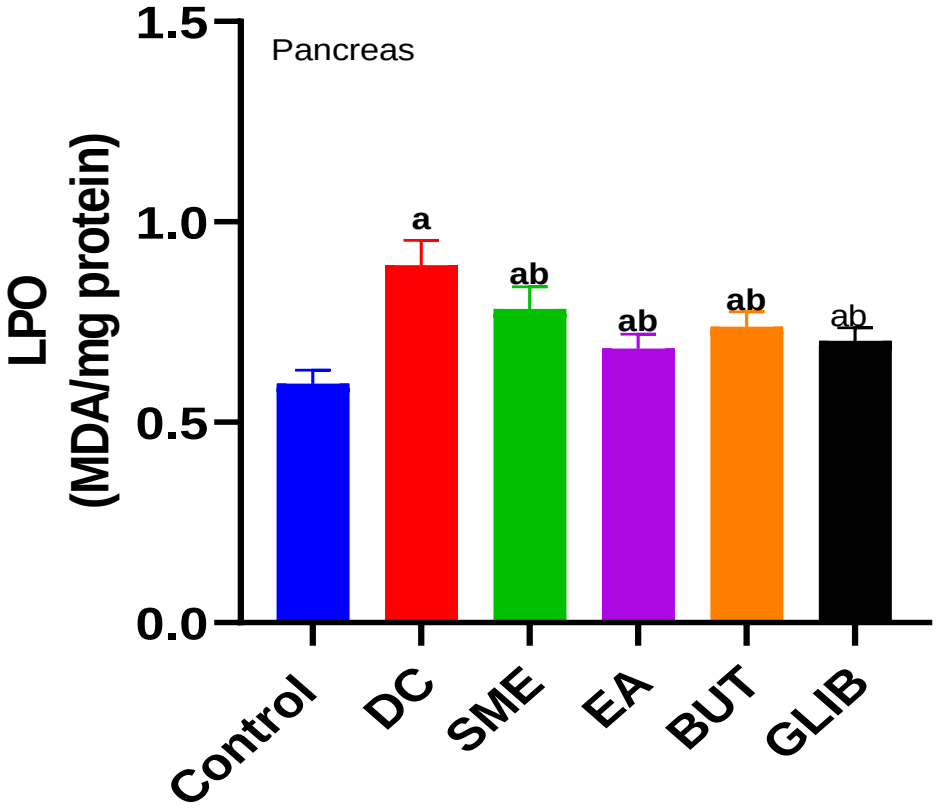
Figure 4.29: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Alkaline Phosphatase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

4.1.8 Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hyperglycemia Mediated Oxidative Stress on Streptozotocin-Indued Diabetic Male Wistar Rats

Figures 4.30 - 4.53 showed that hyperglycemia mediated oxidative stress were ameliorated in pancreas, kidney and liver of diabetic rats upon administration of EA

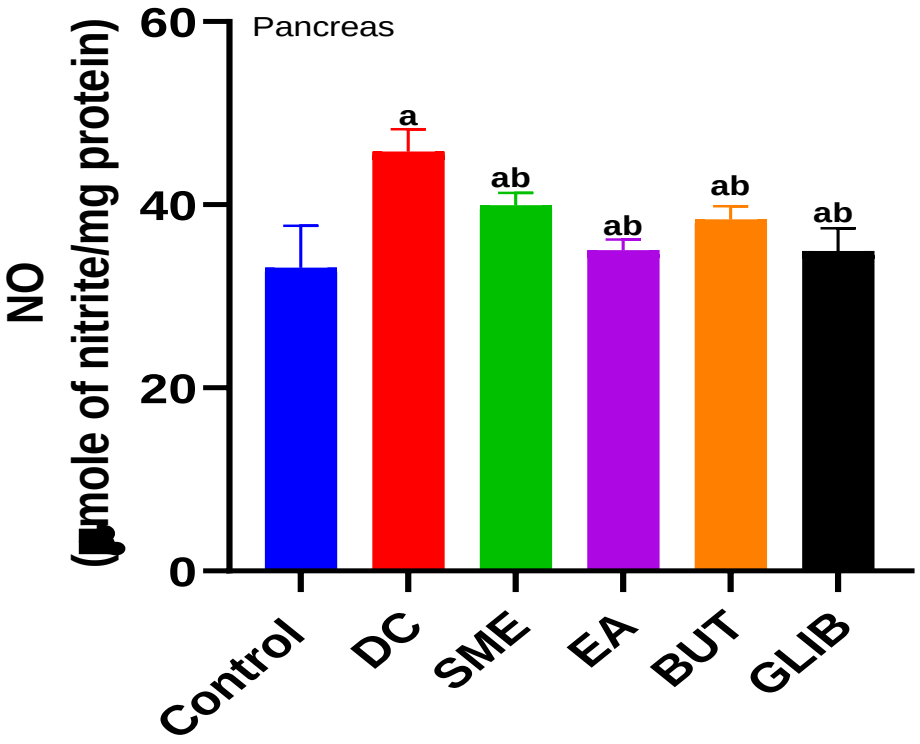


***Solanum macrocarpon* extract and fractions**

Figure 4.30: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic Lipid Peroxidation of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

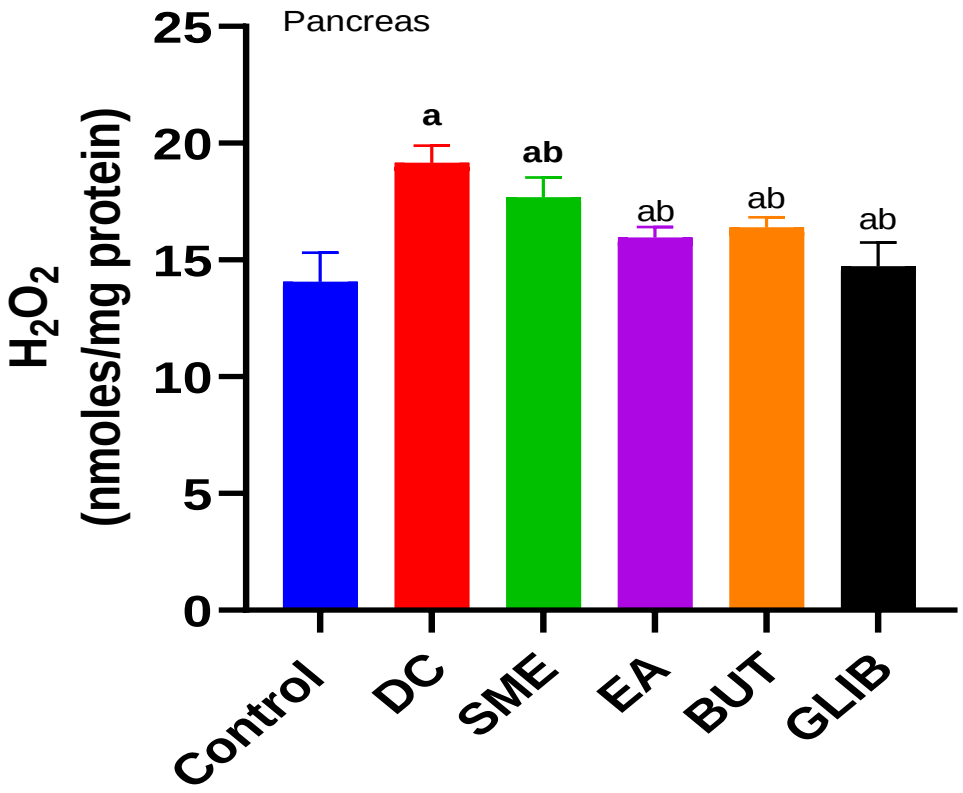


***Solanum macrocarpon* extract and fractions**

Figure 4.31: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic Nitric Oxide of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

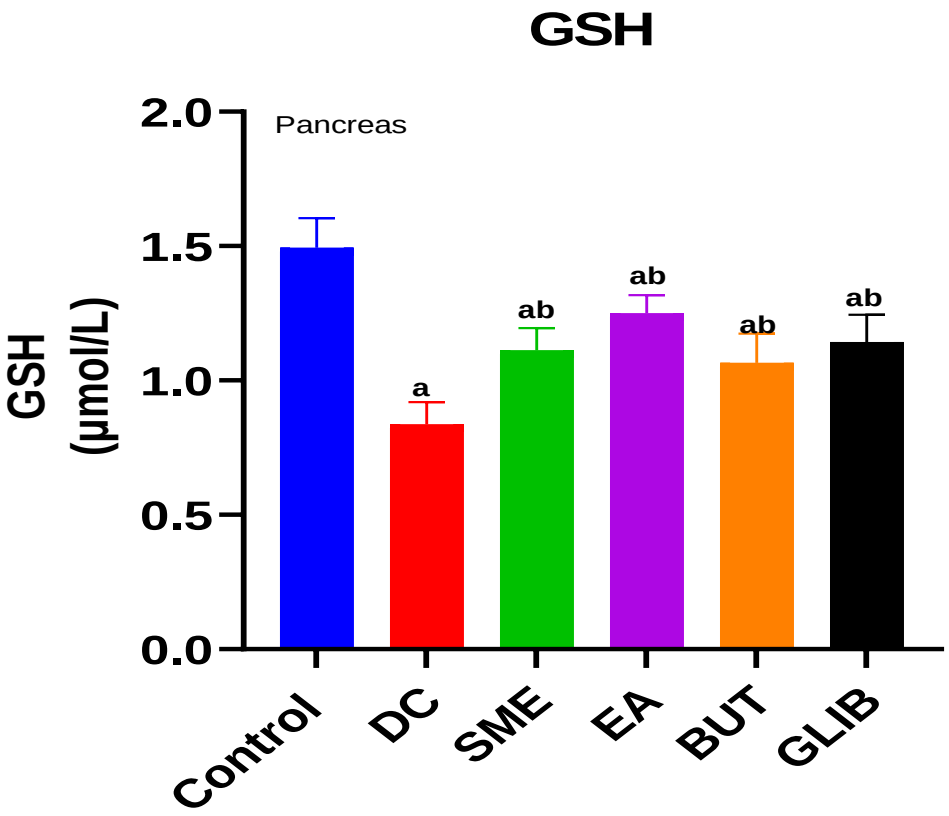


***Solanum macrocarpon* extract and fractions**

Figure 4:32: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic H_2O_2 of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

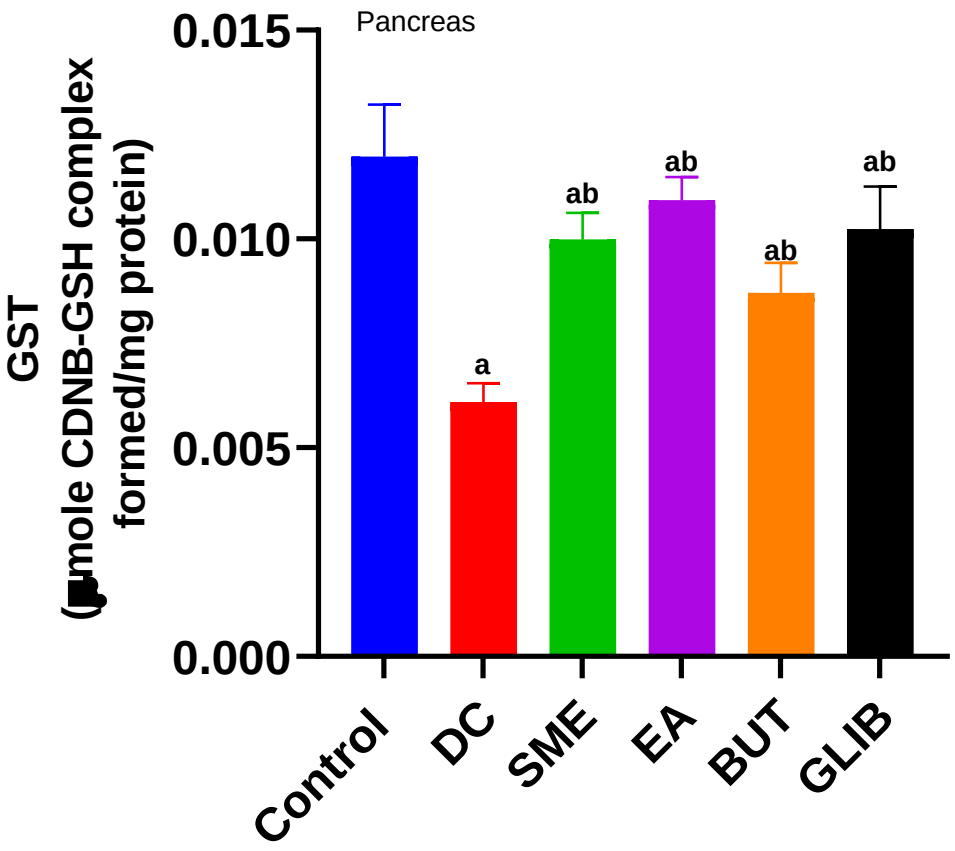


***Solanum macrocarpon* extract and fractions**

Figure 4.33: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic Glutathione of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

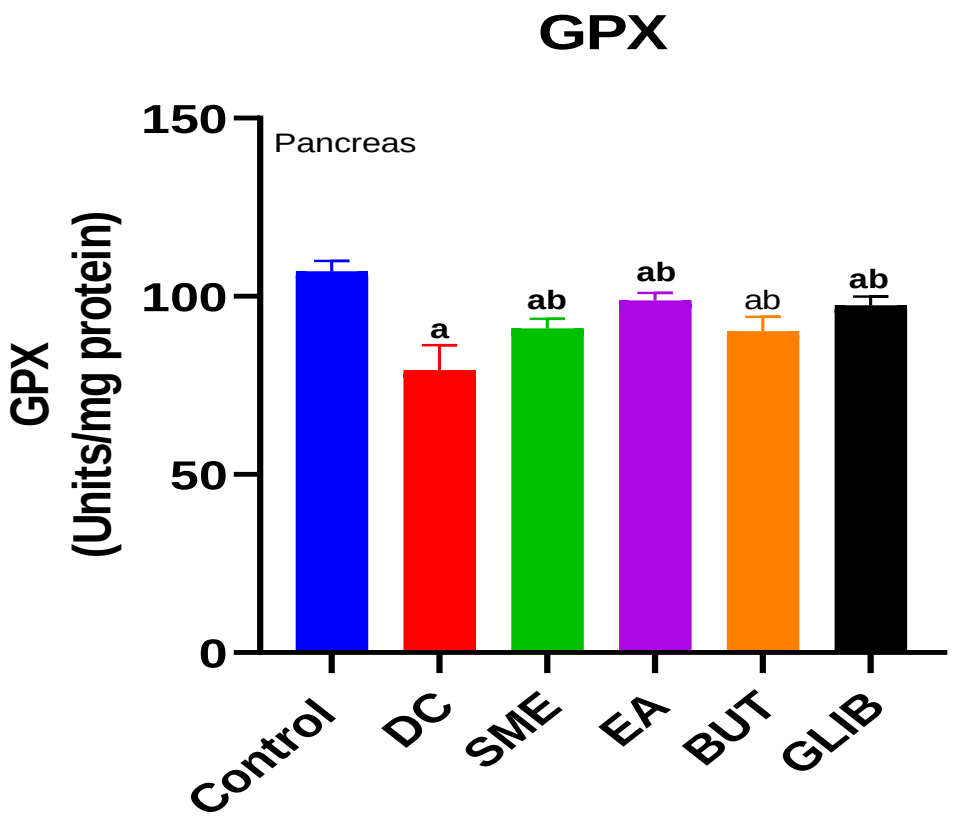


***Solanum macrocarpon* extract and fractions**

Figure 4.34: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic GST of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

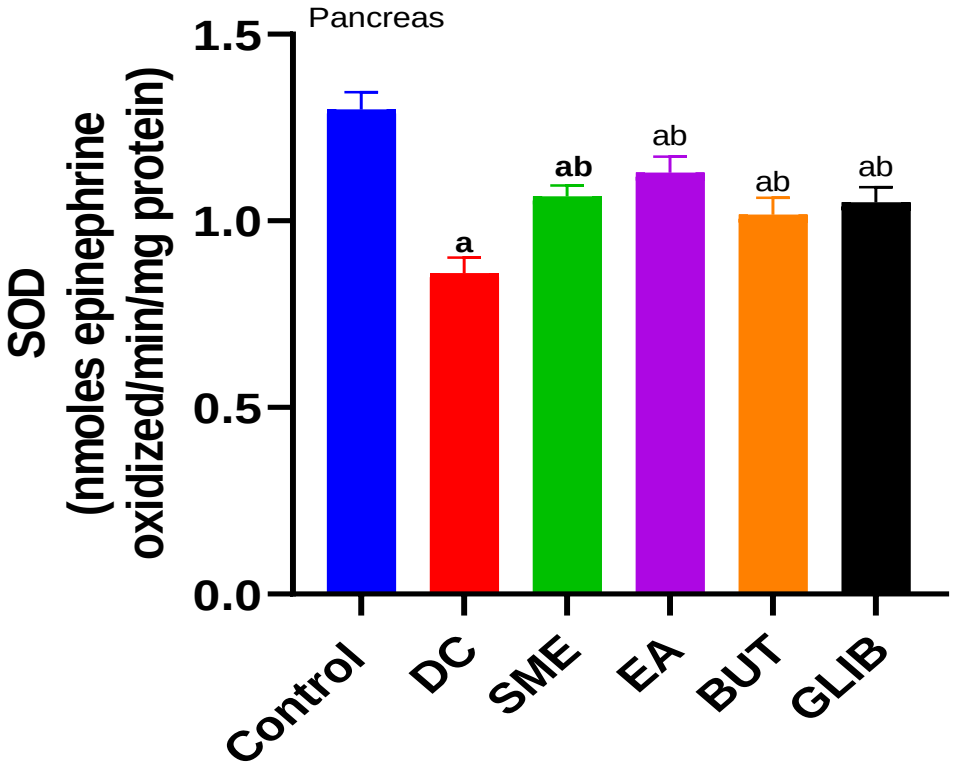
Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

Figure 4.35: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic Glutathione Peroxidase of Diabetic Rats
[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

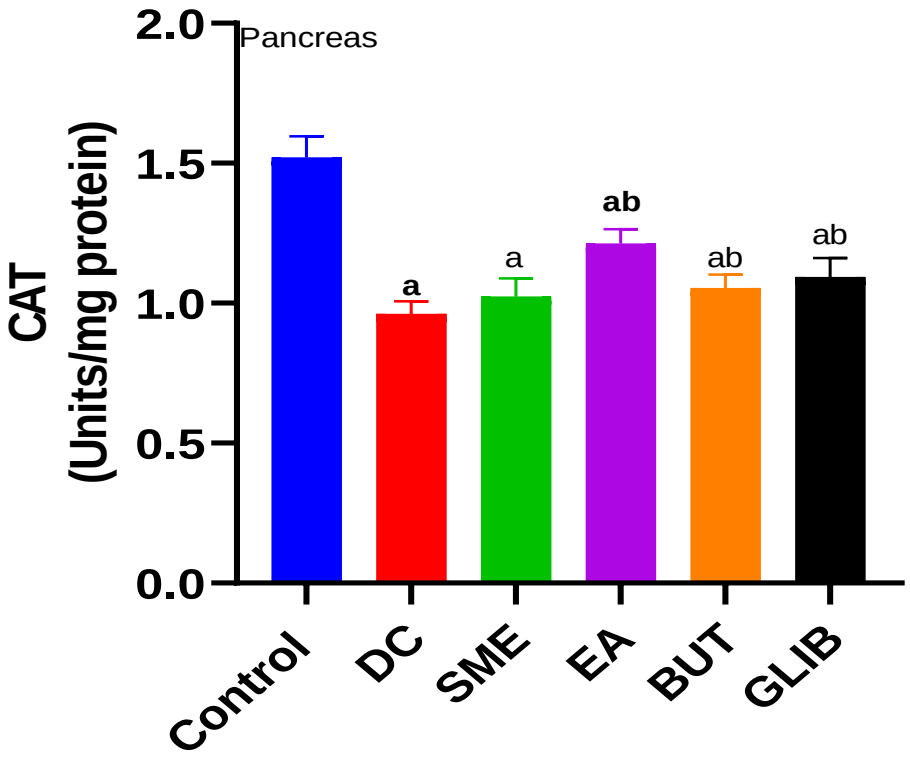


***Solanum macrocarpon* extract and fractions**

Figure 4.36: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic Superoxide Dismutase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

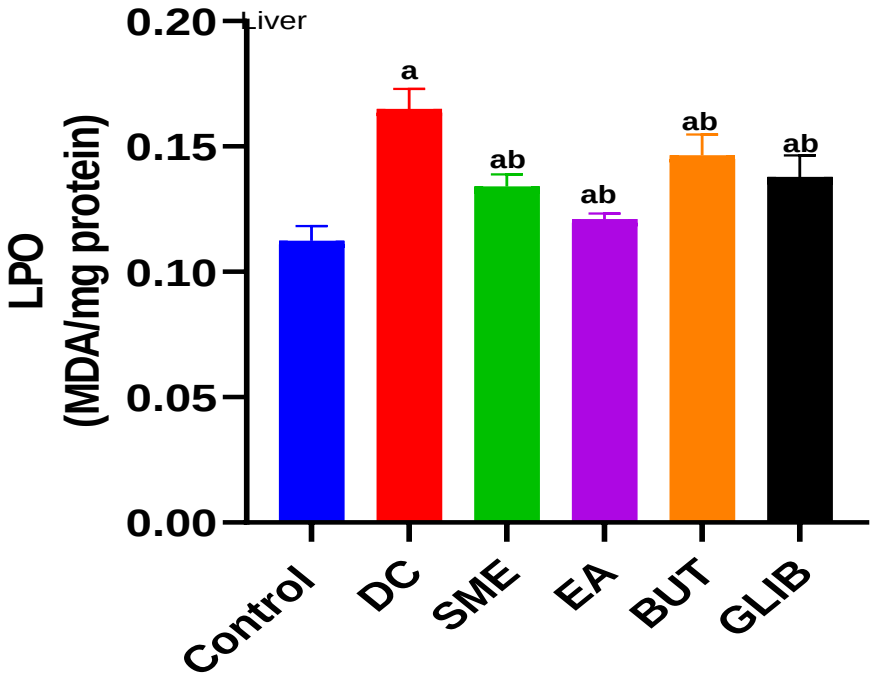


***Solanum macrocarpon* extract and fractions**

Figure 4.37: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Pancreatic Catalase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

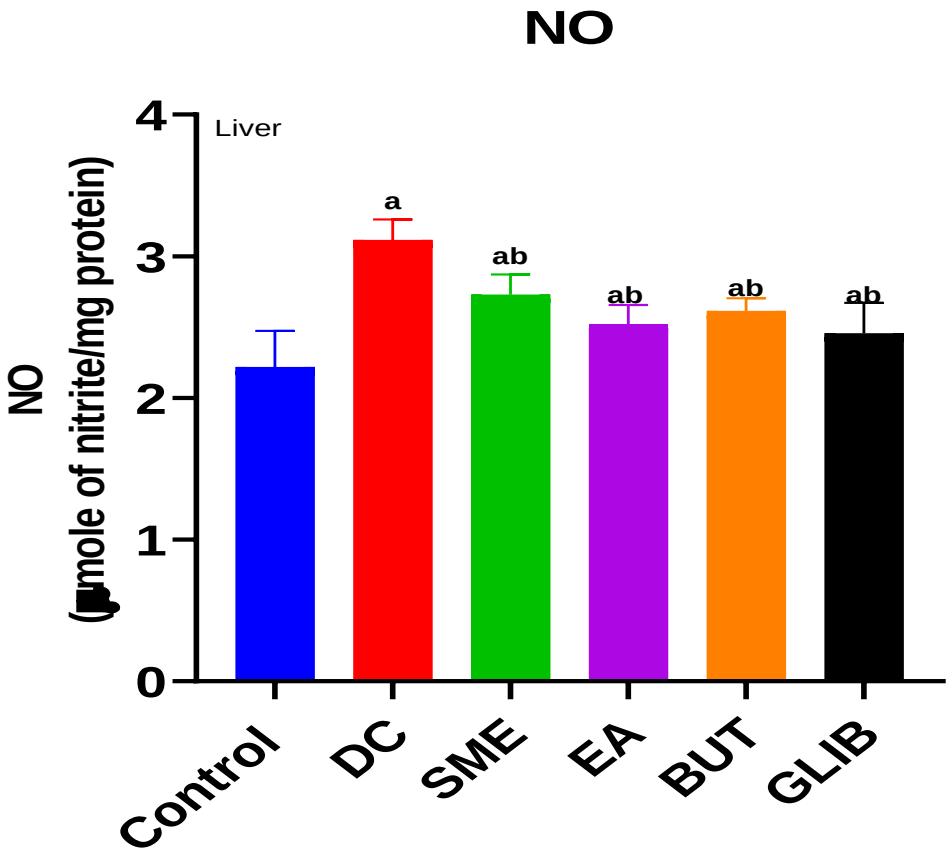


***Solanum macrocarpon* extract and fractions**

Figure 4.38: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic LPO of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

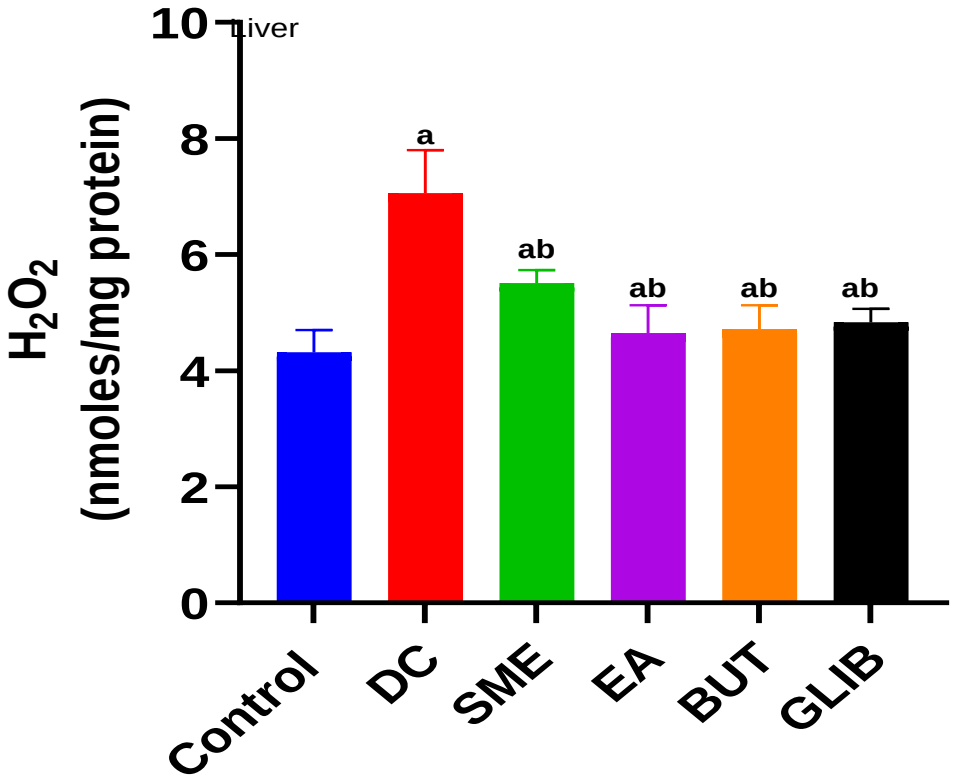


***Solanum macrocarpon* extract and fractions**

Figure 4.39: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic Nitric Oxide of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

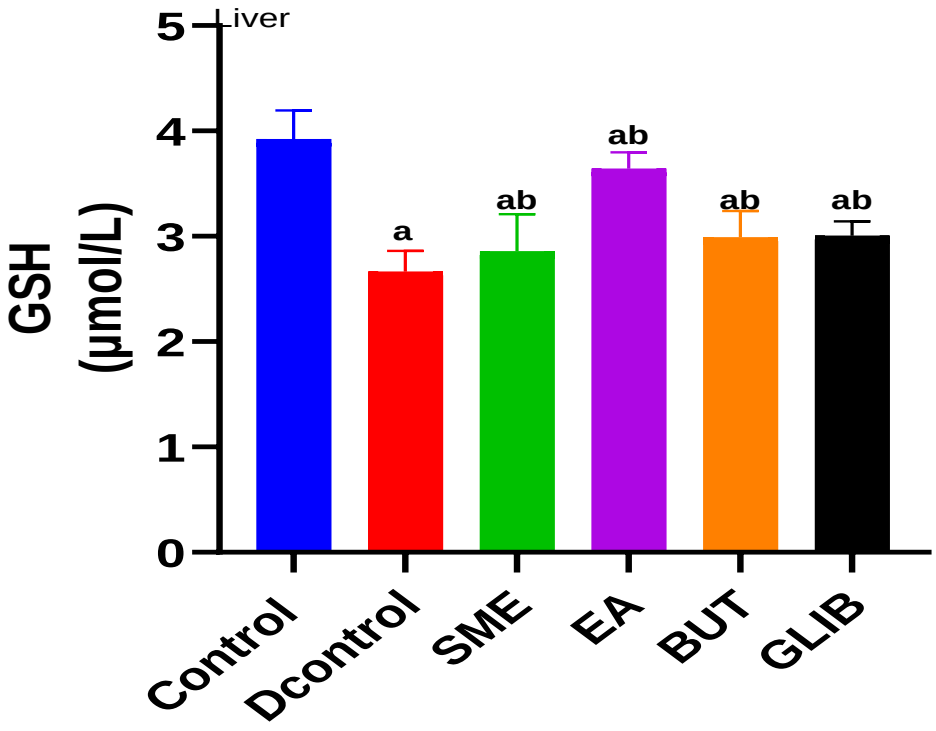


***Solanum macrocarpon* extract and fractions**

Figure 4.40: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic H_2O_2 of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

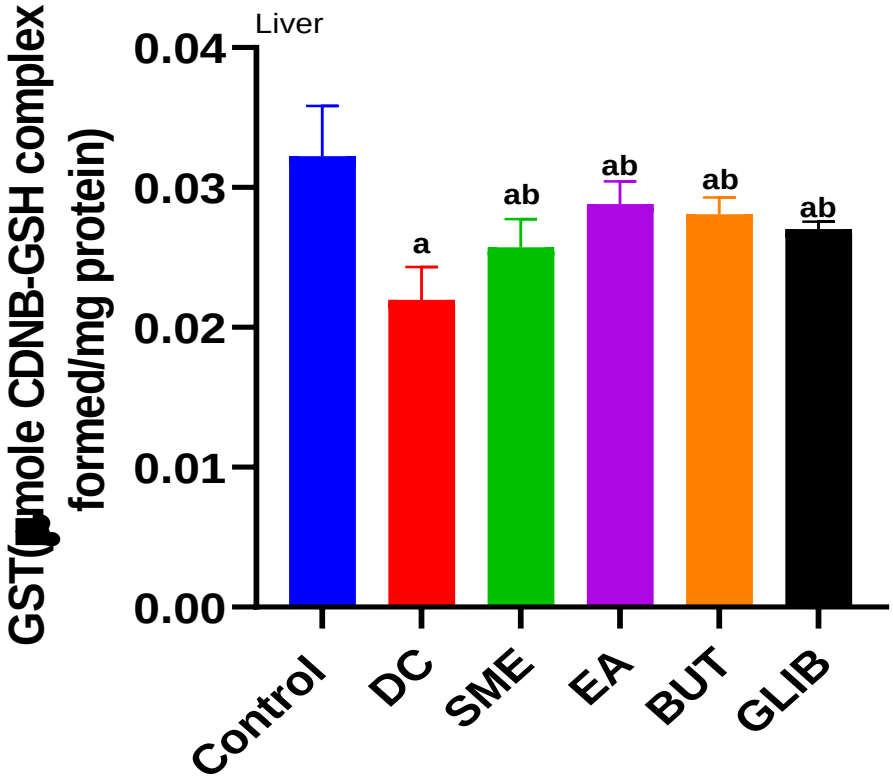


***Solanum macrocarpon* extract and fractions**

Figure 4.41: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic GSH of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

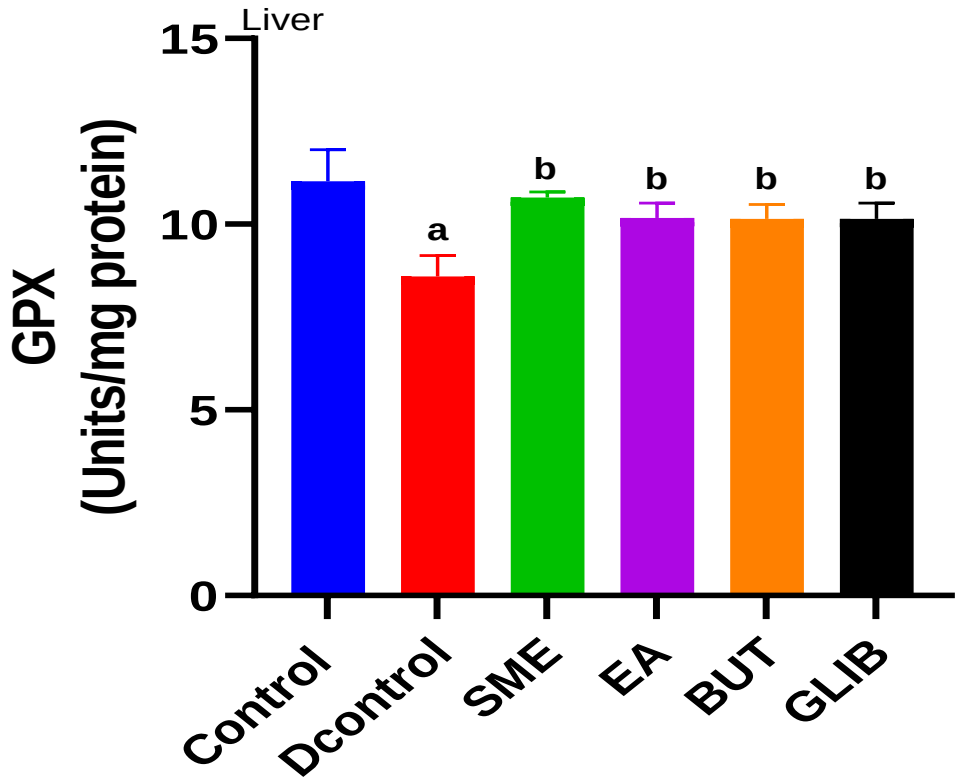


***Solanum macrocarpon* extract and fractions**

Figure 4.42: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic GST of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

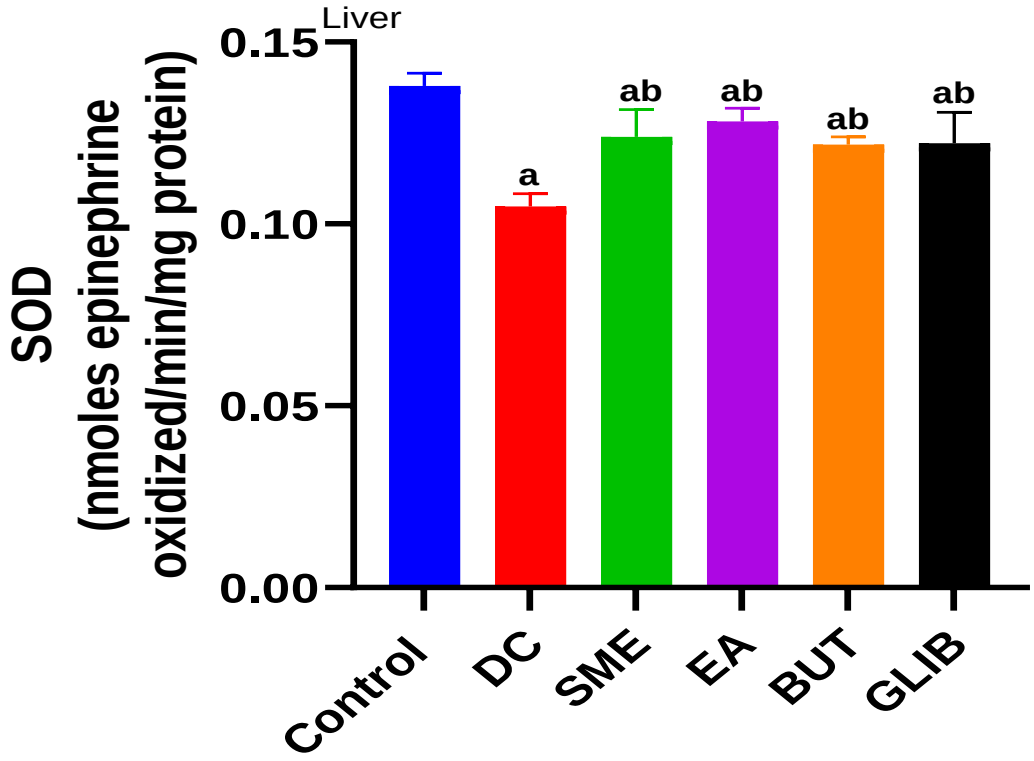


***Solanum macrocarpon* extract and fractions**

Figure 4.43: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic Glutathione Peroxidase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

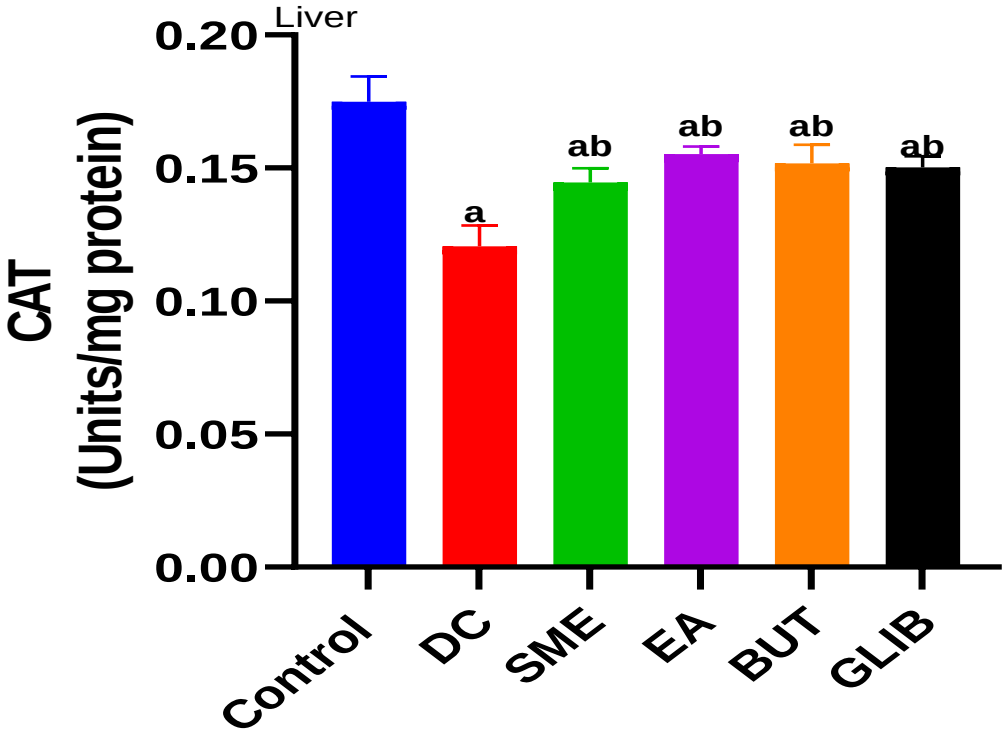
Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* Extract and Fractions**

Figure 4.44: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic Superoxide Dismutase of Diabetic Rats
[a] $p < 0.05$ when control group wwas compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

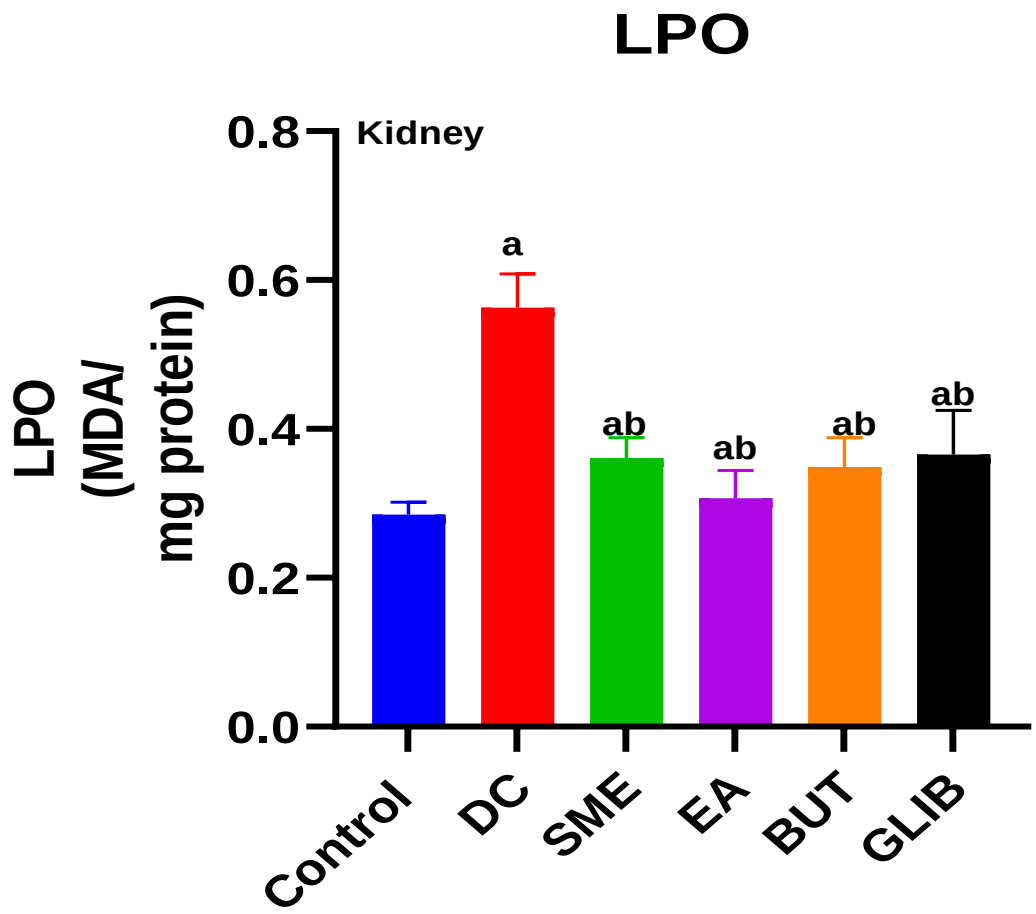


***Solanum macrocarpon* extract and fractions**

Figure 4.45: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Hepatic Catalase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

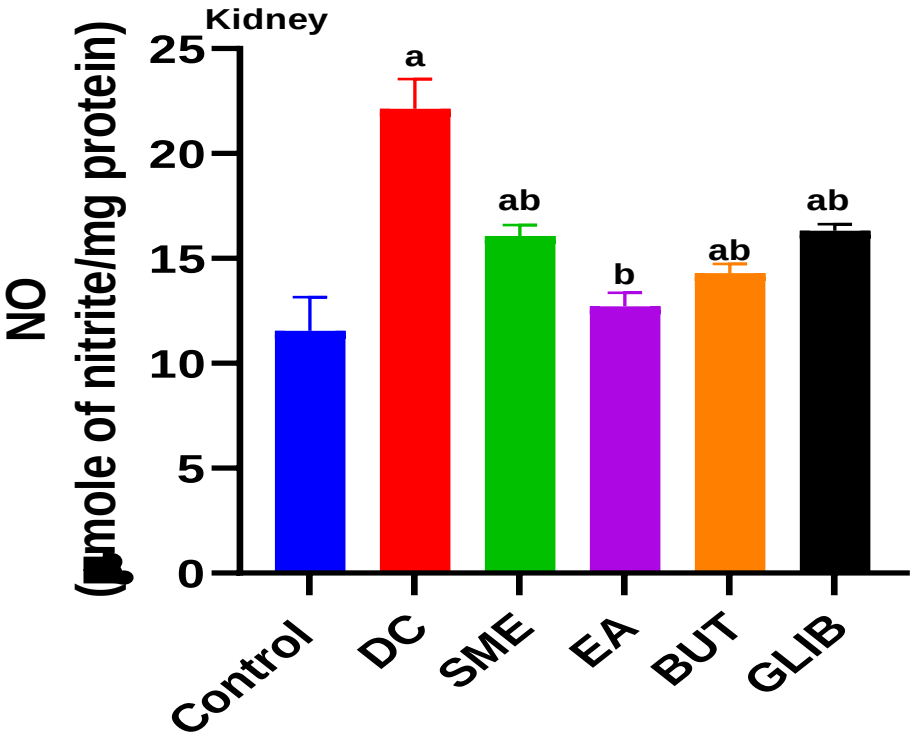


***Solanum macrocarpon* extract and fractions**

Figure 4.46: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Renal LPO of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

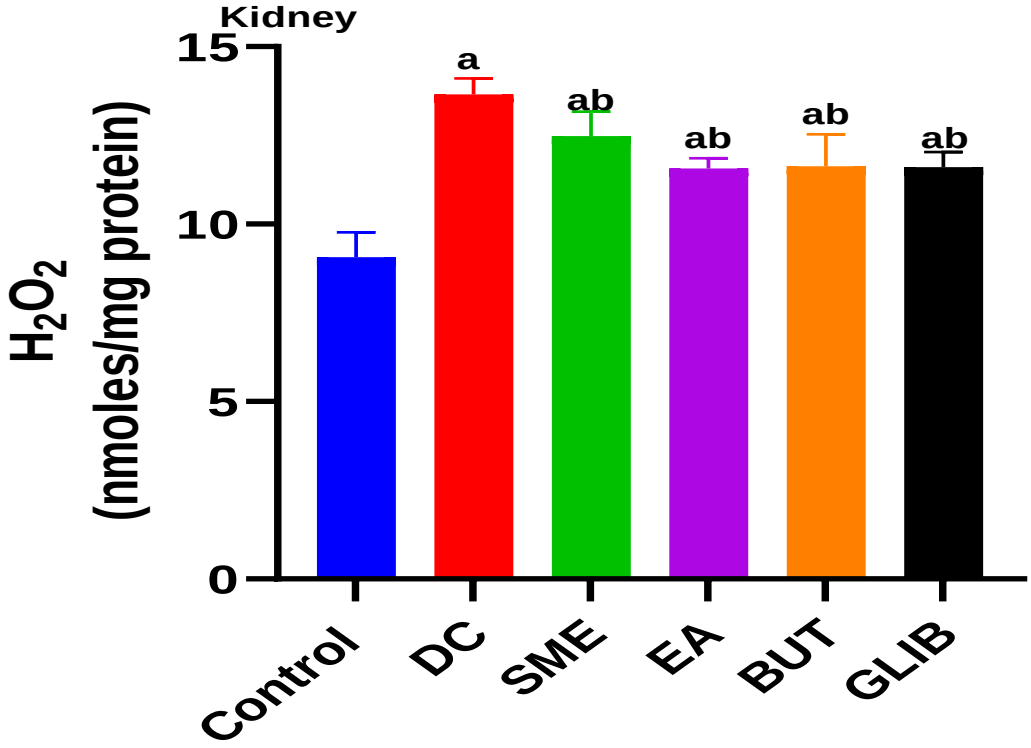


***Solanum macrocarpon* extract and fractions**

Figure 4.47: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Renal Nitric Oxide of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

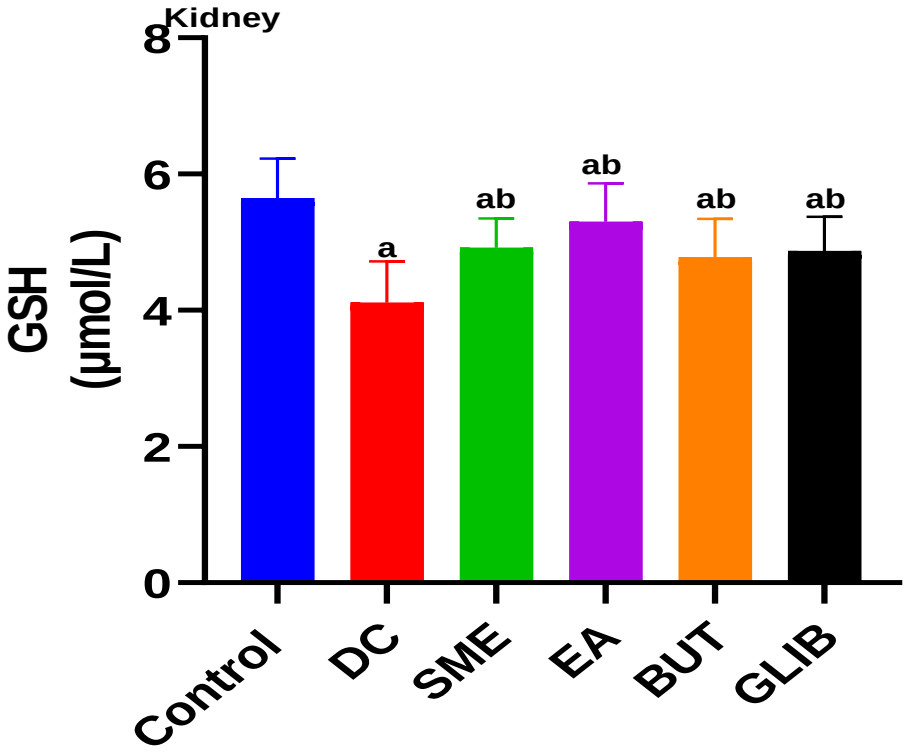


***Solanum macrocarpon* extract and fractions**

Figure 4.48: Effect of *Solanum macrocarpon* linn. Extract and Solvent Fractions on H_2O_2 of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

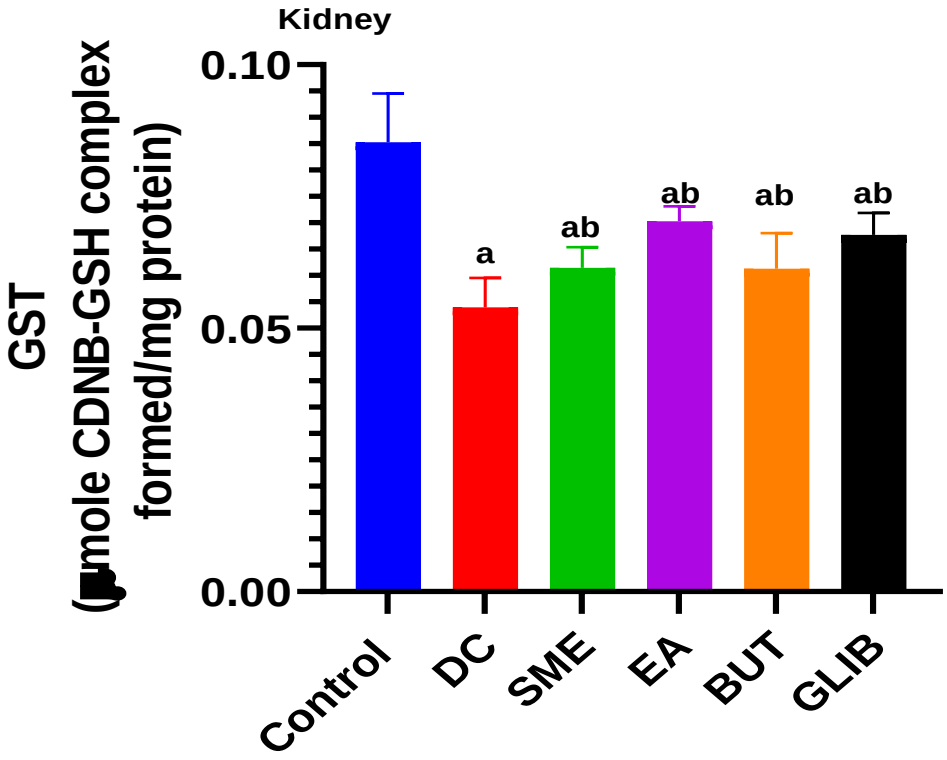


***Solanum macrocarpon* extract and fractions**

Figure 4.49: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Renal GSH of Diabetic Rats

[a] $p < 0.05$ when control group were compared with experimental groups, [b] $p < 0.05$ when diabetic group were compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

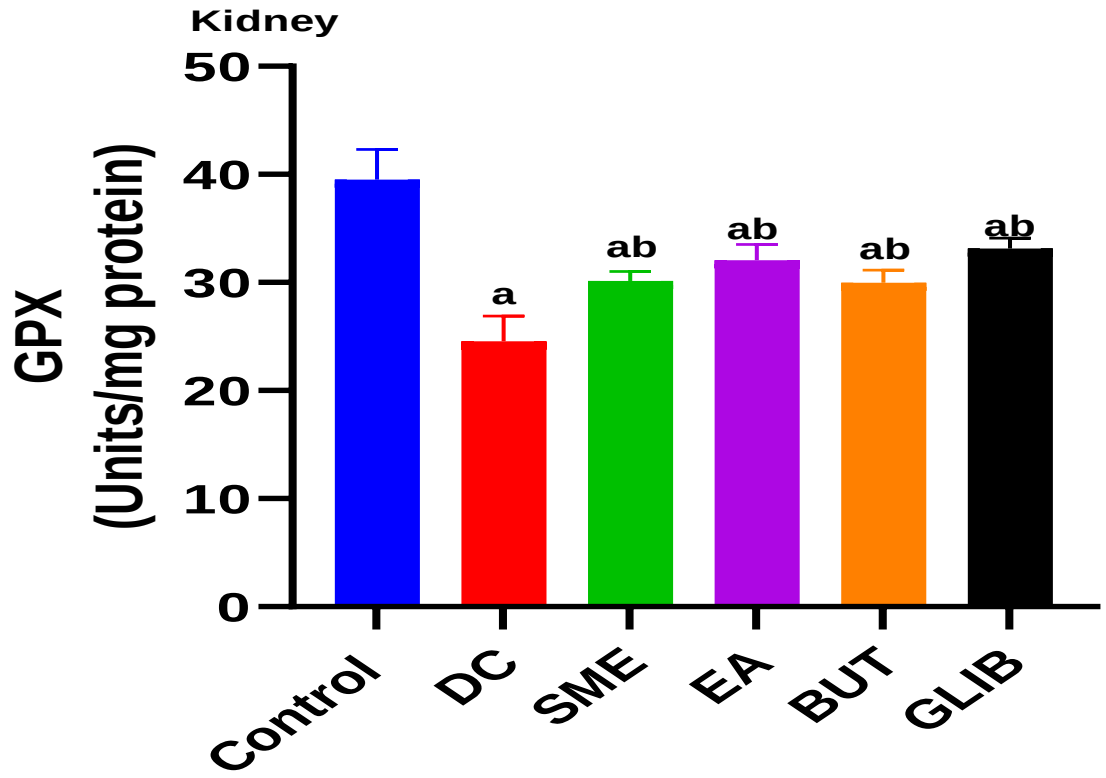


***Solanum macrocarpon* extract and fractions**

Figure 4.50: Effect of *Solanum macrocarpon* linn. Extract and Solvent Fractions on Renal gst of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

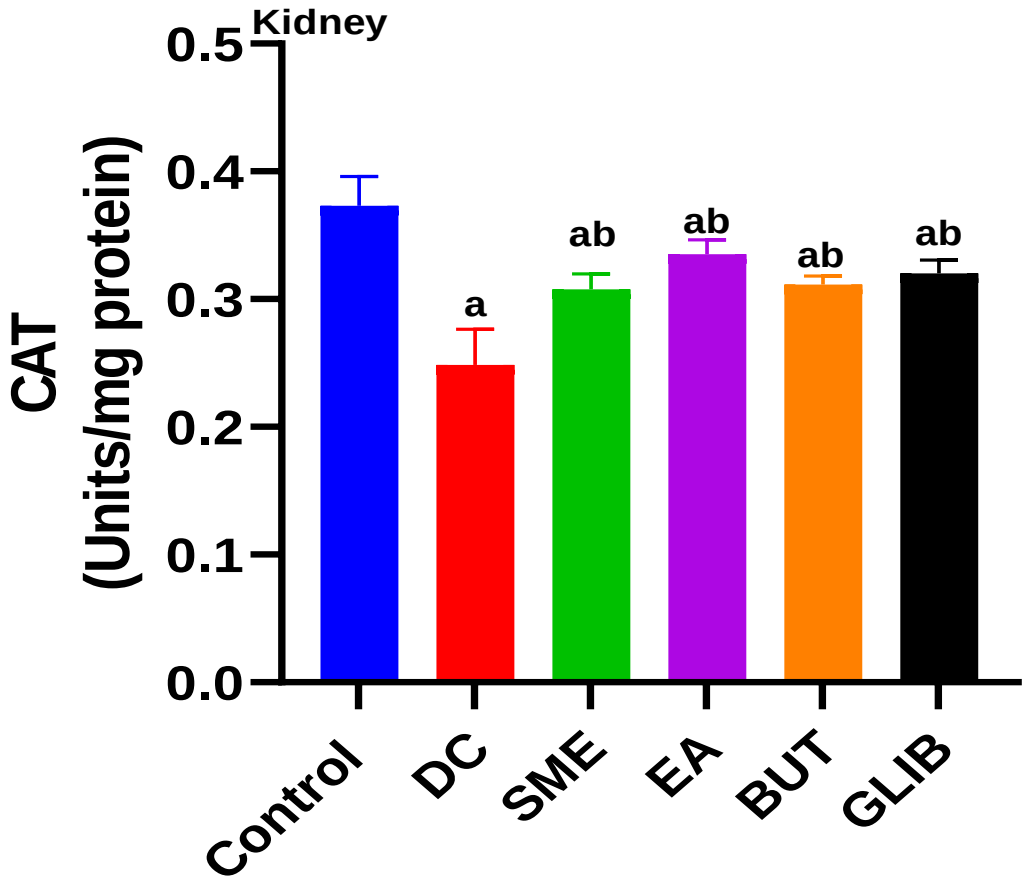


***Solanum macrocarpon* extract and fractions**

Figure 4.51: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Renal Glutathione Peroxidase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

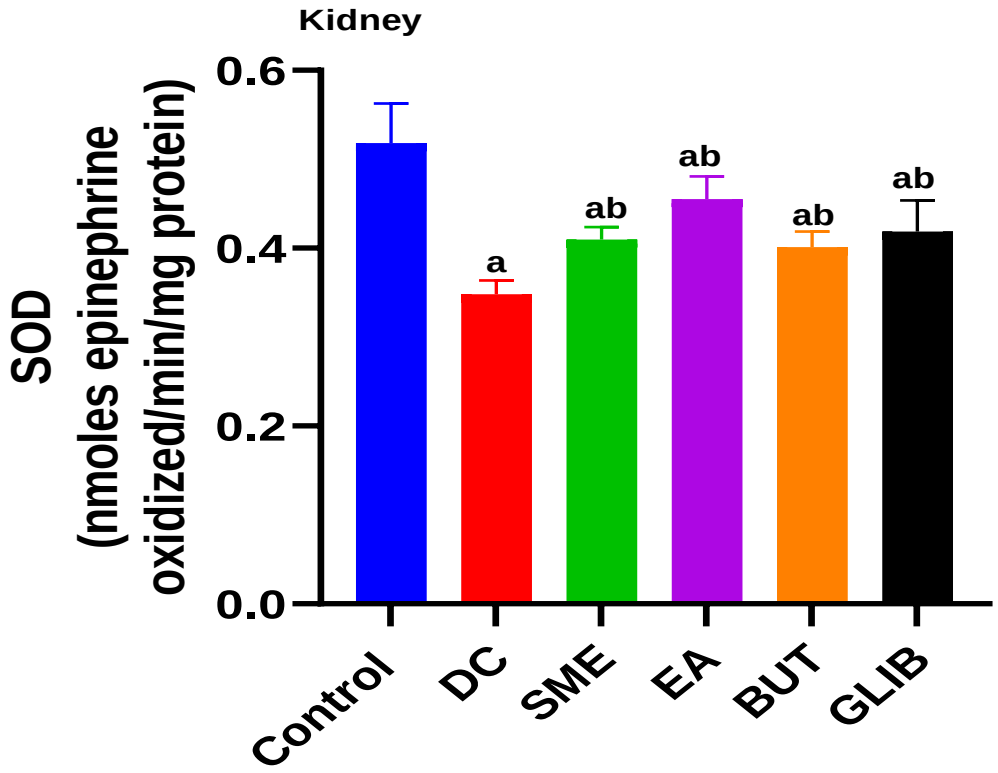


***Solanum macrocarpon* extract and fractions**

Figure 4.52: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Renal Catalase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.



***Solanum macrocarpon* extract and fractions**

Figure 4.53: Effect of *Solanum macrocarpon* Linn. Extract and Solvent Fractions on Superoxide Dismutase of Diabetic Rats

[a] $p < 0.05$ when control group was compared with experimental groups, [b] $p < 0.05$ when diabetic group was compared with treated groups.

Legend: DC: Diabetic control; SME: *Solanum macrocarpon* extract; EA: Ethyl actate fraction; BUT: Butanol fraction; and GLIB: Glibenclamide.

4.1.9 Effect of *Solanum macrocarpon* Linn. Extract And Solvent Fractions on Histopathology on Streptozotocin Induced Diabetic Male Wistar Rats.

Histological examinations of kidneys, pancreas and liver tissues of diabetic rats revealed degenerating changes when compared with the control rats. However, on treatment ethylacetate fraction damages done histologically to these tissues were restored.

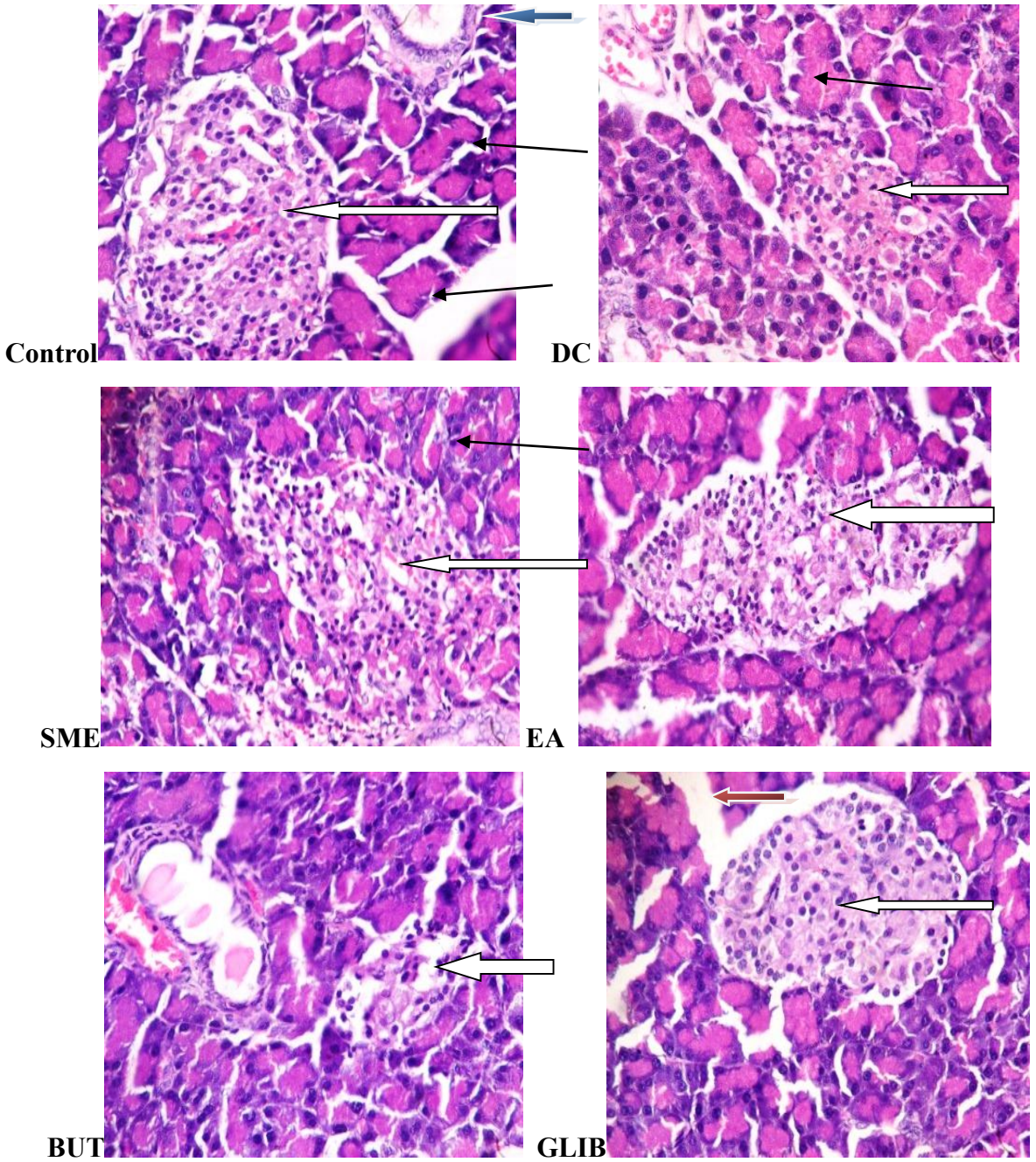


Figure 4.54: Effects of Methanol Extract of *Solanum Macrocarpon* and Fractions on Histopathology of Diabetic Rats. Pancreas

DC=Diabetic control, SME=Methanol extract of *Solanum macrocarpon*, EA= Ethyl acetate fraction of methanol extract of *Solanum macrocarpon* BUT = Butanol fraction of methanol extract of *Solanum macrocarpon* GLIB=Glibenclamide

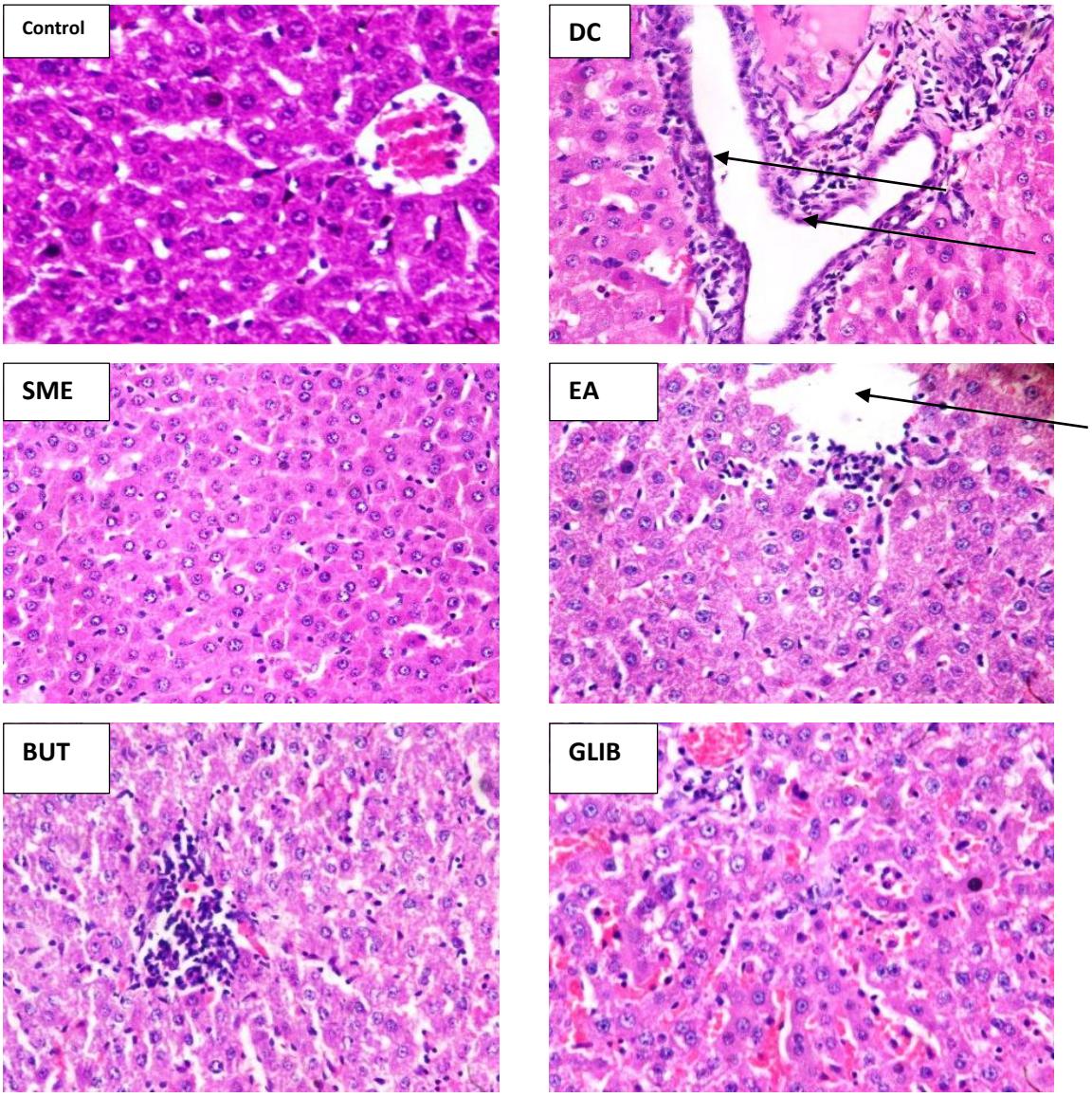


Figure 4.55: Effects of Methanol Extract of *Solanum Macrocarpon* and Fractions on Histopathology of Liver of Diabetic Rats.

DC=Diabetic control, SME=Methanol extract of *Solanum macrocarpon*, EA= Ethyl acetate fraction of methanol extract of *Solanum macrocarpon* BUT = Butanol fraction of methanol extract of *Solanum macrocarpon* GLIB=Glibenclamide

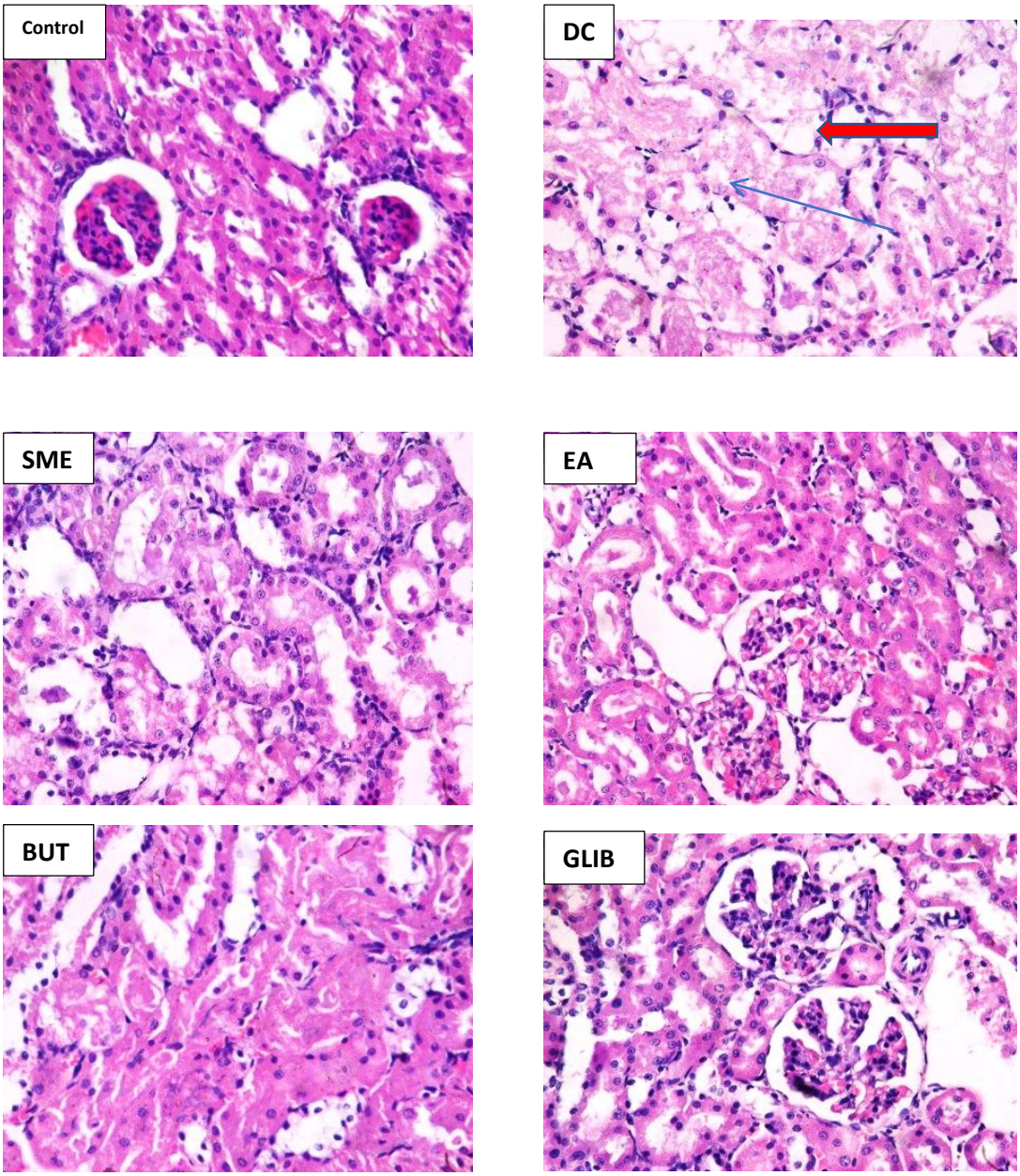


Figure 4.56: Effects of Methanol Extract of *Solanum Macrocarpon* and Fractions on Histopathology of Kidney of Diabetic Rats.

DC=Diabetic control, SME=Methanol extract of *Solanum macrocarpon*, EA= Ethyl acetate fraction of methanol extract of *Solanum macrocarpon* BUT = Butanol fraction of methanol extract of *Solanum macrocarpon* GLIB=Glibenclamide

4.1.10 Identification of the Functional Groups of *Solanum macrocarpon* Linn. Extract and Solvent Fractions Using FT-IR Spectrometry

FT-IR technique was used in determining functional groups present in *Solanum macrocarpon* extract and fractions

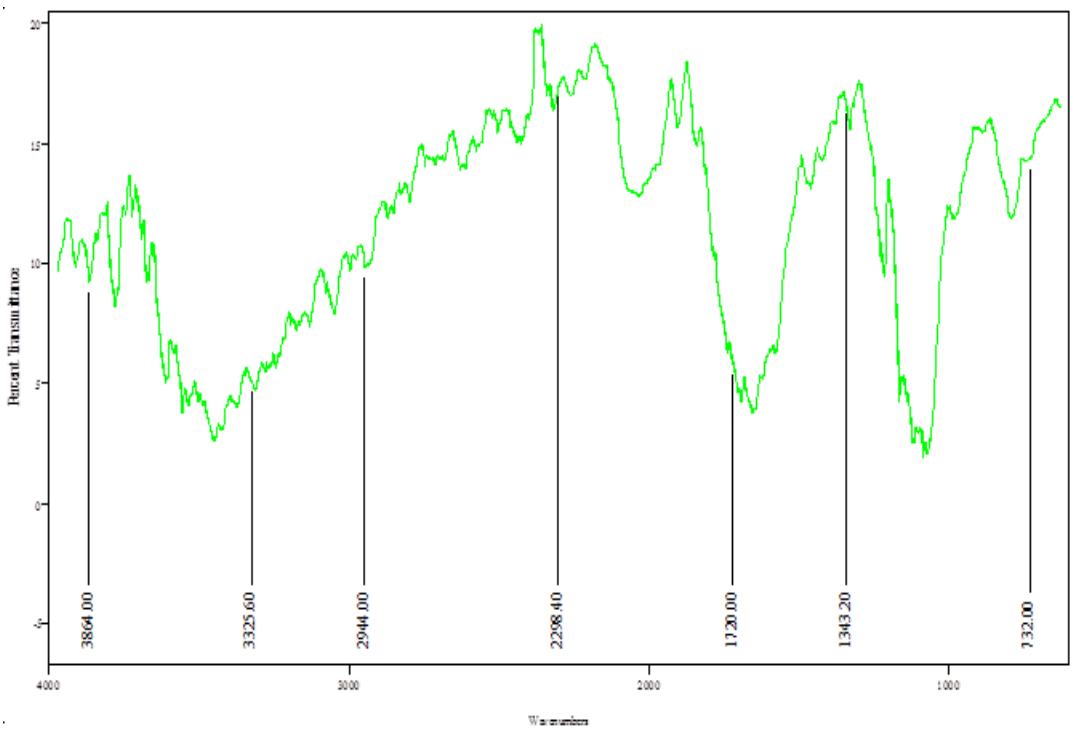


Figure 4.57: FT-IR Chromatogram of Methanol Extract of *Solanum macrocarpon* Crude Extract

Table 4.22: FT-IR Functional Groups Analysis of Methanol Extract of *Solanum macrocarpon* Linn.

Peak at	Peak height	Functional group
732.00	14.36	Methylene
1343.00	16.74	Methyne
1720.00	5.83	Carboxylic acid
2298.40	17.41	Aliphatic cyanide/nitrite
2944.00	9.84	Methyl
3325.60	5.13	Alcohol
3864.00	9.25	Hydroxy

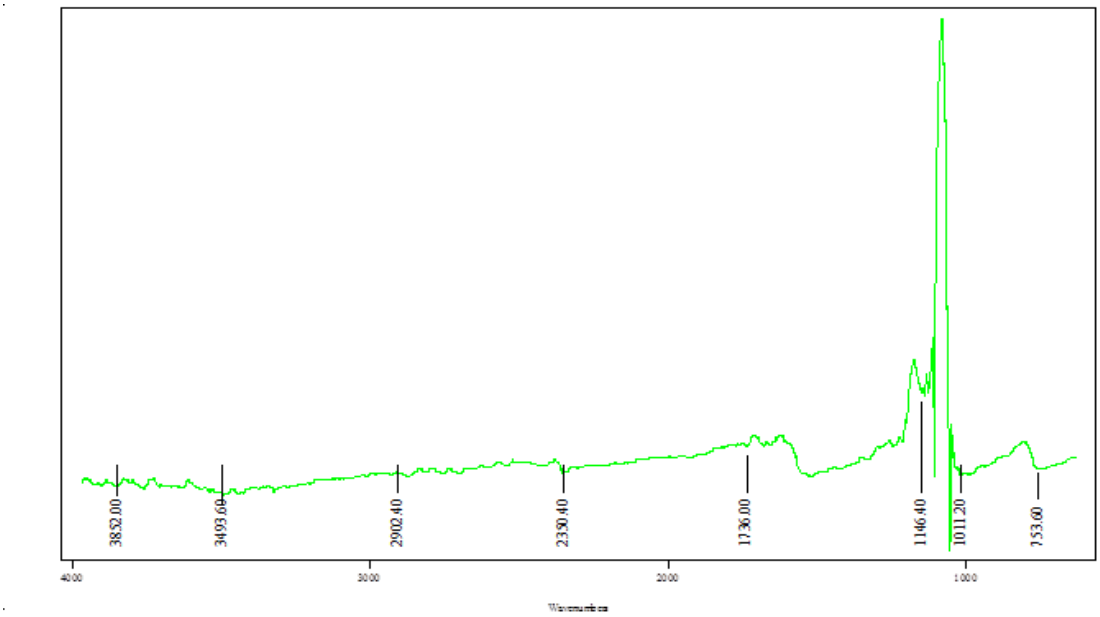


Figure 4.58: FT-IR Chromatogram of Butanol Fraction of Methanol Extract of *Solanum macrocarpon*

Table 4.23: FT-IR Functional Groups Analysis of Butanol Fraction of Methanol Extract of *Solanum macrocarpon* Linn.

Peak at	Peak height	Functional group
753.60	12.89	Aryl
1011.20	10.65	Cyclohexane
1146.40	39.99	Ether
1736.00	20.84	Esther
2350.40	11.68	Alkyne
2902.40	11.71	Methyne
3493.00	3.64	Aromatic primary amine
3852.00	6.82	Hydroxy

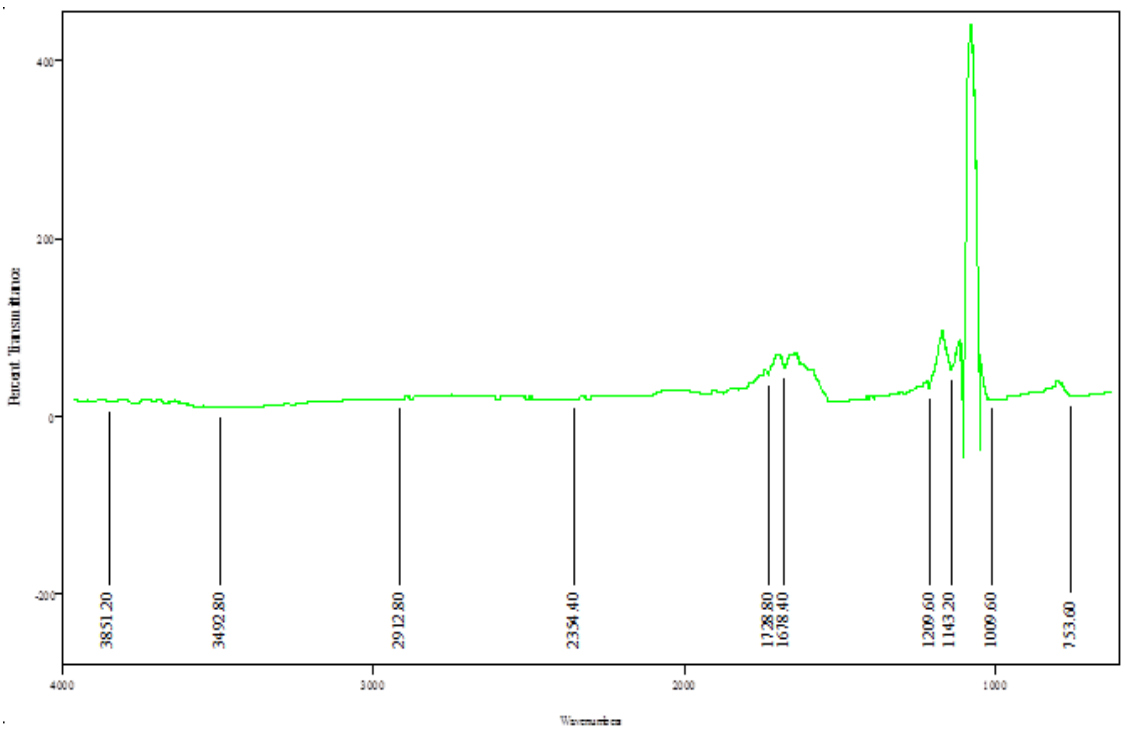


Figure 4.59: FT-IR Chromatogram of Ethyl Acetate Fraction of *Solanum macrocarpon* extract

Table 4.24: FT-IR Functional Groups Analysis of Ethyl Acetate Fraction of Methanol Extract of *Solanum macrocarpon*

Peak at	Peak height	Functional group
753.60	20.66	Aryl
1009.60	19.44	Cyclohexane
1143.20	51.72	Ether
1209.60	32.07	Tertiary amine
1678.40	52.91	Amide
1728.80	46.69	Ester
2354.40	18.47	Alkyne
2912.80	19.25	Methyne
3492.80	8.25	Aromatic Primary Amine
3851.20	16.12	Hydroxy

4.1.11 Identification of the Constituents of *Solanum macrocarpon* Linn. extract and Solvent Fractions Using GC-MS

GC-MS method was employed in identifying active constituents present in *Solanum macrocarpon* extract and fractions

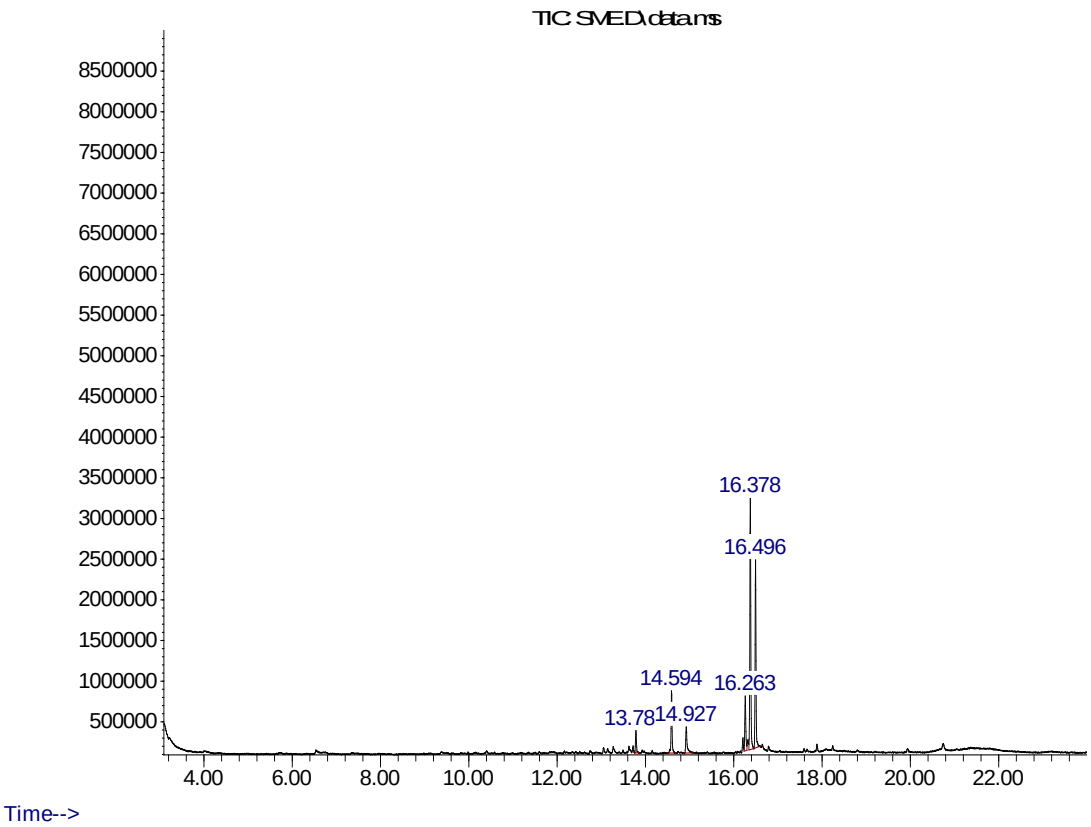
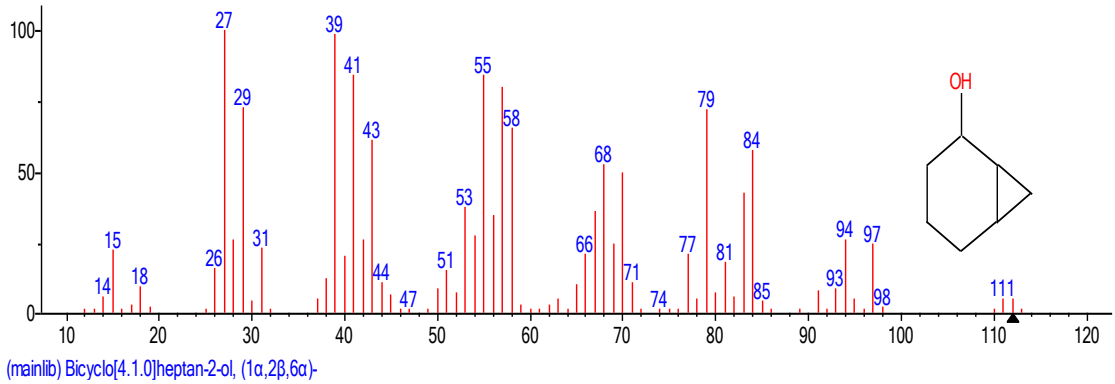


Figure 4.60: GC-MS Chromatogram of Methanol Extract of *Solanum macrocarpon*



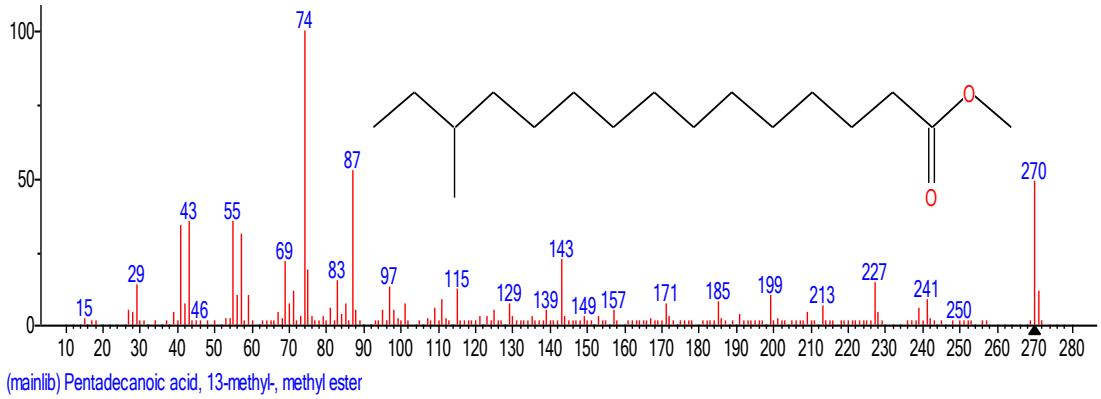
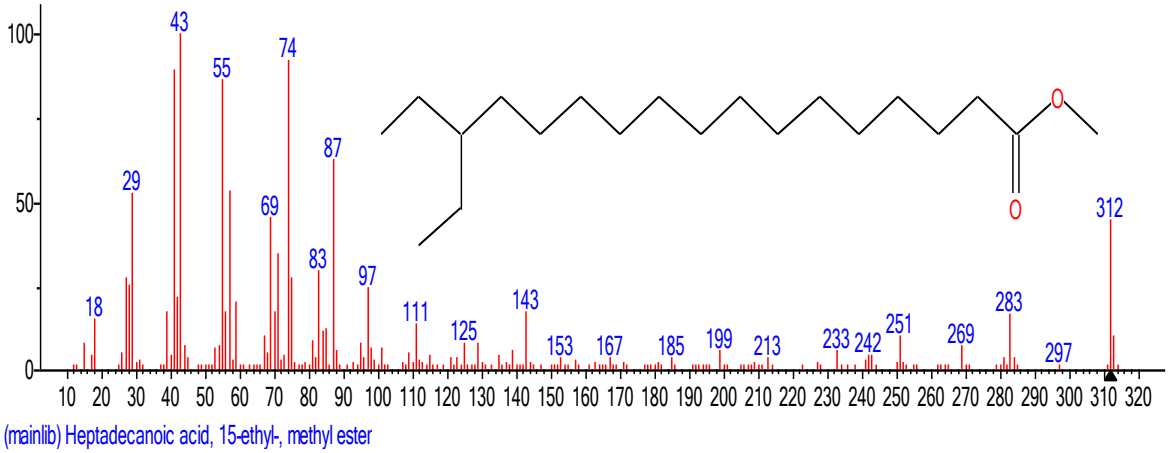


Figure 4.61: Compounds Identified in Methanol Extract of *Solanum macrocarpon* Linn.



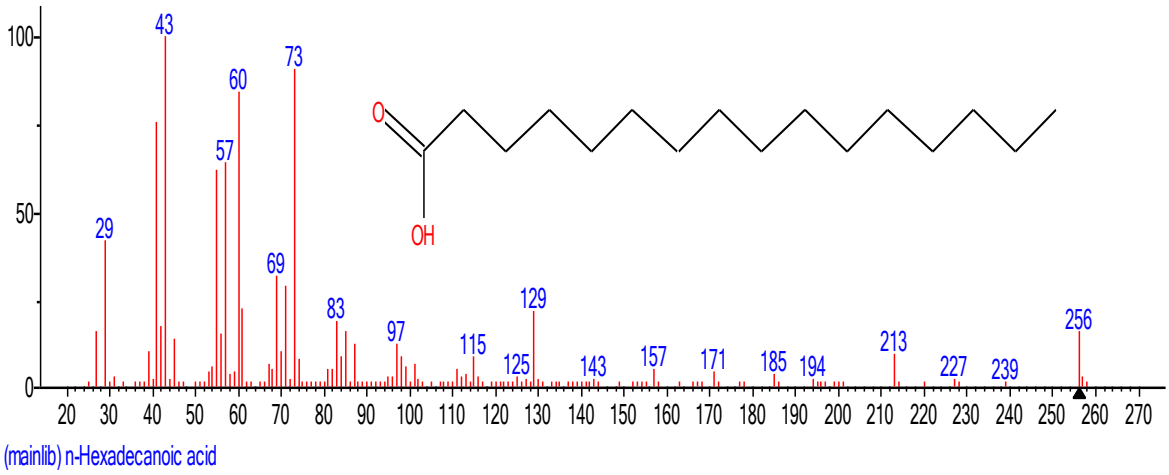


Figure 4.62: Compounds Identified in Methanol Extract of *Solanum macrocarpon* Linn.

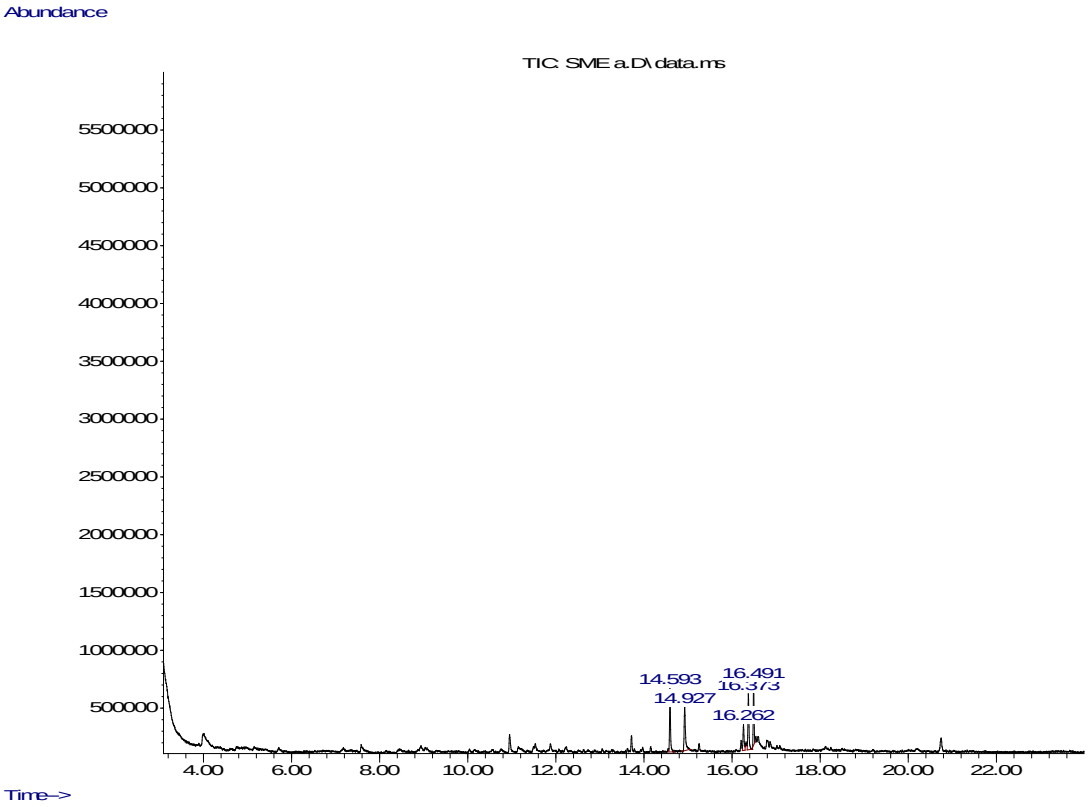
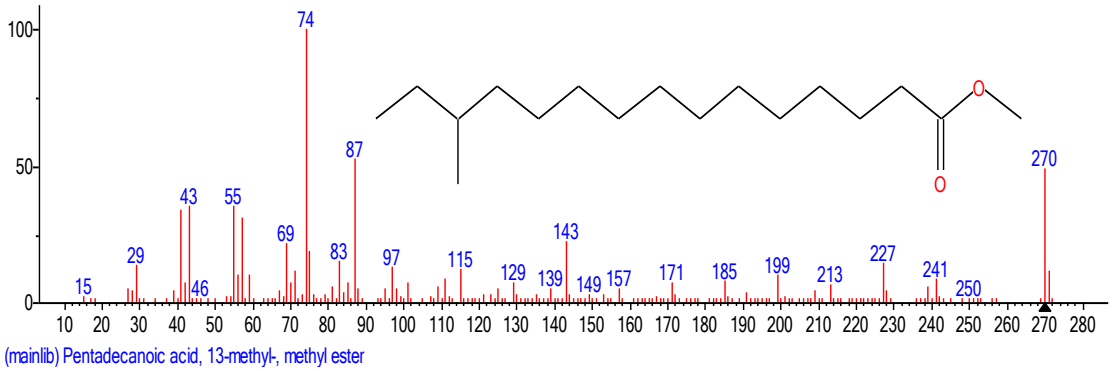


Figure 4.63: GC-MS Chromatogram of Butanol Fraction of Methanol Extract of *Solanum macrocarpon* Linn.



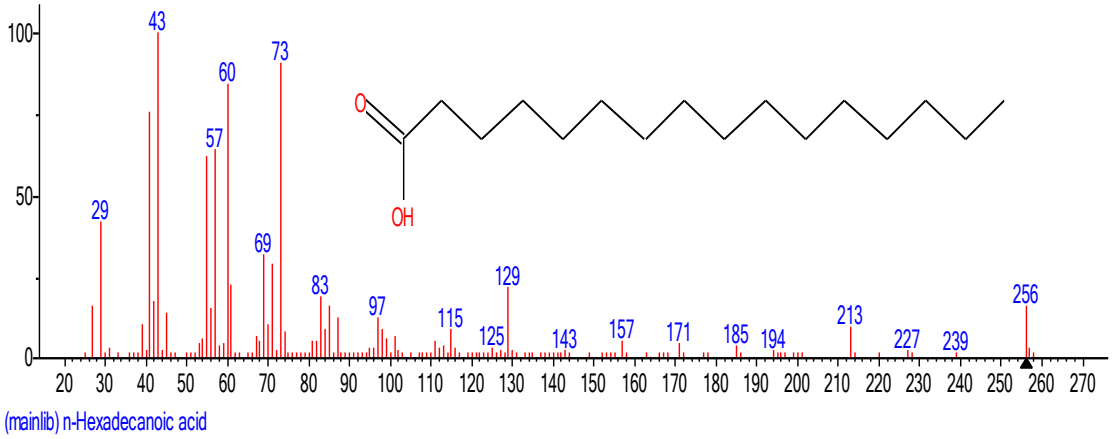


Figure 4.64: Compounds Identified in Butanol Fraction of Methanol Extract of *Solanum macrocarpon* Linn.

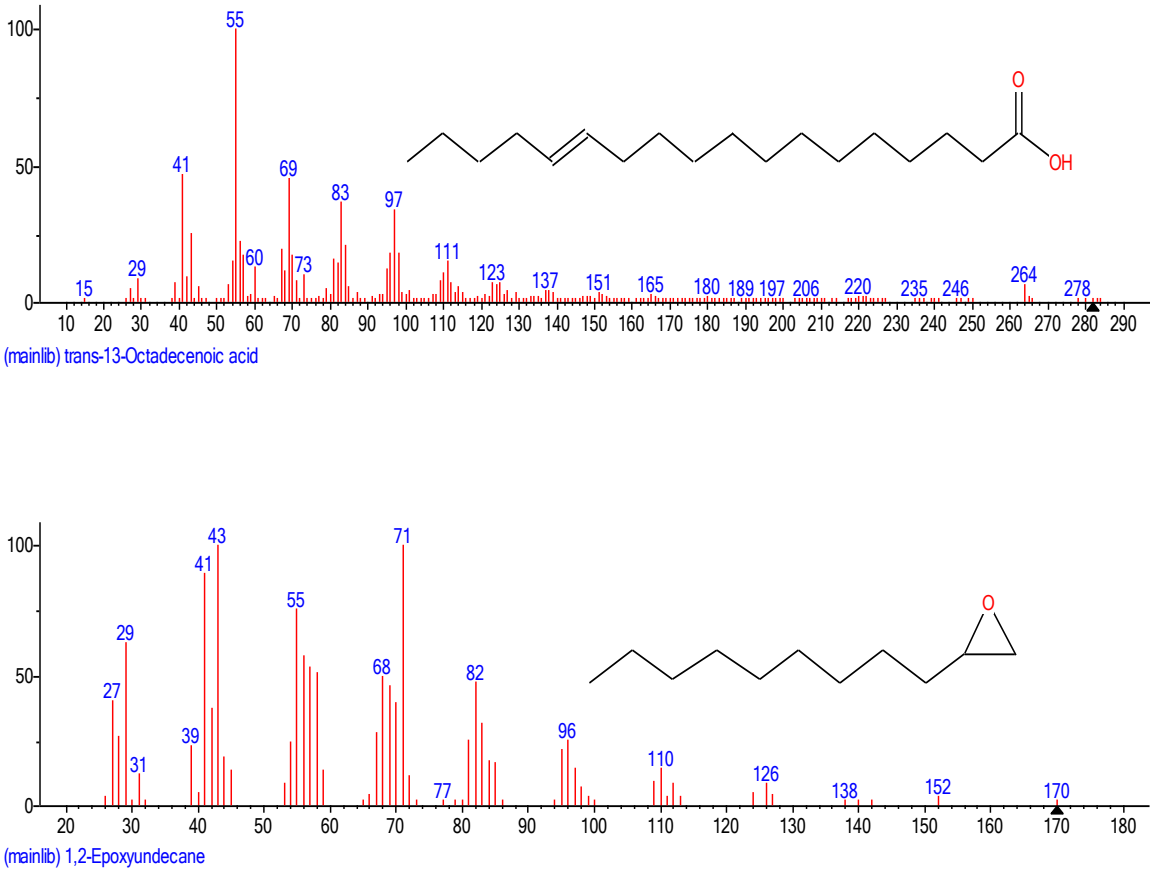


Figure 4.65: Compounds Identified in Butanol Fraction of Methanol Extract of *Solanum macrocarpon* Linn.

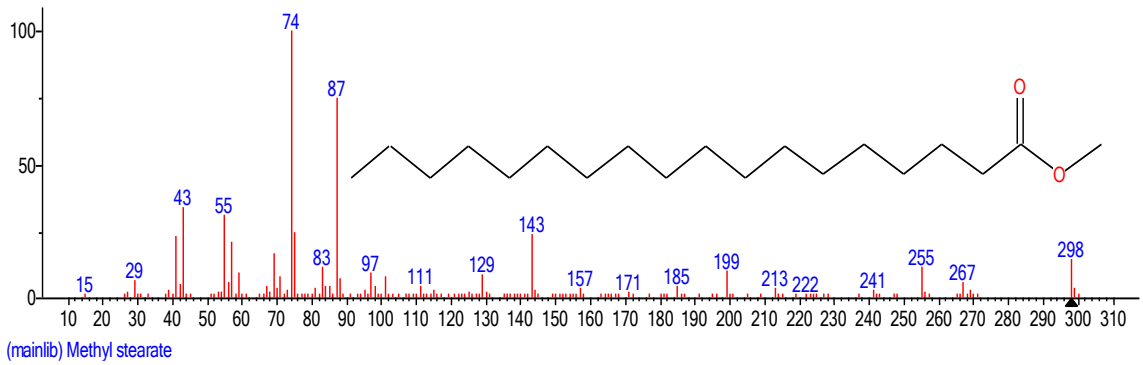


Figure 4.66: Compounds Identified in Butanol Fraction of Methanol Extract of *Solanum macrocarpon* Linn.

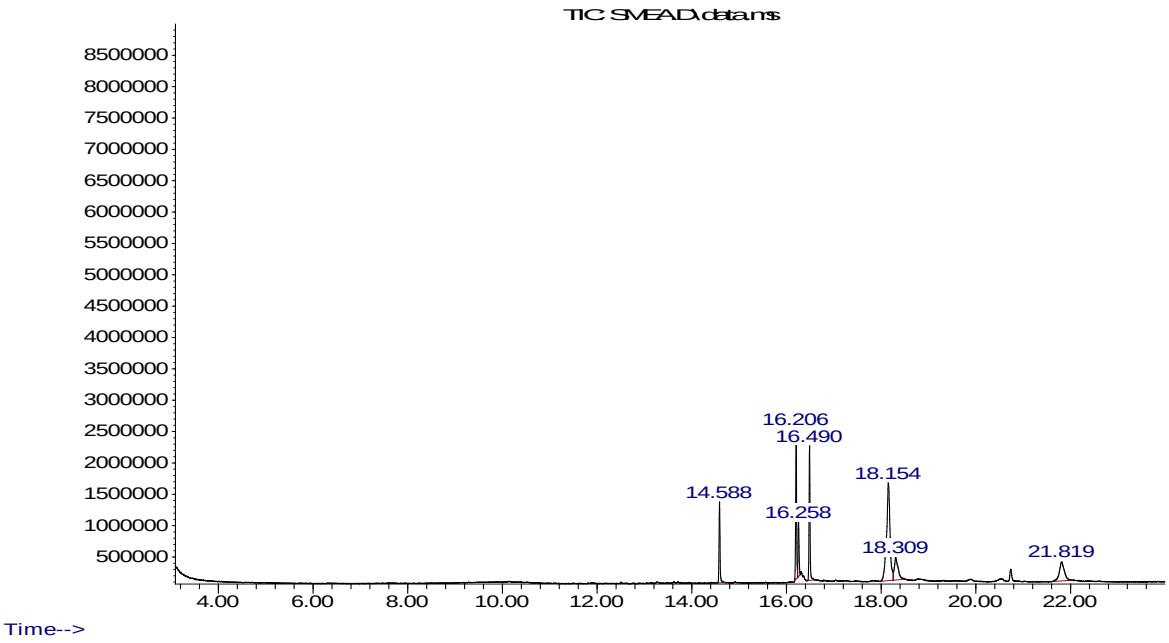
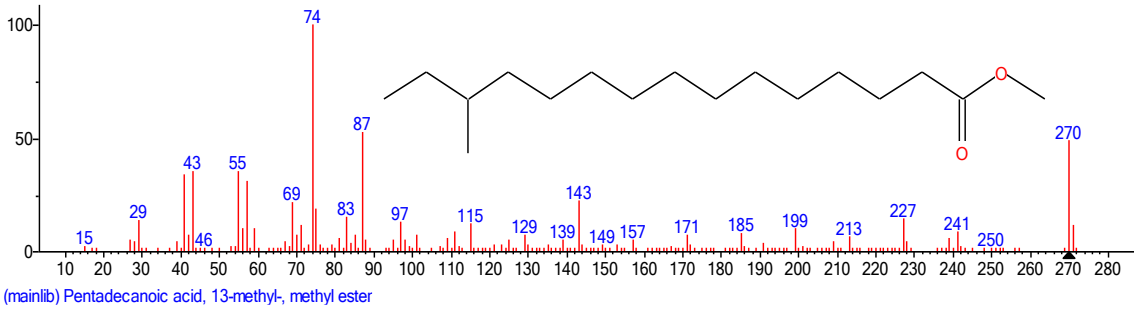


Figure 4.67: GC-MS Chromatogram of Ethyl Acetate Fraction of Methanol Extract of *Solanum macrocarpon* Linn.



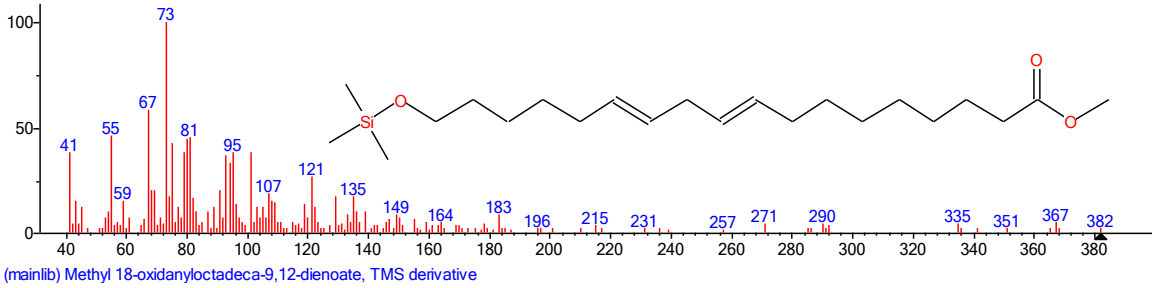


Figure 4.68: Compounds Identified in Ethyl Acetate Fraction of Methanol Extract of *Solanum macrocarpon* Linn.

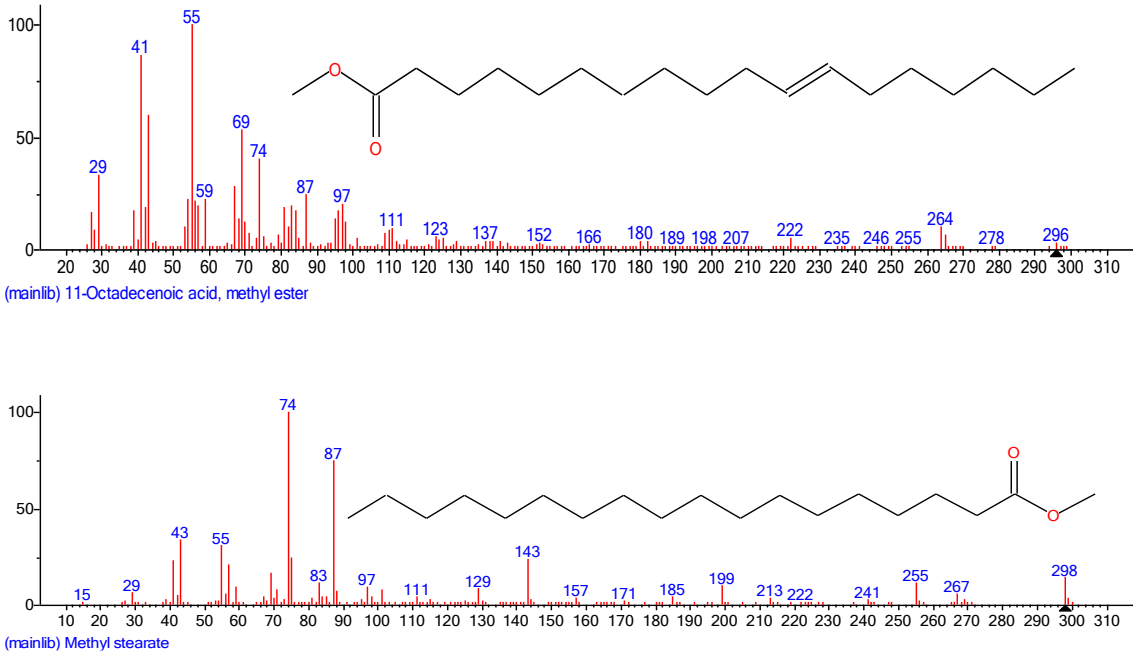
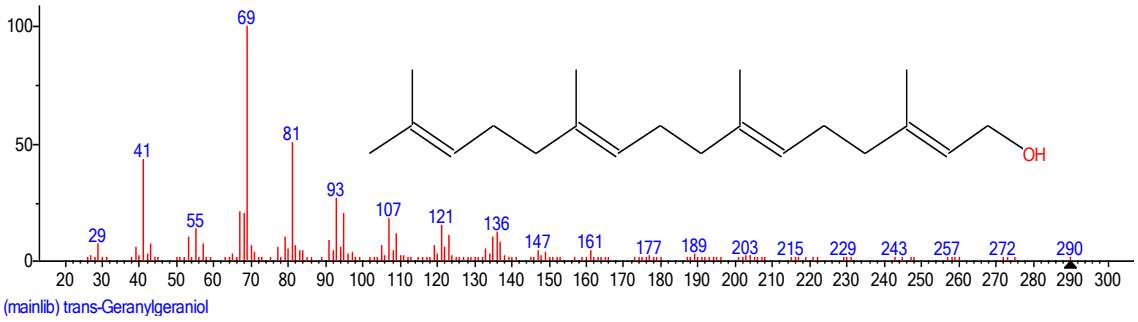


Figure 4.69: Compounds Identified in Ethyl Acetate Fraction of Methanol Extract of *Solanum macrocarpon* Linn.



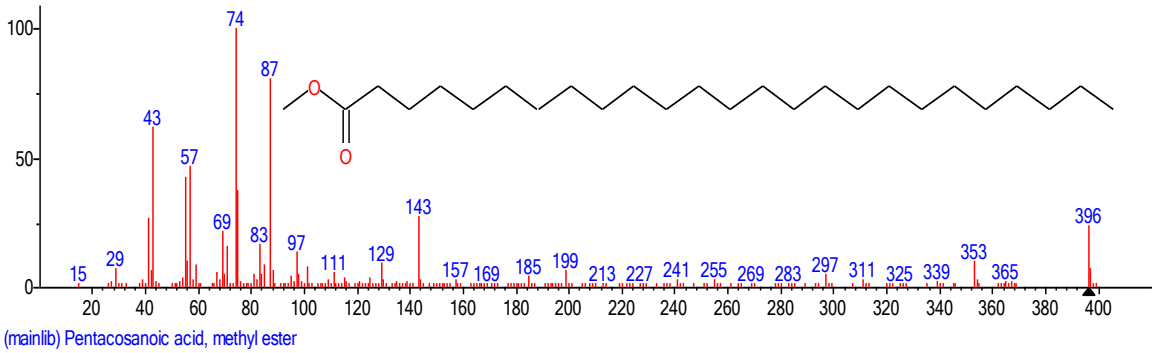


Figure 4.70: **Compounds Identified in Ethyl Acetate Fraction of Methanol Extract of *Solanum macrocarpon* Linn.**

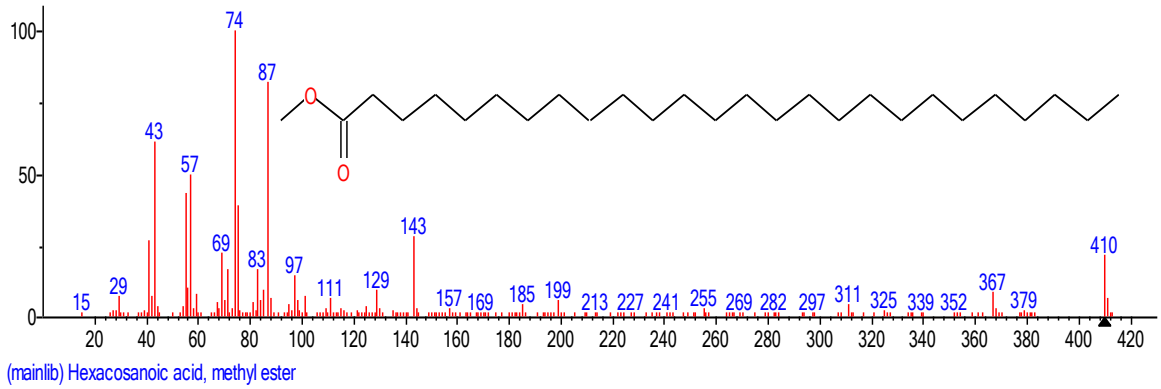


Figure 4.71: Compounds Identified in Ethyl Acetate Fraction of Methanol Extract of *Solanum macrocarpon* Linn.

Table 4.25: Compounds Identified by GC-MS

Crude extract of the *Solanum macrocarpon* Linn.	**Butanol fraction of the *Solanum macrocarpon* Linn extract**	**Ethyl acetate fraction of the *Solanum macrocarpon* Linn extract**
Bicyclo [4,1,0]heptan-2-ol, (1α, 2β, 6α)	Pentadecanoic acid, 13-methyl-methyl Ester	Pentadecanoic acid, 13 methyl, methyl Ester
Pentadecanoic acid, 13 methyl, methyl Ester	*n*-Hexadecanoic acid	Methyl 18-oxidanyloctadeca-9,12 dienoate, TMS derivatives
n-Hexadecanoic acid	trans-13-octadecenoic acid	11- octadecanoic acid, methyl, methyl Ester
Heptadecanoic acid, , 15 ethyl, methyl Ester	1,2-epoxyundecane	Methyl Stearate
	Methyl Stearate	trans-Geranylgeraniol
		Pentacosanoic acid, methyl Ester
		Hexacosanoic acid, methyl Ester

CHAPTER FIVE

DISCUSSION

The oldest type of healthcare that mankind has ever known is traditional medicine (Loha *et al*., 2019). Plants have historically played a vital part in the global health maintenance because all civilizations and cultures employ remedies or medications made from plants (Loha *et al*., 2019). Nowadays, a lot of common drugs have herbal origins or roots. Because modern medicine has failed to effectively treat chronic conditions like diabetes and with the advent of multidrug-resistant bacteria and parasites, the use of traditional medicine has increased in wealthy or industrialised nations as well (Capela *et al*., 2019). In this investigation, Streptozotocin-induced diabetic male Wistar rats were used as test subjects to determine both antioxidative and anti-diabetic potentials of *Solanum macrocarpon* Linn. methanol leaf extract and solvent fractions.

5.1 Phytochemical Screening

The antecedents of this vegetable in traditional medicine practice motivated research. Preliminary phytochemical screening was conducted. Following the standard methodology for extraction and fractionation, phytochemical screening was done on both crude methanol leaf extract (SME) and solvent fractions such as hexane fraction (HEX), chloroform fraction (CHL), ethyl acetate fraction (EA), methanol fraction (MET), and butanol fraction (BUT). Alkaloids, anthraquinones, saponins flavonoids, phytodterols, diterpenes, steroids, phenols, tannins, and terpenoids were all detected in crude extract and solvent fractions using simple procedures in basic qualitative phytochemical screening, but cardiac glycosides and carbohydrates were not (Table 4.10). This discovery is consistent with research findings from Ilodibia *et al.,* 2016 who stated that tannins, flavonoids, saponins, and alkaloids were present; Ajiboye *et al.,* 2014 who noted absence of inulin but stated that saponins, alkaloids, flavonoids and tannins were present; Doughnon *et al.,* 2012 who reported that alkaloids, tannins and saponins were present. Differences of numbers in phytochemicals reported by these workers might be due to variations of the edaphic

and other factors. Since polarity, solubility, and nature of antidiabetic and that of antioxidant bioactive components in *Solanum macrocarpon* leaf were unknown, sequential extraction approaches employing various solvents in rank of polarity (n-hexane, chloroform, ethyl acetate, n-butanol, and methanol) were utilised in this study to separate solvent fractions from the crude extract.

Typically, low polarity substances notably fatty acids, terpenoids, waxes, and certain alkaloids are extracted using n-hexane (Azwanida, 2015) while medium polarity and polar chemical components, notably some terpenoids, flavonoids, and tannins are extracted using chloroform and ethyl acetate (Azwanida, 2015). Highly polar compounds including complex sugars, amino acids, and substances derived from them are extracted using n-butanol (Azwanida, 2015). The reported bioactivities of this vegetable could be caused by the presence of these phytochemicals.

The biosynthesis of phytochemicals, which are secondary metabolites, involves four different types of metabolic pathways: the shikimic-acid, malonic-acid, mevalonic-acid, and methylerythritol-phosphate (MEP) pathways (Azwanida, 2015). The phrase "secondary metabolites," also referred to as "specialised metabolites," was originated by Albrecht Kossel, a 1910 Nobel laureate in medical science. Other names given include natural goods, secondary goods and toxins. Any life-form including fungi, bacteria, animals, and plants, produces organic chemicals known as secondary metabolites. Secondary metabolites arbitrate environmental interplay that could give a creature a selective edge through survival boosting including prolificacy rather than straight involvement in the creature's normal developmental processes (Ramakrishna and Ravishankar, 2011). The antibiotic, antifungal, and antiviral properties of these secondary intermediates shield and protect them from pathogens, UV damage (Bourgault *et al.,* 2011). Since phytochemicals are primarily used by plants for defence, efforts have been made to employ them therapeutically (Rai *et al.,* 2013). Since antiquity, humans have been aware of physiological impacts of phytochemicals that originated from plants. Over two thousand years ago, Chinese herbal medicine commenced using the plant *Artemisia annua*, which is endowed with the active ingredient artemisinin (Arsenault *et al.,* 2008). Alkaloids, one of the prime groups of plant constituents are available in many medicinal plants and they function as anesthetic agents (Wadood *et al.,* 2013). They are noted to be one of the most numerous and varied classes of natural substances and endowed with complex

molecular structures. Saxena *et al.,* 2013, suggested that alkaloids possess avalanche of therapeutic activities including antimalarial (quinine), antiarrhtmic, antihypertensive (many indole alkaloids) and anticancer activities.

According to reports, Saponins display a variety of physiological functions, encompassing anticholesterolemic effect because they form a complex with cholesterol in the digestive tract thus inhibiting or preventing its absorption (Sharma and Paliwal, 2013). There have been reports of spanonis having biological actions that are analgesic, sedative, antihelmintic, antibacterial, antitumor, anti-inflammatory, haemolytic, pesticidal, insecticidal, and molluscidal (Hassan *et al.,* 2012). Numerous studies have demonstrated how saponins have anticancer, hypocholesterolemic, membrane-permeabilizing, and immunostimulant characteristics (Francis *et al.,* 2002).

Sterols- Humans cannot synthesise phytosterols; as a result, they must consume food that contains them (Boukes *et al.,* 2008). The fascinating pharmacological, medical, and agrochemical properties of sterols have been described. These properties include antibacterial, antihelmintic, anticancer, hepatoprotective, immunosuppressive, cytotoxic, plant growth hormone regulator, and cardiotonic or inotropic properties (Boukes *et al.,* 2008).

Glycosides-The vast arrays of different chemicals that make up glycosides are found in almost all plants. Many plants store medicinally important substances in the form of active glycosides, which function as cardiac medicines, anti-tuberculosis, anti-inflammatory, anti-rheumatics, analgesics, emollients, renal antiseptics, laxatives, and expectorants (Hemmalakshmi *et al.,* 2016; Gomathi *et al.,* 2012).

Flavonoids - Flavonoids, the most well-known important phenolic chemicals, have a variety of chemical and biomedical effects (Kumar *et al.,* 2014). According to Ugochuckwu *et al.,* 2013, phenolic compounds, also flavonoids from natural products have been demonstrated to elicit a number of physiological effects, among which are anti-inflammatory, anti-carcinogenic, antioxidant, and free-radical scavenging capabilities. According to reports, flavonoids are efficient and formidable free-radical scavengers of dioxygen together with numerous other ROS and RNS linked to DNA damage and cancer, making them useful treatments for a variety of disease states like cardiovascular, cancer, and neurodegenerative (Rudrapal *et al*., 2022).

Polyphenol- Plant foods high in polyphenols have been shown to have effects similar to those of insulin and can act as potent inhibitors of certain enzymes, including –α-amylase and pancreatic lipase, which are linked to type 2 DM, obesity, and lipid peroxidation (Santhiya *et al.,* 2016). Polyphenols, which are divided into groups like flavonoids, tannins, and stilbenes, have been shown to have health-promoting properties such as the competence to scavenge ROS and RNS, block hydrolases, oxidases, and peroxidases, act as an anti-inflammatory, and have anti-diabetogenic potential (Patel *et al.,* 2011). Researchers have firmly entrenched that naturally occurring phenolics have anti-cancerous, anti-microbial, and neuroprotective properties. They also help the body produce more insulin, and help to reduce excess body fat (Kaur *et al.,* 2021).

Natural products separated into terpenoids, alkaloids, flavonoids, phenolics and other groups have exhibited antidiabetic ability via different mechanisms of action (Patel *et al.,* 2012).

Medicinal plants have produced more than 50,000 secondary metabolites (Teoh*,* 2015). Secondary metabolites perform specialist activities or serve as defensive compounds in plants. Secondary metabolites are endowed with anti-diabetic properties. Flavonoids, alkaloids, phenolics, terpenoids, saponins, xanthones, polysaccharides, and other substances have been found to have antidiabetic effect (Salehi *et al.,* 2019).

Unique components of *Solanum* species, particularly *Solanum macrocarpon* and other species like *Solanum torvum* and *Solanum aethiopicum*, include phenolics, flavonoids, and flavonols. These compounds may be responsible for playing a crucial role in the adsorption and deactivation of ROS, RNS, quenching singlet oxygen, and degrading peroxides (Ilodibia *et al.,* 2016). All these activities have exposed the potentiality of *Solanum macrocarpon* as a valid candidate for drug discovery. In research institute, and pharmaceutical companies, phytochemical testing of medicinal plants is essential, especially with regard to the production of novel pharmaceuticals employed in treating a variety of disorders (Wadood *et al.,* 2013).

5.2 *In Vitro* Antiradical and Antioxidant Activity

From literature review and as discussed above, phenolics, flavonoids, alkaloids and saponins are the major groups of phytochemicals with anti-oxidative potentials and anti-diabetic properties. As a result, they have been authenticated to be significant in

DM. They have the capacity and dependability to remove ROS and RNS, guard against oxidative damage, and reduce the generation of lipid peroxide during DM.

This work examined *in vitro* both anti-radical and antioxidantive properties of the methanol leaf crude-extract of SM along with its solvent fractions utilising a variety of different protocols, such as metal chelating, DPPH free radical scavenging, and nitric oxide scavenging activities. Citric acids, along with ascorbic acid, two natural antioxidants used as reference antioxidants in this investigation, were used. The results of DPPH, nitric oxide, and metal chelating assays were assessed as % inhibition of the free radicals produced, with respect to various concentrations, respectively. Effects that depended on concentration were observed in each example, meaning that higher doses were shown to exhibit more inhibition in each assay. Significant evidence of suitability to scavenge offenders is demonstrated by sharp drop in absorbance of reaction. We assessed the IC50 and DPPH-% inhibition.

Consuming antioxidant-rich vegetables is incredibly beneficial to human health because they protect the body from damaging ROS and RNS and slow the progression of numerous chronic diseases, including diabetes (Khan *et al*., 2016).

The ability of the antioxidants to donate hydrogen, as described in chapter 3 (Baumann, 1979), as well as proton donation or election transfer, affects how active they are against DPPH. Free radicals are a major factor in the harm done to biomolecules, and DPPH has been employed to detect the free radical scavenging abilities in medicinal plants with antioxidantive properties.

DPPH emits a violet or purple colour when mixed with methanol; in the presence of an antioxidant, it turns to various colours of yellow. The characteritically stable free radical DPPH is nitrogen-centreed, and can be distinguished by its distinctive absorbance of 517 nm. Due to their hydrogen donating or election releasing abilities, antioxidants react with stable DPPH and transform to reduced form at a very quick rate (Yamaguchi *et al.,* 1998). The reduction in absorption, which occurs as antioxidants release or donate protons to this radical, is interpreted as a measure of the antioxidants' capability to scavenge ROS and RNS. According to Brand-Williams *et al.,* 1995, the reducing caability of antioxidants against DPPH is determined by observing absorbance at 517nm against varied concentrations of the plant extract. It follows that at higher concentration, plant extract trapped more free-radicals generated by DPPH

leading to decrease in absorbance and increase percentage of DPPH scavenging effect. One unique characteristic of this method is the ability to permit testing of both hydrophilic and lipophilic compounds (Koleva *et al.,* 2002) when compared with other methods that are limited in the nature of the antioxidants that they can be employed to quantify. Given all of these facts, the DPPH test is among the extensively employed methods for evaluating antioxidative properties of extracts from plant origin (Nanjo *et al.,* 1996). Due to its speed, simplicity, and repeatability (Ozcelik *et al.,* 2003), as well as the fact that reactions in this approach are unaffected by side reactions (Livani *et al*., 2013), the DPPH protocol has been utilised to detect the antioxidative potentials of numerous substances. In this investigation, the addition of SM extract and its solvent fractions reduced the purple coloration of DPPH to a yellow-colored product, 1,1-diphenyl-2-picryl hydrazine, dose-dependently. At the least concentration of the standard/crude extract and solvent fraction (0.2 mg/mL), percentage DPPH scavenging activities of ascorbic acid, SME, HEX, CHL, EA, BUT and MET 94.97 ± 8.15; 62.08 ± 7.90; 53.28 ± 9.78; 59.32 ± 5.90; 60.73 ± 9.84; 98.89 ± 8.54 and 49.81 ± 12.00 respectively, while at highest concentration (1.0 mg/mL) the corresponding activities were 95.37 ± 9.87; 94.54 ± 8.56; 74.43 ± 9.79; 87.79 ± 9.76; 92.61 ± 9.85; 95.82 ± 10.00 and 96.14 ± 12.98 proportionately. At (1.0 mg/mL); the radical scavenging activities for MET, BUT and SME were slightly greater, compared to that of ascorbic acid. All of the participants' activity was seen to vary at various concentrations. These findings indicated that at 0.2 mg/mL, there was no appreciable difference between BUT and ascorbic acid in terms of activity, but there was a discernible difference between BUT and other fractions. At 1.0 mg/mL, however, there was no detectable difference between the activities of BUT, EA, MET, including SME when compared with ascorbic acid. It can be inferred that the activities of the extracts and fractions are relatively comparable to that of ascorbic acid (Table 4.11). This finding suggests the presence of phenolic chemicals in crude extract and solvent fractions, which may account for the antioxidative effects exhibited. Phenolic compounds and flavonoids have been revealed to show scavenging effect for free-radicals (Matthew *et al.,* 2015). Ascorbic acid is a very powerful free-radicals scavenger and therefore comparing to such a pure compound, the methanol crude extract and solvent fractions exhibited significant scavenging activity.

From DPPH assay, IC_{50} value, the inhibition capacity at 50%, a measure of the extract (including fractions) concentration needed for 50% inhibition of free-radical DPPH was assessed. IC_{50} was calculated using Graphpad Prism version 5. In this study, the results of IC_{50} values for DPPH radicals of extract and fractions were EA, BUT, CHL, HEX, SME and MET, 0.04046 ± 0.18, 1.033 ± 0.57, 3.225 ± 1.22, 6.651 ± 1.28, 7.432 ± 0.71, 143.1 ± 0.27 µg/mL respectively as compared to ascorbic acid 428.2 ± 0.79 µgmL (Table 4.12). The level of antioxidant activity of the sample is inversely correlated with the IC_{50} value (Li *et al.,* 2012; Xiao *et al.,* 2011). Surprisingly, the IC_{50} data for crude and fractions exhibited were all lower than those for ascorbic acid. This is consistent with the outcome of the research by Phongpaichit *et al*. (2007), that found that IC_{50} concerning various extracts from fungi isolated from *Garcinia* spp. ranged from 2 to 4 µg/mL and were roughly 1000 times less potent than tannin, which was utilised as the standard substance and had an IC_{50} of 2000 µg/mL. Also Ilahi *et al.,* 2013 reported that the IC_{50} of *Pistacia integerrima* was 5.75 ppm as compared to ascorbic acid having IC_{50} -15.09 ppm. Other researchers have reported IC_{50} value of extract being lower than that of ascorbic acid and many researchers have reported different IC_{50} value for ascorbic acid including James *et al.,* 2011. According to Geleti, 2017, the value of ascorbic acid could be affected by the environment condition such as temperature since DPPH is involved, also DPPH is 1-electron oxidant while ascorbic acid is 2-electron reductant and that there is no single standard IC_{50} value for ascorbic acid. According to the current findings, EA possesses the best antioxidative effect and fractions together with crude extract are more effective free radical scavengers than ascorbic acid. Pongpaichit *et al.* (2007) also revealed that extract which are not effective in DPPH system may have strong activity towards hydroxyl radicals due to iron chelation, than straight quenching of radicals. Also extract activity may be due to the presence of superoxide dismutase – like properties. According to Phongpaichit *et al.* (2007) in the DPPH assay, extract with IC_{50} > 250 µg/mL is inert; with IC_{50} > 100 - 250 µg/mL is weakly active; with IC_{50} > 50 - 100 µg/mL is passably effective; with IC_{50} from 10-50 µg/mL is significantly active; and with IC50 10 µg/mL is energetically effective. From this study, EA, BUT, CHL, HEX, and SME can be classified as strong antioxidant while MET and ascorbic acid as weak antioxidant. The current result indicates that both the crude extract and fractions contain phytochemicals that can donate hydrogen to free radicals to scavenge potential offenders and possibly also have additional qualities like super-oxide-dismutase-like activity.

The extract's ability to scavenge nitric oxide was measured as a percentage of inhibition, and at 500 µg/mL this percentage was 77.42 % (Table 4.6).

SME's % inhibition evaluation of its capacity to chelate metals revealed that it was dosage dependant. The main significant pro-oxidant in lipid oxidation according to research is Fe. Iron in its ferrous state accelerates lipid peroxidation via the reaction called Fenton which converts both lipid and hydrogen peroxides into reactive oxygen and nitrogen species.

$Fe^{2+} + H_2O_2$ .....Fe^{3+}+ OH + $^{-}$OH

In addition, at a lower rate of 10%, Fe^{3+} can also generate free radicals from peroxides (Loba *et al*., 2010). DNA damage, protein alteration, and peroxidation are possible outcomes of this. In addition to rendering metal ions inert, chelating agents can severely impair processes that require metals (c. By stabilising transition metals, chelating agents prevent the generation of radicals, hence minimising the harm caused by free radicals. Inhibition percentage of SME is about the same strength as that of the citric acid which was used as the standard. While the standard has inhibition % of 64.40 at 500 µg/mL, SME has 48.83% (Table 4.7). Some phenolic substances have been found to show antioxidant capacity through the chelating of metal ions (Zengin and Aktumsek, 2008). Though it was reported that methanol extract of fruit from Solanum macrocarpon has reduced Fe^{2+}/H_2O_2 induced degradation of deoxyribose by Fenton reaction (Nwanna *et al.,* 2013), there has not been much research on the metal chelating activity of the leaves of *Solanum macrocarpon* in literature. Phenolics are plant-derived antioxidants with the ability to chelate metals and possess radical scavenging qualities (Lopes *et al.,* 1999; Bors and Saran, 1987) thus antioxidant potential can be determined by Fe^{2+} chelating ability. Steinera-Asiedu *et al.,* 2012, reported that the leaves of *Solanum macrocarpon* are good sources of antioxidant; and as well Komlaga *et al.,* 2014; Salawu *et al.,* 2013; Olajire and Azeez, 2011; Odukoya *et al.,* 2007 have reported antioxidant activities of *Solanum macrocarpon* leaves.

Evaluation of the extract's reducing power served as an indirect indicator of its antioxidant capacity. It was discovered that the outcome was dose dependent. It has been established that reducing properties function as an antioxidant by hydrogen atom donation in order to disrupt the free radical chain (Gordon, 1990). Absorbannce increase at 700 nm is indicative of increase in reducing ability. The reducing assay

result suggested that the crude extract and some of solvent fractions possess ability to reduce ferric ion. The reducing power ranking is in the order BUT 0.164µg/gAAE, MET 0.141µg/gAAE, SME 0.117µg/gAAE, EA 0.105µg/gAAE, CHL 0.85µg/gAAE and HEX 0.063µg/gAAE respectively.

The TAC of crude extract and fractions were evaluated. Highest overall antioxidant capability for phosphomolybdate reduction was shown by EA. The order reflects the rating EA 0.046µg/gAAE, CHL 0.023µg/gAAE, SME 0.021µg/gAAE, BUT 0.012 µg/gAAE, MET 0.013µg/gAAE and HEX 0.004µg/gAAE respectively (Figure 4.3).

Numerous investigations have shown that the phosphomolybdate scavenging action of medicinal plants is significantly aided by flavonoids and related polyphenols (Sharififar *et al.,* 2009).

Total flavonoid content result is in the order EA 0.231µg/gQE, BUT 0.190µg/gQE, SME 0.090µg/gQE, HEX 0.081 µg/gQE, CHL 0.053µg/gQE and MET 0.051µg/gQE respectively (Figure 4.2).

The results of evaluating the total phenolic content are in the following numerical order: BUT 0.331, µg/gGAE, EA 0.204 µg/gGAE, SME 0.139 µg/gGAE, MET 0.118 µg/gGAE, CHL 0.074 µg/gGAE, and HEX 0.024 µg/gGAE, respectively (Figure 4.1).

The result showed that *Solanum macrocarpon* extract and solvent fractions reduced the rat pancreas homogenate lipid peroxidation caused by Fe^{2+}. Fe2+'s inherent power to catalyse reaction whereby one-electron is transferred, that produces reactive oxygen species, particularly reactive hydroxyl, which is formed from H_2O_2 via the reaction known as Fenton, may be the reason for the MDA value increase in the presence of pro-oxidant Fe2+. Fe is a crucial metal in normal cellular physiological function; however excessive iron could result into cell destruction. Iron can react with $O2^*$ and H_2O_2 to generate OH* and different free radicals that can destroy body cells. When iron is present in its free state, it can also degrade lipid peroxide or molecules, thereby producing peroxyl and alkoxyl radicals that let lipid oxidation spread or propagate (Tangvarasittichai, 2015). Lipid peroxidation has been found to potentially trigger and exacerbate various clinical disorders, including DM (Perez de Nanclares *et al.,* 2000); neuro degenerative disorder (Berg *et al.,* 2000; Sayre *et al.,* 2000); Liver and heart disease (Milman *et al.,* 2001); Cancer (Beckman *et al.,* 1999).

The fact that *Solanum macrocarpon* extract together with solvent fractions reduced MDA levels produced in rat pancreas homogenate dose-dependently throughout duration of the peroxidatio raises the possibility that Fe^{2+} may have formed complexes with the phytochemical components of *Solanum macrocarpon,* inhibiting Fe^{2+} oxidation. It is possible that *Solanum macrocarpon* crude extract and its solvent fractions have anti-oxidative properties because MDA production in rat pancreatic homogenate was inhibited when pro-oxidant (Fe^{2+}) was present (Figure 4.4). Another factor enhancing inhibition is the *Solanum macrocarpon* extract's capacity to chelate. In rat liver and brain, Olusola *et al.,* 2014 found that leaves of *Solanum macrocarpon* provided protection against iron-induced lipid peroxidation. It demonstrated greater radical scavenging activity and a higher percentage prevention of lipid peroxidation caused by iron sulphate. *Solanum macrocarpon* was found to have inhibitory action by Salawu *et al.,* 2013. Komlaga *et al*., 2014; Famuwagun *et al*., 2017; Okesola *et al*., 2020; and Osei-Owusu *et al*., 2023 have reported *Solanum macrocarpon's* anti-oxidant activity. In prostrate of experimental rats given diets enrihed with *Solanum macrocarpon*, levels of glutathione, glutathione-s-transferase, and super-oxide dismutase all dramatically increase (Iweala and Ogidigo, 2015). According to Iweala and Ogidigo, 2015, prostatic histo-architecture significantly improved in histological examinations of rats fed diets supplemented with *Solanum macrocarpon,* suggesting that these diets may prevent or reduce the growth of Benign Prostatic Hyperplasia (BPH).

5.3 *In Vitro* Antidiabetic Activity

This investigation showed that the extract inhibited carbohydrate hydrolyzing enzymes dose-dependently, thus depicting a plausible dietary plan in treating postprandial high blood sugar level associated with type 2 DM. These enzymes are responsible for breaking down of big carbohydrate molecules into smaller glucose units. A number of scientific workers have stated leafy vegetables' capability to inhibit these enzymes (Jimoh, 2018). At 150 µg/mL the extract inhibited α-amylase at 59.93% with IC_{50} value of 48.73 µg/mL (Table 4.9) and α-glucosidase at 41.74% with IC50 value of 48.73 µg/mL (Table 4.8). The % inhibition of these enzymes employing acarbose is shown in Table 4.9a. This inhibitory capability provides proof that the extract can minimise or diminish generationn of AGEs (Ige *et al.,* 2010; Jung *et al.,* 2009; Tsuji-Naito *et al.,* 2009). It also indicates that *Solanum macrocarpon* extract can exert

antidiabetic effect (Onuora and Okafor, 2016; Pereira *et al.,* 2011; Oboh *et al*., 2005). *Solanum macrocarpon's* inhibitory impact and antioxidant capabilities raise the possibility of using this vegetable in dietary treatment of postprandial hyperglycemia connected to type 2 DM (Kadiri and Olawoye, 2015). Methanol extract of SM revealed radical scavenging activity IC50 of 2.4 mg/ml (Kadiri and Olawoye, 2015). Generally speaking, numerous researches have indicated that *Solanum macrocarpon* has great antioxidant capacity, which is attributable to high phenolic compounds' concentration. According to studies, secondary metabolites including flavonoids and phenolics are likely linked to blocking of carbohydrate hydrolyzing enzymes (Wang *et al.,* 2019)

5.4 Acute Toxicity Study

Acute toxicity in plant extracts deals with the ability of a particular extract or compound from a plant to cause deleterious effects on an organism when it is administered in a single or limited dose. Lethal dose (LD_{50}), which is the amount of the extract or compound that will cause 50% of the test population to die within a specific time frame, can be used to evaluate this toxicity. Organ damage, metabolic abnormalities, and even motility are just a few examples of how acute toxicity manifests. Acute toxicity severity is influenced by a couple of conditions, such as organism being exposed, its species, age, and the dose and administration method. Because plant extracts are frequently employed in traditional medicine and herbal medicines, it is crucial to assess their acute toxicity. This evaluation assists in making sure that the plant extract is safe for use and it's not of any risk to human or animal health. According to Patel *et al.,* 2012, every plant material is not safe; therefore, the toxic effect of these plants should be investigated before consumption. *Solanum macrocarpon* is a vegetable that is eaten by human and animals, therefore it is imperative to determine its level of safety. Acute toxicity research assesses the negative consequences occurring soon after a single dosage of a test substance has been administered. The purpose of this inquiry, which is generally conducted mostly on rodents, is to provide information on the possible toxicity of a novel medicine or remedy early in the development process (Parasuraman, 2011). OECD guideline 425 (2008) was used in the current investigation to assess acute toxicity. No group in the experiment displayed any toxicity at any of the levels applied. At dosages 100, 300, 500, 1000, 2000, and 5000 mg/kg bw, respectively, no morbidity or fatalities observed in all the groups that were treated (Table 4.14)

Consequently, the extract's LD_{50} could be higher than 5000 mg/kg. Therefore, on acute exposure, methanol extract of *Solanum macrocarpon* may be regarded as relatively safe. Plants having an LD_{50} of more than 1000 mg/kg b.wt by oral route are either harmless or have little toxicity (Abid and Mahmood, 2019). This current finding concurs with the reports of earlier researchers that methanol leaf extract of *Solanum macrocarpon* up to 5000 mg/kg is not hazardous or non-toxic. The current findings are consistent with Dougnon *et al.,* 2013, which provide comprehensive reports on SM's safety using body weight, hepatic parameters, renal parameters, haematological parameters, and histological examination. This outcome corresponds with Chidiebere *et al*. (2019) report on their research with the methanol fruit extract of *Solanum macrocarpon*.

A key indicator for assessing toxicity is change in body weight (Vahalia *et al.,* 2011). Average body weight of groups of treated rats in current investigation increased consistently normally, exactly like it did for the normal control. The difference in weight gain of the treated group and control group, however, was statistically insignificant (Figure 4.5). In this study, a gross pathological investigation of treated rat groups' pancreas compared to control group's did not disclose any significant differences in size, shape, colour, or texture (Table 4.17; Figure 4.9).

Biochemical parameters play significant roles as markers in toxicological evaluation because of how they respond to the clinical symptoms and signs that toxicants cause. According to Glassman and Muzykantov (2019), determining how harmful extracts and medicaments are requires a careful study of renal and hepatic functioning. All biochemical markers in this investigation never demonstrated significant alterations. The liver and kidneys play supreme role modulating drug catabolism and excreting plant extracts. According to Zhang, 2018, exogenous chemicals and their metabolites have the potential to cause cell damage or toxicity in these organs. In current investigation, biochemical markers ALT, AST, and ALP showed statistically minimal changes in comparison to the control group, showing the extract had no negative effects on any of the indicators (Loha *et al*., 2019). The electrolytes status, protein state, creatinine, urea, and haematological indices all showed similar findings (Table 4.16; Table 4.15; Figure 4.6; Figure 4.7; Figure 4.8)

5.5 Induction of Diabetes

In order to comprehend pathogenesis or etiology, complications and testing of various therapeutic agents, choosing the right or appropriate experimental model is a sine qua non. Diabetes mellitus models have been discussed in chapter 2.

When making a decision, the past is important to consider. Alloxan has been used to cause experimental diabetes from its initial discovery of alloxan-induced β-cell necrosis in rabbits in 1943. Insulin insufficiency, hyperglycemia, and ketosis are caused by the uric acid derivative, alloxan, which works by selectively destroying the pancreatic islets. Due to alloxan's low stability, brief half-life, and acidic nature of the solution, intravenous administration is the preferred method of administration (Srinivasan and Ramarao, 2007). Similar to alloxan, STZ primary cytotoxic impact on pancreatic β-cells induces hyperglycemia. The irreversible damage to the pancreatic β-cells, which causes degranulation and a reduction in the ability to secrete insulin, is the direct cause of the diabetogenic activity of STZ (Gu *et al.,* 2007). In streptozotocin, the nitrosourea moiety facilitates transport across the cell membrane. Similar to how alloxan causes free radicals to be produced; streptozotocin-induced diabetes has been connected to the alteration or modification of endogenous scavengers of these reactive species (Patel *et al.,* 2012). Alloxan and streptozotocin-induced diabetes models are used as antidiabetic medication screening techniques. Alloxan's and STZ's harmful effects are mediated by ROS and DNA alkylation, respectively. Due to its chemical makeup and improved stability, streptozotocin is commonly employed to repeatedly induce DM experimentally (Lenzen, 2008).

Streptozotocin-induced diabetes shares similar characteristics with that of human diabetes and can be used to study pathogenesis of diabetes, and assess the efficacy of the potential therapeutic intervention. The complexity and diversity of human diabetes may not be adequately replicated by streptozotocin, and different animals may respond differently to the induction of diabetes, making it challenging to achieve consistent result. Other organs, such as the liver, kidney, and spleen, may potentially suffer damage or toxicity induction. Streptozotocin has a wide range of biological effects, such as causing various damages to cells, oncogenesis, tetratogenesis, and chromosomal mutation (Magee and Swann, 1969). Animal models induced by streptozotocin and alloxan are employed for the screening of anti-diabetic drugs, and OGTT.

5.6 Glibenclamide

Glibenclamide, a sulfonylurea medicine that is frequently used to treat type 2 DM served as the reference anti-diabetic drug used for this study. Glibenclamide works by promoting the pancreatic release of hormone insulin then enhancing perceptivity of peripheral tissues to this hormone, which ultimately ends in reduction of blood sugar concentration.

5.6.1 Mechanism of Action of Glibenclamide

The way glibenclamide works is by attaching to certain specialised pancreatic β-cell receptors known as sulfonylurea receptors (SURs). By blocking or shutting KATP channels on the cell membrane, this binding brings about changes in the cell and influences opening of the voltage-gated calcium channels. Insulin is discharged from β-cells into the bloodstreams in response to the entry of calcium ions. Additionally; glibenclamide increases perceptivity of the peripheral tissues to hormone insulin, which enhances their ability to absorb or uptake and use glucose. The activation of a particular enzyme known as protein kinase C (PKC) is thought to be the mechanism behind this action (Luzi and Pozza, 1997). It is well-rooted that the improvement in β-cell activity of the pancreas, which leads to an increase in insulin production, is what causes effect of Glibenclamide on glucose tolerance (Kotha *et al.,* 2017). Oral anti-diabetic medicament glibenclamide has been used as blood sugar lowering medication since 1973 to treat type 2 DM (WHO, 2007). Its main mechanism of action is stimulation of insulin production (Serrano-Martin *et al*., 2006).

5.7 Effect of Ethylacetate Fraction on Body Weight

Depending on type, DM can generally have an impact on body weight both directly and indirectly. Diabetes can cause weight loss, especially when type 1 diabetes is poorly controlled. This happens because the body is incompetent to synthesis adequate insulin to move glucose from bloodstream into the cytoplasm for energy, which forces the body to start destroying fat and muscle instead. Even if the diabetic continues to consume the same number of calories, this may result in weight reduction.

Strangely, diabetes is linked with weight gain or problem losing weight, especially in cases of type 2 DM. This is due to cells of the body developing resistance to insulin, which forces generation of more insulin by pancreas so as to keep blood sugar concentration under control. Excess insulin can cause the body to store more fat, particularly in the abdominal region. Additionally, cravings for sweet foods or an

increase in appetite are two other factors that might result in weight gain in diabetics. Furthermore, several DM medications might cause weight gain. Also encouraging the storage of fat is insulin (Baptist health).

Due to the toxic and destructive actions of STZ, which caused alkylation of DNA, produced hyperglycemia, and caused necrotic lesions, the induction of experimental diabetes using STZ in animal models typically results in weight loss (Piyachaturawat *et al.,* 1988). Numerous studies have linked high blood sugar levels to diabetic animals' lower body weight (Zafar and Naqvi, 2010). The results of this investigation showed that giving the diabetic rats EA for 28 days improved body weight maintenance and animal survival since the weight loss was nearly reversed (Table 4.19). The observed weight loss of diabetic control rats could be accountable to protein waste brought on by a lack of available carbohydrates for usage as an energy source (Ramkumar *et al.,* 2011). Low insulin levels cause muscle tissue's protein content to decrease as a result of proteolysis (Vats e*t al*., 2004). According to the results of the current investigation, weight loss is dramatically promoted by fat mobilisation in voluntary muscle in streptozotocin-induced diabetic animals (Stearns *et al*., 1979; Besse *et al.,* 1993). According to Hammeso *et al.,* 2019, the capacity of EA to prevent weight loss may be attributable to the reversal of glycogenolysis, gluconeogenesis, and proteolysis. This revelation concurs with the findings of Mohan *et al.,* 2013.

5.8 Hypoglycemic Effect of Ethylacetate Fraction

Induction of diabetes using streptozotocin (STZ) causes death of pancreatic β- cells as explained earlier. However, there could be surving β-cells even after treatment with STZ and regeneration also possible (Gomes *et al.,* 1995). The findings from this investigation suggest that insulin-producing cells were not completely shut down but were still operating, and that the majority of the metabolic effects may have been caused by the stimulation of insulin release. The components of EA may have mechanisms that mimic those of insulin, improving insulin sensitivity, boosting glucose-dependent insulin secretion, and promoting islet of Langerhans regeneration in streptozotocin-induced diabetic rats' pancreas. By the reason of glucose concentration being significantly reduced in groups treated with EA suggested that the EA might be promoting or improving pancreatic release of insulin from beta cells. According to the current study, stimulating insulin release from pancreatic cells lowers blood glucose levels similarly to investigations of Gebremesket *et al.,* 2020 and Zangeneh *et al.,*

2018. This is suggesting that EA has the ability to restore loss function of the pancreas or enhancement of remnant from islet of Langerhans cells in order to synthesis more hormones. With very significant drop in blood glucose levels of diabetic rats admimistered EA, in comparison to diabetic control, this study is verifying hypoglycemic effects by EA (Table 4.18).

5.9 Hepatoprotective Effect of Ethylacetate Fraction

The liver might be regarded as one of the most pivotal organs in the body because of its numerous biological functions in catabolism and anabolism of primary metabolites. It plays a crucial role in break down and excretion of medicamentsas well as natural goods, making it the main metabolic organ in charge of maintaining glucose and lipid homeostasis in human body. It is responsible for detoxification by filtering toxins and other harmful materials from the blood stream, including drugs, alcohol and environmental toxins. Liver detoxifies by converting the chemical nature of many toxins. It converts nutrients from food into energy and stores excess glucose as glycogen. It makes bile, a digestive juice which helps in breaking down fats in the small intestine. Liver produces proteins that are crucial for blood clotting, immune function and other processes. It stores important vitamins and minerals including iron and vitamin B12. It controls the amounts of hormones in the body, such as insulin and thyroid hormones, as well as cholesterol and other lipids. The liver is crucial in the process of eliminating bacteria, viruses, and other foreign things from the blood. ALT, AST, ALP, GGT are some of the proteins known as liver enzymes that are produced by hepatocytes and play different parts in several biochemical events taking place in the human body. When liver cells are harmed, these enzymes are released into the blood. These enzymes serve as indicators of both healthy liver function and liver disease (Kalra *et al.,* 2022). Diabetes can raise the risk NAFLD, which may lead to inflammation of liver as well as scarring. NAFLD may progress to NASH, which could lead to cirrhosis and failure of liver. Other liver functions are impacted by diabetes

The levels of AST and ALT are routinely used to determine the severity of liver damage brought on by STZ. Because of necrosis or membrane damage, the enzymes are released into the bloodstream and are therefore detectable in serum. Increased blood enzyme levels are a symptom of liver cell membrane leakage and a loss of functional soundness (Drotman and Lawhan, 1978). In the current study, diabetic rats

had higher serum AST and ALT activity when compared to control. According to Navarro *et al.,* 1993, who demonstrated STZ's hepatotoxic action, this rise may be caused by enzyme leakages from the hepatocytes into the blood stream. In comparison to diabetic control, diabetic rats administered EA had reduced levels of AST and ALT, demonstrating the protective role of EA in STZ-induced liver injury. Total protein, bilirubin, and serum ALP levels all have an impact on how well hepatic cells work. Growing level of ALP in serum may be attributable to increased production as a result of increasing fat digestive enzyme (Muriel and Garcipiana, 1992). It might also be the result of membrane leaking. The serum level of ALP in the current study significantly increased following STZ treatment. Tissue damage brought on by STZ or hyperglycemia may be the cause of this elevated activity. After STZ-induced DM induction, Rai *et al.,* 2010 and Singh *et al.,* 2013 found increase of ALP activity. In the current investigation, administration of EA resulted in a striking reduction in serum ALP activity. Patrick *et al.,* 2008 had reported decrease in transaminases activity on treatment with plant extract. Increased serum enzyme activity caused by STZ-induced liver damage was reversed by EA plausibly due to its capability to stabilise membranes, which prevents the release of intracellular enzymes. This agrees with the universally held idea stating that serum level of transaminase goes back to normal as a result of improvement or amelioration of hepatic parenchyma and rejuvenation of cells of the liver (Thabrew and Joice, 1987). In comparison to the control group, in this investigation, STZ dramatically improved the level of total bilirubin and reduced the amount in total protein. Increased hemolysis, conjugation, or reduced liver absorption can all lead to hyperbilirubenemia, or an increase in plasma bilirubin (Rana *et al*., 1996). Rats given EA had lower serum bilirubin levels when compared to diabetic control. Decrease of bilirubin level may be due to hepatoprotection and decreased oxidative stress during EA therapy. Earlier research by Abd El-Baky *et al*. (2009) described a reduction in bilirubin following treatment with plant extract. ALP, bilirubin, and total protein levels must be properly controlled and managed, which calls for an early improvement in the hepatic cells' secretory system. Any hepatoprotective drug's effectiveness or potency depends on its ability to either minimise the negative or adverse effects or restore the normal physiology of the liver that has been altered by a hepatotoxin. In the test groups, EA reduced the STZ-induced raised enzyme levels, demonstrating the preservation of the hepatocytes' cell membrane's structural integrity or the repair of liver cells that had been injured. EA's

antioxidant properties in protecting liver cells from oxidative stress as a result of its phytochemical composition may be the processes by which it functions as a hepatoprotective agent to shield liver cells from harm (Ali *et al.,* 2019). (Table 4.21; Figures 4.26,4.27 and 4.28).

5.10 Nephroprotective Effect of Ethylacetate Fraction

The kidneys have a variety of roles in maintaining the body's health and homeostasis including the following:

(a) Regulating the amount of fluid in the body by adjusting the amount of urine produced.

(b) Detoxification through the secretion of toxins or the filtration of toxins from the blood into urine.

(c) Eliminating waste substances, such as urea, creatinine, and other waste products, as well as extra fluid from the blood.

(d) Controlling the amounts of electrolytes in the body, including sodium, potassium, and calcium

(e) Controlling blood pressure by manufacturing the hormone RENIN, which activates the renin-angiotensin-aldosterone system.

(f) By producing erythropoietin, that prompts bone marrow to synthesis erythrocytes, erythrocyte production can be achieved.

(g) Stimulating vitamin D, which facilitates the body's absorption of calcium from food. DKD, commonl reffered to as diabetic nephropathy is a serious disease of kidneys influenced by DM. Excessively high blood glucose concentrationr can harm cells of kidneys and blood vessels, which can result in DKD. Damage to the blood arteries brought on by DM can reduce blood flow to the kidneys and compromise kidney function. Diabetes mellitus can raise blood pressure, which makes the kidneys work harder and eventually suffer damage. Diabetes can harm the kidney's blood vessels and nephrons, causing proteinuria, which can develop into kidney disease. Kidney function may diminish as a result of DM, making the kidney less effective at removing waste. Diabetes can result in ESRD, or end-stage renal disease.

The kidneys filter creatinine, a waste product made by muscles, from the blood.

Additionally, as the liver breaks down proteins, urea is created as a waste product, and the kidneys also filter it out of the blood. Creatinine and urea levels in the blood can

rise as a result of DM. If diabetic nephropathy is left untreated, kidney failure will eventually occur. In DM severe renal damage is seen as a result of improper glucose control, including elevated levels of sugar and glycosylated protein in tissue, altered circulatory dynamics in kidneys, and elevated redox imbalance (Amorim *et al*., 2019).

In this work, STZ-induced diabetic rats had dramatically increased levels of serum urea and serum creatinine when compared to control. According to numerous researches, diabetic disorders significantly escalate the chance of destruction of kidney cell (nephropathy) (Ahmed and Osman, 2006). By increasing the amount of ROS and RNS generated by glucose auto-oxidation process, hyperglycemia raises the risk of kidney cell injury (Sharma *et al*., 2006). According to Hansi *et al*. (2012), STZ-induced DM in rats is accompanied with hyperglycemia and an elevated blood creatinine level. Alterations in urea and creatinine levels in serum are signs that DM has impaired kidney function. On treatment with EA, urea and Creatinine levels both reduced. This agrees with findings from research by Kade *et al.,* 2010, Manikanda and Dosa, 2010, and Zangeneh *et al.,* 2018, which employed plant extract to reduce urea and creatinine levels in diabetic rats. It's conceivable that EA's primary effect is due to its ability to increase insulin production because insulin decreases glucose levels and normalises glucose in STZ-induced diabetic rats (Kumar *et al*., 2010). In treated rats, the glucose metabolism was improved, and it was demonstrated that EA may preserve kidney integrity throughout DM. In 33% of diabetic patients, diabetic nephropathy that progresses to end-stage renal disease is seen (Atkins and Zimmet, 2010). Proteinuria and albuminuria are also employed as markers of diabetic nephropathy in animal models of diabetes (Islam and Choi, 2008). Serum creatinine levels that are higher than normal, a reduced Glomerula Filtration Rate (GFR), and protein presence in urine are signs of nephropathy (Figure 4.24; Figure 4.25).

5.11 Protective Effect of Ethylacetate Fraction on Electrolytes

Minerals having an electrical charge known as electrolytes are essential for many bodily functions, such as transmission of nerve impulses, sustaining fluid balance, and regulating or controlling muscle contractions. Electrolytes are impacted by DM in a variety of ways.

Diabetes can:

(a) Cause hyponatremia, a condition marked by a low amount of sodium in the blood. Body may lose salt becausof increased urine, which might lead to this.

(b) Lead to hyperkalemia, a condition marked by an elevated potassium level in the blood. This will happen as a result of low insulin levels, which might make it difficult for cells to absorb potassium.

(c) Lead to hypochloremia, a condition characterised by a low level of chloride in the blood. Dehydration can lead to this because it can make the body lose chloride through sweat and urine.

(d) Cause hypocalcemia, a situation whereby blood has a low amoun of calcium. High blood glucose levels can cause this condition by increasing the excretion of calcium in the urine.

(e) Cause hypomagnesemia, a condition in which blood has a low concentration of magnesium and this can occur due to increased urinary excretion of magnesium, which can result from high blood glucose levels (Liamis *et al.,* 2014).

Diabetes is usually linked with electrolyte disorders and electrolyte imbalance in diabetes is mostly caused by high blood sugar, hence measuring them is important in DM to lessen their consequences (Khan *et al.,* 2019). According to Sotirakopoulos *et al.,* 2012, even when renal function is normal, patients with DM may experience acid-base and electrolyte abnormalities as a result of DM complications and diabetic out-patient care. The most frequent problems are metabolic acidosis and alkalosis (the incidence rises as renal function declines), while the most usual electrolyte abnormalities are hypernatremia and hypokalemia. A set of electrolyte problems are frequently developed in diabetics. These diabetics usually have low levels of potassium, magnesium, and phosphate, particularly when they have non-ketotic hyperglycemic hyperosmolar syndrome and diabetic ketoacidosis, based on Liamis *et al*. (2009). Diabetes mellitus is associated with chronic hyperkalemia, which can result from hyporeninemic hyperaldosteronism, as well as hypo- and hypernatremia (Liamis *et al.,* 2014; Liamis *et al.,* 2006). Electrolyte problems are common in hospitals and are linked to greater rates of death and morbidity (Liamis *et al.,* 2013). Electrolyte problems typically have multiple contributing factors. Numerous pathophysiological factors, such as gastro intestinal absorption capacity, nutritional status, concurrent acid-base imbalances, pharmaceutical drugs, other comorbid disorders (solely renal

disease), or acute illness, either alone or in combination, play a crucial role (Liamis *et al*., 2009). In this investigation, STZ-induced diabetic rats had significantly greater sodium, chloride, potassium, and bicarbonate levels than control rats (Figures 4.20, 4.21, 4.22, and 4.23). According to Mohamed *et al.* (2019), diabetic ketoacidosis, which caused a drop in blood pH and further upset the acid-base balance, may be to blame for the elevated chloride concentration. The concentrations of Na^+, Cl^-, K^+, and HCO_3 were considerably ($P \leq 0.05$) brougnt back to nearly normal after administration of EA for 28 days when compared to diabetic control rats. EA has demonstrated the ability to increase the bodily system's ability to act as a buffer for diabetics through this reversal of the electrolyte imbalance.

Though the modus operandi is not fully established but according to Azmi *et al.* (2019), the distorted regulatory mechanism of electrolytes could be due to high glucose level, thus it is possible that EA has the potential to be explored as the agent for a regulatory mechanism of electrolytes towards ameliorating diabetes complications.

5.12 Hematoprotective Effect of Ethylacetate Fraction

According to Iniaghe *et al*. (2000) and Adeneye *et al.,* 2006, haematological parameters play supreme role in research as well as in diagnosis of illness since they are useful indicators of a human or animal's physiological or pathological condition. Consuming some plant substances with beneficial therapeutic or medicinal properties, whether in their raw or extract form has been linked to anaemia because of erythrocyte separation by spleen, reduced erythrocyte synthesis, or malfunctioning of primary lymphoid organs (Miles and Calder, 2021; Mishra and Tandon, 2012).

Any adverse effects of foreign substances, including plant extracts, on the constituents of animal blood can be discovered through the study of haematological parameters. They are efficient at identifying probable changes in quantities of complex molecules including enzymes, metabolic outputs, haematology, typical physiological function, and the histomorphology of the organs (Magalhaes *et al*., 2008). Chemically induced nephrotoxicity has previously been linked to abnormalities in a number of immune system cells as well as haematological indicators (Huff-Lonergan and Lonergan, 2005). A study has demonstrated that the administration of toxic chemicals, such as STZ, results in a condition which is characterised by a type of anaemia whereby circulating erythrocytes are smaller than the common size of erythtrocytes, possessing decreased red colouration, and whereby there is remarkable decrease in number in

almost all cell types (RBC, WBC, platelets.). The amplified non-enzymatic glycosylation of erythrocyte membrane proteins that is linked to hyperglycemia is said to be the cause of anaemia in diabetics (Huff-Lonergan and Lonergan, 2005). According to Saba *et al*. (2010), in uncontrolled DM with persistent hyperglycemia, oxidation of membrane proteins increases the rate of lipid peroxidation which causes erythrocytes hemolysis and reduces their survival. Numerous studies have shown a connection between acute stress caused by toxic compounds and an increase in WBC and platelet counts, as well as a considerable rise in the number of neutrophils and the neutrophil/lymphocyte ratio (Huff-Lonergan and Lonergan, 2005). In order to explore the preventative effect of EA in anaemic condition in rats that are diabetic, red blood cell paramaters including PCV, Hb, and RBC where observed. The RBC, Hb, and PVC levels in the untreated rats declined dramatically ($p \leq 0.05$), which may be related to infections on the normal bodily systems. On rats with DM induced by streptozotocin, antihyperglycemic effect in *Rubia cordifolia* aqueous root extract was revealed (Baskar *et al.,* 2006). This finding is in line with their publication. Anaemia can be brought on by changes to these factors in people (Balasubraimanian *et al.,* 2009). The levels of RBC and related indicators considerably increased following administration of EA on diabetic rats. These specifics suggest that EA may include phytochemicals that can increase the release or synthesis of erythropoietin in animal stem cells. According to Ohlsson and Aher (2000), the glycoprotein hormone erythropoietin instructs the bone marrow's stem cells to create more red blood cells. Flavonoids, tannins, and other phytochemicals found in EA have been demonstrated to have effective anti-oxidative power (Akah *et al*., 2007). Consequently, they may be able to prevent peroxidation of PUFA in cell membranes and hemolysis of erythrocytes in diabetic animals (Torell *et al*., 1986). The common knowledge is that DM can result in anaemia from a reduction in erythropoietin, and that anaemia is accompanied by weakness, exhaustion, and shortness of breath. White blood cells are harmed by DM, which impairs the immune system's response. Urinary tract infections, skin infections, and gum disease can result from having elevated blood glucose concentrations because they impair immunity making it harder for the body to combat diseases. Damage to platelet function brought on by DM increases the chance of blood clots, which can result in problems including heart attack and stroke. Streptozotocin is known to suppress the immune system by harming WBC and several bodily organs. According to research by Oyedemi *et al*., (2010), intraperitoneal injection of STZ dramatically lowers WBC count in rats.

When rats induced with DM were compared to control in this study, glycated haemoglobin level was much higher, but it was significantly lower in diabetic rats given EA. This is consistent with Kotha *et al*. (2017) findings, which showed that the extract of *Anisomeles malabarica* restored the amount of glycated haemoglobin in streptozotocin-induced diabetic rats. According to Lyons and Basu (2012), the levels of GHB in red blood cells (HbA1C) are utilised as indicators of glycemic management in people with DM. GHB serves as a gauge of hyperglycemia severity in DM animal models. The prolonged hyperglycemia that leads to glycated haemoglobin causes the levels of HbA1C in diabetic rats to rise. Diabetes-related challenges including diabetic retinopathy, nephropathy, and neuropathy are all diagnosed and prognosticated using the HbA1C concentration (Palsamy and Subramanian, 2008). Rats with DM have decreased haemoglobin production (Prabhu *et al*., 2008). (Table 4.20; Figure 4.14)

5.13 Protective Effect of Ethylacetate Fraction on Lipid Profile

Elevated TGL and LDL cholesterol levels, as well as reduced HDL cholesterol levels, can be used to identify the lipid profile of a diabetic.

Diabetes can have a significant impact on lipid profile, leading to alterations in blood levels of different types of lipids. Triglyceride levels can rise due to DM since the hallmark of type 2 DM, impaired insulin sensitivity, can increase the generation of triglycerides by the liver, which raises the risk of coronary disease. HDL or "good" cholesterol levels can drop due to DM. Low HDL levels can raise the risk of coronary disease because they lend to the removal of excess cholesterol from bloodstream. DM may cause an increase in LDL also known as "bad" cholesterol. LDL can lend to arterial plaque development, which increases the chance of cardiac disease and CVA (Bhowmik *et al*., 2018; Ma and Li, 2018). High amounts of tiny density LDL particles, low levels of HDL-C, high levels of TGL, and high levels of T-Chol are all signs of diabetic dyslipidemia.

According to Sztalryd and Kraemer (1995) and Briones *et al.* (1984), insulin stimulates the lipolytic hormone's action on peripheral fat in healthy normal rats, causing it to hydrolyze triglycerides and halt or inhibit the mobilisation of free fatty acids. Despite this, insulin shortage inhibits lipoprotein lipase, which promotes free fatty acid breakdown by liver to cholesterol and phospholipids that are then pumped into the bloodstream and contribute to an elevated serum phospholipid level (Pushparaj *et al.,* 2007). Lower HDL levels and higher levels of LDL, TGL and cholesterol are usually

exhibited by diabetics, according to the findings of Howard *et al.* (2000). Diabetic rats administered EA showed considerably lower levels of TGL, total cholesterol, VLDL, and LDL and higher levels of HDL when compared to diabetic control. Through this action, EA showed how well it lowers cholesterol levels in diabetic rats, and it has since shown that it can avoid complications related to DM. This outcome is consistent with what Singh *et al.,* 2010 found (Figures 4.15, 4.16, 4.17, 4.18, and 4.19)

5.14 Preventative Effect of Ethylacetate Fraction Against Hyperglycemia Mediated Oxidative Stress

According to Pizzino *et al.,* 2017, redox imbalance is a situation that develops when there is an imbalance between the capacity of an organism to clean up certain ROS (from environment) and the synthesis and accumulation of ROS in tissues as well as cells. The phenomena by which elevated blood glucose levels (hyperglycemia) cause a rise in the generation of ROS in the body is known as hyperglycemia-mediated oxidative stress. When excess glucose travels through various metabolic pathways, such as sorbitol-aldose reductase pathway, advanced glycation end products pathway, and HBP, ROS may be produced. The resultant oxidative stress can harm or damage cellular constituents like lipids, protein, and DNA, causing inflammation and tissue damage. Diabetes can have significant impact on the body's oxidative stress balance.

Diabetes can:

1. Enhance ROS generation, which can damage cell and lend to emerging numerous complication.
2. Result to lowering of antioxidant activity, which affects the body's capacity to manufacture and use antioxidants such glutathione and superoxide dismutase. This decline may make oxidative stress worse.
3. Cause mitochondrial dysfunction
4. Lead to chronic low-grade inflammation
5. Lead to endothelial dysfunction.

Chronic hyperglycemia decreases antioxidant state and increases lipids, resulting in oxidative stress. The increase in oxygen free radicals associated with DM may be mostly attributable to elevated blood glucose levels, which cause free radical production by auto-oxidation. Karpen *et al.,* 1982 reported a higher level of lipid peroxides in plasma of diabetic rats induced by streptozotocin. Insulin production is tightly linked to peroxides produced by lipoxygenas (Walsh and Pek, 1984).

In the current investigation, diabetic rats induced with STZ had higher levels of LPO in their pancreas, liver, and kidneys. The amount of LPO in the three tissues was reduced in diabetic rats treated with EA. This result is consistent with the outcome of research by Singh *et al.* (2013). Additionally, NO levels in liver, kidneys, and pancreas of diabetic rats induced with streptozotocin were greater. Within diabetic rats given EA, the level of NO was reduced. This is consistent with the judgements made by Okesola *et al.* (2020). According to Saddala *et al*. (2013), the elevated LPO and NO levels may be linked to deterioration in the antioxidant enzymes' defence system. EA treatment for diabetic rats reduced H_20_2 levels in kidney, liver, and, pancreas (Figures 4.9, 4.30; 4.37, 4.38; 4.45, 4.46).

SOD is a key enzyme in preventing cellular damage from ROS and accelerates the transformation of O^-_2 into oxygen and H_2O_2; substances less harmful to cells. According to Celi (2010) and Searle and Wilson, (1980), the heme protein CAT accelerates breaking down of hydrogen peroxide into H_2O and oxygen, shielding cells from its harmful effects. In comparison to control rats, diabetic rats had reduced SOD and CAT activity. This could be as a result of the enzyme getting glycated as a result of hyperglycemia or because antioxidant enzymes were struggling to neutralise the impact of ROS and RNS (Dia *et al.,* 2014) (Figures 4.35, 4.36; 4.43, 4.44; 4.51, 4.52).

Increased ROS generation in diabetic mice may be the explanation for the decline in SOD and CAT effects in liver and kidneys during a diabetic condition (Kaleem *et al.,* 2006). SOD and catalase actions in liver, kidneys, and pancreas were enhanced in diabetic rats treated with EA. It's likely that the secondary metabolites found in EA are either removing the poisonous STZ substances from the body or reducing oxidative stress by lowering blood sugar levels. The ability of the antioxidant enzyme to do this could potentially be the cause. This result is consistent with Okesola *et al.* (2020) report. While Palanisamy *et al.* (2011) found enhancement in SOD action pancreas following ellagic acid treatment; Dangi and Mishra (2011), reported a shoot up in SOD actions in kidneys and liver, using plant extract. For the purpose of degrading lipid peroxide and hydrogen peroxide, which can lead to oxidative stress, GPx uses glutathione. It is an enzyme with selenium that works with GST to convert H_20_2 and organic hydroperoxides into a non-toxic byproduct at the expense of GSH (Freeman and Crapo, 1982). Hodgson and Fridovich (1975) suggested that the glycation and deactivation of the enzyme, resulting from ROS and RNS, may be a reason for

diminished GPx activity. In this study, diabetic rats' kidney, liver, and pancreas exhibited decreased GPx activity as compared to the control group. EA therapy dramatically raised GPx activity. This corroborates the findings of the study by Ahmadvand *et al*. (2014) that *C. aphylla* extracts increased the amount of GPx action in kidneys and liver. Diabetic rats' liver, pancreas, and kidneys all had considerably higher levels of GSH after receiving EA treatment.

In this investigation, STZ-induced hyperglycemia decreased GST activity. According to Andallu and Varadacharyulu, (2011), this drop may be the result of an excess of radicals reducing GST's mRNA. GST activity increased in pancreas, kidneys, and liver of diabetic rats given EA. Prior research by Okesola *et al.,* 2020 noted an uptick in SOD and GST activity following treatment with plant extracts. Numerous xenobiotics and endobiotic substances are detoxified and processed in large part by GST (Oztetik, 2008). GST offers protection against oxidative stress because it has peroxidase and isomerase activity, binds non-covalently to molecules that are lipophilic and covalently to reactive metabolites made from xenobiotics (Semiz and Sen, 2007).

The diabetic rats' kidney, liver, and pancreas had lower GSH levels, which returned to normal after EA therapy, demonstrating EA's antioxidant capacity. The decrease in GSH in diabetic rats may be due to increased hepatocyte utilisation in an effort to counteract the increased lipid peroxide generation upon STZ treatment. Additionally, operating as a twin-substrate in detoxification of peroxide via GPx and GST, GSH is a direct free-radical scavenger (Winterbourn, 2008). The idea that STZ causes diabetes by generating oxygen free radicals is supported by the observation that rats with diabetes induced by STZ displayed a drop in GSH, most likely, consequent upon streptozotocin’s destruction of β-cells of pancreas. In diabetic rats, EA therapy greatly raised GSH level in pancreas, kidneys and liver. This implies that EA had effects that either increased GSH production, decreased oxidative stress, which in turn decreased GSH conjugation, or both of these effects. Kade *et al.,* 2010; Chakraborty and Das, 2010 had earlier reported increase in GSH level on treatment with plant extracts (Figures 4.32, 4.33, 4.34, 4.40, 4.41, 4.42, 4.48, 4.49, and 4.50).

5.15 Effect of Ethylacetate Fraction on Diabetes Symptoms

Diabetes can have a substantial impact on a diabetic's ability to consume food, produce urine, and drink water.

1. Water consumption – High blood sugar levels in diabetics may cause greater thirst, which can cause them to drink more water in an effort to quench their thirst. A severe condition called water intoxication or hyponatremia can result from excessive water intake.
2. Food intake – Diabetics may experience fluctuations in blood sugar levels, which can cause hunger.
3. Urine output- Diabetics may experience high urine production. Elevated blood glucose concentration can hinder kidneys ability to filter blood, which results in more urine being produced, which can be disruptive to day-to-day activities (Hebden *et al.,* 1986).

The observation of extremely high water, food, and urine production in these experimental animals are indicators of a diabetic state brought on by the injection of STZ. The feed, water intake, and urine production in diabetic rats, all considerably greater than that of control. These signs and symptoms are common markers of type 2 DM in both humans and animal models, and they are a direct result of inadequate insulin (Meshkani and Adeli, 2009). Administration of EA was highly effective in preventing polyuria, polyphagia and polydipsia conditions. The feed intake, water consumption and urine output were significantly reduced after administration of EA (Figures 4.10, 4.11, 4.12). This result is consistent with Kim and Kim (2006) publication, which demonstrated the role of *Morus albas* in regulating the desire for food and water intake in diabetic conditions. Shetty et al.'s 2005 report on *Momordica charantia* in diabetic rats is also consistent with this result. This present study confirms that *Solanum macrocarpon* ethyl acetate fraction (EA), which has been shown to have antidiabetic activity, significantly improves the relief of the "3 polys"—polyuria, polyphargia, and polydipsia—classical diabetic symptoms. The observed efficacy is comparable to or superior to that of glibenclaamide, the standard hypoglycemic drug. When the course of treatment is extended, it is anticipated that EA will demonstrate a more pronounced effect on reducing diabetes symptoms by enhancing the overall metabolism in diabetic animals.

5.16 OGTT

OGTT, one of the assessments used to maintain tabs on diabetic patients, provides crucial information for DM progression. The oral glucose tolerance paradigm was employed (Tesfaye *et al*., 2016) to assess changed carbohydrate metabolism following

post-glucose administration. High blood sugar promotes insulin release from β- cells of pancreas, boosting peripheral sugar uptake, including controlling glucose homeostasis via a number of processes (Jarald *et al.,* 2013). When someone has DM, this becomes out of control and leads to glucose intolerance. In comparison to diabetic control, diabetic rats administered EA had their blood glucose levels back to nearly normal within 120 minutes. As seen in Figure 4.13, this shows that EA has a beneficial impact on how glucose is utilised. EA's effect on glucose tolerance may be caused by its capacity to stimulate left over pancreatic β-cells, β-cell regeneration, or an insulin-like activity. PPARγ stimulation or availability of secondary metabolites notably flavonoids (Bashir *et al.,* 2018), which have demonstrated their ability to inhibit α-glucosidase, capacity to rejuvenate pancreatic β-cells, increase utilisation of sugar in peripheral tissues, and inhibition of activity of glucose transporter in intestine (Jadhav and Puchchakayala, 2012), could also be responsible.

5.17 Proximate and Valuable Nutrients of *Solanum macrocarpon*

In the contemporary time, demands for food have increased geometrically with the exponential human population growth. One of the viable options to fill the gap created by the demands for food is to pay attention to and patronize vegetables, particularly the forgotten and the underutilised indigenous vegetables (UIVs) which are cheap sources of food (Kadiri and Olawoye, 2015). Vegetables are not only nutritious; they are also medicinal for good health or wellness because of the presence of phytochemicals. A number of researchers have highlighted the chemical and nutritional benefits and potentials of fruits and vegetables (Asaolu *et al.,* 2012; Abiodun and Adeleke, 2010). According to estimates from the Food and Agricultural Organisation (FAO), 840 million people worldwide were estimated to be undernourished between 1988 and 2000, with 799 million of those individuals residing in developing nations, 30 million in transitional nations, and 11 million in wealthy or industrialised nations. Though there has been a decline in this figure in the last two decades because of technology and other means of improving food production, however, the number of undernourished people has grown by as many as 150 million people from 2019 – 2022 (World Hunger Fact). Globally, 821.6 million people are considered undernourished or starving today (Global Hunger Index). The prevalence of undernourishment in Nigeria 2019 – 2021 is 12.7% (World Bank collection of development indicator). The world is roving now between hunger and food insecurity. Interests have moved to harnessing,

quantifying, and using food plants, especially vegetables, in order to better comprehend the current situation (Dini-Andreote and Raaijmakers, 2018). Because they make up a higher portion of the average person's diet and are a less expensive source of energy, vegetables are abundant in the carbohydrates, lipids, and proteins that make up human diet. Numerous scholars, such as Hussain *et al.* (2010) and Sreedevi and Chaturvedi (1993), have discussed the significance of these main metabolites. In addition to the primary metabolites, the moisture, crude fibre, ash content, and energy values of the various vegetables and plant species are thought to be significant for human health (Hussain *et al.,* 2010). According to Roger *et al.,* 2005, the protein content of green leafy vegetables ranges from 20.48 to 41.66%. It has also been noted that protein-calorie malnutrition shortages are a major contributor to nutritional pathology. A plant food is said to be considered a good source of protein if more than 12% of its calories come from protein (Pearson, 1976). Additionally, according to reports, adults, pregnant women, and breastfeeding moms require 34–56 g, 13–19 g, and 71 g of protein on daily basis proportionately (Kominiarek and Rajan, 2016). According to the current findings, *Solanum macrocarpon* contains 21.73% of the recommended daily allowance of protein (Table 4.2).

The fat content of most green leafy vegetables has been reported to be from 8.3-27% DW (Ifon and Bassir, 1980). The result of this present study indicates that SM is not too fatty, 3.8%. The crude fibre content of most green leafy vegetables have been reported to be from 8.5 – 20.9% (Ifon and Bassir, 1980). The result of this present work shows that SM has adequate amount of crude fibre, 16.77%. Dietary fibre consumption is associated with decreased risk of colon; breast, as well as liver cancer, additionally, lower blood cholesterol levels, hypertension, constipation, and coronary heart disease (Ishida *et al.,* 2000). The Recommended Dietary Allowances (RDAs) for youngsters, grown-ups, expecting women, also nursing moms viz 19 - 25%, 21-38%, 28%, and 29%, respectively. Based on the results of the current investigation, for human nutrition, SM is still regarded as a genuine source of dietary fibre, with 16.77% crude fibre.

Most leafy green vegetables are said to have an energy value of 248.8-307.1 kcal/100g (Isong *et al.,* 1999). Ash content, a measure of the amount of minerals in biota, has been reported to range between 15.09-15.86% DW (Asibey-Berko and Tayie, 1999;

Lockeett *et al.,* 2000). According to current investigation, the SM's 8.74% ash level is still an excellent origin of minerals.

Moisture content of SM is 10.65% while that of carbohydrate is 38.24%. SM is rated a nutritious and valuable vegetable. The leaves of SM have been reported to be rich in protein, crude fibre, fat, calcium and zinc (Oboh *et al.,* 2005). Additionally, it has been claimed that the nutritional value of the leaves is the same as that of *Moringa oleifera*, a vegetable that is widely used to treat sick children and people with weakened immune systems (Tete-Benissan *et al.,* 2012; Dougnon *et al.,* 2012). Result of macromineral and micromineral analyses reveal the presence of some minerals that are beneficial to human health, such as iron, calcium, potassium, manganese, magnesium, copper, sodium, and zinc (Tables 4.3 and 4.4), while the quantitative proximate analysis (Table 4.2) expresses the status of fat, ash, protein, crude fibre, and moisture contents. Production of enzymes, hormones, and other substances by the body that are necessary for human growth, illness prevention, and wellbeing is made possible by microminerals. People with low iron levels may experience fatigue because their bodies' cells won't receive adequate oxygen. While low sodium levels can cause lightheadedness, manganese and zinc are essential for the body's function. Iron is also required for cell metabolism, growth, and development.

Antioxidant and hypoglycemic potential of *Solanum macrocarpon* extract and solvent fractions are extremely high. Using SM in herbal medicine for treating and managing DM is now supported by scientific evidence. The current investigation shows that EA, which has the largest total flavonoid content, the highest antioxidant capacity, and a high phenolic content, exhibits the best performance in terms of controlling hyperglycemia, reducing the three polys, and improving oxidative stress status. These may have taken place as a result of reduced insulin synthesis, increased insulin sensitivity, inhibition of carbohydrate-digesting enzymes, and free radical scavenging.

5.18 FT-IR Screening of Ethylacetate Fraction

For identifying the functional groups present in plant samples, FT-IR is thought to be a valid and accurate approach. It employs an IR spectrum between 400 and 4000 cm. Comparing the spectra of an unknown molecule to the library of known compounds allows for the identification of many plant compounds (Griffiths and de Haseth, 1986; Okereke *et al*., 2017).

Results of FT-IR audit of EA revealed presence of hydroxy, aryl, the cyclohexane ring, ether, tertiary amine, amide, ester, alkyne, and methynes (Table 4.24 and Figure 4.58). For the identification of bimolecular composition, FT-IR is a dependable and sensitive method (Kumar and Prasad, 2011). The current study's findings also demonstrate that the FT-IR analysis of the EA separated the component's functional groups based on its peak ratio, which identified the chemical components. The existence of aryl, cyclohexane rings, ether, tertiary amines, amides, ester, alkynes, methynes, aromatic primary amines, and hydroxy might be the cause of EA's varied therapeutic characteristics. Ten functional groups have been identified as being crucial.

5.19 GC-MS Screening of Ethylacetate Fraction

For quantitative purposes, GC coupled with MS may identify pure chemicals contained at less than 1ng in biological specimens (Florence and Jeeva, 2015). By interpreting the spectra and comparing them to the reference spectra, one can determine the unknown chemical components in a complex (Hites, 1997). One of the most trustworthy and dependable methods for locating bioactive substances in volatile essential oils, fatty acids, and lipids is GC-MS. From EA, seven components were found. Determined from peak area, chemical notation and retention time, as shown (Figures 4.66, 4.67, and Table 4.25), the identity of these ingredients was verified. The GC-MS investigation of EA revealed presence of pentadecanoic acid, 13 methyl methyl ester; methyl 18-oxidanyloctadeca-9,12, dienoate, TMS derivatives; 11-octadecanoic acid, methyl methyl ester; methyl stearate; trans-geranyl geraniol; pentacosanoic acid, methyl ester; and haxacosanoic acid, methyl ester. With the exception of methyl 18-oxidanyloctade-9, 12, dienoate, tms, an unsaturated fatty acid, and tran-geranylgeraniol, an unsaturated terpenoid, these molecules are predominantly saturated fatty acids. According to research, unsaturated and saturated fatty acids are both advantageous and support antidiabetic activity by enhancing insulin sensitivity, decreasing inflammation, raising the perceptivity of PPAR-receptors as well as enhancing insulin release from β-cells of pancreas (Ahn *et al.,* 2013). Numerous investigations have demonstrated that FAs, particularly UNFAs, have positive effects on the persistence of DM, blood sugar levels, levels of insulin, antioxidant parameters in tissues of diabetic people and animals, improved insulin synthesis, increased GLUT4, and decreased impaired insulin sensitivity (Kotha *et al.,* 2017). According to Yang *et al*. (2011), palmitoleic acid shows positive effect in reducing body weight

gain, high blood glucose, too many triglycerides in blood, impaired insulin sensitivity, and buildup or accumulation of lipids in liver. Pentadecanoic acid, 13-methyl has also been reported to have the ability to improve insulin sensitivity, and trans-geranylgeraniol is a precursor to vitamin K2, which is essential for blood clotting and bone metabolism. In addition, it affects how cells divide and expand as well as how some cancer cells grow and proliferate (Hiruma *et al.,* 2004). This study used GC-MS to be the first to document these chemicals on EA fraction of SM. It is implied that EA fraction contains substances that can help people with DM.

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1 Summary

Diabetes mellitus, a complex endocrine disorder, is a growing health challenge, with roughly 600 million people age 20-79 years currently affected globally (IDF, 2021), representing a global prevalence of 8.5%. Diabetes mellitus is currently responsible for an estimated 1.5 million deaths globally which represents about 2.2% of all death annually (WHO, 2021) and has maimed several hundreds of people across the globe. Due to its multifactorial nature, it has defiled interventions of allopathic drugs, which are even unavailable and costly; hence there is an urgent need for cost – effective source of intervention and the area of focus now is herbal treatment.

S*olanum macrocarpon*, a perennial herb and one of the forgotten and underutilised indigenous vegetables (UIVs), is used in different traditional dishes. Outside its curlinary uses, SM is employed in medicine to treat diabetes and other ailments including constipation, worm infestation, gastrointestinal disorder, skin infections, respiratory tract infections, and others. Numerous researches have shown that SM has anti-inflammatory, antioxidant, and anti-microbial activities. In current investigation, methanol leaf extract of SM was prepared fractionated into HEX, CHL, EA, BUT, and MET fractions. The crude extract (SME) and the factions were engaged in antioxidant assays via *in vitro* models (DPPH, RP, NP, MC and TAC). SME and the fractions exhibited antioxidant ability and they possess polyphenolic compounds including flavonoids and phenolics. SME showed inhibitory activities against α-amylase and α-glucosidase enzymes and induced lipid peroxidation in pancreas homogenate. The most effective fractions, EA and BUT, were utilised *in vivo* to treat male Wistar rats that had been made diabetic by STZ. Rats were separated into several groups, including control, diabetic that received no therapy, diabetic group treated with the reference medication glibenclamide (GLIB), and diabetic groups treated with SME, EA, and BUT, respectively. The dose of the extract and fractious was 300 mg/kg while that of the GLIB was 1 mg/kg. Blood glucose levels, oxidative stress markers, lipid panels, body weight, haematological indices, other biochemical parameters, and

evaluation of the 3 Polys were among the DM-related measures that are examined. Investigations using FT-IR and GC-MS were conducted on SME, BUT, and EA. The findings of this study showed that SME, EA, and BUT all have antidiabetic action, with EA being the most powerful. When compared to diabetic control, EA was able to significantly lower blood glucose levels and markers of oxidative stress in treated rats, suggesting potential preventive effects against diabetic complications; improve body weight loss; and bring the 3 Polys back to nearly normal levels.

Diabetes-related derangement in HDL, total Cholesterol, triglyceride, RBC and its indices, WBC and its indices, glycated hemoglobin, PCV, hemoglobin and Platelets were modulated on treatment with EA. The FT-IR revealed the functional groups while the GC-MS indicated identified bioactive compounds. EA is a potent promising therapeutic agent showing hepato-renal protective effect.

6.2 Conclusion

The results of this investigation allow us to draw the conclusion that the EA fraction of *Solanum macrocarpon* leaf extract is a safe, effective, and potentially therapeutic drug. Its constituent phytochemicals are likely the driving force behind its enormous antioxidant and antidiabetic effects. In this study, EA showed exceptional antioxidant activity in *in vitro* and *in vivo* assays as well as in daily dose of 300 mg/kgbw 28 days treatment. It also exhibited antidiabetic activity to significantly and effectively lower elevated fasting blood sugar, improve glucose uptake and insulin sensitivity, and ameliorate all abnormal biochemical parameters, including the 3 polysyndrome symptoms of DM and other measures of health, in STZ-induced diabetic male Wistar rats. The impact and outstanding effects EA has on liver and kidneys suggest that it may be helpful to treat and prevent DM-related problems such as retinopathy, neuropathy, and nephropathy.

This study emphasizes worth of looking at herbal plants as potential means of fresh treatments in DM and other illnesses. In order to prepare for drug discovery, it has been possible for the first time to identify the functional groups of EA by FT-IR analysis. Additionally, the active compounds have also been screened using GC-MS

This study, therefore, clearly supports the conventional use of this vegetable in treating DM, even though it is constrained by its use of an animal model and requires further research to draw conclusions on clinical correlations to people.

6.3 Recommendations

1. In this study, for the encouraging performance of EA, it is recommended that it can be used either as a prophylactic or curative agent in diabetes treatment and subsequently developed into antidiabetic drug.
2. It is recommended that it can be used in combination with other diabetes therapies such as insulin or other oral medication to enhance the overall management of diabetes. An adjunct
3. It is recommended for isolation, identification and characterization are done.
4 Further studies - To confirm these findings and understand better mechanisms of action.
5 Clinical trials – To assess the effectiveness and harmlessness of the extract in treating DM, experiments involving human subjects should be carried out.
6 Dosage and administration – The optimal dosage and method of administration should be determined ensure maximum effectiveness and minimum side effects.
7 Public education and enlightenment – Diabetics and healthcare providers should be educated about the potential benefits, as well as any potential side effects or interaction with other medications.
8 Regulation – If confirmed safe, it should be regulated and made available to patients through the appropriate channels.

6.4 Contributions to Knowledge

(1) For the first time, EA has been demonstrated in the current investigation as a prospective and promising therapeutic antidiabetic medication that is safe, inexpensive and accessible. It can lessen the severity of complications specific to type 2 DM, which are caused by DM.

(2) This investigation has revealed that SM extract is an effective postprandial control candidate by successfully suppressing carbohydrate hydrolyzing enzymes.

(3) Empolying FT-IR investigation, it was possible to identify for the first time the number and variety of functional groups, and the GC-MC was used to screen for the types and quantity of bioactive chemicals. These are excellent milestones towards the discovery of medications.

(4) This study has shown that, in addition to secondary metabolites, *Solanum macrocarpon* also contains minerals and other wellness-promoting compounds

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APPENDIXES

Plate S1: Blending of the Leaves of *Solanum macrocarpon*

Plate S2: Filtration and Extraction of Plant Samples

Plate S3: Fractionation Using Vacuum Liquid Chromatography

Plate S4: Elemental Analysis of Palnt Extract Using AAS

Plate S5: *In Vivo* Experimental Set-Up in Metabolic Cages

Plate S6: Experimental Rats Inside Plastic Cage

Plate S7: Urine Samples of Rats Collected Through Metabolic Cages

Plate S8: Administration of Extracts to Plant

Plate S9: Homogenisation of Experimental Animal Organs