CHAPTER ONE

INTRODUCTION

1.1 Background

Metabolic syndrome (MetSyn) denotes the conglomerate of several cardio-metabolic risk factors, such as hyperglycemia, dyslipidemia, abdominal obesity, elevated blood pressure and insulin resistance (Alberti *et al*., 2009). MetSyn is one of the most important public health challenge both in the developed and developing countries of the world (Alberti *et al*., 2009) leading to an increasing rate of mortality and morbidity among individuals with MetSyn (Mottillo *et al*., 2010). It is characterized by a combination of risk factors for diabetes and cardiovascular disease (CVD) that are generally linked to insulin resistance and central obesity (Duncan and Schimidt, 2001).

International Diabetes Federation (2006) projected that about 20-25% of the adult population in the world is living with MetSyn. Williams *et al.* (2004) predicted that individuals living with MetSyn have two fold increased risk of death from CVD and five-fold increased risk for the onset of type-2 diabetes in comparison with individuals without MetSyn. This would increase the population of individuals living with type-2 diabetes that has already been ranked the fourth leading cause of mortality in the world (IDF, 2006). The risk factors for CVD that characterizes MetSyn is now believed to be the force driving the increasing prevalence of CVD.

The etiology of MetSyn is multi-factorial including the genetic factors, lifestyle and environment. However, convincing evidence exists that nutritional factors are central to the etiology of MetSyn (O'Toole *et al*., 2008). The rising prevalence of MetSyn has been linked to westernised dietary habits and sedentary lifestyle (NCEP, 2002) which often leads to overweight and obesity. Evidence from large population-based studies propose that strong association exists between the consumption of plant-based foods such as nuts, fruits and vegetables, plant-derived beverages, whole grains and reduced incidence of cardio-metabolic risk factors (Dauchet *et al.*, 2010; Chanet *et al*., 2012). A prospective study reported that the intake of fruits such as grapes, apples, strawberries, chocolate, pears, and bran among premenopausal women reduced death rate from CVD (Mink *et al*., 2007). Numerous studies have

also reported that certain dietary patterns, food groups or nutrients may lower the incidence of MetSyn, providing improved prevention strategies and prognosis among MetSyn subjects (Djousse *et al*., 2010).

For a long period of time, the favourable and positive effects of plant foods have been linked to the presence of vitamins and carotenoids that are known for to possess antioxidant properties (Habuzit and Morand, 2012). Conversely, there has been disappointing reports from large number of intervention studies conducted with these micronutrients which presented no positive effect but rather increased CVD risk (Bjelakovic *et al*., 2007). As a result, researchers later investigated other possible useful compounds available in plant foods such as polyphenols. Special consideration has been given to polyphenols, probably because of the preventive role against various degenerative diseases, including cardiovascular diseases and cancer (Toma´s-Barbera´n and Andre´s-Lacueva, 2012).

Plant foods contain several biologically active molecules such as polyphenols. They are widely distributed in nature and are the most prominent antioxidants commonly found in plant foods such as fruits and vegetables, spices, nuts, herbs and plant derived beverages (Chang *et al*., 2010). Legumes and cereals also contain significant amount of polyphenols (Scalbert *et al*., 2005). Numerous polyphenols have been identified, belonging generally to the four main classes, i. e. phenolic acids, flavonoids, lignans and stilbenes (Spencer *et al*., 2008). Flavonoids and phenolic acids are the major polyphenols in human diet. They contribute to one third and two-thirds of total polyphenol consumption per day (Perez-Jimenez *et al*., 2011). Flavonoids are classified into six subclasses based on their structure: flavanones, isoflavones, flavonols, anthocyanidins, flavanols (catechins and proanthocyanidins) and flavones (Chanet *et al.,* 2012). Some of the subclasses are widely distributed in wide range of planrt foods while others are found in specific plant food.

Citrus was considered a food and a medicinal plant due to its high nutritional value and unique flavour. It is consumed by majority either as a whole fresh fruit or in form of juice. Consumption of citrus juice has been reported to have positive effects in the prevention of cardiovascular related diseases (Dugo and Giacomo, 2002). They are recognized to possess potent anti-inflammatory, anti-bacteria, anti-tumor and antioxidant properties (Abeysinghe *et al*., 2007). The

beneficial effects of citrus fruit have primarily been linked to the biologically active compounds such as polyphenols present in citrus (Abeysinghe *et al*., 2007). Grapefruit (*Citrus paradisi*) had been reported to have antiviral, antibacterial and antifungal properties that were attributed to the antioxidant activity of polyphenols present in *C. paradisi* juice (Brzozowski *et al.*, 2005).

1.2 Statement of the problem

Universal action plan for the prevention of non-communicable diseases (2013 - 2020) is to lower the preventable burden of morbidity, mortality and disability owing to NCDs (WHO, 2013). This is to ensure that individuals can attain the highest possible level of health and productivity at every stage of life and as well ensure that diseases are no longer barriers to well-being or socioeconomic development. However till date, significant number of individuals are still living with MetSyn with increase in incidence and prevalence in both developed and developing countries of the world (Nolan *et al.,* 2017).

Various levels of prevalence of MetSyn have been reported in different countries of the world. Gavrila *et al*. (2011) reported MetSyn prevalence of 6.2% in Spain, from the reports of the National Health and Nutrition Examination Survey (2003-2012), in the USA prevalence of 34.7% was documented (Aguilar *et al*., 2015). Sy *et al*. (2014) reported 9.1% among the Philippines and Martins *et al*. (2015) reported 11.9% among the Brazilian population. In Kenya, Kaduka et al. (2012) indicated a prevalence of 10.0%, Tran *et al*. (2011) reported 17.9% in Ethiopia, Motala *et al.* (2011) documented a prevalence of 26.5% in South Africa, Longo-Mbenza *et al.* (2011) reported 27.1% in Congo, Garrido *et al.* (2009) documented 34% in Botswana. Various level of prevalence has also been reported among different populations in Nigeria; Puepet *et al.* (2009) documented a prevalence of 63.6% in Jos, Ogbera *et al*. (2010) indicated a prevalence of 86% in Lagos, Akintunde *et al.* (2011) showed a prevalence of 42.9% in Osogbo, Ulasi *et al.* (2012) documented a prevalence of 20.5% in Sokoto. In southwestern Nigeria, a prevalence of 36.7% and 16.3% were reported by Ghazali and Sanusi (2010) and Charles Davies *et al.* (2012) respectively. In addition, report from a systematic review carried out by Oguoma *et al.* (2015) indicated a prevalence of 31.7% in Nigeria. The public health concern of metabolic syndrome is premised on the increasing incidences of both type-2 diabetes

and cardiovascular disorders that have reached epidemic proportions worldwide even in Nigeria (WHO, 2003). Although, both developed and developing countries are undergoing rising prevalence of non-communicable diseases, the impact is more devastating in developing countries such as Nigeria where there is existing burden of infectious diseases coupled with the under-resourced public health service.

Several pharmacological and non-pharmacological intervention strategies have been employed in the management and control of MetSyn (Grundy, 2005); however, each of the intervention has one limitation or the other. For instance, current pharmacological management of MetSyn involves treatment of the individual components of MetSyn and this could not address at once the multiple CVD risk factors that typify MetSyn. The ineffectiveness of the pharmacological approach currently in use in the management of MetSyn is exacerbated by low drug compliance among the patients owing to the adverse effects of the drugs, unaffordable costs of medical care by the majority, limited and poor access to drugs and health care services (WHO, 2009). Likewise, lifestyle interventions have been shown to have positive effects on components of MetSyn, nevertheless it is difficult to sustain over time as long-term adherence to lifestyle changes by the general population has been indicated to be poor (WHO, 2003).

In developing countries such as Nigeria, research into intervention strategies has become necessary to control, prevent and halt the rising prevalence of MetSyn and its related complications such as CVDs and type-2 diabetes.

1.3 Justification for the study

Metabolic syndrome remains a major public health challenge that demands rapid evaluation of new, accessible and affordable interventions to prevent and control the incidence of MetSyn to halt the prevalence of non-communicable diseases among the population. World Health Organization (2013) reported that nearly 42% of all deaths from NCDs happened before 70 years of age globally and 48% of deaths from NCD occurred in individuals aged less than 70 years in low- and middle-income countries.

Owing to the shortcomings in the current pharmacological and non-pharmacological management of MetSyn, there is need to develop new, accessible, affordable and

sustainable therapeutic approach that would address at once the multiple CVD risk factors that typify MetSyn. Matfin (2008) suggested a "poly-pill" therapeutic approach for managing MetSyn as this would address the multiple risk factors at once. The use of supplement containing biologically active phytochemicals with established positive effects on human health has been proposed to be a possible successful approach. This is because the relevant risk factors for MetSyn are biologically inter-related in terms of their effect on disease progression (Coca *et al.*, 2017).

A general agreement has been reached to support the hypothesis that the consumption of plant foods including fruits, vegetables that possess reasonably high levels of polyphenols may contribute meaningful to reducing cardiovascular disease risk (Sies *et al*., 2005; Vita, 2005). However, intervention studies on the influence of local and tropical fruits on biomarkers of MetSyn that are strong predictors of CVDs and type-2 diabetes mellitus are comparatively few in numbers in Nigeria. Even in the developed countries, majority of dietary intervention studies have focused on polyphenolic-rich beverages such as green and black tea, red wine etc, and have found them to have beneficial effect on CVD risk factors (Basu *et al*., 2010, Dohadwala *et al*., 2011).

In Nigeria, grapefruit seed extracts have been documented to have antifungal, antibacterial, wound healing and antioxidant properties (Oyelami *et al*., 2005). In Nigerian folk medicine, ethanol decoction of powdered grapefruit seeds has been used in the management of throat infections and high blood pressure (Adeneye, 2008). Despite its indigenous use, few scientific reports could be found in the literature as regards its effects on metabolic and inflammatory diseases.

Researchers have reported that the polyphenol content of fruits depends on the part of the fruit (Jang *et al.*, 2010). Citrus fruit is mostly rich in flavanone, with the whole fruit containing up to five-times more flavanones than the corresponding juice because the non-edible parts of citrus fruit have higher concentration of these compounds (El Gharras, 2009). This claim has been supported by the reports of several researchers. The non-edible parts of citrus fruit, principally the peels, seeds, the albedo (the white spongy portion) and the membranes which are normally discarded as wastes has been reported to have higher polyphenol contents when

compared to the juice vesicles (pulp) (Tripolid *et al.,* 2007; Wang *et al.,* 2008; Samonte and Trinidad, 2013).

The peels obtained from citrus fruits constitute between 50 and 65% of the total mass of the fruits. When not processed further, this by-product becomes a very worrisome waste capable of causing serious environmental pollution. In Nigeria, fruit peels resulting from consumption and industrial processing of the edible parts of fruits alone, are discarded and treated as wastes. Fruit wastes are generated in large quantities and they constitute one of the major source of municipal solid waste. The two main disposal techniques, land-fill and incineration that are currently being used results in emissions of methane and carbon dioxide that contributes to the current severe pollution problem. In addition, during incineration pollutants and secondary wastes such as dioxins are formed and released into the atmosphere posing environmental and health hazards.

Based on this rationale, there is need to find value-added use for fruit wastes such as retrieving the biologically active substances in them for use in food processing to serve as natural sources of antioxidants that are safe for human consumption. This will be useful in replacing the synthetic antioxidants such as 2, 6-di-tert-butyl-p-hydroxytoluene, tertbutyl-4-hydroxyanisole and propyl gallate that are being used to prolong the shelf life of products in food industries.

Therefore, there is need to search for natural and safer substitutes of antioxidants. In general, studies have reported that, fruit peels and seeds which are considered as fruit wastes contain higher levels of phytochemicals and antioxidants than the edible parts of fruits. As such, fruit wastes could be utilized as cost effective and natural source of antioxidants in food industries. They may also be used as natural colorants and preservatives for foods in food industries. If the grapefruit wastes are found to have possess antioxidants and anti-inflammatory properties, it could be effective in preventing and lowering the mortality and morbidity arising from cardiovascular diseases and type-2 diabetes. This would thereby improve health, wellbeing, quality of life and longevity of Nigerians. This could also in turn lead to increased productivity and economic development in Nigeria.

In this era of nutrition transition characterized by sedentary lifestyle, western diets poverty, limited access to drugs coupled with the rising prevalence of MetSyn, the

use of grapefruits could be an affordable, accessible, and low-cost therapeutic strategy in managing MetSyn. It could be effective in preventing and reducing the morbidity and mortality from cardiovascular diseases and type-2 diabetes. This would address multiple risk factors at once, thereby improving health, well-being and quality of life of Nigerians. This could also in turn reduce the significant burdens on the under-resourced public healthcare system in Nigeria.

1.4 Research questions

1. What are the quantities of major phytochemical constituents of extracts from the edible and non-edible of pink and white varieties of grapefruits?
2. What are the antioxidant capacities of the edible and non-edible parts of pink and white varieties of grapefruits?
3. What are the phytochemical constituents of the extracts from the edible and non-edible parts of pink and white varieties of grapefruits?
4. Do edible and non-edible parts of pink and white varieties of grapefruits have influence on the cardio-metabolic markers in male wistar rats?
5. Do edible and non-edible parts of the pink and white varieties of grapefruits have influence on the plasma inflammatory and oxidative stress markers in male wistar rats?
6. Do edible and non-edible parts of pink and white varieties of grapefruits have effect on the kidney and liver function markers of the experimental rats?

1.5 Hypothesis

1. **H_0**=Edible and non-edible parts of grapefruits will not significantly reduce the cardio-metabolic markers in experimental rats
2. **H_0=** Edible and non-edible parts of grapefruits will not significantly reduce the plasma inflammatory and oxidative stress markers in experimental rats
3. **H_0=** Edible and non-edible parts of grapefruits will not have protective effect on the functional parameters of the liver of the experimental rats.
4. **H_0=** Edible and non-edible parts of grapefruits will not have protective effect on the functional parameters of the kidney of the experimental rats.
5. **H_0=** Edible and non-edible parts of grapefruits will not have protective effect on the liver and kidney structure of the experimental rats

1.6 Objectives of the study

General Objective: The general objective of this study is to determine the efficacy of edible and non-edible parts of pink and white grapefruits (*Citrus paradise* Macf.) on biomarkers of Metabolic Syndrome in male wistar rats

Specific objectives of the study are to:

1. Quantify the major phytochemicals and determine the antioxidant capacities of the extracts from edible and non-edible parts of grapefruits
2. Quantify the individual phenolic and flavonoid contents of the extracts from edible and non-edible parts of grapefruits
3. Determine the efficacy of edible and non-edible parts of grapefruits on cardio-metabolic parameters (anthropometric variables, blood pressure, lipid profile, fasting plasma glucose, fasting plasma insulin and insulin resistance) in the experimental rats.
4. Determine the efficacy of edible and non-edible parts of grapefruits on selected plasma oxidative stress and inflammatory markers (MDA, TNF-α, CRP, Adiponectin and IL-6) in the experimental rats.
5. Evaluate the potential effect of the edible and non-edible parts of grapefruits on the functional markers of the liver (ALT, AST, ALP, LDH, total bilirubin and albumin) and kidney (Urea, creatinine, Uric acid, Na^+, K^+, Ca^{2+}, Cl^-) in the experimental rats.
6. Evaluate the effect of the edible and non-edible parts of the two varieties of grapefruits on hepatic and renal structure of the experimental rats

CHAPTER TWO

LITERATURE REVIEW

2.1 History, definition and diagnostic criteria of Metabolic Syndrome

Metabolic syndrome initially was described as the combination of hypertension, gout and hyperglycemia by a Swedish physician and this was the first effort made to define the syndrome. (Kylin, 1923). By 1974, it was reported that a relationship existed between the upper body fat deposition and the metabolic abnormalities and this was prevalent among type 2 diabetes mellitus and cardiovascular diseases patients (Vague *et al*., 1979). Reaven (1989) eventually termed the metabolic disturbances as "Syndrome X" and established that a resistance to insulin was a critical pathophysiological characteristic of the syndrome. Raeven's description of syndrome X seemed alright, however it was later found that he did not consider obesity as one of the characteristics of this syndrome even though majority of the patients with syndrome X also suffered from obesity. Due to these observations, obesity was now considered one of the important characteristics of the syndrome. As time went by, several findings and researches were carried out and based on premises and findings raised by several researchers, the disease had names such as the Deadly Quartet according to (Kaplan, 1989) and Insulin Resistance Syndrome according to DeFronzo and Ferrannini (1991).

Metabolic syndrome has series of definitions and over the years, several expert committees and groups have combined efforts to agree on an overall all-encompassing definition. The first consensus on a global definition of metabolic syndrome by a consultative group of the World Health Organization was published in 1999 (Alberti and Zimmet, 1998). This effort was fuelled by the lack of any global acceptable definition as at the time. The group comprised The European Group for the Study of Insulin Resistance (EGIR), World Health Organization (WHO), and the National Cholesterol Education Program—Third Adult Treatment Panel (NCEP ATP III). They agreed on essential features of MetSyn and these included insulin resistance, dyslipidaemia, obesity and hypertension. However, they had differing clinical yardsticks for the proper identification of the cluster. It was stated that the definition that would be agreed on during the meeting would only serve as a working

definition to allow further research on that topic leading to more information (Alberti and Zimmet, 1998; WHO, 1999).

Since formation, the WHO definition has faced series of criticisms due to several limitations that were raised. The most important limitation was related to the measurement of insulin sensitivity using the euglycemic clamp technique. This limitation made the adoption of the definition very challenging in both clinical practice and epidemiological studies.

Based on this challenge, the definition by the WHO was modified by EGIR. The modification addressed the limitation in the use of euglycemic clamps in measuring insulin sensitivity and incorporated the determination of fasting plasma insulin which is quite easier (AACE, 2003). Due to the fact that insulin resistance cannot be easily and reliably be measured especially among the diabetics because of β-cell dysfunction (an important characteristics of type 2 diabetes); its use has been restricted to only those who can easily and reliably measure insulin resistance despite being one of the main component of the EGIR definition. The EGIR definition came up with a modified cut-points for waist circumference as an important determinant of fat accumulation and other components like plasma triglycerides and HDL-c.

The definition proposed by Adult Treatment Panel III (ATP-III) was introduced by the US National Cholesterol Education Program (NCEP) in 2001. This definition was premised on a "glucose-centric" approach in which all components are of same significance, and thus excludes all other precise determination of insulin resistance. It was established by the panel that the presence of three out of these five abnormalities (blood pressure, plasma triglycerides, fasting glucose levels, HDL-c and waist circumference) in a person would lead to a diagnosis of the disease. Like the EGIR definition, the ATP-III definition retained the use of waist circumference as the indicator of excessive fat accumulation, but with increased threshold, (ATP-III threshold is 102 cm and 88 cm for men and women, respectively versus EGIR-94 cm and 80 cm for men and women, respectively). This was because the ATP-III definition recommended that a lower circumference at the level of the cut-off for the EGIR could still be appropriate for the Asian-American populations especially because they are more prone to developing abdominal fat due to their small skeletal frames and they are also genetically predisposed.

The ATP-III panel also gave recommendations on how to determine the waist circumference accurately. Due to the ease of the ATP-III definition, it later became very popular and generally adopted for use in the clinics and for research purposes. However, inflammatory and haemostatic variables were not incorporated in the definition as was done in the WHO definition.

In 2003, the American Association of Clinical Endocrinologists (AACE) came up with its diagnostics benchmarks for MetSyn by making modifications to the definition of ATP-III. In AACE description of MetSyn, insulin resistance was regarded as the central component with the remaining identified features. The remaining features of importance include elevated blood pressure, elevated fasting and post-load glucose, elevated triglycerides and HDL-c). AACE suggested that unhealthy lifestyle practices is central to the development of MetSyn. Some other factors listed are: obesity, gestational diabetes, hypertension, CVD or family history of diabetes, non-European ancestry, sedentary lifestyle and age above 40 years. Obesity was excluded as a feature of MeytSyn by AACE because they considered obesity risk-factor for insulin resistance. This steered up a lot of criticism especially due to increasing scientific proof that obesity is a principal risk-factor for type-2 diabetes mellitus and CVD (Zimmet *et al.,* 2001).

Over time, due to various definitions put up by various expert groups with different cut-off points and different combinations of MetSyn components, confusion arose for the researchers and the clinicians with regards to the diagnostic criteria to define MetSyn among patients and which of the definitions to use to define prevalence. As a result, reports on prevalence of the syndrome varied across different places due to varying criteria used for diagnose (Eckel *et al.,* 2005). Due to these contrasting definitions leading to confusion, there was a need for an internationally agreed description on MetSyn that is amenable for both clinical practices and research. The introduction of a standard description would enable comparison of prevalence and incidence across various groups and determining its association with other health challenges such as CVD or type 2-diabetes. This need fuelled the International Diabetes Federation (IDF) to hold an expert workshop to modify and improve current definitions and also reach an agreement for the introduction of a new, harmonised and globally accepted definition in May 2004.

The clinical conditions released by the IDF were similar to that of the ATP-III with some components like HDL-c, triglycerides and plasma glucose etc. even having same thresholds. However, unlike the ATP-III, the IDF definition proposes that the threshold for waist circumference should be based on the peculiarities of each ethnic group.

2.2 Different Definitions of the Metabolic Syndrome

2.2.1 World Health Organization (WHO) Definition

WHO (1999) defined Metabolic syndrome as the conglomerate of diabetes or impaired glucose or insulin resistance with additional two from any of the following:

Obesity,

Dyslipidaemia (elevated triglycerides above150 mg/dL, HDL-c < 35 mg/dL for males and 39 mg/dL for females),

Hypertension (Blood pressure ≥140/90 mm Hg or medication) and;

Microalbuminuria (Albumin ≥ 20 μg/min or albumin/creatinine ratio ≥ 30 mg/g)

2.2.2 The European Group for the Study of Insulin Resistance (EGIR) Definition

EGIR (1999) defined Metabolic syndrome as the presence of insulin resistance or hyperinsulinemia in non-diabetic subjects plus two or more of the following:

Central obesity: waist circumference ≥ 94 cm (male) and 80 cm (female)

Dyslipidaemia: triglycerides greater than 177 mg/dL or HDL-c less than 30 mg/dL

Hypertension: Blood pressure greater than or equal to 140/90 mmHg

Fasting plasma glucose greater than or equal to 110 mg/dL

2.2.3. National Cholesterol Education Program-Third Adult Treatment Panel (NCEP ATP-III)

Metabolic Syndrome was defined by ATP-III (2001) as the existence of three or more of the following;

Central obesity: waist circumference greater than 102 cm (male) and 88 cm (female)

Hypertriglyceridemia: triglycerides greater than or equal to 150 mg/dL

Low HDL-C: less than 40 mg/dL for male, 50 mg/dL for female

Hypertension: Blood pressure greater than or equal to 130/95 mm Hg

Fasting plasma glucose greater than or equal to 110 mg/dL

2.2.4 American Association of Clinical Endocrinology (AACE) definition

Metabolic Syndrome diagnosis was proposed with the co-existence of the following

Triglycerides =150 mg/dl

HDL-c = 40 mg/dl for men and 50 mg/dl for women

Blood pressure ≥ 130/85 mmHg

Fasting plasma glucose = 110–125 mg/dl) and 2-h post-glucose=140–200 mg/dl

Other factors for consideration include:

Presence of overweight or obesity

Family history of type-2 diabetes

Presence of polycystic ovary syndrome

Lack of physical activity

2.2.5 Harmonised definition of metabolic syndrome as suggested by IDF

IDF (2005) suggested that metabolic syndrome exists when there is central obesity with any other two of the following:

Elevated triglycerides -150 mg/dL and above

Decreased HDL-c - 40 mg/dL and above for men and 50 mg/dL and above for women

Elevated systolic blood pressure - 130 mm Hg or diastolic - 85 mm Hg and above or confirmed hypertension

Elevated fasting plasma glucose - 100 mg/dL and above or confirmed type-2 diabetes

2.3 Additional criteria for research purpose

The IDF Workshop in support of the new unified description of MetSyn emphasized additional factors that may be associated with MetSyn to enable further modifications as these additional variables are used for research.

The adoption of these other variables by researchers will enable further amendments to the description and establishment of ethnic-specific clinical definition of MetSyn. Some of the parameters identified by the IDF (2005) are as follows:

2.3.1 Abnormal fat distribution

Body composition

Visceral fat accumulation

Biomarkers of adipose tissue such as leptin and adiponectin

Hepatic fat concentration

2.3.2 Atherogenic dyslipidaemia

Measurement of Apo B and small LDL particles

2.3.3. Dysglycaemia

Oral glucose tolerance test

2.3.4 Insulin resistance

Fasting insulin/proinsulin levels

HOMA-IR

Insulin resistance (Bergman minimal model)

Raised free fatty acids (fasting and during oral glucose tolerance test)

M-value from clamp

2.3.5 Vascular dysregulation

Endothelial dysfunction measurement

Microalbuminuria

2.3.6 Pro-inflammatory state

a. Raised high sensitivity C-reactive protein (CRP)
b. Raised inflammatory cytokines (e.g. tumour necrosis factor-alpha, Interleukin-6)
c. Reduced adiponectin plasma levels

2.3.7 Pro-thrombotic state

a. Fibrinolytic factors (plasminogen activator inhibitor-1, etc.)
b. Clotting factors (fibrinogen, etc.)

2.3.8 Hormonal factor

a. Pituitary-adrenal axis

2.4 Role of Inflammation in Metabolic Syndrome and its components

The concept of inflammation can be defined as the reaction of the body to an injury. It is the natural response of immune system to infections and injury in an attempt to repair, heal, or fix itself; this can also be referred to as acute inflammation. Chronic inflammation on the other hand occurs when repairs cannot be achieved leading to

a constant low-grade inflammation. The chronic inflammation has been considered as the origin of many non-communicable chronic diseases (Kassi *et al.*, 2011) such as insulin resistance, type 2 diabetes mellitus, ,and cardiovascular diseases, MetSyn, dementia, osteoporosis and some cancers. Hypotheses made in the last few years have generally led to a belief that the process of inflammation is fundamental to the onset of most diseases that can be positively influenced by frequent intake of fruit (Pan *et al.*, 2010). Research on polyphenols prevalently found in fruits and vegetables have been made and results have shown that these polyphenols play positive roles in attenuating inflammation (Recio *et al.*, 2012).

In recent times, the burden of overweight and obesity has drastically increased with public health significance in the developed and developing countries of the world. Obesity stands at the heart of many of the common lifestyle related chronic NCDs such as atherosclerosis, hypertension, type-2 diabetes and hyperlipidemia thus making most of these conditions and other components of MetSyn like insulin resistance, dyslipidemia, hypertension etc. existing in people with obesity. Obesity therefore increases the risk factors for chronic non communicable diseases.

For a long time, the adipose tissue has been recognized only as a storage site and heat insulator. However, it is also an endocrine organ that produces and secretes several pro-inflammatory cytokines that are markers of inflammation. Some of these pro-inflammatory cytokines includes adiponectin, tumor necrosis alpha (TNF-α), interleukin-6 (IL-6), vascular endothelial growth factor and leptin (Lee and Pratley, 2005). These cytokines have been discovered to play roles in the etiology of many chronic non-communicable diseases (Lee and Pratley, 2005).

Consistent reports have shown that strong relationship exists between MetSyn and low-grade chronic inflammation. The type of inflammation that occurs in metabolic syndrome do not fall into chronic or acute inflammation especially because an injury in the tissue or an infection is usually not present, however it is usually called low-grade chronic inflammation. In 2006, Hotamisligil made an attempt to name the type of inflammation that characterized MetSyn as ‘metaflammation’ which means an inflammation that was metabolically triggered. In 2008, Medzhitov also named it para-inflammation which explains the intermediate state between the basal and inflammatory states.

2.5 Contibution of obesity to inflammation

Obesity has been linked with higher level of non-esterified fatty acids (NEFA) in the blood, as well as peripheral insulin resistance (IR) and these have been regarded as the causative factor of the impaired glucose metabolism (Stumvoll *et al.*, 2005) and MetSyn. In obesity, the body also undergoes an increased synthesis of cytokines and white blood cells that consequently leads to the chronic inflammatory state associated with obesity. McArdle *et al.* (2013) asserted that the coexistence of chronic inflammation and peripheral IR in obesity always results into increased rate of breakdown of fat that subsequently leads to elevated plasma concentration of NEFA and pro-inflammatory cytokines. Hotamisligil *et al.* (1993) first described this association between obesity and inflammation.

It was discovered that different models of obesity shows an elevated level of TNF-α and when this cytokine is eliminated, IR is ameliorated (Hotamisligil *et al.*, 1993). This cytokine (TNF-α) has been discovered to be secreted by white adipose tissue which was once known to be only a storage site for triglyceride but has now been found to be an active secretory organ. Some of these cytokines are called adipokines (such as leptin, TNF-α, adiponectin, Interleukin-1 β, Interleukin-6, Interleukin-10, etc.) and are produced by the adipose tissue. In obesity, there is expansion of the adipose tissue and this is linked with the increased secretion of pro-inflammatory factors that induces IR.

In insulin resistance, the potential of insulin to regulate lipid metabolism is reduced, leading to increased plasma circulating NEFA. These adipokines (pro-inflammatory) and NEFA produced by the adipose tissue cause series of effects on the liver such as the stimulation of lipogenesis and increase in the formation of new glucose molecules from non-carbohydrate sources (Brown and Goldstein 2008). These actions in the liver (increased NEFA and lipogenesis) subsequently leads to elevated synthesis of very low-density lipoprotein (VLDL) and a condition called nonalcoholic fatty liver disease (NAFLD) with atherogenic consequences (Choi and Ginsberg, 2011). Besides dyslipidemia and impaired glucose metabolism, individuals with obesity have high levels of cholesterol, VLDL-triacylglycerol, LDL-Apo and VLDL-Apo in their blood (Chen *et al.*, 2011).

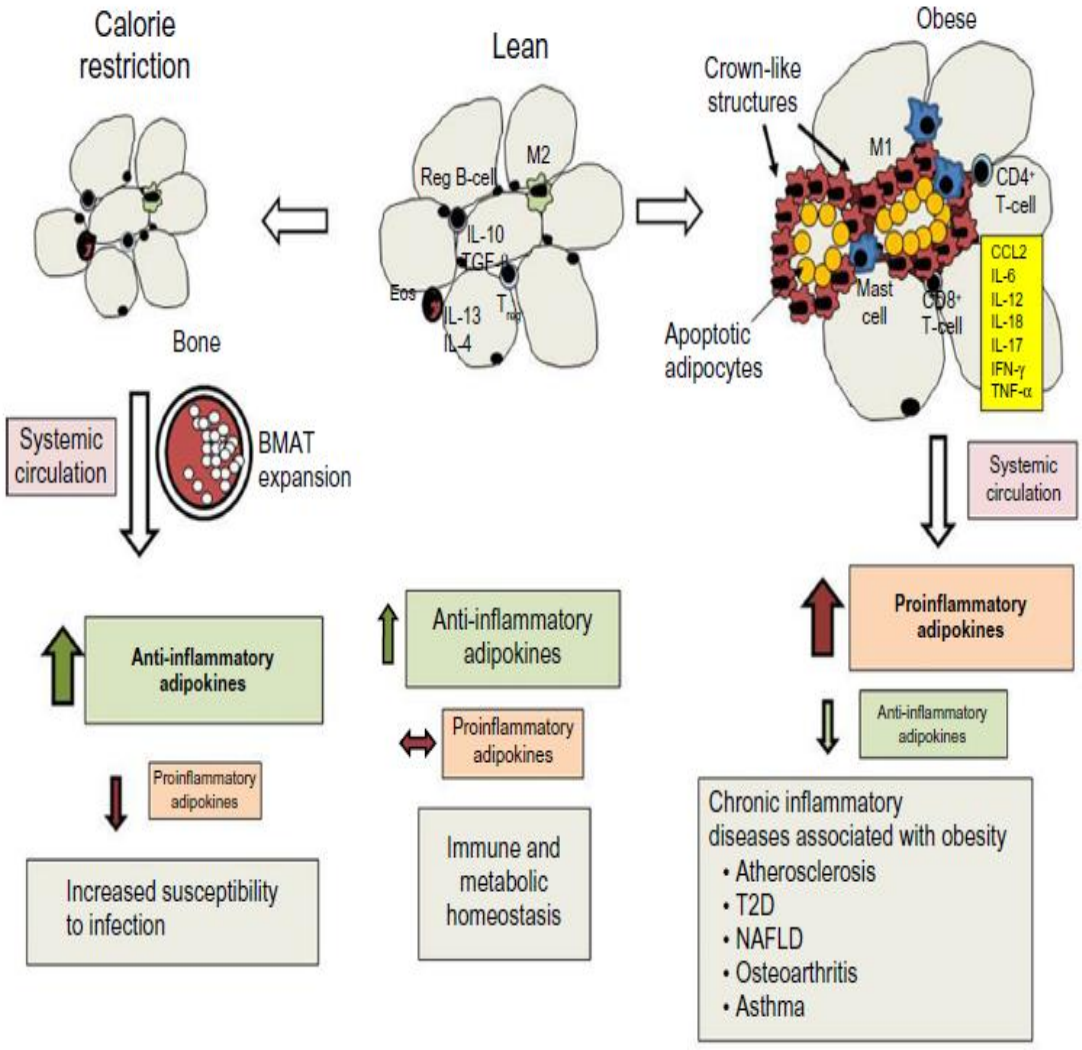


Figure 2.1. Relationship between obesity, adipokines, inflammation and metabolic diseases

Calorie restriction: VAT and SCAT adipocyte size declines but BMAT increases. Increased anti-inflammatory adipokines with greater risk of infectious disease. Lean state: IL-4, IL-13, IL-10 and TGF-b maintain M2 macrophage phenotype with normal metabolic and immune homeostasis. Obese state: hypertrophy promotes rarefaction and apoptosis. M1 macrophages engulf necrotic adipocytes forming crown-like structures. Proinflammatory cytokines and adipokines promote inflammation and diseases associated with obesity. Abbreviations: BMAT, bone marrow adipose tissue; T2D, type 2 diabetes; NAFLD, nonalcoholic fatty liver disease; SCAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; Eos, eosinophils.

Source: Mancuso, 2016

2.6 Roles of Oxidative Stress in Metabolic Syndrome

The function of oxidative stress in cellular injury is fundamental. Oxidative stress (OS) takes place due to the weakness of the body's defense mechanism and the inability of the body to act against increased generation of reactive oxygen species (ROS), causing a shift in the balance between ROS and their protection (Pandey *et al.*, 2010). It is described as a condition that takes place when ROS generated within the cell reduce the capacity of antioxidants, destructing the cellular macromolecules (Silva *et al.*, 2008). ROS are normally the by-products of normal cellular breakdown that are produced during the process of electron transport to generate cellular energy. They are generated by macrophages and neutrophils during the process of respiration in order to remove antigens (Freitas *et al.*, 2010). They are also involved in the stimulation of some genes that encode for transcription, differentiation and development of cells as well as inducing cell-to-cell adhesion, cell signaling, vaso regulation, proliferation of fibroblasts, and promotion of antioxidant enzyme expressions (Gomes *et al.*, 2012). ROS are useful in the body however, uncontrolled and/or over production is detrimental to human health. OS can cause poor regulation of function in cells, lipid peroxidation and damage to DNA molecules, proteins and membrane phospholipids (Silva *et al.*, 2008).

ROS-induced OS is reported to be linked to ageing and some non-communicable diseases like diabetes, cardiovascular diseases, cancer etc (Khurana *et al.*, 2013). OS can trigger resistance to insulin which may progress to intolerance to glucose, and subsequently lead to diabetes mellitus. This could therefore promote the development of athero-sclerotic complications, and elevate the macro- and micro vascular complications (Negre-Salvayre *et al.*, 2009. Apart from cellular damage induced OS, ROS interferes with homeostasis of calcium by increasing the let out of calcium ions in the mitochondria causing elevated levels of calcium-dependent enzymes like nucleases, proteases and phospholipases (Gomes *et al.*, 2012).

2.7 Lipid peroxidation

The significant function of cellular oxidation by free radicals in CVD has been identified as far back as the time of the proposed theory of oxidative atherogenesis (Chisolm and Steinberg, 2000). Studies from animals and humans therefore support the important contributions of lipid oxidation in the prediction of CVD development

and response to treatments. Lipids are at risk of oxidation due to the abundance of reactive double bonds in their molecular configuration (Porter *et al.,* 1995). Among the well-researched indicators of lipid peroxidation are the malondialdehyde (MDA) and isoprostanes (IsoPs). Other biomarkers that have been documented are the oxidation resistance assays, oxysterols and lipid hydroperoxides.

IsoPs are prostaglandin-like compounds that are readily stable and are produced from the peroxidation of arachidonic acid, a polyunsaturated fatty acid (Morrow *et al.,* 1992). The free radicals for IsoPs formation are generated through the transport chain of mitochondrial electrons (Griffiths *et al.,* 2002). IsoPs are afterward released into circulation by phospholipases from the cell membrane (Stafforini *et al.,* 2006). The concentration can then be determined in blood, urine and tissue. Urine and plasma levels of IsoPs have significant relationship with cellular OS both in studies conducted among humans and animals (Griffiths *et al.,* 2002; Fam and Morrow, 2003). Increased production of IsoPs is linked with cigarette smoking, elevated cholesterol, adiposity, hyper-homocysteinemia, diabetes mellitus (Morrow, 2005).

2.8 Malondialdehyde (MDA)

It is a product of polyunsaturated fatty acids peroxidation (Slatter *et al.,* 2000). Plasma and serum concentrations of MDA are determined using colorimetric assay that is hinged on the reaction between MDA and thio-barbituric acid (TBA) and it is measured as thiobarbituric acid reactive species (TBARS). The TBARS assay is generally regarded as a biomarker of OS in many models of CVD. Plasma level of TBARS has been reported by several researchers to increase significantly in streptozotocin-induced diabetic rat models (vanDam *et al.,* 2001). However, its level reduced to normal following administration of antioxidants in the experimental model.

2.9 Oxidative Stress and Obesity

Obesity is the predominant underlying factor of the lifestyle related diseases such as dyslipidemia, diabetes, elevated blood pressure etc. The rising prevalence of obesity, with special reference to deposition of fat in the abdominal region, is central to the development of MetSyn. Numerous studies have proven that obesity is a common phenomenon among patients with MetSyn (Skalicky *et al.,* 2008; Furukawa *et al.,*

2004; Cardona *et al.,* 2008a). Several mechanism of actions have been postulated to explain the relationship between obesity and oxidative stress. Bays *et al.* (2008) suggested excessive accumulation of fat leads to the development of OS. It was further explained that, OS occurs as a result of abnormal functioning of the adipocyte. The presence of adipocyte dysfunction leads to uncontrolled production of adipocytokines that are involved in the onset of obesity-associated MetSyn.

Studies have pointed to the fact abdominal fat accumulation increases the synthesis of the pro-inflammatory markers in the adipocytes and this subsequently leads to the development of OS (de Ferranti and Mozaffarian, 2008). This was also confirmed by the study of Furukawa *et al.* (2004) who reported strong association between obesity and systemic OS. In addition, it was noted that the activities of NADPH oxidase was increased whereas activities of the antioxidant enzymes declined markedly. It is perceived that the increase in the levels of OS and inflammatory factors involves the elevation of superoxide anion synthesis through the NADPH oxidase pathway. This pathway is responsible for the regulation of adipokines, once the activities of NADPH oxidase increases, there would be uncontrolled synthesis of adipokines which eventually would result into the development of OS (Furukawa *et al.,* 2004).

Leptin is a hormone secreted by the adipocytes that controls satiety and energy output. Plasma level of leptin has been demonstrated to have a direct relationship with the percent body fat. Leptin contribute significantly to the development of OS in obese patients because it induces the synthesis of ROS directly. In addition, leptin promotes the synthesis of inflammatory factors that enhances the secretion of cytokines such as TNF-α, and IL-6 which leads to enhanced activity of NADPH oxidase and synthesis of superoxide anion (Vincent and Taylor, 2006).

2.10 Oxidative stress, insulin resistance and type-2 diabetes in Metabolic Syndrome

Insulin resistance and reduction in insulin production are major characteristics of type-2 diabetes. Type-2 diabetes occur due to insulin resistance and it is widely regarded as a main contributor to the onset of diabetes. When the needed amount of insulin secreted can no longer act appropriately on the target tissues (adipose tissue and skeletal muscle), and the body keeps secreting insulin to act on the elevated

blood glucose level, insulin resistance results. Hyperglycaemia has been shown to contribute significantly to the development of complications in diabetes (Martyn *et al.*, 2008). It has been shown consistently that uncontrolled elevated blood glucose for a long time leads to the destruction of various cells especially, β-cell and adipocytes through diverse processes that involved OS. Evidence showed that hyperglycemia or free fatty acids (FFA) induced OS take place before the clinical observation of its complications. Elevated blood glucose directly results into increased production of ROS. It also activates the synthesis of superoxide anion via stimulation of NADPH oxidase (Evans *et al.,* 2003; 2005).

In addition, findings have indicated that elevated FFA levels leads to insulin resistance and the mechanism suggested could be the association between obesity and diabetes. Increased concentration of FFA have negative consequences on the functions of mitochondrial as ROS is generated from the process of oxidative phosphorylation which occurs in the mitochondrial. Besides the ability of FFAs to stimulate OS directly, they are also able to damage the natural ability of the cells to fight free radicals by lowering the level of glutathione within the cell. This could explain how FFAs are able to stimulate Protein Kinase C (Maassen *et al.,* 2007). In addition, the constant competition that exists between fatty acid and glucose for metabolism enables the prevention of pathway for insulin simulation that should enhance glucose uptake by fatty acid metabolites.

Mitochondrial dysfunction in relation to fat metabolism results into retention of fat and its metabolites in the muscle, resulting into the development of insulin resistance. Cardona *et al.* (2008b) reported elevated OS following intake of high fat diet in individuals with MetSyn. Furthermore, oxidative stress destroys β-cell as increased blood glucose level and or FFAs for a long time results in abnormal functioning of the β-cell. Evans *et al*. (2003) postulated that β-cell function are damaged by constantly elevated blood glucose via OS and existence of oxidative stress leads to destruction of the tissue. It has been established that increased blood glucose and high FFA concentrations leads to the formation of ROS (Robertson *et al.*, 2007).

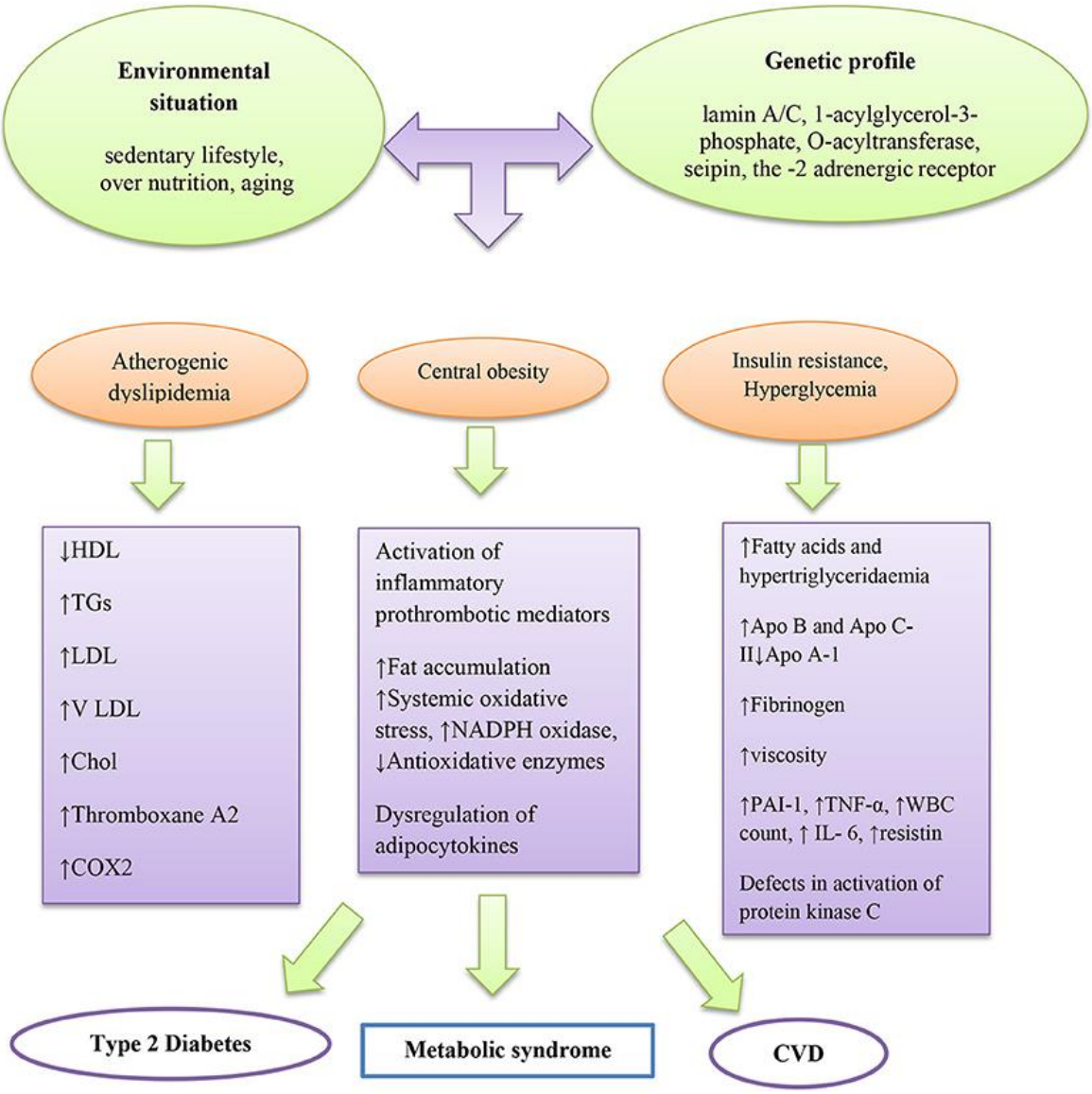


Figure 2.2. Summary of pathogenesis of Metabolic Syndrome

Source: Spencer *et al.*, 2008

2.11 Phytochemicals

Phytochemicals are secondary metabolites that are naturally occurring in plants (Perez-jimenes *et al.*, 2011). They are widely found in vegetables, fruits, beans, whole grains, nuts and seeds. They have been reported to have therapeutic roles and also responsible for the colour, aroma and taste of the plants (Chanet *et al.*, 2012).

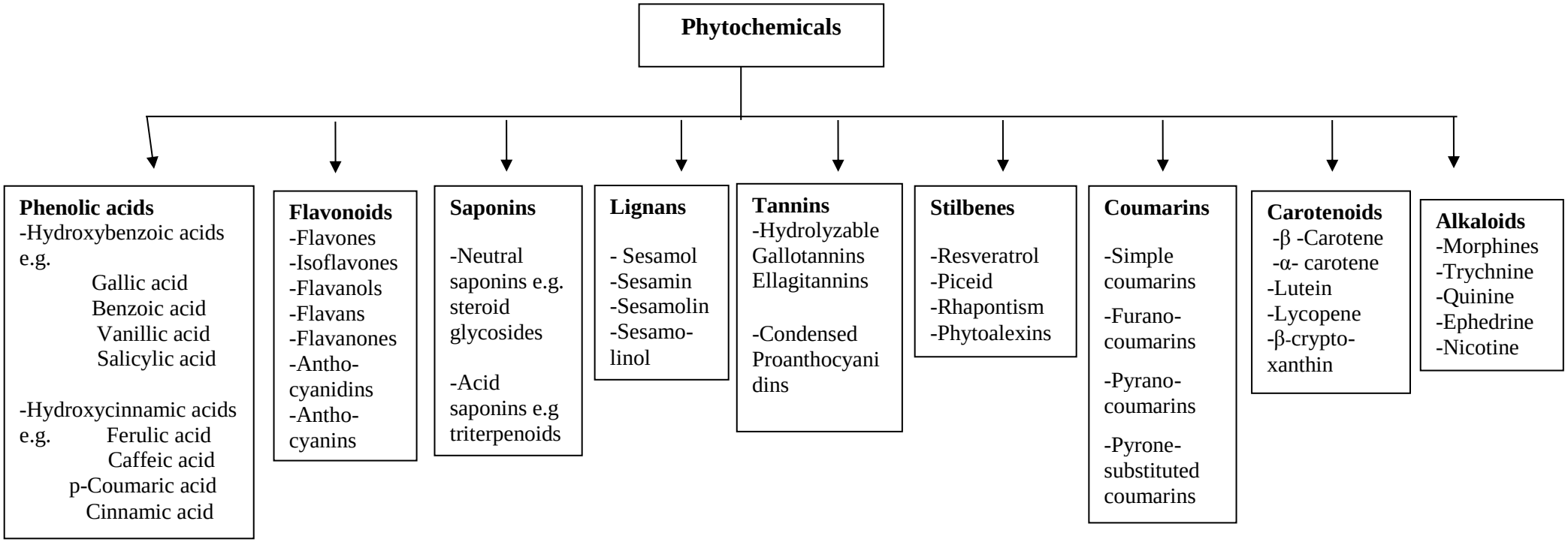


Figure 2.3. Major classes of phytochemicals in plants

Source: Field survey, Ogunkunle 2019

Table 2.1. Food sources of different types of phytochemicals

Phytochemical class	Sub-classes	Examples	Food sources
Phenolic acids	Hydroxybenzoic acids	Gallic acid Benzoic acid Vanillic acid Salicylic acid Ellagic acid	Grape seeds, raspberries, blackberries, pomegranate, vanilla, tea
	Hydroxycinnamic acid	Caffeic acid Chlorogenic acid Coumaric acid Ferulic acid Sinapic acid	Papaya, Peach, Avocado, Kiwi fruit, Passion fruit, Blueberry, Cherry, Orange, Blackcurrant, Mango, Orange, Pineapple, Apple
Flavonoids	Flavonones	Hesperidin Naringenin Eriodictyol Hesperitin Naringin Narirutin	Citrus fruits: oranges, lemons, grapefruit, lime, tangerine
	Flavonols	Quercetin Kaempherol Myricetin Rutin Kaempferid	Cherry, tomato, onion, broccoli, tea, red wine, berries, Citrus fruits, apples, blueberry, passion fruit, pomegranate, blackcurrant, fig, Apple, papaya, red grape, prunes, blueberry, apricot, apple
	Flavones	Apigeninn Luteolin	Cereals, parsley, Celery, Mango, Lemon, pineapple, plum, watermelon, orange

Table 2.1. Food sources of different types of phytochemicals continued

Phytochemical class	Sub-classes	Examples	Food sources
	Isoflavones	Daidzein Genistein Glisitein Daidzin Genistin	Soy products, peas
	Flavanols	Catechin Epicatechin	Chocolate, tea, coffee, beans, apricot, red wine, cherry, apple, Grapes, apricot, peach, blackberry, avocado
	Anthocyanins	Cyanidin Pelargonidin Peonidin Delphinidin Malvidin Petunidin	Berry, pomegranate, black currant, strawberry, raspberry, mangosteen, blueberry, black grape, grapefruit, strawberry, plum, apple, red wine, red cabbage
Lignans		Sesamol Sesamin Sesamolin Sesamolinol	seeds (flax, pumpkin, sunflower, poppy, sesame), whole grains (rye, oats, barley), bran (wheat, oat, rye), beans, garlic fruit (particularly berries), and vegetables,
Stilbenes		Resveratrol Piceid Rhapontism	Wine, grapes, blueberries

Table 2.1. Food sources of different types of phytochemicals continued

Phytochemical class	Sub-classes	Examples	Food sources
Alkaloids			Tea, coffee, cocoa and honey, Solanaceae family such as tomatoes, eggplant, Potatoes, peppers (bell peppers, chili peppers etc)
Saponins		Steroid glycosides Triterpenoids	Legumes (including beans, peanuts, and soy), kidney beans, garlic, Red wine
Carotenoids		Lutein	spinach, kale, broccoli, Brussel sprouts
		Zeaxanthin	egg yolks, maize, spinach
		b-cryptoxanthin	citrus fruits, avocado, papaya, pepper
		α-carotene	carrots, pumpkin, maize
		Lycopene	tomato and its products, water melon, guava
		β-carotene	carrots, spinach, parsley
Tannins		Condensed tannins (proanthocyanidins)	Grapes and pomegranates contain some tannins even as they ripe. Berries, cumin, thyme, apples, sorghum, barley, nuts, chocolate, rhubarb, squash and legumes such as chickpeas and beans,
		Hydrolizable tannins	
Coumarins			strawberries, apricots, and cherries

Source: *Field survey, Ogunkunle 2019*

2.12 Classes of phytochemicals

2.12.1 Phenolic acids

Phenolic acids are secondary metabolites that are widely distributed in plant. Phenolic compounds are known to have distinctive taste, flavour, and health promoting properties attributed to fruits and vegetables (Tomas-Barberan and Adrew, 2012). They are important for growth and reproduction in plants, and are synthesized as a form of reaction to environmental stressors; they also act to protect damaged plants (Valentine *et al.,* 2003). The two principal phenolic acids are: Hydroxycinnamic and hydroxybenzoic acids. Phenolic acids are found both in free and in bound forms. Hydroxycinnamic acid are synthesized as simple esters with glucose or hydroxy carboxylic acids. There are different molecular structures for each of the numerous phenolic compounds in various plant and they differ from one another by the hydroxylated aromatic rings (Mandal *et al.,* 2010). Free phenolic acids are found in vegetables and fruits, while the bound forms are found in the hull, bran and seeds of fruits (Hussain *et al.,* 2004). Bound phenolic acids are loosen from the different parts of plant or fruit through extraction with acids and or alkali and their concentrations are relatively low in plant-based foods compared to free phenolic acids. Numerous studies have shown the impact of environmental stressors on the phytochemical composition and antioxidant content in connection with adaptation of plants in fruits and vegetables. Pennycooke *et al.* (2005) documented that chilling being a type of environmental stressor resulted to increased total phenolic content and antioxidant activity in petunia. Likewise, high irradiation and cold stress was found to increase the flavonoid contents in plants (Tattini *et al.,* 2005). Phenolic acid compounds and their purposes have been the subject of importance in research recently.

2.12.2 Tannins

Tannins are phenolic compound that bind and form strong complex with proteins with distinct astringent taste. They are found in plants. They have moderate to high molecular weight that distinguish them from the groups of low molecular weight plant phenolics. Their molecular weight ranges from 500 to greater than 3000. Hydrolyzable and condensed tannins are the main types of tannin (Santos-Buelga and Scalbert, 2000). One of the main characteristics of tannins is the formation of insoluble complexes with proteins leading to the formation of astringent taste perculiar to tannin-rich foods. The astringency felt in the mouth when foods rich in tannins are consumed is due to the the

reaction with salivary proteins, they bind to proteins in the tongue and mucous membrane in the oral cavity and denature the proteins to produce a shrinking effect (Scalbert and Williamson, 2000). Some fruits are highly astringent because they contain hydrolysable tannins, but the astringency reduces due to dryness or as the fruit ripes (Murkovic *et al.,* 2000).

2.12.3 Saponins

Saponins are plant glycosides that gives foamy lather when agitated with water. They are broadly dispersed and have been documented to exist in at least 500 species of plants. Saponins are generally water soluble but not soluble in non-polar solvents. When saponins are hydrolised, they release a compound free of sugar attachement called "sapogenin." Saponins can be used as enhancers in food and cosmetics, as surface-active agent for use in agricultural practices and in photography, and as additive for effective management in the pharmaceutical industries (San-Martin and Briones, 1999).

Saponins possess various biological activities, viz. antioxidant, immunostimulant, antihepatotoxic, antibacterial, anticarcinogenic, antidiarrheal, antiulcerogenic, antioxytocic, hypocholesterolemic, anticoagulant, hepatoprotective, hypoglycemic, neuroprotective, anti-inflammatory activity, useful in diabetic retinopathy, prevention of dental caries (Rao and Gurfinke, 2000).

2.12.4 Flavonoids

Flavonoids are essential group of naturally existing compounds belonging to plant secondary metabolites that are broadly dispersed in fruits, vegetables and certain beverages. Vegetables use them for their growth and defense against plaques (Havsteen, 2002). Flavonoids are available freely in plant food sources; they have various subclasses that have unique food sources. For instance, flavanones are mainly found in citrus. Flavonoid contributes significantly to the beneficial and health promoting activities attributed to plant foods. Generally in plants, flavonoids are responsible for the color and aroma of flowers to attract pollinators and facilitate seedling's growth and development (Griesbach, 2005). They also shield plants from stressors such as excessive cold and heat etc (Samanta *et al.,* 2011). Flavonoids are recognized as compounds that possessed beneficial health effects on humans and are currently being investigated for therapeutic purposes and prevention of diseases. At present, more than

6000 flavonoids that play significant roles in the health of both humans and plants have been identified.

Flavonoids have various positive and promising beneficial effects that has been linked with diseases such as cancer, diabetes, metabolic syndrome etc (Lee *et al.*, 2009; Ovando *et al*., 2009). Flavonoids can be sub-grouped into different classes based on their structural arrangement. The flavonoid subclasses are: flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones.

2.12.5 Flavones

Flavones are generally available in the plants as glucosides. Main sources include parsley and red peppers; and citrus peels are also rich in flavones such as nobiletin. Luteolin, apigenin and tangeritin are examples of flavonoids that belong to this subclass (Kumar and Pandey, 2013). They are consist of double bond at the C-2 and C-3 with a single ketone group at C-4. Flavones that are of vegetable and fruit origins possessed a hydroxyl group at the C-5 position.

2.12.6 Flavanols

Flavonols are a group of flavonoids that consist of ketone as a functional group, and are mostly monomers of proanthocyanins. They occur readily in several species of vegetables and fruits with examples as quercetin, kaempferol, fisetin and myricetin. Plants that are rich in flavonols are kale, lettuce, apple, onions, grapes and berries. Aside fruits and vegetables, flavonols are also abundant in red wine and tea. The dietary consumption of flavonols has been reported to be associated with a lot of health benefits due to the presence of antioxidants that help in reducing the threat of cardiovascular problems. Flavonols have been reported to be the most abundant subgroup of flavonoids in plants (vegetables and fruits), especially quercetin that is found in several plants (Iwashina, 2013).

2.12.7 Flavanones

Another important subgroup of flavonoids is the flavanones which are present in citrus species like the lemons, oranges and grapefruits in the forms in the forms of naringenin, hesperitin and eriodictyol. Perterson *et al*. (2006) reported that flavonones constituted 98% of the overall flavonoid contents in fruits of citrus plants. Flavonones serves beneficial role in human health due to the presence of free radical-scavenging

properties. These compounds are responsible for the bitter taste of the juice and peel of citrus fruits. Flavonones in several citrus species play significant health benefits in pharmacology, acting as antioxidant, anti-inflammatory, blood lipid-lowering and cholesterol-lowering agents (Iwashina, 2013). They are also known as dihydroflavones with saturated C-rings making it structurally different from other subgroups of flavonoids.

2.12.8 Isoflavonoids

Isoflavonoids are different subgroup of the flavonoids with limited presence in plant species such as legumes and soybeans. Matties *et al*. (2008) have also reported the presence of some isoflavonoids in micro-organisms. Isoflavonoids are significantly involved in the synthesis of phytoalexins by plants during pathogen invasions. (Dixion and Ferreira, 2002), and play important role in the prevention of diseases in plants. Important isoflavones that are known as phyto-oestrogen due to their oestrogenic potential in animals are genistein and daidzein. Szkudelska and Nogowski (2007) have also opined to the fact that isoflavones such as genistein possessed potentials to induce shift in hormones and metabolism.

2.12.9 Flavanols, flavan-3-ols or catechins

Flavanols are referred to as dihydroflavonols/catechins which are the 3-hydroxy surrogate of flavanones. The hydroxyl-group in flavanol always binds to C-3 positon with no double bond between C-2 and C-3 positions. They are largely abundant in apples, pears, peaches and blueberries

2.12.10 Anthocyanins

Anthocyanins are photosynthetic pigments (in plants, fruits and flowers) that are responsible for colourations. The most abundantly studied examples of anthocynins are the dlphinidin, cyanidin, pelargonidin, peonidin and malvidin. Anthocyanins are abundantly present in the pericarps of fruits like black currants, red grapes, blueberries and raspberries. Due to their stability and potential benefits to human health, anthocyanins are readily used in food and drink sector (Giusti and Wrolstad, 2003). Iwashina (2013) asserted that the colouration of anthocyanins is dependent on the level of acidity (pH) of the medium and the methylation of the OH^- group on the A and B rings (Iwashina, 2013).

2.12.11 Chalcones

Chalcones are subgroup of flavonoids that are identified by the absence of C-ring of the basic flavonoid structure, and otherwise called the open-chain flavonoids. Important examples of chalcones are the arbutin, phloretin, chalconaaringenim and phloridzin. They are majorly found in large concentrations in pears, tomatoes, strawberries wheats and bearberries.

2.12.12 Alkaloids

Alkaloids are large group of natural phytochemicals, mostly of microbial, plant and animal origin. Most alkaloids in their pure form are colourless, nonvolatile, crystalline solids with a characteristic bitter taste. Alkaloids have been synthesized for the development of several antibacterial drugs in the pharmaceutical research such as the synthesis of quinine that produced the quinolones, azomycin that led to the production of metronidazole, and quinoline which produced bedaquiline. The development of antibacterial drugs from alkaloids remains the focus of many researchers (Hraiech *et al*., 2012; Parhi *et al*., 2012). Alkaloids is an important class of natural compounds containing nitrogen in a heterocyclic ring (Bogatcheva *et al*., 2011). The use of alkaloids as antibiotic, narcotics, stimulants and anticancer agents has greatly improved the human health sector (Kainsa *et al*., 2012).

2.12.13 Lignans

Lignans are fiber-like compounds that are also referred to as phytoestrogens. They are polyphenols derived from phenylanaline found in plants. Lignans are abundant in foods, especially the legumes and whole-grain cereals. In addition, lignans are also present in some vegetables and fruits (Durazzo *et al*., 2018) and flaxseed in large concentration. Lignans act as antioxidants and can bind to estrogen receptors in the breast tissue. Also, Hazra and Chattopadhyay (2016) reported the potential ability of bacteria in the intestine to convert plant-sourced lignans into enterodiol, enterolacton and enterolignans. Enterolignans have a variety of biological roles such as estrogen-receptor activation, and anti-inflammatory and apoptosis which influence disease risk in humans.

2.12.14 Carotenoids

Carotenoids are natural pigments consisting of various structures and functions (Landrum, 2010). They are universally present in photosynthetic plants and are referred to as precursors of retinol (vitamin A). They also function in organisms that are not photosynthesizing by acting as photo-protective agents. Carotenoids important phytochemicals that are involved in the sequestration of singlet oxygen (O_2^-) and some reactive oxygen species (Edge and Truscott, 2010). This is of great importance, because elevated synthesis and production of ROS level in organismal cells leads to in oxidative stress which is a major factor in the advancement of chronic non-communicable diseases. Fiedor and Burda (2014) asserted that carotenoids and metabolites are very important protective capacity in several ROS-facilitated diseases like the type-2 diabetes, some cancer or neurological-related diseases and eye-related disorders (Fiedor and Burda, 2014). Up to date, about 700 carotenoids are known (Brittton *et al*., 2004) with close to 50 of the carotenoid-types are recognized as nutrients in human foods (Khachik, 2006), while approximately 20 are constituents of bloods and tissues of humans. Breecher and Khachik (1992) have identified β-carotene, α-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, α-cryptoxanthin, γ-carotene, neurosporene, ζ-carotene, phytofluene and phytoene as the most important carotenoids.

2.12.15 Stilbenes

Stilbenes are forms of phenols found in various families of plants like the grapevine, berries and peanuts (Rivie`re *et al*., 2012). Stilbenes are significant group of non-flavonoids which specific polyphenolic structure and consists of a 1, 2-diphenylethylene nucleus. Stilbenes is important for the prevention and therapy of some diseases like the cancer because of the presence of qualities like antioxidant action, anti-inflammatory property and activation of cell death with minimal toxicity. Among all the identified stilbene compounds, resveratrol has been extensively studied due to its wide-spread biological activities like antioxidant, anti-inflammatory and anti-proliferative potentials (Vang *et al*., 2011). Jang *et al.* (1997) initially reported the potential anticancer activity of resveratrol and since then it has received attention of researchers to investigate further their chemo-preventive and chemotherapeutic properties. Resveratrol has been subjected to assays for *in-vitro* and *in-vivo* carcinogenesis for several cancer-types. Such cancers include cancer of the breast (Chatterjee *et al*., 2014), lung (Malhotra *et al*., 2014), colon (Mazue *et al*., 2014) and

non-melanoma and melanoma skin cancer (Back *et al*., 2012). Resveratrol therapy on such cancer as prostate (Hsieh *et al*., 2012), ovarian (Lin *et al*., 2011), liver (Rajasekaran *et al*., 2011), oral cavities (Shrotriya *et al*., 2015), thyroid (Kang *et al*., 2011), and leukemia (Tsan *et al*., 2002) have been studied extensively as well. The chemo-preventive potential of resveratrol is well known to be linked to its antioxidant activity.

2.12.16 Coumarins

Coumarins consist of a large group of phytochemicals in plant species. They are also present in large quantities in some essential oils, especially the cassia leaf, lavender oil and cinnamon bark oil. Coumarin is also present in green tea, fruits, and other food types. Although, coumarins are widely-distributed in tissue parts of plant, highest concentrations are reportedly found in plant' fruits, and gradually reduce in roots, stems and leaves, respectively (Jain and Joshi, 2012). The type of coumarin present in different parts of a plant and the amount can be affected by the environmental conditions and seasonal changes. The coumarins are compounds that attracts the interest of researchers due to their biological properties to further screen them as novel therapeutic agents. As a result, they have been suggested for use in clinical medicine, with further evaluation for therapy of some clinical issues.

2.13 The Grapefruits

2.13.1 The internal and external structure of grapefruits

The grapefruit (*Citrus paradisi*) is a type of citrus species found in the subtropical region and it is well-known for its sour or semisweet taste. Grapefruits are a hybrid species that originated from the Barbados as unintentional product of breeding between sweet oranges (*C. sinesis*) and pomelos, or shaddocks (*C. maxima*). Generally, the internal structure of all citrus fruits is the same and is more complex compared to other fruits. A typical citrus, including grapefruit contained the peel (flavedo, oil gland, albedo, and vascular bundle), the seeds, central core and the segments (segment membrane, juice vesicles or sac) (Ye and Liu, 2005).

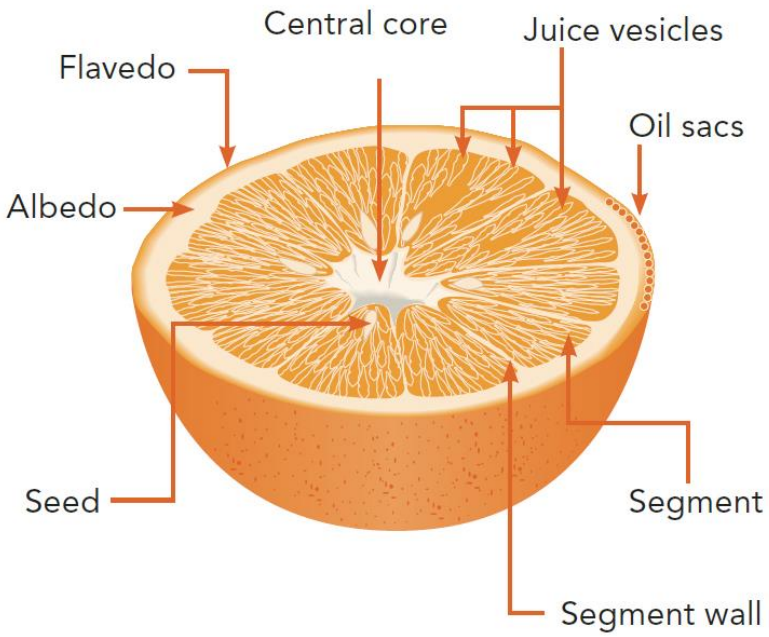


Figure 2.4. Internal structure of a grapefruit

2.13.2 Phytochemical compositions of grapefruits

Grapefruit is a type of citrus fruit that has been shown to contain macronutrients, micronutrients, minerals, vitamins and bioactive compounds such as flavonoids, phenols and coumarins and terpenic substances like the carotenoids and limonoids (Kelebek, 2010; Zou *et al*., 2015). Grapefruit possesses nutritional benefits to humans because of its low sodium, high potassium, folic acid and high level of vitamin C (Rampersaud, 2007).

Several studies in different countries have investigated the phytochemical composition of the different parts of grapefruits such as the peels, seeds, juice, segments etc. and reported that grapefruits contained various phytochemicals such as phenols, flavonoids, carotenoids and alkaloids. Of the different sub classes of flavonoids, grapefruits have been reported to contain substantial concentration of different types of flavanone mainly narirutin, naringin, eriocitrin, hesperidin, and naringenin (Sun *et al*., 2013). Other phenolics often reported to be present in grapefruits include ferulic, p-coumaric, gallic acid, caffeic and sinapic acids (Sun *et al*., 2013). As of now, greater than 60 varieties of flavonoids are known in citrus. The phytochemical screening for grapefruit showed the presence of about 13 flavanoid compounds such as noeriocitrin, narirutin, naringin, poncirin, didymin, hesperidin, eriocitrin and neohesperidin (Neveu *et al*.,

2010), and 20 types of carotenoids, especially lycopene and β-carotene in pink and red grapefruits. Phytofluene and zeta-carotene are also known compounds in grapefruits (Khan and Mackinney, 1953). Manners (2007) has also noted the presence of limonoid compounds like the nomilin and limonin that possess potential benefits to human health. In addition, Bailey *et al*. (1991) also found furanocoumarin in grapefruits and has been studied extensively due to its drug interactions, and has been reported to interact with cytochrome P450, CYP3A4.

2.14 Ameliorative potentials of grapefruit polyphenols on components of Metabolic Syndrome

There has been several reports from epidemiological studies on the ameliorative potentials of different citrus fruit polyphenols particularly the flavanones on NCDs such as CVD, type-2 diabetes, and MetSyn. Many clinical trials and animal studies have also documented beneficial effects of grapefruit polyphenols on different biomarkers and components of MetSyn. A randomized controlled crossover trial conducted by Rizza *et al.* (2011) among individuals with MetSyn receiving 250 mg hesperidin daily for three weeks, showed marked decline in the blood glucose concentration, systolic blood pressure, total cholesterol, high sensitive C-reactive protein with no effects on the triglycerides, LDL-c, HDL-c. Similar reports was documented by Demonty *et al*. (2010) who administered a higher dose of 400mg/day of hesperidin for four weeks among hypercholesterolemic men and women. Supplementation of 222mg/day of naringin among hypertensive men and women showed marked decline in the diastolic blood pressure, lipid profile characterized by lowered LDL-c, triglycerides and total cholesterol, and significant elevation in the HDL-c (Jung *et al.,* 2004). In animal studies, supplementation of 50mg/kg body weight of hesperidine in spontaneously hypertensive rat model led to a reduced systolic blood pressure, improved endothelia function and decreased Malondialdehyde (Yamamoto *et al.*, 2008a; Yamamoto *et al.*, 2008b). In addition, 0.02% hesperidine fed to db/db diabetic rat model reduced plasma concentration of triglycerides, total cholesterol, hepatic triglycerides and cholesterol (Jung *et al.*, 2006).

The beneficial effect of various doses of naringin (one of the abundant flavonoid in grapefruits) on biomarkers of MetSyn have been documented in many studies. Li *et al.* (2004) in their study administered 0.02% naringin to cholesterol-fed mice, documented

a significant reduction in the hepatic 3-hydroxy-3-methyl coenzyme A (HMG-CoA) reductase activity, and increased fecal sterol excretion. Alam *et al*. (2013) also reported decreased total cholesterol, reduced fat deposition in abdominal region, triglycerides, NEFA and blood glucose concentrations following daily supplementation of 100mg/kg body weight (b.w) naringin to a group of wistar rats fed with high fat-high carbohydrate diets. Narigrin supplementation (0.02%) has been reported to show positive effects on the lipid profile particularly the total cholesterol and triglycerides, reduced insulinemia, insulin resistance and blood glucose concentration in rats fed with diabetogenic diet (Chanet *et al.,* 2012; Pu *et al.,* 2012; Jung *et al.,* 2006). Activities of superoxide dismutase, glutathione peroxidase and glutathione reductase in the plasma were significantly increased while inflammatory markers (C-reactive protein, tumor necrosis alpha, interleukin-6) were significantly reduced in rats that were fed with naringin (100mg/kg b.w) in rabbits and rats receiving diets high in cholesterol (Chanet *et al.,* 2012, Alam *et al.,* 2013). Supplementation of naringenin, a predominant flavanone in grapefruits at 0.003, 0.006 and 0.012g/100g of feed for six weeks in rat induced with diabetogenic diet reduced visceral fat and plasma glucose level, total cholesterol and triglycerides (Cho *et al.,* 2011). Naringenin fed to mice on high fat diets at 1.0% has been shown to reduce triglycerides, cholesterolemia and tumor necrosis alfa with no difference in the antioxidant enzymes statistically (Mulvihill *et al.,* 2009). However supplementation of naringenin at 3.0% significantly improved the antioxidant enzymes (Mulvihil *et al*., 2009). These data suggests that grapefruit flavanones have anti-inflammatory and oxidative stress effects.

2.15 Non-pharmacological approaches to the management of metabolic syndrome

Nutritional risk factors for MetSyn are mainly overweight and obesity. Therefore, decrease in fat accumulation remains the central of any therapeutic or preventive approach for MetSyn. Relatively few studies have evaluated the contributions of nutrition to the development of MetSyn intrinsically and those that did employed various definition of MetSyn, thus making comparison difficult.

2.15.1 Weight loss and low energy diets

Weight loss has been proven to be beneficial in alleviating several of the metabolic anomalies linked with MetSyn, such as insulin resistance, raised blood pressure,

elevated uric acid, pro inflammatory factors, dyslipidemia (McBride *et al.*, 2008; Shai *et al.*, 2008). Many studies have reported better insulin sensitivity due to weight reduction, increase in physical activity and modification of diets. This has encouraged investigations into the effects of weight loss, increased physical activity and dietary modification on MetSyn components using randomized controlled trials among various population.

The first three intervention studies on the effects on the combination of lifestyle interventions on the parameters were carried out by Pan *et al.* (1997), Tuomilehto *et al.* (2001) and Knowler *et al*. (2002). The reports of Pan *et al.* (1997) in the Chinese Da Qing study revealed that the majority of the control group developed impaired glucose tolerance and type-2 diabetes during the six years of the intervention compared to the three interventions groups that received alteration in their diets, physical activity plus modification in diets and physical activity alone respectively. Tuomilehto *et al*. (2001) and Knowler *et al.* (2002) reported similar observation in their studies. Similar report was documented by Ramachandran *et al.* (2006) among Japanese and Indian population, results showed that lifestyle intervention programmes comprising reduced body weight, decline in the consumption of fat and carbohydrates, increased intake of fruits and vegetables and increased physical activity effectively reduced components of MetSyn and abdominal fat deposition among the intervention group. Studies have proven that reduced energy intake and weight reduction have positive impacts on the features on MetSyn. Kirk *et al.* (2009) and Markovic *et al.* (1998) investigated the short and long-term effects of energy restriction on components of MetSyn respectively. Participants were randomized to two intervention groups of high (>180g/day) and low (<20 g/day) carbohydrate intake. After the short-term phase of the study, Kirk *et al.* (2009) reported reduced fasting plasma glucose and increase liver insulin sensitivity coupled with reduced glucose synthesis. However, after the long- term phase, fasting blood glucose continues to decrease irrespective of the carbohydrate restriction levels. Markovic *et al.* revealed that the long term energy restriction reduced abdominal fat and improved insulin sensitivity. Hence, weight loss and exercise may efficiently enhance insulin sensitivity compared to carbohydrate restrictions alone. Weight loss especially from abdominal fat is fundamental to the prevention and management of MetSyn.

2.15.2 Mediterranean diets

Mediterranean diet is an eating pattern that has been proven to have several health benefits, such as reduced risk of developing various chronic NCDs such as cardiovascular diseases, type-2 diabetes and certain types of cancer (Knoops *et al.,* 2004). Mediterranean diet is typified by intake of larger amount of antioxidants, polyphenol-rich foods, monounsaturated fatty acids, and low glycaemic index foods (Abete *et al.,* 2011). Hence, individual on this dietary pattern had intake of large amounts of fruits, vegetables, whole grains and cereals with reduced intake of red meat that is often exchanged for fish and low fat dairies. Olive oil is the major form of lipids in Mediterranean diet. There are indications that Mediterranean diet in conjunction with regular physical activity may have a favourable impact on features on MetSyn (Abete *et al.,* 2011). Pitsavos *et al.* (2003) in their study reported reduced odds of coronary risk by 35% among the studied participants who were on regular consumption of Mediterranean diet. In addition, the reports of Panagiotakos *et al.* (2004) revealed a reduced risk of developing MetSyn among individuals who complied with the Mediterranean dietary regimen. Prevalence of MetSyn was found to decline significantly with regular intake of Mediterranean diet among the Mediterraneans with high cardiovascular disease risk (Babio *et al.,* 2009). Gouveri *et al*. (2011) also had similar reports; they found marked reduction in the burden of MetSyn among the population that complied strictly with the Mediterranean dietary pattern. Reports of a randomized controlled trial carried out by Esposito *et al.* (2011) indicated a decline in the level of circulating pro-inflammatory mediators (IL-8, IL-6, IL-7, CRP) and enhanced endothelial function in participants placed on Mediterranean dietary pattern for a period of two years compared to the controls that were not on this dietary regimen. Decreased circulating cytokines were also found to have direct association with decreased body mass index, insulin resistance, plasma glucose and lipid profile. The high fiber content of Mediterranean diet coupled with the high vitamins and polyphenol contents that were known to have potent antioxidant capacity were proposed to be accountable for the reported positive impacts of Mediterranean diet on health. The use of Mediterranean diet appears to be an efficacious and promising approach to truncate the rising prevalence of MetSyn and its features. Nevertheless, the adoption of this eating pattern among the non-Mediterranean individuals is still difficult.

2.15.3 Dietary Approach to Stop Hypertension (DASH)

Evidence suggests that adherence to DASH regimen among hypertensive patients significantly lowered the blood pressure values for systolic and diastolic. Many other studies have examined the effectiveness on DASH regimen on other features of MetSyn. Obarzanek *et al.* (2001) in their DASH study reported that following DASH regimen markedly lowered the total cholesterol and both low- and high density lipoproteins, no significant effect was observed on the triglycerides compared to the controls on normal diet. Obarzanek and colleagues concluded that DASH regimen had minute influence on the lipid profile. In dissimilarity, Azadbakht *et al.* (2011) in a randomized crossover clinical trial conducted among the type-2 diabetics in the Iranian population indicated improved high-density lipoproteins and reduced low-density lipoprotein and triglycerides among the study participants. The study also demonstrated a greater weight loss among participants on DASH regimen. Blumenthal *et al*. (2010) reported similar observation; reduced lipid profile were observed in participants on DASH regimen compared to the control.

2.16 Polyphenols in the management of metabolic syndrome

Polyphenols are biologically active compounds dispersed widely in plants and have proven to have potent antioxidant and anti-inflammatory benefits both in vitro and in vivo (Chiva-Blanch and Visioli, 2012). In recent times, attention has focused on study of polyphenols in health and diseases mainly because of their biological activities and potential health benefits. Different mechanism of action for polyphenols' health benefits have been suggested by various studies.

2.16.1 Effect of Polyphenol Intake on abdominal obesity

Regular consumption of tea have been shown to reduce percent body fat and waist-to-hip ratio among Chinese population compared to the controls (Wu *et al.,* 2003). Additionally, higher intake of flavonoids (flavones, flavonols, and catechins) was reported to have an inverse relationship with body mass index (BMI) (Hughes *et al*., 2008). Consumption of catechin extracts at 690mg were shown to significantly reduce BMI, body weight and waist circumference of adult men (Nagoa *et al.,* 2005). Sulibursk*a et al.* (2012) also confirmed that green tea supplementation have reducing effects on BMI and waist circumference in men. Green tea is generally rich in tannins particularly epigallocatechin and various other flavonoids. Cocoa flavonoids did not

significantly reduce waist circumference, body weight and body mass index of the studied population (Berry *et al.,* 2010). However, Davidson *et al.,* (2008) and Maki *et al.,* (2009) indicated that the percent bod fat, body weight, body mass index and waist circumference was significantly lowered with consumption of cocoa and exercise. Lehtonen *et al.* (2011) reported significant decrease in weight, BMI and abdominal obesity in patients with MetSyn when their diets were accompanied with 480ml of cranberry juice for 8 weeks. Citrus-based fruits were also found to reduce waist circumference among MetSyn subjects (Mulero *et al.,* 2012).

2.16.2 Effect of Polyphenol intake on Blood Pressure

Various studies have reported strong association between increased intake of fruits and vegetables and reduced prevalence of hypertension (Davinelli and Scapagnini, 2016). Serban *et al.* (2015) reported reduced blood pressure with the consumption of hibiscus tea. In addition, a meta-analysis study has also displayed that strong association exists between increased consumption of green tea and reduced blood pressure level (Onakpoya *et al.,* 2014). Similar reports was also made from another meta-analysis (Peng *et al.,* 2014). Consumption of blueberries and strawberries (good sources of anthocyanins) has been linked with decline in blood pressure levels (Cassidy *et al.,* 2011). In addition, supplementation with cocoa yielded a marked decline in the systolic and diastolic blood pressure (Davidson *et al.,* 2008; Berry *et al.,* 2009). However, Lehtonen *et al.* (2011) reported that berry fruit consumption did not affect blood pressure levels significantly.

2.16.3 Effects of polyphenol intake on dyslipidemia

Onakpoya *et al.* (2014) carried out a meta-analysis study and revealed that intake of green tea have positive influence on the lipid profile by reducing total cholesterol and LDL-c levels while HDL-c remained unaffected. However, some studies reported increased levels of HDL-c with intake of green tea (Imai and Nakachi, 1995). In contrast, lipid profile remained unchanged with the consumption of black tea (Wang *et al.,* 2014) and cocoa (Berry *et al.,* 2010). In 2013, Di Renzoand and colleagues reported a direct relationship between frequent intake of dark chocolate and HDL-c among obese women with MetSyn. Strawberries and Chokeberries were shown to have positive influence of lipid profile by lowering the low-density lipoprotein cholesterol and total cholesterol without altering the triglycerides, HDL-c and very low-density lipoprotein

cholesterol levels (Broncel *et al.,* 2010); whereas intake of cranberry did not have any significant influence on the lipid profile.

2.16.4 Effect of polyphenol intake on blood glucose and insulin resistance

Polyphenols have been reported to have inverse association with insulin resistance and type-2 diabetes. Total polyphenol and flavonoid intake particularly the flavanones and flavonols, and stilbenes have been reported to have significant relationship with reduced risk of type-2 diabetes in a study among Mediterraneans with increased risk of CVDs (Tresserra-Rimbau *et al.,* 2016). Vieira *et al.* (2012) and Belcaro *et al.* (2013) showed that intake of green tea has reducing effects on blood glucose but not insulin resistance. Supplementation with 902mg of cocoa flavonols coupled with exercise for a period of 6 weeks was reported to have reducing effects on the blood glucose levels and insulin resistance (Davison *et al.,* 2008). A longitudinal study conducted by Sun *et al*. (2015) among Nurses revealed that intake of hesperetin, naringenin, isorhamnetin, quercetin, and caffeic acids were inversely associated with risk of developing type-2 diabetes. In addition, greater intakes of anthocyanins were found to have significant relationship with lowered risk for type-2 diabetes (Wedick *et al.,* 2012), whereas no relationship was observed between total flavonoid intake and blood glucose.

2.16.5 Effect of polyphenol intake on oxidative stress markers

It has been reported in the literature that polyphenols have reducing influence on several oxidative stress markers such as oxidized LDL-C (ox-LDL), isoprostanes and thiobarbituric acid-reactive substances (TBARS) as well as malonyldialdehyde. Basu *et al.* (2011) in their study reported significant increase in the plasma antioxidant capacity of plasma and reduced ox-LDL and malondialdehyde following the daily supplementation of cranberry juice (480 mL) for 8 weeks among women with MetSyn. Reduced plasma concentration of ox-LDL and malondialdehyde were reported with intake of blueberries among MetSyn patients (Basu *et al.,* 2010). In addition, supplementation of 300 mL/ day of pomegranate juice for 6 weeks was reported to also reduce the concentrations of TBARS in erythrocytes (Kojadinovic *et al.,* 2017). Consumption of citrus fruit juice daily also reduced ox-LDL plasma concentration among subjects with MetSyn (Mulero *et al.,* 2012). Reduced ox-LDL was also reported with consumption of 150mg of quercetin for six weeks among MetSyn patients. Adherence to Mediterranean diet for a period of 3 months was reported to reduce lipid

oxidation and increased antioxidant enzymes in the plasma, erythrocyte, and platelet Bekkouche *et al.*, 2014).

2.16.6 Effect of polyphenol intake on inflammatory markers

Systemic inflammation is considered to occur due to accumulation of visceral fat, and several studies have proven that there is strong association between adipose tissue enlargement and increase in the production of pro-inflammatory cytokines such as interlekin-6 etc.

Recently, diet rich in polyphenols are being prescribed due to their positive favorable effects on inflammation (Murillo and Fernandez, 2017). Kolehmainen *et al.* (2012) revealed that consumption of 400g/day of fresh blueberries for eight weeks among patients with MetSyn decreased plasma concentrations of interleukin-12, C-reactive protein, interleukin-6. It also reduced the activities of moocyte to macrophages differentiation factor (MMD) and C-C-Chemokine receptor type-2 (CCR2) in monocytes. Stull *et al.* (2015) also reported improved endothelial function among subjects with MetSyn following the supplementation of blueberry powder at 45 g/day for six weeks. Conversely, Yang *et al.* (2016) documented that there was no alteration in the levels of circulating CRP, adiponectin and IL-6 as assessed in their study, following the consumption of 50g of berry powder per day for eight weeks among individuals with MetSyn. In addition, intake of 4 cups/day or two capsules/day of green tea extracts for 56 days showed no significant influence on the plasma concentrations of adiponectin, CRP, leptin, IL-6 and also in leptin to adiponectin ratio in patients with MetSyn (Basu *et al.*, 2011). A parallel control trial conducted by Esposito *et al.* (2004) among subjects with MetSyn revealed that adherence to Mediterranean diet for two years led to decreased plasma levels of IL-18, CRP, IL-7 and IL-6. Endothelial function was also enhanced significantly among the patients on Mediterranean diet compared to the controls. Moreover, consumption of Mediterranean diet for 3 months was also reported to lower serum levels of IL-7, CRP, IL-6 and IL-18 in healthy subjects and in MetSyn patients (Bekkouche *et al.*, 2014).

2.17 Grapefruit and drug interaction

It has been extensively reported in literature that grapefruit interacts with the metabolic pathway of diverse drugs. Grapefruit consumption was found to markedly elevate the blood concentration of felodipine (Bailey *et al.*, 1991). Similar occurrence was reported

with other drugs and especially in drugs that act as calcium channel blockers and cholesterol reducing drugs called statins. Studies reported that the increased drug bioavailability was as a result of eradication of P450 cytochrome enzyme (CYP3A4) from the intestine. They are known to contribute significantly to metabolism of various drugs particularly, the xenobiotic (Lewis, 2003). The major compounds accountable for this effect in grapefruit is known as furanocoumarins, which are compounds synthesized in various plants and found in fruits and vegetables particularly in grapefruit. The mechanism of grapefruit - drug interaction has been explained, after grapefruit consumption, furanocoumarins readily got absorbed by the enterocytes that line the intestines, and undergo enzymatic reaction with CYP3A4 to generate numerous metabolites. The metabolites produced get bound irrevocably to CYP3A4 and the formed enzyme is destroyed by rapid catabolism (Li *et al.*, 2006). After the destruction of metabolite-CYP3A4 enzyme, it takes an average of 72 hours for regeneration of new CYP3A4. During this period, any ingested drug that requires CYP3A4 for metabolism reaches the blood stream at high concentrations and tenfold rise in bioavailability has been reported (Ohnishi *et al.*, 2000). Interestingly, very few of the oral medications are metabolized by CYP3A4, large number of drugs are metabolized through other pathway. Moreover, the effect of grapefruit on CYP3A4 metabolized drugs also differs. For instance, statins: pravastatin could be slightly altered while atovastatin is moderately altered but simvastatin was greatly affected (Lin and Lu, 2001).

Another factor that was reported to influence the grapefruit-drug interaction is the level of cytochrome P450 available in the intestinal lumen of an individual. This varies greatly from one person to another and it declines as one advance in age (Paine *et al.* 1997). Individuals with elevated level of P450 in the gut could be subjected to grapefruit drug interaction (Sotaniemi *et al.*, 1997). Also, there is variation in the level of furanocoumarins in grapefruit and this can be as a result of variation in cultivar and or postharvest handling. Study have shown that the concentrations of furanocoumarins in various fruits are affected by processing and it decreased during storage (Cancalon *et al.*, 2011). Though, the negative effect of grapefruit-drug interaction is limited to few drugs but it is important to properly address it. CYP3A4 levels vary largely among individuals to an extent that grapefruit only affect the individuals with high concentrations of P450 in the intestine.

2.18 Animal models of metabolic syndrome

The multifactorial nature of the aetiology of MetSyn makes it difficult and challenging to ascertain appropriate experimental model that best mimics the functional changes that accompany MetSyn in humans. Several studies have attempted to use different methods to induce MetSyn that typifies what is obtainable in humans, some of the numerous methods used to induce MetSyn in animals include dietary modification, genetic modification and use of drugs. A systematic review by Panchal and Brown (2011) suggested that inducing animal models with high-carbohydrate high fat diet resulted in MetSyn that closely mimic the MetSyn in humans.

2.19 Dietary modifications to induce metabolic syndrome

Studies have reported different dietary manipulations that are able to induce MetSyn in experimental animals. These include the use of diets high in fructose, diets high in sucrose, diets high in fat, or combinations of diets such as high fructose-high fat diet, high sucrose-high fat diet, and high-carbohydrate high fat diets. Table 2.2 below summarizes the ability of different diets to promote the development of MetSyn in different animal models. Thirunavukkarasu *et al*. (2004) reported that intake of 60% fructose in the diet of the experimental animals for three weeks induced hyperglycemia and hypertension only but not obesity and dyslipidemia. Sanchez-Lozada *et al.* (2007) documented that addition of fructose (10%) to drinking water increased blood pressure and induced dyslipidemia only. However, Mahmoud and Elshazly (2014) and Mamikutty *et al.* (2014) documented that addition of fructose (10%) to the drinking water of male Wistar rats for at 56 days significantly increased all the components of MetSyn in the experimental animals. On the other hand, consumption of high sucrose diets at 30% for 21 weeks induced obesity, high blood pressure and dyslipidemia but not hyperglycemia in male Wistar rats (Aguilera *et al.,* 2004). Pang *et al.* (2008) also indicated that the consumption of 77% of sucrose significantly increased the blood pressure, and triglycerides as well as hyperglycemia. Vasanji *et al*. (2006) also opined that administration of 32% of sucrose in the drinking water of experimental rats led to increased body weight, hyperglycemia, and increased plasma concentration of total cholesterol. A reduced concentration of fructose in drinking water (as low as 10%) is capable of inducing MetSyn in wistar rat strain within a short period, however it takes a longer period of time for equivalent amount of sucrose or sucrose-rich diet to induce some of the features of MetSyn.

Different types and concentration of high fat diets have been used in various studies to induce MetSyn in experimental animal models. Concentration used ranged from 20% to 60% of entire energy consumed while the type of fat used include plant oils such as corn oil, olive oil etc. and animal fats majorly lard and beef tallow (Buettner *et al.,* 2007). Animal derived fats are used extensively to promote obesity and MetSyn in different animal models. Various researchers have documented that high fat diet particularly the animal derived fats is capable of stimulating glucose intolerance, dyslipidemia and reduced insulin sensitivity (Zivkovic *et al.,* 2007). Ghibaudi *et al.* (2002) determined the effect of different levels of fat on adiposity, lipid profile, circulating free fatty acids, leptin, fasting insulin as well as insulin resistance. They reported that the level of adiposity and the other parameters measured depend on the amount of fat ingested. As the amount of fat ingested increases, the fat mass and total fat pads, plasma triglycerides, total cholesterol, circulating free fatty acids, glucose, leptin and insulin resistance also increases. Graham *et al.* (2010) fed male C57BL/6 mice with 60% fat diet for 40 weeks and documented increased LDL-c, triglycerides, un-esterified cholesterol. Gancheva *et al.* (2015) also indicated that consumption of diet high in fat for 8 weeks resulted into obesity, hyperglycemia and dyslipidemia in male wistar rats.

Effects of different combination of diets in the development of MetSyn in different animal models have been explored. Intake of diets high in carbohydrate (mainly fructose) high fat diet for 16 weeks minimally have been proven to promote obesity, hyperglycemia, hypertension and dyslipidemia in male wistar rats (Panchal *et al.,* 2011; Hao *et al.,* 2015). High fat high fructose diets for 32 weeks promoted excess fat deposition, dyslipidemia and elevated blood glucose in model animals (Dissard *et al.,* 2013), whereas intake of diet high in fat and fructose for 8 weeks induced obesity, hyperglycemia and dyslipidemia in male wistar rats. Zhou *et al.* (2014) also reported the ability of diets high in both fat and sucrose fed to model animals for 42 weeks to induce obesity, hyperglycemia and dyslipidemia.

Table 2.2 Efficacy of different types of diets to induce Metabolic Syndrome

Reference	Type of diet	Length of treatment	Type of animal model	Features of Metabolic Syndrome			
				Obesity	Hyperglycemia	High blood pressure	Dyslipidemia
Thirunavukkarasu *et al.* (2004)	60% fructose in diet	3 weeks	Male Wistar rats	X	√	√	X
Sanchez-Lozada *et al.* (2007)	10% fructose in drinking water	8 weeks	Male Sprague rats	X	X	√	√
Shahraki *et al*. (2011)	High fructose diet	8 weeks	Male wistar rats	X	√	X	√
Mahmoud and Elshazly (2014)	10% fructose in drinking water	12 weeks	Male wistar rats	√	√	√	√
Mamikutty *et al.* (2014)	10% fructose in drinking water	8 weeks	Male wistar rats	√	√	√	√
Oron-Herman *et al.* (2008)	High sucrose diet	7 weeks	Male spontaneously hypertensive rats	X	√	√	X
Aguilera *et al.* (2004)	30% sucrose drinking water	21 weeks	Male wistar rats	√	X	X	X

Table 2.2 Efficacy of different types of diets to induce Metabolic Syndrome continued

Reference	Type of diet	Length of treatment	Type of animal model	Features of Metabolic Syndrome			
				Obesity	**Hyperglycemia**	**High blood pressure**	**Dyslipidemia**
Vasanji *et al.* (2006)	32% sucrose in drinking water	10 weeks	Male Sprague-Dawley rats	X	√	X	X
Pang *et al.* (2008)	77% sucrose diets	6 weeks	Male Sprague-Dawley rats	X	X	√	√
Ghibaudi *et al.* (2002)	High fat diet	24 weeks	Male Sprague-Dawley rats	√	X	√	√
Graham *et al.* (2010)	High fat diet	40 weeks	Male C57BL/6 J mice	X	X	X	√
Podrini *et al.* (2013)	High fat diet	12 weeks	Female C57BL/6 J mice	√	√	X	√
Gancheva *et al.* (2015)	High fat diet	8 weeks	Male wistar rats	√	√	X	√
Li *et al.* (2015)	High fat diet	16 weeks	Male C57BL/6 J mice	√	√	X	√
Suman *et al.* (2016)	High fat diet	10 weeks	Male wistar rats	√	√	√	√

Table 2.2 Efficacy of different types of diets to induce Metabolic Syndrome continued

Reference	Type of diet	Length of treatment	Type of animal model	Features of Metabolic Syndrome			
				Obesity	**Hyperglycemia**	**High blood pressure**	**Dyslipidemia**
Poudyal *et al*. (2010)	High carbohydrate high fat diet	16 weeks	Male wistar rats	√	√	√	√
Panchal *et al.* (2011)	High carbohydrate high fat diet	16 weeks	Male wistar rats	√	√	√	√
Hao *et al*. (2015)	High carbohydrate high fat diet	14 weeks	Male wistar rats	√	√	√	√
Senaphan *et al.* (2015)	High carbohydrate high fat diet	16 weeks	Male Sprague-Dawley rats	X	√	√	√
Dissard *et al.* (2013)	High fat high fructose diet	32 weeks	Male C57BL/6 J mice	√	√	X	√
Barrios-Ramos *et al.* (2014)	High cholesterol diet + fructose in drinking water	4 weeks	Male wistar rats	√	√	√	√

Table 2.2 Efficacy of different types of diets to induce Metabolic Syndrome continued

Reference	Type of diet	Length of treatment	Type of animal model	Features of Metabolic Syndrome			
				Obesity	**Hyperglycemia**	**High blood pressure**	**Dyslipidemia**
Gancheva *et al.* (2015)	High fat high fructose diet	8 weeks	Male wistar rats	√	√	X	√
Zhou *et al.* (2014)	High sucrose high fat diet	48 weeks	Male Sprague-Dawley rats	√	√	X	√

Source: Field survey, Ogunkunle 2019

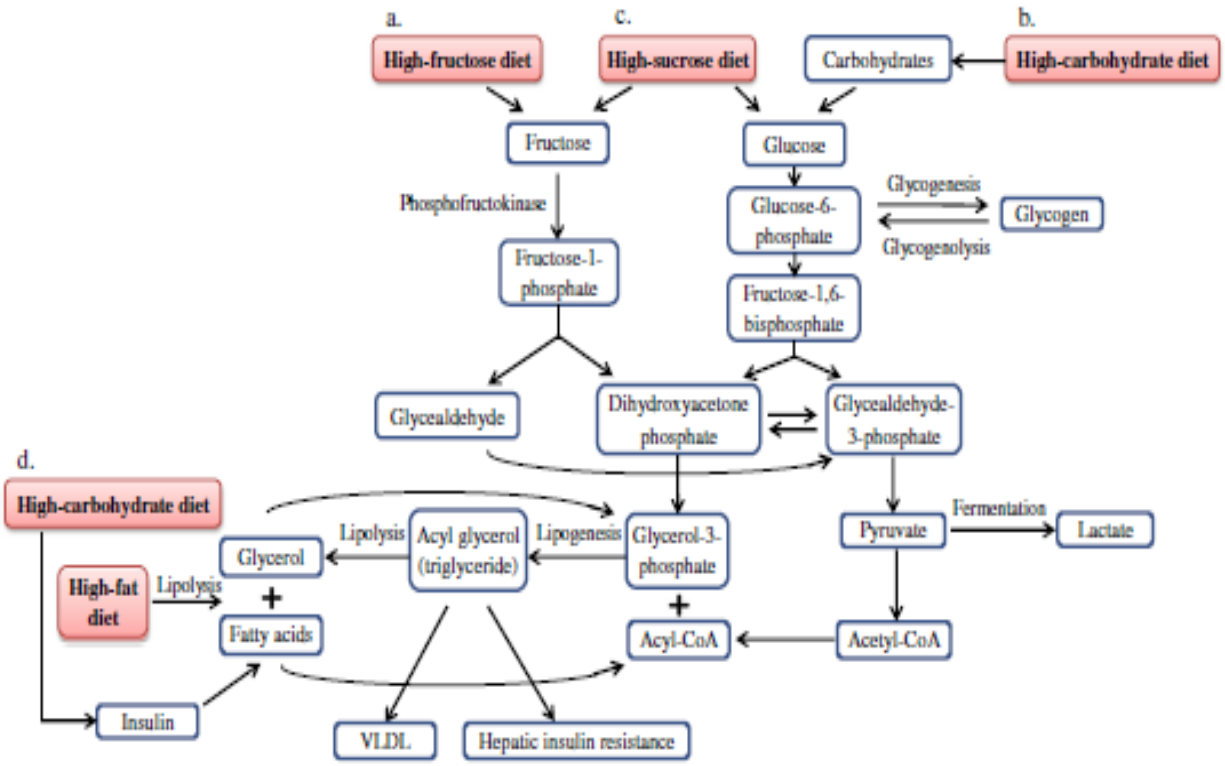


Figure 2.5. Summary of metabolic pathway of different diets used in inducing metabolic syndrome

Source: Wong et al., 2016

a- Represents the metabolic pathway of high fructose consumption, it bypasses the rate-controlling pathway to yield pyruvate, lactate, glycerol and acyl-glycerol continuously. This can subsequently results into the buildup of hepatic cholesterol and triglycerides due to the fat producing features of fructose. This is due to the fact that fructose is a preferred substrate for synthesis of fatty acids to glucose. Afterwards, this can lead to reduced insulin sensitivity and glucose intolerance.

b- Explains the metabolic pathway of high carbohydrate diets. With availability of much glucose due to intake of high-carbohydrate diet initiates glycolytic pathway. This includes glycolysis, glycogenesis and insulin synthesis to enhance fatty acid production by acting on the adipose tissue.

c- Symbolizes the metabolic pathway of sucrose. Sucrose, a disaccharide is broken down to fructose and glucose and each molecule then follows their metabolic pathway.

d- Describes the metabolic pathway of high fat diet. During lipolysis, glycerol and fatty acids produced are re-esterified to produce triglyceride. Abundance of various nutrients resulting from excessive consumption of food promotes overproduction and accumulation of hepatic triglyceride, which eventually results to reduced insulin sensitivity and glucose intolerance.

2.20 Fruit consumption pattern in Nigeria

Fruit and vegetable consumption is important for the provision of micronutrients to the body, as these food items are rich sources of vitamins and minerals required for the growth, development, and normal functioning of the human body (Park, 2005). Consumption of fruits and vegetables is a critical route to good health and longevity because fruits and vegetable are rich sources of vitamins, minerals, dietary fiber and phytochemicals (Rekhy and McConchie, 2014). High fruit and vegetable consumption prevents several non-communicable and chronic diseases such as cardiovascular diseases, hypertension, diabetes, gastrointestinal diseases, and obesity (Blanchard *et al.,* 2009; De Bruijn, 2010). Due to the high antioxidant content of fruit and vegetables, numerous studies have also revealed a strong link between their consumption and the prevention of colon and prostate cancers (Southon, 2000).

Despite these positive benefits attributed to high intake of fruits and vegetables, the World Health Organization (WHO) estimates that approximately 1.7 million (2.8%) deaths per annum worldwide are linked to inadequate intake of fruits and vegetables (WHO, 2013). The WHO subsequently ranks low fruits and vegetable consumption as the sixth main risk factor for mortality in the world (Ruel *et al.,* 2006). In spite of the growing body of evidence highlighting the protective effects of fruits and vegetables, intakes are still inadequate in both developed and developing countries (IARC, 2003). Data on fruit and vegetable intake from the developed countries showed that only three countries (Israel, Italy and Spain) reached the average intakes

of WHO/FAO minimum recommended intakes of five servings per day (IARC, 2003). This consumption pattern was also reported in various developing countries among different populations. For example, a study conducted by Silva *et al.* (2017) in Lagos Nigeria among adolescents, revealed that 5.48% of the respondents consumed five portions of fruits and vegetables daily. Ogunkunle and Oludele (2014) also reported that 87.7% of the older adults studied did not reach the WHO/FAO minimum recommendation for consumption of fruits and vegetables.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The study is laboratory based and experimental in design.

3.2 Study location

Grapefruit samples were prepared in the laboratory of the Department of Human Nutrition, University of Ibadan and the Multicenter research laboratory, University of Ibadan. Analysis of samples were carried out at the laboratory of Phytochemicals and Nutrition, Autonoma University of Queretaro, Queretaro, Mexico; while the experiment was carried out at the central animal house, University of Ibadan.

3.3 Sampling procedure

Fresh white and pink varieties of grapefruits were harvested at maturity from several plants selected at random within a field at the National Horticultural Research Institute, Ibadan and botanical identification of the fruits was carried out in the Department of Botany, University of Ibadan with voucher number UIH/001/2015/995. The grapefruit samples were harvested in November, 2015.

3.4 Sample preparation

Freshly harvested fruits were immediately transported to the laboratory where they were sorted to remove the damaged, bruised and spoilt samples. All fruits were rinsed thoroughly with distilled water. The whole fruit was cut into four equal halves and the edible (pulps and segments) and non-edible parts (peels [flavedo albedo] and seeds) were manually separated using a stainless knife (Appendix 2). The edible and non-edible parts were separately packed into air tight plastics with lids. Each sample (edible and non-edible) were lyophilized separately in a freeze-drier for an average of 14 days (Pressure: 2×10^{-4}Torr; Temp: -60°C) until the desired dryness is achieved. The lyophilized samples were grinded into powder with a laboratory grinder and kept at -18^{O}C until further use. Below is the flow chart of sample preparation:

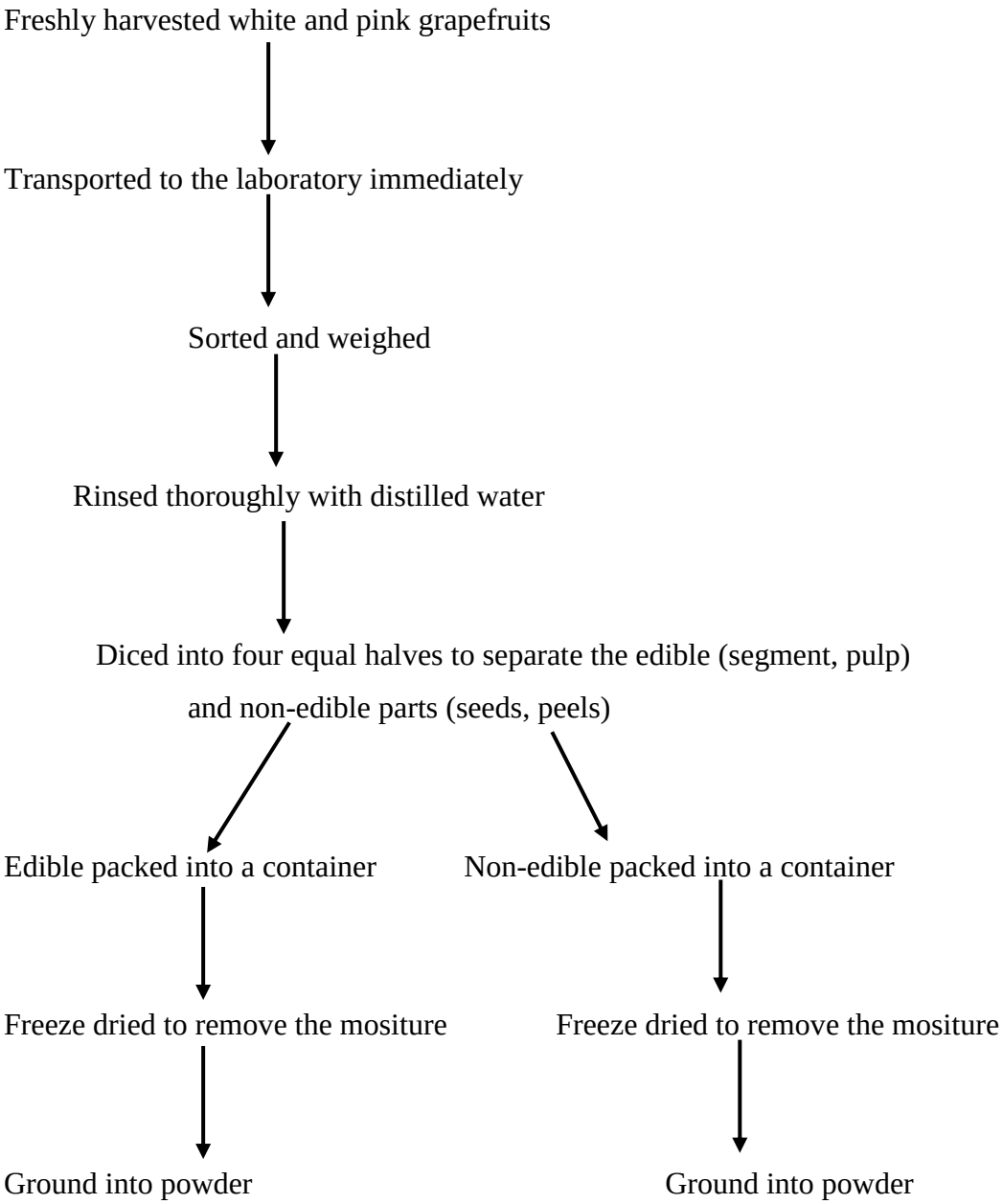


Figure 3.1: Flow chart showing the processes involved in sample preparation

3.5 Extraction procedure

Ultrasonic extraction was carried out separately on the edible and non-edible parts of the two varieties using 72% ethanol following the procedure of Xi *et al.* (2015). To 1g of dry sample, 10 ml of 72% ethanol was added, the mixture was vortexed for 1minute and homogenized using homogenizer (Model: IKA® T-25 Ultra-Turrax Digital High-Speed Homogenizer, China). The homogenized samples were put in Bransonic® Ultrasonic sonicator for 5 minute and centrifuge at 19,600 (rcf) for 10 minutes at 4ºC. The supernatant was decanted and filtered using Whatman No. 42

filter paper. Extraction process was repeated and volume obtained was measured. The extracts were stored at 4˚C prior to analysis. Preparation of the extracts was done in triplicate.

3.6 OBJECTIVE I: Quantification of major phytochemicals and determination of antioxidant capacities of the extracts

3.6.1 Determination of total phenolic content using the Folin−Ciocalteu colorimetric method as described by McDonald *et al.* (2001)

Extracts were diluted with distilled water using a dilution factor of 10. 15µL of extract/ gallic acid/blank was measured into a 96 well ELISA plate and 75µL of Folin-Ciocalteu reagent was added to each of the well. Solution in each well was mixed thoroughly with pipetting and kept in the room at 15-25ºC for 7 minutes. Then, 225 µL of 7.5% sodium bicarbonate solution was added to the mixture. Following 90 min incubation at 25ºC, optical density was measured at 765nm. Gallic acid (25mM) was used as standard, serial dilution was used to prepare different concentrations of gallic acid and the total phenolic amount was measured by calibration curve of gallic acid standard. The obtained values were expressed as mg of gallic acid equivalents (GAE) per g of dry weight indicating the concentration. The total phenolic assay of each extract was measured in triplicates.

3.6.2 Determination of total flavonoids content using Aluminum chlorite colorimetric method as described by Chang *et al.* (2002)

Extracts were diluted with distilled water (5 fold dilution) and an aliquot (150 µL) of the extract/ quercetin/ blank was pipetted into a 96 well ELISA plate. Then 9 µL of 5% $NaNO_3$ was pipetted to each of the well.
For five minutes, the solution was allowed to incubate and at the 5^{th} minute, 45 µL of 10% $AlCl_3$ was added, at 6th minute, we added 60 µL of 1M NaOH. 36 µL of distilled water was added to make up the total volume to 300 µL. Resultant solution was mixed well and optical density was measured at 510nm. Quercetin standard (1mg/ml) was prepared in varying concentration using serial dilution and the total flavonoid content was quantified by calibration curve obtained from different concentrations of quercetin. Results were expressed as mg quercetin equivalents/ g dry weight (DW). Total flavonoid assay of each extract was measured in triplicates.

3.6.3 Determination of total tannins content as described by Obadoni and Ochuko (2001)

Total tannin content of the samples were quanitified using colorimetric method. In a 250ml conical flask, 0.5g of each sample was weighed and 75 µL of distilled water was added. The conical flask was heated gently and allowed to boil for 30minutes. Resultant solution was centrifuge at 2,000 rpm for 20 minutes. The supernatant was collected in 100ml volumetric flask and distilled water was used to make up the volume to 100ml. Into another 100 ml volumetric flask, 75ml of distilled water was measures and 1ml of the sample extract was added. Thereafter, 5ml of Folin-Denis reagent and 10ml of 35% Na_2CO_3 were added to the solution in the volumetric flask. Distilled water was used to make up the volume to 100ml. Resultant solution was shaken very well and incubated for 30 minutes. Optical density of the solutions was measured against a blank sample at 700 nm. Tannic acid standard (1mg/ml) was prepared in different concentrations using serial dilution and the total tannin concentartion of the samples were measured using the calibration curve obtained from different concentrations of tannic acid. Results were expressed as mg tannic acid equivalents/g dry weight. Determination of the tannin content in the samples was carried out in triplicates

3.6.4 Determination of total alkaloid in the extracts using Fazel *et al.* (2008) method

Total alkaloid content of the samples were quanitified using colorimetric method Soxhlet apparatus was used to extract 10g of grapefruit powder successively for 24 hours. The mixture was sieved and rotary evaporator was used to completely remove the methanol leaving a residue. The residue was reconstituted in 2N HCl and sieved. From the filtrate, 1ml was taken into a separatory funnel and extracted with 10 ml chloroform, this procedure was repeated three times and 0.1N NaOH was used to bring the pH to neutral. To this solution, 5 ml of Bromocresol Green and 5 ml of phosphate buffer were added, the solution was thoroughly mixed and further extracted with chloroform (4 ml) and filtered. The filtrate was transferred into a 10ml volumetric flask and chloroform was used to make up the volume to 10ml. Optical density of the solution was measured at 470 nm. Atrophine standard (0.1mg/mL)

was was prepared in different concentrations using serial dilution and results were expressed in mg atrophine equivalent/g dry weight. Determination of alkaloid content of the samples was carried out in triplicates.

3.6.5 Determination of total saponin using the method of Zhang *et al.* (2013)

Total saponin content of the samples were determined using colorimetric method. Soxhlet apparatus was used to defat 2 g of the samples using hexane for 3 hours and samples were allowed to air dry. Two grams of the defatted samples were dissolved in 20mL of 50% aqueous MeOH and the resulting solution was put in a sonicator for 30 minutes at 25°C. This was later centrifuged at 3000 rcf for 10 minutes and the filtrate was removed. The process was repeated once and the extracts combined. Watsman paper (No 1) was used to filter the combined extract and methanol was evaporated from the solution under vacuum using rotary evaporator at 40ºC, leaving the saponin in aqueous solution. The aqueous phase was then centrifuged at 3000 xg for 30 minutes. The aqueous phase was then taken to the separating funnel and 10mL chloroform was used to extract the solution to remove pigments. This process was repeated three times. The concentrated saponin was further extracted with 10mL n-butanol and the n-butanol was evaporated under vacuum at 45ºC. The dry fraction containing saponins was reconstituted in distilled water and freeze dried. Resultant was redissolved in 50% methanol (aqueous) to a concentration of 10mg/ml. From this aliquot, 250 µL was measured and 8% vanillin reagent was added followed by 2.5 mL of 72% (v/v) sulphuric acid. The mixture was mixed with a vortex and placed in water bath at 60ºC for 10 minutes. The mixture was then cooled in ice-cold water bath for 3-4 minutes. Optical density was measured at 544 nm. Diosgenin standard (1mg/ml) was prepared in different concentrations using serial dilution and the total saponin concentration of the samples was quantified using the calibration curve obtained from the different concentrations of diosgenin. Results were expressed as mg diosgenin equivalents/g dry weight. Determination of the saponin content in the samples was carried out in triplicates.

3.6.6 Determination of Total Carotenoid using the method of Doka *et al.* (2013)

Total carotenoid content of the samples were determined using spectrophotometric method. To 0.5 g of the dry sample, 10mL of the mixture of hexane/ toluene/ acetone/ ethanol (10:7:7:6 v/v) was added and 1mL of 40% potassium hydroxide in

methanol was also added. The mixture was gently shaken and agitated in water bath at 56 ºC for 20 minutes at 65rpm. The mixture was cooled with water and 10mL of hexane was added and shaken vigorously. Ten mL of 10% Na_2SO_4 was also added and the mixture was allowed to incubate for 10 minutes and the supernatant was decanted. Absorbance was measured at 450nm. β-carotene standard (1mg/ml) was prepared in different concentrations using serial dilution and the total carotenoid concentration was quantified using the calibration curve obtained from different concentrations of β-carotene standard. Results were expressed as mg β-carotene equivalents/g dry weight. Determination of the total carotenoid content in the samples was carried out in triplicates.

3.6.7 Determination of Antioxidant activities of the extracts using Ferric reducing antioxidant potential (FRAP) assay

The method measures the capacity of the extracts to diminish ferric-2,4,6-tri-2-pyridyls-triazine complex (Fe^{3+} TPTZ) to the ferrous form Fe^{2+}, to produce an intense blue colour. FRAP Assay was prepared using the acetate buffer, TPTZ and $FeCl_3.6H_20$ in ratio 10:1:1 respectively. 20 µl of sample/ standard was measured into the 96 well ELISA plate in triplicates using a pipette and 280 µl of FRAP reagent was added. The samples were incubated in the dark at 37°C for 30 minutes after the addition of the reagent. Water served as blank and optical density of the samples was measured at 593 nm. This absorbance was compared to 0 - 1 mM Fe^{2+} standard curve. The results were expressed the FRAP value in µmol/l using the formula below:

$$\frac{\text{A593 nm of test sample reaction mixture}}{\text{A593 nm of } Fe^{2+} \text{ standard reaction mixture}} \text{ X } Fe^{2+} \text{ standard conc.(µmol/l)}$$

3.6.8 Determination of Antioxidant activities of the extracts using 2, 2-diphenyl-1-picrylhydrazyl radicals (DPPH assay)

Serial dilutions of the standard (Trolox, 2mM) were prepared in methanol. DPPH (1, 1-diphenyl-2-picrylhydrazyl) was also prepared in methanol at 100 µM concentration. 20 µl of sample/ standard was measured into the 96 well ELISA plate in triplicates using a pipet and 280 µl of DPPH reagent was added. The solution was well mixed and kept at 25°C in the dark for 10 minutes, optical density was measured

at 517 nm. DPPH solution was used as blank. Percentage inhibition was determined for individual experiment using the formula below and the concentration at which 50% of the initial and remaining DPPH concentration was calculated from standard DPPH graph.

Percent Inhibition of DPPH Activity =

$$\frac{\text{Optical density (control)} - \text{Optical density (test sample)}}{\text{Optical density (control)}} \times 100$$

3.7 <u>OBJECTIVE 2:</u> Quantification of individual phenolic acid and flavonoids content of the crude extracts

Individual phenolic and flavonoid were quantified using a Reverse phase (RP) C_{18} column High Performance Liquid Chromatography (HPLC) coupled to Photo Diode Array. Reference standard stock solutions of the compounds to be quantified using HPLC analysis was prepared in methanol (HPLC grade) at a concentration of 1.0 mg/mL and stored in a refrigerator at -20˚C until use. All standard solutions were filtered through 0.45µm filters and diluted as necessary with methanol.

The methods of Zhang *et al.* (2012) was used to quantify the phenolic compounds. The samples were filtered using filter membrane (0.45 µm filters; 0.22 µm) after which 10µL of the filtrate was injected into the HPLC system. Reverse phase column (C18, 250 mm and 4.6 mm-internal diameter) was used for chromatographic separation. The mobile phase used were as follows: (A) 0.1% aqueous formic acid and (B) methanol. Gradient elution was performed as follows: from 0 to 21 min, 38–50% B; from 21 to 36 min, 50–80% B; from 36 to 41 min, 80–100% B; from 41 to 51 min, 100% B; from 51 to 60 min, 37–50% B. Flow rate was 0.7 mL/minute and column temperature was steady at 25ºC. At detection of gallic acid, narirutin, naringin, naringenin, hesperedin, and eriocitrin was at wavelengths of 330 nm, caffeic acid, chlorogenic acid and ferulic acid were detected at 320 nm, *p*-coumaric acid was detected at 310 nm and quercetin at 370 nm. Retention time and spectral characteristics of each of the standards was compared with the samples for identification and quantification.

3.7.1 Quality control

To ensure maximum reliability of results, experiments were carried out in triplicate. The stability of baseline and response linearity of the detector were examined to assure precision and accuracy. Before starting the analysis instrument were calibrated using standards and the same HPLC operating conditions were used all through the analysis. Each sample was run in triplicates.

3.8 OBJECTIVES 3, 4 & 5: Experimental protocol

3.8.1 Care and management of the experimental animals

One hundred and fourteen adult male wistar rats (9-10 weeks old) weighing between 270-290 g were obtained from the University of Ibadan Veterinary Physiology animal house. The rats were kept in the College of Medicine Central Animal Facility of the University of Ibadan and were preserved under standard laboratory conditions. Rats were kept in cages (40 × 25 × 17 cm) with three animals per cage under natural conditions of light-dark cycles. The rats were kept for a 7-day period to acclimatize to the environment with access to food and water *ad* libitum. The experiment lasted for a period of 20 weeks.

3.8.2 Induction of Metabolic Syndrome in the experimental rats

Definition of MetSyn as stated by IDF harmonized criteria for MetSyn (Alberti et al., 2005) was used in this study. To induce MetSyn in the experimental rats, high-carbohydrate-high-fat diet (HCHF) supplemented with 25% fructose in drinking water was fed to the experimental rats for a period of 12 weeks. Pancha *et al.* (2011) suggested that the use of high-carbohydrate and high-fat diet to induce MetSyn in animal models is more applicable because it imitates the type of diet accountable for human MetSyn and CVD complications as a basis to investigate potential therapeutic interventions. The table below shows the composition of the diet of the experimental and control groups**.**

Table 3.1: Diet composition of the experimental and control diets

Ingredients (g/kg)	Control diet	HCHF diet
Fructose	-	195g
Corn starch	470g	-
Powdered Milk (Popular® full cream powdered milk)	-	250g
Beef tallow	-	250g
Powdered rat chow	255g	222g
Mineral mix	32g	32g
Vitamin mix	820mg	820mg
Water	250ml	50ml

3.8.3 Experimental animal grouping

The experiment was carried out in two phases: these include induction of MetSyn and the treatment phase that involved the administration of grapefruit powder.

In the first phase of the experiment, MetSyn was induced in the experimental animals hence, animals were randomly grouped into two:

Group 1(control): Normal rats receiving cornstarch-rich diet + distilled water (n=14)

Group 2: Experimental rats receiving HCHF + 25% fructose in drinking water (n=90)

Following 12 weeks of feeding, blood samples were obtained from all the rats through orbital vein to assess diagnostic criteria for metabolic syndrome before the commencement of treatment. The diagnostic criteria used for the animals in this study is the International Diabetes Foundation harmonized criteria (Alberti et al., 2005). Thereafter, six rats were randomly selected from group 1(control) and eighteen rats from group 2 to determine fat deposition, body composition and organ weights prior to intervention. The remaining animals in group 2 were randomly

redistributed based on their weights into 9 groups of 8 rats in each group as below to commence the phase two (treatment):

Experimental rats were randomly divided into 10 groups of 8 rats in each group.

Group 1(Negative Control): Cornstarch- rich diet + tap water

Group 2 (Positive Control): HCHF + 25% fructose in drinking water

Group 3 ($EW_{0.8g}$): HCHF +25% fructose in drinking water + 0.8g EW

Group 4 ($EW_{1.6g}$): HCHF + 25% fructose in drinking water + 1.6g EW

Group 5 ($NEW_{0.5g}$): HCHF +25% fructose in drinking water + 0.5g NEW

Group 6 ($NEW_{1.0g}$): HCHF+ 25% fructose in drinking water + 1.0g NEW

Group 7 ($EP_{0.5g}$): HCHF+ 25% fructose in drinking water + 0.5g EP

Group 8 ($EP_{1.0g}$): HCHF + 25% fructose in drinking water + 1.0g EP

Group 9 ($NEP_{0.3g}$): HCHF + 25% fructose in drinking water + 0.3g NEP

Group 10 ($NEP_{0.6g}$): HCHF+ 25% fructose in drinking water + 0.6g NEP

EW = Edible White; NEW = Non-Edible White; EP = Edible Pink; NEP = Non-Edible Pink

3.8.4 Estimation of quantity of grapefruit for administration

To estimate the amount of grapefruit that was administered to the experimental animals, the amount of polyphenol in rat that correspond to 1g and 2g polyphenols per day in a 70-kg human was estimated using the scaling equation of Bachmann *et al.* (1996) below:

$$Dose_{Human} = Dose_{Rat} \left\{ \frac{Body\ weight_{human}}{Body\ weight_{rat}} \right\}^{0.667}$$

$Body\ weight_{human}$= 70kg

$Body\ weight_{rat}$= 400g=0.4kg

$Dose_{human}$= 1g or 2g

$Dose_{rat}$=????

Using the scaling equation above,

$Dose_{rat}$ that corresponds to 1g polyphenols per day in 70kg human = 0.0318g/day

$Dose_{rat}$ that corresponds to 2g polyphenols per day in 70kg human = 0.0638g/day

The amount of grapefruits that supplied the above estimated polyphenol was calculated from the results obtained from previous determination of total phenols in the different parts and varieties of grapefruits (presented in Table 4.1). Hence, the

amount of edible white grapefruit that will supply 0.0318 g and 0.0638 g polyphenols per day is 0.8 g and 1.6 g respectively. This is also applicable to the other parts and varieties of grapefruits as stated below.

- $EW_{0.8g}$ = 0.8g grapefruit/ day
- $EW_{1.6g}$ = 1.6g grapefruit/ day
- $NEW_{0.5g}$ = 0.5g grapefruit/ day
- $NEW_{1.0g}$ = 1.0g grapefruit/ day
- $EP_{0.5g}$ = 0.5g grapefruit/ day
- $EP_{1.0g}$ = 1.0g grapefruit/ day
- $NEP_{0.3g}$ = 0.3g grapefruit/ day
- $NEP_{0.6g}$ = 0.6g grapefruit/ day

EW = Edible White; NEW = Non-Edible White; EP = Edible Pink; NEP = Non-Edible Pink

Therefore, the amount of grapefruit that supplied 0.0318g and 0.0638g of polyphenols per day was added to the feeds that were given to the experimental animals daily for 8 weeks to treat the metabolic syndrome

3.8.5 Collection of blood samples and plasma preparation

Blood samples about 4-5ml were obtained via orbital vein in rats anaesthetized with di ethyl ether and kept in heparinized sample bottles. The samples were centrifuged at 3000rcf for 10 minutes. The supernatant was aspirated with sterile needle and syringe and kept in plain sample bottles at -20ºC until analysis. Before the blood samples were drawn, experimental rats were fasted overnight and the fructose drinking water in the experimental groups was replaced with tap water *ad libitum*.

3.9 Food, water and energy intake measurement

Food, water and energy intakes were determined daily and mean values were obtained. The food and water intake for each rat was measured by subtracting the remaining amounts in the cage from the measured amount provided to the rat (Abdulla *et al.*, 2011). The energy intake (kJ/day) was calculated based on the quantity of food and water intake and the corresponding constants. Energy intake (kJ/day) = mean food consumption x dietary metabolizable energy (Novelli *et al.*, 2007). The following constant were used for the dietary metabolizable energy: Cornstarch 15.97; powdered milk, 11.50; beef tallow, 37.75; powdered rat food, 13.82; and fructose, 15.42 (Pancha *et al.*, 2011). High-carbohydrate-high-fat diet has

17.98 kJ/g of food and additional 3.95 kJ/mL in drinking water while cornstarch-rich diet has 11.53 kJ/g of food. Feed efficiency was calculated using the formula below as:

$$\frac{\text{Mean body weight gain in grams}}{\text{Daily energy intake in kilojoules}} \times 100$$

(Cope *et al.*, 2007)

3.10 Body composition/anthropometric measurement

At the end of the 12th and 20th weeks of the experiment, the animals were euthanized. The wet weights of the heart, pancreas, liver and kidneys (g/100g body weight) were weighed, and stored for histological analyses. The retroperitoneal, epididymal and mesenteric fat pads were dissected and weighed (g/100g body weight). The experimental animals' body weight were measured weekly in grams using a top loader digital weighing balance. The increment of body weight was calculated by subtracting the initial weight from the final weight and the percentage increase in body weight was determined. Body length (nose to anus length) and abdominal circumference were measured every four weeks using a standard non-stretchable tape.

3.11 Determination of cardio-metabolic parameters

Systolic blood pressure was measured using the tail-cuff plethysmography, plasma concentration of triglycerides (TG), high- and low- density lipoprotein-cholesterol (HDL-c and LDL-c) and total cholesterol (TC) were determined by enzymatic method using analytical kits obtained from Randox®, U.K following the procedures stipulated by the manufacturer. Fasting plasma glucose was measured by glucose oxidase method using glucose assay kit, fasting plasma insulin was quantified using commercial double-antibody enzyme-linked immunosorbent assay (ELISA) from Abcam®, USA. Insulin Resistance was calculated using the Homeostasis Model Assessment for insulin resistance (HOMA-IR).

3.11.1 Determination of Blood pressure

Systolic blood pressure was measured invasively in awake rats by tail plethysmography using automated blood pressure monitor (CODA S1, Kent Scientific Corporation, Torrington, CT). The measurement was taken every 4 weeks

and with a minimum of three readings per measurement following acclimatization and the mean was determined and regarded as the final reading for systolic blood pressure values.

3.11.2 Quantification of plasma concentrations of triglycerides

Randox kit, UK was used to quantify the plasma triglycerides and the procedure used was as stipulated by the manufacturer. To 1ml of reagent, 10µL of sample was added this was allowed to incubate for 10 minutes at room temperature. Colour changed from colourless to pink or violet and the optical density was read at 546nm. The procedure was repeated for standard and blank using standard and distilled water respectively. Plasma triglyceride was calculated as follows

$$\text{Plasma triglyceride} = \frac{\text{Optical density (sample)} \times \text{concentration of standard}}{\text{Optical density (standard)}}$$

3.11.3 Quantification of total cholesterol in the samples

Total plasma cholesterol was determined using Randox kit, UK following the procedure as stipulated by the manufacturer. To 1ml of reagent, 10µL of sample was added this was incubated for 10 minutes at room temperature. Colour changed from colourless to pink or violet and the optical density was read at 546nm. The procedure was repeated for standard and blank using standard and distilled water respectively. Plasma cholesterol was calculated as follows:

$$\text{Plasma cholesterol} = \frac{\text{Optical density (sample)} \times \text{concentration of standard}}{\text{Optical density (standard)}}$$

3.11.4 Determination of fasting plasma glucose in the samples

Fasting plasma glucose level was quantified by glucose assay kit using glucose oxidase method with optical density measured by visible spectrophotometer at 505nm following the manufacturer's procedure.

3.11.5 Quantification of Insulin in the samples

Fasting plasma insulin was quantified using commercial double-antibody enzyme-linked immunosorbent assay (ELISA) from abcam®, USA, following the procedure stipulated by the manufacturer. Briefly, 100 µL of biotin conjugated anti insulin was added to all the wells on the ELISA plate and the plate shaken gently. To the wells designated for samples, 10 µL of sample was added and 10 µL of standard solution to the wells designated for standards. The plate was gently shaken and sealed for 2 hours at 20-25ºC to allow the content incubate. The reaction mixture was discarded and wells were rinsed, then 100 µL of HRP conjugated streptavidin was added to all the wells and shaken gently. The plate was sealed and allow to incubate for 30 minutes at 20-25ºC. The reaction mixture was discarded and the plate was then washed. To each of the well, 100 µL of substrate chromogen reagent was added and shaken and the plate was sealed to allow incubation for 30 minutes at 20-25ºC. Thereafter, 100 µL of the reaction stopper was added to all wells and shaken. Optical density was measured using ELISA plate reader at 450 nm within 30 minutes.

3.11.6 Determination of Insulin Resistance

Insulin Resistance was estimated using the Homeostasis Model Assessment for insulin resistance (HOMA-IR) using this formula stated below:

$$\frac{\text{Fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)}}{22.5}$$

3.12 Measurement of plasma concentration of oxidative stress and inflammatory markers of the experimental rats

Malondialdehyde (MDA), Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), C-reactive protein (CRP) and Adiponectin were measured in the plasma of the experimental rats using commercial kits purchased from Abcam® USA following the manufacturer's standards and protocols.

3.12.1 Quantification of Malondialdehyde in the samples

An aliquot of plasma (250 mL) was passed into a five mL Nunc CryotubeE, followed by successive additions of 25 mL 0.2% butylated hydroxytoluene (dissolved in ethanol) and 1mL 15% aqueous trichloroacetic acid. The mixture was centrifuged at

4000*g* for 15minutes at 4 °C. The deproteinized filtrate (stock) was stored at –20°C. From that stock, an aliquot of 500 µL was transferred into a 5mL CryotubeE and 1mL tertiary butyl alcohol (0.375% in 0.25M HCl) was added. It was then heated at 100°C for 15 minutes. The solution was allowed to cool and analyzed spectrophotometry at 535 nm.

3.12.2 Quantification of Tumor Necrosis-alpha in the samples

Prior to use, all reagents were thoroughly mixed carefully not to produce foam in the containers. 100µL each of standard/ sample/ blank (controls) were added to the wells designated for standard/sample/ blank as appropriate and 50µL of Biotinylated anti-TNF alpha was added to all the wells. The wells were sealed and the content were allowed to react with one another at room temperature for 3 hours. The cover was removed and the plate was washed by aspirating the liquid from each well and adding 300µL of wash buffer. The washing process was repeated three times. Thereafter, 100µL of Streptavidin- Horseradish Peroxidase solution was added into all the wells and the content were allowed to react with each other for 30 minutes at room temperature. The wells were then washed following the procedure described above. 100 µL of Chromogen 3,3',5,5'-Tetramethylbenzidine substrate solution was later added into each well and reaction was allowed to take place for 20 minutes in the dark at room temperature. Direct contact with light was avoided by covering the plate with aluminium foil. Stop reagent (100 µL) was added into each well and optical density of each cell was measured immediately on a spectrophotometer at 450 nm. All samples were assayed in duplicates.

3.12.3 Quantification of Interleukin-6 in the samples

To each of the well, 50 µL of Assay Diluent RD1-54 was pipetted as well as 50 µL of standard /control/ sample to designated wells. The solution was mixed by mildly touching the frame of the plate for a minute. The samples were sealed and allowed to react at 25ºC for 2 hours. The liquid in each well was removed and the wells were washed repeatedly for five times. Thereafter, 100 µL of IL-6 conjugate was added and the wells were sealed. Reactions was allowed to take place in the mixture for 2 hours at room temperature. Each well was aspirated and washed again.100µL of substrate solution was added to each well and incubated for 30 minutes at 25ºC in the dark. Then, 100 µL of stop solution was added to each well. The plate was tapped

mildly at frame to ensure that the content mixed absolutely. Absorbance of each plate was measured at 450nm spectrophotometrically using a microplate reader. All samples were assayed in duplicates.

3.12.4 Quantification of C-Reactive Protein in the Samples

Samples were diluted in wash buffer at ratio 1:4,000 prior to assay. 100 µL of standard/ sample was added to each well of the micro-well plate, the plate was sealed with an adhesive strip and allowed reaction to occur for 2 hours at 25ºC. Thereafter, wash buffer was used to rinse the wells using a wash bottle, the washing process was repeated five times. Following the final wash, the plate was turned upside down and tapped on a paper towel to remove remaining buffer. Horseradish peroxidase-conjugated antibody was diluted (1:100) with wash buffer and 100 µL of the diluted conjugate was added to each well of the plate. The plate was enclosed with adhesive film and allowed to react for one hour at 25ºC. The plate was rinsed with wash buffer for five times using a wash bottle and the residual was drained using a paper towel. To each well of the washed plate, 100 µL of the 3,3,5,5 Tetramethylbenzidine substrate solution was added and a blue color indicated a positive reaction. The reaction was allowed to proceed at 25ºC 10 minutes and then stopped by adding 100 µL of stop solution per well. The reaction mixture turned to yellow and the absorbance was read on an ELISA plate reader at 570 nm.

3.12.5 Quantification of Adiponectin in the Samples

To the first well, 100 µL dilution buffer was added. Subsequently 100 µl standard or 100 µl of diluted control / diluted samples were added. The plate was covered with adhesive tape and allowed to react for one hour at 25ºC (shaken at 350 rpm). After the reaction, the solution in the wells was aspirated and each well was rinsed three times with 300 µL wash buffer. The wash buffer was allowed to react for 15 seconds per cycle. Following the last washing, 100 µl of the Antibody-POD-Conjugate AK was added to each well and the plate was sealed to allow reaction for one hour at 25ºC (shaken at 350 rpm). Following the reaction, wash buffer was used to rinse the well three times and 100 µl of 3,3,5,5 Tetramethylbenzidine substrate solution was added to each well in the plate. The plate was incubated in the dark for 30 minutes at 25°C. 100 µL of stop solution was added to stop the reaction optical density was measured at 450nm within 30 minutes.

3.13 Determination of plasma Hepatic and Renal functional markers of the experimental rats

Activities of aspartate transaminase (AST), lactate dehydrogenase (LDH), alanine transaminase (ALT) and alkaline phosphatase (ALP) in the plasma were measured using commercial assay kits purchased from Randox®, UK while albumin and total bilirubin in the plasma were determined by photometric colour test to assess hepatic functions. To assess the renal functions, urea, creatinine and uric acid levels were determined in the plasma using commercial assay kits. Sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-) ions were determined in the plasma by enzymatic reactions using commercial assay kits while creatinine concentration was measured by Jaffe reaction as described by Tietz et al. (1994).

3.14 Histopathological study of the kidney and liver of the experimental animals

The kidney and liver of the animals were removed and immediately treated with 10% buffered neutral formalin solution. Five μm thick of the treated organs were cut and stained with hematoxyline and eosin (H&E) following this procedure. The cross section removed was deparaffinised by flaming the slide on burner and placing it in the xylene. This procedure was repeated the second time. The tissue was then hydrated by passing it through decreasing concentration of aqueous ethanol in water bath starting with 100.0% ethanol followed by 90.0%, 80.0% and 70.0%. The tissue was then stained in hematoxyline for 3 minutes and then rinsed under a running tap water until the colour was turned blue. The tissue was later differentiated in the mixture of 1% Hydrochloric acid and 70% alcohol for 5 minutes, after which it was rinsed under a running tap water and fixed in ammonia water until the colour turned blue again. The tissue was then stained with 1% Eosin Y for 10 minutes and later rinsed under tap water for another 3 minutes. The rinsed tissue was dehydrated in aqueous ethanol by dipping it in increasing concentration of aqueous ethanol starting with 70.0%, 80.0%, 90.0% and 100.0%, the tissue was later cleared in xylene. The stained tissues were put under the microscope to study the architectural damage and the inflammatory process of the tissues. Fat deposits in the hepatocytes was also assessed. The investigator that performed the histological test was not privy to results of the biochemical assessment and to allocation of treatments of the experimental animals. Observation of the stained tissue on slides were done under a digital microscope (Digital microscope, Model VJ-20005DN, BIO-

MISCROSCOPE, China) at x 100 connected to Leica DF 300 FC camera in RGB mode.

3.15 Statistical analysis

Data obtained were presented as mean with their standard deviation. For multiple comparison of the mean values obtained for each group, one-way analysis of variance (ANOVA) was employed. Duncan's Multiple-range post hoc test was used to separate the statistically significant means among the experimental groups. The ten groups were tested for the effects of treatments and their interactions using two-way analysis of variance (ANOVA). Level of significance level was $\alpha_{0.05}$. All statistical analyses were performed using IBM SPSS version 20 for Windows.

3.16 Ethical Consideration

Ethical approval was obtained from the University of Ibadan/University College Hospital Ethic Review Committee. Experimental procedures involving the use and care of experimental rats was in accordance with the recommendations published by the Canadian Council for care of experimental animal in biomedical research.

CHAPTER FOUR

RESULTS

OBJECTIVE I: Quantification of major groups of phytochemicals and determination of antioxidant activities of crude extracts of grapefruit samples.

4.1 Quantification of phytochemical content and antioxidant activities of edible and non-edible parts of pink and white grapefruits

Phytochemical content and antioxidant activities of the grapefruit varieties are as presented in Table 4.1. Non-edible parts (both pink and white varieties) contained considerably higher ($p<0.05$) total phenols and flavonoids compared to the edible parts. The non-edible parts also contained significantly higher saponin content than the edible parts ($p<0.05$) with higher concentration in the pink variety. Contrastingly for alkaloids, contents in edible parts and white variety were significantly higher than the non-edible and the pink variety, respectively ($p<0.05$). Non-edible parts (both white and pink varieties) have significantly higher tannin content than the edible parts with highest concentration in pink variety. Carotenoid concentration was significantly higher in the pink variety of both edible and non-edible parts. Non-edible part and the pink variety have higher FRAP and DPPH values than the edible parts and white variety.

Table 4.1. Phytochemical content and antioxidant activities of pink and white varieties of grapefruits

Phytochemicals	**Edible White**	**Non-Edible White**	**Edible Pink**	**Non-Edible Pink**
Total phenols (mg GAE/g)	$38.19^{d} \pm 0.50$	$67.84^{b} \pm 0.08$	$62.2^{c} \pm 0.04$	$111.1^{a} \pm 0.45$
Total flavonoid (mg quercetin equivalent/g)	$18.61^{d} \pm 0.09$	$28.16^{b} \pm 0.12$	$25.97^{c} \pm 0.12$	$32.33^{a} \pm 0.12$
Total saponin (mg diosgenin equivalant/g)	$0.46^{c} \pm 0.01$	$8.18^{b} \pm 0.03$	$1.23^{c} \pm 0.12$	$9.79^{a} \pm 0.01$
Total alkaloids (mg atrophine equivalent/g)	$2.90^{a} \pm 0.01$	$2.33^{b} \pm 0.03$	$1.42^{c} \pm 0.03$	$0.29^{d} \pm 0.001$
Total tannin (mg tannic acid equivalent/g)	$235.6^{c} \pm 5.82$	$248.9^{b} \pm 6.17$	$124.1^{d} \pm 2.35$	$351^{a} \pm 9.64$
Total carotenoids (mg β-carotene equivalent/g)	$7.91^{d} \pm 0.16$	$10.85^{c} \pm 0.10$	$17.42^{b} \pm 0.28$	$22.93^{a} \pm 0.03$
Anti-oxidant activities				
FRAP (mM)	$1.00^{c} \pm 0.17$	$3.87^{b} \pm 0.06$	$2.70^{b} \pm 0.32$	$9.43^{a} \pm 0.91$
DPPH (%)	$26.60^{c} \pm 1.80$	$56.79^{b} \pm 1.84$	$56.34^{b} \pm 2.54$	$79.20^{a} \pm 1.37$

Note: mean values with the same letters are not statistically different at $p<0.05$ using DMRT, FRAP =Ferric reducing antioxidant power, DPPH=2, 2-diphenyl-1-picrylhydrazyl radicals

OBJECTIVE 2a: Quantification of individual phenolic acid content in crude extracts of the two grapefruit varieties

4.2 Phenolic acid content of pink and white varieties of grapefruits

Table 4.2 presents the contents of phenolic acid in the grapefruit varieties. Chlorogenic acid content was makedly higher at $p<0.05$ in the non-edible parts than the edible parts, and the pink variety had higher concentration than the white variety. Ferulic and *p*-coumaric acids were also significantly higher in the non-edible parts and white variety than the edible parts and pink variety at $p<0.05$. Contrastingly, concentration of gallic acid was statistically higher in the edible part and pink variety compared to the non-edible part and white variety.

Table 4.2. Phenolic acid content of pink and white varieties of grapefruits

Phenolic acids	**Edible pink (µg/g DW)**	**Non- edible pink (µg/g DW)**	**Edible white (µg/g DW)**	**Non- edible white (µg/g DW)**
Chlorogenic acid	$35.58^{c} \pm 2.53$	$117.18^{a} \pm 2.32$	$27.97^{d} \pm 2.12$	$69.78^{b} \pm 0.34$
Ferulic acid	$11.87^{d} \pm 0.06$	$31.95^{b} \pm 0.16$	$21.0^{c} \pm 1.56$	$45.26^{a} \pm 0.48$
Gallic acid	$295.38^{a} \pm 17.8$	$205.52^{b} \pm 2.19$	$150.74^{d} \pm 8.55$	$166.28^{c} \pm 0.63$
Caffeic acid	$24.08^{a} \pm 1.34$	$11.24^{c} \pm 0.45$	$20.2^{b} \pm 0.06$	$9.17^{d} \pm 1.74$
p-coumaric acid	$0.38^{c} \pm 0.43$	$0.65^{b} \pm 0.12$	$0.34^{c} \pm 0.04$	$0.72^{a} \pm 1.01$

Note: mean values with the same letters are not statistically different at $p<0.05$ using DMRT
Data presented are means ± standard deviation of triplicate samples. DW= dry weight.

OBJECTIVE 2b: Quantification of individual flavonoid content in crude extracts of the two varieties of grapefruit

4.3: Distribution of various flavonoids in pink and white varieties of grapefruits

Table 4.3 showed the distribution of various flavonoids in the grapefruit varieties. The main flavonoid subclass present in considerable amount in the analysed grapefruit samples is the flavanones, and naringin was the principal flavanone. Non-edible portion of the pink and white varieties contained significantly higher ($p<0.05$) concentrations of naringin compared to the edible portion. The pink variety also had higher naringin content compared to the white variety. Similar variation was also observed for narirutin, naringenin and eriocitrin. In contrast, the level of hesperidine and quercetin were higher significantly in the edible portion of both varieties compared to the non-edible portion. The pink variety had higher hesperidine content compared to the white variety whereas quercetin was higher in the white variety.

Table 4.3. Distribution of various flavonoids in the pink and white varieties of grapefruits

Flavonoids	Edible pink (µg/g DW)	Non- edible pink (µg/g DW)	Edible white (µg/g DW)	Non- edible white (µg/g DW)
Naringin	$3560.6^{c} \pm 8.7$	$10136^{a} \pm 17.37$	$1083.7^{d} \pm 4.66$	$4516.32^{b} \pm 9.83$
Narirutin	$2.17^{b} \pm 3.62$	$14.03^{a} \pm 0.39$	$1.89^{c} \pm 0.11$	$13.75^{a} \pm 1.79$
Naringenin	$10.39^{b} \pm 0.84$	$20.67^{a} \pm 1.69$	$2.33^{d} \pm 0.04$	$7.6^{c} \pm 0.16$
Hesperidine	$56.89^{a} \pm 4.65$	$21.16^{c} \pm 0.37$	$35.72^{b} \pm 2.29$	$13.86^{d} \pm 0.22$
Eriocitrin	$30.79^{c} \pm 1.82$	$58.72^{a} \pm 0.94$	$23.11^{d} \pm 3.18$	$43.91^{b} \pm 0.14$
Quercetin	$0.67^{b} \pm 0.22$	$0.13^{d} \pm 0.18$	$0.96^{a} \pm 0.03$	$0.24^{c} \pm 0.14$

Note: mean values with the same letters are not statistically different at $p<0.05$ using DMRT
Data presented are means ± standard deviation of triplicate samples. DW= dry weight.

OBJECTIVE 3: To determine the efficacy of edible and non-edible parts of two varieties of grapefruits on cardio-metabolic parameters in experimental rats (anthropometric variables, blood pressure, lipid profile, fasting plasma glucose, fasting plasma insulin and insulin resistance.

4.4 Effect of grapefruit treatments on the nutritional parameters of the experimental rats

Table 4.4 described the effect of grapefruit treatment on daily food, water and energy intake of the experimental rats at 12 and 20 weeks of the experiment. Food intake was markedly reduced ($p<0.05$) in PC group at 12 and 20 weeks compared to NC group. Similarly, there was a decline in food intake in all grapefruit treated groups compared to NC group at 20 weeks ($p<0.05$). Likewise, a decline was observed in water intake across all the grapefruit treated groups compared to the NC group ($p<0.05$). Energy intake of grapefruit treated groups and the negative control did not differ statistically ($p<0.05$).

Table 4.4. Effect of grapefruit treatment on the nutritional parameters of the experimental rats

Parameters	NC @ 12 weeks	PC @ 12 weeks	NC @ 20 weeks	PC @ 20 weeks	$EW_{0.8g}$ @ 20 weeks	$EW_{1.6g}$ @ 20 weeks	$NEW_{0.5g}$ @ 20 weeks	$NEW_{1.0g}$ @ 20 weeks	$EP_{0.5g}$ @20 weeks	$EP_{1.0g}$ @20 weeks	$NEP_{0.3g}$ @ 20 weeks	$NEP_{0.6g}$ @ 20 weeks	F value	p-value
Food intake (g/d)	27.91^{a} ± 0.62	19.23^{b} ± 0.97	27.35^{a} ± 0.89	19.74^{b} ± 0.36	14.55^{c} ± 0.98	13.91^{c} ± 0.32	14.01^{c} ± 0.76	14.06^{c} ± 0.84	14.35^{c} ± 0.45	14.16^{c} ± 0.92	14.0^{c} ±0.73	13.86^{c} ± 0.84	32.53	0.00
Water intake (mL/d)	32.34^{a} ± 0.34	18.96^{b} ± 0.45	31.45^{a} ± 0.56	18.47^{b} ± 0.87	17.86^{b} ± 0.74	18.23^{b} ± 0.83	18.42^{b} ± 0.76	18.24^{b} ± 0.48	17.69^{b} ± 0.75	17.93^{b} ± 0.91	18.05^{b} ± 0.87	18.31^{b} ± 0.68	40.15	0.00
Energy intake (kJ/d)	375.6^{b} ± 8.2	425.6^{a} ± 9.4	384.6^{b} ± 9.4	433.6^{a} ± 7.3	377.2^{b} ± 6.7	392.8^{b} ± 5.5	380.6^{b} ± 4.9	385.8^{b} ± 6.2	382.3^{b} ± 6.8	387.7^{b} ± 5.4	388.9^{b} ± 11.2	385.5^{b} ± 9.3	36.40	0.00
Energy efficiency (g/kJ)	2.37^{b} ± 0.11	2.76^{a} ± 0.20	2.37^{b} ± 0.17	2.78^{a} ± 0.20	2.12^{c} ± 0.20	2.11^{c} ± 0.32	2.18^{d} ± 0.43	2.12^{c} ± 0.21	2.18^{d} ± 0.23	2.11^{c} ± 0.53	2.12^{c} ± 0.42	2.18^{d} ± 0.73	36.74	0.00

Note: mean values with the same letters are not statistically different at $p<0.05$ using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control**

4.5 Effect of grapefruit treatments on the anthropometric parameters of the experimental rats

Table 4.5 presents the effect of grapefruit supplementation on the anthropometric features of the experimental animals. No significant difference was observed in the naso-anal length and initial body weight of the controls and the grapefruit treated groups. The abdominal circumference of PC group was significantly elevated at week 20 compared to values at week 12 of experiment. However, a significant decrease in the abdominal circumference and body weight was observed among all the groups that received grapefruit treatment compared to values obtained for PC group.

Table 4.5. Effect of grapefruit treatments on the anthropometric parameters of the experimental rats

Parameters	**NC @ 12 weeks**	**PC @ 12 weeks**	**NC @ 20 weeks**	**PC @ 20 weeks**	**$EW_{0.8g}$ @ 20 weeks**	**$EW_{1.6g}$ @ 20 weeks**	**$NEW_{0.5g}$ @ 20 weeks**	**$NEW_{1.0g}$ @ 20 weeks**	**$EP_{0.5g}$ @ 20 weeks**	**$EP_{1.0g}$ @ 20 weeks**	**$NEP_{0.3g}$ @ 20 weeks**	**$NEP_{0.6g}$ @ 20 weeks**	**F value**	**p-value**
Naso-anal length (cm)	23.16 ± 0.63	23.78 ± 0.35	24.2 ± 0.37	24.7 ± 0.52	24.4 ± 0.73	24.8 ± 0.78	24.5 ± 0.45	24.8 ± 0.67	24.5 ± 0.78	24.58 ± 0.44	24.8 ± 0.50	24.8 ± 0.20	0.63	0.76
Abdominal circumference (cm)	14.83^{d} ± 0.26	18.2^{b} ± 0.50	14.92^{d} ± 0.20	20.08^{a} ± 0.86	16.25^{c} ± 0.98	16.33^{c} ± 0.75	16.33^{c} ± 0.60	15.50^{cd} ± 0.55	15.51^{cd} ± 0.56	15.67^{cd} ± 0.52	15.75^{cd} ± 0.27	15.58^{cd} ± 0.66	34.04	0.00
Initial body weight (g)	283.0^{a} ± 6.35	283.0^{a} ±4.85	281.0^{a} ± 5.86	280.0^{a} ± 8.31	285.0^{a} ± 6.20	282.0^{a} ± 7.47	284.0^{a} ± 4.36	275.0^{a} ± 6.21	281.0^{a} ± 5.47	279.0^{a} ± 5.26	284.0^{a} ± 5.66	280.0^{a} ± 6.87	0.58	0.67
Final body weight (g)	307.3^{e} ± 8.73	368.6^{c} ± 14.3	349.3^{d} ±16.67	435.0^{a} ± 6.66	405.0^{b} ± 8.29	403.0^{b} ± 2.61	403.2^{b} ± 2.72	403.7^{b} ± 4.50	403.8^{b} ± 3.43	403.3^{b} ± 2.42	402.7^{b} ± 0.83	404.7^{b} ± 3.20	48.54	0.00
% weight gain (%)	7.90	30.03	24.19	55.36	42.10	42.91	41.90	43.41	43.42	43.42	41.55	44.29		

Note: mean values with the same letters are not statistically different at $p<0.05$ using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control**

4.6 Effect of grapefruit treatments on the body composition and organ weights of the experimental rats

Effect of grtreatment on the body composition and the organ weights of the experimental animals are presented in table 4.6. Mesenteric, retroperitoneal and epididymal fat deposition were significantly increased in the PC group in comparison with the NC group. Grapefruit treatment significantly reduced the mesenteric, retroperitoneal, epididymal and subcutaneous fat deposition in the experimental rats to NC group status. Wet weights of the liver and kidney was increased significantly in the PC group compared to the NC group. In addition, the wet weight of the liver and kidney of the grapefruit treated groups were significantly reduced close to values obtained for the NC status ($p<0.05$). There was no significance difference in the wet weight of the pancreas and heart of the experimental animals across all groups.

Table 4.6. Effect of grapefruit treatment on the body composition and organ weights of the experimental rats

Parameters	NC@ 12 weeks	PC@ 12 weeks	NC @ 20 weeks	PC @ 20 weeks	$EW_{0.8g}$ @ 20 weeks	$EW_{1.6g}$ @ 20 weeks	$NEW_{0.5g}$ @ 20 weeks	$NEW_{1.0g}$ @ 20 weeks	$EP_{0.5g}$ @ 20 weeks	$EP_{1.0g}$ @ 20 weeks	$NEP_{0.3g}$ @ 20 weeks	$NEP_{0.6g}$ @ 20 weeks	F value	p-value
Mesenteric fat (g/100g)	0.82^{d} ± 0.32	1.53^{b} ± 0.27	1.02^{c} ± 0.28	2.82^{a} ± 0.14	1.39^{c} ± 0.11	1.38^{c} ± 0.12	1.35^{c} ± 0.12	1.33^{c} ± 0.23	1.30^{c} ±0.18	1.31^{c} ±0.37	1.30^{c} ± 0.32	1.29^{c} ± 0.16	37.352	0.00
Retroperitoneal fat (g/100g)	2.23^{d} ± 1.93	3.78^{b} ± 0.13	2.35^{d} ± 0.17	4.59^{a} ± 0.15	3.32^{c} ± 0.14	3.27^{c} ± 0.17	3.18^{c} ± 0.55	3.20^{c} ± 0.55	3.03^{c} ±0.66	2.86^{c} ±0.50	2.73^{c} ± 0.26	2.52^{c} ± 0.75	38.39	0.00
Epididymal fat (g/100g)	1.65^{d} ± 0.29	2.66^{b} ± 0.02	2.24^{c} ± 0.07	3.73^{a} ± 0.42	2.25^{c} ± 0.38	2.22^{c} ± 0.14	2.21^{c} ± 0.21	2.25^{c} ± 0.73	2.27^{c} ±0.32	2.26^{c} ±0.56	2.25^{c} ± 0.12	2.23^{c} ± 0.11	34.94	0.00
Subcutaneous fat (g/100g)	1.34^{e} ± 0.32	3.97^{b} ± 0.16	1.58^{d} ± 0.13	4.72^{a} ± 0.18	2.52^{c} ± 0.12	2.48^{c} ± 0.14	2.45^{c} ± 0.13	2.48^{c} ±0.17	2.52^{c} ± 0.12	2.49^{c} ±0.26	2.47^{c} ± 0.17	2.52^{c} ±0.18	47.83	0.00
Liver wet weight (g/100g)	2.94^{d} ± 1.02	3.79^{b} ± 0.02	3.23^{c} ± 0.07	4.08^{a} ± 0.09	3.33^{c} ± 0.11	3.42^{c} ± 0.01	3.25^{c} ± 0.29	3.24^{c} ± 0.52	3.28^{c} ± 0.17	3.15^{c} ±0.04	3.21^{c} ± 0.12	3.20^{c} ± 0.18	42.37	0.00
Kidney weight (g/100g)	0.26^{c} ± 0.01	0.31^{b} ± 0.02	0.29^{c} ± 0.01	0.36^{a} ± 0.20	0.27^{c} ± 0.11	0.21^{c} ± 0.32	0.27^{c} ± 0.01	0.28^{c} ± 0.06	0.26^{c} ± 0.03	0.27^{c} ±0.16	0.28^{c} ± 0.33	0.28^{c} ± 0.11	32.58	0.00
Pancreas weight (g/100g)	0.74 ± 0.01	0.75 ± 0.03	0.84 ± 0.06	0.79 ± 0.04	0.74 ± 0.16	0.79 ± 0.02	0.75 ± 0.01	0.77 ± 0.04	0.80 ± 0.13	0.79 ±0.16	0.74 ± 0.02	0.81 ± 0.05	0.65	0.76
Heart weight (g/100g)	0.26 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.27 ± 0.02	0.28 ± 0.02	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.27 ±0.02	0.28 ± 0.02	0.27 ± 0.01	0.34	0.53

Note: mean values with the same letters are not statistically different at $p<0.05$ using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control**

4.7 Effect of grapefruit treatment on the cardio-metabolic features of the experimental rats at 20 weeks of experiment

Table 4.7 presents the cardio-metabolic parameters of the experimental rats. Concentrations of the fasting plasma glucose, systolic blood pressure, fasting insulin and insulin resistance were markedly higher in the PC group compared to the NC group ($p<0.05$); however, grapefruit treatment significantly reduced all the parameters close to NC status in all treated groups. Non-edible pink variety was the most efficacious in reducing the fasting plasma glucose, insulin, insulin resistance and systolic blood pressure while edible white variety was the least effective.

Figure 4.1 expresses the effect of grapefruit treatment on the lipid profile of the experimental rats. Concentrations of plasma total cholesterol, triglycerides and LDL-c were significantly increased in the PC group compared to the NC group. Also, a significant decrease was observed in high density lipoprotein cholesterol (HDL-c) in PC group when compared with the NC group. Grapefruit treatment significantly reduced plasma total cholesterol, triglycerides and LDL-c and increased HDL-c in all the treated groups close to NC status. Non-edible pink variety was observed to be most effective in reducing LDL-c, total cholesterol, triglycerides and improving the HDL-c while edible white variety was the least effective.

Table 4.7. Effect of grapefruit treatment on the cardio-metabolic parameters of the experimental rats at 20 weeks of experiment

Parameters	NC @ 20 weeks	PC @ 20 weeks	$EW_{0.8g}$ @ 20 weeks	$EW_{1.6g}$@ 20 weeks	$NEW_{0.5g}$ @ 20 weeks	$NEW_{1.0g}$ @ 20 weeks	$EP_{0.5g}$ @ 20 weeks	$EP_{1.0g}$ @ 20 weeks	$NEP_{0.3g}$ @ 20 weeks	$NEP_{0.6g}$ @ 20 weeks	F-value	p-value
Fasting plasma glucose (mmol/L)	5.25^{fg} ± 0.08	8.36^{a} ±0.06	6.91^{b} ± 0.12	6.06^{cd} ± 0.05	5.99^{d} ±0.03	5.79^{e} ±0.07	6.22^{c} ±0.38	5.81^{e} ±0.06	5.38^{f} ±0.05	5.18^{g} ±0.03	281.04	0.00
Interaction/Potency: NC= NEP< NEW= EP< EW< PC F (5;50)= 475.68; p= 0.000												
Systolic blood pressure (mmHg)	121.67^{g} ± 1.63	156.33^{a} ±3.08	134.33^{b} ±1.03	132.0^{c} ±0.89	131.67 ±1.21	129.67^{d} ± 1.03	129.67^{d} ± 0.52	128.83^{de} ±0.75	127.33^{e} ±0.51	124.67^{f} ±1.03	285.00	0.00
Interaction/potency: NC< NEP< EP< NEW< EW< PC F (5;50) = 507.48; p= 0.000												
Fasting Insulin (µg/L)	2.15^{f} ±0.06	4.31^{a} ±0.10	3.52^{b} ±0.04	3.04^{c} ±0.04	2.89^{d} ±0.06	2.36^{e} ±0.10	2.24^{f} ±0.09	2.25^{f} ±0.06	2.20^{f} ± 0.04	2.17^{f} ±0.15	468.16	0.00
Interaction/potency: NC< NEP= EP< NEW< EW< PC F(5;50)= 797.44; p= 0.000												
HOMA- IR	10.68^{g} ± 0.03	34.07^{a} ± 0.04	23.0^{b} ± 0.13	17.42 ^{c}± 0.05	16.37^{c} ± 0. 02	12.92^{d} ± 0.03	13.17^{e} ± 0.27	12.49^{d} ± 0.03	11.19^{fg} ± 0.04	10.63^{g} ± 0.01	332.54	0.00
Interaction/potency: NC= NEP< EP< NEW= EW< PC F(5;50)= 797.44; p= 0.000												

Note: bars with the same letters are not statistically different at p<0.05 using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control; HOMA-IR= Homeostasis Model Assessment for Insulin resistance**

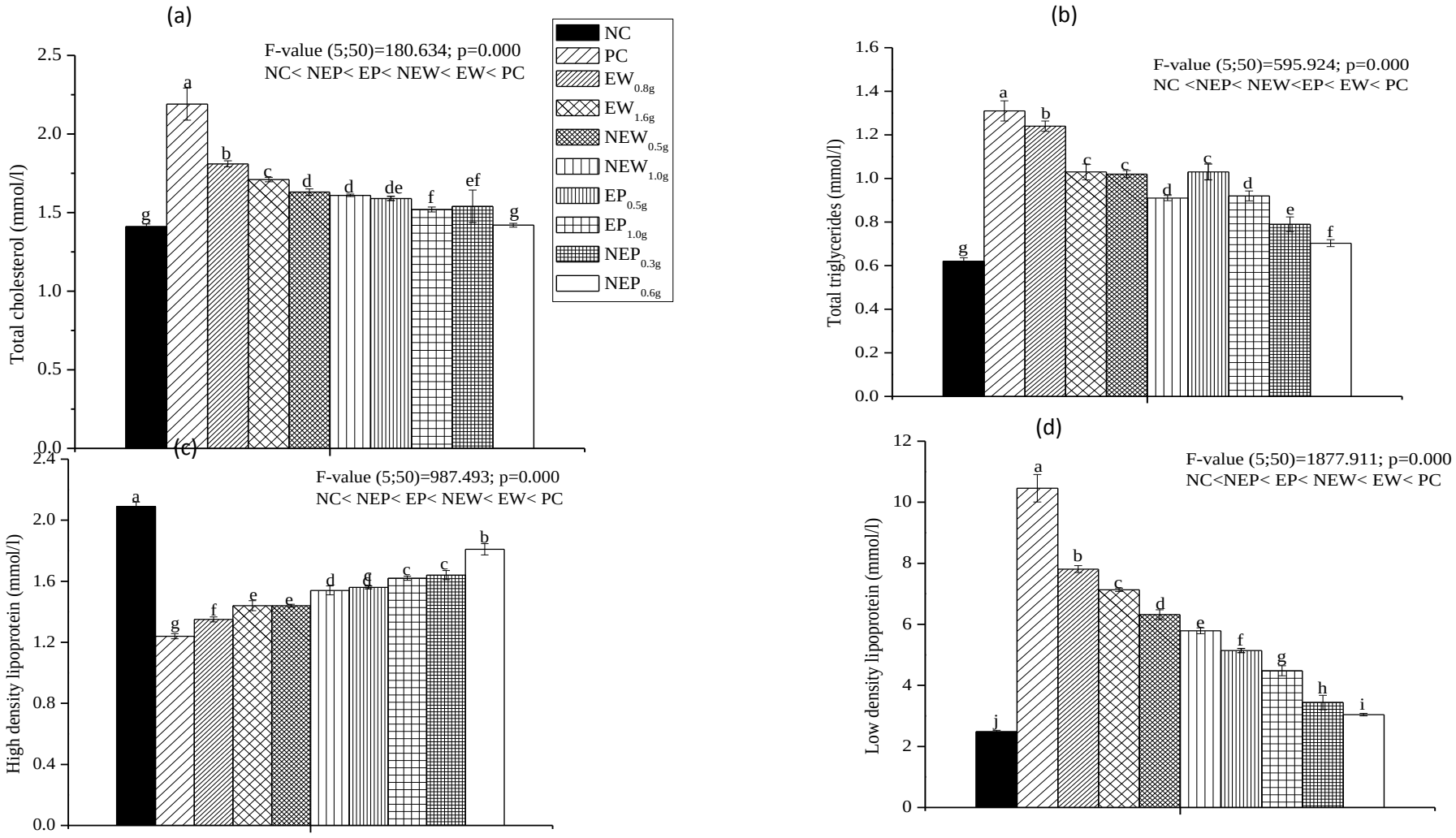


Figure 4.1. Effect of grapefruit treatment on lipid profile of the experimental animals at 20 weeks of experiment

Note: bars with the same letters are not statistically different at p<0.05 using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control**

OBJECTIVE 4: To determine the efficacy of the edible and non-edible parts of pink and white grapefruits on selected plasma oxidative stress and inflammatory markers in experimental rats

4.8 Effect of grapefruit treatments on plasma oxidative stress and inflammatory markers of the experimental rats

Effect of grapefruit treatment on the plasma oxidative and inflammatory markers of experimental rats is presented in Table 4.8. MDA, CRP, TNF-α and IL-6 were elevated significantly in PC group compared to NC group while adiponectin was reduced significantly in PC group compared to NC group. However, plasma concentrations of MDA, CRP, TNF-α and IL-6 were declined significantly in all grapefruit treated groups very close to NC status and adiponectin was significantly increased. Non-edible pink variety was most efficacious in reducing the plasma levels of MDA, TNF-α, CRP and IL-6; and increasing plasma concentration of adiponectin while edible white part was the least efficacious.

Table 4.8. Effect of grapefruit treatments on plasma oxidative stress and inflammatory markers of the experimental rats

Parameters	NC @ 20 weeks	PC @ 20 weeks	$EW_{0.8g}$ @ 20 weeks	$EW_{1.6g}$ @ 20 weeks	$NEW_{0.5g}$ @ 20 weeks	$NEW_{1.0g}$ @ 20 weeks	$EP_{0.5g}$ @ 20 weeks	$EP_{1.0g}$ @ 20 weeks	$NEP_{0.3g}$ @ 20 weeks	$NEP_{0.6g}$ @ 20 weeks	F-value	p-value
MDA (µmol/L)	23.11^{h} ± 3.49	53.70^{a} ± 0.94	34.67^{b} ± 1.68	32.44^{c} ± 0.42	30.24^{d} ± 0.27	28.35^{e} ± 0.28	26.59^{f} ±0.23	25.44^{fg} ± 0.33	24.41^{gh} ± 0.33	22.44^{h} ± 0.31	305.52	0.00
	Interaction/potency NC=NEP<EP<NEW<EW<PC							**F=544.95; p=0.000**				
CRP (µmol/L)	60.34^{k} ± 1.26	105.09^{a} ± 2.70	83.32^{b} ± 1.16	78.52^{c} ± 1.56	75.33^{d} ± 0.68	72.25^{e} ± 0.93	71.27^{ef} ± 0.46	70.0^{f} ± 0.39	68.10^{i} ± 0.57	64.20^{j} ± 1.04	596.32	0.00
	Interaction/potency NC<NEP<EP<NEW<EW<PC							**F=1054.63; p=0.000**				
Adiponectin (ng/mL)	78.45^{a} ± 1.45	41.66^{j} ± 0.87	50.57^{i} ± 0.69	52.70^{h} ± 0.58	54.73^{g} ± 0.46	57.56^{f} ± 0.35	59.41^{e} ± 0.51	61.24^{d} ± 0.84	63.97^{c} ± 1.07	68.12^{b} ±0.37	992.17	0.00
	Interaction/potency NC<NEP<EP<NEW<EW<PC							**F=1753.81; p=0.000**				
TNF-α (pg/mL)	22.54^{j} ± 0.86	66.26^{a} ± 0.96	43.88^{b} ± 0.80	40.35^{c} ± 0.80	37.52^{d} ± 0.39	35.81^{e} ± 0.56	33.87^{f} ± 0.66	31.12^{h} ± 0.64	32.04^{g} ± 0.50	28.07^{i} ± 0.87	1612.85	0.00
	Interaction/potency NC<NEP<EP<NEW<EW<PC							**F=2859.08; p=0.000**				
IL-6 (pg/mL)	22.55^{i} ± 0.59	76.56^{a} ± 1.27	45.86^{b} ± 0.40	43.08^{c} ± 0.58	38.86^{d} ± 0.52	38.86^{d} ± 0.73	37.02^{e} ± 0.49	34.97^{f} ± 0.64	32.14^{g} ± 0.66	29.55^{h} ± 1.10	2384.53	0.00
	Interaction/potency NC<NEP<EP<NEW<EW<PC							**F=4271.07; p=0.000**				

Note: mean values with the same letters are not statistically different at p<0.05 using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control; MDA=** Malondialdehyde**; TNF-α=** Tumor necrosis factor-α**; CRP=** Cell reactive protein**; IL-6= Interleukin-6;**

OBJECTIVE 5a: To evaluate the potential effect of the edible and non-edible parts of grapefruits on the functional markers of the liver (ALT, AST, ALP, LDH, total bilirubin and albumin) in the experimental rats

4.9 Effect of grapefruit treatments on plasma liver function markers of the experimental rats

Table 4.9 describes the effect of grapefruit treatment on liver functional markers of the experimental animals. Activities of ALT, AST, ALP, LDH and total bilirubin in the plasma were significantly elevated in PC group compared with NC group. Grapefruit treatment significantly reduced the activities of ALT, AST, ALP, LDH and total bilirubin in the plasma close to NC status. Whereas, no significant change was observed in the albumin status of the experimental rats. Non-edible part of pink variety was most potent in reducing the plasma activities of ALT, AST, ALP, LDH and total bilirubin while the edible part of white variety was the least potent.

Table 4.9. Effect of grapefruit treatment on plasma liver function markers of the experimental rats

Parameters	NC @ 20 weeks	PC @ 20 weeks	$EW_{0.8g}$ @ 20 weeks	$EW_{1.6g}$@ 20 weeks	$NEW_{0.5g}$ @ 20 weeks	$NEW_{1.0g}$ @ 20 weeks	$EP_{0.5g}$ @ 20 weeks	$EP_{1.0g}$ @ 20 weeks	$NEP_{0.3g}$ @ 20 weeks	$NEP_{0.6g}$ @ 20 weeks	F-value	p-value
Albumin (g/L)	28.39^{ab} ± 0.22	28.83^{ab} ± 0.28	28.45^{ab} ± 0.37	28.18^{ab} ± 0.51	28.25^{ab} ± 0.49	28.47^{ab} ± 0.67	28.52^{ab} ± 0.67	28.51^{ab} ± 0.68	28.77^{a} ± 0.36	28.11^{b} ± 0.11	1.25	0.29
Interaction: NC= PC= EW= NEW= EP= NEP								**F=0.570;**	**p=0.723**			
ALP activity (U/L)	175.96^{j} ± 2.03	289.42^{a} ± 7.33	264.70^{b} ± 6.32	254.63^{c} ± 3.58	246.04^{d} ± 2.53	236.59^{e} ± 5.09	222.95^{f} ± 3.69	213.82^{g} ± 4.78	197.18^{h} ± 5.11	183.16^{i} ± 3.54	370.44	0.00
Interaction: NC< NEP< EP< NEW< EW< PC					**F= 653.843; p= 0.000**							
LDH activity (U/L)	214.14^{i} ± 10.03	432.27^{a} ± 14.24	371.07^{b} ± 7.96	336.11^{e} ± 10.98	308.86^{d} ± 5.58	295.88^{e} ± 3.43	283.87^{f} ± 4.65	275.62^{f} ± 3.73	261.44^{g} ± 3.10	248.65^{h} ± 3.96	408.28	0.00
Interaction: NC< NEP< EP< NEW< EW< PC					**F=718.448; p= 0.000**							
Total Bilirubin (µmol/L)	1.52^{g} ± 0.05	2.57^{a} ± 0.08	1.99^{b} ± 0.05	1.94^{c} ± 0.02	1.91^{c} ± 0.02	1.86^{d} ± 0.03	1.92^{c} ± 0.02	1.81^{d} ± 0.02	1.75^{e} ± 0.04	1.60^{f} ± 0.05	292.69	0.00
Interaction: NC< NEP< EP= NEW< EW< PC					**F= 512.538; P=0.000**							
ALT activity (U/L)	37.21^{f} ± 0.12	58.12^{a} ± 0.37	44.64^{b} ± 1.25	41.94^{c} ± 0.14	40.61^{cd} ± 0.16	41.77^{c} ± 4.05	39.78^{de} ± 0.15	39.13^{de} ± 0.09	38.66^{ef} ± 0.12	38.13^{f} ± 0.14e	120.82	0.00
Interaction: NC= NEP= EP< NEW< EW< PC					**F= 214.398; p=0.000**							
AST activity (U/L)	75.39^{i} ± 0.46	104.83^{a} ± 1.52	86.31^{b} ± 0.27	84.58^{e} ± 0.32	82.88^{d} ± 0.18	81.08^{e} ± 0.36	80.23^{f} ± 0.22	78.25^{g} ± 0.42	77.93^{gh} ± 0.92	77.33^{h} ± 0.33	1057.18	0.00
Interaction: NC< NEP< EP< NEW< EW< PC					**F= 1887.318; p= 0.000**							

Note: mean values with the same letters are not statistically different at p<0.05 using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control; ALP= alkaline phosphatase; ALT= alanine transaminase; LDH= lactate dehydrogenase; AST= aspartate**

OBJECTIVE 5b: To evaluate the potential effect of the edible and non-edible parts of grapefruits on the plasma function markers of the kidney (Urea, creatinine, Uric acid, Na^+, K^+, Ca^{2+}, Cl^-) in the experimental rats

4.10 Effect of grapefruit treatments on plasma kidney function markers of the experimental rats

Table 4.10 presents the effect of grapefruit supplementation on kidney function markers of the experimental animals. Plasma levels of uric acid, creatinine, sodium and calcium were strikingly increased in PC group compared to NC group whereas urea concentration was reduced significantly in PC group compared with NC group. There was significant decline in the plasma concentrations of uric acid, creatinine and sodium in all grapefruit treated groups close to NC status. Plasma concentration of potassium in PC and NC groups did not differ statistically, but grapefruit treatment significantly increased the plasma concentration of potassium and urea in grapefruit treated groups. All grapefruit treatments significantly reduced plasma concentration of calcium close to NC status. Conversely, no statistically difference was observed in the plasma level of chloride across all the groups.

Table 4.10. Effect of grapefruit treatment on kidney function markers of the experimental rats

Parameters	NC @ 20 weeks	PC @ 20 weeks	$EW_{0.8g}$ @ 20 weeks	$EW_{1.6g}$@ 20 weeks	$NEW_{0.5g}$ @ 20 weeks	$NEW_{1.0g}$ @ 20 weeks	$EP_{0.5g}$ @ 20 weeks	$EP_{1.0g}$ @ 20 weeks	$NEP_{0.3g}$ @ 20 weeks	$NEP_{0.6g}$ @ 20 weeks	F-value	p-value
Uric acid (µmol/L)	47.74^{f} ± 0.49	62.63^{a} ± 1.57	52.36^{b} ± 1.15	50.50^{c} ± 0.38	50.14^{cd} ± 0.19	49.77^{c} ± 0.23	49.40^{d} ± 0.22	49.03^{d} ± 0.11	48.62^{e} ± 0.12	48.09^{ef} ± 0.19	259.96	0.00
Interaction: NC= NEP< EP< NEW< EW< PC							**F= 462.42;**		**p= 0.000**			
Creatinine (µmol/L)	39.98^{f} ± 0.24	51.77^{a} ± 1.11	45.96^{b} ± 0.27	44.20^{c} ± 0.08	43.72^{c} ± 0.13	42.13^{de} ± 0.07	42.61^{d} ± 0.66	42.56^{d} ± 0.14	42.20^{de} ± 0.08	41.88^{e} ± 0.16	337.25	0.00
Interaction: NC< NEP< EP= NEW< EW< PC							**F= 588.85;**		**p=0.000**			
Na^{+} (mmol/L)	135.17^{i} ± 0.44	159.21^{a} ± 0.58	147.99^{b} ± 0.43	146.15^{c} ± 0.27	144.31^{d} ± 0.30	142.39^{f} ± 0.24	143.37^{e} ± 0.27	142.68^{f} ± 0.27	140.37^{g} ± 0.19	138.35^{h} ± 0.16	2208.87	0.00
Interaction: NC< NEP< EP< NEW< EW< PC							**F= 3915.01;**		**p= 0.000**			
Ca^{2+} (mmol/L)	2.14^{e} ± 0.07	2.41^{a} ± 0.02	2.33^{b} ± 0.02	2.33^{b} ± 0.01	2.32^{b} ± 0.02	2.32^{bcd} ± 0.01	2.33^{b} ± 0.01	2.32^{bd} ± 0.01	2.31^{cd} ± 0.01	2.29^{d} ± 0.02	53.08	0.00
Interaction: NC< NEP= EP= NEW< EW=PC							**F=94.10;**		**=0.000**			
Cl^{-} (mmol/L)	102.0^{ab} ± 3.74	103.83^{a} ± 2.93	101.67^{ab} ± 0.82	100.67^{ab} ± 1.75	101.67^{ab} ± 1.63	101.01^{ab} ± 1.78	100.33^{ab} ± 2.16	101.0^{ab} ± 2.83	99.83^{b} ± 5.03	101.67^{ab} ± 2.80	0.93	0.51
Interaction: NC= NEP= EP= NEW= EW=PC							**F= 1.277;**		**p= 0.289**			
Urea (mmol/L)	6.39^{a} ± 0.18	2.58^{g} ± 0.15	4.32^{f} ± 0.33	4.60^{e} ± 0.11	5.06^{d} ± 0.15	5.56^{c} ± 0.05	5.09^{d} ± 0.20	5.47^{c} ± 0.08	5.83^{b} ± 0.06	6.21^{b} ± 0.10	256.43	0.00
Interaction: NC>NEP>EP=NEW>EW>PC								**F= 648.23;**		**p= 0.000**		
K^{+} (mmol/L)	4.84^{b} ± 0.08	4.86^{b} ± 0.07	6.76^{a} ± 0.13	8.85^{a} ± 0.06	6.82^{a} ± 0.24	6.75^{a} ± 0.14	6.88^{a} ± 0.17	6.71^{a} ± 0.08	6.85^{a} ± 0.08	6.73^{a} ± 0.07	319.59	0.00
Interaction: NEP= EP= NEW= EW> PC>NC							**F= 546.68;**		**p= 0.000**			

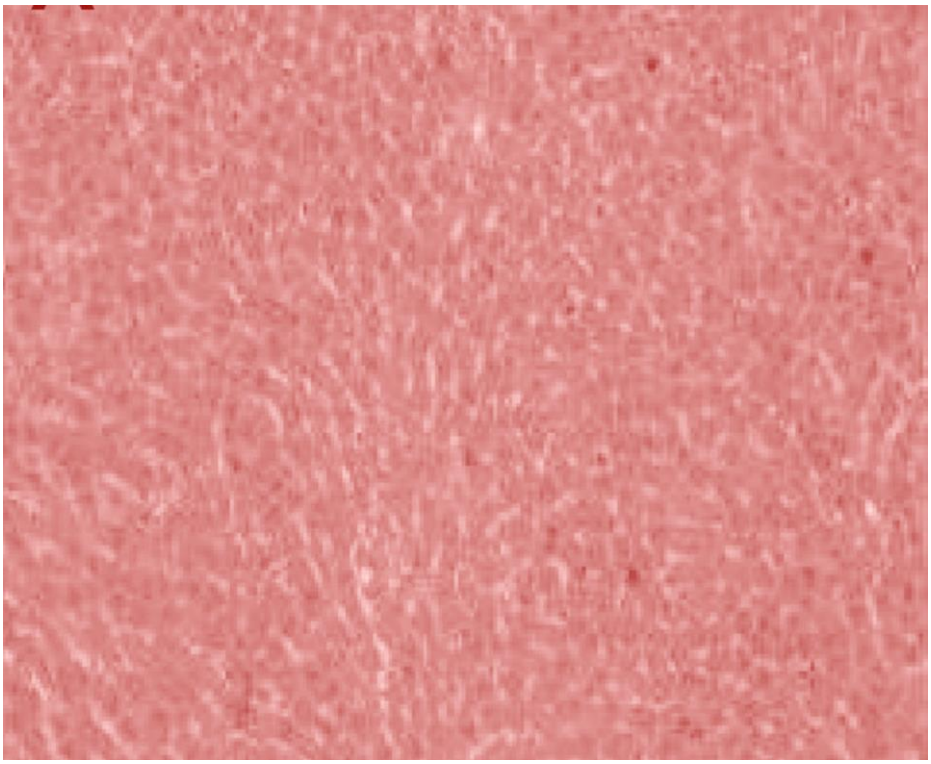
Note: mean values with the same letters are not statistically different at p<0.05 using DMRT. **EW= Edible white; NEW= Non-edible white; EP= Edible pink; NEP= Non-edible pink, NC= Negative control; PC= Positive control**

OBJECTIVE 6a: To evaluate the potential effect of the edible and non-edible parts of the two varieties of grapefruits on the hepatic structure of the experimental rats

4.11 Effect of grapefruit treatments on the hepatic structure of the experimental rats

The effects of the varieties of grapefruits on the hepatic structure after 20 weeks of grapefruit administration are presented in Figure 4. The liver structure of NC group showed normal structural design of the central vein, peripheral vein and the hepatocytes at 20 weeks (Figure 4.2ai). Contrarily in PC group at 20 weeks, there was degeneration of the hepatocytes, presence of scattered necrotic cells and evidence of congestion in the liver (Figure 4.2aii). All other groups ($NEP_{0.3g}$, $NEP_{0.6g}$, $EP_{0.5g}$, $EP_{1.0g}$, $NEW_{0.5g}$, $NEW_{1.0g}$, $EW_{0.8g}$ and $EW_{1.6g}$) presented improved features in the liver structure after 20 weeks of grapefruit treatments (Figures 4.2b-e).

(i)



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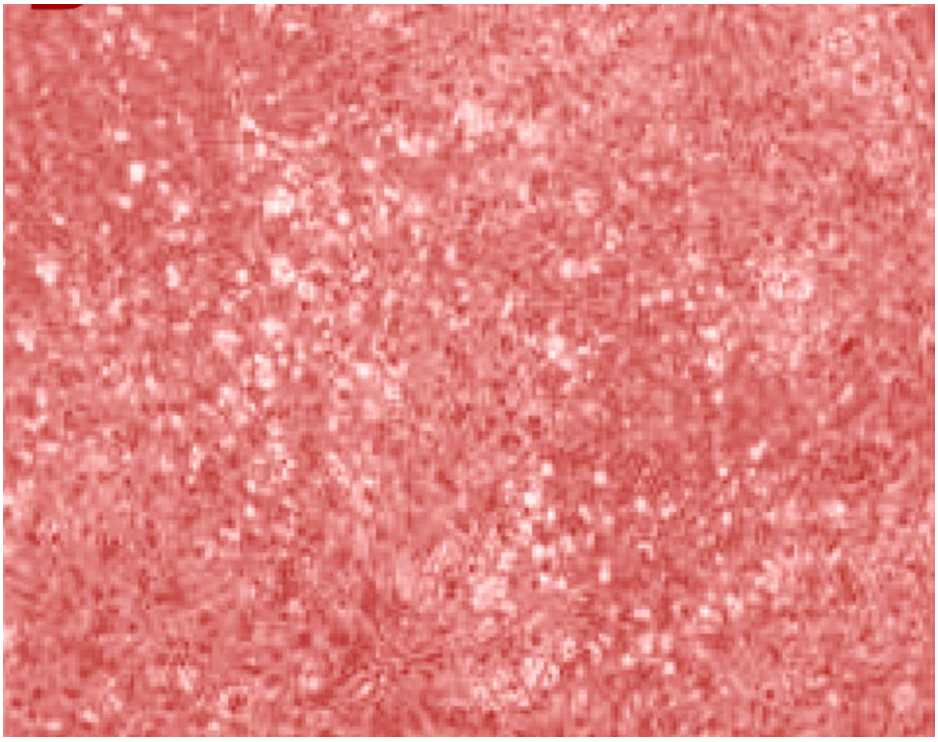
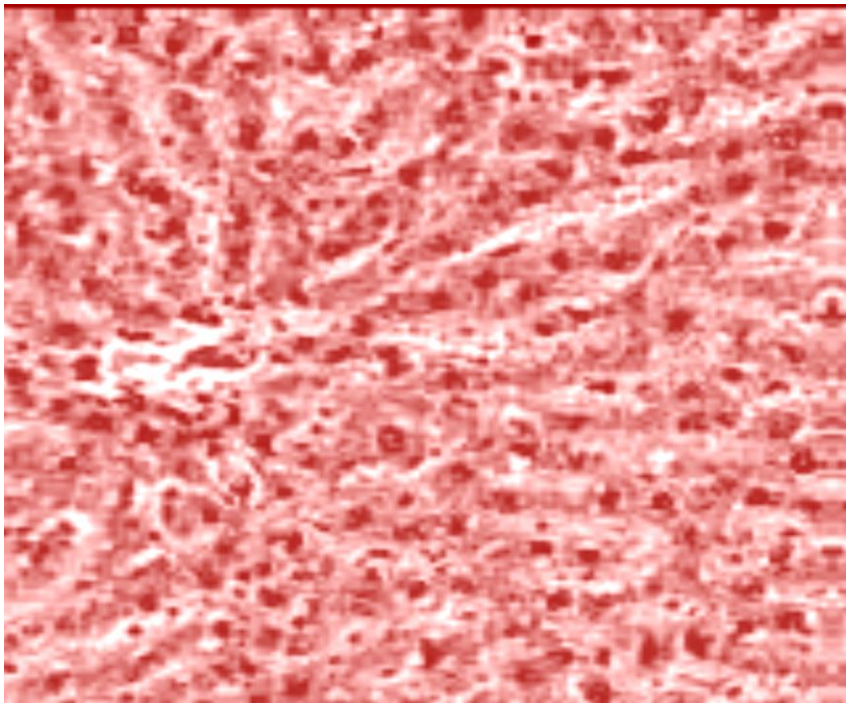


Figure 4.2a. Histopathology of the liver of the experimental rats in (i) NC and (ii) PC groups

(i)



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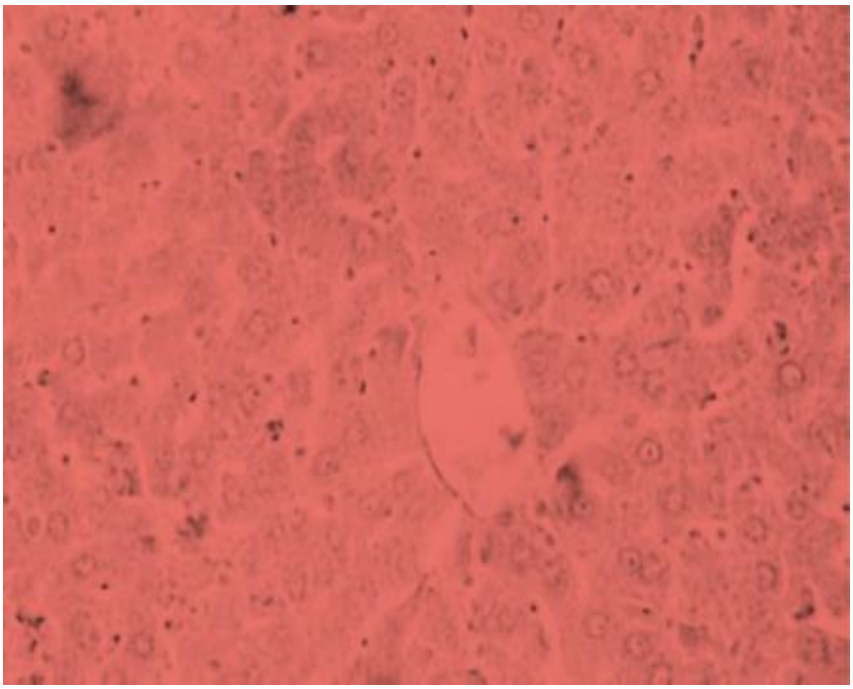
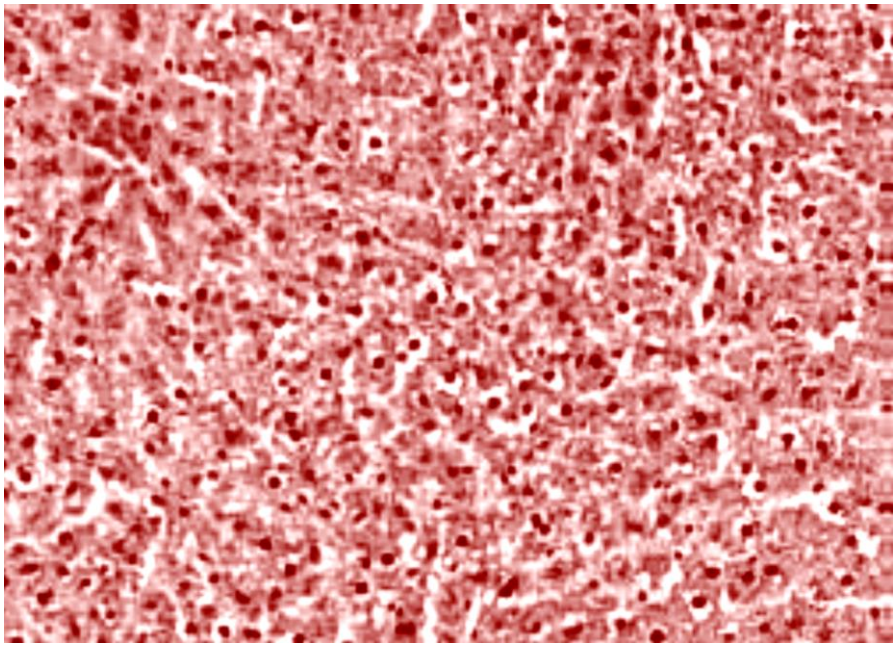


Figure 4.2b. Histopathology of the liver of the experimental rats in (i) $NEP_{0.3g}$ and (ii) $NEP_{0.6g}$ groups

(i)



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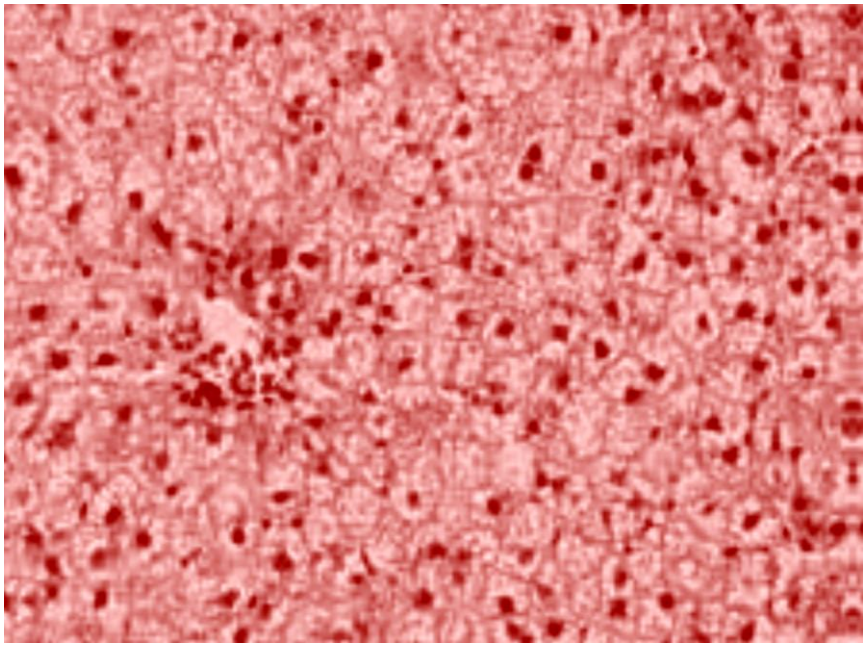
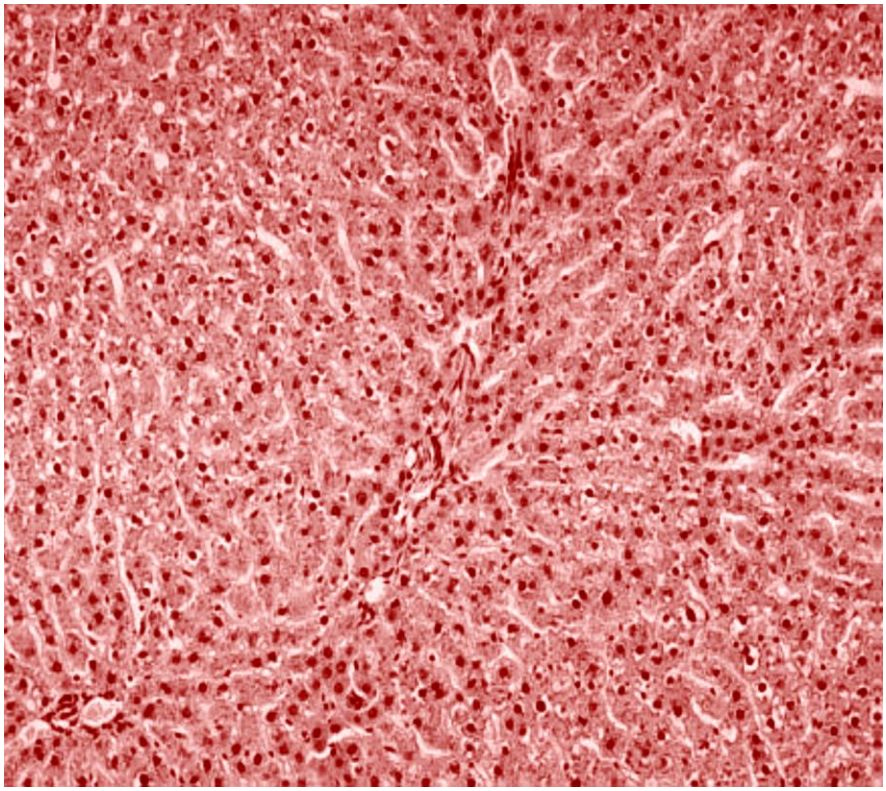


Figure 4.2c. Histopathology of the liver of the experimental rats in (i) $EP_{1.0g}$ and (ii) $EP_{0.5g}$ groups

(i)



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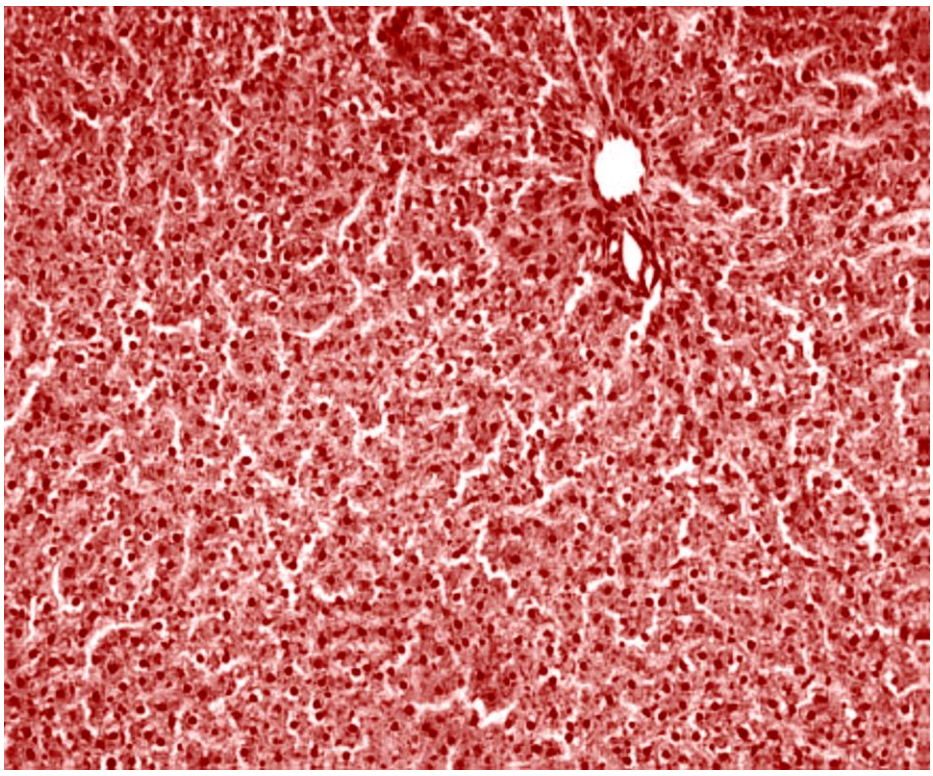
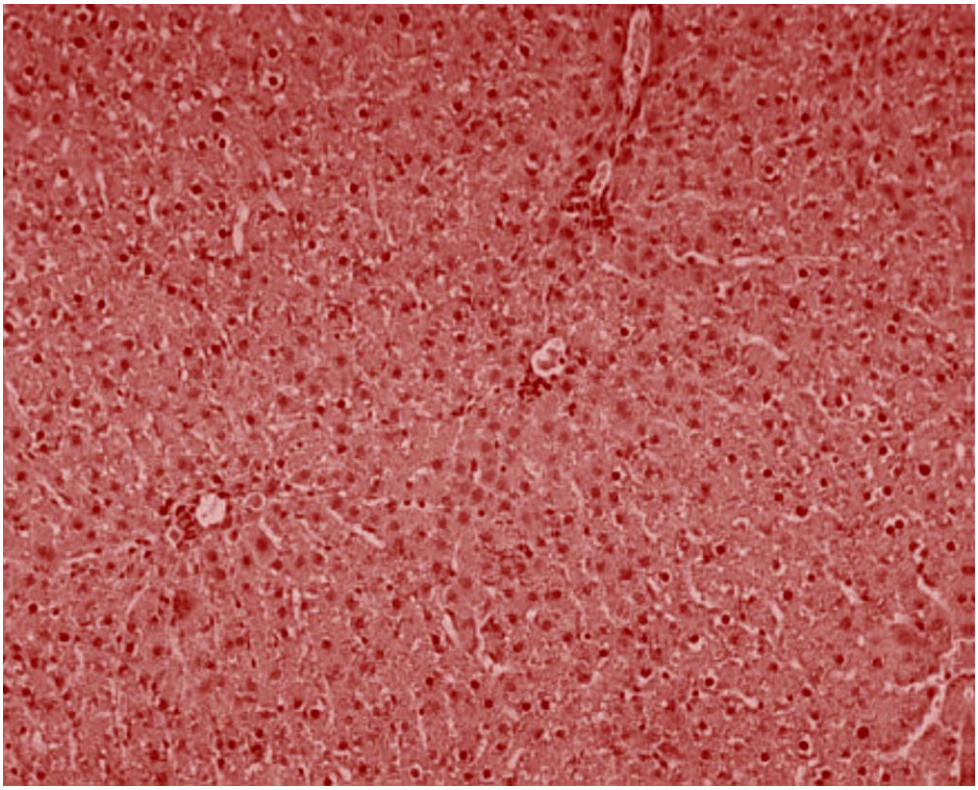


Figure 4.2d. Histopathology of the liver of the experimental rats in (i) $NEW_{0.5g}$ and (ii) $NEW_{1.0g}$ groups

(i)



(ii)

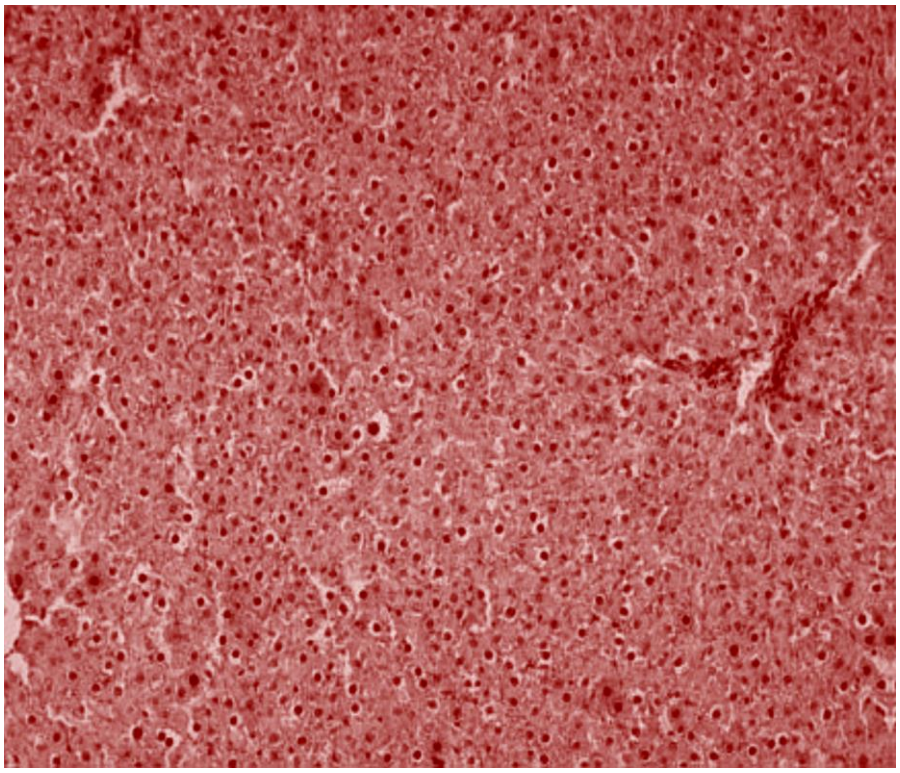


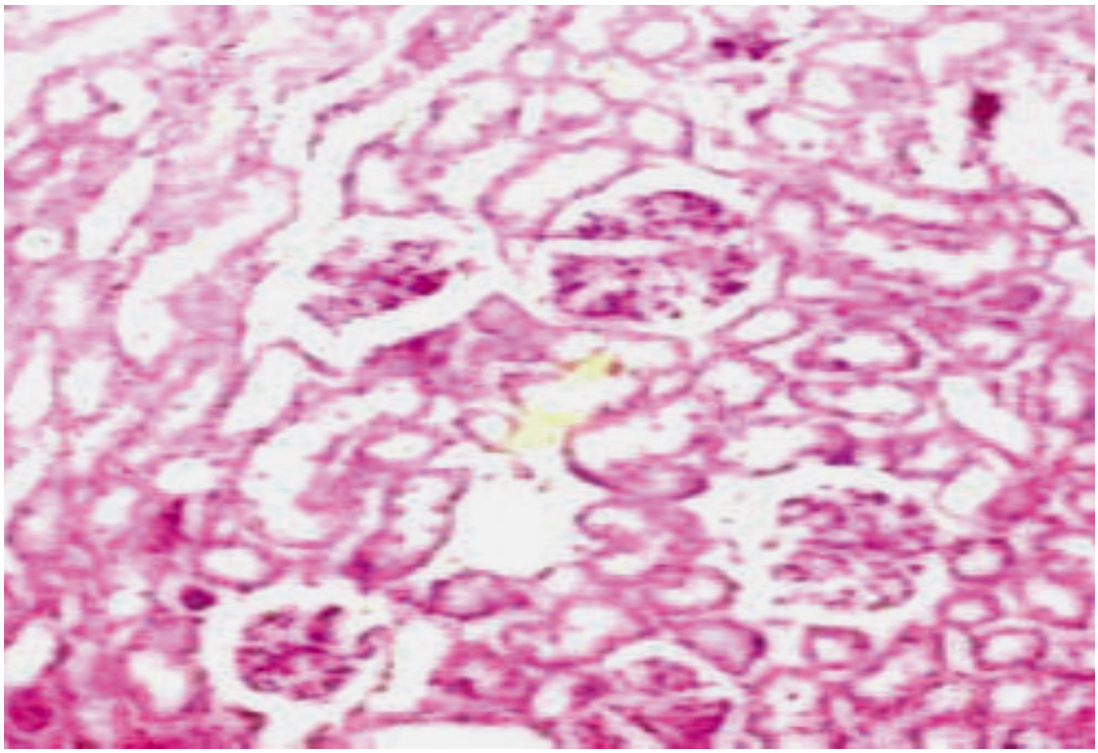
Figure 4.2e. Histopathology of the liver of the experimental rats in (i) $EW_{0.8g}$ and (ii) $EW_{1.0g}$ groups

OBJECTIVE 6b: To evaluate the potential effect of the edible and non-edible parts of the two varieties of grapefruits on the structure of the kidney of the experimental animals

4.12. Effect of grapefruit treatments on the structure of the kidney of the experimental animals

The effects of the varieties of grapefruits on the kidney structure after 20 weeks of grapefruit administration are presented in Figure 4.3. The kidney structure of NC group presented normal structure of the kidney. It features normal glomeruli and tubules, Bowmen's capsule, proximal tubule and distal convoluted tubular (Figure 4.3ai). Contrarily in PC group at 20 weeks, there was the presenece of obstruction of blood flow within the glomerular, damaged tubules, deterioration in tubules with signs of inflammation and dilation of Bowman's space (Figure 4.3aii). All other groups ($NEP_{0.3g}$, $NEP_{0.6g}$, $EP_{0.5g}$, $EP_{1.0g}$, $NEW_{0.5g}$, $NEW_{1.0g}$, $EW_{0.8g}$ and $EW_{1.6g}$) showed improved features in the kidney structure after 20 weeks of grapefruit treatments (Figures 4.3b-e).

i



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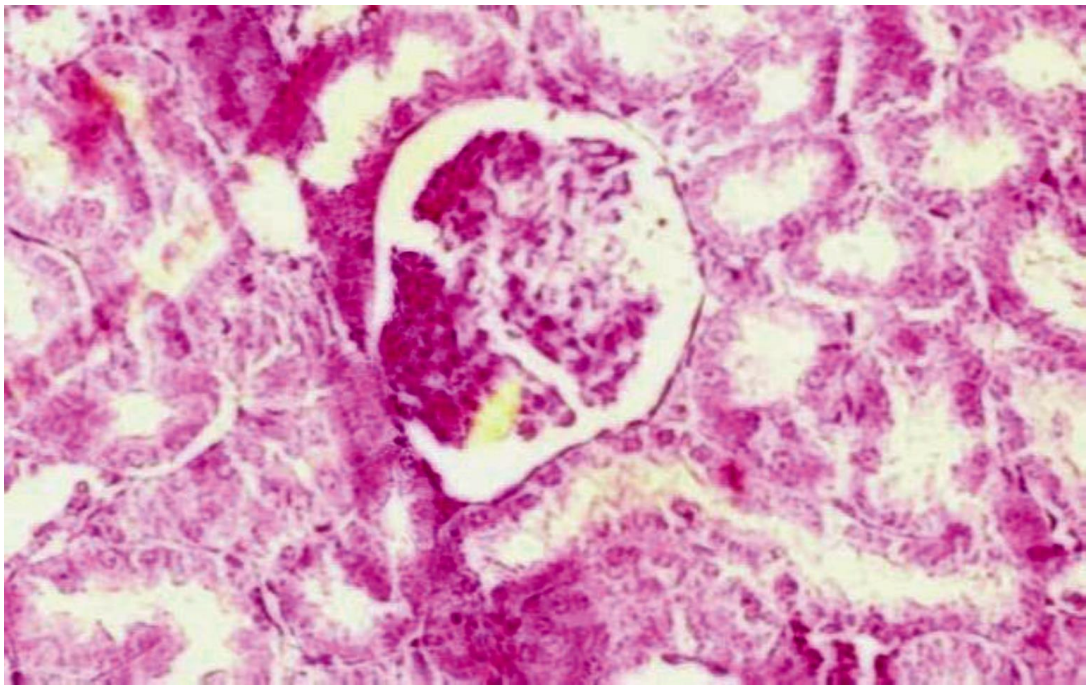
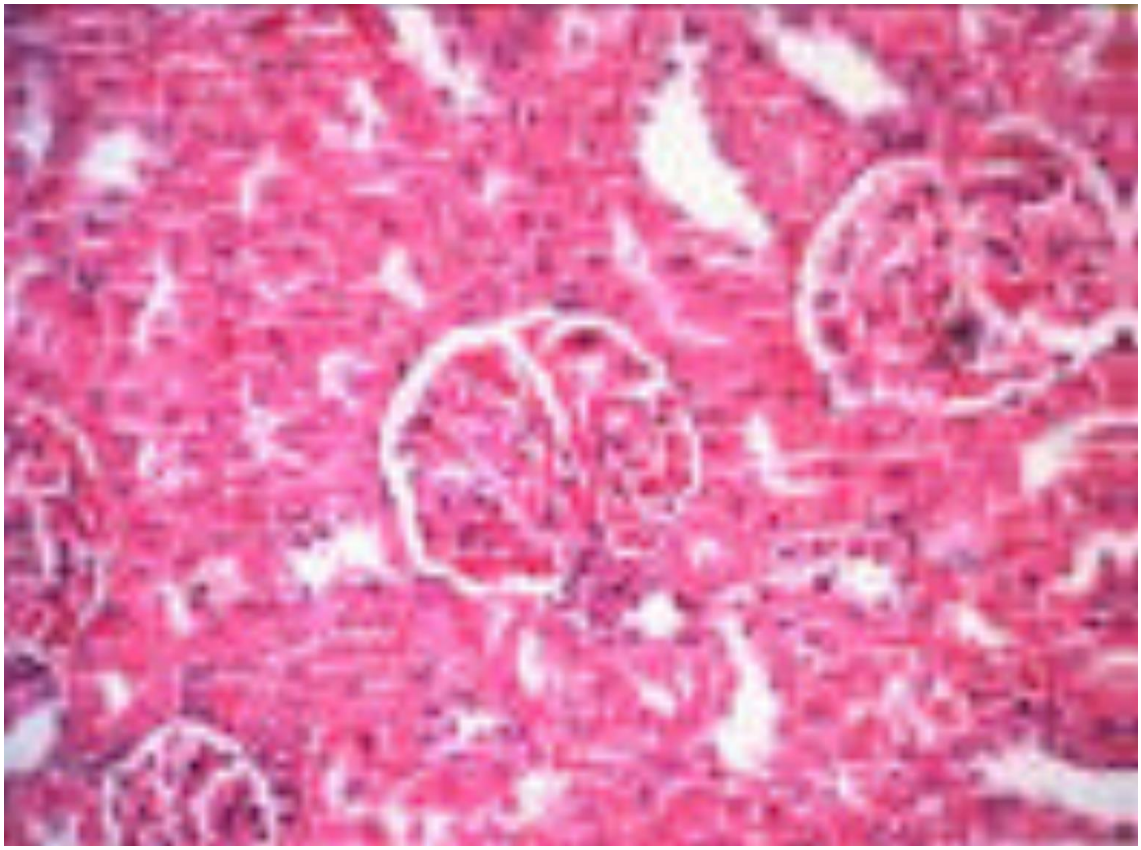


Figure 4.3a. Histopathology of the kidney of the experimental rats in (i) NC and (ii) PC groups

i



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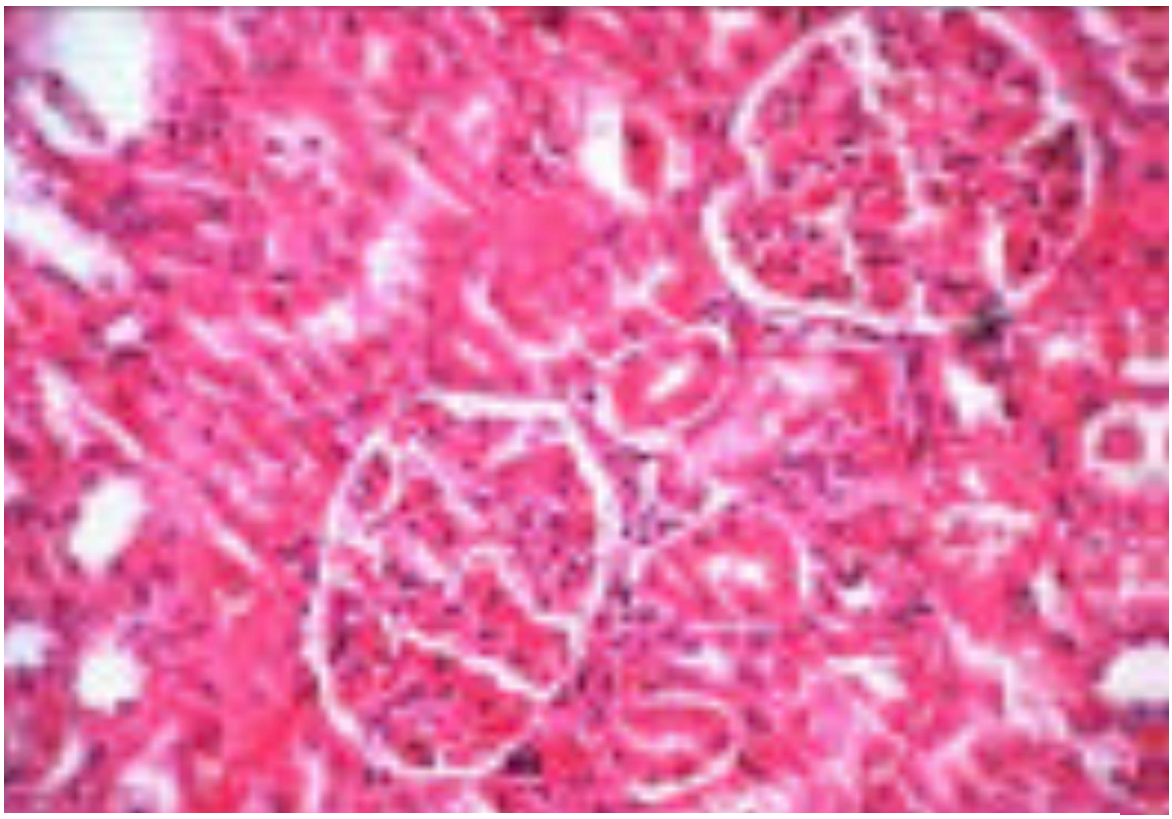
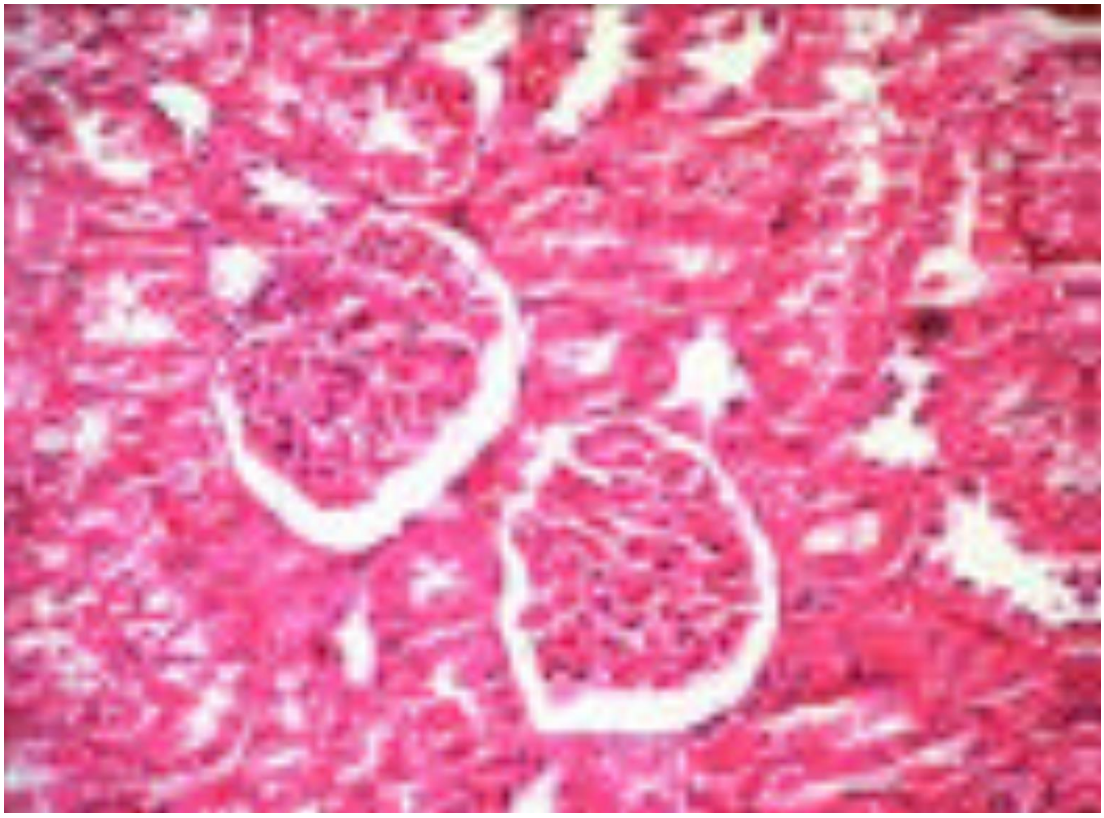


Figure 4.3b. Histopathology of the kidney of the experimental rats in (i) $NEP_{0.3g}$ and (ii) $NEP_{0.6g}$ groups

i



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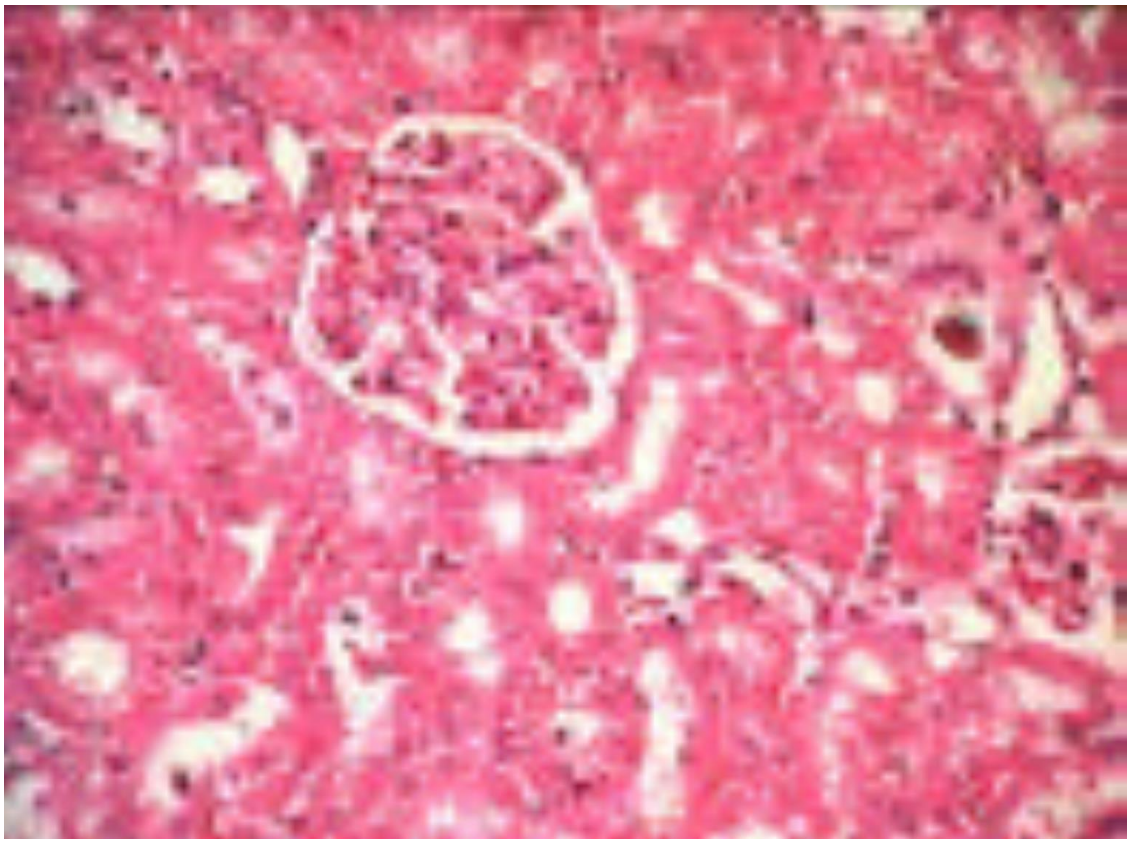
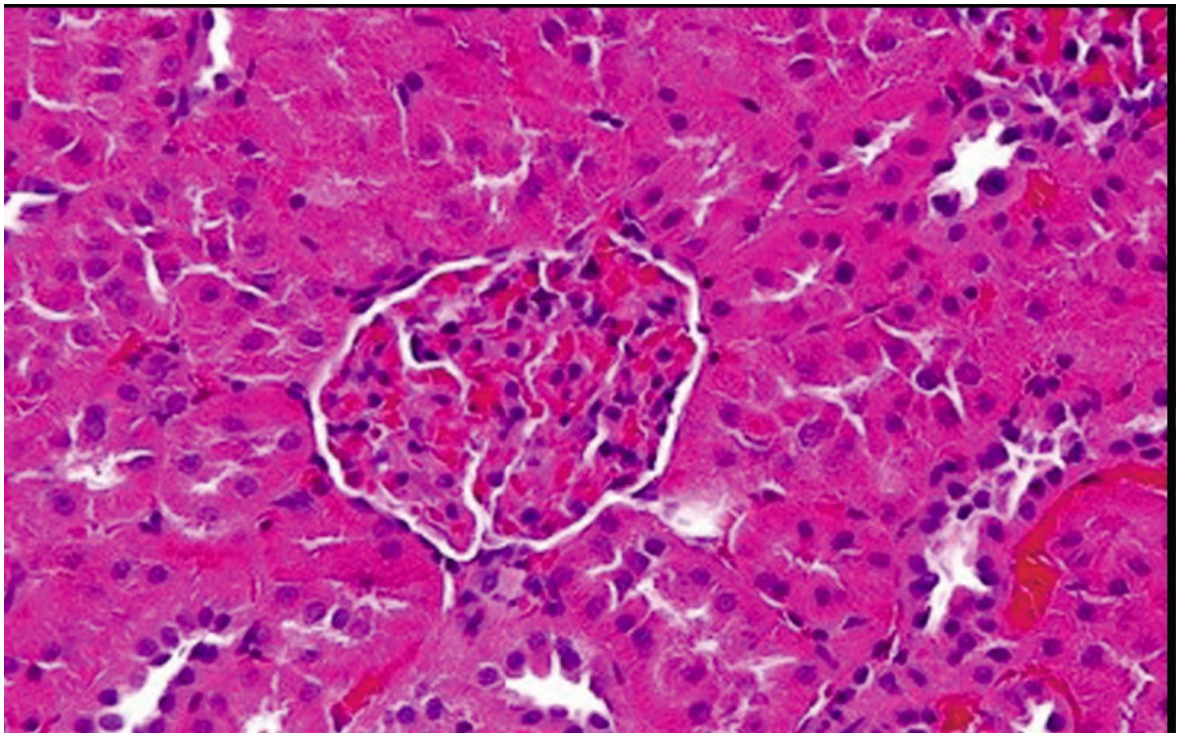


Figure 4.3c. Histopathology of the kidney of the experimental rats in (i) $EP_{0.5g}$ and (ii) $EP_{1.0g}$ groups

i



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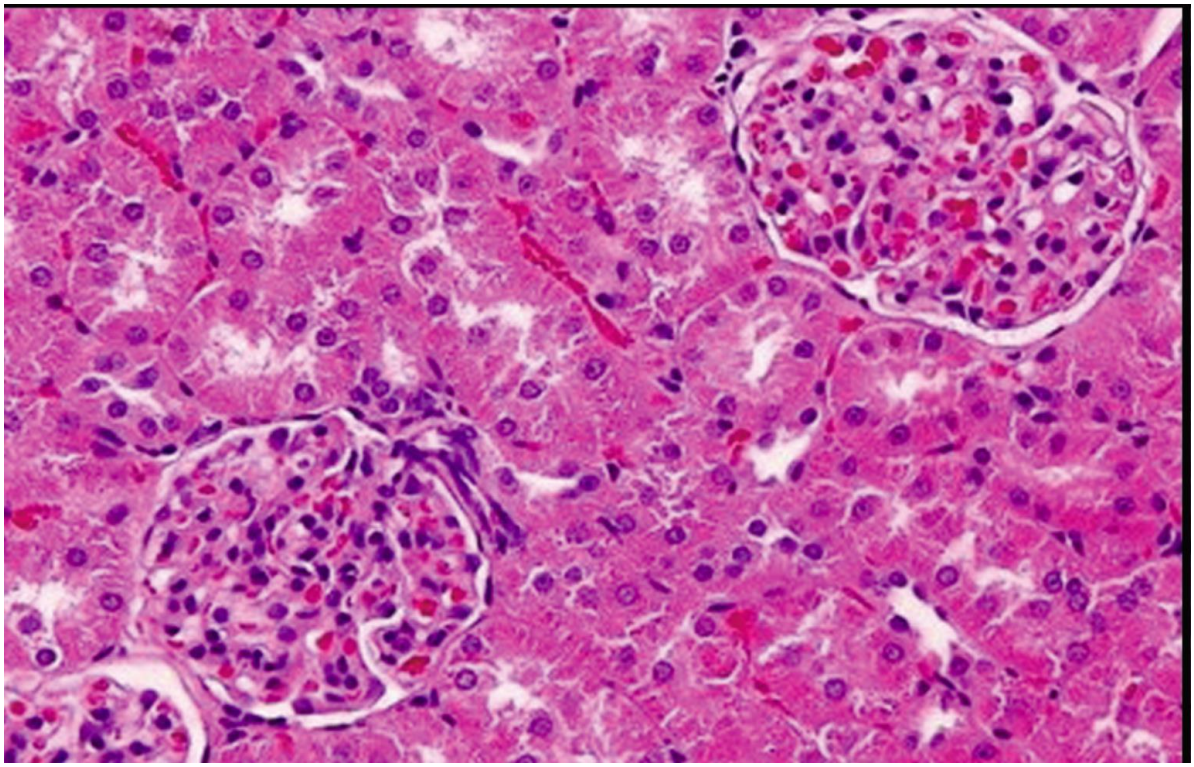
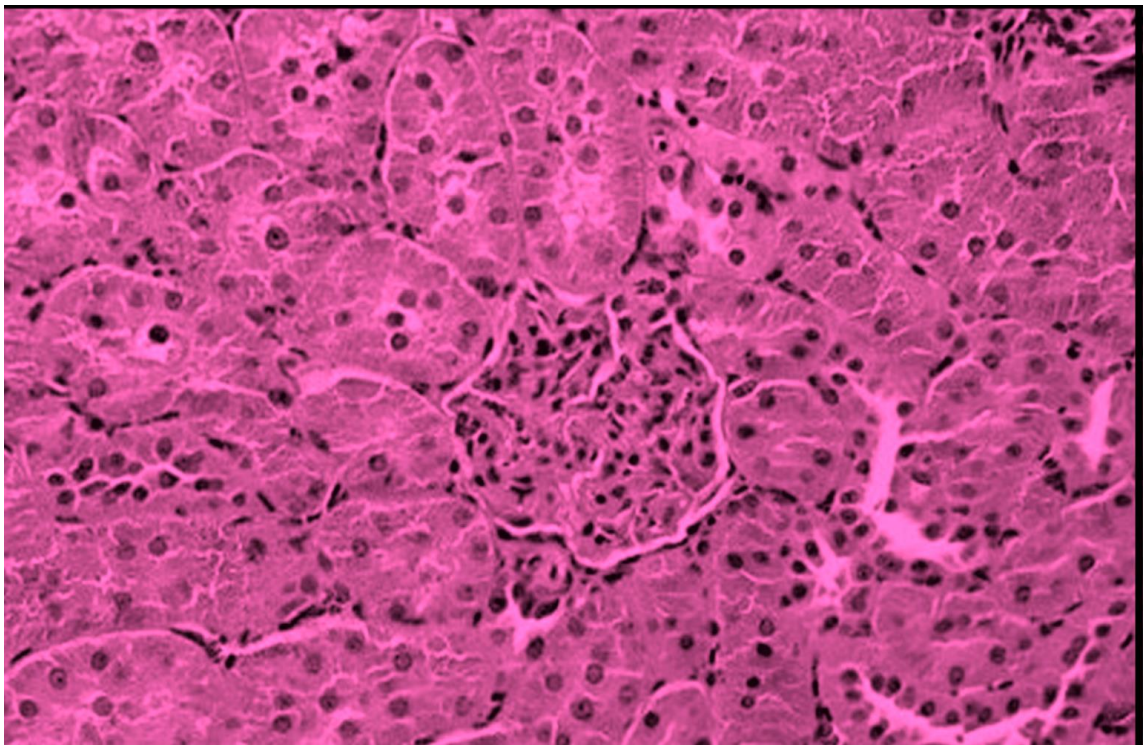


Figure 4.3d. Histopathology of the kidney of the experimental rats in (i) $NEW_{1.0g}$ and (ii) $NEW_{0.5g}$ groups

i



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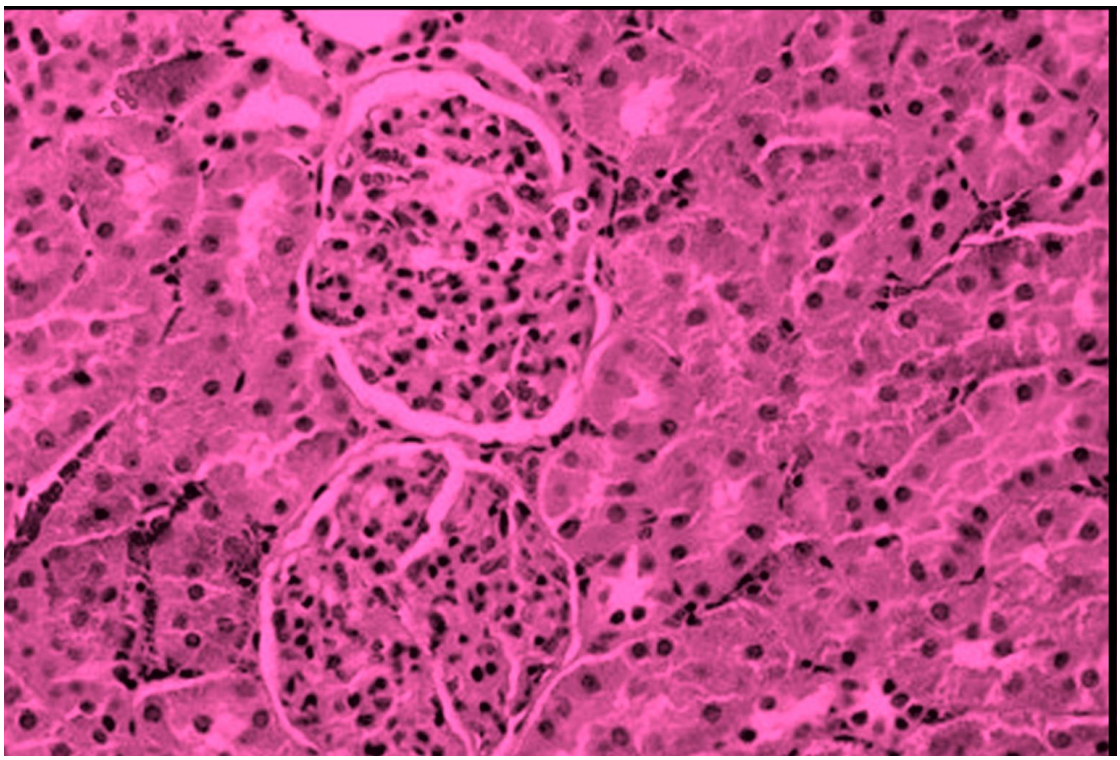

Figure 4.3e. Histopathology of the kidney of the experimental rats in (i) $EW_{1.6g}$ and (ii) $EW_{0.8g}$ groups

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

OBJECTIVE 1: Quantification of the major phytochemicals and determination of the antioxidant capacities of the extracts from edible and non-edible parts of pink and white grapefruits

5.1. DISCUSSION

5.1.1 Phytochemical composition of edible and non-edible parts of pink and white grapefruits

Phytochemicals are bioactive compounds that are generally found in plant sources. They are commonly found in cereals, vegratables, fruits, tea etc in large quantities. One of the major group of phytochemical is the polyphenols which are antioxidants found in plants as well (Xi *et al.,* 2017). Recently, polyphenol research has attracted much attention because of their beneficial roles against many of the non-communicable diseases. Citrus fruit has been identified to possess abundant polyphenols mainly flavonoids and phenolic acids.

In this study, total phenol and flavonoid contents of edible and non-edible parts of white and pink varieties of grapefruits were analysed. It was observed that the non-edible parts of the white and pink varieties contained higher total phenol than the edible parts. This result was consistent with the findings of Fejzić and Ćavar (2014) and Gorinstein *et al.* (2007). The study showed that grapefruit peels contained significantly higher amount of total phenols and flavonoids than the juice. Ghasemi *et al.* (2009) reported that the peels of grapefruits contained twice the amount of total phenols and flavanoids in the tissue. Petchlert *et al.* (2013) and Sir Elkhatim *et al.* (2018) also affirmed that the peels of grapefruits contained more phenolic compounds compared to the juice or the tissue.

Phenols and flavonoids are synthesised in plants as defence mechanism against insects, infectious agents, predators, and environmental stressors such as excessive hot or cold temperature, exposure to UV radiation particularly from the sunlight etc. Environmental stressor are recognised to heighten the production of phenolic compounds in plants in order to prevent oxidative damage that could affect the plant cellular structures due to exposure to environmental stressors (Chanwitheesuk et al., 2005). Generally, the peels of fruits are exposed to the environmental stressors than

the tissue or the pulp that are well protected by the peels. Hence, exposure of the peels to environmental stressors could have aggravated the synthesis of the phenolic compounds, and may explain the rationale for higher content of phenols in the peels of grapefruits compared to the pulp.

In addition, the phenol and flavonoid contents in the pink variety was significantly higher than the amount in white variety. This observation agrees with the report of Sicari *et al.* (2018). They also found that the pink grapefruit possesed strikingly higher total phenols and flavonoids than the white variety in their study. In contrast, Castro-Vazquez *et al.* (2016) reported a higher concentration of total phenol and flavonoids in the freeze-dried white grapefruit variety compared to the pink variety. This could be due to various factors that have been proven to greatly affect the total concentration of phenolic compounds in any fruit sample. Such factors include postharvest conditions, handling and storage (Tiwari and Cuminns, 2013), geographical variation (Leonardo *et al.,* 2013), type of cultivars, level of maturity of the fruit (Maria-John *et al.,* 2006) and extraction methods (Garcia-Salas *et al.,* 2010).

Moreover, in this study, analysis revealed that the total saponin, tannin and carotenoid contents of the non-edible part of both varieties were significantly higher than the contents in the edible parts. Contrastingly, the edible parts particularly of the white variety showed higher alkaloid content compared to the non-edible. There are relatively very limited studies that quantified the total saponin, tannin and carotenoids and alkaloids available in grapefruit samples. Hence, this makes comparison with other studies limited as well. However, few studies concentrated on the qualitative screening of grapefruits to determine the presence of saponins, alkaloids, tannins and carotenoids in the peels and pulps of grapefruits. Ezeabara *et al.* (2014) conducted a study on the qualitative and quantitative analysis of saponin content in various parts of six different citrus fruits showed that saponins were present in the peels and pulps of grapefruits with the peels showing higher saponin content than the juice. Pallavi *et al* (2018) in their qualitative phytochemical screening study also reported the existence of saponins both in the peels and pulp of grapefruit samples screened. Okwu and Emenike (2007) also gave similar report that saponins were present in the peels and pulp of grapefruits.

5.1.2 Antioxidant activities of the edible and non-edible parts of pink and white grapefruits

The antioxidant capacity of a fruit is meant to indicate the free radical scavenging properties and capabilities of such fruit sample. Several methods are being used for the estimation of antioxidant capacity of plants. Such methods include 2, 2-Azino-Bis (3 ethylbenzothiazoline-6-Sulphuric acid) (ABTS), 2-2 Di Phenyl-1-Picryhydroxyl radical (DPPH), Ferric Reducing Antioxidant Power (FRAP), Total Antioxidant Activity (TAA), Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP), Nitric oxide radical scavenging activity (NO), Oxygen Radical Absorbance Capacity (ORAC), Superoxide anion radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC) among others. Due to the limitation of each of the methods used, it has been suggested that at least two methods should be used in determining antioxidant activities of any sample (Gorinstein *et al.,* 2001a; Yu *et al.,* 2002). Therefore, in this study the antioxidant activities of the edible and non-edile parts of two varieties of grapefruits were assessed using the DPPH and FRAP assays.

Both DPPH and FRAP assays revealed that the non-edible parts of pink and white grapefruit varieties had increased antioxidant activity compared to the edible parts. In addition, the pink variety also displayed increased free radical scavenging ability than the white variety. This was consistent with other studies such as Pallavi *et al.* (2017) that reported higher free radical scavenging activity in grapefruit peels compared to the pulp as assessed by DPPH and NO scavenging activity assays.

Sir Elkhatim *et al* (2018) also reported that the peels contained significantly higher antioxidant activity than the tissue or juice. Castro-Vazquez *et al.* (2016) determined the antioxidant activities of white and pink varieties of grapefruits using FRAP and DPPH assay and indicated that the pink variety expressed higher antioxidant activity than the white variety. This result further substantiates the quantitative results of the total phenols and flavonoids content of the grapefruits, given that the non-edible portion of grapefruits that were observed to possess higher phenol and flavonoid content also displayed higher free radical scavenging ability. The antioxidant activities of the different varieties and parts of grapefruits might be a function of the amount of phenol and flavonoids present in each sample. The free radical scavenging ability of majority of fruits have been linked to the existence of the naturally occurring phenolic compounds such as flavonoids, tannins, phenolic acids and alkaloids as well as

carotenoids that are present in fruits (Jamuna *et al.,* 2010). In addition, the antioxidant capacity of these phenolic compounds is adduced to their capability to perform as a reducing agent, ROS suppressors and extinguisher, and metal chelators (Jamuna *et al.,* 2010). Thus, in this study, the free radical scavenging ability of the different varieties and parts of the grapefruits could be linked to the existence of phenolics (flavonoids, phenolic acids, alkaloids, carotenoids). Additionally, numerous investigators have documented strong positive association between total phenol contents and free radical scavenging capacity of fruits and vegetables (Jayaprakasha *et al.,* 2008; Ghasemi *et al.,* 2009).

Objective 2: Quantification of individual phenolic acids and flavonoids in the edible and non-edible parts of pink and white grapefruits grapefruits

5.1.3 Distribution of phenolic acid content in the edible and non-edible parts of pink and white grapefruits

Grapefruits are well-known as good sources of bioactive compounds such as phenolic acids and flavonoids. In this present study, among the quantified phenolic acid, gallic acid was observed to be the most abundant in the non-edible and edible parts of the two varieties tested while others were ranked based on their phenolic content as follows: chlorogenic acid, caffeic acid, ferulic acid and *p*-coumaric acid. Xi *et al.* (2017) documented similar observation in their study where they evaluated the phenolic compositions and antioxidant activities of nine grapefruit varieties available in China. Gallic acid was reported to be the main phenolic acid present in all the nine varieties assessed followed by chlorogenic acid, caffeic acid and ferulic acid respectively.

On the contrary, the reports of Wang *et al.* (2008) showed that chlorogenic acid was the major phenolic acid found in grapefruit. Whereas, Alu'datt *et al.* (2017) reported that *p*-coumaric was the main bound phenolic acid found in grapefruits accounting for 58.0% of the total phenol content while vanillic acid was reported as the major free phenolic acid accounting for 46.5% of the total phenols. Gorinstein *et al*. (2004) also reported ferulic acid as the major phenolic acid component found in the peels and pulp of white grapefruit. These variations reported regarding the major phenolic acid content of grapefruits could be due to the variation in the genetics, cultivars and species as well as environmental factors.

Our results also showed that the non-edible portion of both varieties contained markedly higher concentration of chlorogenic acid compared to the edible parts, the pink variety also had higher concentration compared to the white variety. Similar variation was observed for gallic acid, ferulic acid and *p*-coumaric acid. However, the white variety showed higher ferulic acid and *p*-coumaric acid concentration than the pink variety. Edible parts of the pink and white varieties indicated significantly higher levels of caffeic acid compared to the non-edible parts. It has often been stated that the distribution of polyphenols varies in different parts of citrus fruits. Xi *et al.* (2017) found that the concentration of the phenolics varies in the different parts of various cultivars of grapefruits. They noted that the phenolic acids and flavonoid were widely dispersed in the various parts of the fruits such as the flavedo, segment membrane, albedo, seeds and juice vesicles with flavedo presenting the highest content followed by the segment, juice, albedo and the seeds. Jang *et al.* (2010) also documented that the peels had more phenols and flavonoids that the pulp. In addition, Xi *et al.* (2014) found that the peels are 1-3 times richer in phenolic acids and flavonoids than the pulp or juice. Nogota *et al* (2006) documented hierarchical form of different fruit parts based on their phenolic acid and flavonoid content, the peels was documented to richer in phenolic acids and flavonoids compared to the pulp or juice.

5.1.4 Distribution of flavonoid content in the edible and non-edible parts of pink and white grapefruits

The most prominent flavonoid subclass that are abundantly available in grapefruit is the flavanone and it accounted for 97% of the total flavonoids in grapefruits (Peterson et al., 2006). In this study, six flavonoids of which five belonged to the subclass of flavanones were identified and quantified using HPLC-DAD; the flavonoids are naringin, hesperidin, naringenin, eriocitrin, narirutin and quercetin.

The result indicated that the most abundant flavonoid present in the two varieties of grapefruit tested was naringin, this was followed by hesperidine, eriocitrin, naringenin and narirutin, while quercetin ranked lowest based on their concentrations in the grapefruit samples. It was also observed that the non-edible parts of the pink and white varieties displayed significantly higher level of naringin, narirutin, naringenin and eriocitrin than the edible parts. Likewise, the pink variety showed higher concentration of the above-mentioned flavonoids than the white variety. In dissimilarity, hesperidine

and quercetin were abundant in the edible parts compared to the non-edible parts. Much higher level of quercetin was found in the white variety compared to the pink variety. These observations support the findings from several other studies. Xi *et al.* (2017) reported that naringin was the principal flavonoid found in grapefruits. In another study by Xi *et al.* (2014) to quantify the concentration of flavanones in four varieties of grapefruits and 28 locally available pommelos in China. It was found that all the varieties of grapefruits had 1 to 3.98 times higher level of naringin than the pummelos. Sicari *et al.* (2018) also showed that naringin was the most abundant flavanone in the two varieties of grapefruits assessed in their study.

Objective 3: Efficacy of edible and non-edible parts of two varieties of grapefruits on cardio-metabolic parameters in experimental rats

5.1.5 Effects of grapefruit treatment on nutritional parameters of the experimental rats

Grapefruit treatment significantly reduced food and water intake among the experimental rats to NC status with no significance difference in their daily energy intake. The observed effect on food intake of experimental animals could be linked to the fiber constituents of grapefruits, as dietary fibers are known to give satiety without necessarily increasing the total energy intake. This observation has been documented in other studies. Chudnovskiy *et al.* (2014) reported reduced food intake among the groups of rats on high fat diet and various forms of grapefruit supplementation. The mechanism could also be because of grapefruit's ability to delay evacuation of gastrointestinal content due to its high level of acidity that eventually altered the pH of the gastric content (Chaw *et al.*, 2001). Several studies have proven that there could be delay in evacuation of gastrointestinal content as a result of reduced pH leading to reduced motility of the gastric content to give satiety (Chaw *et al.*, 2001; Ofem *et al.*, 2012; Lin *et al.*, 1990). Another possible mechanism could be the secretion of glucagon-like peptide-1 hormones that synthesized in the intestines in response to the presence of chyme. They are known to inhibit evacuation of gastrointestinal content by regulating the metabolism of nutrients and suppressing appetite (Young *et al*., 2003).

5.1.6 Effects of grapefruit treatment on anthropometric parameters, body composition and organ weights

Excessive weight gain and abdominal fat deposition has been acknowledged to contribute significantly to the observed metabolic derangement such as oxidative stress, chronic low-grade inflammation, dyslipidemia, hyperglycemia and insulin resistance that accompanies MetSyn (Furukawa *et al.,* 2004; Shoelson *et al.,* 2006). It features accumulation of fat particularly the visceral fat in the abdominal region that resulted in the disproportionate discharge of adipokines leading into upsurge of available fatty acids in the blood, thereby increasing the risk of complications (IDF, 2006).

In this study, intake of high-carbohydrate high-fat diet by the animals significantly increased the body weight, abdominal circumference, and fat accumulation in the animals compared to the negative control. This could be because the high-carbohydrate high-fat diet contained more sugar and fat, delivering excess energy than what the animals need. The extra energy is deposited in the adipose tissue leading to expansion and proliferation of the hepatocytes. This eventually resulted in increased body weight gain and fat accumulation (Maury *et al.,* 2010). However, grapefruit supplementation significantly reduced the abdominal circumference and body weight across all the grapefruit treated groups. Several other studies in experimental animals as well as clinical trials documented similar observation.

Chudnovskiy *et al.* (2014) and Farouk *et al.* (2015) noted a striking weight loss among the experimental animals that had high fat diet and grapefruit supplementation. Fujioka *et al.* (2006) in their study among patients with MetSyn to determine the impact of daily intake of fresh whole grapefruit, grapefruit juice, grapefruit capsule and placebo on body weight in relation to metabolic syndrome. They documented marked reduction in the body weight of all the subjects that received grapefruit treatment in comparison with the group that received placebo. The highest body weight reduction was recorded among the subjects that had fresh whole grapefruit supplementation. Murase *et al.,* (2010) explained that this observation could be accredited to a component of grapefruit called nootkatone. It acts as a stimulating agent for adenosine mono phosphate activated protein kinase that prevents weight gain and obesity by increasing the rate at which the body uses energy (Stapleton *et al.,* 1996). Stimulation of AMPK prompt fatty acid oxidation and synthesis of ketone bodies in the liver, reduced production of cholesterol, fats and triglycerides (Thomson *et al.,* 2007). Murase *et al.* (2010) reported that

nootkatone significantly reduced body weight gain that was induced from the intake of high fat and high sucrose diet. It was also noted in this study that the animals on grapefruit supplementation of varying types and doses had lesser mesenteric, epididymal and subcutaneous fat pads that are not statistically different from the NC groups. Experimental animals on grapefruit supplementation also exhibited reduced liver and kidney wet weights. The mechanism of action could also be due to activation of AMPK leading to decline in the production of cholesterol, fats and triglycerides in the liver as explained above.

This observation is in consonance with the reports of other researchers that used model of MetSyn similar to what we used in this study. Reduced wet liver weights was also reported. Naringin, a major flavanone found in grapefruit was found to reduce liver weight, mesenteric and epididymal adiposiity in high fat diet induced rats (Pu *et al.*, 2012). In a similar animal study, 95.4±2.2 mg/kg of naringin was fed daily to high-carbohydrate high-fat diet induced rats for 8 weeks, visceral fat pads and abdominal circumference was observed to be significantly decreased (Alam *et al.,* 2013). Similar reports were obtained when rats induced with high carbohydrate high fat diet were treated with ferulic acid (Senaphan *et al.*, 2015) and quercetin (Panchal *et al.*, 2012).

In this study, the edible and non-edible parts of the white and pink grapefruits contained naringin in abundant and appreciable amount of ferulic acid and quercetin as well. The presence of these bioactive compounds in grapefruits may play a part in reducing the mesenteric, epididymal and subcutaneous fat pads as well as the liver wet weight as observed in this study. Sergeev *et al.* (2014) explained that the reduction in fat accumulation might be due to natural death of adipocyte cells that occurs as a function of growth or development - a phenomenon referred to as apoptosis. Sergeev and colleagues further explained that the presence of naringin in the body would initiate calcium upsurge in the intracellular region. This would subsequently leads to elevated concentration of proteins associated with the scheduled apoptosis, thus promoting weight loss and obesity.

5.1.7 Effect of grapefruit treatment on fasting plasma glucose, insulin and insulin resistance

Hyperglycemia, one of the diagnostic criteria for MetSyn is a pathological condition that resulted from the aberration of macronutrient (carbohydrate, fat and protein)

metabolism. Impaired glucose tolerance, inadequate production of insulin synthesis and insulin resistance are other pathological features that are linked with hyperglycemia (de Koning *et al.,* 2008). Insulin is an essential hormone that is responsible for regulating glucose balance. Insulin regulates body glucose through the stimulation of glucose clearance in the muscle and adipose tissues and preventing hepatic glucose synthesis by obstructing the breakdown of glycogen to glucose and formation of new glucose molecules.

Insulin resistance is a situation in which adequate amount of insulin produced is incapable to respond appropriately in the tissue of interest such as the adipose, muscle and liver. The inability of insulin to respond appropriately to regulate glucose balance will result into hyperglycemia. Given this situation, the pancreatic β-cells will continue to produce more insulin to cater for hyperglycemia, this will eventually lead to increased circulating insulin in the blood and produce adverse outcome on tissues and organs that are sensitive to insulin such as kidney (Peterson *et al.,* 2006).

In this study, diets fed to the experimental rats was able to significantly elevate the plasma level of glucose and insulin, and promote insulin resistance in the experimental rats. However, various doses of grapefruit supplementation significantly attenuated the concentration of fasting plasma glucose and insulin as well as insulin resistance very close to NC status. In addition, the non-edible part of the pink variety of grapefruit was found to be most efficacious in lowering the plasma glucose, insulin and insulin resistance. The concentration of plasma glucose, insulin and insulin resistance of the animals fed with the 0.3g and 0.6g/day of the non-edible part of pink variety of grapefruit did not differ significantly. This observation could be due to the fact that non-edible part of pink variety contained the highest amount of total phenols and flavonoids among other samples tested. In addition, non-edible parts of pink variety was also found to possess large amount of naringin, a principal component of grapefruits that has been conferred to possess beneficial biological functions in relation to health. Several other studies have documented the use of whole grapefruit and or grapefruit constituents such as naringin, naringenin, hesperidin, quercetin, narirutin among others on rats and human in varying doses. Fujioka *et al*. (2006) indicated a significant reduction in the plasma glucose in patients with MetSyn receiving grapefruit treatment (fresh whole grapefruit or grapefruit capsule or grapefruit juice) compared to placebo. In a study conducted on high fat diet induced diabetic rats, supplementation of

naringin at 50 and 100 mg/kg daily for 28 days alleviated impaired glucose tolerance and improved insulin resistance (Sharma *et al.*, 2011).

In addition, intake of 50 mg/kg of naringin for 4 weeks resulted into significantly lowered serum insulin concentration, improved oral glucose tolerance test and blood glycosylated haemoglobin in diabetic rats. Continuous treatment with naringin for 30 days further lowered the serum insulin concentration and enhanced insulin sensitivity (Jagetia *et al.,* 2012). According to Ahmed *et al.* (2012), narigin and hesperidin supplementation at a dose of 50 mg/kg for 56 days fed to high-fat diet induced diabetic rats, significantly decrease the fasting blood glucose and enhanced insulin sensitivity. However, no significant increase on OGTT levels of the rats. Naringin supplementation at 0.2 g/kg for 10 weeks significantly reduced insulin resistance by 20.3% as assessed by HOMA-IR. Also, fasting blood glucose and serum insulin concentrations were markedly reduced following supplementation of naringin in high fat diet fed rats (Pu *et al.*, 2012).

One of the proposed mechanisms through which the principal flavanone in grapefruit, naringin acts as hypoglycemic agent is by increasing the breakdown of glucose to release energy in the liver by way of reducing the activity of glucose-6-phosphatase, an enzyme in charge of hydrolysis of glucose-6-phosphate to produce glucose and phosphate in the liver (Junk *et al.,* 2004). In addition, naringin evidently decrease the formation of new glucose molecules in the liver by reducing the activity of phosphoenolpyruvate carboxykinase, an enzyme in the metabolic pathway of glucose formation from non-carbohydrate sources in the liver (Junk *et al.,* 2004). Also, Zygmunt *et al.* (2010) proposed that the ability of naringin to inhibit hyperglycemia is facilitated by uptake of glucose in the skeletal muscle through AMPK.

5.1.8 Effect of grapefruit treatment on lipid profile

Hyperlipidemia also called dyslipidemia is a pathological state that features increased lipids in the blood. Such lipid includes increased circulating plasma triglycerides and cholesterol such as LDL-c (Nirosha *et al.*, 2014). Dyslipidemia is a common occurrence among individuals with obesity, type-2 diabetes and MetSyn. It contributes significantly to the development of CVD (Wold *et al*., 2005).

In this study, dyslipidemia was evident among the experimental rats that are fed with high carbohydrate high fat diet. However, varying doses of the edible and non-edible parts of pink and white varieties was observed to strikingly lowered the plasma levels of LDL-c, total cholesterol, triglyceride and increased the HDL-c level to the values obtained for the negative control group. The non-edible part of the pink variety was found to be most potent in reducing the plasma lipids compared to the other parts and varieties of grapefruit tested. This could be attributed to the higher concentration of grapefruit flavonoids such as naringin, naringenin, narirutin and phenolic acids such as *p*-coumaric, ferulic acid etc found in the non-edible parts of pink variety compared to the other parts and varieties of grapefruits tested.

A study conducted by Xulu *et al.* (2012), showed that treatment with naringin (50 mg/kg daily) for 45 days significantly lowered the plasma concentrations of LDL-c and elevated the HDL-c levels compared with the negative control group. In addition, significant reduction was reported for hepatic triglyceride and cholesterol. Bodas *et al.* (2011) reported a markedly lowered plasma triglyceride and cholesterol following intake of naringin at a dosage of 1.5 g/kg for 7 weeks. Moreover, among a group of high fat diet-induced diabetic rats subjected to consumption of 50 and 100 mg/kg of naringin for 28 days, significant decline in the total cholesterol, triglyceride, and LDL levels was observed and plasma concentration of HDL-c was significantly elevated (Sharma *et al.*, 2011).

Pu *et al.* (2012) reported a significant decrease in the total cholesterol and LDL concentration following the consumption of 0.2 g/kg naringin for 10 weeks. However, concentration of HDL-c was evidently elevated and the total triglyceride levels remained unchanged. Naringin supplementation (50 mg/kg) for 30 days decreased the concentrations of free fatty acids, total cholesterol, triglycerides, LDL and VLDL, while HDL-c level in the serum was significantly elevated compared to the controls. In addition, hepatic activity of HMG-CoA reductase was reduced strikingly (Ahmed *et al.*, 2012). Senaphan *et al.* (2015) reported a marked decrease in the plasma concentration of total cholesterol, LDL-c and triglycerides. Experimental animals fed with various concentartions of ferulic acid, quercetin, *p*-coumaric and naringenin showed increased concentration of HDL-c and reduced levels of total cholesterol, LDL-c, triglycerides (Shruti and Vijay, 2011; Shuang *et al.* 2016).

Various mechanisms have been hypothesized through which grapefruit polyphenols exert the beneficial roles on blood lipids. It was suggested that the decrease in the LDL-C plasma concentration as reported in this study and by many other researchers could have been because of the decline in the VLDL and purification process of the liver to get rid of the LDL precursors (Knekt *et al.,* 2002). Improved concentrations of HDL reported might also be as a result of the prevention of the rho-signaling pathways by stimulating Peroxisome Proliferator Activated Receptor-α (a transcription factor that controls lipid metabolism) and reduction in the plasma protein that is involved in the interchange of cholesteryl esters to triglycerides and vice versa (Raposo *et al.,* 2014).

Reduced activity of HMG-CoA reductase, an enzyme that controls the rate of hepatic cholesterol synthesis is another suggested mechanism through which grapefruit phenols exert its positive actions on dyslipidemia which is similar to the effect of statins (blood lipid lowering agent drugs) (Ali *et al.,* 2017). Similarly, naringin was found to significantly reduced the activity of Acyl-CoA:cholesterol acyl Transferase (ACAT). Acyl-CoA:cholesterol acyltransferase is an enzyme that promotes the synthesis of cholesteryl esters from cholesterol and long-chain fatty-acyl-coenzyme A. It functions in the control of cholesterol balance within the cell (Chang *et al.,* 2009).

5.1.9 Effect of grapefruit treatment on blood pressure

High blood pressure also referred to as hypertension can be described as the persistent elevation of blood pressure above normal. Hypertension is central to MetSyn and an important risk factor for CVDs (Abdulla *et al.,* 2011). Consumption of plant diets particularly fruits and vegetable have been established to contribute significantly to the prevention and management of various non-communicable diseases based on the profitable roles of the phytoconstituents of plant (Ejim *et al.,* 2011; Visnagri *et al.,* 2015).

In this study, groups of rats on high-carbohydrate high-fat diet demonstrated significant increase in the systolic blood pressure. Supplementations of various doses of edible and non-edible parts of white and pink grapefruits significantly declined the systolic blood pressure of the rats compared to the negative control. Studies have demonstrated that naringin, a major flavonoid in grapefruit possess antihypertensive properties similar to what is obtainable with drugs. This may partly explain the antihypertensive properties

exhibited by the edible and non-edible parts of the white and pink grapefruits. Visnagri *et al.* (2015) reported noticeable reduction in the systolic and diastolic blood pressure of high fat diet induced rats following the daily intake of 40mg/kg dosage of naringin for 8 weeks. Additionally, the mean arterial blood pressure was also lowered compared to the controls.

Ikemura *et al.* (2012) treated diet induced obese rats with naringin at 250, 500 and 1000 mg/kg daily for 4 weeks, significant decrease in systolic blood pressure and enhanced endothelia function were observed in the genetically modified rats that are prone to hypertension. Alam *et al.* (2013) observed marked decline in the systolic blood pressure and elevated metabolites of nitric oxide in the urine of rats placed on high fat diet following the intake of 100 mg/kg naringin for 8weeks in stroke-prone hypertensive rats. Fallahi *et al.* (2012) documented similar report following the administration of 10 mg/kg dosage of naringenin daily in streptozotocin-induced diabetic rats. Fallahi and colleagues reported that naringenin have vasodilatation effect by increasing the endothelium-dependent relaxation in naringenin treated rats.

Futhermore, in humans, supplementation of 677 mg/L naringin and narirutin in 0.5 liter of grapefruit juice daily was shown to have reducing effect on the diastolic blood pressure of patients with stage I hypertension (Reshef *et al.*,2005). The hypertensive properties of the grapefruit flavonoids was suggested to have resulted from its ability to reduce the synthesis of nitric oxide metabolites which perform an essential function in regulating blood pressure. The amount of nitric oxide available have been shown by Thent and Das (2015) to be proportionate to dilatation of blood vessels. Hence, reduction in the concentrations of nitric oxide will result in relaxation of smooth muscles that will eventually lead to reduced blood pressure (Thent and Das, 2015). Moreover, Visnagri *et al.* (2015) also suggested that the antihypertensive potential of naringin was due to its strong radical scavenging ability by elevating superoxide dismutase activity and upregulating its renal expression.

5.1.10 Effect of grapefruit treatment on inflammatory markers

Pro-inflammatory cytokines and adipokines perform vital functions in the onset of metabolic syndrome. Adipokines are secreted in the adipose tissue and can function either as a pro-inflammatory or anti-inflammatory marker. They control metabolic and inflammatory activities in the body (Kang et al., 2016). Imbalance between the

activities of pro- and anti-inflammatory adipokines occurs as a result of excessive body fat accumulation and enlargement of the adipose tissue and this favour the formation of pro-inflammatory factors. As they increase in concentration, systemic inflammation and insulin resistance is stimulated particularly in the obese patients resulting into the onset of metabolic diseases such as MetSyn (Ouchi *et al.*, 2011). Examples of pro inflammatory markers includes IL-6 and TNF-α while adiponectin is regarded as anti-inflammatory markers. Apart from the pro- and anti-inflammatory activities of adipokines, they also function in the regulation of insulin resistance and as well participates in the metabolism of fats and carbohydrates.

In this study, effect of varying doses of edible and non-edible parts of white and pink varieties of grapefruits were tested on the pro- and anti-inflammatory markers such as TNF-α, IL-6, adiponectin and CRP. High carbohydrate high fat diet significantly elevated the levels of TNF-α, IL-6 and CRP in the plasma but lowered adiponectin plasma level. Following the supplementation of various doses of edible and non-edible parts of white and pink varieties, significant reduction was observed in the levels of TNF-α, IL-6 and CRP in the plasma compared to the negative control. Moreover, adiponectin was markedly elevated across all the treated groups compared to the negative control group. The non-edible part of the pink variety was found to be most efficacious in alleviating the pro-inflammatory adipokines. Ability to significantly reduce the pro-inflammatory markers and increase the anti-inflammatory makers proves that grapefruit has anti-inflammatory properties. This observed beneficial effect of grapefruit could be linked to the existence of phenolics and flavonoids such as naringin, narirutin, hesperidin, naringenin etc that are present in considerable amount in grapefruits, coupled with the free radical scavenging ability of those phenolics and flavoniods. Phenolics and flavonoids are potent anti-inflammatory substances and studies have demonstrated that naringin, naringenin and other citrus flavonoids are effective anti-inflammatory agents (García-Lafuente *et al.*, 2009).

Naringin and hesperidine were demonstrated to markedly lower the inflammatory markers such as the TNF-α, IL-6, leptin concentration in different models of inflammation (Jain *et al.*, 2011; Mahmod *et al.* 2012; Ke *et al.* (2017).
The proposed mechanism for the anti-inflammatory properties of grapefruit phenolics is by preventing the liberation of TNF-α-induced free fatty acid and hinder the initiation

of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a protein complex that controls DNA transcription and synthesis of cytokine (Yosida *et al.*, 2010; 2013).

5.1.11 Effect of grapefruit treatment on oxidative stress markers

Free radicals also called reactive oxygen species are metabolites that are released in the cause of normal metabolism in the body and play vital roles in the onset of various metabolic diseases by inducing oxidative stress (Fuhrman and Aviram, 2001). Oxidative stress occurs when there is increased generation of reactive oxygen species and the body's ability to neutralize their injurious consequences is reduced. When there is oxidative stress, lipid peroxidation occurs due to elevated levels of reactive oxygen species as indicated by excessive secretion of malondialdehyde (MDA), a strong biomarker of oxidative stress (Fiorentino *et al.*, 2013). The body is able to overpower the effects of free radicals and the resulting oxidative stress with the aid of antioxidants (Niki, 2008). Antioxidants contribute significantly to the capability of the cells to fight the generated free radicals by donating hydrogen atom to break the chain reaction, thus, boosting the ability of the cells against reactive oxygen species-induced injury (Francisqueti *et al.*, 2017).

In this study, intake of high-carbohydrate high-fat diet significantly elevated the concentrations of plasma MDA in the experimental rats. However, supplementation of various doses of edible and non-edible parts of white and pink grapefruits significantly reduced the plasma level of MDA across all the treated groups compared to the controls. The non-edible part of pink variety was found to be most potent, as the concentration of plasma MDA values obtained in the group of animals treated with non-edible part of pink variety of grapefruit did not differ from the plasma MDA level in the negative controls statistically. The ability of the administered grapefruit to significantly reduce the level of plasma MDA showed that it possess the capability to fight the generated free radicals, hence it is potent in reducing lipid peroxidation and oxidative stress. This observed effect could be attributed to the antioxidant capacity of grapefruit flavonoids and phenolics as reported in this study. Flavonoids particularly from citrus fruits and its products are known to have high antioxidant capacity by protecting the cells from the injurious activities of the free radicals (Fuhrman *et al.*, 2001; Guimarães *et al.*, 2010).

Results obtained from this study is similar to the observation of other researchers. Jagetia and Lalnuntluangi (2016) documented significant increase in the activity of enzymatic antioxidants (glutathione, superoxide dismutase, catalase and glutathione-s-transferase) following naringin supplementation at dosage of 2 mg/kg. Also, significant decrease was observed for lipid peroxidation in the doxorubicin induced oxidative stress rats. In addition, supplementation of 0.2 g/kg naringin for 10 weeks significantly enhanced the activities of superoxide dismutase, glutathione peroxidase, catalase and total antioxidant capacity while significant decrease was observed for MDA in high-fat diet induced mice (Pu *et al.,* 2012). Akondi *et al.* (2011) showed that 10 mg/kg of naringin treatment for 46 days significantly lowered the concentrations of MDA and elevated levels of superoxide dismutase and catalase in diet induced diabetic rats. Similarly, Murunga *et al.* (2016) reported that, inclusion of 50 mg/kg naringin for 42 days in the diets of diabetic rats significantly improved glutathione activity in the serum and the liver, and decrease the concentration of MDA. Adebiyi *et al.* (2016) documented a marked improvement in the activities of superoxide dismutase by 65.0% in the diabetic rats treated with 50 mg/kg naringin for 56 days.

5.1.12 Protective effects of grapefruit treatment on the functional markers and structure of the liver

Consumption of fructose is one of the main origin of oxidative stress and non-alcoholic fatty liver disease (Lee *et al.,* 2010). It is recognised to fuel retention of fat in the liver by elevating fat production and inhibiting its oxidation (Ackerman *et al.,* 2005). This process is known to occur even in the absence of intake of energy (Roncal-Jimenez *et al.,* 2011). Studies carried out among humans have shown significant association between overconsumption of fructose and onset of non-alcoholic liver disease (Abdelmalek *et al.* 2012).

In this study, feeding of the experimental rats with the high-carbohydrate high-fat diet significantly increased the wet weight of the liver and increased the plasma activities of aspartate transaminase, lactate dehydrogenase, alanine transaminase, alkaline phosphatase and alkaline phosphatase compared to the negative control. This is an indication that the diet induced liver damage. This is evident by the histopathology of the liver structure that showed features of inflammation and abnormal retention of lipids in the cell. Supplementation of various doses of edible and non-edible parts of pink and

white grapefruits significantly reduced the wet weight of the liver and the elevated plasma activities of aspartate transaminase, lactate dehydrogenase, alanine transaminase and alkaline phosphatase close to NC status. Furthermore, hepatic structure of the grapefruit treated groups showed improved architecture of the liver. This observed result is an indication that the edible and non-edible parts of white and pink grapefruit possess hepato-protective properties that could have been conferred by the flavonoids and phenolic components of grapefruit as well as their anti-oxidant properties.

Several other researchers have documented the hepato-protective activity of grapefruits and its phyto-constituents such as naringin and naringenin (Pari et al., 2011; Renugadevi et al., 2010; Kannappan *et al*., 2010; (Pu *et al.*, 2010; Alam *et al.* 2013).

5.1.13 Protective effects of grapefruits on the functional markers and structure of the kidney

Fructose intake is recognized as one of the causes of hypertension and kidney injury. The method through which fructose induce high blood pressure is multifaceted. One of the ways suggested by which fructose induce high blood pressure is by elevating intestinal absorption of sodium that eventually resulted into the activation of the sympathetic nervous system and prevention of systemic endothelial function. Another hypothesized mechanism is by elevation of uric acid concentration in the cells and blood. Sanchez-Lozada *et al.* (2007) reported a significant reduction in the blood pressure of hypetersive rats induced with fructose when the uric acid concentration was markedly reduced. Sanchez-Lozada *et al.* suggested that the increased concentration of uric acid might lead to afferent arteriolopathy and subsequently glomerular hypertension. Cirillo *et al.* (2009) also proposed that fructose infliteration into the urine can be trapped in the proximal tubule resulting into increased cioncentration of the intracellular uric acid coupled with inflammation and oxidative stress.

Generally, fruits are natural sources of fructose, antioxidants, polyphenols, fiber as well as vitamins and minerals. Research questions have been raised as to whether the naturally occurring fructose in fruits can induce high blood pressure and the associated complications. Reungjui *et al.* (2007) hypothesized that the antioxidants, polyphenols as well as the soluble and insoluble fiber constituents of fruits may douse the effects of fructose. This hypothesis was confirmed in a study conducted by Forman *et al.* (2009)

among individuals whose main source of fructose is fruits. Forman and colleagues documented that, there was no correlation between consumption of fructose consumed from fruit sources and blood pressure. Also, Jalal *et al.* (2010) observed a strong correlation between fructose consumption and blood pressure when the amount of fructose obtained from fruits was left out of the analysis.

In this study, consumption of high carbohydrate high fat diet significantly elevated the plasma concentration of uric acid, creatinine and sodium in the experimental rats. Fructose being the main type of carbohydrate in the formulated diet might be the mediator of the observed elevated systolic blood pressure through the increased concentration of sodium and uric acid as shown in the results. This could have been the cause for the observed glomerular congestion, inflammation and kidney injury as shown by the histopathology of the kidney.

Nevertheless, administration of various doses of edible and non-edible parts of white and pink grapefruits significantly reduced the elevated concentrations of plasma uric acid, creatinine and sodium in all the grapefruit treated groups. This also affirms the hypothesis of Reungjui *et al.* (2007) and Vasdev *et al.* (2002), that the phytoconstituents of fruits ameliorates the kidney injury mediated by excessive consumption of fructose. The edible and non-edible parts of white and pink grapefruits have been shown to possess considerable amount of phenols and flavonoid as well as antioxidant properties as shown in this study. This may partly explain the ability of the edible and non-edible parts of white and pink grapefruits to attenuate the plasma concentrations of uric acid, creatinine and sodium, and as well reversed the damages done to the kidney. This observation is in consonance with the reports of other researchers. Attalla *et al*. (2009) reported significant reduction in the elevated uric acid and creatinine concentrations following naringin supplementation at 300 mg/kg body weight /day orally in glycerol-induced hypertensive rats. Significant improvement was also observed in the kidney tubule injury as shown by histopathology of the liver of the rats. A study conducted by Gelen *et al.* (2018) showed that naringin supplementation significantly reduced the serum concentrations of creatinine and BUN following administration of 100 mg/kg naringin intraperitonially for 14 days in 5-fluorouracil-induced nephrotoxicity in rats. Values obtained in the naringin treated groups were similar to the controls.

5.2 CONCLUSION

In conclusion, it is evident from the study that the non-edible parts of white and pink grapefruits have higher content of phenols and flavonoids compared to the edible parts.

They also portend higher antioxidant activities than the edible parts.

Of the two varieties, the pink variety had higher amount of phenols and flavonoids than the white variety and also displayed higher antioxidant activities.

Consumption of high-carbohydrate high-fat diet induced the features of metabolic syndrome.

Supplementation with edible and non-edible parts of pink and white grapefruit is effective in managing cardio-metabolic parameters, especially the lipid profile, fasting plasma insulin, blood pressure and insulin resistance.

The edible and non-edible parts of pink and white grapefruit ameliorated oxidative stress and inflammatory markers induced by the high carbohydrate high-fat feeding and showed anti-oxidant and anti-inflammatory properties.

Also, supplementation with edible and non-edible parts of pink and white grapefruit have protective effects on the liver and kidney functional markers and as well as the structure.

In addition, the non-edible parts of the pink and white grapefruits showed higher efficacy than the edible parts.

Of the two varieties of grapefruits, pink variety showed higher efficacy in ameliorating metabolic syndrome biomarkers compared to the white variety. We propose that grapefruits decreased renal and hepatic injury through the antioxidant and anti-inflammatory effects of polyphenols, mainly naringin that is abundant in the edible and non-edible parts of white and pink grapefruits.

5.3 RECOMMENDATIONS

Based on the finding from this study, the following are recommended

- Based on antioxidant properties exhibited by the non-edible parts of pink and white grapefruits that are usually discarded as waste, they could be useful in the food processing industries serving as natural sources of antioxidants for use in food preservation and thereby reducing the amount of solid waste generated from fruit peels.

- The peels that are usually discarded as waste could be processed futher in form of tea bags to enable the population benefit from the richness of the non-edible parts.

- Consumption of grapefruits is generaly recommended for induvials who are overweight and obese, and those showing the features of metabolic syndrome. Moreover, individuals who do not exhibit any of the listed features above could aslo freely consume grapefruits so as to benefit from the health promoting properties of grapefruit for prevention purposes.

- In addition, reports on health benefits of grapefruits have been obtained largely from animal research, hence human clinical studies are needed to corroborate the health benefits of grapefruits on humans.

5.4 Contribution to Knowledge

- Edible and non-edible parts of white and and pink varieties of grapefruit contain appreciable amount of phenolic acid, flavonoids, saponins, alkaloids, tannins and carotenoids.
- The phenolic composition of edible and non-edible parts of white and pink varieties of grapefruit include gallic acid, chlorogenic acid, ferulic acid, caffeic acid and *p*-coumaric acid.
- The most abundant phenolc acid in the edible and non-edible parts of white and pink varieties of grapefruit is gallic acid

- The flavanoid composition of edible and non-edible parts of white and pink varieties of grapefruit include naringin, narirutin, naringenin, hesperidine, eriocitrin and quercetin in considerable amount
- The most abundant flavonoid in edible and non-edible parts of white and pink varieties of grapefruit is naringin
- The non-edible parts of white and pink grapefruits have higher concentrations of phytochemicals than the edible parts
- The pink variety of grapefruit contained higher phytochemicals than the white variety
- Consumption of grapefruit promote weight loss, reduces fat accumulation and abdominal obesity
- Grapefruit consumption lowers blood pressure, plasma glucose and reduces insulin resistance
- Consumption of grapefruits ameliorates dyslipidemia by lowering LDL-c, total cholesterol, total triglycerides and improving HDL-c
- Grapefruits possesses antioxidant properties by reducing the oxidative stress markers
- Grapefruit exhibits anti-inflammatory properties by reducing the pro-inflammatory factors (CRP, IL-6, TNF-α) and enhancing anti-inflammatory markers (Adiponectin)
- Grapefruits displays hepatoprotective properties by reducing the activities of the liver functional markers and reverses the hepatic ijury induced by inflammation
- Consumption of grapefruit showed protective effects on the kidney by reducing the plasma uric acid, sodium and creatinine.

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APPENDIX 1

Appendix 1: Explanatory Summary

Metabolic Syndrome (MetSyn) is a public health challenge and predictor of type-2 diabetes and cardiovascular diseases. Shortcomings in current pharmacological and non-pharmacological management of MetSyn demand evaluation of affordable and sustainable therapeutic approach. Evidence suggest that antioxidant properties of polyphenols from citrus fruits may reduce risks of these diseases. This study therefore assessed the efficacy of edible and non-edible parts of two varieties of grapefruits on metabolic syndrome biomarkers in male Wistar rats.

The experimental study involved two phases. Phase one: edible (pulps) and non-edible (peels, seeds) parts of freshly harvested white and pink grapefruits were manually separated, freeze-dried and pulverised. Edible white (EW), non-edible white (NEW), edible pink (EP) and non-edible pink (NEP) were analysed for phytochemicals (total phenols, flavonoids, tannins, alkaloids, saponins) and carotenoids using standard procedures. Antioxidant activities were determined using DPPH and FRAP assays. Phenols (cholorogenic, gallic and caffeic acids) and flavonoids (naringin, narirutin, narigenin) were quantified using HPLC. Phase two involved grouping 80 mature male wistar rats into 10. Group 1(Negative control) received corn starch-rich diet, group 2 (positive control) and groups 3 to 10 received MetS-inducing diet for 12 weeks. Groups 3 to 6 diets were supplemented with 0.8gEW, 0.4gNEW, 0.6gEP and 0.3gNEP grapefruit powder delivering 0.032g polyphenols while groups 7 to 10 had 1.6gEW, 0.8gNEW, 1.2gEP and 0.6gNEP supplying 0.064g polyphenols daily for 8 weeks respectively. Blood pressure (BP) was determined. Blood samples were analysed for lipid profile (total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein), insulin resistance (IR), Tumor necrosis factor-α (TNF-α), Interleukin-6 and Malondialdehyde using standard procedures. Data were analysed using ANOVA at $\alpha_{0.05.}$

Highest total phenols, flavonoids, tannin, saponins and carotenoids were obtained in NEP variety. Highest total alkaloids was obtained in EW variety. Antioxidant activities was highest in NEP and lowest in EW variety. Chlorogenic acid was highest in NEP, gallic acid and caffeic acid were highest in EP variety. Naringin, narirutin and naringenin were highest in NEP variety. Significant reduction was observed for BP, lipid profile, IR, TNF-α, Interleukin-6 and Malondialdehyde in all grapefruit treated groups. Highest and lowest reduction in BP, total cholesterol, triglyceride, low-density

lipoprotein were observed in 0.6gNEP and 0.8gEW respectively. High-density lipoprotein was increased by 33% and 11% in 0.6gNEP and 0.8gEW respectively. Highest and lowest reduction in IR, TNF-α, Interleukin-6, Malondialdehyde were observed in 0.6gNEP and 0.8gEW respectively. Significant difference was observed for BP, lipid profile, IR, TNF-α, Interleukin-6 and Malondialdehyde between high and low dosages administered.

Edible and non-edible parts of white and pink grapefruit attenuated biomarkers of metabolic syndrome, non-edible of pink variety was most efficacious. Grapefruit could serve as potential functional food in prevention and management of metabolic syndrome.

Keywords: Oxidative stress markers, Inflammatory markers, Antioxidants, Grapefruits

Word count: 438

APPENDIX 2

Appendix 2: Picture showing the edible and non edible parts of grapefruits

Picture showing the edible parts of grapefruits

Picture showing the non-edible parts of grapefruits

APPENDIX 3

Appendix 3: Physical properties and yield of fresh weight of the two varieties of grapefruits

	Titratable acidity (TA) g/l	**TSS (ºBrix)**	**pH (at room temp)**	**% juice**	**Yield (g/kg)**
Pink variety juice	0.95±0.01	13.80±0.19	3.61±0.07	57.83	-
White variety juice	0.92±0.04	12.10±0.19	3.61±0.07	46.68	-
Edible white	-	-	-	-	126.76
Edible pink	-	-	-	-	99.19
Non- edible pink	-	-	-	-	140.05
Non- edible white	-	-	-	-	124.14

APPENDIX 4

Appendix 4: Diagnostic criteria for the experimental rats after 12 weeks of feeding

Parameters	Control (n=6)	Experimental group (n=36)	t-value	P value
Fasting plasma glucose (mmol/L)	4.64±0.53	7.21±0.50	36.991	0.000
Total triglyceride (mmol/L)	0.44± 0.03	1.41±0.09	12.217	0.000
Systolic blood pressure (mmHg)	121.50± 1.05	147.11±3.31	18.452	0.000
Total cholesterol (mmol/L)	1.45±0.02	1.93±0.07	9.590	0.000
Low Density Lipoprotein (mmol/L)	1.95±0.34	8.93±1.04	9.101	0.000
High Density Lipoprotein (mmol/L)	1.34±0.25	1.22±0.21	15.358	0.000
Abdominal circumference (cm)	14.83±0.26	20.2±0.50	11.281	0.000
Body weight (g)	307.3±8.73	368.6±14.3	9.869	0.000

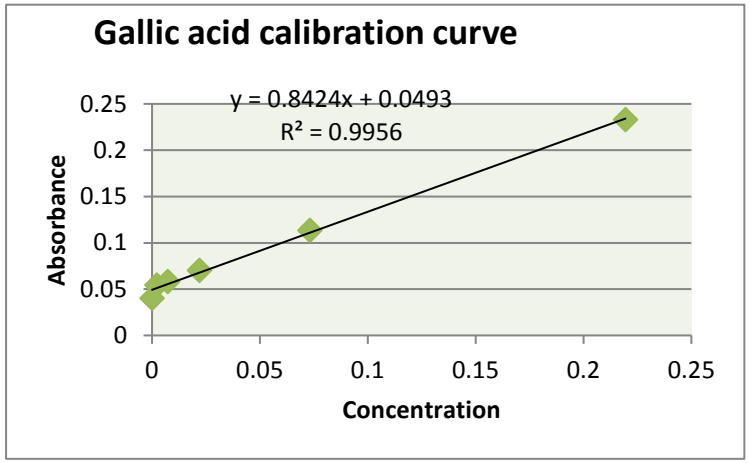
APPENDIX 5

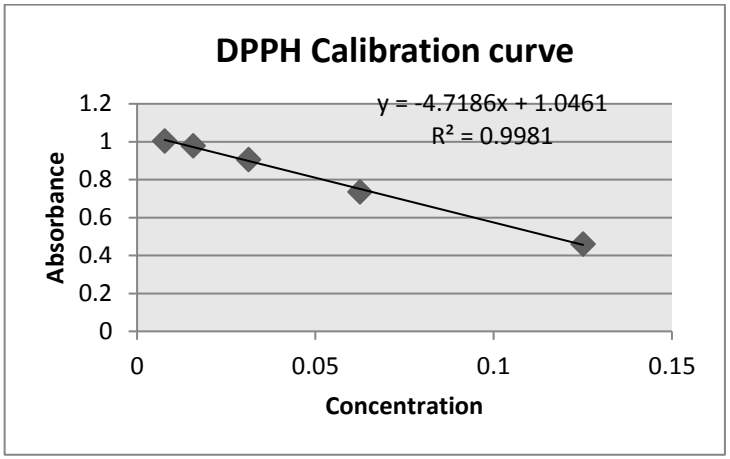
Appendix 5: Efficacy of the diet to induce cardio-metabolic, oxidative stress and inflammatory parameters in the experimental rats after 12 weeks of feeding

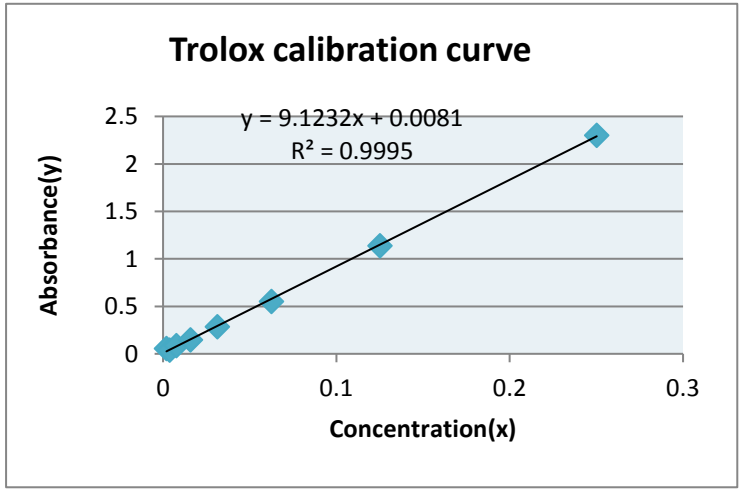
Parameter	Control group (n=14)	Experimental group (n=90)	t-value	p-value
Insulin (µg/L)	2.03 ± 0.06	4.23 ± 0.03	34.87	0.000
HOMA-IR	10.36 ± 0.03	25.14 ± 0.12	22.87	0.000
MDA(µmol/L)	20.79 ± 0.17	37.32 ± 3.54	18.53	0.000
CRP(µmol/L)	52.86 ± 0.32	92.57 ± 1.20	63.56	0.000
Adiponectin (ng/mL)	72.31 ±2.39	32.63 ± 1.32	72.18	0.000
TNF-α (pg/mL)	22.38 ± 0.34	52.74 ±0.26	73.26	0.000
IL-6 (pg/mL)	22.86 ± 0.46	55.31 ±0.15	72.85	0.000

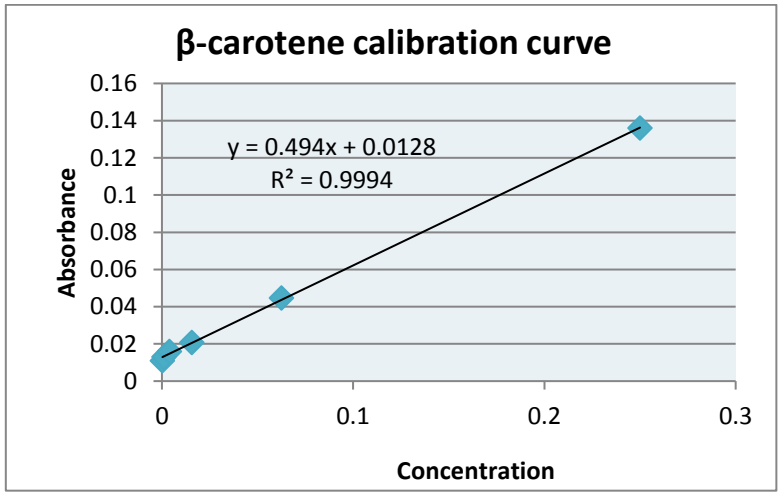
APPENDIX 6

Appendix 6: Calibration curve of standards

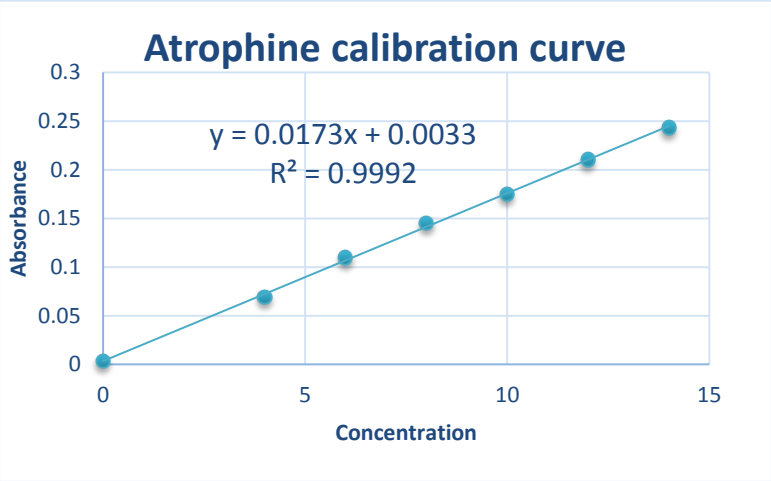


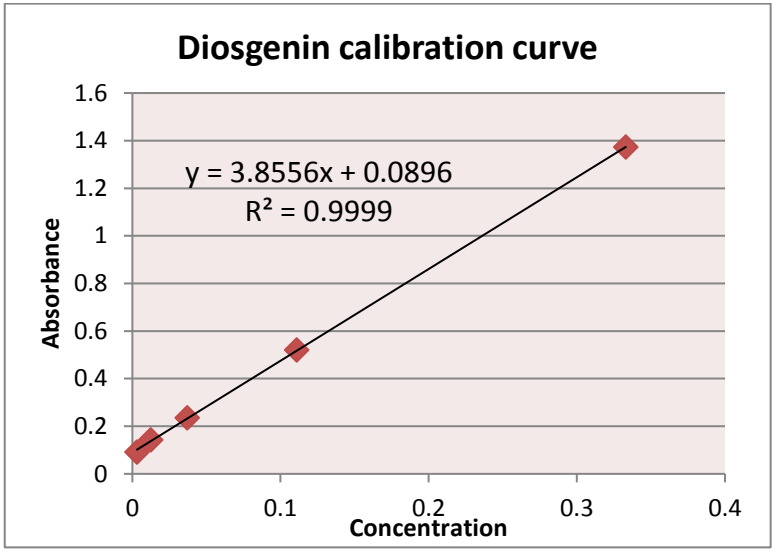


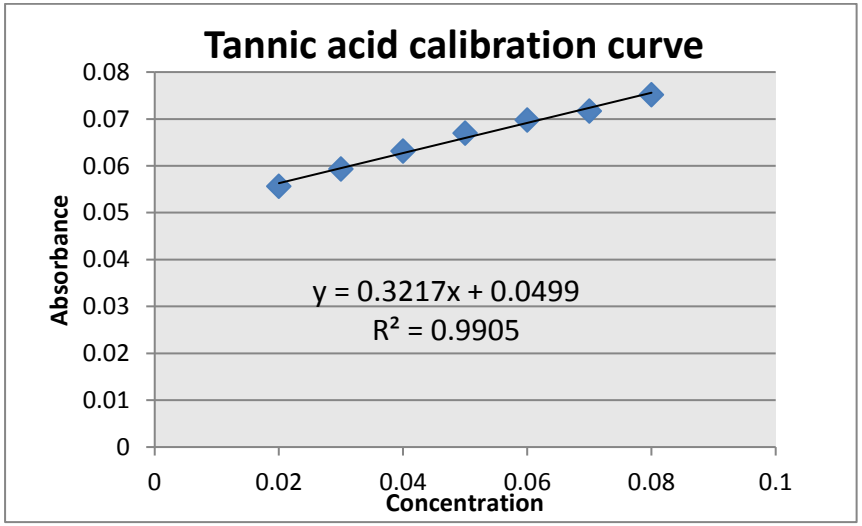




Appendix 6 continued: Callibration curve of standards

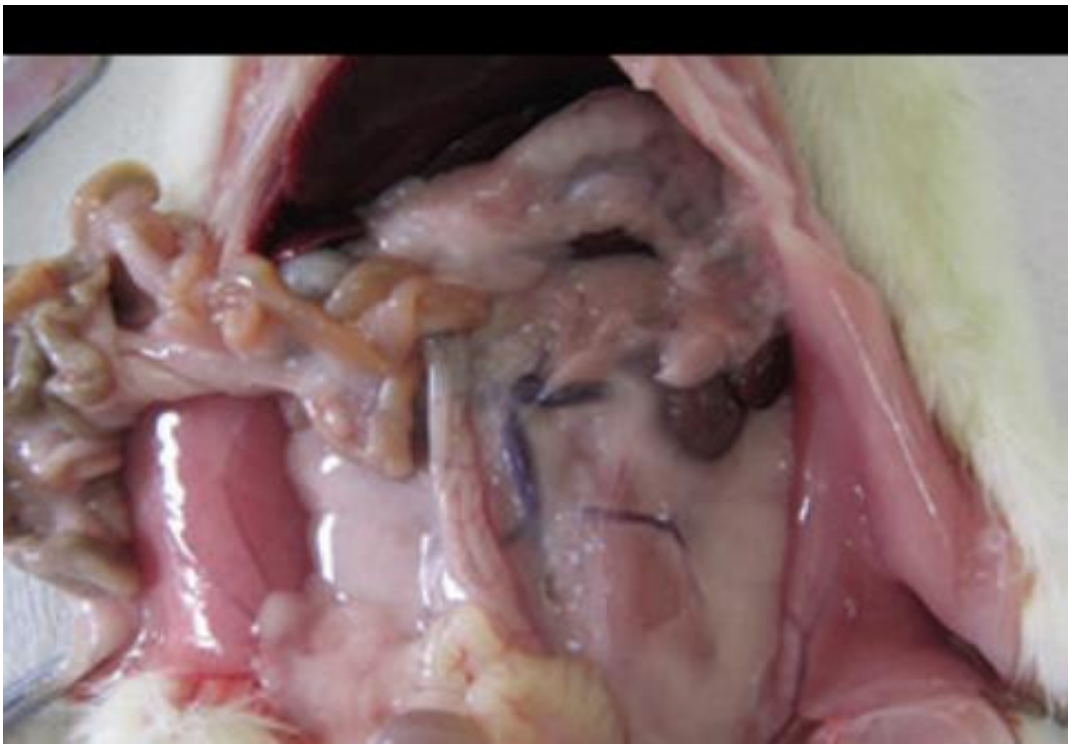




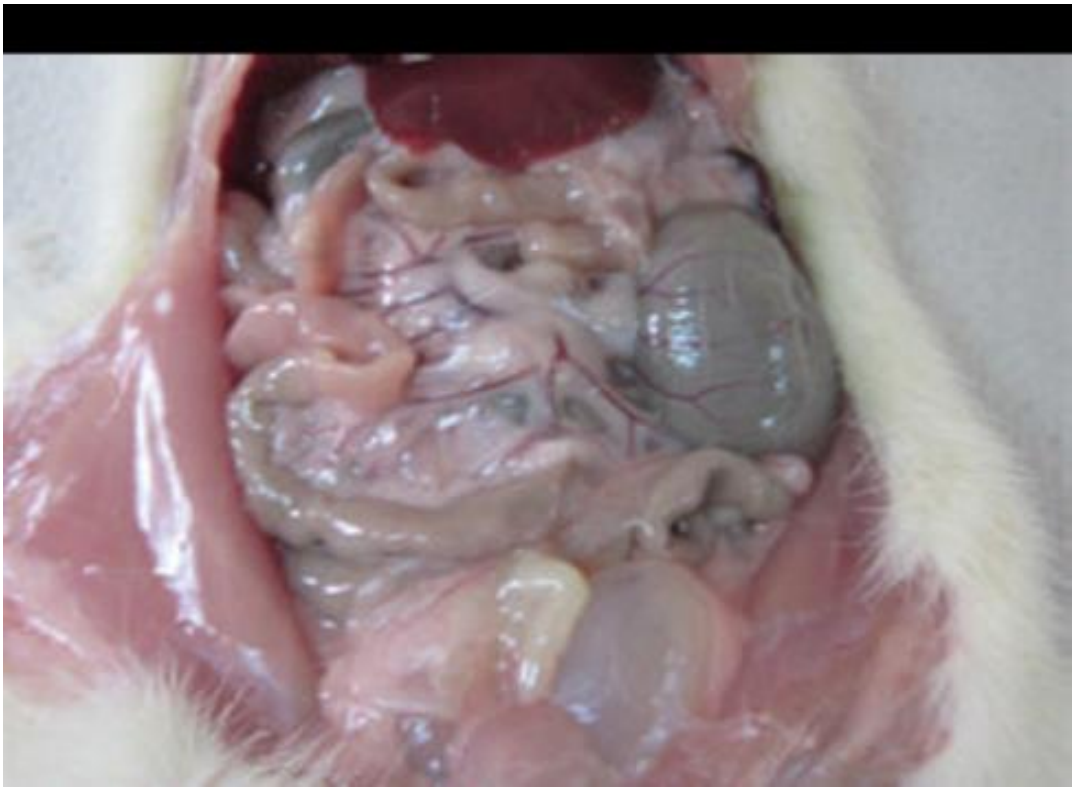


APPENDIX 7

Appendix 7: Fat deposition in the experimental rats

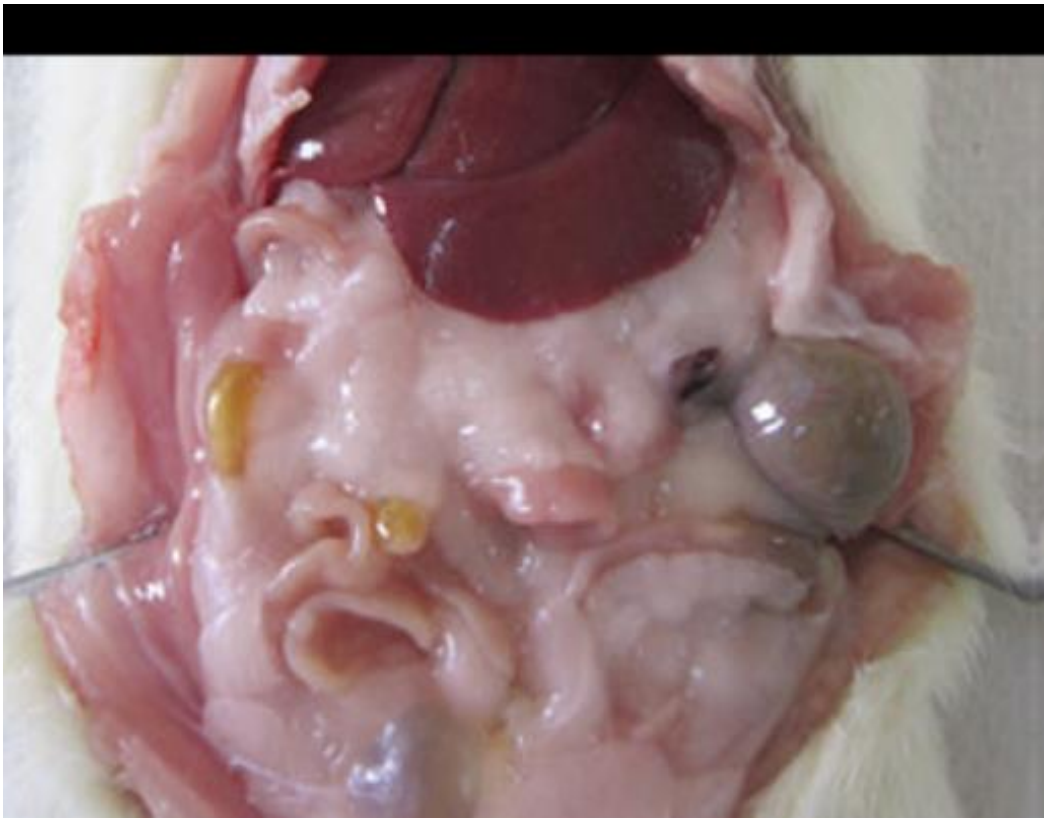


a) Mesentery, epididymal, and retroperitoneal for positive control group at 12 weeks

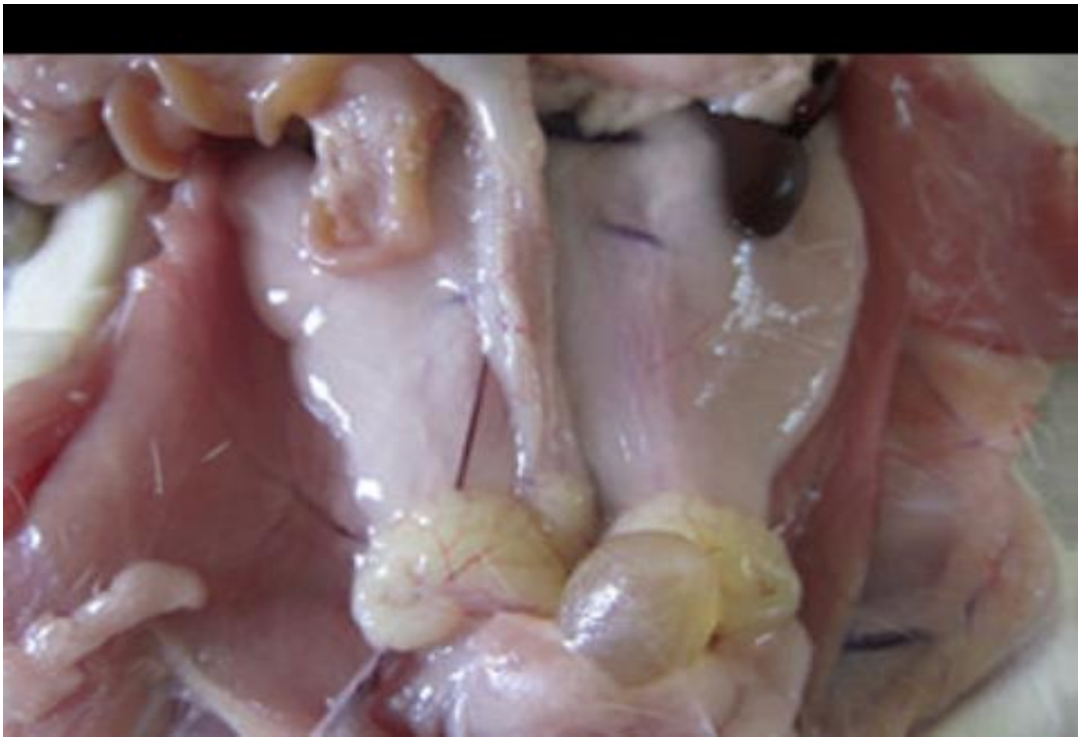


b)Mesentery, epididymal, and retroperitoneal fat deposit for negative control group at 12 weeks

Appendix 7 continued: Fat deposition in the experimental rats



(c) Mesentery, epididymal, and retroperitoneal fat deposit in the positive control group at 20 weeks



(d) Deposition of abdominal adipose tissue in the positive control group at 20 weeks