# PROTECTIVE EFFECTS OF *CORCHORUS OLITORIUS* LEAF EXTRACT AND FRACTIONS AGAINST ISOPROTERENOL-INDUCED ACUTE MYOCARDIAL INFARCTION IN WISTAR RATS.


**BY**

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# ABSTRACT

Acute Myocardial Infarction (AMI) is a leading cause of death associated with Heart Failure (HF) worldwide. Oxidative stress, inflammation and apoptosis are important underlying pathogenesis of AMI, which are not target of currently available drugs. *Corchorus olitoriusleaf* (COL) is useful in ethnomedicine to treat HF. However, there is paucity of scientific information on its effects on AMI. Therefore, the protective effects of COL extract and fractions against isoproterenol-induced AMI in Wistar rats were evaluated.

*Corchorus olitorius leaf* (COL) was obtained from Moniya market, Ibadan and authenticated at Forestry Research Institute of Nigeria (FHI; 110410). Air-dried COL was extracted with 70% ethanol (COE), concentrated and partitioned with N-hexane (COhex), dichloromethane (CODCM), ethyl acetate (COEtOAc) and ethanol (COEtOH) resulting in four fractions.Isoproterenol (100 mg/kg, i.p) was used to induce AMI in rats. Thirty-five animals (n=7) were pre-treated orally for nineteen days with; distilled water (1.0 mL/kg/day, non-AMI control), distilled water (1.0 mL/kg/day, AMI control), COE (250 and 500 mg/kg/day) or enalapril (10 mg/kg/day) before induction of AMI on days 20 and 21. Another 11 groups of rats (n=6) were pre-treated for twelve days with the four fractions; COhex, CODCM, COEtOAc and COEtOH (50 and 100 mg/kg/day), distilled water and enalapril before induction of AMI on days 13 and 14. Blood volume (BV) and flow (BF) were measured using tail cuff with QRS- and p-interval determined electrocardiographically. Thereafter, serum biomarkers of inflammation: advance oxidised protein product (AOPP), myeloperoxidase (MPO), C-reactive protein (CRP), creatine kinase-MB (CKMB), lactate dehydrogenase (LDH), nitrite and cardiac markers of oxidative stress (malondialdehyde, reduced glutathione and non-protein thiol (NPT) were determined using standard techniques. Nuclear Factor-$_k$B (p65NFKappaB), Bcl-2 protein, Bax protein and p53 expression were determined using immunohistochemistry. Constituents of CODCM were identified using GC-MS. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$

The AMI increased QRS interval (21.0±1.0m/s vs 13.0±0.5m/s), reduced p-interval (12.0±0.5m/s vs 21.0±0.5m/s), BF (1.4±0.3cm/s vs 32.0±0.5cm/s) and BV (25.0±1.0m/s vs 107.0±2.0m/s) compared to non-AMI control. The COE (250 mg/kg and 500 mg/kg) and enalapril reduced the interval of QRS (12.0±0.5m/s, 15.0±0.3m/s, 15.4±0.4m/s), increased

p-interval (19.2±1.6, 19.2±0.6, 22.8±0.5m/s), enhanced BF (17.0±1.1cm/s, 8.0±0.4cm/s, 12.0±0.7cm/s) and BV (57.0±3.2m/s, 30.0±3.3m/s, 16.0±1.6m/s) significantly compared to AMI. There was a significant increase in nitrite, GSH, NPT in AMI rats compared to normal control, however, COE reversed it. The COE also prevented the increase in serum level of LDH, AOPP, MPO and malondialdehyde. Compared to AMI rats, COE fractions reduced serum level of CRP, CK-MB and tissue level of p53 and increased the expressions of p65NF-KappaB. The CODCM (50 & 100 mg/kg) and enalapril (10 mg/kg) decreased tissue expression of Bax and increased Bcl-2 expression (Bax; 1.00±0.01, 1.00±0.01, 1.00±0.01 vs 16.00±0.05 and Bcl-2; 8.00±0.01, 4.00±0.01, 5.00±0.01 vs 1.00±0.05). The highest activity was observed in CODCM fraction. Cystamine, 3-methoxyflavone were major components.

The protective effects of *Corchorus olitorius* against acute myocardial infarction in rats may be through modulation of thiol-related antioxidants and p65NFKappaB-dependent antiapoptotic pathways.

**Keywords:** *Corchorus olitorius*, Myocardial infarction, Oxidative stress, NFkappaB

**Word count:**493

# CERTIFICATION

This is to certify that this research work was originally carried out by Mr. Alabi Babatunde Adebola in the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan

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# DEDICATION

My Project is dedicated to the Godhead three in one, the beginning and the end, your spirit is the source of my life and inspiration, also to my one and only Adesola and Deborah Alabi.

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To my friends and colleagues, Ogunleye Fisayo, Ayobami Dare, Onasebu Damilare Tomiwa, Ben Azu Benneth, Eni Ebi Ayaebi God bless you all.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Myocardial infarction is a main cause of heart attack occurring due to diminished blood flow through coronary artery which results from coronary obstruction. It isthe most important cause of death associated with heart failure worldwide (Ramon *et al*., 2014) and this ischemic heart disease was responsible for about 7.3 million deaths all over the world in 2008. Review evidence has shown that the prevalence of acute myocardial infarction is greater in wealthier countries than developing countries (Julian *et al*., 2013) but gradually becoming prevalent in Nigeria and responsible for about 150,000 death in the country as at 2017 (Adewole, 2017). Acute myocardial infarction was described to be deadlier than Human Immunodeficiency Virus and cancer except lung cancer (Ogba, 2017). Coronary artery blockade can be either fixed (as a result of arteriosclerotic calcified plaque which can occur alone or arteriosclerosis superimposed with excoriation, aggregation of platelet and thrombosis) (Moise*et al*., 1984) or non-fixed, (which is as a result of spastic constriction of coronary artery alone or spasm superimposed on degrees of static obstruction (Bruschke*et al*., 1989), or both. Whether fixed or static obstruction, heart attack is a non-static process with series of cellular event that constitute injury occurring at different time. Oxidative stress, inflammation, calcium overload, ATP depletion are basic pathways of ischemic insult to myocardial tissue that eventually results into cardiomyocyte death (Hausenloy, 2013). Cardiomyocyte death is due to apoptosis and necrosis with apoptosis occurring within 6 – 8 hours after severe ischemia and secondary necrosis occurring between 12hours to 4 days (Jack *et al*., 1999). Important biomarkers of myocardial injury include cardiac troponin-c, lactate dehydrogenase and creatine kinase MB type. During the last decades, pharmacological intervention in use have shown a significant decline in deathof patients with myocardial infarction (Liew *et al*., 2006). The pharmacological therapies include; thrombolytic drugs, anti-platelet drugs, antithrombin, beta blockers, statins, organic nitrates and angiotensin converting enzyme inhibitor. Despite the availability of the currently used drugs against myocardial infarction, incidence of mortality recorded is still high and the mechanism of actions of these drugs is limited to improvingthe reperfusion of myocardial tissue which eventually cause reperfusion injury and decreasing the activity of the myocardial tissue.Most of these drugs are non-target of important underlying pathogenesis like apoptosis,

oxidative stress and inflammation which are known to be major cause of myocyte death. Current studies on how to improve pharmacological interventioninclude other role of angiotensin II receptor blockers. Angiotensin II receptors which are located on the myocardial tissue has been shown totrigger apoptosis and oxidative stress during myocardial infarction. This further revealedthe antioxidant and anti-apoptotic effect of ACE blockers aside its blood vessel and blood volume (Preload and Afterload effect) effect, (Chopra *et al.*, 1996). Substantial research has been focused on plant phytochemicals as a potential therapeutic target in cardiovascular disease due to theirprotective role against oxidative stress, inflammation, apoptosis and necrosis. In addition,current drugs against myocardial infarction is veryexpensive,thereby limiting the availability of these drugs in developing countries. The protective role of plant phytochemicals against oxidative stress, inflammation, apoptosis and necrosis is basically due to abundant presence of polyphenols especially (Swapner *et al*., 2015). According to Weisburger, (2002),occurrence of cardiovascular disease is inversely proportional to fruit and vegetable consumption. In fact, it has been recommended by WHO thataverage consumption of fruits and vegetable in a day should not be less than 500 g. Experimental studies on the role of polyphenols like; bioflavonoids (lutein, quercetin, lichocalone D,), tannin, curcumin, xanthine have been shown to protect the myocardial tissue against acute myocardial infarction whether through effect against inflammation or production of reactive oxygen species (Lee *et al*., 2002, Xuan *et al*., 2015).

*Corchorus olitorius* plant is from the family of Tiliaceae, with the plant leaves screened phytochemically and shown to possess antioxidants, which include ascorbic acid, β-carotene, α-tocopherol, glutathione containing compounds and polyphenols (Mavengahama*et al*., 2013). Traditionally, extract from the leaf of this plant has been usedto treat gonorrhea, pyrexia and malignant tumor (Ndlovu *et al*., 2008). InWest Africa, the leaves are used for ameliorating megaloblastic anemia.*Corchorus olitorious* leaf extract at varying concentration of 250 and 500 mg/kg revealed a significant hepato-protective effect in hepatotoxic rats (Salawu, 2007). Traditionally, the leaf twigs of this plant was reported to be used against heart trouble (Denton, 1997; Fondio and Grubben, 2004) and Mahbubul, (2013), reported that the administration of olitoriside during heart failure can improve cardiac insufficiency to the extent of replacing strophanthin. Based on these evidence, *Corchorus olitorius* known to possess abundant polyphenols and other

compounds was chosen to investigate its protective and ameliorative effect against acute myocardial infarction.

## 1.1 AIM

The study was designed to evaluate the anti-apoptotic, anti-inflammatory and anti-oxidative effect of *Corchorus olitorious* leaf extract in rats induced with myocardial infarction.

## 1.2 OBJECTIVES.

Specific objectives of the study are as follows:

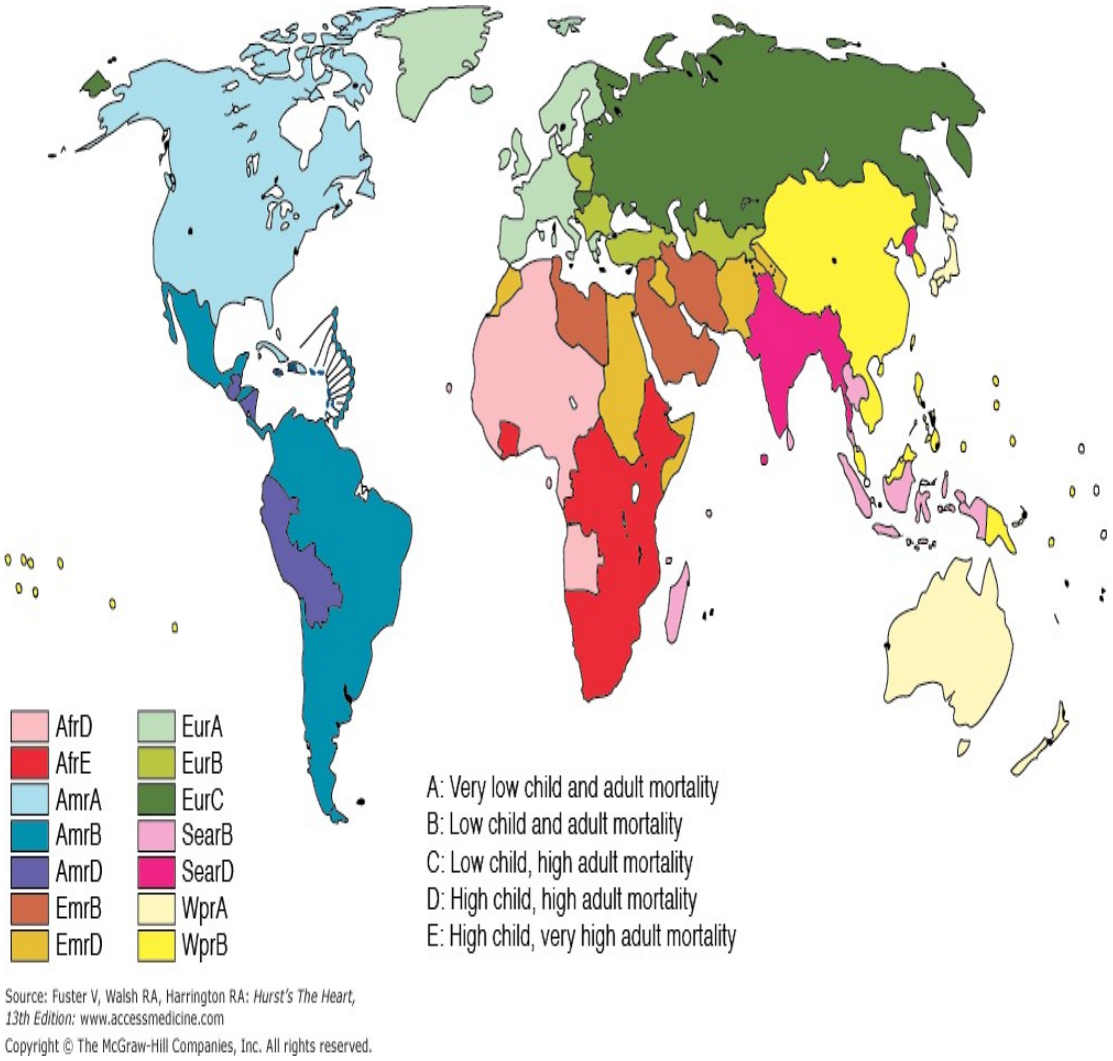
- To assess the effect of *Corchorus olitorious* leaf extract on haemodynamic parameter and electrocardiographic events.
- To estimate the effect of *Corchorus olitorious* extract on the extent of myocardial injury.
- To estimate the effecton nitric oxide derivative
- To measure the level of antioxidants and pro-oxidant bio-markers like MDA, GSH, GPX, GST and AOPP
- To estimate the level of inflammation markers like MPO and AOPP
- To measure the level of LDH using ELISA kit
- To Fractionate the extract using polar and non-polar solvents
- Enzyme linked immunoassay to detect Creatine Kinase-MB level.
- Enzyme linked immunoassay assay to detect inflammation markers like CRP and p65$NF_k$B.
- Immunohistochemistry assay on apoptotic related proteins like Bax, Bcl-2, p65$NF_k$B and p53
- Mass Spectroscopy (GC-MS) to detect active component.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 MYOCARDIAL ISCHEMIA

To obtain constant oxygen and nutrient, the heart requires that the two main coronary arteriesperfuse the myocardium. If any of the twocoronary arteries or its terminal is blocked, that portion of the heart experience poor perfusion, a condition referred to as "cardiac ischemia. If hypoxia lasts longer, the oxygen deprived myocardiumoften dies(heart attack). Histological cell swelling and clumping is not immediate after the onset of myocardial ischemia, but takes a period of about 20 minute or less in lower animal models (Jennings, 1991). Buja, (2013) defines myocardial infarction as a condition associated witha sudden blockage of the main coronary artery, resulting in a complex series of cellular events and pathways causing apoptosis, necrosis and functional impairment of heart. Acute myocardial infarction (AMI) is a maincause of death in humans (Ramon *et al*., 2013) and if previous trends do not change, this ischemic heart disease will be animportant cause of death associated with heart failure in 2030 (Mathers, 2006). In 2008, this disease was responsible for 7.25 million deaths all over the world (12.8%), according to the WHO. Evidence review showed that the prevalence of acute myocardial infarction is more in developed countries than underdeveloped countries (Julian, 2014) however, the incidence of this disease in some West African countries like Nigeria is becoming great. Adewole, (2017) reported that acute myocardial infarction is responsible for about 150000 death in that same year. Acute myocardial infarction or heart attack has been reported to be deadlier than HIV and all forms of cancer except lung cancer (Ogha, 2017).




Source: Fuster V, Walsh RA, Harrington RA: *Hurst's The Heart, 13th Edition:* www.accessmedicine.com



**Figure 2.1: Map showing the incidence of Acute Myocardial Infarction (Jayaraj*et al*., 2018).**

Common symptoms include pain in the chest, arm, back, neck, or jaw.Other symptoms may include difficult breathing, nausea, weak feeling, and cold sweat. Chest pain caused by poor perfusion and resulting in lack of oxygento the heart muscle is called angina.This pain usually spreads to the left arm, but can also spread to the lower jaw, right arm, neck, back, and upper abdomen (Van de *et al*., 2008), which resemble heartburn. Difficult breathing usually occurs when aninjured heart restricts the output of the left ventricle, causing left ventricular hypertrophyand subsequent pulmonary edema(Mallinson, 2010).

Weakness, lightheadedness and palpitations are also associated. These symptoms areassociated with an abundant release of neurotransmitters from the sympathetic part of autonomic nervous system (Little *et al*., 1986), in response to pain and abnormal blood flow resulting from a malfunction of the heart muscle. Without proper ambulatory or emergency attention, severe acute myocardial infarction which progress from stable to unstable angina can lead to loss of consciousness (mainly due to the development of ventricular fibrillation as arrhythmia) (Van de Werf *et al*., 2008). In women, chest pain cannot be an important prognostic tool for coronary ischemia compared to men due to many physiological changes (McSweeney *et al*., 2003). Episodes of backache and jaw pain along with chest pain in Women can be the best prognostic measure.

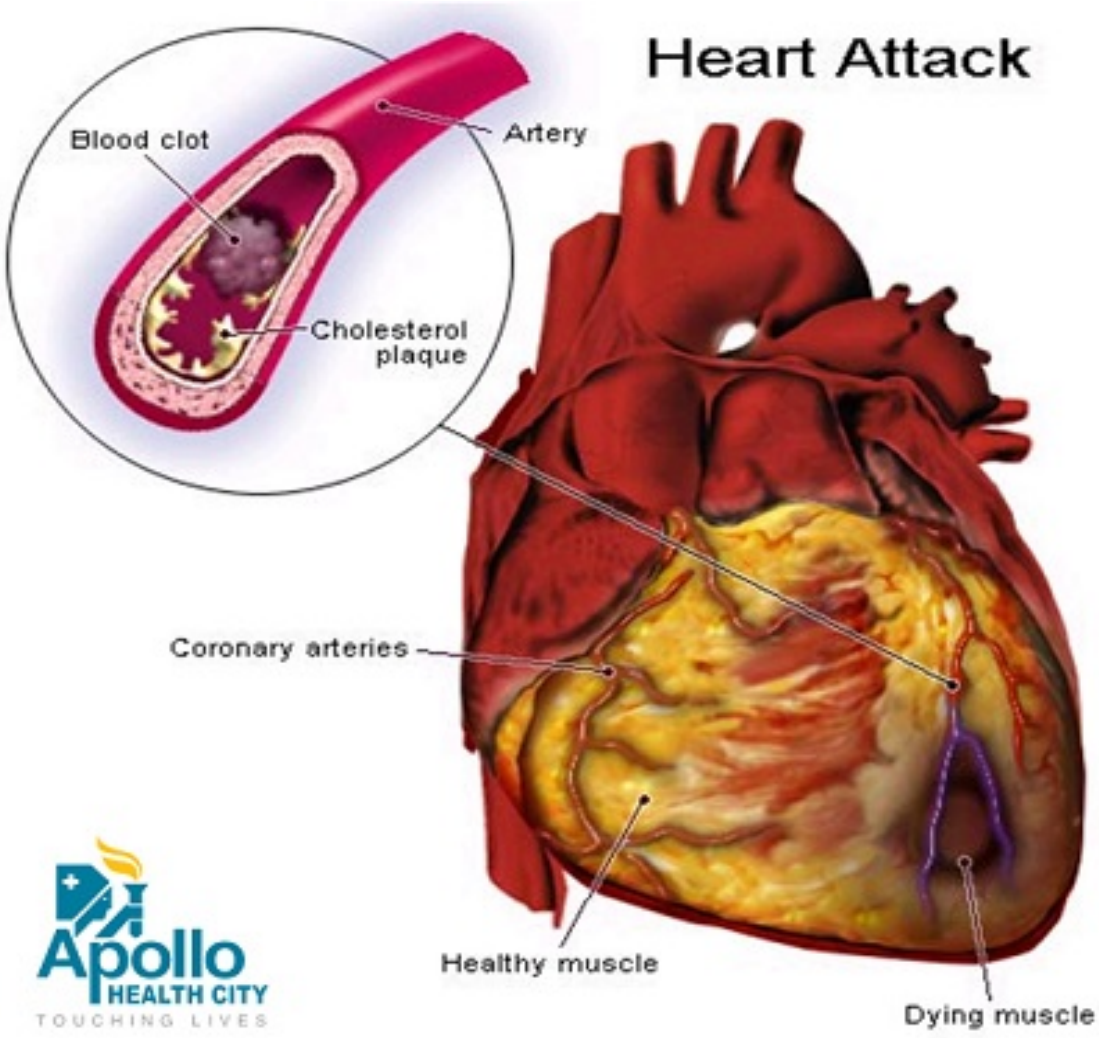



**Figure 2.2: Structure of the pathological coronary vessel, heart and Infarction area, Warne, (2019).**

**Classification of Myocardial Infarction**

Myocardial infarction is usually classified as an increase inST interval MI and non-perception of ST MI (Moe, 2010). Whenever ST interval increases with myocardial infarction, there isa combination of symptoms associated with hypoxia of the heart tissue with elevation of the ST segments on the electrocardiogram, followed by an increase in protein in the blood-associated death of the cardiac muscle andrepresents 25 to 40 percent of cases in hospital (Morawieck*et al*., 2019).

From anatomicalpoint of view, two types of myocardial infarction are transmural and non-transmissible. The main feature of transmural myocardial infarction is that the segment of ischemic death passes through the entire thickness of the myocardium,so that it progressed from the endocardium through the myocardium to the epicardium. In the case of non-transmissible MI, the ischemic necrosis zone does not spread throughout the thickness of myocardial segments. During transmissible MI, the area of ischemic necrosis is restricted to either endocardium or to the endocardium or myocardium. Since the endocardial and subendocardial zones of the myocardial wall are always poorly perfused, they are most susceptible to ischemia conditions (Dotan *et al*., 2011).

## Pathophysiology

Acute myocardial infarction (MI) is usually referred to as regional myocardial necrosis, usually based on endocardium, secondary occlusion of the epicardial artery. In contrast, concentric subendocardial necrosis can be caused by global ischemia and reperfusion in cases of prolonged cardiac arrest during resuscitation(Dotan *et al*., 2011). The areas of myocardial infarction can be subepicardial if the occlusion of small vessels by thromboembolism originates from a coronary thrombus.Arterosclerotic plaque deposition in the artery of epicardium is the most common triggering event, leading to coagulation of a cascade that can sometimes lead to a complete blockage of the artery (Tsujita *et al*., 2010). Atherosclerosis refers to the gradual increase of low-density cholesterol and fibrous tissue in the wall of arteries in the form of plaques (Woollard *et al*., 2010). The plaquesprovide stable angina as major symptom, canend unstable angina characterized by broken plaque and also contribute to the formation of blood clot that will further close the artery. A disintegrated coronary artery plaque can lead to

myocardial infarction (Tsujita *et al*., 2010) and about a trillion myocardial cells are planned to be lost in a typical myocardial infarction (Laflame *et al*., 2005). Poorblood flow to the heart lead to a process called the ischemic cascade if it last long enough; cardiac cells in the region of the closed coronary artery die (mainly by necrosis) and do not grow. It is replaced by the formation of extracellular matrix. Previous studies of the last decade have shownin addition to necrosis, myocardial infarction is associated with, apoptosis(Olivetti*et al*., 1997). In fact, apoptosis has been described to be first form of myocardial cell death to appear within 6-8 hours after infarction in rats and humans (Cleutjens *et al*., 1999). Based on the evidence that the cell death is usually associated with apoptosis because it happens 6-8 hours after infarction, it has been regarded to as a major cause of myocardial infarction but necrosis also occur immediately after apoptosis between 12 hours to 4 days. This secondary necrosis was described to evoke inflammatory response associated with cytokine release attracting more leukocytes (Lagrand *et al*., 1997).

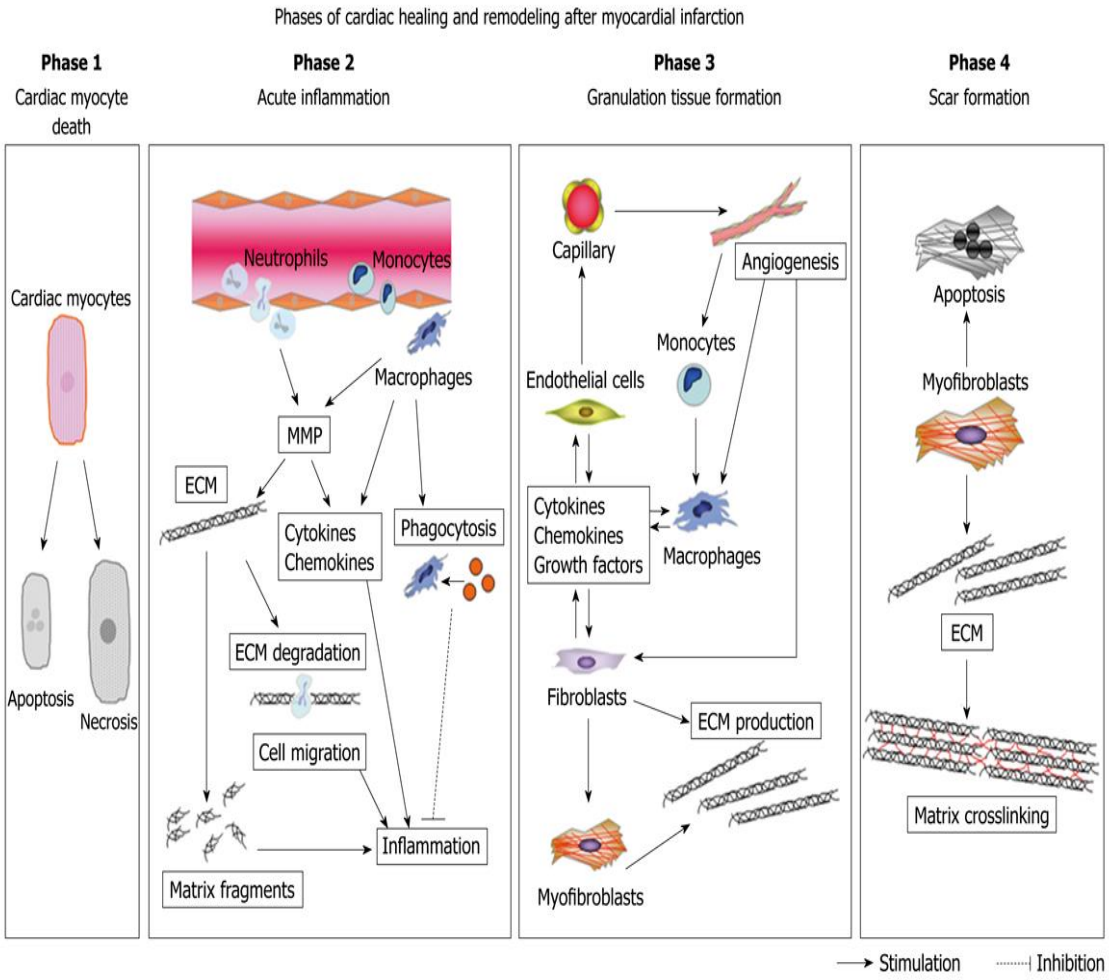



**Figure 2.3: Stages of Myocardial Infarction (Cleutjens *et al*., 1999)**

Apoptosis differs from necrosis in the following ways (Valen *et al*., 2003):

- Apoptosis is a programmed cell death cascade while necrosis is not
- Apoptosis can be pathologic or physiologic but necrosis is always pathologic
- Apoptosis require ATP depletion, which is not needed in necrosis
- Plasma membrane almost intact until late during apoptosis while plasma membrane is destroyed early during necrosis
- No cellular content leakage during apoptosis but this leakage is a major characteristic of necrosis
- In apoptosis, there is no mitochondrial swelling and cell shrinkage, while mitochondrial swelling and oncosis are part of necrosis
- No inflammation associated within minutes of apoptosis occurrence while inflammation is always triggered by necrosis
- Change in nuclear morphology is associated with apoptosis and no change in nuclear morphology during necrosis
- There is selective protein degradation via caspases in apoptosis while necrosis does not entail protein degradation through caspases.

The inflammatory response caused bynecrosis result from the release of cytokines, such as interleukin 6 and 8 within 12 – 16 hours after the onset of ischemia. 2-3 days after heart attack, a new extracellular matrix (ECM) proteins (like fibrin fibronectin, collagen and metalloproteinase (MMPs) which degrade collagen synthesis) are laid in the borderline infarction zoneat first and then in the central infarction region. C-reactive protein (CRP) is a sensitive marker for inflammation and blood level increase was observed during myocardial infarctionand some drugs for myocardial infarction revealed serum CRP level reduction (Lagrand*et al*., 1997).The functionof CRP is binding to destroyed-phosphatidylcholine present on the surface of either dead or dying myocardium to stimulate the complement

cascade system (Thompson *et al*., 1999) thereby causing the necrosis and apoptosis of myocardial cell andengulfment by macrophages. Serum rise in this protein is a quick response phase occurring due to increased serum level of interleukin-6 produced by macrophages and myocardial tissue injury (Lau *et al*., 2005). Also, the sensitization of pro-inflammatory transcription factor called Nuclear Factor KappaB ($NF\text{-}_kB$) is included. This redox factor is called a sensitive transcription factor,which can be stimulated by increased production of reactive oxygen species, hypoxia, hyperoxia, anoxia, cytokines, protein kinase-C activator, bacterial or viral products likelipopolysaccharide (LPS), dsRNA or the leukemia virus T-cellsof type 1 Tax protein, and UV-radiation (Chengjuang*et al*., 2002). The transcription factor NF-κB is an importantregulator of the acute early response gene, which is involved in regulating cellular responses and apoptosis in response to various injuries (Chengjuang *et al*., 2002). The NF-κB transcription factor consists of five family members called p50 type, p52 type, p65 (RelA) form, c-Rel and RelB form. All these family member can be in different homodimeric and heterodimeric form (Karin, 2000). The most predominant heterodimeric form is p65 (Rel A) - p50 NFKappaB usually in the form of p105NF-KappaB inactivated by IKB either alpha or beta (Zandi *et al*., 1997). Upon activation, p105NF-KappaB is cleaved to p50NF-KappaB which forms dimer with p65 (Rel A) through the enzymatic action of IKB kinase that is known as IKK. IKKα and IKKβ are the two catalytic subunits in the complex that phosphorylate IKB on the serine residue 32 and 36 leading to ubiquination and degradation of IKB so that the NF-KappaB can migrate from cytosol to the cell nucleus binds to DNA-binding site (Scherer *et al*., 1995). Binding to DNA in the presence of co-associated or co-repressor protein such as histone acetyltransferase or histone deacetylase enhance the transcription of cytokines and apoptotic related genes. An alternative pathway, but less studied mechanism of NF-κB activation, involves tyrosine phosphorylation of IκBα causing the dissociation of NF-κB from IκBα without proteolytic degradation (Fan *et al*., 1999). Imbert *et al*., (1996), observed that activation of T-cell with a protein phosphatase inhibitor or hypoxia or hyperoxia caused phosphorylation of IκBα at tyrosine residue 42 and subsequent NF-κB nuclear translocation without IκB degradation. Similar discovery was also found in neuron cell model, where nerve growth factor treatment leads to NF-κB activation through IκBα tyrosine phosphorylation without degradation (Bui *et al*., 2001). When hypoxia and hyper-

oxygenation was used to induce cellular injury by Chengjuang *et al*., (2002), with the involvement of tyrosine phosphorylation associated with no IκB proteolytic degradation, apoptosis was inhibited. Increased serum TNF-α level syncronising with increased NF-KappaB expression was described by Antwerp *et al*., (1996) to possess anti-apoptotic effect. Protective role of this transcription factor was also seen in p65/RelA knockoutmice, the mice died from severe apoptosis of the liver from embryonic stage (Beg *et al*., 1995).

In addition, Nuclear Factor KappaB has been observed to be triggered during ischemic heart preconditioning and pharmacological inhibition of this factor diminish cardio-protection in classic and non-classic models.

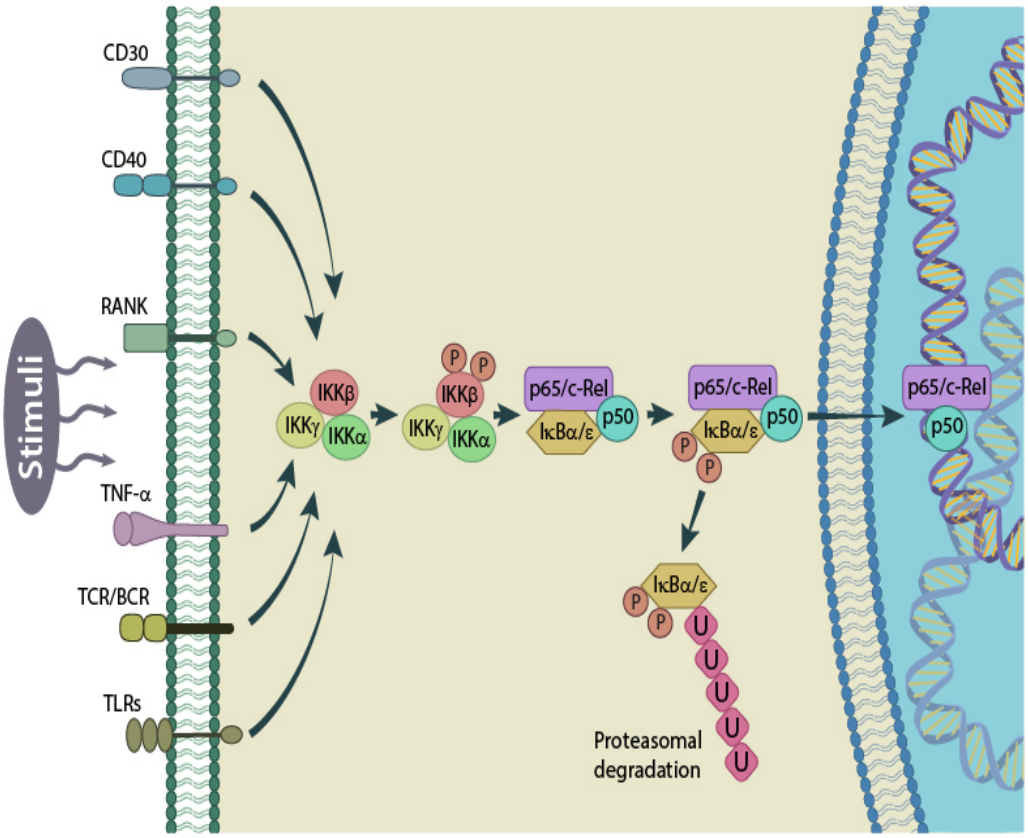



**Figure 2.4: $NF_kB$ Pathway (Dolcet *et al.*, 2005)**

In addition, metabolic activity disruption in myocytes during infarction has been linked with elevated tissue level of histamine, arachidonic acid, linoleic acid, inosine and methylhistamine while compounds like **creatine**, alanine, threonine, xantosine were decreased (Yue *et al*., 2013). Disruption of metabolism in the pathophysiology of ischemic infarction is associated with a decrease in the production of ATP due to disruption of oxidative phosphorylation associated with impaired mitochondria (Yue *et al*., 2013). Physiologically, 60 – 90 % of the fuel for ATP synthesis is obtained from free fatty acid while 10 – 40 % from glucose and lactate. During ischemia, changes in beta-oxidation of fatty acids in the mitochondria lead to the transfer of ATP synthesis to glucose and lactate only by anaerobic respiration, which ultimately leads to the accumulation of lactate in the cytosol. During severe ischemic condition, ATP synthesized through glucose and anaerobic pathway is not enough and the myocardium will increase the catabolism of creatine through creatine kinase to resynthesize ATP leading to increased level of creatine kinase MB type in the serum. It was also explained that prolonged low oxygen tension and the increased duration of positive inotropic effect of isoproterenol on the cardiac muscle also triggered oxidative stress and $Ca^{2+}$ overload during isoproterenol induced myocardial infarction (Chagoya de Sanchez *et al*., 2012). The mechanism behind $Ca^{2+}$ overload starts with the accumulation of lactate and $H^{+}$ leading to a fall in intracellular pH leading to increased activity of $Na^{+}$- $H^{+}$ channel counter transporter thereby increasing the level of intracellular $Na^{+.}$ Increased intracellular $Na^{+}$ level activate $Na^{+}$ - $Ca^{2+}$ exchanger channel which increase intracellular calcium level and more ATP depletion for active transport of the $Ca^{2+}$ out of the cytosol through $Ca^{2+}$ pump of the sacroplasmic reticulum which eventually weakens the contractile work of the myocardium. The glutathione cycle, studied by measuring the level of myocardial tissue in a reduced form of glutathione, glutathione enzymes (peroxidase and glutathione reductase), showed reduced concentration. This confirms the importance of the ROS generation, as well as thereduction of antioxidant defense mechanism (Díaz-Muñoz *et al*., 2006).

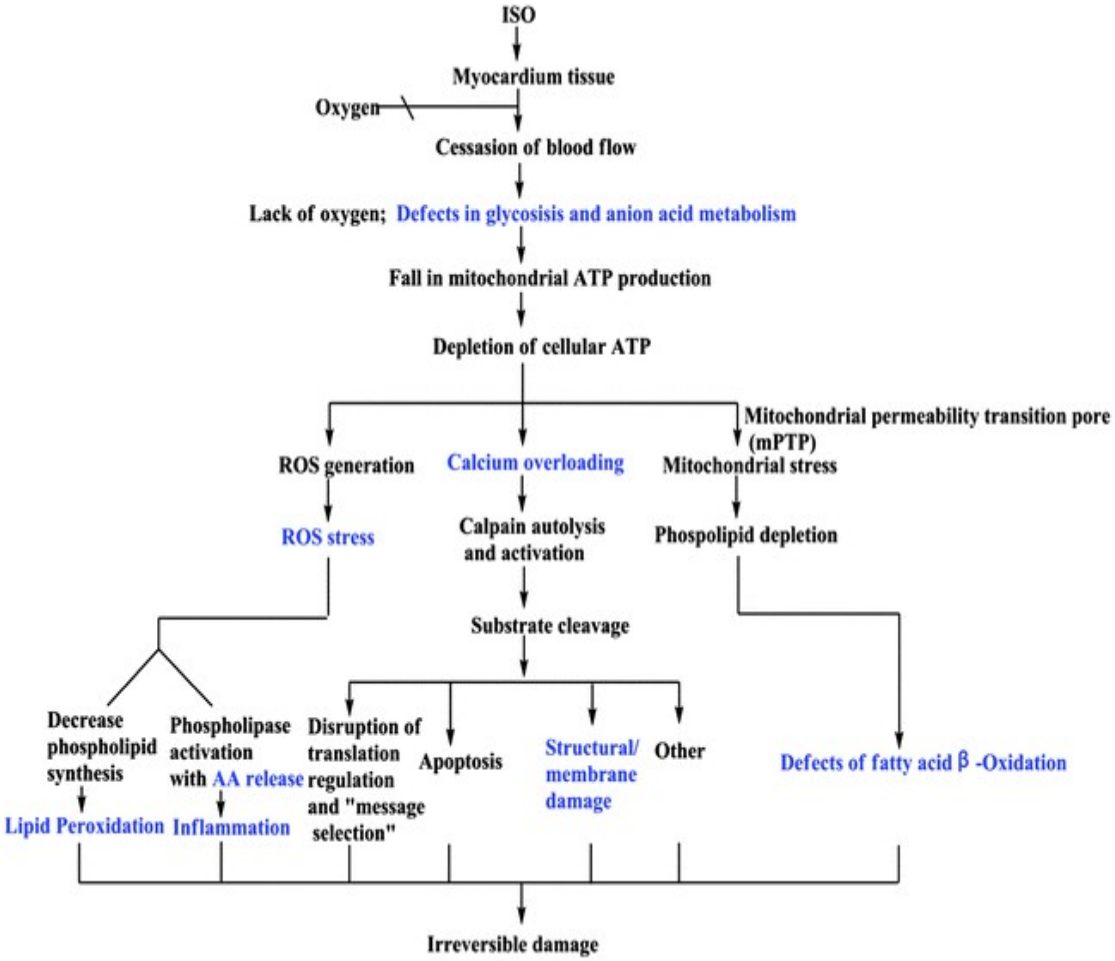



**Figure 2.5: Chart on Metabolic Disturbance During Acute Myocardial Infarction (Yue *et al*., 2013).**

## Apoptosis

Apoptosis is a Greek word derived (leaves or flower dropping off tree) can be called genetically determined cell death or suicide (Yellon *et al*., 2000). Physiologically, this process controls the number of cells in multicellular organisms, maintaining a balance between cell division and cell death (Sabbah *et al*., 1998). Examples of physiological use of apoptosis include removalof destroyed or stagnant cell through this process, proper formation of an organ in an embryo (Sabbah *et al*., 1998). Pathologically, apoptosis contribute to cell death in chronic disease conditions like neurodegenerative diseases, autoimmune diseases, diabetes, cancer and acute tissue insult like myocardial infarction and even ischemia–reperfusion injury (Valen *et al*., 2003). Previous studies has revealed that during apoptosis, there is upregulation of proapoptotic proteins like Bax, downregulation of anti-apoptotic proteins like Bcl-2, activation of Fas or TNF-alpha receptors, activation of p53 and c-Jun Kinase pathways, tissue infiltration with neutrophils and macrophage activation (Borutaite *et al*., 2003). The pathways regulation of genetically induced cell death can be classified into intrinsic pathway and extrinsic pathway.

The intrinsic pathway which is always the main pathway activated, requires the binding of apoptotic activating factor (Apaf-1) to cytochrome C in the presence of ATP to activate pro-caspase 9 and is converted to caspase 9 (Hoffman *et al*., 2004). Cytochrome C, released from mitochondria, is caused by a change in cellular respiration, which is usually associated with hypoxia during ischemic insult (Kremastinos *et al*., 2007). Caspase 9, along with other caspases, such as caspase 2, 8 and 10 are considered initiators which helps to proteolytically activate the execution caspases like caspase 3, 6 and 7 to initiate programmed cell death. Bcl-2 proteins control apoptosis by inhibiting the binding of Apaf-1 with cytochrome C as well as controling the cytochrome C release from mitochondria (Hoffman *et al*., 2004). The ratio of Bcl-2 to Bax protein expression has been linked to death of tissue after stimulating apoptosis (Zhao *et al*., 2000) and the family of Bcl include Bcl-2, Bcl-x, Bcl-w, Bid and Boo. The pro-apoptotic protein families include Bax, Bak, Bik, Bim (Zhao *et al*., 2000). The extrinsic pathway require the binding of specific death ligand like Fas ligand of TNF- alpha to death receptors which include Fas receptors and TNF receptors, which cleave procaspase 8 to caspase 8 which activate execution caspases like caspase 3, caspase 6 and caspase 7 (Pandey *et al*., 2000).

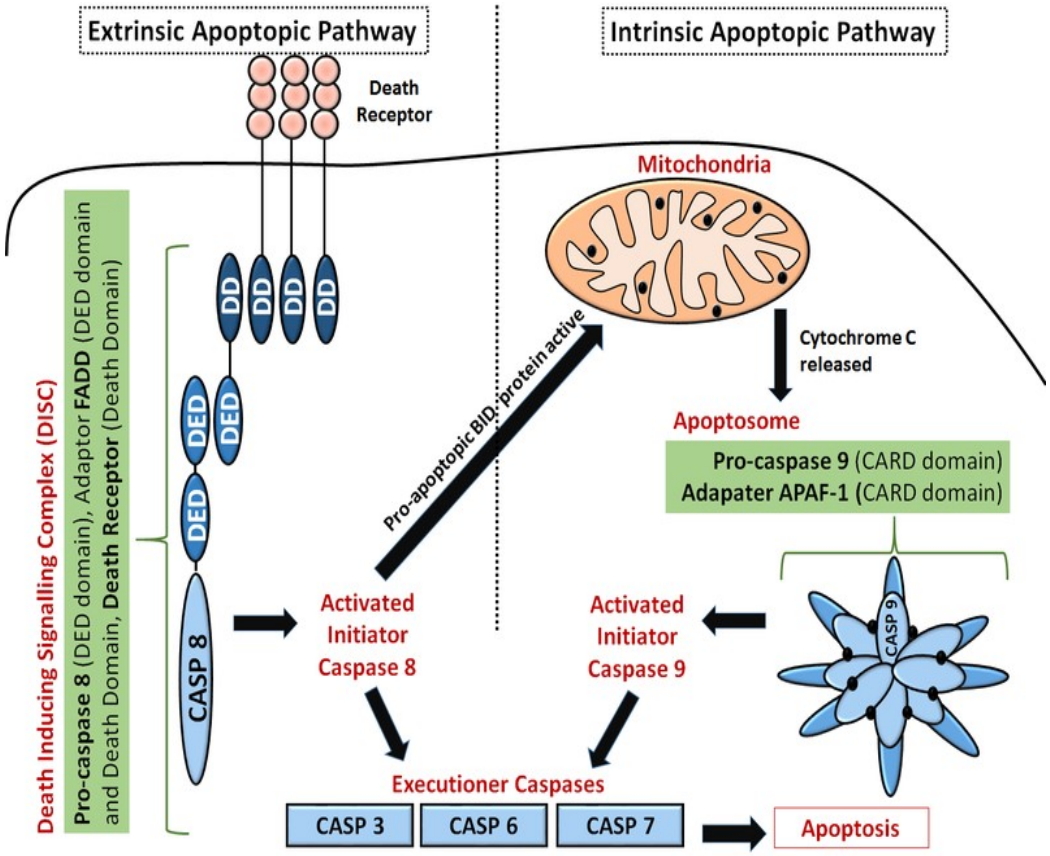



**Figure 2.6: Apoptotic pathway, (Jess, 2018)**

**Risk Factors**

Risk factors for acute Myocardial Ischemiawere established by the findings of the Framingham Heart Study in the 1960s (Brian *et al*., 2015). Sufficient information about factors that can increase myocardial ischemia is important for the prevention of cardiovascular morbidity and mortality. The classification of factors according to Brian *et al*., 2015 was based on conventional risk factors, modifiable risk factors, non-traditional risk factor, and chronic disease leading to coronary artery disease. Other risk factors include; low serum testosterone levels, hysterectomy, imbalance of clot dissolving enzymes (tissue plasminogen activator) and their inhibitors (plasminogen activator inhibitors-1) and lack of sleep. There can also be modifiable risk factors which was derived based on life style. High level of low density lipoprotein cholesterol, high blood pressure, cigarette smoking, diabetes, obesity and insufficient physical activity all falls under this modifiable risk factors.

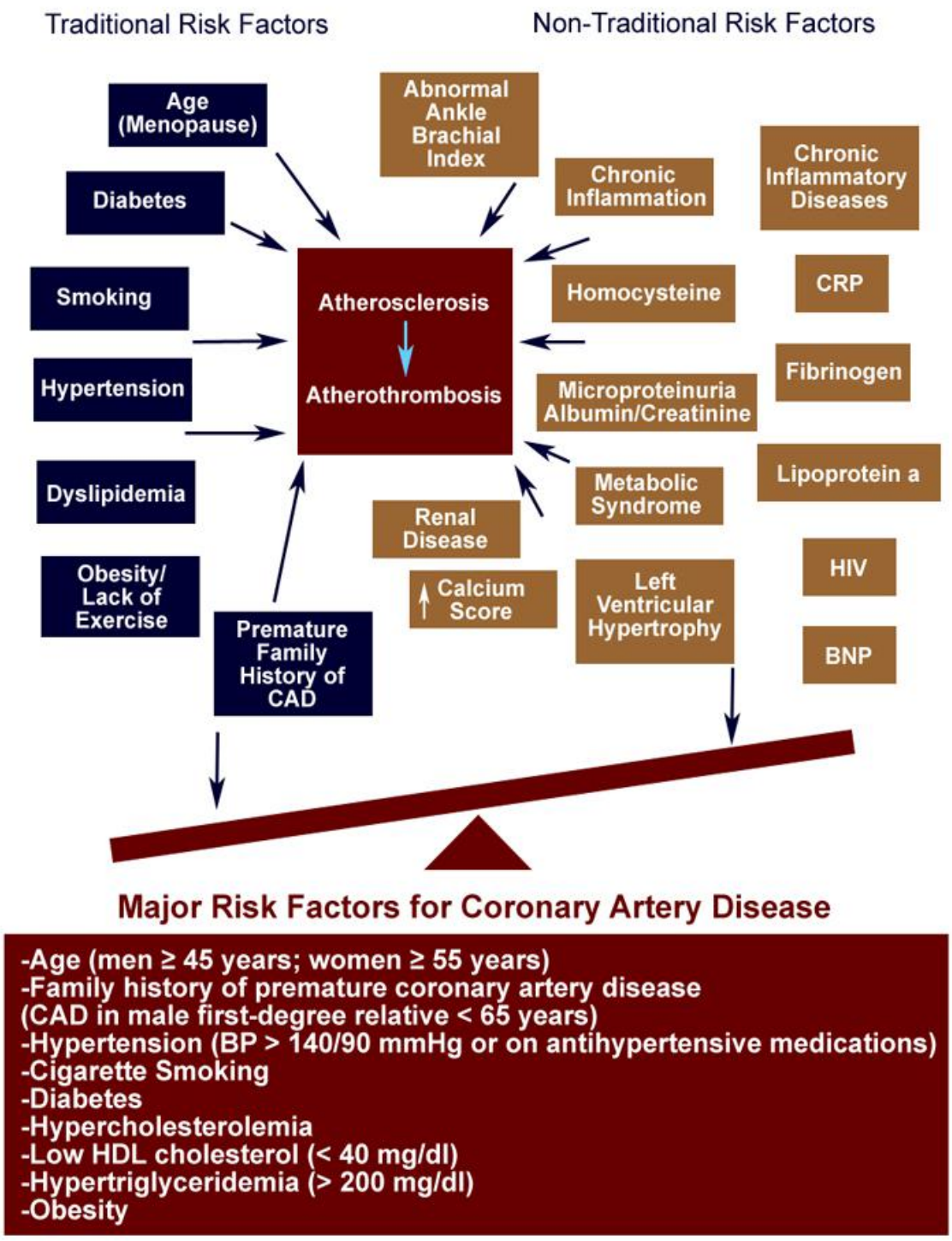



**Figure 2.7: Risk factors for arteriosclerosis, (Brian *et al*., 2015)**

## Prevention and Management

Myocardial infarction and other associated cardiovascular diseases can be prevented to a large extent by a lot of lifestyle changes. In addition, certain treatments can also prevent myocardial infarction.

## Prevention

One of the best wayto prevent acute myocardial infarction is lifestyle.Adequate intake of wholegrain starch, less sugar intake, consuming fruit and vegetables on a daily basis, consuming 4–5 servings of unsalted nuts, seed and legumes a week can help to reduce the incidence of myocardial infarction (National Institute for Health and Clinical Incidence, 2014). Following a diet lifestyle in which less than 30% of the energy consume comes from fat, a diet that contains less than 7% of the energy intake in the form of saturated fat, and a diet that contains less than 300 mg/day of cholesterol (National Institute for Health and Clinical Incidence, 2014).Replacing saturated with mono- polyunsaturated fat is also recommended (Mozaffarian *et al*., 2010). Olive oil, rapeseed oil and related products can be used instead of saturated fat.

## Pharmacological Intervention

Aspirin is one of the most widely used and studied drug against myocardial infarction. Aspirin was regarded and preferred as first line of treatment in patient with the cardiovascular risk due to low risk of bleeding even though it is antiplatelet drug (Hodis, 2014). In addition toaspirin, Cholesterol-lowering drugs,derived from statin may be used in individualswho are susceptible to cardiovascular disease (National Institute for Health and Clinical Incidence, 2014). After an attack due to acute myocardial infarction, administration of organic nitratesand inhibitors of the angiotensin converting enzyme was shown toreduceassociated death risk (Perez *et al*., 2009). Other therapy used in myocardial infarction risk patients include thrombolytic drugs, anticoagulants, β-blockers.**It has been shown that cardiac renin angiotensin system (RAS) and not the circulatory system may help in the amelioration of myocardial infarction.** In addition, decreased production of prostaglandin and free radical clearance of **ACE inhibitors** have been suggested to reduce myocardial infarction (Chopra *et al*., 1996).

## Compensatory Mechanisms of Myocardial Infarction

The compensatory mechanism that is developed during acute myocardial infarction can be classified into factors within the heart and factors outside the heart. Factors within the heart often regard to as cardiac factor entails increased heart rate, over-time ventricular dilation or eccentric hypertrophy and increase in the force of myocardial contraction associated with increased ventricular filling (Frank-Starling explained mechanism). Another important compensatory mechanism is the involvement of autonomic nervous system which is associated with enhanced activity of sympathetic nervous system to trigger tachycardia and reduced activity of parasympathetic nervous activity. Hormonal response to myocardial infarction include the activation of renin that enzymatically act on angiotensinogen and convert it to angiotensin 1. Angiotensin 1 is converted to angiotensin 2 which is the most potent of the renin-angiotensin system. Angiotensin 2 is a known potent vasoconstrictor and strong stimulator of mineralocorticoid like aldosterone. Aldosterone is widely known to increase blood volume thereby increasing preload.

Other hormones that was shown to be associated with myocardial infarction include natriuretic peptide and catecholamine.

The activation of sympathetic nerves,angiotensin 2-aldosterone system, increased release of antidiuretic hormone (vasopressin) and atrial natriuretic peptide is associated with acute response to acute myocardial infarction. Generally, activation of nervous system and hormones is called neuro-humoral response and the overall action of these responses is to produce arterial vasoconstrictionand venous constriction thereby increasing afterload and preload with the increase inblood volumeincreasing preload through increased ventricular filling. In general, these neuro-humoral responses can be considered as compensatory mechanisms, but worsen myocardial infarction leading a chronic type of heart failure called congestive heart failure which is associated with left-ventricular hypertrophy, pulmonary edema and general swelling of the body as a result of stagnated tissue fluid.

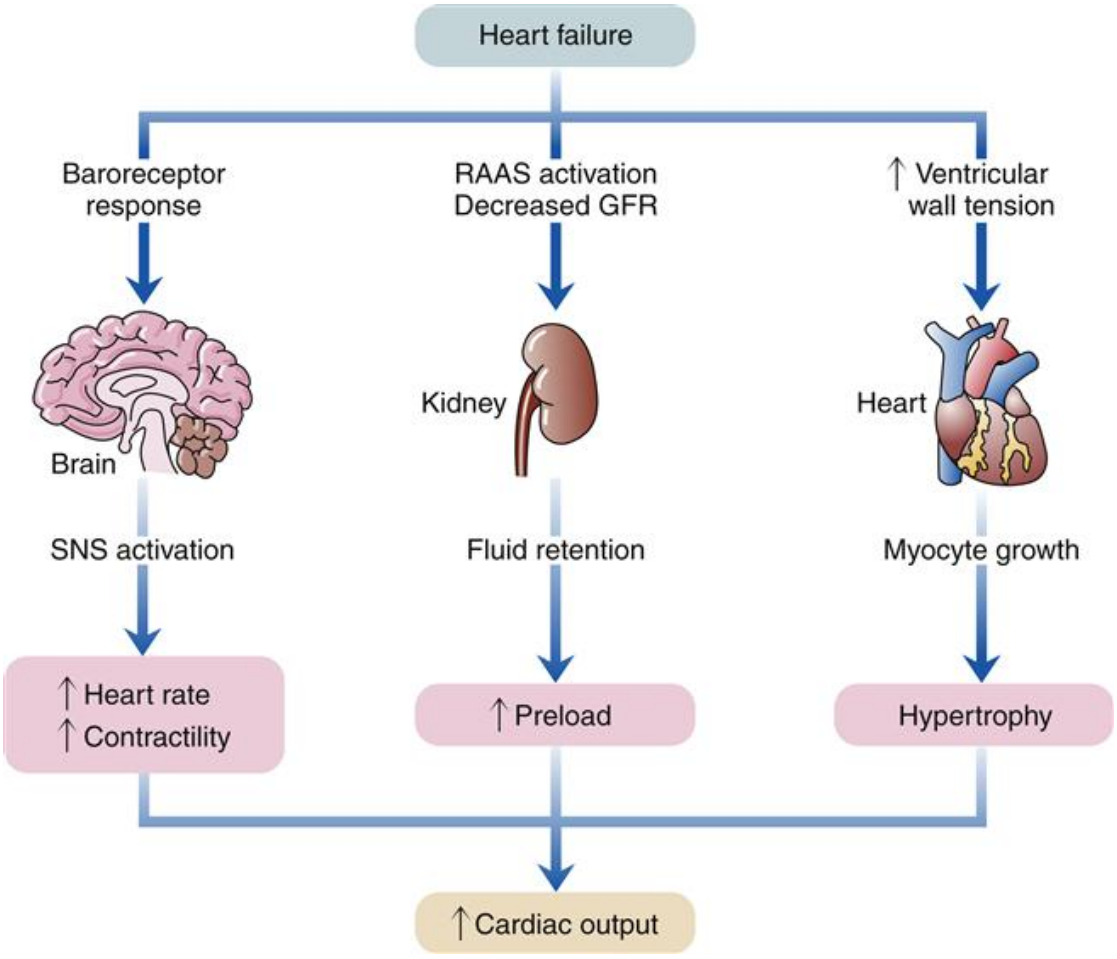



**Figure 2.8: Compensatory mechanism in Acute Heart Failure (Shann, 2018)**

**Acute Myocardial Infarction and Nitric oxide effect (Massion *et al*., 2003)**

The nitric oxide action in the myocardium is not static, but dynamic and depends on the state of the heart. It is described that Nitric oxide regulates the myocardium through its vascular-dependent and vascular-independent effects. The vascular-dependent effect is based on the vasodilation, which leads toa decrease in preload and afterload. The vascular independent effect is based on direct effect of nitric oxide on heart by finely regulating induction–contraction coupling, modulation of vegetative signal and mitochondrial respiration. During baseline (no change in load or application of inotropic agent), the effect of nitric oxide on myocardium is called bi-modal,which has positive inotropic effects at low level and negative effect at higher levels. During exercise, endogenous nitric oxide showed a negative inotropic effect through force frequency ratio.The lack of contraction of the heart muscle stretches and weakens the positive inotropic response to isoproterenol, and is thus described as having a beta-adrenergic antagonist effect. It has been described that eNOS reduces the conductivity and excitation of a beta adrenergic agonist to protect the heart from arrhythmias.

In such diseases as infarction caused by ischemia, there was a decrease in the activity of nitric oxide synthase from the endothelium and a decrease in the level of endogenous physiological NO. It is in tandem with the best expression of inducible nitric oxide synthase released by cytokines and inflammatory heart failure cells.

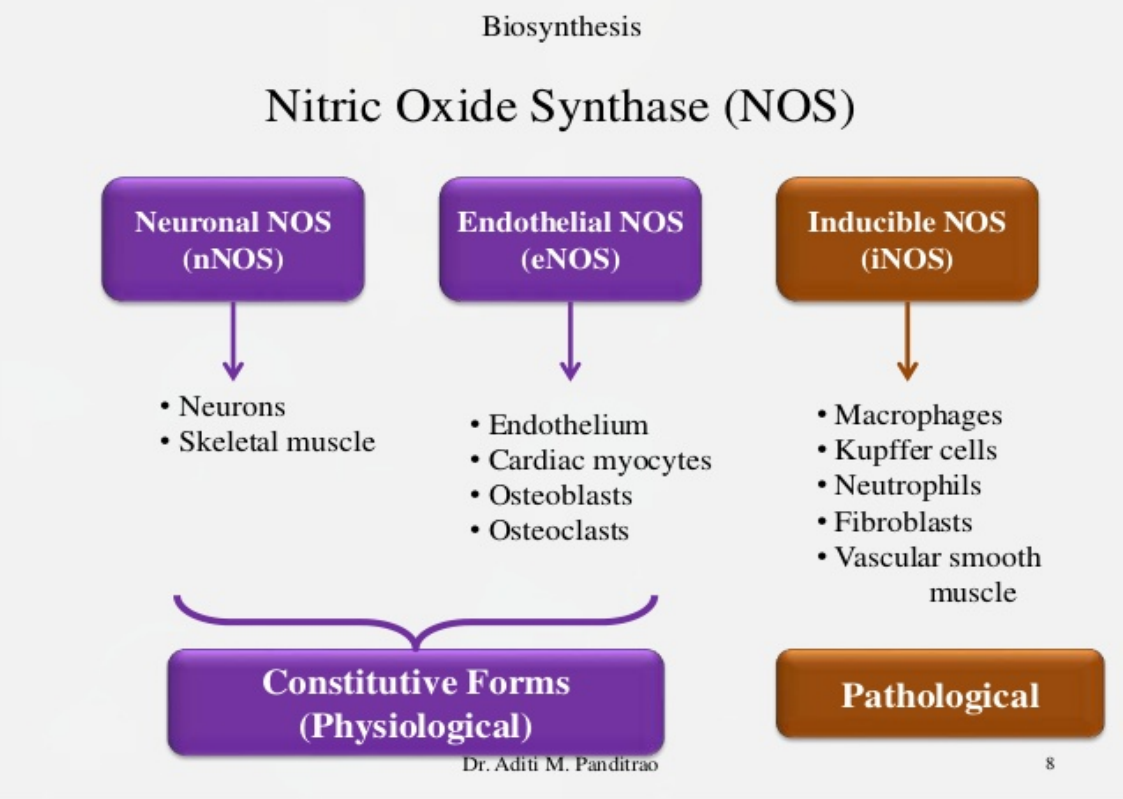



**Figure 2.9: Role of Nitric Oxide (Aditi, 2016)**

**Natural Product a Promising Approach in the Management of Myocardial Infarction**

Herb as a source of medicine is gradually gaining acceptance from the public and medical profession due to advance in the understanding of the mechanisms by which herbs positively influence health and quality of life (Berman, 2000). Herb as potent free radical scavenger, antioxidants is an important mechanism studied thoroughly. It has been shown that natural antioxidants, especially phenolic and flavonoids, are safe and also bioactive. Therefore, serious attention has been directed towards credentials of plants with antioxidant ability in current years. Below is the list of natural components and herbal formulations which has been shown to prevent isoproterenol-induced myocardial infarction:

**Natural ComponentsProtecting against Isoproterenol-Induced Acute Myocardial Infarction.**

| Agents | Dose | Parameters Protected |
|---|---|---|
| Salvianolic acid A from dried root of *Salvia miltiorrhiza* (Family Lamiaceae | 0.3, 1 and 3mg/kg, i.v for 8days | Improved cardiac marker enzymes (LDH, CK-MB, AST), antioxidant enzymes (Malodialdehyde, SOD, CAT, GPX), Mitochondrial respiratory dysfunction, left ventricular function, ECG and histopathological alteration (Wang *et al*., 2009). |
| Gallic acid an endogenous plant phenol | 15 mg/kg for 10 days | Protect cardiac marker enzymes (LDH, CK-MB, AST), antioxidant enzymes (Malodialdehyde, SOD, CAT, GPX), troponin-T and histopathological alteration of lysosomal enzymes: The activities of ß- glucuronidase, ß-galactosidase, cathepsin-B and D were significantly ($P<0.05$) protected in the serum and heart of rat (Kahkeshani*et al*., 2019) |
| Ethanolic extracts of *Curcumis trigonus* Roxb. (Family Cucurbiateace) | 75 and 100 mg/kg was administered orally for 2 weeks | Serum marker enzymes, electrocardiographic changes (ST elevation, QRS complex, P wave, RR interval and heart rate) and histopathological changes were well protected aftergiving the |

| | | |
|---|---|---|
| | | extract (Thippeswamy *et al*., 2009) |
| Ethanol extract of stem-bark and stem-wood of *Premna serratifolia* Lin, (Family Verbenaceae) | 100 mg per100 g, given through i.p. route for four weeks | Maintained ECG changes in rat heart, serum protein, serum Albumin/ Globulin ratio, Rajendran, (2009) |
| Quercetin a bioflavonoid | 10 mg/ kgbw given through oral rout for 2weeks | Significantly protects serum and heart lipid profile (TC, TG, HDL, PL, FFA), the activity of plasma and liver 3-hydroxy-3-methylglutaryl-coenzyme-A reductase and the activity of plasma and liver lecithin cholesterol acyl transferase. Significantly reduced the levels of hexose, hexoamine, fucose and sialic acid in the serum and heart (Lee *et al*., 2011). |
| Echinocystic acid (EA), isolated and identified from the fruits of *Gleditisa sinensis* Lam (Family: Fabaceae) | 30 and 15 mg/kg, i.v | EA prevents the ST-segment changes and Bcl-2 mRNA level (Muhamed *et al*., 2013) |
| Aqueous extract of *Embelia ribes.* Burm fruit (Family: Myrsinaceae) | 100 mg/kg, p.o. for 1 month | Heart rate, systolic blood pressure, endogenous antioxidants levels, histopathological changes, serum marker enzymes were improved. (Bhandari *et al*., 2008) |
| Green tea | 100 mg $kg^{-1}$, 30 days | Prevented increased heart weight, body weight , andbiomarkers likeAST, ALP, ALT, LDH and CK-MB, lipid peroxidation, enhanced membrane endogenous antioxidants (SOD, CAT, GPX and GST.) and membrane bound ATPases ($Na^+/K^+$ATPase, $mg^{2+}$ATPase and $Ca^{2+}$ATPase), serum and heart profile (TC, TG, HDL, PL, FFA, VLDL and LDL), lipid metabolizing enzymes (LCAT, LPL, and CES), histopathological alteration. (Upaganlawar *et al*., 2009) |
| Hydroalcoholic extracts of leaves of *Sida cordifolia* L | 100 and 500 mg/kg for 1 month | Biomarkers of cardiac injury (LDH and CK-MB) and antioxidants (SOD and catalase) were |

| | | |
|---|---|---|
| (Family: Malvaceae) | | maintained in serum and heart tissue homogenate. (Kubavat and Asdaq., 2009) |
| Aqueous extracts of *Oxalis corniculata* (Family: Oxalidaceae) | 30 days | Cardiac injury marker enzymes (LDH), serum lipids was reduced. It also maintained the activity of activities of antioxidant enzymes (CAT, SOD, Malodialdehyde), vitamin C, protein sulfhydryl groups and reduced glutathione (GSH) and histopathological alteration were prevented. (Abhilash *et al*., 2010) |

## 2.2 CORCHORUS OLITORIUS

*Corchorus olitorius* (Linn) is a vegetable belonging to the family Tiliaceae and in English it is often called jute mallow, and in south-west Nigeria- “ewedu”. It is an important green vegetable in many tropical areas, such as Egypt, Sudan, India, Bangladesh, in tropical Asia such as Philippine and Malaysia, as well as in tropical Africa, Japan, the Caribbean and Cyprus (Samra *et al*., 2007). The leaves (fresh or dried) are preparedand turnedinto a thick soup that can be added to stew.It is described as a rich source of vitamins and minerals (Tindal, 1983).

**Figure 2.10: *Corchorus olitorius* plant, (Adebo *et al*., 2018)**

**Figure 2.11: *Corchorus olitorius* plant, (Adebo *et al*., 2018)**

**Medicinal Uses**

The plant *Corchorus olitorius* have several applications as a medicinal plant which include the following:

- The extract from the leaf of this plant is usedin traditional medicine for the treatment of gonorrhea, pain, fever and tumor (Ndlovu *et al*., 2008);
- Its leaves and roots are also used as herbal medicine in South East Asia to treat cystitis, dysuria, fever and gonorrhea (Ndlovu *et al*., 2008).
- In part of Nigeria, plant leaves are used as decoction for thetreatment of anemia andas well as blood purifier (Aiyeloja, 2006)
- Leaflets are used for heart problems (Denton, 1997), while cold leaf fusion is used to restore appetite and strength, leaves are used for ascites, pains, piles, tumors, gonorrhea and pyrexia (Fasinmirin, 2009).

**Previous Studies on *Corchorus olitorius***

The protective effect of ethanol extract of *Corchorus olitorius* against $CCl_4$ induced hepatic damage in rats was studied (Salawu, 2007) and the result showed a significant hepatoprotective effect by reducingserum and liver ALT, AST, and total protein at doses of 250 and 500 mg/kg in carbon-tetrachloride induced hepatotoxic rats (Salawu, 2007). This study also showed significant inhibition of lipid peroxidation as evidence by a decrease in MDA Values.

Phenolic antioxidants in leaves of *Corchorus olitorious*has been found to include phenolic [5-caffeoylquinic acid (chlorogenic acid), 3, 5-dicaffeoylquinic acid, quercetin 3galactoside, quercetin 3-glucoside, quercetin 3-(6-malonylglucoside), and quercetin 3-(6-malonylgalactoside) (tentative)] using NMR and FAB-MS (Keiko *et al*., 1999). The contents of these phenolic compounds, ascorbic acid, and alpha tocopherol in *C. olitorius* leaves were determined, and their antioxidant activity was measured using radical-initiated peroxidation of linoleic acid. The results showed that 5-caffeic acid is the dominant phenolic antioxidant in *C. olitorius* leaves (phenolic antioxidants from leaves of the *Corchorus olitorius* leaves (Keiko *et al*., 1999). None of these phenolic compounds is in a recent study of chemical composition and antioxidant in in-vitro properties of selected vegetables (Salawu *et al*., 2007) with the exception of caffeic acid using GC-MS

analysis. Caffeic acid is a phenolic compound, usually present among many plants that have been carefully studied and they are known for their physiological activities including anti-inflammatory anti-allergic and anti tumour (Kimata *et al*., 2000). They also investigate the ability to inhibit lipid peroxidation of *Corchorus olitorius* among other vegetables and they decided that all estimated vegetables are able to inhibit lipid peroxidation, and thevegetable consumption of*Vernonia amygdalina* and *Corchorus olitorious*provide better cytoprotective effects. Further results from this study showed that ethanol extract of *Corchorus olitorious* and other vegetables possesshigh superoxide and hydrogen peroxide scavenging capacityprobably due to the presence of caffeic acid and other flavonoids. Oboh *et al*., (2009) conducted a comparative study of the antioxidant properties of water soluble component extract and non-polar soluble component of the extract of the *Corchorious olitorius*. Water soluble component and non-polar soluble component of the leaf were prepared using water and hexane, respectively and their antioxidant properties were determined. Polar solvent extract showed a significantly higher (1,1-diphenyl-2picrylhydrazyl radical-scavenging ability, reducing power, trolox equivalent antioxidant capacity than non-polar. However, non-polar extract showed significantly higher hydroxyl clearance activity than polar extract while there was no significant difference in their Fe(II) chelating ability. The higher 1,1-diphenyl-2-picrylhydrazyl radical-reducing ability, reducing power and trolox equivalent antioxidant capacity of the hydrophilic extract could be as a result of higher total phenol (630.8 mg/100 g), total flavonoid (227.8 mg/100 g) and non-flavonoid polyphenols (403.0 mg/100 g), and its high ascorbic acid content (32.6 mg/100 g). While the higher OH reducing ability of non-polar extractcan be due to high carotenoid composition (42.5 mg/100 g). Therefore, the synergistic antioxidant activities of the hydrophilic and lipophilic constituents may contribute to the medicinal properties of *C. olitorius* leaf (Oboh *et al*., 2009).Further work,showed a protective effect of aqueous extract of *Corchorus olitorius* leaves (AECO) against sodium arsenite-induced toxicity in experimental rats (Das *et al*., 2010). Significant inhibition of hepatic and renal antioxidant enzymes such as superoxide dismutase, catalase, glutathione-S-transferase, and glutathione peroxidase and glutathione reductase has beennoticed. The level of reduced glutathione decreased, while the levels of oxidized glutathione and thiobarbituric acid reactive substances in selected tissue increased after arsenic poisoning. Treatment with AECO in doses of

50 and 100mg/kg body weight p.o. for 15days after arsenic poisoning significantly improved hepatic and renal antioxidant markers based on varying dose. AECO treatment also significantly reduced the arsenic-induced DNA fragmentation of liver and kidney. Histological studies on the ultrastructural changes of liver and kidney confirm the protective activity of the AECO (Das *et al*., 2010). Thus aqueous extract of *Corchorus olitorius* leaves is significant in protecting animals from arsenic induced hepatic and renal toxicity.

# CHAPTER THREE

# 3.0 MATERIALS AND METHOD

## 3.1 MATERIALS

**CHEMICALS and MATERIALS**: Distilled water, ethanol, enalapril, soxchlet extractor, microplate reader, homogenizer, cold centrifuge, bucket centrifuge, ketamine, xylazine, foil, heparinized capillary tube, water heater, pH meter, unichannel and multichannel micropippete, microplate, thiobarbituric acid, Kent incorporation non-invasive blood pressure machine, Rats Electrocardiographic machine, hydrogen peroxide, reduced glutathione, Sodium azide, Tris-KCL, Trichloroacetic acid, DTNB 5′, 5′-Dithiobis- (2-nitrobenzoate) , $K_2HPO_4$(Phosphate buffer), 0.1$MPO_4$ (Myeloperoxidase), Griess reagent, Sulphosalicyclicacid, HCL (Hydrochloric acid), $H_2SO_4$ (Sulphuric acid), Sorbitol, Adrenaline, bicarbornate buffer, Biurett reagent, Dichloromethane, n-Hexane, ethyl-acetate, Randox LDH Elisa Kit, Bioway ELISA Kit for CRP, fortress Diagnositc CK-MB ELISA Kit and Bioassay Technology lab. ELISA Kit for NFKappaB, 10% formaldehyde solution, Bioss P65$NF_kB$, Bcl2 and Bax antibodies, and spectrophotometer.

## 3.2 METHOD

### 3.2.1 PREPARATION OF PLANT EXTRACT (Kupchan extraction protocol 1969).

Fresh healthy leaves of *Corchorus olitorius* was collected and authenticated at forestry research institute of Nigeria Jericho, Ibadan. The voucher specimen was given FHI number 110410 at Forest Herbarium. The leaves were air dried for 21 days and pulverized into powdery form with high capacity grinder and soaked into 70% aqueous ethanol at the ratio of 1:10 (w/v, that is 5 gm in 100 ml) for 3 days. The extract was filtered using Whitman filter paper (90 mm) and dried through evaporation under reduced pressure until only water layer remains. Remaining water was removed by rotary evaporator and semi-solid form was obtained.

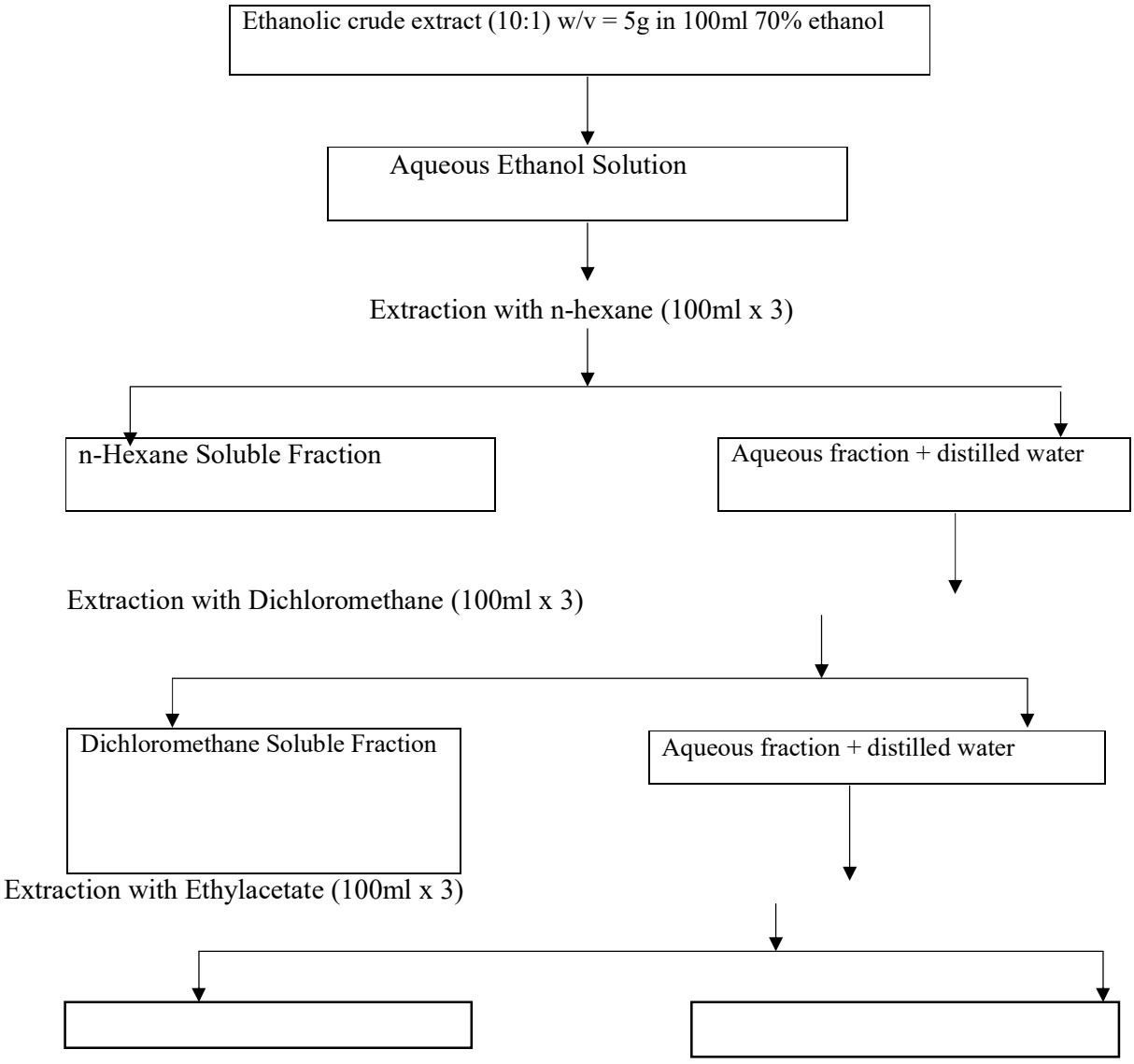
Percentage yield obtained using formula $\frac{W2}{w1} \times 100\%$.

Where $W_2$ (180g) the final weight and $W_1$ (2000g) is the initial weight.

Crude extract percentage yield was 9%

### 3.2.2 SOLVENT FRACTION

Using weight-volume ratio of 5gm of crude extract triturated with 90% ethanol (1:10 w/v that is 5 gm in 100 ml), the solution prepared was then partitioned using solvent of increasing polarity such as n-hexane (53gm), dichloromethane (2gm), ethyl-acetate (5gm), and ethanol (64gm). All these partitions were dried using rotary evaporator ata temperature of 39$^{o}$C.




**Flow chart showing the steps of liquid-liquid extraction (Modified Kupchan partitioning by VanWagenen *et al*., 1993).**

### 3.2.2 ANIMALS

Male and female Wistar rats, with the weight range of 120-150g, were used for this study. They were kept in a departmental animal shelter (Department of Veterinary Pharmacology and Toxicology) under controlled temperature and light/dark cycle conditions for 12 hours, each ratwas provided laboratory feed and water ad-libitum after approval from University of Ibadan Animal Ethics Committee and the ethical approval number given was UI-ACUREC/18/0057.

### 3.2.3 EXPERIMENTAL DESIGN

The first phase of the experimental study was designed for *Corchorus olitorius* ethanol extract (70%)Pre-induction treatment, Post-induction treatment and acute toxicity study. The second phase of the study involved the pretreatment of rats with polar and non-polar fraction and the third phase of the study was designed to identify the active components of the fraction using Gas Chromatography-Mass Spectroscopy.

## 3.2.3.1 Acute toxicity study

Acute toxicity for *Corchorus olitorius* leaf extract was carried out based on the OECD (Organization for Economic Co-operation and Development) guideline no. 423. Feed was withdrawn from Wistar rats weighing between 120-150 gm overnight before oral administration for an acute toxicity test. After fasting, the animals were weighed and the extract was orallygiven at the dose of 2000 mg/kg. Followed immediately after administration ofextract, rat feed wasfurther withheld for 3-4hrs.This is followed by observations at 30minutes, 1h, 2h, 3h, 4h, 24h, and once a day until day 14. Rats were evaluated for changes in weight, tremors, diarrhea, lethargy, sleep, coma and death. There were alsoobjective observations like changes in the skin, fur, eyes and behavioral pattern was carried out. Although the extract did not produce any harmful effect at the dose of 2000 mg/kg and was required to dose for 21 days,a further toxicity stage 2 was performed at a dose of 5000 mg/kg according to the same procedure as stage 1. It was found that the extract was safe at 5000 mg/kg.

### 3.2.3.2 Pre-treatment Study (Roza *et al*., 2015 experimental model)

Rats were randomly divided into five groups consisting of sixanimals in each group and treated as follows:

**Group I**: control (distilled water 1.0 mL/kg/day, p.o) for twenty one days

**Group II**: control of MI (distilled water 1.0 mL/kg/day, p.o.) for 19 days and given ISO (100 mg/kg, s.c.) on 20$^{th}$ and 21$^{st}$ day

**Group III** – *C. olitorious* (250 mg/kg/day, p.o.) for 19 days and given ISO (100 mg/kg, s.c.) on the 20$^{th}$ and 21$^{st}$ day

**Group IV** – *C. olitorious* (500 mg/kg/day, p.o.) for 19 days and given ISO (100 mg/kg, s.c.) on the 20$^{th}$ and 21$^{st}$ day

**Group V** – Enalapril (10 mg/kg/day, p.o.) for 19 days and given ISO (100 mg/kg, s.c.) on 20th and 21st day

### 3.2.3.3 Post-treatment Study

Rats were randomly divided into four groups comprising of six animalsper group and post-treated in the following way:

**Group I**: control without MI (distilled water,1.0 mL/kg/day p.o.) for 21 days

**Group II**: ISO (100mg/kg, s.c.) on 1$^{st}$ and 2$^{nd}$ day and given (distilled water1.0 mL/kg/day, p.o.) for 21 days

**Group III**: ISO (100mg/kg, s.c.) on 1$^{st}$ and 2$^{nd}$ day and given *Corchorus olitorious* (500 mg/kg, p.o.) for remaining 19 days

**Group IV**: ISO (100mg/kg, s.c.) on 1$^{st}$ and 2$^{nd}$ day and given enalapril(10 mg/kg, p.o.) for remaining 19 days.

### 3.2.3.4 FractionPre-treatment Study

Animals were randomly divided into eleven groups comprising of six animals in each group and treated in the following way:

**Group I**: control without MI (distilled water, 1.0 mL/kg/day p.o.) for 14 days.

**Group II**: MI control (distilled water, 1.0 mL/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group III** – *Corchorus olitorious* ethanol fraction(50 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group IV** – *Corchorus olitorious* ethanol fraction(100 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group V** – Dichloromethane fraction (50 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group VI** – Dichloromethane fraction (100 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group VII** – Ethyl-acetate fraction (50 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group VIII** – Ethyl-acetate fraction (100 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group IX** – n-Hexane fraction (50 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group X** – n-Hexane fraction (100 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day.

**Group XI** – Enalapril (10 mg/kg/day, p.o.) for 12 days and given ISO (100 mg/kg, s.c.) on $13^{th}$ and $14^{th}$ day

### 3.2.4 Acute myocardial infarction induced with Isoprenaline in rats.

In pre-induction treatment experimental design, group III, IV and V animal were administered 250, 500 mg/kg extract and 10 mg/kg enalapril respectively for 19 days. ISO (100 mg/kg) was administered subcutaneously (S.C.) to group II, III, IV, and V animals for 2 days. On day 21, blood pressure and ECG were checked between 6-8 hours after the Isoproterenol administration.

Group II, III, and IV animals in post-treatment design were given isoproterenol (100 mg/kg) subcutaneously on day 1 and 2. 500 mg/kg extract and 10 mg/kg extract were administered 24 hours later to group III and IV animals and this continue till day 21.

#### 3.2.4.1 Hemodynamic Measurement

Systole, diastole, mean pressure, tail blood flow, tail blood volume and heart rate were measured using Kent Incorporation non-invasive blood pressure monitoring machine. A tail cuff was inflated to block the flow of blood to the tail. This cuff opens slowly, and the other tail cuff that was connected to the VPR (Volume Pressure Recorder) sensor, estimate the hemodynamics of the countercurrent blood flow. After returning the blood to the tail, the sensor cuff measures tail swelling caused by arterial blood flow pulsations. Systolic blood pressure is measured at the first appearance of swelling ofthe tail. Diastolic bloodpressureis measured automatically when theincrease in swelling in the tail stops. All blood pressure parameters areshown as output signal on the computer that received the information from the machine controller. The controller received signal from the VPR of the tail occlusion cuff which is also coupled to the animal control. Four ECG limb leads were connected to the subcutaneous portions of anesthetized (Ketamine and xylazine) rats. The lead send heart electrical activity to the animal ECG machine which is connected to output display unit to observe the wave pattern.

### 3.2.5 Animal Sacrifice and Sample Collection

Feed was withdrawn from the rats 12 hours before sacrifice. Blood was collected from the eye through the retro-orbital plexusand left for 1hr at room temperature. The serum was separated by centrifugation at 5000rpm for 15min and kept at -80$^{o}$C for subsequent biochemical analysis. Heart tissue was immediately removed and washed with cold

saline. Heart tissue was dissected longitudinally, weighed and a portion was kept in 10% formalin for histopathological examination. The other longitudinal section was immediately homogenized in cold 0.1M Tris-HCl buffer (pH 7.4, 1:10 w/v). The homogenate was then centrifuged (Cold centrifuge) at 13,000 rpm for 10min at 4$^{o}$C and the supernatant stored at -80$^{o}$C for biochemical analysis.

### 3.2.6 Lactate Dehydrogenase assay

The levels of serum lactate dehydrogenase were determined by Randox LDH elisa kit. 0.02 ml of the serum from each animal in all groups was incubated with 1.0ml reagent at 37$^{o}$C, mixed and read four times (0 min, 1, 2, 3 min) at spectrophotometric wavelength of 365nm. Reagent composition include Buffer/substrate (Phosphate buffer and pyruvate) and NADH.

**Calculation**

To calculate the LDH activity, the following formulae was used

U/I 15000 x ΔA Hg 365nm/min

Where, ΔA = change in absorbance (0min – 4min)

### 3.2.7 Estimation of NO Activity (By Indirect method of nitrite estimation)

Nitrite was measured using Greiss reagent and is an indicator of the formation of nitric oxide. A 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthylamine diamine dihydrochloric acid in water) was added to 100 µl of animal serum and the optical density was measured at 490 nm (Green *et al*., 1982)

**Calculation**

$$\text{Nitrite value} = \frac{Ab - 0.0082}{0.269}$$

Where Ab = Absorbance at 490nm.

### 3.2.8 Estimation of Tissue Non Protein Thiol and Protein Thiol

**(a) Non Protein Thiol:** 500µl of trichloroacetic acid was added to 500µl of tissue sample, centrifuged at 4000r/min for 10 minute. 20µl of the supernatant was removed and mixed with 170µl of 0.1M phosphate buffer saline along with 10µl DTMB and read using microplate reader at 400 nm wavelength.

**Calculation**

$$NPT = \frac{\text{Absorbance at 400nm} - 0.0062}{0.0047} = X$$

$$NPT = \frac{X}{\text{Total protein}}$$

$$\text{Total Protein} = \frac{\text{Absorbance at 630nm} + 0.0057}{0.1601}$$

**(b) Protein Thiol:** 196µl of buffer added with 10µl sample with 6µl of DTNB. This was followed by 30 minute incubation and measurement at 405nm wavelength using microplate reader.

**Calculation**

$$PT = \frac{\text{Absorbance at 405nm} - 0.0062}{0.0047} = Y$$

$$NPT = \frac{Y}{\text{Total protein}}$$

$$\text{Total Protein} = \frac{\text{Absorbance at 630nm} + 0.0057}{0.1601}$$

### 3.2.9 Tissue Total Protein Assay

50µl of tissue sample was pipetted into the well plate and 150µl of Biuret reagent added into the wells. The mixture was incubatedfor 30minute at 30$^{o}$C and measured at 630nm wavelength with microplate spectrum.

**Calculation**

$$\text{Total Protein} = \frac{\text{Absorbance at 630nm} + 0.0057}{0.1601}$$

## 3.2.10 Serum Myeloperoxidase Protocol

A solution of O-dianisidine dihydrochloride (O-diamistidine) was prepared by dissolving 16.7 mg of o-dianisidine dihydrochloride, 90 mg of distilled water and 10ml potassium phosphate buffer and was freshly prepared for the test. 10µl of serum was transfer into the 96-well plate, 50µl of diluted hydrogen peroxide (4µl of 30% hydrogen peroxide diluted in 96µl distilled water) was then added along with 200µl of o-dianisidine. Four readings were taken at 1minute interval at 450nm.

**Calculation**

$$\text{MPO activity} = \frac{\text{change in absorbance (2min – 0min)}}{0.0113}$$

## 3.2.11 Measurement of Malondialdehyde

The test was done by the method of (Wright *et al*., 1981) with minor changes. The reaction mixture contains 100µl myocardial tissue homogenate, 125µl of trichloroacetic acid (10%), and 125µl thiobarbituric acid (0.67%). All tubes were placed in a boiling water bath for 45 minutes. The tubes were transferred to ice bath and then centrifuged for 25 minutesat 2500×g. The amount of malondialdehyde (MDA) formed in each of the samples was estimated by measuring the optical density of the supernatant at 450 nm in a microplate reader. Results are expressed in nmol of MDA formed/ mg protein.

**Calculation**

$$\text{MDA formed} = \frac{\text{Optical measurement x } 0.28}{156000 \text{ x } 0.02 \text{ x Total Protein}}$$

### 3.2.12 Estimation of Reduced Glutathione Level

The level of this parameter in the myocardial tissue was estimated using the method of Jollow et al., (1974). 1 ml of PMS (10%) was mixed with 1.0 ml of sulphosalicylic acid (4%). Incubation of tissue sample was carried out at $4^oC$ for 1 h and was spinned at 4000r/m for 5 minute at $4^oC$ using a cold centrifuge. The assay mixture consists of 20µl supernatant with 180µl DTNB (4 mg /1 ml). Color is determined and measuredinstantly at 412 nm using a microplate reader. The concentration of GSH was calculated as nmol DTNB conjugate formed/gm tissue.

**Calculation**

$$\text{GSK level} = \frac{\text{Absorbance level} - 0.0062}{0.0042}$$

### 3.2.13 Antioxidant enzymes

**Glutathione Peroxidase Assay:** The following reagents were added to 100µl of myocardial tissue sample: 100µl Phosphate buffer ($K_2HPO_4$), 20µl sodium azide, 40µl reduced glutathione, 20µl hydrogen peroxide and 120µl distilled water. It was then incubated at $37^oC$ for 5minute, and 100µl trichloroacetic acid was then added. It was then spinned at 3000r/m for 5minute. 50µl supernatant was removed and pippeted into 96 micro well along with 100µl Phosphate buffer ($K_2HPO_4$) and 50µl DTNB. GPX – GSH dependent activity was detected with microplate reader at 405nm spectrum.

**Enzyme activity was calculated using the formula:** $\frac{\text{GSH Concentration}}{\text{Total Protein}}$

**GSH concentration** = 245.34 – Remained GSH

**Remained GSH** = $\frac{\text{Absorbance level} - 0.0062}{0.0042}$

Unit of GPX level is nmol/min/ mgprotein

**Glutathione-S-Transferase Assay:** 10µl of myocardial tissue sample was transferred into microwell along with 140µl Phosphate Buffer Saline (pH 6.5), 10µl GSH, 100µl cDNB (Chloro-Dinitro-Benzone). Four readings were taken at 1 minute interval (0min, 1min, 2min, 3min) with microplate reader at 450nm. Based on change in absorbance per time GST level was estimated using a formula:

$$\textbf{GST Levels} = \frac{T4\text{-}T0}{3} = Y = \frac{Y}{9.6 \times 0.01 \times \text{Total Protein}}$$

## 3.2.14 Estimation of Serum Advanced Oxidized Protein Product (AOPP)

100µl Buffer Saline (Phosphate pH 7.5) was added with 50µl of serum. 2 minutes later, 12.5µl potassium iodide and 25µl acetic acid was added with the serum and measured at 370nm wavelength using a microplate reader.

**Calculation**

$$\text{Serum AOPP (/ mg protein)} = \frac{\text{Absorbance at 370nm}}{\text{Total Protein}}$$

## 3.2.15 Estimation of serum CRP Level

**TEST PRINCIPLES**

The principle behind using the Kit to determine CRP level in the seum was based on latex-activating immunoturbidimetry. Here, serum CRP attach witha specificCRP agglutinin that waslacedon the latex materials as a coat leading to agglutination. The turbidity caused by clumping wassensed through optic lightof spectrophotometer. Thechange in absorbance is proportional to CRP level present inside the sample. The real concentration was obtained by comparism with a standard curve of a known concentrations.

**Reagents: R1:** Glycine buffer solution. Sodium azide < 0.1%,

**R2:** Latex suspension, anti-CRP antibodies, glycine buffer, sodium azide < 0.1%

**Procedure**

2 µl of serum sample was added with 225 µl of R1 (Glycine buffer solution. Sodium azide < 0.1%).Aftermixing, incubation at 37°C for 1~5 minutes was done. Addition of 75 µl of R2 (Latex suspension, anti-CRP antibodies, glycine buffer, sodium azide < 0.1%) takes place, thoroughly mixedand at 37°C, it was incubated for 10 seconds. Optical density measurement OD 1 was taken by a spectrophotometer and optical density measurement OD 2 was taken 5 minutes after the first measurement. Optical density change was measured (ΔOD) through a formula;**ΔOD = OD 2 – OD 1**

### 3.2.16 Estimation of serum CK-MB Level

**Reagents: R1:** Imidazole Buffer, pH 6.7 (110 mmol/l), Glucose (21mmol/l), mg Acetate (11mmol/l), EDTA (4.2mmol/l),N-Acetylcysteine (24mmol/l), CK-MM (2000U/l)

**R2:** Imidazole Buffer, pH 6.7 (110 mmol/l), Creatinephosphate (186mmol/l).

**Procedure**

Working reagent was prepared through adding R1 with R2 at the ratio of 5 to 1 (V:V). This working reagent was stabled up 24 hours at +20°C to +25°C. Maintaining a temperature of 37°C, 500 µl of reagent was added with 20 µl of serum inside the tubes using a micro-pipette. After mixing, incubation occur for 5 minute with the first absorbance A1 taken at 340nm using a spectrophotometer. 5 minute later second absorbance A2 was read at 340nm using a spectrophotometer.

**Calculation**: Change in absorbance (ΔA) = A2 – A1 was applied to a formula: ΔA/min. x 1651 to determine the CK-MB level.

### 3.2.17 Estimation of tissue P65NF-KappaB Level

**Procedure**

| Components | Amount |
|---|---|
| Control Solution (24ng/ml) | 0.5ml |
| Diluent ( Standard) | 3ml |
| Streptavidin-HRP | 6ml |
| Stopping Solution | 6ml |
| Solution A with Substrate | 6ml |
| Solution B with substrate | 6ml |
| Buffer wash solution (30x) | 20ml |
| Biotin-Conjugate with Anti-Rat NF-$_k$B Antibody | 1ml |
| Guideline for user | 1 |
| Plate Sealer | 2 pieces |
| Zipper bag | 1 |

Before homogenization, myocardial tissue was washed with Phosphate Buffer Saline (pH 7.4). The tissue was homogenized usingphosphate buffer saline (pH7.4) with a glass homogenizer on ice. It was later centrifuged at 5000 RPM for 15 minutes.

50µl control solution was added to the control. 40µl was taken from the homogenized myocardial tissueand added to sample wells along with 10µl $NF_kB$ agglutinin, this was followed by the addition of 50µl streptavidin-HRP to sample wells and standard well. After the micro-well was shaken to ensure mixture, it was covered with a sealer and incubated for 60 minutes at 37°C. The sealer was removed and the plate washed 5 times with the buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute per each wash. This was followed by 50µl substrate solution A addition to each well, along with 50µl substrate solution B. The plate was incubated covered with another sealer for 10 minutes at 37°C in the dark. 50µl Stop Solution was added after 10 minutes to each well so that the blue color will change into yellow immediately. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

### 3.2.18 Immunohistochemical Staining Protocol

Immunohistochemistry was used to detect expression level of myocardial tissue bcl2, bax, p65NFK$_k$B and p53 using their respective antibodies. Myocardial tissues were preserved with 10% formlaldehyde andpieces of tissue 5 microns thickwere cut into glass slide with polylysine coating. To extract antigens,sections were boiled in 10 mM citrate buffer (pH 6.5) for 20 minutes. The products of this stage from section were incubated with hydrogen peroxide for 15 minutes to reduce non-specific staining then washed three times (5 minutes each) with 1X PBST (0.05% Tween-20). A blocking solution was injected for 10 minutes, then the sections were incubated with diluted bioss primary antibodies of bcl2, bax, p53 and p65NF$_k$B. Anti-NF-$_k$B (p65) (dilution1:300), anti BCL-2(1:200), ant-BAX (1:200) and anti-p53 (dilution 1:200) overnight at 4°C. Further processing was carried out using the anti-polyvalent system Ultra Vision plus Detection, HRP/DAB (ready for use) from Thermo-scientific system. The peroxidase complex was visualized using 3, 3'-diaminobenzidine (DAB). Finally, the slides were counterstained with haematoxylin, cleaned in xylene, dehydrated with ethanol and microscopic analysis was performed (Olympus BX-51) after DPX assembly.

### 3.2.19 Histological examination

Hearts obtained from all experimental group were immediately stored in 10% formalin solution. After that, the heartsare processed, dehydrated and immersed in paraffin. Then the tissues were stained with hematoxylin and eosin and examined under a high power microscope and photomicrographs were taken.

### 3.2.20 Gas Chromatography - Mass Spectroscopy Principle

An Agilent instrument with 7890 GC technology was used for analysis, and an Agilent detector model with 5975 MSD technology (Mass Spectroscopy Detector) was used. In the methods of chromatographic separation, there are two phases that include mobile and stationary phases. The mobile phase was the carrier gas (Helium, 99.99% purity), and the stationary phase was the column. Model HP5 MS column with a length of 30 m, an internal diameter of 0.320 mm and a thickness of 0.25 microns. The oven temperature program is an initial temperature of $80^{o}$c which must be maintained for 1 minute. It increases by $10^{0}$ per minute to the final temperature of $240^{0}$c for 6 minutes. The injection volume was 1 microlitre and the temperature of heater or detector was $250^{0}$C.

Operation: The sample extracted was placed in a vial bottle and the vial bottle was placed in auto injector sample compartment. The automatic injector introduced the sample into the liner. The mobile phase pushed the sample of the liner into the column, where the separation into different component occurs at different retention times. The MS interprets the MZ spectrum (mass to charge ratio) with molar mass and structures.

## 3.3 Data Analysis

Results arepresented as means ± SEM (Standard error of mean). Between two groups, comparison was done using paired t-test. For comparison within the groups, one way analysis of variance (ANOVA) was usedwith Tukey's post-hoc test. Result analyses was done using SPSS software version 21. All statistical significance set as $p < 0.05$.

# CHAPTER FOUR

## 4.0 RESULTS

For all results, the values were expressed as mean ± SEM for all the rats in each group. *Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The sign # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).

### 4.1 BLOOD PRESSURE EFFECT

#### 4.1.1 SYSTOLIC, DIASTOLIC AND MEAN PRESSURE EFFECT

In the infarction control rats, there was a significant decrease in systolic, diastolic and mean arterial pressure (p <0.05) compared with non-infarction control rats. There was a significant increase inblood pressure parameters in the pre-treated rats (250 mg/kg, 500 mg/kg *C. olitorius* 70% ethanol extract and 10 mg/kg enalapril) compared with the infarction control rats.

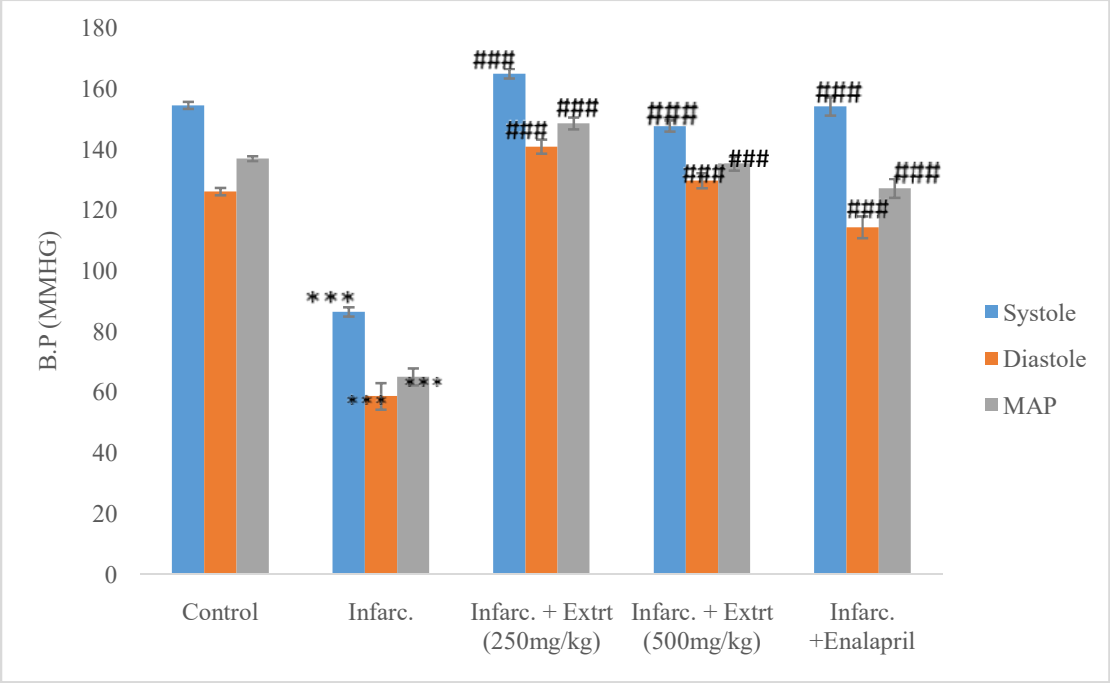



**Fig. 4.1.1:The effect of 70% ethanol leaf extract of*C.olitorius* on the systolic, diastolic and mean arterial pressure inisoproterenol-induced AMI in rats.Bar chartrepresents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001)**

### 4.1.2 EFFECT ON THE VOLUME OF ARTERIAL BLOOD

Arterial blood volume was reduced ($p<0.05$) in rats with infarction compared to the non-infarction control. Blood volume increased significantly when 250 mg / kg*C. olitorius* extract was administered to rats. A slight increase in blood volume was observed at 500 mg/kg pre-treatment group but insignificant. Pre-treating the rats with enalapril (10 mg/kg) does not increase the blood volume.

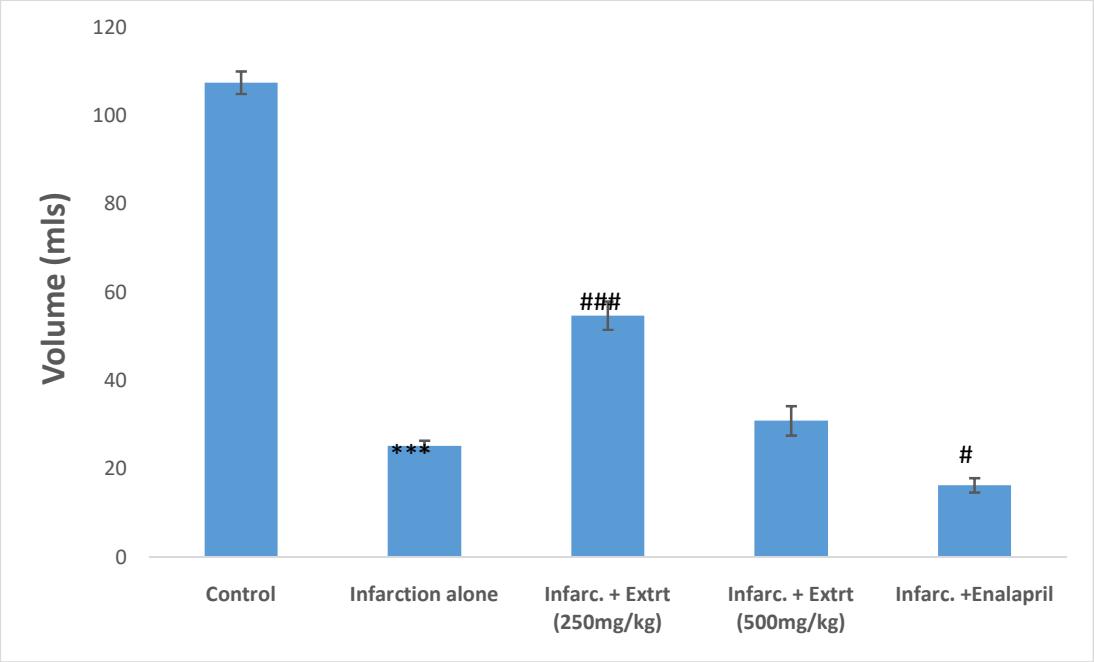



**Fig. 4.1.2**: **The effect of 70% ethanol leaf extract of*C.olitorius* on the arterial blood volumeinisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001)**

### 4.1.3 EFFECT ON BLOOD FLOW

Isoproterenol-induced acute myocardial infarction reduced blood flow significantly ($p<0.05$). When rats were pre-treated with 250 mg/kg, 500 mg/kg *C. olitorius*ethanol extract and 10 mg enalapril, the blood flow was significantly increasedalthough not to the level of normal control rats.

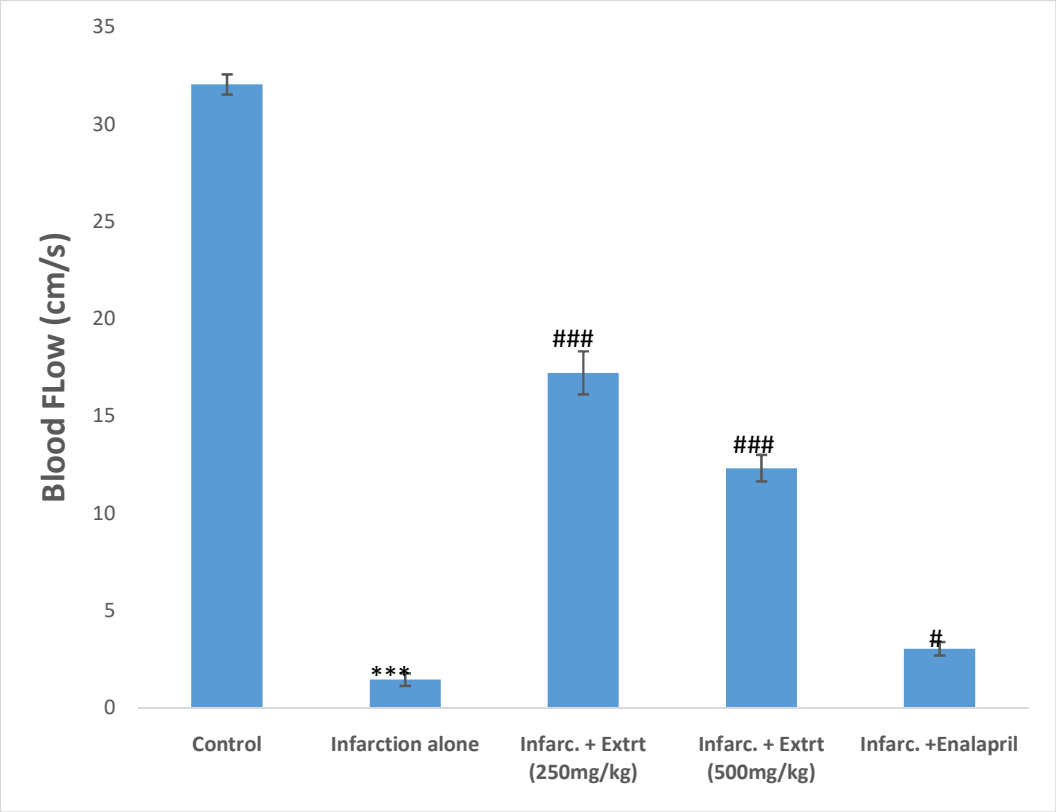


**Fig. 4.1.3:The effect of 70% ethanol leaf extract of*C.olitorius* on the blood flowinisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.1.4 EFFECT ON HEART RATE

The heart rate was significantly decreasedin the infarction control ratscompared with non-infarction control rats, and the rats pretreated with250 mg/kg, 500 mg/kg *C. olitorius* ethanol extract and 10 mg/kg enalapril showed increase in the heart rate.

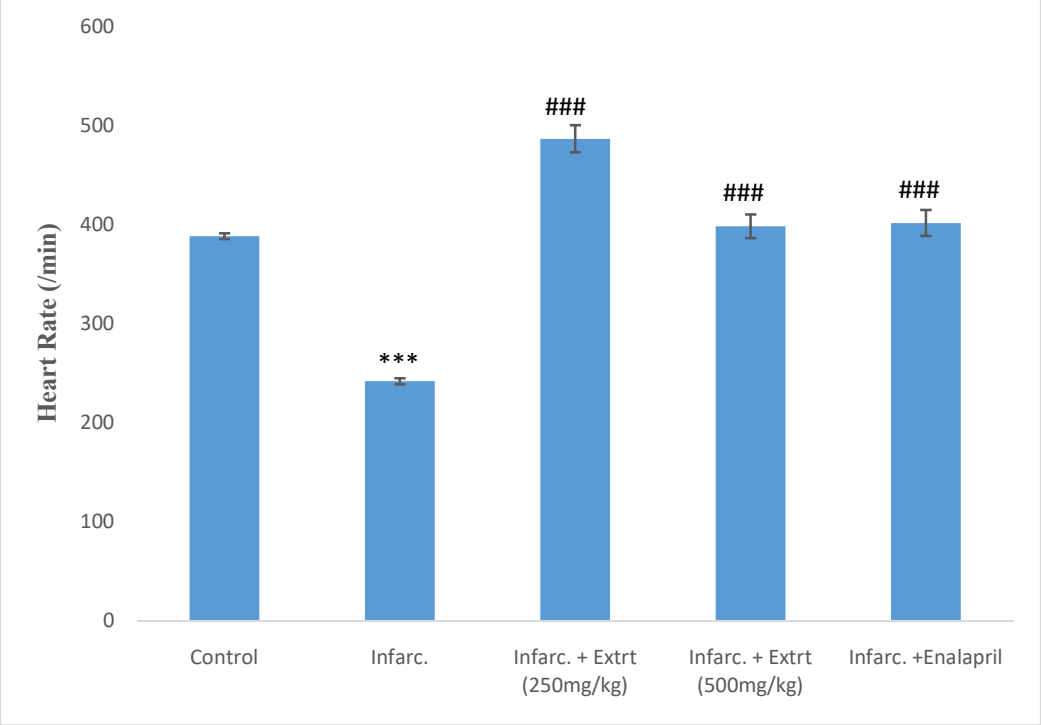



**Fig. 4.1.4: The effect of 70% ethanol leaf extract of*C.olitorius* on theheart rateinisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.2 EFFECT ON ELECTROCARDIOGRAM

### 4.2.1 EFFECT ON P-INTERVAL AND QRS COMPLEX

Isoproterenol-induced acute myocardial infarction increased the QRS interval in the infarction control rats compared to the normal control rats. Pre-treating the rats with 250 mg/kg extract significantly reduced QRS interval ($p<0.05$) while the rats pre-treated with 500 mg/kg extract and 10 mg/kg enalapril also significantly ($p<0.05$) narrowed the QRS interval. The reduced p-intervalobservedin the myocardial infarction controlrats was increased ($p<0.05$) in the pre-treatment groups.

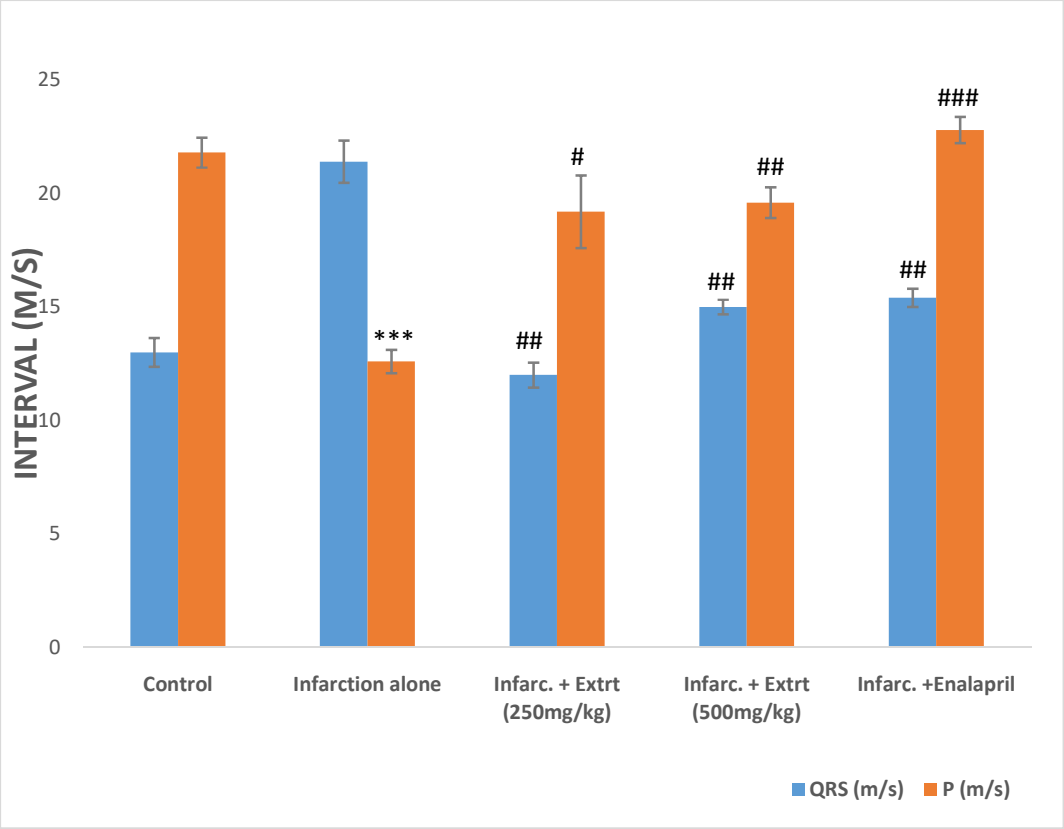



**Fig. 4.2.1: The effect of 70% ethanol leaf extract of*C.olitorius* on the QRS and P-interval (m/s) inisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001)**

### 4.2.2 EFFECT ON R-AMPLITUDE

The R-amplitude was significantly reduced in the infarction control rats compared with the non-infarction rats. Pre-treating with 250 mg/kg ethanol extract significantly increased the amplitude. No significant effect was observed in the 500 mg/kg and enalapril pre-treatment groups.

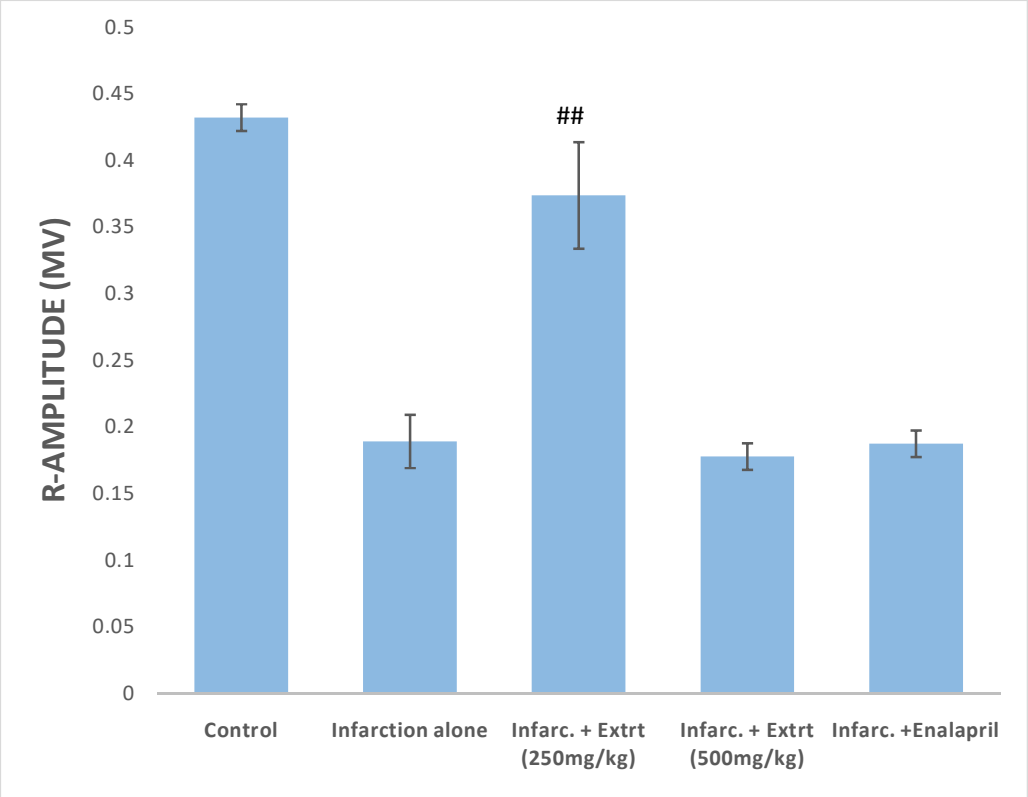



**Fig. 4.2.2:The effect of 70% ethanol leaf extract of*C.olitorius* on the R-amplitude (mv)inisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001)**

## 4.3 EFFECT ON SERUM CARDIAC INJURY MARKER LEVEL

The serum level of lactate dehydrogenase and creatine kinase-MB was significantly increased during myocardial infarction without pre-treatmentwhilethe serum levelof these cardiac injury biomarkers weredecreased when rats were pre-treatedwithextract and enalapril.

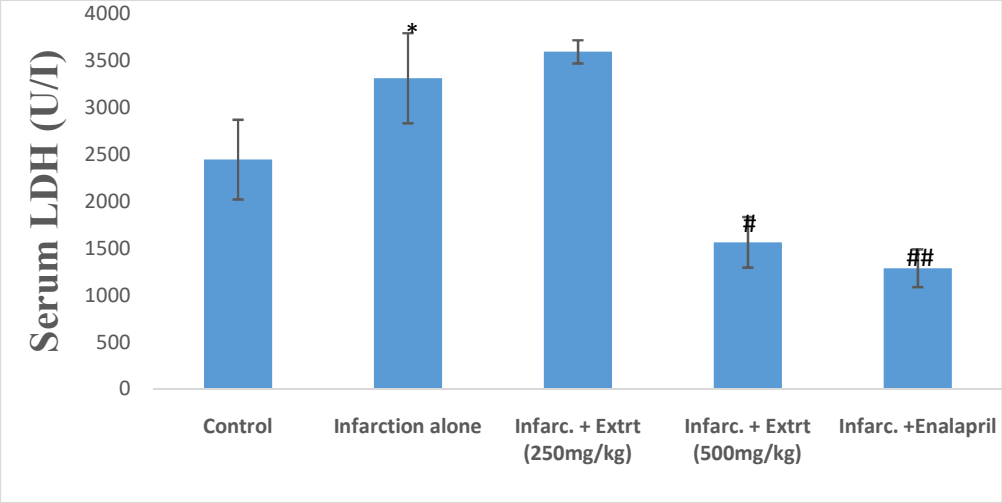



**Fig. 4.3.1:The effect of 70% ethanol leaf extract of*C.olitorius* on the serum lactate dehydrogenaseinisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

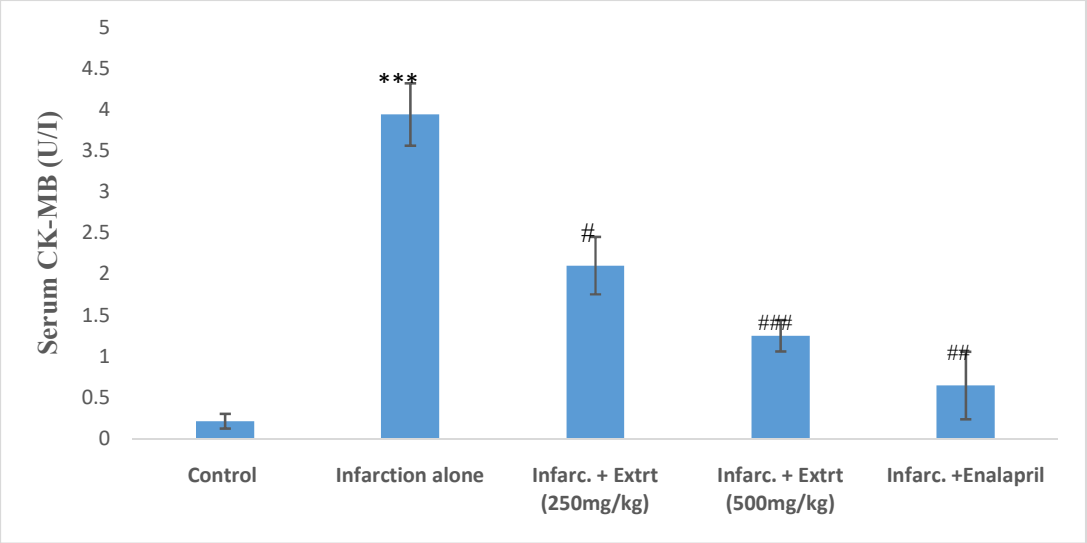



**Fig. 4.3.2:The effect of 70% ethanol leaf extract of*C.olitorius* on the serum creatine kinase-MB levelinisoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.4 EFFECT ON SERUM NITRITE LEVEL

### 4.4.1 PRE-INFARCTION STUDY

TheSlight decrease in serum nitrite level in the infarction group was not significant. The level of nitrite in the ratspre-treated with extract(250 mg/kg and 500 mg/kg) was increased significantly compared with acute infarction ratswithout treatment. Enalapril at a dose of 10 mg/kg also improved serum nitrite level, but insignificantly.

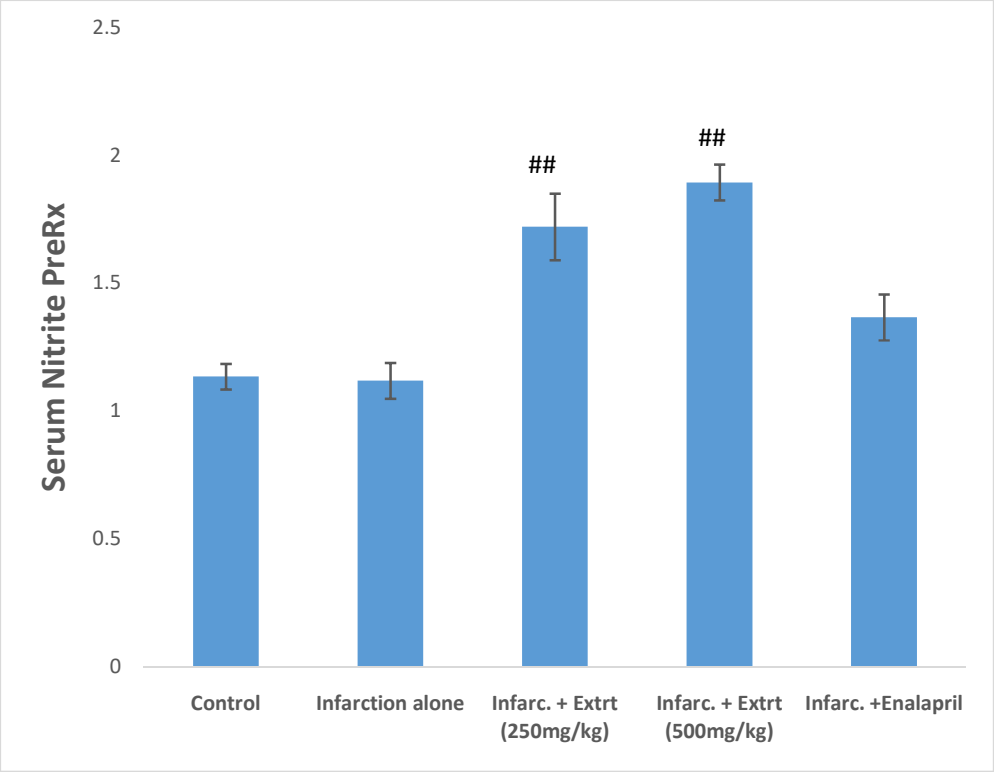



**Fig. 4.4.1: The effect of 70% ethanol leaf extract of*C.olitorius* on the serum nitrite level inisoproterenol-induced AMI inrats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.4.2 POST-TREATMENT

Serum nitrite was significantly reduced in the infarction control rats compared with normal control during post-treatment study. Post-treatment with 500 mg/kgextract increased the serum nitrite level significantly ($p<0.05$).

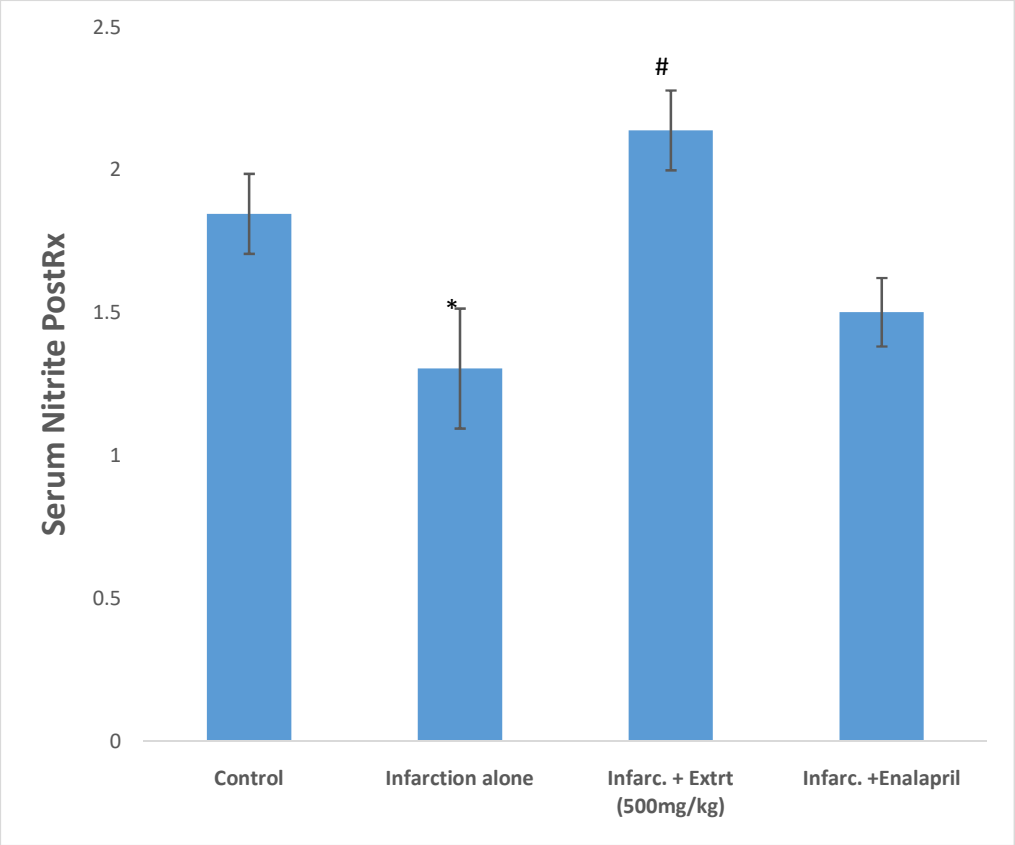



**Fig. 4.4.2:The effect of 70% ethanol leaf extract of*C.olitorius* on the serum nitrite levelin isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.5 EFFECT ON SERUM ADVANCED OXIDIZED PROTEIN PRODUCTS

### 4.5.1 PRE-TREATMENT STUDY

Compared with normal control group, AOPP level in the infarction control rats was very high. Pre-treating the ratswith *C.olitorius* ethanol extract (250 mg/kg and 500 mg/kg) and enalapril (10 mg/kg) significantly reduce AOPP level.

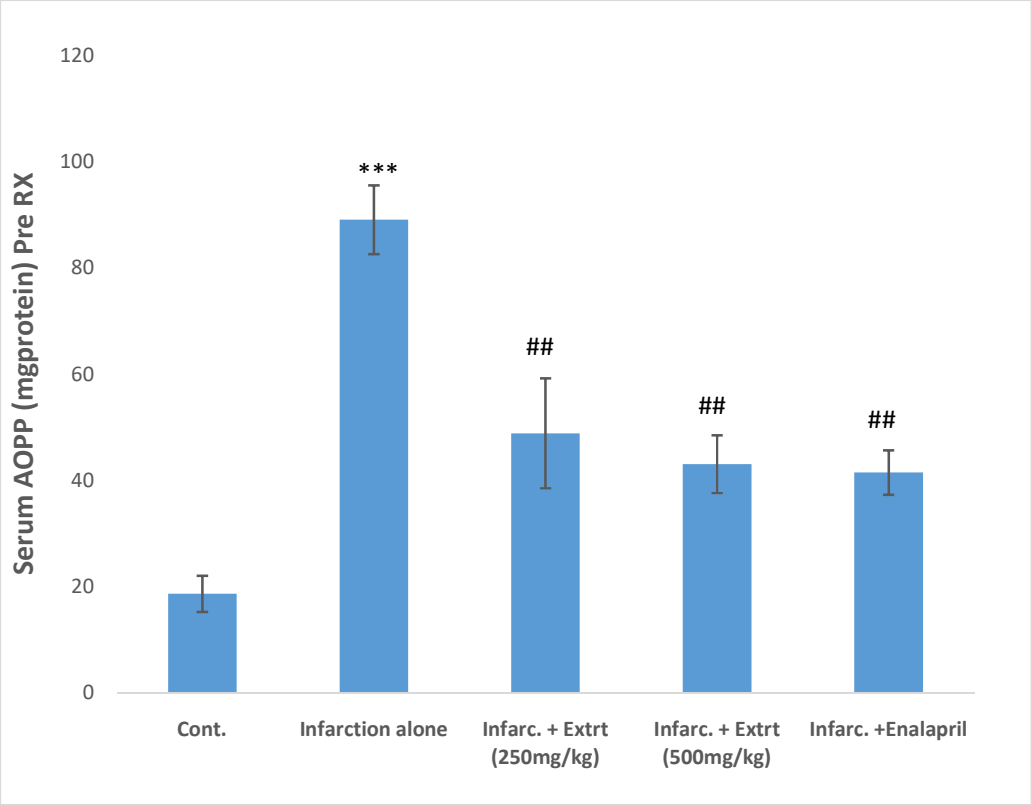



**Fig. 4.5.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the serum AOPP level in Isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.5.2 POST-TREATMENT STUDY

AOPP level was slightly increased in the infarction group but not significant, whereas the level of AOPP in the extract and enalapril treated rats were reduced significantly.

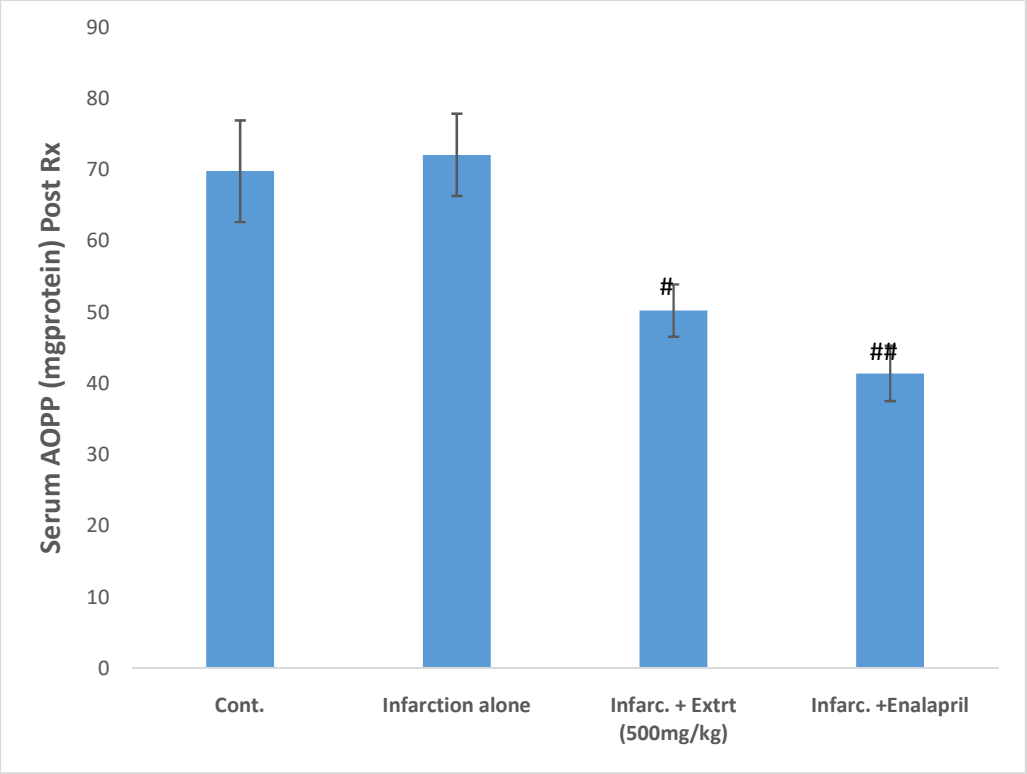



**Fig. 4.5.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the serum AOPP level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.6 EFFECT ON TISSUE PROTEIN AND NON-PROTEIN THIOL

### 4.6.1 PRE-TREATMENT STUDY

Tissue protein thiol level was not affected by either the isoproterenol-induced acute myocardial infarction or the extract but non-protein thiol level in the myocardial tissue was reduced significantly in the infarction group. A significant increase was observed in the pretreatment rats (500 mg/kg extract concentration and 10 mg/kg enalapril).

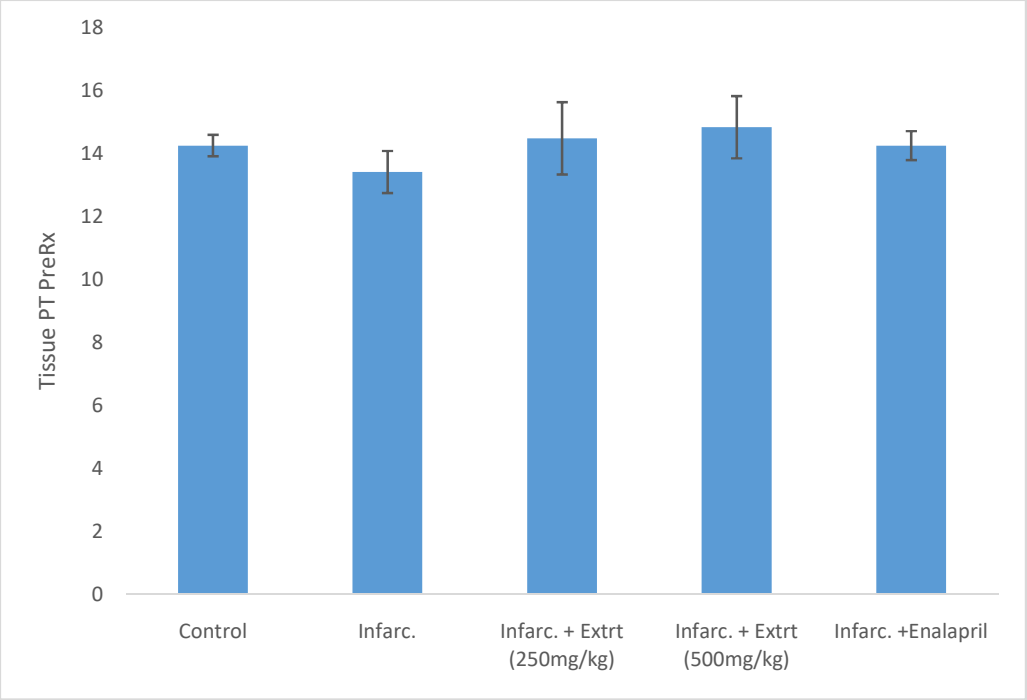



**Fig. 4.6.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue protein thiol level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

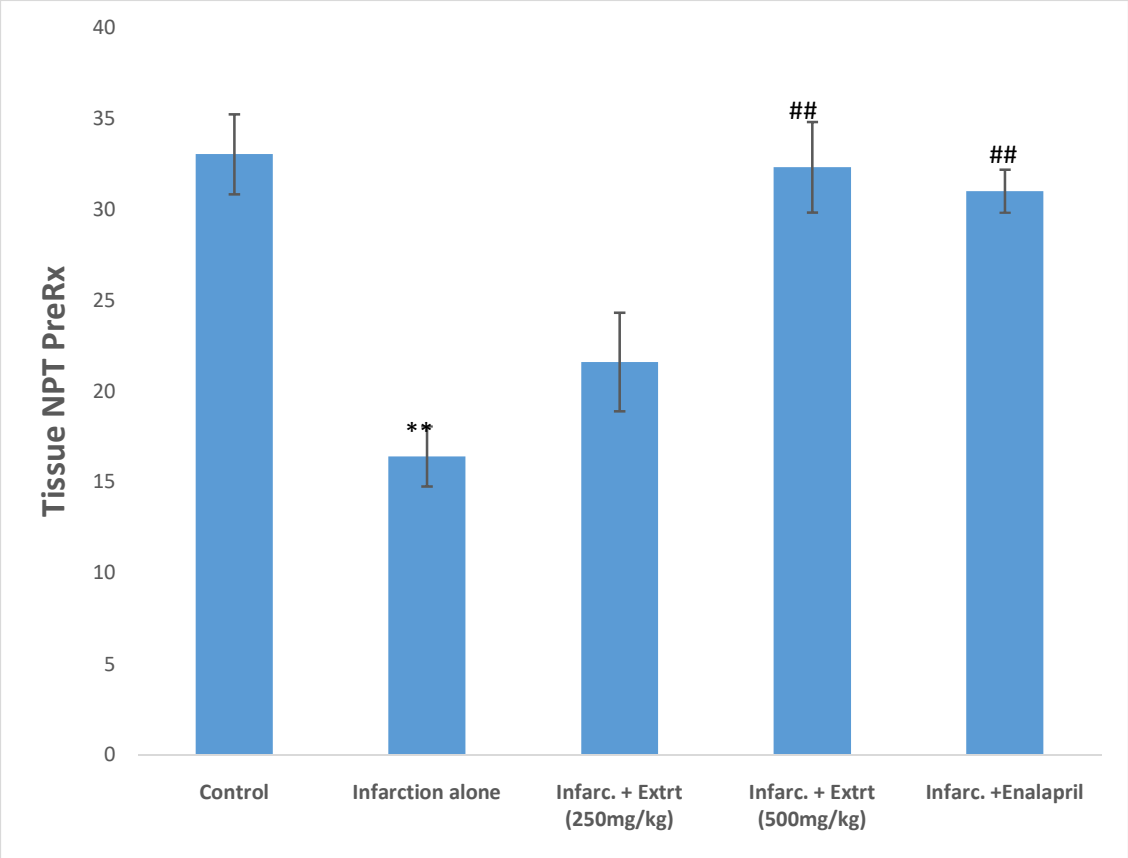



**Fig. 4.6.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue non-protein thiol level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.6.2 POST-TREATMENT STUDY

Protein and non-protein thiol level in the myocardial infarction animal tissue was reduced significantly. A Slight increase in PT and NTP levels was observed in the groups after treatment (500 mg/kg extract and 10 mg/kg enalapril) although it was only enalapril that was significant ($p<0.05$).

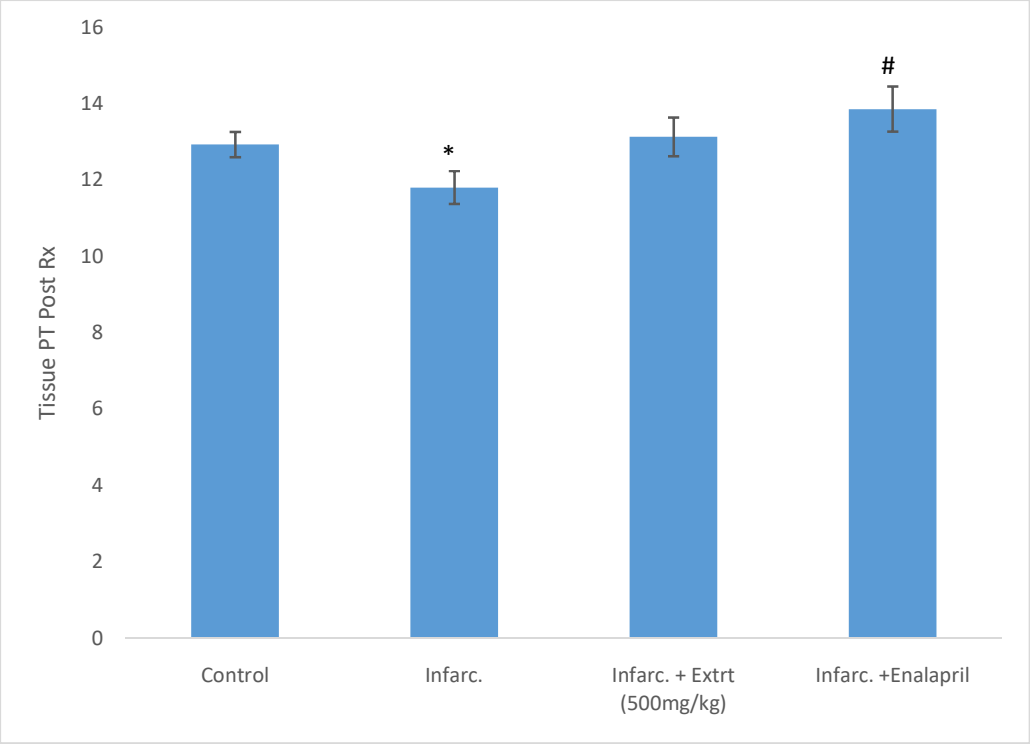



**Fig. 4.6.3: The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue protein thiol level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

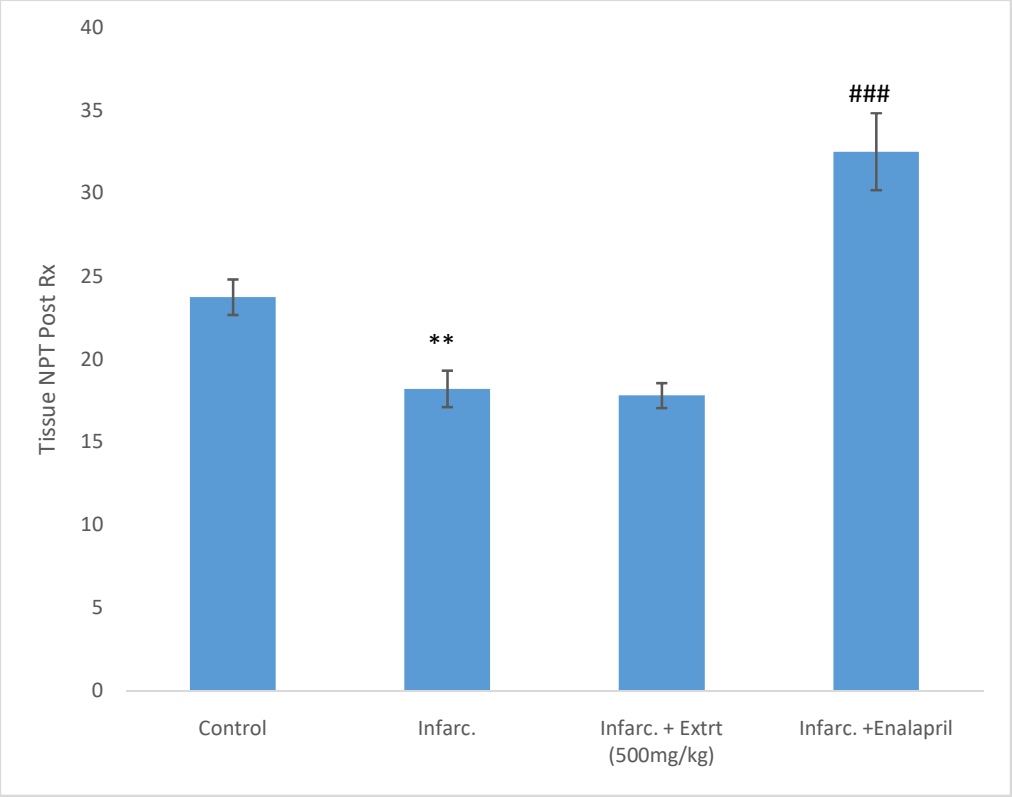



**Fig. 4.6.4:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue non-protein thiol level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.7 EFFECT ON TISSUE REDUCED GLUTATHIONE (GSH)

### 4.7.1 PRE-TREATMENT STUDY

Infarction control rats revealed a significant reduction in tissue level of GSH. Pre-treating the rats with 250 mg/kg & 500 mg/kg extract and enalapril increased the GSH level.

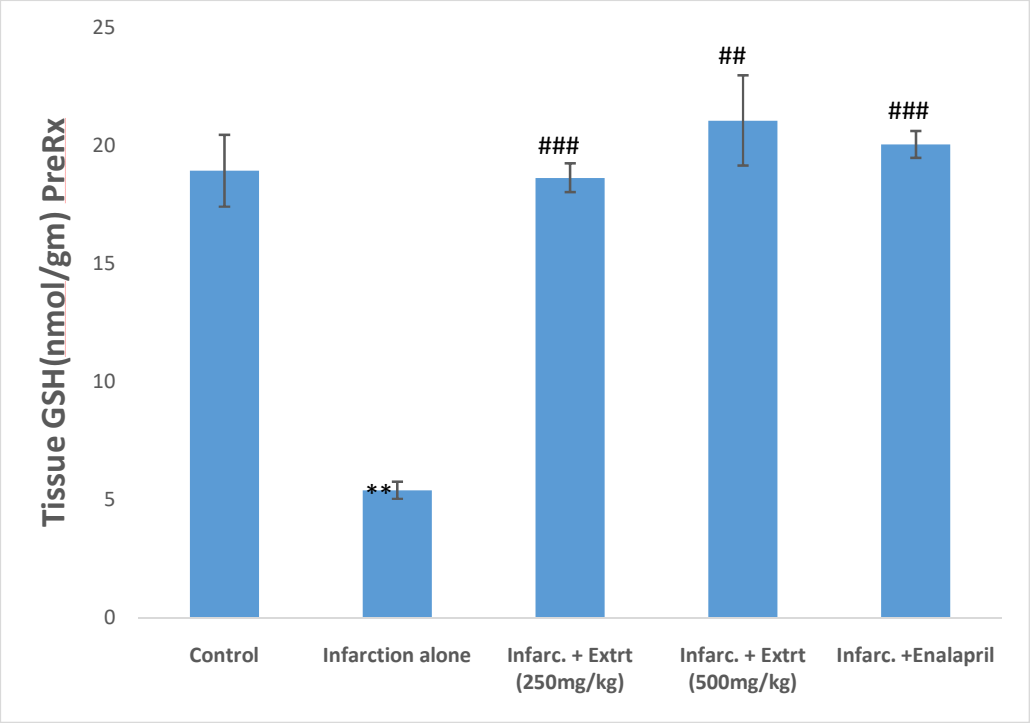



**Fig. 4.7.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue reduced glutathione level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

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### 4.7.2 POST-TREATMENT STUDY

The level of GSH was also reduced in the myocardial infarction rats of post-infarction treatment study and the level was enhanced when post-treated with 500 mg/kg extract.

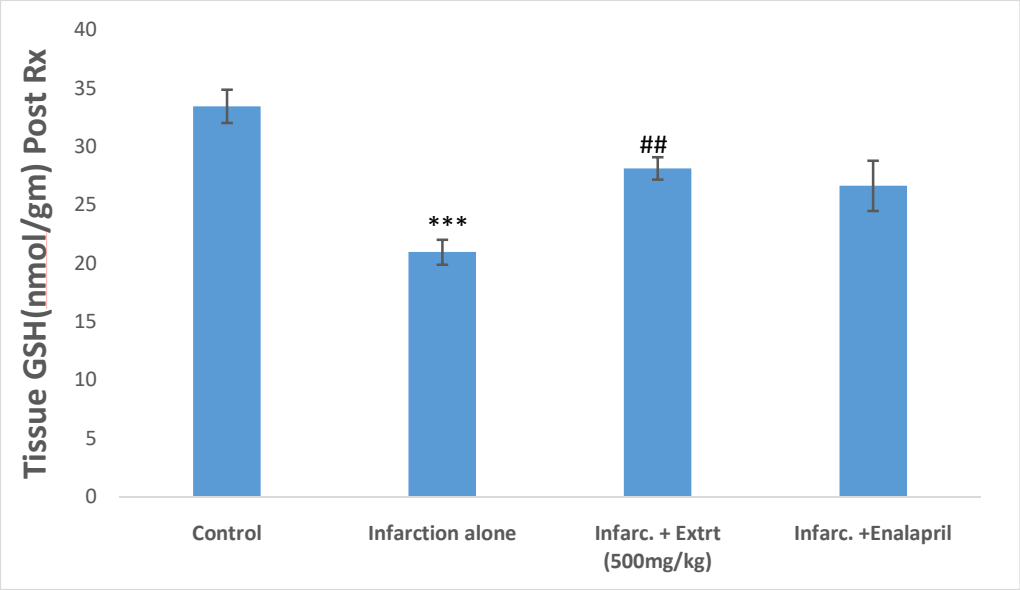


**Fig. 4.7.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue reduced glutathione level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.8 EFFECT ON ANTIOXIDANT ENZYMES

### 4.8.1 PRE-TREATMENT STUDY

The tissue level of glutathione peroxidase was slightly decreasedin the infarction control experimental rats. There was a slight increase ($p>0.05$)in GPx level in the pre-treatment rats (250 mg/kg & 500 mg/kg) but enalapril significantly increase ($p<0.05$) GPx level in myocardial tissue. Glutathione-S-transferase level was also reduced in the infarction control group butonly 500 mg/kg pre-infarction treatment group enhanced myocardial tissue GST level significantly.

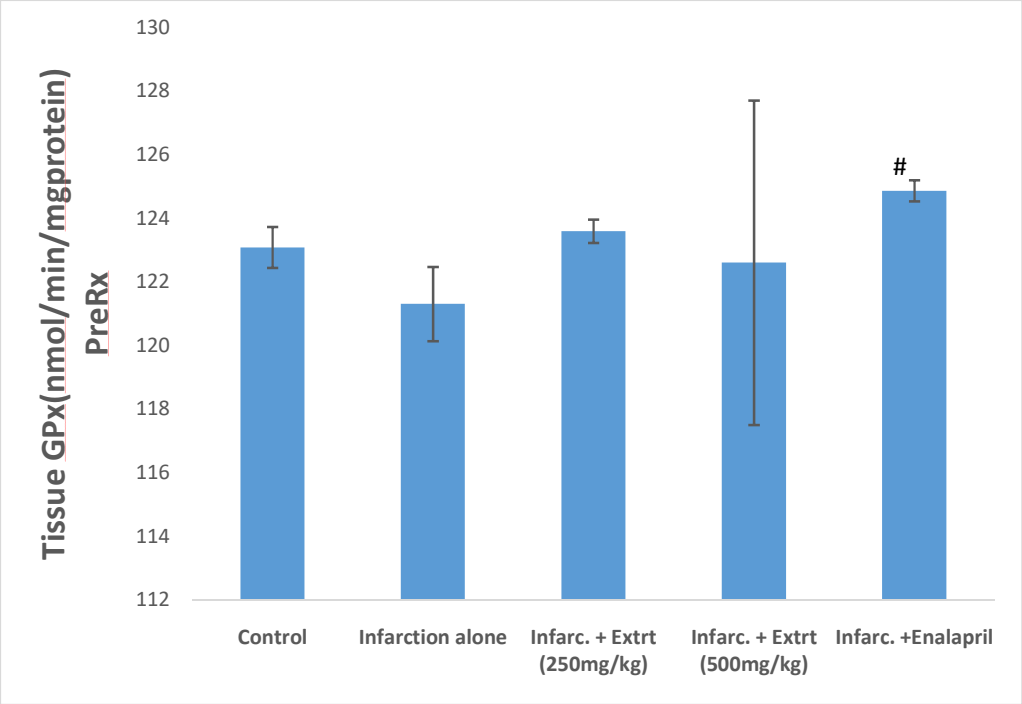


**Fig. 4.8.1.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue glutathione peroxidase level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

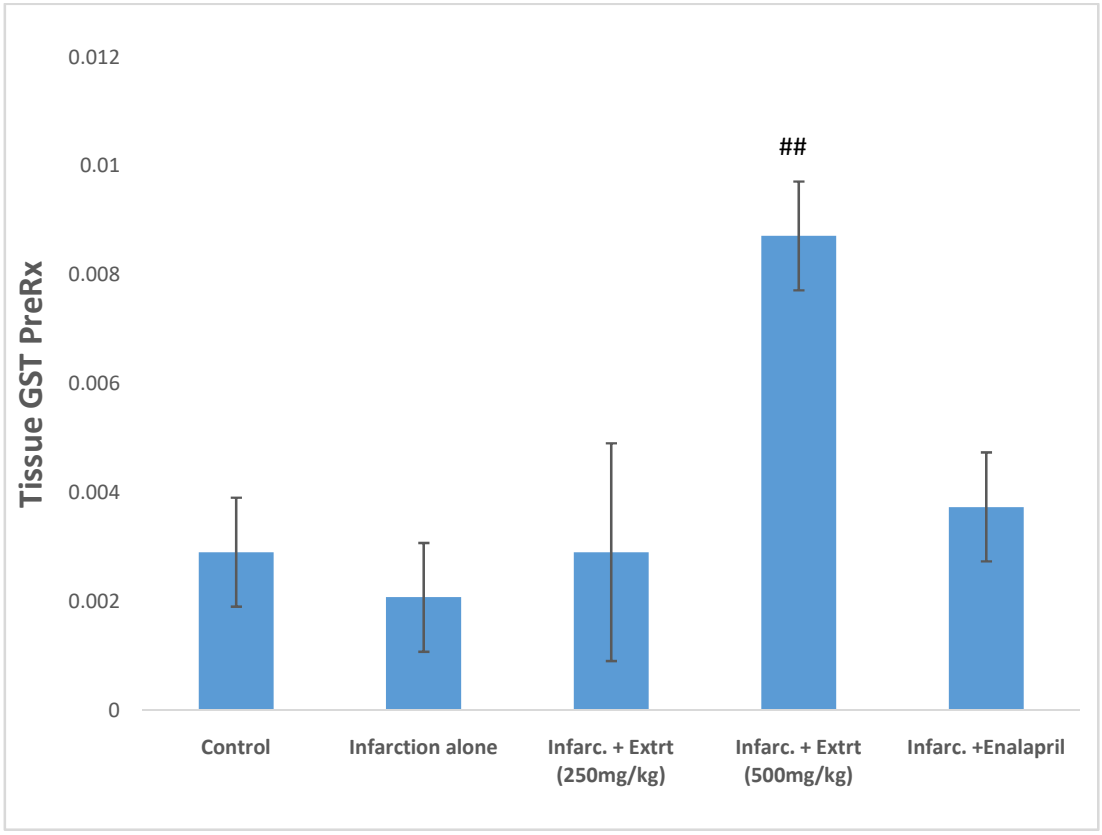


**Fig. 4.8.1.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue glutathione-S-transferase level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.8.2 POST-INFARCTION TREATMENT STUDY

Myocardial tissue GPX level was low in the infarction control group. Post-treatment groups (500 mg/kg extract and 10 mg/kg enalapril) showed no effect on tissue GPx level. Also, GST level was reduced in the infarction control group although not significant and its increased tissue level in the post-treatment groups was not significant ($p>0.05$).

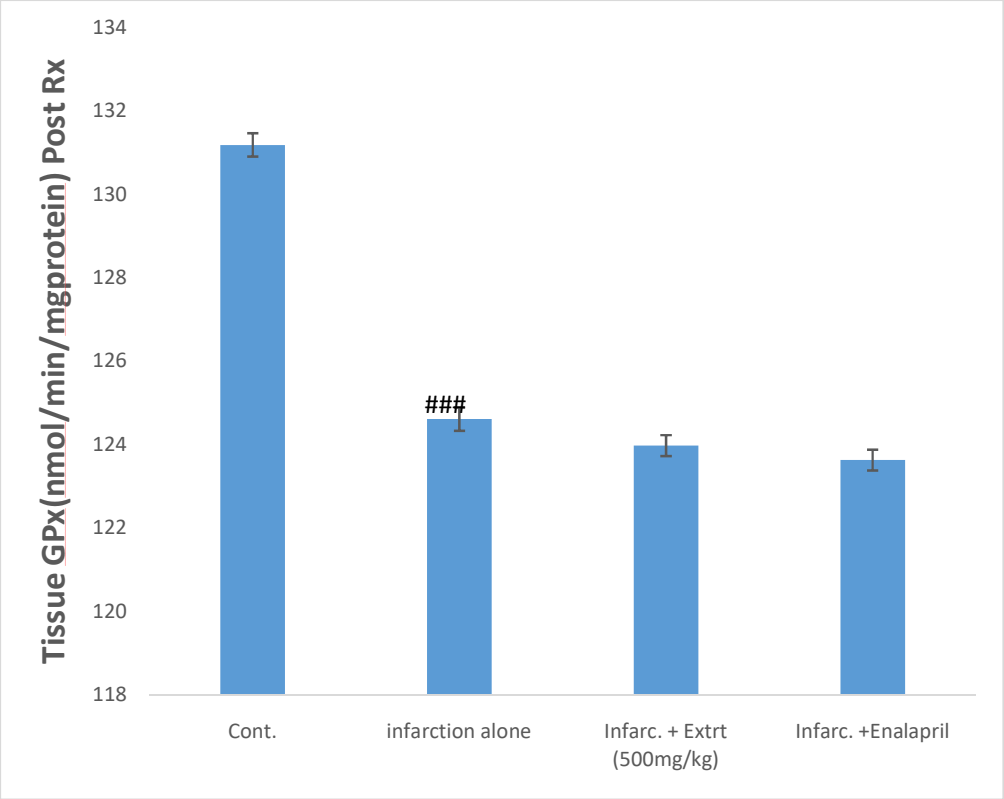


**Fig. 4.8.2.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue glutathione peroxidase level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

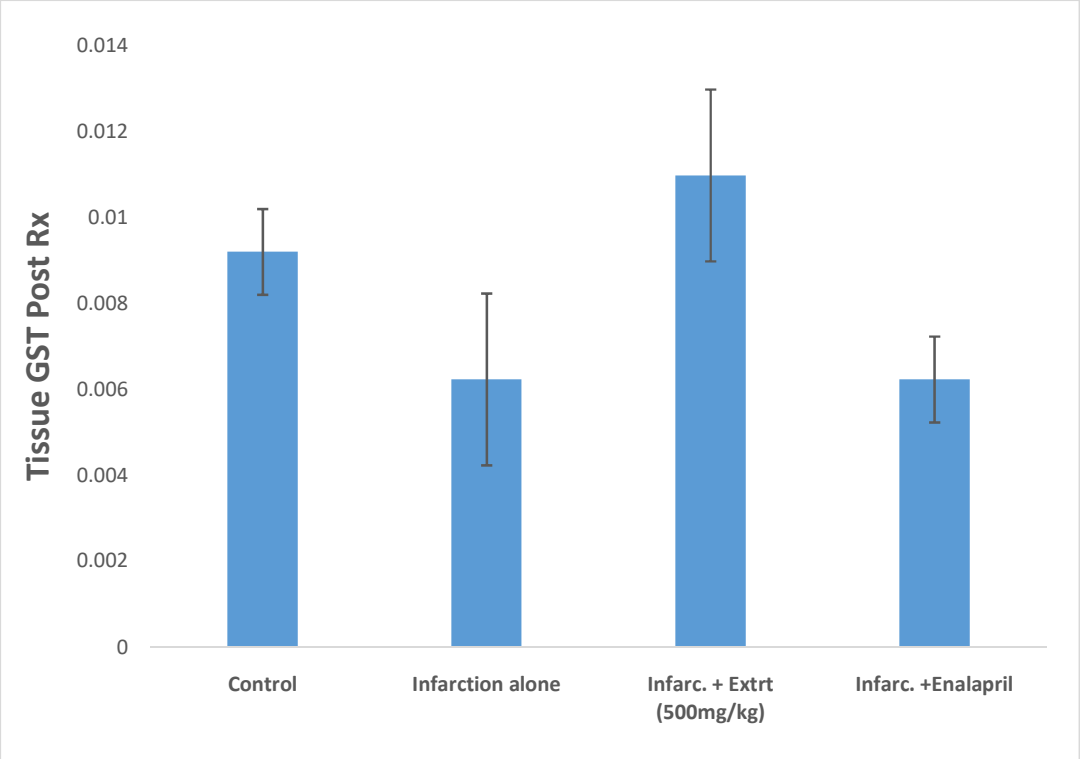


**Fig. 4.8.2.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue glutathione-S-transferase level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.9 EFFECT ON OXIDANTS MARKER

### 4.9.1 PRE-TREATMENT STUDY

Serum myeloperoxidase (MPO) level increased significantly in the infarction control experimental rats. Pre-treating with extracts and enalapril significantly decreased the serum MPO level ($p<0.05$).

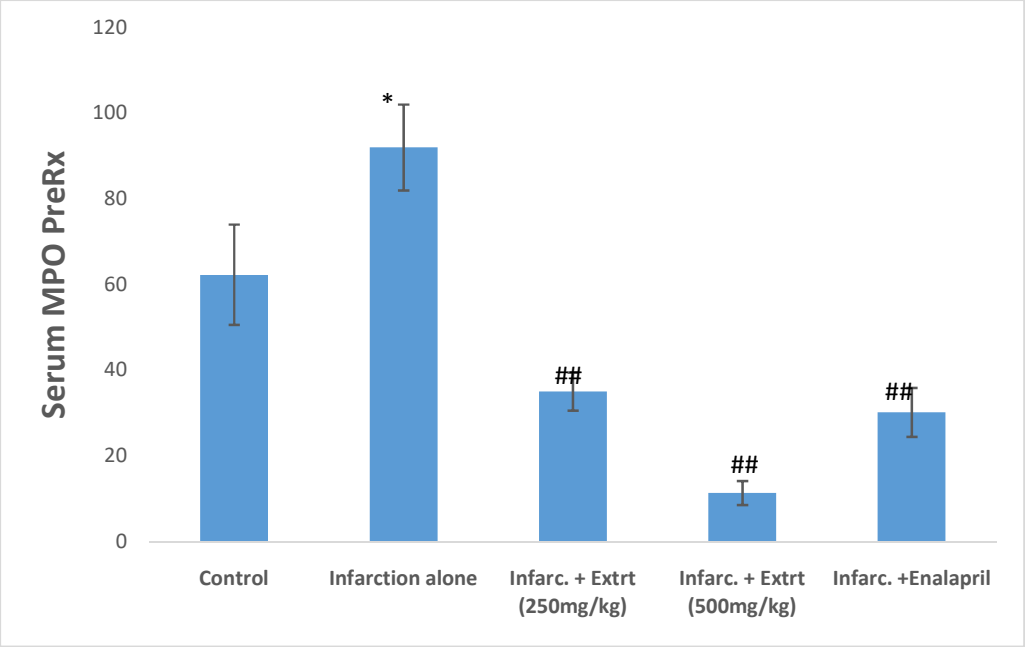


**Fig. 4.9.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the serum myeloperoxidase level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.9.2 POST-INFARCTION TREATMENT STUDY

There was no difference between the serum level of MPO in the infarction and non-infarction ratsbut significant decrease in serum level was observed in rats post-treated with extract and enalapril.

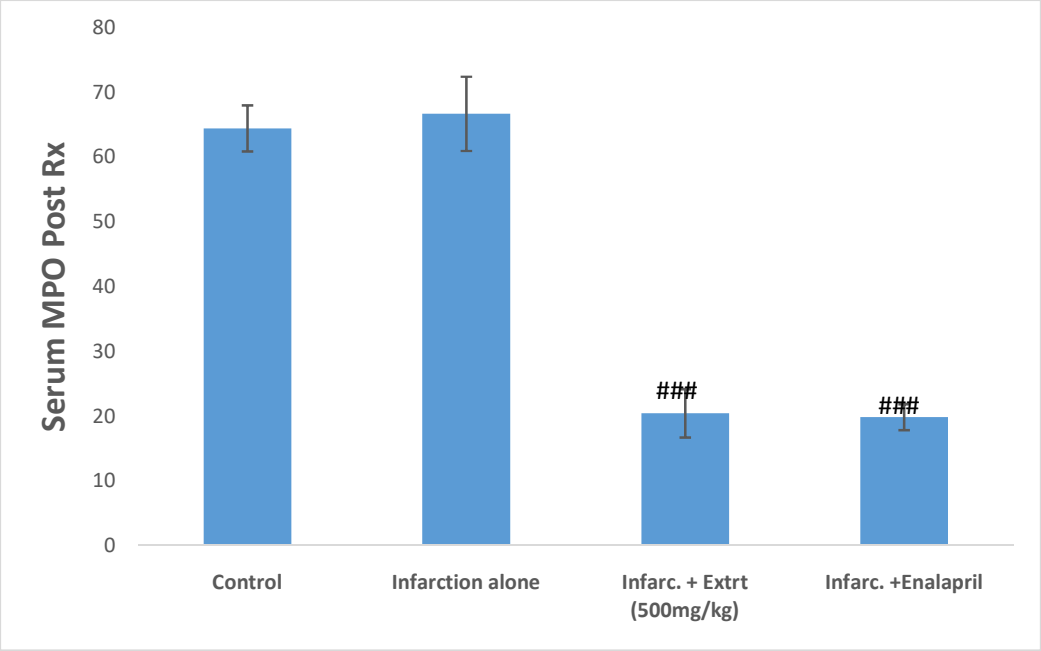


**Fig. 4.9.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the serum myeloperoxidase peroxidase level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.10 EFFECT ON LIPID PEROXIDATION

### 4.10.1 PRE-TREATMENT STUDY

Tissue malondialdehyde (MDA) level was significantly increased in myocardial infarction and MDA level was reduced in the pre-treatment groups.

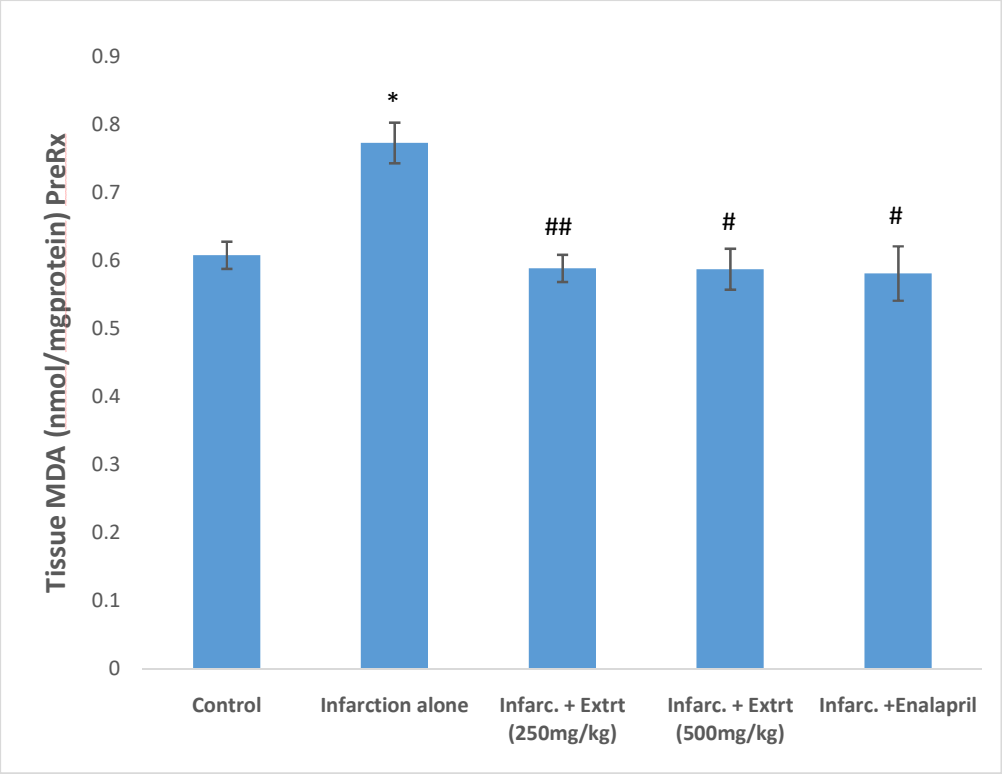


**Fig. 4.10.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue malondialdehyde level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.10.2 POST-INFARCTION TREATMENT STUDY

The increase in tissue MDA level was observedin the infarction treatment group and significant decrease was also observed in enalapril post-treatment group alone ($p<0.05$).

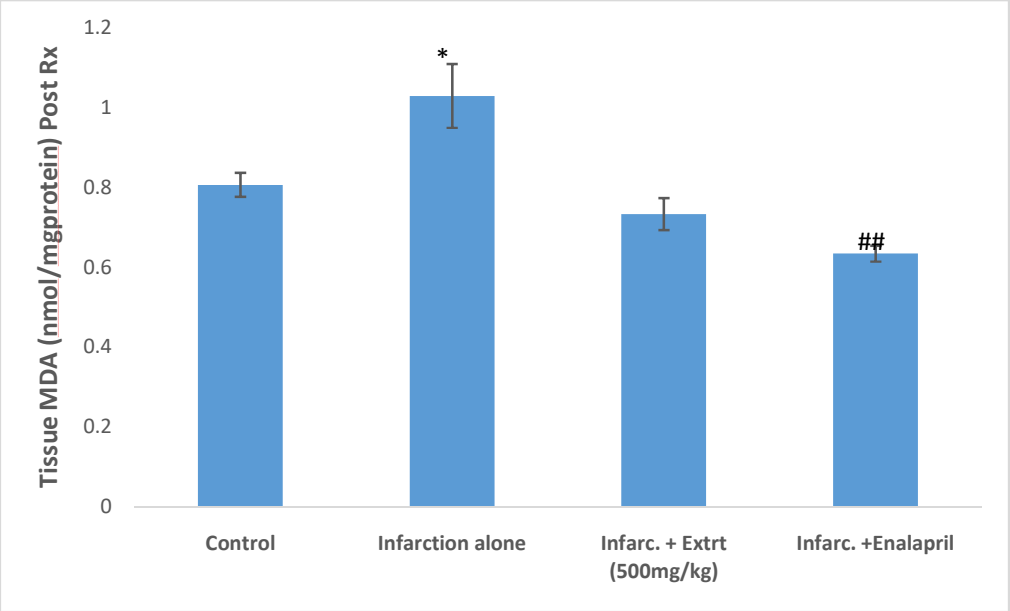


**Fig. 4.10.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue malondiadehyde level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.11 EFFECT ON SERUM AND TISSUE TOTAL PROTEIN

### 4.11.1 PRE-TREATMENT STUDY

Serum and myocardial tissue total protein level was reduced in the infarction rats. 250 mg/kg extract pre-treatment rats revealed,increase in serum and tissue total protein. Serum and tissue level of total protein in the 500 mg/kg extract pre-treatment group was also high. Only the tissue of rats pre-treated with enalapril before infarction showed increase in total protein.

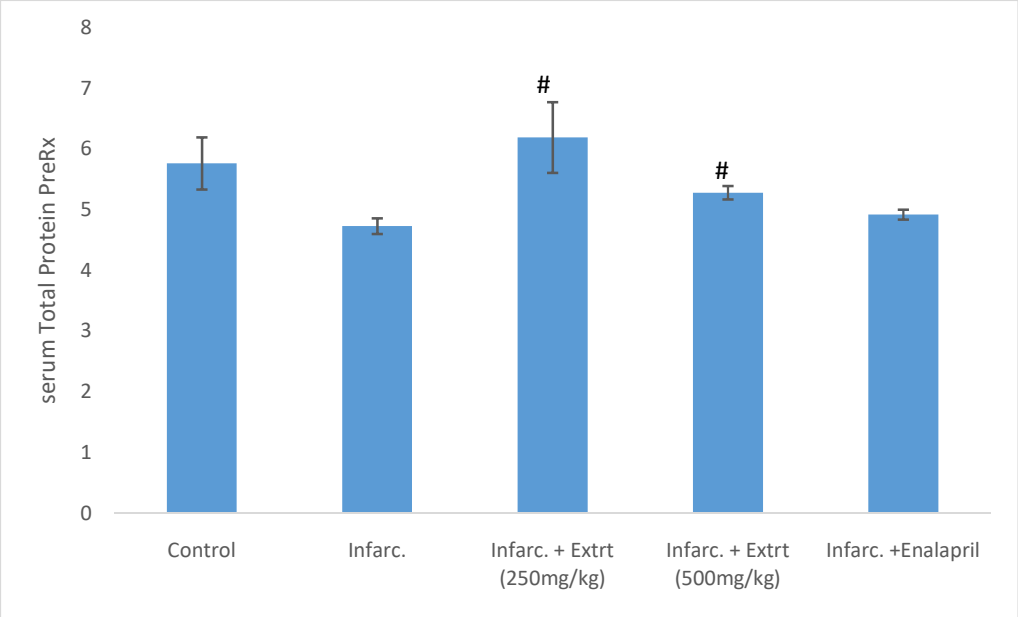


**Fig. 4.11.1.1:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the serum total protein level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

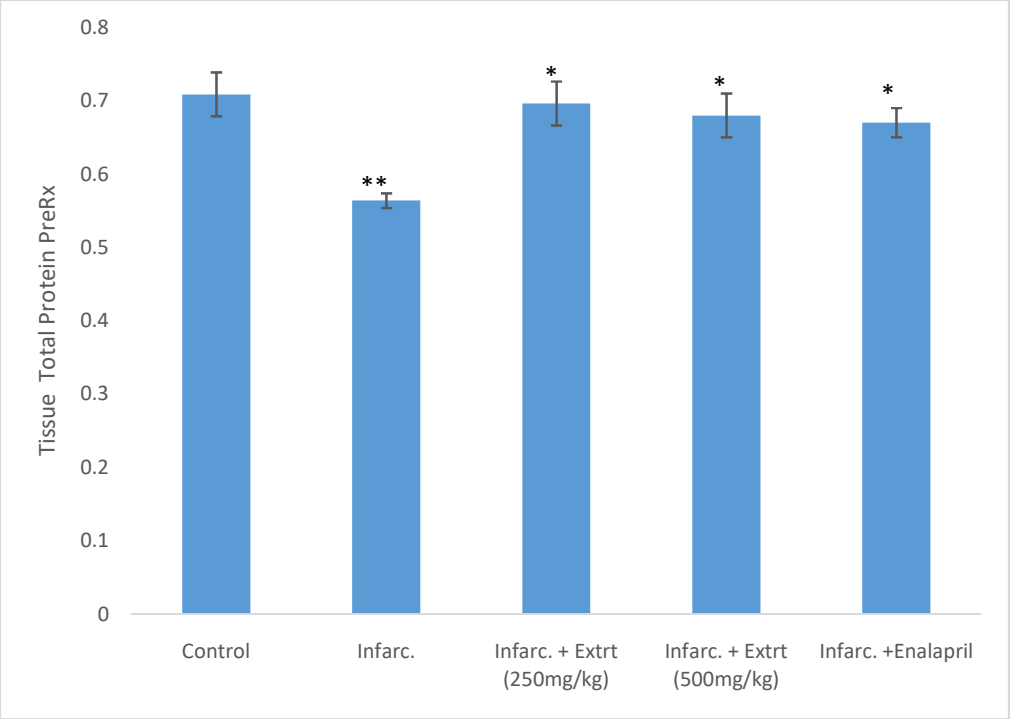


**Fig. 4.11.1.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue total protein level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

### 4.11.2 POST-TREATMENT STUDY

Serum and tissue total protein level was reduced in the infarction group just like pre-treatment study. No significant effect of extract and enalapril was observed on total protein in serum but increase in tissue protein was observed at 500 mg/kg extract and enalapril post-treatment infarction rats.

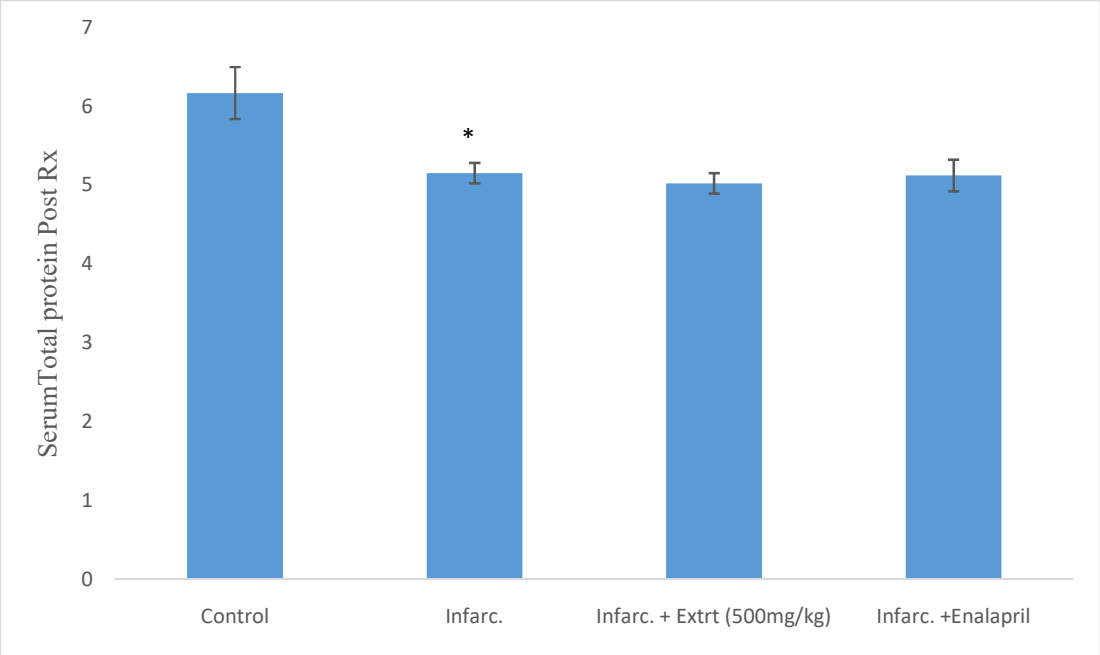


**Fig. 4.11.2.1: The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the serum total protein level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

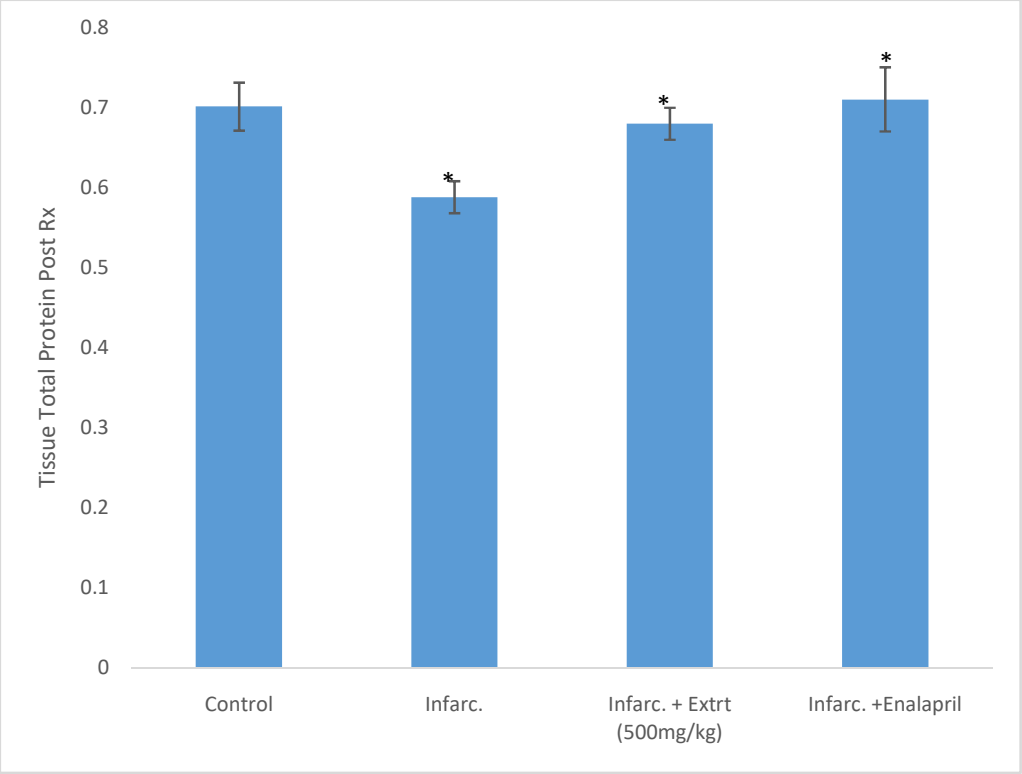


**Fig. 4.11.2.2:The effect of 70% ethanol leaf extract of*C.olitorius* and enalapril on the tissue total protein level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.12 PHOTOMICROGRAPH OF HISTOLOGICAL EXAMINATION

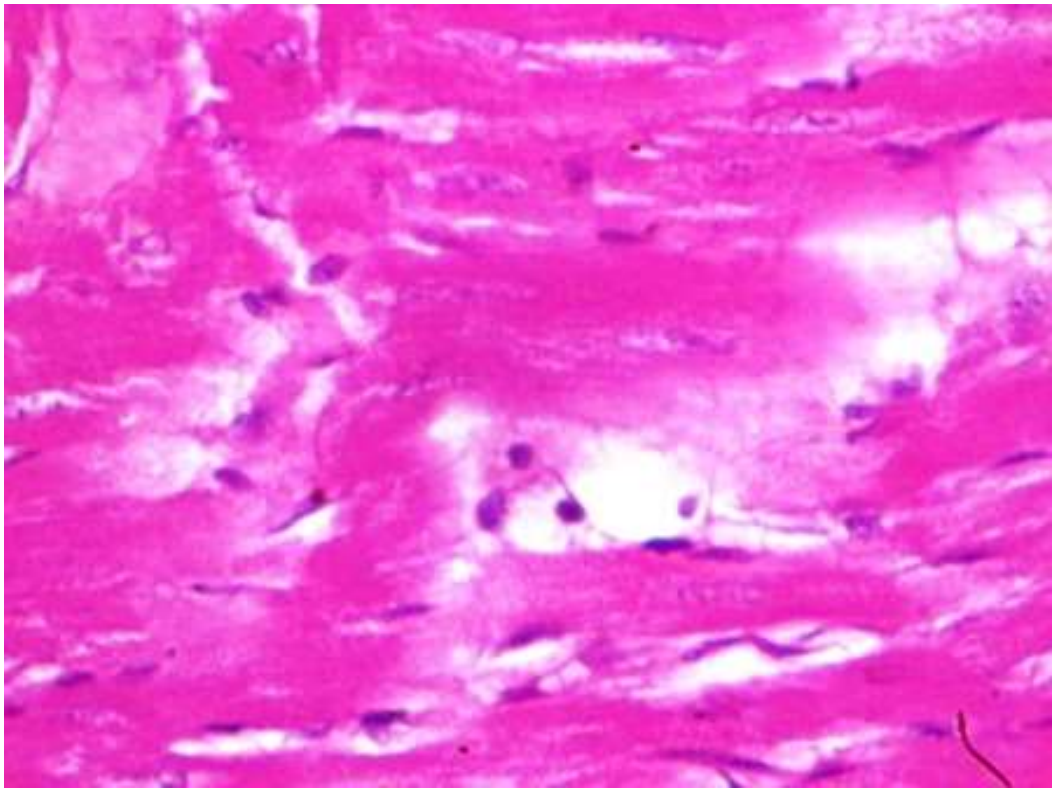

**Fig. 4.12.1 (Normal control group for pre-treatment study): Plate showed no lesion**

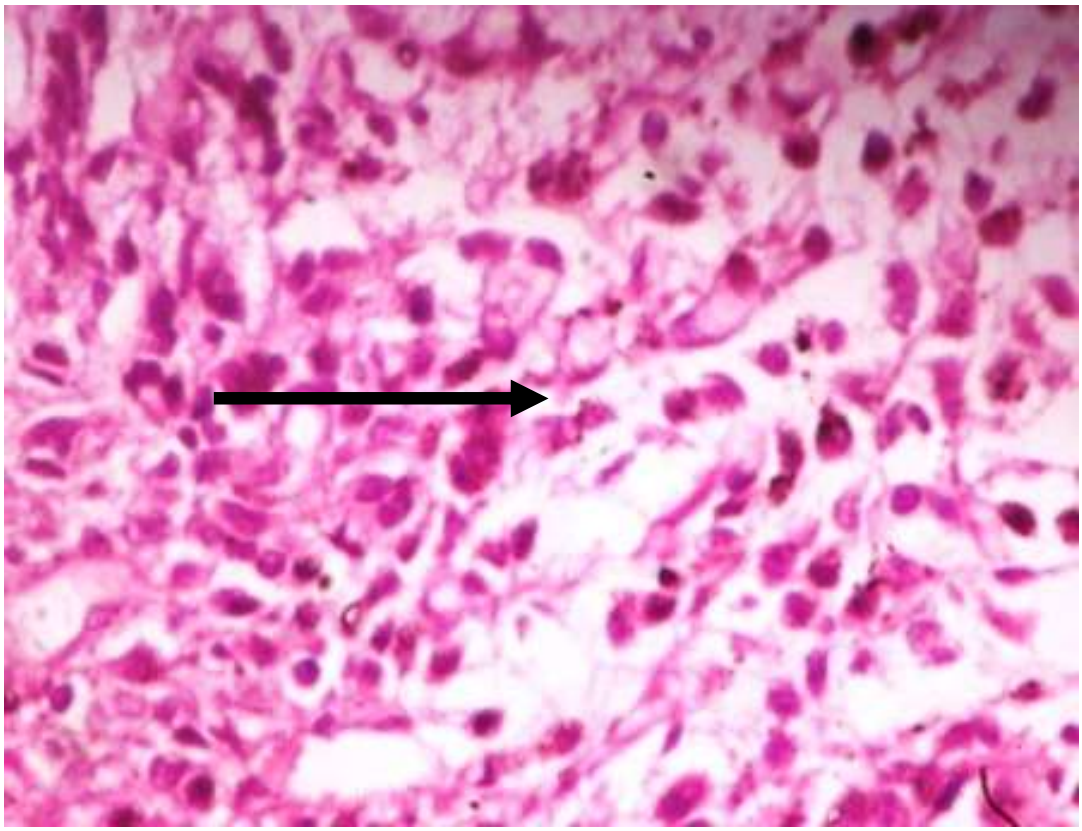

**Fig. 4.12.2 (Myocardial Infarction Alone): Plate showed severe, extensive multifocal area of myocardial infarction with marked infiltration of the myocardium and pericardium with inflammatory cells (black arrow)**

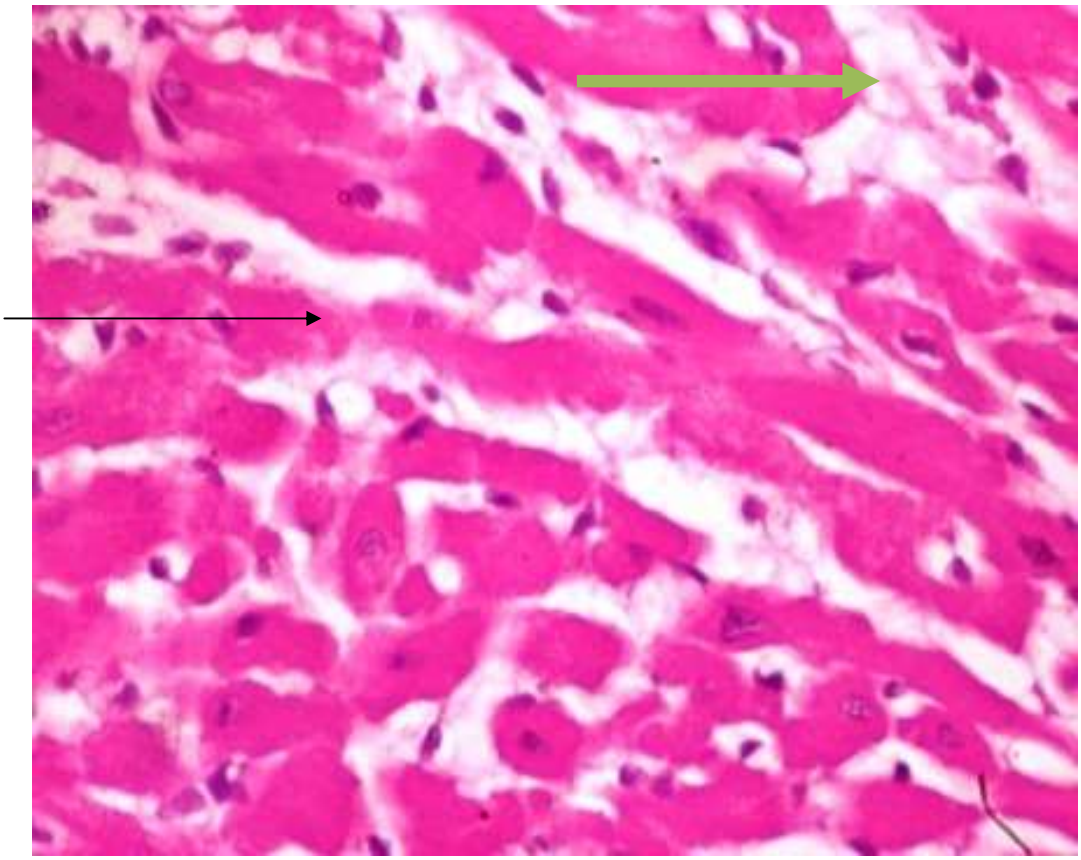

**Fig. 4.12.3 (Pre-Treatment with 250 mg/kg *C. olitorius* ethanol extract): Plate showed moderate disseminated infiltration of the myocardium (slender arrow) with inflammatory cells and mild fatty infiltration (green arrow).**

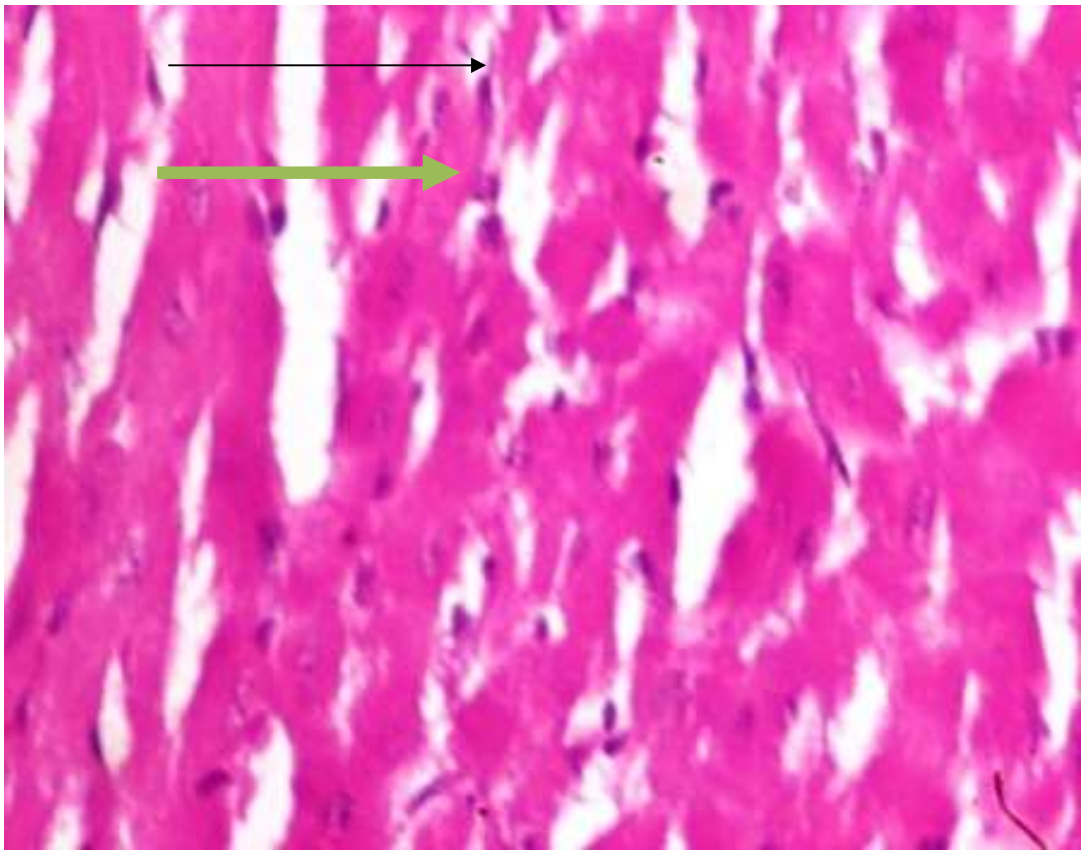

**Fig. 4.12.4 (Pre-Treatment with 500 mg/kg *C. olitorius* ethanol extract): Plate showed moderate disseminated infiltration of the myocardium (slender arrow) with inflammatory cells and mild fatty infiltration (green arrow).**

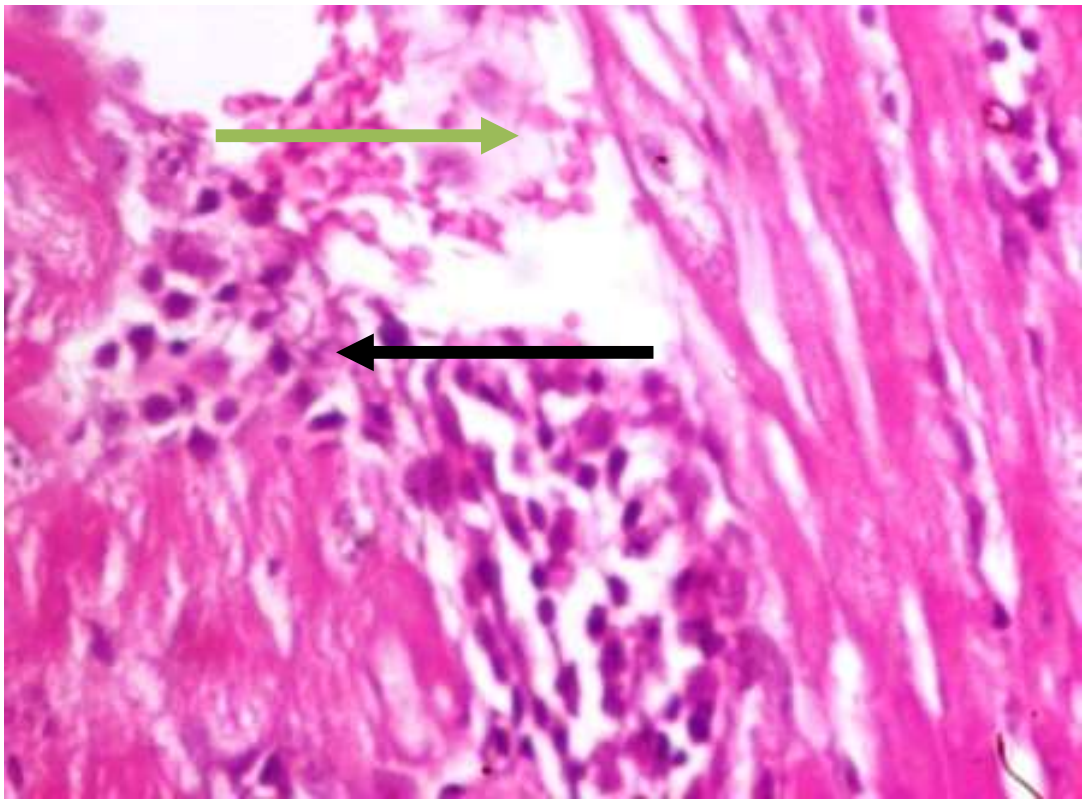

**Fig. 4.12.5(Positive Control, 10mg/kg enalapril): Plate showed moderate focal area of myocardial infarction with marked infiltration of the myocardium and pericardium by inflammatory cells (black arrows) and area of fatty infiltration (green arrows).**

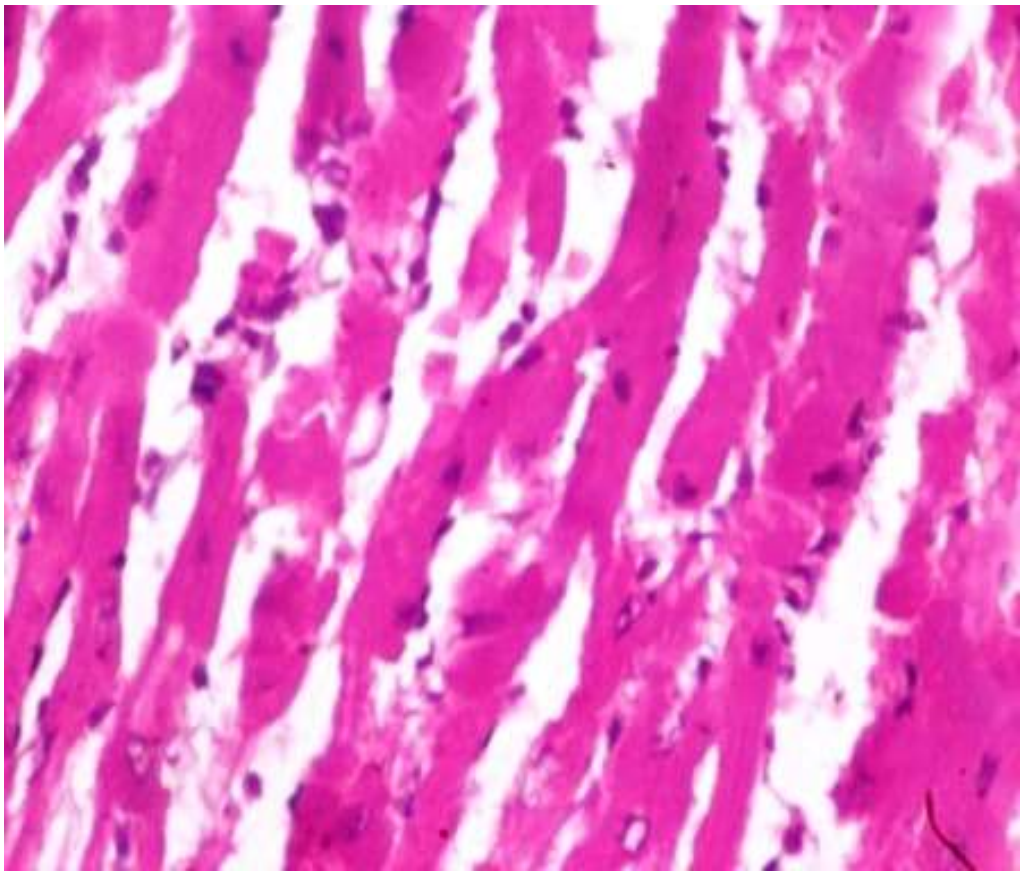

**Fig. 4.12.6 (Normal control group for post-treatment study): Plate showed no lesion.**

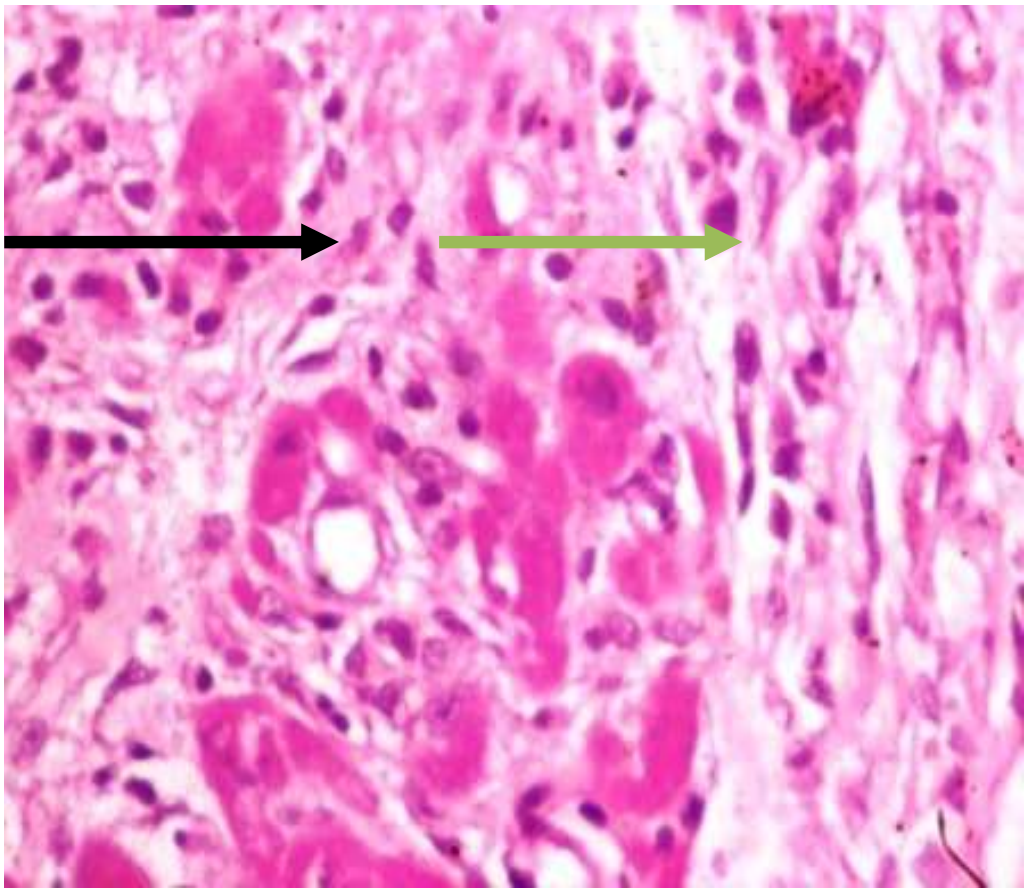

**Fig. 4.12.7 (Myocardial Infarction Alone): Plate showed myocardial infarction with infiltration by inflammatory cells (black arrow) and area of fatty infiltration (green arrow)**

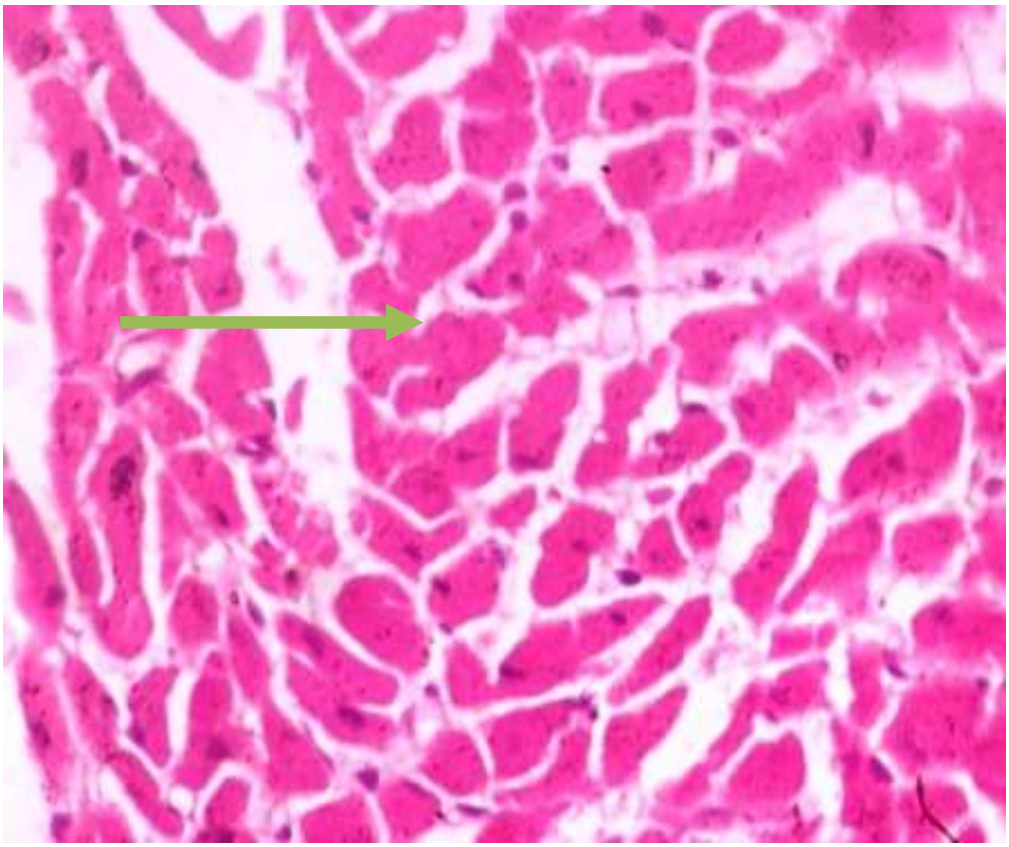

**Fig. 4.12.8 (Post-treatment with 500mg/kg *C. olitorius* ethanolic extract): Plate showed mild area of fatty infiltration (green arrow)**

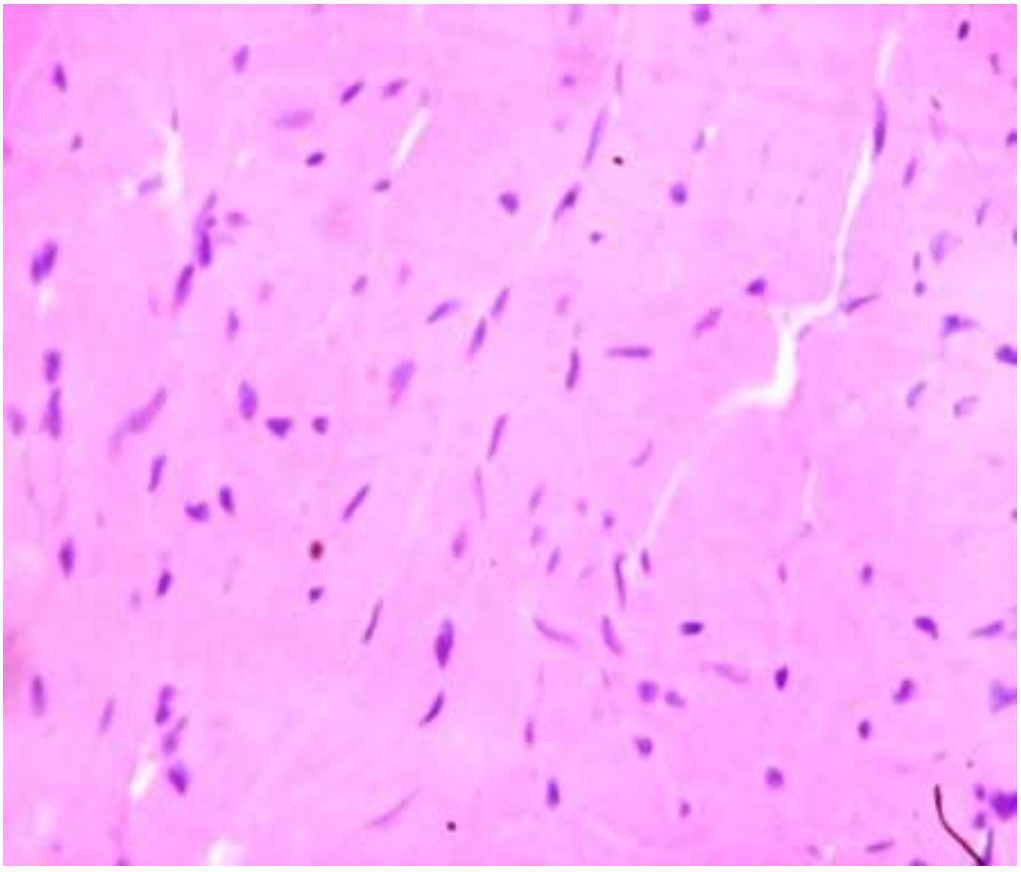

**Fig. 4.12.9 (Positive Control, 10mg/kg enalapril): Plate showed no lesion.**

## 4.13 EFFECT OF *CORCHORUS OLITORIUS* LEAF FRACTIONS ON SERUM C-REACTIVE PROTEIN.

Serum CRP was increased in the myocardial infarction rats compared to non-infarction control. Both the polar solvents (ethanol and ethyl-acetate) and non-polar solvents (dichloromethane and hexane) pre-treatment group significantly reduce CRP level in the serum ($p<0.05$) except 50 mg/kg n-hexane pre-treatment group. Better activity (Reduction in Serum CRP level) was observed in 100 mg/kg DCM and n-hexane pre-treatment group.

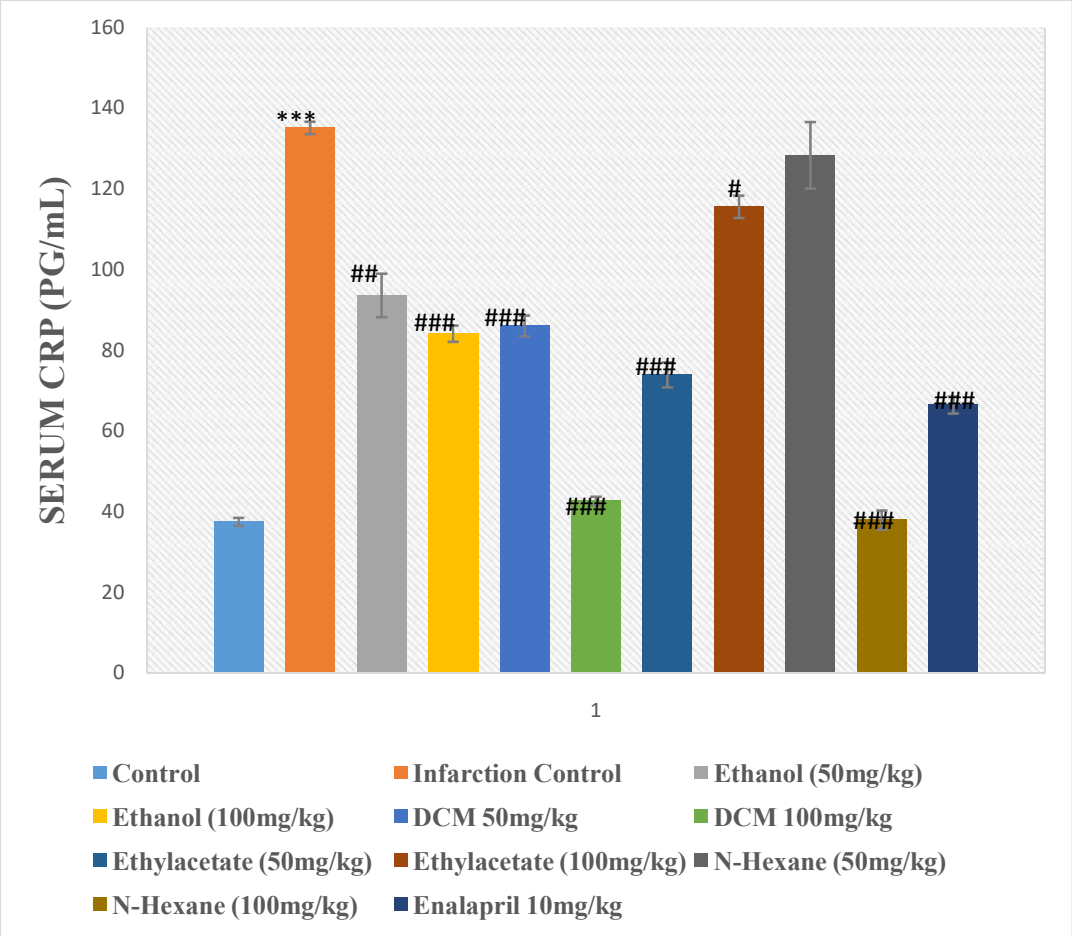


**Fig.4.13: The effect of ethanol, dichloromethane, ethyl-acetate, n-Hexane fractionof*C.olitorius* and enalapril on the Serum CRP level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

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## 4.14 EFFECT OF *CORCHORUS OLITORIUS* LEAF FRACTIONS ON SERUM CREATINE KINASE-MB.

Acute myocardial infarction group showed a significant increase in the serum level of creatine kinase-MB when compared with normal control rats. Except 50 mg/kg and 100 mg/kg n-hexane fraction pre-treatment group, ethanol fraction, ethyl-acetate fraction and dichoromethane fraction (50 mg/kg and 100 mg/kg) pre-treatment groups all reduced serum CK-MB level. Although better activity was again showed in dichloromethane pre-treatment group.

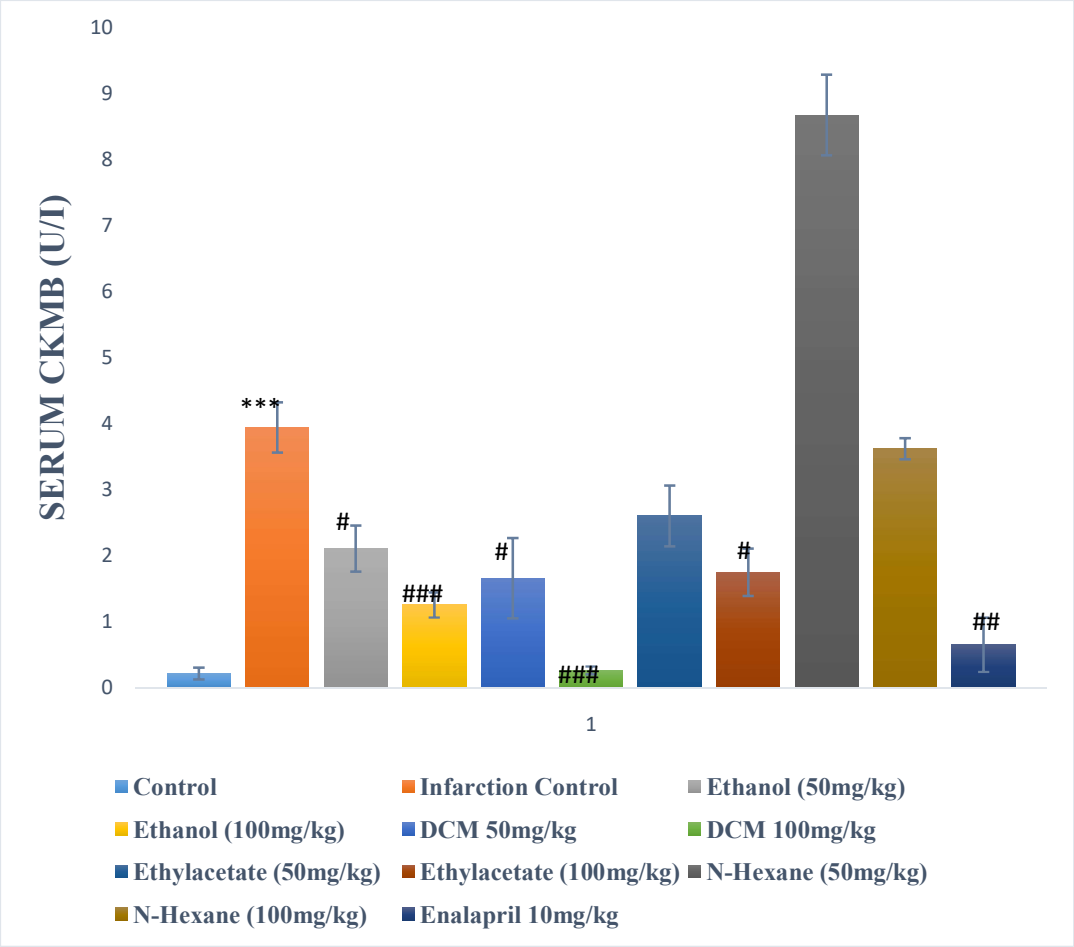



**Fig. 4.14: The effect of ethanol, dichloromethane, ethyl-acetate, n-Hexane fractionof*C.olitorius* and enalapril on the Serum CK-MB level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.15 EFFECT OF *CORCHORUS OLITORIUS* LEAF FRACTIONS ON TISSUE p65NFKappaB.

Tissue p65NFKappaB level was reduced in the acute myocardial infarction group compared with the normal control group. Tissue p65NFKappaB level was enhanced in all fraction pre-treatment group except 50 mg/kg ethanol and DCM.

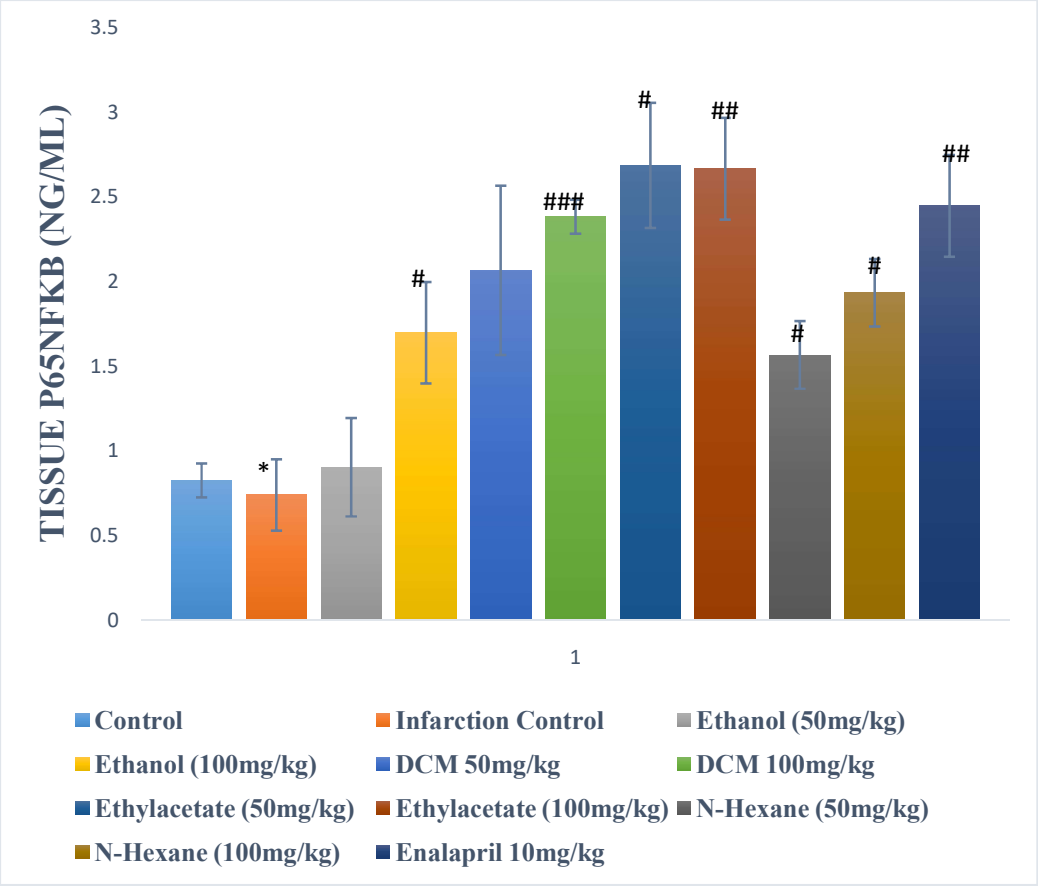



**Fig. 4.15: The effect of ethanol, dichloromethane, ethyl-acetate, n-Hexane fractionof*C.olitorius* and enalapril on the tissue p65NF$_k$B level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

## 4.16 EFFECT OF *CORCHORUS OLITORIUS* LEAF DCM FRACTION ON TISSUE p65NFKappaB.

Increase in tissue p65NFKappaB expression ($p<0.05$) was observed in dichloromethane pre-treatment groups (50 mg/kg and 100 mg/kg) and enalapril pre-treatment group (10 mg/kg).

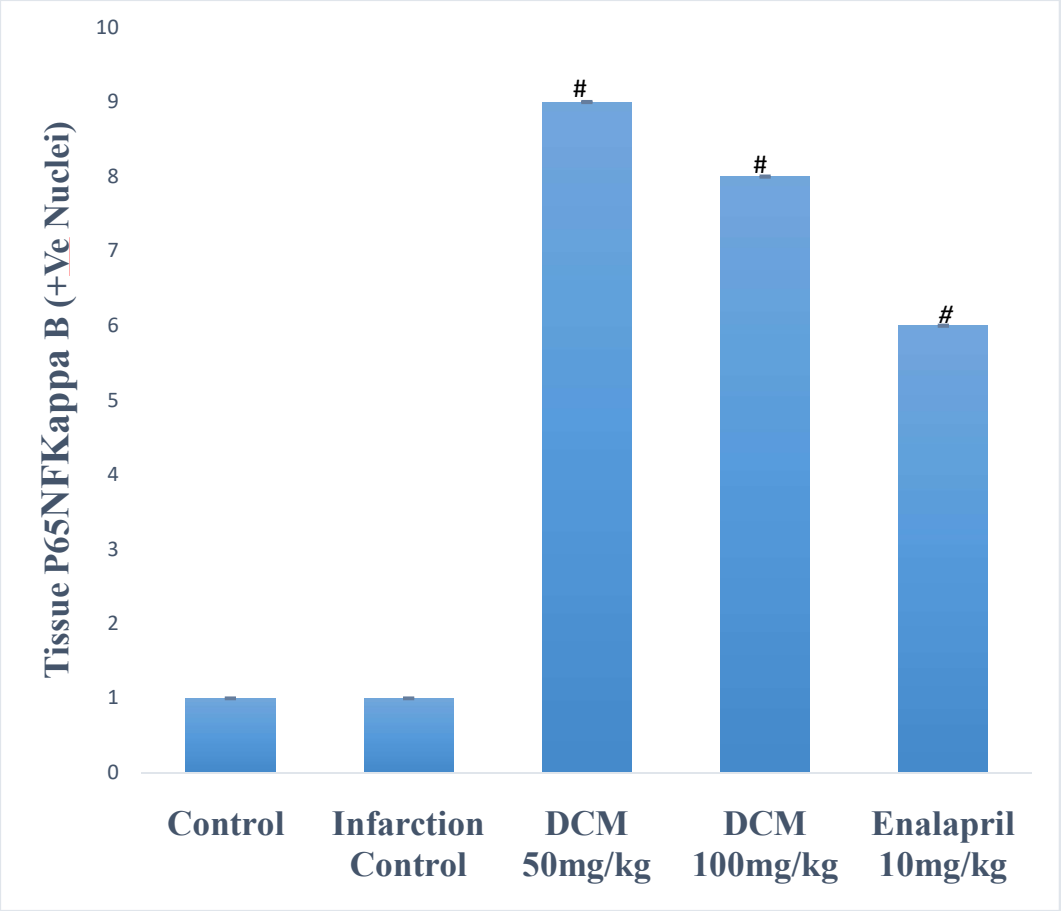



**Fig. 4.16: The effect of dichloromethane (DCM) (50 and 100 mg/kg) fraction of*C.olitorius* and enalapril (10 mg/kg) on the Tissue p65NFKappaB expression level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

**4.17 PHOTOMICROGRAPH SHOWING p65NFKappaB EXPRESSION OF MYOCARDIAL TISSUE.**

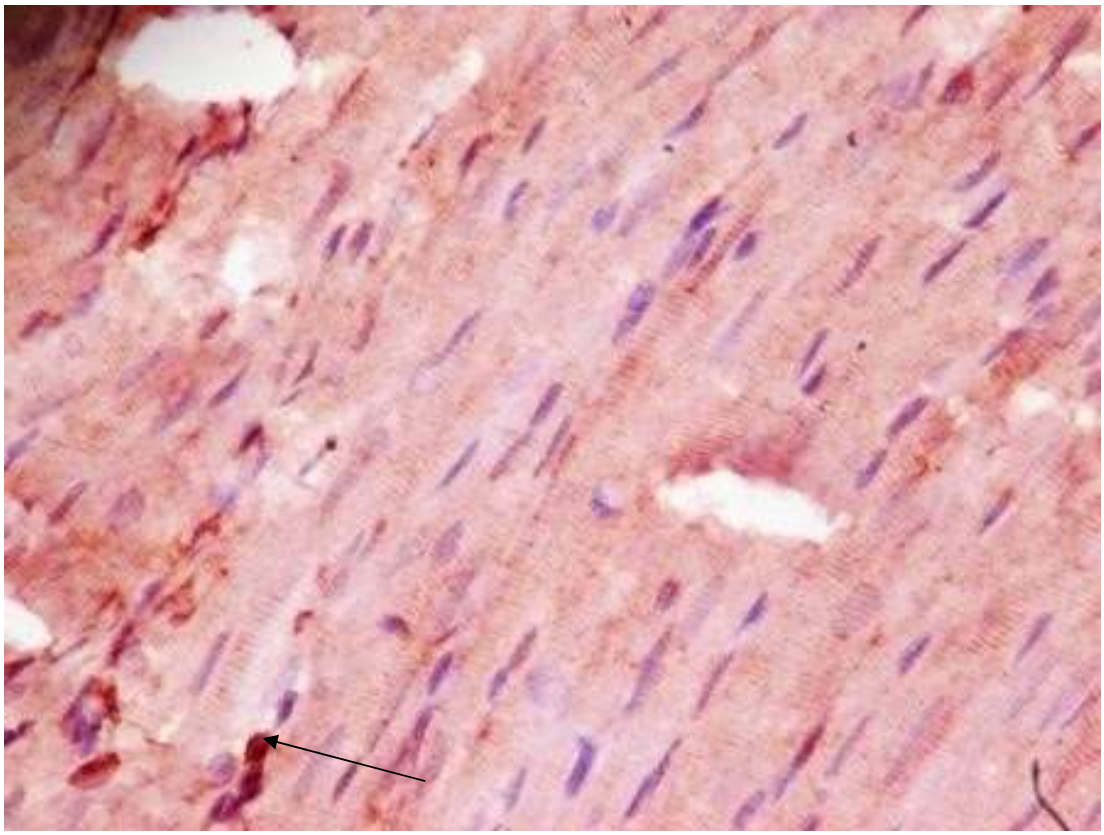


**Fig. 4.17.1: (Normal Control): Moderate expression of $NF_kB$ with positive nuclei**

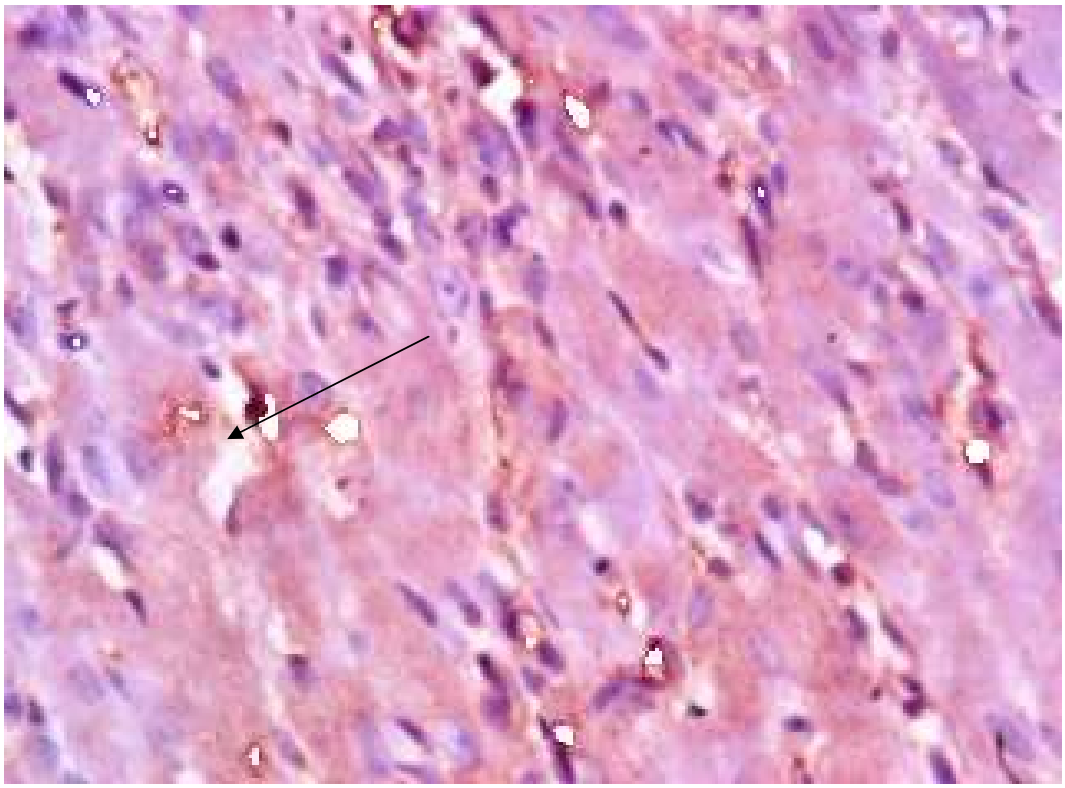


**Fig. 4.17.2: (Infarction Alone): Moderate expression of $NF_kB$ with positive nuclei**

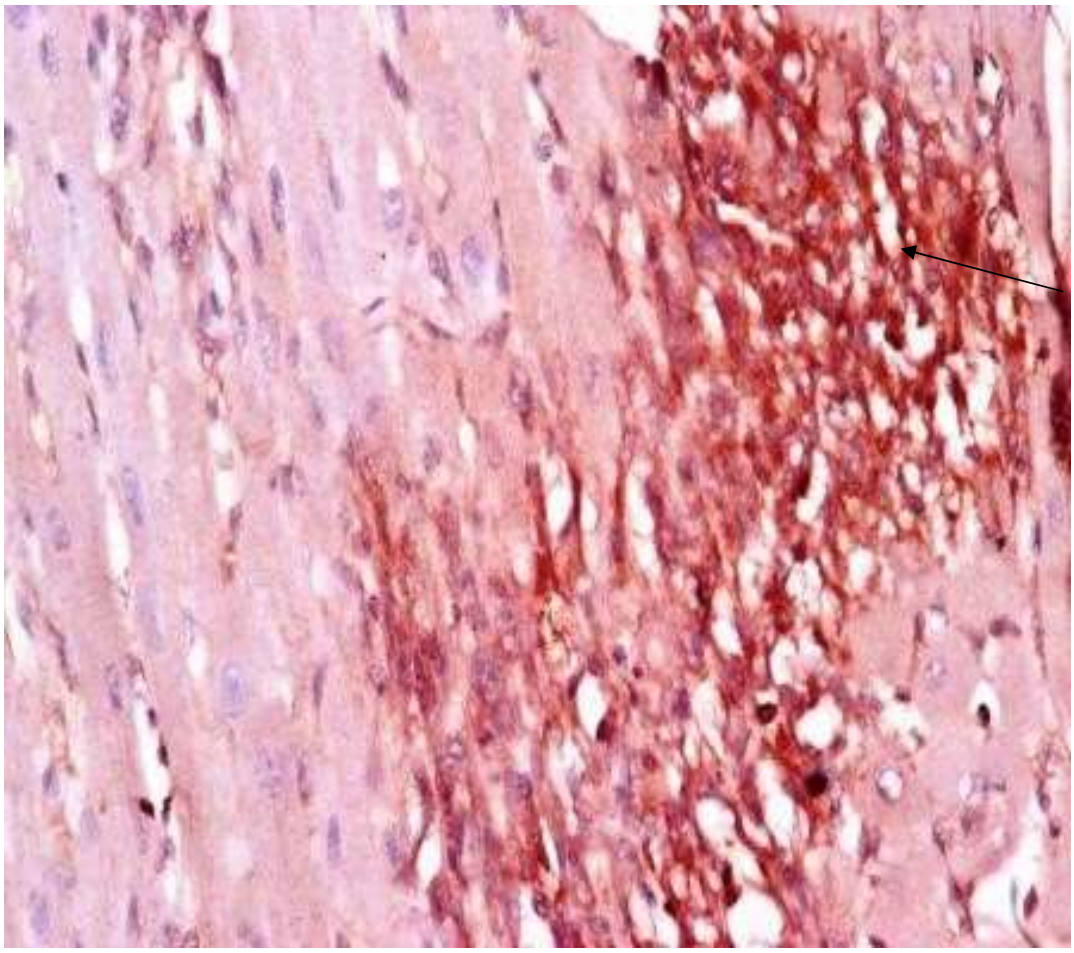


**Fig. 4.17.3: (DCM Pre-Treatment 50mg/kg): Severe expression with some positive nuclei.**

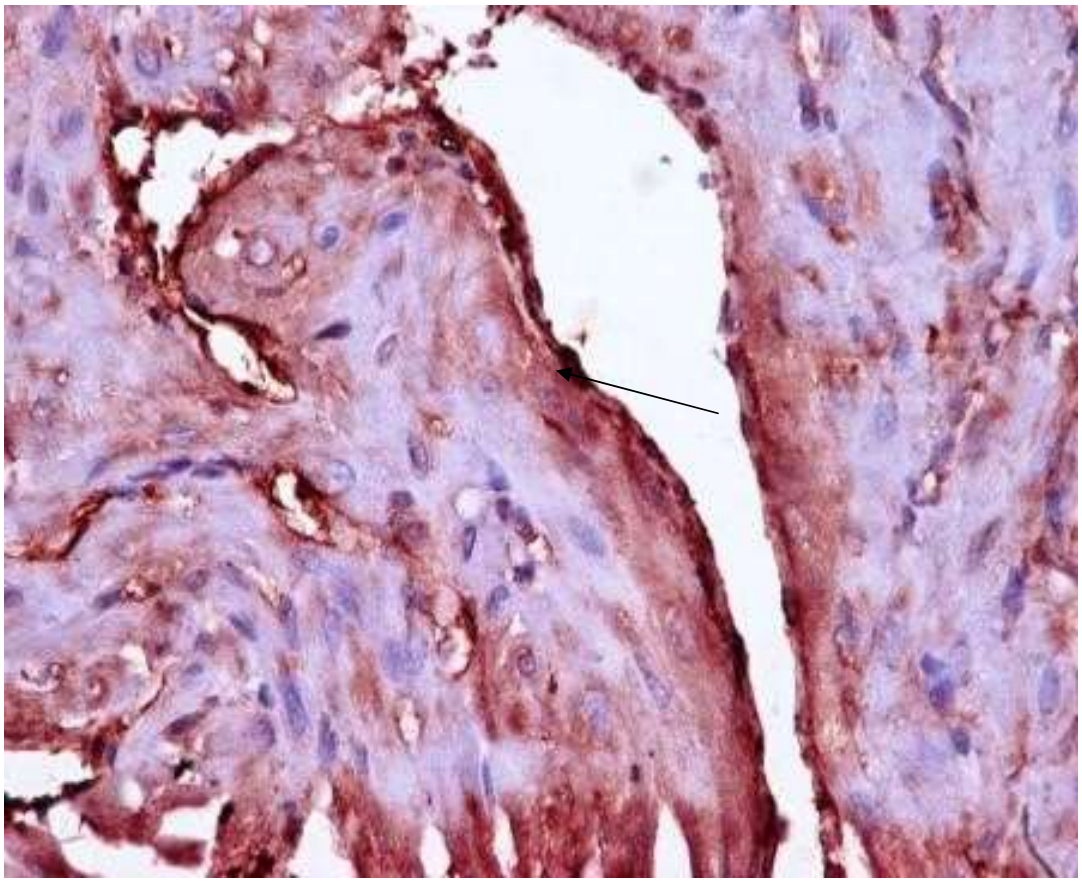


**Fig. 4.17.4: (DCM Pre-Treatment 100mg/kg): Very moderate expression with positive nuclei**

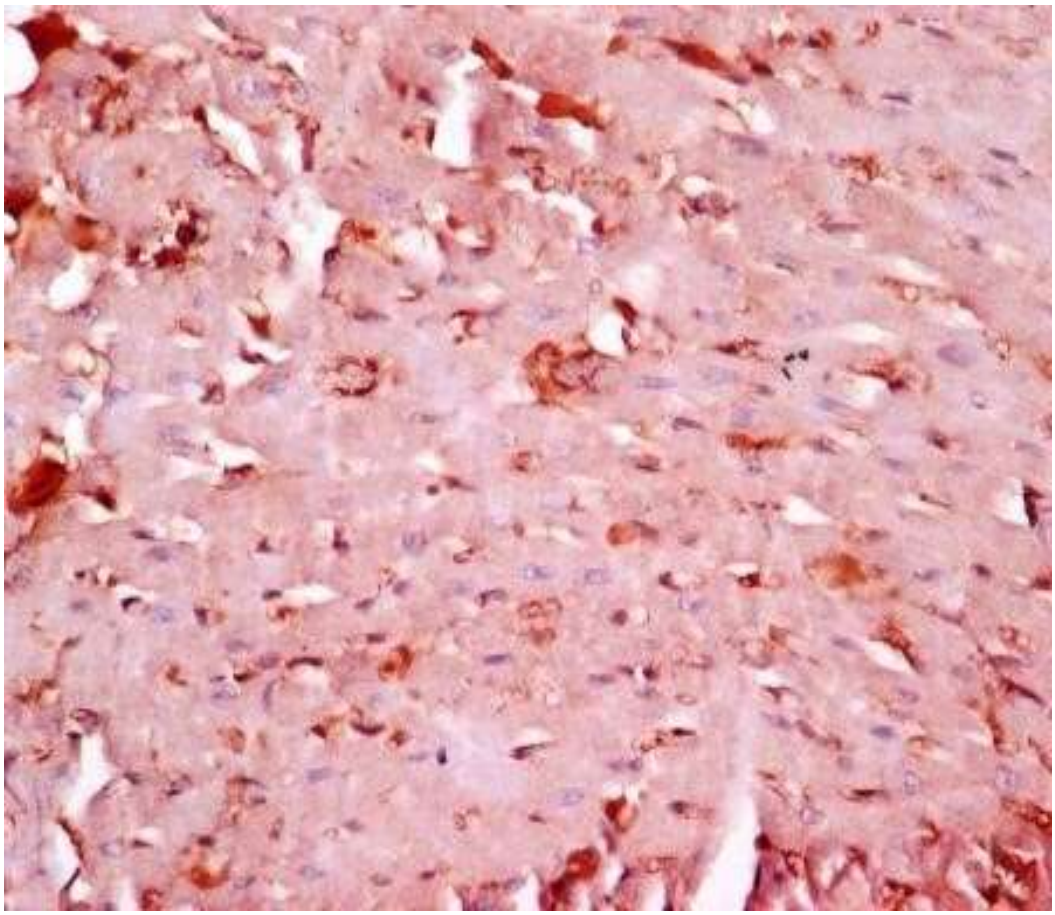


**Fig. 4.17.5 (Enalapril Pre-treatment 10mg/kg): Severe and diffuse expression with some positive nuclei.**

## 4.18 EFFECT OF *CORCHORUS OLITORIUS* LEAF DCM FRACTION ON TISSUE BCL2 EXPRESSION

The myocardial expression of bcl2 was low in the acute myocardial infarction rats when compared with normal control rats. Also, Dichloromethane and enalapril pre-treatment groups significantly enhanced tissue expression of bcl2 with better expression observed in 50 mg/kg DCM fraction pre-treatment group.

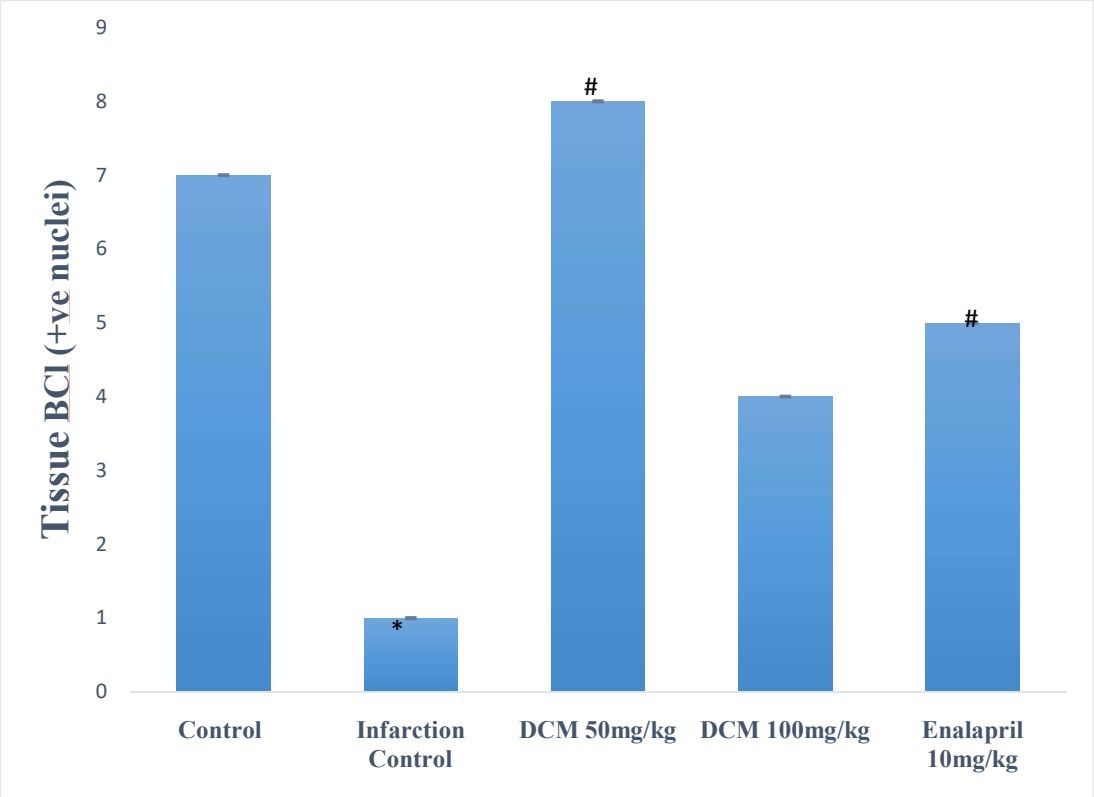



**Fig. 4.18: The effect of dichloromethane (DCM) (50 and 100 mg/kg) fraction of*C.olitorius* and enalapril (10 mg/kg) on the Tissue bcl2 expression level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

**4.19 PHOTOMICROGRAPH SHOWING BCL2 EXPRESSION OF MYOCARDIAL TISSUE.**

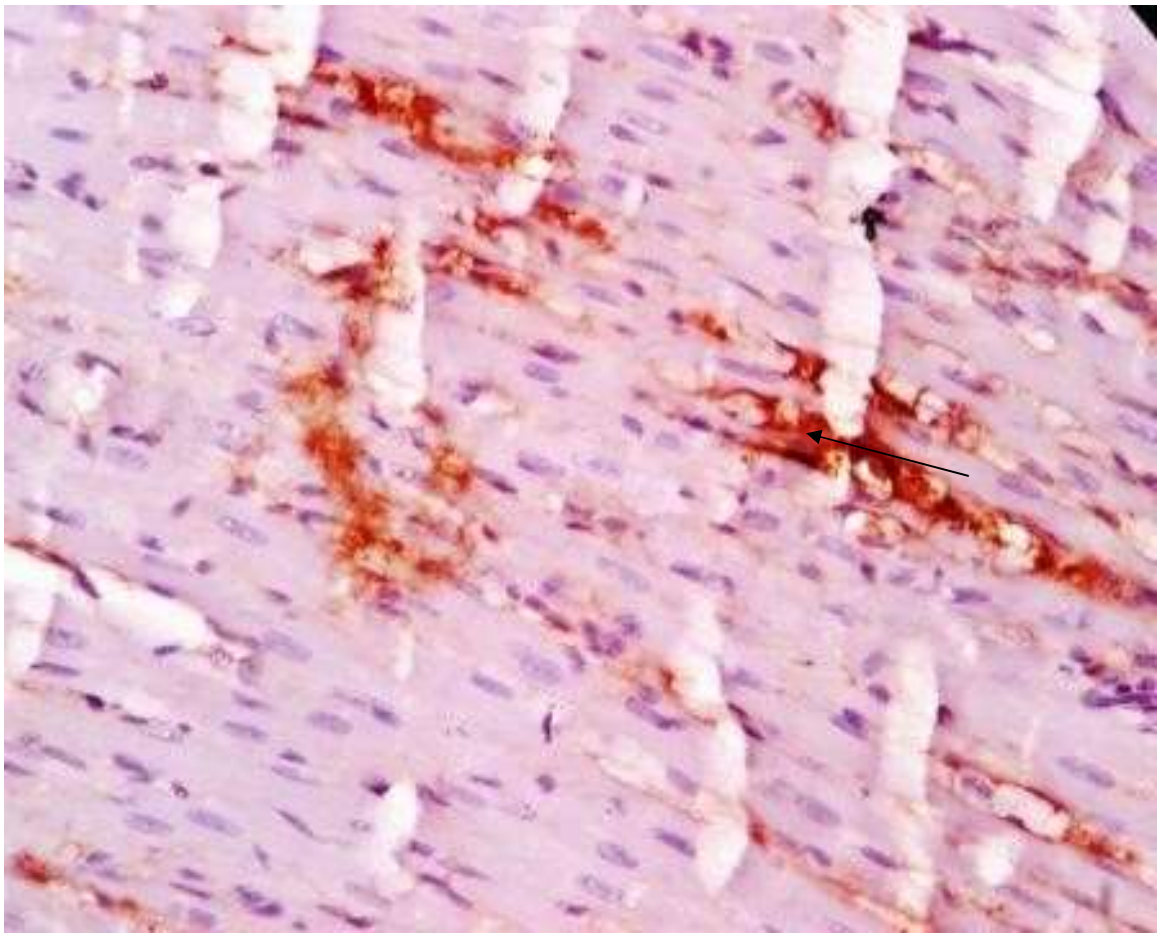


**Fig. 4.19.1 (Normal control): Moderate expression of Bcl2 with positive nuclei.**

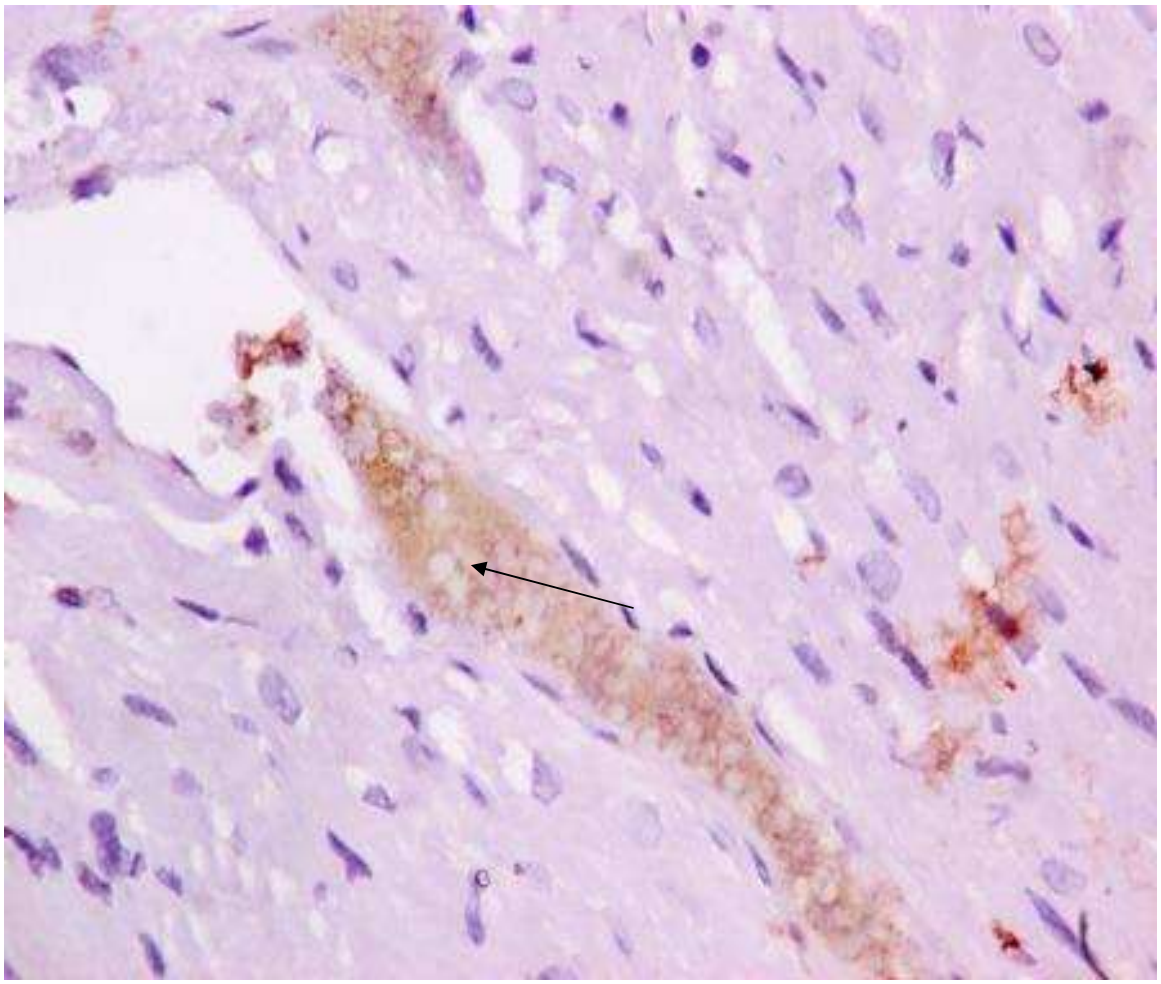


**Fig. 4.19.2 (Infarction Alone): Very mild expression**

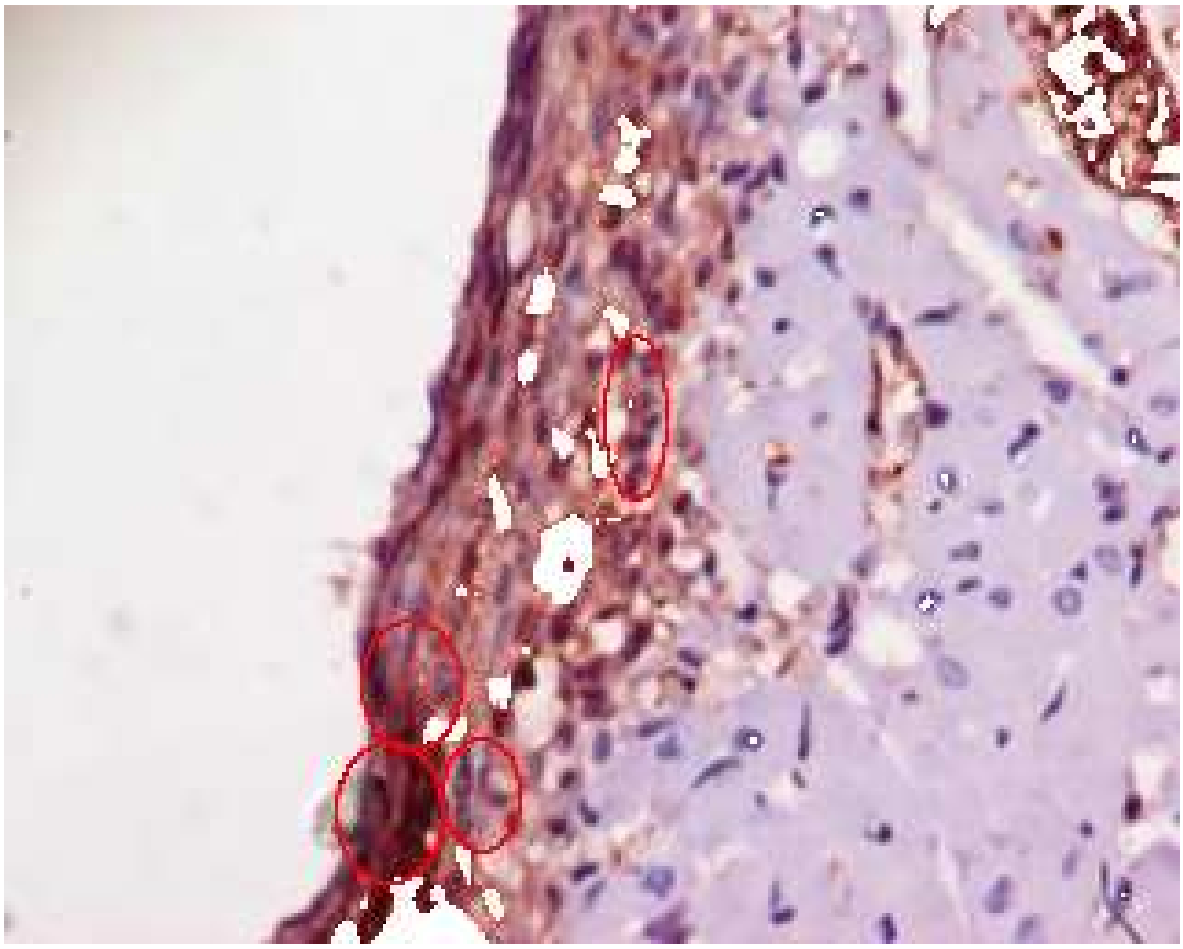


**Fig. 4.19.3 (DCM Pre-Treatment 50mg/kg): Severe expression with some positive nuclei.**

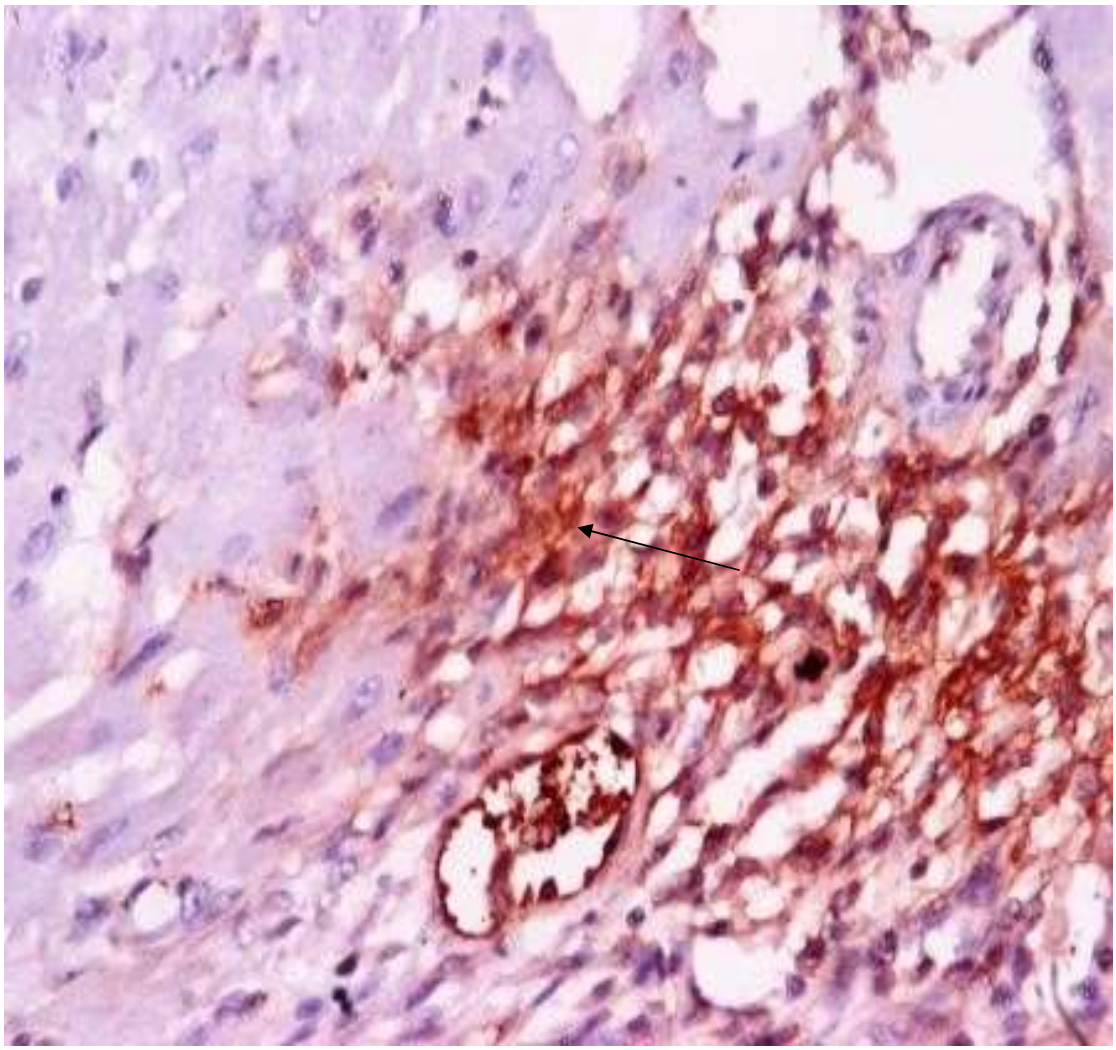


**Fig. 4.19.4 (DCM Pre-Treatment 100mg/kg): Very moderate expression with positive nuclei.**

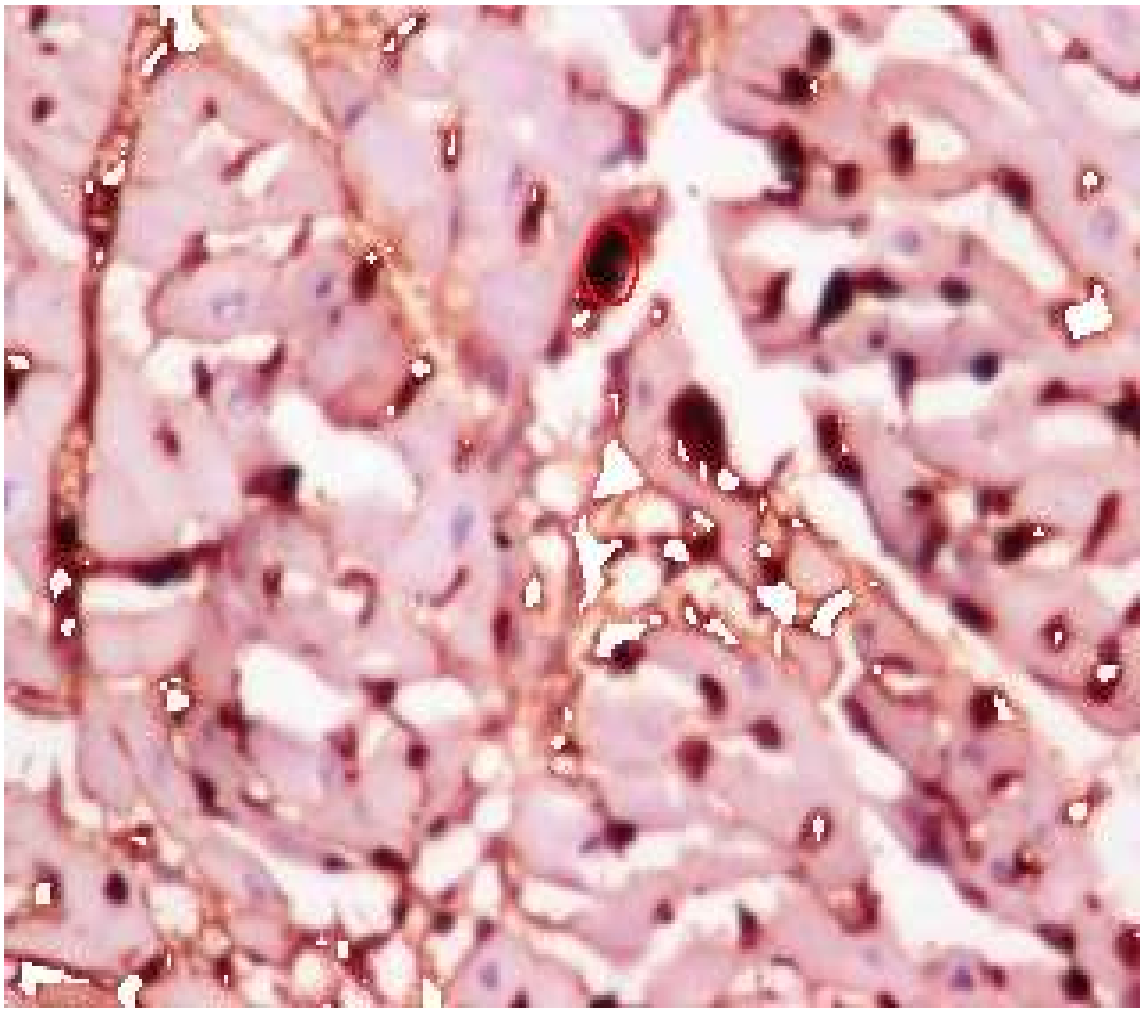


**Fig. 4.19.5 (Enalapril Pre-treatment 10mg/kg): Severe expression with some positive nuclei.**

## 4.20 EFFECT OF *CORCHORUS OLITORIUS* LEAF DCM FRACTION ON TISSUE BAX EXPRESSION.

Myocardial tissue bax expression was significantly increased during acute myocardial infarction compared with normal control rats. Pre-treating the rats with DCM (50 mg/kg and 100 mg/kg) and 10 mg/kg enalapril significantly reduced the expression.

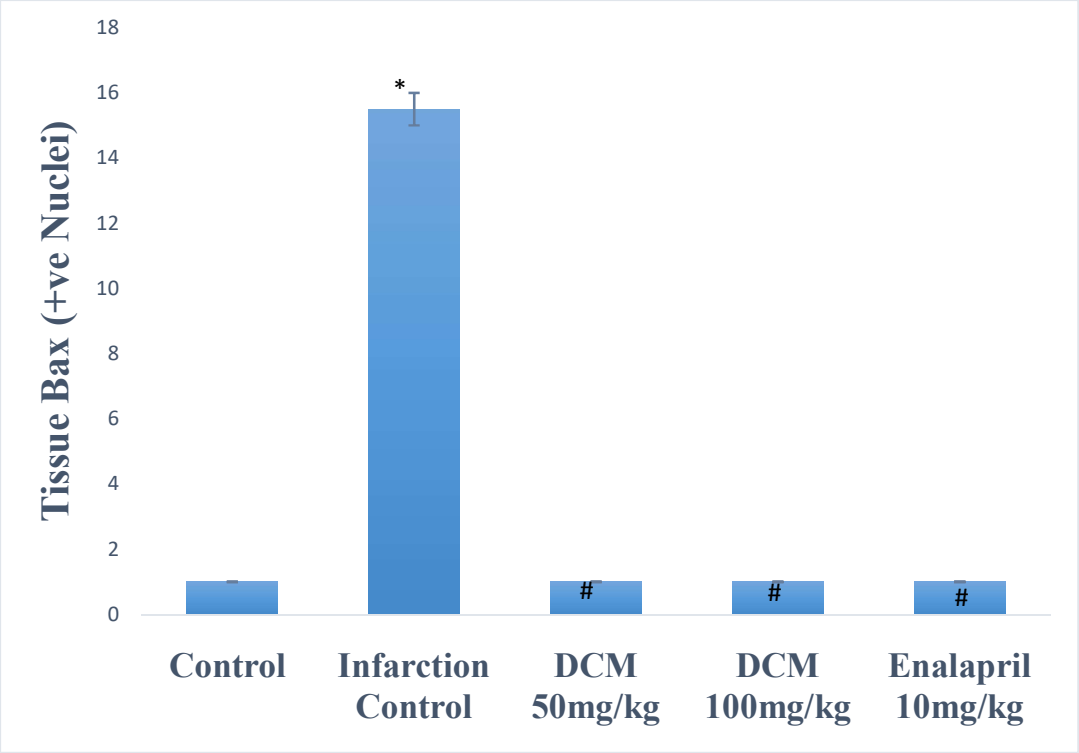



**Fig. 4.20: The effect of dichloromethane (DCM) (50 and 100 mg/kg) fraction of*C.olitorius* and enalapril (10 mg/kg) on the Tissue bax expression level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

**4.21 PHOTOMICROGRAPH SHOWING BAX EXPRESSION OF MYOCARDIAL TISSUE.**

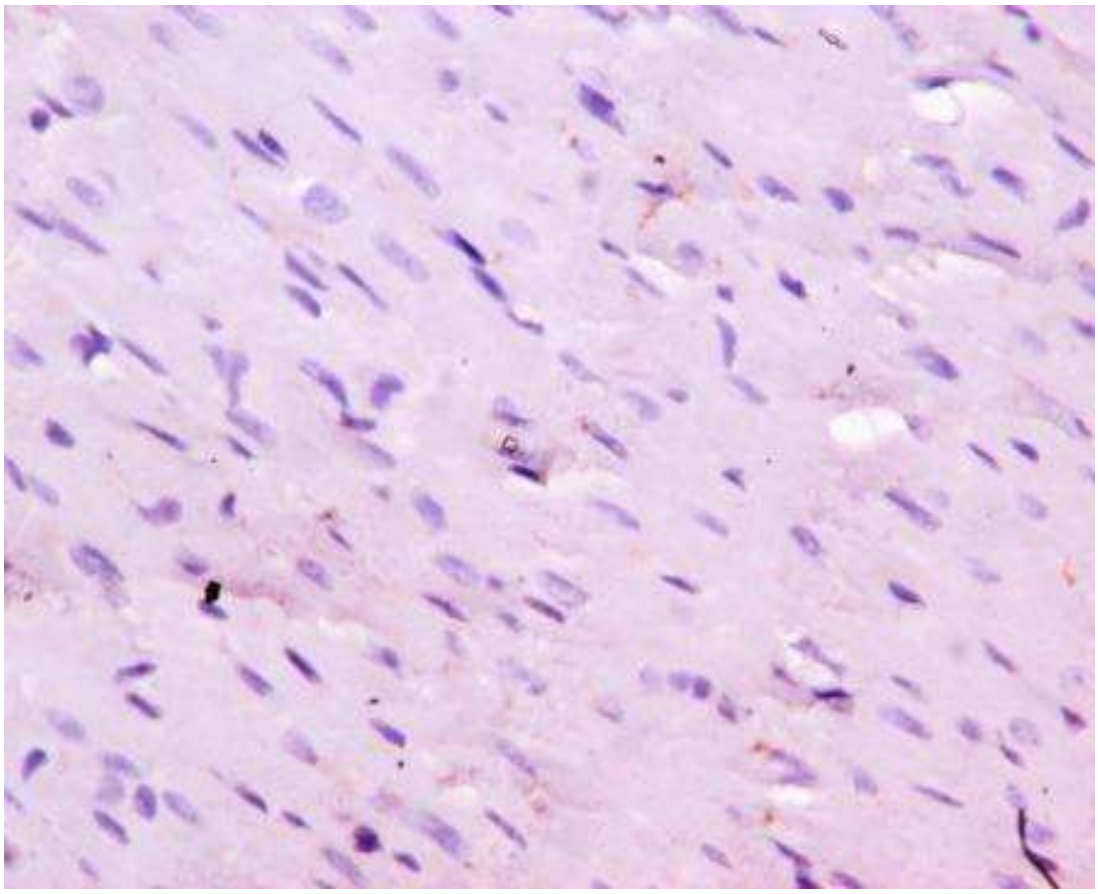


**Fig. 4.21.1 (Normal control): no expression of bax**

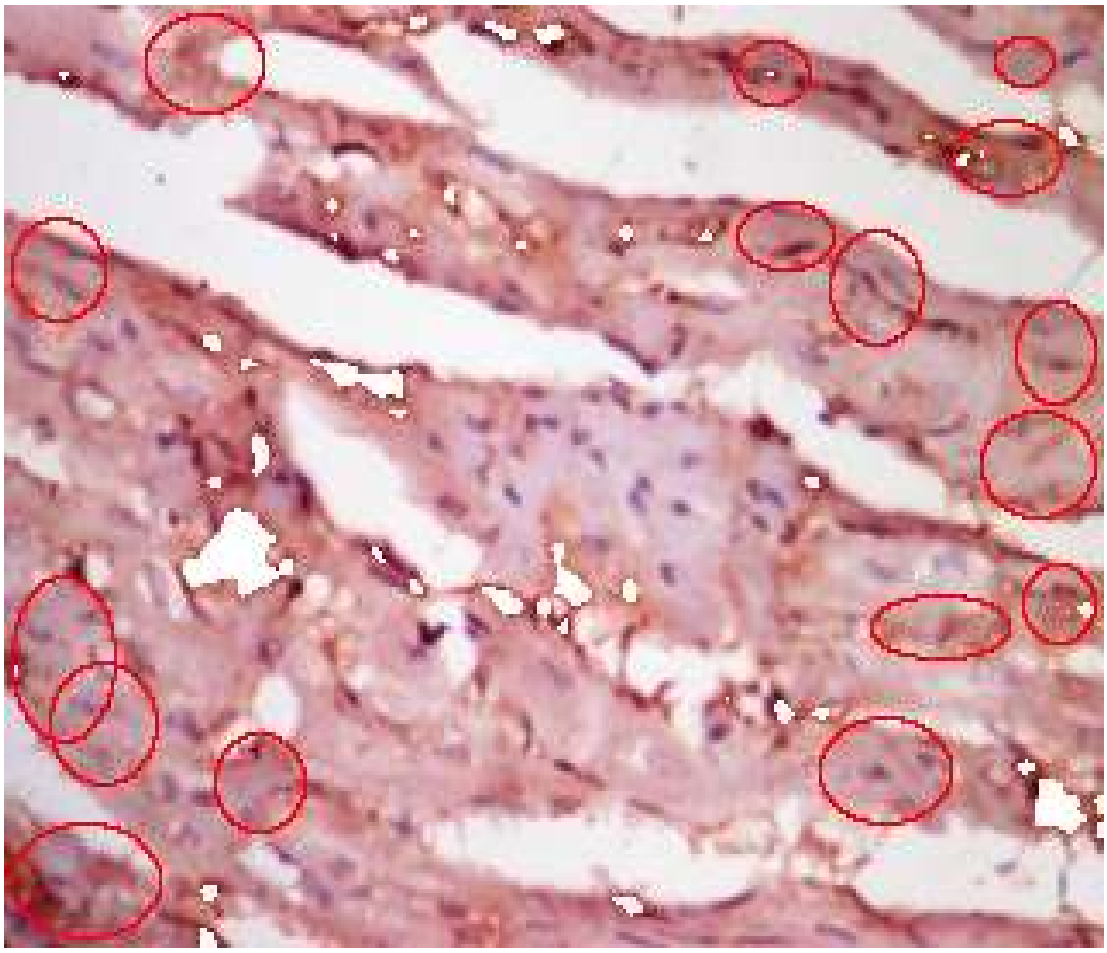


**Fig. 4.21.2(Infarction Alone): severe expression with some positive nuclei**

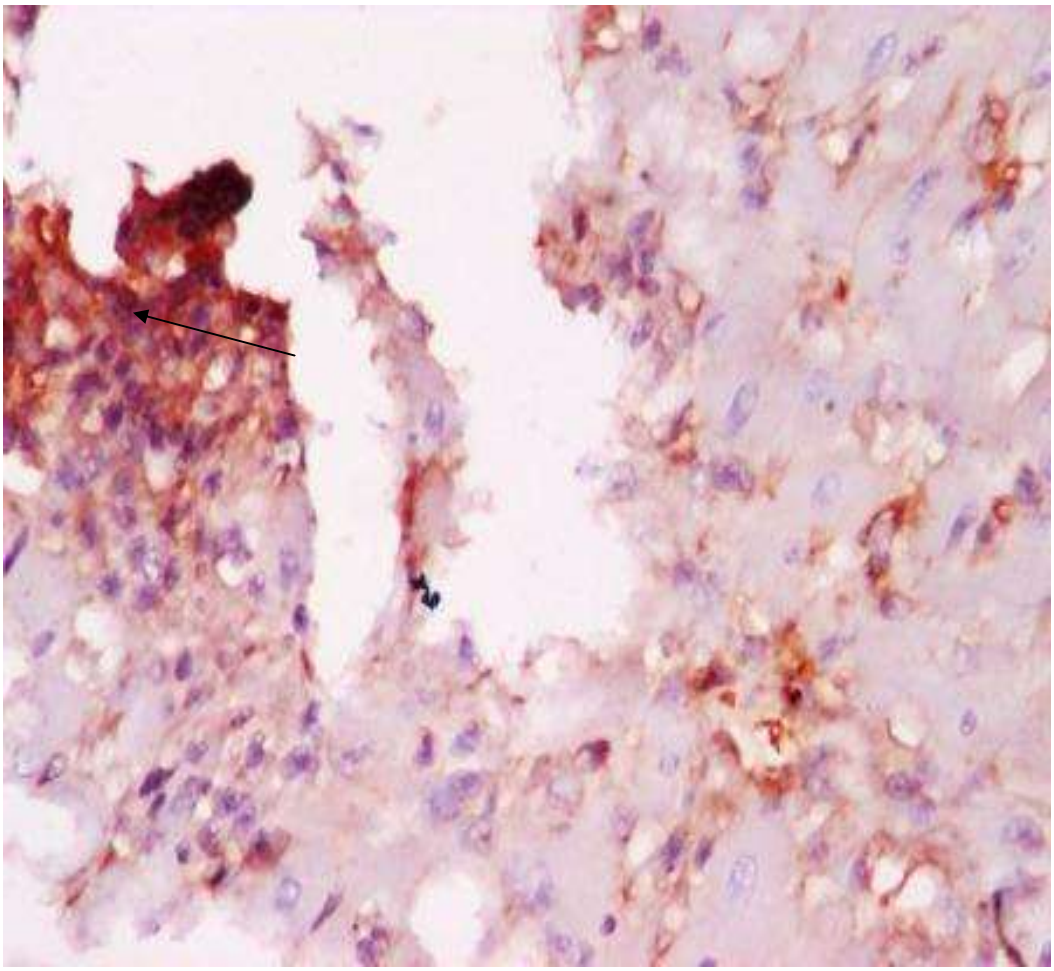


**Fig. 4.21.3 (DCM Pre-Treatment 50mg/kg): Very mild expression with positive nuclei.**

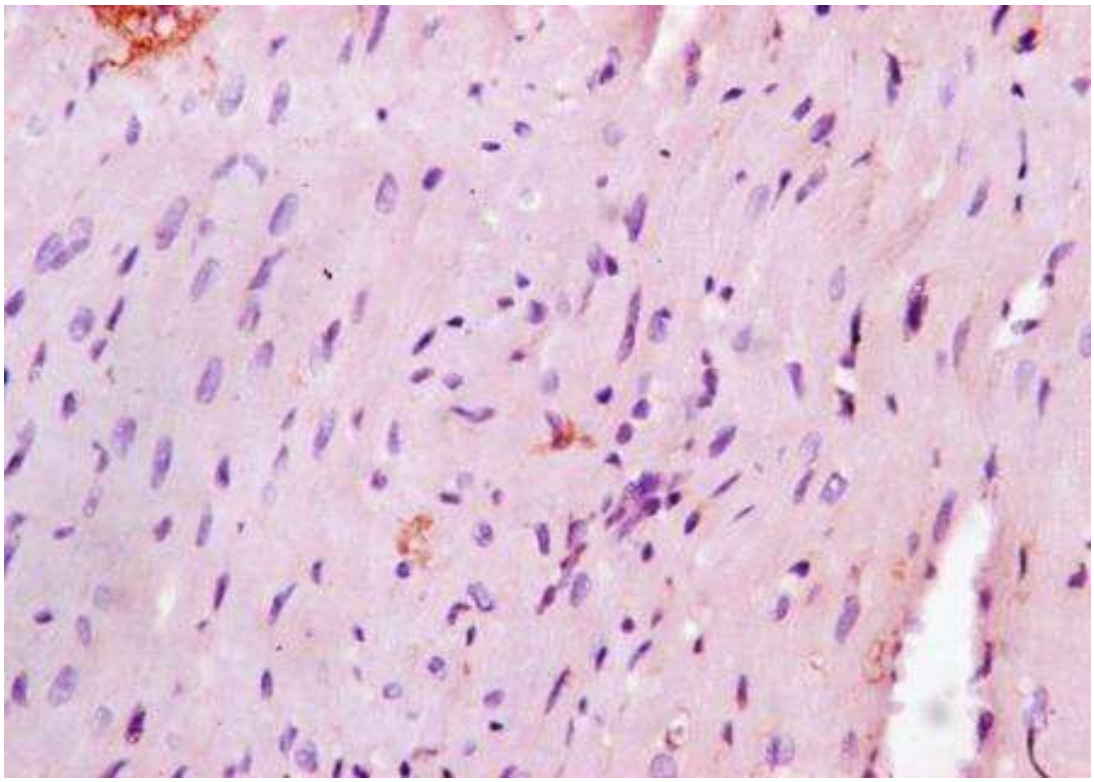


**Fig. 4.21.5 (Enalapril Pre-treatment 10mg/kg): Show a very mild expression with positive nuclei.**

## 4.22 EFFECT OF *CORCHORUS OLITORIUS* LEAF DCM FRACTION ON TISSUE p53 EXPRESSION.

Myocardial tissue p53 expression was also significantly increased during acute myocardial infarction compared with normal controlrats. Pre-treating with DCM fraction(50 mg/kg and 100 mg/kg) and 10 mg/kg enalapril significantly reduce the expression.

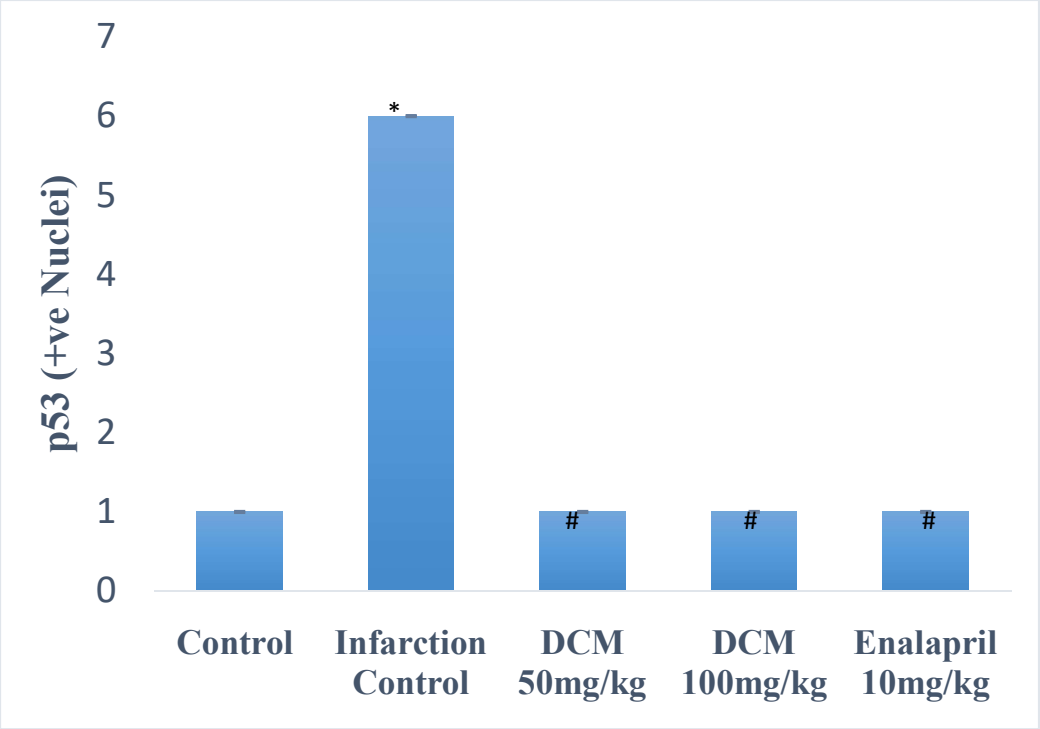
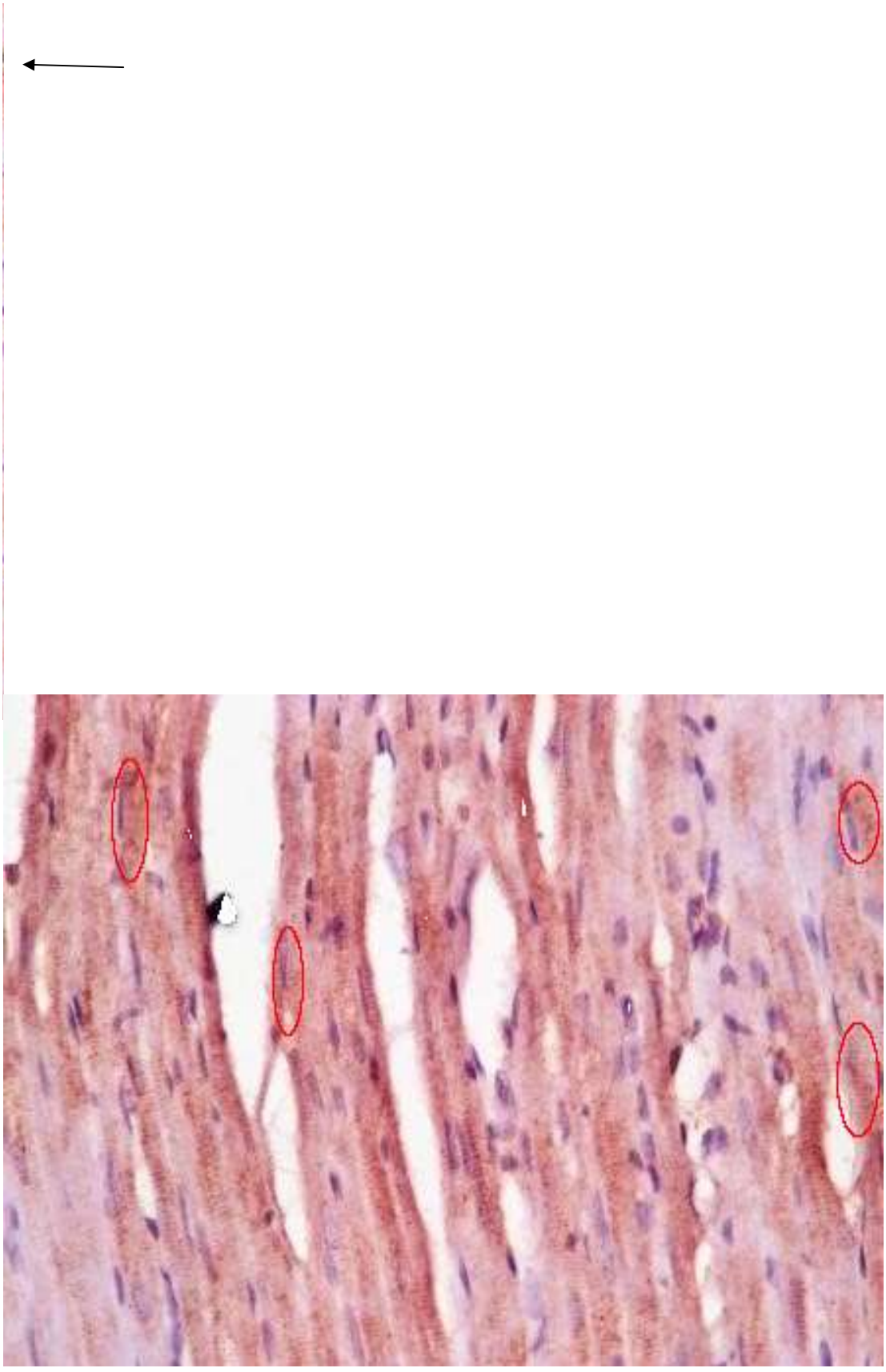



**Fig. 4.22: The effect of dichloromethane (DCM) (50 and 100 mg/kg) fraction of*C.olitorius* and enalapril (10 mg/kg) on the Tissue p53 expression level in isoproterenol-induced AMI in rats.Bar chart represents mean ± SEM. The * Represent a significant difference in values from the normal control (*p<0.05, **p<0.01, *** p<0.001). The # represents significant value difference from infarction alone (#p<0.05, ##p<0.01, ### p<0.001).**

**4.23 PHOTOMICROGRAPH SHOWING p53 EXPRESSION OF MYOCARDIAL TISSUE.**



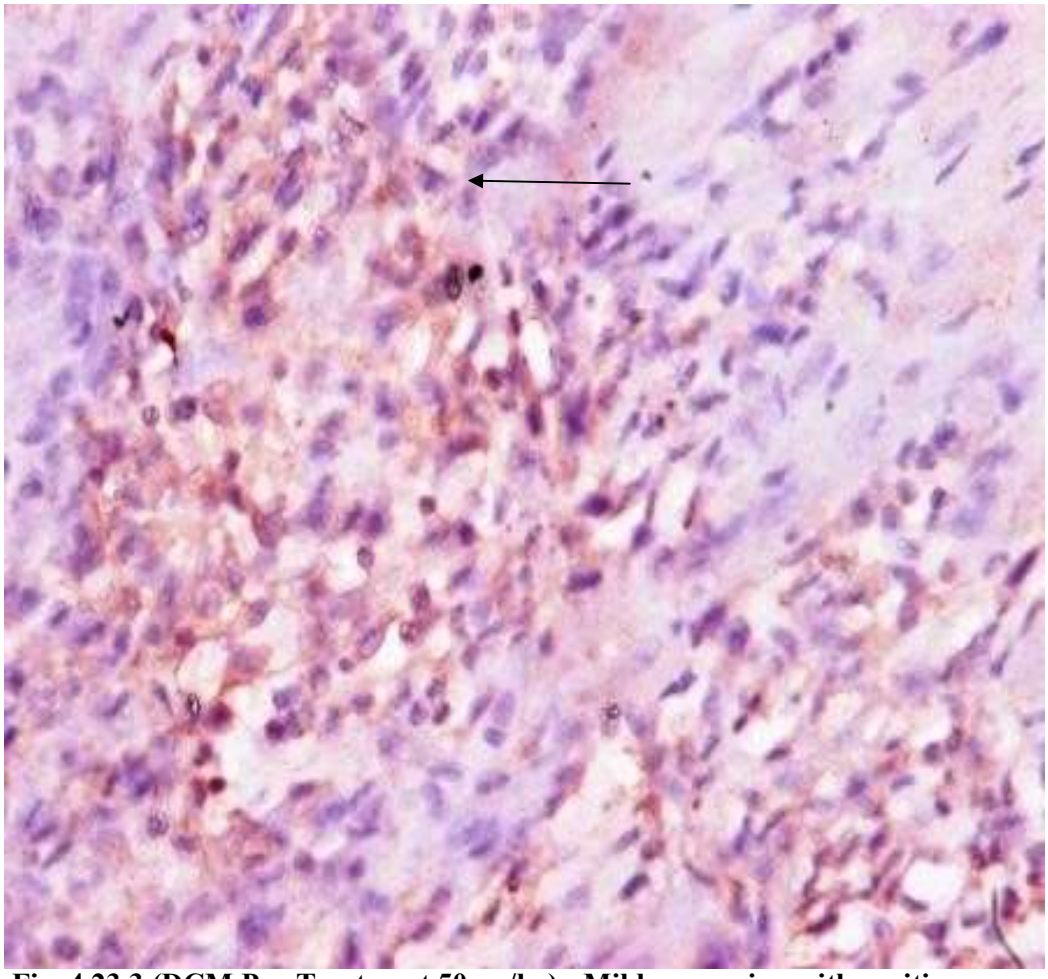


**Fig. 4.23.3 (DCM Pre-Treatment 50mg/kg): Mild expression with positive nuclei.**

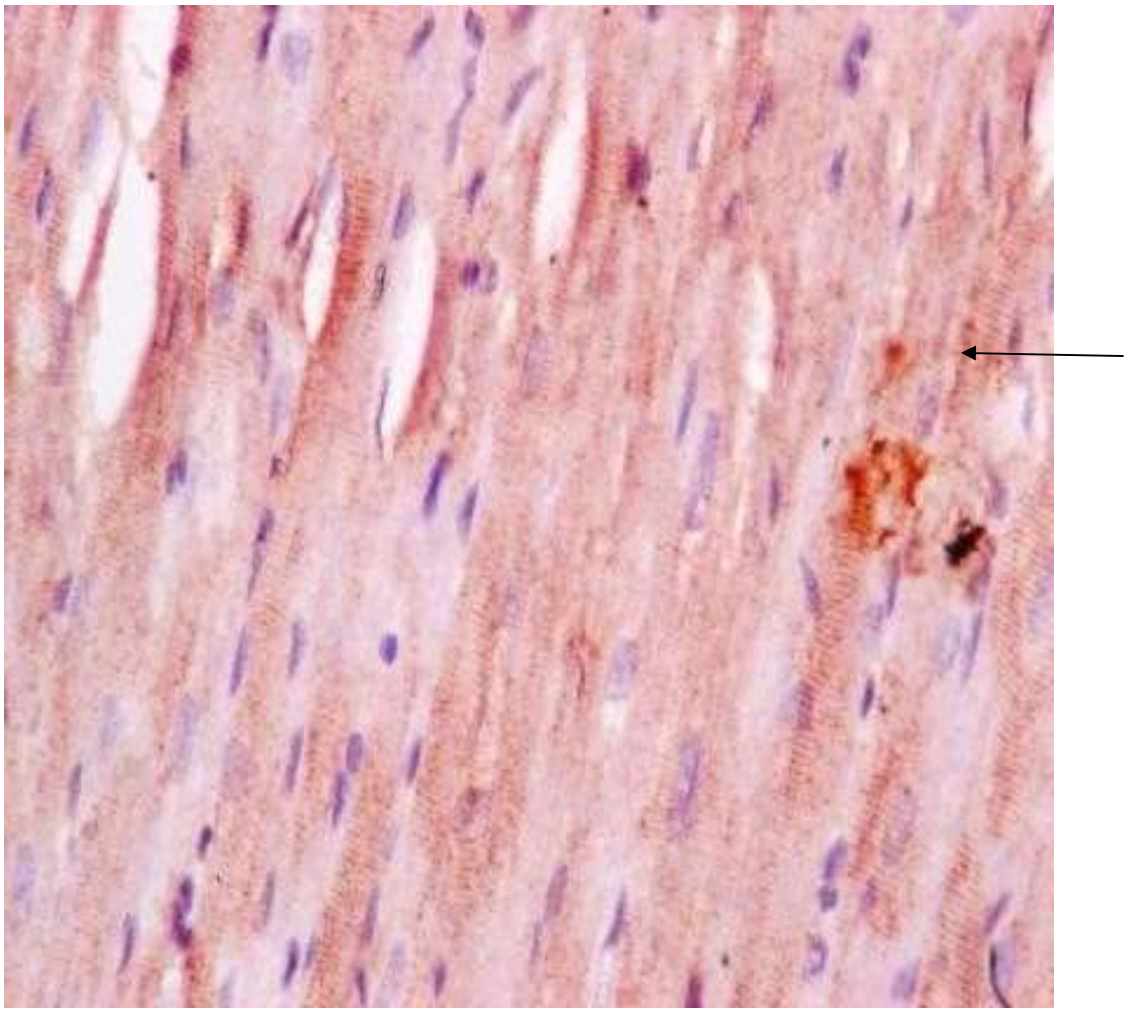


**Fig. 4.23.4 (DCM Pre-Treatment 100mg/kg): Very mild expression with positive nuclei.**

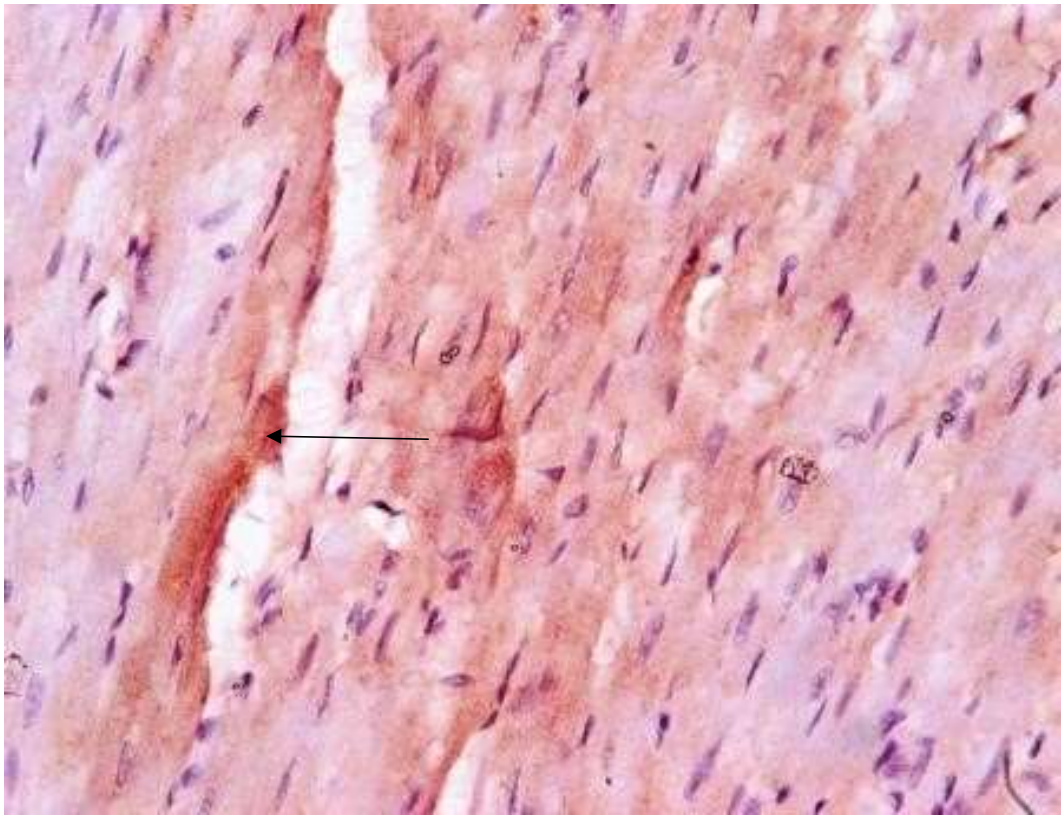


**Fig. 4.23.5 (Enalapril Pre-treatment 10mg/kg): Mild expression with positive nuclei.**

## 4.24. GC-MS Analysis of Dichloromethane Fraction

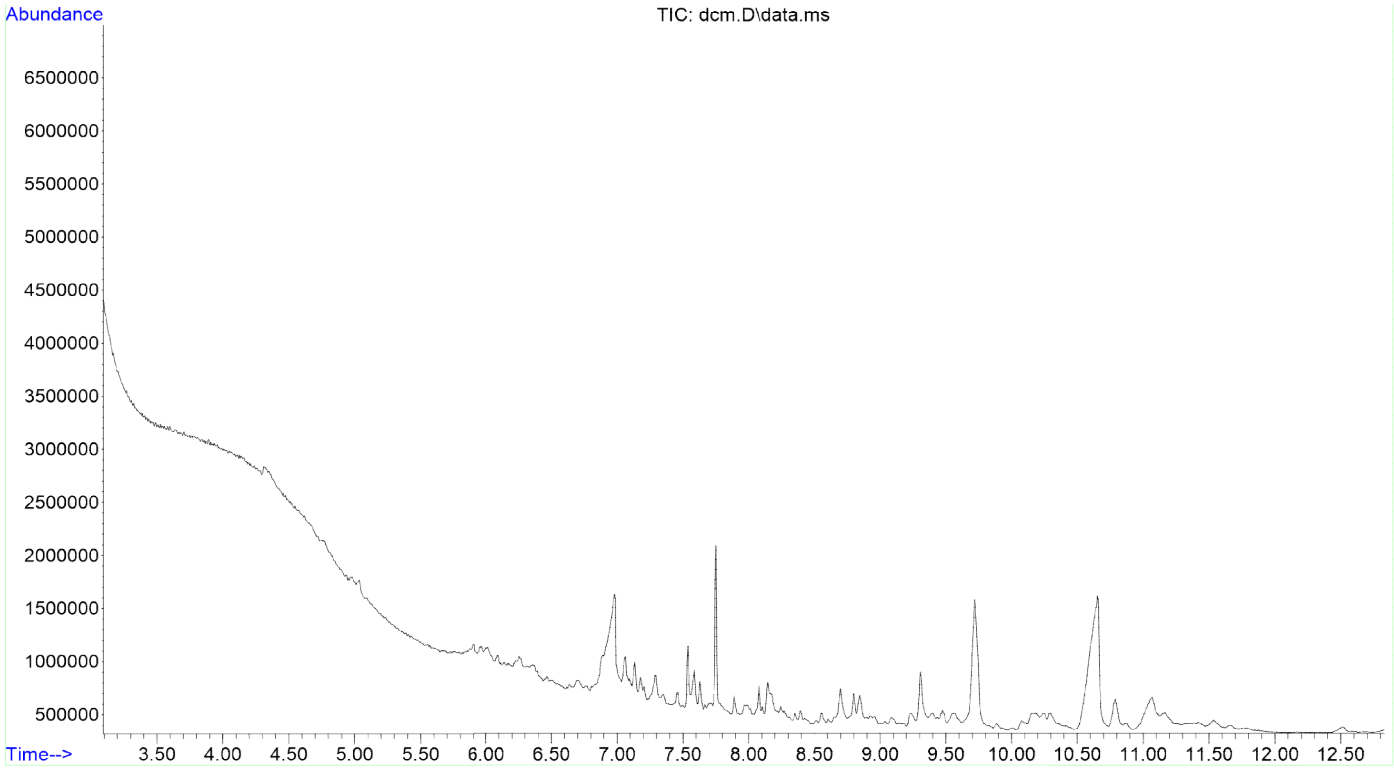



**Figure 4.24: GC-MS analysis spectra of compounds present in Dichloromethane fraction of *Corchorus olitorius***

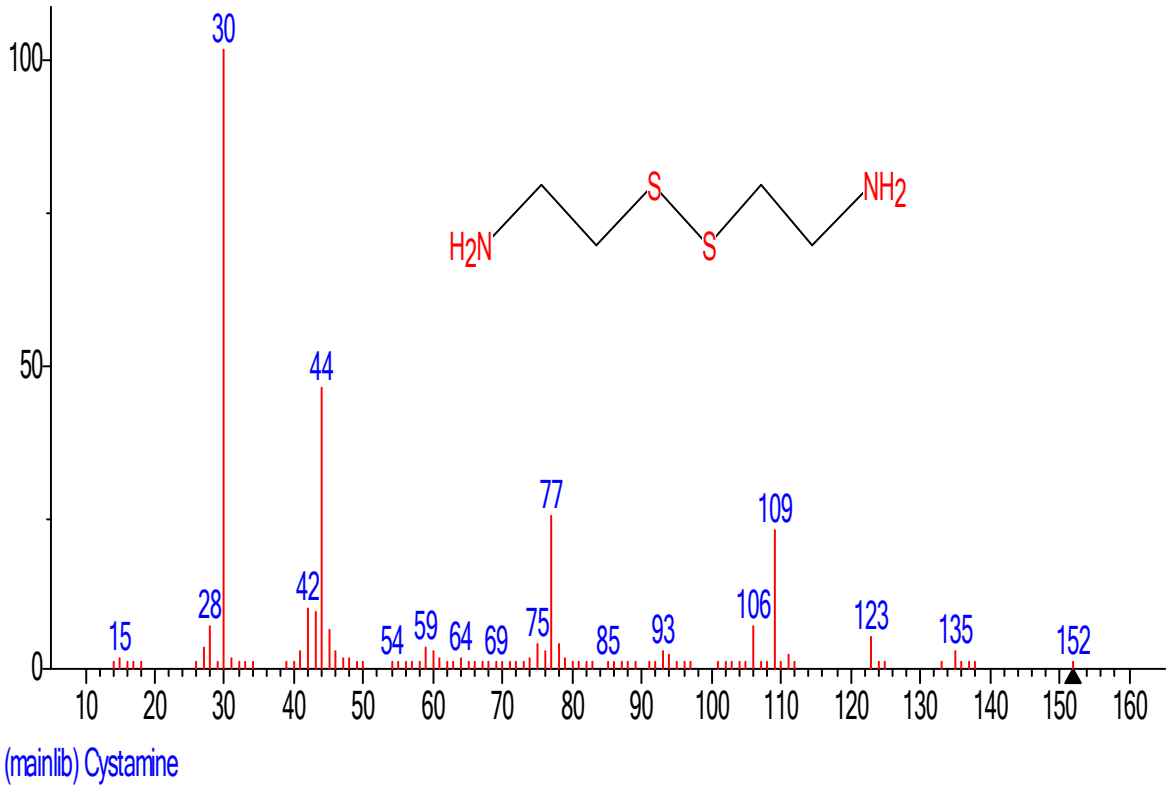



**Figure 4.24.1: TheGC-MSspectrum of cystamine obtained from dicloromethane fraction of *C. olitorius*,with x-axis as m/z ratio and y-axis asrelative intensity on the chromatogram.**

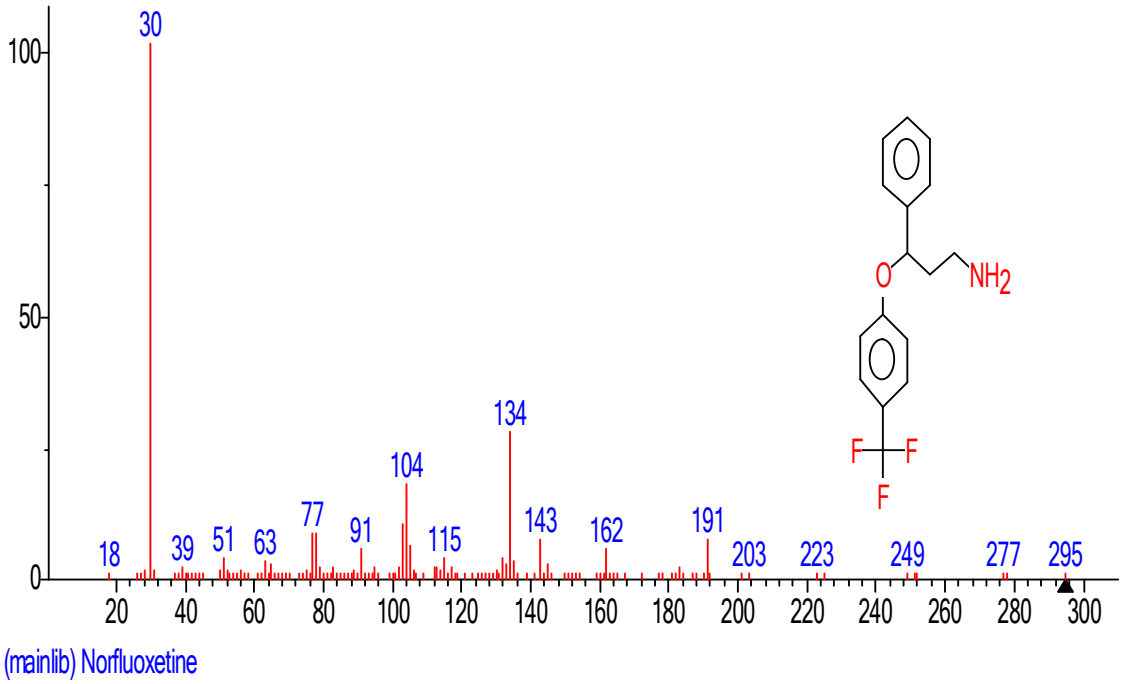



**Figure 4.24.2: The GC-MS spectrum of norfluoxetine obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

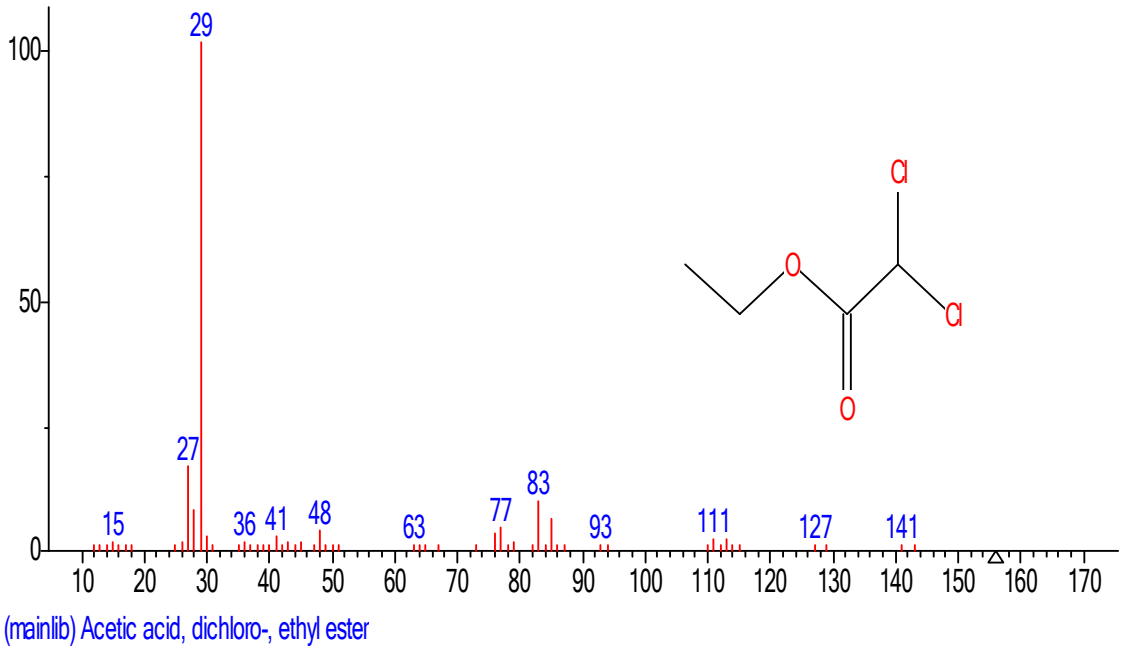



**Figure 4.24.3: The GC-MS spectrum of acetic acid, dichloro-, ethyl ester obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

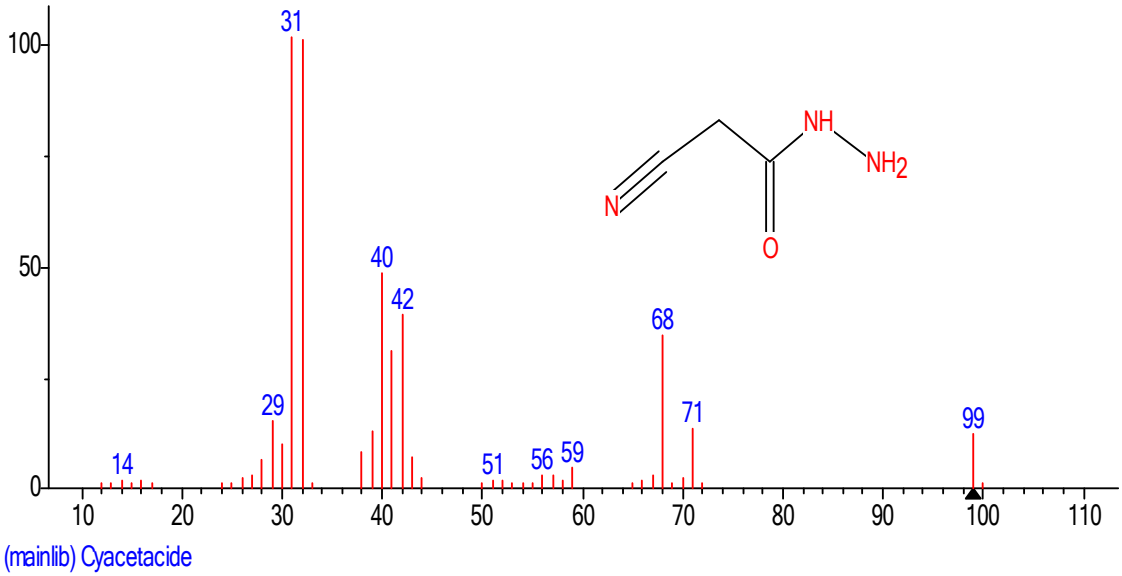



**Figure 4.24.4: The GC-MS spectrum of cyacetacide obtained from diclorometh**ane **fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

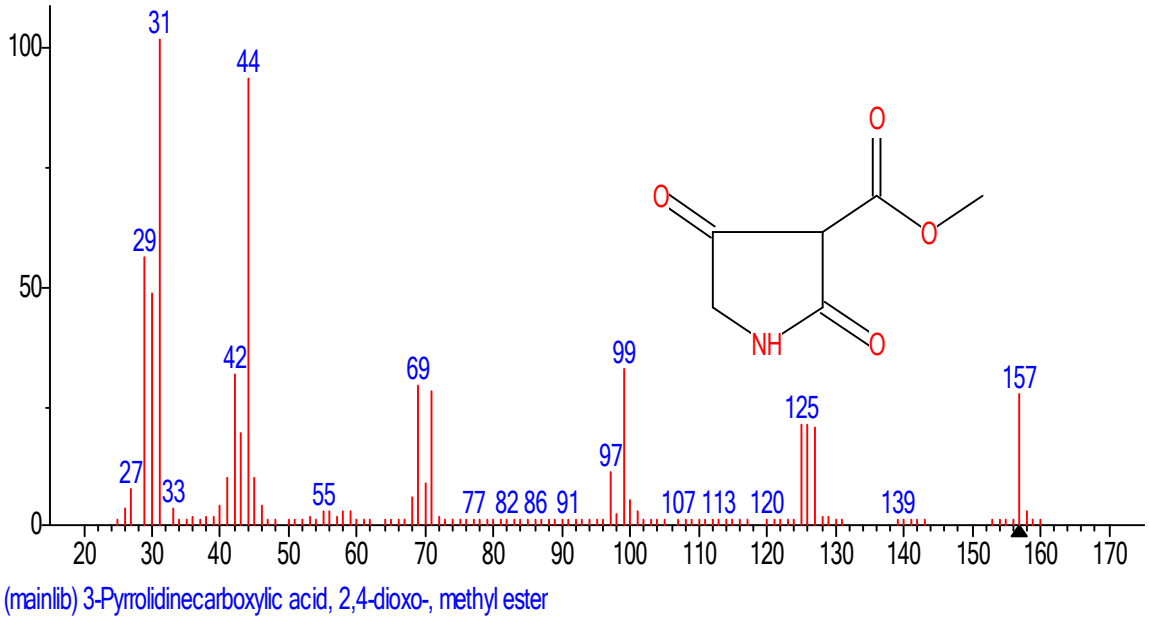



**Figure 4.24.5: The GC-MS spectrum of 3-pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl ester obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

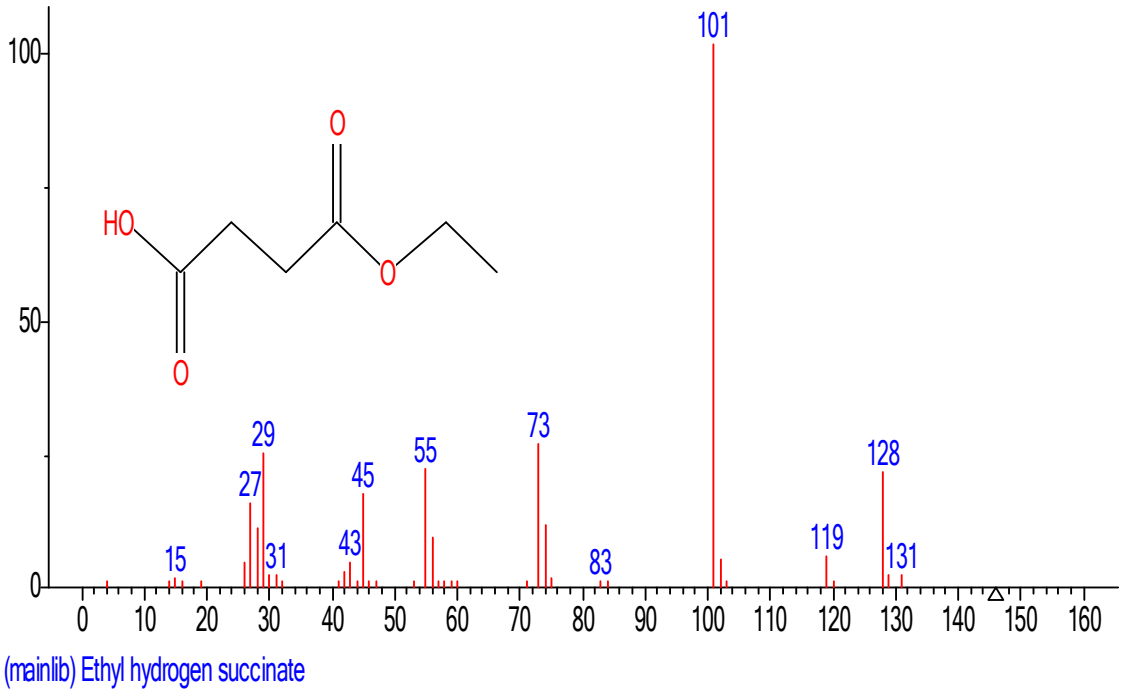



**Figure 4.24.6: The GC-MS spectrum of ethyl hydrogen succinate obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

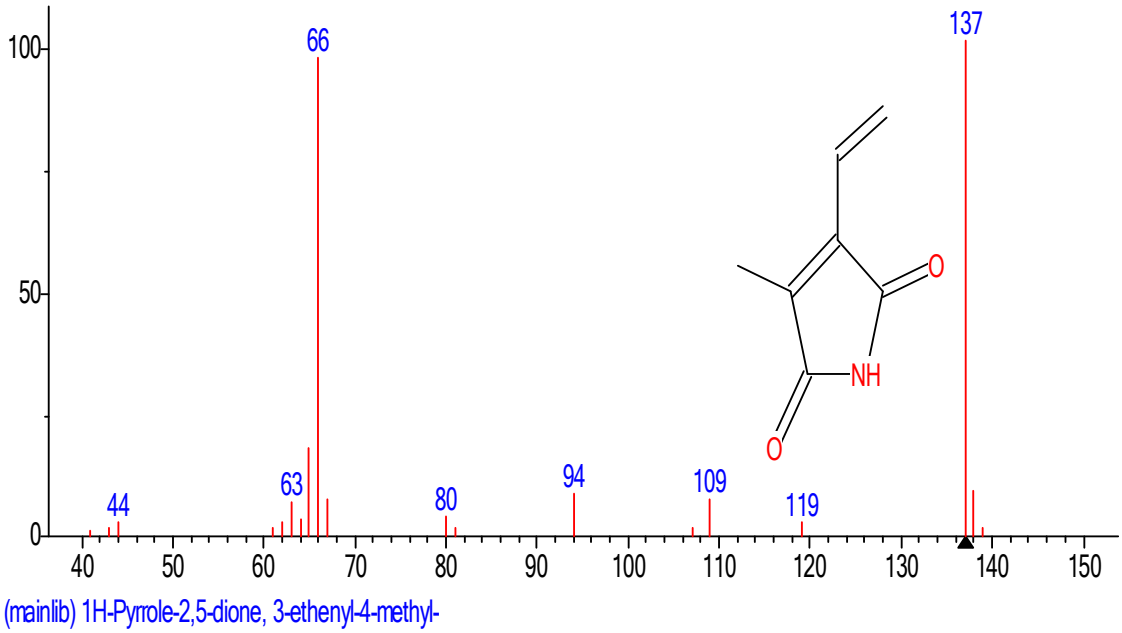



**Figure 4.24.7: The GC-MS spectrum of 1H-pyrrole-2,5-dione, 3-ethenyl-4-methyl- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

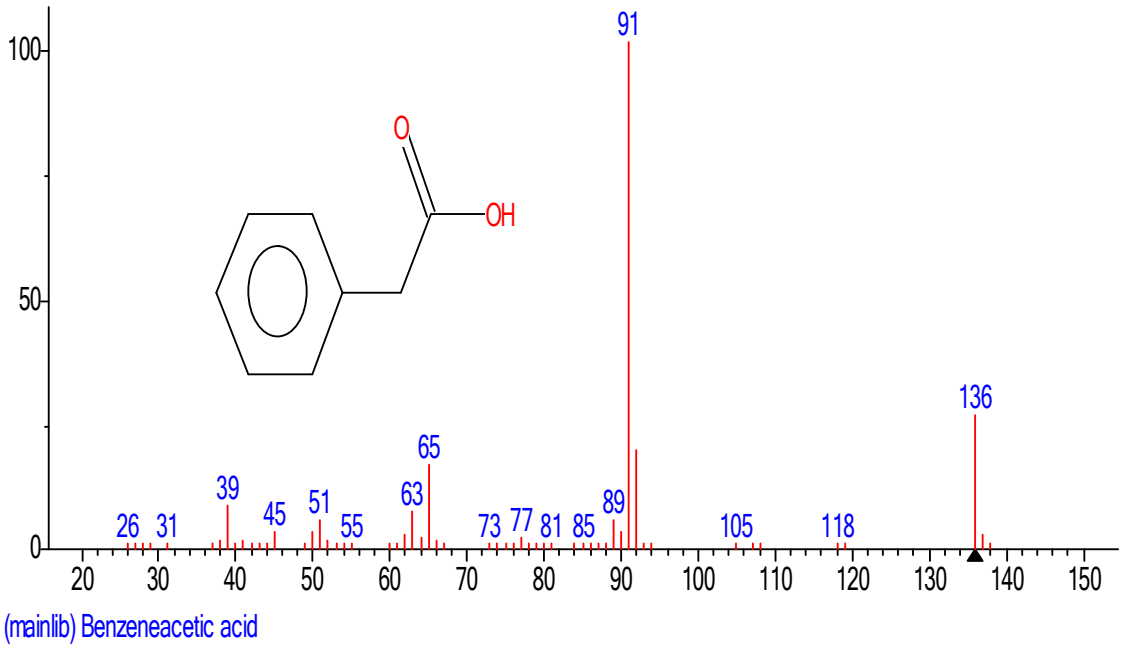



**Figure 4.24.8: The GC-MS spectrum of benzeneacetic acid obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

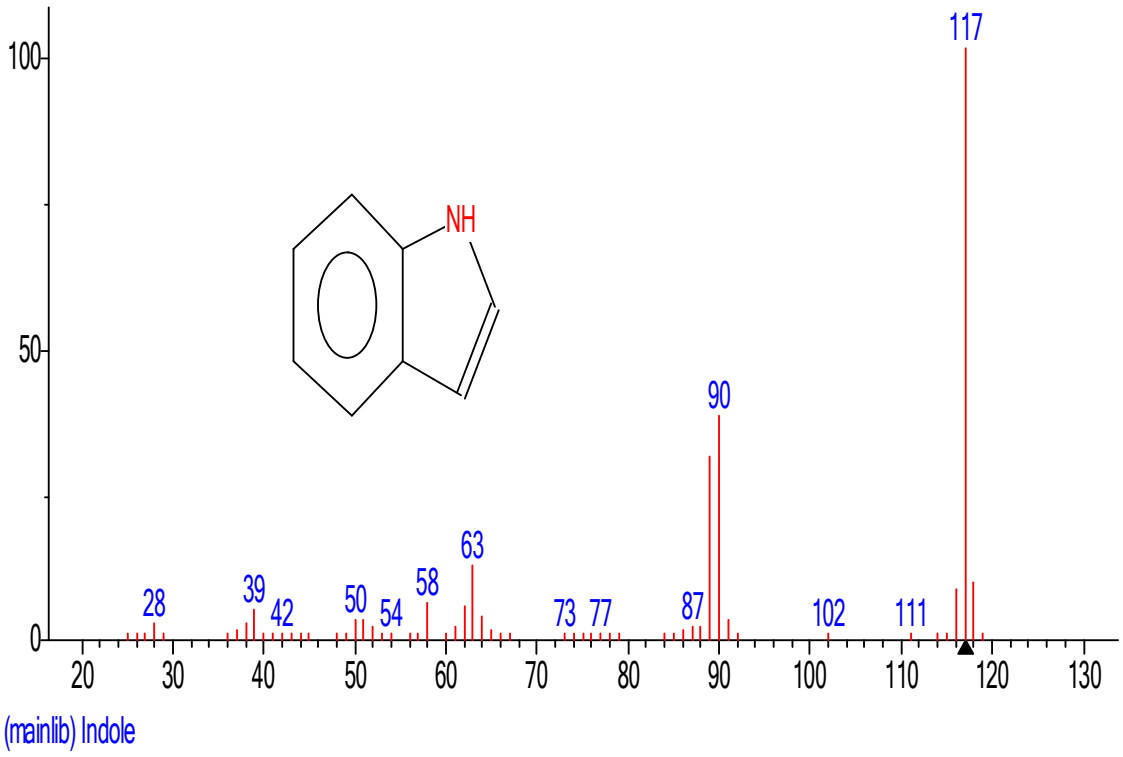



**Figure 4.24.9: The GC-MS spectrum of indole obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

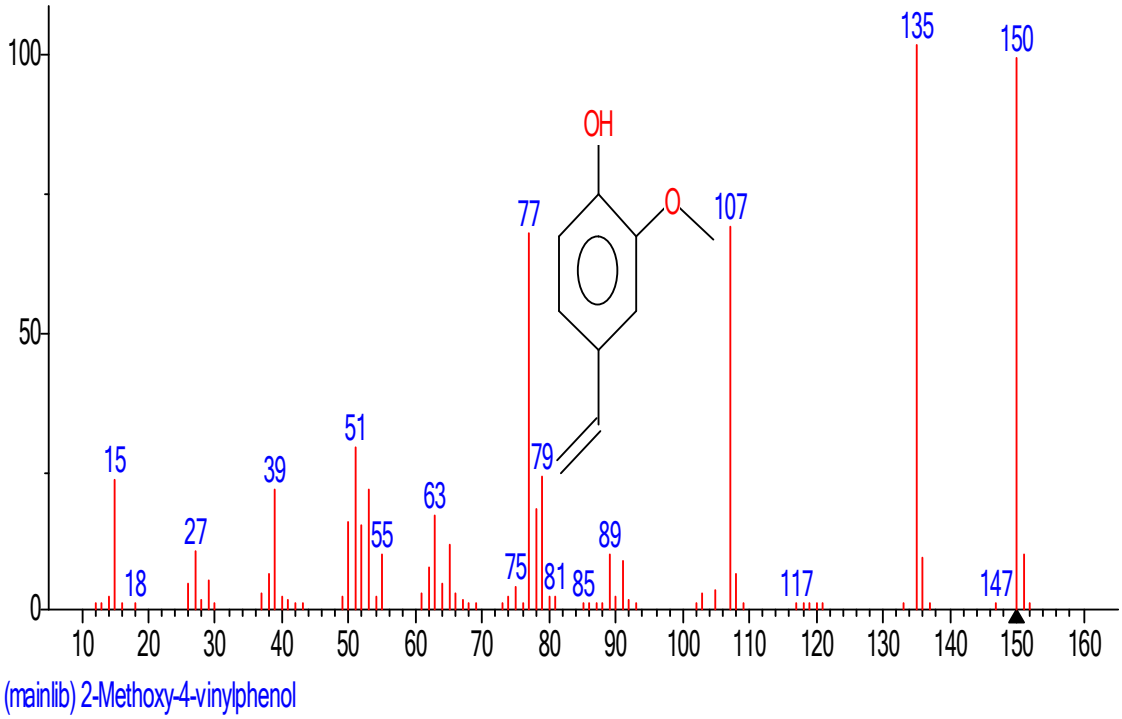



**Figure 4.24.10: The GC-MS spectrum of benzeneacetic acid obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

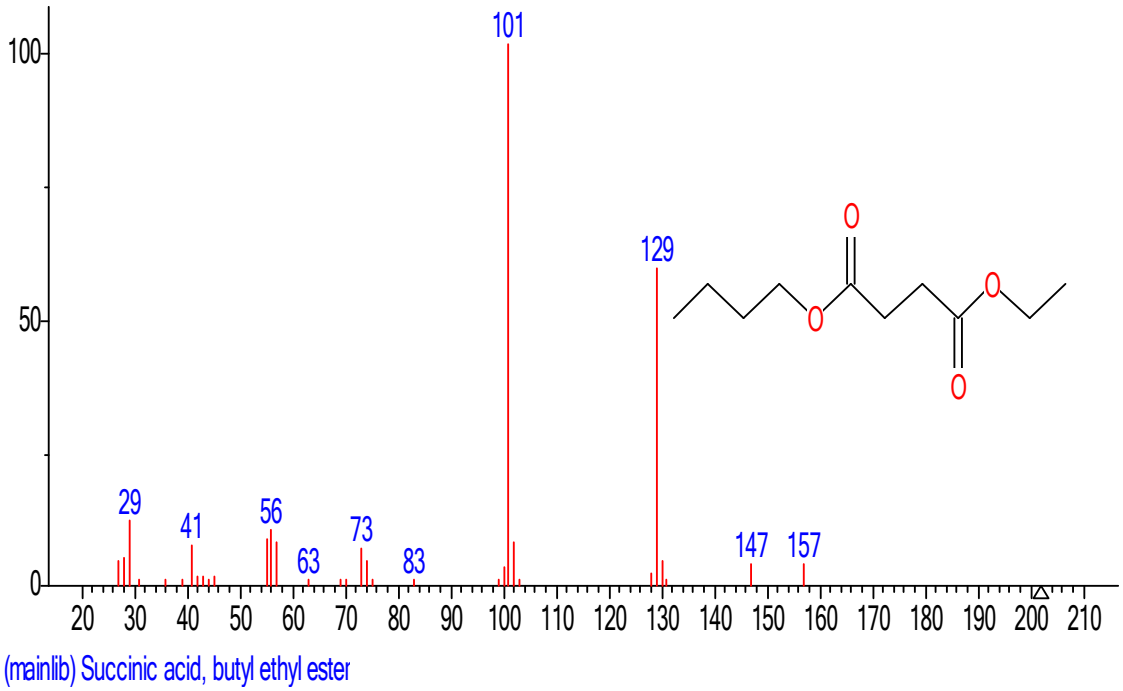



**Figure 4.24.11: The GC-MS spectrum of succinic acid, butyl ethyl ester obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

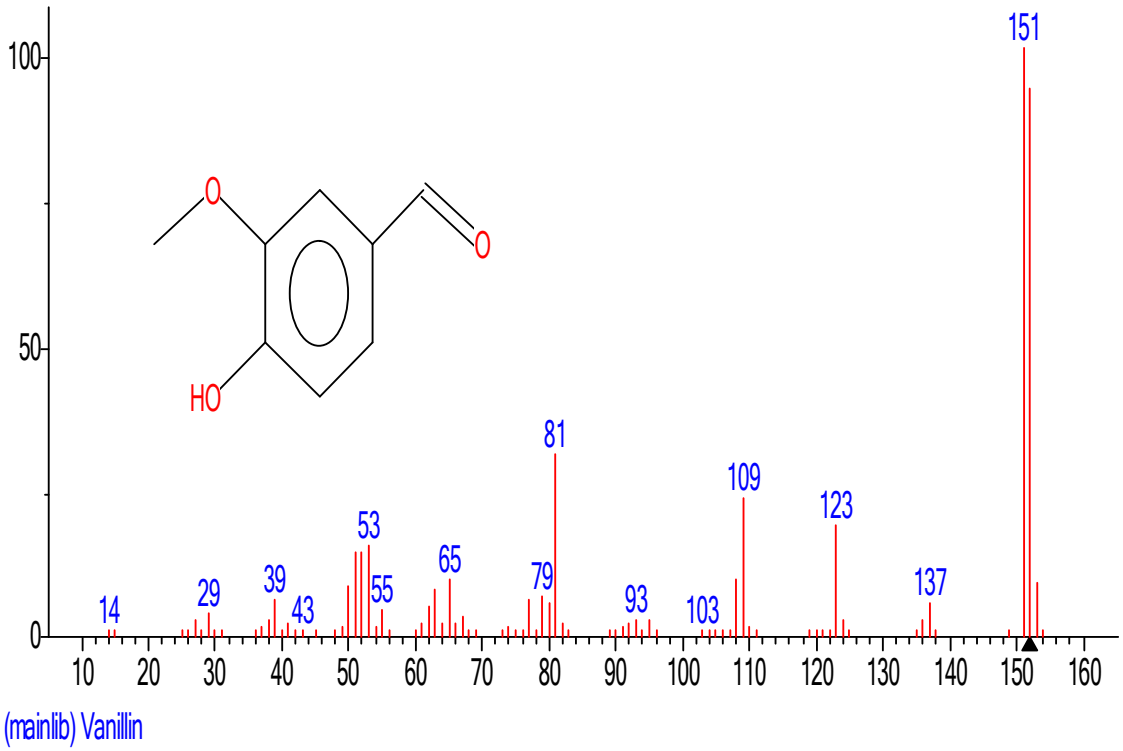



**Figure 4.24.12: The GC-MS spectrum of vanillin obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

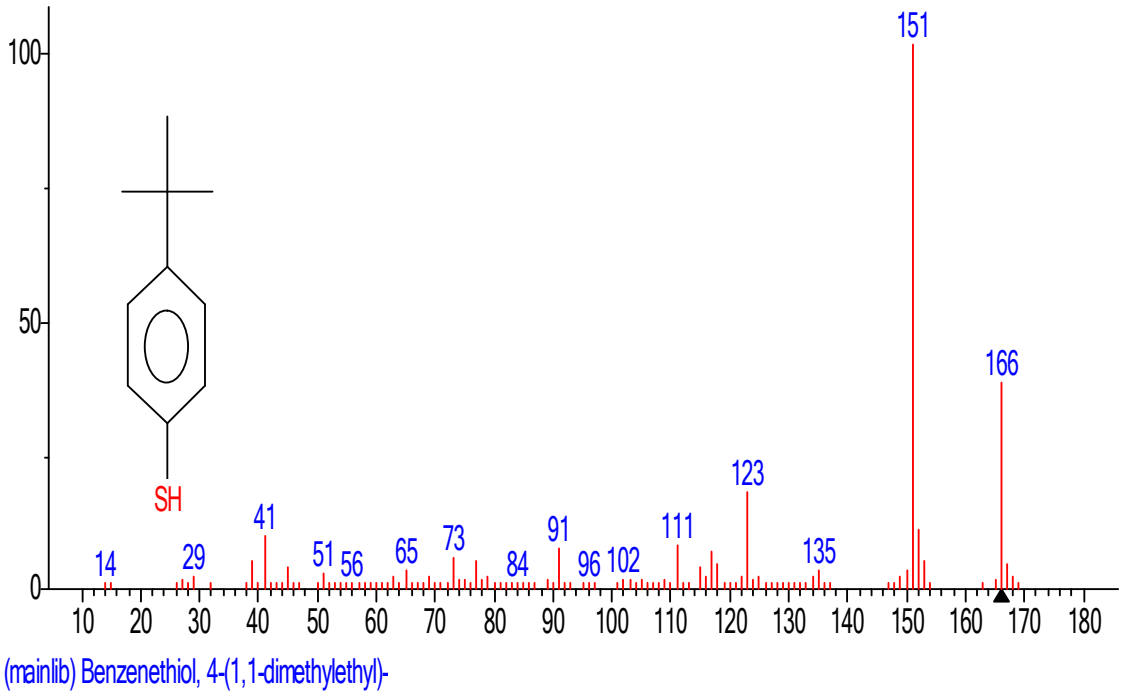



**Figure 4.24.13: The GC-MS spectrum of benzenethiol, 4-(1, 1-dimethylethyl)- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

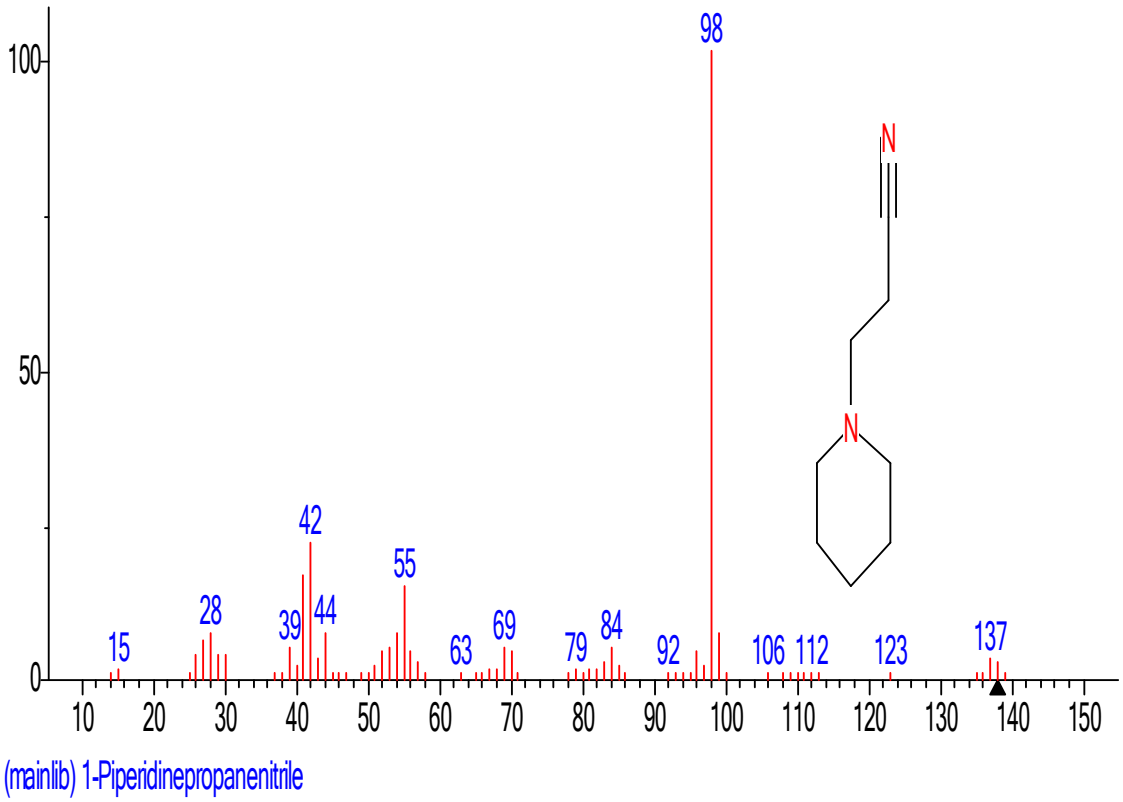



**Figure 4.24.14: The GC-MS spectrum of 1-piperidinepropanenitrile obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

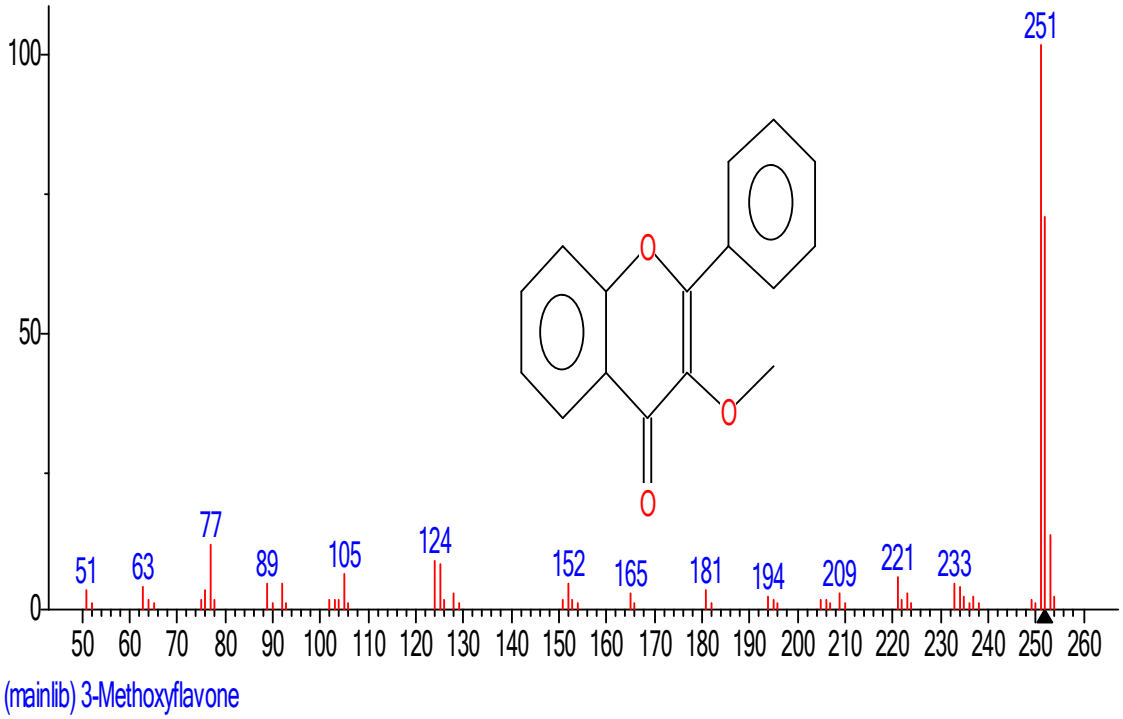



**Figure 4.24.15: The GC-MS spectrum of 3-methoxyflavone obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

6

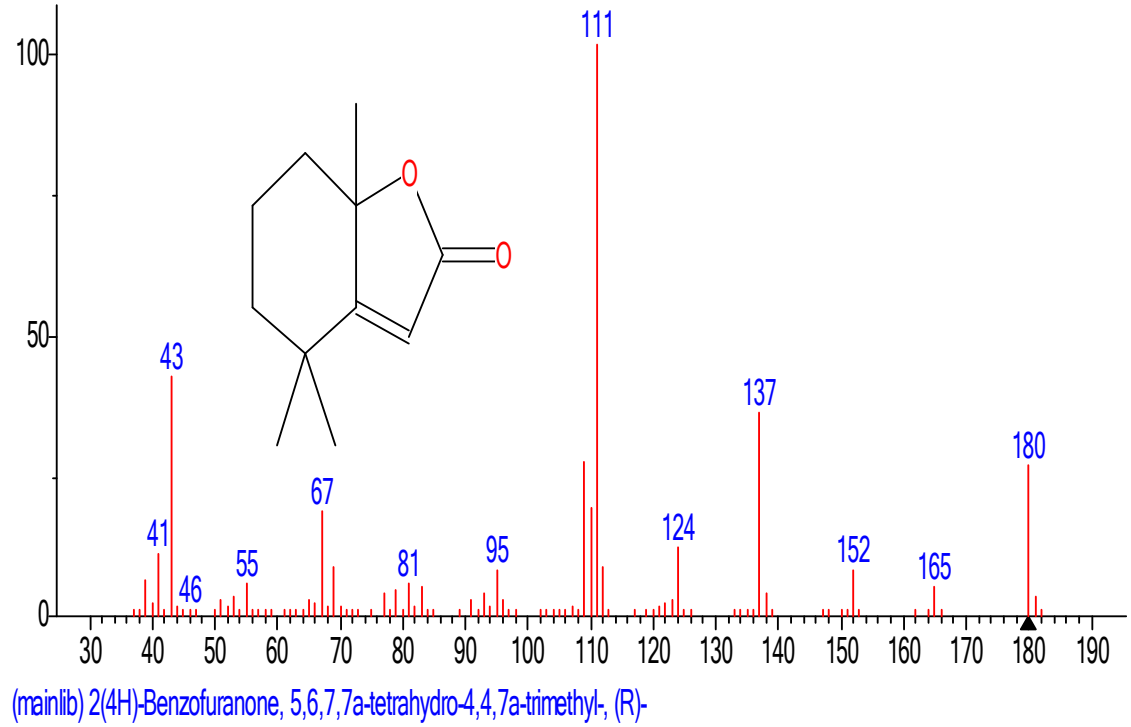



**Figure 4.24.16: The GC-MS spectrum of 2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,(R)- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

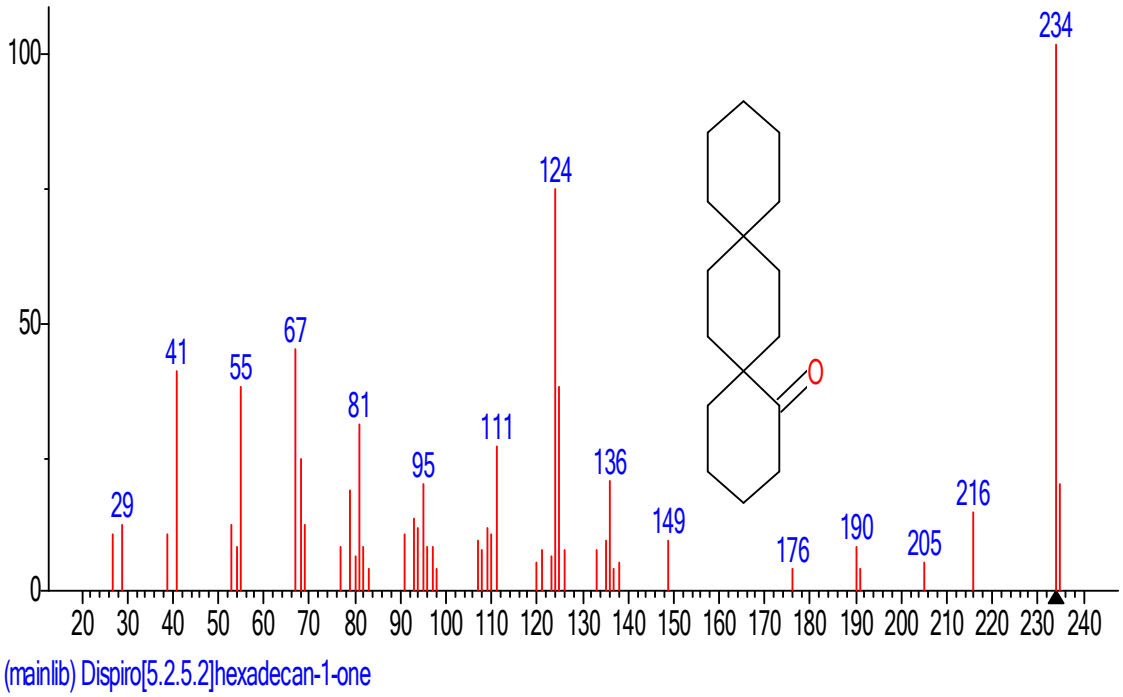



**Figure 4.24.17: The GC-MS spectrum of Dispiro(5.2.5.2)hexadecane-1-one obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

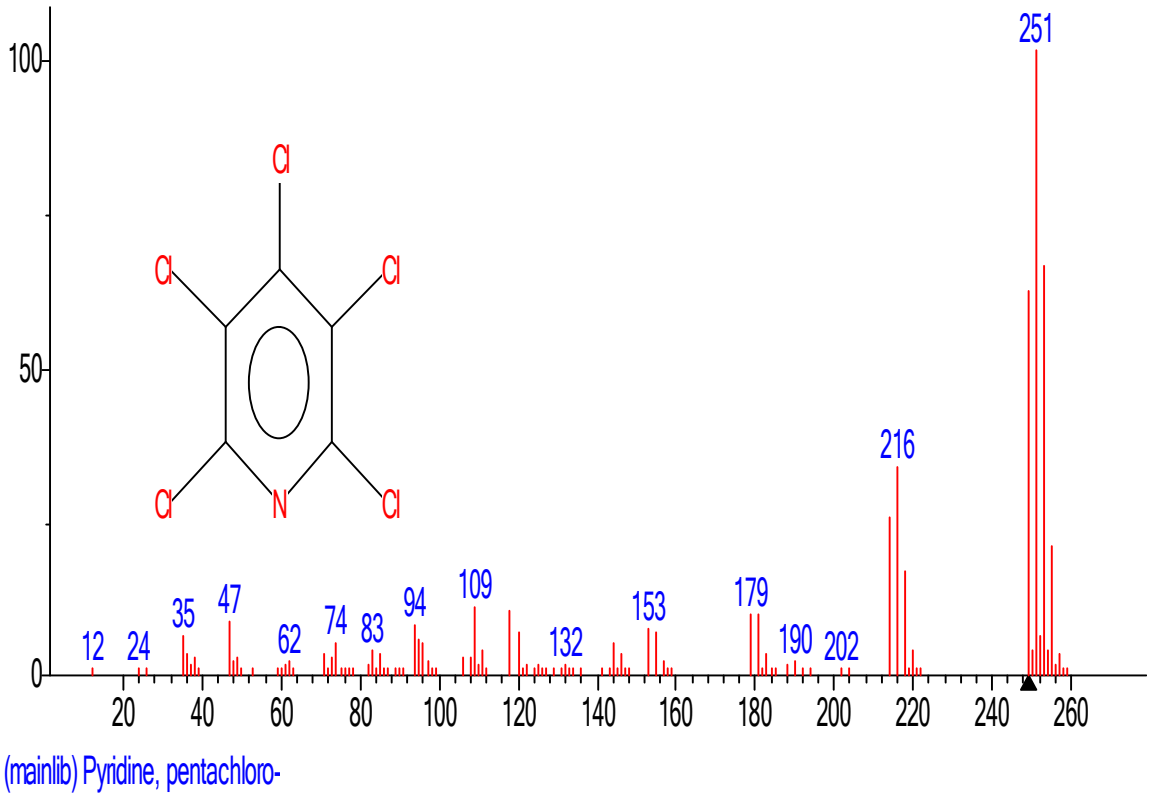



**Figure 4.24.18: The GC-MS spectrum of pyridine, pentachloro- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

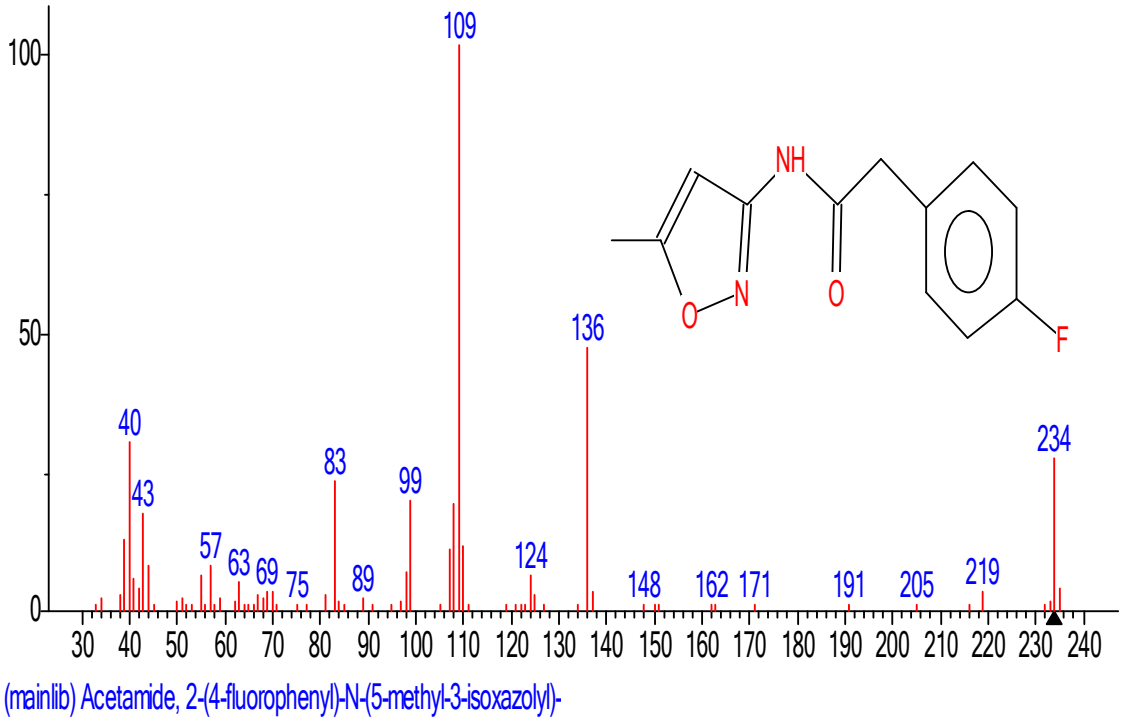



**Figure 4.24.19: The GC-MS spectrum of acetamide, 2-(4-fluorophenyl)-N-(5-methyl-3-isoxazolyl)- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

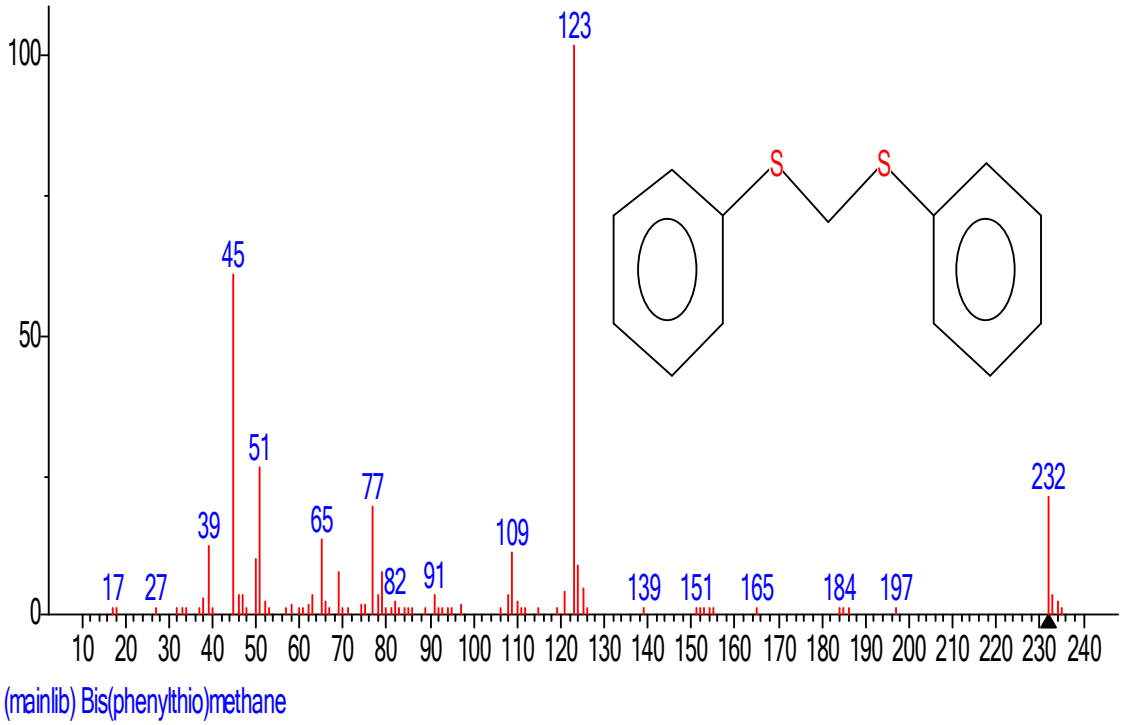



**Figure 4.24.20: The GC-MS spectrum of bis(phenylthiol)methane obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

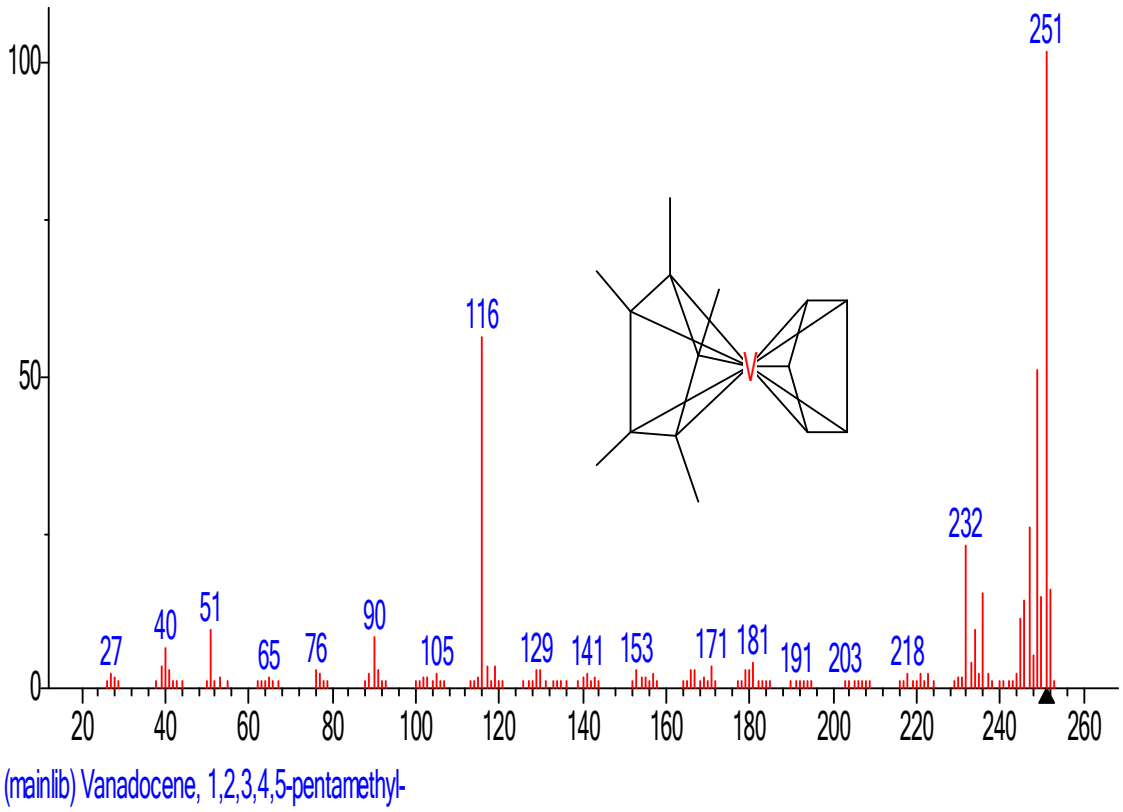



**Figure 4.24.21: The GC-MS spectrum of vanadocene, 1,2,3,4,5-pentamethyl- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

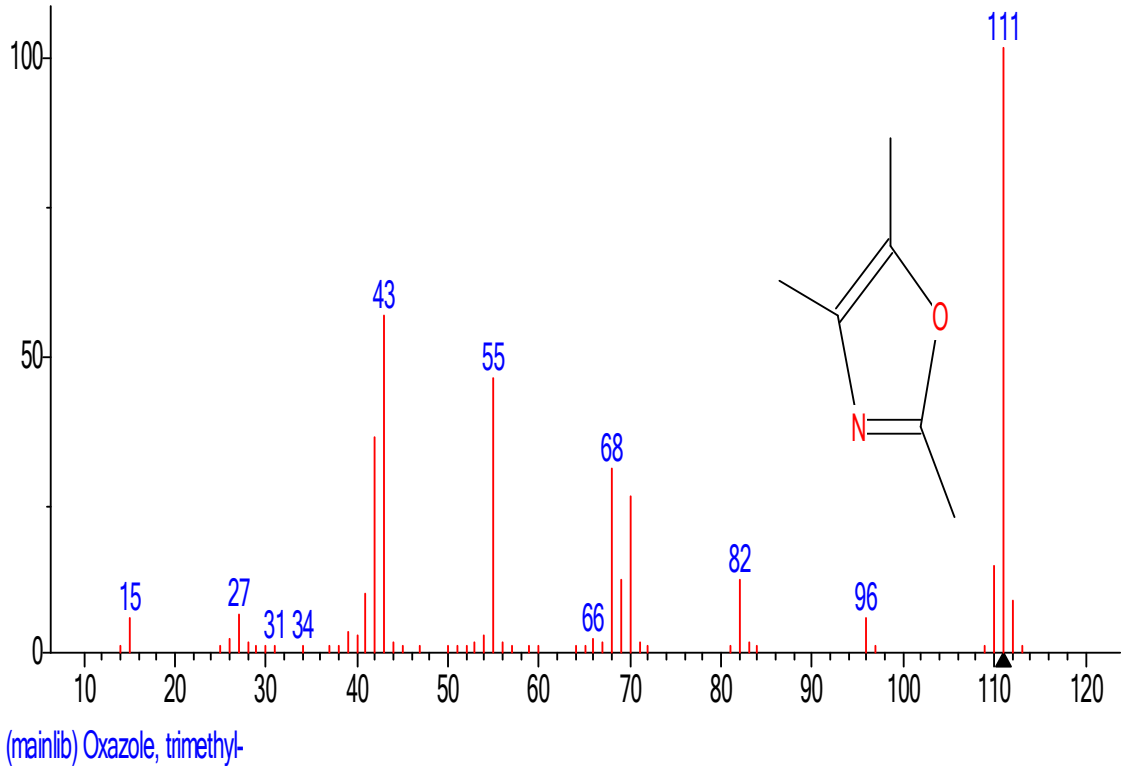



**Figure 4.24.22: The GC-MS spectrum of oxazole, trimethyl- obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

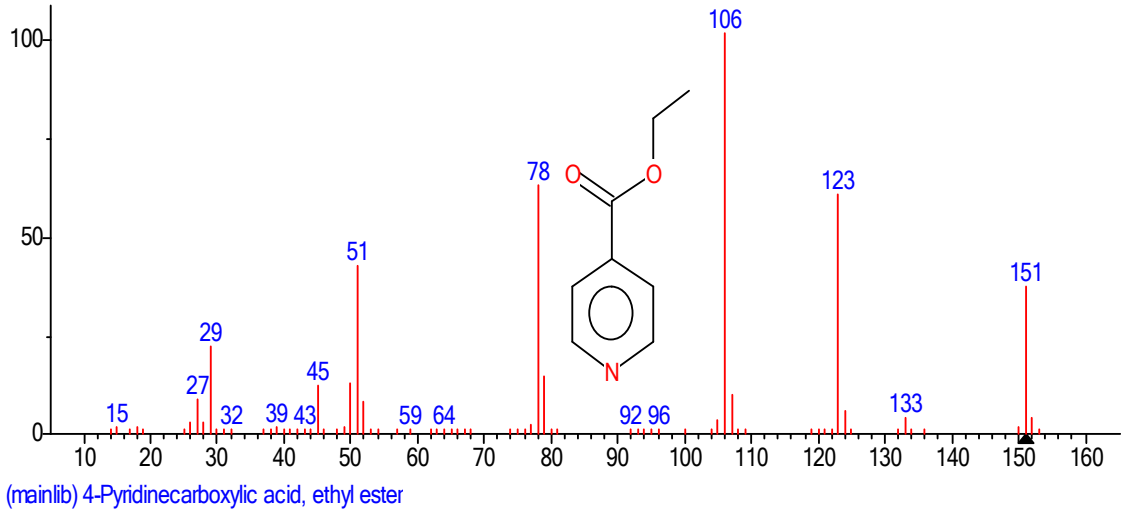



**Figure 4.24.23: The GC-MS spectrum of 4-pyridinecarboxylic acid, ethyl ester obtained from dicloromethane fraction of *C. olitorius*, with x-axis as m/z ratio and y-axis as relative intensity on the chromatogram.**

**Table 4.1: Chemical constituents of *Corchorus olitorius* Dichloromethane Fraction, based on GC-MS Analysis**

| PK | RT | Area Pct | Library/ID | CAS |
|---|---|---|---|---|
| 1 | 4.3097 | 59.3064 | Cystamine | 000051-85-4 |
| 2 | 5.7579 | 0.9203 | 2-propoxy-urea | 000592-31-4 |
| 3 | 5.8944 | 1.1513 | Norfluoxetine | 056161-73-0 |
| 4 | 5.9656 | 1.5409 | Acetic acid, dichloro-, ethyl ester | 000535-15-9 |
| 5 | 6.2564 | 3.8991 | Cyacetacide | 000140-87-4 |
| 6 | 6.7016 | 0.2678 | 3-Pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl ester | 051925-57-6 |
| 7 | 6.9805 | 3.8443 | Ethyl hydrogen succinate | 001070-34-4 |
| 8 | 7.1349 | 1.6009 | 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- | 020189-42-8 |
| 9 | 7.2951 | 0.9906 | Benzeneacetic acid | 000103-82-2 |
| 10 | 7.4554 | 0.2232 | Indole | 000120-72-9 |
| 11 | 7.5385 | 1.3703 | 2-Methoxy-4-vinylphenol | 007786-61-0 |
| 12 | 7.7521 | 1.7122 | Succinic acid, butyl ethyl ester | 1000324-85-1 |
| 13 | 7.9658 | 0.3964 | Vanillin | 000121-33-5 |
| 14 | 8.1439 | 1.977 | 1-Piperidinepropanenitrile | 003088-41-3 |
| 15 | 8.3931 | 0.2402 | Benzenethiol, 4-(1,1-dimethylethyl )- | 002396-68-1 |
| 16 | 8.5534 | 0.2129 | 3-Methoxyflavone | 1000306-41-0 |
| 17 | 8.7018 | 0.6446 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | 017092-92-1 |
| 18 | 8.7967 | 0.6883 | Dispiro[5.2.5.2]hexadecan-7-one | 001781-82-4 |
| 19 | 9.7881 | 0.7125 | Pyridine, pentachloro- | 002176-62-7 |
| 20 | 9.3072 | 1.3366 | Acetamide, 2-(4-fluorophenyl)-N-(5-methyl-3-isoxazolyl)- | 351060-75-8 |
| 21 | 9.7167 | 3.5413 | Bis(phenylthio)methane | 003561-67-9 |
| 22 | 10.1856 | 3.7465 | Vanadocene, 1,2,3,4,5-pentamethyl- | 088271-58-3 |

| | | | | |
|---|---|---|---|---|
| 23 | 10.6545 | 6.1215 | Oxazole, trimethyl- | 020662-84-4 |
| 24 | 10.791 | 0.9971 | Isonicotinic acid, octyl ester | 1000299-88-4 |
| 25 | 11.07 | 2.7614 | Benzenamine, 2,4-dibromo- | 000615-57-6 |
| 26 | 12.5122 | 0.259 | Pyridine, 3,5-dibromo-2-methyl- | 038749-87-0 |

**PK: Peak, RT: Retention time Area PCT: Area percentage (%) CAS NO: Chemical Abstract Service based on based on Scientific Library Identity.**

# CHAPTER FIVE

## 5.1DISCUSSION

From the results obtained during this study, high dose of isoproterenol was used to induce acute myocardial infarction in rats(non-invasive method). Hemodynamic parameters, electrocardiographic wave patterns, biomarkers of cardiac injury, endogenous tissue antioxidants, oxidative stress markers, inflammation markers and apoptotic related proteins obtained in theacute myocardial infarction rats compared to normal control, pre-treatment, post-treatment and fraction pre-treatment groups support the role of isoproterenol as a standard non-invasive experimental technique of inducing acute myocardial infarction. Isoproterenol-induced acute myocardial infarction was also used in previous study of Benita *et al*., (2016), Mahendra, (2010). In addition, O Brian, (2006), Balaz, (1978), report that when administered at high dosage,isoproterenol can cause myocardial infarction in Wistar rats, which is similar to those present in human myocardial infarction. After theadministration of isoproterenol at a dose of 100 mg/kg for two days, severe hypotension and abnormal ECG wave patternswas observed in the control group with infarction. Compared with the normal control group, systolic, diastolic and mean arterial pressure dropped sharply from (154.4 ± 1.21, 126.0 ± 1.18, 136.8 ± 0.80) in the normal control group to (86.4 ± 1.50, 58.6 ± 4, 37.65 ± 2.77) of the infarction control group (figure 4. 1.1). In addition, other hemodynamic parameters, such as arterial tail blood volume, tail blood flow and heart rate, were significantly reduced (Fig. 4.1.2, 4.1.3 and 4.1.4) compared with the normal control group. Isoproterenol, an adrenergic non-selective beta-receptor agonist is known to increase the force of contraction of the myocardium and heart rate. In addition, stimulation of the beta-receptorby agonist in smooth muscle of arterioles will cause vasodilation. This increase in myocardial strength and heartbeat raise blood pressure of the systole while its blood vessel dilation effect reducethe blood pressure during diastole and eventually results in decreased mean arterial pressure. Severe hypotension observed during this study is associated with excessive oxygen demand due to increased workload and reduced oxygen supply due to reduced diastolic coronary filling (Boyete*et al*., 2019)leading to cardiac muscle weakness thereby affecting cardiac output. The excess vasodilating effect has also been described as a main cause of hypotension, reduced blood volume and blood flow (Avinash *et al*., 2009) while myocardial weakening could be the suggested

explanation behind decreased heart rate. Aside severe hypotension being part of symptoms of acute myocardial infarction, alteration in electrocardiogram wave pattern are also major diagnostic technique used to detect acute infarction (Morris, 2002). Non-invasive ECG system showed abnormal pattern in the infarction control group of the present study. QRS interval of the infraction group was wide (21.4±0.93m/s) compared with (13.0±0.63m/s) of the normal control group. Also, reduction in P-wave interval of infarction group (12.6±0.51m/s) compared with 21.8 ±0.66) of normal control was obvious and a significant reduction in R-amplitude of infarctionrats was also observed.

R-amp average value for the normal control group was (0.4324 ± 0.01), and it was significantly reduced to (0.1892 ± 0.002 mV) in the infarction control rats. QRS complex is located between Q and S waves and its duration shows the time it takes for ventricular depolarization to spread (Morris, 2002). The wide QRS complex is an important reflection of intra-ventricular conduction barrier that can be observed in right and left bundle branch blocks, heart failure and myocardial ischemia. Broad QRS complex can also be seen in the treatment of rats with various drugs, such as doxorubicin(Kelishonni *et al*., 2008), disopyramide (Krol *et al*., 2015) azithromycin (Atli *et al*., 2015). In the current study, 100 mg/kg of isoproterenol, administered for 2 days, also increased the QRS interval which is a main symptom of acute heart attack. In the ECG recording, P-wave connotes depolarization of the atria and its absence in rats is characterized by atrial fibrillation similar to that of humans (Haugan *et al*., 2010).

Although in higher animals,clinical analysis of the length and shape of the P-wave provides clinically important information about rats, experimental data are not enough to dispense the changes in the shape and length of P wave. Although the current study shows a decrease in P-wave interval below the normal, however Milliez and collaborators reported that prolongation of the P-wave may be associated with increased susceptibility to supraventricular arrhythmia in Wistar rats after myocardial infarction (Milliez *et al.*, 2005). In addition to the abnormal ECG patterns discussed above, low amplitude was also observed in the infarction control group (0.1892±0.02) which is below the value (0.4324±0.01) recorded for normal control group.Other infarction groups pre-treated with varying concentrations of *C. olitorious* ethanol extract (250 mg/kg and 500 mg/kg) and 10 mg/kg enalapril showed improvement when their hemodynamic parameters were checked. Pretreatment of animals for 19 days prevented severe hypotension observed in infarction control animals. The systole, diastole and

mean pressure were raised to normal control group level (154±1.21, 126±1.18 and 136.8±0.80). Although the mechanism by which the ethanol extract and enalapril reversed the hypotension is not clear, cardiac output improvement may be a suggested explanation. Cardiac output depends on stroke volume and heart rate. Tail arterial volume and blood flow that was significantly improved in 250 mg/kg and 500 mg/kg infarction plus extract pre-treatment group (Fig. 4.1.2 and 4.1.3) support the explanation that improved CO may be the basic cause of the reversed hypotension. Also reduced heart rate observed in the infarction group (Fig. 4.1.4) that was reversed in extract and enalapril pretreated group may also contribute to the improved cardiac output. It is a bit difficult to explain and attribute the anti-hypotensive effect of *C. olitorious* extract and enalapril to its vaso-effect. Basically due to the fact that enalapril is a basic vasodilator (angiotensin converting enzyme inhibitor), it is used in treating hypertension and some types of heart failure (consensors study group, 1987). Also, non-peptide ACE inhibitor identified as nicotianamine and polyphenols were identified and purified from C. *olitorous* leaves using High Pressure Liquid Chromatography and Nuclear Magnetic Resonance (Koichi *et al*., 1988). These evidence further supports the explanation that the anti-hypotensive effects may be due to improved cardiac output.

The interval between the QRS wave was reduced when rats were pre-treated with varying extract doses and enalapril (10 mg/kg). BetterQRS interval reducing effect was observed in 250 mg/kg extract pretreatment group (Fig. 4.2.1a). By prolonging ventricular depolarization (wide QRS interval), it was explained that myocardial infarction increases susceptibility to ventricular arrythmia (Krol *et al*., 2015), and the results obtained from this study further confirm the antiarrythmic effect of the extract and positive control drug on pre-treatment rats. Other abnormal ECG patternsassociated with myocardial infarction andprevented by the pre-treatment include prolonged p-interval (Fig. 4.2.1) and increased ventricular amplitude especially in 250 mg/kg extract pre-treatment group (Fig. 4.2.2). Depolarization of the Cardiac muscle is almost synchronized with the contraction of heart muscle. Without depolarization there cannot be myocardial contraction. Prolonged atria P wave depolarization indicates prolonged atrial contraction. Probably due to the prolongation of the atrial contractile period and improving ventricular contraction associated with an increased amplitude of QRS, cardiac output of infarcted hearts was improved by the extract and enalapril.

Biochemical analysison serum and tissue parameters of myocardial infarctionalso showed important results. Pre-treatment with500 mg/kgextractrevealed a significant increase in serum level of lactate dehydrogenase although 250 mg/kg pretreatment ratsshowed no effecton serum LDH level. The enalapril pretreatment group demonstrate a further decrease in serum level of lactate dehydrogenase. Lactate dehydrogenase is an important and specific enzyme biomarker for myocardial infarction (Miller *et al*., 2010). Lactate dehydrogenase is responsible for the conversion of pyruvic acid to lactic acid. The LDH-1 isoenzyme is usually found in myocardium and LDH-2 predominates in the serum. A high level of lactate dehydrogenaseindicates a myocardial infarction. Previous research by Roza *et al*., (2015), Mahendra *et al*., (2010), Sunanda, (2015) support an increase in serum lactate dehydrogenase level associated with myocardial infarction. LDH are enzymes in the cytosol, where they catalyze anaerobic and aerobic cellular respiration. Once there is ischemic damagecaused by introduction ofisoproterenol, there was a violation of the integrity of cell membrane and intracellular proteins,which led to the release into the blood circulation, causing a rise in the serum levels of these lactate dehydrogenase during acute necrosis phase (Sobel, 1972). Upaganlawa*et al*., (2009) revealed that,elevatedserum LDH are associated with functional ischemia, hypoxia and hyperactivity associated with increased enzymatic metabolism. This explanation facilitate the conclusion that an excess serum LDH may be associated with altered myocardial metabolism and myocardial necrosis. Probably,due to the membrane stabilizing effect of ethanol extract and enalapril, serum LDH level was reduced. The effect of stabilization of the membrane may be associated with inhibition of the ability of lipid peroxidationof the membrane. The crude extract and enalapril pre-treatment and post-treatment study were used to determine serum nitric oxide derivative level during acute myocardial infarction. Pre-treatment and post-treatment study reveals a reduction in serum NO derivatives in the myocardial infarction group. A gradual increase in their serum concentration was associated with varying extract doses but enalapril seems to have no effect on serum NO derivative. Nitric oxide can be an endogenous biological molecule that is released as gaseous free radical that are involved in a lot of normal and abnormal process. This molecule was referred to as double edge sword by Otani, (2009). In fact, its action can be direct or indirect (Miranda*et al*., 2000). Due to the direct effect, blood vessel dilation, relaxation of smooth muscle and diminished heart contraction occur (Kroncke, 1997). Indirect actions are associated with the generation of peroxylnitrite ($ONOO^-$). Some researchers believe that NO is a cardiomyocyte second

messenger expressed by nitric oxide synthase of the heart (eNOS, iNOS, nNOS) and at physiological concentration, enhance cardiac contractility(Kopincova*et al*., 2011). It was further explained that eNOS express physiological NO concentration and further explanation by Krenek, (2009) that physiological amount of NO may help to sustain myocardial contraction supported this. Present study showed a NO enhancement by the extract which also support the study by Kopincova*et al*., (2011). This observation contradicts the study of Sanchez *et al*., (2002) who suggest that NO plays a significant role in the aggravation of myocardial infarction. Significance of NO concentration in serum of infarction animal group can further support the suggested explanation that NO enhancement is a major means of protection against infarction most especially in the pre-treatment study and to some extent in post-treatment study although there seems to be a need to do further investigation on the effect of the extract and control drug on tissue expression of eNOS and iNOS in the myocardium. Increased R-amplitude in pre-treatment group and reverse occurrence in the myocardial infarction group also support NO positive inotropic effect. In addition, this may also improve cardiac output against severe hypotension. In addition,larger dose of isoproterenol from this study, may results in increased blood vessel and myocardial tissue NO production resulting into severe hypotension and also reacting with membrane lipid to form peroxylnitrite eventually causing reduced serum NO concentration in infarction non-treated animals.

Specific serum and tissue antioxidant systems were enhanced in the pre-treatment and post-treatment study animals. There was an increase in reduced glutathione (GSH) level in pre-treatment and post-treatment study. Enalapril enhanced glutathione peroxidase in the pretreatment study and 500 mg/kg extract pre-treatment group also shows increased glutathione transferase level. There was no effect of the extract and enalapril on the tissue enzymatic antioxidant concentration suggesting that GSH may be the major antioxidant against myocardial infarction. Other biochemical parameter test in the pre and post-infarction treatment study groups explained the importance of thiol (SH) containing compound especially GSH in the antioxidant defense mechanism against free radicals. There was a reduced non-protein thiol level in the infarction group and 500 mg/kg extract, 10 mg/kg enalapril increased non-protein thiol level more than 250 mg/kg extract. The same increase was observed in the post-treatment study groups. Based on the antioxidant test, it was observed that (SH) containing compounds like GSH and other non-protein thiol compounds are the main antioxidant protective source of

myocardiocytes against acute infarction from the present study. Sulphydryl containing compounds also called thiols, are organic compounds containing sulphydryl group and make up a large proportion ofantioxidants in the body. The level of protein thiols including protein sulphydryl groups and protein mixed disulphides like cysteine, homocysteine, cysteine-glycine (CysSH, HcySH, CysGlySH) were assayed in myocardial tissue but acute infarction seems not to affect their tissue level in the pre-treatment and post-treatment study. It was also observed that the tissue level of protein or enzyme related antioxidants like glutathione reductase, glutathione peroxidase in the normal control rats were not different from the infarction, pretreatment and post-treatment study rats.

There was increase in serum level of myeloperoxidase (MPO) in the myocardial infarction group, and the serum level of this inflammatory marker was greatly reduced in 250 mg/kg extract pre-treatment group, with 10 mg/kg enalapril rats and 500 mg/kg extract pre-treatment group showing better activity. Similar effect was observed in the post-treatment study. MPO is a popular enzyme released by neutrophils, and known for itsstrong pro-oxidative and pro-inflammatory properties. The increase in activity of this enzyme has been linked with consumption of endothelial-derived NO thereby reducing the blood level of NO (Valentina *et al*., 2008). The implication of this from this present study is that enhanced serum nitrite in the pre-treatment groups is inversely proportional to MPO activity in the pre-treatment group. Also, tissue MDA level was reduced to normal in infarction pre-treated animal groups with higher MDA level observed in the infarction control group and post-treatment study also reveals similar property. Malondialdehyde tissue increase is a sign of lipid peroxidation due to increase in tissue free radical level ($ONOO^-$) leading to further explanation that antioxidant effect is a major defense mechanism in necrosis related myocardial infarction. In addition, increase in theserum level of Advanced Oxidation Protein Products (AOPP), animportant biomarker of oxidative stress and inflammation within the infarction, pre-treatment and post-treatment study rats is an eye opener on the role of enalapril and *Corchorus olitorius* leaf extract against inflammation and oxidative stress. AOPP was proposed to be a possible marker of oxidative injury (Marcela *et al*., 2005), which originates from oxidative stress and inflammatory activities. Its serum level was largely reduced within the treatment (Pre & Post) study rats and increased in the infarction control rats. These product is a result of free radicals action on proteins and may act as inflammatory

mediators that can trigger neutrophils, T-lymphocytes and monocytes release thereby leading to up-regulation and over-stimulation of dendrites (Alderman *et al*., 2002). Effect of the extract and enalapril on MPO and AOPP suggest their strong anti-inflammatory effect as another protective means in myocytes against acute myocardial infarction. Slight increase in serum and tissue total protein level was observed in pre-treated animals while the infarction experimental rats showed decrease in serum and tissue total protein level with similar result noticed in the post-treatment study design. This result supports previous studies relating acute myocardial infarction with acute renal failure. Lekston, (2009) explain that in acute myocardial infarction, impaired renal activity may be a result of acute renal failure existing along with it. Previous study by Gautam *et al*., (2012) also support this. Hypoproteinemia is one of the symptoms of renal failure which was observed in the present infarction control rats.

From the pre-treatment and post-treatment study, pre-treating the rats with ethanol extract seems to produce better anti-inflammatory and anti-oxidant effect than post-treating the animals although enalapril is very effective during both pre and post-treatment study. Also, pre-treatment concentration of 250 mg/kg ethanol extract produced consistent effect on all observed parameters during pre-treatment study. This lead to limiting the fraction study to pre-treatment, using two polar solvents (ethanol and ethyl-acetate), two non-polar solvents (dichloromethane and n-hexane). Serum level of C-reactive protein was severely elevated in the acute myocardial infarction rats and the serum level of this important biomarker in the pre-treatment groups was reduced except in n-hexane 50 mg fraction. 100 mg/kg Dichloromethane fraction and n-hexane fraction showed better activity than other fractions and 10 mg/kg enalapril against serum C-reactive protein level. The role of this protein is to attach to lysed-phosphatidylcholine expressed on the surface of either dead or dying myocardial cells to stimulate the complement cascade system (Thompson *et al*., 1999) thereby promoting necrotic and apoptotic myocardial cell engulfment by macrophages. Serum rise in this protein is called acute response phase occurring due to increase in serum interleukin-6 level produced by macrophages and myocardial tissue injury (Lau *et al*., 2005). This serum reduction effect could be due to inhibition of inflammatory biomarkers release, necrosis and apoptosis. Although Enalapril (10 mg/kg) almost reduced serum level of creatine kinase-MB to normal control group level, 100 mg/kg dichloromethane fraction pre-treatment group once again showed better effect than the positive control drug. The

serum level of this enzyme in the myocardial infarction rats was very high but all the pre-treatment fraction groups reduced the serum level except 50 mg/kg n-hexane pre-treatment group. Reducing the activity of creatine kinase-MB suggest that the fractions also produced cardio-protective effect through inhibition of severe depletion of Adenosine Triphosphate level in the myocardial tissue. Creatine Kinase-MB, that is always found in almost all tissues of the body but highly abundant in the myocardial cells (Roza *et al*., 2015) is an enzyme that the heart depends on during acute and severe ischemia after depletion of ATP (the major high energy phosphate donating compound). This enzyme act on creatine which donate a high energy phosphate compound for recycling of ADP to ATP.

The result also reveals the effect of the fractions on nuclear factor kappa B which is usually referred to as major pro-inflammatory transcription factor. There was an increase in myocardial tissue level of this transcription factor in all fractions and there seems to be no difference between nuclear factor kappa B level of the control group and myocardial infarction group. Increased myocardial level of this transcription factor during ischemic heart condition is a paradox and lead to repetition of estimating the level of the nuclear factor using immunohistochemistry technique to determine the tissue expression level. Severe expression of tissue of nuclear factor kappa B level was observed in dichloromethane pre-treatment group and reduced tissue expression in myocardial infarction group was also found which is similar to result obtained when enzyme linked immunosorbent assay was used. It is expected of nuclear factor kappa B to trans-locate into the nucleus during canonical activation associated with phosphorylation by IKK (inhibitory kinase) on the serine n-terminal and enhance the transcription of inflammatory cytokines like interleukin-6, cyclo-oxygenase-2, inhibitory nitric oxide synthase which further promotes inflammation (Guro *et al*., 2001, Chenguang *et al*., 2002,). From this study, cardio-protective effect of the fractions thatcorrelates with increased myocardial tissue level of nuclear factor kappaB support previous study by Morgan *et al*., (2000), Chenguang, (2002) Liu *et al*., (1996), which reveals that NFKappaB possess beneficial anti-apoptotic protective role during acute cellular stress. It was revealed by Guro *et al*., (2001) that NFKB activation was the main mechanism of cardio-protection during ischemic preconditioning against ischemic reperfusion injury. Chenguang, (2002), suggested that anti-apoptotic effect could be due to tyrosine kinase terminal phosphorylation of IKB alpha instead of serine n- terminal phosphorylation.

Thus, the role of inflammatory cytokines and nuclear factor kappa B was described to possess double- edged role in ischemic heart related disease like reperfusion injury and other chronic disease like cancer.

p65RelA-P50NFkappaB which represent the most common heterodimeric type of myocardial NFKppaB has been linked to its binding with DNA transcription specific site in the presence of histone-acetyltransferase and histone-deacetylase to increased transcription, over-expression of Bcl-2 family (anti-apoptotic related proteins) and inhibit the transcription, protein expression of Bax, p53 which are pro-programmed cell death related proteins (Leon *et al*., 2006). Result obtained from this study on the expression of Bcl-2, Bax and p53 using immunohistochemistry assay technique support these explanation. The expression of Bcl-2 was very high in 50 mg/kg, 100 mg/kg dichloromethane pre-treatment group and 10 mg/kg enalapril pre-treatment group. This protein expression was very low in acute myocardial infarction group. Also, Bax and p53 expression in myocardial tissue was very severe in acute infarction rats and very mild in the pre-treatment group (50 mg/kg, 100 mg/kg dichloromethane and 10 mg/kg enalapril). The role of Bcl-2 in apoptosis is very crucial and has been described to be a major determinant of apoptotic pathway (Zhao *et al*., 2000). Bcl-2 proteins are believed to control apoptosis by inhibiting the binding of apoptotic activating factor-1 (Apaf-1) with cytochrome-C released from mitochondria leading to activation of different caspases and also control the release of the cytochrome -C (Hausmann*et al*., 2000). The ratio of bcl-2 to bax proteins have been explained to determine the survival or death of myocardial tissue after acute and severe ischemic induced apoptosis (Krijnen*et al*., 2002). In normal myocardial cells, p53 was explained to be kept at low levels by the E3 ubiquitin ligase Mdm2 through proteosomal degradation (Tak *et al*., 2017). From this study, it seems nuclear factor kappa B was enhanced by the pre-treatment fractions to increase the transcription of anti-apoptotic Bcl-2 proteins, and inhibit the expression of Bax and p53 proteins. In addition, results obtained from a study of Chenguang*et al*, (2002) suggested that the timing of p65$NF_kB$ activation during the acute phases of injury can significantly influence apoptotic outcomes in an injury-stimuli-dependent manner. The study by Chenguang, revealed that the anti-apoptotic role of p65$NF_kB$ is due to acute injury response like hypoxia/reoxygenation, UV radiation or TNF-α and this has also been linked with tyrosine phosphorylation of $I_kB\alpha$ and not the conventional serine phosphorylation on position 32 and 36. From the present study, increase in p65$NF_kB$

activation associated with fraction and enalapril pretreatment and the low tissue level of p65$NF_kB$ observed in non-treatment infarction control rats support the anti-apoptotic role of the transcription factor during the acute severe phase of myocardial ischemic injury.

From the results obtained during fraction study, dichloromethane fraction showed better activity. This prompt the gas chromatography- mass spectroscopy (GCMS) analysis of the DCM fraction to determineits active component fraction. GCMS revealedthe presence of compounds with pharmacological importance. These compounds include Cystamine, Norfluoxetine, Pyrroles (3-Pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl ester, 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- and Indole), Vanadocene derivative (Vanadocene, 1,2,3,4,5-pentamethyl-), Carboxylic acid derivative (Benzene acetic acid, Isonicotinic acid octyl ester), Polyphenols (Methoxylphenol), Pyridines (Pyridine, pentachloro-, Pyridine, 3,5-dibromo-2-methyl-), Oxazoles (Acetamide, 2-(4-fluorophenyl)-N-(5-methyl-3-isoxazolyl)-, Oxazole, trimethyl-). Isoxazole is a core ring in the structure of many anticancer drugs (Tyrosine kinase inhibitor of HER-2 oxazole), antibiotics (cloxacillin, dicloxacillin, oxacillin), anti-inflammatory (Cyclooxygenase-2 inhibitor like valdecoxib), antihyperglycemic agents, HIV- inhibitory drugs and glutamate receptor antagonist (Jayaroopa *et al*., 2013). Vanadocene derivatives along with other transition metals like titanium, platinum (Cisplatin) has been described to be very useful in treating testicular cancer (Fatih *et al*., 2000).Indole nucleus is the main backbone of a lot of tubulin polymerization inhibitors like vincristine (Brancale*et al*., 2007) and COX-2 inhibitors like 3- arymethylindole (Zarghi*et al*., 2011) and other anti-inflammatory like indomethacine. Benzene acetic acid or Phenylacetic acid is also a major ring and a metabolite of alkylating agent like chlorambucil used in treatment of solid tumor (Alberts*et al*., 1980), Lumiracoxib a COX-2 inhibitor (Barzatt, 2014). Also, Isonicotinic acid derivative is a major component of isoniazide a drug used against tuberculosis which inhibit the synthesis of mycolic acid. Informations about the pharmacological use of these compounds suggest that the dichloromethane fraction of the *Corchorus olitorius* possess a strong anticancer and anti-inflammatory potential.

A major compound of this GC-MS analysis that is highly relevant to the present study is cystamine. Percentage composition of this compound is about 60% of the entire dichloromethane fraction. Structurally, this compound possessdisulfide link with primary amines group at the terminal ends which can be used to introduce sulfhydryl residues in proteins, nucleic acids and other molecules, or as active species in disulfide exchange

crosslinking reactions (Hersch*et al*., 2006). The end-product of this is formation of thiol related compounds that are important anti-oxidants. The presence of cystamine support the increased tissue level of sulfhydryl related compounds like reduced glutathione (GSH) and non-protein thiol observed during pre-treatment with ethanolic extract and suggest that thiolation of different intra-molecular components of myocardial tissue can help to protect the heart against acute myocardial infarction. Also detected, but with very low percentage composition and no record of its Pharmacological use is benzene-thiol that could act synergistically with cystamine. Another compound suspected to be active component of dichoromethane fraction is a ketone derivative and polyphenolic compound referred to as 3-methoxylflavone with percentage composition of 0.21. This flavonoid that seems soluble in non-polar solvent (DCM) after series of fractionation could be a major lipophilic flavonoid with improved bioavailability to elicit pharmacological effect on distant target tissue of the heart. This compound could act in concert with other low quantity phenols like Vanillin and 2-Methoxy-4-vinylphenol to elicit anti-oxidant, anti-inflammatory and anti-apoptotic in myocardial tissue.

Finally, non-polar soluble compounds suspected to be active include cystamine, benzene thiol, polyphenols, pyrroles, oxazoles and benzene acetic acid. These compounds, which has been shown to possess anti-oxidant and anti-inflammatory effect seems to be effective against reactive oxygen species production, lipid peroxidation, ATP depletion, inflammatory mediators production that is usually associated with acute ischemic injury induce apoptosis and necrosis during acute myocardial infarction.

## 5.2 CONCLUSION AND RECOMMENDATION

From this study, pre-treatment with *Corchorus olitorius* which produced better cardioprotective effect than post-treatment against acute myocardial infarction support the role of phytochemicals and natural products as apreventive regimen. From the study,*Corchorus olitorius*leaf extract and fractions protect the myocardium of Wistar rats against arrhythmia, apoptosis, inflammation, necrosis and oxidative stress associated with acute myocardial infarction. In addition, the current study has shown that p65Nuclear Factor KappaB which is known to be a pro-inflammatory transcription factor was shown to exert anti-apoptotic effect during acute myocardial infarction.

Based on the results obtained from this study, there is need to investigate the role of active suspected components like cystamine, 3-methoxyflavone, 2-Methoxy-4-

vinylphenol as therapeutic strategy against acute myocardial infarction. This research also suggest a need for further studies on the role of transcription factors during acute myocardial infarction.

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# Appendix

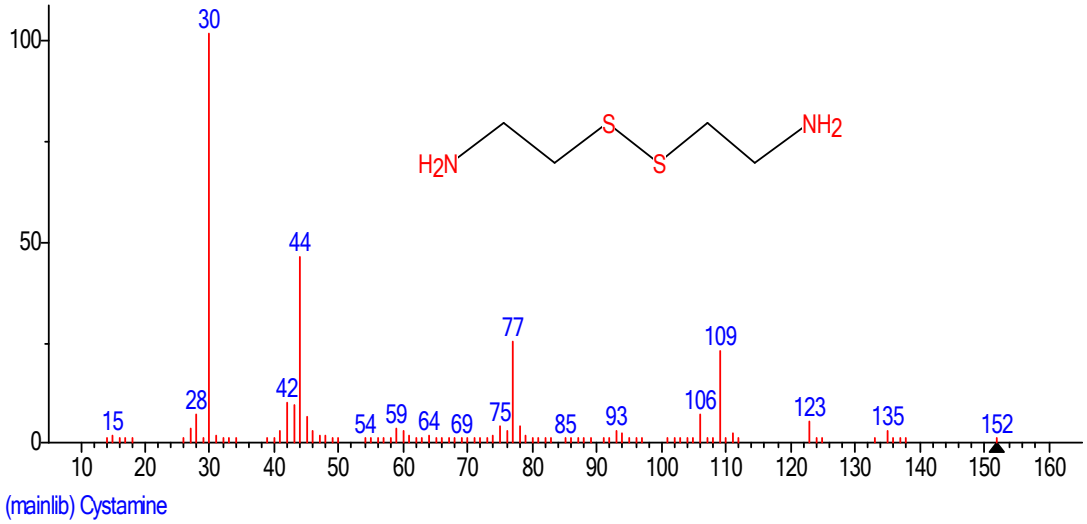
## Table 4.2 Classification of Chemical constituents based on GC-MS Analysis

| Classification | PK | RT | Area PCT | Compound | CAS |
|---|---|---|---|---|---|
| **Carboxylic acid** | 4 | 5.97 | 1.54 | Acetic acid, dichloro-, ethyl ester | 000535-15-9 |
| | 6 | 6.70 | 0.27 | 3-Pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl ester | 051925-57-6 |
| | 7 | 6.98 | 3.84 | Ethyl hydrogen succinate | 001070-34-4 |
| | 9 | 7.30 | 1.00 | Benzeneacetic acid | 000103-82-2 |
| | 12 | 7.75 | 1.71 | Succinic acid, butyl ethyl ester | 1000324-85-1 |
| | 24 | 10.79 | 1.00 | Isonicotinic acid, octyl ester | 1000299-88-4 |
| **Thiol** | 158.39 | 0.24 | | Benzenethiol, 4-(1,1-dimethylethyl )- | 002396-68-1 |
| **Pyrrole** | 6 | 6.70 | 0.27 | 3-Pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl ester | 051925-57-6 |
| | 8 | 7.13 | 1.60 | 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- | 020189-42-8 |
| | 10 | 7.46 | 0.22 | Indole | 000120-72-9 |
| **Oxazole** | 20 | 9.31 | 1.34 | Acetamide, 2-(4-fluorophenyl)-N-(5-methyl-3-isoxazolyl)- | 351060-75-8 |
| | 23 | 10.65 | 6.12 | Oxazole, trimethyl- | 020662-84-4 |
| **Pyridine** | 19 | 9.79 | 0.71 | Pyridine, pentachloro- | 002176-62-7 |
| | 26 | 12.51 | 0.26 | Pyridine, 3,5-dibromo-2-methyl- | 038749-87-0 |
| **Alkane** | 14 | 8.14 | 1.98 | 1-Piperidinepropanenitrile | 003088-41-3 |
| | 21 | 9.72 | 3.54 | Bis(phenylthio)methane | 003561-67-9 |
| **Ketone** | 16 | 8.55 | 0.21 | 3-Methoxyflavone | 1000306-41-0 |
| | 17 | 8.70 | 0.64 | 2(4H)-Benzofuranone, 5,6,7,7 a-tetrahydro-4,4,7a-trimethyl-, (R)- | 017092-92-1 |
| | 18 | 8.80 | 0.69 | Dispiro[5.2.5.2]hexadecan-7-one | 001781-82-4 |
| **Amine** | 1 | 4.31 | 59.31 | Cystamine | 000051-85-4 |
| | 25 | 11.07 | 2.76 | Benzenamine, 2,4-dibromo- | 000615-57-6 |
| | 3 | 5.89 | 1.15 | Norfluoxetine | 056161-73-0 |
| **Amide** | 2 | 5.76 | 0.92 | 2-propoxy-urea | 000592-31-4 |
| | 20 | 9.31 | 1.34 | Acetamide, 2-(4-fluorophenyl)-N-(5-methyl-3-isoxazolyl)- | 351060-75-8 |
| | 5 | 6.26 | 3.90 | Cyacetacide | 000140-87-4 |
| **Phenol** | 11 | 7.54 | 1.37 | 2-Methoxy-4-vinylphenol | 007786-61-0 |
| | 16 | 8.55 | 0.21 | 3-Methoxyflavone | 1000306-41-0 |
| | 13 | 7.97 | 0.40 | Vanillin | 000121-33-5 |
| **Transition Metal** | 22 | 10.19 | 3.75 | Vanadocene, 1,2,3,4,5-pentamethyl- | 088271-58-3 |

**PK: Peak, RT: Retention time Area PCT: Area percentage (%) CAS NO: Chemical Abstract Service based on based on Scientific Library Identity.**

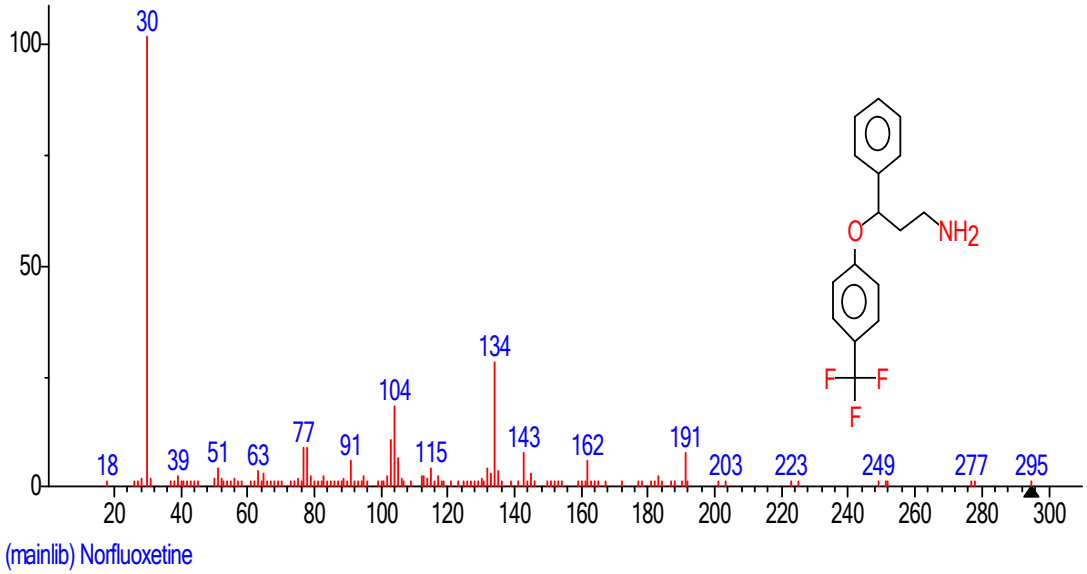
**Reported Pharmacological Activity of the GC-MS Chemical Constituents**

**Cystamine**



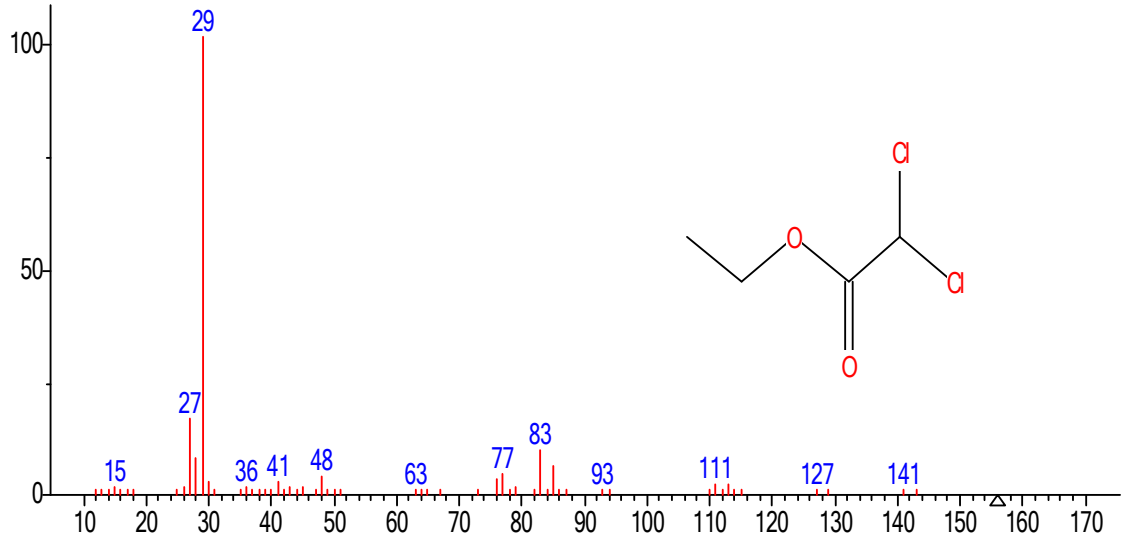

Cystamine is a disulphide containing compound that possess a strong antioxidant property. Cystamine is a changed molecule of cystine, into a disulfide-containing compound with the presence of primary amines at the two terminals and it's useful in the introduction of sulphhydryl group in proteins, nucleic acid, and other molecules. By donating SH group to different intracellular molecules, the level of free radicals can be reduced through redox reaction.

**Norfluoxetin**




Norfluoxetin is an active metabolite of fluoxetine also known as seproxetine. Pharmacologically, it is known to selectively inhibit serotonine reuptake and thus used as antidepressant.

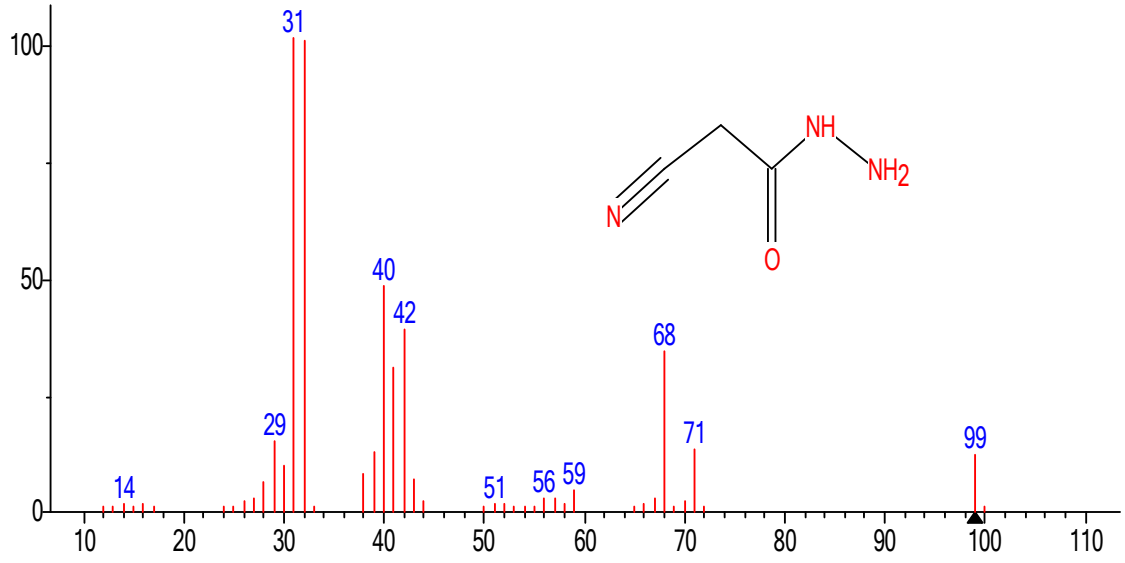
**Acetic acid, dichloro- ethyl ester**




(mainlib) Acetic acid, dichloro-, ethyl ester

There was no major pharmacological activity reported for this compound.

**Cyacetacide**

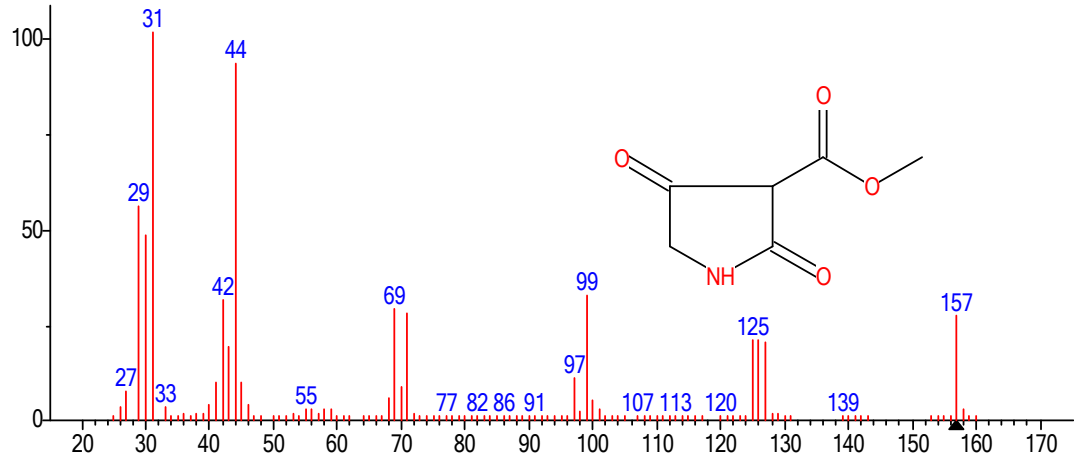



(mainlib) Cyacetacide

There was no pharmacological activity reported for this compound.

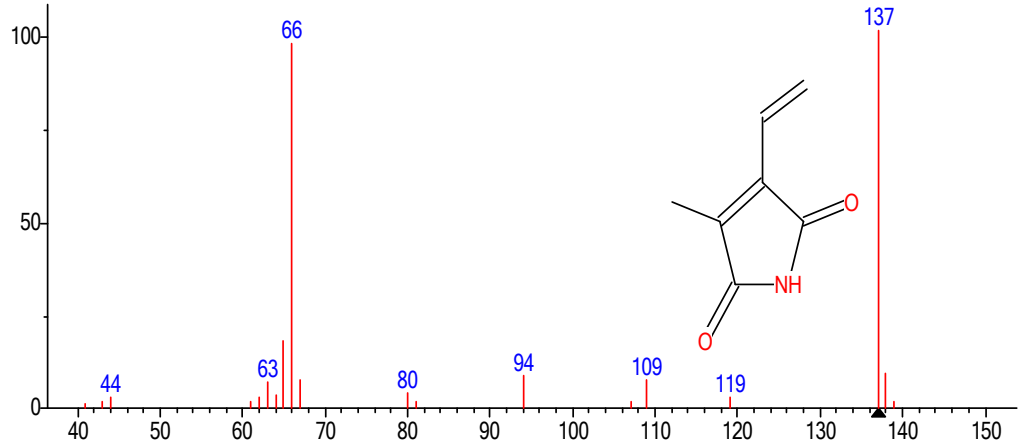
**Pyrrole Derivatives**

**3-Pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl esther**




(mainlib) 3-Pyrrolidinecarboxylic acid, 2,4-dioxo-, methyl ester

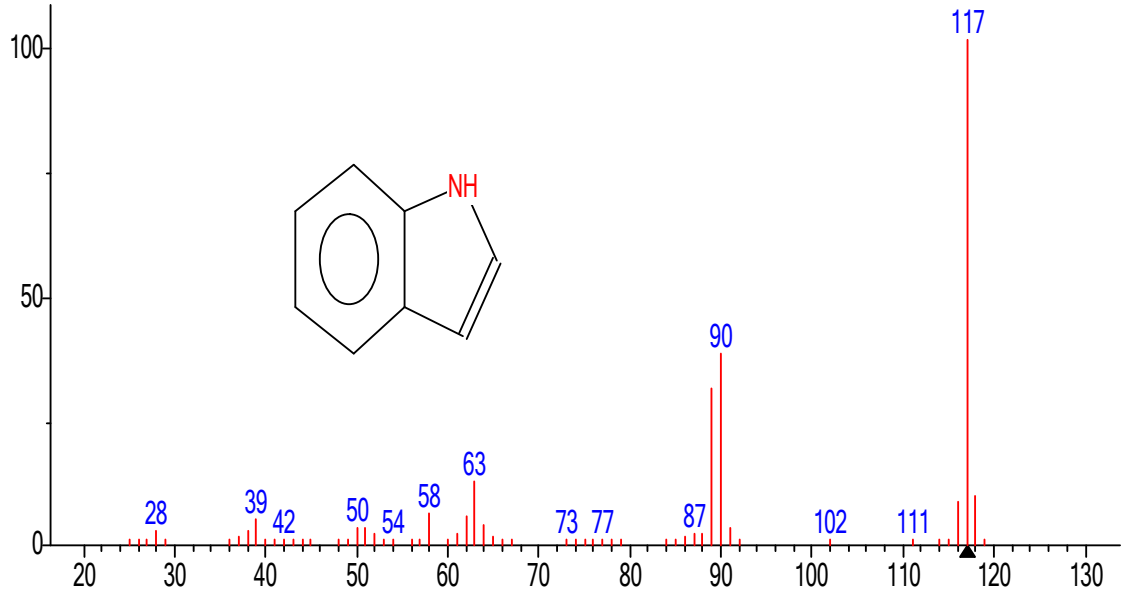
**1H-Pyrrole-2,5-dione, ethenyl-4-methyl-**




(mainlib) 1H-Pyrrole-2,5-dione, 3-ethenyl-4-methyl-

Pyrrole is a five membered ring with formular $C_4H_4NH$ existing naturally as porphyrines portion of heme, vitamin B12, or bilirubin. Due to its inability to exist alone, **indoles**, carbaxoles, **oxazoles** are all pyrrole containing compound and they all are active ingredients of standard drug. The ondasetron and tropisetron known to be 5-hydroxyltryptamine receptor antagonist ($5\text{-}HT_3$) are indoles. Several indolylmaleimides are powerful blockers of protein kinase C (PKC) with antitumor potentials. In addition, devazepibe is a cholecystokinin (CCK) receptor blocker involved in anxiety that structurally possess indole derivative of pyrrole ring.

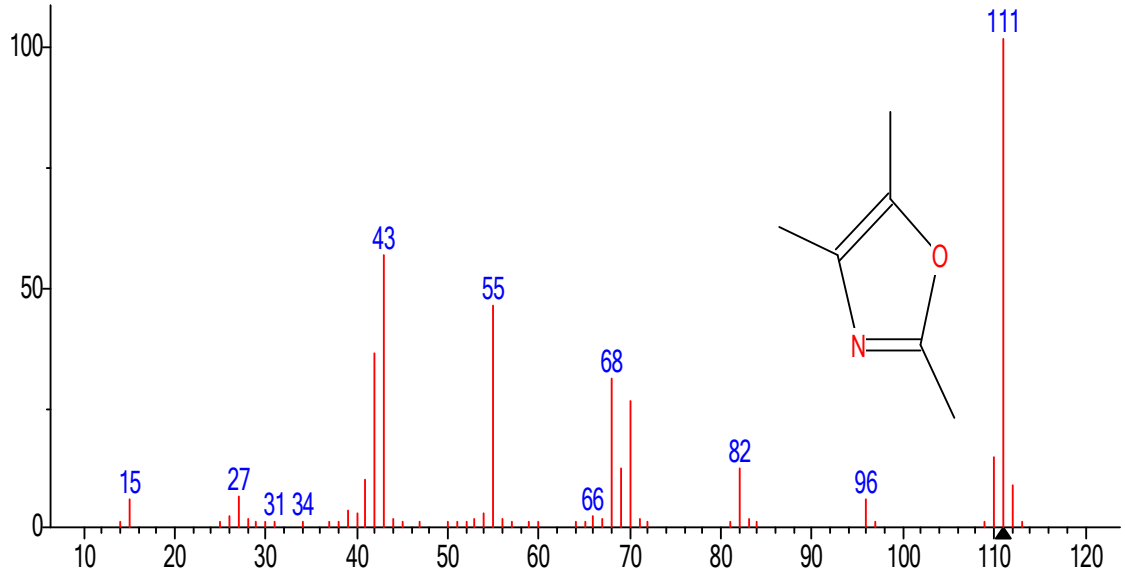
**Indole**



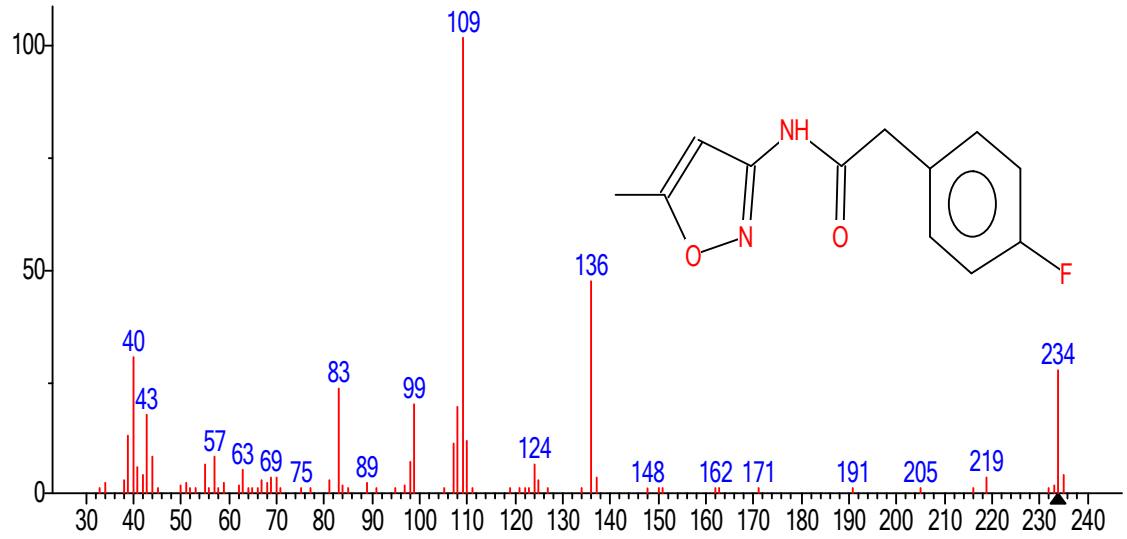

(mainlib) Indole

This is a six membered benzene ring with five membered pyrrole ring. In addition to aforementioned pharmacological use of indole, 3-arylmethylindoline is a cyclo-oxygenase 2 inhibitor (COX-2), Famitinib is a pyrrole containing drug known to inhibit tyrosine receptor kinase and currently under development for the treatment breast cancer and indomethacin is also another anti-inflammatory drug. This compound has been described to be one of the most naturally occurring compounds in many drug.

**Oxazole Derivatives**
**Oxazole, Trimethyl**



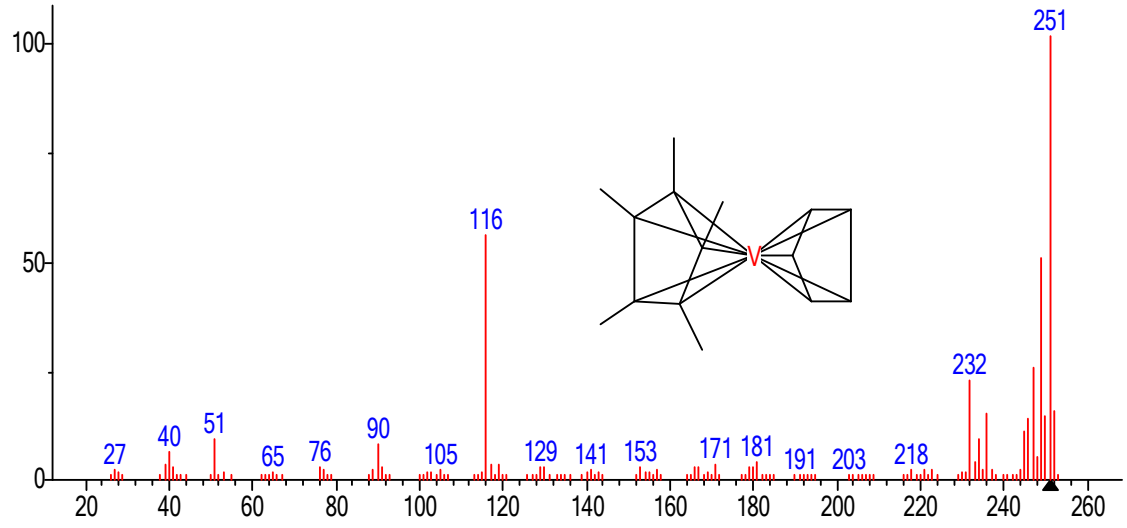

(mainlib) Oxazole, trimethyl-




(mainlib) Acetamide, 2-(4-fluorophenyl)-N-(5-methyl-3-isoxazolyl)-

Oxazole is a structural related compound of pyrrole with minor difference of a five membered heterocyclic compound containing oxygen and nitrogen atoms in the position 1 and 2, whenever isoxazole its derivative compound is saturated partly, structural related isoxazolines are formed and when fully saturated, analog isoxazolidines are formed. Isoxazole derivatives are used as COX-2 inhibitor and anti-inflammatory drugs.

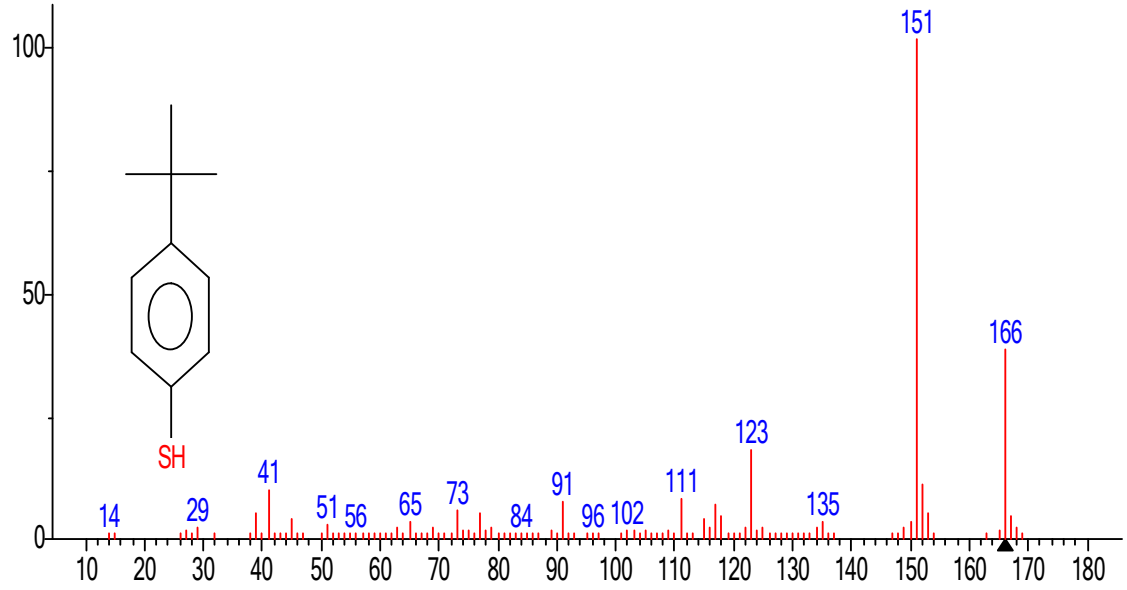
**Vanadocene 1,2,3,4,5-pentamethyl-**




(mainlib) Vanadocene, 1,2,3,4,5-pentamethyl-

Apart from cisplatin a platinum derivative, vanadocene 1,2,3,4,5-pentamethyl a vanadium derivative is another transition metal that has been reported to be very important in the treatment of testicular cancer. This compound has also been linked to possess spermicidal effect through.

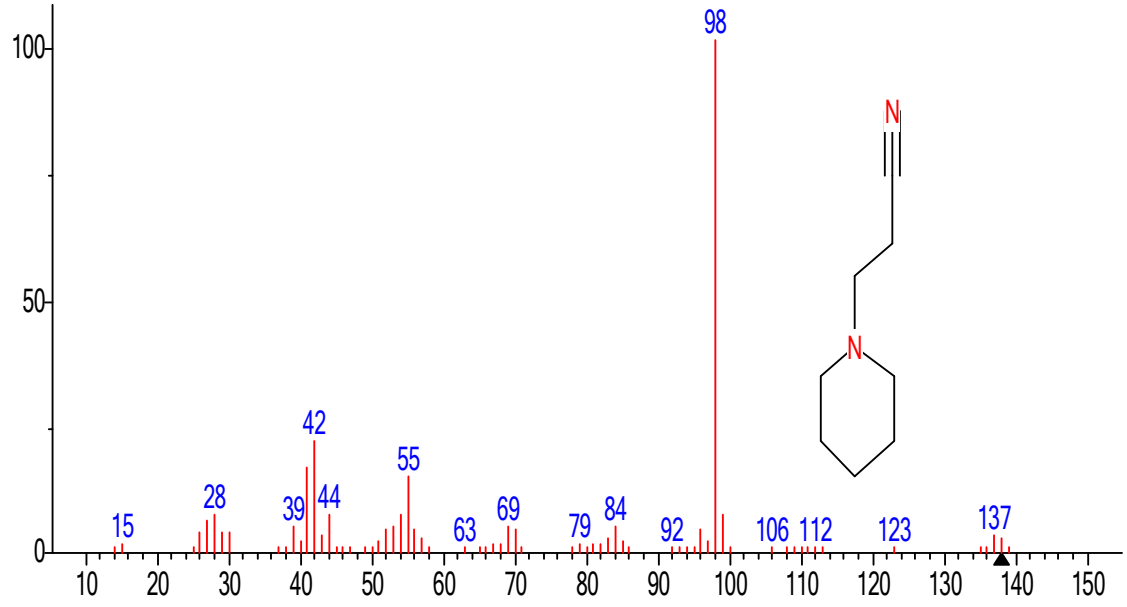
**Benzene Thiol**




(mainlib) Benzenethiol, 4-(1,1-dimethylethyl)-

This benzene ring sulfhydryl containing compound has not been linked with any pharmacological effect but could be potential anti-oxidant due to the presence of thiol group attachment on position 4.

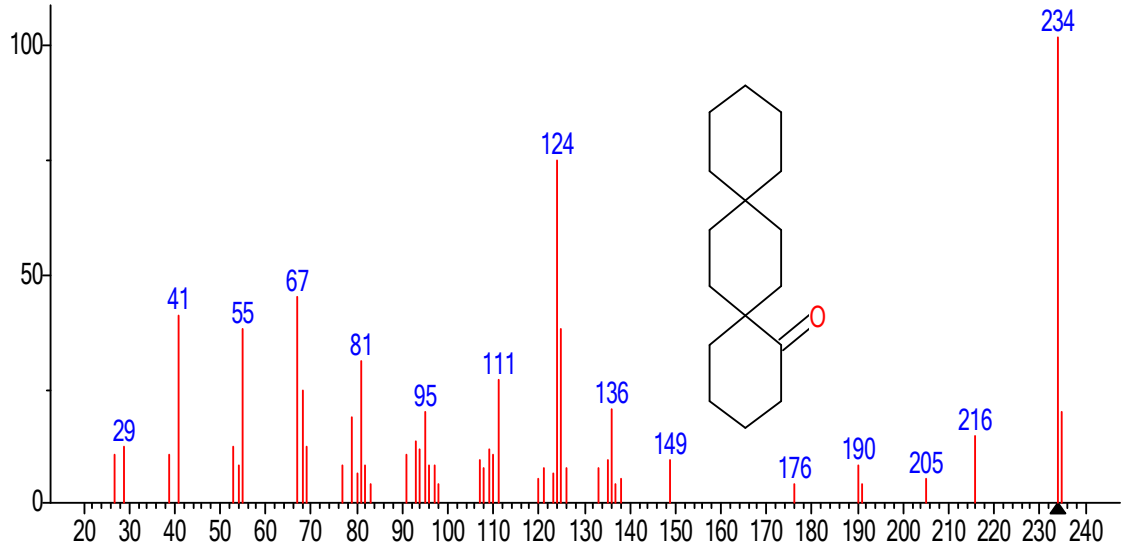
**1-Piperidinepropanenitrile**




(mainlib) 1-Piperidinepropanenitrile

There was no reported pharmacological effect of this compound yet.

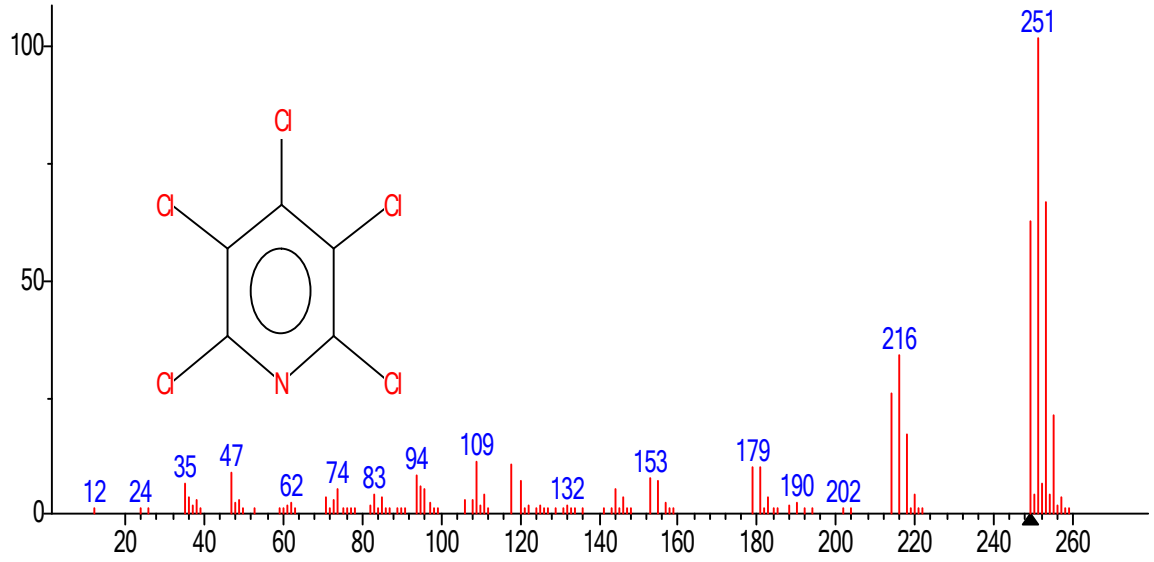
**Dispiro (5.2.5.2)hexadecan-1-one**




(mainlib) Dispiro[5.2.5.2]hexadecan-1-one

There was no reported pharmacological effect of this compound yet.

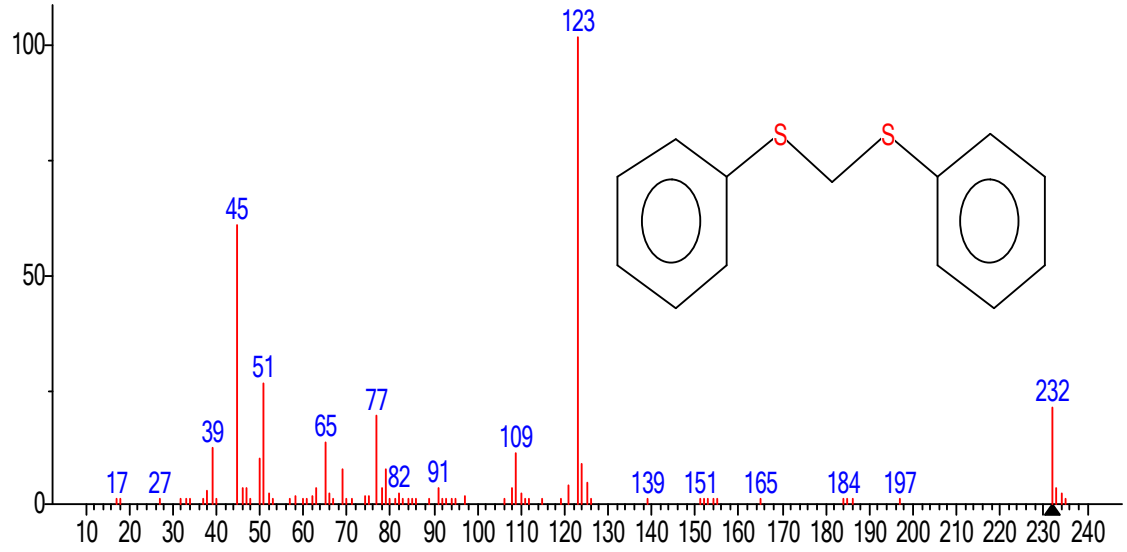
**Pyridine, pentachloro-**




(mainlib) Pyridine, pentachloro-

There was no reported pharmacological effect of this compound yet.

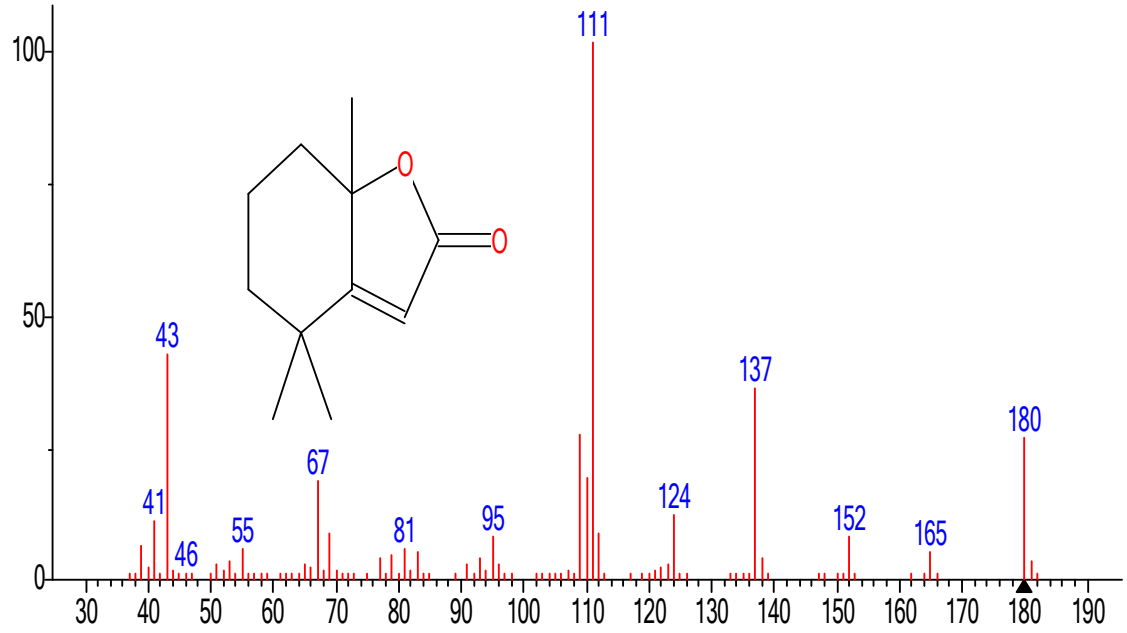
**Bis (Phenylthio)methane**




(mainlib) Bis(phenylthio)methane

There was no reported pharmacological effect of this compound yet.

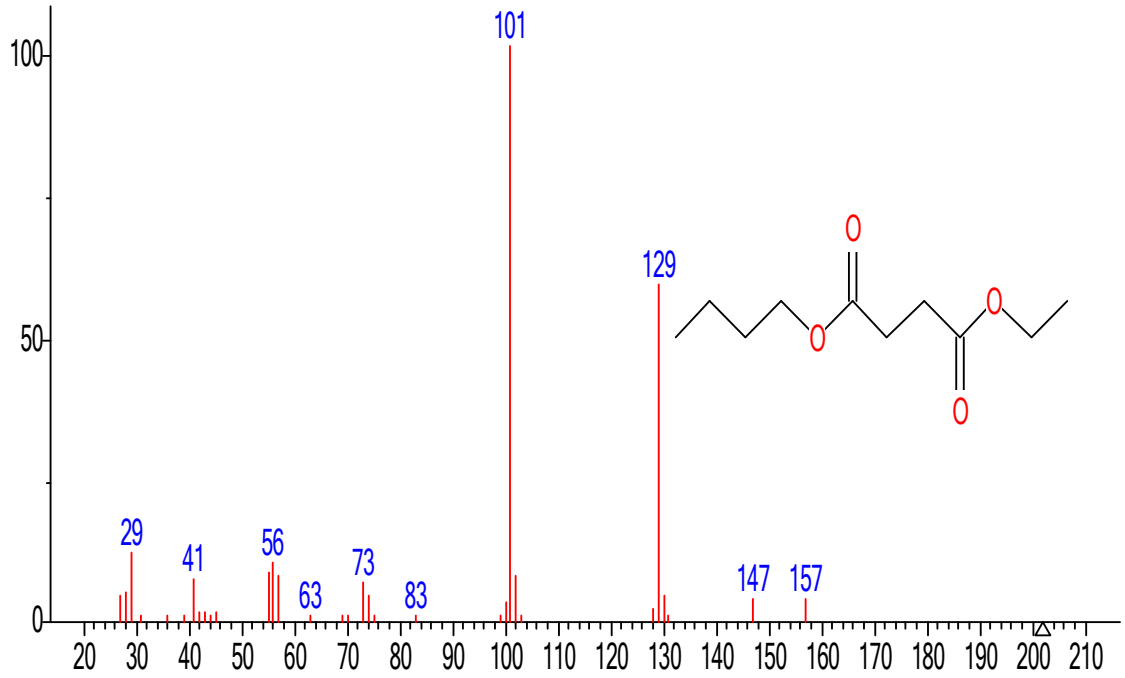
**2(4H)-Benzofuranone, 5,6,7, 7a-tetrahydro-4,4,7a-trimethyl- R-**




(mainlib) 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-

There was no reported pharmacological effect of this compound yet.

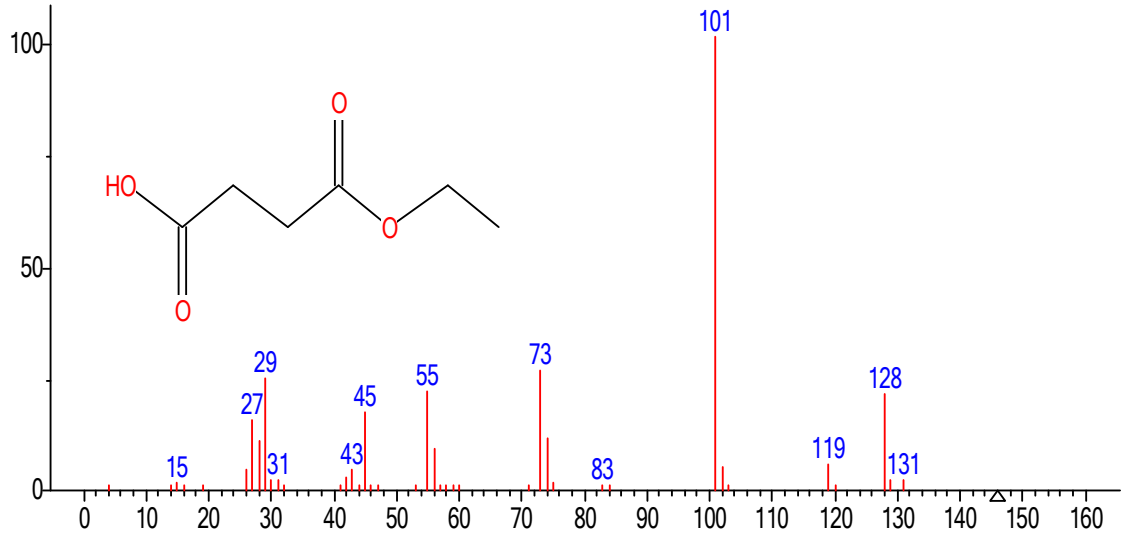
**Succinic acid, butyl ethyl ester**




(mainlib) Succinic acid, butyl ethyl ester

There was no reported pharmacological effect of this compound yet.

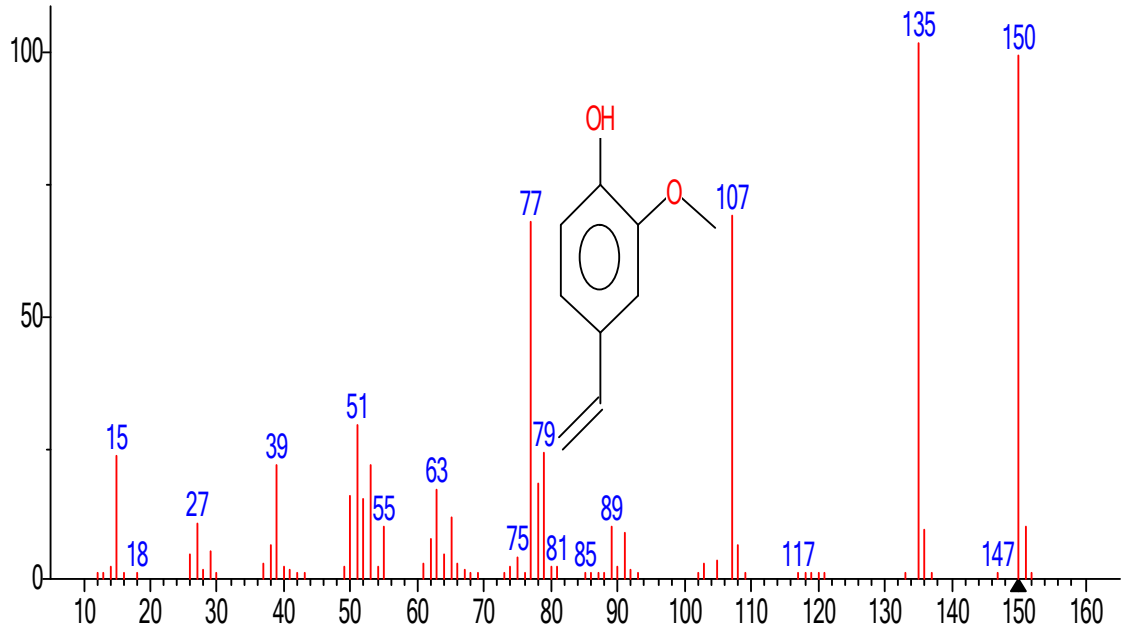
**Ethyl hydrogen succinate**




(mainlib) Ethyl hydrogen succinate

There was no pharmacological activity linked to this compound yet.

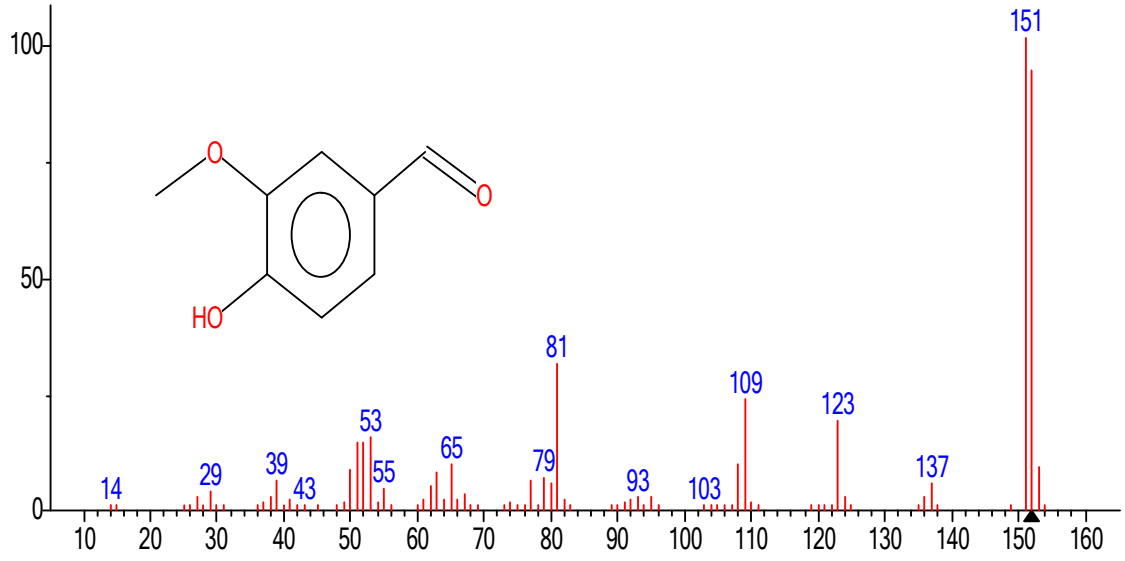
**2-Methoxy-4-VinylPhenol**




(mainlib) 2-Methoxy-4-vinylphenol

This is a compound with aromatic ring and commonly used to add flavor and part of the natural aromatic substance of buckweat.

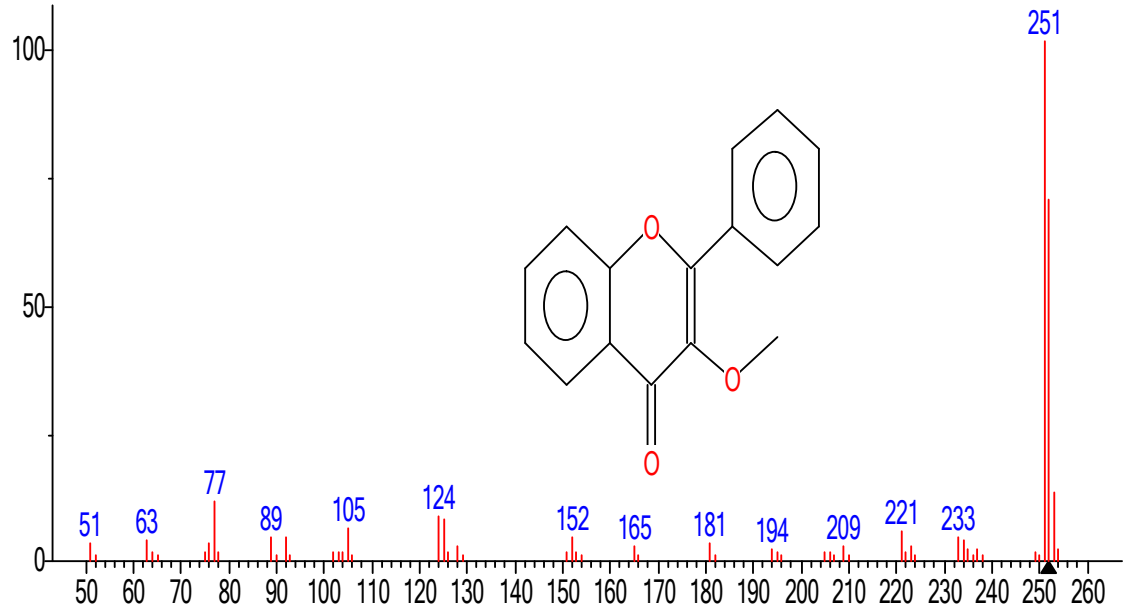
**Vanillin**




(mainlib) Vanillin

Vanillin is a phenolic aldehyde that can be synthesized instead of natural vanilla and used as flavouring agent in foods, beverages and pharmaceuticals. The pharmacological use of this agent include its use as antioxidant, anticonvulsant and anti-muscarinic.

**3-Methoxyflavone**




(mainlib) 3-Methoxyflavone

3-methoxyflavone is a structural analog of 3-O-methoxylgalangin or dihydroxyl-3-methoxylflavone is a flavone derivative of flavonoid. This compound has been reported to be used along with coumarin in the pharmacological treatment of arteriosclerosis