EVALUATION OF THE EFFECTS OF HYDROMETHANOL EXTRACT AND FRACTIONS OF *Crassocephalum rubens* ON BLOOD COAGULATION AND CARDIAC PARAMETERS IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTED RATS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES,

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DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

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ECHEHOMOS CORNELIUS REHOBOTH

……..

DATE

CERTIFICATION

This is to certify that the content of this project entitled '**Evaluation of the Effect of Hydromethanol Extract and Fractions of *Crassocephalum rubens* on Blood Coagulation and Cardiac Parameters in Isoproterenol -induced Myocardial Infarcted Rats'** was prepared and submitted by **ECHEHOMOS CORNELIUS REHOBOTH** in partial fulfillment of the requirement for the degree of **BACHELOR OF SCIENCE IN BIOCHEMISTRY.**

The original research work was carried out by him under my supervision and is hereby accepted.

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DEDICATION

This project is dedicated to God almighty, the Alpha and Omega and also to my parents, Mr. and Mrs. Echehomos Paul and family.

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My utmost gratitude goes to the Almighty GOD, who in his infinite mercies inspired the conception of this project write-up and also made it possible to be a great success. My special thanks also goes to Dr. (Mrs.) Ayodele O.O. who supervised my project and also put me through with my project write-up.

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ABSTRACT

Myocardial infarction is a serious cardiac condition that is considered a significant reason for death and morbidity across the world. Atherosclerosis, or the emergence of blood clots in the coronary arteries over time, is the reason. Blood coagulation is a fast and effective process that results in clot formation that needs control. The studies on the effects of hydromethanol extract and fractions *Crassocephalum rubens* (*C. rubens*) against blood coagulation profile and concentration of cardiac markers induced by drugs and therapeutic agents remain insufficient, especially its effects on Isoproterenol (ISO.) Special emphasis was given to the impact of *C. rubens* in blood coagulation cascade and cardiac enzymes concentration where sixty adult male albino rats were used in this study.

The animals were divided into twelve equal groups at random. Group I served as control and received 1ml phosphate buffer saline. Group II received ISO and aspirin orally, Group III received ISO without pretreatment, followed by intraperitoneal injection of ISO (100 mg/kg), to induce myocardial infarction. Group IV-XII received *C. rubens* extracts and fractions at different doses -100mg/kg, 150mg/kg and 200mg/kg. Plant administration was done by oral gavage daily for 14 days, followed by MI induction on day 15.

The result of showed significant increase in the blood coagulation profile (clotting and prothrombin time) and elevation of cardiac markers (alkaline phosphatase and total protein) in the plasma and decrease in the bleeding time, activated partial thromboplastin time and Lactate dehydrogenase concentration when compared to the normal control group, with the exception of certain concentrations of the plant extract which indicates that *C.rubens* has procoagulant activity.

Keywords: Blood coagulation, *C. rubens*, Isoproterenol, Myocardial Infarction and Pro-coagulant.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Ischemic heart disease, a non-communicable disease that has become a major concern, with myocardial infarction (MI) being the most severe, and responsible for around 20% of all deaths worldwide each year (Prabha et al. 2014). This occurs when the coronary blood flow and myocardial demand are out of control, resulting in ischemia and myocardial death. Myocardial damage resulted by free radicals is an etiological pathway linked to elevated levels of reactive oxygen species and a weak antioxidant defense function (Ansil et al. 2014).

MI, which is caused by occlusion of blood that blocks the coronary artery, occurs in starved condition of oxygen and nutrient supply to the downstream myocardium. Particular mechanisms involving MI have been linked to apoptosis, inflammation, and oxidative stress, both of which contribute to cardiac failure (Geeta et al. 2016). According to Islam et al. 2020, Isoproterenol (L-b-(3, 4-dihydroxyphenyl)-2-isopropyl amino ethanol hydrochloride) is a synthetic beta-adrenergic receptor agonist that causes irreversible cellular damage and infarct-like necrosis of the myocardium in rats when provided subcutaneously. The ISO-induced myocardial necrosis in rat model is a well-accepted standardized model for testing cardiac dysfunction, blood coagulation parameters, and studying the effectiveness of natural and synthetic cardioprotective agents (Sumaira et al. 2014).

Blood coagulation is a quick and efficient process that causes clots to develop, which necessitates regulation. Many diseases have a problem with blood coagulation, which involves many proteins (factors) that must act in a specific order to produce clot formation (Sumaira et al. 2014). A change in the equilibrium between clot formation and coagulation inhibition, favoring either pro or anticoagulation, can lead to potentially fatal bleeding (Ayodele et al. 2019). Controlling this mechanism necessitates drug therapies aimed at reducing tissue damage caused by decreased blood flow, which happens as the coagulation process restricts blood flow to a tissue region.

Cardiovascular diseases, with MI as an example, are the primary cause of mortality globally (Aditya et al. 2018). Despite substantial progress in prevention and control, the cause of

cardiovascular disease is not completely understood, which pose as challenge in developing new cardiovascular therapeutics (Aditya et al. 2018).

Myocardial infarction is caused by blood clots. Plaque (an accumulation of cholesterol, fibrous tissue, and inflammatory cells) can form in the coronary arteries over time, a condition known as atherosclerosis (Sheu et al. 2010). The primary cause of a sudden blockage in a coronary artery is a blood clot (thrombus). It usually originates inside the constricted coronary artery due to atherosclerosis. Without blood, tissue loses oxygen and dies. This blood clot blocks the flow of blood through the artery to the heart muscle, results to starve the heart muscle of oxygen and nutrients (Cheng et al. 2010).

Medicinal plants have been discovered to be important sources of novel therapeutic agents. It has been found to contain bioactive compounds that elicit several biological activities such as antioxidant, anticoagulant, antibacterial, etc. (Khorrami et al. 2012). *C. rubens* also known as fire weed, is an annual edible plant native to Africa. *C. rubens* was detailed to have anti-inflammatory, antioxidant, antibiotic, and hepatoprotective properties in literature (Ayodele et al. 2019).

Therefore, it is essential to investigate the effects of *C. rubens* leaf hydromethanol extract and fractions on blood coagulation and cardiac markers parameters of isoproterenol-induced myocardial infarction in rats.

1.2 Statement of the Problem

Acute myocardial infarction (MI) has a 30% fatality rate, with nearly half of the deaths occurring before reaching the hospital in Nigeria. Approximately 5-10% of survivors experience early death in the first year following a myocardial infarction (Oren et al. 2021). Acute MI has led to the loss of viable myocardium, followed by adverse cardiac remodeling, in spite of the success of therapies for acute MI, adverse cardiac remodeling and heart failure still develop (McLaughlin et al., 2019). With the growing prevalence of MI-induced heart failure, new medicines and therapies are needed, which can simultaneously prevent or manage remodeling and protect cardiac function (Afroz et al., 2016). There is a high need for this study because myocardial infarction has constituted a lot of heart issues in the society, therefore *C. rubens* leaves was detailed to possess antihyperlipidemic, anticoagulant and antioxidant activity (Ayodele et al.,

2019). In this study, it is important to explore and confirm *C. rubens* leaves as a possible source of novel hepatoprotective and antihyperlipidemic drugs.

1.3 Aim and Objectives of the Study

This study aims at investigating the effects of hydromethanol extract and fractions of C. *rubens* on the blood coagulation and cardiac parameters of rats induced with myocardial infarction by achieving the specific objectives which are to:

i. determine the effect of hydromethanol extract of *C. rubens* on the blood coagulation parameters (blood clotting time, bleeding time) of rats induced with myocardial infraction.
ii. determine the effect of hydromethanol extract of *C. rubens* on blood coagulation parameters (activated partial thromboplastin time, Prothrombin time) of the experimental rats.
iii. determine the effect of hydromethanol extract of *C. rubens* on the levels of cardiac and hepatic markers [Total protein (TP), Lactate dehydrogenase (LDH), Alanine transferase (ALT), Aspartate transferase (AST) and alkaline phosphatase (ALP)] in the experimental rats.
iv. characterize the bioactive compound in ethyl acetate fraction of *C. rubens* using GC-MS / LCMS.

1.4 Scope of the Study

This study entails whether hydromethanol extract of *C. rubens* will suppress the ISO induced-myocardial infarction in the rat model or not. Also, blood coagulation and cardiac marker parameters will be a factor in the determination of the study and a positive control for check.

1.5 Significance of the Study

Although the hydromethanol extract of *C.rubens* was detailed to improve human health and minimize the risk of both blood lipid level and cardiovascular disorders such as atherosclerosis, hyperlipidemia, thrombosis, and hypertension (Aditya et al. 2018), there are however, insufficient literature reporting its effects on blood coagulation parameters.

This study will provide information on the effects of hydromethanol leaf extract on blood coagulation parameters and cardiac markers in MI rats model.

1.6 Definition of Terms

Isoproterenol: a drug used to treat bradycardia (slow heart rate), heart block, and asthma in rare cases.

Myocardial infarction: refers to heart attack, a life threatening emergency that arises when blood flow to the heart is blocked by a blood clot. Tissue lacks oxygen and dies lacking blood.

Blood coagulation: The mechanism through which blood transforms from fluid to solid, creating a thrombus, is also known as clotting.

Cardiac markers: are indicators which are used to assess cardiac function and can help in illness prediction and diagnosis.

Crassocephalum rubens: Medicinally, it's used as a stomachic and to alleviate liver problems and colds, as well as cure burns.

Cardiovascular diseases: are a range of cardiac - related problems.

Hydromethanol: is a solvent matrix consisting of a combination of water and methanol for extraction.

CHAPTER TWO

LITERATURE REVIEW

Crassocephalum rubens (Jacq. S. Moore), Asteracaea commonly called fireweed or Redflower ragleaf, is an annual edible plant that is widespread in tropical and sub-tropical regions. It is eaten by humans in many countries of Africa. In south-western and south eastern Nigeria, they are found most commonly in markets during the rainy season. The leaves possess antihyperlipidemic and anticoagulant properties which serve as a phytomedicine (Ayodele et al. 2019).

2.1 General Overview of *Crassocephalum rubens*

C. rubens is a wild or semi-domesticated aromatic traditional green vegetable consumed the most in South west, Nigeria and mildly used in South East, Nigeria. It is a member of the family of Asteraceae (sunflower family) and taxonomically known as *Crassocephalum rubens* (Grubben *et* al. 2018). Its English names are fireweed, thickhead, red flower ragleaf and Okinawa spinach while the Yoruba tribe in South-West of Nigeria calls it "Efo Ebolo or Ebire" (Grubben et al. 2018) and the Igbos in the south-East of Nigeria call it erimionu (Denton et al. 2018). The vegetable was detailed to have medicinal values and locally used as nutraceutical; the leaves are used for indigestion, headaches, fresh wounds, nose bleeding and sleeping sickness. They are also rich in fibers, vitamins, minerals for example include carotene (provitamin A), ascorbic acid, riboflavin, iron, iodine, calcium (Adjatin et al. 2013). The roots are used in the remedy of swollen lips (Grubben et al. 2018). Fireweed is either of the neglected and under-utilized vegetables among the Yorubas and Igbos in Nigeria (Grubben et al. 2018). The foul odor is the utmost reason for ignoring the plant, among other things (aroma) that is inherent in the vegetable which might be because of certain phytochemical constituent(s) of the plant (Denton et al. 2018).

Considering the scientifically documented potential toxicity of several vegetable species to people and animals, leaf extracts of C. rubens are submitted to qualitative phytochemical screening and cytotoxicity testing (Dansi et al. 2013).

2.1.1 Habitat

C. rubens is a perennial growing to 0.8m (2ft 7inchs) by 0.3m (1ft) at a fast rate. it is considered as weed, commonly found in abandoned farmlands, wastage places, plantations and backyard gardens that are rich in organic matter (Grubben et al. 2018). The plant's temperature requirement is 23- 30^0C and annual rainfall of 600-1500 is suitable. It prefers well-drained soil, dogged condition and grows well under shade in cocoa or tea plantation (Entaz et al. 2018). It is suitable for light (sandy) and medium (loamy) soils and suitable pH for soils that are acidic, neutral, or basic (alkaline) (Entaz et al. 2016).

2.1.2 Biological Classification of *Crassocephalum rubens*

Kingdom-Plantae

Subkingdom-Viridiplantae

Infra-kingdom-Streptophyta

Super- division-Embryophyta

Division-Tracheophyta

Sub-division-Spermatophytina

Class-Magnoliopsida

Super order-Asteranae

Order-Asterales

Family-Asteraceae

Genus-Crassocephalum Moench

Species-Crassocephalum rubens

Other species of Crassocephalum rubens include: *Crasssocephalum biafrae*, *Crassocephalum*

buchiense, *Crassocephalum aurantiacum* (Grubben *et al*. 2018).

2.1.3 Nutritional Composition of *Crassocephalum rubens*

The nutritional value of the species of ebolo (*C. rubens*) was evaluated through AOAC-recommended method of analysis for proximate composition, vitamin C and mineral profile (Arawande et al. 2013). The analysis includes the contents in moisture, raw protein, total lipids, ash, fibre, carbohydrates for *C. rubens*. The mineral composition of *C. rubens* include sodium, Manganese, phosphorus, iron, magnesium, Zinc, calcium, Copper and potassium contents. The sample powder of *C. rubens* contains flavonoid, phenol, oxalate, tannin, saponin, phytate, alkaloid, ascorbic acid (Arawande et al. 2013).

2.2 Phytochemical Profiling

This involves the screening of phytochemicals of *C. rubens* in which there are pharmacologically important compounds such as coumarins, tannins, mucilage, heterosides, flavonoids, reducing compounds and anthocyanins, Steroids, alkaloids, quinone derivatives, saponins, cyanogenic derivatives, triterpenoids and cardiac glycosides which tend to exhibit biological properties such as anticoagulant effect, antihyperlipidemic effect which are present in *C. rubens* (Azokpota et al. 2013).

2.3 Blood Coagulation

Blood coagulation is a fast and effective process that results in clot formation, which is a coagulation disorder that need control (Ayodele et al., 2019). Coagulation is the process where blood loses its fluidity externally while still maintaining constant flow in the blood vessels.

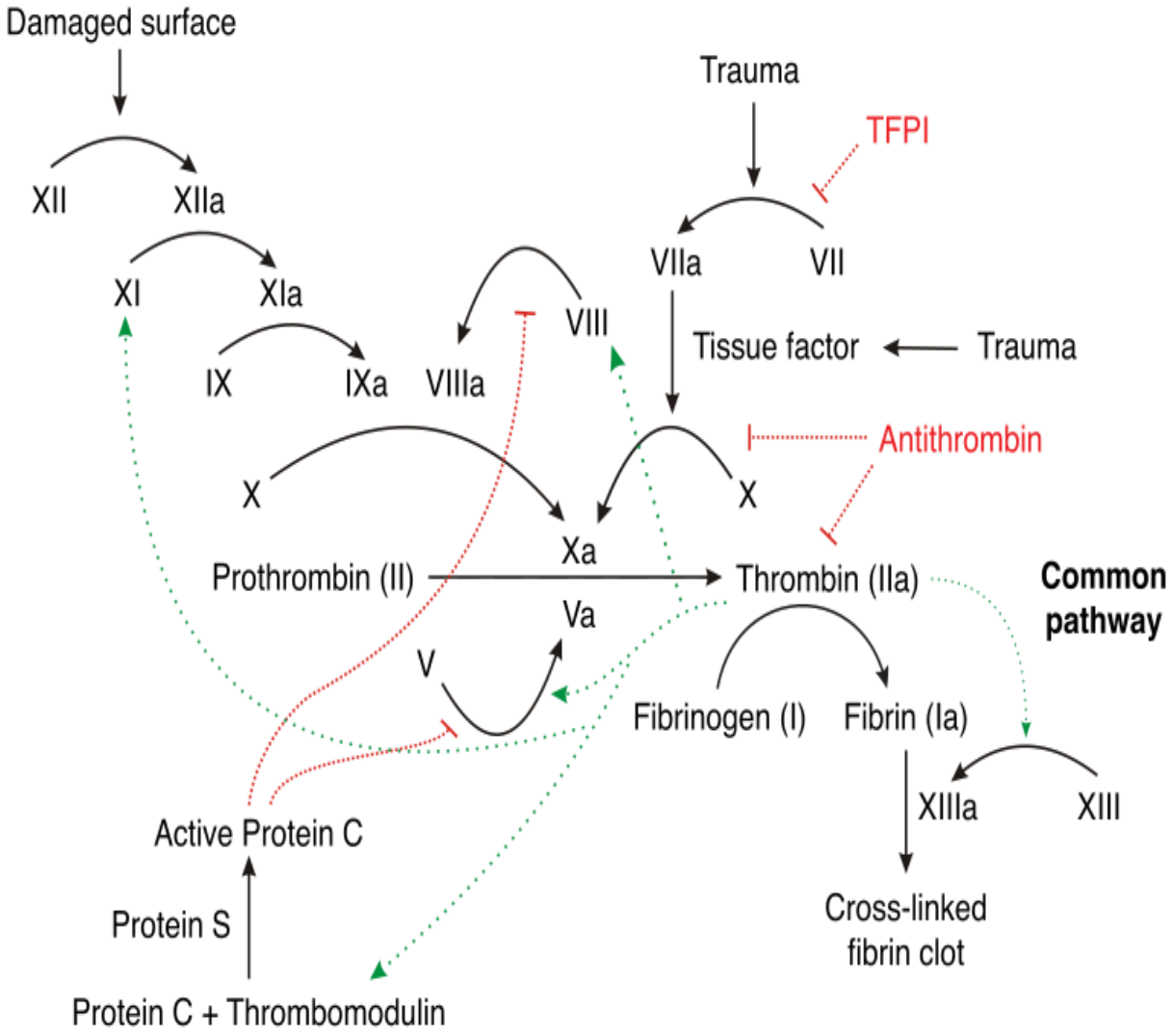

Figure 2.1: Diagram showing the clotting factors of the coagulation cascade (Boer et al. 2011).

Fibrinogen (Factor 1), Thromboplastin (Factor 3), Prothrombin (Factor 2) Labile Factor or Stable Factor (Factor 6), Antihemophilic Factor (Factor 8), Proaccelerin (Factor 5), Christmas Factor (Factor 9), Calcium (Factor 4), Stuart - Power Factor (Factor 10), Plasma Thrombin antecedent (Factor 11), Hegman Factor (Factor 12) and Fibrin Stabilising Factor (Factor 13) are thirteen clotting factors known as shown in fig 2.1 (Betsaida et al. 2018).

The coagulation cascade is caused by the interaction of several factors (Boer et al. 2011).

The clotting factors are dormant and must be awakened in order for blood to clot. Production of prothrombin activator, conversion of prothrombin to thrombin, and conversion of thrombin to fibrin are the three steps involved in blood clotting formation. (Boer et al. 2011). The fibrin generated stabilizes the platelet block, which is referred to as a 'clot.' The clot is removed and destroyed by an enzyme called plasmin once the vessel has repaired (Betsaida et al. 2018). It's critical that the body manages hemostasis effectively since it may lead to thrombosis when there's too much and haemorrhage when there's not enough. (Betsaida et al., 2018).

Many diseases are characterized by a disturbance in blood coagulation In order to keep the cardiovascular system in control, blood coagulation parameters comprise a sequence of responses that decrease or stop blood flow (Christersson et al. 2009). Spontaneous vasoconstriction, platelet aggregation, fibrinolysis (clot dissolution) and blood clotting are examples of these reactions. Because a shift in the balance between blood coagulation and inhibition of coagulation to favor either pro- or anticoagulation might result in spontaneous bleeding, this quick and efficient process requires control (Christersson et al. 2009). Control of this process under many clinical situations requires drug interventions like phytochemicals existing in plants that aim at preventing tissue damage caused by reduced blood flow that when the coagulation process prevents blood from reaching a tissue or organ. Important blood coagulation parameters include the blood clotting time, Prothrombin time and the activated partial Thromboplastin time (Ayodele et al. 2019).

2.3.1 Blood Coagulation Disorders

Blood coagulation disorder is a disease that prevents blood from clotting normally. Blood is transformed from liquid to solid during the clotting event, known as coagulation. When injured, the blood normally begins to clot to prevent a massive loss of blood. Certain diseases might cause blood to clot improperly, resulting in excessive or persistent bleeding (Kahn et al. 2018). It causes abnormal bleeding either external or internal bleeding. Some diseases cause a significant increase in the volume of blood that leaves the body for example when a person has a cut on the skin. Other disorders can cause bleeding to occur under the skin or in critical organs including the brain. It is also a state that influences the blood's clotting activities. Hemophilia, Von Willebrand disease, clotting factor deficiencies, hypercoagulable states and deep venous

thrombosis are all coagulations disorders. Hemophilia and Von Willebrand disease are among the best known (Kahn et al. 2018).

2.3.2 Classes of Blood Coagulation Disorders

There are two classes of blood coagulation disorders; Hyper-coagulation and Hypo-coagulation.

2.3.2.1 Hyper-Coagulation

Hypercoagulation or a hypercoagulable state is increased tendency to form thrombus. Platelets can collect or aggregate and attach to vessel walls due to blood flow difficulties or changes in blood vessel walls, triggering a pathological clotting process. Congestive heart failure, immobility and artificial body surfaces, such as oral contraceptives, artificial heart valves, the post-partum period and pregnancy are all linked to clotting system issues. Thrombus development occurs due to platelet abnormalities as well as clotting system abnormalities. Abnormal platelet function leading to thrombus formation as an outcome of arteriosclerosis, diabetes mellitus, elevated blood cholesterol and lipids, and smoking (Darien et al. 2013).

2.3.2.1.1 Inherited Hyper-Coagulation

Protein C, antithrombin III and protein S deficiency are all inherited hypercoagulable disorders. (Penner et al. 2020). To prevent excessive coagulation, normal quantities of these compounds are required. Each of these three diseases is autosomal dominantly inherited. Deficiency of protein S is by far the most prevalent hereditary coagulation inhibitor disorder (Penner et al. 2020). This inherited trait results in superficial thrombophlebitis, deep vein thrombosis, and pulmonary emboli (Wedro et al. 2019).

2.3.2.1.2 Acquired Hyper-Coagulation

Acquired diseases that are not diagnosed precedes an occlusion event that produces an unexplained percent of thromboses. Thromboembolism is frequently linked with bone marrow diseases such as polycythemia vera, in which unusually high amounts of platelets are generated. A hematologic problem is frequently identified before a thrombus develops. Hughes syndrome (Antiphospholipid antibodies syndrome) and cancer, or Trousseau syndrome, are two acquired diseases linked with thrombosis that aren't easily visible. (Penner et al. 2020). Infection,

autoimmune disorders including systemic lupus erythematosus, cancer, and medicines like chlorpromazine, procainamide and quinidine are all linked to antiphospholipid antibodies. Antiphospholipid antibody titers over a certain threshold have been linked to a raised thrombosis risk in studies, however the cause for this is unknown (Darien et al. 2013). It is possible that antiphospholipid antibodies are markers for other antibodies or agents that promote thrombosis.

2.3.2.2 Hypo-Coagulation (Haemophilia)

Haemophilia is an occasional state in which blood delays to clot appropriately. It mostly affects men (Choi et al. 2017). This leads to excessive bleeding Clotting factors are 13 different types of proteins that assist platelets to help blood clot. Platelets are small blood cells that form in tissues of the bone (Mauro et al. 2017).

Clotting factors are proteins that interact with platelets to halt bleeding at the injury site. Hemophiliacs produce lower amounts of either Factor VIII or Factor IX than those without the condition. This means the person tends to bleed for a prolonged time after an injury, and they are more susceptible to internal bleeding (Choi et al. 2017). If the bleeding happens within a critical organ, such as the brain, it can be deadly.

Hemophilia is usually a genetic condition, it is something that a person is born with. It occurs due to a mutation in one of the X chromosome's clotting factor genes. Because the gene may be transferred from mother to son, hemophilia mostly affects men (Mauro et al. 2017). Due to the lack of second X chromosome in males, they are unable to compensate for the faulty gene. XX chromosomes are found in females whereas most males have XY sex chromosomes.

Females can carry hemophilia but are unlikely to develop the disease. To develop hemophilia, a both female's X chromosomes must carry the defective gene, which is extremely uncommon. Sometimes, hemophilia is acquired because of a spontaneous genetic mutation. The disorder can also develop if the body forms antibodies to clotting factors in the blood then stop the clotting proteins from working (Crosta et al., 2017).

2.3.3 Risk Factors of Blood Coagulation Disorders

Risk factors of blood coagulation disorders include prolonged immobility (such as long plane or car rides), smoking, certain medications including birth control pills, inherited blood-clotting disorders (Wedro et al. 2019).

2.3.4 Symptoms of Blood Coagulation Disorders

The range of symptoms depends on extremity of the factor deficiency. Persons with mild deficiency may bleed in the case of trauma while people with severe deficiency bleed for no reason, which is called spontaneous bleeding (Choi et al. 2017). Which includes Chest pain, redness, swelling, shortness of breath, rapid pulse and breathing, profound dizziness and abdominal pain (Wedro et al. 2019).

2.3.5 Causes of Blood Coagulation Disorders

When blood delays clotting appropriately, bleeding problems can occur. The body requires clotting factors and platelets for blood to clot. Platelets cluster together at the location of a broken or wounded blood vessel to create a clog. A fibrin clot is formed when the clotting components come together. This retains the platelets and prevents blood from flowing out of the artery (Betsaida et al. 2018).

In people with bleeding disorders, however, the clotting factors or platelets are inactivated or are in short supply. Excessive or persistent bleeding can occur when blood delays to clot. It can also cause bleeding in joints, muscles and other areas of the body, which might be spontaneous or unexpected.

Most bleeding diseases are hereditary, meaning they are transferred from generation to generation. However, some disorders evolve due to medical conditions, as liver disease (Wedro et al. 2019).

Bleeding disorders are due to low red blood count, vitamin k deficiency and side effects from certain medications (Penner et al. 2020). Thrombosis form when blood seeps from a blood vessel, and this is usually a good thing because the thrombus helps to prevent further

haemorrhage at the wound site (Moake et al. 2019). Atrial fibrillation is a process where blood clot in the heart due to the upper chamber of the heart does not beat in an organized manner and blood tends to become stagnant along the atrium's surface in which this causes blood clot formation (Bethsaida et al. 2018). It is caused when plaque deposits form along the lining of the artery and grow which causes the vessel to narrow especially in patients with atherosclerotic disease (wedro et al. 2019). Also, when one is completely motionless and their muscular tissues do not contract, blood does not return to the heart. The blood becomes stagnant and begins to form small clot along the vein's surface, in which the clot can grow and occlude the vein thereby preventing blood from returning to the heart (Bethsaida et al. 2018).

2.3.6 Treatment of Blood Coagulation Disorders

The 2016 guidelines of 'Chest Physicians of American college' recommended that pulmonary embolus patients (PE) and Deep vein thrombosis (DVT) should be treated with anticoagulation medications depending on their situation (Wedro et al. 2019). Pulmonary embolus (PE) and Deep vein thrombosis (DVT) patients with no active cancer are treated with novel oral anticoagulant also called direct oral anticoagulant (Wedro et al. 2019). Pulmonary embolus (PE) and Deep vein thrombosis (DVT) patients with active cancer are treated with low molecular weight heparin (enoxaparin) (Kahn et al. 2018). Critically sick patients who show signs of cardiac strain or shock are treated using thrombolytic agents called tissue plasminogen activators which is injected into the vein in the upper arm to thin the blood immediately and alteplase (TPA, Activase, Cathflo Activase) (Moake et al. 2020). Surgery may be attempted to remove the clot depending on the situation (Kahn et al. 2018). Medications that can interfere with the clotting of the blood are called anticoagulants.

2.4 Myocardial Infarction

Myocardial infarction is a stoppage or obstruction of flow of blood to the cardiac muscle, result to medical predicament (Sheu et al. 2010). Blood clot stops flow of blood to the heart, a heart attack ensues. With no blood, oxygen is lost and tissue damaged. This blood clot can block the flow of blood through the blood vessel to the cardiac muscle, due to the cardiac muscle starved of oxygen and nutrients. When a segment of the cardiac muscle is damaged or dies due to ischemia, it is called a cardiac attack, or myocardial clog (Sheu et al. 2010). A blood clot is the

primary cause of a sudden blockage in a coronary blood vessel (thrombus). A blood clot usually originates inside a coronary blood vessel that has already been constricted by atherosclerosis, a condition in which fatty deposits (plaques) grow up along the inside walls of blood vessels, narrowing them (Balogobin et al. 2020). The vascular system comprises of heart and blood vessels, which circulate blood throughout the body. It is responsible for nutrients, hormones and oxygen transport to body and removes cellular waste products from the body (Lusis et al. 2015).

The term cardiovascular disease [CVD] refers to a collection of diseases that attack the atria and its components, although MI [Myocardial infarction], angina pectoris, hypertension, stroke, and other circulatory diseases are most often associated with CVD. (Lusis et al. 2015). The common heart diseases that have been reported are coronary artery diseases, congestive heart failure, cardiac arrest, arrhythmias, and peripheral artery diseases. It was known worldwide, the ruling cause of death is due to cardiovascular diseases because annually more people die from heart diseases than other grounds (Luo et al. 2018).

Approximately CVDs claimed the lives of 17.5 million individuals in the year 2012, fatalities accounted for 31% of all deaths worldwide. Of these deaths, Coronary heart disease claimed 7.4 million lives and 6.7 million deaths were linked to heart stroke (Sheu et al. 2010). Due to non-Communicable diseases, 16 million deaths were recorded under the age of 60, 85% of which were from low- and middle-income countries in which 40% of the death were caused by CVDs (Sheu et al. 2010). Cardiovascular disorders come in a variety of forms. Among them based on the prevalence of diseases across the world, the most considerable CVD are like Atherosclerosis, Myocardial infarction, Ischemia, and Cardiomyopathy (Lusis et al. 2015).

MI may compromise diastolic and systolic function, making the patient vulnerable to arrhythmias. Furthermore, MI might result in a variety of severe consequences such as ventricular septal rupture, left ventricular aneurysm, arrhythmias, and emboli; while the risk factor includes Diabetes, hypertension and obesity (Rutger et al. 2020).

Essentially, Reperfusion therapy is used to treat acute MI, and it should be started within 12 hours after symptoms begin. Shorter periods between symptoms and reperfusion result in decreased mortality. A longer interval, leads to greater mortality, mechanical problems, and morbidity (Rutger et al. 2020).

According to Luo et al. 2018, myocardial infarction parameters include the Serum total cholesterol level (TC), triglyceride level, very low-density lipoprotein cholesterol level (VLDL-c), low density lipoprotein cholesterol level (LDL-c) and (HDL-c) high density lipoprotein cholesterol level.

2.4.1 Risk Factors of Myocardial Infarction

Myocardial infarction's risk factors include High blood cholesterol (Hypercholesterolemia) in which cholesterol is a major component of the plaques deposited in the arterial walls, a high level of cholesterol in the blood is linked to an high heart attack risk (kulick et al. 2020). Cholesterol cannot be broken down in the blood until it interacts with proteins called lipoproteins, without cholesterol interacting with lipoproteins the cholesterol will not dissolve. VLDL-very low-density lipoprotein, LDL-low density lipoprotein, and HDL-high density lipoprotein are lipoproteins that combine cholesterol throughout the blood (high density lipoprotein). LDL cholesterol, a "bad" cholesterol that transports cholesterol esters from the liver to surrounding tissues in arterial plaques, LDL cholesterol levels that are too high raise the risk of heart disease. HDL cholesterol is a healthy cholesterol that extracts cholesterol esters from peripheral tissues and transfers them to the liver, HDL cholesterol levels that are too low raise the risk of heart disease. (kulick et al. 2020).

Hypertension (High blood pressure) is a risk factor whereby heart attack can develop due to high blood pressure. High systolic (blood pressure when heart contracts) and diastolic (blood pressure when the heart relaxes) blood pressure both raise heart disease risk. (kuli*ck* et al. 2020**)**. Tobacco and tobacco products contain chemicals that weaken blood vessel walls, speed up the progression of atherosclerosis, and raise heart disease risk. (Roshan et al. 2018). Type 1 and 2 diabetes are linked to raise atherosclerosis risk in the body. Diabetes mellitus patients are at increased risk due to decreased flow of blood to legs and other areas of the body, as elevated blood sugar can allow cholesterol to be synthesized in arterial plaque. (kuli*ck* et al. 2020**)**. Individuals with a genealogy of heart diseases have a raise of heart attack risk, the risk is higher if there is a genealogy of heart disease (Roshan et al. 2018).

2.4.2 Symptoms of Myocardial Infarction

Symptoms such as chest discomfort, shortness of breath, nausea, heartburn, indigestion, general malaise (vague feeling of illness) and abdominal pain (Balogobin et al., 2020).

2.4.3 Causes of Myocardial Infarction

Causes of myocardial infarction include, Atherosclerosis which is also known as cardiovascular disease, this state is the primary cause of cardiac attacks in which buildup of fat and cholesterol occurs and develops plaque on the coronary artery's surface. If one of the plaques is ruptured blood clot will be formed on top of the ruptured plaque in which a large blood clot is enough to completely block blood flow through an artery, triggering a heart attack ((Roshan et al. 2018). Another cause is coronary artery spasm which can be a rare blockage; spasms of the coronary vessels cause them to become temporarily constricted. This constriction block the blood flow to the atrium and cause a heart attack if it lasts long enough (Balogobin et al. 2020).

Coronary artery tear also called spontaneous coronary artery dissection, a tear in coronary artery that prevents blood from reaching the heart thereby causing a heart attack (Roshan et al. 2018).

Saturated fats also presents to plaque growth in the coronary blood vessels. They are usually found in meat, dairy products, butter and cheese. By raising the quantity of bad cholesterol in the circulatory stream while decreasing the amount of good cholesterol, these lipids cause artery obstruction (Luo et al. 2018).

2.4.4 Treatment of Myocardial Infarction

Heart attack needs immediate treatment, to unclog the arteries that provide blood to the heart, a technique known as angioplasty is done. Blood vessel bypass graft can also be performed in some cases, where the patients' veins and arteries are rerouted so that blood can flow around the blockage of the artery's coronary vessel (Luo et al. 2018).

A number of medications are considered as therapeutic agent for cardiac attack. Such as blood thinners, examples are aspirin that dissolves thrombus and improves blood circulation via restricted arteries, Thrombolytics are medications that dissolve blood clots. Antiplatelet medicines like Clopidogrel can halt new thrombus from developing and existing clots from spreading, while Nitroglcerin can widen blood arteries and Beta-blockers can reduce blood pressure and mitigate the heart muscle. This also aids in limiting the severity of cardiac damage,

ACE inhibitors used to nether blood pressure and decrease stress on the atrium (Prachar et al. 2011).

Other basic treatments include: exercising regularly, eating healthfully, maintaining a healthy weight, not using tobacco products, controlling your blood pressure, lowering your LDL cholesterol.

2.5 Association of Blood Coagulation and Myocardial Infarction

Blood coagulation plays a role in the progression of myocardial infarction because anomalies in the coagulation system, whether provoked or spontaneous, influence risk (Aditya et al. 2018).

Increased synthesis and activation of coagulant proteins such as antithrombin, protein C, and protein S, reduced synthesis of anticoagulants, and inhibition of fibrinolysis are some of the ways through which inflammatory interactions affect the coagulation system. However, coagulation is activated by inflammation, and inflammatory feedback is maintained by coagulation. (Aditya et al. 2018).

Thrombosis is an important step in the pathogenesis of ischemic heart disease (IHD), involving platelets and a coagulation cascade. Specifically, after injury, platelets adhere to collagen and vWF (von Willebrand factor) which is a long polypeptide chain having a molecular mass of 270 kDa (Spronk et al. 2014). This multi-subunit plasma protein is found in bloodstream and platelets, as well as endothelium, megakaryocytic, and subendothelial tissues. In sub-endothelial tissue, the vWF acts as a bridge between endothelial collagen and surface of platelet receptors. Activated platelets release stored granules, such as ADP and thromboxane A_2, stimulating platelet aggregation. Coagulation is triggered by the activation of proteases; factor Xa (activated factor X) begins the cascade's last common route, which leads to the production of thrombin (Spronk et al. 2014). The clotting process relies heavily on thrombin: it converts soluble fibrinogen to fibrin; activates FV, FVIII, and FXI generating more thrombin; stimulates platelets; and favors the formation of cross-linked bonds among the fibrin molecules by activating FXIII, which stabilizes the clot (Spronk et al. 2014).Cofactors are also involved in the cascade, including vitamin K, and in the fibrinolysis, such as PAI (plasminogen activator inhibitor), fibrinogen, and D-dimer (Christersson et al. 2009).

A fissure of atherosclerotic plaque causes platelet activation and aggregation, as well as the production of thrombin and fibrin, which leads to the development of a thrombus. During a

myocardial infarction, the endothelium becomes unstable, platelets get activated, and the coagulation system becomes activated, putting a person at risk for future thrombotic events (Christersson et al. 2009).

Activated blood clotting is a critical factor in determining the likelihood of atherothrombotic events like myocardial infarction (Spronk et al. 2014). Atherosclerosis has two phases: in the first, it develops under the influence of "classical" risk factors, such as genetic and acquired factors (Lipinski et al. 2011). While fibrinogen/fibrin molecules play a role in early plaque wound, raised systemic coagulation activity has little impact on arterial thrombosis risk, unless a number of specific procoagulant forces collide (Lipinski et al. 2011). Despite the presence of the tissue factor–factor VII complex, all fibrin in the atherosclerotic plaque is unlikely to be the direct result of local clotting activity (Lipinski et al. 2011). The main cause of clotting in this phase is anticoagulant plasmin. The second step is marked by progressing atherosclerosis and a larger impact of inflammation, as seen by a raised plasma C-reactive protein level, which is the result of raised production affected by interleukin-6 (Spronk et al. 2014). Inflammation supersedes anticoagulant defenses, which may have become less effective due to thrombomodulin and endothelial cell protein C receptor expression dysregulation (Spronk et al. 2014). The inflammatory drive causes repeated stimulation of tissue factor and assembly of catalytic complexes on aggregated cells and micro particles throughout this phase, which keeps thrombin and fibrin synthesis at a constant level (Spronk et al., 2014). Systemic and arterial wall-driven clotting become more crucial in progressive atherosclerosis, and raised levels of D-dimer particles should be read as indications of this thromboembolism (Spronk et al. 2014).

Platelet adherence, stimulation, and accumulation, fibrin production, and fibrinolysis are the three essential components of the clotting cascade system (Spronk et al. 2014). These proteins react to one another and to the blood artery wall, ensuring that blood flow to tissues is unaffected by clotting under physiological environment. These components associate with one another and with the blood artery wall, ensuring flow of blood to tissues is unaffected by clotting under physiological conditions. Blood coagulation is triggered, indicating that thrombosis (the development of an intraluminal thrombus) is always the result of the interplay of three factors: a modified artery wall, an impeded or changed blood circulation pattern, and a changed blood composition (Spronk et al. 2014).

2.6 Isoproterenol

Isoproterenol is an agonist for the beta-1 and beta-2 adrenergic receptors used to treat bradydysrhythmias, which induce a rise in heart rate, heart contractility, and smooth muscle relaxation in the bronchial, gastrointestinal, and uterine systems (Desmine et al. 2018). It is a non-selective β-adrenoreceptor agonist that is isopropylamine analog of epinephrine. It has a molecular weight of 211.258 g/mol, a half-life of two (2) minutes and a chemical formula $C_{11}H_{17}N0_3$ (Szymanski et al. 2021).

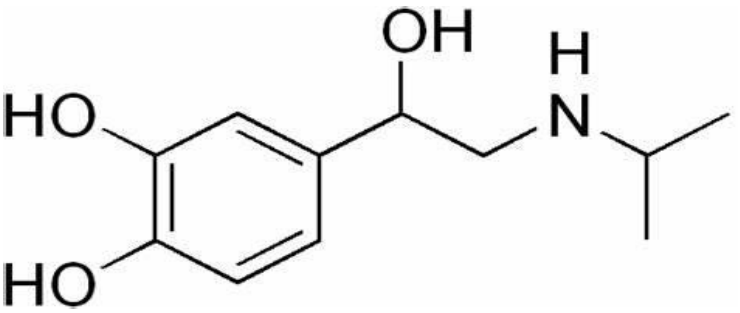

Figure 2.2: Diagram showing the molecular structure of Isoproterenol (Szymanski et al. 2021).

2.6.1 Adverse Effects of Isoproterenol

Adverse effects incude nervousness, headache, nausea, visual blurring, hypertension, hypotension, pulmonary edema, angina e.t.c. (Szymanski et al. 2021).

2.6.2 Mechanism of Action

The effects of beta-1 and beta-2 adrenergic receptors are mediated by the G-alpha stimulatory second messenger mechanism (Desmine et al. 2018). A seven-transmembrane-spanning protein makes up the structure of G-protein coupled receptors. The ligand-binding site is found in the extracellular domain. The intracellular domain binds to a G-alpha stimulatory protein bound to a GDP molecule in the inactive state (Desmine et al. 2018). When ligand binds to beta-1 receptor's

extracellular domain, the alpha subunit swaps a GDP molecule for a GTP molecule and becomes activated. The intracellular domain of the G-alpha protein dissociates, causing adenylate cyclase to be activated. After that, activated adenylate cyclase converts intracellular ATP to cAMP (Desmine et al. 2018). cAMP, the pathway's primary second messenger, activates protein kinase A. (PKA). In cardiac myocytes, activated PKA phosphorylates L-type calcium channels, due to raise in intracellular calcium. PKA also allows ryanodine receptors on the sarcoplasmic reticulum to release more calcium (Desmine et al. 2018).

2.7 D-dimer

D-dimers are formed when cross-linked, insoluble fibrin molecules are cleaved. The fibrinogen molecule contains two D-domains on the outside and an E-domain in the center (Armaghan et al. 2016). Each molecule is a dimer in terms of ultrastructure, consisting of three polypeptide chains called alpha, beta, and gamma that are bound together by two disulfide bonds (Armaghan et al. 2016). The other half of each dimer is bound together by three disulfide bonds. In the emergency room, D-Dimer is used as an initial screening procedure to diagnose patients with signs or symptoms of venous thromboembolism. D-dimer is an identifier of endogenous fibrinolysis that can be detected in patients with deep venous thrombosis (Linkins et al. 2017).

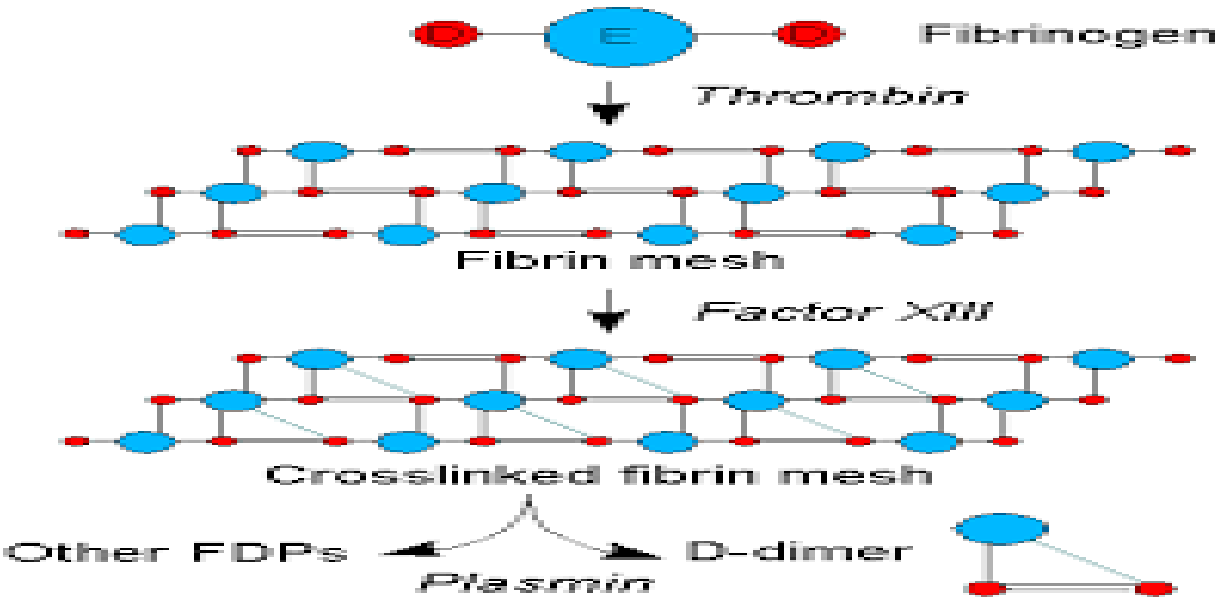

Figure 2.3: Diagram showing formation of D-dimer (Armaghan et al. 2016).

Plasmin cleaves cross-linked undissolved fibrin units to release fibrin byproducts as endovascular thrombosis progresses (FDPs). The residue of two neighboring fused D-domains from cross-linked fibrils, now known as D-dimers, is one of these products (Armaghan et al. 2016). The D-dimer present in blood samples forms a stable D-dimer/E-complex by non-covalently bonding to E-fragments. Assays based on antibodies interacting with epitopes specifically present on the D-dimer molecule have been designed to detect serum D-dimer antigen as shown in (figure 2.3). These epitopes are formed when factor XIII crosslinks fibrin polymers, and they are not found on other FDPs. Because commercial kits use unrelated antibodies, D-dimer assays are not same (Armaghan et al. 2016).

D-dimer concentrations in the blood rise due to fibrinolysis, making them direct indicators of thrombotic activity disorders. However, findings suggest that this biomarker's possible effects include atherosclerosis, myocardial infarctions, stroke, and aortic dissections (Armaghan et al. 2016). Serum D-dimers decide whether and which kind of analysis for imaging is needed, for identifying and curative purposes. Despite the fact that increased D-dimer levels at the time of diagnosis are associated with the worst short-term mortality outcomes and prognosis, findings suggest that increased D-dimer levels at the time of diagnosis are associated with the worst long-term mortality outcomes and prognosis (Linkins et al. 2017).

2.8 Platelet Aggregation

Human platelets are anucleated cells that take part in hemostasis and thrombosis, clot retraction, and vessel constriction, among other pathophysiological processes (Martel et al. 2019).

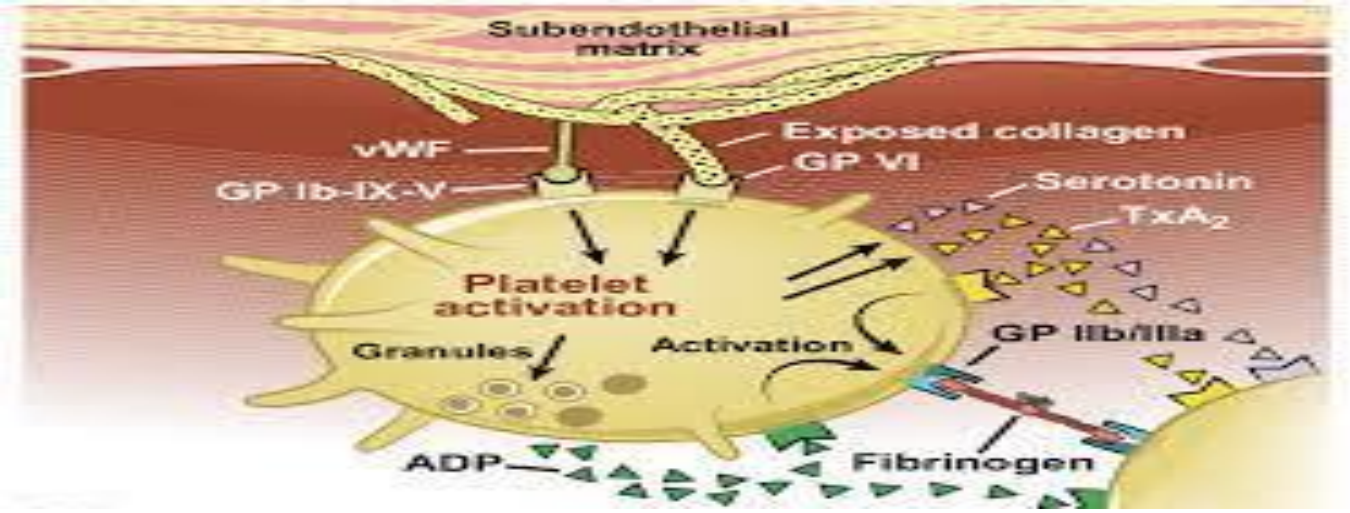

Figure 2.4: Diagram showing platelet aggregation (Martel et al. 2019).

The essential occurrence in CVD and atherosclerosis are platelet aggregation and thrombosis. Platelets adhere to the wounded artery wall to create a plaque, then aggregate and release thromboxane A2 (TXA2) and adenosine diphosphate (ADP), both of which encourage further aggregation as shown in (figure 2.2). The ensuing platelet plug causes partial or total occlusion at the lesion site, as well as distal embolization, resulting in ischemia/infarction of the affected organ. In practical practice, antiplatelet medications work by blocking the essential process of platelet aggregation (Martel et al. 2019).

Platelet aggregation in the presence of flow emerged a combined process that includes: vWf exposure on immobilized platelets' surfaces, GPIb binding to vWf on the surface of immobilized platelets induces a reversible process of platelet aggregation and an inevitable aggregation process involving integrin IIb3.

Platelet thrombus formation in vivo shows that this multistep adhesion mechanism is needed for platelet aggregation in arterioles and also appears to facilitate platelet aggregate formation in venules, according to studies (Suhasini et al. 2015). The main hemostatic plug's development and the growth of diseased embolism at sites of atherosclerotic plaque breakup are also dependent on platelet aggregation at sites of cardiovascular injury. Platelet adhesion (platelet interaction with the damaged vessel wall) is a complicated mechanism involving several adhesive substrates (von Willebrand factor [vWf], collagen) and receptors on the surface of platelet (GPIb/V/IX, integrins

IIb3 and IIb2) (Suhasini et al. 2015). The platelet surface contact linking GPIb and matrix-bound vWf acts as a necessary stage for laminin-mediated cell delay by tethering platelets to the site of vascular damage, especially under high shear pressure. Although the molecular occurences that underpin platelet adhesion under various shorten states have been well described, the mechanism(s) by which platelets in free-flowing blood attach to the first layer of adhering platelets (platelet adhesion or clumping) under flow have yet to be established (Martel et al. 2019).

However, the process by which platelets bind to the layer of suspended platelets in free-flowing blood is unknown (Suhasini et al. 2015). This is a vital concern since platelet accumulation at regions of cardiovascular damage is normally caused by platelets accruing from free-flowing blood onto the luminal layer of a growing thrombus, rather than platelets in suspension (Suhasini et al. 2015). Platelets suspended at sites of artery wall injury thus have a highly receptive surface for platelet recruitment from circulating blood. Despite its critical role in seizure and surgery, the structure of this reactive surface is still poorly known (Rumbaut et al. 2010). *In vivo* platelet aggregation, a complicated process in which platelets continually tether, detach and translocate from a growing thrombus' luminal layer, with only a tiny fraction of tethering platelets creating stationary adhesion connections., according to reports (Suhasini et al. 2015). The binding of GPIb on free-flowing platelets to vWf expressed on the layer of suspended platelets mediates the tethering/translocation process. This tethering mechanism is required for platelet adhesion contacts to form at arterial shear rates, and it may also improve the efficiency of platelet aggregation at venous shear rates (Rumbaut et al. 2010).

2.9 Cardiac Markers

A biomarker is a trait that is scientifically assessed and analyzed as a predictor of inherent biological processes, clinical processes, or chemical feedbacks to a curative intervention, according to the National Institutes of Health consensus meeting. A biomarker is described in variety of ways. (Jarolim et al. 2014). To be clinically effective, an ideal biomarker should meet the following criteria such as a reasonable cost and with a reasonable processing period, a reliable, repeatable assay should be possible, the test result should clarify a fair proportion of the outcome regardless of proven predictors and the assay should be highly responsive and specific

for the outcome it is intended to define. The procedure must be satisfactory and clear to the patient, and the test findings must be simple for physicians to interpret.

Awareness of the biomarker's degree, in particular, should alter patient management. Biomarkers can range in complexity from basic medicinal chemistry and hematology tests to sophisticated laboratory tests conducted in blood, urine, other body fluids, or diverse tissues. The majority of existing cardiovascular biomarkers are tested in plasma or serum using more advanced medicinal chemistry assays, such as immunoassays. (Chan et al. 2015). Tiny molecules and metabolites, nucleic acid sequences, RNAs, and microRNAs are all examples of biomarkers. Cardiovascular biomarkers are classified as either proven or new, depending on which they are used to diagnose an acute incident, predict short- or long-term outcomes, or guide therapy (Chan et al. 2015). Inflammatory response, hypoxia and cytotoxicity, smooth muscle cell damage, raised lipid peroxidation, plaque instability, and volume overload, and extracellular matrix remodeling are all pathophysiological processes that cardiac biomarkers capture. Plaque instability and raised lipid peroxidation may be linked by an inflammatory biomarker. Finally, cardiovascular biomarkers should be classified based on the diseased individual for which they are most useful: acute coronary syndrome, heart attack, and hypertrophic Cardiomyopathy (HCM) (Jarolim et al. 2014).

2.9.1 Plasma Troponin T (cTnT)

The cardiac regulatory proteins troponin T (cTnT) and troponin I (cTnI) regulate the calcium-mediated relationship between myosin and actin. Relevant genes code for the cardiac variants of these regulatory proteins (Sharma et al. 2014).

Monoclonal antibodies to cTnI and cTnT epitopes are used to detect cardiac troponins in the serum. According to Sharma et al. 2014, these antibodies have very little crossreactivity with skeletal muscle troponins and are particularly selective for cardiac troponin.

Electrocardiographic indications of myocardial ischaemia in people who do not have them, the lack of cardiac troponins in the blood twelve hours after the emergence of chest pain is linked with minimal chance of an adverse effect in terms of myocardial infarction which mortality, which allows for early discharge (Tibbles et al. 2014).

A wide variety of disorders linked to raise cardiac troponin levels have the potential to create medical ambiguity and therapeutic dilemmas in case care (Tibbles et al. 2014).

2.9.2 Aspartate Aminotransferase (AST)

The aspartate aminotransferase (AST), an enzyme mostly present in the hepatic tissue, found in red blood cells, heart cells, and kidneys (Yoon et al. 2016). AST levels are mainly used to diagnose liver diseases, but they can be used together with other enzymes to monitor the progression of different liver disorders. When bodily tissue or an organ, such as hepatic tissue or heart, is sick or damaged, AST is released into the circulation, causing enzyme levels to increase. As a result, the AST blood level is proportional to the degree of tissue injury (Saikrishna et al. 2020).

Aspartate aminotransferase was the first biomarker used to help diagnose acute MI (AST). Ladue proposed in 1954 that AST released from necrotic cardiomyocytes could be used to diagnose acute MI. During an acute MI, AST levels rise for 3 to 4 hours, plateau for 15 to 28 hours, and then fall down to baseline within 5 days (Saikrishna et al. 2020). Since AST is not a unique identifier for cardiac myocytes, it has lost popularity in existing clinical practice for diagnosing acute MI (Sharma et al. 2014). As a consequence, AST blood levels are elevated in hepatic disease (e.g., hepatitis, hepatic congestion), pericarditis, pulmonary embolism, and shock, and are no longer used to diagnose acute MI.

2.9.3 Alanine Aminotransferase (ALT)

Pyruvate glutamate transaminase is an enzyme also known as ALT. In alanine cycle, alanine aminotransferase stimulates the transition of an amino compound from L-alanine to alpha-ketoglutarate, forming pyruvate and glutamate (York, 2017). Pyridoxal phosphate, a coenzyme that proceed as an amino carrier, is needed for ALT. It is important for gluconeogenesis and amino acid metabolism. Serum activation is proportional to the amount of infected hepatocytes, with large increases indicating permanent cell loss and necrosis and small increases indicating membrane blebbing and reversible cell damage (Dzoyem et al. 2014). The transaminase enzyme ALT is present in organ tissues and serum, particularly liver, although large concentrations are present in kidney, skeletal muscle and myocardium. Minimal levels of ALT are found in

pancreas, spleen and lung. ALT is an indicator of liver activity that is raised in serum when there is severe cellular necrosis (Dzoyem et al. 2014).

Hepatitis, congestive heart disease, and myopathy all cause elevated ALT levels. (York, 2017).

2.9.4 Lactate dehydrogenase (LDH)

Another possible biomarker for identifying myocardial ischemia is lactate dehydrogenase (LDH) (Saikrishna et al. 2020). LDH levels rise 6 to 12 hours after an acute MI, peak in twenty-four to seventy-two hours, and back to normal in 8 to 14 days. Previously, a ratio of LDH1 (a heart isoform) to LDH2 greater than 1 was thought to be unique for an acute MI. LDH is no longer performed in the prognosis of myocardial infarction because it is not a unique identifier for cardiac myocytes and its levels can rise in a variety of other conditions. (Anadon et al. 2014). LDH is now only used to distinguish people with acute from sub-acute MI with elevated troponin levels but normal creatine kinase (CK) and CK-MB levels in the recognition of acute MI (Saikrishna et al. 2020). LDH blood levels are also useful for identifying erythrocyte hemolysis and assessing the treatment and prognosis of tumors including testicular germ cell tumors (Anadon et al. 2014).

2.10.0 Principles of Assay

2.10.1 Bleeding time

Principle: On the tail, a standardized incision is performed. The length of time the incision bled was calculated. The development of a haemostatic plug, which is based on the number of platelets present and their capacity to attach to the subendothelium, is indicated by the cessation of bleeding.

2.10.2 Clotting Time

Principle: Clotting Time is the length of time neccessary for blood to clot *in vitro*. The test is based on the fact that when entire blood is revealed to a foreign layer, such as a glass tube, it forms a solid clot. In the presence of no tissue factors, it is an approximate estimate of the total intrinsic clotting proteins.

2.10.3 Prothrombin Time

Principle**:** In showing of Ca^{++}, tissue thromboplastin initiates the extrinsic route of the human blood coagulation cascade. The concentration of specific clotting elements present in the clotting system determines the activation time. This aids in determining the etiology and scope of the hemorrhagic disease. The clotting cascade is started when thromboplastin reagent is introduced to citrated plasma, resulting in the emergence of a gel clot. If no factor activity is present in the extrinsic route of the coagulation cycle, the time it requires to produce a clot will be longer.

2.10.4 Activated Partial Thromboplastin Time

Principle: The APTT is founded on the idea that adding a platelet replacement, factor XII activator, and $CaCl_2$ to citrated plasma leads to the rise of a stable clot. The time it requires for a stable clot to develop is documented in seconds shows the real aPTT result.

2.10.5 Total Protein Concentration

Principle: Cupric ions interact with protein peptide bonds in an alkaline media, resulting in the creation of a colored complex.

2.10.6 Lactate Dehydrogenase (LD) Concentration

Principle: L-Pyruvate + NADH + H^+ $\Longrightarrow$ L-lactate + NAD^+

This reaction is catalyzed by LD.

2.10.7 Alkaline Phosphatase (ALP) Concentration

Principle: Alkaline phosphatase from the sample hydrolyzes the substrate p-nitrophenyl to produce p-nitrophenol, which has a yellow colour and can be measured with a UV spectrophotometer at 405nm.

The amount of colour generated is related to the sample's alkaline phosphatase activity.

p-nitrophenyl phosphate + H_2O $\Longrightarrow$ phosphate + p-nitrophenol

This reaction is catalyzed by ALP.

CHAPTER THREE

METHODOLOGY

3.1 Materials and Chemicals

This study's reagents were all analytical grade.

Volumetric flask, weighing balance, Filter paper, Funnel, Dropper, Test tubes, Test tube racks, Beaker, Measuring cylinder, Rotary evaporator, Spatula, Water bath, Nose mask, Hand gloves, Distilled water, Chloroform, Sodium citrate, Sodium chloride, n-Hexane, n-Butanol, Ethyl acetate, Methanol, Aspirin, Glass slide, Pin, Scapel, Filter paper, Micropipettes and tips. Isoproterenol was obtained from Get well pharmaceuticals, New Delhi, India. Reagent kits for activated partial thromboplastin time (aPTT) and Prothrombin time (PT) were purchased from Diagen Diagnostic Reagents Ltd., Thame, Oxon, UK. Reagent kits for total protein (TP), alkaline phosphatase (ALP) and lactate dehydrogenase (LD) activity were purchased from Randox Laboratories Ltd, UK.

3.1.1 Collection of Plant Materials

C. rubens (Jacq.S.Moore) of family Asteraceae was obtained from farm in Ekiti state, South-Western Nigeria. The plant was authenticated at the Department of Plant Biology, University of Lagos, Nigeria. A voucher specimen 8788 was assigned and a sample dropped at the Department's Herbarium.

3.1.2 Preparation of Plant Sample

C. rubens leaves were oven-dried at 40 °C and ground into powder using an electric blender and stored in the refrigerator at 4 °C until further use. The ground sample was soaked with 70% hydro methanol (1:8 w/v) for 72hrs accompanied by intermittent shaking. After that, fine muslin cloth and Whatman No. 1 filter paper were used to filter the suspension. The hydromethanol extract was concentrated at low pressure using a rotary evaporator, at 40 °C and kept at 4 °C in the refrigerator until further use. The concentrated crude extract was reconstituted with water and subjected to solvent partitioning using Hexane, Ethyl acetate and Butanol sequentially (Otsuka, 2006). GCMS was used to characterize the phytochemicals.

3.2 Study Design: The study was an *in vivo* animal study.

3.2.1 Experimental Animals

The Animal Facility at Mountain Top University's Department of Biological Sciences provided sixty male albino wistar rats for the study. The rodents were kept in cages at Mountain Top University's Animal Facility, where they were fed a regular rat diet and had unlimited access to water. They were acclimatized for two weeks.

Animal handling procedure were in accordance with ethical guide for the care of Laboratory animals and usage based on the guidelines of the Institutional Animal Ethics Committee (IAEC) and ethical clearance was obtained from University of Lagos, Akoka.

3.2.2 Grouping of Experimental Animals

Experimental animals were randomly allocated into 12 groups of 5 animals each. The description of the groups are as follows:

Group 1: Normal control (given 1ml phosphate buffered saline (PBS).

Group 2: Negative control (ISO without pretreatment).

Group 3: Positive control; ISO + Aspirin (75 mg/kg)

Group 4: ISO + pretreatment with the crude hydromethanol extract of *C. rubens* (100 mg/kg).

Group 5: ISO + pretreatment with the crude hydromethanol extract of *C. rubens* (150 mg/kg).

Group 6: ISO + pretreatment with the crude hydromethanol extract of *C. rubens* (200 mg/kg).

Group 7: ISO + pretreatment with the hexane fraction of *C. rubens* (100 mg/kg).

Group 8: ISO + pretreatment with the hexane fraction of *C. rubens* (150 mg/kg).

Group 9: ISO + pretreatment with the hexane fraction of *C. rubens* (200 mg/kg).

Group 10: ISO + pretreatment with ethyl acetate fraction of *C. rubens* (100 mg/kg).

Group 11: ISO + pretreatment with ethyl acetate fraction of *C. rubens* (150 mg/kg).

Group 12: ISO + pretreatment with ethyl acetate fraction of *C. rubens* (200 mg/kg).

The animals were kept under monitoring and administration of plant extract was done orally once daily for 14 days prior to induction of myocardial infarction.

3.2.3 Induction of Myocardial Infarction

Myocardial infarction was induced by single intraperitoneal injection of isoproterenol (ISO; 100 mg/kg body weight) in phosphate buffer (pH 7.4) (Syrifah et al. 2018). Induction was carried out on day 14 On day 15, after an overnight fast, the rats were sacrificed under anesthesia, dissected and blood was collected by cardiac puncture. The heart and liver tissues were excised, washed free of blood in normal saline, and blotted with tissue paper for the preparation of homogenate.

3.3 Preparation of Blood Plasma, Serum and Tissue

The collected blood sample was transferred into centrifugation tubes containing 3.2% sodium citrate which was used for blood coagulation assays. The plasma was separated. Plasma from the same group was pooled together and then kept at 4^{o}C in the refrigerator until use. Heparinized blood was used for other assays.

The blood samples were centrifuged at 2500 revolution per minute (RPM) for period of 15 minutes using Thermo Scientific Centrifuge (Heraeus Megafuge 8).

The sera were aspirated using Microflux pipette into dry, clean sample bottles and were stored frozen at (4^0 C) overnight. The animals were quickly dissected, the cardiac tissues were excised, cleaned of fatty layers, weighed and transferred into a phosphate buffer solution. Thereafter, each organ was blotted with blotting paper, cut thinly with a sterile blade and homogenized separately in a cold phosphate buffer solution (1:5 w/v). The homogenates obtained were centrifuged at 3000 revolution per minute (RPM) for period of 10 minutes to obtain the supernatants which were then gently collected into sample bottles, stored frozen (4^0 C) overnight before being used for the various biochemical assays.

3.4 Assay Methods

3.4.1 Bleeding Time

Bleeding time was determined as described by Shrivasta and Das (1987) as reported by (Raof et al. 2013).

Procedure: On the tip of the tails of the experimental animals, a conventional incision was made, and the tip of the tail was put on clean glass slide and filter paper. A stopwatch was used to record the bleeding time at 10 second intervals.

3.4.2 Clotting Time

Clotting time was determined using Ivy's method reported by Ibu and Adeniyi (1989).

Procedure**:** A drip of blood from the tail of each rat was placed on clean glass slide and a stopwatch started reading at the same time. A pin was passed across once every 15 seconds. As soon as a thread of fibrin was noticed, the stopwatch was stopped and the time was recorded.

3.4.3 Prothrombin Time

The assay was done according to the method of Brown (1988)

Procedure: The reagent vial was swirled before use in which the reconstituted PT reagent was dispensed into a clean dry test tube. Then the dispensed PT reagent was pre warmed at 37^0c for

10 minutes. After that 100 µl of plasma was transferred into the test tube and incubated in the water bath in which the timer began and the clotting time was documented in seconds.

3.4.4 activated Partial Thromboplastin Time (aPPT)

The assay was done according to the method of Brown (1988)**.**

Procedure: The reagent vial was swirled before use in which the 100 µl reconstituted aPPT reagent was dispensed into a clean dry test tube. Then the dispensed aPPT reagent was pre warmed at 37^0c for 10 minutes. After that 1000 µl of plasma was transferred into the test tube and 100 µl 0f $cacl_2$ solution was transferred to each test tubes incubated in the water bath in which the timer began and the clotting time was documented in seconds.

3.4.5 Cardiac Markers Assay

3.4.5.1 Total Protein (TP) Assay

Procedure: Sterile test tubes were used which were labelled appropriately, 20µl of enzyme source (heart) was transferred to the samples test tubes, 20µl of water was added to the blank test tube and 20µl of standard was transferred to the standard test tube. 1ml of Reagent 1 was transferred to the sample test tubes, blank test tube and standard test tube, which the solution was combined and incubated in the hot-air oven for 30 minutes at 25 ^{0} C. The samples absorbance was read against the blank using UV-Visible spectroscopy (Auto-palmer Ltd, UK) at 546nm wavelength.

Total protein concentration was calculated as

$$\text{Total protein concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration (5.95g/dl)}$$

3.4.5.2 Lactate Dehydrogenase (LD) Assay

Procedure: The Reagent1b was reconstituted with 3mls of Reagent1a which was taken as the reagent. Sterile test tubes were used which were labelled appropriately, 40µl of enzyme source (heart) was transferred to the sample test tubes and 1ml of water was transferred to the blank test tube. 1ml of reagent was transferred to the sample test tubes and blank test tube, which the

solution was mixed and the samples absorbance was read against the blank at 30 second (A_1), 1minute (A_2), 2 minutes (A_3) and 3 minutes(A_4) intervals using UV-Visible spectroscopy (Auto-palmer Ltd, UK) at 340nm wavelength.

LD activity was calculated using the formulae at +25/+30^{o}c as

U/I = 4127 X change in absorbance 340 nm/min

3.4.5.3 Alkaline Phosphatase (ALP) Assay

Procedure: Sterile test tubes were used which were labelled appropriately, 20µl of enzyme source (plasma) was transferred to the sample test tubes and 20µl of water was transferred to the blank test tube. 1ml of Reagent 1 was transferred to the sample test tubes and blank test tube, which the solution was mixed and the samples absorbance was read against the blank at 1minute (A_1), 2 minutes (A_2) and 3 minutes (A_3) intervals using UV-Visible spectroscopy (Auto-palmer Ltd, UK) at 405nm wavelength.

ALP activity was calculated using the formulae as

U/I = 2742 X change in absorbance 405 nm/min

3.4.6 Phytochemical Characterization

The phytochemical characterization of the crude extract of the *C. rubens* was done using Gas Chromatography-Mass Spectrometry (GC-MS) for the presence of alkaloids, glycosides, cardiac glycosides, steroids, coumarin, tannins, flavonoids, saponins and reducing sugar (Entaz et al., 2016)

Procedure: A gas chromatograph by Hewlett Packard (Model 6890 series) with aflare photoelectric detector and a Hewlett Packard 7683 series 250 °C MS transfer line temperature injector were employed in GC-MS experiment. An HP-5MS merged silicone packed column (30 x 0.25 mm) was placed in the GC with a surface area of 1.0 m.. Using helium gas (99.999 percent) as a gas phase at a steady flow rate of 22 cm / s, the temperature of the oven was held at 50 ° C for 5 minutes and then raised at a speed of 2 ° C / min from 50 to 250 ° C. At a 1:30 split ratio, a 1.0micron extract (1 mg diluted in 1 ml absolute alcohol) was injected. Agilent Science Network Mass Spectrometer (Model 5973 series) coupled with gas chromatograph by Hewlett Packard (Model 6890 series) and with NIST08 Library software database was studied by MS.

Mass spectra were drawn at 70 eV/200 °C, 1 scan/s scanning rate. Compound recognition was done using the NIST08 Library database. The mass spectrum of each unidentified molecule was matched to the compounds that were already contained in the library software database.

3.5 Waste Disposal

The rat carcasses were buried at the designated burial site, while sample bottles containing unused blood and other biological samples were incinerated.

3.6 Statistical Analysis

Data obtained from the study was statistically analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple comparisons (post-hoc) using GraphPad prism 9.2.0 Results were expressed as a mean ± standard error of mean (SEM). P values less than 0.05 ($p < 0.05$) were considered statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Percentage yield of crude extract and other Fractions of *C. rubens*

Table 4.1 percentage yield of crude extract and other fractions of *C. rubens*

Name of the sample	Hydromethanol Extract	Hexane Fraction	Ethyl acetate Fraction
Percentage Yield	13.85%	16.42%	7.44%

4.2 Effect of Hydromethanol fractions and extract of *C. rubens* on Bleeding Time in Myocardial Rats

Presented in figure 4.1 is the effect of hydromethanol fractions and extract of *C. rubens* on bleeding time in myocardiac rats. The result revealed significant ($p < 0.05$) increase in bleeding time of normal control rats when compared with the isoproterenol only induced rats. There was significant decrease ($p < 0.05$) in bleeding time of groups administered 100 mg/kg, 150 mg/kg, 200 mg/kg of the methanol crude extract, fractions when compared to aspirin treated group with the exception of both methanol extract and ethyl acetate 100 mg/kg. The best activity was observed in methanol extract 100 mg/kg by increasing bleeding time when compared to other test fractions.

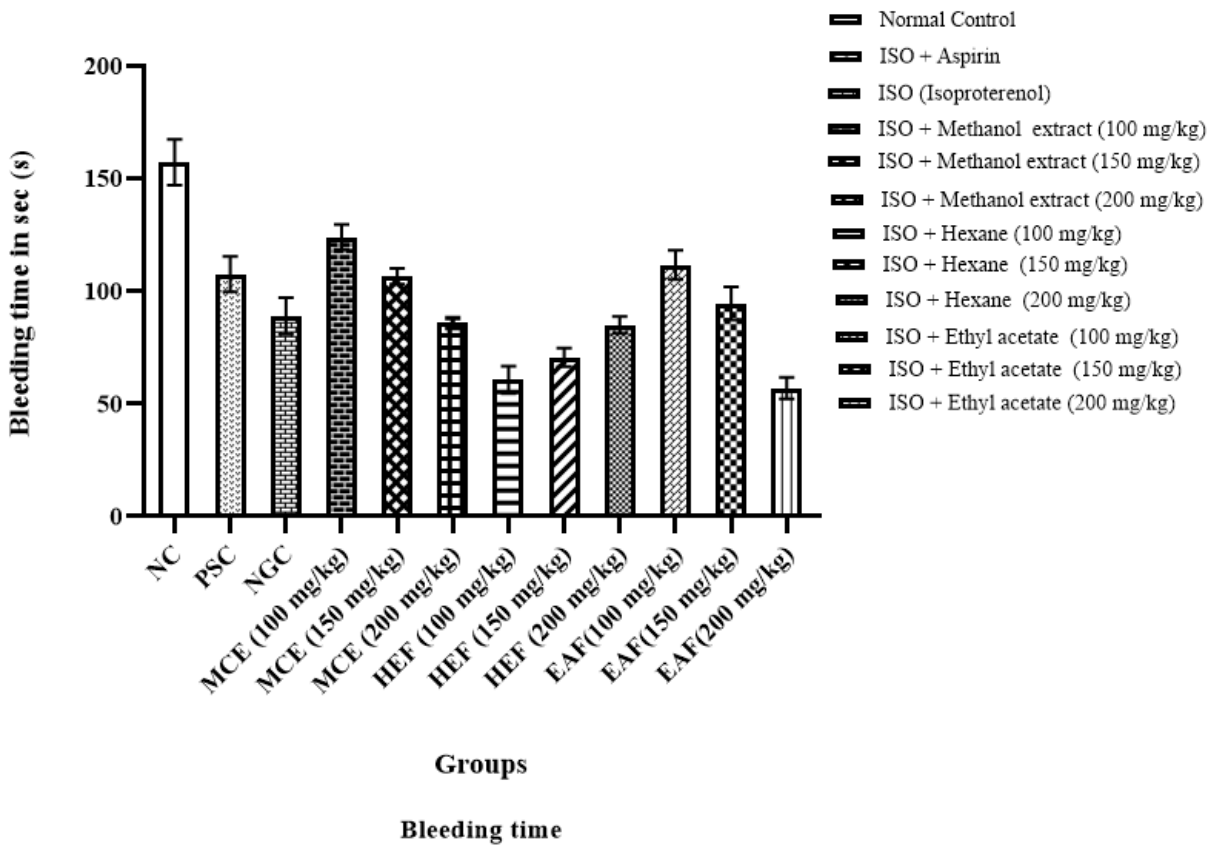

FIGURE 4.1: Bleeding time of hydromethanol fractions and extract of *C. rubens* in control and myocardial rats

Values are presented as mean ±SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control

4.3 Effect of Hydromethanol Fractions and extract of *C. rubens* on Clotting Time in Myocardial Rats

Presented in figure 4.2 is the effect of hydromethanol fractions and extract of *C. rubens* on clotting time in myocardial rats. The result revealed significant ($p < 0.05$) increase in clotting time of aspirin treated rats when compared with the normal control and isoproterenol only induced groups. There was significant decrease ($p < 0.05$) in clotting time in the groups that were administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol extract, and fractions when compared to aspirin treated group with exception of methanol extract 100 mg/kg and ethyl acetate 200 mg/kg. The best activity was observed in methanol extract 100 mg/kg and ethyl acetate 200 mg/kg by increasing the clotting time when compared to other test fractions.

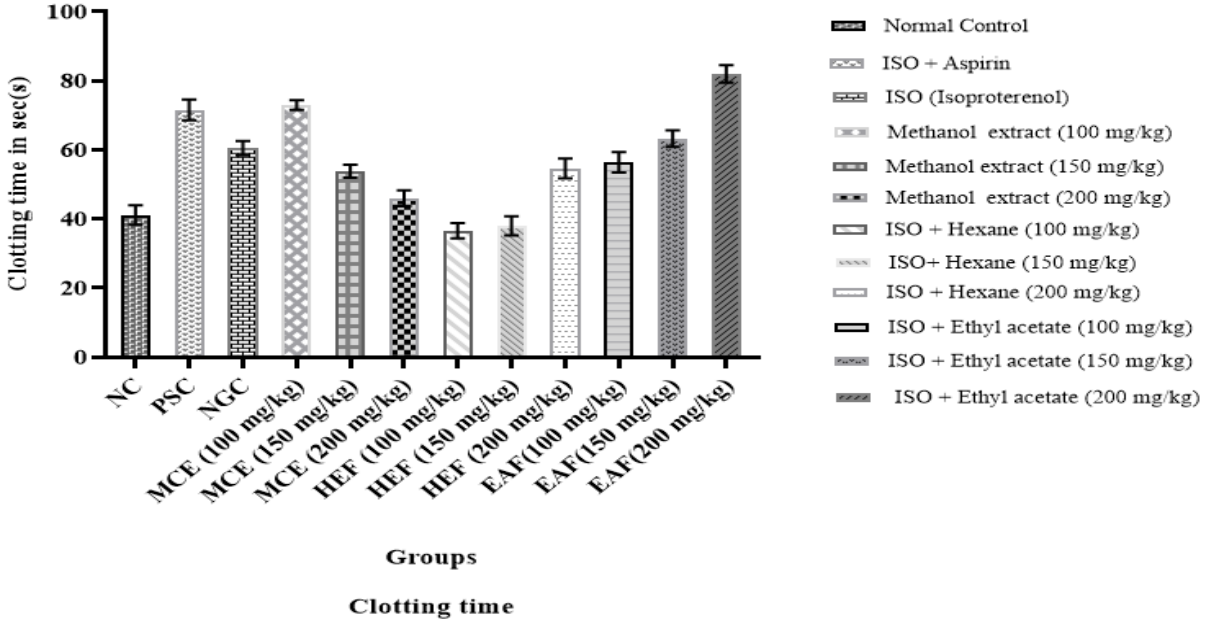

FIGURE 4.2: Clotting time of hydromethanol Fractions and extract of *C. rubens* in control and myocardial rats

Values are presented as mean ± SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control

4.4 Effect of Hydromethanol Fractions and extract of *C .rubens* on Prothrombin Time in Myocardial Rats

Figure 4.3 depicts the effect of hydromethanol fractions and extract of *C. rubens* on prothrombin time in myocardial rats. The result showed no significant ($p < 0.05$) decrease in prothrombin time of normal control against isoproterenol only induced groups. There was significant ($p < 0.05$) decrease in prothrombin time of the groups that were administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol extract and fractions, when compared to aspirin treated group with the exception of methanol extract 200 mg/kg and ethyl acetate 150 mg/kg, 200 mg/kg. The best activity was observed in methanol extract 100 mg/kg and 150 mg/kg by decreasing the prothrombin time when compared to other test fractions.

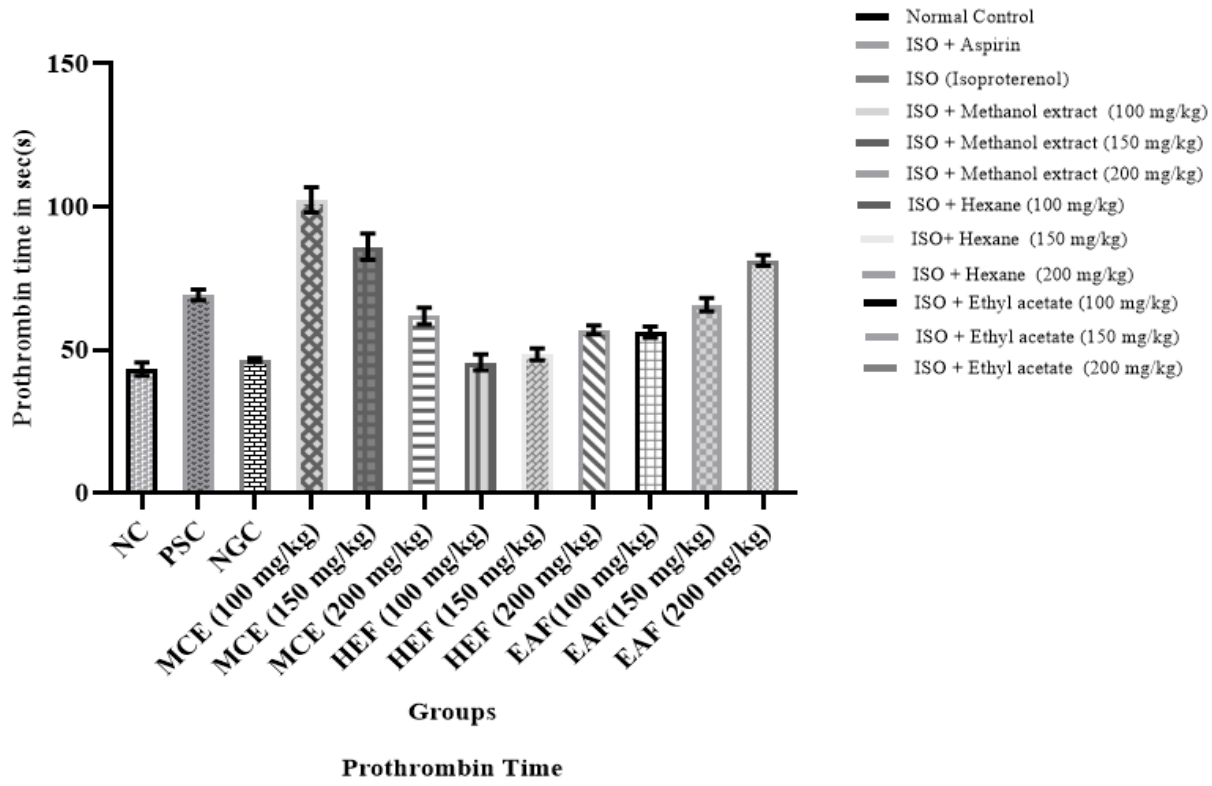

FIGURE 4.3: Prothrombin time of hydromethanol Fractions and extract of *C. rubens* in control and myocardial rats

Values are presented as mean ± SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control

4.5 Effect of Hydromethanol Fractions and extract of C .rubens on Activated Partial Thromboplastin Time in Myocardial Rats

Presented in figure 4.4 is the effect of hydromethanol fractions and extract of *C. rubens* on Activated partial thromboplastin time in myocardial rats. The result showed no significant ($p < 0.05$) decrease in activated partial thromboplastin time of normal control against isoproterenol only induced group. There was significant ($p < 0.05$) decrease in the activated partial thromboplastin time in groups that were administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol extract and fractions, when compared to aspirin treated group with the exception of methanol extract 100 and 150 mg/kg. The best activity was observed in hexane 100 mg/kg by decreasing the activated partial thromboplastin time when compared to other test fractions.

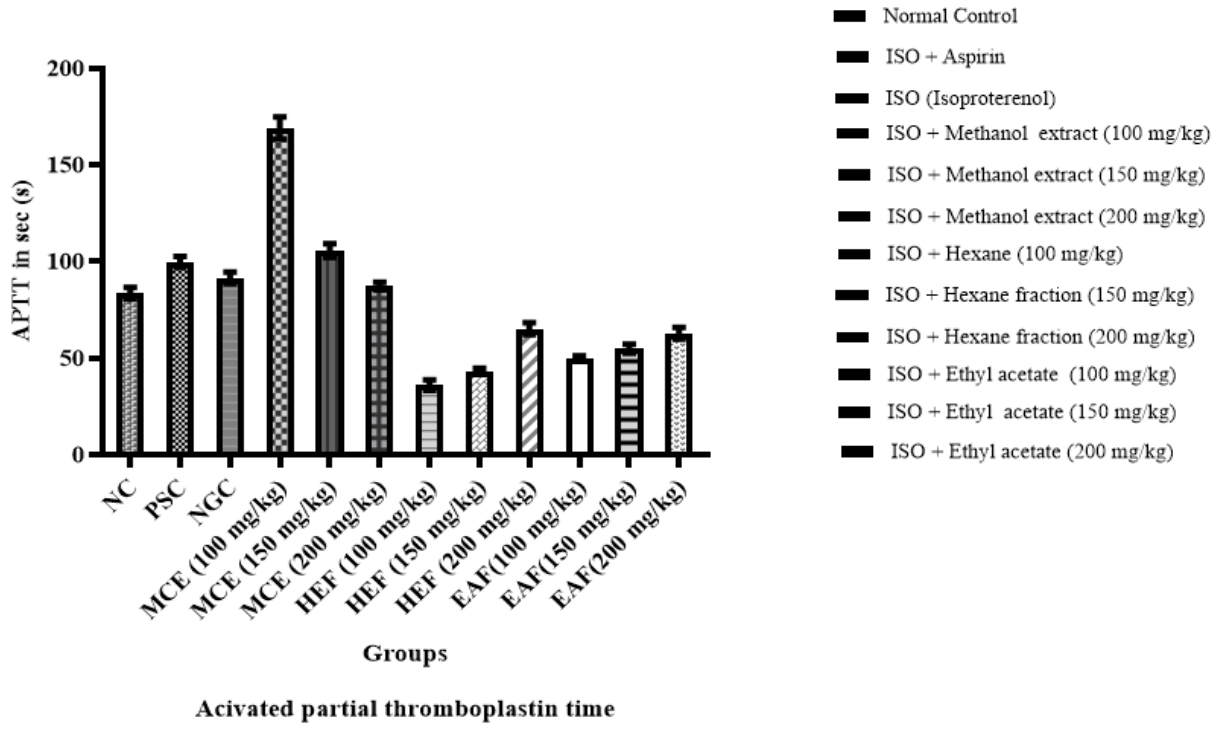

FIGURE 4.4: Activated partial thromboplastin time (aPPT) of hydromethanol Fractions and extract of *C. rubens* in control and myocardial rats

Values are mean ± SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control.

4.6 Effect of Hydromethanol Extract and Fractions of *C .rubens* on Total Protein Concentration in Myocardial Rats

Presented in figure 4.5 is the effect of hydromethanol extract and fractions of *C. rubens* on total protein concentration in myocardial rats. The result showed significant ($p < 0.05$) decrease in the total protein concentration of normal control against isoproterenol only induced group. There was significant ($p < 0.05$) decrease in total protein concentration in the groups administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol extract and fractions when compared to aspirin treated group. The best activity was observed in methanol extract 100 mg/kg by decreasing total protein concentration when compared to other test fractions.

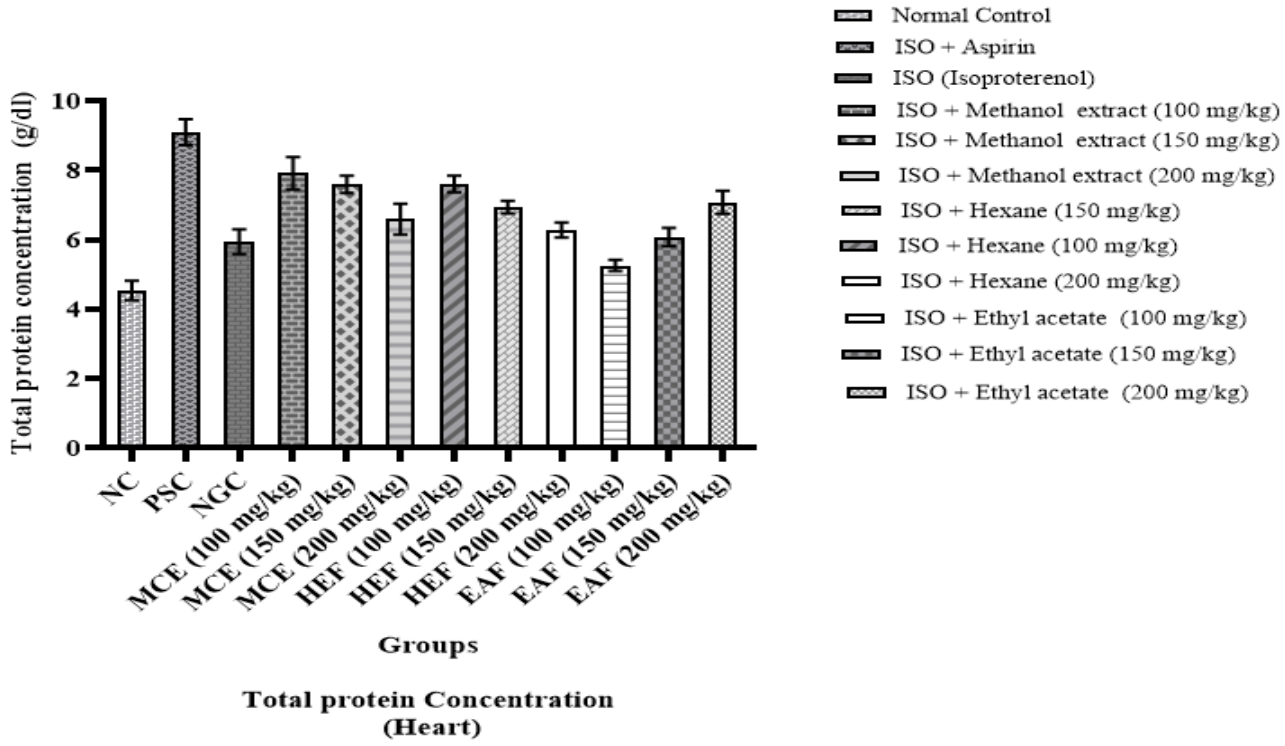

FIGURE 4.5: Total protein concentration of hydromethanol Fractions and extract of *C. rubens* in control and myocardial rats

Values are mean ± SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control

4.7 Effect of Hydromethano Fractions and extract of *C .rubens* on Lactate Dehydrogenase (LD) Concentration in Myocardial Rats

Presented in figure 4.6 is the effect of hydromethanol fractions and extract of *C. rubens* on LD concentration in myocardial rats. The result showed significant ($p < 0.05$) increase in LD concentration of normal control rats against isoproterenol only induced group. There was significant ($p < 0.05$) increase in LD concentration in the groups administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol extract and fractions groups when compared to aspirin treated group with the exception of hexane 100 mg/kg and ethyl acetate 150, 200 mg/kg. The best activity was observed in methanol extract 200 mg/kg by increasing LD concentration when compared to other test fractions

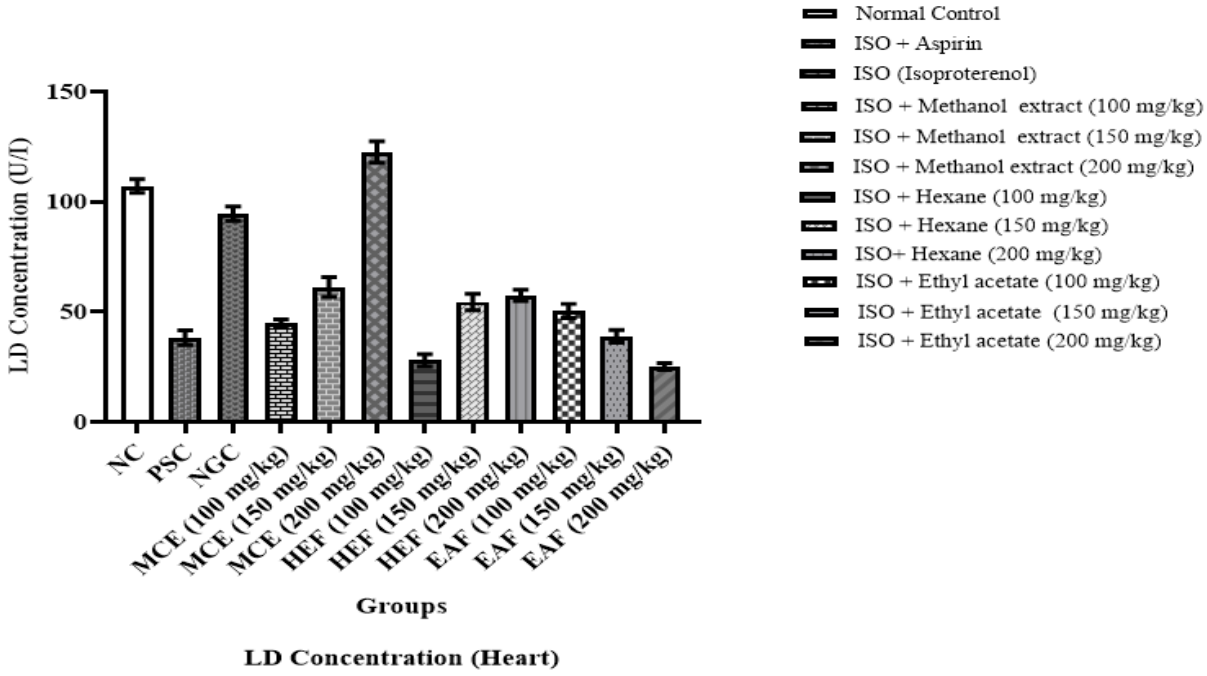

FIGURE 4.6: LD concentration of hydromethanol Fractions and extract of *C. rubens* in control and myocardial rats

Values are mean ± SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control

4.8 Effect of Hydromethanol Fractions and extract of *C. rubens* on Alkaline Phosphatase (ALP) Concentration in Myocardial Rats

Presented in figure 4.7 is the effect of hydromethanol fractions and extract of *C. rubens* on ALP concentration in myocardial rats The result showed significant ($p < 0.05$) decrease in ALP concentration of normal control against isoproterenol only induced group. There was significant ($p < 0.05$) decrease in ALP concentration in the groups administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol fractions and extract groups when compared to aspirin treated group. The recorded ALP concentration in the aspirin treated group is almost the same with that of methanol extract 200 mg/kg. The best activity was observed in ethyl acetate 100 mg/kg by decreasing ALP concentration when compared to other test fractions

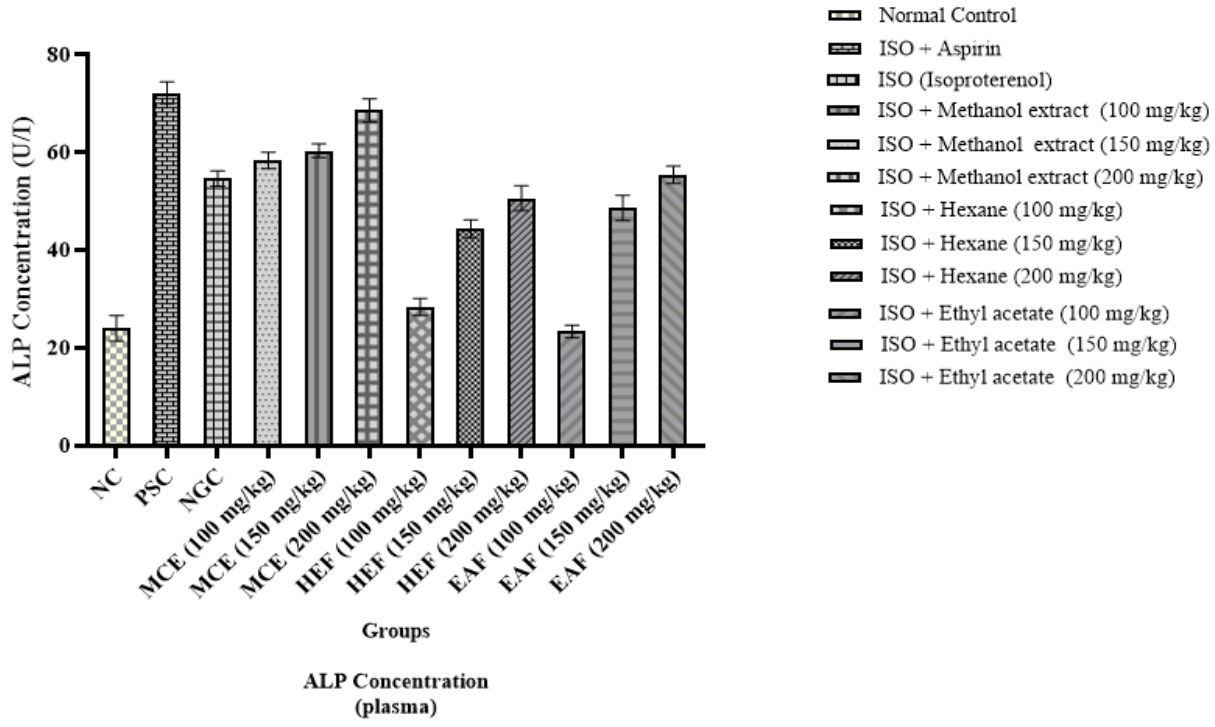

FIGURE 4.7: ALP concentration of hydromethanol Fractions and extract of *C. rubens* in control and myocardial rats

Values are mean ± SEM; n=5

NGC: negative control; ISO: Isoproterenol, PSC: positive control

4.9 GCMS Identified Phytochemical components of ethyl acetate fraction of *C. rubens*

Table 4.2: GCMS Identified Phytochemical components of ethyl acetate fraction of *C. rubens*

Serial Number	R/T (min)	Area (%)	Name of Compound	M/F	Reported biological Activity
1	2.325	0.50	2-Ethoxyethyl isobutyl carbonate Tetraetheleneglycol monomethylethe 2,5,8,11,14,17-Hexaoxanonadecan-19 –ol	$C_9H_{18}O_4$	Acetylcholinergic
2	2.519	1.26	Boric acid, trimethyl ester Boric acid, trimethyl ester Boronic acid, ethyl-, dimethyl ester	$C_3H_9BO_3$	Uric acid inhibitor, Arachidonic acid inhibitor, acidulant
3	2.925	0.42	Methanamine, N-hydroxy-N-methyl- Methanamine, N-hydroxy-N-methyl- Cyanogen chloride	C_2H_7NO	Neurostimulant, anaphylactic, narcotic, neuroparalytic, nitric oxide inhibitor
4	3.519	0.18	3-Amino-2,2-dimethyl-1-propanol 1,4-Dioxaspiro[2.4]heptan-5-one, 7 ,7-	$C_5H_{13}NO$	Increase aromatic amino acid carboxylase activity

			dimethyl-3,3-Diethyldiaziridine		
5	4.239	0.08	O-Butylisourea 2-Butanone, 4-hydroxy-3-methyl- Methanamine, N-hydroxy-N-methyl-	$C_8H_{18}ON_2O$	Anticancer, antitumor, NADH oxidase inhibitor, osteolytic, stimulate osteoblast activity
6	7.097	0.03	Tetradecane, 2,6,10-trimethyl- Decane, 3-methyl- Eicosane	$C_{15}H_{32}$	Methyl donor, methyl-guanidine inhibitor
7	7.961	0.05	Sulfurous acid, butyl tridecyl ester Heptadecane Nonane, 4,5-dimethyl	$C_{17}H_{36}O_3S$	Urinary acidulant, arachidonic acid inhibitor, uric acid inhibitor
8	8.592	0.02	Hexadecane Disulfide, di-tert-dodecyl Heptadecane,9-hexyl-	$C_{16}H_{35}S_2$	Coronary dilator, diuretic, digitalic, diaphoretic
9	9.118	0.23	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{11}H_{18}O_2$	Oligosaccharide provider
			9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	Increase zinc bioavailability,

10	10.419	-0.17	Hexadecane, 1-(ethenyloxy)- Dodecane, 2,5-dimethyl-		arachidonic acid inhibitor, methyl donor, acidifier, aromatic amino acid activator
12	10.963	0.26	3,5-Dimethoxytoluene	$C_9H_{12}O_2$	Antioxidant, antimicrobial
13	11.282	0.14	(+)-4-Carene1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- Cyclohexene, 4-ethenyl-4-methyl-3-1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	$C_{15}H_{24}$	Methyl donor, methyl guanidine inhibitor, catechol-O-methyl transferase inhibitor
14	11.370	0.07	Adipic acid, isobutyl 2,3,5,6-tetrachlorophenyl ester Adipic acid, isobutyl 2,3,4,6-tetrachlorophenyl ester 3-Aminooxy-4-chlorobutyric acid, ethyl ester	$C_{16}H_{19}Cl_4O_4$	Acidifier, Acidulant, arachidonic acid inhibitor, uric acid inhibitor
15	11.451	0.03	2H-Pyran,5,6-dihydro-2-methyl- 1,3-Butadiene,2,3-	$C_6H_{10}O$	Hepatoprotective, HMG-CoA inhibitor, herbicide, histamine

			dimethyl-Ethane,1,2-dichloro-1,1,2-trifluoro-		inhibitor, hypoglycemic, hypolipidemic, hypouricemic
16	11.658	0.45	.alpha.-Cubebene alfa.-Copaene Copaene	$C_{15}H_{24}$	Alpha-amylase inhibitor, alpha glucosidase inhibitor, alpha reductase inhibitor
17	11.789	1.62	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1.alpha.,2.beta.,4.beta.)]Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-1,5,9-Cyclododecatriene, 1,5,9-tri methyl	$C_{15}H_{24}$	Triglycerigenic, methyl donor, methyl guanidine inhibitor, trichuricide
18	12.120	9.44	(E)-.beta.-Famesene cis-.beta.-Farnesene-1,6,10-Dodecatriene,7,11-dimethyl -3-methylene-	$C_{15}H_{24}$	Beta-galactosidase inhibitor, beta-glucronidase inhibitor, beta-2-receptor agonist, beta blocker.
19	12.358	0.56	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-	$C_{15}H_{24}$	Increases zinc bioavailability

			Humulene		
20	12.590	8.74	.beta.-Bisabolene .beta.-Bisabolene .alpha.-Farnesene	$C_{15}H_{24}$	Beta-blocker, beta-adrenergic receptor blocker, beta-galactosidase inhibitor, 5-alpha reductase inhibitor
21	12.746	2.59	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]- Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- cis-.beta.-Farnesene	$C_{15}H_{24}$	Antimicrobial, antifungal
22	12.971	4.62	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-1,2-Dimethoxy-4-(2-methoxy-1-propenyl)benzene Benzenamide, N-cyano-3,4,5-trimethoxy-	$C_{12}H_{16}O_3$	Increases norepinephrine activity, tumor necrosis factor inhibitor, anaphylactic, natriuretic, antitumorr, neuromuscular blocker

23	13.540	13.41	Apiol 1,3-Benzodioxole, 4,5-dimethoxy-6- (2-propenyl)-	$C_{12}H_{14}O_4$	Antimicrobial, antifungal, anti-proliferative
24	13.959	6.29	1-Butanamine, N-(2-furanylmethylen e)-3-methyl-1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-cyclopentyl-Acetic acid, [(Z,Z)-3,7,11-trimeth yl-2,6,10-dodecatrien-1-yl] ester	$C_8H_{19}N$	GABAnergic, antitumor, increases norepinephrine production, neurostimulant, nitric oxide inhibitor, expectorant
25	14.310	6.77	Phthalic acid, butyl 3-phenylpropyl ester Diethylmalonic acid, di(3-phenylpropyl) ester 2-Oxazolidinone, 4-(hydroxymethyl)-5-phenyl-	$C_{17}H_{24}0_4$	Diaphoretic, Diuretic, Increases superoxide dismutase activity
26	14.447	1.88	1,2-Benzenedicarboxylic acid, bis(4-methylpentyl) ester Bis(tridecyl)phthalat	$C_{16}H_{22}0_4$	Increases aromatic acid decarboxylase activity, uric acid inhibitor, acidifier

			e Phthalic acid, 3,3-dimethylbut-2-yl tridecyl ester		
27	14.554	2.72	1,2-Benzenediacetonitril-N'-[(2-Hydroxy-1-naphthyl)methylene]-2-(4-octyloxyphenoxy)acethydrazide 1,3-Benzenediamine, 4-methyl-5-nitro-	$C_{20}H_{30}O_4$	Acidifier, arachidonic acid inhibitor, urinary-acidulant, uric acid inhibitor
28	14.685	4.19	Ethyl-4-[tetrahydropyran-2-yloxy]-2-butynoate 2-Butenediamide, 2-methyl-, (Z)- 22 Isoxazole, 3,5-dimethyl-4-bromo-	$C_{11}H_{16}0_4$	Methyl donor, methyl-guanidine donor, catechol-o-methyl transferase inhibitor
29	14.904	0.94	Propanenitrile, 3-chloro-2,3-dihydroximino- 4-Methyl-2,3-hexadien-1-ol 3-Octyn-1-ol	C_3H_4CIN	Oligossacharide provider, methyl donor, methyl-guanidine donor
30	14.967	1.25	Furan-2-carboxylic acid,4-diethyl	$C_5H_4O_3$	Anagelsic, anti-inflammatory,

			aminomethyl-5-ethyl-Melochinin 1,3,6-Triazahomoadamantane		antiplatelet, uric acid inhibitor
31	15.104	6.34	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Antibacterial, Urinary-acidulant, urine-acidifier, methyl donor, hypercholesterolemia, hemolytic, nematicide, pesticide
32	15.661	12.09	Ethamivan Butyldimethylsilyloxybenzene	$C_{12}H_{20}OSi$	Pesticide, tocopherols
33	16.487	7.97	9,12-Octadecadienoic acid, methyl ester 7-Octadecenoic acid, methyl ester 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	Increases zinc bioavailability, uric acid inhibitor, arachidonic acid inhibitor, increases aromatic acid decarboxylase activity
34	17.294	1.57	Solanidan-3-one 2-Benzothiazolamine Isosolanidine	$C_{27}H_{43}NO$ $C_7H_6N_2S$	Antibacterial, antifungal activity
			Lumazine 5,7-Dihydroxy-4-	$C_{10}H_8O_4$	Antioxidant and antimicrobial activity

35	17.525	2.32	methylcoumarin 1-(1-(2-Thienyl)cyclohexyl) pyrroli dine		
36	18.151	0.41	5-Decyne 5-Tridecene 5-Dodecyne	$C_{10}H_{18}$ $C_{13}H_{26}$ $C_{12}H_{22}$	Antibacterial and allelopathic activity(Mohammed; Asif, 2013)
37	18.301	0.52	Pyridine, 4-methyl-, 1-oxide Tungsten, [(2,3-.eta.)-bicyclo[2.2.1]hepta-2,5-diene]pentacarbonyl - Pyrazole-3-carboxylic acid, 5-nitro-	C_6H_7NO $C_{11}H_8O_4$ $C_4H_3N_3O_4$	Nitric acid synthase inhibitor, methyl donor methyl-guanidine donor, arachidonic acid inhibitor, uric acid inhibitor
38	18.645	0.14	Thiophene, 2-ethenyl- Mitozolomide 1-Cyclopentenylpheny lmethane	C_4H_4S $C_5H_8N_4O_3S_2$ $C_{12}H_{14}$	Antimicrobial activity (Maddila; Jonnalagada, 2012)

Main activity sources: Duke (2013, 2016).

Ethyl acetate GC-MS analysis of *C. rubens* fraction reveals the existence of compounds together with their relative abundance (area), molecular formulas, and weights. R/T stands for retention time and M/F stands for molecular formula.

The NIST library was used to get the systematic names and molecular formulas of the detected components, as indicated in table 4.2. On the basis of Duke's Phytochemical and Ethnobotanical Databases, the biological actions of substances were substantially predicted (Duke, 2013, 2016).

4.10 DISCUSSION

This experiment examined the effects of hydromethanol fraction and extract of *C. rubens* on blood coagulation and cardiac parameters in isoproterenol-induced myocardial infarction in rat models.

The *C. rubens* plant is rich in proteins, essential amino acids, minerals, flavonoids as well as isothiocyanates and contains natural antioxidants (Arawande et al. 2013), which is a source of bioactive compounds such as flavonoids and phenolic compounds. The fraction and extract from *C. rubens* exhibit antioxidant, anti-inflammatory, anti-hyperlipidemic and hepatoprotective functions (Ayodele et al. 2019). Current and ongoing researches have revealed *C. rubens* is a significant plant with multifunctional applications in human nutrition, medicines and products (Vafaie, 2016).

Isoproterenol-induced MI has proved to be a standardized model for the study of Several medications have favorable impacts on heart function (Syrifah et al. 2018). At supramaximal doses, isoproterenol, an artificial catecholamine, causes extreme stress in the heart, leading to cardiomyopathy (Syrifah et al. 2018). Catecholamines are released neurotransmitters following stimulation of sympathetic system and they play important role in the regulation of myocardial contractility and metabolism. Excessive exposure of the cardium to norepinephrine results in cellular damage, seen in clinical conditions such as angina pictoris and myocardial infarction. When injected with isoproterenol, animals develop myocardial necrosis which leads to alteration of cardiac function through an increase in the level of myocardial lipids, activities of cardiac enzymes and anticoagulants (Afroz et al. 2016).

Due to increase platelet aggregation and anti-hemorrhagic condition of the cells which constitute a low prolongation effect in the normal control group. The findings from this study showed that the fractions of *C. rubens* decreased significantly the clotting and bleeding time in myocardial rats as compared with asprin treated group with the exception of methanol extract 100, 150 mg/kg, ethyl acetate 100 mg/kg and for bleeding time methanol extract 100 mg/kg, ethyl acetate

200 mg/kg for clotting time, with ethyl acetate fraction having the best activity. These findings corroborate with earlier reports in which *C.rubens* was demonstrated to have similar effects (Ayodele et al. 2019; Bamidele et al. 2015). However, From the results obtained, there was significant decrease in clotting time as compared to the aspirin treated group with the exception of methanol extract 100 mg/kg and ethyl acetate 200 mg/kg, reflecting that there was an increase in one or more coagulation factors that are engaged in the intrinsic route which thrombin will play a crucial role in abnormal coagulation formation. It aids in the transformation of aqueous fibrinogen into solid fibrin during the coagulation cascade and also activate coagulation factors V, XIII, and XI that triggers mobilization of activated platelets into the blood circulation (Mauro et al. 2017). These processes further stimulate the arachidonic pathways to release phospholipids which activate more platelets. Whereby low clotting time decreases platelet aggregation which leads to bleeding disorder. increased bleeding time leads to blood vessel defect thst is clumping problems with platelets which decreases platelet aggregation in which blood fail to clot (Lind, 2014).

According to Ayodele et al. (2019) and Osunsanmi et al. (2018), in Prothrombin time and activated partial thromboplastin time methanol extract 100 and 150 mg/kg, and 200 mg/kg ethyl acetate showed high prolongation effect as compared to aspirin treated group which is similar to the findings of this study where there is significant increase in methanol extract 100mg/kg which has the highest prolongation effect. The present study however, shows that methanol extract 100mg/kg is the less active procoagulant. Since prothrombin time is an extrinsic clotting system screening test, that is, factor VII and can also detect deficiencies of factor V, X, prothrombin and fibrinogen, it indicates that the decrease in prothrombin time by the extract as compared to the aspirin treated group with the exception of methanol extract 100 and 150 mg/kg and ethyl acetate 200 mg/kg in which other groups apart from the groups in exception may be as a result of decrease in the concentration of prothrombin or one of the other extrinsic clotting factors. Which as a result, indicates that it takes longer time for the blood to clot. This may be happening because the production of blood clotting proteins in decreased in the liver. Reduced aPTT indicates that clotting will take less time than predicted (but is linked to a lower risk of blood clots owing to a lupus anticoagulant) and can be caused by a variety of circumstances.

Based on total protein concentration according to (Romana et al. 2017 and Muralidharan et al. 2018) there is increase in concentration of total protein in isoproterenol only induced group when compared with normal control which is similar to the findings of this study where there is significant increase in 100mg/kg, 150 mg/kg and 200 mg/kg of methanol extract, fraction, aspirin treated and isoproterenol only induced groups when compared to the normal control groups. However, physiological and genetic variances could be at work in this case, putting it into consideration of the number of days the rats were pretreated with the extract for 14 days, which may be the reflection of consequences of cellular injury due to lipid peroxides. Elevated total protein concentration indicates: dehydration, inflammation or infections, such as viral hepatitis B or C, HIV, a certain type of cancer, such as multiple myeloma, that causes protein to accumulate abnormally (Dimeski; Barnett, 2015).

Inadequate oxygen or nutrient availability to cardiac tissues, or pharmacologically induced cardiac malfunction, increases permeability or even ruptures the cardiac tissue, allowing cytosolic enzymes such as AST ,CK-MB, LDH, and ALP (clinical identifiers of MI) to circulate into the bloodstream and rise in serum concentrations (Romana et al. 2017). According to Barman et al. 2013, this increase in LD in the heart and decrease in ALP in the plasma when compared to the standard drug and also according to Lobo et al. (2015), there is increase in LD and decrease in ALP concentration in the plasma when compared with the standard drug which is similar to the findings of this study where there is significant decrease in 100mg/kg, 150 mg/kg and 200 mg/kg of methanol extract, fraction when compared to the aspirin treated group with the exception of methanol extract 200 mg/kg of LD due to its high dose and protection is concentration dependent. However, at that dose they might be a high turnout of pyruvate being transformed to lactate in the cardiac muscle which could be inferred that methanol extract was able to preserve the structural and functional integrities of the contractile apparatus, preventing cardiac damage and increase of LD level suggests an increased leakage of lactate dehydrogenase from mitochondria due to toxicity induced by treatment with ISO while low level of LD concentration could be because of genetic variances like mutation of the gene that codes for lactate dehydrogenase and inhibits the body's ability to degrade sugar for use as cellular energy (Klein et al. 2020).

Based on ALP concentration according to Isidro et al. 2012, raised levels of ALP concentration when compared to the normal control and decreased level when compared with standard drug are associated with increased osteoblastic activity and bone formation rates**.** Which is similar to the findings in this study which shows significant increase in all the groups when compared to the normal control group with the exception of ethyl acetate 100 mg/kg that is almost the same. However, there is little or no bone formation rates or osteoblastic activity occurring in ethyl acetate 100 mg/kg group. This could be because of increased breakdown of blood proteins which may be an indicative of diseases ranging from gallstones and thyroid disease to hepatitis and cancer and also increased levels of ALP in myocardial toxicity may be due to the leakage of the alkaline phosphatase into the blood (Wolf, 2018). *C. rubens* pre-treatment caused in the dropped concentration of the marker enzyme (ALP) in plasma when compared with the aspirin treated group. Which validated that *C. rubens* could sustain cell membrane integrity, thereby limiting the leakage of these enzymes.

Herbal medicine involves the use of plants and plant products in treatment of various ailments due to free radical generation (Prince et al. 2005). *C. rubens* contain several medicinal activities, which include antibiotic, anti-diabetic, anti-helminthic, anti-inflammatory and blood regulation properties (Khorrami et al. 2012). The use of *C. rubens* locally in treatment of indigestion (Ayodele et al.2019), and basal diets fortified with the leaf in the chemoprevention of myocardial infarction in rats (Olusola et al. 2020), have been reported. These properties could be linked to secondary compounds inherent in the plant. Polyphenols present in plants and plant products are secondary metabolites, and detailed to demonstrate pharmacological effects which could be related to their antioxidant potential (Khorrami et al. 2012). Presence of this identified phytochemical compound, Furan-2-carboxylic acid 4-diethylaminomethyl-5-ethyl-Melochinin-1, 3,6-Triazahomoadamantane in this study possess antiplatelet activity which may be responsible for the procoagulant activity of the plant. Antiplatelet therapy has become a crucial pharmacological technique in the determent and management of cerebrovascular, cardiovascular and peripheral arterial diseases after a number of controlled clinical trials demonstrated that inhibiting and decreasing platelet aggregation reduces the Ischemic atherothrombotic events: mortality and morbidity (Papp et al. 2013). The findings of this study thus suggest the procoagulant activity of *C. rubens*.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The findings of this study suggest that *C. rubens* methanol leaf fractions and extract is a good plant that has procoagulant effect, increases the concentration of cardiac marker enzymes. *C. rubens* could thus be a good therapeutic agent for managing myocardial infarction rather than treating it.

5.2 RECOMMENDATION

It is recommended that future research should be conducted on *C. rubens* extract to elucidate its possible mechanisms of action on cascade of blood clotting and cardiac marker enzymes, as well find out the active compounds responsible for the observed effects.

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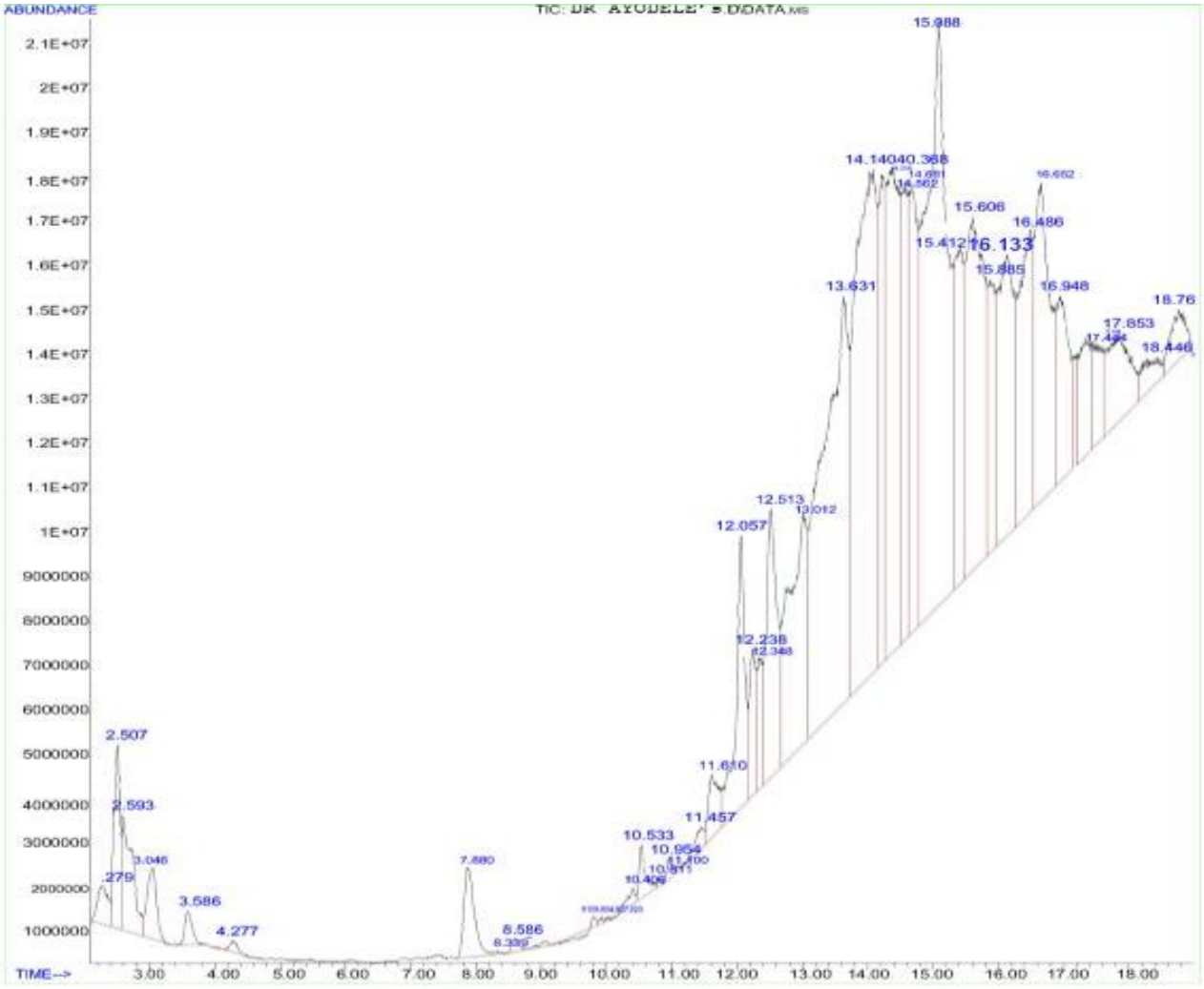
Appendices

Appendix I: GCMS chromatogram of ethyl acetate fraction of *C. rubens* Leaf extract



Appendix II: The Data obtained from Animal study.

Selected dose	**Animal body weight (g)**	**Average weight (g)**	**Stock solution (conc.)**	**Daily dose in Ml**
Group 1 (normal control)	162 U	154	1 mL of PBS daily; 70 mL for 14 days	1.0
	151 H			1.0
	187 T			1.0
	159 B			1.0
	112 R			1.0
Group 2 (positive control) /75 mg/kg (Aspirin	169 U	174	913.5 mg in 70 mL PBS (13.05 mg/mL) for 14 days	1.0
	204 H			1.2
	133 T			0.8
	162 B			0.9
	202 R			1.2
Group 3 (negative control)	234 U	217	1 mL of PBS daily; 70 mL for 14 days	1.0
	246 H			1.0
	233 T			1.0
	195 B			1.0
	177 R			1.0
Group 4 (crude extract)/ 100 mg /kg	179 U	172	1204 mg in 70 mL PBS (17.2 mg/mL) for 14 days	1.0
	185 H			1.1
	175 T			1.0
	128 B			0.7
	193 R			1.1
Group 5 (crude extract)/ 150 mg	183 U		1743 mg in 70 mL PBS (24.9 mg/mL)	1.1
	177 H			1.1

/kg	155 T	166	for 14 days	0.9
	166 B			1.0
	148 R			0.9
Group 6 (crude extract)/ 200 mg /kg	179 U	176	2464 mg in 70 mL PBS (35.2 mg/mL) for 14 days	1.0
	216 H			1.2
	191 T			1.1
	153 B			0.9
	139 R			0.8
Group 7 (Hexane extract)/ 100 mg /kg	182 U	169	1183 mg in 70 mL PBS (16.9mg/mL) for 14 days	1.1
	213 H			1.3
	133 T			0.8
	179 B			1.1
	138 R			0.8
Group 8 (Hexane extract)/ 150 mg /kg	183 U	180	1890 mg in 70 mL PBS (27 mg/mL) for 14 days	1.0
	129 H			0.7
	202 T			1.1
	162 B			0.9
	225 R			1.3
Group 9 (Hexane extract)/ 200 mg /kg	180 U	162	2268 mg in 70 mL PBS (32.4 mg/mL) for 14 days	1.1
	134 H			0.8
	153 T			0.9
	159 B			1.0
	182 R			1.1
Group 10 (Ethyl	117 U		798 mg in 70 mL	1.0

acetate extract)/ 100 mg /kg	112 H	114	PBS (11.4 mg/mL) for 14 days	1.0
	116 T			1.0
	109 B			0.9
	121 R			1.1
Group 11 (Ethyl acetate extract)/ 150 mg /kg	107 U	102	1071 mg in 70 mL PBS (15.3 mg/mL) for 14 days	1.1
	87 H			0.9
	106 T			1.0
	105 B			1.0
	106 R			1.0
Group 12 (Ethyl acetate extract)/ 200 mg /kg	80 U	67	938 mg in 70 mL PBS (13.4 mg/mL) for 14 days	1.2
	69 H			1.0
	63 T			0.9
	59 B			0.8
	62 R			0.9

Appendix III: The values for the weight of the petri dishes before and after drying of the crude extract using the hot air oven

Weight of petri dishes before drying (g)	Weight of petri dishes after drying (g)	Difference in weight (g)
A (7.41)	8.33	1.42
B (7.40)	8.74	1.34
C (7.40)	9.58	2.18
D (7.42)	10.98	3.56
E (6.60)	8.62	2.02
F (7.40)	10.66	3.26
G (6.60)	11.00	4.40
H (7.40)	11.69	5.07
I (6.67)	8.03	1.36
J (6.60)	7.97	1.37
K (5.99)	6.77	0.78
L (6.01)	6.96	0.95
M (6.44)	7.11	0.67
N (6.41)	6.74	0.33
O (5.99)	6.72	0.73
P (6.02)	6.90	0.88
Q (6.00)	6.77	0.77

R (6.58)	7.37	0.79
A1 (31.50)	36.94	5.44
B1 (34.80)	39.21	4.41
C1 (31.56)	35.87	4.31
D1 (35.38)	38.14	2.76
E1 (35.32)	39.79	4.47
F1 (39.19)	42.28	3.09
G1 (37.88)	41.63	3.75
Total		71.91

Appendix IV: The values for the weight of the petri dishes before and after drying of the ethyl acetate fraction using the hot air oven

Weight of petri dishes before drying (g)	Weight of petri dishes after drying (g)	Difference in weight (g)
A (28.38)	28.70	0.32
B (30.26)	30.89	0.63
C (30.90)	31.41	0.51
D (30.94)	31.47	0.53
E (29.29)	30.25	1.06
F (31.73)	31.97	0.24
G (32.44)	33.45	1.01

Total		4.3

Appendix V: The values for the weight of the beakers before and after drying of the hexane fraction using hot air oven

Weight of beaker before drying (g)	Weight after beaker after drying (g)	Difference in weight (g)
A (99.11)	100.83	1.72
B (104.15)	105.70	1.55
C (98.33)	104.54	6.21
Total		9.48

Appendix VI: Results of Bleeding time in seconds

	Group1 (normal control)	Group2 (positive control)/ (75mg/kg aspirin)	Group3 (Negative control)	Group4 (crude extract)/100 mg/kg
Unmarked	157	136	73	124
Head	165	107	111	115
Tail	185	97	89	114
Back	157	108	83	122
Right leg	122	90	90	145

	Group5 (crude extract)/150 mg/kg	Group6 (crude extract)/200mg/kg	Group7 (Hexane extract)/100mg/kg	Group8(Hexane extract)/150mg/kg
Unmarked	120	94	39	71

Head	103	82	67	56
Tail	100	82	60	76
Back	103	85	61	80
Right leg	107	86	76	70

	Group9 (Hexane extract)/200mg/kg	Group10 (Ethyl acetate extract)/100mg/kg	Group11 (Ethyl acetate extract)/150mg/kg	Group12 (Ethyl acetate extract)/ 100mg/kg
Unmarked	73	92	98	65
Head	96	110	97	71
Tail	85	133	74	46
Back	88	111	109	55
Right leg	83	112	89	48

Appendix VII: Results of clotting time in seconds

	Group1 (normal control)	Group2 (positive control)/ (75mg/kg aspirin)	Group3 (Negative control)	Group4 (crude extract)/100 mg/kg
Unmarked	35	74	63	77
Head	48	72	55	76
Tail	44	77	60	71
Back	34	60	64	70
Right leg	45	75	53	71

	Group5 (crude extract)/150 mg/kg	Group6 (crude extract)/200mg/kg	Group7 (Hexane extract)/100mg/kg	Group8(Hexane extract)/150mg/kg
Unmarked	55	47	35	45
Head	60	46	36	30
Tail	54	42	45	41
Back	51	54	32	33
Right leg	49	41	35	41

	Group9 (Hexane extract)/200mg/kg	Group10 (Ethyl acetate extract)/100mg/kg	Group11 (Ethyl acetate extract)/150mg/kg	Group12 (Ethyl acetate extract)/ 200mg/kg
Unmarked	46	54	69	85
Head	52	48	58	76
Tail	58	58	65	84
Back	63	56	61	76
Right leg	54	66	71	89

Appendix VIII: Results of prothrombin time (PT) in seconds

	Group1 (normal control)	Group2 (positive control)/ (75mg/kg aspirin)	Group3 (Negative control)	Group4 (crude extract)/100 mg/kg
Unmarked	43	72	45	98
Head	43	69	47	119
Tail	48	71	48	104
Back	47	62	46	95
Right leg	35	72	49	96

	Group5 (crude extract)/150 mg/kg	Group6 (crude extract)/200mg/kg	Group7 (Hexane extract)/100mg/kg	Group8(Hexane extract)/150mg/kg
Unmarked	80	72	45	47
Head	76	62	49	45
Tail	95	62	50	56
Back	80	55	35	49
Right leg	99	58	49	45

	Group9 (Hexane extract)/200mg/kg	Group10 (Ethyl acetate extract)/100mg/kg	Group11 (Ethyl acetate extract)/150mg/kg	Group12 (Ethyl acetate extract)/ 200mg/kg
Unmarked	62	57	70	80
Head	57	52	69	78
Tail	57	53	60	77
Back	57	63	64	84
Right leg	52	56	65	87

Appendix IX: Results of Activated partial thromboplastin time (APTT) in seconds

	Group1 (normal control)	Group2 (positive control)/ (75mg/kg aspirin)	Group3 (Negative control)	Group4 (crude extract)/100 mg/kg
Unmarked	87	99	91	149
Head	83	103	95	164
Tail	93	90	97	180
Back	77	100	83	178
Right leg	78	107	80	174

	Group5 (crude extract)/150 mg/kg	Group6 (crude extract)/200mg/kg	Group7 (Hexane extract)/100mg/kg	Group8(Hexane extract)/150mg/kg
Unmarked	114	90	38	38
Head	105	93	28	45
Tail	110	84	35	47
Back	106	87	34	42
Right leg	93	82	45	44

	Group9 (Hexane extract)/200mg/kg	Group10 (Ethyl acetate extract)/100mg/kg	Group11 (Ethyl acetate extract)/150mg/kg	Group12 (Ethyl acetate extract)/ 200mg/kg
Unmarked	54	48	48	56
Head	68	49	55	58
Tail	72	49	58	72
Back	67	48	58	61
Right leg	65	55	52	67

Appendix X: Results of assay for Total protein in Heart

	Abs 1	Abs2	Mean abs
Standard	0.456	0.460	0.458

	Group1 (normal control)				Group2 (positive control)/75mg/kg aspirin				Group 3 (negative control)			
	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)
Unmarked	0.410	0.402	0.406	5.141	0.640	0.632	0.636	8.262	0.436	0.453	0.444	6.738
Head	0.340	0.363	0.351	4.560	0.761	0.767	0.764	9.925	0.330	0.333	0.333	5.038
Tail	0.340	0.364	0.352	4.573	0.781	0.750	0.765	9.938	0.330	0.332	0.333	5.038
Back	0.380	0.378	0.379	4.924	0.641	0.633	0.637	8.275	0.477	0.477	0.477	6.205
Right leg	0.230	0.310	0.270	3.508	0.700	0.700	0.700	9.100	0.437	0.454	0.445	5.781

	Group4 (crude extract)/100mg/kg				Group5 (crude extract)/150mg/kg				Group 6(crude extract)/200mg/kg			
	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)
Unmarked	0.599	0.599	0.599	7.786	0.622	0.622	0.622	8.081	0.504	0.511	0.508	6.600
Head	0.547	0.547	0.547	7.106	0.510	0.520	0.515	6.691	0.431	0.440	0.435	5.651
Tail	0.746	0.746	0.746	9.699	0.581	0.588	0.585	7.600	0.450	0.460	0.455	5.911
Back	0.597	0.597	0.597	7.759	0.610	0.622	0.616	8.003	0.632	0.632	0.632	8.210
Right leg	0.557	0.558	0.558	7.249	0.580	0.588	0.584	7.587	0.504	0.511	0.508	6.600

	Group7 (Hexane extract)/100mg/kg				Group8 (Hexane extract)/150mg/kg				Group 9 (Hexane extract)/200mg/kg			
	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)
Unmarked	0.530	0.521	0.525	6.820	0.494	0.510	0.502	6.522	0.520	0.520	0.520	6.762
Head	0.614	0.614	0.614	7.977	0.563	0.563	0.563	7.321	0.474	0.467	0.471	6.119
Tail	0.613	0.614	0.614	7.977	0.494	0.510	0.502	6.522	0.441	0.430	0.435	5.651
Back	0.560	0.563	0.561	7.288	0.562	0.562	0.562	7.302	0.474	0.467	0.471	6.119
Right leg	0.614	0.615	0.615	7.990	0.551	0.530	0.540	7.015	0.474	0.474	0.474	6.163

	Group10 (Ethyl acetate extract)/100mg/kg				Group11 (Ethyl acetate)/150mg/kg				Group 12 (Ethyl acetate extract)/200mg/kg			
	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)	Abs1	Abs2	Mean abs	T.protein Conc (g/dl)
Unmarked	0.450	0.450	0.450	5.846	0.520	0.530	0.525	6.820	0.650	0.640	0.645	8.379
Head	0.407	0.403	0.405	5.261	0.466	0.467	0.467	6.067	0.515	0.514	0.515	6.688
Tail	0.380	0.370	0.375	4.872	0.450	0.450	0.450	5.846	0.516	0.513	0.514	6.678
Back	0.406	0.403	0.405	5.261	0.467	0.466	0.467	6.067	0.540	0.530	0.535	6.950
Right leg	0.390	0.390	0.390	5.067	0.430	0.430	0.430	5.586	0.517	0.513	0.515	6.691

Appendix XI: Results of Assay for Lactate dehydrogenase activity in the heart

	Group1 (Normal control)					Group2 (positive control)/75mg/kg aspirin					Group 3 (negative control)				
	Abs 0.5 min	Abs 1 min	Abs 2min	Abs 3 min	LD activity (U/I)	Abs 0.5 Min	Abs 1 min	Abs 2 min	Abs 3 min	LD activity (U/I)	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 min	LD activity (U/I)
Unmarked	0.785	0.807	0.817	0.865	110.1	0.726	0.732	0.736	0.748	30.2	0.436	0.496	0.504	0.508	99.1
Head	0.611	0.620	0.633	0.693	112.8	0.726	0.732	0.737	0.747	30.3	0.330	0.360	0.371	0..392	85.2
Tail	0.610	0.621	0.633	0.693	112.8	0.657	0.664	0.670	0.687	41.3	0.331	0.360	0.371	0.393	85.3
Back	0.579	0.599	0.613	0.650	97.4	0.720	0.735	0.740	0.752	44.0	0.584	0.632	0.646	0.653	94.9
Right leg	0.420	0.455	0.477	0.495	102.6	0.785	0.795	0.812	0.818	45.4	0.436	0.496	0.503	0.508	99.1

	Group4 (crude extract)/100 mg/kg					Group5 (crude extract)/150 mg/kg					Group 6 (crude extract)/200 mg/kg				
	Abs 0.5 min	Abs 1 min	Abs 2min	Abs 3 min	LD activity (U/I)	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 Min	LD activity (U/I)	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 min	LD activity (U/I)
Unmarked	0.587	0.596	0.602	0.618	42.6	0.483	0.490	0.505	0.526	58.6	0.603	0.623	0.625	0.696	127.9
Head	0.587	0.595	0.601	0.617	41.3	0.673	0.681	0.685	0.730	78.4	0.603	0.624	0.626	0.695	129.3
Tail	0.375	0.386	0.390	0.406	42.7	0.571	0.580	0.593	0.614	59.2	0.497	0.508	0.531	0.595	132.1
Back	0.751	0.761	0.766	0.787	49.5	0.558	0.570	0.588	0.596	52.3	0.627	0.693	0.698	0.714	118.2
Right leg	0.625	0.640	0.648	0.660	48.1	0.572	0.580	0.592	0.614	57.8	0.618	0.658	0.674	0.694	105.6

	Group7 (Hexane extract)/100 mg/kg					Group8 (Hexane extract)/150 mg/kg					Group 9 (Hexane extract)/200 mg/kg				
	Abs 0.5 min	Abs 1 min	Abs 2min	Abs 3 min	LD activity (U/I)	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 Min	LD activity (U/I)	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 min	LD activity (U/I)
Unmarked	0.839	0.840	0.845	0.855	22.0	0.605	0.621	0.628	0.648	59.1	0.437	0.450	0.462	0.474	50.9
Head	0.507	0.507	0.507	0.845	32.2	0.617	0.622	0.633	0.658	56.4	0.437	0.450	0.463	0.475	52.3
Tail	0.777	0.784	0.794	0.800	31.6	0.604	0.611	0.629	0.639	48.1	0.621	0.637	0.651	0.673	59.2
Back	0.685	0.695	0.697	0.700	20.6	0.940	0.945	0.966	0.987	64.7	0.251	0.272	0.283	0.298	64.7
Right leg	0.776	0.784	0.794	0.800	33.0	0.257	0.266	0.267	0.289	44.0	0.449	0.471	0.482	0.493	60.5

	Group10 (Ethyl acetate extract)/100 mg/kg					Group11 (Ethyl acetate extract)/150 mg/kg					Group 12 (Ethyl acetate extract)/200 mg/kg				
	Abs 0.5 min	Abs 1 min	Abs 2min	Abs 3 min	LD activity	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 min	LD activity(U/I)	Abs 0.5 min	Abs 1 min	Abs 2 min	Abs 3 min	LD activity (U/I)
Unmarked	0.700	0.712	0.728	0.734	46.8	0.626	0.628	0.635	0.650	33.0	0.740	0.744	0.751	0.760	27.5
Head	0.700	0.712	0.727	0.734	60.5	0.540	0.555	0.572	0.578	46.5	0.377	0.379	0.383	0.397	27.5
Tail	0.806	0.818	0.824	0.846	55.0	0.388	0.400	0.405	0.416	38.5	0.566	0.569	0.572	0.580	19.3
Back	0.725	0.738	0.740	0.759	46.8	0.543	0.555	0.571	0.577	46.7	0.571	0.576	0.583	0.589	24.8
Right leg	0.569	0.580	0.588	0.599	42.6	0.617	0.626	0.632	0.644	37.1	0.562	0.569	0.579	0.581	26.1

Appendix XII: Results of Assay of alkaline phosphatase activity in the plasma

	Group1 (Normal control)					Group2 (positive control)/75 mg/kg aspirin					Group 3 (negative control)				
	Abs initial	Abs 1 min	Abs 2 Min	Abs 3 min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 Min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 min	ALP activity (U/I)
Unmarked	0.349	0.362	0.367	0.369	18.3	0.350	0.369	0.416	0.431	74.0	0.201	0.217	0.242	0.261	54.8
Head	0.341	0.352	0.363	0.365	21.9	0.291	0.302	0.325	0.378	79.5	0.256	0.289	0.301	0.314	50.6
Tail	0.236	0.246	0.251	0.262	24.0	0.276	0.291	0.296	0.340	67.6	0.236	0.252	0.280	0.297	55.8
Back	0.342	0.352	0.363	0.366	21.9	0.304	0.327	0.359	0.383	72.2	0.331	0.371	0.375	0.394	57.6
Right leg	0.440	0.453	0.465	0.477	33.8	0.306	0.337	0.369	0.390	67.6	0.256	0.287	0.299	0.311	50.3

	Group4 (crude extract)/100 mg/kg					Group5 (crude extract)/150 mg/kg					Group 6 (crude extract)/200 mg/kg				
	Abs initial	Abs 1 min	Abs 2 Min	Abs 3 min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 min	ALP activity (U/I)
Unmarked	0.286	0.302	0.339	0.353	61.6	0.312	0.315	0.337	0.372	64.0	0.329	0.360	0.379	0.398	63.1
Head	0.206	0.239	0.250	0.270	58.4	0.460	0.484	0.488	0.522	56.7	0.330	0.360	0.380	0.399	63.1
Tail	0.263	0.281	0.317	0.328	59.4	0.460	0.485	0.488	0.523	57.6	0.205	0.216	0.244	0.283	71.3
Back	0.263	0.281	0.318	0.329	60.3	0.617	0.649	0.651	0.675	62.2	0.610	0.621	0.632	0.683	74.9
Right leg	0.298	0.302	0.344	0.355	52.1	0.462	0.490	0.497	0.531	61.2	0.213	0.244	0.273	0.290	70.4

	Group7 (Hexane extract)/100 mg/kg					Group8 (Hexane extract)/150 mg/kg					Group 9 (Hexane extract)/200 mg/kg				
	Abs initial	Abs 1 min	Abs 2 Min	Abs 3 min	ALP Activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 Min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 min	ALP activity (U/I)
Unmarked	0.371	0.373	0.385	0.397	24.5	0.588	0.611	0.624	0.636	44.4	0.354	0.358	0.374	0.376	47.5
Head	0.427	0.432	0.445	0.460	30.2	0.440	0.466	0.478	0.487	43.0	0.422	0.438	0.448	0.453	46.6
Tail	0.469	0.474	0.484	0.485	23.8	0.299	0.327	0.349	0.365	51.2	0.396	0.442	0.453	0.462	60.3
Back	0.427	0.433	0.446	0.460	30.2	0.440	0.466	0.479	0.487	43.0	0.421	0.439	0.449	0.453	47.5
Right leg	0.442	0.451	0.468	0.498	32.9	0.453	0.461	0.463	0.467	40.2	0.515	0.516	0.519	0.521	51.2

	Group10 (Ethyl acetate extract)/100 mg/kg					Group11 (Ethyl acetate extract)/150 mg/kg					Group 12 (Ethyl acetate extract)/200 mg/kg				
	Abs Initial	Abs 1 min	Abs 2 Min	Abs 3 min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 Min	ALP activity (U/I)	Abs initial	Abs 1 min	Abs 2 min	Abs 3 Min	ALP activity (U/I)
Unmarked	0.239	0.240	0.243	0.249	24.7	0.362	0.363	0.365	0.368	42.0	0.310	0.316	0.329	0.333	52.1
Head	0.390	0.393	0.405	0.415	23.3	0.407	0.439	0.442	0.449	51.4	0.249	0.254	0.263	0.266	61.2
Tail	0.359	0.363	0.370	0.373	27.4	0.219	0.221	0.291	0.293	47.5	0.250	0.254	0.264	0.266	51.2
Back	0.329	0.332	0.339	0.351	20.2	0.329	0.341	0.366	0.368	53.9	0.224	0.229	0.244	0.245	55.8
Right leg	0.329	0.332	0.339	0.352	21.0	0.408	0.438	0.441	0.444	51.2	0.215	0.217	0.218	0.221	56.7