CARDIOPROTECTIVE AND ANTI-ATHEROSCLEROTIC EFFECTS OF *Solanum incanum* (*Linnaeus*.) AND *Rhamnus prinoides* EXTRACTS IN ANIMAL MODELS

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DECLARATION

Student

This being my original work, it has not been presented for a degree or any other award in any university.

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DEDICATION

Dedication of this work goes to my family, my mother Lucy Wanjiku, my father Peter Kahiga and our very own siblings for their prayers, financial and emotional support, and encouragement during the course of this study.

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May the Divine deluge you with ultimate blessings.

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ACRONYMS AND ABBREVIATIONS

ALT	**:**	Alanine aminotransferase
ANOVA	**:**	Analysis of variance
CIMT	**:**	Carotid Intima Media Thickness
CVD	**:**	Cardiovascular disease
DCM	**:**	Dichloromethane
FDA	**:**	Food Drug Administration
GGT	**:**	Gamma glutamyl transferase
HDL	**:**	High density lipoprotein
HCHF	**:**	High cholesterol high fats
IMT	**:**	Intima media thickness
LDL	**:**	Low density lipoprotein
LYMP	**:**	Lymphocytes
MCHC	**:**	Mean Cell Hemoglobin Concentration
MON	**:**	Monocytes
NEUT	**:**	Neutrophils
OECD	**:**	Organization for Economic Cooperation and Development
OxLDL	**:**	Oxidized form of low-density lipoproteins.
PLT	**:**	Platelets
RDW	**:**	Red cell distribution width
SD	**:**	Standard Deviation
SEM	**:**	Standard Error of Mean
TC	**:**	Total cholesterol

ABSTRACT

Cardio protection is a mechanism that serve to protect the heart and its vessels from injury, diseases or malfunction. In Kenya, plant material extracts have been applied as a remedy in diseases such as, hepatitis, malaria etc. Nonetheless, use of these traditional remedies gathers a compendium of risks to consumers ascribed to scantiness of information on safety and inclusive of their antihyperlipidemic ability. Application 10% methanol and normal saline was used to reconstitute the materials. Mature Albino Wistar rats three months old were fed with HCHF diet (10% egg York (5.6g/bw),10% lard (5.6g/bw),0.2% cholic acid (0.112g/bw) and 0.59% propylthiouracil (0.28g/bw), for 28 days. Onset of 28th day, the rats were euthanized and bioassays done. Body weights and organ weights were recorded. For cardiotonic studies,10 New Zealand male rabbits were used. They were injected with 1000 units of heparin to avoid clot formation. Chest was opened through cardiac thoracotomy and heart placed in a dish containing Tyrode solution. This was followed by Langerdorff method using a kymograph for ionotropic and chronotropic effects. In toxicity studies, male mice of age 6-7 weeks were given oral doses of plant extract for 28 days of the experiment. On 29th day of the experiment, animals were sacrificed through cardiac puncture and blood sample collected for biochemical assays. Mice's were ruminated with rodent pellets and water *adlipitum*. OECD 407 precepts were followed when conducting toxicity studies. One way ANOVA was used in data analysis. This was followed by Tukey as post hoc and statistical significance at $P<0.05$.Both plant extracts exhibited positive ionotropic and negative chronotropic effects. *R. prinoides* extracts had significant reduction on total percent changes of the heart rate. Significance reduction on low density lipoproteins and total cholesterol was exhibited by both plant extracts, following a high cholesterol high fat diet (HCHF). Total body weights were significantly reduced. Extracts of *S. incanum* showed presence of saponins, alkaloids, glycosides, flavonoids, terpenoids, steroids and phenolics. Extracts of *R. prinoides* displayed presence of phenolics, glycosides, terpenoids, alkaloids steroids and saponins. Further investigation should be done to quantify the number of glycosides present in both plant extracts.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The heart is a muscular hollow organ that is responsible for pumping of the blood (Davis, 2021). The blood is received from the veins, into the arteries and eventually throughout the body. The mammalian heart is also contemplated as “two pump that functions in series” (Katz, 2011).The right atrium and ventricle transports blood from systemic circulation into pulmonary circulation, the left atria and ventricles pumps blood from the pulmonary circulation to the systemic circulation. heart contains valves in each of the four chambers that prevents the backflow of the blood.

The heart is involved in various functions like, getting rid of the body's metabolic waste, pumping mechanisms to the lungs, and eventually pumping hormones and other vital chemicals in the body (Allarakh, 2020).Various diseases affects the heart resulting to abnormal functioning. These includes cardiomyopathy, which is caused by prolonged high blood pressure, nutritional deficiency among other causes. Cardiac arrythmias affects the rhythm of the heart actively and it can be caused by various factors which includes, genetics, medical, and emotional factors (Klein, 2020).

Atherosclerosis is a disease in which fats builds up in the arteries and other blood vessels. (Brazier, 2020) It occurs when the endothelium (a thin layer of the cells lining of blood vessels) becomes desecrate. This damage can be as a result of factors such as elevated levels of fats in the blood. Other factors may include smoking, hypertension and hyperglycemia (Yvette, 2020). Atherosclerosis is an imminent cause of cardiovascular disease (CVD) (Frostegard, 2013).This is a chronic inflammatory constrain in which the

immune competent cells lead to production of pro-inflammatory cytokines, where there is abundance of apoptotic cells and low-density lipoproteins (oxLDL) which are oxidized. In ruptured atherosclerotic plagues, CVD can appear as a direct cause.

The risk factors associated with Atherosclerosis includes; high cholesterol levels, smoking, and obesity, family history of cardiovascular diseases, insulin resistance and high blood pressure. (Gibbons, 2020).Cardiac output (CO) refers to the total amount of blood a heart can pump and the mediums used in the transport of blood to body organs and brain (Lowery, 2020).Determinants of cardiac output includes the body's need for oxygen variation, heart rate and ejection fraction/stroke volume.

Myocardial contractility and heart force symbolizes the intrinsic ability of muscles of the heart to contact (Grossman, 2020).Myocardial infarction (MI) results from the irreversible damage of the muscles of the heart due to lack of oxygen. This eventually leads to cardiac arrhythmias and impairment of systolic and diastolic functionality (Gavin, 2020).

Various important conventional and therapeutic approaches are used in the management of atherosclerosis. They include drugs for the treatment of hypercholesterolemia and lipid-lowering drug statins such as Avastatin (Lipitor), Fluvastatin (lescol) also used in the management of atherosclerosis (Ogbru, 2019). However, high percentage of these drugs compositions are made from medicinal plants and a lot of emphasis on prevention and management of diet has been recommended in fighting atherosclerosis.

Nutritional strategies are important in the management of atherosclerosis (Torres, 2015).Dietary fiber, plant sterols, taurine and olive oil has important hypocholesterolemia

effects and reduces the risk of coronary artery diseases (Soliman, 2015).High soluble fiber foods supplements have been postulated to decrease serum cholesterol by 15% to 19% (Anderson, 2012).This results to a decrease of Coronary Artery Disease by 30%.

In Kenya, several plant material extracts have been used as a traditional remedy in management of, anti-platelet aggregate, weight loss, and antihyperlipidemic aspects (Waweru, 2017). Some of the plants used include *Solanum incanum* and *Rhamnus prinoides*. These plants are soup flavonoids and are thought to be remedies for cardiovascular disorders (Kirichenko, 2020). nevertheless, a little is known on their efficacy and safety. This study aimed at evaluating the effects of these plant extracts on their cardioprotective and anti- atherosclerotic potential.

1.2 Statement of the Problem

Atherosclerosis and hypertension are the most prevalent type of heart diseases (Sabrin, 2017).Approximately about 18.2 million adults age 20 and above have coronary artery disease (CAD) due to atherosclerosis, which is about 6.1% killing 365,914 people in the recent years (CDC, 2017).Coronary Vascular Diseases (CVD) due to atherosclerosis shows a high severe impact and capacity of causing almost half of all deaths worldwide occurring from low to middle-income countries (Keates, 2017).Sub-Saharan Africa is the major contributor of Coronary Vascular Disease being a home of about one billion people. This constitutes to about half of all the global deaths and 11.3% of all deaths to CVD in Africa (Yuyun *et al.*, 2020). Increased Carotid Intima Media Thickness (CIMT) due to atherosclerosis and accumulation of cholesterol has been measured. This stands at 59.0 million resulting to 6.21% of the tested participants (Tatuene, 2020).In Kenya,

cardiovascular disease studies featured atherosclerosis in over 20% of carotid arteries (Onge'ngo 2017).

Currently, the management of atherosclerosis and coronary artery diseases is by surgical procedures which include angioplasty also called Percutaneous Coronary Artery Intervention (PCAI) which helps to open the clogged and blocked arteries (Chen, 2021).These surgical procedures are very expensive and can lead to other severe conditions such as stroke, changes in the circadian rhythm ,and allergic reactions to anesthesia. The surgical procedure requires expertise (cardiovascular surgeons) and the drugs used are also expensive, not readily available and not very efficient and are associated with adverse side effects.

Traditional medicinal remedies have been viewed to be effective in management of atherosclerosis and other coronary artery diseases. *Solanum incanum* and *Rhamnus prinoides* plant extracts has shown to produce antihyperlipidemic and antihypercholesterolemic effects to the body. However traditional medicinal plants are also associated with toxicities in the body and currently there is little research that has been done on their safety and cardio protective effects hence raising a need to determine the body biochemical and hematological changes in the body on their due use, which this study aims to investigate.

1.3 Justification

More attention has been directed towards prevention measures and panacea on "fighting" dyslipidemia that leads to atherosclerosis and CADs. This is by the use of these traditional medicinal plants. These disease conditions are managed by various chemotherapeutic agents and surgical intervention which are usually expensive, relatively unavailable, and

are associated with numerous adverse side effects. This study therefore has an advantage, consummation of traditional medicinal plants in therapeutic approaches are thought to be safer, cheaper, easily available and having fewer and not severe side effects. This study aimed to investigate cardio protective and anti-atherosclerosis effects of methanol extracts of *S. incanum* and *R. prinoides* in animal models.

1.4 Null Hypothesis

Extracts of *Solanum incanum* roots and *Rhamnus prinoides* barks do not have cardio protective and anti-atherosclerotic effects in animal models.

1.5 Significance of the Study

From this study, information will be provided in the society and scientific community on the safety and anti- hyperlipidemia effects of using *Solanum incanum* and *Rhamnus prinoides* and also contribute to world health assembly on cardiovascular diseases call to research on solutions to coronary artery diseases (CAD).

1.6 Objectives

1.6.1 General Objective

To evaluate the cardioprotective and anti-atherosclerotic effects of methanol extracts of *Solanum incanum* roots *and* barks of *Rhamnus prinoides* in animal models.

1.6.2 Specific Objectives

i. To determine ionotropic and chronotropic effects of methanol extracts of *S. incanum* and *R. prinoides* using isolated rabbit heart.

ii. To determine effects of methanol extracts of *S. incanum* and *R. prinoides* on biochemical and hematological parameters on mice.

iii. Qualitative phytochemical composition of methanol extracts of *Solanum incanum* and *Rhamnus prinoides.*

CHAPTER TWO: LITERATURE REVIEW

2.1 Atherosclerosis, Hypertension and Coronary Heart Disease

Atherosclerosis is the building up of fats, cholesterol and other substances, which then restricts blood flow (Beckermann, 2019). The plague can burst triggering the blood clot which is called atherosclerotic plaque. Atherosclerosis develops in a gradual manner leading to mild atherosclerosis which do not have any major clinical manifestations. This causes the Symptoms not to be experienced until the artery is narrowed or clogged, to a point it cannot supply adequate blood to the organs and tissues. In majority cases, deposition of clots in the blood prevents proper blood flow hence triggering a heart attack and stroke. (Boileau, 2019)**.** Approximately about 18.2 million adults age 20 and above have coronary artery disease (CAD) due to atherosclerosis, which is about 6.1% killing365, 914 people in the recent years.

Hypertension or high blood pressure results in high amount of pressure building up inside the arteries (Alexander, 1995).During the heartbeat, blood is pushed through the arteries in the whole body, resulting in arteries throughout the body stretching more than normal. These overstretching results to harm or damage to the endothelium which is the delicate lining of the arteries. This eventually causes the arteries to become stiffer on course of time. (Beckermann, 2019).

Hypertension and coronary artery disease occurs in atherosclerosis. (Kramer, 2020). Hypertension is elucidated as being a major factor for risk of developing atherosclerosis.

The endothelium is the central focus point of effect for both atherosclerosis and coronary artery diseases. Atherosclerosis is a cardinal manifestation to inflammatory disease. The thermogenic stimuli in particular, dyslipidemia, activates the inflammatory response which results in causing the articulation of the mononuclear leucocyte recruiting channels (Alexander, 1995).The vascular cell adhesion molecule-1 which is the preliminary gene, is controlled by transcription factors which are being regulated by oxidative stress. This then results to redox state of endothelial cell to be modified (Ntsekhe, 2017).This then results to vascular smooth cell growth. As chronic and acute hypertension can increase the risk of atherosclerosis, high diastolic pressure may also significantly contribute to atherosclerosis. Low density lipoprotein receptors can show molecular defect similar to hypercholesterolemia. (Escobar, 2002).The left ventricular atrophy promotes the decrease of coronary artery reserves hence increasing the myocardial oxygen demand contributing to myocardial ischemia.

2.2 The Physiology of Heart Rate

The mammalian heart is a diversified system that has a phenomena of continuous contractions (Ademi et al., 2009). This ensures blood transportation throughout the body. The pumping mechanism is as a result of electric impulses throughout the heart leading to repetition of cycles called the Heart Pulse Rate (James & Sunil, 2020). This continuous measure of pulsation is achieved by the counts of contractions per unit time. This is achieved by heart rate variability (HRV) calculation in the Electrocardiogram (ECG) recording.

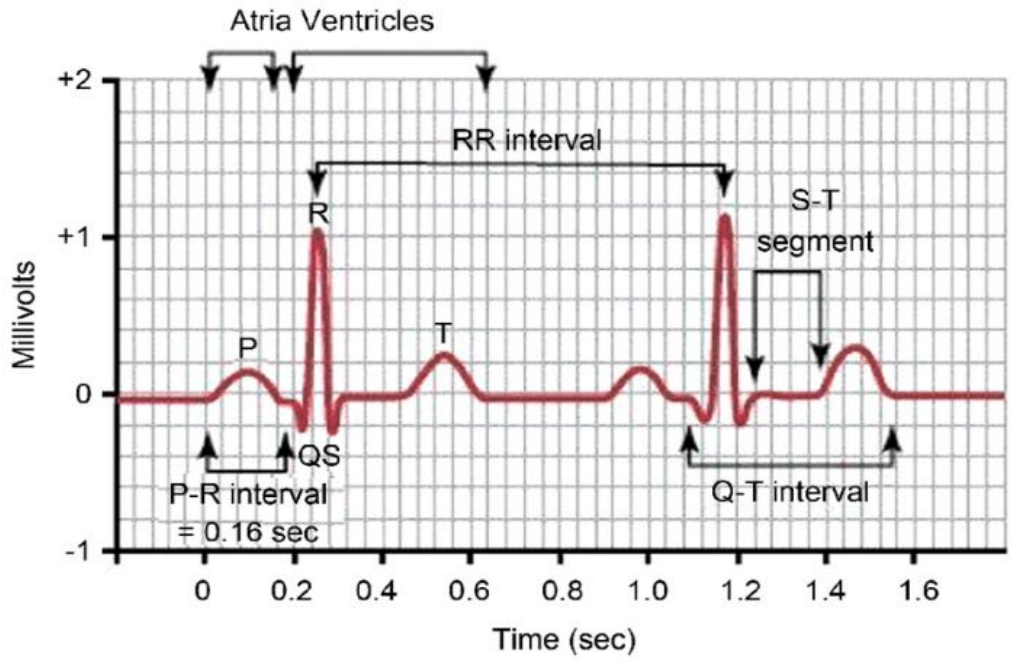

Plate 2.1: A normal ECG, waves, intervals and segment in an electrical activity. The waves indicate an electrical event during a heartbeat.
P-shows an upward movement, QRS shows upward larger deflection, S- represents downward wave.PR indicates the transition period for electrical signal travelling to the ventricle of the heart, T-wave denotes ventricular repolarization.

2.3 Factors Affecting Heart Rate

Various factors are associated with heart variability in atherosclerosis (Marlin *etal*, 2018).Various factors are associated with effect in heart rate changes. These includes; hormones, nervous system, physical activities, drugs, blood volume, body temperature, stress and emotions among others. Elevation in heart rate enhances tensile stress on the arterial wall and extends the exposure further to the coronary endothelium. This further affects both systolic and oscillatory shear force (Breijo-Márquez, 2018). Increase in heart variability boosts the pulsative motion of the heart (Soosaimanickam Carmel, 2016). This therefore changes the geometry of the epicardial arteries and affects the hemodynamic environment.

2.3.1 Nervous System and Heart Rate Variability

The nervous system has a significant effect on the variability of heart rate (Lusis, 2000). The Visceral Nervous System (VNS) substitutes to pathological heart conditions such as infarction of the myocardium and heart failure (Falk, 2006).ANS activity leads to perturbations which enhances increase in sympathetic activity that results to tachy-arrthymias and sudden cardiac death.

The electrical and Myocardial contractility is largely regulated by the Autonomic Nervous System (Becker *et al*., 2015). This neural regulation is labored through interplay of sympathetic vagal outflows. Additionally, vagal stimulation of cardiac pacemaker cells causes hyperpolarization and sympathetic stimulation, therefore resulting to chronotropic effects of the heart(Medić, 2016).

2.3.2 Effect of Hormones on Heart Rate

Thyroid hormone has a consequential impact on heart function (Becker *et al*., 2015). Low and high number of thyroid hormones causes a significant change on cardiac output. The expression of sarcoplasmic reticulum and calcium activated ATPase is done through thyroid hormones monitoring. In addition, Hyperthyroidism results in the increase of Resting Heart Rate (RHR), Stroke Volume and Myocardial Contractility; on the other hand, Hypothyroidism leads to a decreased heart rate and weakening myocardial contractility thus prolonging the systolic and diastolic rate(Shen *et al*., 2020).

2.3.3 Effect of Adrenaline on Heart Rate

Epinephrine also known as adrenaline has a significant effect on the heart rate (James & Sunil, 2020).Adrenaline causes an increase inward flow of Ca^{2+} and Na^{2+} channels. This however results to deceleration of cardiac pacemaker depolarization. The complexity of atherosclerosis is manifested through imbalances of total cholesterol, low density lipoproteins and high-density lipoprotein (Hackam, 2010).Exposure to increased concentration of LDL to the blood vessels causes the endothelium to allow entry of lymphocytes and monocytes. This in turn causes the transhumance or resettling deeper in the blood vessel walls, hence causing reactions that results to plague formation with inflammation. However, this is in comparison with HDL which causes a protective result in atherosclerosis. Suppression and repression of LDL assemblance inside the atherosclerotic plagues leads to reduction of cytotoxicity and oxidation (Lauren *etal*, 2015).

2.4 Management of Coronary Heart Disease

Accurate and prompt management of atherosclerosis is important. This helps to reduce severity of other cardiovascular diseases and complications (Hackam, 2010).Drugs approaches target potential cellular areas including the vascular smooth muscles, monocytic cells, thrombocytes and the endothelial cells (Donovan, 2018).Current drugs in the management of Atherosclerosis works by the mechanism of slowing or even reversing the effects of the disease. They include statins such as Lovastatin Atorvastatin, Pravastatin, Simvastatin and Rosuvastatin (Vaughan, 2000),which act by competitively inhibition of HMG-COA enzyme. This enzyme is a rate limiting factor in formation of cholesterol from the body tissues and liver. This enzyme works by the response of a negative feedback

mechanisms, thus regulating the decrease of reductase gene expression of both sterol and non-sterol components by metabolism of mevalonate (Libby & Theroux, 2005).

The statins drugs decrease the content of cholesterol in the hepatocyte which in turn increases low-density lipoprotein receptors expression responsible for cholesterol uptake. This is done through receptor-based endocytosis. (Ward, 2019).Aspirin reduces the risk of platelet clump in narrowed arteries to prevent further blockage (Kawahito, 2002).Blood pressure medications are also used. They help to prevent further complications related to atherosclerosis, hence reducing the risk of heart attack (Libby & Theroux, 2005). Other conventional methods includes surgery to open the blocked arteries (Ademi *et al*., 2009; Weirick & Anderson, 2009).

2.5 Alternative Methods for the Management of Atherosclerosis

They include acupuncture and Electro-acupuncture (Shen *et al*., 2020).Lifestyle changes has also been associated with decreased risk to atherosclerosis(Spring *et al*., 2014). Not smoking cigarettes, low alcohol consumption and healthy diet (low intake of saturated fatty acids and high intake of calcium, potassium and fiber), decreases the risk of atherosclerosis. Omega 3 fatty acids promotes resolution to atherosclerotic inflammation (Carracedo *et al*., 2019).In addition, this process requires modulation of immune responses on vascular walls. Red wine consumption reduces the incidences of coronary artery diseases (Aviram, 2006). Its consumption increases the activity of paraoxonase, which can hydrolyze lipid peroxides in oxidized LDL (Sharpe, 2011).

Some behavioral phenomena like sleep deprivation and psychosocial stress may be associated with atherosclerotic cardiovascular disease(Lechner *et al*., 2020).This is in relation to nutrient sensing pathways, autophagy and endocrine signaling activities.

2.6 Plant Atherosclerotic Effects

Various plants material extracts are postulated to possess cardioprotective mechanisms. *(Ahmed et al*., 2018).They includes extracts of *Lannea edulis* (Banda *et al*., 2018), Mushroom extracts (Carmel,2016)(*Agaricomycetes*) which have cardio protective effects (Badalyan *et al*., 2021). Traditionally, oral activated charcoal has been used to fight atherosclerotic lesions. (Yamamoto, 2011). In Kenya among the Marakwet community, traditional medicinal plants extracts treated 41 diseases including treating atherosclerosis, meningitis, arthritis, trachoma, small pox and fevers are known to 10% of the people (Kipkore, 2020).

Plant Extracts of *Acacia nilotica* is used as a traditional therapeutic remedy (Ali *et al*., 2012).These effects includes, antihyperlipidemic, antimicrobial, antifungal, angina, treatment of venereal diseases, fever, coughs, headaches, stomach ulcers and malaria (Farzana & Tharique, 2014).Fresh pods extracts are effective in spermatorrhea, loss of viscidity of sperm and premature ejaculation (Roozbeh & Darvish, 2016).The plant has been shown to have antimicrobial effect on *Staphylococcus aureus*, *E.coli, Bacillus subtilis*, and *Shigella sonnei* employing diffusion agar method (Ali *et al*., 2012).

2.6.1 *Solanum incanum*

Solanum incanum (*Solanaceae*), Sodom apple, bitter apple is consumed in Africa as a traditional therapy for treatment of various ailments (Mwonjoria *et al.*, 2014).Bushy perennial herb which in Kenya it has extensive distribution. It's considered a weed though sometimes often cultivated (Dakone & Guadie, 2016). *S. incanum* is a herb of soft wooden shrub up to 1.8m in height and grows 1-3 meters tall, with an oval shaped leaves of 2-3 cm

in diameter. Local names include *Mutongu/Muturere* in Kikuyu, *Entulele* in Maasai (Lusweti, 2011).It consist of green fruits with white spikes and white colored cluster flowers. They are edible as cooked vegetables and the roots, leaves flavors the food (Dakone & Guadie, 2016). They are bitter when raw with yellow-orange appearance when ripe (Dakone & Guadie, 2016).

In African tropical countries, *S.incanum* is used as a traditional remedy for body aches, inflammation of the ears, abdominal pains, angina, toothaches, and dysmenorrhea *(Mwonjoria et al*., 2014).It treated disorders like neoplastic pyrexia and rheumatism, microbial diseases, pneumonia and venereal diseases. A decoction from leaves, roots and fruits were made for drinking. Painful sores were treated by applying a paste or ash from the leaves (Mwonjoria *et al*., 2014; Dakone & Guadie, 2016). In addition, the root infusion was also made and cleaned dried roots chewed.

S. incanum contain phytochemicals such as alkaloids, risins, glycosides, flavonoids, steroids, Solasodine, triterpenes, and phenolic.(Dakone & Guadie, 2016). Studies conducted has reported antimalarial, antimicrobial, antifungal, antidiabetic, anticarcinogenic, and anti-Schistosomal effects on the plant. The aqueous root extract showed antiplasmolytic activity (Urga *et al.*, 2008).

The plant is associated with toxicity effects that includes anorexia, coughing, staggering gait, shivering, lateral recumbency, and bleating (Thaiyah, 2007).The fruits contains Dimethyl nitrosamine which contains carcinogenic effects (Mwonjoria *et al*., 2014b).Studies on antimicrobial activities shows effect against gram negative (*E.coli* & *S.typi*) and gram positive (*Bacillus subtilis* & *Staphylococcus aureus*) bacteria's, with a

zone of inhibition of 0.00 to 16.06 (Sbhatu & Abraha, 2020).Studies of its inflammatory and antinociceptive effects shows a mechanism of action of high phenolic and flavonoid phytochemicals content (Mwonjoria *et al*., 2014; Wang *et al*., 2014).The phytochemicals in *Solanum incanum* exhibits hypolipidemic effects in complications related to diabetes (Banda *et al*., 2018). Various studies reports that, the levels of total cholesterol (TC) and triglycerides (TG) are greatly decreased from the consumption of the fruit extracts of the plant (Banda *et al*., 2018; Sbhatu & Abraha, 2020).

Plate 2.2: *Solanum incanum Linnaeus* (photo taken at Nova Pioneer Kiambu County), sept 2021.

2.6.2 *Rhamnus prinoides*

Rhamnus prinoides (Rhamnacea), Orkonyil/ Olkokola in Maasai and *Ngukura* in Kikuyu, are small trees or shrubs which are rigid and branched growing to five meters in height (Amare, 2018). The leaves are small and narrow clustered on short side branches having a lead tip pointed which can be rounded or notched. The plant is widely distributed in East, central and South African countries being native to Kenya (Gebre, 2012).In Kenya the plant is majorly distributed in Rift valley and Central provinces of Kenya (Moller, 2016). The decoction of the roots is mixed with milk or even taken orally and have been active against pneumonia, stomachache, back pains, gonorrhea, and malnutrition (Nedi, 2016).

Studies conducted has reported *R.prinoides* have effects on anti-inflammatory, anti-oxidant wound healing, blood purifier, treatment of waterborne diseases, sexually transmitted diseases and malaria (Nigussie *et al*., 2021).In Kenya the barks of *Rhamnus prinoides* is consumed as a folk medicine in the management of sexually transmitted infections and backaches (Nankaya, 2019).*R.prinoides* contains phytochemicals such as anthraquinones, saponins, steroids, tannins, terpenoids, alkaloids and flavonoids (Nigussie *et al*., 2021). Study shows potential antioxidant and anti-inflammatory activities on *R.prinoides* (Gui-Lin, 2020). The anti-microbial activity of *R. prinoides* against *E. coli* and *Staphylococcus spp* has also been reported (Molla, 2016).

The plant compounds thought to contribute to antimalarial and parasitemia suppression include triterpenes, oxygenated sesquiterpenes, flavones, and vernolic acid (Muregi *et al*., 2007).*R.prinoides* is used as a hopping agent in the making of traditional alcoholic drinks

(Nigussie *et al*., 2021).This is characterized alongside other active traditional uses such as soil conservation, dyes in textile, making of ornaments and nectar for bees.

The herb has also been associated with treatment of Alzheimer's disease (Soriano, 2021). This works by the mechanisms of acetylcholinesterase inhibition. Among the Maasai community, the traditional healers identified *R.prinoides* as a very important herb in the management of diabetes and was frequently sought (Kamau *et al*., 2017).

Plate 2.3: *Rhamnus prinoides* (photo taken in Olendeem, Narok County) sept 2021

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

Rhamnus prinoides barks were collected at Olendeem in Narok County. Narok is situated 143 kilometers west of Nairobi under the Great Rift Valley. It is inhabited by the Maasai community and neighbors the Kilgoris region, Nyamira and Kisii counties (Figure 1). *Solanum incanum* roots was collected in Kamiti corner area, Nova Pioneer Kiambu County. It's located about 16 kilometers North from Nairobi Metropolitan region and greatly inhabited by the Kikuyu community. It borders regions such as Kabete, Ruiru, Gatundu and Limuru (Figure 2). Plant materials were collected month of April morning hours of the day.

Plants were randomly selected. The Maasai and the Kikuyu communities uses these plants in different ways. There are a few medical health facilities in Olendeem Narok region and this leads to relying of medicinal plants for disease management. Information on the uses, collection and how they are administered was obtained from the local herbalist.

Plate 3.1: Map of Kenya indicating study area. Narok County
Source: (google maps https://opencounty.org/county-about.php?com=8&cid=33)

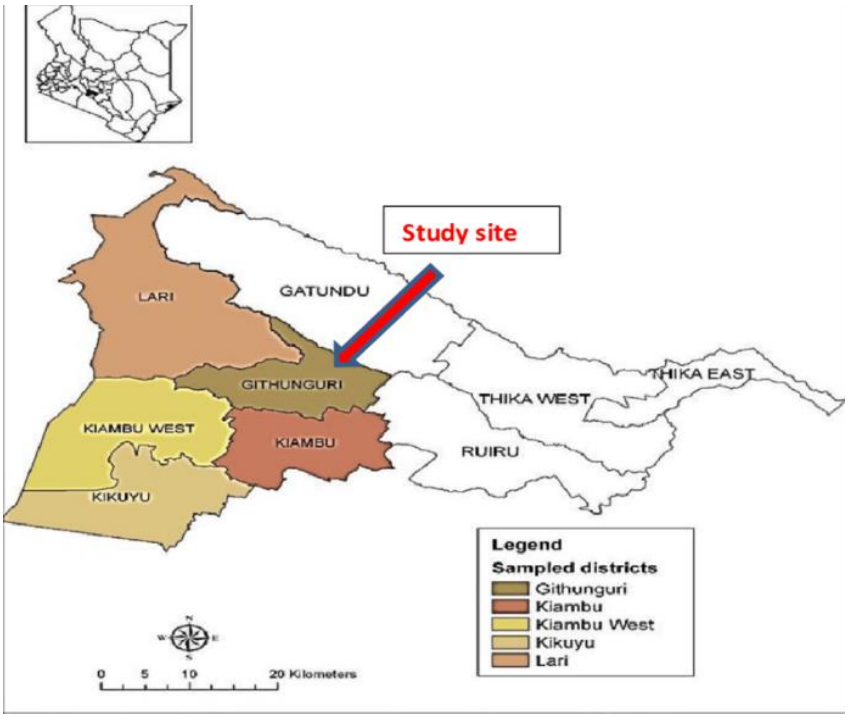

Plate 3.2: Map of Kenya indicating study area. Kiambu County
Source: (google maps https://opencounty.org/county-about.php?com=8&cid=22)

3.2.1 Inclusion Criteria

Plants within Olendeem region of Mau division Olchorro, Narok County. *Rhamnus Prinoides* plants are found in dry semi-arid areas. The vast areas within this region are dominated by the Maasai community. *Solanum Incanum* was collected in Kamiti corner area near Nova pioneer Kiambu County dominated by the Kikuyu community. Only matured plants were used. *Roots of Solanum incanum* and barks of *Rhamnus Prinoides* were used. Ten male New Zealand rabbits weighing about 2-3 kilograms were used for ionotropic and chronotropic studies. Male rats six weeks old with an average weight of 150 grams were used. For sub-acute toxicity study, male mice of about 25grams were used.

3.2.2 Exclusion Criteria

Plants outside Olendeem region, Mau division were excluded. *Solanum incanum* outside Kamiti corner area was excluded. Immature and over aged plants were also excluded. Female Rabbits and rabbits exceeding 2-3 kilograms were excluded. Female and male mice out of the age bracket of 6-7 weeks were excluded.

3.3 Study Design

Controlled randomized laboratory study design was used. Animal Breeds were randomly purposively selected from University of Nairobi Kabete campus animal house. They were placed in polyethylene cages. The selection involved purely healthy animals. This was achieved by accessing their behavior's, fur alignment, as well as the food intake. Temperatures were also taken to confirm healthy status. Food pellets and water were given ad libitum at Kenyatta University Animal House Department (biochemistry, microbiology and biotechnology laboratories). Hyperlipidemia was confirmed on weight gain after 30 days of HCHF (High Cholesterol High Fat) diet.

3.4 Sample Size

Various studies that uses experimental animals applies adequacy of six animals per group (Charan & Biswas, 2013).In toxicity studies, six mice's were used per experiment. For hyperlipidemia studies five rats were used per group which was in compliance with animal ethic principle of replacement, reduction and refinement (Cheluvappa et al., 2017).Ten male rabbits (five in each experimental groups) were used for ionotropic and chronotropic studies. The use of rabbits was advantageous as rabbits have been used actively for several non-infectious disease studies such as atherosclerosis (Esteves et al., 2018).

3.5 Sampling Technique

Sampling technique applied the use of purposeful and random selection of the plant materials. *Solanum incanum* roots was collected from Nova pioneer and *Rhamnus Prinoides* from Olendeem village. The two places were purposively selected. Experimental animals were acclimatized before commencement of the experiments. Random selection was applied when assigning the animals to various treatments.

3.6 Sample Collection

Using a Viking hatchet *Rhamnus prinoides* barks were randomly removed from the plant and packed into a nylon gunny bag. A hand pick hoe and a shovel were used to extract *Solanum incanum* roots from randomly selected *Solanum incanum* plants. They were then placed in a nylon gunny bag. Collected plant materials were separately cleaned with running tap water to remove dust, dirt and other foreign agents attached to the surface of the plant. They were packaged in mafuko brown khaki bags and shipped to Kenyatta University Biochemistry and Biotechnology laboratory for drying and crushing. The

collected parts were dried in the laboratory away from direct exposure to sun light. A qualified taxonomist identified them and a voucher specimen number (SM2022/01TB) was allocated from the University of Nairobi Herbarium.

Collection of blood and tissue samples was done under deep general anesthesia. This was done in both hyperlipidemic rats and male mice for sub-acute toxicity study. This was done after 28th day of daily drug administration.

Lard for hyperlipidemic studies was collected in Thika Pigs Community Slaughterhouse near Makongeni area along Thika-Garissa Highway. They were cleaned to remove top debris, wrapped with baking paper and air removed inside then covered with home cling film. They were frozen at -15 degree Celsius for later use.

3.6.1 Plant Extraction

Roots of *Solanum Incanum* were randomly selected from the plants. The roots were naturally desiccated at ambient temperature for two weeks. The dried plant parts were grounded using an electric mill into powder. The barks of *Rhamnus Prinoides* were chopped into small pieces, then air dried at ambient room temperature for three weeks then followed milling into fine homogeneous powder by the use of electric mill and later sieved through a mesh (Zhengzhou Yize machinery co., Ltd. Henan, China).

Standard weighing scale (*Scout pro SPU 402*) was used to weigh about 100g of the powdered plants. They were soaked in absolute methanol repeatedly for 72 hours. Immediately after adding the solvent agitation was done for about eight seconds this increased extraction efficiency by breaking the plant's cell wall thus resulting to enhanced release of the soluble phytochemicals. They were later decanted.

After overnight incubation, the supernatant was filtered using Whatman No 1 filtering paper. The filtered substance was then concentrated using a Rotor evaporator at reduced pressure to obtain the extracts. The final product was a solid mass devoid of the solvent used. The dried mass was then stored at room temperature in airtight bottles which were later used for desired pharmaceutical formulations and also phytochemical testing. Dimethylsulfoxide (DMSO) and normal saline in the ratio mixture of 1:10 was used to reconstitute the dried extracts *(*Mwonjoria *etal., 2011).*

3.7 Experimental Animals and Design

Hyperlipidemic study consisted of six treatments (n=6). The negative control was administered to treatment 1. They were fed with a high cholesterol high fat diet. The high cholesterol high fat diet consisted of 10% egg York (5.6g/bw),10% lard (5.6g/bw),0.2% cholic acid (0.112g/bw) and 0.59% propylthiouracil (0.28g/bw). Propylthiouracil increased efficacy by lowering the normal rats metabolic rate and maintaining rate of body weight gain (Nawale *et al*., 2018).

A standard therapeutic dose of *Solanum incanum* and *Rhamnus prinoides* was administered to treatments 4,5,6 with 25,50, and 100mg/kg respectively. Baseline was the second treatment; the rats were fed ad libitum. The last treatment was the positive control where 40mg/kg of Avastatin was administered. Oral gavage was used to administer 0.25mls of the drug daily. Throughout the period of drug administration, animals were monitored for any behavior changes which could arise due to toxicity such as vomiting, diarrhea, ataxia or even death. Body weights were recorded weekly.

On 28th day, the animals were sacrificed. Body weights were recorded before the sacrifice. Organ weights of the liver, kidney, spleen, lungs, heart and brain were also recorded. (Appendix III)

Sub-acute toxicity study involved 35 male mice's age between 6-7 weeks with average body weight of 22gms.Mice's were acclimatized in the cages at ambient room temperature conditions for about one week before the experiment commenced. Standard commercial rodent pellets from Unga Limited were provided ad libitum. After one week of acclimatization, animals were sorted into seven groups (n=5) as follows; group 1 *Solanum incanum* 100mg/kg, group 2 *Solanum incanum* 174mg/kg, 3rd group *Solanum incanum* 300 mg/kg, 4th group, *Rhamnus prinoides* 100mg/kg, group 5 *Rhamnus prinoides* 174mg/kg, group 6 *Rhamnus prinoides* 300mg/kg and group 7 Baseline which was the normal control.

3.7.1 Animal Preparation for Cardiotonic Studies

For ionotropic and chronotropic studies, 10 male White New Zealand Rabbits of 4-5 months age were used. Rabbits have been shown to respond well to cardiotonic drugs and ease of handling for cardiotonic studies. The rabbits were prepared for the study by injection of 1000 units of heparin. It was to assist in avoiding formation of clots in the heart of the rabbits. Heparin was induced through the ear region. The heart was removed carefully after the chest was opened through a cardiac thoracotomy procedure. After the removal of the heart, it was placed quickly and carefully in a dish containing Tyrode solution. This was all done under room temperature conditions. Excess blood was removed through gently squeezing. Dissection of the aorta followed and trimming of all the tissue fascia connected on the heart was done.

Drugs and standard chemicals used in the study were kept at recommended storage conditions. This maintained their stability hence reducing the chances of contamination that would affect the results of the study. Biohazard liquid, solid wastes and sharps were handled in compliance with ISO 15190 guidelines.

3.7.2 Kymograph Preparation and Organ Bath

Following heparinization and anesthesia, the rabbit hearts were isolated through a midsternal thoracotomy procedure. The heart was quickly removed and placed in cold Tyrode solution (NaCl 130, KCl 4.7, CaCl 2.2, MgCl2 0.6, NaH2PO4 1.2, NaHCO3 24.2, and glucose 12) for further preparation. Ph was kept stable and maintained at 7.4 by equilibration by a mixture of 95% oxygen and 5% carbon iv oxide. The temperature of the Tyrode solution was maintained constant throughout the experiment at 37ºC.The perfusion pressure was kept at 60mmHg.

Acetylcholine reduces heart rate and contraction. To check if the parameters could be restored by the plant extracts, acetylcholine was administered. The plant extracts then followed in a stepwise manner. Stock solution was prepared from the Tyrode solution and the working solution were prepare from the initial stock solutions. On each of the prepared concentrated solutions one ml was added to the organ bath and used as the negative control.

Adrenaline causes an increase in heart rate (HR) and contraction force. To confirm whether the plant extracts would restore this parameter, adrenaline was administered then the plant extracts followed. Model SRD-001 Kymograph from brand of Orchid Scientific was used in recording of cardiac contractility. It contained a revolving drum which had a kymograph paper to record the readings (Mbida *et al*., 2022).

3.7.3 Langendorff Method

To screen for cardiotonic (ionotropic and chronotropic) effects of Solanum *incanum* and *Rhamnus prinoides,* Langendorff method was used (Langendorff 1895). Cutting of the aorta was done just below a section where it divides. When the heart was ready, a solution containing Tyrode was used to transfer the heart to it. The aorta was tied on the glass cannular and temperature was kept constant at 37 degrees Celsius with continuous oxygenation. Extra care was made a priority to ensure air bubbles do not enter the aorta. Those that entered were immediately released.

Attached to the ventricle was a fine nylon thread having a hook and a small spring clip to the auricles. Threads were then connected to the levers of the spring for recording of the heart contractions. Stabilization of the heart was allowed first for a period of 20 minutes. Recordings of the heart rate and coronary flow was taken conveniently over 30 seconds.
An injection to the rubber tubing was done in order to add the drugs (Acetylcholine and Adrenaline) and also the plant extracts. Any noted heart block reversal was done by the administration of 0.1µg atropine.

When conducting procedures, personal protective equipment's (PPEs) were donned. Sharps used during the procedure were disposed in leak proof sharp containers. Organs collected for analysis were heart, liver kidneys, and pancreases.

3.8 Toxicity Assay

3.8.1 Sub-Acute Toxicity Effects Assay of Plant Extracts in Mice.

3.8.1.1 Hematological Analysis

Recommended Organization for Economic Cooperation and Development (OECD-407) sub-acute toxicity studies protocols were used. Animal weights were monitored and recorded on a weekly basis through day 28. The study determined the effect of the drugs on hematological and biochemical blood parameters (Onwuliri *et al*., 2010). Total blood collected from the cardiac puncture was about 2mls and it was immediately placed in tubes containing EDTA (Ethylene Diamine Tetra Acetic Acid) anticoagulant. The complete blood count was done using a Sysmex XP 300 Hematology analyzer machine. After testing for hematological indices, blood was then centrifuged and plasma was collected. Using standard pipettes, plasma was transferred to clean appropriately labelled cryovials and stored at -20ºC for subsequent testing. Hematological parameters evaluated included; hemoglobin level, erythrocyte count, thrombocytes count, Leukocyte blood cell differentials, mean cell volume (MCV) and mean corpuscular hemoglobin (Silva-Santana *et al*., 2020).

3.8.1.2 Biochemical Assay

Cobas c111 chemistry analyzer machine was used for analysis of biochemical samples. The machine used the principle of absorbance photometry and photoelectric colorimetry to measure a specific chemical composition in the sample (Jang *et al*., 2011). Tested biochemical parameters included; Liver function parameters (alanine aminotransferases, aspartate aminotransferases, gamma glutamyl transferases, alkaline phosphatase, bilirubin), Renal function tests (Creatinine, Urea, Uric-acid, sodium, potassium, chloride,

calcium, phosphorous) and Lipid profile tests (Total cholesterol, Low density lipoproteins, high density lipoproteins, triglycerides).

3.9 Qualitative Phytochemical Analysis

The procedures included mixing of crude plant extract with certain chemical in specific ratios as per the approved testing protocols. Tested phytochemicals included; alkaloids, saponins, cardiac glycosides, flavonoids, tannins and terpenoids. Qualitative phytochemical tests included but not limited to, Hager's test for alkaloids, Sodium hydroxide test for coumarins and Sodium bicarbonate test for saponin (Shaikh *et al*., 2020). The mixtures were allowed to stand for considerable amount of time and temperature for the reaction to take place. Color change was used as a determining factor for the results of the reactants.

3.9.1 Alkaloid (Wagner's test)

Two (2mls) of the plant extract was added into a clean test tube. Along the sides of the tube, 2drops of Wagner's reagent was added. The formation of a brown to reddish precipitate was considered positive for the alkaloid test (Morsy, 2014).

3.9.2 Cardiac Glycoside (Keller-Killani test)

One (1 ml) of the plant extract was added into a clean test tube, followed by addition of 1.5mls of glacial acetic acid. One (1) drop of ferric chloride followed by acidification with two (2) drops of conc sulphuric acid on the sides of the test tube was added. Immediately after the addition of the sulphuric acid, formation of a blue color in acetic acid layer was denoted as positive for the presence of cardiac glycosides (Kamau *et al*., 2017).

3.9.3 Flavonoids (Alkaline reagent test)

One (1ml) of the plant extract was added into a clean test tube. Three (3) drops of 10% ammonium hydroxide solution was then added on the sides of test tube. Formation of a yellow fluorescence was indicative of the presence of flavonoids in the mixture (Shaikh and Patil, 2020).

3.9.4 Saponin (foam test)

Two (2mls) of the plant material was placed into clean tube. 1ml of distilled water was added and the solution was shaken vigorously for 30seconds. After shaking, the solution was left to stand for 10 minutes. Foam formation denoted the presence of saponin (Shaikh and Patil, 2020).

3.9.5 Tannins (Braymer's test)

One (1ml) filtrate (plant extract) was added into a clean test tube. Three (3mls) of distilled water was put into the tube,then three (3) drops of ferric chloride solution along the wall of the test tube. Formation of blue green color was indicative for the presence of tannins (Shaikh and Patil,2020).

3.9.6 Coumarin (NaOH test)

One (1ml) plant extract was added into a clean test tube followed by addition of three (3) drops of 10% NaOH solution. Along the side of the tube, three (3) drops of chloroform was added. Formation of a yellow color was indicative for the presence of coumarin (Shaikh and Patil, 2020).

3.9.7 Terpenoids (Salkowski test)

Two (2mls) of the plant material extract was added into clean tube. Acidification of the extract was done by adding three (3) drops of anhydrous sulphuric acid. The mixture shaken and allowed to stand. Presence of golden yellow layer at the bottom was indicative of the presence of terpenoids (Shaikh and Patil, 2020).

3.10 Ethical Approval and Confidentiality

All studies were conducted following compliance with the guidelines for use and care for laboratory animals (Wolfensohn *et al*., 2013).

3.11 Data Analysis

Raw data was tabulated into Excel Spreadsheet, organized. A descriptive statistic's analysis was done using the mean and standard error of the mean (SEM). Inferential statistics, One-way analysis of variance (ANOVA) and Tukey's post hoc test was used to analyze for statistical differences among the groups. An independent *Student*s' t-test was analyzed as comparison of the effects of two extracts. The significance levels were set at value of $p < 0.05$.

CHAPTER FOUR: RESULTS

4.1 Cardiotropic Effects on Extracts of *Solanum incanum* and *Rhamnus prinoides*

4.1.1 Chronotropic Effects of *Solanum incanum* extracts

As depicted in table 4.1, effects of extract of *S. incanum* in concentrations of 25, 50 and 100mg/ml caused alterations on heart rate of rabbit's heart in the 1st, 2nd, 3rd, 4th and 5th minutes of the experiment. The effect of *S. incanum* extract at the three concentrations, as well as the acetylcholine, lead a significant reduction of the heart rate from minute 0 to the 5th minute of the experiment ($p<0.05$). Nevertheless, the effect of adrenaline caused a considerable increase in heart rate from minute 0 to the 5th minute ($p<0.05$). The heart rate of heart isolated from normal control rabbits was insignificant throughout the experiment ($p>0.05$).

At the three tested concentrations, the effects of Solanum incanum plant extracts on heart rate were not significantly different in minute 0 and 1st minute of the study ($p>0.05$). However, on 2nd, 3rd, 4th and 5th minute of experiment, a significant difference was observed on the heart rate ($p<0.05$; Table 4.1). The extract lowered the heart rate in a concentration-dependent manner (Table 4.1). The effect of adrenaline increased the heart rate from the 1st minute of the study in comparison to the effect of *S. incanum* at the three concentrations ($p<0.05$). Effect of acetylcholine significantly lowered the heart rate from the 2nd minute of the experiment, this was in comparison with other treatment groups ($p<0.05$; Table 4.1). Heart rate in normal control of isolated heart was significantly greater than the heart rate seen in the heart perfused with the extract at the three concentrations $p<0.05$; (Table4.1).

Table 4.1: Chronotropic effects of *S. incanum* extracts

	Heart rate (percentage change in heart rate)					
Treatment	**0 min**	**1st**	**2nd**	**3rd**	**4th**	**5th**
Baseline	210.00±2.89^{aA}	210.00±2.89^{bA}	208.33±1.67^{bA}	208.33±1.67^{bA}	210.00±2.89^{bA}	211.67±1.67^{bA}
	(0.00±0.00)	(0.00±0.00^{b})	(0.00±0.00^{b})	(0.00±0.00^{b})	(0.00±0.00^{b})	(0.00±0.00^{b})
Acetylcholine	210.00±2.89^{aA}	178.33±1.67^{dB}	161.67±1.67^{eC}	141.67±1.67^{eD}	131.67±1.67^{eE}	121.67±1.67^{eF}
	(0.00±0.00)	(-15.08±0.79^{d})	(-22.40±0.80^{e})	(-32.00±0.80^{e})	(-37.3±0.79^{f})	(-42.52±0.79^{e})
Adrenaline	211.67±1.67^{aD}	230.00±2.89^{aC}	245.00±2.89^{aB}	250.00±2.89^{aAB}	260.00±2.89^{aA}	260.00±2.89^{aA}
	(0.00±0.00)	(9.52±1.37^{a})	(17.60±1.39^{a})	(20.00±1.39^{a})	(23.81±1.37^{a})	(22.83±1.36^{a})
25mg/ml	208.33±1.67^{aA}	196.67±1.67^{cB}	188.33±1.67^{cBC}	185.00±2.89^{cCD}	176.67±1.67^{cDE}	171.67±1.67^{cE}
	(0.00±0.00)	(-6.35±0.79^{c})	(-9.60±0.80^{c})	(-11.20±1.39^{c})	(-15.87±0.79^{c})	(-18.90±0.79^{c})
50mg/ml	210.00±2.89^{aA}	193.33±1.67^{cB}	181.67±1.67^{cdC}	171.67±1.67^{dD}	161.67±1.67^{dDE}	156.67±1.67^{dE}
	(0.00±0.00)	(-7.94±0.79^{c})	(-12.80±0.80^{cd})	(-17.60±0.80^{d})	(-23.02±0.79^{d})	(-25.99±0.79^{d})
100mg/ml	211.67±3.33^{aA}	188.33±1.67^{cdB}	175.00±2.89^{dC}	163.33±1.67^{dD}	151.67±1.67^{dE}	148.33±1.67^{dE}
	(0.00±0.00)	(-10.32±0.79^{c})	(-16.00±1.39^{d})	(-21.60±0.80^{d})	(-27.78±0.79^{e})	(-29.92±0.79^{d})

Means and SEM that do not share a lowercase letter along the column, as well as means of percentage change in heart rate (within parenthesis) that does not share a letter along the column are, significantly different. Means that do not share an uppercase superscript letter along the row, are significantly different ($p<0.05$).

On other hand, the effect of *R. prinoides* at concentrations of 25, 50 and 100 mg/ml resulted in alterations in heart rate on isolated rabbit's heart (Table 4.2). Extract at the three concentrations, including acetylcholine caused a significant decline in heart rate from minute 0 to the 5^{th} minute of the experiment ($p<0.5$). However, the effect of adrenaline showed a significant increase ($p<0.05$) in heart rate from minute 0 to minute 5^{th} minute of the experiment (Table 4.2). The heart rates of the normal control isolated rabbit's heart were non-significant ($p>0.05$) in the entire study (Table 4.2).

Rhamnus prinoides effects at all three concentrations on the heart rate was not significantly different in the 0, 1^{st}, 2^{nd} and 3^{rd} minutes of the experiment ($p>0.05$). However, heart rate at the three concentrations had a significant difference ($p<0.05$) in the 4^{th} and 5^{th} minutes of the experiment (Table 4.2). Activity of acetylcholine on heart rate was insignificant compared to the effect of the extract at the concentration of 100mg/ml in the 1^{st}, 2^{nd} and 4^{th} minutes of the study ($p>0.05$), as well as at the concentration of 50mg/ml at the 1^{st} minute of the experiment (Table 4.2). The effect of adrenaline had a significant increase ($p<0.05$) in the heart rate from the 1^{st} minute of the treatment onwards compared to those seen in the other experimental groups (Table 4.2).

Table 4.2: Chronotropic effects of *R. prinoides* extracts on isolated rabbit's heart.

	Heart rate (percentage change in heart rate)					
Treatment group	**0 min**	**1st**	**2nd**	**3rd**	**4th**	**5th**
Baseline	210.00±2.89^{aA}	210.00±2.89^{bA}	208.33±1.67^{bA}	208.33±1.67^{bA}	210.00±2.89^{bA}	211.67±1.67^{bA}
	(0.00±0.00)	(0.00±0.00^{b})	(0.00±0.00^{b})	(0.00±0.00^{b})	(0.00±0.00^{b})	(0.00±0.00^{b})
Acetylcholine	210.00±2.89^{aA}	178.33±1.67^{dB}	161.67±1.67^{dC}	141.67±1.67^{dD}	131.67±1.67^{eE}	121.67±1.67^{eF}
	(0.00±0.00)	(-15.79±0.79^{d})	(-22.40±0.80^{d})	(-32.00±0.80^{d})	(-37.30±0.79^{e})	(-42.52±0.787^{e})
Adrenaline	211.67±1.67^{aD}	230.00±2.89^{aC}	245.00±2.89^{aB}	250.00±2.89^{aAB}	260.00±2.89^{aA}	260.00±2.89^{aA}
	(0.00±0.00)	(9.52±1.37^{a})	(17.60±1.39^{a})	(20.00±1.39^{a})	(23.81±1.37^{a})	(22.83±1.36^{a})
25mg/ml	210.00±2.89^{aA}	190.33±2.67^{cB}	180.00±2.89^{cC}	165.00±2.89^{cD}	156.67±1.67^{cD}	155.00±2.89^{cD}
	(0.00±0.00)	(-7.94±0.79^{c})	(-13.60±1.39^{c})	(-20.80±1.39^{c})	(-25.40±0.79^{c})	(-26.77±1.36^{c})
50mg/ml	211.67±1.67^{aA}	186.67±1.67^{cdB}	175.00±2.89^{cC}	163.33±1.67^{cD}	150.00±2.89^{cdE}	146.67±1.67^{cdE}
	(0.00±0.00)	(-11.11±0.79^{c})	(-16.00±1.39^{c})	(-21.60±0.80^{c})	(-28.57±1.37^{cd})	(-30.71±0.79^{cd})
100mg/ml	211.67±1.67^{aA}	185.00±2.89^{cdB}	171.67±1.67^{cdC}	155.00±2.89^{cD}	141.67±1.67^{deE}	141.67±1.67^{dE}
	(0.00±0.00)	(-11.90±1.37^{cd})	(-17.40±0.80^{cd})	(-25.60±1.39^{c})	(-32.54±0.79^{d})	(-33.07±0.79^{d})

Means of heart rate that do not share a lowercase letter along the column, as well as means of percentage change in heart rate (within parenthesis) that does not share a letter along the column are, significantly different, ($p<0.05$). Means that do not share an uppercase superscript letter along the row are significantly different ($p<0.05$).

In comparison, the effect of *R. prinoides* at various concentrations of 25, 50 and 100mg/ml noted a significant reduction in the total percentage change in heart rate on the rabbit's heart compared to the effect of *S. incanum* at the corresponding concentration ($p<0.01$; Figure 4.1).

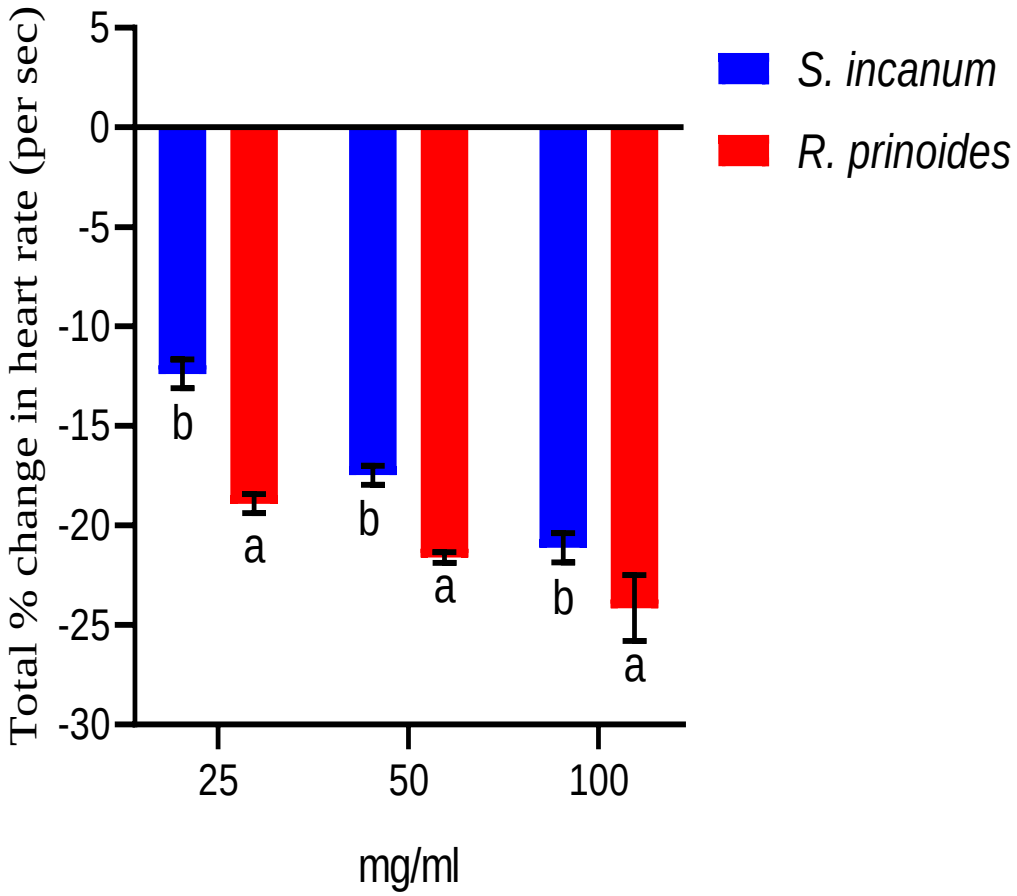

Figure 4.1: Comparison of chronotropic effects of *Solanum incanum* and *Rhamnus prinoides* extracts on isolated rabbit's heart.
Bars that do not share a letter are statistically significant using an independent t-test in the same concentration ($p<0.05$).

4.2 Effects of *Solanum incanum* and *Rhamnus prinoides* on Ionotropic Activity (height of force of contraction) on Isolated Rabbit's Heart

Effect of S. *incanum* at the concentrations of 25, 50 and 100mg/ml revealed changes in height of force of contraction of isolated rabbit's heart (Table 4.3). The effect of the *S. incanum* at the three concentrations, as well as adrenaline resulted in a significant increase ($p<0.05$) in the height of force of contraction of the isolated heart from minute 0 to the 5th minute of the experiment (Table 4.3). However, the effect of acetylcholine on the height of force of contraction caused a significant increase, from minute 0 to the 5th minute of the experiment ($p<0.05$; Table 4.3). The heights of the force of contraction of hearts isolated from normal control rabbits were insignificant in the entire experiment ($p>0.05$; Table 4.3). In minute zero, the heights of the force of contraction of all treatment groups were statistically insignificant ($p>0.05$; Table 4.3).

Effect of *S. incanum* at concentrations of 25, 50 and 100mg/ml was insignificant on the height of force of contraction of rabbit's heart ($p>0.05$) in the 1st, 3rd, and 4th minutes of the experiment. Nonetheless, the effect of the extract at the three concentrations on the height of force of contraction differed significantly in the 2nd and 5th minutes ($p<0.05$) of the experiment (Table 4.3).

The effect of acetylcholine had a significantly lower height of force of contraction ($p<0.05$) relative to those observed at the three concentrations of extract in the 1st, 2nd, 3rd, 4th and 5th minutes of the treatment period (Table 4.3). From the 1st minute onwards, the effect of adrenaline resulted in a significant increase in the height of force of contraction ($p<0.05$) compared to values seen in other treatment groups (Table 4.3). From the 1st minute

onwards, the normal control isolate hearts had a significantly lower height of force of contraction ($p<0.05$) in comparison to those noted in the extract-treated hearts at the three concentrations, as well as acetylcholine (Table 4.3).

Table 4.3: Ionotropic effects of *S. incanum* extracts on isolated rabbit's heart

	Height of force of contraction (mm)					
Treatment group	**0 min**	**1st**	**2nd**	**3rd**	**4th**	**5th**
Baseline	2.93 ± 0.07^{Aa}	3.00 ± 0.12^{cA}	3.00 ± 0.00^{dA}	3.00 ± 0.12^{cA}	2.87 ± 0.13^{cA}	2.93 ± 0.07^{dA}
Acetylcholine	2.93 ± 0.07^{aA}	2.73 ± 0.03^{dAB}	2.65 ± 0.03^{eB}	2.53 ± 0.03^{dBC}	2.40 ± 0.03^{dC}	2.38 ± 0.04^{eC}
Adrenaline	2.93 ± 0.03^{aD}	3.97 ± 0.03^{aC}	4.13 ± 0.03^{aBC}	4.30 ± 0.03^{aB}	4.51 ± 0.04^{aA}	4.70 ± 0.06^{aA}
25mg	2.90 ± 0.06^{aE}	3.23 ± 0.03^{bcD}	3.42 ± 0.04^{cCD}	3.58 ± 0.04^{bBC}	3.72 ± 0.04^{bB}	4.00 ± 0.04^{cA}
50mg	2.97 ± 0.03^{aE}	3.33 ± 0.03^{bD}	3.62 ± 0.04^{bC}	3.77 ± 0.04^{bBC}	3.92 ± 0.04^{bB}	4.15 ± 0.03^{bcA}
100mg	2.92 ± 0.06^{aE}	3.48 ± 0.02^{bD}	3.70 ± 0.03^{bC}	3.83 ± 0.04^{bBC}	3.97 ± 0.03^{bB}	4.30 ± 0.03^{cA}

Means and SEM that do not share a lowercase superscript letter along the column are significantly different ($p<0.05$). Means and SEM that do not share an uppercase superscript letter are significantly different.

Effects of *S. incanum* at the three studies concentrations on the total percentage change in height of force of contraction of heart isolated from the rabbits differed significantly ($p<0.05$; Figure 4.2). The effect of adrenaline caused a significant increase ($p<0.05$) in the total percentage change in height of force of contraction compared to the total percentage changes noted in the other treatment groups (Figure 4.2). The effect of acetylcholine had a significantly lower total percentage change in height of force of contraction ($p<0.05$) compared to those noted in the other treatment groups (Figure 4.2). The total percentage change in height of force of contraction observed in normal control group was significantly lower than those reported in extract-treated and adrenaline-treated isolated hearts ($p<0.05$; Figure 4.2).

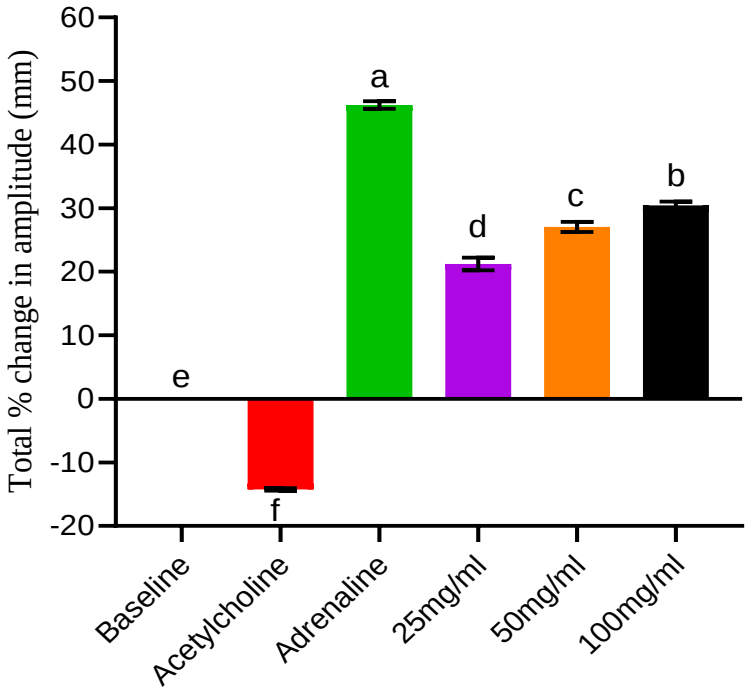

Figure 4.2: Effect of methanol extract of *Solanum incanum* on total percentage change in height of force of contraction of rabbit's isolated heart.
Bars that do not share a letter are statistically significant ($p<0.05$).

In addition, effects of *R. prinoides* at the concentration of 25, 50 and 100mg/ml revealed alterations in height of force of contraction of isolated hearts in the entire experiment (Table 4.4). Therapy with the extract at the three concentrations, as well as adrenaline resulted in a significant increase in amplitude (height of force of contraction) ($p<0.05$) from minute zero to the fifth minute of the experiment (Table 4.4). However, the effect of acetylcholine caused a significant decline in the height of force of contraction from minute 0 to the 5^{th} minute of the study ($p<0.05$; Table 4.4). The height of force of contraction of the normal control group did not differ significantly from minute 0 to minute 5 of the experiment ($p>0.05$; Table 4.4). The height of force of contraction of all the treatment groups was not significantly different ($p>0.05$) in minute zero (Table 4.4).

Therapeutic effects of *R. prinoides* at concentrations of 25, 50 and 100mg/ml on the height of force of contraction never differed significantly in the 1^{st} minute ($p>0.05$) of the experiment. However, the effect of the extract at the three concentrations was significantly different in 2^{nd}, 3^{rd}, 4^{th} and 5^{th} minutes ($p<0.05$) of the experiment (Table 4.4). The extract increased the height of the force of contraction in a concentration-dependent manner (Table 4.4). The effect of adrenaline on the height of force of contraction statistically matched the effect of the extract at the concentration of 100mg/ml in the 1^{st}, 3^{rd}, and 5^{th} minutes ($p>0.05$) of the experiment (Table 4.4). The effect of acetylcholine had a significantly lower height of force of contraction compared to those noted in the other groups from the second minute onwards ($p<0.05$). The height of force of contraction of the heart isolated from normal control rabbits was considerably lower than those seen in extract-treated isolated hearts ($p<0.05$) in entire experiment (Table 4.4).

Table 4.4: Ionotropic effects of *Rhamnus prinoides* extracts on rabbit's isolated heart.

Means and SEM that do not share a lowercase superscript letter along the column, are significantly different. Means and SEM that do not share an uppercase superscript letter, are significantly different ($p<0.05$).

	Height of force of contraction (mm)					
Treatment group	**0 min**	**1st**	**2nd**	**3rd**	**4th**	**5th**
Baseline	2.93 ± 0.07^{aA}	3.00 ± 0.12^{dA}	3.00 ± 0.00^{dA}	3.00 ± 0.12^{cA}	2.87 ± 0.13^{dA}	2.93 ± 0.07^{dA}
Acetylcholine	2.93 ± 0.07^{aA}	2.73 ± 0.03^{dAB}	2.65 ± 0.03^{eB}	2.53 ± 0.03^{dBC}	2.40 ± 0.03^{eC}	2.38 ± 0.04^{eC}
Adrenaline	2.93 ± 0.03^{aD}	3.97 ± 0.03^{aC}	4.13 ± 0.03^{aBC}	4.30 ± 0.03^{aB}	4.51 ± 0.04^{aA}	4.70 ± 0.06^{aA}
25mg	2.97 ± 0.03^{aE}	3.32 ± 0.04^{bcD}	3.58 ± 0.03^{cC}	3.92 ± 0.04^{bB}	3.95 ± 0.03^{cB}	4.15 ± 0.03^{cA}
50mg	2.93 ± 0.07^{aD}	3.57 ± 0.03^{bcC}	3.75 ± 0.02^{bC}	4.05 ± 0.05^{abB}	4.15 ± 0.03^{bcB}	4.42 ± 0.03^{bA}
100mg	2.90 ± 0.06^{aB}	3.72 ± 0.04^{abB}	3.85 ± 0.02^{bB}	4.20 ± 0.03^{aB}	4.30 ± 0.03^{abB}	4.50 ± 0.03^{abA}

Effects *R prinoides* at the three concentrations differed significantly on the total percentage change in height of force of contraction of the heart isolated from rabbit in the entire experiment ($p<0.05$; Figure 4.3). The total percentage change in the amplitude (height of force of contraction) of the heart perfused with adrenaline was significantly higher than those ($p<0.05$) in the other treatment groups (Figure 4.3). Effect of acetylcholine noted a significantly lower total percentage change in height of force of contraction compared to those seen ($p<0.05$) in the other treatment groups (Table 4.3). Total percentage change in height of force of contraction in the heart isolated from normal control rabbits was significantly lower than those reported ($p<0.05$) in extract-treated and adrenaline-treated isolated rabbits' heart (Figure 4.3).

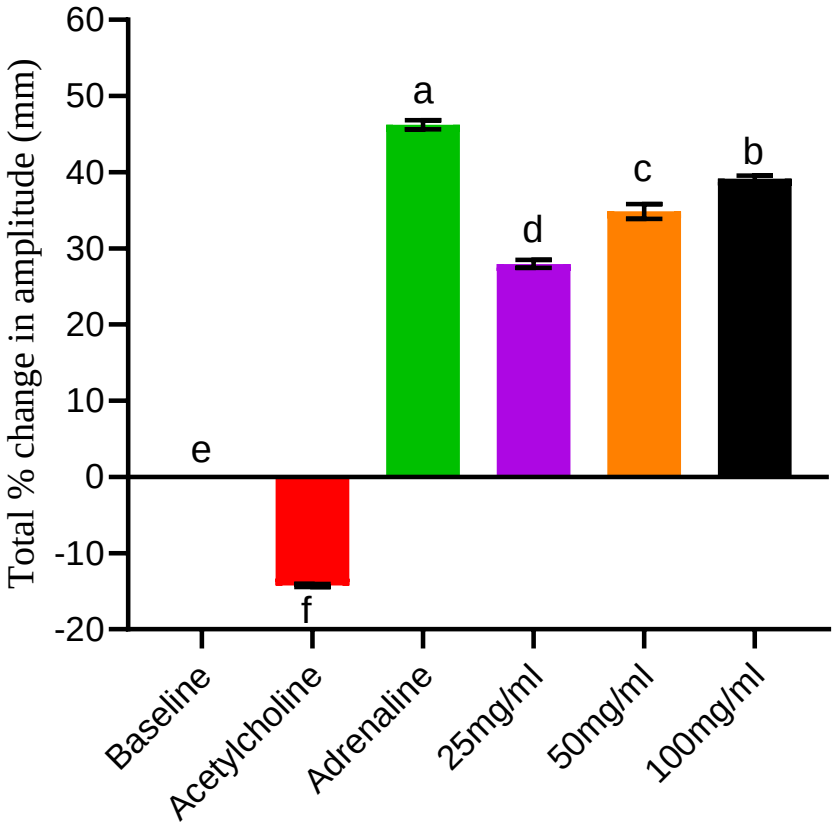

Figure 4.3: Effect of extracts of *Rhamnus prinoides* on total percentage change in height of force of contraction of rabbit's isolated heart.
Bars that do not share a letter are statistically significant. ($p<0.05$).

In comparison, the effect of *S. incanum* extract at the concentrations of 25, 50 and 100mg/mg had a significantly lower total percentage change in height of force of contraction of rabbit's isolated heart compared to percentage changes noted in *R. prinoides* extract at the same concentration ($p<0.05$; Figure 4.4).

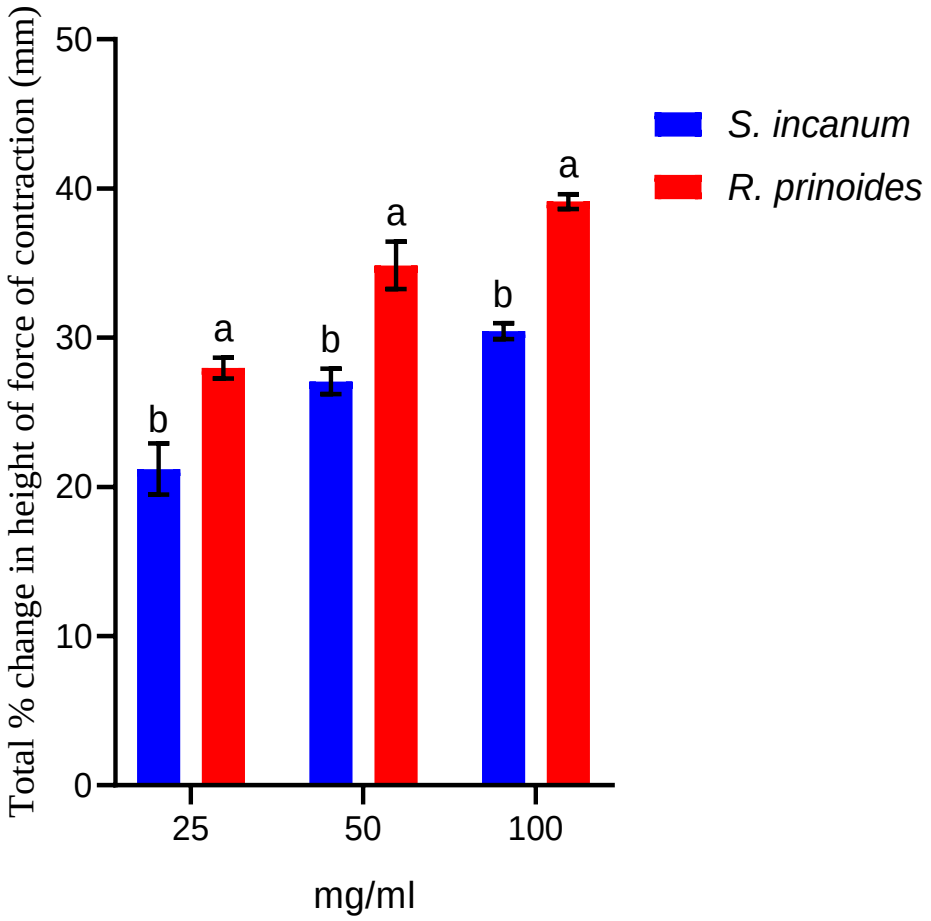

Figure 4.4: Comparison of ionotropic effects *Solanum incanum* and *Rhamnus prinoides* extracts on isolated rabbit's heart.
Bars that do not share a letter are statistically significant using an independent t-test in the same concentration ($p<0.05$).

4.3 Anti-hyperlipidemic Effects of *Solanum incanum* and *Rhamnus prinoides* on high-fat-diet in rats

4.3.1 Effect of Extracts of *Solanum incanum* and *Rhamnus prinoides* on Body Weight Following High-Fat-Diet-Induced Hyperlipidemia in Rats

There were changes in body weights of rats that were given methanolic extracts of *S. incanum* dosages of 25, 50 and 100mg/kg bw following high-fat-diet (HFD)-induced hyperlipidemia (Table 4.5). The body weights of lipidemia control rats were significantly higher compared to those of the other treatment groups ($p<0.05$) in the entire experiment (Table 4.5). Therapy with *S. incanum* extract at the three doses, as well as the reference drug, avastatin reduced the body weights of rats relative to those observed in the hyperlipidemia control rats (Table 4.5). Throughout the experiment, the body weights of normal control rats were significantly lower than those recorded ($p<0.05$) in other treatment groups (Table 4.5). Body weights of rats in all treatment groups significantly increased in the entirely in the study ($p<0.05$; Table 4.5).

Body weights of rats that were administered with *S. incanum* extract at the three doses differed significantly from day 14 of the experiment onwards ($p<0.05$; Table 4.5). The extracts exhibited a dose-dependent response in the reduction of the body weights of rats (Table 4.5). On days 7 and 21 of the experiment, the body weights of rats that received the reference drug, avastatin were not significantly different from those of rats that were administered with *S. incanum* extract at the three studied doses ($p>0.05$; Table 4.5). In addition, on day 14, effect of avastatin on body weights matched statistically with the effect ($p>0.05$) of the extract at the doses of 25mg/kg bw (Table 4.5). Further, on day 28, the body

weights of rats that received avastatin was statistically similar compared to those of rats that were administered with extract ($p>0.05$) doses of 50 and 100mg/kg bw (Table 4.5).

The total percentage weekly change in body weights of rats that received extract at the doses of 50, and 100 mg/kg bw was significantly lower than those of the extract ($p<0.05$) dose of 25mg/kg bw (Table 4.5). The effect of the reference drug, avastatin on the total percentage weekly change in body weights statistically matched with the effect of the extract ($p>0.05$) dose of 25mg/kg bw. The negative control rats had significantly higher total change in the body weights compared to those of ($p<0.05$) the other treatment groups (Table 4.5).

Table 4.5: Effect of *S. incanum* extracts on body weights following high-fat-diet-induced in rats

	Percentage change in body weights (g)				
Treatment	**Day-7**	**Day-14**	**Day-21**	**Day-28**	**Total change**
Baseline	11.32 ± 1.23^{cD}	30.27 ± 0.59^{dC}	54.65 ± 1.06^{dB}	68.85 ± 1.25^{dA}	41.27 ± 0.63^{e}
Negative control	26.52 ± 0.69^{aD}	52.49 ± 0.90^{aC}	81.61 ± 1.21^{aB}	95.34 ± 0.88^{aA}	63.99 ± 0.55^{a}
Avastatin	21.06 ± 0.72^{bD}	42.13 ± 0.94^{bC}	62.31 ± 0.88^{bcB}	79.98 ± 1.00^{cA}	51.37 ± 0.10^{c}
25mg	21.59 ± 0.95^{bD}	45.06 ± 0.72^{bC}	64.62 ± 1.00^{bB}	84.50 ± 0.53^{bA}	53.94 ± 0.37^{b}
50mg	21.38 ± 1.31^{bD}	38.18 ± 0.58^{cC}	60.82 ± 0.64^{bcB}	79.61 ± 0.71^{cA}	50.00 ± 0.25^{cd}
100mg	19.60 ± 0.86^{bD}	36.39 ± 0.58^{cC}	59.63 ± 0.88^{cB}	76.88 ± 0.90^{cA}	48.13 ± 0.46^{d}

Means and SEM that do not share a letter along the column are significantly different. Means and SEM that do not share an uppercase superscript letter are significantly different ($p<0.05$).

HFD = high-fat-diet.

Moreover, following high fat diet induced hyperlipidemia, rats that were given *Rhamnus prinoides* extract doses of 25, 50, and100 mg/kg bw revealed alterations in body weights throughout the experiment (Table 4.6). The negative control rats had significantly higher body weights compared to those ($p<0.05$) of the other treatment groups throughout the experiment (Table 4.6). Treatment with the extract, as well as avastatin reduced the body weights of rats close to that of the normal control rats (Table 4.6). The normal control, negative control, positive control and extract-treated rats revealed a significant elevation ($p<0.05$) in the body weights throughout the experiment (Table 4.6).

The effect of *R. prinoides* extract at the three doses on the body weights differed significantly ($p<0.05$) from day 14 onwards (Table 4.6). The body weights of the rats declined in a dose-dependent manner after therapy with the plant extracts (Table 4.6). The effect of the reference drug, avastatin was statistically equivalent to the effect of the extract at the three doses ($p>0.05$) on day 7 of the experiment (Table 4.6). Similarly, the effect of avastatin on the body weight was not significantly different compared to the effect of the extract at the dosages of 25 and 50mg/kg bw on days 21 and 28 of the experiment ($p>0.05$; Table 4.6).

The total change in percent body weights of rats that received the extract at the three doses differed significantly ($p>0.05$; Table 4.6). The effect of avastatin on the total change in body weights statistically matched with ($p>0.05$) the effect of the extract dose of 25mg/kg bw (Table 4.6). The total percentage weekly change in body weights of the negative control rats was significantly higher compared to ($p<0.05$) those of the other treatment groups (Table 4.6). The normal control rats had significantly lower total percentage change in body

weights compared to the total percentage change ($p<0.05$) of other treatment groups (Table 4.6).

Table 4.6: Effect of *R. prinoides* extract on body weight following high-fat-diet-induced rats

	Percentage change in the body weight (g)				
Treatment	**7th day**	**14th day**	**21st day**	**28th day**	**Total change**
Baseline	11.32 ± 1.23^{cD}	30.27 ± 0.59^{dC}	54.65 ± 1.06^{dB}	68.85 ± 1.25^{dA}	41.27 ± 0.63^{e}
Negative control	26.52 ± 0.69^{aD}	52.49 ± 0.90^{aC}	81.61 ± 1.21^{aB}	95.34 ± 0.88^{aA}	63.99 ± 0.55^{a}
Avastatin	21.06 ± 0.72^{bD}	42.13 ± 0.94^{bC}	62.31 ± 0.88^{bB}	79.98 ± 1.00^{bA}	51.37 ± 0.10^{b}
25mg	22.05 ± 0.46^{bD}	44.09 ± 0.84^{bC}	62.02 ± 0.58^{bB}	82.87 ± 0.69^{bA}	52.98 ± 0.58^{b}
50mg	20.48 ± 0.72^{bD}	37.42 ± 0.58^{cC}	59.20 ± 0.62^{bcB}	79.22 ± 0.67^{bA}	49.08 ± 0.28^{c}
100mg	19.77 ± 0.69^{bD}	34.08 ± 0.82^{cC}	56.99 ± 0.71^{cdB}	72.89 ± 0.71^{cA}	45.93 ± 0.35^{d}

Means and SEM that do not share a letter along the column are significantly different. Means and SEM that do not share an uppercase superscript letter are significantly different ($p<0.05$).

HFD = high-fat-diet.

In comparison, the effects of *S. incanum* and *R. prinoides* at the doses of 25 and 50mg/kg bw on total percentage weekly change in body weights were not significantly different following HFD-induced hyperlipidemia ($p>0.05$) in rats (Figure 4.5). However, the effect of *R. prinoides* extract dose of 100mg/kg bw significantly lowered the total percentage weekly change in body weights compared to the weekly change recorded in *S. incanum* at the same dosage ($p<0.05$; Figure 4.5).

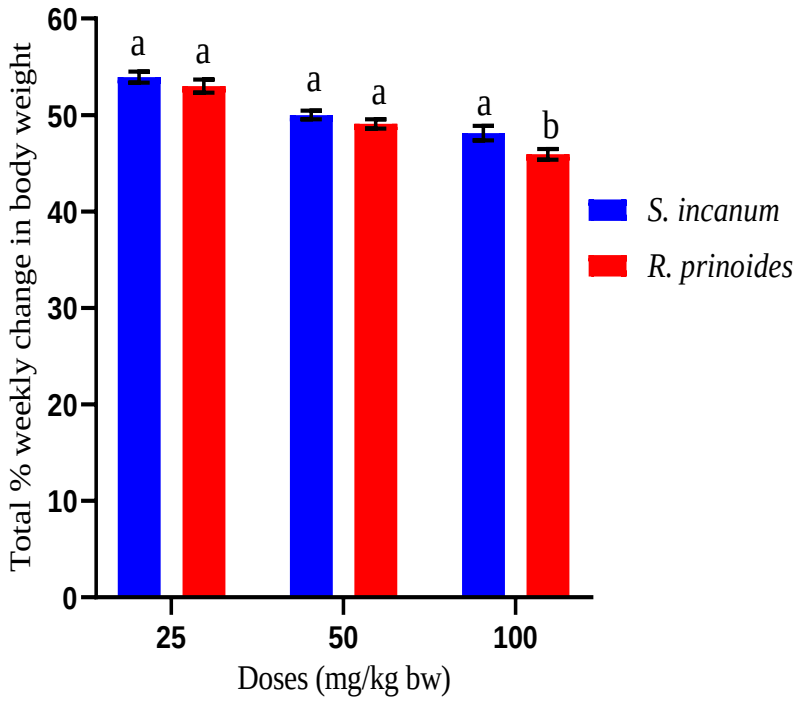

Figure 4.5: Comparison of the effects of extracts of *S. incanum* and *R. prinoides* on total percentage weekly change in body weight following HFD-induced hyperlipidemia in rats. Bars with different letters at the same dosages are significantly different ($p<0.05$).

4.3.2 Effects of *Solanum incanum* and *Rhamnus prinoides* extracts on lipid profiles following high-fat-diet-induced hyperlipidemia in rats.

The effect of *Solanum incanum* caused alterations in levels of high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TGs) and low-density lipoprotein (LDL) following HFD-induced hyperlipidemia in rats (Table 4.7). After induction of hyperlipidemia, the hyperlipidemia control rats revealed a significant reduction in levels of HDL, as well as a substantial increase in the levels of TC, TGs and LDL relative to levels noted ($p<0.05$) in the other treatment groups. However, after therapy with the extract as well as reference drug, the levels of HDL increased, while levels of TC, TGs and LDL reduced close to levels that were observed in normal control rats (Table 4.7). The normal control rats noted significantly lower levels of TC, TGs and LDL ($p<0.05$) compared to other treatment groups (Table 4.7).

The effect of *S. incanum* extract at the dosages of 25, 50 and 100mg/kg bw revealed no significant variations in the levels of HDL, TGs and LDL following HFD-induced hyperlipidemia ($p>0.05$) in rats (Table 4.7). Nonetheless, the effect of the extract at the three tested doses on the levels of TC differed significantly following HFD-induced hyperlipidemia ($p<0.05$) in rats (Table 4.7). The extract ameliorated abnormal levels of HDL, TC, TGs and LDL in a dose-dependent response (Table 4.7). The effect of avastatin matched statistically with ($p>0.05$) the effect of the extract at the three doses on the levels of TC and TGs. Besides, the levels of HDL and LDL in avastatin-treated rats were not significantly different compared to levels of extract-treated rats at the dosages of 25 and 50mg/kg bw ($p>0.05$). Similarly, the HDL levels in normal control rats were non-

significant compared to those extract-treated rats ($p>0.05$) at the doses of 25 and 50mg/kg bw (Table 4.7).

Table 4.7: Effect of extract of *Solanum incanum* on lipid profiles following high-fat diet.

	Lipid profiles (mmol/L)			
Treatment	**HDL**	**TC**	**TGs**	**LDL**
Baseline	1.63 ± 0.12^{a}	1.57 ± 0.11^{d}	0.71 ± 0.70^{c}	0.41 ± 0.07^{d}
Negative control	0.40 ± 0.02^{d}	11.57 ± 0.24^{a}	4.21 ± 0.19^{a}	9.02 ± 0.18^{a}
Avastatin	1.11 ± 0.05^{c}	6.83 ± 0.22^{bc}	2.21 ± 0.23^{b}	4.98 ± 0.28^{b}
HFD + 25mg	1.26 ± 0.10^{bc}	7.38 ± 0.24^{b}	2.23 ± 0.23^{b}	4.77 ± 0.10^{bc}
HFD + 50mg	1.42 ± 0.10^{abc}	7.16 ± 0.17^{b}	1.55 ± 0.19^{b}	4.51 ± 0.14^{bc}
HFD + 100mg	1.52 ± 0.11^{ab}	6.00 ± 0.28^{c}	1.54 ± 0.16^{b}	3.93 ± 0.35^{c}

Means and SEM that do not share a letter along the column are significantly different ($p<0.05$).

On the other hand, therapy with methanol extract of *R. prinoides* doses of 25, 50 and 100mg/kg bw resulted in changes in levels of HDL, TC, TGs and LDL on HFD-induced hyperlipidemia in rats (Table 4.8). The negative control rats had significantly lower levels of HDL and considerably higher TC, TGs and LDL levels compared to those of rats ($p<0.05$) in the other treatment groups (Table 4.8). The effects of the extract at the three tested dosages, as well as the reference drug, avastatin increased the HDL levels and lowered the levels of TC, TGs and LDL close to those of normal control rats (Table 4.8). The therapeutic effect of *R. prinoides* extract at the three doses on the levels of HDL, TC, TGs and LDL differed significantly following HFD-induced lipidemia ($p<0.05$). The effect of *R. prinoides* extract dose-dependently alleviated aberrant levels of HDL, TC, TGs and LDL (Table 4.8). The levels of HDL, TC and LDL in rats treated with avastatin were not significantly different compared to levels seen in rats treated with ($p>0.05$) the extract dose

of 50mg/kg bw. The levels of HDL in normal control rats were statistically equivalent compared to those reported ($p>0.05$) in extract-treated rats at the dose of 25 and 50mg/kg bw (Table 4.8).

Table 4.8: Effect of *R. prinoides* extracts on lipid profiles following high-fat-diet-induced hyperlipidemia in rats

	Lipid profiles (mmol/L)			
Treatment	**HDL**	**TC**	**TRIG**	**LDL**
Baseline	1.63 ± 0.12^{b}	1.57 ± 0.11^{d}	0.71 ± 0.70^{d}	0.41 ± 0.07^{d}
Negative control	0.40 ± 0.02^{d}	11.57 ± 0.24^{a}	4.21 ± 0.19^{a}	9.02 ± 0.18^{a}
HFD + Avastatin	1.11 ± 0.05^{c}	6.83 ± 0.22^{b}	2.21 ± 0.23^{b}	4.98 ± 0.28^{b}
HFD + 25mg	1.44 ± 0.05^{bc}	6.31 ± 0.16^{b}	1.55 ± 0.10^{c}	4.14 ± 0.12^{b}
HFD + 50mg	1.75 ± 0.10^{b}	4.40 ± 0.40^{c}	1.10 ± 0.05^{cd}	3.04 ± 0.43^{c}
HFD + 100mg	2.19 ± 0.12^{a}	3.54 ± 0.08^{c}	0.94 ± 0.05^{d}	2.23 ± 0.26^{c}

Means and SEM that do not share a letter along the column are significantly different ($p<0.05$).
HFD: High fat diet

Comparatively, the effects of *S. incanum* and *R. prinoides* extracts dose of 25mg/kg bw on HDL were not significantly different following HFD-induced hyperlipidemia in rats ($p>0.05$). Nonetheless, at the doses of 50 and 100mg/kg bw in rats treated with *R. prinoides*, the levels of HDL were significantly higher compared to those observed in *S. incanum* extract at the equivalent dose ($p<0.05$; Figure 4.6). Further, the levels of TC, TGs and LDL in rats treated with *S. incanum* doses of 25, 50 and 100mg/kg bw were significantly higher than those of rats treated with *R. prinoides* at the same dosage ($p<0.05$; Figure 4.6).

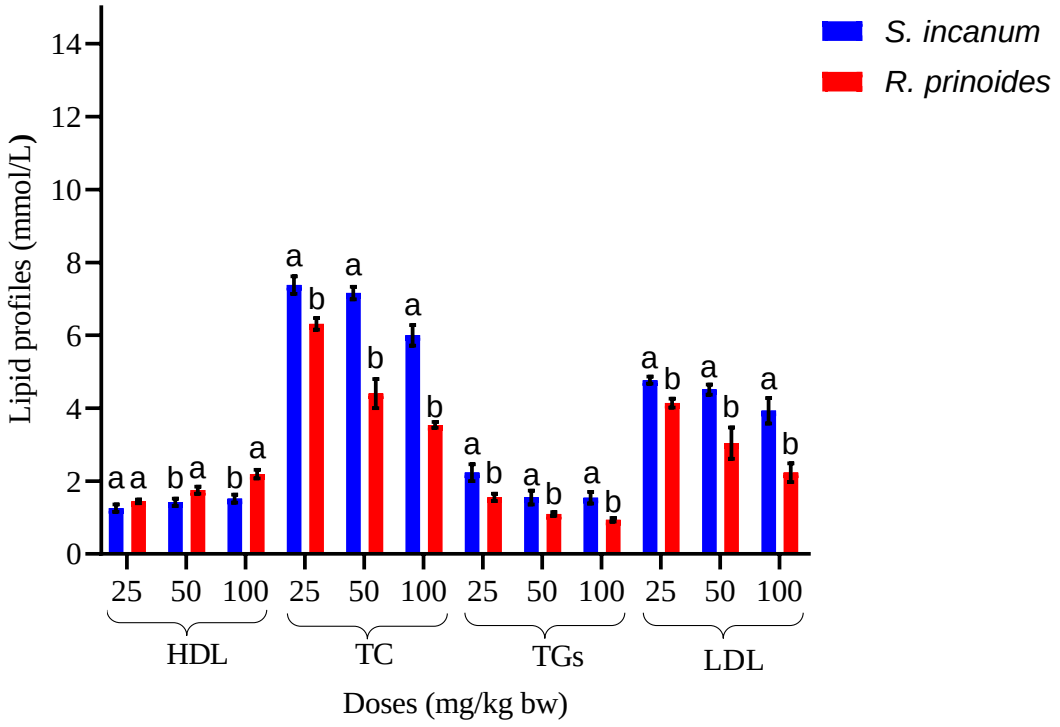

Figure 4.6: Comparison between effects of *S. incanum* and *R. prinoide*s extracts on lipid profiles following HFD-induced hyperlipidemia in rats.
Bars with different letters at the same dosages are significantly different ($p<0.05$).

4.4 Acute and Sub-Acute Toxicity Profiles of Methanol Extracts of *Solanum incanum* and *Rhamnus prinoides* in mice

4.4.1 Acute Toxicity Effects of *Solanum incanum* and *Rhamnus prinoides* Extracts in Mice

Mice that were administered with methanol extracts of *solanum incanum* and *Rhamnus prinoides* doses of 1000 and 2000mg/kg bw did not show any fatalities or signs and symptoms of toxicity after a single dose exposure for 14 days. The extracts did not exhibit any toxicological effects, including diarrhea, altered skin and fur texture, change in

breathing pattern, or drug-related behavioral abnormalities such as agitation, aggression, convulsions, paralysis, clumping together, and atypical movement.

4.4.2 Sub-acute Toxicity Effects of Methanol Extracts of *Solanum incanum* and *Rhamnus prinoides* in Mice

4.4.2.1 Effects *Solanum incanum* and *Rhamnus prinoides* extracts on body weights of mice

There were changes in body weights of mice that were administered with methanol extract *S. incanum* doses of 100, 174 and 300mg/kg bw for 28 consecutive days (Table 4.9). The body weights of all the experimental animals increased significantly during the entire experiment ($p<0.05$; Table 4.9). On days 7 and 14 of the experiment, the body weights of mice that received *S. incanum* extract at the three doses were not significantly different from those ($p>0.05$) of normal control mice (Table 4.9). However, on days 21 and 28, the body weights of mice that were given the extract dose of 300mg/kg bw were significantly lower than those ($p<0.05$) of normal control mice (Table 4.9). The total percentage weekly change in body weights of mice that received extract at the three tested doses were statistically insignificant from those ($p>0.05$) of normal control mice (Table 4.9).

Table 4.9: Effect of *S. incanum* extracts on body weight in mice

Percentage change (body weights) (g)				
DAYS	**Baseline**	**100mg/kg**	**174mg/kg**	**300mg/kg**
7th day	7.88 ± 0.84^{aD}	5.91 ± 1.07^{aD}	6.09 ± 1.00^{aD}	5.91 ± 1.00^{aD}
14th day	14.08 ± 1.04^{aC}	15.12 ± 0.99^{aC}	14.91 ± 1.06^{aC}	16.12 ± 0.92^{aC}
21st day	28.12 ± 1.30^{aB}	25.24 ± 0.39^{abB}	25.44 ± 0.77^{abB}	24.56 ± 0.74^{bB}
28th	35.13 ± 0.58^{aA}	33.58 ± 0.93^{abA}	32.48 ± 1.11^{abA}	31.34 ± 0.85^{bA}
Total change	21.30 ± 0.50^{a}	19.96 ± 0.38^{a}	19.73 ± 0.42^{a}	19.48 ± 0.63^{a}

Means and SEM that do not share a letter along the row are significantly different. Means and SEM that do not share an uppercase superscript letter along the column are significantly different ($p<0.05$).

Moreover, the mice that were given the *R. prinoides* extract doses of 100, 174, and 300mg/kg bw had alterations in body weights in the entire experiment (Table 4.10). The body weights of mice in the normal control, as well as mice administered with extract at the three doses significantly increased from day 7 to day 28 ($p<0.05$) of the experiment (Table 4.10). The effect of *R. prinoides* extract at the three doses showed no significant difference in body weights on days 7, 14 and 21 ($p<0.05$) of the experiment (Table 4.10). However, on day 28, the body weights of mice that were administered with extract at the dose of 300mg/kg bw were significantly lower than those ($p<0.05$) of the normal control mice (Table 4.10). The total percentage weekly change in body weights of mice that received the extract at the three doses were statistically insignificant ($p>0.05$; Table 4.10).

Table 4.10: Effect of *R. prinoides* extracts on body weight in mice

Percentage change in body weight (g)				
DAYS	**Baseline**	**100mg/kg**	**174mg/kg**	**300mg/kg**
7th day	7.87±0.84^{aD}	6.29±1.04^{aD}	6.26±1.09^{aD}	6.17±1.04^{aD}
14th day	14.08±1.04^{aC}	15.15±0.94^{aC}	15.22±1.13^{aC}	14.20±1.02^{aC}
21st day	28.12±1.30^{aB}	25.95±1.15^{aB}	25.02±0.92^{aB}	24.76±0.91^{aB}
28th	35.13±0.58^{aA}	33.07±1.18^{abA}	31.24±0.98^{abA}	31.85±0.46^{bA}
Total change	21.30±0.50^{a}	20.12±0.50^{a}	19.43±0.72^{a}	19.24±0.65^{a}

Means and SEM that do not share a letter along the row are significantly different. Means and SEM that do not share an uppercase superscript letter along the column are significantly different ($p<0.05$).

4.4.2.2 Effects of *Solanum incanum* and *Rhamnus prinoides* on Relative Organ Weights in Mice

The mice that received *S. incanum* extract doses of 100, 174 and 300mg/kg bw revealed no significant variations in relative organ weights of the spleen, kidney, kidney, brain, lungs, and heart and were comparable with those ($p>0.05$) of the normal control mice (Table 4.11).

Table 4.11: Effects of *S. incanum* on relative organ weights in mice

Relative organ weights (%)						
Treatment	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Heart**	**Brain**
Baseline	4.90±0.41	1.42±0.11	0.63±0.04	0.68±0.04	0.47±0.40	1.01±0.05
100mg/kg	4.91±0.43	1.45±0.86	0.95±0.22	0.66±0.05	0.51±0.03	0.96±0.20
174mg/kg	4.37±0.22	1.52±0.12	1.14±0.10	0.63±0.01	0.45±0.03	0.87±0.10
300mg/kg	5.33±0.27	1.18±0.63	1.13±0.13	0.67±0.02	0.47±0.30	0.98±0.03

Values in the same column are not statistically significant using one- way ANOVA ($p>0.05$).

On the other hand, the mice that received *R. prinoides* extract at the dosages of 100, 174 and 300mg/kg bw showed no significant variations in the relative organ weights of the liver, lungs, spleen, kidney, heart and brain and were statistically similar compared to those ($p>0.05$) of normal control mice (Table 4.12)

Table 4.12: Effects of *R. prinoides* extracts on relative organ weights in mice

Relative organ weight (%)						
Treatment	**Liver**	**Kidney**	**Spleen**	**Lungs**	**Heart**	**Brain**
Baseline	4.90±0.41	1.42±0.11	0.63±0.43	0.67±0.04	0.47±0.04	1.01±0.05
100mg/kg	5.20±0.33	1.36±0.12	0.91±0.73	0.74±0.46	0.58±0.05	0.98±0.01
174mg/kg	5.32±0.47	1.30±0.09	0.84±0.14	0.75±0.50	0.55±0.06	1.04±0.04
300mg/kg	6.03±0.34	1.32±0.12	0.97±0.14	0.83±0.70	0.60±0.07	1.05±0.07

Values in the same column are not statistically significant ($p>0.05$).

4.4.2.3 Effects of Methanol Extracts of *Solanum incanum* and *Rhamnus prinoides* on hematological Parameters in Mice

The mice that were administered with methanol extract at the doses of 100, 174 and 300mg/kg bw showed no significant variations ($p>0.05$) in the levels of total WBC, RBCs, HGB, HCT, MCV, RDW, PLT, MPV, PDW and PCT. The levels of these parameters statistically matched with those observed ($p>0.05$) in the normal control mice (Table 4.13).

Table 4.13: Effect of *S. incanum* on hematological parameters in mice

Treatment	Baseline	100 mg/kg	174 mg/kg	300 mg/kg
WBCs (*10^9/L)	10.12±0.59	9.83±0.41	10.37±0.52	11.23±0.50
RBCs (*10^12/L)	11.11±0.27	10.65±0.33	10.72±0.33	10.57±0.44
HB (g/dL)	16.10±0.42	16.28±0.54	15.90±0.42	16.12±0.37
HCT (%)	60.73±1.43	60.63±1.73	60.87±1.50	60.00±2.19
MCV (fL)	54.58±0.76	56.98±0.78	56.84±0.77	56.78±0.46
RDW (%)	19.79±0.48	20.38±0.66	20.34±0.57	19.62±0.46
PLT (*10^9/L)	63.58±1.21	63.80±0.77	64.47±1.81	62.45±2.07
MPV (fL)	7.56±0.26	7.74±0.23	7.60±0.14	7.54±0.21
PDW (fL)	10.30±0.41	9.93±0.27	9.56±0.57	9.06±0.80
PCT (%)	0.68±0.06	0.70±0.06	0.81±0.05	0.80±0.02

Values in the same row are not statistically significant ($p>0.05$).

The levels of total WBC, HB, RBC, HCT, RDW, MCV, PLT, MPV, PCT and PDW did not differ significantly in mice that were administered with *R. prinoides* extract doses of 100, 174, and 300mg/kg bw and were comparable to levels seen in normal control mice ($p>0.09$; Table 4.14).

Table 4.14: Effect of *R. prinoides* on hematological parameters in mice

Treatment	Baseline	100mg/kg	174mg/kg	300mg/kg
WBC (*10^9/L)	10.12±0.59	9.54±1.07	11.21±0.23	11.43±0.49
RBC (*10^12/L)	11.11±0.27	10.64±0.37	10.32±0.17	10.25±0.16
HGB (g/dL)	16.10±0.46	15.56±0.15	16.00±0.44	16.40±0.38
HCT (%)	60.73±1.43	59.93±2.32	57.69±1.31	58.16±0.97
MCV (fL)	54.68±0.76	56.38±0.78	55.38±0.52	56.70±0.38
RDW-CV (%)	19.74±0.48	21.27±0.33	20.32±0.93	19.52±0.92
PLT (*10^9/L)	63.58±1.21	63.30±1.9	64.36±1.02	66.00±0.94
MPV (fL)	7.56±0.26	7.34±0.23	5.21±1.79	7.44±0.29
PDW (fL)	10.30±0.41	9.64±0.40	10.35±0.32	10.46±0.19
PCT (%)	0.68±0.06	0.74±0.02	0.73±0.03	0.78±0.02

Values in the same row are not statistically significant ($p>0.05$).

4.4.2.4 Effects of *Solanum incanum* and *Rhamnus prinoides* on Liver Function and Renal Function Tests in Mice

The levels of ALP, AST, ALT, TP, ALB, TBIL, DBIL, CREAT, urea, Na^+, K^+, Cl^-, Ca and P did not differ significantly in mice that were administered with *S. incanum* at the doses of 100, 174 and 300mg/kg bw and were comparable to levels seen ($p>0.05$) in the normal control mice (Table 4.15).

Table 4.15: Effect of *S. incanum* on liver function and renal function tests in mice

Treatment	**Baseline**	**100mg/kg**	**174mg/kg**	**300 mg/kg**
ALP (U/L)	88.00±0.83	89.20±0.37	87.40±0.92	87.20±0.58
AST (U/L)	274.80±1.30	275.80±0.58	275.60±0.81	278.80±1.16
ALT(U/L)	117.80±0.73	117.20±0.73	117.40±0.67	118.60±0.74
TP(U/L)	6.70±0.11	6.72±0.05	6.82±0.12	6.94±0.10
ALB(U/L)	3.55±0.11	3.48±0.14	3.56±0.03	3.66±0.34
TBIL(U/L)	25.40±0.51	26.20±0.37	26.80±0.49	27.20±0.66
DBIL(U/L)	10.72±0.18	10.80±0.22	11.28±0.26	11.12±0.38
CREAT (µmol/L)	64.18±0.90	66.22±0.84	64.84±0.23	63.28±0.50
Urea (µmol/L)	524.20±7.15	518.20±1.74	517.40±3.19	525.49±6.40
Na^+ (µmol/L)	143.20±1.08	142.80±0.58	142.80±0.80	144.40±0.60
K^+ (µmol/L)	5.10±0.34	4.68±0.23	4.82±0.03	5.18±0.07
Cl^-(µmol/L)	105.80±0.58	105.60±0.40	105.20±0.49	106.00±0.44
Ca (µmol/L)	2.31±0.09	2.17±0.06	2.21±0.03	2.56±0.12
P (µmol/L)	1.45±0.12	1.41±0.11	1.67±0.08	1.67±0.10

Values in the same row are not statistically significant ($p>0.05$).

The mice that were given *R. prinoides* extract at the dosages of 100, 174 and 300mg/kg bw did not differ significantly on levels of ALP, AST, ALT, TP, ALB, TBIL, DBIL, CREAT, urea, Na^+, K^+, Cl^-, Ca and P and were comparable to levels ($p>0.05$) seen in the normal control mice (Table 4.16).

Table 4.16: Effects of *R. prinoides* on liver function and renal function tests in mice

Treatment	Baseline	100 mg/kg	174 mg/kg	300 mg/kg
ALP (U/L)	88.00±0.83	89.20±0.37	87.40±0.92	87.20±0.58
AST (U/L)	274.80±1.30	275.80±0.58	275.60±0.81	278.80±1.16
ALT(U/L)	117.80±0.73	117.20±0.73	117.40±0.67	118.60±0.74
TP(U/L)	6.70±0.11	6.72±0.05	6.82±0.12	6.94±0.10
ALB(U/L)	3.55±0.11	3.48±0.14	3.56±0.03	3.66±0.34
TBIL(U/L)	25.40±0.51	26.20±0.37	26.80±0.49	27.20±0.66
DBIL(U/L)	10.72±0.18	10.80±0.22	11.28±0.26	11.12±0.38
CREAT (µmol/L)	64.18±0.90	64.30±0.31	64.40±0.60	65.50±0.32
Urea (µmol/L)	524.20±7.15	525.00±7.45	518.80±4.93	530.40±5.66
Na^+ (µmol/L)	144.80±1.08	144.80±0.49	143.80±0.37	144.60±0.51
K^+ (µmol/L)	5.10±0.34	5.60±0.26	4.76±0.09	5.08±0.03
Cl^-(µmol/L)	105.80±0.53	105.40±0.24	105.00±0.01	106.20±0.37
Ca (µmol/L)	2.31±0.09	2.40±0.09	2.57±0.14	2.74±0.17
P (µmol/L)	1.45±0.12	1.37±0.02	1.31±0.09	1.67±0.17

Values in the same row are not statistically significant (p>0.05).

4.4.2.5 Effect *S. incanum* and *R. prinoides* Extracts on Lipid Profiles in Mice

The mice that received *S. incanum* extract at the doses of 100, 174 and 300mg/kg bw did not significantly alter the levels of HDL, LDL, TGs and TC and were comparable to levels observed (p>0.05) in the normal control mice (Table 4.17).

Table 4.17: Effect of *S. incanum* extracts on lipid profiles in mice

Treatment (mmol/L)	Baseline	100 mg/kg	174 mg/kg	300 mg/kg
CHOl	1.70±0.12	1.47±0.10	1.74±0.14	1.32±0.09
HDL	0.86±0.05	0.97±0.05	0.94±0.08	1.10±0.05
TRIG	0.68±0.05	0.64±0.04	0.83±0.08	0.68±0.06
LDL	0.36±0.03	0.16±0.01	0.20±0.03	0.24±0.02

Values in the same row are not statistically significant (p>0.05).

The levels of TC, HDL, TGs and LDL did not differ significantly in mice that were administered with *R. prinoides* at the doses of 100, 174 and 300mg/kg bw and were compared to levels seen (p>0.05) in the normal control mice (Table 4.18).

Table 4.18: Effects of *R. prinoides* on lipid profiles in mice

Treatment (mmol/L)	**Baseline**	**100mg/kg**	**174mg/kg**	**300mg/kg**
CHOL	1.69±0.12	1.49±0.07	1.29±0.16	1.24±0.12
HDL	0.86±0.05	0.82±0.07	0.88±0.46	0.90±0.04
TGs	0.68±0.05	0.60±0.05	0.51±0.08	0.49±0.05
LDL	0.36±0.03	0.30±0.05	0.29±0.04	0.26±0.03

Values in the same row are not statistically significant (p>0.05).

4.5 Qualitative Phytochemical Analysis of *Solanum incanum* and *Rhamnus prinoides* extracts

The qualitative analysis of *S. incanum* noted the presence of flavonoids, alkaloids, terpenoids, saponins, glycosides, phenolics and steroids. Similarly, the analysis of *R. prinoides* showed presence of terpenoids, alkaloids, saponins, glycosides, phenolics and steroids (Table 4.19).

Table 4.19: Qualitative phytochemical analysis of *Solanum incanum* and *Rhamnus prinoides* extracts.

Secondary metabolites	***Solanum incanum***	***Rhamnus prinoides***
Saponins	+	+
Alkaloids	+	+
Terpenoids	+	+
Flavonoids	+	-
glycosides	+	+
Steroids	+	+
Phenolics	+	+

+ = presence; - = absence

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Globally, cardiovascular diseases are the leading causes of mortality (Tsao *et al*., 2023; WHO, 2023). Estimates of World Health Organization (WHO), 17.9 million fatalities were attributed to cardiovascular diseases in 2019 globally, representing 32% of total deaths. More than 75 percent of the victims are in middle and low-income nations (WHO, 2023). Congestive heart failure is one of the most predominant clinical manifestations of cardiovascular diseases. It occurs when the cardiac output is insufficient to supply nutrients and oxygen to organs and tissues in the body (Rajendar *et al*., 2017).

Hyperlipidemia, including hypertension and obesity, increases the risk for congestive heart failure (Alembagheri *et al*., 2022). Hyperlipidemia is a medical disorder characterized by elevated levels of blood lipids such as low-density lipoproteins, cholesterol and triglycerides, as well as reduced levels of high-density lipoproteins (Hassan *et al*., 2022). A deficit in lipoprotein lipase function or a lack of surface apoprotein C-II, increment in ingestion of high-fat-eating regimens, genetic anomalies, hypothyroidism, and environmental factors are some of the causes of hyperlipidemia (Ibukun and Omoregie, 2018; Mengistu *et al*., 2019; Hassan *et al*., 2022).

Several drugs with cardiotonic effects such as digitoxin, enoximone, amrinone, piroximone, and milrinone are highly prescribed to treat heart failure (Schure and DiNardo, 2019; Satria *et al*., 2023). Cardiotonic medications improve the efficiency and contraction of the heart muscles, thereby increasing blood flow to all body tissues (Satria *et al*., 2023). On the other hand, anti-hyperlipidemia drugs such as avastatin, fibrates, atorvastatin,

Fluvastatin and lovastatin are often prescribed to treat hyperlipidemia (Huang *et al*., 2018; Ibukun and Omoregie, 2022). However, synthetic cardiotonic and anti-hyperlipidemia drugs are associated with severe effects, thus limiting their use (Huang *et al*., 2018; Alembagheri *et al*., 2022). These necessitate the need for alternative cardiotonic and anti-hyperlipidemia agents.

Over the years, medicinal plants have served as essential bioresources for the management of many diseases and disorders (Hassan *et al*., 2022; Keihanian *et al*., 2023). It is therefore reasonable to refer to medicinal plants as a sleeping giant for potential development of novel therapeutic agents. The Kikuyu and Maasai communities residing in Kiambu and Narok Counties in Kenya use *Solanum Incanum* and *Rhamnus prinoides* in the management of both cardiovascular diseases and hyperlipidemia, respectively. However, there was a paucity of scientific evidence to validate these claims. This study aimed at determining the cardiotonic and anti-hyperlipidemia activities, as well as acute and subacute toxicity effects of methanol extracts of *Solanum Incanum* and *Rhamnus prinoides*.

The effect of *S. Incanum* and *R. prinoides* noted a significant decrease in heart rate (negative chronotropic effect) and an increase in height of force of contraction (positive ionotropic effect) on rabbit isolated heart compared to values noted in the normal control from the first minute of the experiment. According to these findings, the two extracts had cardiotonic effects without causing cardiac arrest even at high doses. The effect of adrenaline revealed a significant elevation of the heart rate and amplitude of contraction compared to the effect of the extracts at the three concentrations from the first minute of the experiment onwards. The effect of acetylcholine reported a significant decrease in the

heart rate and the amplitude (height of force of contraction) relative to the effect of the extracts from the first minute of the study onwards.

The effects of the two extracts enhanced the height of force of contraction and reduced the heart rate in a concentration-dependent manner. This implied that an increase in concentration enhanced contractility and lowered the heart rate. Similar studies have also reported similar findings. For instance, a study by Mohire *et al*. (2007) reported a significant decrease in heart rate with an increase in force of contraction upon the increase in the concentrations of aqueous extract of *Pterocarpus marsupium*. Besides, a study by Akhtar *et al*. (2013) also showed that an increase in the concentration of methanol extract of *Saussurea lappa* resulted in a significant increase in the height of force of contraction with decrease in heart rate, although contractility started decreasing at higher doses. Further, Rajendar *et al*. (2017) also demonstrated that an increase in the concentration of methanol extract of *Moringa oleifera* had a significant increase in the amplitude and a decrease in heart rate.

The spatiotemporal regulation of intracellular Ca^{2+} levels is crucial for cardiac function. When the plasma membrane depolarizes, voltage-dependent Ca^{2+} channels are opened, allowing an influx of Ca^{2+} into the cell to initiate the contraction cycle. Ca^{2+} is released from the SR (sarcoplasmic reticulum) when Ca^{2+} levels in the dyadic cleft, the small area between the plasma membrane and the SR, rise. This is accomplished by the resident Ryanodine receptor (RyR) in the SR opening. The contraction mechanism of cardiac cells is activated as a result of the rise in cytoplasmic Ca^{2+}. To relax, intracellular Ca^{2+} levels must fall to their resting level. The plasma membrane Na^{+}-Ca^{2+} exchanger (NCX) and the

SR Ca^{2+}-ATPase (SERCA) work together to achieve this activity. The Ca^{2+} ions can be moved by both transporters despite gradients in concentration. To complete this process, NCX is fueled by the electrochemical gradient of Na^{+} to remove Ca^{2+} from the cell, while SERCA hydrolyzes ATP to replenish the SR with Ca^{2+} (Ottolia *et al*., 2021).

Congestive heart failure occurs due to improper utilization of Ca^{2+} or Ca^{2+} insufficiency in the cells (Ottolia *et al*., 2021). Drugs that raise intracellular Ca^{2+} levels therefore usually demonstrate beneficial cardiotonic effects. Various drugs' positive inotropic and negative chronotropic effects can be mediated via phosphodiesterase (PDE) inhibition, direct activation of β1-adrenoceptors, or inhibition of Na^{+}/K^{+} ATPase (Mahmood *et al*., 2013; Khan *et al*., 2016; Bbosa *et al*., 2019). The positive ionotropic effects that were exhibited by the studied extracts were therefore attributed to cardiac stimulation or through increased availability of intracellular Ca^{2+} by opening membrane L-type Ca^{2+} channels or via other mechanisms such as inhibition of potassium channels and β1-adrenoceptors. This demonstrates the possibility that the extract's mechanism of action was through reservation of the $Na^{+/}K^{+}$ pump activities. This inhibition raises the levels of Ca^{2+} available for the contraction of the heart muscles, improving cardiac output and lowering heart rate.

Cardiac insufficiency is the inability of the heart to pump enough blood to adequately provide organs and tissue with oxygen and nutrients. This condition causes shortness of breath and fatigue (Satria et al., 2023). Cardiotonic agents are drugs-agents that causes an increase on the contractile strength of muscle of the heart (myocardium) and enhance its capability and efficiency. Positive inotropic and negative chronotropic effects are directly related to cardiotonic activities. The total Blood volume (volemia) exiting the left heart

ventricle increases with an increase in the force of myocardial contraction. As a result, the cardiac output (the amount of blood exiting the left ventricle with each contraction) also increases, which causes an increased blood flow in all body organs (Somade *et al*., 2017; Satria *et al*., 2023). A decrease in heart rate upon administration of the extracts could be explained by an elevation of amplitude (height of the force of contraction).

The cardiotonic activities of the two extracts can be attributed to the classes of phytocompounds that were identified using qualitative analysis. The two extracts noted the presence of glycosides, flavonoids, phenolics, saponins, alkaloids, terpenoids and steroids, except flavonoids in *R. prinoides* extract. This could explain why *S. incanum* extract had a better cardiotonic effect compared to *R. prinoides* extract. The phytochemical classes of flavonoids, steroids, glycosides phenolics and terpenoids have been associated with cardiotonic effects (Akhtar *et al*., 2013; Khan *et al*., 2016; Somade *et al*., 2017; Satria *et al*., 2023). For instance, glycosides inhibits Na^+/K^+ ATPase resulting in increased intracellular Ca^{2+} concentrations via Na^+/Ca^{2+} exchange. This causes both the transient and slow inward Ca^{2+} influx to rise (Karumuri *et al*., 2011). Besides, flavonoids (such as kaempferol and quercetin) have also been shown to increase cardiac muscle contraction (Satria *et al*., 2023).

In this study Adrenaline was used as a positive control. Adrenaline caused a significant cardiotonic activity by significantly increasing the contractility of the heart muscle and heart rate compared to the effect of the extract. However, adrenaline usually overstimulates and overworks the heart causing a drop in contractility of heart muscle and heart rate (Bbosa *et al*., 2019). An exceptionally high dose of adrenaline may result in cardiac arrest

or even tissue death due to a lack of oxygen and nutrients (Shattock *et al*., 2015; Bbosa *et al*., 2019).

Conversely, acetylcholine was used as the negative control. Acetylcholine typically slows the sinus node's rate of rhythm and the irritability of the fibers at the Atrioventricular node junction, which slows the transit of cardiac impulses. Acetylcholine causes a rapid potassium efflux out of the conductive fibers thus increasing the ease at which potassium ion can pass through the plasmalemma or the muscle nerve fibers. As a result, the fibers become hyperpolarized and considerably less excitable. This reduces the intensity and frequency of cardiac contractions. In the sinus node, hyperpolarization reduces the resting potential difference across the membrane. This leads to lengthening the time to cross for excitation to occurs. The sinus rhythm may be completely stopped at high acetylcholine concentrations (Shattock *et al*., 2015; Bbosa *et al*., 2019).

The current research study noted cardiotonic activities of *S. incanum* and *R. prinoides* on rabbit isolated hearts. The extracts produced negative chronotropic and positive ionotropic activities. The possible mechanism of the two extracts could be due to the inhibition of the Na^+/K^+ ATPase pump. The cardiotonic effects of the extracts were associated with the phytocompounds such as saponins, alkaloids, glycosides, flavonoids, terpenoids, steroids and phenolics that were detected using qualitative analysis. Additionally, research on bioassay fractionation and purification may reveal pharmacologically active phytoconstituents responsible for cardiotonic effects, as well as elucidate the mechanism of action underlying the increased height of force of contraction and the negative chronotropic effects.

This study also found that methanol extract of *S. incanum* and *R. prinoides* had anti-hyperlipidemia activity following HFD-induced hyperlipidemia in rats. This was noted through a reduction in body weights as well as amelioration of aberrant levels of lipid profiles following HFD-induced hyperlipidemia in rats. Similarly, the reference drug, avastatin also revealed anti-hyperlipidemia activity and its effect was comparable to the effects of the two studied extracts.

Hyperlipidemia is a lipid metabolism disorder manifested through elevation of levels of plasma TGs, cholesterols, cholesterol esters, lipoproteins, and phospholipids lipoproteins including low-density lipoprotein (LDL) and very low-density lipoproteins (vLDL) and reduced levels of circulating high density lipoproteins (Hassan *et al*., 2022). It is known as a huge factor in the onset and progression of coronary heart disease, arteriosclerosis, ischemic heart disease, or cardiovascular ailments, as well as myocardial infarction (Adekiya *et al*., 2018). Two types of lipidemia exist: primary and secondary lipidemia. Genetic factors affecting lipoprotein transport, receptors, and enzymes are the cause of primary hyperlipidemia. Secondary hyperlipidemia, also known as acquired hyperlipidemia, is caused by modifications in blood lipid levels as a result of other dysfunctions such as nutrition disturbances, diabetes, hypertension, hypothyroidism, nephrosis, and the prolonged use of corticosteroids and oral contraceptives (Hassan *et al*., 2022).

Endogenous and exogenous lipids are main source of blood lipids. Endogenous lipids are made in the hepatocytes while the exogenous lipids are ingested and eventually absorbed. A lipid panel test is used preliminarily for clinical screening of lipid-based abnormalities,

this is useful in diagnosis of specific disease conditions such as to ascertain disease risk factors, and to assess therapeutic agents (Sinaga *et al*., 2022). Due to modern lifestyles that increase the consumption of high-fat eating habits, the prevalence of hyperlipidemia has drastically increased throughout the world. Globally, the prevalence of hyperlipidemia is about 39% with a prevalence of 26% in developing countries (Mengistu *et al*., 2019). According to reports, hyperlipidemia affects approximately one-fourth of myocardial infarction patients (Ashfaq *et al*., 2017).

In many research studies, HFD is used to induce hyperlipidemia in animal models (Ibukun and Omoregie, 2022; Liu *et al*., 2023). Cooking oil, egg yolk and bile salts are fed to rats to raise their cholesterol (Sinaga *et al*., 2022). In this study, the induction of hyperlipidemia using HFD resulted in increased body weights and elevated levels of TGs, cholesterol and LDL, with reduced levels of HDL in hyperlipidemia control rats relative to values noted in the normal control rats. Animals fed on HFD may have elevated LDL levels due to alterations in hepatic LDL receptors. Additionally, it results in oxidative stress, which raises the levels of oxidized LDL (Ashfaq *et al*., 2017; Ibukun and Omoregie, 2022). A high-fat food supplemented and slowed LDL clearance due to a deficiency in LDL receptors are likely to result in higher serum TC levels that are above normal ranges (Hassan *et al*., 2022)

Avastatin is a highly prescribed drug in the treatment of dyslipidemia and therefore the choice as reference drug in this study (Dagli-Hernandez *et al*., 2022). Avastatin belongs to a class of drugs called "statins." It enhances the biosynthesis of HDL, as well as lower the levels of low-density lipoproteins, total cholesterol, triglycerides, and very low-density lipoproteins. Statin drugs usually suppress HMG-CoA reductase (Sizar *et al*., 2022). Drug statins lower the synthesis of cholesterol in the liver by suppressing the conversion of HMG-CoA. Besides, statin drugs increase the percentage of LDL receptors on hepatic cell surfaces (Bouitbir *et al*., 2020; Schweitzer). However, avastatin is associated with several side effects such as liver cirrhosis, muscle weakness and high blood sugar (Ward *et al*., 2019).

Therapy with the methanol extracts of *S. incanum* and *R. prinoides* as well as reference avastatin demonstrated improvements in levels of lipid profiles and body weights of rats close to those observed in the normal control rats. The lipid-lowering effects of the extracts in hyperlipidemic control rats could results to decreased bile acid excretion and suppression of cholesterol biosynthesis. This could be attributed to the phytochemicals that were detected through qualitative phytochemical analysis. The qualitative phytochemical analysis of the two plant extracts noted presence of glycosides, phenolics, terpenoids, flavonoids, saponins, steroids and alkaloids, except for flavonoids in *R. prinoides* extract. Through activation of cellular antioxidants and lowering cellular oxygenase, flavonoids can lower macrophage oxidative stress and LDL lipid oxidation (Sinaga *et al*., 2022). Besides, flavonoids have been reported to enhance the levels of HDL as well as affect the function of reverse cholesterol transport (Lacorte *et al*., 2021).

Saponins can bind to dietary cholesterol to form insoluble complexes, as well as colic acid (bile) in the formation of micelles. This enhances the ability of the fiber to bind to cholesterol, these limits the gut from absorbing cholesterol (Mengistu *et al*., 2019; Sinaga *et al*., 2022). A study by Zalar *et al*. (2022) reported that alkaloids can lower plasma levels of low-density lipoproteins, triglycerides and total cholesterol, while increasing high density lipoproteins levels. This is achieved through the inhibition of pancreatic lipolytic enzymes, which helps in the conversion of triglycerides into glycerol and fatty acids, terpenoids and flavonoids have been documented to lower levels of low-density lipoproteins, total cholesterol, triglycerides, and very low-density lipoproteins (Ashfaq *et al*., 2017).

In this study, the rats that were fed HFD noted a substantial increment in body weight. However, upon treatment with the extract, the extract-treated treated rats showed a significant decrease in body weight. This decrease could be due to the presence of bioactive compounds such as saponins and phenolics, which can reduce the food appetite and malabsorption of nutrients in the intestines of animals. A research study by Mengistu *et al*. (2019) agreed and corresponds with the results of this particular study on the reduction of body weight after therapy with *Calpurnia aurea* extract on HFD-induced hyperlipidemia.

The current study also revealed elevated levels of triglycerides, low density lipoproteins and total cholesterol in HFD control rats relative to levels seen in normal control rats. The effect of the extracts significantly lowered these indices in contrast to those noted in the hyperlipidemia control rats. Lipoproteins are specialized particles whose responsibility is to transport lipids throughout the bloodstream. They serve as transporters for cholesterol,

fatty acids, and their esters. Lipoproteins are grouped depending on their lipid/protein proportions into low density lipoproteins, very low-density lipoproteins, high density lipoproteins and chylomicrons. The distinctive lipoproteins coupled with apolipoprotein protein molecules, serve as lipid transfer proteins (Huang *et al*., 2018; Hassan *et al*., 2022).

Mechanisms that regulate cholesterol homeostasis include cholesterol biosynthesis, dietary cholesterol absorption, and biliary secretion, which removes cholesterol from the body (Duan *et al*., 2022). HDL plays a significant role in transfer of extra cholesterol from the cells to the liver. The cholesterol is subsequently released into the bile via ATP-binding cassette transporters as a free sterol or transformed into bile acids with the aid of cholesterol 7-hydroxylase, an enzyme for bile biosynthesis. After being secreted into the small intestine with bile, bile acids are subsequently reabsorbed into the blood, with the remaining 5% being secreted into feces (Grefhorst *et al*., 2019; Aladaileh *et al*., 2019; Feingold, 2021).

As cholesterol and esters are transported from tissues to the liver cells to be converted to bile acids, HDL is crucial in this process. As a result, HDL performs the crucial task of lowering cholesterol levels in the blood and peripheral tissues as well as preventing the development of atherosclerotic plaque in the aorta (Luo *et al*., 2020). Through the control of lipoprotein interactions, TGs are critical component in preserving healthy lipid metabolism. Higher serum TGs levels are associated with a higher incidence of coronary artery diseases (Duan *et al*., 2020). LDL transports cholesterol from the liver to the arteries' smooth muscle cells and peripheral cells. Thus, an increase in LDL levels can result in cholesterol buildup in the arteries and aorta, thereby causing atherosclerosis. Oxygen free

radicals cause oxidation of LDL in the artery walls, resulting in oxidized LDL, which attracts the macrophages. These macrophages ingest oxidized LDL particles and then accumulate near the artery wall causing atheromatous arteriosclerosis plaques (Aladaileh *et al*., 2019; Feingold, 2021).

The two extracts therefore could have enhanced the biosynthesis of HLD, thereby reducing the levels of cholesterol in the tissues. The extracts also considerably reduced LDL levels, and this effect could attribute to an increase in endothelium-bound lipoprotein lipase, an enzyme that hydrolyzes triglycerides into fatty acids (Surya *et al*., 2017). Further, a noteworthy result of this study was the drop in serum TGs levels upon treatment with the extracts. The extracts' TGs lowering effects could be attributed to an increase in plasma lecithin cholesterol acyltransferase and HMG-CoA reductase activities, which enhance hepatic bile acid biosynthesis and speed up the conversion of cholesterol and triglycerides to bile acids, which are excreted in the feces (Hassan *et al*., 2022).

Similar anti-hyperlipidemia studies have reported consistent results on lipid profiles. A study by Ibukun and Omoregie (2022) reported that methanol extract of *Chrysophyllum albidum* noted a significant decrease in LDL, TC and TGs, as well as an increase in HDL following HFD-induced hyperlipidemia in rats. In addition, Sinaga *et al*. (2022) demonstrated that ethanol extract of *Coriandrum sativum* significantly increased levels of HDL and reduced levels of TC, TGs and LDL on HFD-induced hyperlipidemia in rats. Further, Mengistu *et al*. (2019) noted that *Calpurnia aurea* leaf extract lowered the levels of TGs, TC and LDL following HFD-induced hyperlipidemia.

The result from this study showed that methanol extracts of *S. incanum* and *R. prinoides* significantly reduced levels of TC, LDL and TGs as well as increased HDL levels in rats following HFD-induced hyperlipidemia. The two medicinal plants can therefore be novel candidates for developing antihyperlipidemic agents.

Use of traditional medicinal plants especially as source of medications has gained popularity worldwide, especially in developing nations where they are considered safe due to their natural origin (Salmerón-Manzano, *et al*., 2020). One of the demerits of medicinal plants is that they are indiscriminately ingested without any scientific evidence of associated safety or toxicity risk (Mofana *et al*., 2023). Numerous scientific studies have been done on therapeutic potential of medicinal plants, and results indicate that there is a significant potential for usage of medicinal plants for treatment of variety of diseases and disorders. However, relatively few medicinal plants have been extensively evaluated for their potentially harmful effects (Hemalatha *et al*., 2019). As a result, it is imperative to carry out research on toxicological effects of medicinal plants as this information is essential for evaluating any potential detrimental effects. This study, therefore, aimed to determine the acute and sub-acute effects of methanol extracts of *S. incanum* and *R. prinoides* in mice.

Acute toxicity study evaluates the adverse outcomes after oral single dose exposure of a test drug in a brief period. This test, which primarily involves rodents, is typically done to ascertain the potential toxicity effect of a novel drug. Acute toxicity also helps the researcher to plan the dosages for sub-acute toxicity. The animals are observed for any behavioral changes or general toxic symptoms, including mortality, for 14 days (OECD,

2022; Pauahi *et al*., 2023). The acute toxicity effects of methanol extract of *S. incanum* and *R. prinoides* at doses of 1000 and 2000mg/kg bw never showed any toxicity signs or behaviors after a single dose exposure in mice. The extracts never revealed toxicity signs such as diarrhea, altered skin and fur texture, change in breathing pattern, aggression, convulsions, or drug-related behavioral abnormalities such as agitation, paralysis, clumping together, and abnormal movement.

Since the LD_{50} of the two studied extracts was above 2000mg/kg bw of mice, the extracts were considered relatively safe according to the classification of acute toxicity (Hemalatha *et al*., 2019). The findings of acute toxicity agreed with those of Ugwah-Oguejiofor *et al*. (2019) who reported that aqueous extract of *Caralluma dalzielii* had an LD_{50} of greater than 200mg/kg bw in mice. Besides, Hemalatha *et al*. (2019) noted that methanol extract of *Trema orientalis* (L.) had an LD_{50} of greater than 2000mg/kg bw in rats. Further, a study by Muhammed *et al*. (2021) documented that ethanol extract of *Ficus deltoidei* had an LD_{50} of more than 200mg/kg bw in mice.

Sub-acute toxicity tests are designed to assess a chemical's toxicity for 28 days repeated exposure. It assists in determining doses for longer-term sub-chronic toxicity. After 28 days, the animals (rodents) are euthanized, and blood collected for both hematological and biochemical parameters analysis. Other indices that are assessed include body weights, relative organ weights and histopathology of vital organs (OECD, 2008; Mofana *et al*., 2023). In this study, effect of methanol extract of *S. incanum* and *R. prinoides* at the doses of 100, 174 and 300mg/kg bw were non-toxic on the body weights, relative organ weights,

hematological parameters, liver function test, renal function test and lipid profile test in mice.

Changes in body weight is also influenced by relative internal organ weights. This is alongside initial total food intake and food content parameters. Recurrent body weight variations can be used in the assessment of body toxicity (Loha *et al*., 2019). Adverse effects of chemicals or drug intake can alter body weight. Variations in weights of the body following exposure to potentially harmful chemicals or drugs may be a sign of toxicity. If more than 10% of the initial body weight is lost following administration of the crude extract, the body weight may display toxic effects (Reduan *et al*., 2021). In this study, there was a continuous elevation in body weights of the mice administered with the extract at the three doses, as well as normal control mice. In addition, on days 21 and 28 of the study, the body weights of mice that were administered with *S. incanum* extract at the dose of 300mg/kg bw were significantly lower in contrast to the body weights of the normal control mice. Similarly, the effect of *R. prinoides* at the same dose significantly reduced the body weights of mice compared to those of normal control on day 28.

Although there was a significant reduction in the body weights of mice after administration of extract at dose of 300mg/kg bw, the percentage decline was not more than 10%. This implied that the extracts were non-toxic. The body weight decrease could be attributed to the presence of phytocompounds such as saponins that were detected in qualitative analysis of both extracts. These phytocompounds are associated with nutrient malabsorption in the intestines and loss of appetite, thus resulting in weight loss (Abraham and Ahmad, 2021).

Organs which are vital such as the liver, lungs, spleen, kidney, heart and brain are also affected by toxicants and therefore used to evaluate for toxicity. The vital organs may reveal acute organ injury, or physiologic disturbance resulting in inflammation (Hasan *et al*., 2020). In this study, relative organ weights of all vital organs were statistically non-significant after administration of the extracts and were compared to those of normal control mice. It was therefore noted that administration of two extracts did not show any toxic effects on the vital organs.

The hematopoietic system is more susceptible to toxic substances. These toxic substances target the bone marrow where the synthesis of new blood cells such as leucocytes, erythrocytes and thrombocytes take place (Nalimu *et al*., 2022). Therefore, determining the effect of plant extracts on the animal requires an evaluation of hematological parameters. The primary roles of WBCs and their differential are to protect the body from infections and toxins. An increase in white blood cell counts, especially granulocytes (neutrophils, eosinophils, and basophils) may indicate immune system activation, inflammation, infection or necrosis. Lymphocytes participate in adaptive immunity processes, which aid the body's immune system in combating bacteria, viruses, and cancer (Pouaha *et al*., 2023). In this study, the WBCs and differential counts of the mice administered with the extracts were non-significant compared to those of control, suggesting that they did not interfere with the leucopoiesis activity.

Erythrocytes and related parameter such RBCs, HGB, HCT, MCV and RDW are involved in the transport of respiratory gases into as well as in the maintenance of acid-base balance. A rise in erythrocytes can indicate oxygenation and no anemia (Pouaha *et al*., 2023). In the

current study, the levels of RBCs, HGB, HCT, MCV and RDW in mice that were administered with the extracts did not exhibit significant variations as to those of normal control, clear indication that the effects of the extract were non-toxic to the erythropoiesis activity and did not cause anemia.

Toxic substances can affect thrombocytes either by increasing (thrombocytosis) or reducing (thrombocytopenia) their levels beyond normal ranges (Obakiro *et al*., 2021). A reduction in levels of thrombocytes can result in excessive bleeding, while the increase in thrombocytes can cause thrombosis (Unuofin *et al*., 2018). In this study, the levels of PLT, MPV, PDW and PCT were not significantly affected by the extracts and the levels were comparable to those of normal control mice. This suggests that the two studied extracts were safe since they did not cause thrombocytosis or thrombocytopenia.

In the metabolism and excretion of medications or plant products, the liver and kidneys play crucial roles. On these organs, exogenous substances and their metabolites may cause toxicity or cell damage (Loha *et al*., 2019). As a result, while assessing the safety of medications, examinations of renal and hepatic functions are crucial. The liver is the primary organ for drug metabolism, making it a prime location for drug-related diseases (Hemalatha *et al*., 2019).

The abnormal rise of the liver panel parameters as well as the decline in protein and albumin, is typically linked to liver injury (Pouaha *et al*., 2023). The most sensitive marker of the liver is ALT, which is mostly present in the liver (Hemalatha *et al*., 2019). AST leaks into the blood when the liver cells are injured. AST is also primarily found in RBCs, kidneys and skeletal and cardiac muscles. Unlike ALT, AST is not a liver-specific marker

(Akanmu *et al*., 2020). A rise in ALP levels is an indicator of cholestatic disease or biliary duct obstruction (Iserhienrhien and Okolie, 2020).

Serum bilirubin levels rise in response to RBCs lysis or liver damage (Pouaha *et al*., 2023). To examine the secretory and synthetic functional capacity of the liver, the serum protein level must be determined. Dehydration due to severe vomiting and diarrhea, as well as liver damage, can result in a reduction in serum total protein and albumin levels (Loha *et al*., 2019). In this study, the levels of ALT, AST, ALP, direct and indirect bilirubin, total protein and albumin were not significantly affected by extracts and were comparable to the normal control ranges. This implies that the extracts were non-toxic to the liver biomarkers.

The function of the kidney is the excretion of waste products, drugs, and toxins through urine. The kidney plays a role in maintaining homeostasis throughout the body by controlling blood pressure, electrolyte levels, extracellular fluid volume, and acid-base balance (Pouaha *et al*., 2023). Urea, creatinine, and serum electrolytes such as potassium, sodium, calcium and chloride are measured in the typical blood test that evaluates renal function (Ugwah-Oguejiofor *et al*., 2019; Iserhienrhien and Okolie, 2020).

Creatinine is a byproduct of muscles as a result of regular daily activity. The glomerular filtration rate is assessed using plasma creatinine levels. Lack of clearance of the creatinine by the kidney results in a buildup of the creatinine in the blood (Ugwah-Oguejiofor *et al*., 2019). Urea is a byproduct of protein metabolism and tends to accumulate in the blood due to the lack of clearance by the kidney (Iserhienrhien and Okolie, 2020). Electrolytes are necessary for basic life processes, such as maintaining electrical neutrality in cells and producing and conducting action potentials in the nerves and muscles. Important

electrolytes include calcium, phosphate, sodium, potassium, chloride, and bicarbonates (Shrimanker and Bhattarai, 2023). Despite changes in the body system, the kidneys maintain electrolyte levels constant. An imbalance in electrolyte levels is an indicator of kidney damage (Nalimu *et al*., 2022).

In the present study, the effects of the two extracts did not significantly affect the levels of creatinine, urea, $Na^{+,}$ K^{+}, Cl^{-}, Ca and P and were statistically similar compared to those of normal control mice. This suggests that the kidney's structural and functional integrity were not comprised after administration of the extracts for 28 days.

The reduced levels of HDL and elevated levels of TGs, TC and LD may be indicators of alteration in lipid metabolism that can be caused by toxicants (Arunsi *et al*., 2020). This can result in the development of cardiovascular diseases, obesity, diabetes, hypercholesteremia and hypertension (Iserhienrhien and Okolie, 2020). The Lipid profile test is therefore important when evaluating the toxicity of a substance. The dysregulation of lipid metabolism shown by changes in increased LDL and decreased HDL levels could be caused by a substance interfering with lipolysis and the mobilization of free fatty acids from peripheral depots (Obakiro *et al*., 2021). Higher TGs levels usually suggest inadequate clearance or overproduction of cholesterol, which could raise the risk of cardiovascular disease (Pouaha *et al*., 2023). In this study, the two extracts did not significantly alter the levels of HDL, LDL, TGs and TC, and the levels were comparable to those of the normal control mice. This suggests that the two extracts had a non-toxic effect on lipid metabolism.

The sub-acute toxicity findings on this study were consistent with a study done by Hemalatha *et al*. (2019) which found that methanol extract of *Trema orientalis* had no toxic effect on body weights, organ weights, hematological parameters, liver and renal function tests, and lipid profiles in rats. A similar study by Mpofana *et al*. (2023) reported that the effect of methanol extract of *Cassipourea flanaganii* was non-toxic to body weights, relative organ weights, hematological, liver function and renal function tests and lipid profiles in rats. Nevertheless, study by Pouaha *et al*. (2023) documented that methanol extract of *Momordica foetida* demonstrated a significant increase in TC and serum urea, as well as a decrease in serum total protein levels in rats. Similarly, study by Mkangara *et al*. (2019) showed that methanol extract of *Aloe rabaiensis* at the dose of 1000mgkg bw caused a significant elevation in AST and ALT levels in mice.

In this study, the acute toxicity effects of methanol extracts of *S. incanum* and *R. prinoides* did not reveal toxic signs or fatalities in mice. The LD_{50} of the two extracts was therefore greater than 2000mg/kg bw in mice. The sub-acute effects on toxicity of the two plant extracts at doses of 100, 174 and 300mg/kg bw were non-toxic to the body weights, relative organ weights, levels of hematological parameters, renal test, liver function tests, and lipid profiles in mice. The two extracts at the tested doses were therefore considered safe and this could be the reason they are widely used by different communities in Kenya in the management of diseases and disorders.

5.2. Conclusions

It is therefore concluded that:

i. Methanol extracts of *S. incanum* and *R. prinoides* at the concentrations of 25, 50 and 100mg/ml have cardiotonic effects on rabbit isolated heart.

ii. The methanolic extracts of *S. incanum* and *R. prinoides* at the doses of 25, 50 and 100mg/kg bw have antihyperlipidemic effects on HFD-induced hyperlipidemia in rats.

iii. The methanol extracts of *S. incanum* and *R. prinoides* at the doses 100, 174 and 300mgkg bw have no acute and sub-acute toxicity effects in mice.

iv. The methanol extracts of *S. incanum* and *R. prinoides* possess classes of phytocompounds associated with cardiotonic and anti-hyperlipidemic effects.

The research questions of the current study were therefore answered affirmatively.

5.3 Recommendations

5.3.1 Recommendations from This Study

The following are recommendations from this study:

i. The methanol extracts of *S. incanum* and *R. prinoides* may be used as alternative candidates in the development of novel cardiotonic agents with few side effects.

ii. The methanol extracts of *S. incanum* and *R. prinoides* may be used as alternative sources for the development of antihyperlipidemic therapeutics with minimal side effects.

iii. The methanol extracts of *S. incanum* and *R. prinoides* are safe at a maximum single dose exposure of 2000mg/kg bw and at maximum repeated dose exposure (28 days) of 300mg/kg bw

iv. The methanol extracts of *S. incanum* and *R. prinoides* possess classes of phytocompounds that can be used as alternative agents in the development of cardiotonic and anti-hyperlipidemic drugs.

5.3.2 Recommendation for Further Studies

i. Bioassay-guided fractionation and purification of bioactive phytoconstituents responsible for cardiotonic and hyperlipidemic effects

ii. Elucidate the mechanism of action underlying the positive ionotropic, negative chronotropic and hyperlipidemic effects.

iii. Determine the sub-chronic toxicity effects of the two extracts.

iv. Carry out the quantitative phytochemical composition of the extracts.

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APPENDICES

Appendix I: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **411801**

Date of Issue: **18/November/2021**

RESEARCH LICENSE

This is to Certify that Mr.. STEPHEN NGIGI MBURU of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: EVALUATION OF CARDIO PROTECTIVE AND ANTI-ATHEROSCLEROTIC EFFECTS OF Solanum incanum Linnaeus AND Rhamnus prinoides EXTRACTS IN ANIMAL MODELS for the period ending : 18/November/2022.

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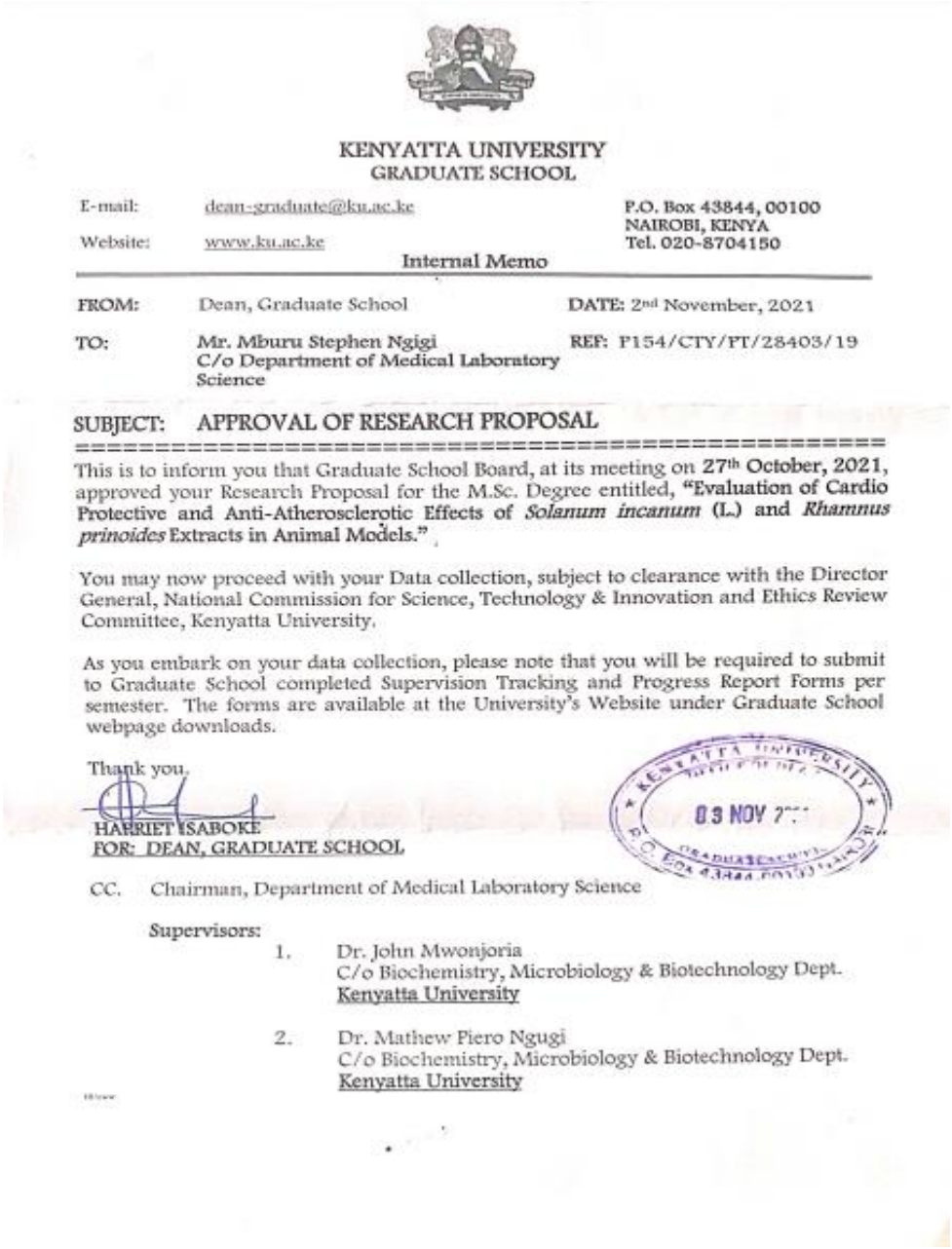
Appendix II: Institutional Research Approvals



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Internal Memo

FROM: Dean, Graduate School

DATE: 2nd November, 2021

TO: Mr. Mburu Stephen Ngigi
C/o Department of Medical Laboratory Science

REF: P154/CTY/PT/28403/19

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 27th October, 2021, approved your Research Proposal for the M.Sc. Degree entitled, **"Evaluation of Cardio Protective and Anti-Atherosclerotic Effects of *Solanum incanum* (L.) and *Rhamnus prinoides* Extracts in Animal Models."**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation and Ethics Review Committee, Kenyatta University.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

HARRIET ISABOKE
FOR: DEAN, GRADUATE SCHOOL

KENYATTA UNIVERSITY
03 NOV 2021
P.O. BOX 43844 - 00100 GRADUATE SCHOOL

CC. Chairman, Department of Medical Laboratory Science

Supervisors:

1. Dr. John Mwonjoria
C/o Biochemistry, Microbiology & Biotechnology Dept.
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2. Dr. Mathew Piero Ngugi
C/o Biochemistry, Microbiology & Biotechnology Dept.
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Appendix III: Research Publication

Check for updates

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Research Article

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Cardioprotective and anti-atherosclerotic effects of *Rhamnus prinoides* extracts in animal models

Stephen Ngigi Mburu, Mathew Piero Ngugi, John K. Mwonjoria

ABSTRACT

Rhamnus prinoides (*R. prinoides*) are small trees or shrubs which are rigid and branched. Its barks and roots are used for treatment of various ailments and diverse diseases. It is commonly distributed in rift valley and central provinces of Kenya. A little is known about its safety and antihyperlipidemic effects in management of atherosclerosis. 10% DMSO and normal saline was used to reconstitute the *R. prinoides* extracts because of its stability and reliable solvent for extraction in organic and inorganic application. Mature Albino Wistar rats three months old were fed with HCHF diet (10% egg York (5.6g/bw),10% lard (5.6g/bw),0.2% cholic acid (0.112g/bw) and 0.59% propylthiouracil (0.28g/bw), for 28 days. Onset of 28th day, the rats were euthanized and bioassays done. Both body weights and organ weights were recorded. For cardiotonic studies, 5 New Zealand male rabbits were used. They were injected with 1000 units of heparin to avoid clot formation. Chest was opened through cardiac thoracotomy and heart placed in a dish containing Tyrode solution. Langerdorff method was used using a kymograph in the study of ionotropic and chronotropic effects. In toxicity studies, male mice of age 6-7 weeks were given oral doses of plant extract inclusive of the control for 28 days of the experiment. On 29th day of the experiment, animals were sacrificed through cardiac puncture and the blood sample collected was used for hematological and biochemical assays. Mice were ruminated with rodent pellets and water without cease. OECD 407 precepts were followed when conducting toxicity studies. One way ANOVA was used in data analysis. This was followed by Tukey as post hoc and statistical significance at $p<0.05$. Extracts of *R. prinoides* showed presence of saponins, alkaloids, glycosides, terpenoids, steroids and phenolics. *R. prinoides* plant extracts exhibited positive ionotropic and negative chronotropic effects. Significance reduction on low density lipoproteins and total cholesterol was exhibited by *R. prinoides* plant extracts, following a high cholesterol high fat diet.

Keywords: Atherosclerosis, Hyperlipidemia, *Rhamnus prinoides*, LDL:HDL ratio, Cardiovascular, Coronary vascular disease.

INTRODUCTION

Plant extracts have been used in treatment and management of various ailments from time immemorial. One of the most used plants for treatment of these ailments is *Rhamnus prinoides*. Belongs to the family *Rhamnaceae*, also called *Orkonyil/ Olkokola* in Maasai and *Ngukura* in Kikuyu. It is a small tree or shrubs which are rigid and branched growing to five meters in height [1]. It is used as a traditional remedy for body aches [2] inflammation of the ear, abdominal pains, angina, toothaches and dysmenorrhea. The plant is widely distributed in East, central and South African countries being native to Kenya [3]. In Kenya the plant is majorly distributed in Rift valley and Central provinces of Kenya [4]. The decoction of the roots is mixed with milk or even taken orally and have been active against pneumonia, stomachache, back pains, gonorrhea, and malnutrition [5]. *R. prinoides* have effects on anti-inflammatory, anti-oxidant wound healing, blood purifier, treatment of waterborne diseases, sexually transmitted diseases and malaria [2]. In Kenya the barks of *R. prinoides* is consumed as a folk medicine in the management of sexually transmitted infections and backaches [1]. *R. prinoides* contains phytochemicals such as anthraquinones, saponins, steroids, tannins, terpenoids, and flavonoids [2]. Study shows potential antioxidant and anti-inflammatory activities on *R. prinoides* [6]. The anti-microbial activity of *R. prinoides* against *E. coli* and *Staphylococcus spp* has also been reported. This study aimed at evaluating the cardioprotective and anti-atherosclerotic effects of *R. prinoides* extracts and their safety.

MATERIAL AND METHOD

Collection and preparation of plant materials

The roots of *Rhamnus prinoides* barks was collected in Olendeem Narok County. They were cleaned, chopped, and dried away from direct exposure of sunlight. This was done in biochemistry laboratory

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Appendix IV: Treatment protocol in evaluation of Antihyperlipidemic effects of *S. incanum* and *R. prinoides* in rats.

Animal groups	**Treatment**
I. Baseline	Distilled water
II. Negative control	High cholesterol high fat diet +Distilled water
III.Positive control	Standard drug- Avastatin
IV. Experiment A_1	*S. incanum* 25mg/kg bw
V. Experiment B_1	*S. incanum* 50mg/kg bw
VI. Experiment C_1	*S. incanum* 100mg/kg bw
IV. Experiment A_2	*R. prinoides* 25mg/kg bw
V. Experiment B_2	*R. prinoides* 50mg/kg bw
VI. Experiment C_2	*R. prinoides* 100mg/kg bw

KEY: bw-Body Weight

Appendix V: Treatment protocol in evaluation of toxicity effects of *S. incanum* and *R. prinoides* in mice

Animal groups	**Treatment**
I. Baseline	Distilled water
II. Experiment A_1	*S. incanum* 100mg/kg bw
III. Experiment B_1	*S. incanum* 173mg/kg bw
IV. Experiment C_1	*S. incanum* 300 mg/kg bw
II. Experiment A_2	*R. prinoides* 100mg/kg bw
III. Experiment B_2	*R. prinoides* 173mg/kg bw
IV. Experiment C_2	*R. prinoides* 300 mg/kg bw

KEY: bw-Body Weight

Appendix VI: Preparation of Reagents

Preparation of stock solution (*S incanum and R prinoides*) 500mgs

Requirements:

i. 500gs powdered S *incanum and R prinoides* plant extract

ii. Methanol

iii. Diethyl ether

iv.10%DMSO

Procedure:500g of the plant extract was put into 250ml of highly volatile diethyl ether to defat. Whatman filter paper was used to filter and cover the dried extract and allowed to stand for 30 mins. Dissolved the 500gs of S *incanum and R prinoides* plant extract into the 200ml of the methanol Gently shaked the mixture to dissolve. Decanted when the methanol became saturated and was let stand for 24hrs.Rota vap used for removal of solvent and the prepared drug was transferred to a clean dry McCartney bottle.

Preparation of the working solution (*S incanum and R prinoides)* 500mgs

Average weight of the rats in this group was taken which averaged to 150gms. 0.5mls of the treatment was administered twice daily for 28days.

Calculation of total volume need for the one month

Total number of animals in this group (n=6), Volume of drug administered per rat per day = 0.5*2=1ml. Total volume need for the whole month = (6*1.0*28) = 168ml. To compensate for pipetting errors 40mls of the working solution was prepared.

Concentration of the stock solution = 500mgs while the average weight of rats = 150gm

Formula:

500mgs---------- 1000gms

? mgs---------- 150gms

(150*500)/1000 = 75mgs (concentration of the working solution).

$C_1V_1 = C_2V_2$

C1 = 500, V1= ? C2= 75, V2= 40

V1= (C2*V2)/C1

V1 = (75*40)/500

V1=6

To prepare 40mls of the working solution, 6ml was pipetted from the stock solution and added into a beaker.

Appendix VII: High Fat diet and standard drug calculations

Components per amount required per week 168ml (24ml*)

1.Egg York-10%

10g---------100ml

56g??

10*56/100=**5.6g** in 56ml of the negative control

2.Lard -10%

10g---------100ml

56g??

10*56/100=**5.6g** in 56ml of the negative control

3.Cholic Acid -0.2%

200mg--------100ml

56ml

200*56/100mg=112mg in 56ml=0.112g

4.**Thiouracil -0.5%**

500mg--------100ml

56ml

500*56/100ml=280mg in 56ml=0.28g

5.Atorvastatin (positive control) -40mg/kg

40mg--------1000g

150mg

150mg*40/1000=6mg

6mg dissolved in 0.5 ml. In one week, it will be 0.5*6=3*7(for one week) =21ml

If 6mg-------0.5ml

?? 21ml

21*6/0.5ml

=0.252g of Atorvastatin

Appendix VIII: Toxicity Doses Calculation

Highest dose used in atherosclerosis was 100 which was used as the lowest dose in toxicity. This was multiplied by a factor of 3 to get the highest dose for toxicity studies. The middle dose was calculated using the following formula:

Middle dose=$R\sqrt{highest\ dose \div lowest\ dose}$

R=N-1 log doses

R=3-1

2

Thus

$22\sqrt{300 \div 100}$

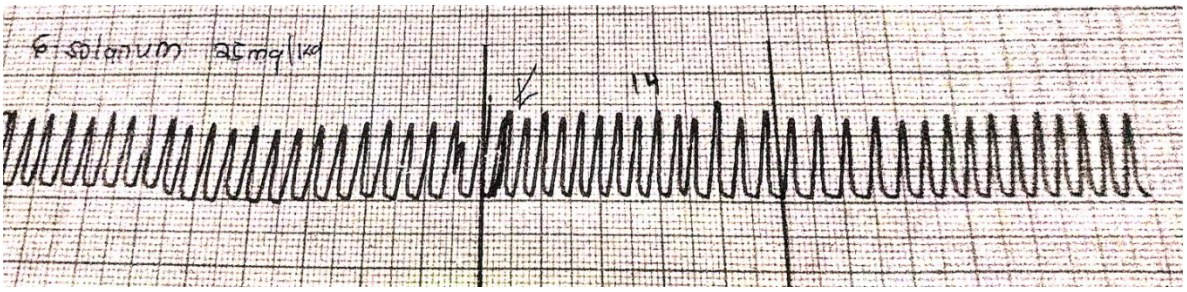
=1.732

1.732*100

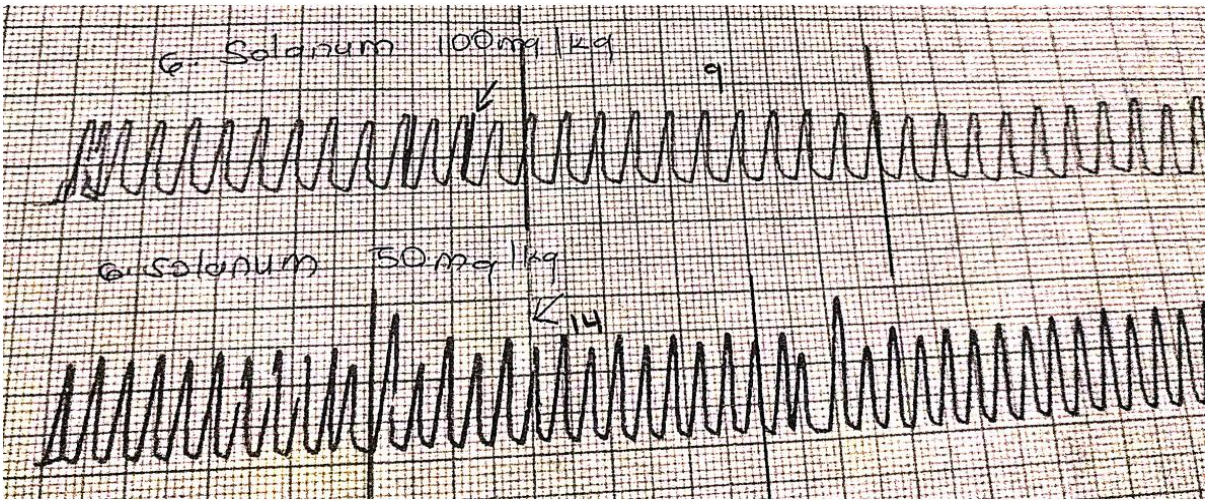
Middle dose =173mg/kg

Appendix IX: Kymograph electrocardiogram waves of various plant doses and controls.

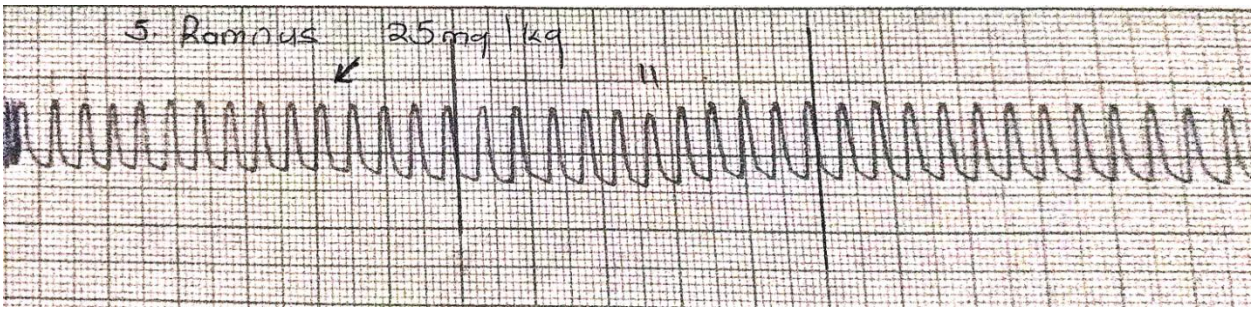
i) *Solanum incanum* 25mg/kg.



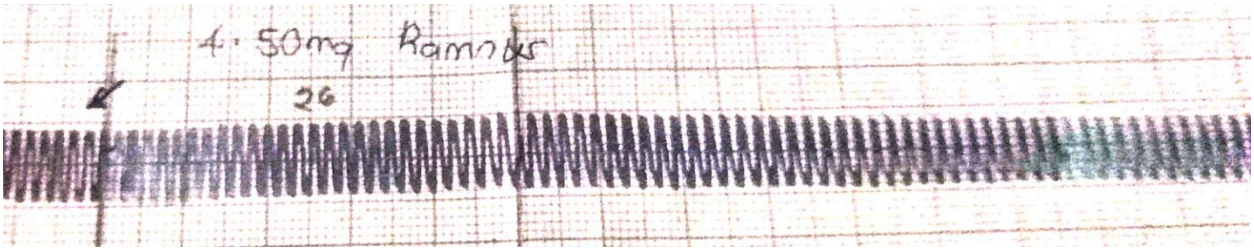
ii) *Solanum incanum* 50mg/kg and 100mg/kg



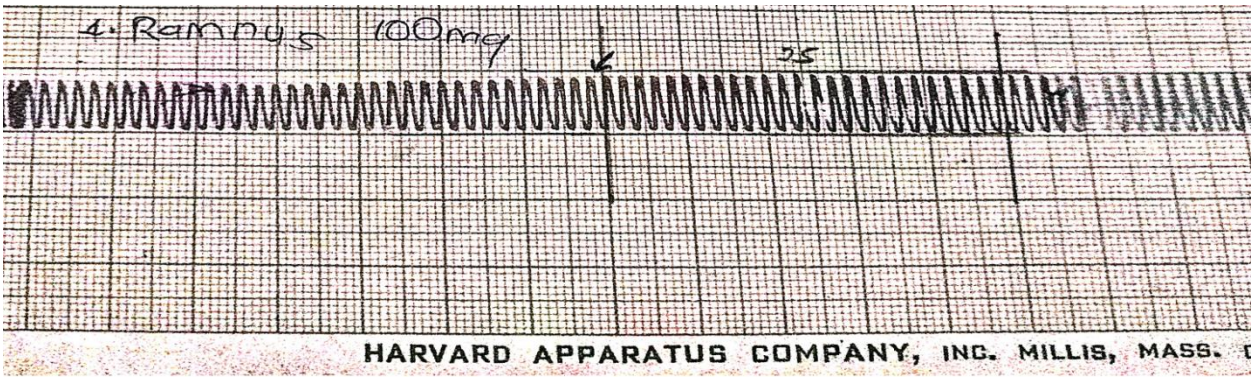
iii) *Rhamnus prinoides* 25mg/kg



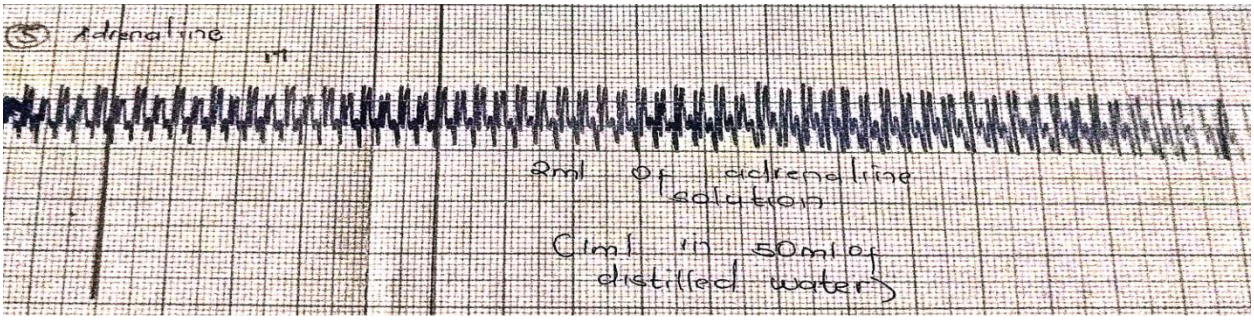
iv) *Rhamnus prinoides* 50mg/kg



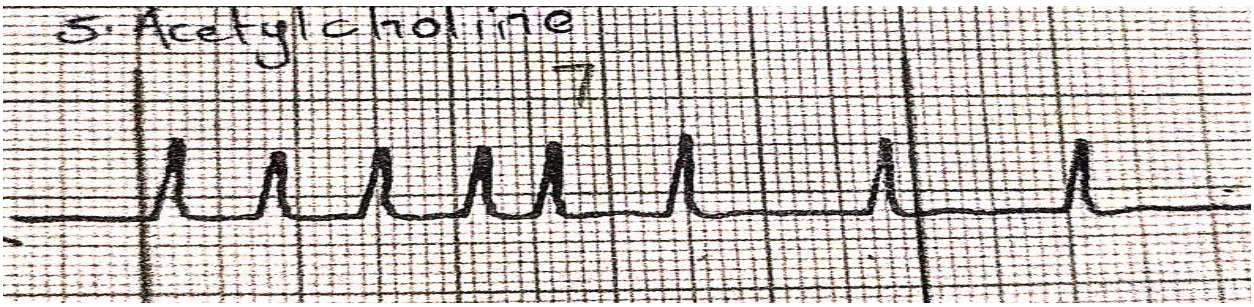
v) *Rhamnus prinoides* 100mg/kg



vi) Adrenaline 2ml (Positive control)



vii) Acetylcholine (Negative control)



Appendix X: Reagents and Materials

Reagents and materials used in the study included:

i. Dichloromethane

ii. Methanol

iii. Distilled water

iv. 40% formalin

v. Normal saline,

vi. Bouin's fluid

vii. Absolute alcohol

viii. Paraffin wax

ix. EDTA tubes

x. Chloroform

xi. Heparin

xii. Atropine

xiii. Acetylcholine

xiii. Adrenaline

xiii. Standard chemicals in qualitative phytochemical analysis (Ammonia hydroxide, suphuric acid, glacial acetic acid, Ferric chloride Sodium hydroxide).

xii. Equipments (Weighing balance, Rotor evaporator, Centrifuge, Kymograph and water bath Microscope, Hematology analyzer, Chemistry analyzer)