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EVALUATIONS OF SUB-CHRONIC TOXICITY OF HYDRO-ETHANOLIC SEED EXTRACTS OF *ALBIZIA GUMMIFERA* AND *MILLETTIA FERRUGINEA* ON BLOOD, HEART AND SMALL INTESTINE OF ALBINO WISTAR RATS

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This is to certify that the thesis prepared by Molla Getu, entitled:

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List of abbreviations

ALP ----------Alkaline phosphatase

ALT----------Alanine aminotransferase

AST----------Aspartate aminotransferase

BASO ----------Basophils

CCK----------Cholecystokinin

CK----------Creatin kinase

EOS ----------Eosinophils

EPHI----------Ethiopian Public Health Institute

FC----------Female rats control group

HCT----------Hematocrit

HGB ----------Hemoglobin

LD_{50}----------Lethal dose for 50 percent of the population

LYMPH ----------Lymphocytes

MC----------Male rats control group

MCH ----------Mean corpuscular hemoglobin

MCHC ----------Mean corpuscular hemoglobin concentration

MCV----------Mean cell volume

PCV----------Packed cell volume

PLT ----------Platelets

RBC----------Red blood cells

RDW-CV ----------Red cell distribution width

SEM ----------Standard error of the mean

SPSS ----------Statistical package for social sciences

ABSTRACT

Background: In Ethiopia there are large numbers of plants having medicinal values; among them are *Albizia gummifera* and *Millettia ferruginia*. The effect of seeds extracts of these plants on blood parameters and organs such as heart and small intestine is not fully investigated.

Objective: This study aimed to evaluate the sub-chronic toxic effects of hydro-ethanolic (70%) seed extracts of *A. gummifera* and *M. ferruginea* on the heart, small intestine and on some blood parameters in albino wistar rats.

Method: Seeds were collected from different areas of Ethiopia. They were dried and crushed to powder and macerated with hydro-alcohol and placed in orbital shaker. The extract was then filtered, evaporated to dryness by Rota vapor and further concentrated by water bath at 40^{o}c. The gummy residue extract was weighed and packed in air tight brown glass bottles with proper label and kept at 4^{O}c. The sample size for the study was 158 rats.To determine the LD_{50} of the extract, 108 female rats were treated with different doses of the extracts. For the sub-chronic toxicity study, 50 rats (25 male and 25 female) were administered at doses of 125 mg/kg/day and 250 mg/kg/day for 90 days. Hematological analysis was performed usingautomatic hematology analyzer, cell-DYN-3700 (Abbott Diagnostic Division, USA) and clinical chemistry analyzer, Human star 80 (Human GmbH, Germany) was used to determine biochemical parametrs. Data was cleaned, entered and analyzed using SPSS 16.Finally, the animals were quickly dissected. Heart and small intestine were carefully removed. Portions of these organs were fixed in 10% formalin for histopathological examination.

Result: The LD_{50} of *A. gummifera* and *M. ferrugina* were found to be 4000 mg/kg and 3500 mg/kg, respectively. The seed extract of *A. gummifera* decreased the MCH ($P<0.05$) in the male rats at 125 mg/kg and 250 mg/kg doses; MCHC at both doses in the female rats; and MCH in the female rats at higher dose but increased RDW-CV in the male rats at all doses. Furthermore, it increased neutrophil at the highest dose in both female and male. The seed extract of *M. ferruginea* decreased the MCHC and monocyte ($p < 0.05$) of female rats at the highest doses. But CK, ALP, ALT and Urea were significantly elevated in female rats administered with 250 mg/kg of *M. ferruginea* seed extract. Whereas, seed extract of *A. gummifera* increased CK and urea in male rats at 250 mg/kg. Some histopathological changes in heart and small intestine were also observed for both plants extracts.

Conclusion: This study suggests that 70% hydro-ethanolic seed extracts of *A. gummifera* and *M. ferruginea* may slightly induce anemia and inflammation, maybe phytotoxic to the heart, liver and kidneys that resulted in a rise in serumCK, ALP, ALT and Urea at high dose and cardiac and small intestinal tissue damage at all doses. Further studies would be needed to isolate the active ingredients which cause the toxicities.

Key words: *A. gummifera, M. ferruginea*, Seed, hydroethanolic extract, Toxicity, Wistar Albino Rats.

1. INTRODUCTION

1.1. Background

Before the beginning of scientific medicine, with all the limitations like being toxic to blood cells and other organs, traditional cultures around the world employed the use of medicinal plants as disease remedies for centuries [1].

Global traditional medicine usage is widespread and growing in various parts of the developing world such as Africa, China, India, Japan and Latin America in which about 80%, 40%, 65%, 60-70% and 40%-71% respectively of their population use traditional medicine to meet their primary health care needs [2] since they are often more available and affordable than western medicine. To this effect, there are researches on toxicological effects of different medicinal plant extract on blood and different organs of administered animals so as to be cautious not to use them as a medicine unless their safety is proved by appropriate scientific evidences [3-6].

In Africa, more than 2,000 plants have been identified and used as remedies to treat several ailments, but very few of these plants have been screened for their safety [7].

Ethiopia has a long history of using plants as traditional medicine, but knowledge about the extent and characteristics of traditional healing practices is limited and it has even been ignored in the national health care system [8]. The folk knowledge and traditions of Ethiopia utilize the herbal resources available in nature. This knowledge is transferred from generation to generation orally as guarded secrets [9]. *Albizia gummifera* and *Millettia ferruginea* are among the many plants used for traditional medicine in Ethiopia.

One of the plants used as traditional medicine in Ethiopia is *A. gummifera (*peacock flower- in English, Sessa- in Amharic, Ambabesamuka- in Oromifa*)*. It belongs to the family Leguminosae, and a sub family Mimosaideae. *A. gummifera* is a large deciduous tree with flattened canopy, growing up to 35m high and trunk up to 75cm in diameter. It is found in east Africa, the Democratic Republic of Congo, Madagascar, and West Africa, ranging from dry or wet lowlands to upland forest edges, and in riverine forest, at an altitude of 2400m above sea level. It is indigenous in few countries namely; Angola, Cameroon, Democratic

Republic of Congo, Ethiopia, Kenya, Madagascar, Nigeria, Tanzania, Uganda, and Zambia. It is, however, exotic in Brazil [10].The picture of the plant is shown in figure1.

Figure 1: Photograph of *A. gummifera* showing the leaves and its pods

Various parts of *A. gummifera* are used in traditional medicine. Its bark is used to treat malaria, head ache, scabies, and psoriasis. It also hastens partrusion. Its root is used to treat pain, skin diseases, diarrhea, eye diseases and sleeping sickness. Their leaf is used in the treatment of diarrhea, eye troubles, asthma, sores, and fractures. An extract of crushed pods is drunk to treat stomach-ache [11].

A. gummifera has shown effects against different bacteria at different gradient of dilution [12]. It has also shown potential molluscidal activities against *Biomphalaria pfeifferi, Bulinus sp.* and *physaacuta* [13]. The seed extract of *A. gummifera* shows larvicidal activities against *Aedes agypti, Aedes africanus,* and *Culex quinque fasciatus* [14]. Furthermore, crude hydro-alcoholic (20-80 %) extracts of *A. gummifera* was effective against reference strain of *N. gonorrhoeae* [15].

*M. ferruginea (*birbira- in Amharic, sotallo- in Oromiffa, sari- in OromiffaArsi, Yego- in OromifaHarar, Enghediksho- in Sidama, Zaghia- in Wallayita*),* belongs to the family Leguminosae and a sub family papilionoideae.It is an indigenous plant species found only in Ethiopia. There are two sub-species known to be found in this country. These are*: M. ferruginea* which is confined to the northern part of the country and *M. darasana* which is found in southern provinces, particularly Sidama region. The tree is umbrella-shaped or

flattened at top, and grows up to a height of 25-35m [16, 17]. The picture of the plant seeds is shown in figure 2.

Figure 2: Photograph of seeds of *M. ferruginea*

The seed extract of *M. ferruginea* showed promising larvicidal activities against *Aedes agypti*, *Aedes africanus* and *Culexquinque fasciatus* [14]. Traditionally, bark and mature fruit and seeds of *M. ferruginea* are used as fishing poison [18]. The fruits, leaves, seeds and stem decoction of *M. ferruginea* are used for the treatments of pain, earache & bacterial infection of nails, insecticidal properties and toothaches respectively [18]. Furthermore, *Millettiaferruginea* leaf has anti bacteria effect [19]. Acute toxicity studies of these plants on mice showed medium lethal dose values ranging from 150 mg/kg- 450 mg/kg when the aqueous extracts were administered intraperitonealy [13, 14]. Several toxicity studies on blood and organs using animal models are being carried out to strengthen the body of knowledge and ensure their safe use [3-6].

1.1.1 Bioactive Ingredients of *A. gummifera* and *M. ferruginea*

The constituents of a given plant species determines its therapeutic effects. Phytochemicalstudies have shown the presence of various bioactive ingredients in some traditionally used plant extracts, which are responsible for their medicinal uses. Aqueous seed extracts of *A. gummifera* contain chemical constituents such as alkaloids, polyphenols, unsaturated sterol/or triterpens, saponins, glycosides and carbohydrates [13]. Pytochemical screening showed that aqueous seed extracts of *M. ferruginea* contain chemical constituents such as polyphenols, tannins, unsaturated sterol/or triterpens, glycosides and carbohydrates [13].

1.1. 2. Blood Composition and Functions

The two components which are formed elements and plasma make the blood. As liquid connective tissue, blood transports many substances through the body and helps to maintain homeostasis of nutrients, wastes and gases. In humans the relative volume of blood cells and plasma in whole blood is approximately 45% and 55%, respectively. Total blood volume in the average adult person is about 5 to 6 L or 7% to 8% of the total body weight [20]. Whereas, the mean blood volume for male rats was found to be 55.6µl/gm and the corresponding value for female rats is 53.1µl/gm [21].

The erythrocytes make up about 45% of blood volume. An estimate of the volume of packed erythrocytes per unit volume of blood (hematocrit) has various clinical applications [20, 22]. Erythrocytes transport oxygen in the blood through the red pigment hemoglobin. Hemoglobin contains iron and proteins which increase the oxygen carrying capacity of erythrocytes [23, 24].

The WBCs, also known as leukocytes, make up a very small percentage of the total number of cells in the blood stream, but have important functions in body's immune system. Based on the presence of chemical filled vesicles in their cytoplasm that give them their function leukocytes can be classified in to granulocytes(neutrophils, eosinophils, and basophils) and agranulocytes (monocytes and lymphocytes). Neutrophils contain digestive enzymes that neutralize bacteria during inflammation. Eosinophils contain digestive enzymes specialized for digesting viruses that have been bound to by antibodies in the blood. Basophils release histamine to intensify allergic reactions and help protect the body from parasites. During inflammation, granular leukocytes leave the blood stream by migrating between the endothelial cells of the venules and capillaries by a process known as diapedesis, and enter the connective tissue spaces to perform their function [22, 23].

Lymphocytes include T cells and natural killer cells that fight off infection caused by viruses and intracellular pathogens and B cells that produce antibodies against infections by pathogens. Whereas, circulating monocytes undergo further maturation up on leaving the vasculature and migrating into the various tissues and body cavities [25]. Inside body tissues, monocytes are differentiated into macrophages [25].

Macrophages play role in immune system of the body. They engulf and ingest parasites, microbes and the dead cells from wound and infections. They regulate lymphocyte activation and proliferation and they are essential in the activation process of T- and B- lymphocytes by antigens and allogenic cells.The mean age of the granulocyte is 8.7 to 9.4 days [26]. The majority of the granulocytes enter the blood stream at an age of about 6 days. Less than 5 % of the granulocytes in the blood stream are less than 5 days old, and a negligible percentage is older than 3 weeks. That of lymphocytes form two groups; the Younger have a mean age of about 3 to 4 days while the others have a mean age of about 100 to 200 days [27].

Megakayrocytes inside the red bone marrow periodically rupture and release thousands of pieces of membrane or small cell fragments (Platelets or thrombocytes) responsible for the clotting of blood and the formation of scabs. Platelets do not contain nucleus and only survive in the body for up to a week before macrophage capture and digest them. Their transport towards the vessel wall is influenced by the hematocrit, red blood cell (RBC) size, and shape. They are the primary cells responsible for the control of bleeding and under normal circumstances their activation in response to bleeding triggers the clotting process [23, 28]. A study on the analysis of normal rat blood using KX-21 Symex hematological analyzer showed a platelet value of 943.14 -946.96 $\times 10^3/\mu L$ [29].

Plasma which is the non-cellular or liquid portion of the blood makes up 55% of the bloods volume. Components of plasma include water, proteins, and dissolved substances. Water makes 90% of plasma. The proteins within plasma include antibodies, albumin and other plasma proteins. Antibodies which are part of the immune system bind to antigens on the surface of pathogens that infect the body. Albumin helps maintain the body's osmotic balance by providing isotonic solution for cells of the body. Many different substances can be found dissolved in the plasma, including glucose, oxygen, carbon dioxide, electrolytes, nutrients, and cellular waste products. The plasma functions as transportation medium for these substances as they move throughout the body [20].

1.1. 3 Heart: structure and Functions

The anatomical organization of human and rat hearts are almost similar. The heart of the rat is a four chambered organ like other mammals. There is no normal communication between the left and the right chambers. The heart is enclosed within a thin, transparent pericardium that is attached to the major arteries and veins at the base of the heart [30].

The topographic features of the heart are similar to those of other mammals, with a clearly defined atrio-ventricular groove separating the atria from the ventricles. Anterior and posterior papillary muscles in the left ventricle projecting into the ventricular lumen anchor the chordae tendineae of the mitral valve. The anterior papillary muscle of the rat is located more laterally than that in the man. Papillary muscles in the ventricle are slender, elongated structures varying from 2 to 5 or more in number [30].

The cardiac valves of rat are similar to those in other species. The aortic and pulmonary valves have 3 leaflets; the mitral valve, anterior and posterior leaflets; and the tricuspid, a posterior septal leaflet and 2 lateral leaflets on the free wall portion of the right ventricular wall. There are multiple thin chordae tendineae connecting the mitral and tricuspid leaflets to the papillary muscle [30].

1.1. 4 Small intestine: Structure and Functions

The small intestine of rats is made up of the duodenum, the jejunum and the ileum. The duodenum, the first part of the small intestine occupies the right dorso-lateral part of the abdominal cavity and had the largest diameter. The jejunum is located distal to the duodenum and makes the longest segment of the small intestine. It can be differentiated from the duodenum by its folding nature and is made up of many folds held together by mesentery. The jejunum was related craniomedially to the caecum. The ileum extends distal to the jejunum with the same diameter as the jejunum and only differed from the jejunum by the absence of folds seen on the jejunum [31**].**

These three segments have many common histological features like the villi and some minor structural differences. The duodenum has intestinal villi which were seen in the mucosa. Intestinal glands (Brunner's) are seen in the submucosa and make the major distinguishing features observed in the duodenum. The jejunum has long leaflike villi, which are mucosa

projections with numerous intestinal glands (*crypts of Lieberkuhn*) that opened into pits between the bases of the villi and penetrated the mucosa as far as the muscularis. The ileum also contains villi and the intestinal glands around the mucosa. Mucosal associated lymphoid tissues (Payers patches) are localized in the submucosa, while the tunica muscularis has longitudinal muscles [32].

1.2 Statement of the problem

Despite the presence of solid scientific evidence with regard to the biological activities of most of the natural products used in folk remedy, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to the consumers [11]. Nowadays, there is great concern by health authorities, pharmaceutical industries, and consumers in relation to drug discovery and development because the need for safe and effective drugs is increased by the general public. Because plants having medicinal value are used by the societies for long period, consumers might assume that these plants have little or no side effect. But there are studies which showed the adverse effect of medicinal plants applied in traditional medicine [33]. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects.

1.3 Significance of the study

A. gummifera and *M. ferruginea* are widely used in the treatment of various health problems but the issues of appropriate dosage and harmful side effects of these plants have not been adequately researched. So far, no literature is available on histopathologic implications in sub-chronic oral administration of the seeds extract of these two plants.

This study, therefore, will be an input for researchers, health authorities, pharmaceutical industries, and consumers as it intends to investigate the histopathologic effect of the extract on the blood, heart and small intestine.

2. Literature review

Traditional medicine is widely used to treat several ailments, and is often more available and affordable than western medicine. But these traditional medicines are not without limitations; several studies are conducted to assess the safety of these herbal medicines. Most of the studies are able to demonstrate the toxicological effect of the herbal remedies on organs and tissues of administered animals. This shows that herbal medicines should not be used as medicine unless their safety is proved by appropriate scientific evidences [5, 6].

2.1 Blood and Biochemical markers

Blood offers important profile to study the toxicological impact on animal tissues. Different blood parameters are often subjected to change depending upon stress condition and various other environmental factors. Decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies. A study on the leaf extracts of *A. schimperiana* against trypanosome congolense infection in mice showed that the extract significantly prevent the hemolytic effect of the parasite by increasing the packed cell volume (PCV) [34]. Another study done on the effect of ethanol leaf extracts of *M. aboensis*on hematological parameters of treated Wistar albino rats at the dose 3000-5000 mg/kg body weight showed significant increase in packed cell volume (PCV), hemoglobin concentration of the cell, total white blood cell counts and platelets count compared to control. Results from this study suggest that the ethanolic leaf extract of *M. aboensis* altered the activities of the hemopoietic system [35].

Rats treated with aqueous leaf extract of *Ocimum gratissimum also showed* reduction of platelet and lymphocyte counts and elevation of neutrophil and total white blood cell counts. The result of this study provides caution not to consume quantities of *O. gratissimum* [29**].**
Measurement of blood biomedical parameters can be used as important diagnostic tool for the detection of abnormalities in various body tissues and organs. Following tissue injury biochemicals stored inside the cytoplasm of injured cells are released into the extracellular spaces where they join the circulation. So the type and amount of biochemicals present in the blood shows the type and extent of tissue injury. Presence of large amount of creatine kinase in the blood for example shows extensive damage to the myocardium. But an increase in serum amylase level showed pancreatic damage [36]. A report on the effect of oral administration of *A. lebbeck* aqueous steam bark extract on some liver function indices in rats

indicated a significant increase in the activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) in the test groups compared with that of the control group which is an indicative of hepatotoxicity of the extract [37].

A study on antifatigue effect of *M. speciosae* Champ (Leguminosae) extract in Mice demonstrated a delayed accumulation of bilirubin (BUN) and decreased level of serum creatin kinase (CK) which indicates that the extract might have curative effect on myocardium [38].

2.2 Histopathology of rat heart

Histopathological features observed following toxic insult to the heart may include enlargement of the connective tissue, vacuolation and deposit of serum in the endomysial capillary, vasodilation, area of hemorrhage, diffused and degenerated cardiac muscle fibers, and loss of cellular components and nuclei [39]. A study on the effect of methanolic seed extract of Albizia species on the heart of rat demonstrated area of hemorrhage and vasodilation inside the myocardium which showed the toxic nature of the extract [40].

Histopathological study done on cardio toxicity of doxorubicin drug treated rat showed severe necrotic changes along with inflammatory cells, marked fragmentation of muscle fibers, congestion of myocardial vessels, lack of cross striations in most of the cardiac myocytes, increased cytoplasmic eosinophilia, and pyknosis of nuclei of cardiac myocytes. Whereas administration of doxorubicin sensitized rats with grape seeds extract along with vitamin prevents the DOX induced myocardial toxicity by boosting the endogenous antioxidant activity [41]. So plant extract can even treat organs or tissue insulted by other plant extract.

2.3 Histopathology of rat small intestine

Histopathological lesions observed in the wall of the small intestine following toxic insults by plant extract include mucosal atrophy, hemorrhage, lymphocytic infiltration, villi atrophy and desquamation of the mucosa [32].

The effect of sub-chronic doses of chloroform extract of *Artemisia maciverae* on the histology of the small intestine of male albino rats was studied and the extract caused significant mucosal necrosis in the small intestine of treatment groups at 50, 100 and 200

mg/kg compared with the control group. Number of epithelial cells which are responsible for the synthesis of digestive enzymes was markedly reduced as a result of mucosal necrosis. So the impaired digestive process ultimately caused the observed weight loss in treated groups [42].

Similarly seeds extract of *A. fabaceae* resulted in vasodilation and vascular membrane hyper permeability in the mucosa and submucosa of small intestine of the treated rats. So the observed inflammatory infiltrate of polymorphonuclear neutrophils in histological section of the small intestine are caused by an increase in vascular permeability and vasodilation [40].

3. OBJECTIVE

3.1 General objective

- To evaluate the sub-chronic toxicological effect of hydroethanolic (70%) seeds extract of *A. gummifera* and *M. ferruginea* on the heart, small intestine, and blood of Albino Wistar rats.

3.2 Specific objectives

- To estimate the LD_{50} of seed extracts of *A. gummifera* in rats.
- To estimate the LD50 of seed extracts of *M. ferruginea* in rats.
- To investigate the effects of sub-chronic administration of the extracts on body weight, hematological and biochemical parameters in rats.
- To demonstrate the sub-chronic effect of the extracts on weight of heart and small intestine of rats.
- To investigate gross and microscopic histopathological changes of heart and small intestine after sub-chronic administration of the extract rats.

4 . MATERIALS AND METHODS

4.1. Study place

The study was conducted at different areas; EPHI for animal and plant collection and processing, and hematological and biochemical testing; and at Histology Laboratory of Medical faculty for tissue processing.

4.2. Study Design and period

This is a comparative study using animal model which was carriedout between2014-2016

4.3. Study Population

A total of 154 male and female albino wistar rats were used.

4.4. Sample size and sampling technique

4.4.1. Sample size

A total of 52 female rats were used for acute toxicity test (48 of the female rats were randomly grouped into 12 groups each containing 4 rats, the remaining 4 female rats were used as controls). That is a total of 104 female rats were used for the two extracts.

In addition, 50 rats (25 male and 25 female) were used for sub-chronic toxicity test. These 50 rats were randomly divided into 2 groups, 25 rats for first extract (10 rats for the lower dose, 10 rats for the higher dose and 5 rats for the control) and the other 25 rats were used for the second extract (10 rats for the lower dose, 10 for the higher dose and 5 for the control).

4.4.2. Sampling Technique

Convenient (non-probability) sampling of albino rats was employed.

4.5. Data collection procedures

4.5.1. Plant Collection and Processing

The seeds of *A. gummifera* and *M. ferruginea* were collected in November 2013 in the wild at altitudinal range of 900 – 3900 m. They were identified and confirmed by a taxonomist using standard Flora, and voucher specimens of *A. gummifera* (Voucher No. AG-2006) and of *M. ferruginea* (Voucher No.MF-2049) which were pre-deposited in the Herbarium of the Traditional and Modern Medicine Research directorate, Ethiopian Public Health Institute, Addis Ababa. The seed of *A. gummifera* was collected from Matu 400km west of Addis Ababa. Whereas seeds of *M. ferruginea* was collected from Bodetti, Welayeta soddo destrict

300km south of Addis Ababa.The seeds of the plant were dried and crushed to powder at the Traditional and Modern Medicine Research Directorate of the Ethiopian Public Health Institute (EPHI).

4.5.2. Plant Material Extraction

Hydroalcoholic extract of the seed powder was used for these studies. To this effect, 1250 gm of the powdered material of *A. gummifera* and 1230 gm of the powdered seeds of *M. ferruginea* using wooden-made pestle and mortarwere macerated separately with hydroalcohol (70% ethanolic) in 1:4 solute to solvent ratio. The preparation was placed in orbital shaker at room temperature for 72 hrs. This step was repeated three times to extract exhaustively until the extract gave faint or no coloration. The extract was then filtered through Whatman filter paper No.1 and the filtrate was evaporated to dryness under reduced pressure by Rota vapor and further concentrated by water bath at 40 ^{o}c.

Then, the gummy residue extract was weighed and packed in air tight brown glass bottles with proper label and kept in a refrigerator at 4^{o}c. until used for the preparation of stock solutions required in the subsequent experimental tests. For preparation of tested doses, appropriate amount of the crude extract was weighed and dissolved in 2-5ml distilled water immediately before administration.

4.5.3. Experimental Animals

For acute toxicity study, 108 female rats were used but for the sub-chronic toxicity study 50 rats (25 female and 25 male) were used. All the experimental animals used in this study were bred at EPHI animal rearing unit. The male and female rats were kept in separate cagesand were maintained on a 12hrs light/dark cycle, at room temperature and with free access to water and food, except for overnight fasting prior to drug administration for the acute toxicity study. They were all acclimatized prior to drug administration. The leftover food and water were changed daily and the cages were cleaned with the husk changed every three days. All the animals were apparently seen healthy.

4.6. Acute Oral Toxicity Test of *A. gummifera* and *M. ferruginea* Seed Extracts in Rats

A total of 108 female rats were used for the study. Forty eight female rats were divided into 12 groups (each group contains 4 rats) were treated with seed extracts of the two plants at different doses for acute toxicity study. After overnight fasting, rats in group I- XII were given the extract at single oral doses of 50, 100, 150, 250, 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 mg/kg body weight, respectively for both plants separately.

After treatment, post treatment observation were conducted to look for any sign of acute toxicity related to behavioral and general health alteration such as hyperactivity, ataxia, altered sleep, altered feeding, vomiting and diarrhea. And the other main objective of this acute toxicity study was to determine the LD_{50} of the extracts of the plants. A control group of four rats was given only the vehicle. At the end of two weeks, one animal from each group was randomly sacrificed by cervical dislocation and post-mortem gross observation was carried out on the internal organs (heart and small intestine).

4.7. Sub-chronic Oral Toxicity Test of *A. gummifera* and *M. ferruginea* Seed Extracts in Rats

This study was conducted on 25 male and 25 female rats to investigate the effect of sub chronic treatment with seed extracts of the two plants on general body weight and weight of the organs (Heart and Small intestine); and on blood parameters as well as histopathology of heart and small intestine tissues. The rats were randomly assigned to two groups of ten animals, five males and five females each for one of the plant extract. Similarly, the animals were randomly assigned to two groups of ten animals, five males and five females each for the other plant extract. One group containing ten animals (five male and five females) was assigned as control group. Throughout the experimental period, the female and male rats were housed in separate cages. Animals in one group received single daily dose of 125 mg/kg body weight and the second group was administered with a single daily dose of 250 mg/kg body weight for 90 days of the seeds extract of *A. gummifera.* Similarly, the seeds extract of *M. ferruginea* were given for one group a single daily dose of 125 mg/kg body weight and for the other, 250 mg/kg body weight for a period of 90 days. The control group received only vehicle daily throughout the period of study. The actual dose of the plant extract

corresponding to each group was calculated on the basis of the body weight. The extract was dissolved in distilled water immediately before administration.

Throughout the study period, animals in all the study groups were carefully monitored for any clinical signs of toxicity. The body weight of each rat in each group was measured before the beginning of extract administration and once a week thereafter throughout the study period. From these measurements, mean body weights were calculated and used for analysis to see changes on body weight.

4.7.1. Specimen Collection

On day 91^{th} all surviving animals were fasted overnight and 2-3 ml blood samples were collected into two tubes: EDTA and plane tubes. The EDTA blood was used for a hematological study which included RBC, HGB, HCT, WBC, PLT, MCH, MCHC, RDW and MCV. The plane tube blood was allowed to coagulate before being centrifuged and the serum separated. The serum was assayed for CK, AST, ALT, ALP and urea.

For tissue processing, each of the rat in the treated and control groups was sacrificed by cervical dislocation. After death, the animal was placed in the supine position on dissection board. The limbs were stretched and fixed to make the autopsy of the organs of interest easy. At autopsy heart and small intestine were visually examined for any signs of gross lesions. The organs removed from each rats were blotted on the filter paper. Then each of these organs was weighed on a semi-microbalance. After rinsing in normal saline, organ samples were taken from each of these organs. These specimens were placed in a pre-labeled sample bottles containing 10% formaldehyde solution to fix the tissues for histopathological studies.

4.7.2. Hematological and Biochemical Analyses

Blood analysis (hematology and blood chemistry) was conducted at core laboratories of EPHI, Addis Ababa. Hematological parameters including RBC, HCT, HGB, RDW, PLT count, WBC count, and differential count of each of the WBCs were measured in an automatic hematology analyzer, cell-DYN-3700 (Abbott Diagnostic Division, USA). In addition, red cell indices such as MCV, MCH and MCHC were also analyzed with the automatic analyzer. Similarly, serum biochemical parameters including CK, ALP, ALT,

AST, and urea were determined usingclinical chemistry analyzer, Human star 80 (Human GmbH, Germany)

Principle of automated hematological analysis using CELL-Dina 3700

CELL-DYN 3700 hematology analyzer performs simultaneous impedance and laser measurements on white blood cells. For impedance method cell counting and sizing is based on the detection and measurement of changes in electrical impedance (resistance) produced by a particle as it passes through a small aperture. Particles such as blood cells are non-conductive but are suspended in electrically conductive diluents. As a dilute suspension of cells is drawn through the aperture, the passage of each individual cell momentarily increases the impedance of the electrical path between two electrodes that are located on each side of the aperture.

Whereas, in the optical method, laser light is used and a diluted blood specimen passes in a steady stream through which a beam of laser light is focused. As each cell passes through the sensing zone of the flow cell, it scatters the focused light. Then, scattered light is detected by a photodetector and converted to an electrical impulse. The number of impulses generated is directly proportional to the number of cells passing through the sensing zone [43].

Principle of Human star 80 clinical chemistry analyzer

Serum samples are loaded on tray. Then, a pipette aspirates a precisely measured aliquot of sample and discharges it into the reaction vessel; a measured volume of diluents rinses the pipette. Reagents are dispensed into the reaction vessel. After the solution is mixed, it is either passed through a colorimeter, which measures its absorbance while it is still in the reaction vessel, or aspirated into a flow cell, where its absorbance is measured by a flow-through colorimeter. The analyzed then calculates the analyte′s chemical concentration [44].

4.7.3. Histopathological studies

4.7.3.1. Tissue Processing

Tissue samples taken at autopsy were processed for histopathological study at Histology laboratory, Department of Anatomy, College of Health Sciences, AAU. Each tissue sample taken from heart and small intestine of all extract treated and control animals were fixed separately in 10% neutral buffered formalin (Appendix I).

After fixation, the tissues were rinsed in running water overnight to remove excess fixative. The wet fixed tissues were dehydrated in upgraded ethyl alcohol: in 50% alcohol, 70% alcohol, 90% alcohol, absolute alcohol I, absolute alcohol II and absolute alcohol III (Appendix II). After dehydration the specimens were cleared in xylene I and xylene II (Appendix II). The dehydrated and cleared tissues were infiltrated or impregnated using liquid paraffin wax I and liquid paraffin wax II (Appendix II). The impregnated tissues were embedded to make tissue blocks. All tissue blocks were labeled and allowed to dry at room temperature. These tissue blocks were sectioned with a Leica Rotary Microtome (Leica Rm2125RT, Model Rm2125, China) at 4-6μm thickness. Tissue sections were collected and gently floated on a tissue flotation water bath at a temperature of 20 ^{o}C to unfold the tissue sections. After tissue sections are taken by microscopic slides, these slides were placed in dry oven for overnight heat fixation before staining.

4.7.3.2. Tissue Staining

The supporting structure of the tissue section (paraffin wax) was cleared from the tissue by immersing the slides in bakers containing xylene I and xylene II solutions (Appendix III). Because the staining dyes (hematoxylin and eosin) are water soluble, the dehydrated tissue sections were hydrated using downgraded alcohols: in absolute ethanol I, absolute ethanol II, 95% ethanol, 70% ethanol and 50% ethanol (Appendix III). The slides were then rinsed in distilled water before stained with Harris' hematoxylin. These slides were washed in tap water and dipped into 1% acid alcohol (Appendix I) for differentiation and remove excess stain. After washing with running tape water, the slides were then dipped in bluing solution before stained with counter stain (eosin). To permanently mount the slides with DPX, H and E stained sections were dehydrated by running through increased grade of ethyl alcohols: 50 % ethanol, 70% ethanol, 95% ethanol, absolute ethanol II and absolute ethanol I (Appendix III)

4.7.3.3. Microscopy and Photomicrography

Microscopic slides of organs under study were examined carefully under compound light microscope at Histology Laboratory of Anatomy department, College of Health Sciences, AAU. Slides derived from organs of extract treated groups were evaluated for any histologic

alteration compared to slides from their respective control groups. The presence or absence of histopathological changes in the tissues sections was assessed. Finally, photomicrographs of selected slides were takenusing (LEICA ICC50 HD, Germany) automated built-in digital photo- camera.

4.8. Statistical Analysis

The data were analyzed statistically using SPSS version 20, computer software package. The values of body weight changes were analyzed and the results were expressed as M ± SEM. To compare differences between the treated and control groups one-way analysis of variance (ANOVA) were used, followed by Dunnett's t-test to determine their level of significance. Values at $p<0.05$ were considered statistically significant.

4.9. Ethical Considerations

All the experiments had been conducted following the approval by the school of Medicine, AAU in line with the highest standard for the humane and compassionate use of animals in biomedical research.

4.10. Data Quality Assurance

Samples for hematological and biochemical analysis are collected and analyzed under the standard operational procedures. Commercial controls were used for both hematology and clinical chemistry analyzers as per the manufacturers' recommendations. All reagents and controls are stored and used before the expiry date. Samples of histopathological study were preserved in 10% formaldehyde solution before processed following the standard procedures. Body and organs weight were properly taken and data entry quality was guaranteed by entering SPSS version 16.

5. RESULTS

5.1. Acute Toxicity Study

A single dose toxicity test were done to estimate lethal dose of the 70% ethanolic seeds extract of *A. gummifera* and *M. ferruginea* in rats' model. These extracts at 50, 100, 150, 250, 500, 1000, 1500, 2000 and 2500mg/kg body weight were administered orally to rat models and did not cause death or mortality. One and two rats died among the 3000 and 3500 mg/kg *M. ferruginea* seed extract administered groups, respectively. While two rats died among the 4000 mg/kg *A. gummifera* seed extract treated group. Acute toxicity study showedthat the LD_{50} of *A. gummifera* and *M. ferrugina* were 4000 mg/kg and 3500 mg/kg respectively. No pathological lesions of heart and small intestine were observed.

5.2. Sub-chronic Toxicity Study

5.2.1. Effect of the extract of both plants on general health and body weight

For sub-chronic toxicity evaluation, all the male and female rats were orally administered with the repeated doses of both 125 mg/kg and 250 mg/kg body weight for 90 days. Through the period of administration, there were no extract related noticeable changes in their health and general behavior of the treated rats as compared to the control group for both plant extracts. No abnormal findings on gross observation of the heart and small intestine were observed in this rat. But throughout the period of administration, there were no toxicity related deaths.

The effect of the seeds extracts of *A. gummifera* and *M. ferruginea* on the body weight of male and female rats during the 13 weeks of sub-chronic treatment are summarized in (Tables 1 and 2), respectively. Body weight of both the treated and control groups increased with increasing duration. As it can be seen from Figures 1 to 4, the body weight increase patterns of the male and female rats during the sub-chronic treatment with both of the plant extracts seem to be normal. In the seeds extract of *A. gummifera* administered groups, no significant difference was observed in the mean values of the body weights of male and female rats treated with 125 mg/kg body weight and males treated with 250 mg/kg body weight as compared with their respective controls. However, significant difference was observed in the mean body weight of female rats treated with 250mg/kg body weight as it decreased at the 10^{th} week by 12.8% as compared with the control group. Similarly, in seed

extract of *M. ferruginea* treated groups, the mean body weight difference between the administered and control groups were not significant, except, in the males' rats treated with 125 mg/kg body weight during the 9^{th} and 10^{th} weeks of administration period, where the mean body weight increased by 6.5% in the 9^{th} week and further by 4.2% during the 10^{th} week.

Table 1: Effect of the sub-chronic administration of *A. gummifera* seed extract on the body weight (in gm) of male and female rats during the 13th week

Period	***Sex***	***Control***	***Treatment groups (mg/kg body weight/day)***	
			125	***250***
WK1	Male	166.4±1.75	168.2±3.35(0.24)	166.8±2.49(0.51)
	Female	164.8±2.13	167.6±3.36(0.40)	167.8±3.67(0.32)
WK2	Male	175.2±2.15	179.6±4.13 (0.24)	176±5.09(0.12)
	Female	172.2±3.65	179.2±5.12(0.53)	178.6±3.44(0.91)
WK3	Male	191.8±2.18	191.2±4.08 (0.25)	171.6±5.29(0.11)
	Female	186.6±2.98	189.6±3.97(0.59)	187.2±4.25(0.51)
WK4	Male	201.6±2.80	189.8±3.48(0.68)	196.2± 5.90 (0.18)
	Female	197.6±3.28	196.2±4.12(0.67)	202±4.15(0.66)
WK5	Male	209.8±2.85	205.2±2.08(0.56)	216.8±4.91(0.32)
	Female	205.2±3.06	209.4±5.42(0.29)	225.2±3.47(0.81)
WK6	Male	216.8±2.75	209.4±1.29(0.17)	218.8±3.65(0.59)
	Female	213±2.76	211.6±4.27(0.42)	217.2±2.94(0.90)
WK7	Male	223.6±2.50	214.4±1.89(0.59)	219.8±4.83(0.23)
	Female	220.4±3.76	207.2±4.62(0.70)	226.8±2.08(0.28)
WK8	Male	231±2.51	221.4±1.80(0.54)	220.8±3.80(0.44)
	Female	227±4.89	210.6±4.61(0.91)	229.8±2.39(0.19)
WK9	Male	242.2±2.35	226.6±1.99(0.75)	222.2±4.59(0.22)
	Female	235.6±4.53	212.2±7.77(0.32)	215.2±1.39(0.23)
WK10	Male	252.6±2.42	233.8±2.15(0.83)	234.8±4.53(0.25)
	Female	247.8±3.65	223±6.40(0.32)	216±7.07 (0.04) *
WK11	Male	261.6±6.77	238.8±2.35(0.06)	237.6±3.47(0.22)
	Female	255.2±3.76	219.4±6.52(0.31)	220.6±2.06(0.27)
WK12	Male	265±4.82	249.6± 3.43(0.53)	240.2±5.11(0.43)
	Female	265.4±4.53	225.6±5.81(0.64)	226.4±3.47(0.62)
WK13	Male	269.2±4.22	258.8±3.53(0.74)	248.4±3.33(0.20)
	Female	274.4±4.00	223.8±5.99(0.45)	232.6±2.60(0.42)

Values are given as Mean ± S. E. M. for each male and female subgroup. The figures under the brackets indicate the calculated p-values of the treatment groups as compared to the controls.
=significant ($p<0.05$). The mean difference is considered significant at $p< 0.05$

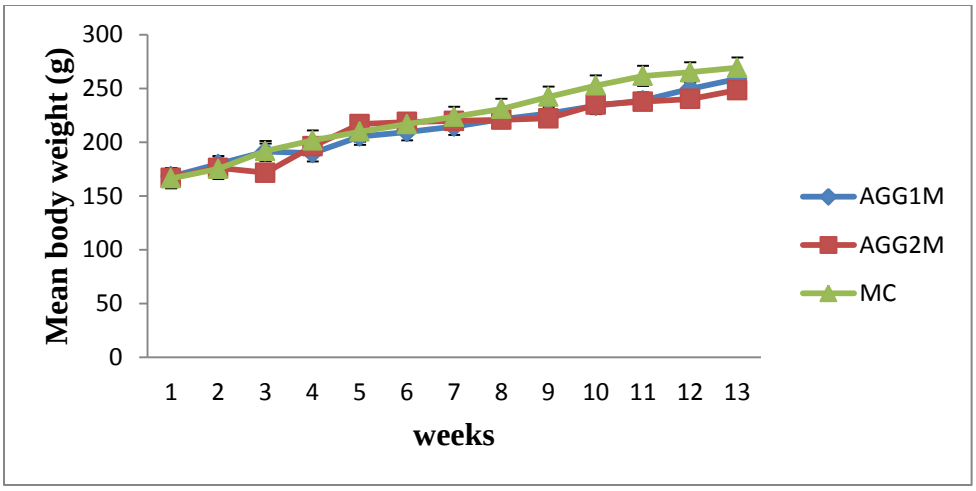

Figure 3: Time course and effect of seeds extract of *A. gummifera* on body growth pattern of male rats treated with 125mg/kg body weight and 250 mg/kg body weight as compared to the controls. *Each value point represents mean ± S. E. M. Note: AGG1M=A. gummifera administered group one male rats, AGG2M=A. gummifera administered group two male rats & MC= male control rats*

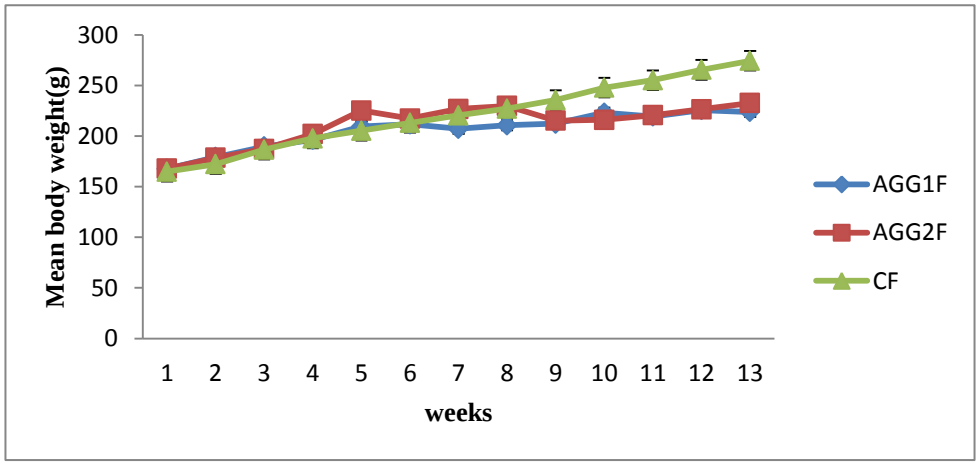

Figure 4: Time course and effect of seeds extract of *A. gummifera* on body growth pattern of female rats treated with 125 mg/kg body weight and 250 mg/kg body weight as compared to the controls. *Each value point represents mean ± S. E. M. Note: AGG1F=A. gummifera` administered group one female rats, AGG2F= A. gummifera administered group two femalerats& CF= female control rats*

Table 2: Effect of seed extract of *M. ferruginea* on the body weight (in gm) of male and female rats during the 13th week

Period	***Sex***	***Control***	***Treatment groups (mg/kg body weight/day)***	
			125	***250***
WK1	Male	166.4 ± 1.75	164.2 ± 1.59 (0.86)	166.8 ± 3.93 (0.15)
	Female	164.8 ± 2.13	163.8 ± 1.99 (0.89)	165.6 ± 3.52 (0.36)
WK2	Male	175.2 ± 2.15	171 ± 2.86 (0.59)	183.4 ± 3.88 (0.28)
	Female	172.2 ± 3.65	171.2 ± 1.91 (0.24)	171.2±3.32 (0.86)
WK3	Male	191.8 ± 2.18	184.6 ± 2.66 (0.71)	195±3.85(0.29)
	Female	186.6 ± 2.98	177.4 ± 2.38 (0.67)	176.2 ± 3.12 (0.93)
WK4	Male	201.6 ± 2.80	200.2 ± 1.11(0.12)	209 ± 3.94 (0.53)
	Female	197.6 ± 3.28	187.4 ± 3.44 (0.93)	183.4 ± 1.94 (0.33)
WK5	Male	209.8 ± 2.85	204.8 ± 3.42 (0.86)	220.8 ± 5.18 (0.27)
	Female	205.2 ± 3.06	195.4 ± 4.89 (0.38)	186.6 ± 3.49 (0.80)
WK6	Male	216.8 ± 2.75	212.4 ± 5.27 (0.32)	232.8 ± 3.65 (0.59)
	Female	213 ± 2.76	205 ± 6.32 (0.14)	199.4 ± 3.31 (0.73)
WK7	Male	223.6 ± 2.50	220.2 ± 5.48 (0.23)	250.6 ± 5.50 (0.16)
	Female	220.4 ± 3.76	212.2 ± 3.59 (0.93)	201.6 ± 1.81 (0.18)
WK8	Male	231 ± 2.51	212.8 ± 5.39 (0.24)	258.2 ± 6.24 (0.11)
	Female	227 ± 4.89	223 ± 4.85 (0.99)	210.6 ± 4.78 (0.97)
WK9	Male	242.2 ± 2.35	258 ± 11.31 * (0.01)	212.4 ± 5.52 (0.19)
	Female	235.6 ± 4.53	230.4±6.62 (0.48)	220.4 ± 5.81 (0.64)
WK10	Male	252.6 ± 2.42	263.2±11.29 *(0.01)	220.2 ± 6.82 (0.11)
	Female	247.8 ± 3.65	238.6 ± 6.45 (0.29)	230.6 ± 6.38 (0.30)
WK11	Male	261.6 ± 6.77	212.8±5.73 (0.68)	258.2 ± 10.78 (0.39)
	Female	255.2 ± 3.76	246.2 ± 6.01 (0.39)	235.4 ± 5.32 (0.52)
WK12	Male	265 ± 4.82	211.4±6.86 (0.72)	252.2 ± 11.09 (0.51)
	Female	265.4 ± 4.53	258.6 ± 5.39 (0.75)	240.2 ± 6.24 (0.55)
WK13	Male	269.2 ± 4.22	221.8 ± 6.52 (0.86)	260 ± 11.02 (0.36)
	Female	274.4 ± 4.00	267.2 ± 4.69 (0.77)	242.2 ± 7.14 (0.29)

Values are given as Mean ± S. E. M. for each male and female subgroup. The figures under the brackets indicate the calculated p-values of the treatment groups as compared to the controls.
***=significant ($p<0.05$). The mean difference is considered significant at $p< 0.05$*

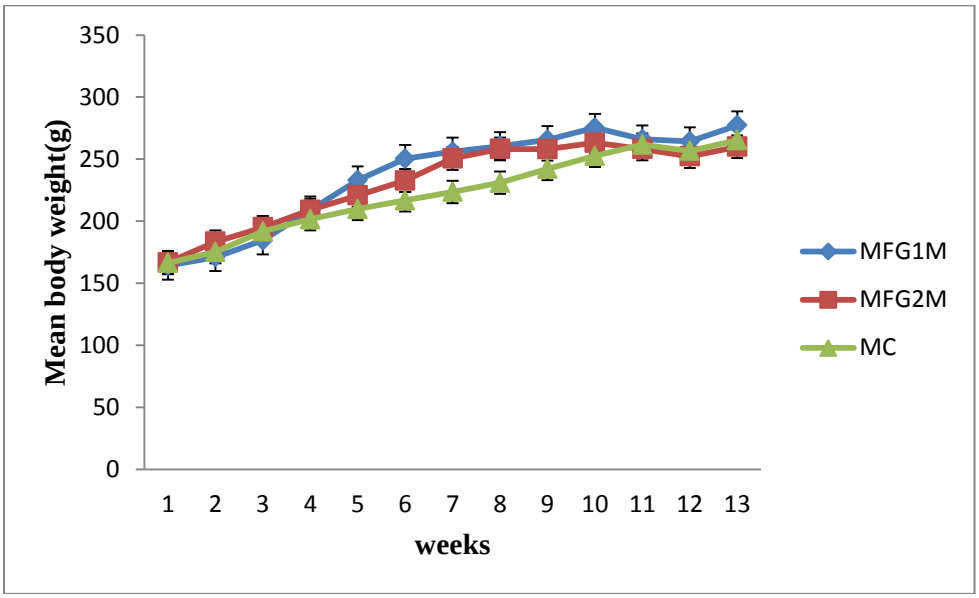

Figure 5: Time course and effect of seeds extract of *M. ferruginea* on body growth pattern of male rats treated with 125 mg/kg body weight and 250 mg/kg body weight as compared to the controls. *Each value point represents mean ± S.E.M. Note: MFG1M= M. ferruginea administered group one male rats, MFG2M= M. ferruginea administered group two male rats & MC= male control rats*

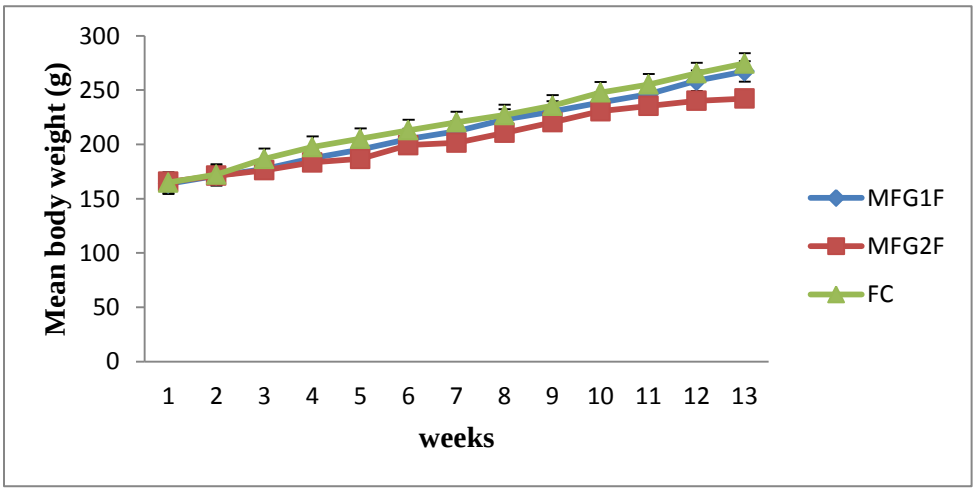

Figure 6: Time course and effect of seeds extract of *M. ferruginea* on body growth pattern of female rats treated with 125 mg/kg body weight and 250 mg/kg body weight as compared to the controls. *Each value point represents mean ± S. E. M. Note: MFG1F= M. ferruginea administered group one female rats, MFG2F= M. ferruginea administered group two female rats & CF= female control rats*

5.2.2. Effect of *A. gummifera* and *M. ferruginea* Hydro alcohol Seed Extract on Hematological Parameters

The effect of *A. gummifera* seed extract on hematological parameters of male rats as compared to the controls after 13 weeks of sub-chronic administration is illustrated in **Table 3**. Male rats administered at the dose of 125 mg/kg and 250 mg/kg body weight seed extract of *A. gummifera* did not show significant change on their hematological parameters. But few hematological parameters (MCH, RDW-CV) showed significant change ($p<0.05$). MCH was significantly decreased by 3.4% and 5.9% for male rats administered at 125 mg/kg and 250 mg/kg respectively. And RDW-CV significantly increased by 7.3% and 8.6% for male rats administered at125 mg/kg and 250 mg/kg doses respectively. Neutrophil also showed significant increase from 15.36±2.01 to 32.70±2.20 for male rat administered at 250 mg/kg.

The effect of *A. gummifera* seed extract on hematological parameters of female rats treated for 13 weeks is shown in **Table 4**. MCH was significantly decreased by 5.6% for female rat treated at 250 gm/kg dose. Whereas MCHC was significantly decreased by 2.0% and 2.7% for female rats treated at 125mg/kg and 250mg/kg doses, respectively. Besides, neutrophil was significantly increased from 12.06±2.14 to 35.00±1.80 for female rats treated at 250 gm/kg dose.

The effect of seed extract of *M. ferruginea* on hematological parameters of male and female rats following sub-chronic administration is illustrated in (**Tables 5 and 6**), respectively. Hematological parameters in both sexes were not significantly affected by sub chronic treatment of these rats at 125 mg/kg and 250 mg/kg doses. But significant ($p<0.05$) decrease in MCHC and monocyte by 2.2% and 66.8% respectively was observed for female rats administered at 250 mg/kg.

Table 3: Hematological parameters of male rats administered with 125 mg/kg and 250 mg/kg body weight of seed extract of *A. gummifera* for 13 weeks

Hematological Parameters	***Control (G3)***	***125 mg/kg body weight (G1)***	***250 mg/kg body weight (G2)***
WBC ($x10^3/\mu L$)	7.99 ± 1.08	12.24 ± 2.18 (0.19)	10.94 ± 1.49 (0.39)
RBC ($x10^6/\mu L$)	9.94 ± 0.17	10.14 ± 0.14 (0.54)	10.00 ± 0.11 (0.93)
HGB (g/dL)	18.50 ± 0 .25	18.26 ± 0.38 (0.80)	17.53 ± 0.23 (0.10)
HCT (%)	54.36 ± 1.12	54.30 ± 1.06 0(0.99)	52.73 ± 0.49 (0.41)
MCV (fL)	54.66 ± 0.44	53.50 ± 0.36 (0.25)	52.73 ± 0.66 (0.06)
MCH (pg)	18.63 ± 0 .06	18.00 ± 0.20 (0.04)*	17.53 ± 0.14 (0.004)*
MCHC (g/dL)	34.03 ± 0.33	33.63 ± 0.18 (0.49)	33.23 ± 0.23 (0.12)
PLT ($x10^3/\mu L$)	901.66 ± 67.49	1101.00 ± 81.22 (0.11)	1079.33 ± 23.53 (0.15)
RDW-CV(%)	20.16 ± 0 .06	21.63 ± 0.21 (0.00)*	21.90 ± 0.15 (0.00)*
NEUT(%)	15.36 ± 2.01	28.30 ± 4.93 (0.05)	32.70 ± 2.20(0.01)*
LYMPH(%)	77.56 ± 1.73	66.50 ± 6.24 (0.14)	64.66 ± 1.46 (0.09)
MONO(%)	5.30 ± 1.44	4.70 ± 1.20 (0.90)	5.60 ± 0.70 (0.97)
EO(%)	1.40 ± 0 .95	0.26 ± 0.08 (0.32)	0.16 ± 0.06 (0.27)
BASO(%)	0.36 ± 0 .17	0.23 ± 0.13 (0.73)	0.20 ± 0.10 (0.63)

*Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. * =significant ($p<0.05$). The mean difference is considered significant at $p< 0.05$. Note: G1=male rates treated with 125 mg/kg body weight A. gummifera, G2=male rates treated with 250mg/kg body weight of A. gummifera, G3=male control group.*

Table 4: Hematological parameters of female rats administered with125 mg/kg and 250 mg/kg body weight of seed extract of *A. gummifera* for 13 weeks

Hematological Parameters	**Control (G3)**	**125 mg/kg body weight (G1)**	**250 mg/kg body weight (G2)**
WBC ($x10^3/\mu L$)	5.27 ± 2.32	9.55 ± 1.71 (0.42)	10.48± 3.17 (0.30)
RBC ($x10^6/\mu L$)	8.66 ± 0.31	8.99 ± 0.18 (0.51)	9.32± 0.14 (0.14)
HGB (g/dL)	16.86 ± 0 .67	17.16 ± 0.55 (0.88)	17.13 ± 0.06 (0.90)
HCT (%)	49.13 ± 1.74	51.36 ± 1.40 (0.42)	50.93 ± 0.34 (0.55)
MCV (fL)	56.76 ± 0.66	57.10 ± 0.40 (0.88)	54.63 ± 0.61 (0.06)
MCH (pg)	19.46 ± 0.23	19.06 ± 0.23 (0.42)	18.36 ± 0.23 (0.02)*
MCHC (g/dL)	34.33 ± 0 .14	33.63 ± 0.12 (0.03)*	33.40 ± 0.20 (0.01) *
PLT ($x10^3/\mu L$)	665.00 ± 218.70	1029.00 ± 12.34 (0.18)	1222.33 ± 92.09 (0.05)
RDW-CV(%)	17.23 ± 0 .98	18.60 ± 0.20 (0.26)	19.43 ± 0.27 (0.07)
NEUT(%)	12.06 ± 2.14	20.90 ± 6.96 (0.32)	35.00 ± 1.80 (0.02)*
BASO(%)	0.03 ± 0 .03	0.10 ± 0.05 (0.48)	0.06 ± 0.03 (0.81)
EO(%)	3.66 ± 3.12	0.50 ±0.05 (0.40)	0.26 ± 0.12 (0.36)
MONO(%)	4.40 ± 0.55	5.13 ± 1.88 (0.94)	6.53 ± 2.51 (0.64)
LYMPH(%)	79.83 ± 3.89	73.36 ± 8.51 (0.67)	58.13 ± 4.16 (0.07)

*Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05. Note: G1=female rates treated with 125 mg/kg body weight of A. gummifera, G2=female rates treated with 250 mg/kg body weight of A. gummifera, G3=female control group.*

Table 5: Hematological parameters of male rats administered with 125 mg/kg and 250mg/kg body weight of seed extract of *M. ferruginea* for 13 weeks

Hematological Parameters	**Control (G3)**	**125 mg/kg body weight (G1)**	**250 mg/kg body weight (G2)**
WBC ($x10^3/\mu L$)	7.99 ± 1.08	9.36 ± 1.23 (0.55)	7.75 ± 0.48 (0.97)
RBC ($x10^6/\mu L$)	9.94 ± 0.17	10.04 ± 0.32 (0.95)	9.82 ± 0.35 (0.94)
HGB (g/dL)	18.50 ± 0.25	18.99 ± 0.40 (0.60)	18.76 ± 0 .48 (0.85)
HCT (%)	54.36 ± 1.12	55.63 ± 0.97 (0.65)	54.63 ± 1.18 (0.97)
MCV (fL)	54.66 ± 0.44	55.46 ± 0.84 (0.68)	55.63 ± 0.88 (0.59)
MCH (pg)	18.63 ± 0.06	18.86 ± 0.23 (0.59)	19.10 ± 0.20 (0.19)
MCHC (g/dL)	34.03 ± 0.33	34.10 ± 0.11 (0.97)	34.33 ± 0.24 (0.62)
PLT ($x10^3/\mu L$)	901.66 ± 67.49	1001.66 ± 59.40 (0.46)	922.66 ± 59.91 (0.96)
RDW-CV(%)	20.16 ± 0.06	20.33 ± 0.54 (0.95)	20.00 ± 0.52 (0.95)
NEUT(%)	15.36 ± 2.01	14.30 ± 0.68 (0.81)	13.30 ± 1.07 (0.50)
BASO(%)	0.36 ± 0.17	0.13 ± 0.03 (0.32)	0.23 ± 0.08 (0.64)
EO(%)	1.40 ± 0.95	0.30 ± 0.05 (0.33)	0.20± 0.10 (0.28)
MONO(%)	5.30 ± 1.44	4.03 ± 0.53 (0.62)	5.33± 0.94 (1.00)
LYMPH(%)	77.56 ± 1.73	81.23 ± 0.43 (0.15)	80.93±1.36 (0.19)

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control.The mean difference is not significant ($p \geq 0.05$). Note: G1=male rates treated with 125 mg/kg body weight of M. ferruginea, G2= male rates treated with 250 mg/kg body weight of M. ferruginea, G3=male rates control group.

Table 6: Hematological parameters of female rats administered with 125 mg/kg and 250 mg/kg body weight of seed extract of *M. ferruginea* for 13 weeks

Hematological Parameters	**Control (G3)**	**125 mg/kg body weight (G1)**	**250m g/kg body weight (G2)**
WBC ($x10^3/\mu L$)	5.27 ± 2.32	6.63 ± 0.49 (0.72)	4.22 ± 0.02 (0.81)
RBC ($x10^6/\mu L$)	8.66 ± 0.31	8.73 ± 0.07 (0.95)	8.66 ± 0.16 (1.00)
HGB (g/dL)	16.86 ± 0.67	17.30 ± 0.10 (0.70)	16.86 ± 0.26 (1.00)
HCT (%)	49.13 ± 1.74	51.53 ± 0.06 (0.29)	49.53 ± 0.85 (0.95)
MCV (fL)	56.76 ± 0.66	57.46 ± 0.03 (0.54)	59.03 ± 0.56 (0.30)
MCH (pg)	19.46 ± 0.23	19.80 ± 0.15 (0.32)	19.60 ± 0.05 (0.79)
MCHC (g/dL)	34.33 ± 0.14	34.20 ± 0.05 (0.72)	33.56 ± 0.17 (0.01)*
PLT ($x10^3/\mu L$)	665.00 ± 218.70	956.00 ± 95.10 (0.30)	1121.66 ± 13.92 (0.09)
RDW-CV(%)	17.23 ± 0.98	17.60 ± 0.81 (0.92)	18.03 ± 0.37 (0.69)
NEUT(%)	12.06 ± 2.14	12.06 ± 2.91 (1.00)	12.83 ± 0.43 (0.95)
BASO(%)	0.03 ± 0.03	0.10 ± 0.05 (0.48)	0.03 ± 0.03 (1.00)
EO(%)	3.66 ± 3.12	0.76 ± 0.26 (0.46)	0.53 ± 0.20 (0.41)
MONO(%)	4.40 ± 0.55	3.16 ± 1.01 (0.38)	1.46 ± 0.14 (0.03) *
LYMPH(%)	79.83 ± 3.89	83.90 ± 2.20 (0.47)	84.96 ± 0.34 (0.33)

*Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. * =significant ($p<0.05$). The mean difference is considered significant at $p< 0.05$. Note: G1=female rates treated with 125 mg/kg body weight of M. ferruginea, G2=female rates treated with 250 mg/kg body weight of M. ferruginea, G3= female control group.*

5.2. 3. Effect of *A. gummifera* and *M. ferruginea* Hydro alcohol Seed

Extract on Serum Biochemicalparameters

Effects of sub-chronic treatment with hydro-alcoholic seed extract of *A. gummifera* on serum biochemical parameters of male and female rats are shown in **(Tables7 and 8)**, respectively. Urea and CK were significantly increased by 28.94% and 12% respectively for male rats administered at 250 mg/kg body weight. CK was also increased significantly by14.59% for female rats administered at 250 gm/kg. But the other parameters measured were not significantly different between the control and extract administered groups at both doses. The effects of sub-chronic treatment with hydro-alcoholic seed extract of *M. ferruginea* on serum biochemical parameters of male and female are shown in **(Tables 9 and 10)**, respectively. Except CK which increased significantly by 9% for male rats treated at 250 mg/kg, all the other parameters measured in male rats which received any of the doses were not significantly different from those of the controls. Urea, ALP, ALT and CK, however, were found to be significantly different in the female rats at 250 mg/kg body weight as increased by 8.84%, 59.40%, 27.45% and 19.56%, respectively.

Table 7: Serum biochemical parameters of male rats administered with 125 mg/kg and 250 mg/kg body weight of seed extract of *A. gummifera* for 13 weeks

Biochemical Parameters	**Control (G3)**	**125 mg/kg dose (G1)**	**250 mg/kg dose (G2)**
Ck(U/L)	239.00 ± 1.95	243.00 ± 1.95(0.323)	267.80 ± 2.29(0.00)*
Albumin(g/dl)	4.70 ± 0.22	4.79 ± 0.26(0.94)	4.45 ± 0.19(0.65)
ALP (U/L	135.75 ± 11.69	168 ± 12.79 (0.44)	160.75 ± 29.26(0.59)
ALT(U/L)	79.75 ± 2.59	94.25 ± 5.15(0.11)	79.75 ± 6.21 (1.00)
AST(U/L)	179.75 ± 5.20	251.5 ± 43.65(0.34)	253.75± 47.42 (0.32)
Urea (mg/dL)	28.50 ± 0.96	33 ± 0.41 (0.09)	36.75± 2.21 (0.005)*

*Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05. Note: G1=male rates treated with 125 mg/kg body weight of A. gummifera, G2=male rates treated with 250 mg/kg body weight of A. gummifera, G3= male rates control groups.*

Table 8: Serum biochemical parameters of female rats administered with 125 mg/kg and 250 mg/kg body weight of seed extract of *A. gummifera* for 13 weeks

Biochemical Parameters	**Control (G3)**	**125 mg/kg dose (G1)**	**250 mg/kg dose (G2)**
CK(U/L)	209.80 ±1.36	219.00 ± 2.00(0.149)	240.40±5.53(0.00)*
Albumin(g/dl)	5.16 ± 0.19	5.01 ± 0.16(0.79)	4.87 ± 0.16 (0.42)
ALP (U/L	75.75 ± 12.49	88.75 ± 8.41 (0.79)	134.75 ± 22.87(0.05)
ALT(U/L)	63.75 ± 5.66	74.75 ± 4.00(0.26)	61.50 ± 5.33 (0.93)
AST(U/L)	194 ± 16.92	237.25 ± 62.26(0.65)	192.25 ± 13.88 (0.99)
Urea (mg/dL)	34.50 ± 2.33	38.75 ± 2.49 (0.29)	37.00 ± 1.08 (0.62)

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. The mean difference is considered significant at p< 0.05. Note: G1= female rates treated with 125 mg/kg body weight of A. gummifera, G2=female rates treated with 250 mg/kg body weight of A. gummifera, G3=female rates control group.

Table 9: Serum biochemical parameters of male rats administered with 125 mg/kg and 250 mg/kg body weight of seed extract of *M. ferruginea* for 13 weeks

Biochemical Parameters	**Control (G3)**	**125 mg/kg dose (G1)**	**250 mg/kg dose (G2)**
CK(U/L)	254.00 ± 2.70	261.00 ± 2.61(0.12)	277.00±3.77(0.00)*
Albumin(g/dl)	4.70 ± 0.22	5.03 ± 0.20(0.39)	4.85 ± 0.10(0.82)
ALP (U/L)	135.75 ± 11.69	158.00 ± 17.56 (0.48)	161.25 ± 13.74(0.39)
ALT(U/L)	79.75 ± 2.59	85.75±4.27(0.39)	80.50± 3.28 (0.98)
AST(U/L)	179.75 ± 5.20	210.25 ± 21.44(0.22)	181.25 ± 5.07 (0.99)
Urea (mg/dL)	28.50 ± 0.96	32.25 ± 1.49 (0.17)	30.00 ± 1.73(0.69)

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. The mean difference was significant (p< 0.05). Note: G1=male rates treated with 125 mg/kg body weight of M. ferruginea, G2= male rates treated with 250 mg/kg body weight of M. ferruginea, G3=male rates control group.

Table 10: Serum biochemical parameters of female rats administered with 125 mg/kg and 250 mg/kg body weight of seed extract of *M. ferruginea for* 13 weeks

Biochemical Parameters	**Control (G3)**	**125 mg/kg dose (G1)**	**250 mg/kg dose (G2)**
CK(U/L)	262.40 ± 2.94	271.80±5.44(.19)	285.60±2.84(0.02)*
Albumin(g/dl)	5.16 ± 0.19	5.34 ± 0.15 (0.62)	5.15 ± 0.10(1.00)
ALP (U/L)	75.75 ± 12.49	87.00 ± 6.01 (0.54)	120.75±2.05 (0.006)*
ALT(U/L)	63.75 ± 5.66	74.25± 4.34 (0.20)	81.25±2.28(0.03)*
AST(U/L)	194 ± 16.92	206.00 ± 20.33 (0.95)	264.00 ± 46.59 (0.24)
Urea (mg/dL)	34.50 ± 2.33	34.25 ± 0.75 (0.99)	41.25 ± 1.38 (0.03)*

*Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. * =significant (p<0.05). The mean difference is considered significant at p< 0.05. Note: G1= female rates treated with 125 mg/kg body weight of M. ferruginea, G2=female rates treated with 250 mg/kg body weight of M. ferruginea, G3= female rates control group.*

5.2. 4. Macroscopic Observations and Organ Weights

After treatment period is completed, both treated and control groups were sacrificed. During dissection, post mortem examination revealed no gross abnormal findings in the heart and small intestine. The mean organ weight of heart and intestine of the seed extract of *A. gummifera* administered groups and control group are shown in **(Tables 11 and 12)** for the male and female rats, respectively. The result showed no significant difference in the organ weights between extract treated and control rats of both sex except for the weight of heart of female rats treated at higher dose which was decreased by 28%. The mean organ weight of heart and small intestine of the seed extract of *M. ferruginea* administered groups and control group are shown in **Tables 13 and 14** for the male and female rats, respectively. No significant difference was noted in the organ weights between extract treated and control rats of either sex except for the weight of heart of female rats which was decreased by 22.5%.

Table 11: Organ weights of male rats administered with 125 mg/kg and 250 mg/kg body weight doses of the seed extracts of *A. gummifera*

Group	Dose (mg/kg)	Heart (g)	Intestine (g)
I	125	0.88 ± 0.02 (0.50)	6.51 ± 0.25 (0.52)
II	250	0.86 ± 0.05 (0.26)	6.16 ±0.27 (0.14)
III	Control	0.94 ± 0.04	6.88 ± 0.27

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control. The mean difference was significant at P<0.05.

Table 12: Organ weights of female rats administered with 125 mg/kg and 250 mg/kg body weight doses of the seed extracts of *A. gummifera*

Group	Dose (mg/kg)	Heart(g)	Intestine(g)
I	125	0.87± 0.03(0.07)	4.91± 0.09(0.07)
II	250	0.69 ± 0.03(0.00)*	4.04 ± 0.03(0.12)
III	Control	0.96 ± 0.04	5.71 ± 0.20

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control.The mean difference was significant at P<0.05.

Table 13: Organ weights of male rats administered with 125 mg/kg & 250 mg/kg body weight doses of the seed extracts of *M. ferruginea*

Group	Dose mg/kg	Heart (g)	Intestine (g)
I	125	0.91 ± 0.03 (0.31)	5.90±.25 (0.06)
II	250	0.90 ± 0.03(0.37)	6.50 ± 0.21 (0.09)
III	Control	0.84 ± 0.04	5.08 ± 0.19

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control.The mean difference was significant at P<0.05.

Table 14: Organ weights of female rats administered with 125 mg/kg & 250 mg/kg body weight doses of the seed extracts of *M. ferruginea*

Group	Dose (mg/kg)	Heart (g)	Intestine (g)
I	125	0.80 ± 0.01 (0.07)	4.32 ± 0.20 (0.65)
II	250	0.69 ± 0.01 (0.00)*	5.00 ± 0.12 (0.07)
III	Control	0.89±.02	4.28 ± 0.18

Values are expressed as Mean ± S. E. M. The figures in brackets indicate the calculated p-values of the treatment groups as compared to the control.The mean difference was significant at P<0.05.

5.2. 5 Microscopic observations

Effect of hydroalcoholic seed extracts *A. gummifera and M. ferruginea* on histopathology of the heart

Hematoxylin and eosin stained heart tissue sections were examined to assess the effect of the 90 days sub-chronic oral administration with 70% ethanolic seed extracts of *A. gummifera* and *M. ferruginea* on this tissue. Light microscopic examination of the heart sections of 125mg/kg of both male and female rats for seed extract of *A. gummifera* administered group showed blood congestion and area of necrosis **(Figure 5)**. The 250 mg/kg administered group also showed congestions of blood in the myocardium and focal cellular necrosis and pykinosis **(Figure 5)**.

In addition, light microscopic examination of the heart sections of 125 mg/kg of both male and female rats for seed extract of *M. ferruginea* administered group showed congestions of blood in vessels, and necrosis **(Figure 6)**. At the 250 mg/kg, besides congestion it has shown major cellular necrosis and inflammation **(Figure 6)**.

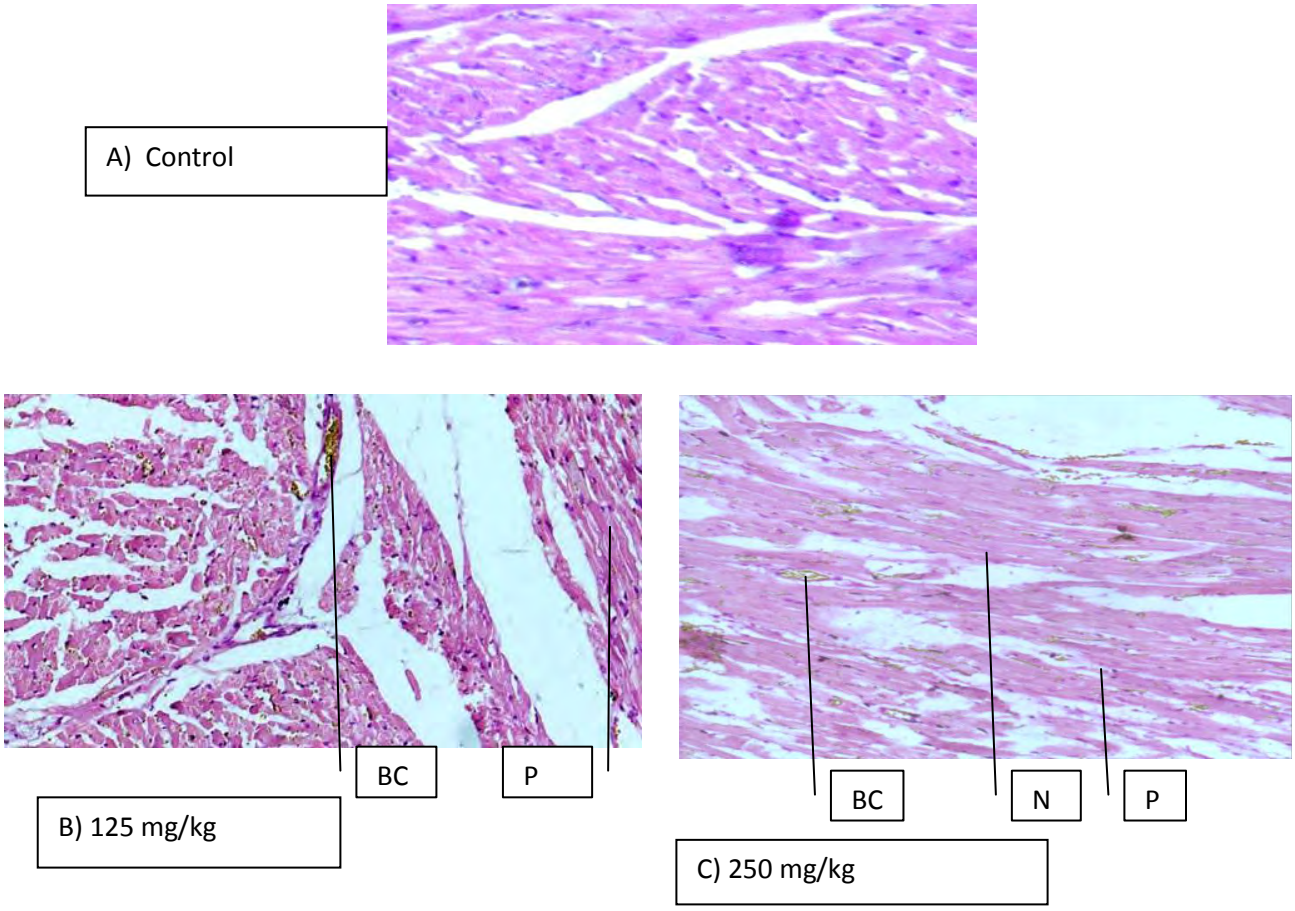

Figure 7: Photomicrograph of H and E stained heart sections from rats administered with hydroethanolic seeds extracts of *A. gummifera at* 125 mg/kg body weight/day (B), 250 mg/kg body weight/day (C), and control (A) rats. *Changes observed in the sections from the hydroethanolic extract administered rat are focal necrosis(N), congestion of blood in the vessels of subendothelial space (BC) in rat administered at 125 mg/ kg body weight/day (B); necrosis(N), congestion of blood in the myocarial vessels (BC), and pykinosis (P) in rats administered 250 mg/k body weight/day (C); While there was no histopathological changes visible in the sections of the control (A) rats. (Magnifications, all ×2000).*

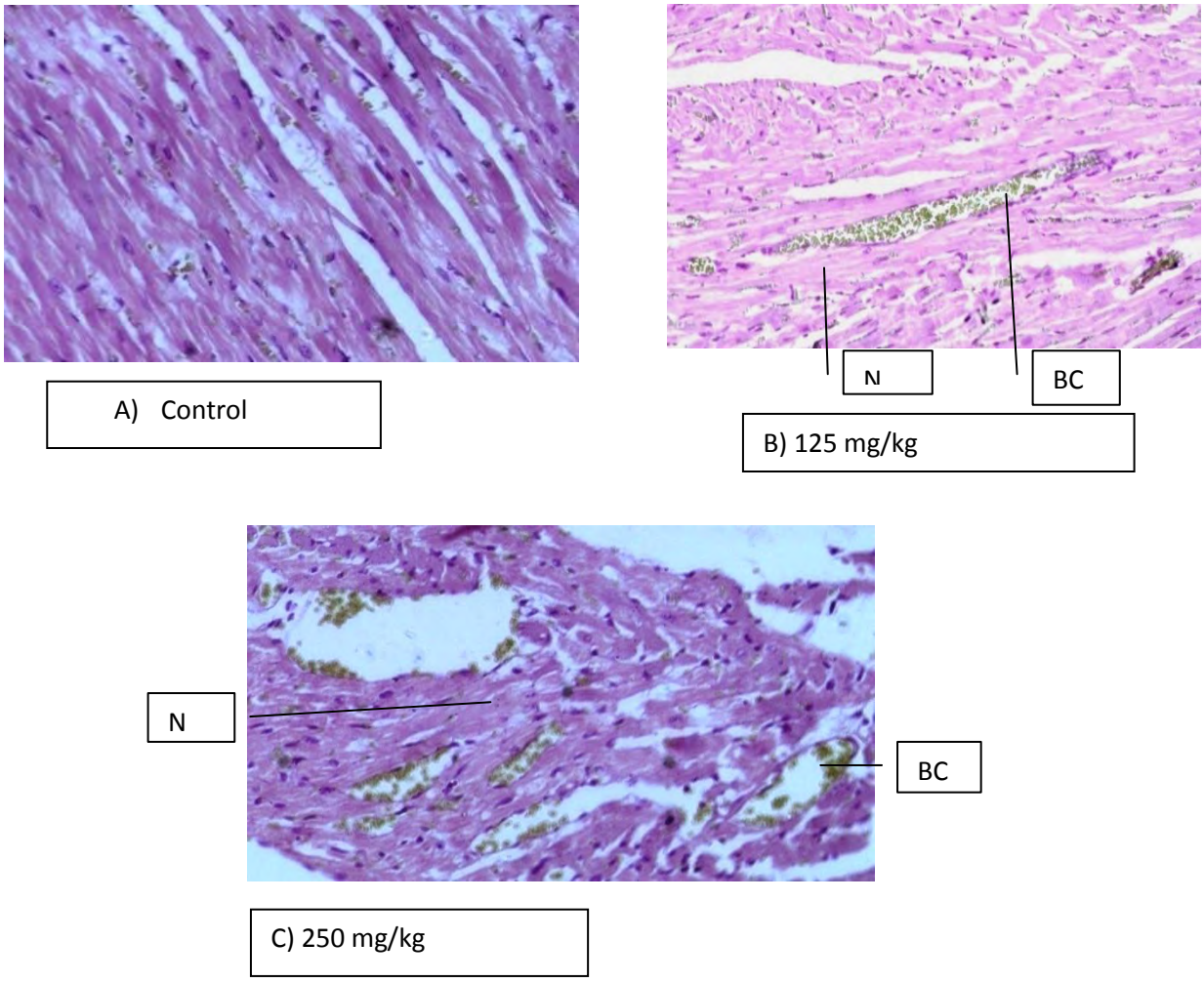

Figure 8: Photomicrographs of H and E stained Heart sections from rats administered with hydroethanolic seed extracts of *M. ferruginea at* 125 mg/kg body weight/day (B), 250mg/kgbody weight/day (C), and control (A) rats. *Changes observed in the sections from the hydroethanolic extract administered rat are congestion of blood in the vessels of myocardium (BC), and area of necrosis (N) in rat administered at 125 mg/ kg body weight/day (B); marked congestion of blood in vessels of myocardium (BC) and areas of necrosis (N) in rats administered 250mg/k body weight/day (C); While there was no histopathological changes visible in the sections of the control (C) rats. (Magnifications, all ×2000).*

Effect of hydroalcoholic seed extract of both plants on histopathology of the small Intestine

Histological examinations of sections of the intestine from rats administered with the seeds extract of *A. gummifera* at 125mg/kg in both male and female rats have shown inflammation (I) and desquamation of the mucosa (D) **(Figure 7)**. Small Intestine histology of both sex administered with 250 mg/kg body weight have also shown desquamation and inflammation of the mucosa, and submucosal atrophy **(Figure 7)**.

Similarly, histological examinations of sections of the intestine from rats administered with the seed extract of *M. ferruginea* at 125mg/kg for both male and female rat showed desquamation and infiltration of the mucosa and submucosal atrophy **(Figure 8)**. Intestine histology of both sexes administered at 250 mg/kg also expressed desquamation and infiltration of the mucosa and marked blood congestions in the submucosa **(Figure 8)**.

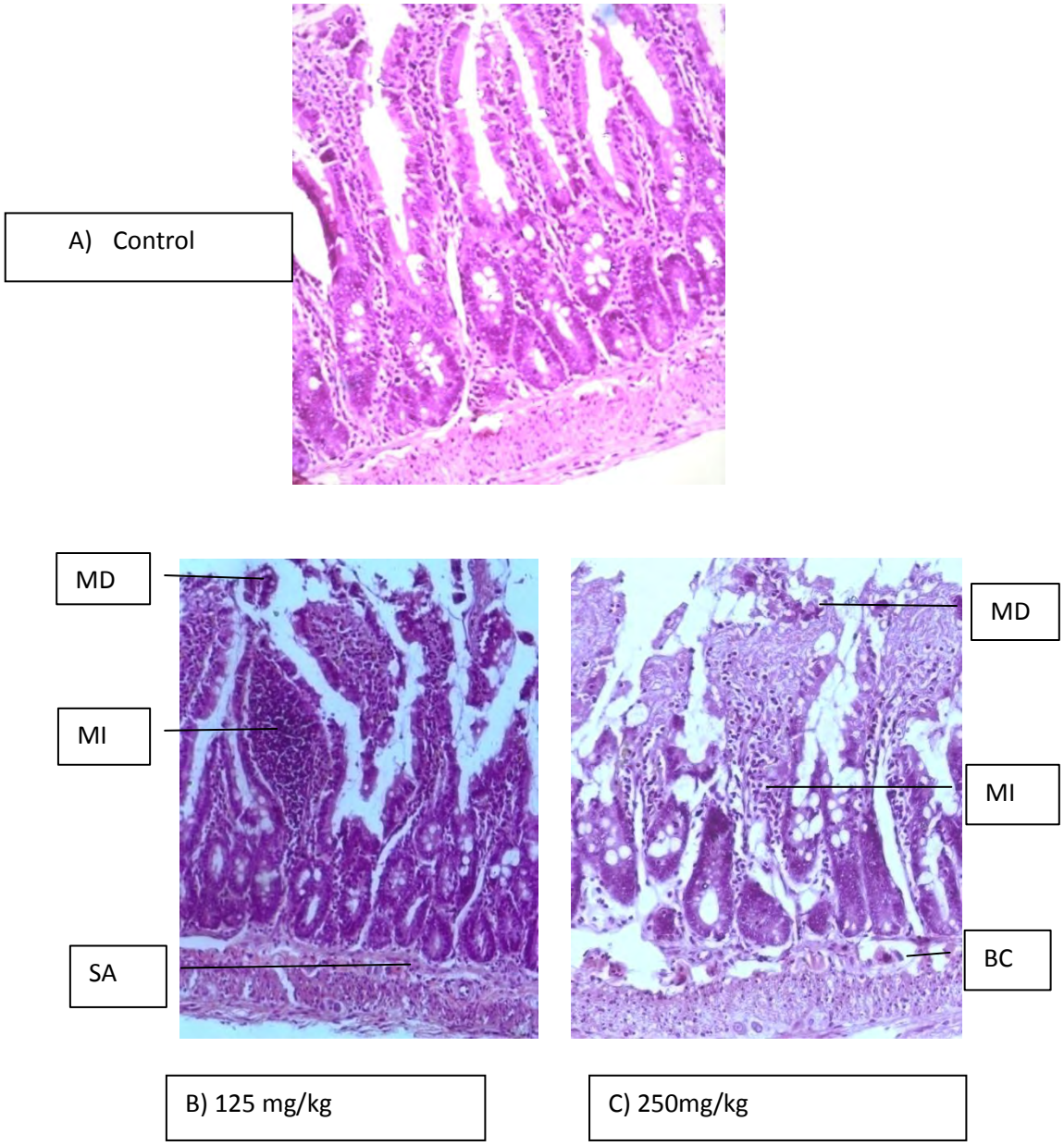

Figure 9: Photomicrograph of H and E stained small intestine sections from rats administered with hydroethanolic seed extract of *A. gummifera* at 125 mg/kg body weight/day (B), 250 mg/kgbody weight/day (C), and control (C) rats. *Changes observed in the sections from the hydroethanolic extract administered rats are mucosal desquamation (MD), mucosal infiltration (MI) and submucosal atrophy (SA) in rat administered at 125 mg/ kg body weight/day (B); mucosal desquamation (MD), mucosal infiltration (MI) , blood congestion in submucosa(BC) in rats administered 250 mg/k body weight/day (C); While there was no histopathological changes visible in the sections of the control rat (C). (Magnifications, all ×2000).*

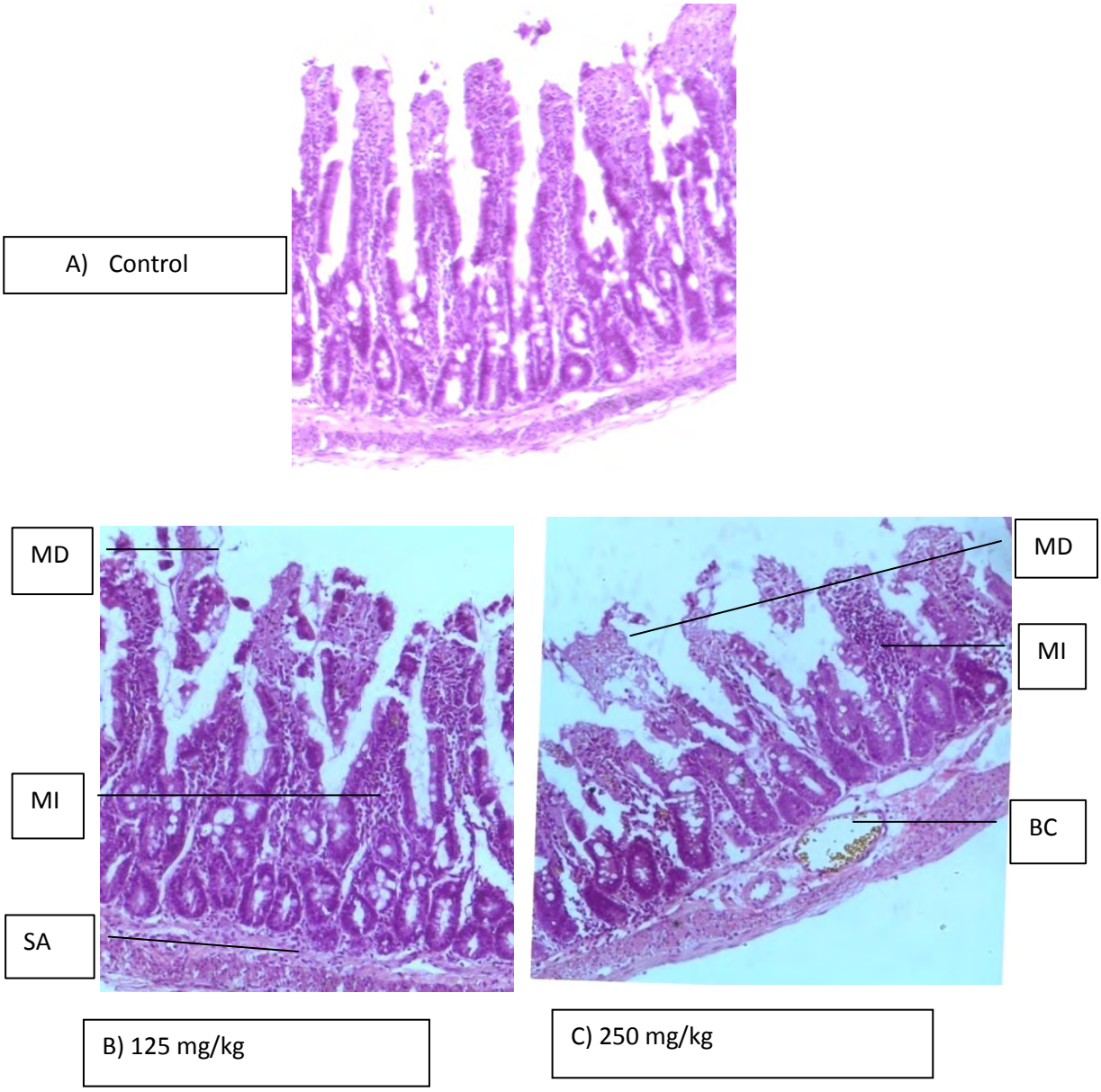

Figure 10: Photomicrograph of H and E stained small intestine sections from rats administered with hydroethanolic seed extract of *M. ferruginea* at 125 mg/kg body weight/day (B), 250 mg/kgbody weight/day (C), and control (A) rats. *Changes observed are: Desquamation of the epithelial layer of the mucosa (MD), mucosal infiltration (MI), submucosal atrophy (SA) in rat administerd at 125 mg/ kg body weight/day (B); atrophy, desquamation and infiltration of the mucosa (MD,MA,MI) and marked blood congestion in the submucosa(BCS) in rats administered 250 mg/kg body weight/day (B); While there was no histopathological changes visible in the sections of the control rat (A). (Magnifications, all ×2000).*

6. DISCUSSION

Albino rats were treated with different doses of seed extracts of *A. gummifera and M. ferruginea* to study acute toxicity test. The result showed no sign of toxicity and death after the aqueous extracts of both plants were orally administered to rats to the level of 3000mg/kg. The present study showed that the LD_{50} of *M. ferruginea and A. gummifera* were found to be 3500 mg/kg and 4000 mg/kg body weight respectively as 50 % of the animals died with 3500 mg/kg and 4000 mg/kg for *M. ferruginea* as well as *A. gummifera* seeds extract treated groups, respectively. In other studies the aqueous leaf extract of *A. chevalieri* also showed LD_{50} of greater than 3000 mg/kg body weight which is almost similar with the hydoethanoic seed extract of *A. gummifera* [45]. Another study conducted on mice also showed the LD_{50} of *A. gummifera* and *M. ferruginea* to be 2300 and 2500 mg/kg body weight respectively [14].

The sub-chronic toxicity was studied by administering seeds extract of *A. gummifera* and *M. ferruginea* to experimental animals (rats) for 90 days and showed no death and abnormal signs throughout the study period. But during the 3rd week of administration of *M. ferruginea*, one male rat was found dead with unknown reason because inspection of the necropsy of the heart and small intestine showed no gross abnormality. Besides, the seeds extracts of both plants had no harmful effect on body growth patterns of test groups. Except for female rats whose body weight insignificantly decreased during the 9th week of administration with seed extract of *A. gummifera* in a dose dependent manner. The body weight of both the test and control groups of both sexes were increased as the duration of treatment increased. Moreover, female rats during the 10th week of treatment with 250 mg/kg body weight of *A. gummifera* seed extract showed statistically significant decrease in body weight. The reason for this significant weight loses might be due to components of the extract which might interfere with the digestive activity. Previous toxicity studies which were done on administration of aqueous extract of *Vernonia amygdalina* [46] and administration of aqueous extract of *Clerodendrum myricoides* on experimental animals also showed similar result [47].

When there is underlying disease condition, it is common to see altered blood and biochemical parameters [48]. So toxicities can be demonstrated by the presence of changes in hematological as well as biochemical parameters [49]. The different hematological

parameters (RBC count, HCT (PCV), HGB, MCV, MCH or MCHC, and RDW-CV) are used to assess types of anemia [27, 50]. RDW-CV, which is an automated parameter providing information on the degree of variation of individual red cell size, has been used with the traditional red cell indices in order to narrow down the possible causes of anemia in an individual patient [51]. Anemia is mainly due to a decrease in RBC count and HGB content. These alterations may be secondary to defective hematopoiesis, inefficient erytheropoiesis or extensive destruction of red blood cells [52, 53].

A. gummifera seed extract administered at 125 and 250gm/kg body weight/ day to male rats for 90 days did not decrease RBC count, PLT, WBC, LYMPH, MONO, EO, BASO, HCT and HGB as compared with the male control group. This finding is in line with the result of other study conducted on related species in which oral administration of saponins isolated from *Albizia lebbeck* bark extract did not decrease hematological parameters. But neutrophil is significantly increased in male rats which are treated with 250 mg/kg *A. gummifera* seed extract as compared to the control. RDW–CV is also significantly increased at both doses as compared to the male control. Even though the extract of this plant did not decrease MCV and MCHC it significantly decreased the level of MCH at both doses as compared to the male control. Based on alterations observed on these hematological parameters, *A. gummifera* may slightly induce anemia in male rats. At both doses, the seed extract of *A. gummifera* did not decrease total RBC count, PLT, WBC, LYMPH, MONO, EO, BASO, HCT, HGB and RDW-CV in the female rats. But it significantly increased the neutrophil at 250 mg/kg as compared to the control. Seed extract of *A. gummifera* did not decrease the level of MCH but, decreased MCV at 250 mg/kg administered group and MCHC at both doses (125 and 250 mg/kg body weight) in the female rats as compared to the female control. These findings also indicate the plant seeds extract might induce anemia in female rats. Further researches on the seed extract of *A. gummifera* are required to validate the present findings [54].

Sub-chronic (90 days) oral administration of *M. ferruginea* seed extract at 125 and 250 mg/kg body weight/day to male rats did not significantly affect total RBC count, WBC, PLT, LYMPH, MONO, BASO, EO, HCT and HGB as compared to the male control group. Besides, the levels of red blood cell indices MCV, MCHC, MCH were not affected and the RDW-CV was not increased following administration of seeds extract of this plant to male

rats at both doses as compared to the male control. Sub-chronic (90 days) oral administration of *M. ferruginea* seed extract at 125 and 250 mg/kg body weight/day to female rats did not alter the blood parameters. Previous study done on the effect of ethanol leaf extract of *Millettia aboensis* on hematological parameters of wister albino rats at dose lower than 2000 mg/kg body weight of the extract also showed similar results. But, at 250 mg/kg body weight of oral administration of seed extract of *M. ferruginea*, MCHC and monocytes have decreased as compared to female control. These may show the presence of a sub-chronic inflammatory process [35].

When cells are exposed to toxic substance persistently they become irreversibly injured and released their components into the surrounding blood vessels. These released cellular components are known as biomarkers. By measuring and quantifying the different serum biochemical profiles one can estimate the extent of tissue injury and know the type of tissue which is injured [55]. The determination of creatine kinase is utilized in the diagnosis and monitoring of myocardial injury. Following injury to the myocardium creatine kinase is released from the damaged myocardial cells [56].

The present study showed that following the sub-chronic oral administration of seed extract of *A. gummifera* at 250 mg/kg to male and female rats, the level of CK is increased by 12% and 14.59%, respectively when compared to the male and female control groups. On the other hand, seed extract of *M. ferruginea* with 125 mg/kg and 250 mg/kg increased serum level of CK by 6.7% and 9.0% respectively in male rats when compared with the male control group. But in female rats, this extract increased CK by 8.8% only at 250mg/kg when compared to female control group. These findings are in agreement with the previous study done on toxicological evaluation of aqueous leaf extract of *Chromolaena odorata* in male Wister albino rats which showed significant increase in CK level at 538.5mg/kg when compared to the control group [57]. The increase in serum level of CK in this study may indicate that seed extracts of both plants have toxic effect on the hearts of male and female rats at higher dose (250 mg/kg). In addition seed extract of *M. ferruginea* at lower dose (125 mg/kg) could also have deleterious effect on the heart of male rats.

ALT which is one of the serum biomarkers is localized in liver tissue, and trace amount is found in skeletal muscle and heart tissue. ALT is localized in cytoplasm and mitochondria of

hepatocytes, skeletal myocytes and cardiomyocytes. If these tissues are injured because of various reasons, ALT could leak out from damaged tissues and appears in the blood [58, 59]. AST is also a biomarker found in liver, heart, muscle, brain and kidney tissues. It is also localized in the cytoplasm and mitochondria of various body cells. Leakage of AST to the blood stream is mainly due to hepatocellular necrosis [58, 60]. ALP is localized in intestine, liver, bile duct, bone, placenta, and kidney tissues. It is manly localized in the cell membrane of various body cells. If there is injury to above mentioned organs there will be over production AST and released in to the surrounding blood vessels [61, 62]. The levels of some cellular products like Albumin are reduced in the blood following damage to their cells of origin [63].

The study showed that sub-chronic (90 days) oral administration of seed extract of *A. gummifera* at 125 and 250 mg/kg body weight to male and female rats did not significantly affect serum biochemical parameters (Albumin, ALP, ALT, and AST) as compared to the control groups. Previous study done on oral administration of aqueous leaf extract of *Albizia chevalieri* and saponins (from *Albizia lebbeck* bark) to experimental rats also showed no significant effect on serum biochemical parameters [45, 54]. The serum urea which was seen increased at higher dose in male rats may show seed extract of *A. gummifera* might induce renal toxicity. Serum urea was increased at 250 mg/kg body weight as compared to the male control. This indicates that the seed extract of *A. gummifera* may induce renal toxicity in male rats at higher doses as supported by previous study in which the serum levels of urea and creatinine in different drug-induced nephrotoxicity were higher in males than females [64-66].

The sub-chronic (90 days) oral administration of seed extract of *M. ferruginea* at 125 and 250 mg/kg body weight/day to male rats showed no significant alteration on serum biochemical levels except for CK which is statistically elevated at both doses. Similar finding is also observed in a study on cardiotoxicicty by oral administration of *Rhododendri Mollis Flos* extract in rats [67]. An increase in the level of CK might show cardiotoxicity of the extract. This is also evidenced by previous study that showed increased serum CK level in Doxorubicin treated mice [68].

Whereas, in female rats, *M. ferruginea* seed extract significantly increases the level of CK, ALP, ALT and Urea at the higher dose. But albumin and AST levels are not affected.
Biochemical parameters (albumin and AST) analyzed for female rats as compared to the control at both doses was also normal. On the other hand, ALP, ALT and Urea were statistically found significant at 250 mg/kg body weight compared to the female control. This shows the seed extract of *M. ferruginea* may induce cardiac, hepatic and renal toxicity in female rats at higher dose. Similar results were also obtained by other researchers with aqueous extract of *Tithonia diversifolia* in rats [69], and *Clerodendrum myricoides* in mice [47].

To assess the effect of these extracts on the weight of the heart and small intestine, the weight of these 2 organs were measured and analyzed. The present study shows statically significant decrease in the weight of the heart of treated rats as compared with the control. This finding is in agreement with previous study done on Doxorubicin treated mice which showed significant decrease in the weight of the heart of treated mice as compared with the control [68]. But the extracts at both doses did not affect the weight of the small intestine of both male and female rats.

To make toxicological study more soundable, besides hematological, biochemical and gross morphological analysis, histopathology of the heart and small intestine was done. Histopathology of the heart and small intestine assesses and investigates cellular injury to these organs. Some of the possible histopathological alterations observed on heart tissue after exposure to toxic agents include: blood congestion, myofibre degeneration, clumped nuclei, eunucleated muscle fibers and pycnotic nuclei [70]. Likewise, histopathological alterations evident in toxic small intestine may include, necrosis, degeneration like dilation of villi, separated basal membrane, degeneration in epithelium and infiltration [71].

In the present study, after treatment of rats with 125 and 250 mg/kg of *A. gummifera* and *M. ferruginea* sees extracts is completed, post mortum inspection and evaluation of organs of rats were made and showed no abnormal gross finding on the heart and small intestine. But light microscopic examination of the heart sections of both male and female rats which are sub-chronically administered with 125 mg/kg of seed extract of *A. gummifera* showed blood congestion and small area of necrosis. Whereas, the 250 mg/kg administered group showed

congestions of blood in the myocardium and focal cellular necrosis and pykinosis. In addition, light microscopic examination of the heart sections of both male and female rats which are sub-chronically administered with125 mg/kg of seed extract of *M. ferruginea* showed congestions of blood in vessels, and small area of necrosis. At the 250 mg/kg, besides congestion has shown major cellular necrosis and inflammation. Histopathological alterations of the heart observed in this study are in agreement with other study done on effect of *Acacia nilotica* pod extract on some biochemical parameters and histopathological features in albino rats which showed marked hemorrhage and myocardial necrosis [72].

Histological examinations of sections of the intestine from rats administered with the seed extract of *A. gummifera* at 125mg/kg in both male and female rats have shown inflammation and desquamation of the mucosa. But at higher dose (250 mg/kg) besides mucosal desquamation and inflammation, tissue sections of small intestine of male and female rats have shown submucosal atrophy. Similarly, histological examinations of sections of the small intestine from rats administered with the seed extract of *M. ferruginea* at 125mg/kg for both male and female rat showed desquamation and infiltration of the mucosa and submucosal atrophy. Intestinal histology of both sex administered at 250 mg/kg also expressed desquamation and infiltration of the mucosa and marked blood congestions in the submucosa. Histopathological alterations of small intestine of rats observed in this study are in line with previous study done on histopathological and biochemical studies after administration of aqueous extract of raw Aloe vera leaves to rats which showed desquamation of the mucosa, mononuclear cellular infiltration and goblet cells hyperplasia [48].

7. CONCLUSION AND rRECOMENDATION

7.1. Conclusion

- Analysis of blood parameters for male rats treated with hydro-alcoholic seed extract of *A. gummifera* at 125 and 250 mg/kg body weight showed decreased MCH and increased RDW-CV, MCH, and increased RDW-CV.
- Decreased MCHC at both doses and MCV at higher dose in female rats which may indicate anemia can be developed in both male and female rats as a result of the extract.
- An increased neutrophil number at 250 mg/kg in male and female rats which may show inflammatory process during the course of the treatment.
- All serum chemistry analyzed in this study for the seeds extract of *A. gummiffera* were found normal in both male and female rats, except for serum CK which is elevated in male and female rats at 250mg/kg body weight.
- An increase in serum urea level in male rats administered at 250 mg/kg body weight of *A. gummiffera* seed extract.
- The histopathological alterations observed in the heart and small intestine tissues of male and female rats are indicatives of the toxic effect of seed extract of *A. gummiffera* on these organs especially at higher doses.
- Hematological parameters analyzed for the hydro-alcoholic seed extract of *M. ferruginea* administered at 125gm/kg and 250gm/kg body weight to male and female rats were found within the normal ranges.
- A decrease in monocyte count in female rats is observed which may be due to infiltration of monocytes to the injured tissue.
- MCHC was also found decreased in female rats at the higher dose which is an indicative of anemia.
- An increased serum CK, ALT, ALP and urea at 250 mg/kg body weight which may have toxic effect on multiple organs at its higher dose.

- The sub-chronic (90 days) administration of seeds extract of *M. ferruginea* at 125 and 250 mg/kg body weight to male and female rats demonstrated histopathological alterations on the heart and small intestines such as congestion of blood in the vessels of myocardium,and area of necrosis at both doses;desquamation of the epithelial layer of the mucosa, mucosal infiltration, submucosal atrophy at the lower dose and atrophy, desquamation and infiltration of the mucosa and marked blood congestion in the submucosa at the higher dose.
- Those hematological, biochemical and histopathological alterations observed on the studied organs and tissues may be resulted from toxic ingredients in the seeds extract of both plants so further studies would be required to isolate the specific components of the plants responsible for the toxicity in order to standardize the plants preparation for maximum therapeutic benefit.

7.2. Recommendation

- Further studies are recommended
  - to isolate the active ingredients responsible for the observedtoxicities
  - to describe the mechanism of action of the extract forthe toxic effects.
  - to examine the toxic effects of these plants on other organs using similar animal model.
  - to assess the toxic effects of these plants on bloodparameters and histopathology of internal organson other animal models.

8. STRENGTH AND LIMITATION OF THE STUDY

8.1. Strength

Effects of the seed extracts were investigated using multiple parameters (Hematological, Biochemical, Histopathological, Acute toxicity)

8.2. Limitation

Troponin which is one of cardiac injury markers is not included because of lack of reagents.

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10. APPENDICES

10.1. Appendix I: preparations of working solutions

10% Neutral Buffered Formalin

10% Formalin (90% distilled H_2O and 37% Formalin) ……...1000ml

Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$)……....4g

Sodium monohydrogen phosphate anhydrous (Na_2HPO_4) ……..6.5g

Harris' Hematoxylin

Hematoxylin Crystal ……..2.5g

Ethanol, 100% ……25ml

Ammonium or Potassium Alum…….....50g

Distilled water …….500ml

Mercuric oxide (red) ……...1.25g

Eosin

Eosin Y (Yellow) ……....0.5g

Ethanol, 95% ……..….100ml

Glaceial acetic acid ……..…0.5ml

1% Acid Alcohol

Ethanol, 70% ……500ml

HCl, concentrated……..5ml

Bluing Solution

Sodium bicarbonate ……...2.5g

Ethanol……1000ml

Distilled water ……500ml

10.2. Appendix II: Tissue processing schedules to form paraffin blocks for manual technique

Fixation

Buffered formalin, 10% ……..24 hrs

Washing

Running tape water…….....24 hrs

Dehydration

Alcohol, 70% ……1 hr

Alcohol, 80% ……1 hr

Alcohol, 95% ……1 hr

Absolute alcohol I ……1 hr

Absolute alcohol II…….1 hr

Clearing

Xylene I ……1 hr

Xylene II ……1 hr

Infiltration (in paraffin oven)

Paraffin wax I 56 $^{o}_{C}$ (52-64$_{o}$c)……1½ hrs

Paraffin wax II 56 $^{o}_{C}$(52-64$_{o}$c)……..1½ hrs

10.3. Appendix III: Routine heamatoxylin and eosin (H and E) staining schedule for tissue sections.

Chemicals	Duration of Staining
Xylene I	5min
Xylene II	5min
Absolute alcohol I	3min
Absolute alcohol II	3min
Alcohol, 95%	3min
Alcohol, 80%	3min
Distilled water	5min
Hematoxylin	4min
Acid alcohol	agitate(1sec)
Bluing solution	1seconds (three dips)
Tap water	5min
Eosin 14	1min
Alcohol, 80%	3min
Alcohol, 95%	3min
Absolute alcohol, II	3min
Absolute alcohol, I	3min
Xylene II	5min
Xylene I	until mounting

10.4. Appendix IV: Routine procedures CELL-DYn 3700 hematology analyzers

- Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
- Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
- Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
- Entering rat code number(ID), sample ID, etc

- With the analyzer in the ready mode, select [RUN], [SPECIMEN TYPE] and then [RAT CODE].
- Enter the following information in the indicated fields:

a. NEXT ID: Manually enter or barcode.

b. PATIENT FIELD: Type in the rat`s code.

c. PARAMETER SET: Enter the number 1.

- Place the sample under the probe and immerse the probe in the specimen.
- Press the touch plate, which is located behind the probe to begin aspiration.
- Remove the specimen from the probe when the beep sounds. The wash block will move down the probe and clean it.
- Upon completion, the wash block returns to the starting position and the specimen results are displayed. Do not begin testing the next sample until the current results are displayed.
- Dilute the sample if White blood cell counts ≥100,000 /mm3 and platelet counts ≥1,000,000 /mm3 are outside the linearity specifications of the instrument.
- Print the displayed results or reports.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: MollaGetu (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Melaku Tamene (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.