EFFECTS OF *ALBIZIA LEBBECK* ON HAEMATOLOGICAL PARAMETERS AND HISTOLOGICAL STRUCTURE OF DIGESTIVE ORGANS IN RATS

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN ANATOMY OF THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2021

ABSTRACT

In Tanzania, *Albizia lebbeck* is among the multipurpose plant used for treatment of various diseases and as a fodder. However, scientific information on the effects of this plant in the animal body is non-existent. Therefore, this study was conducted to evaluate the effects of methanol extracts of stem barks (SB) and leaves of *Albizia lebbeck* on haematological parameters and histological structure of digestive organs in rats. Sixty rats were randomly allotted into ten groups (I-X) of six rats each. Methanol extracts of SB and leaves of *A. lebbeck* were administered orally at doses of 100 mg/kg and 400 mg/kg to the experimental groups and the control groups received distilled water orally for five and 30 days. On days six and 31, rats were sacrificed and blood samples were collected into tripotassium ethylenediamine tetraacetate tubes for haematological analysis and tissue samples from the digestive organs (liver, stomach and jejunum) were immersion-fixed in 10% neutral buffered formalin for histopathological evaluation. Results revealed that, after five days of treatment, both extracts of *A. lebbeck* significantly ($P<0.05$) increased thrombocytes and plateletcrit at 400 mg/kg, and platelets distribution width (PDW) at 400 mg/kg SB compared to the control group. After 30 days of treatment, both extracts significantly ($P<0.05$) increased leucocytes at 100 mg/kg, lymphocytes and eosinophils at 400 mg/kg, also eosinophils and haematocrit at 100 mg/kg leaves, and erythrocytes at 100 mg/kg SB compared to the control group. Both extracts significantly ($P<0.05$) decreased mean corpuscular volume, mean platelet volume, and PDW at 100 mg/kg, monocytes at 100 mg/kg SB, and monocytes and neutrophils at 400 mg/kg leaves compared to the control group. Other haematological parameters changed insignificantly ($P>0.05$) at both doses and treatment durations. Histopathological lesions in the liver including empty spaces in cytoplasm of hepatocytes, sinusoids compression, necrosis, and inflammatory cells infiltration were observed for both extracts. There was no significant

histopathological lesion in the stomach and jejunum at both doses and treatment durations. Therefore, prolonged use of both extracts should be discouraged because may affects haematological parameters and the liver.

DECLARATION

I, Gwakisa Aroni, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Gwakisa Aroni Date

(MSc. Candidate)

The above declaration is confirmed by:

_____

_____ _____

Prof. Remmy J. Assey Date

(Supervisor)

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ACKNOWLEDGEMENTS

I am thankful to The Almighty God for giving me strength, protection, and guidance throughout the course of my study. I would like to thank my supervisor Prof. Remmy J. Assey for his close supervision, inspiration, encouragement, and positive critics which enabled me to accomplish this work on time. My thanks are further extended to Dr. Lusekelo Mwangengwa for his advices throughout the period of my study and his support for chemicals and reagents. I also wish to convey my thanks to the Head of the Department of Anatomy and Pathology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture for his contributions and support towards accomplishments of this work.

It would have been impossible to finish this work without the valuable technical assistances from Mr. Philemon Mkuchu and Mr. Zemu Mbilu. Many thanks to both of them.

Finally, I thank everyone who on one way or another facilitated the accomplishment of this work.

DEDICATION

This work is dedicated to my mother Christina Kajinga and my daughter Gladness Gwakisa.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BA	Basophils
CBC	Complete blood count
CVMBS	College of Veterinary Medicine and Biomedical Sciences
DILI	Drug Induced Liver Injury
E	Eosin
EO	Eosinophils
G	Group
H	Haematoxylin
HB	Haemoglobin
HCT	Haematocrit
HILI	Herb induced liver injury
K3-EDTA	Tripotassium-ethylenediamine tetraacetate
LYM	Lymphocytes
MCH	Mean corpuscular volume
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MON	Monocytes
MPV	Mean platelet volume
NEU	Neutrophils
PCT	Plateletcrit
PCV	Packed cell volume
PDW	Platelets distribution width
PLT	Platelets

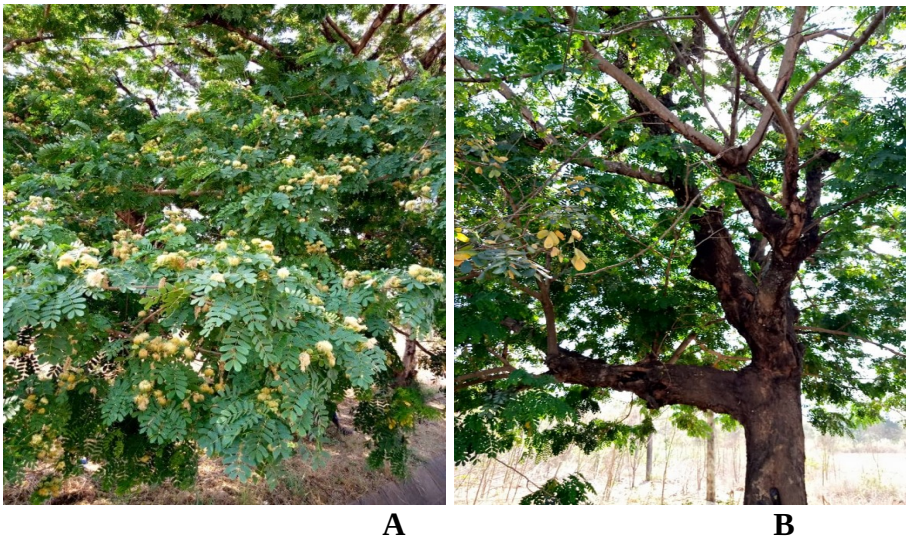
RBC	Red blood cells
RDW	Red blood cell distribution width
SB	Stem barks
SD	Standard deviation
SUA	Sokoine University of Agriculture
THR	Thrombocytes
TM	Traditional medicine
WBC	White blood cells
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Albizia lebbeck is a leguminous plant which belongs to the family of *Fabaceae* (Mishra *et al*., 2010; Danlami and Elisha, 2017). The plant can grow to a height of 18–30 m with a trunk 50 cm to 1 m in diameter (Jayasiri *et al*., 2015; Neeti *et al.,* 2016). The plant is found all over India, Bangladesh, tropical and subtropical Asia and Africa (Islam *et al.,* 2018; Padmanabhan, 2019). It is a multipurpose plant widely used as herbal medicine and forage for animals in many countries. The plant is used in folk remedies for abdominal tumors, boils, cough, eye ailments, flu, lung ailments, leprosy, and scrofulous swellings (Gupta *et al.,* 2006). In Tanzania *Albizia lebbeck* is used for treatment of eye problems, diabetes (Nahashon, 2013), dysmenorrhea, malaria and diarrhoea (Hilonga *et al.,* 2018). The plant is also used as a supplement feed for domestic ruminants (Ndemanisho *et al.,* 2006).



A **B**

Figure 1: Photograph of *Albizia lebbeck* showing: (A) Leaves and flowers (B) Stem and leaves

Haematological parameters are measurements that concern with the blood and organs that are responsible for forming the blood (Bamishaiye *et al*., 2009; Etim *et al*., 2014). Haematological parameters are important and reliable factors used to monitor and evaluate the physiological and pathological status of animals (Ladokun *et al.,* 2015; Debelo *et al.,* 2016). Changes on haematological parameters are often used to determine various states of the body as well as stresses due to environmental, nutritional and pathological factors (Etim *et al*., 2014). The commonly used haematological parameters are erythrocytes (red blood cells, RBC), leucocytes (white blood cells, WBC), haemoglobin concentration (HBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) (Mwale *et al.,* 2014; Fathima *et al*., 2019). Platelets and platelet indices such as plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are also widely used (Giovanetti *et al.,* 2011; Budak *et al.,* 2016).

The digestive system has organs that are responsible for reception, mechanical breakdown, chemical digestion, and absorption of food and elimination of unabsorbed residues. The system runs from the lips to the anus and has parts such as mouth, pharynx, alimentary canal and accessory organs like teeth, tongue, salivary gland, liver and pancreas (Sisson and Grossman's, 1966). The alimentary canal is a tube which extends from the esophagus to the anus. The canal contains several consecutive segments such as esophagus, stomach, small intestine and large intestine (Sisson and Grossman's, 1966). In this study, the digestive organs which were used to evaluate the effects of *Albizia lebbeck* were liver, stomach and jejunum. These organs were purposely selected because of the functions they perform in the animal body. Furthermore, the stomach and jejunum were included in this study due to the fact that there was no study which investigated the effects of *A. lebbeck* in these organs.

1.2 Problem Statement and Justification of the Study

Many people perceive that the use of plants in traditional medicine as well as fodder has no side effects because it is natural, hence safe. However, according to World Health Organization (WHO) (2013) and Mensah *et al.* (2019), the use of traditional medicine under the assumption that "natural means safe" is not necessarily true. The safety of the traditional medicine is of big concern among people and global health authorities because it is used by unlicensed or uncertified practitioners, as well as self-medication (Kasole *et al.*, 2019). Various studies have revealed harmful effects associated with the use of plants or traditional medicines (TM) on tissues, organs, systems, and the whole animal body. For instance, according to WHO (2004) long-term use of kava kava (*Piper methysticum)* can cause serious liver damage and according to Medan *et al.* (2016) *Acacia nilotica* cause toxicity to Nubian goats with postmortem lesions of congested lung, kidney, fatty liver, and intestine. Administration of aqueous pod extract of *Acacia nilotica* to albino rats causes marked haemorrhages in lung, heart and kidney with renal tubular degeneration, hepatocellular necrosis and mononuclear cell infiltrations, mild congestion of the stomach and mild sub mucosal mononuclear cell infiltrations of the small intestine (Umaru, 2012). Debebe *et al.* (2017) demonstrated that administration of the ethanol extract of *Albizia gummifera* seed to albino wistar rats caused congestion of blood in central vein and sinusoids, focal cellular necrosis and pyknosis in the liver, while in the kidneys it caused congestion, atrophy of glomeruli and formation of focal protein cast in the renal interstitium

The benefits of *Albizia lebbeck* are well established and documented throughout the world. In Tanzania, despite the wide use of the locally available species of *A. lebbeck* for treatments of various diseases such as eye problems, diabetes (Nahashon, 2013), dysmenorrhea, malaria, and diarrhoea (Hilonga *et al.*, 2018) as well as the feed-

supplement (Ndemanisho *et al.,* 2006), scientific information on the effects of this plant in the animal body in Tanzania is non-existent. Therefore, the present study was undertaken to evaluate the effects of locally available species of *A. lebbeck* on haematological parameters and various histological structures of the digestive organs in rats.

1.3 Objectives

1.3.1 General objective

To evaluate the effects of *A. lebbeck* on haematological parameters and histological structure of digestive organs in rats.

1.3.2 Specific objectives

i. To determine the effects of various doses of methanol extracts of stem barks and leaves of *A. lebbeck* on haematological parameters such as RBC count, HB, PCV, RBC indices, WBC count and differential WBC counts, platelets and platelets indices in rats.

ii. To assess the histological changes in the liver, stomach and jejunum of rats following exposure to various doses of methanol extracts of stem barks and leaves of *A. lebbeck.*

1.4 Research Questions

i. What are the effects of methanol extracts of the stem barks and leaves of *A. lebbeck* on haematological parameters in rats?

ii. What are the histological changes on the structure of the digestive organs of rats post exposure to the methanol extracts of stem barks and leaves of *A. lebbeck*?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Albizia lebbeck*

2.1.1 Description of the plant

A. lebbeck is a plant belonging to the legume family (Fabaceae) sub-family Leguminosae (South-Australia), Mimosoideae (new South-Wales) (Shakoor *et al.,* 2016). This tree can reach a height of 18–30 m with a trunk 50 cm to 1 m in diameter (Neeti *et al.,* 2016; Singh and Agrawal, 2018). In open circumstances, the tree is spreading and sometime multi stemmed, to 25 meters high and 30 meters in width, with green color lower branches (Shakoor *et al.,* 2016). The leaves are bi-pinnate, 7.5–15 cm long, with one to four pairs of pinnae, each pinna with 6–18 leaflets. The flowers are white, with numerous 2.5–3.8 cm long stamens, and very fragrant. The fruit is a pod 15–30 cm long and 2.5-5.0 cm broad, containing six to twelve seeds (Neeti *et al.*, 2016; Singh and Agrawal, 2018), having pale bark with glabrous young shoots (Sharma and Dubey, 2015; Doshi *et al*., 2016)**.** The plant has many common names such as women tongue and rattle tree, names derived from the noise made by the dry pods of the tree when they are being shaken by wind (Sheyin *et al.,* 2015; Neeti *et al.,* 2016), Shirish (Desai *et al.,* 2014; Sharma and Dubey, 2015), and Siris tree (Danlami and Elisha, 2017; Padmanabhan, 2019). In Tanzania, the plant is known as Mkungu (Nahashon, 2013), Mlongelonge, and Myenjeyenje (Hilonga *et al.,* 2018) and in Swahili is known as Mkungu and Mkingu (Shakoor *et al.,* 2016).

2.1.2 Distribution of the plant

The plant is found all over India, Bangladesh, tropical and subtropical Asia and Africa (Islam *et al.,* 2018; Padmanabhan, 2019). The plant can vary from one area to another on

morphology, growth habit, flower color, leaves, stem and chemical composition (Shakoor *et al.,* 2016).

2.1.3 Phytochemicals

The phytochemicals of the plant are alkaloid, terpenoid, saponin, steroid and cardiac glycoside (Hussain *et al.,* 2008), tannin, flavonoid (Rahul *et al.,* 2010), phlabotannin (Tasnim *et al.,* 2014), anthraquinones and other phenolics (Hussain *et al.,* 2008).

2.1.4 Nutritional components

The leaves have 17% to 26% crude protein; 100 kg of leaves yield11-12 kg of digestible protein and 37 kg of digestible carbohydrate (Mishra *et al.,* 2010; Tasnim *et al.,* 2014).

2.1.5 General uses of the plant

2.1.5.1 Herbal medicine

i) Every part of the plant

Is used for the treatment of bites and stings from venomous animals (Zia-ul-haq *et al.,* 2013; Wati and Khabiruddin, 2017).

ii) Roots

Are used for alleviation of spasms and stimulates cardiovascular system, also have the anticancer and spermicidal properties (Sharma and Dubey, 2015).

iii) Leaves

Are used for treatment of ophthalmic diseases, night blindness, syphilis and ulcer, cold, cough, and respiratory disorders (Sharma and Dubey, 2015).

iv) Barks

Applied locally for healing wound, acne vulgaris, non-healing ulcers, boils, and abscess. It is also used to treat pruritis, scabies, and dermatophytosis locally whereas internally it is used in powder form in blood dyscrasias and Jaundice (Khan *et al.,* 2018). It is also used orally in the management of dementia, psychosis, epilepsy, mania, and paralysis (Khan *et al.,* 2018).

v) Seeds

Its powder is used locally in the diseases of eyes such as corneal opacity and grittiness of eyes along with other medications while internally it has been used in the management of premature ejaculation, aphrodisiac, scrofula, syphilis, epilepsy, mania, cold and cough, sinusitis, constipation, hemorrhoids, and dysentery (Khan *et al.,* 2018).

vi) Flowers

Are used for managements of chronic cough, bronchitis, tropical pulmonary eosinophilia, asthma, inflammation, scrofula, skin diseases, leprosy, leucoderma, chronic catarrh, seminal weakness, opthalmopathy, poisoning, for the treatment of spermatorrhea, and snakebite (Verma *et al.*, 2013).

vii) Pods

Are used as antiprotozoal, antidiabetic and anticancer (Zia-ul-haq *et al.,* 2013**;** Sharma and Dubey, 2015).

2.1.5.2 Fodder

It is grown as fodder for ruminants that feed on leaves, twigs, seeds, and pods in Pakistan (Zia-ul-haq *et al.,* 2013). In Sudan, goats eat the fallen leaves and flowers, also has been

reported that rabbits and bats feed on the leaves (Mishra *et al.,* 2010). Most livestock readily eat leaves and young twigs of this promising fodder tree (Ansari *et al.,* 2016).

2.1.6 Effects on the reproductive organs

A. lebbeck significantly decrease weights of reproductive organs (Gupta *et al.,* 2006, Sharma *et al.,* 2013), for instance the testis, epididymis, seminal vesicle and ventral prostate decreased in a significant manner (Gupta *et al.,* 2004, 2005). The plant has also been shown to decrease the sperm motility as well as sperm density (Gupta *et al.,* 2006, Sharma *et al.,* 2013), deplete significantly surface area of sertoli cells as well as the cells counts, decrease Leydig cells nuclear area and number of mature Leydig cells, and reduce the levels of testosterone in serum (Gupta *et al.,* 2006).

2.2 Haematological Parameters

Investigation of haematological parameters provide valuable information on the determination of the extent of the deleterious effects of foreign compounds such as plant extracts on the blood constituents of an animal (Daradka, 2016; Christian *et al.,* 2017). Differential white blood cells count is useful for establishing the nature of infection due to the facts that relying only from white blood cell count may provide information which can be either partial or in some cases misleading (Saidu *et al.,* 2007). When the haematological parameters are severely altered either by increasing or decreasing in number, it implies the presence of various kinds of diseases or toxicity (Mwale *et al.,* 2014).

The commonly used haematological parameters are erythrocyte (red blood cell, RBC), leucocytes (white blood cells, WBC), haemoglobin (HB), Packed Cell Volume (PCV) and values which include mean corpuscular volume or cell (MCV), mean corpuscular

haemoglobin, mean corpuscular haemoglobin concentration (MCHC) (Fathima *et al*., 2019), platelets and platelet indices such as plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) (Giovanetti *et al.*, 2011).

2.2.1 Red Blood Cells (RBC)

Red blood corpuscles (erythrocytes) are non-nucleated cells in mammals and they have haemoglobin that is responsible for transporting oxygen. The common shape of RBC in most domestic animals is disc or biconcave disc (discoid) leading to high surface area to volume ratio resulting into more deformable red blood cell (Adili and Melizi, 2014). Water account 60% of erythrocytes volume and the remaining 40% represent solids substances, almost 90% of solid material is haemoglobin (Thamer *et al.,* 2016). The size of mature erythrocytes varies from one species to another for instance in bovine and equine is 5-6 µm, in ovine 4-5 µm, in caprine is 2.5-3.9 µm, and in canine is 6-8 µm (Adili *et al.*, 2016). The number of erythrocytes in human are $5x10^6$ per microlitre with the reference range being $4.7x\ 10^6$-$6.1\ x10^6$ in men and $4.2\ x10^6$-$5.4x10^6$ in women (Dzierzak and Philipsen, 2014), cow is 5.0 –10.0 $x10^6/mm^3$ (Etim *et al.*, 2013; 2014), rabbit is 5.0-8.0 $x10^6/mm^3$ (Etim *et al.,* 2014), sheep is 9.0-15.0 $x10^6$/µl and goat is 8.0-$18x\ 10^6$/µl (Jones and Allison, 2007). The number of erythrocytes may elevate above the normal range due to various conditions such as increased erythropoietin production as caused by chronic hypoxemia and disorders of lung or renal, myeloproliferative disorder, exposure to carbon monoxide, chronic kidney disease, liver disease, certain forms of heart disease, lung disease, diarrhoea, vomiting, excessive sweating, dehydration, use of diuretics and the severe burns (Arika *et al.,* 2016). Erythrocytes may drop below the reference range as the result of anaemia, bone marrow failure, erythropoietin deficiency, haemolysis, nutritional deficiencies including iron, copper, folate, vitamin B12, and vitamin B6, over dehydration, and blood sucking parasites (Etim *et al.*, 2013).

Red blood cell count is the determining the number of erythrocytes in the animal and is an integral part of the complete blood cell count (CBC). This process is useful for diagnosis of anaemia as well as other conditions altering the red blood cell. Red blood cell count includes total RBC count, haemoglobin, haematocrit, and red blood cell indices. Red blood cell indices are parameters which are used to determine the size and the content of haemoglobin in RBC. These indices include the mean cell volume or mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration, and red blood cell distribution width (RDW) (Mwale *et al.,* 2014; Fathima *et al*., 2019). Alteration of the reference range of these indices suggests the presence of anaemia and the type of anaemia (Etim *et al.,* 2013).

i. Haemoglobin (HB)

Haemoglobin is the protein substance found in erythrocytes of all vertebrates except in Channichthyldae and in tissues of some invertebrates (Etim *et al.,* 2013). Haemoglobin is produced in the bone marrow and has heme as prosthetic group and globin as the protein part. Heme is formed by joining of iron and porphyrin ring and is present as aprosthetic group in haemoglobin and other substances such as myoglobin, cytochromes, peroxidases, catalases and tryptophan pyrrolases (Arika *et al.,* 2016). It is responsible for transporting respiratory gases such as oxygen from the respiratory organs to other parts of the body tissues and carbon dioxide from the tissues to the respiratory organs for exhalation (Etim *et al.,* 2013; Arika *et al.,* 2016).

The concentration of haemoglobin in health animals differ from one species to another such as in cow is 10 –15 g/dl, in pig is10 –16 g/dl, in sheep is 8 –16 g/dl, in rabbit is 10 – 15 g/dl, in guinea pig is 11 –15 g/dl (Etim *et al.,* 2013), and in adult human is 12.0 - 17.2 g/dl (Arika *et al.,* 2016). The molecular weight of haemoglobin is approximately 67, 000

and each gram has 3.4 mg of iron. Many factors may decrease the concentration of haemoglobin in animals such as bleeding, iron deficiency (Etim *et al.*, 2013; Arika *et al.*, 2016), increase of erythrocytes destruction, and sickle cell disease (Arika *et al.*, 2016). The decreased levels of haemoglobin either accompanied with the decrease of the erythrocytes or not may result into anaemia which in turn impedes the amount of oxygen delivered to the body tissues. The level of haemoglobin may increase due to exposure to high attitudes, dehydrations, and tumours (Etim *et al.*, 2013).

ii. Haematocrit (HCT)

Haematocrit is the proportional occupied by red blood cell in whole blood volume in animals and is important clinically for determination of anaemia (Arika *et al.*, 2016). According to Etim *et al.* (2013) the word haematocrit was derived from Greek words, hema meaning “blood” and criterion. It was coined by Magnus Blix at Uppsala in 1891 as haematokrit and in 1903 haematocrit was coined. Its root stems from the Greek words hema-blood and krites, judge –meaning to gauge or judge the blood (Etim *et al.*, 2013). The haematocrit or packed cell volume (PCV) is one of an important constituent of an animal’s complete blood count result, together with haemoglobin concentration, white blood cell count, and platelet count (Etim *et al.*, 2013). According to Etim *et al.* (2013) the normal values of PCV in various species are 24% to 45% in sheep, 24% to 48% in cattle, 30% to 50% in rabbit, and 37% to 48% guinea pig, while Arika *et al.* (2016) reported that the normal values of PCV in human are 42% to 52% for males and 36% to 48% for females. When the levels of haematocrit are lower than the normal levels it indicates anaemia and when the levels of haematocrit increase above the normal range it imply polycythemia (Arika *et al.*, 2016).

Many factors are responsible for decreasing the packed cell volume such as liver and kidney diseases, malnutrition, vitamin B12 and folic acid deficiencies, iron deficiency, and pregnancy while high levels of haematocrit may results due to dehydration, which is a decreased amount of water in the tissues, and diarrhoea (Etim *et al.,* 2013). According to Etim *et al.* (2013) the use of mean corpuscular volume (MCV) and the red cell distribution width (RDW) can be valuable for assessments haematocrit when decreased below the normal range, because the haematocrit used to measure the percentage of red blood cells within the blood volume. The MCV is the measure of average size of the red cells and the RDW is measure of the heterogeneity in size of the red cell population. When haematocrit decreased below the normal levels together with the decrease of MCV below the normal range with an increase of RDW indicates a chronic-iron-deficient anaemia leading into fault haemoglobin production during erythropoiesis (Etim *et al.,* 2013).

iii. Mean corpuscular volume (MCV)

Mean corpuscular volume is a measure of the average volume or size of a red blood cell. It is expressed in femtolitre (fl, or 10-15L) and the normal values differ from one species to the other such as in cow is 39 –55 fl, in pig is 50 –68 fl, in sheep is 23 –48 fl, in rabbit is 78 –95 fl, in guinea pig is 67 –77 fl (Etim *et al.,* 2014), and in human is 78.5-96.4 fl (Arika *et al.,* 2016). The average red blood cells size influence the rise or fall of MCV for instance small average red blood cells size (microcytic) result into low MCV, normal average erythrocytes size (normocytic) lead to normal MCV, and large average blood cell size (macrocytic) result into high MCV(Arika *et al.,* 2016). MCV can be used to categorize anaemia into microcytic, normocytic or macrocytic anaemia (Etim *et al.,* 2013). Mean corpuscular volume may decrease below the normal values due to iron

deficiency, microcytic anaemia and thalassaemia syndromes while may increase above the normal range because of vitamin B12 deficiency and folate deficiency (Arika *et al.,* 2016). According to Doig and Zhang. (2017) and Paul *et al*. (2017) MCV can be calculated by using the following formula:-

$$MCV\ (fl) = \frac{HCT\ (\%)\ X10}{RBC\ (Million/\mu l)}$$

iv. Mean cell haemoglobin (MCH)

The MCH is the average amount of haemoglobin in erythrocytes and is expressed in picograms (Doig and Zhang, 2017). According to Etim *et al.* (2013) it is obtained by dividing the total mass of haemoglobin by the number of erythrocytes in a volume of blood and multiplies by 10. MCH = (HB x 10)/RBC. The amount of haemoglobin is influenced by the size of the erythrocytes for instance small cells have small amount of haemoglobin and the large cells have large amount of haemoglobin. Therefore, the MCV should be taken into consideration whenever the MCH are interpreted (Doig and Zhang, 2017). These parameters are direct related for example when MCH decreased the MCV also will decrease, but hypochromia cannot be determined using the MCH. Hence, the MCH is not a good indicator compared to the MCHC, which does correlate to visual assessments of hypochromia (Doig and Zhang, 2017). The reference values of MCH in various species are in cow is 13 –17 pg, in pig is17 –23 pg, in sheep is 8 –12 pg (Etim *et al.*, 2014), and in human is 27-33 pg (Arika *et al.*, 2016). The values can be below the normal range as the results of various factors such as iron deficiency, thalassaemias, and in some cases of anaemia in chronic diseases, while elevated values of MCH may results due to macrocytic anaemia (Arika *et al.*, 2016).

v. Mean corpuscular haemoglobin concentration (MCHC)

MCHC is the measure of average concentration of haemoglobin in red blood cell and is expressed in g/dl or percentage (%) (Arika *et al.,* 2016). The primary function of MCHC is for diagnosis of anaemia, also used to determine the capacity of the bone marrow to produce red blood cells (Etim *et al.,* 2013). The normal range of MCHC varies from one species to another such as in human is 32.6-37.7 g/dl (Arika *et al.,* 2016), in cow 30 –36 g/dl, in pig is 30 –36 g/dl, in sheep is 31 –38 g/dl, in rabbit is 27 –37 g/dl, and in guinea pig is 30 –34 g/dl (Etim *et al.,* 2014).

The concentration of MCHC may decrease below the reference range as the result of iron deficiency, abnormal haemoglobin synthesis, failure of blood osmoregulation and plasma osmolarity and may elevate above the reference values due hereditary spherocytosis (Arika *et al.,* 2016). When the concentration of MCHC is reduced is known as hypochromic in microcytic anaemia, when it is normal is known as normochromic in macrocytic anaemia (because of the larger cell size, although the haemoglobin amount or MCH is high, the concentration remains within the reference intervals), and increased MCHC in some cases is known as hyperchromic (Etim *et al.,* 2013). According to Doig and Zhang. (2017) and Paul *et al.* (2017). MCHC can be calculated by the following formula: - MCHC = HB/HCT × 100%. Unequal rise of the HB will result into a false increase of the MCHC. Assessment of the morphology of erythrocytes will indicate that there are no spherocytes, thus, no hyperchromia. On the other hand, when HCT decrease result also into false rise of MCHC (Doig and Zhang, 2017).

vi. Red blood cell distribution width

Red blood cell distribution width (RDW) is the measure of variation in size of erythrocytes. The RDW increase when there is anisocytosis and remain within the normal range when there is no anisocytosis (Doig and Zhang, 2017). This parameter cannot fall

below the reference value because it is a statistical calculation of the heterogeneity of the size of red blood cells (Doig and Zhang, 2017). Red blood cell distribution width can be expressed as either a standard deviation (RDW-SD) or coefficient of variation (RDW-CV), the latter being the SD divided by the MCV. In either case, as the size range between cells increases, the RDW rises (Doig and Zhang, 2017). On the other hand, for the RDW to drop below the normal range the cells produced are supposed to be of the same size than normal which is impossible because cell production is the biological process, therefore, RDW is a parameter that only increase (Doig and Zhang, 2017). Although in rarely conditions sick individual may have an RDW value that is marginally dropped below the normal range, but will not implies as either an instrument error or a pathological condition due to the fact that determination of the normal values leaves about 2.5% of healthy, normal patients' results outside the reference interval on the lower end (Doig and Zhang, 2017).

2.2.2 Leucocytes and related parameters

Leucocytes are granulocytes and agranulocytes cells of immune system that protect animals from infections (Roland *et al.*, 2014). Leucocytes are classified into two major groups of granulocytes and agranulocytes based on the presence or absence of the specific granules into the cytoplasm, respectively. The granulocytes include neutrophils, eosinophils, and basophils while the agranulocytes include the lymphocytes and monocytes. All leucocytes except lymphocytes are formed from myeloid stem cells which are found in the bone marrow while the lymphoid stem cells which are found in lymphoid tissues produce lymphocytes (Jones and Allison, 2007). Leucocytes contribute only number of small percentage of the total population of blood cells and change widely for instance when the blood pressure and blood flow velocity increase will result into increasing number of leucocytes in the circulation due to the fact that the marginal pool of

neutrophils will detach and join the circulatory pool, and when the blood pressure and blood flow velocity decrease will result into decreasing number of leucocytes in the circulation pool (Roland *et al.,* 2014). According to Etim *et al.* (2013) in adult health animal the number of leucocytes is about 7000 per microlitre of blood which contribute about 1% of the total blood volume. When the number of leucocytes rises above the reference range is known as leucocytosis and when drop below the reference range is known as leucopenia (Roland *et al.,* 2014).

Increase in leucocytes count after treatment with plant extracts is an indication that the plant most likely possesses phytochemicals which have an immunomodulatory effects that can boost up immune system of the animals through increased production of leucocytes (Ahmed and Aslam, 2018; Madaki *et al.*, 2019). The immune boosters are used to strengthen and harmonize degenerative body systems and assist the immune system to fight invading agents including bacteria and viruses (Ladukon *et al.,* 2015).

i. Lymphocytes (LYM)

Lymphocytes are cells of immune system that facilitate the phagocytes in protecting the body from pathogens and other foreign invasion (Arika *et al.,* 2016). They are round in shape with the nuclei at the center or slightly at peripheral (eccentric). The chromatin in the nucleus is closed packed and, the nucleus is large which make high ratio to cytoplasm (Mitchell and John, 2008). The primary lymphoid organs such as the bone marrow and thymus are responsible for synthesis of lymphocytes while in the secondary lymphoid organs including lymph nodes, spleen and lymphoid tissues alimentary and respiratory tract is where the specific immune response are produced (Arika *et al.,* 2016). Based on the functions lymphocytes are classified into T- lymphocytes which account 75% of the total lymphocytes of the blood and are involved in cell-mediated immunity and B-

lymphocytes which constitutes 25% of the total lymphocytes and are responsible for humoral immunity (Arika *et al.,* 2016). When the antigen or foreign substance stimulates the B-lymphocytes within the spleen and lymph nodes, they will change into immunoblasts and finally into plasma cells which produce the antibody specific to the antigen (Arika *et al.,* 2016). Majority of the T-lymphocytes produced in the bone marrow are destructed in the thymus so as to choose few cells which recognize self-histocompatibility molecule of human leucocytes antigen but not react with self-tissue antigens.

Lymphocytes can be either of the small size such as 6 to 9 µm in diameter which is found in horse, pig and carnivores or of large size such as 12 to 15 µm in diameter (Dellmann and Brown, 1981). The normal range of lymphocytes population within the WBC for different animals species are 40% to 60% for pig, 40% to 70% for sheep and cow, 40% to 80% for rabbit, 39% to 72% for guinea pig (Etim *et al.,* 2014), and 20% to 40% for dog, cat and horse (Dellmann and Brown, 1981). Lymphocytosis occurs when the lymphocyte count is over the upper limit such as in human when the absolute lymphocyte count is >4000/cmm. Levels are higher in infancy and gradually decrease toward adult levels. Causes of lymphocytosis include acute infections, pertussis, hepatitis, infectious mononucleosis, chronic infections, tuberculosis, congenital syphilis and lymphoma or leukemia (Arika *et al.,* 2016).

ii. Monocytes (MON)

Is one of the types of agranulocytes leucocytes found in the circulation and form an integral part of the phagocytes system (Arika *et al.,* 2016). Monocytes are the largest leucocytes within the normal peripheral blood smear with the diameter of 15 to 20 µm (Dellmann and Brown, 1981). The shape of monocytes is round or amorphous, with the

nuclei that can be either oval, or round, or lobed (Mitchell and Johns, 2008). In the connective tissues the monocytes become macrophages and interact with lymphocytes and play vital functions in the recognition and interaction of immunocompetent cells and antigen (Arika *et al.*, 2016).

The normal ranges of monocytes in different species are 2% to 10% for pig, 0% to 6% for sheep, 1% to 6% for cattle, 1% to 4% for rabbit, and 2% to 6% for guinea pig (Etim *et al.*, 2014). Monocytosis is the situation in which the number of monocytes increases above the reference range because of the various conditions including chronic bacterial infections such as tuberculosis, inflammation and malignant disorders while monocytopenia which is the situation when the number of monocytes decreased below reference range due to corticosteroid treatment (Arika *et al.*, 2016).

iii. Neutrophils (NEU)

Neutrophils are granulocytes polymorphonuclear leucocytes which account 60% to 70% of circulating leucocytes in humans (Arika *et al.,* 2016). The diameter of mature neutrophils is about 10-12 µm and has fine granules within the cytoplasm and a lobed nucleus (Dellmann and Brown, 1981). The primary granules of the neutrophils have myloperoxidase, acid phosphatase and other acid hydrolases while the secondary granules have collagenase, lactoferrin and lysosomes (Arika *et al.,* 2016). The neutrophils protect the body of animals against infections by microorganisms particularly bacteria through oxygen dependent mechanism which involve synthesis of hydrogen peroxide and the superoxide anion, and eliminate infectious agent through intracellular pH or enzymes lysozyme, also through lactoferrin that are contents of the secondary granules (Arika *et al.,* 2016).

Neutrophilia is the condition in which the number of neutrophils increases over the upper limit while neutropenia is the condition in which the number of neutrophils decreases below the lower limit. Various factors are responsible for neutrophilia including bacterial infections, inflammation or necrosis, metabolic disorders (diabetic ketoacidosis, uremia, and eclampsia), Steroid therapy, and acute haemorrhage or hemolysis (Arika *et al.,* 2016). Neutropenia is caused by wide range of inherited and acquired disorders, infections, vitamin deficiencies, and direct toxicity or immune-mediated damage by drugs (Arika *et al.,* 2016).

iv. Eosinophils

Eosinophils are granulocytes that are synthesized in the bone marrow with the main functions being destroying pathogen and modifying the process of inflammation. The diameter of the eosinophils is 10 to 15 µm and has bilobed nuclei surrounded by important acidophilic granules 0.5 to 1.0 µm in size (Dellmann and Brown, 1981). The reference values of eosinophils in different species are 0% to 10% in pig and sheep, 0% to 4% in cattle and rabbit and 0% to 5% in guinea pig (Etim *et al.,* 2013). Eosinophilia is the condition of increased eosinophil count above the normal values which results due to allergic disease, parasite infection, certain fungus infections, asthma, autoimmune diseases, eczema, hay fever, leukemia and other blood disorders such as Chronic myeloid leukemia, Pernicious anaemia and Hodgkin disease while eosinopenia which is the condition of eosinophil count to decrease below the reference values that result due to alcohol intoxication and overproduction of certain steroids in the body (such as cortisol) (Arika *et al.,* 2016).

v. Basophils

Are granulocytes leucocytes with large many dark cytoplasmic granules that obscure the nucleus and has heparin and histamine (Arika *et al.,* 2016). The diameter of basophils is 10 t0 12 µm and the nucleus can be either bilobed or irregular-shaped (Dellmann and Brown, 1981). When the basophils move into the tissues differentiate into mast cells. They are used in immunoglobulin E (IgE) mediated hypersensitivity reactions because of having attachments sites for IgE and their degranulation results into release of histamine (Arika *et al.,* 2016). Consequently, when allergen and IgE reacts results into release of histamine which cause the clinical features of allergy or hypersensitivity (Arika *et al.,* 2016). Basophils have heparin, histamine, and serotonin, and are thought to stimulate blood flow to injured tissues, simultaneously preventing excessive haemorrhage (Arika *et al.,* 2016).

2.2.3 Platelets

Platelets are derived from cytoplasmic fragments of megakaryocytes with its primary function to prevent bleeding (Jones and Allison, 2007). Each platelet formed from the fragmentation of the megakaryocytes is covered by a piece of cell membrane and they range from 250 000 to 400 000 platelets/µL in blood with the life span of only five to nine days (Arika *et al.,* 2016). The size of platelet varies from one species to other with the smallest size observed in bovine (Roland *et al.,* 2014). The total number of circulating thrombocytes is affected by various processes such as production, consumption, sequestration, and loss (Jones and Allison, 2007). Apart from hemostasis and thrombosis, platelets play vital role in inflammatory process, microbial host defence, wound healing, angiogenesis, remodeling, influence the function of the vascular wall and circulating immune cells, and secrete microbicidal proteins and antibacterial peptides (Budak *et al.,* 2016). Also facilitate movement of leucocytes from the circulation to the tissues and play various vital functions in the mechanisms of diseases development (Budak *et al.,* 2016).

Platelets count is the process which involves determination of the total number of the platelets within the blood streams and platelets indices such as plateletcrit, mean platelet volume (MPV), and platelet distribution width (PDW). When all the platelet indices are measured at the same time will give us a valid instrument for measuring disease severity and an insight into the potential etiology that resulted in platelets' indices changes (Budak *et al.,* 2016).

i. Plateletcrit (PCT)

Plateletcrit is the percentage of the volume occupied by platelets in the bloodstream and is obtained by using the formula PCT = platelet count × MPV / 10,000 (Budak *et al.,* 2016). Under physiological conditions, the amount of platelets in the blood is maintained in an equilibrium state by regeneration and elimination. Under normal situations, regeneration and elimination keep the number of platelets in blood constant (Budak *et al.,* 2016).

ii. Mean platelet volume (MPV)

Mean platelet volume is the measure of average size of platelets and useful test for the differential diagnosis of thrombocytopenia (Gao *et al.,* 2014). According to Gao *et al.* (2014) MPV is negative correlated to the degree of PLT maturity. Mean platelet volume levels rise when platelet synthesis is reduced and young platelets become bigger and more active (Budak *et al.,* 2016). The indicator of platelets production rate and activation is the elevated MPV which suggests increased platelets diameter. When the platelets are activated their shape transform into spherical from the biconcave discs, and increased pseudopod formation occurs that results to MPV increase during platelet activation (Budak *et al.,* 2016). High grade inflammation may either increase the levels of MPV because of the presence of the large size of platelets in bloodstream or decrease the levels

of MPV because of the increase utilization and migration of these large platelets in the vascular segments of the inflammatory area (Budak *et al.,* 2016). Various factors such as race, age, smoking status, alcohol consumption, and physical activity are responsible to alter the blood platelet count and MPV (Budak *et al.,* 2016).

iii. Platelet distribution width (PDW)

Platelet distribution width is the measure of platelets heterogeneity and elevates when there is platelet anisocytosis (Budak *et al.,* 2016). The normal range of PDW differs widely from 8.3% to 56.6% (Budak *et al.,* 2016). According to Gao *et al*. (2014) various factors are responsible for the variation of the platelet size such as swelling, destruction, and immaturity which may result into the rise of the PDW above the reference interval. Platelet distribution width directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology (Budak *et al.,* 2016). Mean platelet volume and PDW are directly related and usually change in the same direction under the normal conditions (Budak *et al.,* 2016).

2.3 Liver

The liver is the largest gland in the body and has both endocrine and exocrine roles; it is responsible for synthesis, collection and concentration of bile in the gall bladder before being released in the duodenum (Dawood and Khamas, 2017). The liver is found in the cranial third of the abdominal cavity and has many lobes; however, the nomenclature for the liver lobes differs among authors (Thoolen *et al.,* 2010). Large part of the liver lies to the right in many species, even though may extends across the median plane (Al-Sobayil, 2008). The surface of the liver that faces the diaphragm is more convex and the surface that faces the abdominal organs is concave. These surfaces when they meet ventrolaterally they form a sharp edged border and dorsally they form a blunt border

(Aurich *et al*., 2014). The concave surface has the hepatic porta through which several structures such as portal vein, bile duct, and hepatic vessels enter and leave the organ (Dawood and Khamas, 2017).

The relative weight of the liver varies from one group of animals to the other for instance in carnivores is 3% to 4%, 2% in omnivores, 1% to 1.5% in herbivores (Dawood and Khamas, 2017), and 6% in rats (Vdoviaková *et al.,* 2016). In many species, the liver has four main lobes which are right, left, quadrate and caudate lobe, with a several supporting ligaments. In rats and mice the four lobes are median (middle), right, left, and caudate lobe and all, except the left, are more subdivided into two or more parts (Malarkey *et al.,* 2005).

The morphology and functions of liver in mammals is complex, it is made up with parenchyma cell known as hepatocytes and non- parenchymal cells including the Kupffer cells, sinusoidal endothelial cells, and other cells types that are located in the sinusoidal compartment (Rashad *et al*., 2017). The number of hepatocytes in the liver are many which account 60% of the total liver cells and 80% of the volume of liver, sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and biliary epithelium make up a significant number (3-20% each) of the biologically important each (Malarkey *et al*., 2005)**.** The hepatic lobule is the structural unit of the liver (Rashad *et al.,* 2017). The central vein located in the center of each hepatic lobule and the hepatic sinusoids arise between the plates of the hepatocytes (Madhan and Raju, 2013). The plates of hepatocytes which are organized into roughly hexagonal are partitioned by intervening sinusoids which are radiating outward from the central veins, with portal triad at the vertices of each hexagon **(**Madhan and Raju, 2013; Rashad *et al.,* 2017).

The functional sub-unit of the liver is the acinus which has elliptical or diamond shape (Thoolen *et al.,* 2010). The liver acinus constitutes blood flow and metabolic functions and has three zones which are zone 1 (periportal), zone 2 (transitional; midzonal), and zone 3 (centrilobular) (Thoolen *et al.,* 2010). Hepatocytes in zone 1 are responsible for oxidative liver functions such as gluconeogenesis, β-oxidation of fatty acids, and cholesterol synthesis, while zone 3 cells are more specialized for glycolysis, lipogenesis, and cytochrome P-450–based drug detoxification (Thoolen *et al.,* 2010).

Blood is conveyed to the liver through the hepatic artery and portal vein (Vdoviaková *et al.*, 2016; Rashad *et al*., 2017). According to Malarkey *et al*. (2005) about 70% of the blood flow and 40% of the oxygen are delivered to the liver by portal vein while 30% of the blood flow and 60% of the oxygen are delivered to the liver by hepatic artery. These vessels enter in the liver on the visceral surface through the hepatic porta and form branches along the fibrous septa together with the tributaries of the hepatic duct (Aurich *et al.,* 2014). The blood is taken away from the liver by hepatic vein which release its content into the caudal vena cava (Dyce *et al*., 1996).

2.4 Stomach

Is the dilated part of the gastrointestinal tract in which the process of digestion are initiated, it is located between the esophagus and small intestine (Dyce *et al*., 1996). Stomachs of domestic animals are classified based on the form into simple stomachs with one compartment and complex stomachs with many compartments, and distribution of the mucosa lining into glandular stomachs which are lined by glandular mucosa with simple columnar epithelium and composite stomachs which have the glandular mucosa and non-glandular mucosa that covered with stratified squamous epithelium (Aurich *et al.,* 2014). Cats and dogs have the simple glandular stomachs. The horse and the pigs have the simple, composite stomachs, whereby the majority of the stomach is lined by the

glandular mucosa and small cranial part by a non-glandular mucosa (Aurich *et al.,* 2014). Also rat has the simple, composite stomach, the forestomach (non-glandular stomach) covered with the stratified squamous epithelium and account about 60% of the total area of the stomach and the glandular part account the remaining part (Frantz *et al.,* 1991). Ruminants have a complex, composite stomach, which comprises of four compartments, three of which (rumen, reticulum, and omasum) are lined by non-glandular mucosa and one (abomasum) lined by glandular mucosa (Aurich *et al.,* 2014).

Stomach walls have four layers which are arranged from outside to inside into peritoneum, smooth muscle, submucosa, and mucosa (Dellmann and Brown, 1981; Dyce *et al*., 1996). The mucosa can be either non-glandular or glandular depending on the location for instance where esophagus joins the stomach is non-glandular and the remaining part is the glandular mucosa (Aurich *et al.,* 2014). The species-specific distribution of the gastric gland result into partitioning the stomach into three glandular regions; cardiac (*glandulae cardiacae*), proper gastric (*glandulae gastric propriae*), and pyloric (*glandulae pyloricae*) (Aurich *et al.,* 2014). These glands are named according to the region where they are located for instance the cardiac glands found in the cardia, the pyloric glands are located in the mucosa of the pyloric part of the stomach, and the proper or fundic glands are found in the fundus and body (Dellmann and Brown, 1981). The cardiac and pyloric glands secrete mucus, which protect the mucosa of the stomach against the gastric juice by the lining the inner surface of the stomach and by neutralizing the acid (Aurich *et al.,* 2014). There are three various classes of the cells which are located in the region of the proper (fundic) gastric glands including the neck cells, which are found in the neck of the glands produce mucus and serve as reserve cells to replace epithelial cells, chief cells produce pepsinogen, the precursor for pepsin, and Parietal cells which produce the chloride and hydrogen ions and an intrinsic factor which is the essential for the resorption of vitamin B12 in the ileum (Aurich *et al.,* 2014).

The submucosa consists of a strong, but thin layer of areolar tissue. It is separated from the mucosa proper by plexiform muscularies mucosae. It has the gastric arteries, veins, nerves, fat, lymphatic tissue, and fibres especially the collagenous and elastic (Aurich *et al.*, 2014). The muscular layer has smooth muscles which are arranged into two layers of inner circular and outer longitudinal muscles, these muscles are useful for combining the food with the gastric juice and ultimately conveying it to the small intestine (Aurich *et al.*, 2014). The visceral serosa covers the entire organ, attaching to the underlying muscle except along the curvatures where it is reflected to continue into the omenta (Dellmann and Brown, 1981).

2.5 Small Intestine

Is the part of alimentary canal which extends from the pylorus to the caecum and it has three main parts which are duodenum, jejunum, and ileum (Bello *et al.,* 2019). The small intestine comprises four layers which are mucosa (*tunica mucosa*), submucosa (*tela submucosa*), muscular part (*tunica muscularis*), and peritoneum (*serosa lamina visceralis*) from inside to outside (Dyce *et al*., 1996). The mucosa consists of mucosa epithelium with the columnar cells, lamina propria with the straight tubular intestinal gland, and lamina muscular. The submucosa is made up with the loose connective tissues, blood vessels, lymphatics, lymphatic follicles, and nerves plexuses. Muscular layer has the inner circular layer and outer longitudinal which is relatively thinner compared to the former (Aurich *et al.*, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

This study was conducted at Sokoine University of Agriculture (SUA) in Morogoro region, Tanzania between November 2019 and June 2020. Morogoro region lies between latitude 6° 49' 27" South of the Equator and between longitude 37° 39' and 48" East of Greenwich Meridian. The topography of Morogoro is predominantly plain with rainfall ranging between 900 and 1400 mm annually. The average temperature in Morogoro is 30^0C due to its lowland nature. The area was selected purposely because of the presence of the facilities and experts, therefore, the project was conducted smoothly and cost effectively.

3.2 Plant Materials Collection and Preparation of the Methanol Extracts of Stem barks and Leaves of *Albizia lebbeck*

Stem barks and leaves of the plant were collected from a forest around SUA main campus. The plant materials were chopped into small pieces, dried under shade at room temperature for six weeks, and then grinded into fine powder using wooden mortar and pestle. Five hundred grammes (500g) each of the powdered stem barks and leaves were weighed and put into a separate clean dry conical flask and 1.5 litres of 70% methanol was added in each. The mixture was shaken and left for 72 hours. The extracts were decanted into clean dry conical flasks and then filtered through cotton wool and pieces of clothes. The filtrates obtained were evaporated to semi solid substance by using rotary evaporator at 70^0C and concentrated by using water bath at 46^0C to yield a solid mass of the stem barks and leaves of *A. lebbeck*. Then the solid mass obtained was packed into separate air tight containers and stored in the refrigerator until the time of use.

The use of methanol for extraction of stem barks and leaves of *A. lebbeck* was adopted from other studies. Furthermore, methanol was recovered from the extracts by using rotary evaporator and the solid mass of the extracts obtained were dissolved in distilled water. Therefore, the histopathological lesions observed in the liver may resulted due to the effects of the *A. lebbeck* and not because of methanol.

A **B** **C** **D**

Figure 2: (A) Incubation of the mixture of 70% methanol and powdered stem barks. (B) Filtration of the mixture. (C) Evaporation of the filtrate. (D) Concentrating of the filtrate in a water bath at 46^0C

3.3 Study Animals

Healthy Wistar albino rats (*Rattus norvegicus*) of both sexes were purchased from a breeder in Morogoro town and used in this study. The rats were 15-16 weeks old and weighing 96-135 g. The rats were allowed to acclimatize for a period of two weeks in the small animal unit house at the College of Veterinary Medicine and Biomedical Sciences (CVMBS), SUA prior to the study. The female rats were nulliparous and non-pregnant. The rats of different sexes were put in different cages and each cage had a maximum of four rats. The rats were maintained under standard laboratory conditions of

12 hours light and 12 hours dark, ambient temperature of 28 ± 2^0C and 45-60% humidity. The rats were fed on broiler mash finisher and tap water *ad libitum*. The animal welfare was considered throughout the study period.

3.4 Sample Size

Sample size was estimated by using the following formula by Charan and Biswas, 2013.
Resources equation methods (E) =Total number of animals-Total number of the groups.
The optimum size is 10 to 20.

Since the treatment duration was divided into two major phases, each phase had five groups. Phase one (treatment for five days): E= (5x5)-5= 20
Phase two (treatment for 30 days): E= (5x5)-5= 20
Therefore, 50 rats were required for both treatment duration, but taking into consideration of attrition and power maximization, 60 rats were used in this study.

3.5 Study Design

Sub-acute toxicity study was performed to evaluate the effects of *A. lebbeck* on the haematological parameters and structure of digestive organs in rats. In this design rats were randomly assigned into ten groups of six (n=6) rats each. Each group had four females and two males. Rats in the control groups were orally administered with one ml of distilled water once per day for five and 30 consecutive days, while those in the treatment groups received methanol extracts of stem barks and leaves of *A. lebbeck* orally at different doses once per day for five and 30 days as shown in Table 1. The solid mas of the methanol extracts obtained were dissolved in distilled water so as to facilitate easy administration orally.

Table 1: Summarize the sub-acute toxicity study design

Groups	Doses	Treatments	Days of treatment
I	One ml	Distilled water	Five
II	100 mg/kg	Stem barks extract	Five
III	100 mg/kg	Leaves extract	Five
IV	400 mg/kg	Stem barks extract	Five
V	400 mg/kg	Leaves extract	Five
VI	One ml	Distilled water	30
VII	100 mg/kg	Stem barks extract	30
VIII	100 mg/kg	Leaves extract	30
IX	400 mg/kg	Stem barks extract	30
X	400 mg/kg	Leaves extract	30

3.6 Sample Collection

Rats from both the control and experimental groups were put under general anaesthesia by using chloroform on day six for groups I-V and on day 31 for groups VI-X. Anaesthesized each rat was put on dorsal recumbency or supine position and the abdominal and thoracic cavities were opened. After opening the cavities, blood sample was collected from the heart of each rat into vacutainer tube with anticoagulant tripotassium ethylenediamine tetraacetate (K3-EDTA) for haematological analysis. Following collection of blood sample, each rat was sacrificed by dislocation of the neck and digestive organs (liver, stomach and jejunum) were collected and immersion-fixed in 10% neutral buffered formalin for histological studies.

3.7 Evaluation of Effects

3.7.1 Determination of haematological parameters

Blood samples collected into K3-EDTA vacutainer tube as described in section 3.6 were analyzed by using automated haematological analyzer (NS^{TM} 4S, FRANCE). Red blood cell count, HB, HCT, RBC indices (MCV, MCH, MCHC, and RDW), WBC count, WBC differential counts (LYM, MON, NEU, EO, and BA), THR, and THR indices (MPV, PCT, and PDW) were determined.

A **B**

Figure 3: (A) blood sample collection (B) Blood samples analysis

3.7.2. Histological studies of the digestive organs

3.7.2.1 Tissue processing

The 10% neutral buffered formalin fixed tissues preserved as described in section 3.6 were processed using standard protocols (Kiernan, 1990). These protocols were slightly modified at the Department of Anatomy and Pathology, CVMBS, SUA. Briefly; (i) Dehydration: the fixed tissues were dehydrated with progressively increasing concentration of ethanol from 70%, 95% I, 95% II, absolute I, and absolute II at a temperature of 60^{0}C for twenty minutes each except 70% was for fifteen minutes.

(ii) Clearing: the dehydrated tissues were cleared by using chloroform I and chloroform II at a temperature of 60^0C for 30 minutes each. (iii) Paraffin: wax infiltration, the cleared tissues were impregnated with paraffin wax I and paraffin wax II for forty minutes each.(iv) Embedding: the wax impregnated tissues were embedded in paraffin blocks. All tissue blocks were labeled and allowed to dry at room temperature. (v) Sectioning: the tissue blocks were sectioned by using a Rotary Microtome (Ref. No: 368065/2, Baird

and Tatlock (London) LTD. Essex, England) at 4-5 μm thickness. The ribbons of these sections were collected and gently floated on a tissue flotation water bath at a temperature of 20^0C to stretch the paraffin wax impregnated tissue. The stretched floated ribbons were picked up on microscopic slides. The slides were put in oven overnight at a temperature of 60^0C to help the tissue section adhere to the slides. The slides were allowed to cool at room temperature and kept ready for routine staining steps.

3.7.2.2 Tissue staining

Standard tissue staining protocols (Kiernan, 1990) were used with minor modification as described below:-

Before staining of the tissues, the sectioned tissues were deparaffinised by three series of xylene starting from xylene I, xylene II and xylene III for five minutes each. Then the tissues were hydrated successively by running through decreasing concentration of ethanol from absolute, 95% and 70% for two minutes each. The slides were then rinsed in distilled water followed by Harris' haematoxylin (H) stain for five minutes. These slides were washed in tap water and dipped into 1% acid alcohol for differentiation and remove excess stain. The sections were rinsed briefly in running tap water to remove excess acid.

The slides were then dipped in lithium carbonate (Li_2CO_3, a bluing solution) followed by counter stain with eosin (E) for five minutes. The H and E stained sections were washed fairly quickly in water and dehydrated by running through ethyl alcohol I, ethyl alcohol II and ethyl alcohol III for two minutes in each. Then the slides were run through xylene I, xylene II and xylene III for two minutes in each. Lastly, these slides were mounted using DPX mountant and glass cover slips.

3.7.2.3 Microscopy and photomicrography

Microscopic slides of organs under study were examined carefully with compound light microscope at the Department of Anatomy and Pathology, CVMBS, SUA, starting with magnification of four, followed by 10, 20, and 40 magnifications. Slides from the experimental groups were assessed for any morphological deviation in the respective organs compared to their control counterparts. Lastly, photomicrographs of some selected slides were taken using Olympus BH-2 mounted with Moticam Pro 205A Camera.

3.8 Ethical Considerations

Ethical clearance was issued by SUA ethical committee with reference number RPGS/R/ ETHICS/24 and all experiments were carried out in accordance with SUA guidelines.

3.9 Data Analysis

Quantitative data collected was analysed by one way analysis of variance (ANOVA) using R version 3.5.1 statistical software. Post hoc testing was performed for comparisons between groups by using Dunnett's t-test. All values were expressed as Mean ± standard deviation (SD). P values <0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

None of the experimental animals fell sick or died during the experimentation period

4.1 Effects of *Albizia lebbeck* on Haematological Parameters

4.1.1 Effects of methanol extracts of stem barks and leaves of *Albizia lebbeck* on white blood cells count and differential white blood cells counts following five and 30 days of treatment

Oral administration of methanol extract of stem barks of *A. lebbeck* to rats at doses of 100 mg/kg and 400 mg/kg induced changes on total white blood cells count and differential white blood cells count compared to the control group as shown in Table 2 for five days of treatment and Table 3 for 30 days of treatment**.** The extract of stem barks at doses rates of 100 mg/kg and 400 mg/kg after five days of treatment did not cause significant change ($P > 0.05$) in WBC, LYM, NEU, MON, EO, and BA compared to the control group. However, WBC, NUE, MON, and BA marginally decreased at both doses compared to the control group, while LYM and EO slightly increased compared to the control group. Oral administration of the extract of stem barks of *A. lebbeck* for 30 days caused a significantly ($P<0.05$) increase in WBC at a dose rate of 100 mg/kg compared to the control group, slightly decreased WBC at the dose rate of 400 mg/kg although, was not statistically significant ($P > 0.05$), significantly ($P<0.05$) increased LYM and EO at dose rate of 400 mg/kg compared with the control group, marginally increased LYM and EO at the dose rate of 100 mg/kg compared to the control group even though was not statistically significant ($P > 0.05$), significant ($P<0.05$) decreased in MON at dose rate of 100 mg/kg while at dose rate of 400 mg/k MON insignificantly changed ($P > 0.05$) although, slightly decreased compared to the control group, and NEU as well as BA

insignificantly changed (P> 0.05) at both doses compared to the control group even though both slightly increased at dose rate of 100 mg/kg and both marginally decreased at dose rate of 400 mg/kg compared to the control group.

Similarly, oral administration of methanol extract of leaves of *A. lebbeck* in rats at doses rates of 100 mg/kg and 400 mg/kg induced some changes on total white blood cells count and differential white blood cells counts compared to the control group as shown in Table 4 for five days of treatment and Table 5 for 30 days of treatment**.** The methanol extract of leaves of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg after five days of treatment did not cause significant change (P> 0.05) in WBC, LYM, NEU, MON, EO, and BA compared to the control group. However, WBC, NEU, MON, and BA slightly decreased at both doses compared to the control group, while LYM and EO marginally increased at both doses compared to the control group.

After 30 days of treatment, the leaves extract significantly (P<0.05) increased WBC at dose rate of 100 mg/kg compared to the control group, while at 400 mg/kg marginally decreased compared to the control group, although was not statistically significant (P> 0.05). The extract significantly (P<0.05) increased EO at both doses rates compared to the control group. However, the extract significantly (P<0.05) decreased NEU and MON at dose rate of 400 mg/kg compared to the control group. Neutrophils and MON at dose rate of 100 mg/kg were not statistically significant (P> 0.05), although slightly decreased compared to the control group. Basophils at both doses was not statistically significant (P> 0.05), even though slightly increased at 100 mg/kg and marginally decreased at 400 mg/kg compared to the control group.

Table 2: Effects of methanol extract of stem barks of *A. lebbeck* on WBC count and differential WBC counts after five days of treatment

Haematological parameters	Doses and groups		
	Control (GI)	100 mg/kg(GII)	400 mg/kg(GIV)
WBC (M/mm^3)	7.50 ±3.98	5.29±2.31(0.57)	5.74±1.85(0.22)
LYM (%)	58±17.40	62.38±5.73(0.90)	59.54±8.0(0.36)
NEU (%)	15.05±4.50	14.94±8.63(0.56)	9.39±5.77(0.73)
MON (%)	15.42±3.29	13.50±2.62(0.35)	11.80±4.47(0.42)
EO (%)	9.74±3.26	11.25±4.64(0.08)	10.52±5.35(0.24)
BA (%)	0.30±0.19	0.25±0.19(0.41)	0.26±0.18(0.58)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 3: Effects of methanol extract of stem barks of *A. lebbeck* on WBC count and differential WBC counts at day 30 post treatment

Haematological parameters	Doses and groups		
	Control (GVI)	100 mg/kg (GVII)	400 mg/kg (GIX)
WBC (M/mm^3)	7.06 ±2.73	13.32±0.55**(0.01)	5.99±3.02(0.83)
LYM (%)	56.88±5.27	58.18±6.49(0.36)	66.68±3.5**(0.00)
NEU (%)	20.35±4.61	21.78±5.36(0.60)	12.50±9.71(0.08)
MON (%)	17.40±3.61	12.93±0.90*(0.04)	13±6.84(0.09)
EO (%)	5.01±2.17	9.65±4.81(0.32)	13.60±6.0**(0.01)
BA (%)	0.35±0.21	0.48±0.12(0.65)	0.33±0.14(0.47)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 4: Effects of *A. lebbeck* leaves extract on WBC count and differential WBC counts at day five post treatment

Haematological parameters	Doses and groups		
	Control (GI)	100 mg/kg (GIII)	400 mg/kg (GV)
WBC (M/mm^3)	7.50 ±3.98	5.61±1.79(0.36)	4.92±2.62(0.31)
LYM (%)	58±7.40	63±5.48(0.78)	63.85±5.99(0.34)
NEU (%)	15.05±4.50	13±6.35(0.96)	13.04±9.47(0.85)
MON (%)	15.42±3.29	14±3.64(0.39)	13.16±2.40(0.33)
EO (%)	9.74±3.26	12±5.34(0.67)	9.83±3.34(0.61)
BA (%)	0.30±0.19	0.26±0.18(0.57)	0.23±0.05(0.59)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 5: Effects of *A. lebbeck* leaves extract on WBC count and differential WBC counts after 30 days of treatment

Haematological parameters	Doses and groups		
	Control (GVI)	100 mg/kg (GVIII)	400 mg/kg (GX)
WBC (M/mm^3)	7.06 ±2.73	12.84±2.56**(0.02)	5.72±2.02(0.77)
LYM (%)	56.88±5.27	57.80±4.68(0.50)	66.6±4.21**(0.00)
NEU (%)	20.35±4.61	23.58±5.37(0.90)	9.65±2.45*(0.02)
MON (%)	17.40±3.61	19.95±3.86(0.32)	11.78±1.44*(0.02)
EO (%)	5.01±2.17	11.9±3.86**(0.00)	13.7±5.23**(0.00)
BA (%)	0.35±0.21	0.48±0.36(0.80)	0.3±0.22(0.94)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

4.1.2 Effects of stem barks and leaves extracts of *Albizia lebbeck* on red blood cells count and red blood cells indices after five and 30 days treatment

Treatment of the rats with methanol extract of stem barks of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg caused some changes on RBC count and RBC indices compared to the control group as illustrated in Table 6 for five days of treatment and Table 7 for 30 days of treatment**.** The methanol extract of stem barks of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg after five days of treatment insignificantly changed (P> 0.05) RBC, MCV, HCT, MCH, MCHC, HB, and RDW compared to the control group. The extract slightly increased RBC and HCT at both doses and marginally decreased MCV, MCH, MCHC, HB, and RDW at both doses compared to the control group. After 30 days of treatment, the extract significantly (P<0.05) increased RBC and significantly (P<0.05) decreased MCV at dose rate of 100 mg/kg compared to the control group. Other parameters such as HCT, MCH, MCHC, HB, and RDW insignificantly changed at doses rates of 100 mg/kg and 400 mg/kg after 30 days of treatment compared to the control group. However, HCT and RDW marginally increased at both doses compared to the control group, and MCH, MCHC, and HB slightly decreased at both doses compared to the control group.

In addition, treatment of rats with methanol extract of leaves of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg caused some changes on RBC count and RBC indices compared to the control group as illustrated in Table 8 for five days of treatment and Table 9 for 30 days of treatment**.** Methanol extract of leaves of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg after five days of treatment insignificantly (P>0.05) changed RBC, MCV, HCT, MCH, MCHC, HB, and RDW. However, at both doses the extract marginally increased RBC, HCT and RDW, and slightly decreased MCV, MCH, MCHC, and HB compared to the control group. After 30 days of treatment, the leaves extract significantly (P<0.05) increased HCT and significantly (P<0.05) decreased MCV at the dose rate of 100 mg/kg, while at the dose rate of 400 mg/kg HCT and MCV insignificantly changed, although both slightly increased compared to the control group. RBC, MCH, MCHC, HB, and RDW insignificantly (P>0.05) changed at both dose s after 30 days treatment compared to the control group. MCH, MCHC, HB, and RDW marginally decreased at both doses and RBC slightly increased at both doses compared to the control group.

Table 6: Effects of stem barks extract of *Albizia lebbeck* on red blood cells count and red blood cells indices after five days of treatment

Haematological parameters	**Doses and groups**		
	Control (GI)	**100 mg/kg (GII)**	**400 mg/kg (GIV)**
RBC (M/mm^3)	7.21±0.81	7.50±0.85(0.68)	7.55±0.63(0.69)
MCV (fl)	59.22±0.81	57.88±1.0(0.33)	58.58±0.82(0.65)
HCT (%)	42.62±5.46	43.42±5.20(0.62)	44.18±3.98(0.42)
MCH (pg)	19.08±0.87	18.0±0.76(0.29)	18.64±0.93(0.37)
MCHC (g/dl)	32.32±1.89	31.62±1.28(0.51)	31.90±1.75(0.45)
HB (g/dl)	13.74±1.03	13.72±1.38(0.36)	13.66±0.60(0.58)
RDW	10.46±0.26	10.54±0.64(0.26)	10.72±1.11(0.71)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 7: Effects of *Albizia lebbeck* stem barks extract on red blood cells count and red blood cells indices after 30 days of treatment

Haematological parameters	Doses and groups		
	Control (GVI)	**100 mg/kg (GVII)**	**400 mg/kg (GIX)**
RBC (M/mm^3)	4.39±1.98	7.0±0.22**(0.02)	5.67±2.37(0.08)
MCV (fl)	75.18±10.95	58.83±1.13*(0.03)	69.33±8.27(0.61)
HCT (%)	37.13±8.93 42.87±11.32(0.38)		45.90±0.66(0.54)
MCH (pg)	40.23±11.43 31.70±16.93(0.74)		28.20±13.0(0.40)
MCHC (g/dl)	32.37±7.16	26.15±6.23(0.62)	28.72±4.43(0.31)
HB (g/dl)	15.28±1.10	14.93±0.29(0.21)	14.72±1.14(0.53)
RDW	12.85±1.69	11.38±1.0(0.18)	13.47±2.0(0.90)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 8: Effects of methanol extract of leaves of *Albizia lebbeck* on red blood cells count and red blood cells indices after five days of treatment

Haematological parameters	Doses and groups		
	Control (GI)	**100 mg/kg (GIII)**	**400 mg/kg (GV)**
RBC (M/mm^3)	7.21±0.81	7.53±0.54(0.68)	7.40±0.65(0.69)
MCV (fl)	59.22±0.81	58±0.45(0.17)	59.18±1.47(0.83)
HCT (%)	42.62±5.46	43.56±4.67(0.9)	43±4.52(0.98)
MCH (pg)	19.08±0.87	18.46±0.87(027)	18.92±0.69(0.10)
MCHC (g/dl)	32.32±1.89	32±1.37(0.90)	31.88±1.41(0.97)
HB (g/dl)	13.74±1.03	13±1.23(0.84)	10.20±0.40(0.95)
RDW	10.46±0.26	12±1.21(0.14)	11.34±1.02(0.85)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreas ed compared to the control group. G= Group

Table 9: Effects of *Albizia lebbeck* leaves extract on red blood cells count and red blood cells indices after 30 days of treatment

Haematological parameters	Doses and groups		
	Control (GVI)	**100 mg/kg (GVIII)**	**400 mg/kg (GX)**
RBC (M/mm^3)	4.39±1.98	6.56±4.43(0.09)	4.89±2.37(0.63)
MCV(fl)	75.18±10.95	57±2.43*(0.04)	71±1.08(0.33)
HCT (%)	37.13±8.93	51±2.82**(0.01)	38.48±11(0.69)
MCH (pg)	40.53±15.43	27.85±13(0.16)	36.05±17(0.42)
MCHC (g/dl)	32.37±7.16	29.43±3.48(0.08)	28.93±9.44(0.56)
HB (g/dl)	15.28±1.10	14.90±1.75(0.81)	14.58±0.63(0.47)
RDW	12.85±1.69	12.08±0.75(0.24)	12.73±0.73(0.82)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

4.1.3 Effects of methanol extracts of stem barks and leaves of *Albizia lebbeck* on thrombocytes and thrombocytes indices after five and 30 days treatment

Thrombocytes and THR indices changed in rats that were treated with methanol extract of stem barks of *A. lebbeck* at doses levels of 100 mg/kg and 400 mg/kg compared to the control group (Table 10 for five days of treatment and Table 11 for 30 days of treatment). The stem barks extract significantly (P<0.05) increased THR, PCT and PDW at dose rate of 400 mg/kg after five days treatment compared to the control group. At the dose rate of 100 mg/kg after five days treatment, the stem barks extract insignificantly changed (P> 0.05) THR, PCT and PDW, although marginally decreased compared to the control group. Mean platelet volume (MPV) was not changed significantly (P> 0.05) at both doses after five days treatment even though slightly decreased compared to the control group. After 30 days of treatment, the stem barks extract at dose rate of 100 mg/kg significantly (P<0.05) decreased MPV and PDW. While, the extract at doses rates of 100 mg/kg and 400 mg/kg after 30 days treatment insignificantly changed (P> 0.05) THR and PCT, although both slightly increased compared to the control group.

Also THR and THR indices changed in rats treated with methanol extract of leaves of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg compared to the control group as shown in Table 12 for five days of treatment and Table 13 for 30 days of treatment. Methanol extract of leaves of *A. lebbeck* significantly (P<0.05) increased THR and PCT at dose rate of 400 mg/kg after five days treatment compared to the control group. Thrombocytes and PCT were statistically insignificant (P> 0.05) at dose rate of 100 mg/kg after five days treatment, but both slightly decreased compared to the control

group. Also the extract caused insignificant change (P> 0.05) in PDW at both doses after five days treatment, although at both doses slightly increased compared to the control group. After 30 days treatment, the leaves extract at dose rate of 100 mg/kg significantly (P<0.05) decreased MPV and PDW compared to the control group. Whereas, at the dose rate of 400 mg/kg after 30 days treatment the leaves extract insignificantly changed (P> 0.05) MPV and PDW even though both marginally decreased compared to the control group. Furthermore, at doses rates of 100 mg/kg and 400 mg/kg after 30 days treatment, the extract insignificantly changed (P> 0.05) THR and PCT, although at both doses THR marginally increased and PCT marginally decreased compared to the control group.

Table 10: Effects of methanol extract of stem barks of *Albizia lebbeck* on thrombocytes count and thrombocytes indices after five days of treatment

Haematological parameters	Doses and groups		
	Control (GI)	100 mg/kg (GII)	400 mg/kg (GIV)
THR (M/mm^3)	802.8±47.0	798±75.63(0.89)	957.2±94.41**(0.04)
MPV (fl)	7.34±0.17	7.20±0.14(0.31)	7.24±0.13(0.75)
PCT (%)	0.59±0.03	0.58±0.1(0.79)	0.69±0.05**(0.01)
PDW (fl)	7.12±0.31	7.08±0.43(0.46)	7.82±0.33**(0.02)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 11: Effects of methanol extract of stem barks of *Albizia lebbeck* on thrombocytes count and thrombocytes indices after 30 days of treatment

Haematological parameters	Doses and groups		
	Control (GVI)	100 mg/kg (GVII)	400 mg/kg (GIX)
THR (M/mm^3)	850.6±98.89	1012.0±256.35(0.62)	922.5±236.61(0.82)
MPV (fl)	7.47±0.31	7.03±0.11*(0.04)	7.25±0.45(0.08)
PCT (%)	1.61±1.98	1.62±0.4(0.90)	1.71±0.32(0.71)
PDW (fl)	7.88±1.06	6.33±0.29*(0.03)	6.95±1.38(0.11)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 12: Effects of methanol extract of leaves of *A. lebbeck* on thrombocytes count and thrombocytes indices after five days of treatment

Haematological parameters	Doses and groups		
	Control (GI)	**100 mg/kg (GIII)**	**400 mg/kg (GV)**
THR (M/mm^3)	802.8±47.34	789±68.75(0.10)	974.2±79**(0.03)
MPV (fl)	7.34±0.17	7.21±0.12(0.24)	7.42±0.41(0.66)
PCT (%)	0.59±0.03	0.57±0.08(0.23)	0.72±0.09**(0.00)
PDW (fl)	7.12±0.31	7.16±0.35(0.8)	7.30±0.33(0.82)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

Table 13: Effects of methanol extract of leaves of *A. lebbeck* on thrombocytes count and thrombocytes indices after 30 days of treatment

Haematological parameters	Doses and groups		
	Control (GVI)	**100 mg/kg (GVIII)**	**400 mg/kg (GX)**
THR (M/mm^3)	850.6±98.89	1182.67±177.35(0.62)	1297.5±546.84(0.81)
MPV (fl)	7.47±0.31	6.93±0.22*(0.03)	6.93±0.67(0.04)
PCT (%)	1.61±1.98	1.52±0.62(0.25)	1.58±1.31(0.39)
PDW (fl)	7.88±1.06	6.23±0.4*(0.04)	6.56±1.44(0.11)

All values are expressed as Mean ± SD (n=6). ** Significantly (P<0.05) increased compared to the control group. The figures under the bracket indicate the calculated P values of the treated groups as compared to the control groups. *Significantly (P<0.05) decreased compared to the control group. G= Group

4.2 Effects of *Albizia lebbeck* on Histological Structure of the Digestive Organs

4.2.1 Effects of methanol extracts of stem barks and leaves of *Albizia lebbeck* on the microscopic structures of the liver

Haematoxylin and eosin stained tissue sections of the liver were examined with a light microscope to evaluate the effects of methanol extracts of stem barks and leaves of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg after five and 30 days

treatment. Light microscope examination of the liver sections of rats treated with stem barks extract of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg for five days showed mild change of structures of the liver including presence of empty spaces in some hepatocytes, pyknotic nuclei, sinusoids compression, mild enlargement of some hepatocytes, and nuclei of some hepatocytes were pushed to the periphery compared to the control group which depicted normal architecture of the liver (Figure 4). Moreover, after 30 days treatment of the rats with methanol extract of stem barks of *A. lebbeck* at the dose rate of 100 mg/kg, the liver sections revealed inflammatory cells infiltration at the portal and periportal areas, stratification of epithelium of the bile ducts, necrosis and sinusoids dilatation compared with the control group as illustrated by Figure 5. Furthermore, at the dose level of 400 mg/kg stem barks, the liver sections showed hyperplasia of the bile ducts, and hyaline degenerations compared to the control group which showed normal architecture (Figure 5).

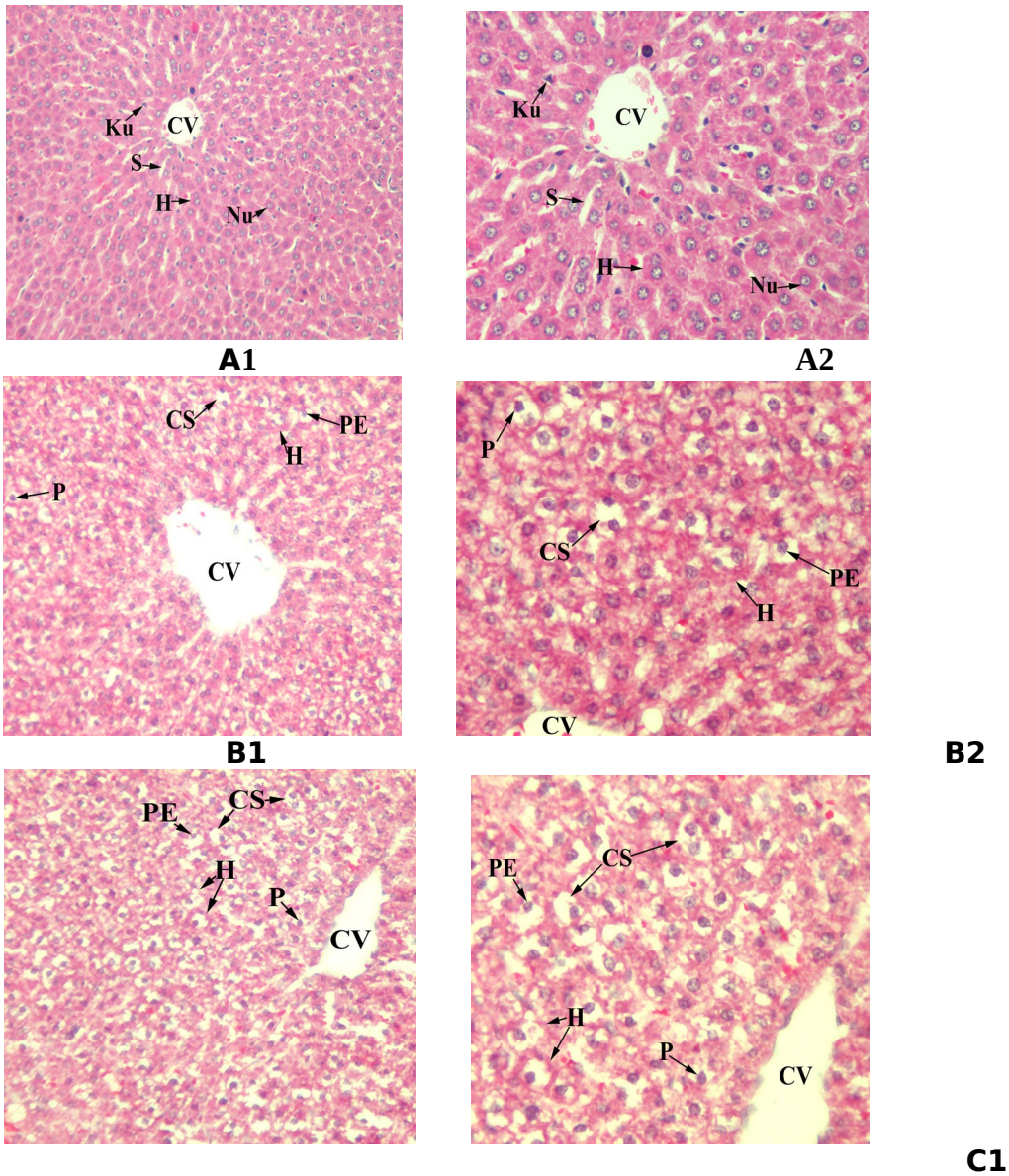


Figure 4: **Light micrographs of the liver of the control group and treated groups with methanol extract of stem barks of *Albizia lebbeck* for five days.**

(A1 at x100 and A2 at x200) Control group showing normal central vein (CV), hepatocytes (H), nuclei of hepatocytes (Nu), Kupffer cells (Ku), and sinusoids (S). (B1at x100 and B2 at x200) 100 mg/kg stem barks treated animals showing empty spaces in cytoplasm of hepatocytes (CS), pyknotic nuclei (P) enlargement of hepatocytes (H), compressed sinusoids (not indicated), and nuclei of hepatocytes pushed to the periphery (PE). (C1 at x100 and C2 at x200) 400 mg/kg stem barks treated animals showing pyknotic nuclei (P), empty spaces in the cytoplasm of hepatocytes (CS), compressed sinusoids (not indicated), nuclei of hepatocytes pushed to the periphery (PE), and enlargement of hepatocytes (H). H and E stained sections.

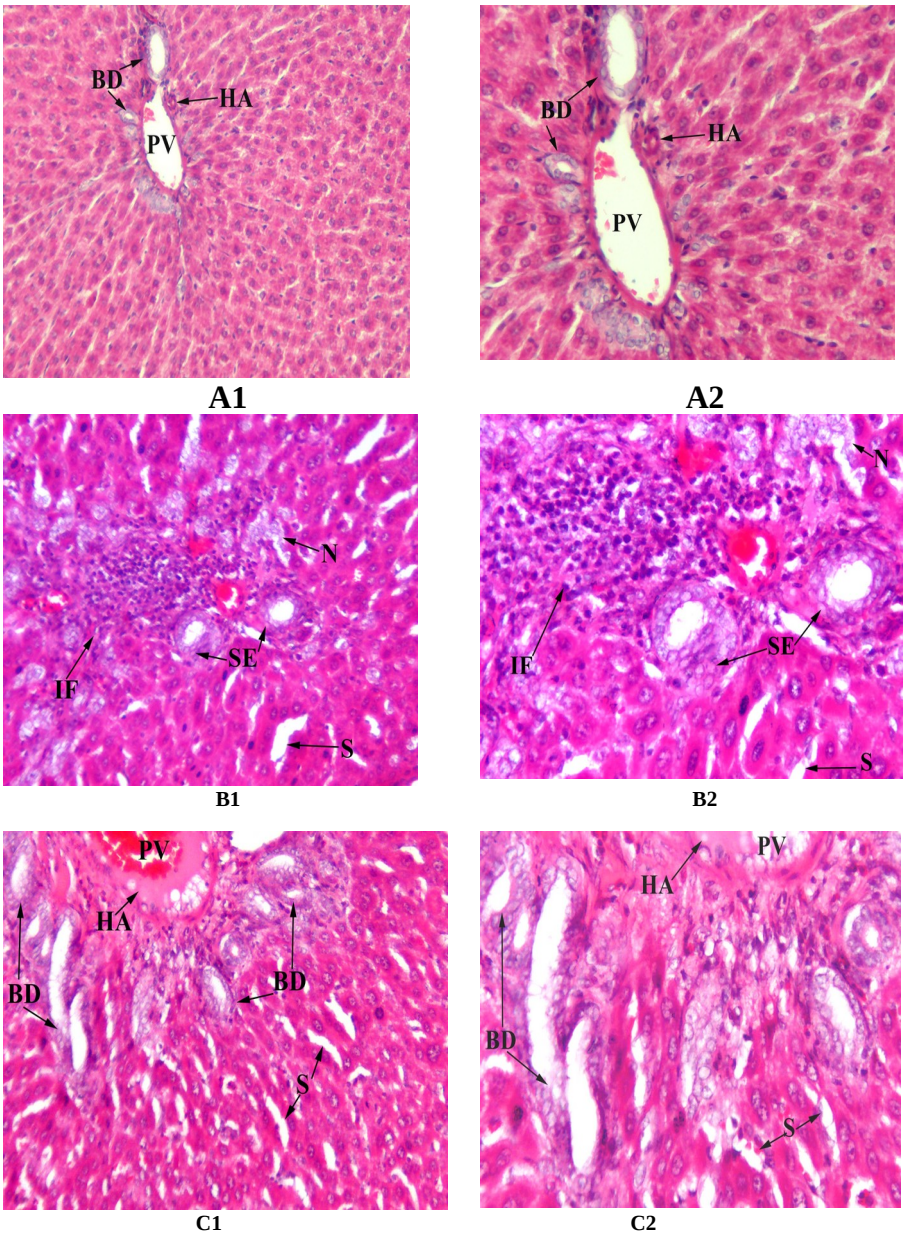


Figure 5: Light micrographs of the liver of the control group and treated groups with methanol extract of stem barks of *Albizia lebbeck* for 30 days.

(A1 at x100 and A2 at x200) Control group showing normal portal vein (PV), bile duct (BD) and hepatic artery (HA). (B1at x100 and B2 at x200) 100 mg/kg stem barks treated group showing inflammatory cells infiltration (IF), sinusoids dilatation (S), stratified epithelia of bile ducts (SE), and necrosis (N). (C1 at x100 and C2 at x200) 400 mg/kg stem barks treated animals showing hyperplasia of the bile ducts (BD), hyaline degeneration (HA) and sinusoids dilatation (S). H and E stained sections.

Moreover, light microscope examination of the liver sections of rats treated with methanol extract of leaves of *A. lebbeck* at the dose rate of 100 mg/kg for five days showed normal light microscopic structures that were similar to that of the control group (Figure 6). However, at the dose rate of 400 mg/kg, the liver sections of the rats treated with methanol extract of leaves of *A. lebbeck* for five days showed mild change on the light microscopic structures such as presence of empty spaces in the cytoplasm of hepatocytes, sinusoids compression, nuclei of some hepatocytes were pushed to the periphery, enlargement of hepatocytes, and pyknotic nuclei compared to the control group which exhibited normal architecture of the liver (Figure 6). Furthermore, after 30 days, liver sections of rats treated with methanol extract of leaves of *A. lebbeck* at the dose rate of 100 mg/kg showed inflammatory cells infiltration, necrosis and sinusoids dilatation compared to the control group (Figure 7). Moreover, liver sections of rats that were treated with leaves extract of *A. lebbeck* at the dose level of 400 mg/kg for 30 days showed inflammatory cells infiltration which extended from one portal triad to the other (bridging inflammation), and necrosis which extended from one portal triad to the other (bridging necrosis) (Figure 7).

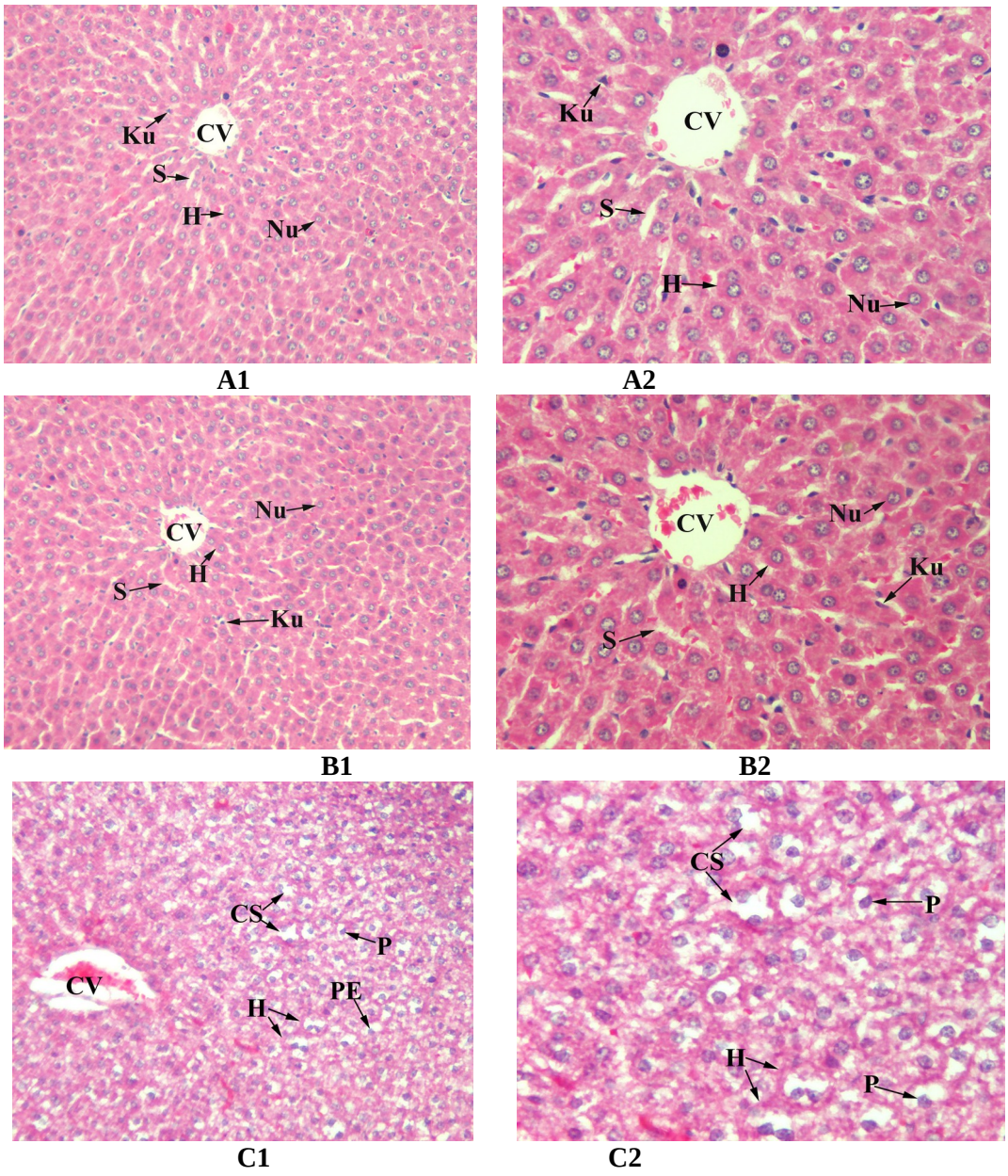


Figure 6: **Light micrographs of the liver of the control group and treated groups with methanol extract of leaves of *Albizia lebbeck* for five days.**

(A1 at x100 and A2 at x200) Control group showing normal central vein (CV), hepatic cords, hepatocytes (H), nuclei of hepatocytes (Nu), Kupffer cells (Ku), and sinusoids (S). (B1 at x100 and B2 at x200) 100 mg/kg leaves treated animals showing normal central vein (CV), hepatocytes (H), nuclei of hepatocytes (Nu), Kupffer cells (Ku), and sinusoids (S). (C1 at x100 and C2 at x200) 400 mg/kg leaves treated animals showing empty spaces in the cytoplasm of hepatocytes (CS), pyknotic nuclei (P), compressed sinusoids (not indicated), periphery located nuclei of hepatocytes (PE), and enlargement of hepatocytes (H). H and E stained sections.

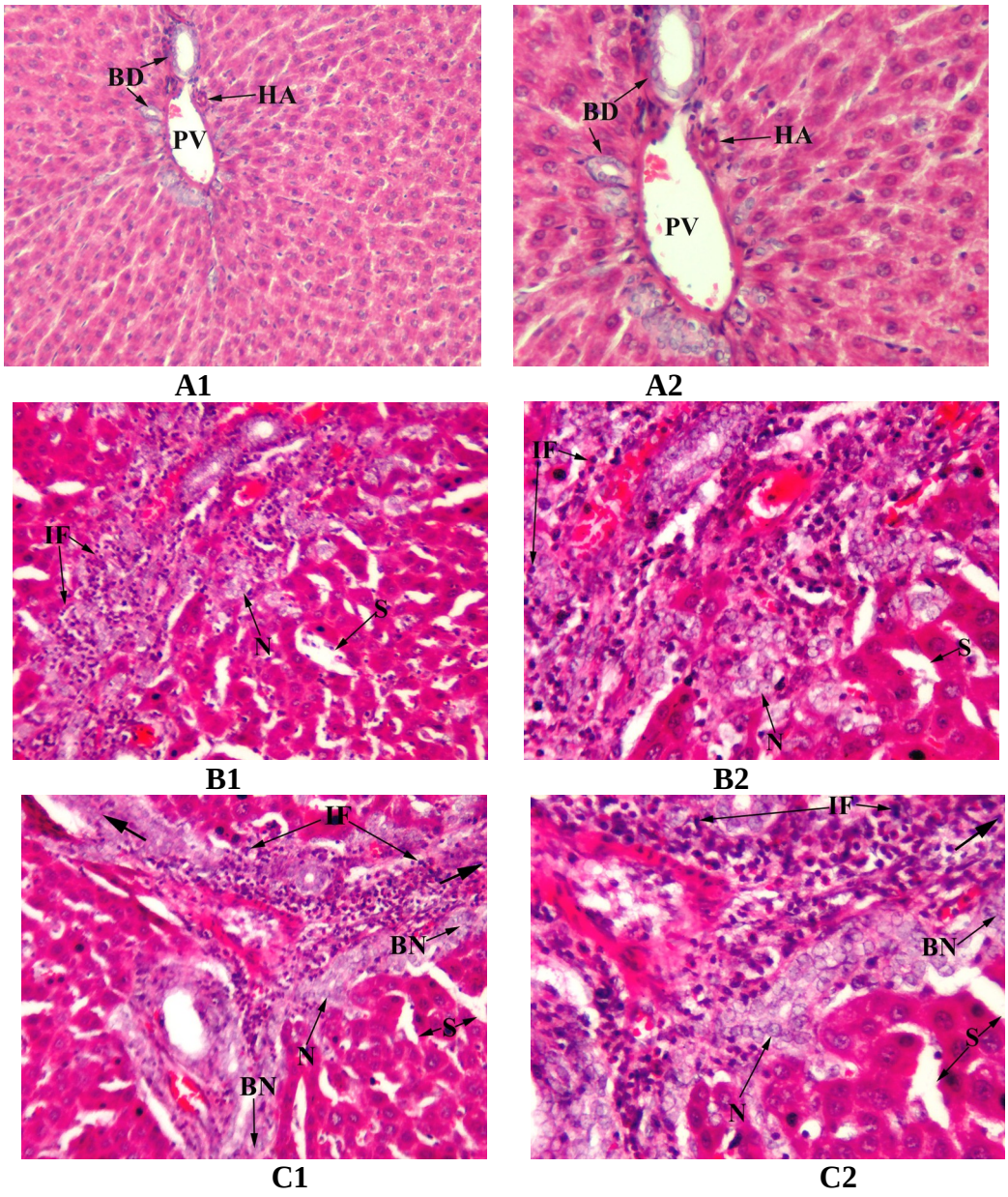


Figure 7: Light micrographs of the liver of the control group and treated groups with methanol extract of leaves of *Albizia lebbeck* for 30 days.

(A1 at x100 and A2 at x200) Control group showing portal vein (PV), bile duct (BD) and hepatic artery (HA). (B1 at x100 and B2 at x200) 100 mg/kg leaves treated animals showing inflammatory cells infiltration (IF), and Necrosis (N). (C1 at x100 and C2 at x200) 400 mg/kg leaves treated animals showing inflammatory cells infiltration (IF), Necrosis (N), bridging inflammatory cells infiltration (thick arrows), and bridging necrosis (BN). H and E stained sections.

4.2.2 Effects of methanol extracts of *Albizia lebbeck* stem barks and leaves on microscopic structures of the stomach

Haematoxylin and eosin stained tissue sections of the stomach were examined with light microscope to evaluate the effects of methanol extracts of stem barks and leaves of *A. lebbeck* on its microscopic structures. Stomach sections of rats treated with methanol extract of stem barks at the doses rates of 100 mg/kg and 400 mg/kg for five days revealed no significant changes on its microscopic structures such as tunica mucosa (epithelium, lamina propria and lamina muscularis), tela sub mucosa, tunica muscularis, and tunica serosa compared to the control (Figure 8). Furthermore, the stem barks extract of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg for 30 days treatment did not cause significant changes on the light microscopic structures of the stomach compared to the control group (Figure 9).

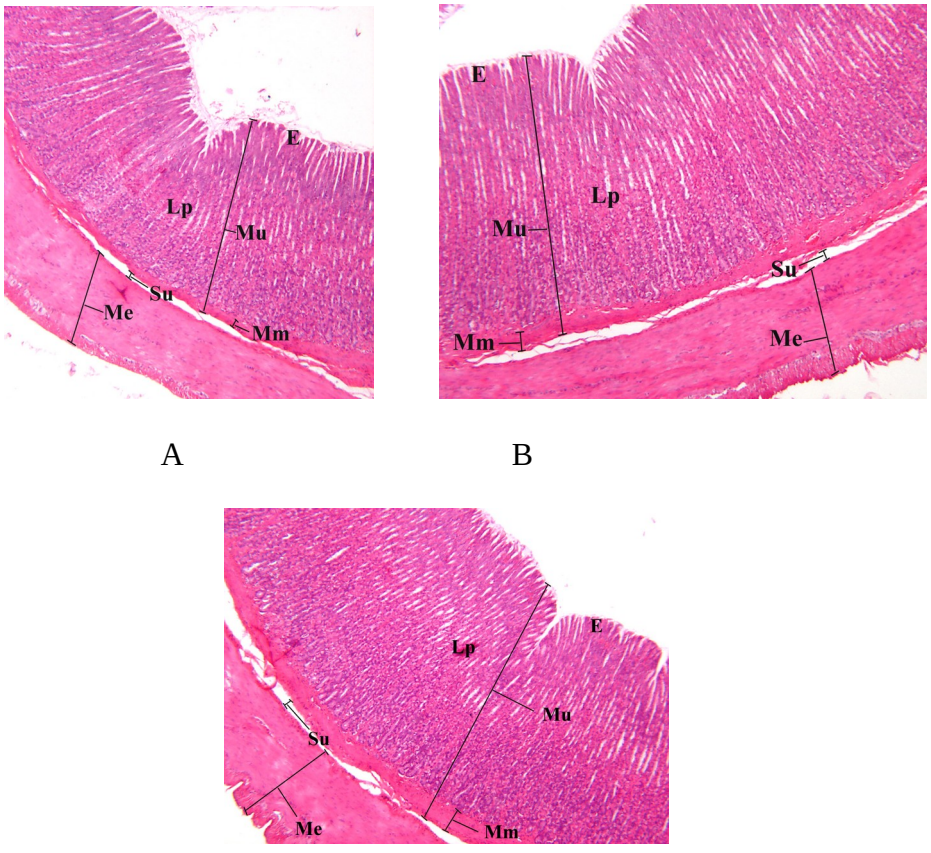


Figure 8: Light micrographs of the stomach of the control group and treated groups with methanol extract of *Albizia lebbeck* stem barks for five days.

(A) Control group showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela submucosa (Su), and tunica muscularis (Me). (B) 100 mg/kg stem barks treated group showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). (C) 400 mg/kg stem barks treated group showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). H and E stained sections x40.

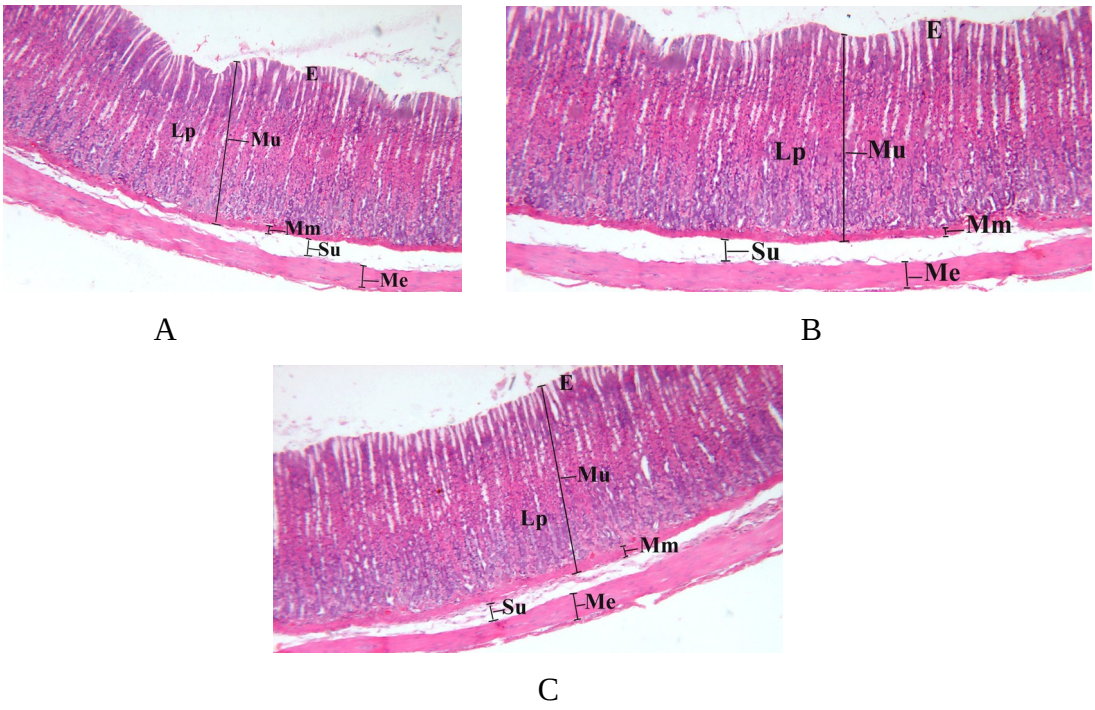


Figure 9: Light micrographs of the stomach of the control group and treated groups with methanol extract of *Albizia lebbeck* stem barks for 30 days.

(A) Control group showing tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). (B) 100 mg/kg stem barks treated animals showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). (C) Showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me) in 400 mg/kg stem barks treated group. H and E stained sections x 40.

Similarly, light microscope examination of the stomach sections of rats treated with extract of leaves of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg for five days revealed no significant changes on the light microscopic structures of the stomach such as tunica mucosa (epithelium, lamina propria and lamina muscularis), tela sub mucosa, tunica muscularis, and tunica serosa compared to the control group (Figure 10).

Furthermore, the stomach sections of rats that were treated with methanol extract of leaves at doses of 100 mg/kg and 400 mg/kg for 30 days showed no significant changes on the light microscopic structures compared to the control group (Figure 11).

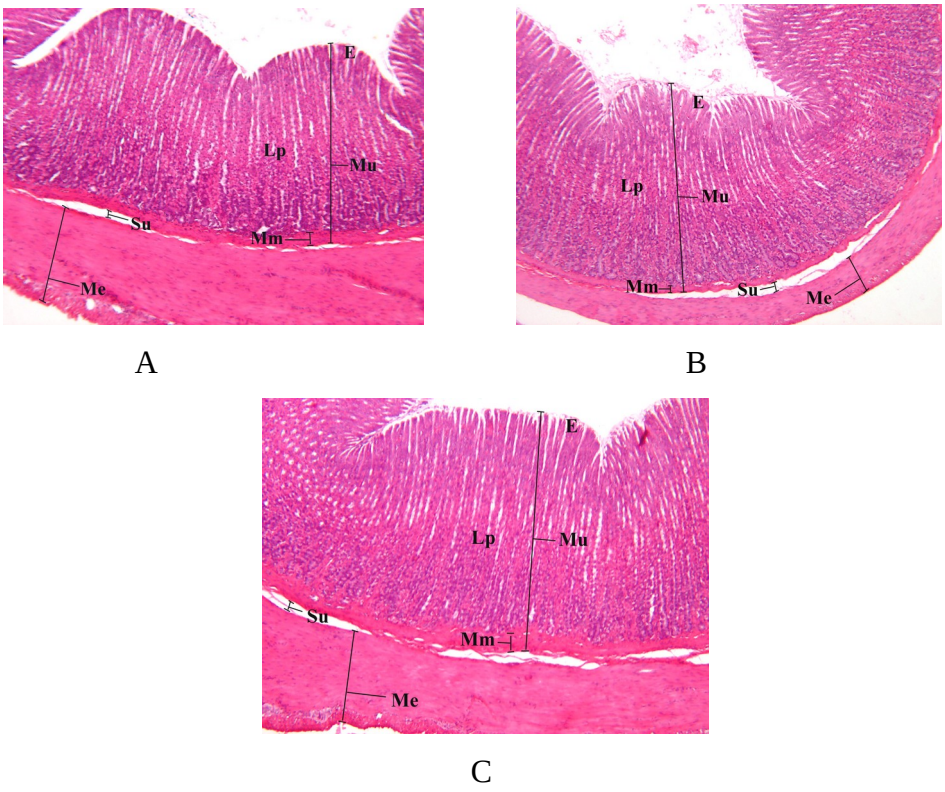


Figure 10: Light micrographs of the stomach of the control group and treated groups with methanol extract of *Albizia lebbeck* leaves for five days.

(A) Control group showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela submucosa (Su), and tunica muscularis (Me). (B) 100 mg/kg leaves treated group showing intact tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela submucosa (Su), and tunica muscularis (Me) epithelium, lamina propria, lamina muscularis, tela sub mucosa, and tunica muscularis. (C) 400 mg/kg leaves treated group showing intact tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela submucosa (Su), and tunica muscularis (Me). H and E stained sections x40.

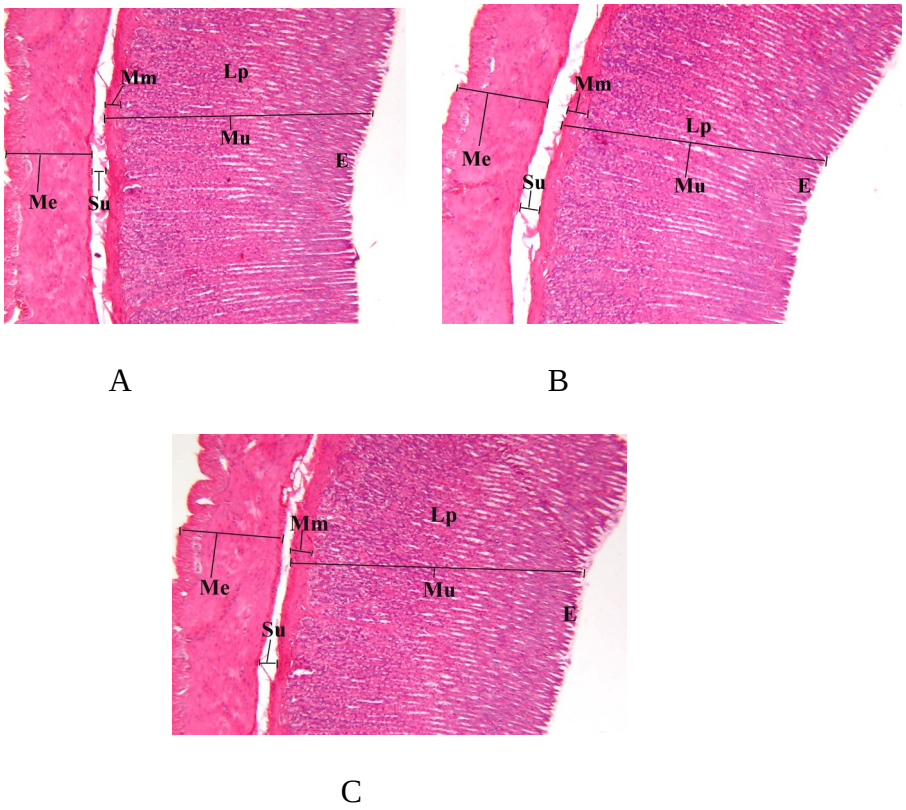


Figure 11: Light micrographs of the stomach of the control group and treated groups with methanol extract of *Albizia lebbeck* leaves for 30 days.

(A) Control group showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). (B) 100 mg/kg leaves treated animals showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). (C) 400 mg/kg leaves treated group showing normal tunica mucosa (Mu), epithelium (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). H and E stained sections x 40.

4.2.3 Effects of methanol extracts of stem barks and leaves of *Albizia lebbeck* on microscopic structures of the jejunum

Haematoxylin and eosin stained tissue sections of the jejunum were examined under light microscope to determine the effects of methanol extracts of stem barks and leaves of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg after five and 30 days treatment. Light microscope examination of the jejunum sections of animals treated with methanol extract of stem barks of *A. lebbeck* at doses of 100 mg/kg and 400 mg/kg for five days revealed no significant changes on the light microscopic structures of the jejunum (Figure 12). Furthermore, jejunum sections of animals that were treated with methanol extract of stem barks of *A. lebbeck* at doses rates of 100 mg/kg and 400 mg/kg for 30 days revealed normal microscopic structures (Figure 13).

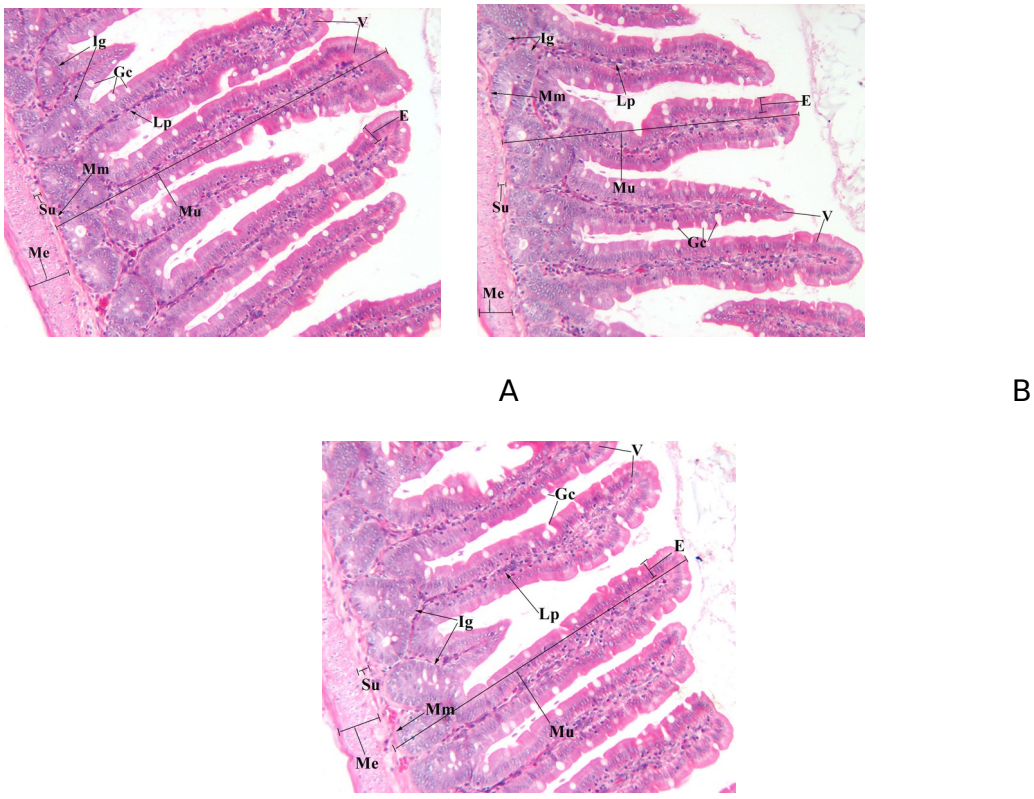


Figure 12: Light micrographs of the jejunum of the control group and treated groups with methanol extract of *Albizia lebbeck* stem barks for five days.

(A) Control group showing tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (B) 100 mg/kg stem barks treated animals showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (C) 400 mg/kg stem barks treated animals showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). H and E stained sections x100.

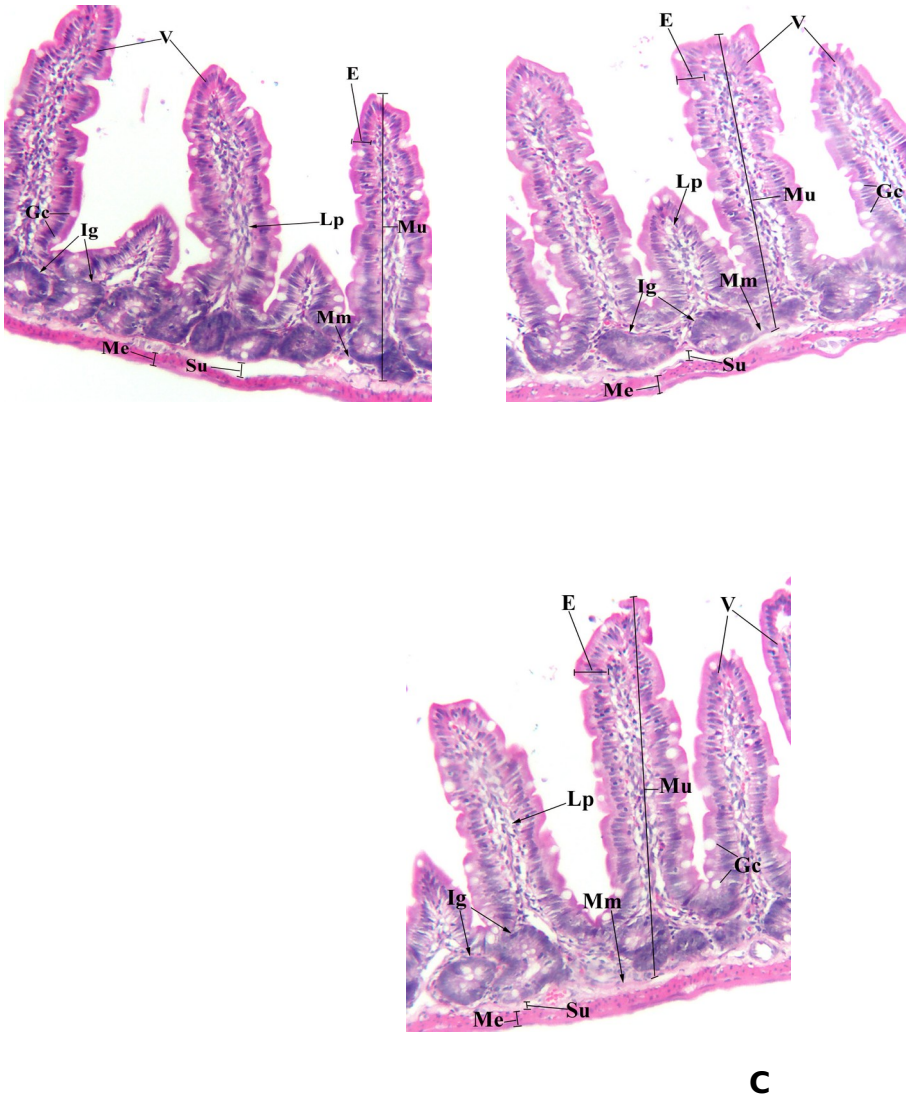


Figure 13: Light micrographs of the jejunum of the control group and treated groups with methanol extract of *Albizia lebbeck* stem barks for 30 days.

(A) Control group showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (B) 100 mg/kg stem barks treated rats showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (C) 400 mg/kg stem barks treated animals showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). H and E stained sections x100.

In addition, light microscope examination of the jejunum sections of animals treated with methanol extract of leaves of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg for five days revealed no significant changes on the light microscopic structures (Figure 14). Furthermore, the jejunum sections of rats that were treated with methanol extract of leaves of *A. lebbeck* for 30 days at the doses rates of 100 mg/kg and 400 mg/kg exhibited no significant changes on the light microscopic structures compared to that of the control group (Figure 15).

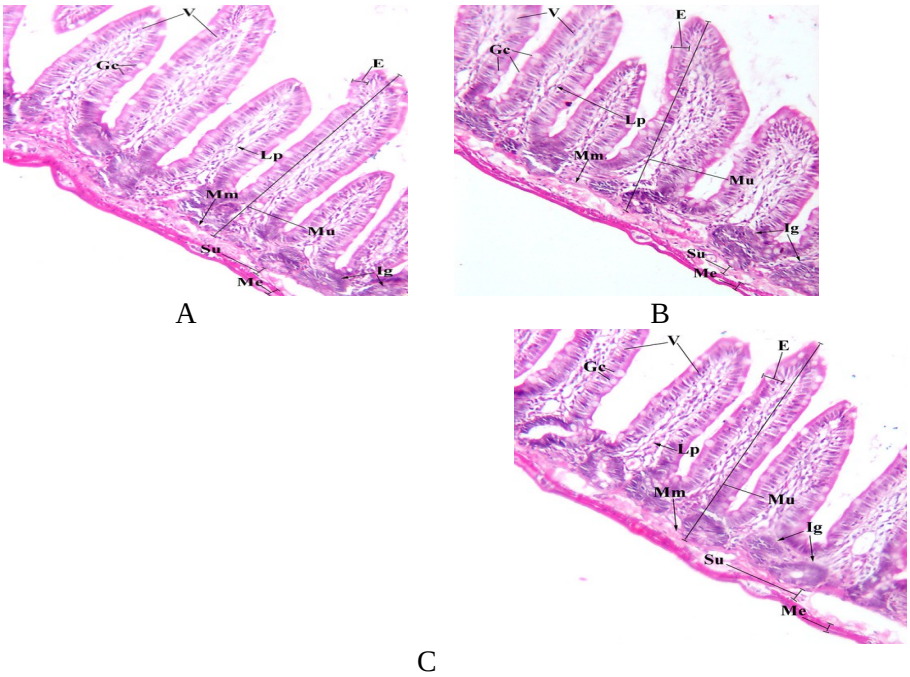


Figure 14: Light micrographs of the jejunum of the control group and treated groups with methanol extract of *Albizia lebbeck* leaves for five days.

(A) Control group showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (B) 100 mg/kg leaves treated group showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (C) 400 mg/kg leaves treated animals showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). H and E stained sections x 100.

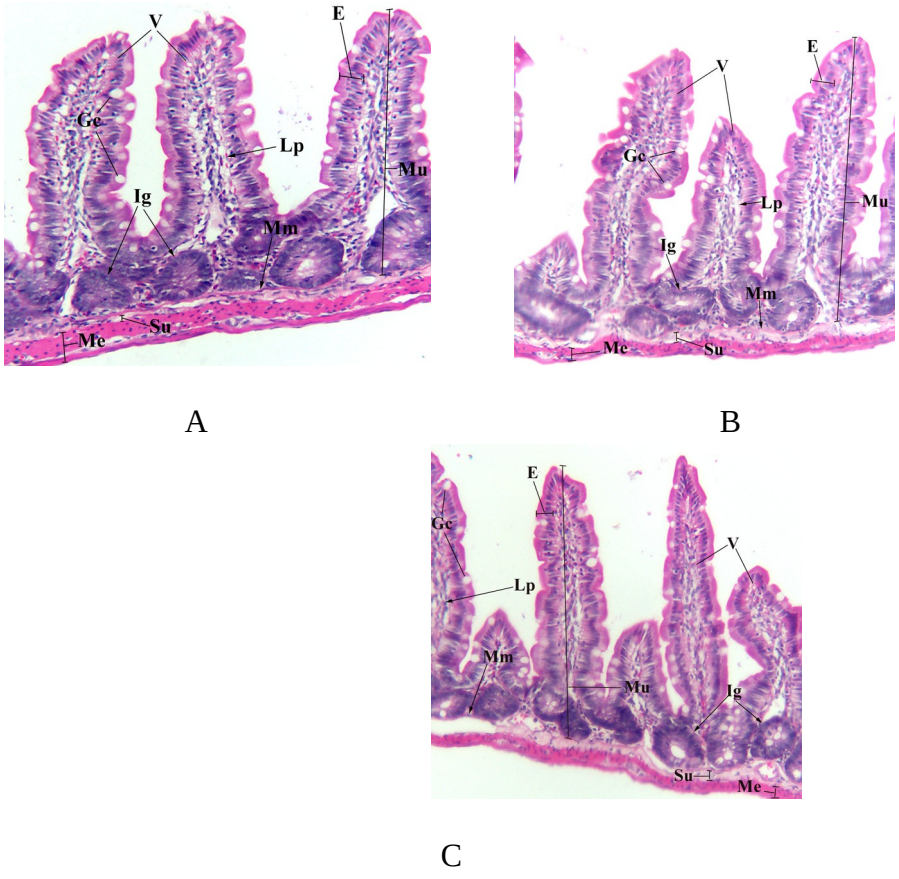


Figure 15: Light micrographs of the jejunum of the control group and treated groups with methanol extract of *Albizia lebbeck* leaves for 30 days.

(A) Control group showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc), and intestinal glands (Ig). (B) 100 mg/kg leaves treated animals showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc) and intestinal glands (Ig). (C) 400 mg/kg leaves treated animals showing normal tunica mucosa (Mu), epithelium (E), villi (V), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), tunica muscularis (Me), goblet cells (Gc), and intestinal glands (Ig). H and E stained sections x100.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Haematological Parameters

In the present study, methanol extracts of stem barks and leaves of *A. lebbeck* caused a significant increase in WBC count specifically at dose rates of 100 mg/kg and 400 mg/kg, respectively, after 30 days of treatment. The changes observed in WBC may be attributed by the reaction to the toxic effects of the plant extracts. This finding is in agreement with findings by Maya *et al*. (2012); Doshi *et al.* (2016) who also reported that *A. lebbeck* rise the number of WBC. Also Kajaria *et al*. (2013) reported similar findings that Shirishadi compound (*Albizia lebbeck, Cyprus rotundus*, and *Solanum surattense*) elevate WBC count.

Eosinophils counts significantly increased at 100 mg/kg leaves and at 400 mg/kg for both stem barks and leaves after 30 days of treatment. The significant increase of EO observed in this study might be attributed by the presence of active principles in these parts of the plant that enhanced production of EO. According to Agiang *et al*. (2017) eosinophils are type of leucocytes that play a vital role in the immune system especially in body's response to allergic reactions, asthma and in fighting parasitic infections. Furthermore, Arika *et al.* (2016) reported that eosinophils are responsible for destroying foreign substances and modifying inflammation. Various studies have shown that *A. lebbeck* can be used in treatment of parasitic infections such as antimalarial (Kalia *et al.,* 2015), antitrypanosomal (Wurochekke and Nok, 2004), anthelminthic (El Garhy and Mahmoud, 2002) as well as anti-inflammatory (Babu *et al.,* 2009). The use of this plant to fight parasitic infection, inflammatory and allergic reactions might be attributed by the increase of the eosinophils count after treatment. Therefore, *A. lebbeck* could be used in

managements of parasitic infections, inflammation, and protect the animals from foreign substances.

Monocytes significantly decreased at 100 mg/kg stem barks and 400 mg/kg leaves as well as NUE at 400 mg/kg leaves after 30 days of treatment. This significant decrease in MON and NUE could be due to the presence of phytoconstituents in *A. lebbeck* that might decrease production of MON and NUE or increase movement into the tissues. According to Arika *et al.* (2016) neutrophils and monocytes have phagocytic activities, they attack and destroy foreign particles, cell waste materials and bacteria. Therefore, the decrease of MON and NEU may impede phagocytosis process which may predispose the animals to infections. Basophils neither decreased nor increased significantly at different doses for both treatment durations.

The red blood cells parameters including RBC, HCT, HB, MCV, MCH, MCHC, and RDW are valuable indicators of the levels of circulating erythrocytes and serves as useful indices for bone marrow capacity to produce RBC in response to the administration of drugs, toxins, or plant extract (Madaki *et al.,* 2019). The present study found a significant increase in RBC and HCT at 100 mg/kg stem barks and 100 mg/kg leaves, respectively after 30 days of treatment. According to Ahmed and Aslam (2018) and Ijioma *et al.* (2019) RBC may increase due to the presence of strong ant-oxidants effect in the plant extracts which inhibit lysis of RBC's from radical formation. Furthermore, Ijioma *et al.* (2019) documented that flavonoids, phenolic compounds, and tannins protect erythrocytes from oxidative damage. Various studies had reported the presence of these phytochemicals (Flavonoids, phenolic compounds, and tannins) in different parts *A. lebbeck* (Kumar *et al*., 2013; Abriham and Paulos, 2016). Also red blood cells may increase due to the action of the erythropoietin which is a glycoprotein hormone that

stimulates stem cells in the bone marrow to produce more erythrocytes (Umaru *et al.,* 2015; Zaruwa *et al.,* 2016). According to the present findings, *A. lebbeck* may contain secondary metabolites which acted either as antioxidants or stimulated release of erythropoietin in the kidneys leading to increase the number of erythrocytes and packed cell volume in rats which in turn would provide better transportation of oxygen to the tissues and carbon dioxide away from the tissues.

The extracts significantly decreased MCV at dose rate of 100 mg/kg after 30 days of treatment. Mean cell volume measures average size or volume of the RBC (Etim *et al.,* 2013). According to Iwuanyanwu and Nkpaa (2015) when RBC decreased in size than normal, the MCV will be below normal and implies microcytic, when RBC increased in size than normal, the MCV will be elevated and indicates macrocytic and when RBC are of the normal size are termed as normocytic. These size categories are used to classify anaemia (Iwuanyanwu and Nkpaa, 2015). Although at the dose levels of 100 mg/kg stem barks and leaves significantly increased the RBC and HCT, respectively compared to the control group after 30 days of treatment, the significant decreased of the MCV at the same dose and treatment duration gave an indication that the extracts may have caused increase synthesis of unhealthy RBC, particularly, it shows that the RBC that were produced had a higher proportional of erythrocytes of small size. Thus, extracts of stem barks and leaves of *A. lebbeck* may be decreased the size of RBC which may result into microcytic anaemia.

Haemoglobin, MCH, MCHC, and RDW insignificantly changed at both doses and treatment durations. This suggests that when methanol extracts of both stem barks and leaves of *A. lebbeck* are administered orally in rats at the doses rates of 100 mg/kg and

400 mg/kg for five and 30 days had insignificant influences in HB, MCH, MCHC, and RDW.

The significant differences observed in RBC and RBC indices were only at 100 mg/kg after 30 days of treatment. This suggests that prolong use of stem barks and leaves of *A. lebbeck* at 100 mg/kg may alter the RBC and some of its indices.

Furthermore, result from the present study showed a significant increase in THR and PCT at dose rate of 400 mg/kg stem barks and leaves as well as PDW at 400 mg/kg stem barks after five days of treatment. While there were significant decrease in MPV and PDW at dose rate of 100 mg/kg stem barks and leaves after 30 days of treatment compared to the control group. Several factors such as amount of production, consumption, sequestration, and loss are responsible in influencing the platelet counts in animals (Jones and Allison, 2007; Roland *et al.*, 2014). According to Umaru *et al.* (2016) plants that had tannins, alkaloids, saponins, and glycosides increase the number of thrombocytes. The present findings suggests that the extracts may have these phytochemicals (tannins, alkaloids, saponins, and glycosides) which increased THR count significantly through various mechanisms such as increase production, decrease consumption, decrease sequestration and loss.

The main functions of platelets is formation of blood-clot during tissue damage, preventing bleeding and microbial infection, enhancing the functions of immune components, and facilitates wound covering as well as initiate wound healing through regeneration of new cells and tissues that serve as covering of the dead-tissue (Agu *et al.*, 2017). Findings from the present study had shown that methanol extracts of both stem barks and leaves of *A. lebbeck* at 400 mg/kg after five days of treatment significantly

increase the total number of platelets and plateletcrit. Thus, *A. lebbeck* stem barks and leaves can play a pivot role in the wound-healing process and management of the thrombocytopenia disorders as well as hemophilia.

Additionally, the present study demonstrated that, at the dose rate of 400 mg/kg stem barks for five days treatment caused a significant increase in PDW, while extracts of both stem barks and leaves of *A. lebbeck* at the dose rate of 100 mg/kg for 30 days of treatment caused a significant decrease in MPV and PDW. Mean platelet volume is the measure of average size of platelets and useful test for the differential diagnosis of thrombocytopenia (Gao *et al.,* 2014). Mean platelet volume level rises when platelet synthesis is reduced and young platelets become bigger and more active (Budak *et al.,* 2016). Therefore, the present findings indicated that both extracts of *A. lebbeck* at dose rate of 100 mg/kg increased platelet production due to the fact that MPV significantly decreased compared to the control group. Platelet distribution width (PDW) is an indicator of the heterogeneity in platelet size (Gao *et al.,* 2014). A high value of PDW suggests a large range of platelet size due to swelling, destruction, and immaturity (Gao *et al.,* 2014). Thus, methanol extract of stem barks of *A. lebbeck* at 400 mg/kg after five days of treatment increased variation in size of platelets while at 100 mg/kg stem barks and leaves after 30 days of treatment decreased variation in size of thrombocytes compared to the control group.

Changes on haematological parameters in response to the treatment observed in this study were inconsistent. There was a difference between doses, parts of the plant used and duration of treatment. Thus, methanol extracts of *A. lebbeck* at different doses, parts used, and treatment durations can either increase or decrease the haematological parameters in rats compared to the control group.

The present findings on haematological parameters particularly in WBC, RBC, and HCT at a dose rate of 100 mg/kg are contrary to what was reported by Gupta *et al.* (2005) who reported that oral administration of saponins of *A. lebbeck* bark at dose level of 50mg/kg/ b.w. per day for 60 days did not cause any significant change in WBC, RBC, HB, and HCT. Furthermore, Gupta *et al.* (2006) stated that oral administration of methanol extracts of *A. lebbeck* bark at the dose level of 100 mg/rat/day to male rats of proven fertility for 60 days did not affect RBC and WBC counts, HB, and HCT. However, at the dose rate of 400 mg/kg the findings were in agreement with what was reported by Gupta *et al.* (2005, 2006). The contrast may be resulted due to difference in doses used. However, this result is in consistent with previous reported by Kajaria *et al.* (2013) who suggested that ethanolic extracts of Shirishadi compound (*Albizia lebbeck*, *Cyprus rotundus,* and *Solanum surattense*) increase levels of total WBC and RBC.

5.2 Histological Structures of the Liver

Liver perform various vital functions such as energy storage, production of protein, regulation of cholesterol metabolism, and detoxifying of toxins or drug in the body (Setyawati *et al.,* 2016). Foreign compounds are mainly metabolized in the liver by the action of drug-metabolizing enzymes such as microsomal cytochrome P450 enzymes, mixed-function mono-oxygenases, glutathione-S-transferases, sulfotransferases and UDP-glucuronosyltrans-ferases (Stickel *et al.,* 2005). The liver work very hard to metabolize poison or toxin materials which enter into it. By doing so the liver can be damaged easily either structurally or impair its functions (Setyawati *et al.,* 2016; Amadi and Orisakwe, 2018). Certain herbals have been identified as the cause of acute and chronic hepatitis, cholestasis, drug-induced autoimmunity, vascular lesions, and even hepatic failure and cirrhosis (Stickel *et al.,* 2005). Liver damage that results due to the ingestion of herbal medicines is known as ‘herb-induced liver injury’ (HILI), which happens infrequently in

only a few susceptible individuals (Amadi and Orisakwe, 2018). The clinical presentations of the HILI are similar to those of drug-induced liver injury (DILI) (Amadi and Orisakwe, 2018). Additionally, HILI and DILI share similar characteristics because either conditions result due to chemical components that can be made either by natural or synthetic processes (Amadi and Orisakwe, 2018).

In the present study, liver of rats that were orally administered with methanol extracts of stem barks and leaves of *A. lebbeck* for five days at both doses for stem barks and at dose rate of 400 mg/kg leaves showed mild change on the microscopic structures such as presence of empty spaces in the cytoplasm of hepatocytes, compressed sinusoids, pyknotic nuclei, enlargement of hepatocytes, and nuclei of some hepatocytes were pushed to the periphery. Furthermore, liver of the rats that were orally administered with methanol extracts of stem barks and leaves of *A. lebbeck* for 30 days at the dose rate of 100 mg/kg revealed inflammatory cells infiltration, sinusoids dilatation and necrosis. Moreover, at the dose rate of 400 mg/kg leaves for 30 days treatment, the liver sections revealed inflammatory cells infiltration, necrosis and sinusoids dilatation. Also liver of the rats that were orally administered with extract of stem barks of *A. lebbeck* for 30 days at dose rate of 400 mg/kg revealed hyperplasia of the bile ducts, hyaline degeneration and sinusoids dilatation. The histopathological lesions observed in the liver sections of the treated rats suggest that phytochemicals of *A. lebbeck* cause injury in the liver.

According to Atsafack *et al.* (2015) the presence of empty vacuole-like spaces in the hepatocytes are caused by either abnormal infiltration of the extracellular substances into the hepatocytes or malfunctioning of the hepatocytes. Furthermore, the presence of clear or empty spaces in the cytoplasm of hepatocytes may result due to accumulation of the glycogen, because the accumulated glycogen usually is washed out during tissues process

leaving empty or clear spaces in the cytoplasm. Thus, oral administration of the methanol extracts of stem barks and leaves of *A. lebbeck* in rats for five days may cause either abnormal infiltration of extracellular substances or impair functions of hepatocytes or accumulation of glycogen due to the fact that there were empty spaces in the cytoplasm of hepatocytes of the treated animals.

Thoolen *et al*. (2010) reported that hepatocellular enlargement or swelling may occur from accumulation of the glycogen, fat or other substances and may also be a feature of degeneration and some forms of hepatocellular necrosis. Furthermore, enlargement of hepatocytes cause sinusoids compression and atrophy of hepatocytes cause sinusoids dilatation (Thoolen *et al.,* 2010). Therefore, methanol extracts of stem barks and leaves of *A. lebbeck* may cause enlargement of hepatocytes in rats after treatment for five and 30 days, respectively due to the fact that there were sinusoids compression in the liver of the treated groups compared to the control group.

According to Thoolen *et al.* (2010) an infiltration of different inflammatory cells is typically a response to parenchymal cell death with causes ranging from infectious agents, exposure to toxicants, generation of toxic metabolites, and tissue anoxia. In the present study there was infiltration of inflammatory cells and necrosis at the portal and periportal areas after treatment of the rats with both extracts for 30 days. Thus, methanol extracts of *A. lebbeck* may cause cell death in the liver of treated rats. The changes on the haematological parameters particularly the decrease of neutrophils and monocytes observed may be related to inflammatory cells infiltration in the liver as revealed by histopathological analysis of this organ. The observed hyperplasia of bile ducts in the liver of treated rats with the methanol extract of stem barks of *A. lebbeck* at higher dose for 30 days may be resulted due to the bioactive compounds of the extract that impaired

bile drainage. The histopathogical lesions caused by both extract were not dose dependent but time of treatment dependent due to the fact that the lesions become more severe after 30 days of treatment.

The finding of the present study on the light microscopic structures of the liver is contrary to what was reported by Kalia *et al*. (2015) who reported that administration of the ethanolic bark extract of *A. lebbeck* has no adverse effects on the liver of mice infected with malaria parasites. The contrast observed may be resulted due to various factors including health status of the animals used, difference in treatment durations, doses, and other factors which are responsible for affecting the amount and quality of the phytochemicals in plant such as difference in soil chemistry, climate, altitude, and seasonal. This finding is in line with various other studies which have documented that administration of the herbal plants to the experimental animals may cause histopathological changes in the liver (Atsafack *et al.,* 2015; Debebe *et al.,* 2017).

5.3 Histological Structures of the Stomach

In the present study, stomach of rats that were treated with the methanol extracts of stem barks and leaves of *A. lebbeck* at the doses rates of 100 mg/kg and 400 mg/kg for five days showed normal structures that were similar to that of the control group. Also stomach of the rats that were orally administered with the extracts of stem barks and leaves of *A. lebbeck* at the dose rate of 100 mg/kg for 30 days exhibited normal light microscopic structures that were similar to that of the control group. Furthermore, at the dose rate of 400 mg/kg for both extracts for 30 days of treatment, the stomach sections of rats revealed normal light microscopic structures that were similar to that of control group. This finding suggests that the use of methanol extracts of stem

barks and leaves of *A. lebbeck* at both doses and treatment duration has no effects on the light microscopic structures of the stomach of animals.

According to Park *et al.* (2019) the stomach is protected from injury by various factors such as gastric mucus, bicarbonate ions, prostaglandins and innate resistance of mucosal cells. Thus, these protective factors might protected the stomach of treated rats from the bioactive compounds of methanol extracts of stem barks and leaves of *A. lebbeck* as why there was no significant histopathological lesions in the stomach sections of treated rats compared to the stomach sections of the control group.

5.4 Histological Structures of the Jejunum

Jejunum is the part of the small intestine which is responsible for chemical digestion and absorption of nutrients. In the course of its functions, the jejunum can be exposed to several chemicals and phytochemicals which may damage it or impair its absorptive function. Hence, jejunum also is the useful part for assessing the effects of herbals and fodder. In the present study, there was no significant change on the light microscopic structures of the jejunum of rats that were treated with the methanol extracts of stem barks and leaves of *A. lebbeck* at both doses and treatment duration. This finding suggests that use of *A. lebbeck* stem barks and leaves at lower and higher dose rate for five and 30 days had no significant effects on the light microscopic structures of the jejunum.

This finding on the light microscopic structures of the stomach and jejunum is contrary to what was reported by Umaru (2012) who documented that prolong administration of aqueous pod extract of *Acacia nilotica* in albino rats cause histopathological lesions in the stomach and small intestines.

The presence of bioactive compounds such as alkaloids, tannins, saponins, oxalate (Nweke *et al.*, 2019), flavonoids, and glycosides (Alebachew *et al.*, 2014) may cause histopathological lesions in the organs. Acidity and toxicity have been reported to be associated with the presence of oxalate in the food (Nweke *et al.*, 2019). Also Olaniyan *et al*. (2016) reported that the presence of glycosides and large intake of tannins may cause lymphocytic infiltration and damage in organs, respectively. Therefore, histopathological lesions observed in the liver of rats after oral administration of methanol extracts of stem barks and leaves of *A. lebbeck* might be attributed by the combined effects of the bioactive compounds such as alkaloids, tannins, saponins, flavonoids, glycosides, and oxalate that might be present in the extracts of *A. lebbeck*. However, these bioactive compounds did not cause significant histopathological lesions in the stomach and jejunum. These organs may have the protective factors that protected them from injury by the bioactive compounds of the extracts.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study revealed that oral administration of methanol extracts of both stem barks and leaves of *A. lebbeck* at lower and higher doses for 30 days increased the number of WBC, LYM and EO. While the extracts decreased the number of MON, furthermore, the leaves extract at 400 mg/kg for 30 days of treatment reduced NUE count.

Oral administration of the plant extracts at dose of 100 mg/kg for 30 days caused microcytic anaemia due to the fact that significantly decreased MCV.

The study has shown that methanol extracts of *A. lebbeck* when administered orally at dose rate of 400 mg/kg for five days can be used for management of thrombocytopenia because the extracts significantly increased THR and PCT.

Treatment of the rats orally with methanol extracts of stem barks and leaves of *A. lebbeck* for five and 30 days induced histopathological lesion in the liver. Therefore, prolonged use of both parts of *A. lebbeck* for treatment of various ailments and as fodder should be discouraged so as to avoid the deleterious effects that might occur in the liver.

Administration of methanol extracts of stem barks and leaves of *A. lebbeck* orally in rats for five and 30 days did not cause significant histopathological lesions neither in the stomach nor in the jejunum.

6.2 Recommendations

The present study evaluated the effects of methanol extracts of both stem barks and leaves of *A. lebbeck* on haematological parameters and structures of the digestive organs in rats. However, further studies are required on the:-

i. Identification of the phytochemicals that are responsible to increase and decrease of some haematological parameters.
ii. Identification of the phytoconstituents that causes the deleterious effects on the light microscopic structures of the liver.
iii. Identification of the mechanisms of action of the phytochemicals on haematological parameters and liver.
iv. Determination of the effects of the methanol extracts of this plant on microscopic structures of the liver, stomach and jejunum by using electronic microscope.
v. Determination of the effects of the methanol extracts of this plant on other body systems.
vi. Assessment of the effects of *A. lebbeck* on biochemical parameters, body weight, and feed intake.
vii. Investigation of the effects of these parts of the plant on the haematological parameters and structures of the digestives organs in other experimental animals.

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