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**Journal of Biology and Life Science**
ISSN 2157-6076
2024, Vol. 15, No. 1

# Effects of Methanol Extract of *Albizia lebbeck* Leaves on the Body Weight, Hematological Parameters and Histological Structures of Digestive Organs in Rats (*Rattus norvegicus)*

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Received: September 17, 2023 Accepted: October 27, 2023 Published: November 12, 2023

doi:10.5296/jbls.v15i1.21453 URL: https://doi.org/10.5296/jbls.v15i1.21453

**Abstract**

Due to its use in traditional medication and its leaves and pods being considered as potential fodder for livestock the toxicity of *Albizia lebbeck* needs to be thoroughly explored. The study evaluated the effects of 70% methanol extract of *Albizia lebbeck* leaves on body weight, hematological parameters and histological structures of digestive organs in Wistar rats. Twenty-one healthy adult female rats were experimented with and divided into two groups of six and fifteen animals. Six rats were randomly subdivided into two groups of three rats each for plant extract $LD_{50}$ determination at 2000 mg/kg and 5000 mg/kg dosages, respectively. Fifteen rats were randomly sub-allotted into three groups of five animals. Group I the control received distilled water by gavaging. Groups II and III received 100 mg/kg and 400 mg/kg of the extract, respectively—treatment covered 30 days. On day 31, the body weight of each rat was recorded before being sacrificed under chloroform. Blood was collected for

hematological analysis. The liver and stomach were trimmed and processed for histopathology. The results revealed the $LD_{50}$ of the extract to be greater than 5000 mg/kg. Extract treatment had no significant effects on the rat's body weight. A significant ($P<0.05$) increase in WBC, Eosinophils, RBC, and Hematocrit was revealed at 100 mg/kg of extract treatment. A dosage of 400 mg/kg of the extract caused a significant increase in Eosinophils and Lymphocytes. However, the plant extract caused a significant ($P<0.05$) decrease in Mean Platelet Volume and Platelet Distribution Width at both doses, Monocytes and Neutrophils at 400 mg/kg and Mean Corpuscular Volume and Mean Corpuscular Hemoglobin at 100 mg/kg compared with the control. Both doses of the extract treatments caused lesions in the liver characterized by, necrosis, sinusoidal dilatation and inflammatory cell infiltration with no significant lesions in the stomach. In conclusion, the extract of *Albizia lebbeck* leaves caused a significant decrease in some hematological parameters and some lesions in the liver at 100 mg/kg and 400 mg/kg.

**Keywords:** Albizia lebbeck, hematological parameters, digestive organs

## 1. Introduction

### *1.1 Background Information*

*Albizia lebbeck* is a leguminous plant that belongs to the family of *Fabaceae* (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 (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 extensively used in the treatment of various diseases and as a fodder for livestock in several countries. The plant is used in the management of wound, boils, abscess, blood dyscrasias, jaundice, dementia, psychosis, epilepsy, mania, paralysis, syphilis, cold and cough, sinusitis, constipation, hemorrhoids, and dysentery (Khan *et al.,* 2018). Various parts of the plant are used as animal feed in Pakistan (Zia-ul-haq *et al.,* 2013), Sudan (Mishra *et al.,* 2010) and many other countries (Ansari *et al.,* 2016). In Tanzania, *Albizia lebbeck* is used to treat various ailments including eye problems, diabetes (Nahashon, 2013), dysmenorrheal, malaria, and diarrhea (Hilonga *et al.,* 2018). Moreover, the plant is used as a supplement feed for domestic animals (Ndemanisho *et al.,* 2006).

Hematological parameters are measurements that concern the blood and organs that are responsible for forming the blood (Bamishaiye *et al*., 2009; Etim *et al*., 2014). Hematological 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 in hematological 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 hematological parameters are erythrocytes (red blood cells), leucocytes (white blood cells), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (Fathima *et al*., 2019). Platelets and platelet indices such as Plateletcrit, mean platelet volume and platelet distribution width are also widely used (Giovanetti *et al.,* 2011; Budak *et al.,* 2016).

The digestive organs are responsible for reception, mechanical breakdown, chemical digestion, absorption of food, and elimination of unabsorbed residues. The organs include the digestive tube or tract and accessory organs. The digestive tube contains the mouth, pharynx, esophagus, stomach, small intestine, and large intestine. The accessory organs include teeth, tongue, salivary gland, liver, and pancreas (Sisson and Grossman's, 1966; Aurich *et al.,* 2014). In this study, the digestive organs that were used to evaluate the effects of *A. lebbeck* were the liver and stomach. These organs were purposely selected because of the functions they perform in the animal body and studies investigating the effects of *A. lebbeck* in these organs are scanty.

In Tanzania, despite the wide use of the locally available species of *Albizia lebbeck* for treatments of various diseases and as the feed-supplement, scientific information on the effects of this plant on the animal body is non-existent. Therefore, the present study was undertaken to evaluate the effects of locally available species of *A. lebbeck* leaves on body weight, hematological parameters and histological structures of the digestive organs in rats.

## 2. Material and Methods

### *2.1 Description of the Study Area*

This study was performed in the Morogoro region, Tanzania. Morogoro region lies between latitude 5º 58" and 10º 0" South of the Equator and between longitude 35º 25" and 35º 30" East of Greenwich Meridian. The topography of Morogoro is predominantly plain with rainfall ranging between 900 and 1 400 mm annually. The average temperature in Morogoro is 30° C due to its lowland nature.

### *2.2 Plant's Materials Collection, Authentication and Preparation of the Methanol Extract of Albizia Lebbeck Leaves*

Leaves of *A. lebbeck* were collected from a forest around Morogoro municipal and transported to the Toxicology laboratory in the College of Veterinary Medicine and Biomedical Sciences (CVMBS) at Sokoine University of Agriculture (SUA). Before the commencement of the processing of the plant leaves, the leaves were authenticated by a botanist from the College of Forestry, Wildlife and Tourism; at SUA. The authenticated leaves were dried under shade at room temperature for seven weeks and then ground into fine powder by using an electrical blender. Extraction was carried out by soaking 500 g of powdered leaves in 1.5 L of 70% methanol (Gupta *et al.,* 2004). The mixture was stored at room temperature for 72 hours and regularly shaken. After three days, the extract from *A. lebbeck* leaves was obtained by filtering the methanol extract through the Whatman filter paper number 1. The filtrate obtained was evaporated to dryness by using a rotary evaporator at $75^0$C and concentrated by using a water bath at $46^0$C to yield a solid mass of the plant leaves. Then the solid mass obtained was put in air tight container and stored in a refrigerator at $4^0$C until the time of use.

### *2.3 Study Animals*

Healthy adult female nulliparous and non-pregnant Wistar albino rats (*Rattus norvegicus*)

were procured from a local breeder in Morogoro municipal and used in this study. The rats were 11-13 weeks old and weighed 120 -150g. The animals were acclimatized for 14 days in the laboratory at CVMBS, SUA before the start of the experiment. The rats were housed in cages and each cage had three animals. They were kept under standard laboratory conditions of 12 hours light and 12 hours dark, ambient temperature of 28 ± $2^0$C and 45 - 60% humidity. They were provided with layers mash and portable water *ad libitum*. Rat's welfare was observed all over the research period.

*2.4 Experimental Setup*

Twenty-one (21) nulliparous and non - pregnant female Wistar albino rats were used in this study. The rats were randomly divided into two groups of six and 15 rats for acute toxicity test and sub - acute effects, respectively. An acute toxicity study was performed to determine the $LD_{50}$ of methanol extract of *A. lebbeck* leaves. It was conducted according to the Organization for Economic Cooperation and Development (OECD) guidelines number 423(OECD, 2001) with minor modifications. Briefly, six rats were fasted overnight but allowed free access to drinking water before the commencement of the experiment. The fasted rats were measured in weight and divided into two groups of three rats ($n = 3$) each. Groups I and II were orally administered once with the methanol extract of *A. lebbeck* leaves at the dose rates of 2000 mg/kg and 5000 mg/kg, respectively. Each animal was closely observed for one hour post - treatment and periodically during the first 24 hours, with close monitoring during the first four hours. Thereafter, observed once a day for 14 days for any behavior changes, feed intake, signs of toxicity, and mortality.

Fifteen (15) rats were used to evaluate the sub - acute effects of methanol extract of *A. lebbeck* leaves on body weight, hematological parameters and histological structures of digestive organs. The rats were randomly assigned into three groups of five rats ($n = 5$) each. Before treatment body weights of the rats were recorded and labeled as initial body weight. Rats in group I labeled as control received 1ml of distilled water as a placebo by gavage for 30 days. Rats in groups II and III were orally administered with 70% methanol extract of *A. lebbeck* leaves at a dosage of 100 mg/kg and 400 mg/kg once daily, respectively, for 30 days. These doses were chosen according to the previous efficacious studies (Doshi *et al.*, 2016; Neeti *et al.*, 2016)

*2.5 Determination of the Final Body Weight*

The body weight recorded after treatment was labeled as the final body weight. The final body weight of each rat was measured by an electronic beam balance model type BL-220H, (SHIMADZU CORPORATION JAPAN). The body weight gain was calculated by subtracting the initial from the final body weight.

*2.6 Samples Collection*

Rats from both control and experimental groups were anesthetized by using chloroform on day 31. Thereafter, each rat was kept in a dorsal recumbence and a blood sample was collected from the heart of each rat into a vacutainer tube with anticoagulant tripotassium ethylene diamine tetra acetic acid ($K_3$-EDTA) for hematological analysis. After collection of

the blood samples, rats were sacrificed by neck dislocation, and the abdominal cavity was opened. Finally, the digestive organs (liver and stomach) were collected from the abdominal cavity of each rat and immersion – fixed in 10% neutral buffered formalin for histological studies.

*2.7 Determination of the $LD_{50}$*

Acute toxicity was determined by recording any mortality, feed intake, behavior changes, and signs of toxicity for 14 days post-treatment.

*2.8 Analysis of Hematological Parameters*

An automated hematological analyzer (NS$^{TM}$ 4S, FRANCE) was used to analyze blood samples collected into $K_3$-EDTA vacutainer tubes as explained in section *2.6*. White blood cell (WBC) count, WBC differential counts (Lymphocytes, Monocytes, Neutrophils, Eosinophils, and Basophils), Red blood cell(RBC) count, Hemoglobin (HB), Hematocrit (HCT), RBC indices (Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Red blood cell distribution width (RDW)), Thrombocytes (THR), and THR indices (Mean platelet volume (MPV), Plateletcrit (PCT) and Platelet distribution width (PDW)) were analyzed.

*2.9 Histological Studies of the Digestive Organs*

The standard histological procedures were employed in tissue histopathology**.** The digestive organs (liver and stomach) which were fixed in 10% neutral buffered formalin as elaborated in section *2.6* were processed and stained using standard protocols (Kiernan, 1990). These protocols were slightly modified at the Department of Veterinary Anatomy and Pathology, CVMBS, SUA. Briefly, tissue processing included (i) Dehydration of the fixed tissues by changing the concentration of ethanol (70%, 95% I, 95% II, absolute I, and absolute ethanol II) at $60^0$C for 20 minutes each. (ii) Cleared the dehydrated tissues by using chloroform I and II for 30 minutes each at $60^0$C. (iii) Paraffin wax I and II were infiltrated into the cleared tissues for 40 minutes each. (iv)Embedded the wax-infiltrated tissues in paraffin blocks. (v) Sectioned the tissue blocks at 3-5 µm thickness by using a Rotary Microtome. The sectioned tissue ribbons were gathered and slowly placed on a tissue flotation water bath at $20^0$C to unfold the paraffin wax-infiltrated tissue. Microscopic slides were used to pick the unfolded tissue ribbons and placed in the oven overnight at $60^0$C to assist the tissue ribbons in attaching well to the slides. Thereafter, the slides were cooled at room temperature and kept ready for routine staining steps. However, before tissue staining, the sectioned tissues were deparaffinized by xylene I, II and III for five minutes each. They were then dehydrated by ethanol starting with absolute, 95% and 70% for two minutes each. Tissue staining: The tissues were stained by Harris' hematoxylin (H) and Eosin (E). Briefly, the process involved staining the tissues by using H for five minutes followed by washing in tape water and putting in 1% acid alcohol for differentiation as well as to take out too much stain. Thereafter, the slides were soaked in lithium carbonate followed by a counter stain with E for five minutes. The H and E stained sections were dipped slightly rapidly in water and dehydrated by ethyl alcohol I, II and III for two minutes in each. Then the slides were passed through

xylene I, II and III for two minutes each. Finally, by using DPX mountain and glass cover slips the slides were mounted and covered, respectively.

Then a compound light microscope was used to carefully examine the microscopic slides of the liver and stomach under study by starting with a magnification of 40, followed by 100, 200, and 400 magnifications. Slides from the treated rats were rigorously evaluated for any histological change in respective organs compared to the control rats. Finally, by using an Olympus BH-2 mounted with Moticam Pro 205A Camera photomicrographs of some chosen slides were taken.

*2.10 Ethical Considerations*

Research was conducted by following SUA guidelines and all the protocols were approved by the SUA ethical committee with reference number RPGS/R/ETHICS/24.

*2.11 Data Analysis*

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test using Statistical Package for Social Sciences (SPSS) version 20 software. All values were expressed as Mean ± standard deviation (SD). P values < 0.05 were considered as significant.

## 3. Results

*3.1 Acute Toxicity Study*

Oral administration of methanol extract of *A. lebbeck* leaves at the doses of 2000 mg/kg and 5000 mg/kg did not cause any mortality or sign of acute toxicity to the study animals.

*3.2 Sub-Acute Effects of Methanol Extract of Albizia Lebbeck Leaves on Body Weight, Hematological Parameters and Histological Structures of Digestive Organs*

3.2.1 Body Weight

Oral administration of methanol extract of *A. lebbeck* leaves at the doses of 100 mg/kg and 400 mg/kg did not cause any significant change (P > 0.05) in the body weight of the treated animals compared with the control group (**Table 1**). The final body weight of both control and treatment groups marginally increased compared to the initial body weight. However, the rate of increase in the treated groups was slightly lower compared to the control group as indicated in **Table 1**.

Table 1. Effects of methanol extract of *Albizia lebbeck* leaves on body weight at day thirty post-treatment

| Body weight in grams: | Doses: | | | |
|---|---|---|---|---|
| | Control group | 100 mg/kg | 400 mg/kg | P-value |
| Initial body weight | 122.20±1.92 | 122.90±1.88 | 123.16±1.55 | 0.69 |
| Final body weight | 144.84±7.17 | 140.90±4.04 | 139.36±3.17 | 0.25 |

| | | | |
|---|---|---|---|
| Body weight gain | 22.64 | 18.00 | 16.20 |

All values are expressed as Mean ± SD (n = 5). One-way ANOVA was followed by Dunnett's test.

3.2.2 Hematological Parameters

The methanol extract of the *A. lebbeck* leaves at dosages of 100 mg/kg and 400 mg/kg caused some changes in total white blood cell count and differential white blood cell counts compared to the control group (**Table 2**). There was a significant ($P < 0.05$) increase in the total WBC at the dosage of 100 mg/kg of the plant extract treatment compared to the control. There was some marginal decrease in the total WBC count in the group of rats treated with 400 mg/kg of the plant extract although the difference was statistically insignificant ($P > 0.05$) when compared to the control group. Differential white blood cell counts revealed both doses of 100 mg/kg and 400 mg/kg of the plant extract caused a significant increase ($P < 0.05$) in the eosinophil count in the treated rats compared to the control group. Neutrophils and monocyte counts decreased significantly ($P < 0.05$) in treated rats at a dosage of 400 mg/kg compared to the control group. A dosage of 100 mg/kg had no significant ($P > 0.05$) effects on neutrophils and monocyte counts although the two blood parameters were slightly decreased in the extract - treated rats compared to the control group. The basophils count insignificantly ($P < 0.05$) changed at both doses compared to the control group.

Moreover, at both doses of 100 mg/kg and 400 mg/kg the extract of *A. lebbeck* leaves caused some changes in RBC count and RBC indices compared to the control group (**Table 3**). More specifically, the plant leaf extract significantly ($P < 0.05$) increased the RBC as well as HCT values while significantly ($P < 0.05$) decreased the MCV and MCH at the dose rate of 100 mg/kg. However, at the dosage of 400 mg/kg of the plant extract, RBC, HCT, MCV, and MCH did not vary significantly in the extract - treated rats although RBC and HCT they were both slightly increased and MCV as well as MCH marginally decreased compared to the control group. Also, no significant ($P > 0.05$) difference in the values of MCHC, HB and RDW was observed between the extract - treated rats at both doses and the control group.

Furthermore, the 70% methanol extract of *A. lebbeck* leaves at both doses of 100 mg/kg and 400 mg/kg significantly ($P < 0.05$) decreased the MPV and PDW compared to the control group. However, both doses of the plant extract did not cause any significant ($P > 0.05$) change in THR and PCT, with only a marginal increase in the two parameters being observed when compared to the control group (**Table 4**).

Table 2. Effects of methanol extract of *Albizia lebbeck* leaves on WBC count and differential WBC counts after thirty days of treatment

| Hematological parameters: | Doses: | | | |
|---|---|---|---|---|
| | Control group | 100 mg/kg | 400 mg/kg | P-value |
| WBC ($M/mm^3$) | 7.00±1.22 | 13.00±1.00** | 5.80±1.48 | 0.00 |
| Lymphocytes (%) | 53.20±3.83 | 55.80±3.49 | 64.80±4.55** | 0.00 |
| Neutrophils (%) | 21.60±1.34 | 19.80±1.48 | 9.60±1.14* | 0.00 |
| Monocytes (%) | 20.00±1.41 | 18.20±1.30 | 12.00± 0.71* | 0.00 |
| Eosinophils (%) | 5.60±2.07 | 11.80±0.84** | 13.60±1.14** | 0.00 |
| Basophils (%) | 0.20±0.45 | 0.20±0.45 | 0.20±0.45 | 1.00 |

All values are expressed as Mean ± SD (n = 5). One-way ANOVA was followed by Dunnett's test.

** Significantly ($P < 0.05$) increased compared to the control group.

* Significantly ($P < 0.05$) decreased compared to the control group.

Table 3: Effects of methanol extract of *Albizia lebbeck* leaves on red blood cell count and red blood cell indices after thirty days of treatment

| Hematological parameters: | Doses: | | | |
|---|---|---|---|---|
| | Control group | 100 mg/kg | 400 mg/kg | P-value |
| RBC ($M/mm^3$) | 4.40 ±0.55 | 6.80±0.45** | 5.20±0.84 | 0.00 |
| HCT (%) | 29.20±1.30 | 44.40±2.30** | 32.60±3.05 | 0.00 |
| HB (g/dl) | 15.40±1.14 | 15.00±1.58 | 14.60±0.55 | 0.57 |
| MCV (fl) | 79.00± 2.24 | 57.40±2.07* | 71.00±10.56 | 0.00 |
| MCH (pg) | 29.20±4.66 | 9.80±0.837* | 19.80±10.50 | 0.00 |
| MCHC (g/dl) | 33.40±10.38 | 21.80±7.23 | 25.00±9.30 | 0.16 |
| RDW | 13.20±1.92 | 12.00±0.71 | 12.80±0.45 | 0.32 |

All values are expressed as Mean ± SD (n=5). One-way ANOVA was followed by Dunnett's test.

** Significantly ($P < 0.05$) increased compared to the control group.

* Significantly ($P < 0.05$) decreased compared to the control group.

Table 4: Effects of methanol extract of *Albizia lebbeck* leaves on thrombocyte count and thrombocytes indices after 30 days of treatment

| Hematological parameters: | Doses: | | | |
|---|---|---|---|---|
| | Control group | 100 mg/kg | 400 mg/kg | P-value |
| THR (M/mm$^3$) | 694.80±81.05 | 772.80±187.03 | 742.60±152.89 | 0.71 |
| PCT (%) | 1.00±0.71 | 1.80±0.45 | 1.60±1.14 | 0.31 |
| MPV (fl) | 7.80±0.45 | 7.00±0.00* | 7.00±0.71* | 0.03 |
| PDW (fl) | 8.40±0.89 | 6.20±0.84* | 6.60±0.55* | 0.00 |

All values are expressed as Mean ± SD (n=5). One-way ANOVA was followed by Dunnett's test.

** Significantly ($P < 0.05$) increased compared to the control group.

* Significantly ($P < 0.05$) decreased compared to the control group.

3.2.3 The Histological Structures of the Liver and Stomach

Histopathology of the liver indicated the presence of lesions in the H&E-stained sections of the liver from the rats receiving a dosage of 100 mg/kg of the plant extract. The lesions were characterized by multiple inflammatory cell infiltration, necrosis, stratified epithelia of the bile ducts, and sinusoid dilatation compared to the control group (**Figure 1**). Moreover, the dosage of 400 mg/kg of the plant extract caused extensive inflammatory cell infiltration which extended from one portal triad to the other (bridging inflammation), necrosis which also extended from one portal triad to the other (bridging necrosis) and sinusoids dilatation (**Figure 1**). On the other hand, the light microscope examination of the stomach sections of rats treated with methanol extract of *A. lebbeck* leaves at the doses rates of 100 mg/kg and 400 mg/kg revealed no significant lesion on the structures of the stomach such as tunica mucosa (lamina epithelial, lamina propria and lamina muscularis), tale sub mucosa, tunica muscularis, and tunica serosa compared to the control group (**Figure 2**).

Journal of Biology and Life Science
ISSN 2157-6076
2024, Vol. 15, No. 1

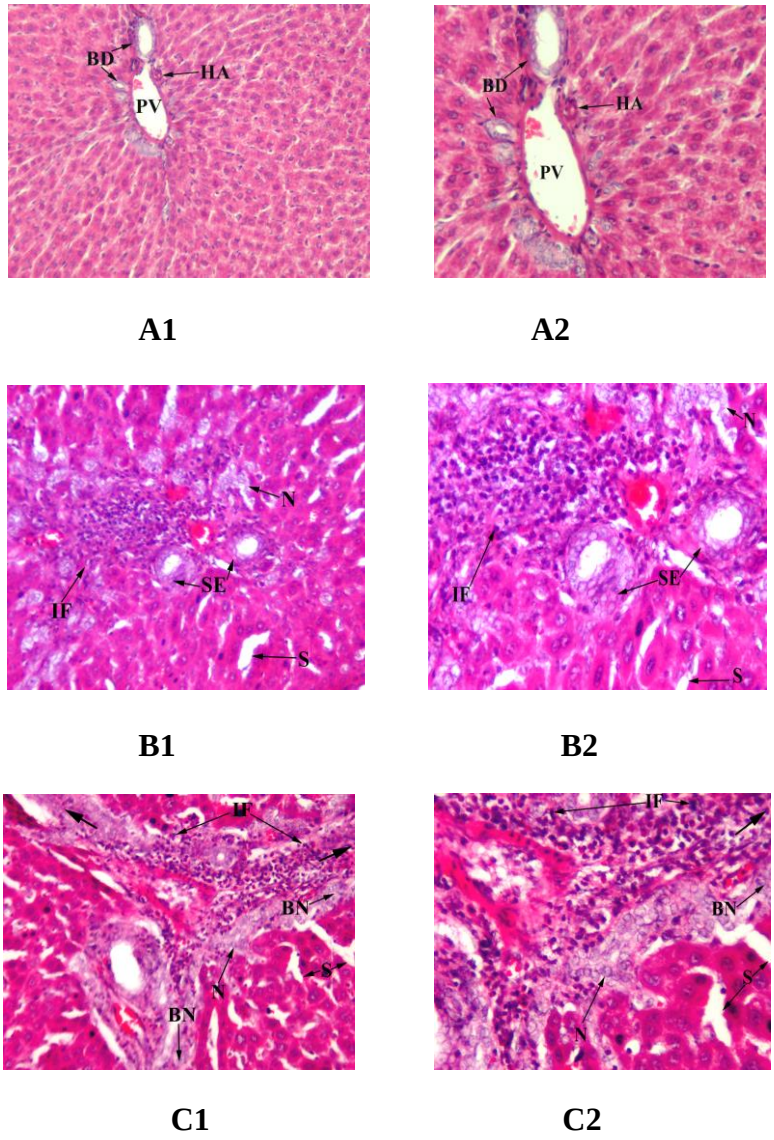
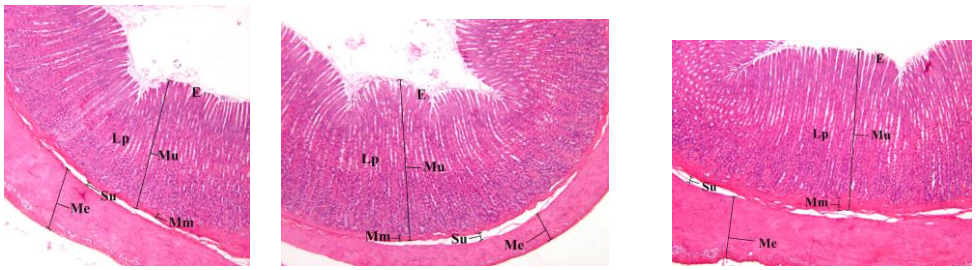



Figure 1: Photomicrographs of the liver sections of the control and treated groups with methanol extract of *Albizia lebbeck* leaves for thirty days

(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 treated animals showing inflammatory cell infiltration (IF), sinusoid dilatation (S), stratified epithelia of the bile ducts (SE), and necrosis (N).

(C1 at x100 and C2 at x200) 400 mg/kg treated animals showing inflammatory cell infiltration (IF), necrosis (N), bridging inflammatory cell infiltration (thick arrows), and bridging necrosis (BN). H and E stained sections.




A B C

Figure 2. Photomicrographs of the stomach sections 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), lamina epithelialis (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me).

(B) 100 mg/kg treated group showing intact tunica mucosa (Mu), lamina epithelialis (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me).

(C) 400 mg/kg treated group showing normal tunica mucosa (Mu), lamina epithelialis (E), lamina propria (Lp), lamina muscularis (Mm), tela sub mucosa (Su), and tunica muscularis (Me). H and E stained section x40.

## 4. Discussion

The study revealed that the $LD_{50}$ of the 70% methanol extract of *A. lebbeck* leaves was greater than 5000 mg/kg body weight. According to Erhirhie *et al.* (2018), any substance with $LD_{50}$ between 5000 – 15000 mg/kg is practically non - toxic. Thus, 70% methanol extract of *A. lebbeck* leaves is practically non - toxic. This result agreed with the study reported by Islam *et al.* (2018), who reported that the extract of *A. lebbeck* leaves has a low toxicity profile with $LD_{50}$ greater than 3000 mg/kg.

Also, a change in body weight which is one of the initial signs of early toxicity (Ghosh *et al.*, 2019) was not evident following extract treatment in the rats. The insignificant change in body weight in the extract-treated rats relative to the control indicates that the leaf extract might not contain any phytochemical that affects the body weight of the treated rats. This finding is similar to what was reported by Gupta *et al.* (2006) and contrary to what was found by Odey *et al.* (2017). The final body weight gain noted in this study for both control and treated groups was just a normal growth pattern.

The present study showed that the plant extract significantly increased WBC count at the dose rate of 100 mg/kg. This implied that at this dose the plant extract may contain some secondary metabolites that can stimulate white blood cell production. This finding is in agreement with findings by Doshi *et al.* (2016) who also reported that *A. lebbeck* rise in the number of WBCs. Additionally, eosinophil count significantly increased at both doses after thirty days of treatment. The significant increase in eosinophils observed in this study might be caused by the presence of active principles in this part of the plant that enhanced the

production of eosinophils. According to Agiang *et al*. (2017), eosinophils are the type of leukocytes that play a vital role in the immune system, especially in the 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. In the present study, the eosinophil count significantly increased, thus, the plant extract can be used in the management of allergic reactions, asthma and parasitic infections, destroying foreign substances and modifying inflammation. On the other hand, monocytes and neutrophils significantly decreased at 400 mg/kg after thirty days of treatment. This significant decrease in monocytes and neutrophils could be due to the presence of phytoconstituents in *A. lebbeck* leaves that might decrease the production of monocytes and neutrophils or increase the movement of these blood cells into the tissues. According to Arika *et al.* (2016) neutrophils and monocytes have phagocyte's activities; they attack and destroy foreign particles, cell waste materials and bacteria. Therefore, prolonged use of the *A. lebbeck* leaves at higher doses may predispose animals to infections by impeding the process of phagocytosis. Basophils neither decreased nor increased significantly at both doses.

The red blood cell parameters including RBC, HCT, HB, MCV, MCH, MCHC, and RDW are valuable indicators of the levels of circulating erythrocytes and serve 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 revealed a significant increase in RBC and HCT at 100 mg/kg after thirty days of treatment. According to Ijioma *et al*. (2019), RBC may increase due to the presence of strong anti - oxidants effect in the plant extracts which inhibit lyses of RBCs from radical formation. Moreover, red blood cells may increase because of the action of the erythropoietin which is a glycoprotein hormone that stimulates stem cells in the bone marrow to produce more erythrocytes (Zaruwa *et al.*, 2016). Therefore, the increase in the RBC and HCT observed in the present study suggests that the plant extract might contain some active principles that can act either as antioxidants or stimulate the release of more erythropoietin or both. The plant extract significantly decreased MCV and MCH at the dose rate of 100 mg/kg after thirty days of treatment. According to Iwuanyanwu and Nkpaa (2015) when RBCs decrease in size than normal, the MCV will be below normal and implies microcytic, when RBCs increase in size than normal, the MCV will be elevated and indicate macrocytic and when RBCs are of the normal size are termed as normocytic. These size categories are used to classify anemia (Iwuanyanwu and Nkpaa, 2015). The amount of hemoglobin is influenced by the size of the red blood cells for instance small cells have a small amount of hemoglobin and large cells have a large amount of hemoglobin. Therefore, the MCV should be taken into consideration whenever the MCH is interpreted (Doig and Zhang, 2017). Although RBC and HCT at the dose level of 100 mg/kg significantly increased in the treated group compared to the control group, the significant decrease in MCV and MCH at the same dose indicated that the plant extract might increase the synthesis of unhealthy RBC, particularly, it shows that high proportional of red blood cells produced were small in size. Therefore, leaves of *A. lebbeck* might have some active ingredients that can cause microcytic hypochromic anemia. Hemoglobin, MCHC and RDW insignificantly changed at both treatment dosages. This indicates that the plant extract did not have any secondary metabolite that significantly influences these hematological parameters.

Furthermore, the extract of *A. lebbeck* at both doses of 100 mg/kg and 400 mg/kg caused a significant decrease in MPV and PDW. Mean platelet volume is the measure of the 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 the extract might contain phytochemicals that increased platelet production because 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 sizes due to swelling, destruction, and immaturity (Gao *et al.*, 2014). Thus, methanol extract of *A. lebbeck* leaves at both doses after thirty days of treatment decreased variation in the size of thrombocytes in the treated groups compared to the control group.

Histopathological study of the plant extract in the liver indicated the presence of some severe histopathological lesions including extensive inflammatory cell infiltration, necrosis, sinusoid dilatation, bridging inflammatory cell infiltration, and bridging necrosis. The lesions observed in the liver of the treated rats could suggest that the plant extract might contain phytoconstituents which are toxic to this organ. According to Nweke *et al.* (2019), the presence of bioactive compounds such as alkaloids, tannins, saponins, and oxalate may cause histopathological lesions in the organs. Also, Olaniyan *et al*. (2016) reported that the presence of glycosides and a large intake of tannins may cause lymphocytic infiltration and damage in organs, respectively. Therefore, the plant extract might contain these bioactive compounds (alkaloids, tannins, saponins, glycosides, and oxalate) which caused histopathological lesions in the liver of the treated animals. The histopathological lesions observed in the liver of the treated animals in this study were contrary to what was reported by Kalia *et al*. (2015). However, these findings were in line with various other studies that have documented that the administration of the herbal plants to the experimental animals may cause histopathological lesions in the liver (Atsafack *et al.*, 2015; Debebe *et al.*, 2017). Whereas, there was no significant histopathological lesion in the stomach of the treated animals at both doses compared to the control animals. The bioactive compounds that caused histopathological lesions in the liver of the treated rats did not cause any significant lesions in the stomach of the treated animals. This might be because of the presence of protective factors including gastric mucus, bicarbonate ions, prostaglandins, and innate resistance of mucosal cells (Park *et al*., 2019) in the stomach of treated rats. This finding is similar to what was reported by Ghosh *et al.* (2019) and contrary to what was stated by Umaru (2012).

## 5. Conclusion

The acute toxicity study of the 70% methanol extract of the *A. lebbeck* leaves did not cause any mortality or sign of toxicity in the treated rats at 2000 mg/kg and 5000 mg/kg. Therefore, the oral $LD_{50}$ of the plant extract was greater than 5000 mg/kg. Moreover, the sub-acute toxicity study of the 70% methanol extract of the *A. lebbeck* leaves did not adversely affect the body weight and histological structures of the stomach of the treated rats at the doses of 100 mg/kg and 400 mg/kg. However, the plant extract at the doses of 100 mg/kg and 400 mg/kg caused a significant decrease in some hematological parameters and histopathological lesions in the liver.

**Acknowledgments**

The authors would like to convey sincere thanks to the Head of the Department of Veterinary Anatomy and Pathology for the provision of space and equipment. Many thanks go to Prof. Claudius Luziga for assistance in taking photomicrographs. We also extend our thanks to Mr. Philemon Mkuchu, Mr. Togolai Mbillu and Mr. Mgaya Mwangalimi for their valuable technical assistance in the laboratory.

**Authors contributions**

Dr. Gwakisa Aroni was responsible for the conception of the idea, design, plant collection and extraction, hematological and histopathological analyses and interpretation, and manuscript preparation. Prof. Remmy Assey supervised the study and proofreading of the manuscript. Dr. Lusekelo Mwangengwa contributed to the hematological analysis and a draft of the manuscript. All authors read and approved the final manuscript.

**Funding**

This study was self-funded

**Competing interests**

The authors declare that they have no competing interests

**Informed consent**

Obtained.

**Ethics approval**

The Publication Ethics Committee of the Macrothink Institute.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

**Provenance and peer review**

Not commissioned; externally double-blind peer reviewed.

**Data availability statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

**Data sharing statement**

No additional data are available.

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