



Effects of Ethanol Extract of *Bridelia ferruginea* (W.) on the Haematological and Histopathological Parameters in Swiss Albino Rats Infected with *Salmonella typhi*

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study and wrote the protocol. Author BTA performed the statistical analysis, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Author EOD guided in the entire research and edited the final draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the effects of ethanol stem bark extract of *Bridelia ferruginea* on the hematological and histopathological parameters in Swiss albino rats infected with *Salmonella typhi*.

Study Design: Experimental design.

Place and Duration of Study: Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. Between January, 2018 and June, 2018.

Methodology: Fresh stem bark of *Bridelia ferruginea* was collected, dried, powered, and extracted using 70% ethanol. Twenty – seven rats of the same age between 90 -120 g in weight were selected and divided into 9 groups containing three each. The infectivity dose (ID) was determined with the clinical *S. typhi*. The dose of the *B. ferruginea* stem bark extract (50-5000 mg/kg) used in this study was administered orally for 7 days. At the end of the treatment period, the rats fasted overnight. Then blood samples were collected by cardiac puncture for haematological studies and

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thereafter sacrificed. Organs (liver and kidney) were excised for relative organ weight analysis and histopathological studies.

Results: The infectious dose (ID) of *S. typhi* on experimental rats in this study was 3.2×10^6 cfu/ml. The weights of liver and kidney in all groups under observation slightly increases (treated with ciprofloxacin and ethanol extract) when compared to the control(uninfected) except for the infected/treated where the weight was significantly reduced. However, The Packed Cell Volume (PCV) and Haemoglobin (Hb) of rats treated with a higher dose of the ethanol (5000mg/ml) extract increased significantly ($p < 0.05$) when compared to the control(uninfected) while the infected but not treated reduced significantly($p < 0.05$) when compared with the uninfected, infected and treated with Ciprofloxacin and extract groups. The infected and untreated group has the highest reduction in red blood cell counts(RBC) with 3.40 ± 0.11 g/l respectively which were significantly different at $p < 0.05$ when compared to other treatment groups while a non-significant decrease was observed in the Red Blood Cell (RBC) of rats administered with various concentrations of ethanol extract and ciprofloxacin after treatment. White Blood Cells (WBC) of rats given the extract significantly reduced when compared with the control (un-infected) group while there was no significant difference in the lymphocyte count of rat administered the extract of *B. ferruginea* and ciprofloxacin. Neutrophils of rats in all groups significantly increased ($p < 0.05$). However, the monocyte and eosinophil level were not significantly different from the control. There was dilation of sinusoids depletion separating the hepatic cord in place lined by kupffer cells and not necrosis with possible deposition of immunological materials observed in the liver of rats infected/treated with the extract at various concentrations. Similarly, there were loss of bowman capsule, severe karyolysis and several tubular drainages with possible deposition of immunological materials in the glomeruli basement of the kidney after treatment with the extract of *B. ferruginea*.

Conclusion: The stem bark ethanol extract of *Bridelia ferruginea* can be found relatively safe. However, excessive use might be toxic to the to the kidney and the liver.

Keywords: *Bridelia ferruginea*; *Salmonella typhi*; haematological; histopathological; rats.

1. INTRODUCTION

A high percentage of people in both developed and developing countries use medicinal plants for therapeutic purposes as reported by [1], this is consistent with the estimation of the World Health Organization (WHO) that about 78–80% of the world's populations rely on medicinal plants for their primary health care as was stated by [2] in his findings. Different plants and their parts are used all over the world for various purposes [3]. Some of which have their constituents and therapeutic properties established scientifically many others are yet to be subjected to investigations. In northern Nigeria variety of plant species are used in diseases management or no consideration to toxicological profile of the plant [4].

Bridelia ferruginea (Willd) is referred to as Ira which belong to family Euphorbiaceae. It a wood shrub that grows in the savannah rainforests of Africa [5]. [3] stated that the stem bark extract is used for milk coagulation and also in lime juice for the formulation of traditional gargle (ogun-efu). The bark extract of the plant possesses antimicrobial activities against some micro-organisms known to cause enteric and

secondary upper respiratory water treatment [6]. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases and herbivorous animals. The ethanol extract of the stem bark of this plant contains tannins, alkaloids, sterols, terpenes flavonoids and saponins [7]. Prevention of metabolic syndrome in type 2 diabetes as reported by [8]. Also, Antimicrobial properties of stem bark of *B. ferruginea* against facultative Gram negatives rods has been reported by [9].

S. typhi causes enteric fever which is a major public health problem both in developing as well as developed economics [10]. It is solely a human pathogen predominately found in the intestinal lumen. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharide (LPS) which protects the bacteria from the environment. The bacteria infect by coming in direct contact with the phagocytic cells. This contact involves the formation of appendages formed which are shorter than flagella but thicker than both flagella and pili. [11] reported that *S. typhi* cells enter the epithelial cells lining the intestine they cause host cell ruffling which temporarily damages the microvilli on the surface of the cell. This causes a

rush of white blood cells into the mucosa, which throws off the ratios between absorption and secretion and lead to diarrhea. However, typhoid *Salmonella* species have increasingly become resistant to conventional antibiotics such as ampicillin, chloramphenicol, cotrimoxazole, and fluoroquinolones in developing countries [12].

However, limited scientific evidence on the safety profile and its efficacy is required to back up the continued traditional therapeutic application of the plant extract. Besides, toxicity is a measure of how poisonous a substance is, it shows the degree of adverse effects caused by the interaction of the toxicants with body cells as reported by [4]. The possible effects produced depend on the chemical properties of the toxic substance and the cell membrane [13]. Similarly, blood components are exposed to significant concentrations of these toxic compounds as they form the medium for their transport. Hence, the evaluation of the toxic effects of stem bark of *B. ferruginea* extract on haematological and histopathological parameters in Swiss albino rats infected with *Salmonella typhi*.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh stem bark of *Bridelia ferruginea* W. was collected from a garden at Igbara-Odo in Ekiti State. The stem bark was identified and authenticated by using the herbarium specimens of the department of crop, soil and pest management, Federal University of Technology, Akure (FUTA).

2.2 Source and Preservation of Bacteria

Pure clinical and typed isolates of *Salmonella typhi* were obtained from the stock culture of The University of Ibadan Teaching Hospital, Ibadan Oyo State and typed isolate of *S. typhi* ATCC19214 was obtained from Pathology and Clinical Laboratory Pathcare of Lagos University Teaching Hospital (LUTH) Lagos State, Nigeria. The bacteria isolates were kept on already prepared nutrient agar slants and transported immediately to the microbiology laboratory of the Federal University of Technology, Akure, Ondo State for further analysis using the methods of [4].

2.3 Animals

Fifty-four (54) Swiss albino rats (90-120 g) between 8-12 weeks were obtained from the

Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria. The rats were fed with standard rat pellets (Livestock Feeds, Ikeja, Lagos State) and water *ad libitum*. The animals were housed under standard laboratory conditions and were acclimatized for 7 days before the treatment started. The experimental procedures involving animals were conducted in conformity with International National and Institutional Guidelines as described by [6].

2.4 Inclusion and Exclusion Criteria

Both sexes of the albino rats between 8-12 weeks old, healthy and with no previous exposure to antibiotics were used while rats that are more 12 weeks old, infected and exposed to antibiotics were excluded from this study.

2.5 Preparation of Standard Inoculum of *S. typhi* for *In-vivo* Assay

About 0.238 g of sodium hydrogen phosphate was dissolved in 0.019 g of potassium dihydrogen phosphate and sodium chloride respectively. The mixture was made up to 100 ml with distilled water and pH was adjusted to neutral Ph and then, standard inoculum of *Salmonella typhi* was inoculated into 1000 ml of nutrient broth and incubated at 35°C for 24 hours. After incubation, the cells were centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded. Pellets were re-suspended in Phosphate buffered saline (PBS) and centrifuged again for four times. The final cell button was re-suspended in PBS and serially diluted 10^1 to 10^6 as employed by [14].

2.6 Determination of Infectious Dose (ID)

The method described by [4] was adopted. Fifteen healthy Swiss albino rats were used to determine the Infectious Dose of *S. typhi*. The rats were divided into five groups of three rats and each group was infected with different concentrations of *Salmonella typhi* suspension. The groups were closely monitored for Five days and the concentration of *S. typhi* suspension that produces the signs like unformed stool, feverish condition, temperature rises, weak, scattered fur, falling of hairs, stool with mucous and weight loss in animals was $3.2 \text{ ml } (10^2 \text{ cfu/ml})$ and was given as the infectivity dose (ID_{50}) of *S. typhi*. Also, corresponding colony-forming units per millilitres (cfu/ml) of the bacterial dilutions were determined using plate count method on *Salmonella-Shigella* agar (SSA).

2.7 In-vivo Assay

The method described by [15,16] was adopted. The Swiss albino rats had free access to feeds and water and they were acclimatized for more than a week prior to the experiment. Twenty-seven (27) Swiss albino rats having an average weight between 90-120 g of 8-12 weeks were infected with *Salmonella typhi* suspension orogastrically and then placed in eighteen (18) different cages for 7 days to determine the anti-typhoid activities using ethanol extracts of *Bridelia ferruginea* stem bark. Group 1a was uninfected/untreated, group 2a was infected/treated with Ciprofloxacin, group 3a was infected/untreated, group 4a was infected/treated with 50 mg/kg of ethanol extract, group 5a was infected/treated with 100 mg/kg of ethanol extract, group 6a was infected/treated with 300 mg/kg of ethanol extract, group 7a was infected/treated with 1000 mg/kg of ethanol extract, group 8a was infected/treated with 2600 mg/kg of ethanol extract and group 9a was infected/treated with 5000 mg/kg of ethanol extract. All the experimental rats in groups 1, 2,3,4,5,6,7,8 and 9 were observed for signs of infection before and after being treated.

2.7.1 Relative organ weight

After the experiment, all the animals were euthanized under chloroform anaesthesia. Organs such as liver and kidney of the sacrificed animals were excised, washed with normal saline, examined for any lesions, and weighed in grams to obtain the relative organ weight as employed by [17]. The relative organ weights were calculated for each rat using;

$$\text{Relative Organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight at sacrifice (g)}} \times 100$$

2.7.2 Haematological assay

Blood samples were collected before treatment through cardiac puncture while after the experiment. Animals were sacrificed and incisions were quickly made in the sacrificed animal's cervical region. Blood samples were collected from the heart were dispensed into EDTA bottles for haematological analyses [17] and various analyses as described by [13] were carried out on the blood samples.

2.7.3 Histopathological assay

The histology of various organs was carried out using the method of [18]. The required organs

were excised, weight and fixed in aqueous Bouin's solution for 48 hours. They were sequentially embedded in paraffin wax blocks according to the standard procedure and sectioned at 5 μ thickness. They were further deparaffined with xylol, and histologic observations were performed after staining with haematoxylin and eosin. The slides were examined under a light microscope and the magnified images of the tissue structure were captured at x400.

2.8 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) while the means were compared by Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences version 23.0. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

3.1 Infectious Dose (ID)

The *Salmonella typhi* dosage that produced infection signs like weakness, scattered fur, feverish condition, stool with mucous and weight loss in the experimental rats was 3.2ml x 10^6 cfu/ml.

3.2 Effect of Treatment on Relative Organ Weights (ROW)

The effect done by the treatment on relative organ weight (g/100 g) of the rats is shown in Table 1. The weights of liver and kidney in all groups under observation slightly increases (treated with ciprofloxacin and ethanol extract) when compared to the control (uninfected/untreated) except for the infected but not treated where the weight was significantly reduced.

3.3 Haematological Analysis

Table 2 and Fig. 1 shows the haematological analysis result of the blood samples collected from the rats after the experimental procedure. In this study, the machine analyzed blood samples to give about seven parameters, which include hemoglobin (HB), packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), neutrophils (NEU),

lymphocytes (LYMP), monocytes (MON), basophils (BAS) and eosinophils(EOS).

The packed cell volume (PCV) and hemoglobin (HB) showed that control (un-infected/untreated) group is $48.33 \pm 0.57\%$ and $19.83 \pm 0.83 \text{ g/l}$ were significantly at $P < 0.05$ higher than the infected/untreated, treated with ciprofloxacin and treatment groups. It was observed that among the treatment groups, group treated with 5000 mg/kg with ethanol extract of *Bridelia ferruginea* stem bark was $46.00 \pm 1.00\%$ and $19.02 \pm 0.14 \text{ g/l}$ were significantly higher at $P < 0.05$ than other treatment groups respectively. However, the infected/untreated had the lowest value of packed cell volume(PCV) and hemoglobin with $31.50 \pm 0.50 \%$ and $10.66 \pm 0.20 \text{ g/l}$.

The white blood cell(WBC) and lymphocyte counts were observed among the rats that were infected with *Salmonella typhi* when compared to the control (uninfected/untreated)group with $10.36 \pm 0.20 \text{ g/l}$ and 41.00% respectively. Among the infected group, the untreated group had the highest increased in white blood cell(WBC)and lymphocytes counts with $12.8 \pm 0.24 \text{ g/l}$ and 56.00% which were significantly $p < 0.05$ different from other infected groups.

The red blood cell (RBC) counts were observed among the rats that were infected with *Salmonella typhi* when compared to the control (uninfected/untreated)group which have a value of $8.30 \pm 0.17 \text{ g/l}$ respectively which were significantly different at $p < 0.05$ between the red blood cell (RBC) counts of the infected groups. The infected/untreated group has the highest reduction in red blood cell (RBC) counts with $3.40 \pm 0.11 \text{ g/l}$ respectively which were significantly different at $p < 0.05$ when compared to other treatment groups. It was observed that the value of the red blood cell(RBC) counts in the rats increase with an increased concentration administered with the highest value of red blood cell (RBC) count observed in the group treated with 5000 mg/kg ethanol extract with $19.02 \pm 0.14 \text{ g/l}$ respectively.

The MCV, MCH and MCHC shows a non-significant increase at $p < 0.05$ except at 1000 mg/kg where a decrease was observed in MCV when compared to the control. However, treatment with ethanol extract does not have any significant difference at $p < 0.05$ on the eosinophil and basophils count when compared with the infected/untreated including control groups.

3.4 Histopathological Analysis

The histopathological results of the kidney of the experimental rats on plates 1A - 7A. Normal architecture of nephron with intact wide glomeruli room, loss of bowman capsule, several karyolysis, severe tubular drainage and possible deposition of immunological material in the glomeruli basement were observed in the groups infected /treated with 50-5000 mg/kg ethanol extract of *Bridelia ferruginea* stem bark when compared with the control (un-infected).

On plate 1B -7B results, dilated and loss sinusoid, possible deposition of immunological material in the hepatocyte with haemorrhage, hepatic sinusoids depletion separating the hepatic cord in place lined by kupffer cell and dot necrosis were observed in groups infected/treated with 50- 5000 mg/kg ethanol extract of *Bridelia ferruginea* stem bark when compared with the control (un-infected).

4. DISCUSSION

This work tested the effect of ethanol extract of *Bridelia ferruginea* stem bark on haematological and histopathological parameters of Swiss albino rats infected with *Salmonella typhi*. The results of the study show that, the stem bark extract of *Bridelia ferruginea* administered at the dosages used and for the duration of the experiment suppress the hemopoietic system.

The relative organ weight was observed in the experimental rats which is an important indicator of physiological and pathological status in animals. It is also instrumental when diagnosing whether the organs were exposed to injury or not. The weights of liver and kidney in all groups under observation slightly increases i.e (treated with ciprofloxacin and ethanol extract) when compared to the control(un-infected/untreated) except for the infected/treated where the weight was significantly reduced. [19] reported that since this is a non- significant increase in organ weights, by extrapolation and implication, the results may be an indication of the low toxicity and relative safety of the extract. Also, the increment in the relative weight of the kidney and liver reported in this study might be due to high presence of cardiac glycoside in the extract of *B. ferruginea*. Hence, this study agreed that its use should be controlled.

In this present study, PCV and Hb of the rats showed a highly significant decrease at all doses

when compared to the control (un-infected/untreated). But at 5000 mg/kg concentration of the ethanol extract, there was a significant increase which is closer to control (un-infected/untreated) corroborated with work [4] who reported an increase in the PCV and Hb of rats treated with the extract of unripe *A. muricata*. However, the result differed from the findings of [20] who reported a non-significant difference in the PCV levels in the Wistar albino rats administered with ethanol leaf extract of *Petroselinum crispum*. [21] stated that packed cell volume (PCV) is involved in the transport of oxygen and absorbed nutrients and so the increment shows better transportation and thus

results in an increased primary and secondary polycythemia while haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body function as well as transport carbon dioxide out of the body of animals. Therefore, the increase in PCV and Hb in this study clearly indicates that the extract of *B. ferruginea* has a stimulatory property which ultimately results in increased blood volume. In addition, the increase in haemoglobin level observed implied an enhancement of the oxygen carrying capacity of the blood in the rats given the stem bark extract of *B. ferruginea*.

Table 1. Effect of treatment on the relative weight of the organs

Groups	Relative organ weight(g/100 g)	
	Liver	Kidney
Un-infected	4.03 ^a ± 0.04	2.00 ^a ± 0.12
Infected/ treated Ciprofloxacin	4.05 ^a ± 0.06	2.02 ^b ± 0.03
Infected/untreated	1.67 ^a ±0.06	1.10 ^{bc} ± 0.02
50 mg/kg ethanol extracts	4.06 ^a ±0.07	2.03 ^a ±0.12
100 mg/kg ethanol extracts	4.05 ^a ±0.05	2.08 ^a ±0.14
300 mg/kg ethanol extracts	4.08 ^a ± 0.06	2.07 ^a ±0.13
1000 mg/kg ethanol extracts	4.08 ^a ±0.03	2.05 ^a ±0.14
2600 mg/kg ethanol extracts	4.07 ^a ±0.05	2.09 ^a ± 0.15
5000 mg/kg ethanol extracts	4.05 ^a ± 0.08	2.09 ^a ±0.14

Data are presented as Mean± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P< 0.05)

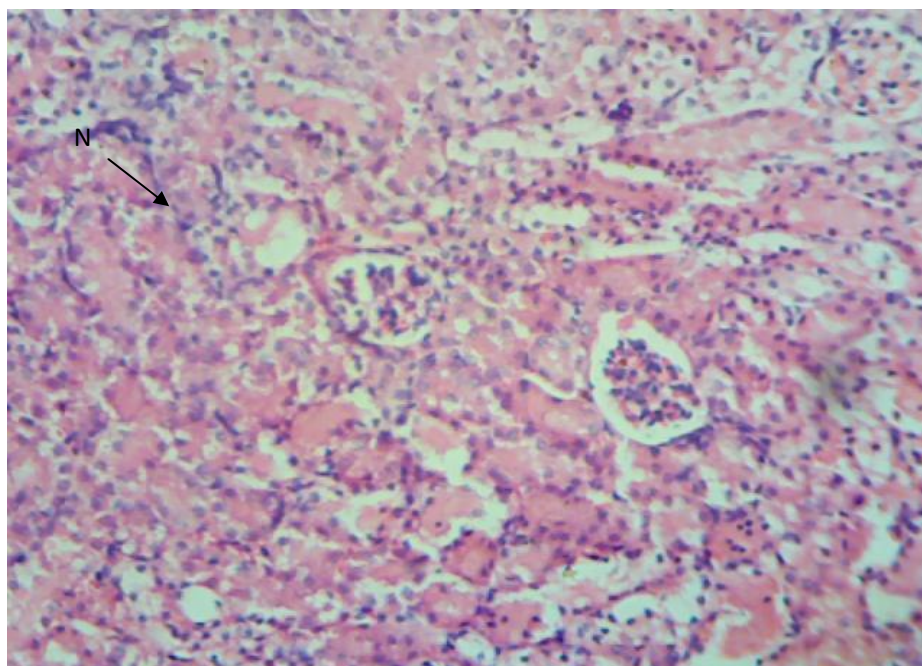


Plate 1a. Photomicrograph of a Section kidney of un-infected rats showing (a) kidney with almost near normal architecture. H&E X400

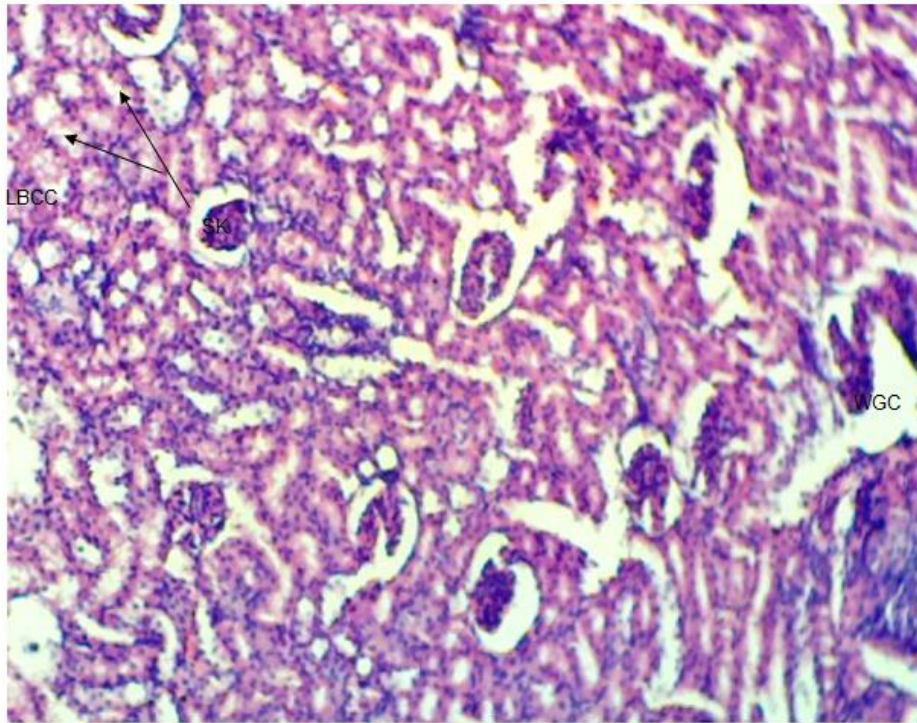


Plate 2a. Photomicrograph of a Section kidney of rats infected/treated with 50mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) normal architecture of nephron with intact wide glomeruli room (b) loss of bowman capsule (c) several karyolysis. H&E X400

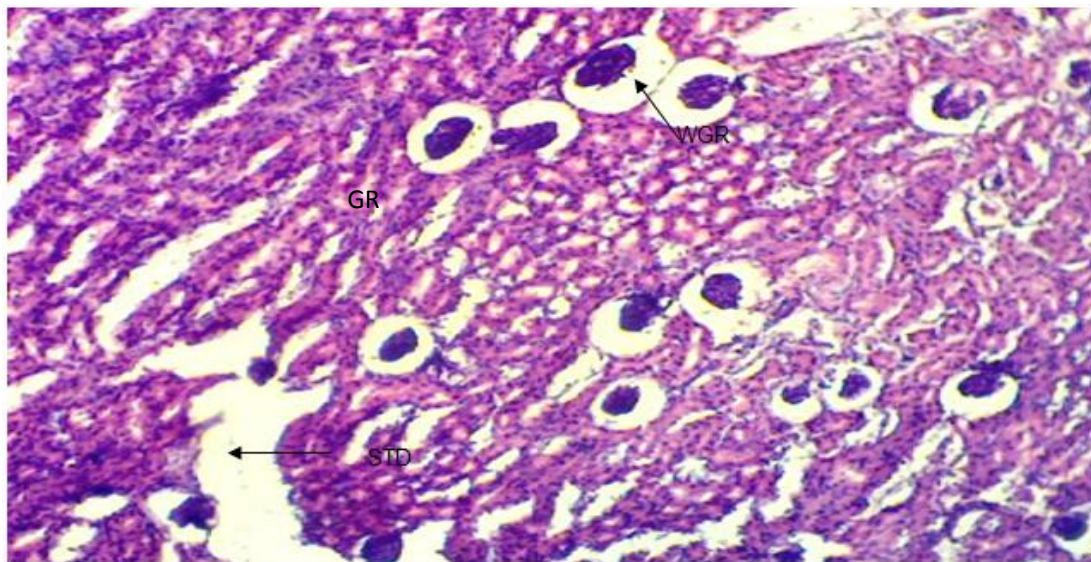


Plate 3a. Photomicrograph of a Section kidney of rats infected/treated with 100mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) normal architecture of nephron with intact wide glomeruli room (b) loss of bowman capsule (c) severe tubular drainage. H&E X400

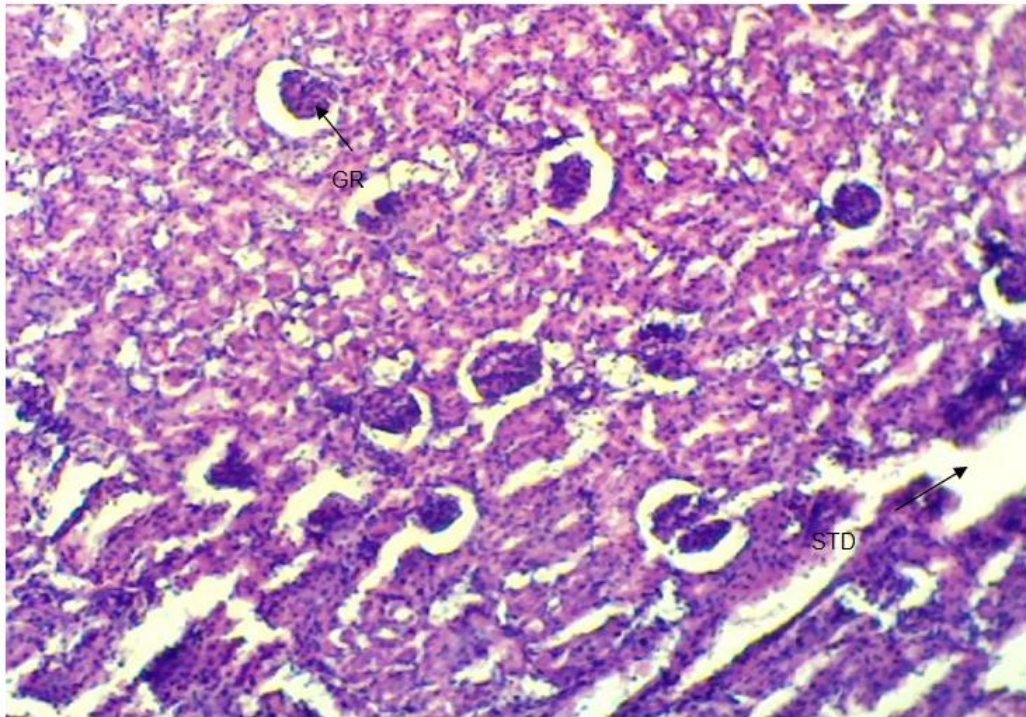


Plate 4a. Photomicrograph of a Section kidney of rats infected/treated with 300 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) normal architecture of nephron with well-formed glomeruli room (b) severe tubular drainage. H&E X400

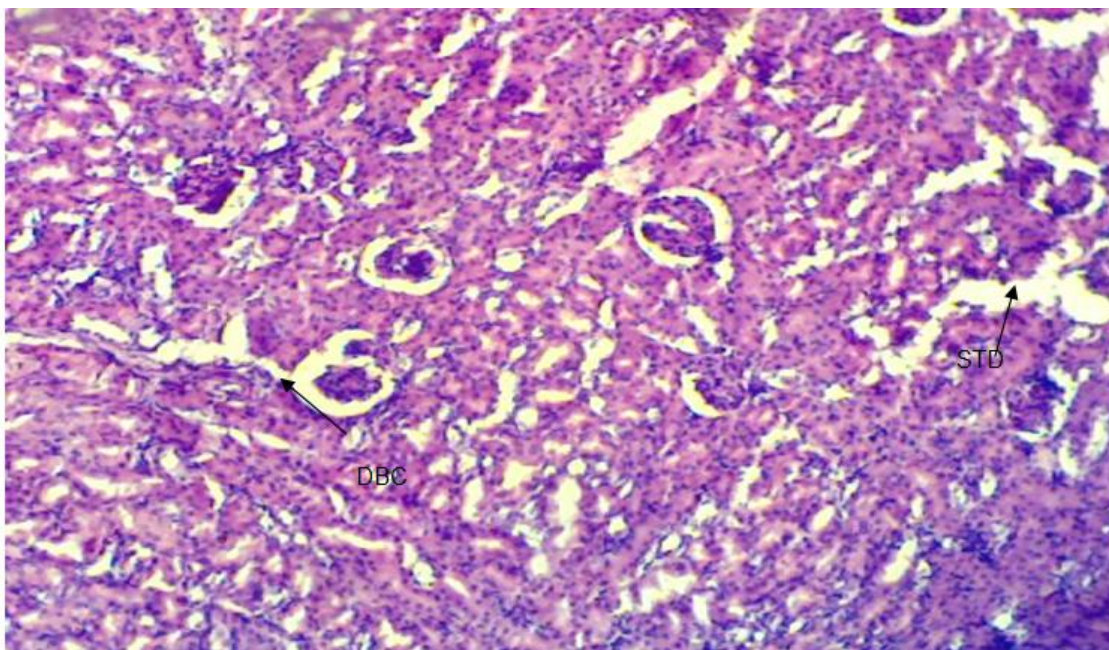


Plate 5a. Photomicrograph of a Section kidney of rats infected/treated with 1000 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) normal architecture of nephron with intact glomeruli room (b) depletion of bowman capsule (b) slight tubular drainage. H&E X400

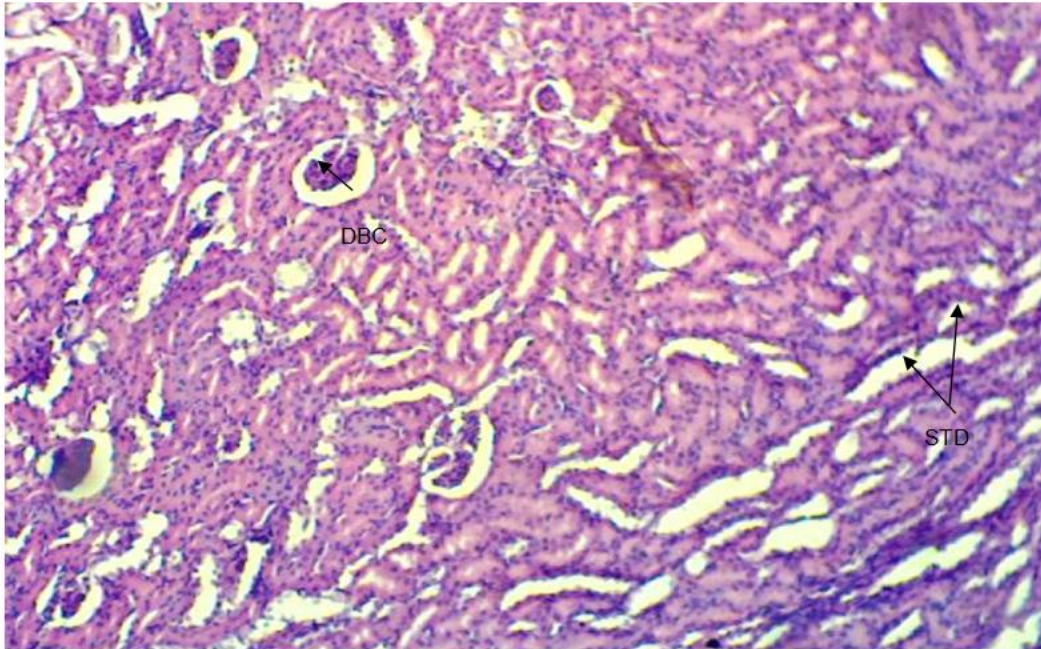


Plate 6a. Photomicrograph of a Section kidney of rats infected/treated with 2600 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) normal architecture of nephron with intact glomeruli room (b) depletion of bowman capsule (b) slight tubular drainage. H&E X400

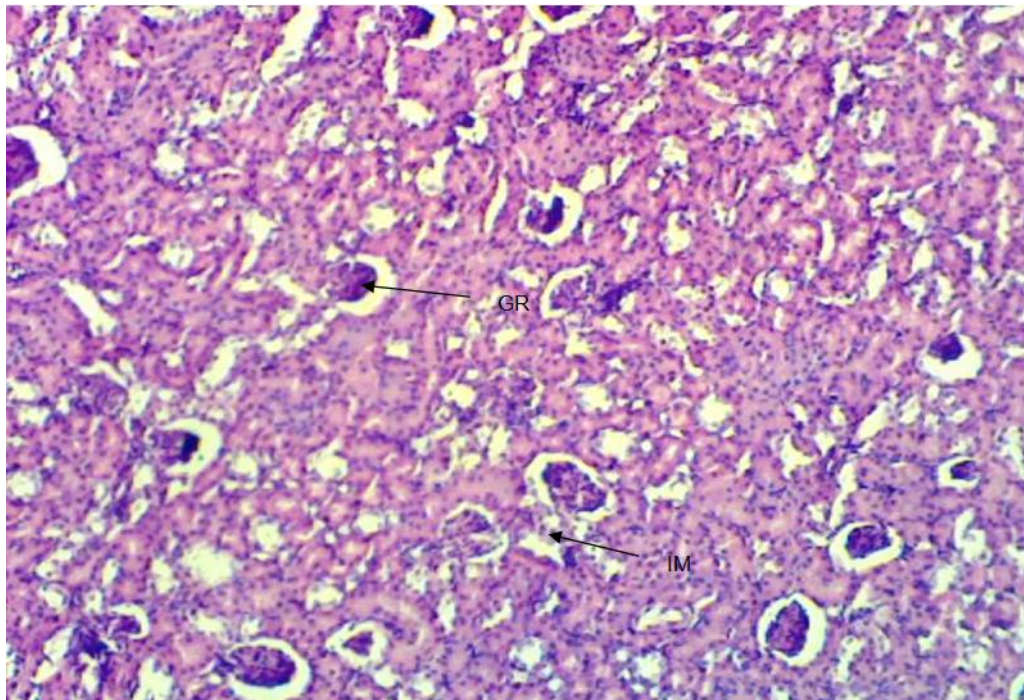


Plate 7a. Photomicrograph of a Section kidney of rats infected/treated with 2600 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) normal architecture of nephron with intact glomeruli room (b) possible deposition of immunological material in the glomeruli basement. H&E X400

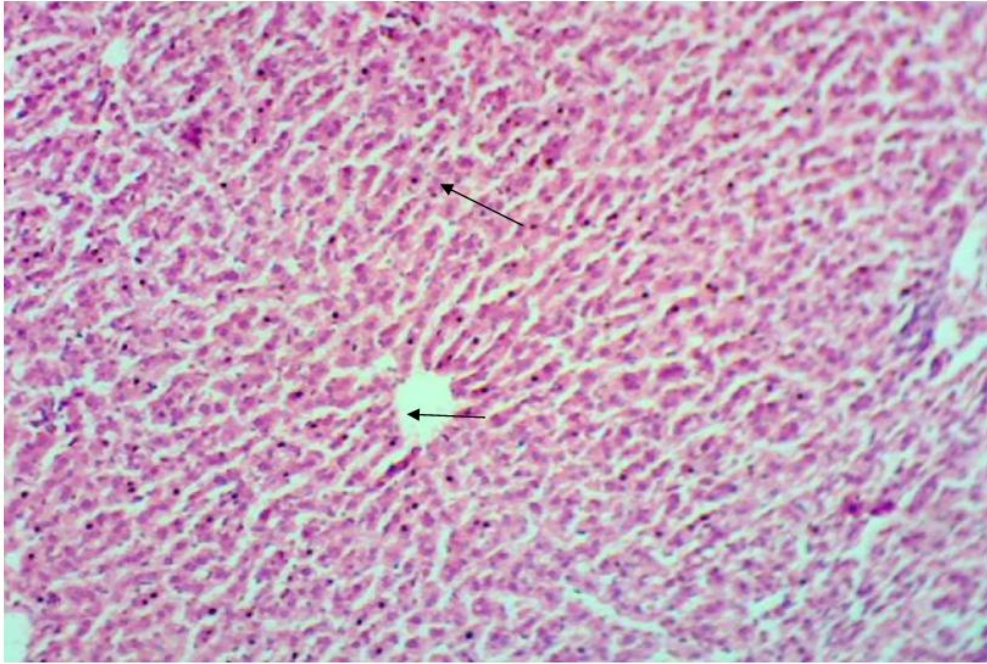


Plate 1b. Photomicrograph of a Section liver of rats control (un-infected) showing (a) Normal architecture of hepatocytes with well organised sinusoids (b) central vein. H&E X400

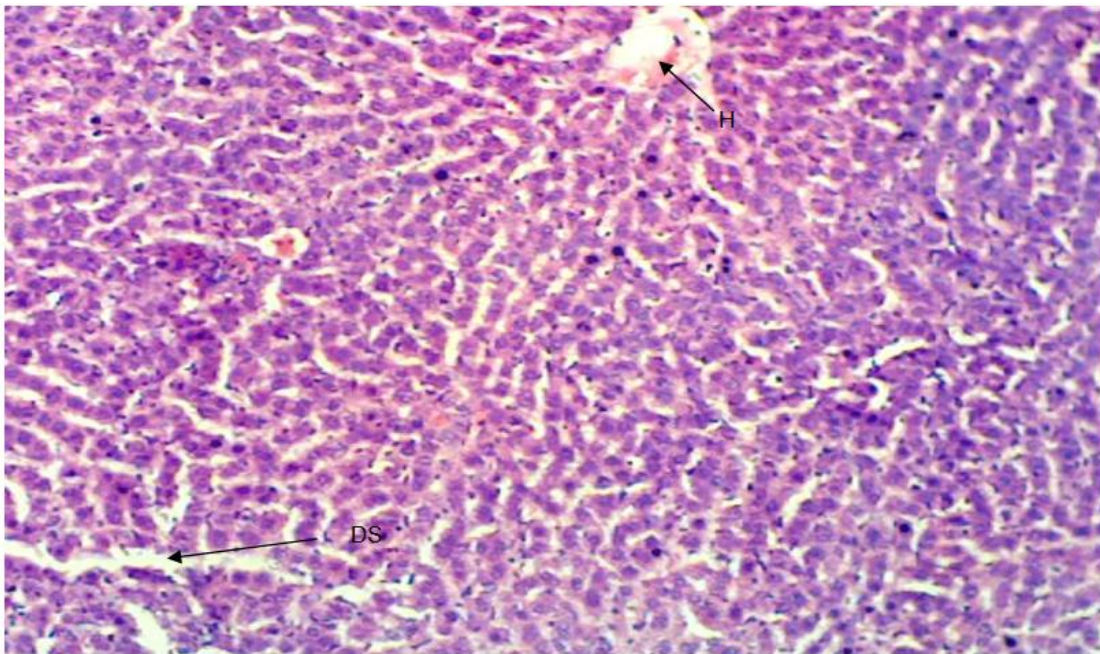


Plate 2b. Photomicrograph of a Section liver of rats infected/treated with 50 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) Dilation of sinusoid and (b) hepatic sinusoids separating the hepatic cord in place lined by kupffer cell (c) possible haemorrhage. H&E X400

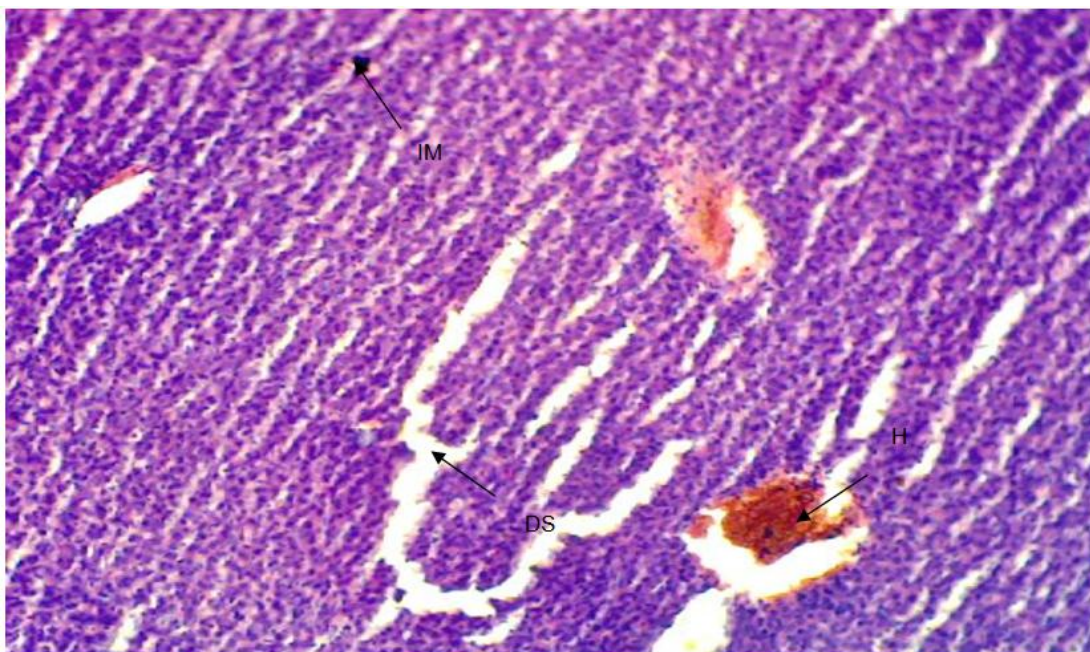


Plate 3b. Photomicrograph of a Section liver of rats infected/treated with 100 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) Dilation of sinusoid and (b) possible deposition of immunological material in the hepatocyte (c) haemorrhage. H&E X400

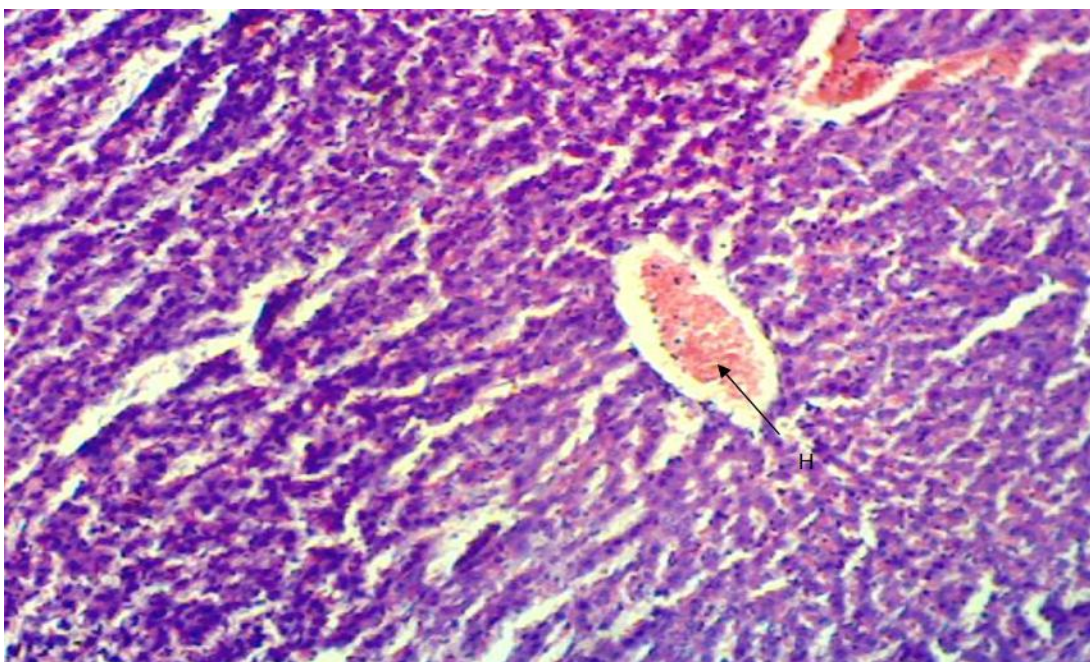


Plate 4b. Photomicrograph of a Section liver of rats infected/treated with 300 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) Dilation of sinusoid and (b) hepatic sinusoids separating the hepatic cord in place lined by kupffer cell (c) possible haemorrhage. H&E X400

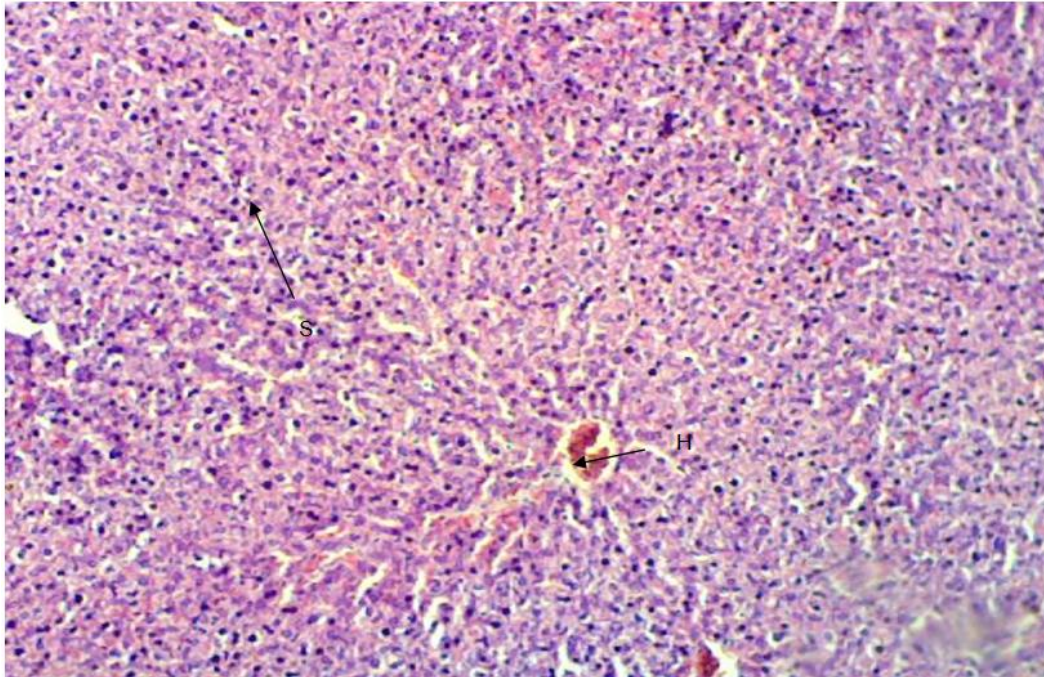


Plate 5b. Photomicrograph of a Section liver of rats infected/treated with 1000 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) the hepatic sinusoids depletion separating the hepatic cord in place lined by kupffer cells (b) possible haemorrhage. H&E X400

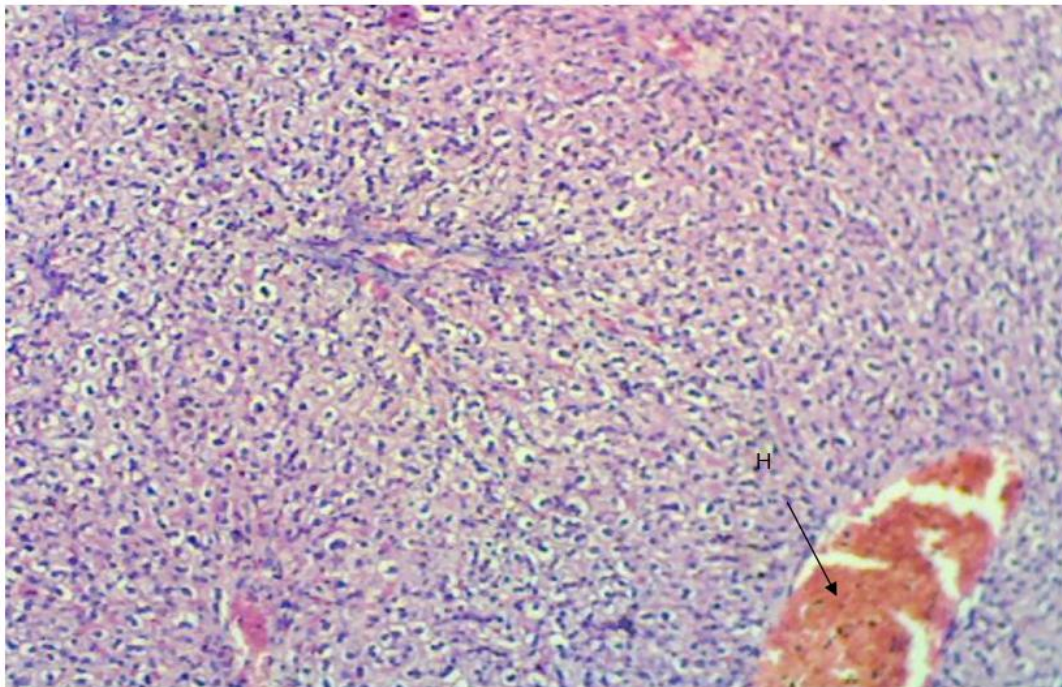


Plate 6b. Photomicrograph of a Section liver of rats infected/treated with 2600 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) Dilation of sinusoid and (b) hepatic sinusoids separating the hepatic cord in place lined by kupffer cell (c) possible haemorrhage. H&E X400

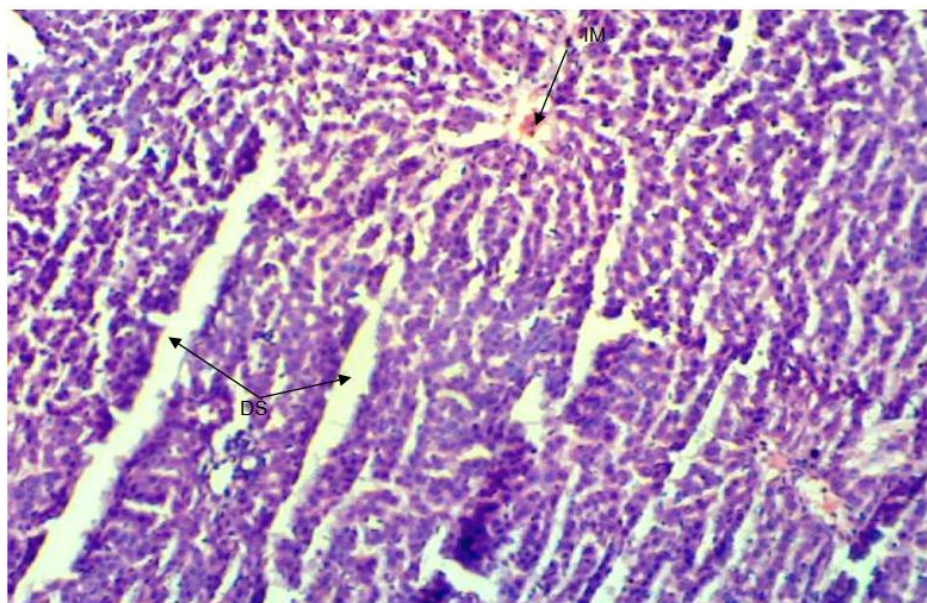


Plate 7b. Photomicrograph of a Section liver of rats infected/treated with 5000 mg/kg ethanol extract of *B. ferruginea* stem bark showing (a) dilation of sinusoids depletion separating the hepatic cord in place lined by kupffer cells (b) possible immunological materials. H&E X400

The insignificant decrease observed in the RBC_s of rats administered with various concentrations of the ethanol extract of *B. ferruginea* after treatment in this study corroborated [22] who reported that the aqueous flower extract of *Hibiscus sabdariffa* has a non-significant decrease in the level of RBC of rats at 400 mg/ml. Red blood cells (erythrocytes) serves as a carrier of hemoglobin and it is involved in the transport of oxygen and carbon dioxide returned to the lungs as stated by [23]. The non-significant decrease experienced in this study, might imply a reduction in the level of oxygen that would be carried to the tissue as well as the level of carbon dioxide returned to the lungs.

The significant ($P < 0.05$) increase in neutrophil count observed across all groups in the in this study is similar to the findings of [4] who reported a significant increase in all groups of rats treated with extract of unripe *A. muricata* fruits and [15] who in his own findings reported a significant increase in all groups of rats treated with the leaf extract of *O. gratissimum*. As neutrophils are part of white blood cell and its differentials, thus its increase observed was been justified as it responds primarily to the infection caused by the bacteria as it is capable of generating antibodies the process of phagocytosis and have a high degree of resistance to diseases [24] and enhances adaptability to local environment and disease prevalent conditions [23].

The WBC of the group of rats infected/treated with various concentration and infected/treated with ciprofloxacin significantly reduced ($P < 0.05$) after treatment. This result agreed with the [4] who reported a decrease in the WBC of Swiss albino rats treated with ethanol unripe fruits of *A. muricata*. However, this result differed from the findings of [25] who reported an increase in the level of WBC in rats given the extract of *Voacanga africana*. [26] stated granulocytes regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC. Therefore, the decrease observed may imply that the ethanol stem bark extract of *Bridelia ferruginea* can cause immune suppression and also an impairment in the ability of the ethanol extract to stimulate the production of WBC_s according to [19]. However, the significant increase ($P < 0.05$) in the group infected/un-treated with *S. typhi* was due to the fact that the rats were able to produced WBC which are capable of generating antibiotics in the process of phagocytosis and have high degree of resistance to diseases [23]. The lymphocytes of the group of rats infected/treated with ethanol extract in various concentrations were significantly increased compared to the control group; this imply challenge on the immune system by the plant extract at various concentrations. According to [27], lymphocytes are the main effect or cells of the immune system and the observed increase in

the test groups except in the infected/treated with ciprofloxacin when compared to the control(un-infected) in this study may specifically be ascribed to the ability of the extract to stimulate these differentials to promote phagocytosis (cellular ingestion of offending agents). High differentials (lymphocytes and neutrophils) counts can be the result of many factors that include bacterial infection, acute inflammation, stress response effect from drugs and splenectomy, among others [28].

Also, the insignificant change observed in the level of eosinophil and monocyte in this study agreed with the findings of [4] who reported a non- significant difference in the eosinophil levels in the Swiss albino rats administered the ethanol unripe extract of *A. muricata* fruits. Eosinophils are important in the defense against protozoan and helminth parasites mainly by releasing cationic peptides and reactive oxygen immediately into the extracellular fluid as reported by [26]. Hence, its non- significant change in infection involving bacteria as obtained in this study.

Histopathological examination was carried out on the liver and kidney of the experimental rats at the end of the administration of the extract and it is indicated on plates (1A-7A and 1B-7B). Normal architecture of nephron with intact wide glomeruli

room (WGR),loss of bowman capsule, several karyolysis (SK), severe tubular drainage, deposition of immunological material(IM) in the glomeruli basement observed in the group of rats infected/treated suggests that the extract has damaging effects on the kidney architecture when compare to the control (un-infected) at various concentrations of the ethanol extract. This result observed suggests that the ethanol extract of *Bridelia ferruginea* stem bark partially improved the architecture of the kidney of nephron despite the damages done. The intact glomeruli room is in agreement with a previous report that the unripe fruits ethanol extract of *A. muricata* and leaf extract of *Moringa oleifera* did not produce an adverse effect on the kidney of experimental rats [4,29]. However, severe damages done is indicative of adverse activity. This result is comparable with the findings of [30] and [4] who reported that standardized methanol extract of *Mitragyna speciosa* and ethanol extract of *A. muricata* revealed some abnormal morphological characteristics in all treated rats. The partial improvement in the kidney structure after treatment may indicate that the period of treatment was not sufficient for good protection/restoration against the adverse effect of infection. It is therefore suggested that a varied period of treatment should be employed in further studies on the plant.

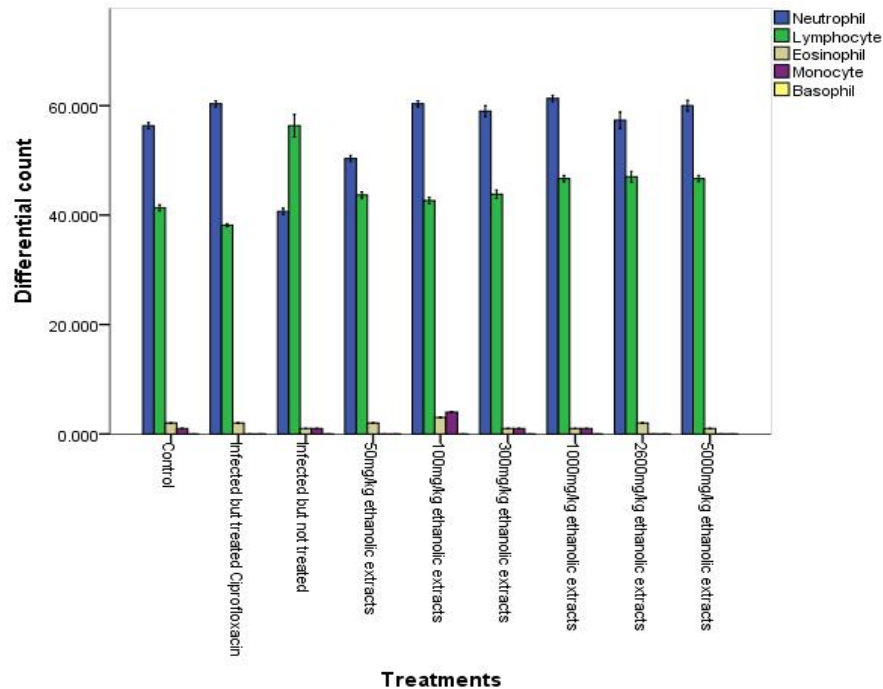


Fig. 1. White blood cells differential counts analysis

Table 2. Haematological analysis after treatment

Treatment	PCV %	HB g/L	WBC (10^9 /g/L)	RBC 10^{12} /g/L	MCV fL	MCH Pg	MCHC g/dl
Un- infected	48.33 ± 0.57 ^f	19.83 ± 0.44 ^f	10.36 ± 0.20 ^b	8.30 ± 0.17 ^e	89.85 ± 0.45 ^b	30.06 ± 0.58 ^b	32.77 ± 0.39 ^{ab}
Infected but treated Ciprofloxacin	44.16 ± 1.25 ^b	14.50 ± 0.28 ^{cd}	8.29 ± 0.15 ^b	6.43 ± 0.29 ^c	90.00 ± 0.57 ^b	30.00 ± 0.57 ^b	32.44 ± 0.44 ^{ab}
Infected/untreated	31.50 ± 0.50 ^a	10.66 ± 0.20 ^a	12.8 ± 0.24 ^f	3.40 ± 0.11 ^a	90.06 ± 0.58 ^b	29.93 ± 0.46 ^b	31.40 ± 0.95 ^a
50 mg/kg ethanol extracts	40.00 ± 0.50 ^e	12.76 ± 0.39 ^b	6.41 ± 0.01 ^a	5.16 ± 0.12 ^b	91.29 ± 0.64 ^b	30.33 ± 0.33 ^b	33.08 ± 0.58 ^b
100 mg/kg ethanol extracts	43.00 ± 1.00 ^c	14.76 ± 0.29 ^c	8.53 ± 0.03 ^c	6.02 ± 0.33 ^c	91.05 ± 0.58 ^b	30.51 ± 0.86 ^b	32.07 ± 0.64 ^a
300 mg/kg ethanol extracts	43.00 ± 1.00 ^c	15.03 ± 0.15 ^{cd}	9.56 ± 0.08 ^{de}	5.76 ± 0.31 ^b	90.23 ± 0.62 ^b	31.09 ± 0.49 ^b	32.00 ± 0.00 ^a
1000 mg/kg ethanol extracts	43.83 ± 0.76 ^c	15.50 ± 0.50 ^d	9.86 ± 0.43 ^{bc}	6.00 ± 0.25 ^{bc}	82.21 ± 0.40 ^a	31.41 ± 0.59 ^b	33.44 ± 0.29 ^b
2600 mg/kg ethanol extracts	44.83 ± 0.76 ^d	17.78 ± 0.14 ^e	9.93 ± 0.48 ^e	6.47 ± 0.38 ^c	91.37 ± 0.31 ^b	27.56 ± 0.29 ^a	33.97 ± 0.02 ^b
5000 mg/kg ethanol Extracts	46.00 ± 1.00 ^e	19.02 ± 0.14 ^f	9.77 ± 0.34 ^e	6.90 ± 0.20 ^{cd}	92.13 ± 0.47 ^b	27.73 ± 0.14 ^a	33.64 ± 0.32 ^b

Data are represented as mean ± S.D. mean values with same superscript are not significantly different. Values are considered significantly different at $P \leq 0.05$

Dilation of sinusoid(DS) and the hepatic sinusoids separating the hepatic cord in place lined by kupffer cells, possible haemorrhage, immunological materials in the hepatocyte and dot necrosis observed in the group of rats infected/treated suggests that the extract has a destructive effect on the liver architecture when compare to the control (un-infected) at various concentrations of the ethanol extract. This result observed suggests that the ethanol extract of *Bridelia ferruginea* stem bark partially damages on the liver. This result is in contrast with the findings of [4] and [21] who reported that the extract of *Annona muricata* and *Tetrapleura tetraptera* did not reveal any gross damages to the tissue of the liver in experimental animals that were treated with various doses of the extract.

5. CONCLUSION

The relative safety of the ethanol stem bark extract of *Bridelia ferruginea* as obtained in the present study is comparable to the report of [31]. However, excessive use must be cautioned and banned from using due to its actions on the liver and kidney. Therefore, it is recommended that further research should be carried out using another solvent apart from ethanol and also various concentrations of the extract lower to the ones used for this study should be considered.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 8523, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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