

Anti-oxidant, Histopathology and LD₅₀ Evaluation of Ethanol Stem Bark Extract of *Enantia chlorantha* in Rodents

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Received date: July 17, 2017; Accepted date: August 01, 2017; Published date: August 06, 2017

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Abstract

Enantia chlorantha is a well-known medicinal plant that is used all over Nigeria. The Ibibios of South-South Nigeria use the stem bark of this plant for the treatment of malaria, stomach upset, toothache and convulsions. Other pharmacological investigations such as anti-inflammatory analgesic, antipyretic and anti-ulcer were also carried out. The extract was evaluated at doses of 32.40 mg/kg, 64.80 mg/kg and 96.20 mg/kg. The LD₅₀ value of 324 mg/kg showed that the extract has moderate toxicity. Saponins, tannins, anthraquinones, cardiac glycosides, terpenes and alkaloids were present on phytochemical screening. The free radical scavenging ability of the extract against 1,1-Diphenyl-2 Picrylhydrazyl (DPPH) was assessed. Results of this study corroborate the use of the extract in febrile illnesses other inflammatory conditions. Subchronic histopathological results of vital organs showed that the extract elicited mild cellular reaction at the doses employed.

Keywords: *Enantia chlorantha*; LD₅₀; Phytochemical; Free radical scavenging; Anti-oxidant

Introduction

Enantia chlorantha is a fair sized ornamental forest tree that can grow to heights of 30 m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits [1]. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, *Moambe Jaune* and *Annika chlorantha*. Locally, the name varies from place to place [2]. The Ibibios of Akwa Ibom call it Uno eto; the Yoruba's call it Osupupa or dokita Igbo. The Edo people refer to it as Erenbav bogo while Ikale and Boki tribes refer to it as Osumolu and Kakerim respectively. The family is Annonaceae and the specie is chlorantha. There are over 40 different species that grow in Nigeria as is seen by the various names that it is called and the varied uses to which it is applied. *Enantia chlorantha* is used to treat a wide variety of conditions using its roots, stem bark, fruit and leaves. In Akwa Ibom it is used to treat malaria fever, typhoid fever, jaundice, dysentery, wounds, infections, high blood pressure and convulsions. It has been used also as anti-viral, anti-candidal and for gastroenteritis [3,4]. A decoction of the stem bark in illicit gin is usually taken to relieve painful and swollen joints, fever, headache and toothache. We aim by this work to use various models to evaluate its anticonvulsive properties and possible mechanism of action.

Materials and Methods

Plant material

The plant *Enantia chlorantha* was collected in January 2012 in Uyo the capital city of Akwa Ibom State, Nigeria. It was identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom state Nigeria. A

voucher specimen (voucher number UUH 018/13) was deposited with the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

Extraction

The plant stem bark was washed and partially air dried for about 2 weeks at ambient temperature (25°C ± 1°C). It was then ground to powder using pestle and mortar. The pulverized sample was divided into two parts. One part was cold macerated in 70% ethanol at room temperature for 72 h and then filtered. The filtrate was dried in a rotary evaporator at 40°C. This extract is referred to as crude. The other part was successively and gradually macerated for 72 h at room temperature in the following solvents: *n*-hexane, chloroform, ethyl acetate, methanol and water to obtain different fractions. The crude extract and the fractions were stored in the freezer at -4°C until required.

Acute toxicity study of the extract

The method of Lorke was used to determine the LD₅₀ of the extract in Swiss albino mice. The extract was administered to three groups of mice containing three mice each at a dose range of 100-1000 mg/kg, (i.p). The animals were observed for physical signs of toxicity and the number deaths in each group within 24 h were recorded. The animals were fasted for 24 h prior to the experiment but allowed water ad libitum. The LD₅₀ was calculated as the geometric mean of the maximum dose producing 0% mortality (A) and the minimum dose producing 100% mortality (B). LD₅₀=√AB.

Phytochemical screening

The phytochemical screening of the extract was done according to the methods of Odebiyi and Sofowora, Harbone, Trease and Evans [5-7]. The following bioactive compounds were screened for their

presence: saponins, tannins and alkaloids. Others were flavanoids, anthraquinones, cardiac glycosides and reducing sugars.

Preparation of reagents

The following reagents were prepared according to standard procedures.

Mayer's reagent: This was constituted as 5 g of potassium iodide in 10 ml of distilled water and 1.35 g of mercuric chloride in 60 ml of distilled water and made up to 100 ml solution with distilled water. The solutions were labeled A and B respectively.

Dragendorff's reagent: A solution of 0.85 g of Bismuth nitrate dissolved in 40 ml of distilled water and 10 ml of acetic acid was made and labeled A. Potassium iodide 8 g was dissolved in 20 ml of water and labeled B, the two solutions were mixed and placed in a dark bottle.

Fehling's solution A: A solution of 0.1 ml of sulphuric acid and 6.93 g of copper (I) sulphate was constituted and made up to 100 ml with distilled water.

Fehling's solution B: Potassium sodium tartrate 35.2 g was mixed with 15.4 g of sodium hydroxide and dissolved in 100 ml of distilled water.

Test for alkaloids

The extract (1 g) was heated with 5 ml of 1% HCl in a small beaker in a boiling water bath. The mixture was cooled and filtered. The filtrate was decanted into two test tubes. One was treated with few drops of Mayer's reagent and the other with Dragendorff's reagent. They were both observed for turbidity or precipitation, which indicates the presence of alkaloids.

A modified form of the thin layer chromatography method described by Farnsworth and Euler was carried out to rule out the presence of non-alkaloidal compounds. 0.5 g of the extract was treated with dilute HCl and heated for 5 min [8]. The solution was allowed to cool and then treated with ammonia until it is distinctly alkaline to litmus paper and was subsequently extracted twice with 10 ml portions of chloroform. The chloroform extracts were combined and concentrated in vacuo and spotted on pre-coated thin layer plates. It was then sprayed with freshly prepared Dragendorff's reagent. An orange or darker coloured spot on the chromatogram indicated a positive reaction which was taken as confirmation of the presence of alkaloids.

Test for saponins

The tests were carried out as follows.

Frothing test: Distilled water (2 ml) was shaken vigorously with 0.5 g of the extract and observed for frothing. The test tube was kept for 15 min and warmed carefully and observed again. Frothing which persisted was taken as preliminary evidence of the presence of saponins.

Test for sugar portion of saponins

Half gram of the extract was shaken with distilled water in a test tube. The mixture was made alkaline by the addition of 2 ml of 5% sodium bicarbonate solution. 2 ml of Fehling's solution A and B was

added and boiled. A brown precipitate indicates a positive result for simple sugar.

Half gram of the extract was shaken with distilled water in a test tube. Fehling's solution A and B were added to the mixture and boiled. A brick red precipitate indicates a positive result.

Blood hemolysis test

To three test tubes were added a mixture of 2 ml of isotonic extract and 5 ml of diluted blood. To another set of three test tubes were added 2 ml of saline and 5 ml of blood. These were left for 6 h and the contents centrifuged. A red colour in the supernatant is taken as evidence of hemolysis.

Test for flavonoids

Half gram was dissolved in 2 ml of methanol by heating in a test tube. Some pieces of magnesium metal and a few drops of concentrated hydrochloric acid was added (Schibatas reaction). A crimson coloured precipitate indicates a positive result.

Half gram of the extract was dissolved in 2 ml of sodium hydroxide in a test tube. A yellow solution that becomes colourless with the addition hydrochloric acid indicates the presence of flavonoids.

Test for tannins

Two grams of the extract was shaken with distilled water and filtered. 2 ml of dilute ferric chloride solution was added to the filtrate. A blue-black precipitate indicates the presence of tannins.

Test for anthraquinones

Bortragers test (Test for free hydroxyl anthraquinones): Five grams of the extract was shaken with 10 ml of benzene and filtered, 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the lower phase was observed. A violet coloured lower phase will indicate the presence of anthraquinones.

Test for combined anthraquinones: Five grams of the extract was boiled with 10% sulphuric acid and filtered while hot. The filtrate was shaken with benzene. The benzene layer was decanted and half its own volume of 10% ammonia solution was added. A violet colour in the lower phase will indicate the presence of combined anthraquinones.

Test for terpenes: The extract (0.5 g) was dissolved in 1 ml of acetic anhydride and then in chloroform. The solution was under laid with 2 ml of concentrated sulphuric acid (Lieberman-Burhards reaction). A reddish brown colored ring at the zone of contact with the supernatant and the color changing from blue to green indicates the presence of sterol and triterpenes.

Test for glycosides

Test for reducing sugar: The extract (0.1 g) was placed in a beaker, 3 ml of 10% sulphuric acid and 15 ml of distilled water were added. This mixture was then boiled and alkalized by adding 10 ml of potassium hydroxide solution. 10 ml of Fehling's solution was added and the mixture was boiled for 3 min. The presence of a reducing sugar of the glycoside was indicated by a brick red precipitate.

Test for cyanogenic glycoside: The extract (0.1 g) was placed in three test tubes labelled A-C. Distilled water was added to test tubes A and sodium picrate paper was suspended in each of the three test tubes.

The test tubes were stoppered immediately. Test tube B was placed in boiling water for 5 min while test tubes A and C were kept at room temperature for 45 min. A change in colour of the sodium picrate paper indicates the presence of cyanogenic glycosides.

Test for cardiac glycosides

Salkowski test: The plant extract (0.5 gm) was dissolved in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added to form a lower layer. A reddish-brown ring observed at the interphase of the two liquids indicated the presence of a steroidal ring which is characteristic of cardiac glycoside.

Lieberman's test: The extract (0.5 gm) was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was under laid with 2 ml of concentrated sulphuric acid. A reddish brown coloured ring at the zone of contact with the supernatant and the colour changing from blue to green indicated the presence of triterpenes and sterols. Steroidal rings characterize the aglycones of cardiac glycosides.

Keller Killani's test: The extract (0.5 gm) was dissolved in 2 ml of glacial acid containing one drop of ferric chloride solution. The mixture was under laid with 2 ml of concentrated sulphuric acid. A brown ring which darkened on standing will indicate the presence of deoxy sugar in cardiac glycosides.

Animal stock

Swiss albino mice weighing 25-30 g of both sexes were used for the experiments. They were obtained from the Department of Pharmacology Animal House in the University of Uyo, Uyo. The animals were housed in standard plastic cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. They were fed with pelleted feeds (Bendel Feeds) and allowed water ad libitum.

Anti-oxidant Activity of the Extract

Effect of extract on free radical scavenging activity using 1, 1-Diphenyl-2 Picrylhydrazyl (DPPH)

The method of Ursini, Maiorino, Morazzoni, Roveri and Pifferi was used to find the free radical scavenging ability of the extract against 1, 1-Diphenyl-2 Picrylhydrazyl (DPPH) [9]. A 1 ml aliquot (0.05 g of the extract was dissolved in 20 ml of methanol) was mixed with 1 ml, 0.4 mM methanol solution consisting DPPH radicals. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nM.

Evaluation of sub-chronic toxicity of extract in rats

Adult albino rats weighing 120-160 g of both sexes were used. They were randomly divided into four groups of six animals per group.

Group 1 received 10 ml/kg, (p.o.) of distilled water. Groups 2-4 were given the extract (32.40, 64.80 and 96.20 mg/kg, i.p.) respectively. The extract was given at 9 am on alternate days for 28 days while toxic manifestations and mortality were being recorded. Weight changes were noted once a week. On day 29, the animals were fasted overnight and light chloroform anesthesia was administered and blood samples collected for hematological and biochemical investigations. Hemoglobin, hematocrit, red blood cell count, white blood cell count, mean corpuscular Hemoglobin concentration and mean corpuscular volume were determined using automatic counter system (K 21, Tokyo, Japan).

The organs were harvested and submitted to the Department of Anatomy in Basic Medical Sciences for the slide preparations while the interpretations of the slides were done in the Department of Histopathology in the University of Uyo Teaching Hospital.

Results

Phytochemical screening

The result of phytochemical screening of the extract is shown in Table 1. Phytochemical screening of the extract revealed the presence of the following secondary metabolites: saponins, alkaloids, terpenes, cardiac glycosides, anthraquinones and flavonoids.

Determination of median Lethal Dose (LD₅₀)

The extract (100-1000 mg/kg) produced physical signs of toxicity such as writhing, gasping, tachycardia, decrease in motor activity, decreased respiratory rate and death. These effects were dose-dependent. All the mice treated with 350 mg/kg and above did not survive. The intraperitoneal LD₅₀ of the extract in mice was calculated according to the method of Lorke to be 324.0 mg/kg. The LD₅₀ was calculated as geometric mean of the maximum dose producing 0% mortality (A) and the minimum dose producing 100% mortality (B). $LD_{50} = \sqrt{AB}$.

In-vitro anti-oxidant activity

Effect of extract on 1,2-diphenyl-2-picrylhydrazyl (DPPH): The DPPH radical scavenging activity of extract (5-50 µg/ml) was detected and compared with ascorbic acid. The percentage inhibition (% inhibition) at various concentrations (5-50 µg/ml) of the extract as well as standard ascorbic acid (5-50 µg/ml) were calculated and plotted in Figures 1 and 2. The IC₅₀ values were calculated from the graph and were found to be 45.60 and 40.76 µg/ml for the extract and ascorbic acid respectively.

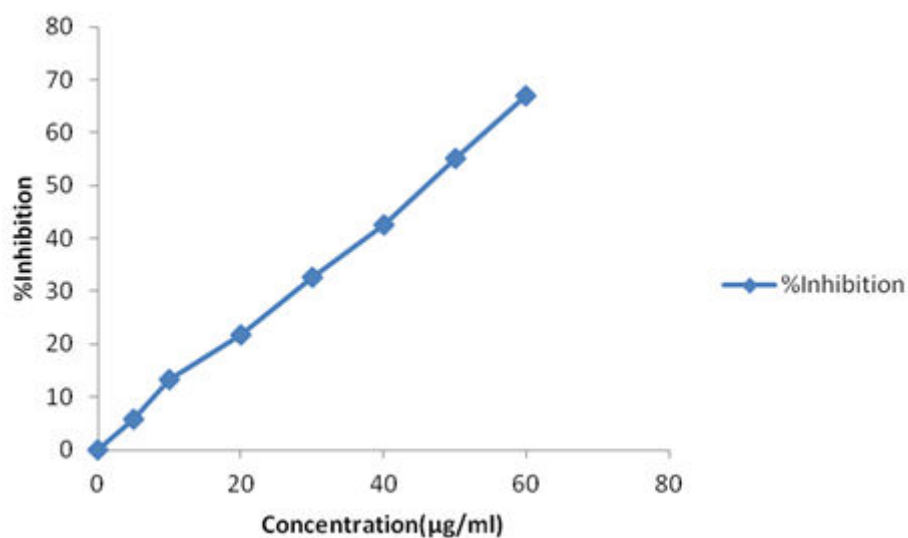


Figure 1: *In-vitro* anti-oxidant activity of ethanol stem bark extract of *Enantia chlorantha*.

Constituents tested	Remarks
Alkaloids	++
Anthraquinones	+
Cardiac glycosides	+
Flavonoids	++
Phlobotannins	+
Saponins	+
Simple sugars	++
Protoberberine alkaloids	+
Palmitine chloride	+++
Jatrorhizine chloride	++
Tannins	++

Table 1: Result of phytochemical screening of extract. (++)-Positive, (+)-Slightly positive, (+++)-Strongly positive.

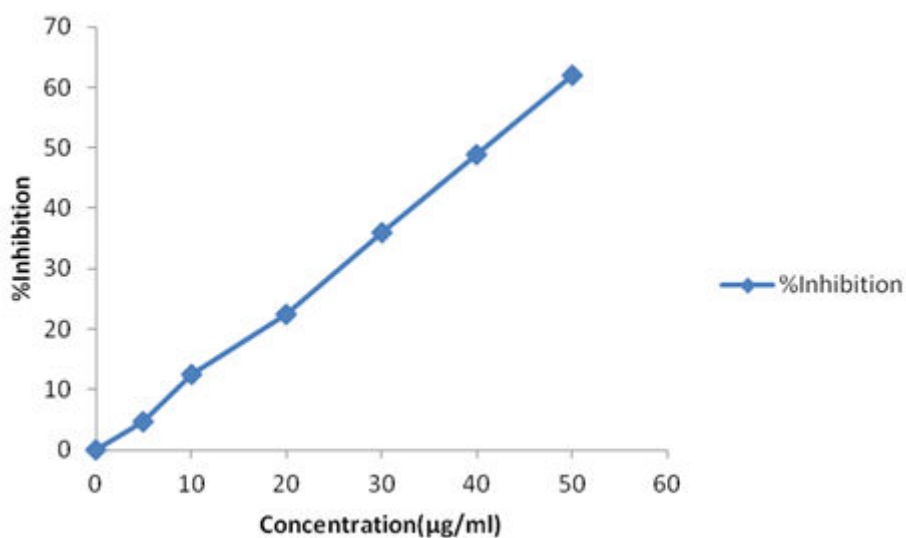


Figure 2: *In-vitro* anti-oxidant activity of Ascorbic acid.

Effect of extract on organ histology in rats

The effects of sub-chronic administration of extract on organ histology in rats are shown in Plates 1-24.

The extract (32.20-96.20 mg/kg) produced mild to moderate cellular proliferation and hyperplasia in most of the organs studied.

Histopathological findings

Control group: Heart.

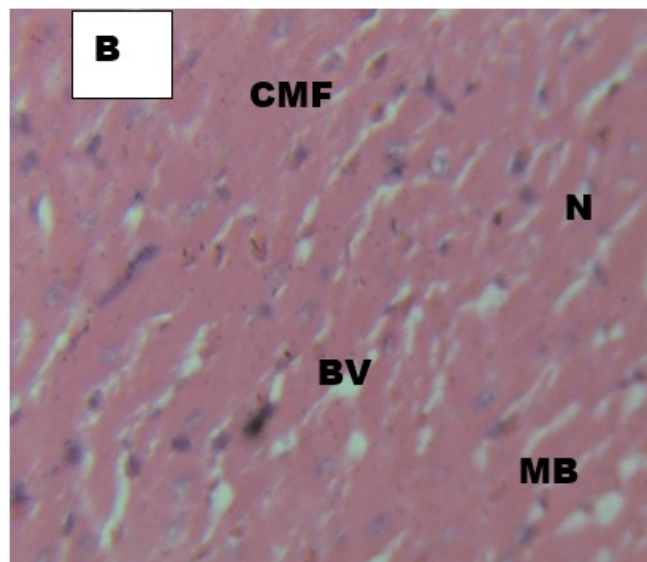
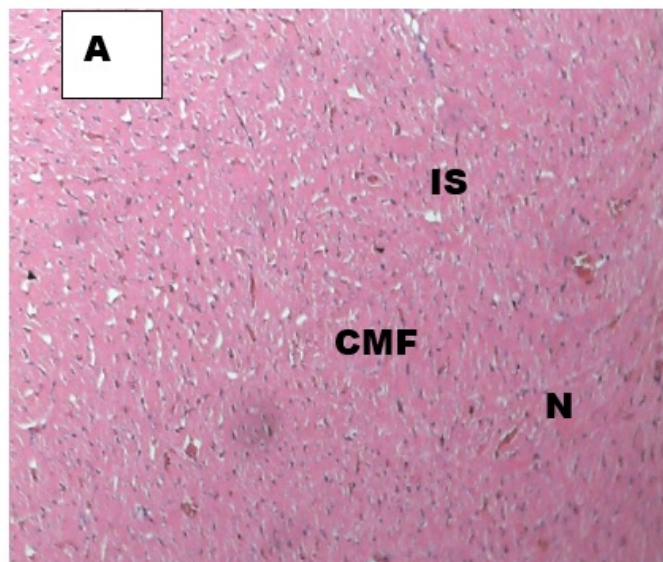


Plate 1: Photomicrographs of normal Heart (control) at magnification A (X100) and B (X400) stained with H & E technique. Key: N-Nucleus, CMF-Cardiac Muscle Fibre, IS-Interstitium, BV-Blood Vessels and MB-Muscle Bundle.

Group-2

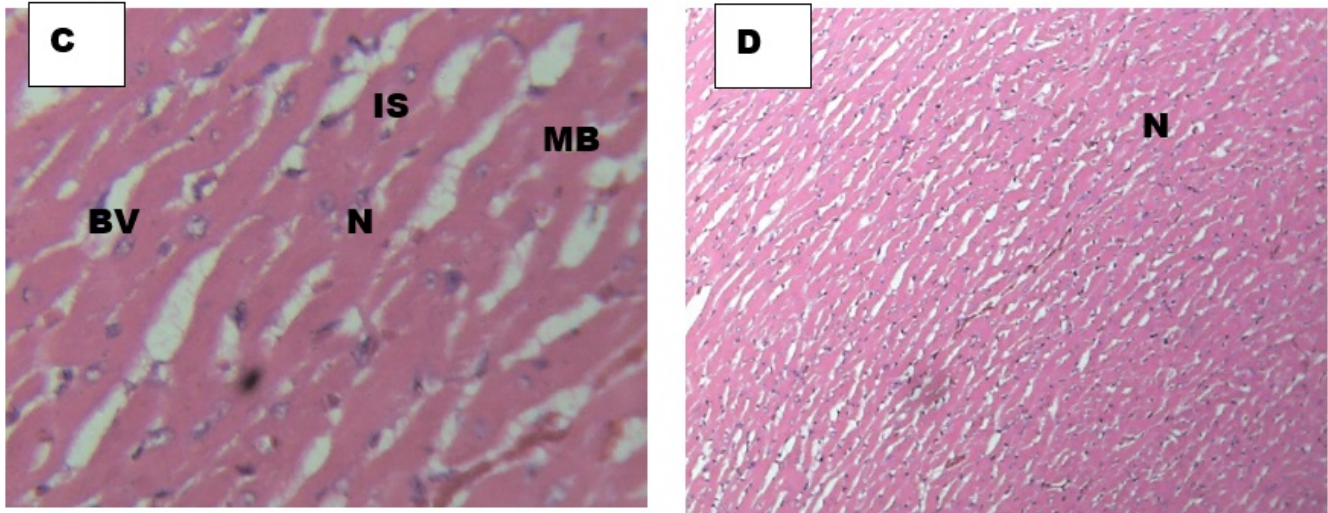


Plate 2: Photomicrographs of Heart treated with 32.40 mg/kg of extract at magnification C (X400) and D (X100) stained with H & E technique. Key: N- Nucleus, CMF- Cardiac Muscle Fibre, IS- Interstitium, BV- Blood Vessels and MB- Muscle Bundle.

Group-3

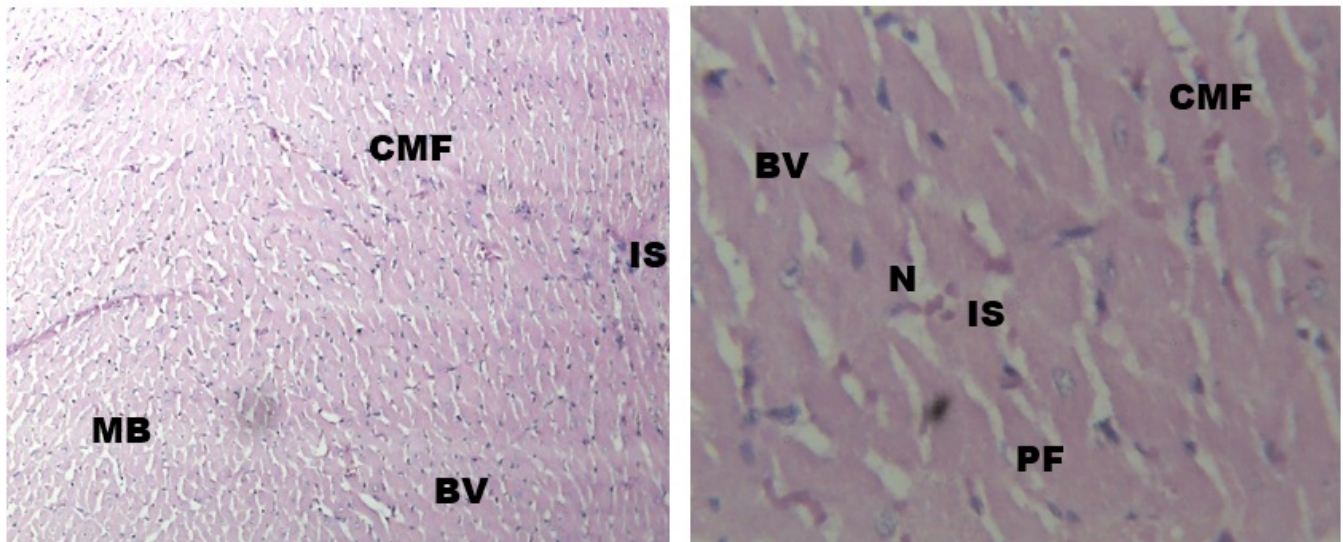


Plate 3: Photomicrographs of Heart treated with 64.80 mg/kg of extract at magnification E (X100) and F (X400) stained with H & E technique. Key: N- Nucleus, CMF- Cardiac Muscle Fibre, IS- Interstitium, BV- Blood Vessels, PF- Purkinje Fibres, MB- Muscle Bundle and I- Inflammation.

Group-4

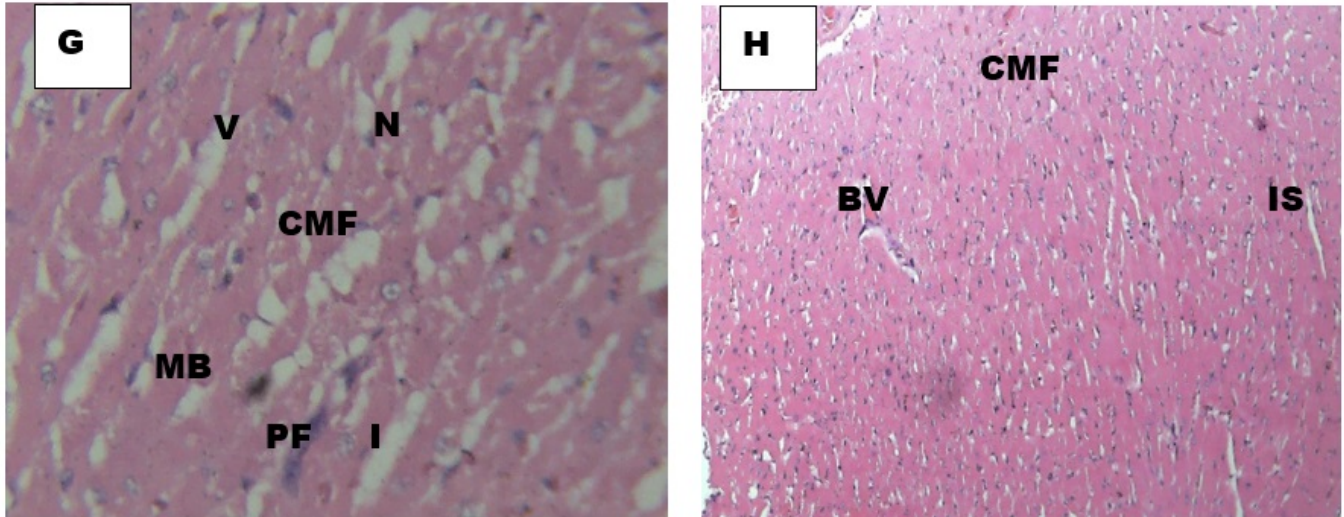


Plate 4: Photomicrographs of Heart treated with 96.20 mg/kg of extract at magnification G (X400) and H (X100) stained with H & E technique. Key: N- Nucleus, CMF- Cardiac Muscle Fibre, IS- Interstitium, BV- Blood Vessels, PF- Purkinje Fibres, MB- Muscle Bundle, V- Vacuolization and I- Inflammation.

Control Group: Liver

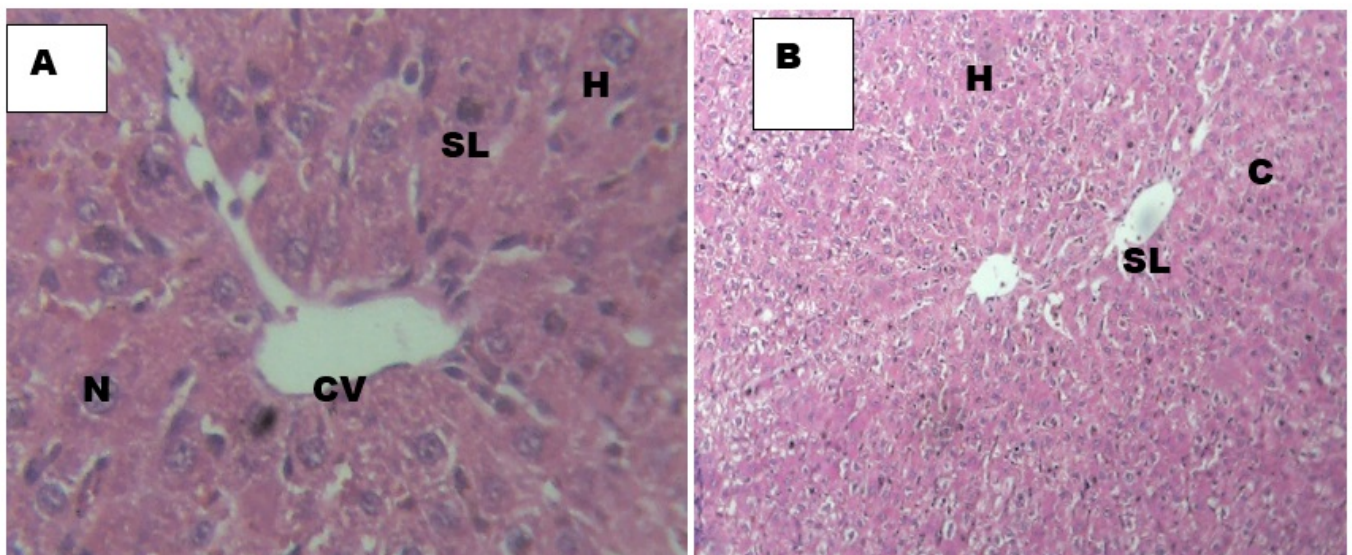


Plate 5: Photomicrographs of normal liver (control) at magnification A (X400) and B (X100) stained with H & E technique. Key: CV-Central Vein, Pt-Portal Triad, H-Hepatocytes, HV-Hepatic vein, SL-Sinusoidal Layer and PN-Pyknotic Nucleus.

Group-2

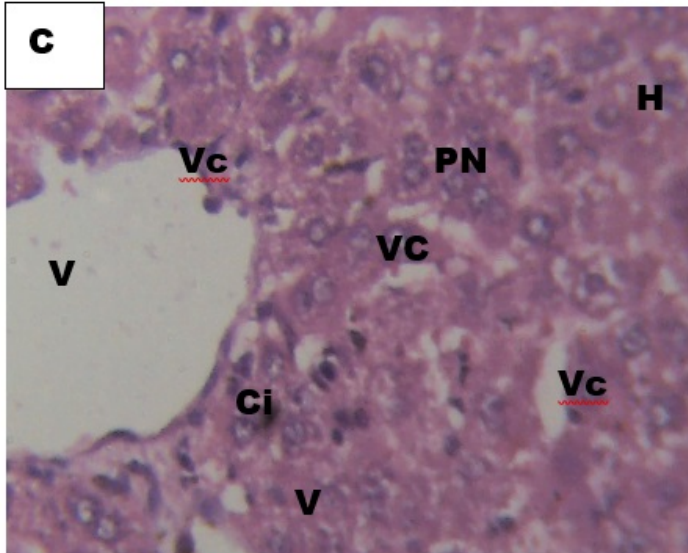


Plate 6: Photomicrographs of Liver treated with 32.40 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: CV-Central Vein, H-Hepatocytes, HV-Hepatic Vein, SL-Sinusoidal Layer, PN- Pyknotic Nucleus, Ci- Cellular infiltration, VC-Vascular congestion and V- Vacuolization.

Group-3

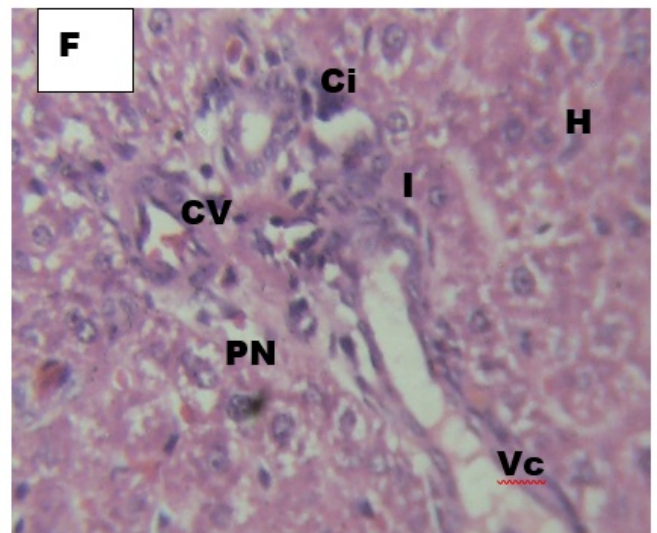
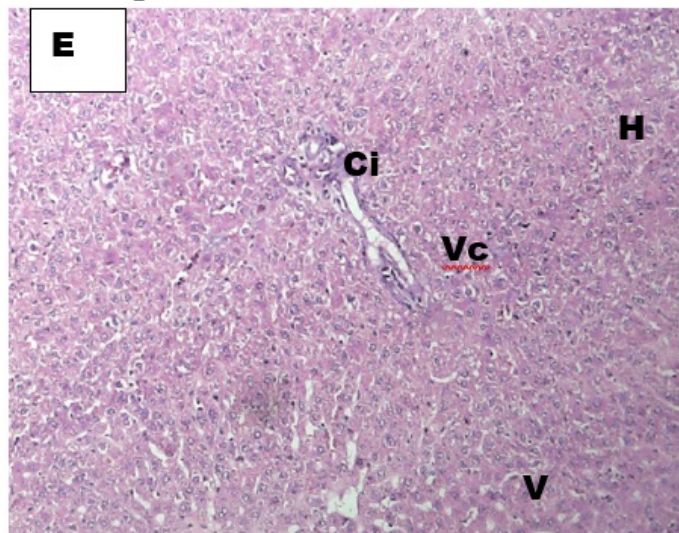


Plate 7: Photomicrographs of Liver treated with 64.80 mg/kg of *Enantia chlorantha* at magnification C (X100) and D (X400) stained with H & E technique. Key: CV-Central Vein, H-Hepatocytes, HV-Hepatic vein, PN- Pyknotic Nucleus, Ci- Cellular infiltration, VC-Vascular congestion, V- Vacuolization and I- Inflammation.

Group-4

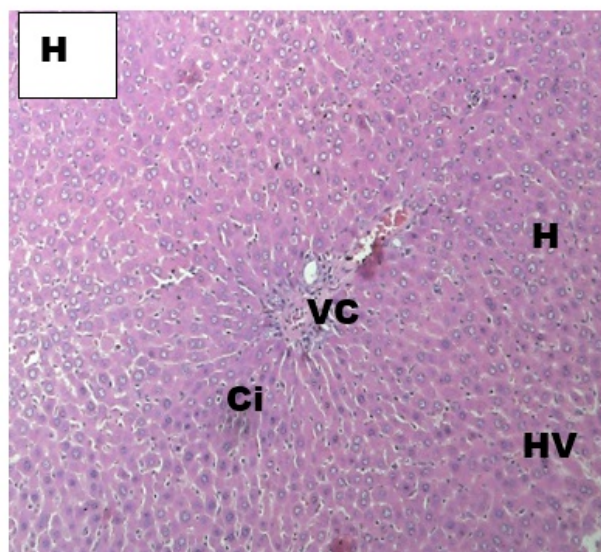
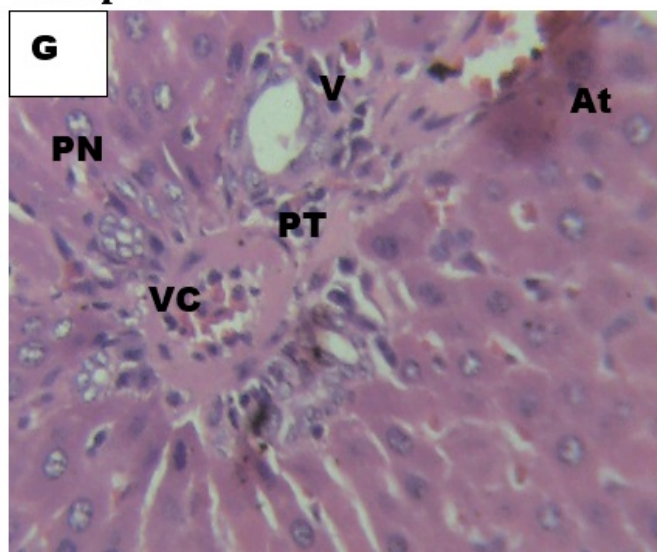


Plate 8: Photomicrographs of Liver treated with 96.20 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: CV-Central Vein, Pt-Portal triad, H-Hepatocytes, HV-Hepatic Vein, VC- Vascular Congestion, PN- Pyknotic Nucleus, CD- Cellular infiltration, V- Vacuolization and At- Artefact.

Control-group: Kidney

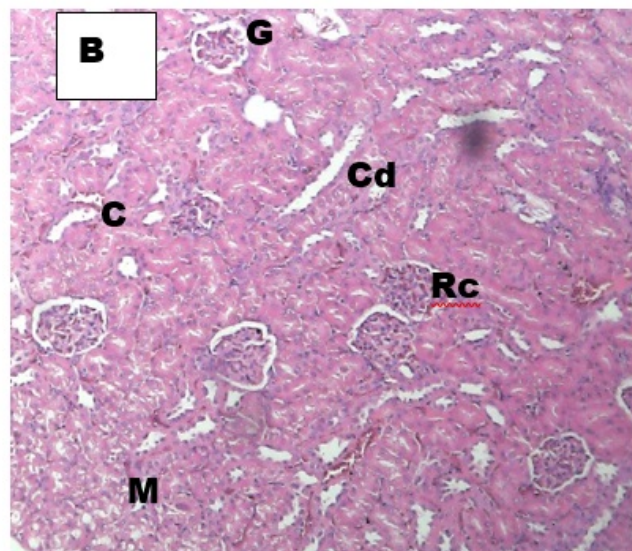


Plate 9: Photomicrographs of Kidney (control) at magnification A (X400) and B (X100) stained with H & E technique. Key: G-Glomerulus, Rc-Renal corpuscle, DCT- Distal Convoluted Tubule, PCT- Proximal Convoluted Tubules Cd-Collecting Ducts.

Group-2

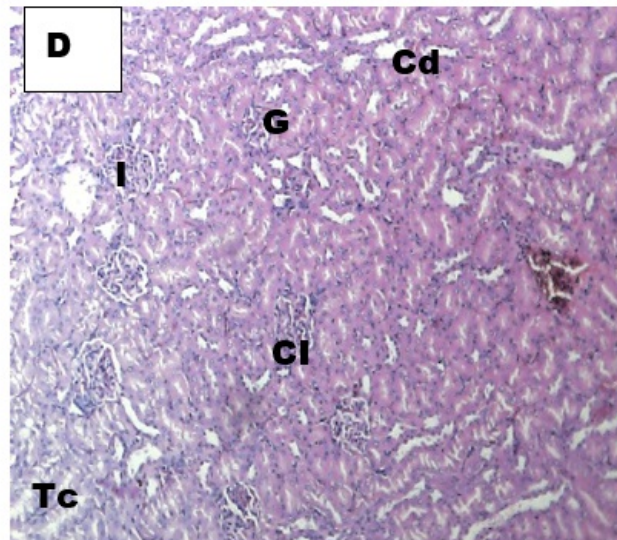
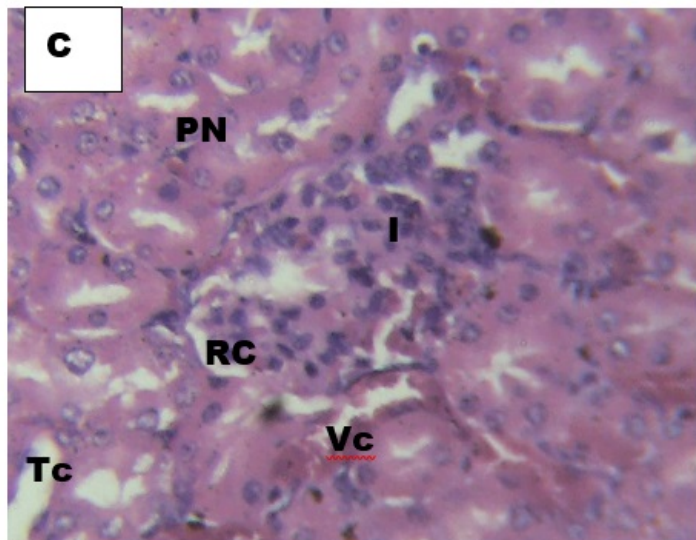


Plate 10: Photomicrographs of Kidney treated with 32.40 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: G-Glomerulus, Rc-Renal corpuscle, VC-Vascular Congestion, Cd-Collecting Ducts. CI-Cellular Infiltration, PN- Pyknotic Nucleus Tc- Tubular Congestion and I- Inflammation.

Group-3

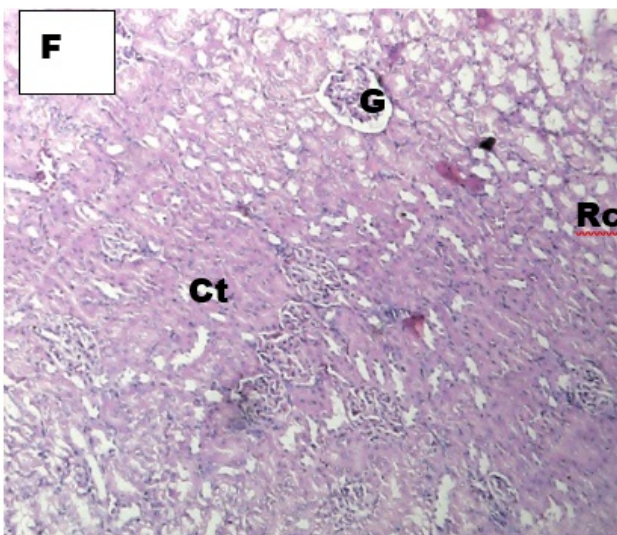
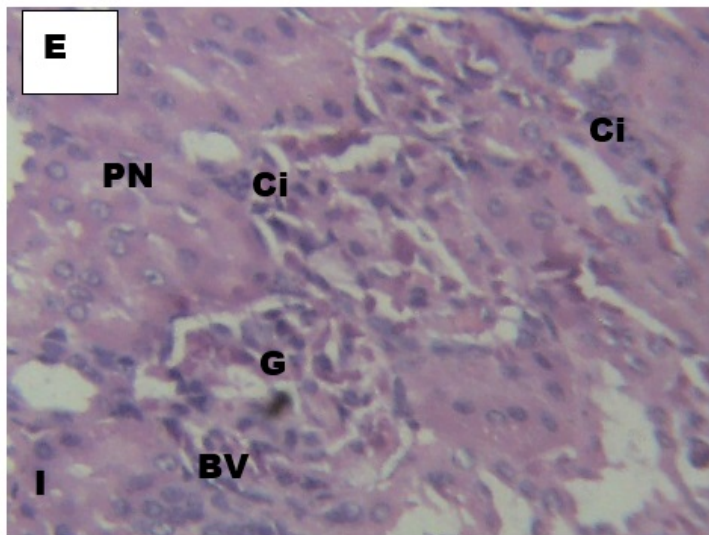


Plate 11: Photomicrographs of Kidney treated with 64.80 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: G-Glomerulus, Rc-Renal corpuscle, Ct-Convoluted tubule, Cd-Collecting ducts. CI- Cellular Infiltration, PN- Pyknotic Nucleus and I-Inflammation.

Group-4

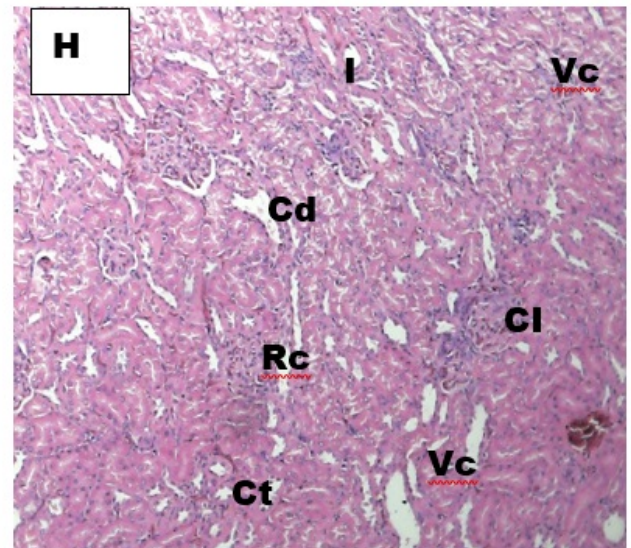
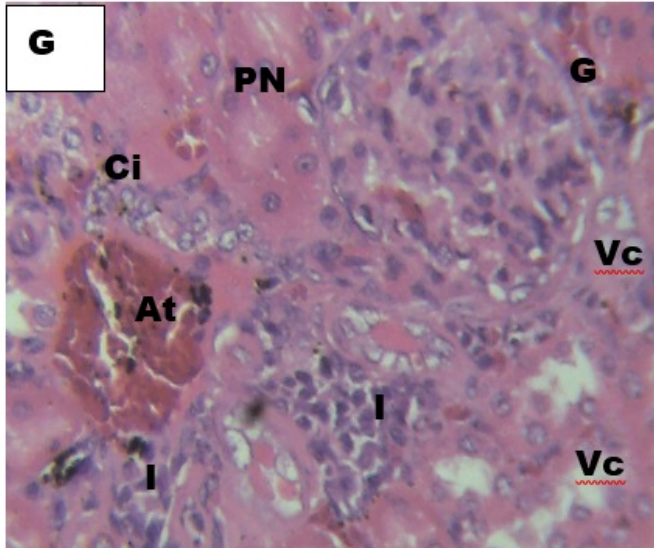


Plate 12: Photomicrographs of Kidney treated with 96.20 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: G-Glomerulus, Rc-Renal corpuscle, Vc-Vascular congestion, Cd-Collecting ducts. CI-Cellular Infiltration, PN- Pyknotic Nucleus I- Inflammation and At- Artifact.

Control-group: Spleen

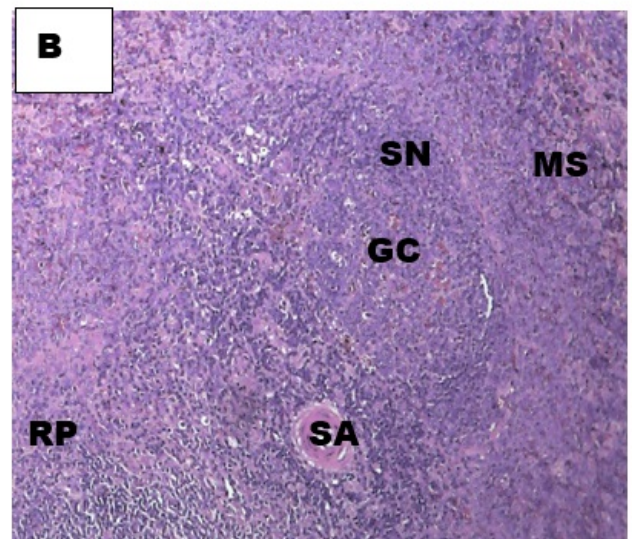
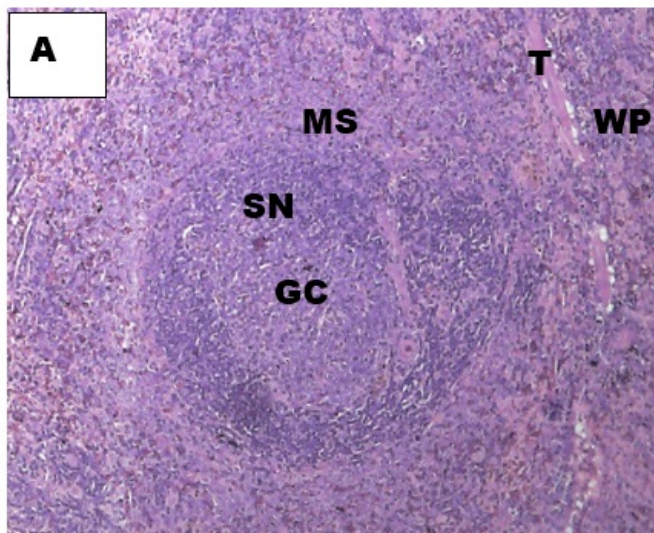


Plate 13: Photomicrographs of normal Spleen (control) at magnification A (X100) and B (X400) stained with H & E technique. Key: SN- Splenic Nodule, MS- Medullary Sinus, T- Trabeculae, SA- Splenic Artery, WP- White Pulp, RP- Red Pulp and GC- Germinal Center.

Group-2

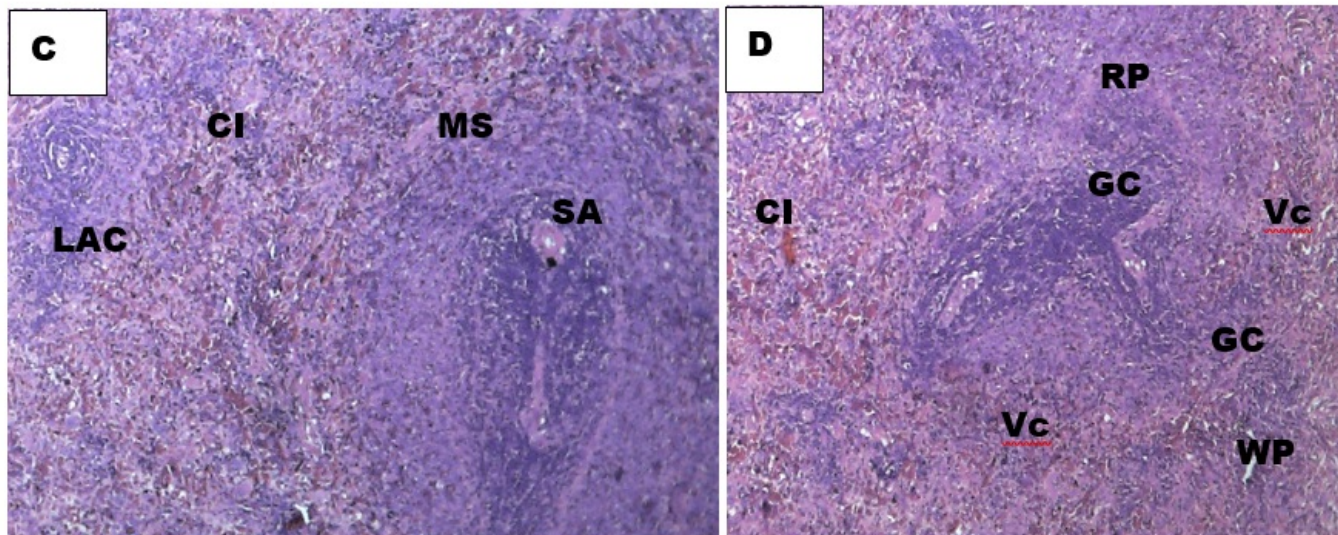


Plate 14: Photomicrographs of Spleen treated with 32.40 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: SN- Splenic Nodule, MS- Medullary Sinus, T- Trabeculae, SA- Splenic Artery, WP- White Pulp, RP- Red Pulp and GC- Germinal Center, LAC- Lymphoid Associated Cells and Vc-Vascular congestion.

Group-3

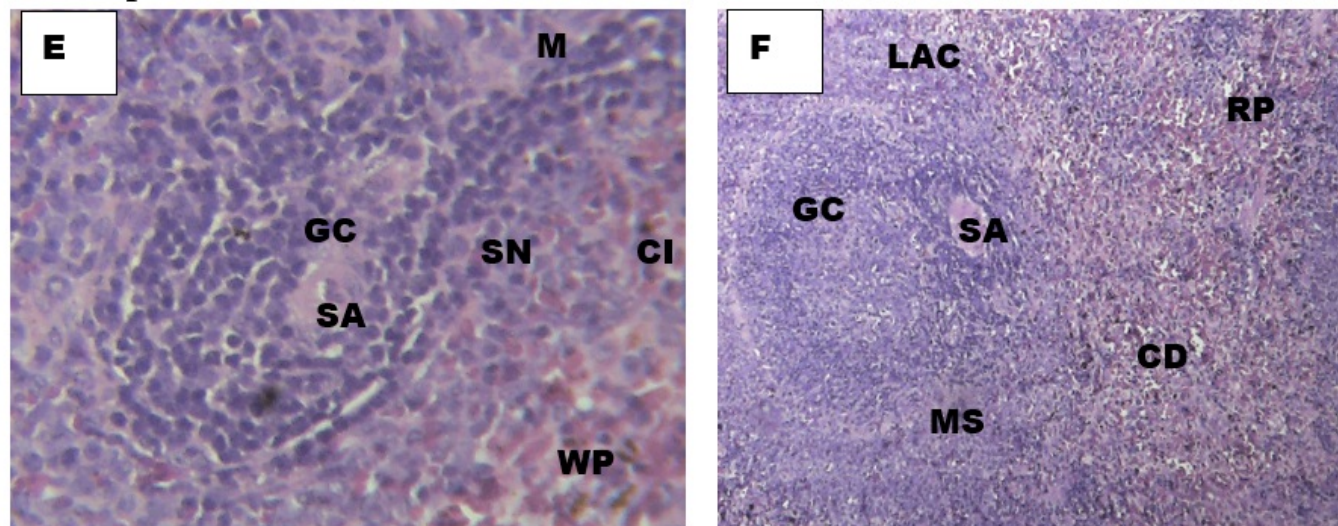


Plate 15: Photomicrographs of Spleen treated with 64.80 mg/kg of extract at magnification E (X100) and F (X400) stained with H & E technique. Key: SN- Splenic Nodule, MS- Medullary Sinus, T- Trabeculae, SA- Splenic Artery, WP- White Pulp, RP- Red Pulp and GC- Germinal Center, Ci- Cellular infiltration and LAC- Lymphoid Associated Cells.

Group-4

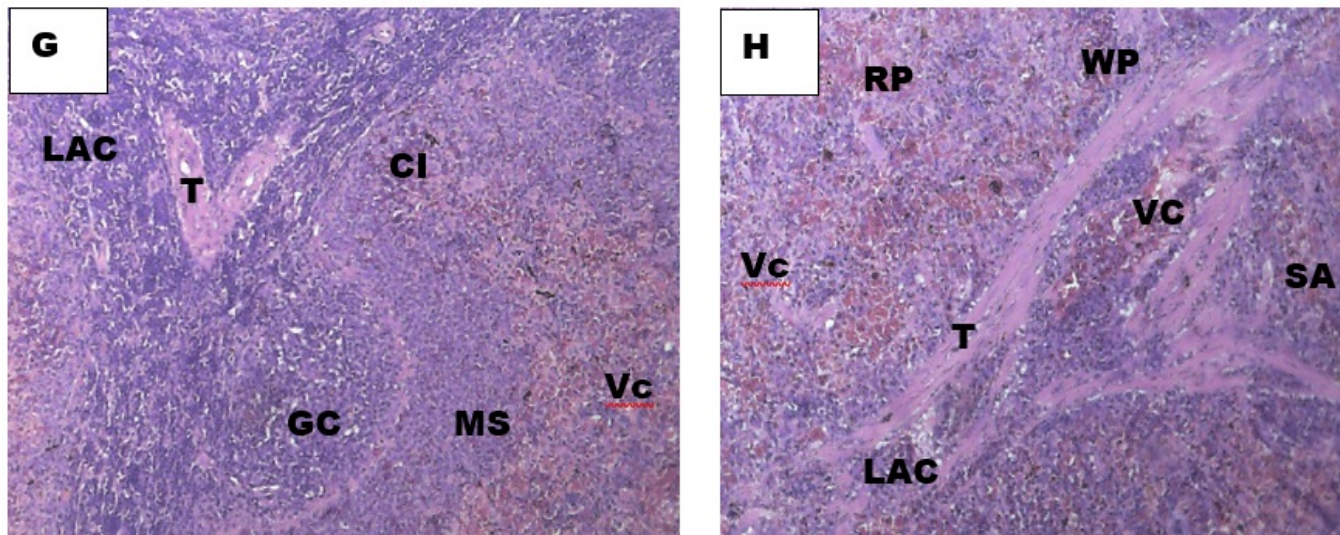


Plate 16: Photomicrographs of Spleen treated with 96.20 mg/kg of extract at magnification G (X100) and H (X400) stained with H & E technique. Key: SN- Splenic Nodule, MS- Medullary Sinus, T- Trabeculae, SA- Splenic Artery, WP- White Pulp, RP- Red Pulp and GC- Germinal Center, Vc-Vascular congestion and LAC- Lymphoid Associated Cells.

Control-Group: Lungs

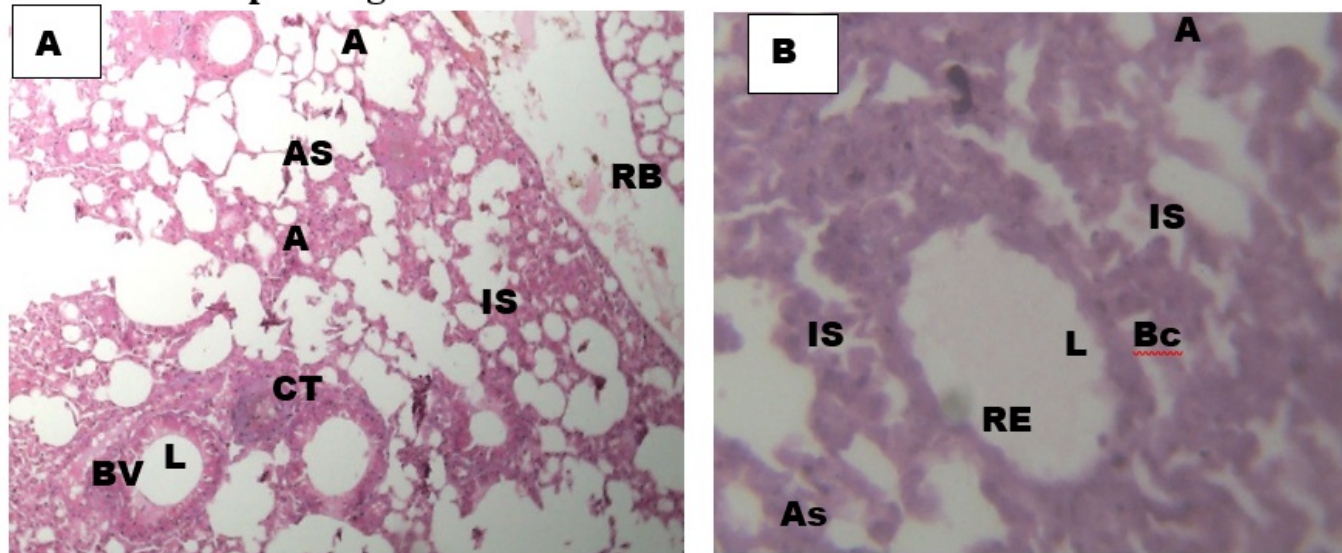


Plate 17: Photomicrograph of normal lung (control) at magnification A (X100) and B (X400) stained with H & E technique. Key: CT- Connective Tissue, BV- Blood Vessel, RB- Respiratory Bronchiole, AS- Alveoli Sacs, Bc- Bronchus, IS- Inter-alveolar Septum, A- Alveoli, L - lumen and RE-Respiratory Epithelium.

Group-2

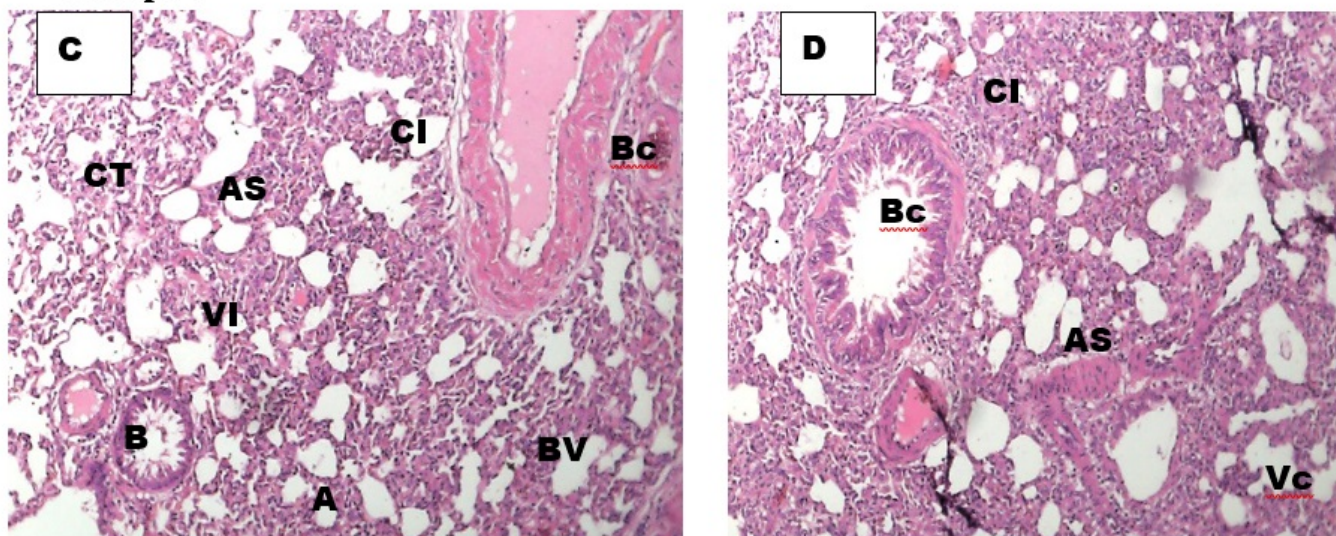


Plate 18: Photomicrograph of lung treated with 32.40 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: CT-Connective Tissue, BV- Blood Vessel, CI- Cellular Infiltration AS- Alveoli Sacs, Bc- Bronchus, B- Bronchiole, VI- vascular infiltration, A- Alveoli and Vc-Vascular congestion.

Group-3

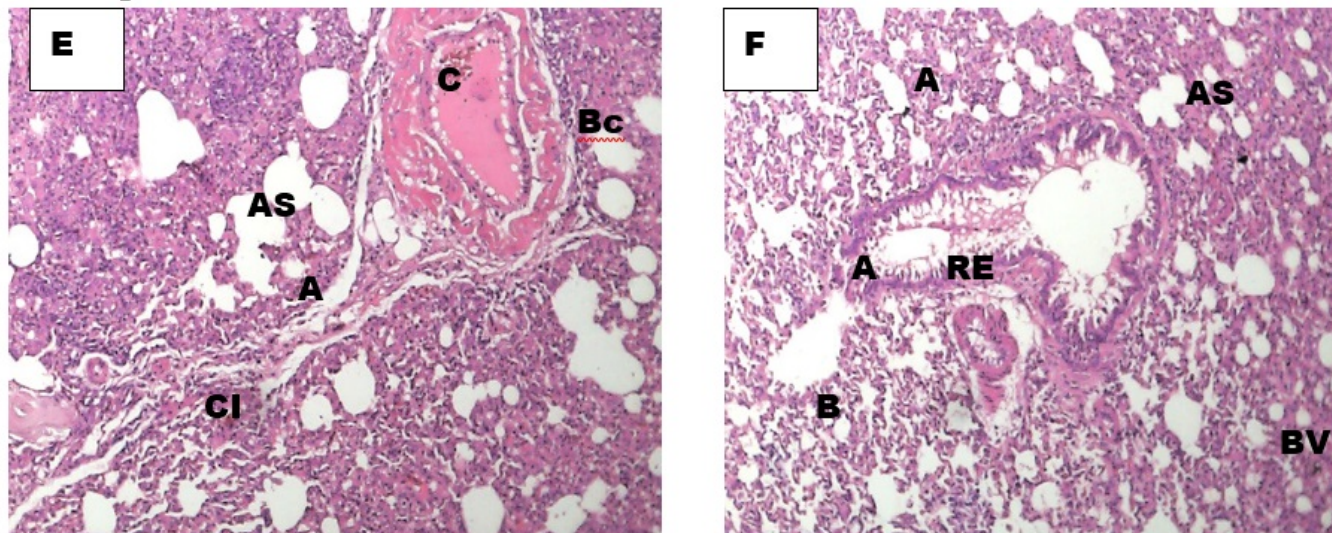


Plate 19: Photomicrograph of lung treated with 64.80 mg/kg of extract at magnification E (X100) and F (X400) stained with H & E technique. Key: CT-Connective Tissue, BV- Blood Vessel, CI- Cellular Infiltration, AS- Alveoli Sacs, Bc- Bronchus, B- Bronchiole, A- Alveoli, RE- Respiratory Epithelium and C- Cartilage.

Group-4

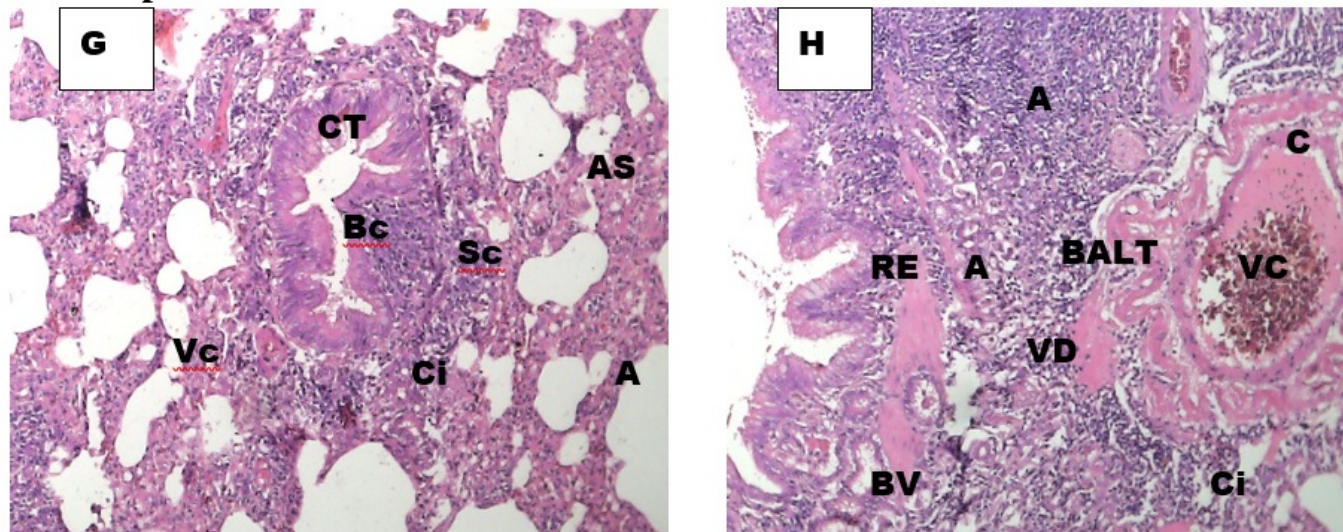


Plate 20: Photomicrographs of lung treated with 96.20 mg/kg of extract at magnification G (X100) and H (X400) stained with H & E technique. Key: CT-Connective Tissue, BV- Blood Vessel, Ci- Cellular Infiltration, AS- Alveoli Sacs, Bc- Bronchus, B- Bronchiole, VC- Vascular Congestion, A- Alveoli, RE- Respiratory Epithelium, C- Cartilage and BALT-Basophilic Associated Lymphoid Cells.

Control-Group: Ovary

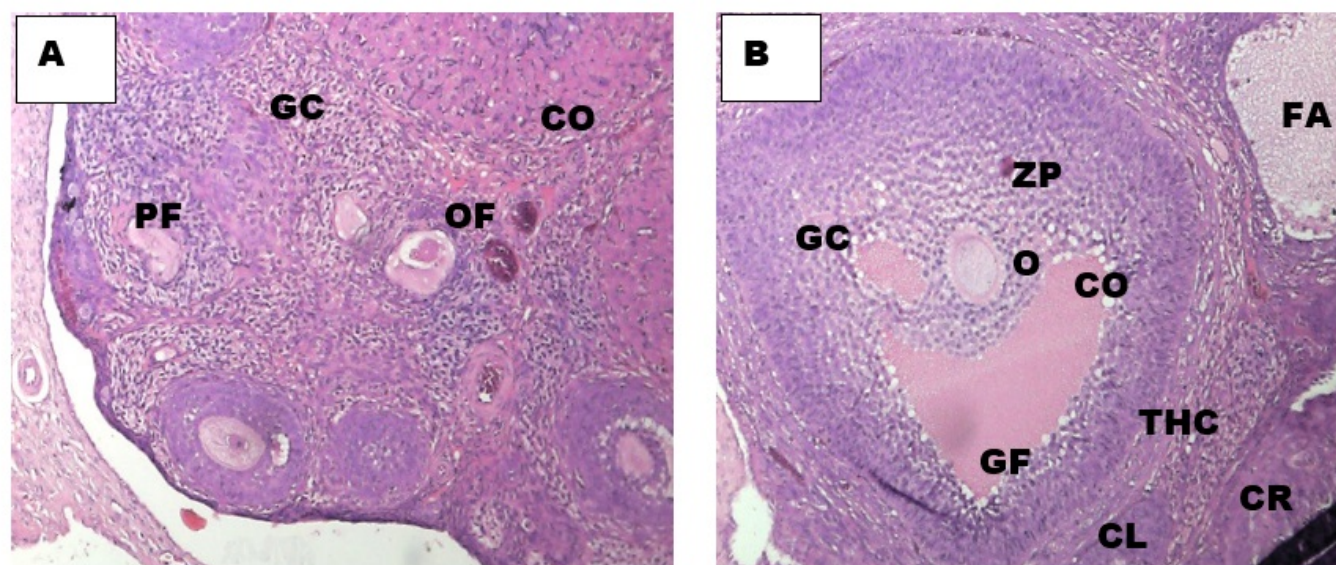


Plate 21: Photomicrographs of normal Ovary (control) at magnification A (X100) and B (X400) stained with H & E technique. Key: GF- Graafian Follicle, THC-Theca Cells, GC- Granulosa Cells, OF- Ovarian Follicle CR- Corona Radiata, CO- Cumulus Oophorus, O- Ovary, FA- Follicular Antrum, ZP- Zona Pellucida, Gc- Germinal Center and CL- Cortical Layer.

Group-2

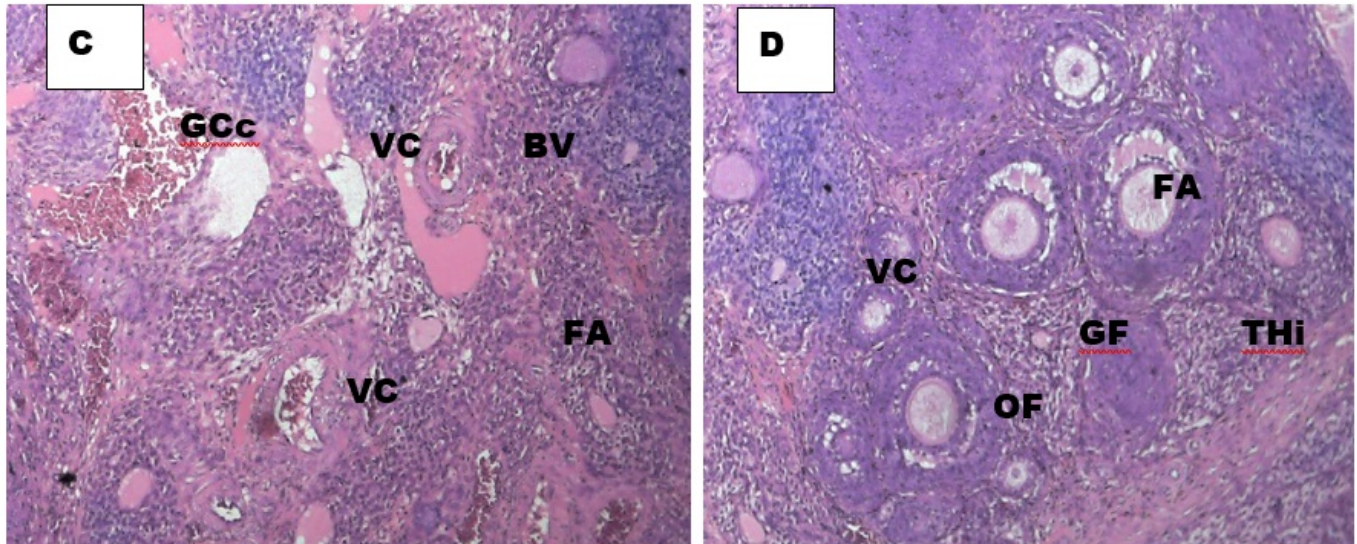


Plate 22: Photomicrographs of Ovary treated with 32.40 mg/kg of extract at magnification C (X100) and D (X400) stained with H & E technique. Key: GF-Graafian Follicle, THi-Theca Cell infiltration, GCc- Granulosa Cell congestion, OF- Ovarian Follicle, BV- Blood Vessel, O- Ovum, FA- Follicular Antrum and VC- Vascular Congestion.

Group-3

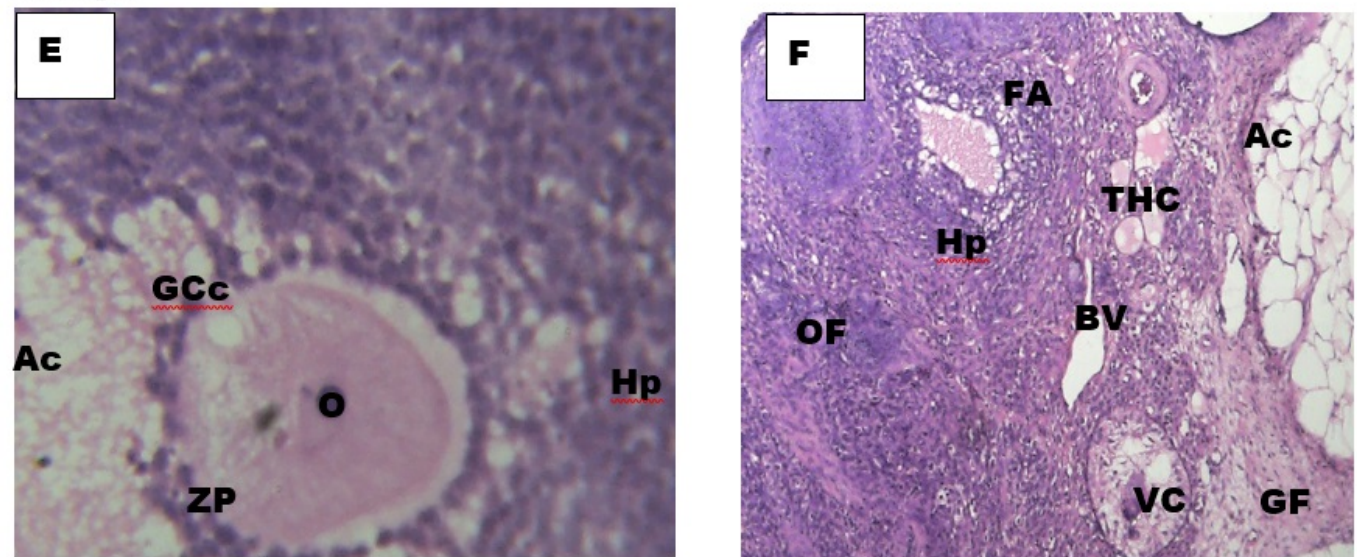


Plate 23: Photomicrographs of Ovary treated with 64.80 mg/kg of extract at magnification E (X100) and F (X400) stained with H & E technique. Key: GF-Graafian Follicle, THC-Theca Cell Congestion, GCc- Granulosa Cell congestion, Hp- Hyperplasia, OF- Ovarian Follicle, BV- Blood Vessel, Ac- Adipocytes, O- Ovum, FA- Follicular Antrum, VC- Vascular Congestion and ZP-Zona Pellucida.

Group-4

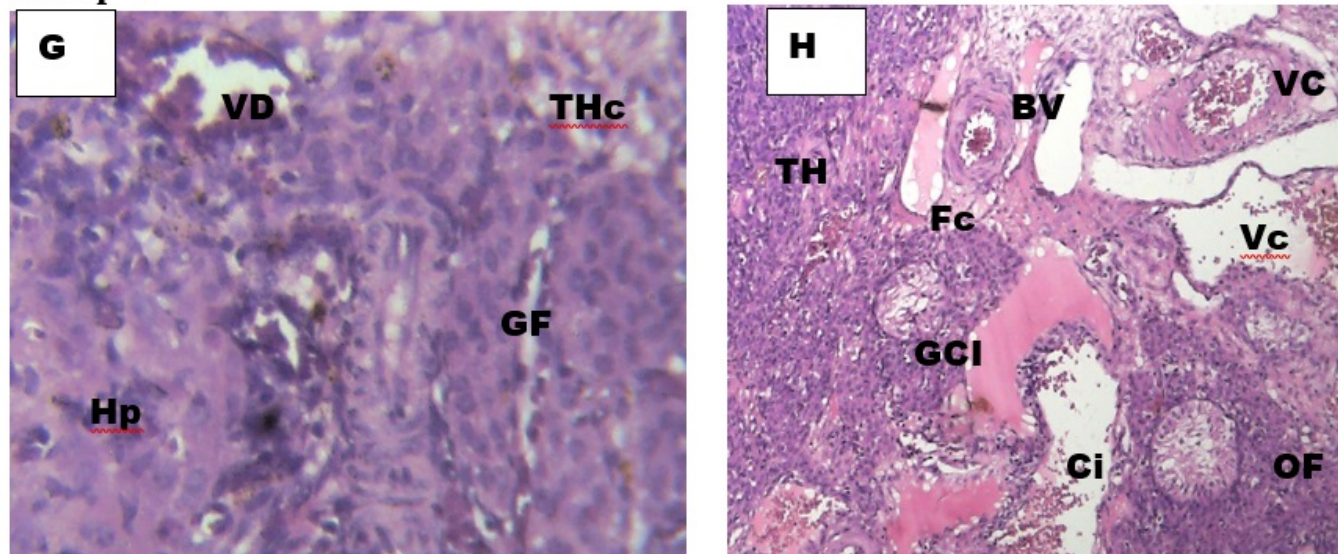


Plate 24: Photomicrographs of Ovary treated with 96.20 mg/kg of extract at magnification G (X100) and H (X400) stained with H & E technique. Key: GF-Graafian Follicle, THc-Theca Cell congestion, GCI- Granulosa Cell Infiltration, Hp- Hyperplasia, OF- Ovarian Follicle, BV- Blood Vessel, O- Ovum, VC- Vascular Congestion, Ci- Cellular infiltration, Fc- Follicular congestion.

Conclusion

In this study, acute toxicity testing, phytochemical screening and anti-oxidant evaluations were carried out. The intra-peritoneal LD₅₀ value of 324 mg/kg showed that the extract has moderate toxicity. The extract was also found to contain saponin, tannins, anthraquinones, cardiac glycosides, terpenes and alkaloids. Earlier studies have reported the isolation of protoberberine (7.8.-dihydro-8-hydroxypalmatine) and berberine alkaloids from the stem bark of the plant [10-12]. These revealed the great medicinal potentials of this plant. Secondary metabolites such as alkaloids and terpenes like sesquiterpenes and monoterpenes have been implicated in anti-plasmodial activity of plants [13,14]. The extract also contains protoberberine and berberine alkaloids which have been reported to be responsible for the anti-malarial activity of this plant [10-12]. That the extract contains the above secondary metabolites suggest that the anti-plasmodial activity exhibited by the plant may in part be due to these active ingredients. Reactive oxygen species such as superoxide anion and hydrogen peroxide have been implicated in almost every inflammatory and febrile disease in recent times. The anti-oxidant effects of this extract when compared to vitamin c have shown the major reason why the plant is so popular with the natives. It has anti-inflammatory, antipyretic and analgesic effects which are necessary components of any drug that will combat fever, pain and swellings [15]. Histological results on vital organs of the body such as heart, spleen, liver, kidney, lungs and ovary showed mild inflammatory changes. Increase in total and conjugated bilirubin levels, without corresponding increases in ALT, AST, ALP and albumin level defines a mild toxic effect of the extract in the liver that maybe associated with the moderate cellular degeneration observed in the histology of the extract treated rats. Serum albumin levels are usually reduced in chronic liver diseases, congestive heart failure and nephritis [16]. The histological findings in the organs corroborate an earlier report by Tan

et al. [12], who reported changes seen in the rats treated with the extract. The mild cellular changes seen in the organs maybe due to the low doses used in this study which is far lower than those of previous reports [17]. The phytochemical constituents may qualitatively and quantitatively change depending on the region of collection of the plant. Therefore, these results taken together clearly demonstrated the reason behind wide acceptability and tolerability of the extract/plant among the Ibibios for the treatment of diseased conditions whose models were employed in the investigation of the effect of the plant. This plant should be characterized and further studies done with the hope of turning this medicinal plant into a useful anti-inflammatory cream for topical use or tablets for internal use.

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