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Sub-chronic Toxicity Study of Hydroethanolic Leaf Extract of *Telfairia occidentalis* Hook. f. (Cucurbitaceae) in Rats

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Abstract: Background: The leaf of *Telfairia occidentalis* Hook f. (Cucurbitaceae) is consumed in different parts of Nigeria because of the numerous nutritional and medicinal attributes ascribed to it. The sub-chronic toxicity of the hydroethanolic leaf extract of *Telfairia occidentalis* (TO) was investigated in this study. **Methods:** Rats in different groups were separately administered 80, 400 and 2000 mg/kg TO *p.o.* for 60 days. Animals were sacrificed and blood samples collected for haematological and biochemical analysis. Vital organs were harvested and evaluated for *in vivo* antioxidants and histopathological changes. **Results:** Results showed no significant changes in the weight of vital organs except in respect of the testes of the group

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treated with 2000 mg/kg extract which showed a significant ($p<0.05$) reduction in weight compared to the control group. There was a significant ($p<0.01$) increase in sperm motility and count of the group administered 80 mg/kg extract and a significant ($p<0.001$) reduction was observed at 2000 mg/kg. There were significant increases in the level of Hb and PCV at 80 and 2000 mg/kg of the extract. In respect of liver function parameters, a significant decrease in AST and ALT levels at doses of 400 and 2000 mg/kg relative to control was observed. A significant reduction ($p<0.05$) in the level of total cholesterol (400 mg/kg) and increase ($p<0.05$) in level of HDL (80-2000 mg/kg) compared to control was observed. There was also significant ($p<0.05$) increase in the level of MDA and significant ($p<0.05$) decrease in SOD level in the testes at 2000 mg/kg. Histopathological assessment of the testes revealed abnormality at this dose. These effects were reversed after 30 days of cessation of administration of TO. **Conclusions:** The findings showed that the hydroethanolic leaf extract of *Telfairia occidentalis* is relatively non-toxic on acute and sub-chronic exposure, with potential to elicit anti-anaemic effect, reduce the risk of atherosclerosis and cardiovascular disease, and enhance antioxidant status in the brain and liver. Although possibly beneficial at low dose, the extract could be harmful to the testes on prolonged oral exposure at high dose.

Keywords: *Telfairia occidentalis*; Cucurbitaceae; subchronic; toxicity; biochemical; haematological

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1. Introduction

The use of herbal remedies by all stratums of people is on the increase worldwide. Phytotherapy is a major form of treatment for more than 70% of the world's population. Herbal preparations have a role to play in modern medicine, and there is clear evidence of their therapeutic benefits [1]. It is a widely held belief that herbal preparations are 'natural'. However, despite the belief and claim of being natural and safe, herbal remedies have been associated with lethal effects which have been attributed to several factors including hepatic toxicity of main constituents and contamination of preparation by heavy metals or microorganisms [2].

Telfairia occidentalis Hook. f. (Cucurbitaceae) is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. Common names of the plant include "fluted gourd" and "fluted pumpkin". In Nigeria, the leaf is consumed in different parts of the country because of the numerous nutritional and medicinal attributes ascribed to it. It has different traditional names; among Igbos it is known as "Ugu"; "Iroko" in Yoruba; "Ubong" in Efik and "Umeke" in Edo [3]. In folkloric medicine, the fresh leaves are used in the treatment of anemia, sudden attack of convulsion and malaria [4-6]. Extracts of TO have been scientifically investigated and reported to possess anti-anaemic [5], hepatoprotective [7], hypoglycemic [8], antinociceptive and anti-inflammatory activities [9].

In view of the importance of toxicological evaluation in drug and standardized herbal remedy discovery and development, the evaluation of the hydroethanolic leaf extract of TO for its acute and sub-chronic toxicological effects, conducted in this study, is worthwhile. Apart from the scientific assessment of the safety of the extract, other potential benefits in the treatment of human diseases, associated with long-term administration, may also be detected.

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2. Materials and methods

2.1. Plant Material

Fresh leaves of TO were obtained from a local herb market in Mushin, Lagos State, Nigeria. Botanical identification and authentication of the plant material was done by Mr. T.K. Odewo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria. A voucher specimen (numbered LUH 5580) was deposited in the institutional herbarium for reference purpose.

2.2. Extraction

Fresh leaves of TO were cut into small pieces and air-dried until a constant weight was obtained. The dried material was milled into fine powder using electric blender to give 925 g of pulverized material. One hundred gram (100 g) of the plant powder was macerated in 500 mL hydroethanol (1:1) for 48 h. In all, the 925 g powdered plant material was macerated in 4.5 L of hydroethanol. The extract was thereafter decanted and filtered twice. The residue was re-macerated (× 2) for exhaustive extraction. At the end of the extraction process, the combined filtrate was evaporated to dryness under reduced pressure at 40 °C. A dark brownish solid extract with a yield of 15% was obtained. The extract was sticky in nature with a sweet smell and dissolved completely in distilled water. The solid extract was reconstituted in distilled water to give different concentrations based on the required dosage before administration to experimental animals.

2.3. Laboratory Animals

Mice (15-20 g) and male albino rats (100-150 g) used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed in plastic cages with wooden shavings as beddings, kept and maintained

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under standard environmental conditions (23-25 °C 12 h/12 h light/dark cycle) with standard rodent diet (Livestock Feeds Plc., Lagos, Nigeria) and water *ad libitum.* Animals were acclimatized for 7 days before the commencement of the experiment. Protocol used in this study was in accordance with the specifications of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria and the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biochemical Research [10].

2.4. Phytochemical Screening

Preliminary phytochemical screening was done according to the methods of Harbone [11], Trease and Evans [12] and Edeoga *et al.* [13].

2.5. Acute Toxicity Test

Mice of both sexes which had been fasted for 12 h prior to the test were administered the extract orally at the dose of 5000 mg/kg and intraperitoneally at 1000 - 5000 mg/kg. Animals were observed for 2 h post-administration for behavioral changes and signs of toxicity. Mortality observed in each group within 24 h was recorded. Surviving animals were observed further for 14 days for any signs of delayed toxicity. The LD_{50} was estimated by the log dose-probit analysis method [14].

2.6. Sub-chronic Toxicity Test

Sixty four male albino rats were randomly divided into 4 groups (n=16). Animals in the different groups were daily treated with distilled water (control; Group I) and TO extract at doses of 80 (Group II), 400 (Group III), and 2000 mg/kg (Group IV) orally. The doses of the extract used in this study represent the sub-therapeutic, therapeutic, and supra-therapeutic doses respectively [15,16]. The therapeutic dose of 400 mg/kg was the most effective dose in a

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previous study on the analgesic and anti-inflammatory activities of the extract [9]. Animals were weighed on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 61. They were also observed for behavioral and morphological changes.

2.6.1. Blood and Tissue Collection

Twenty four hours after the last administration, some animals (n=10) were sacrificed while the rest were sacrificed 30 days later (reversibility studies). The animals were anaesthetized using 1 % chloralose in 25 % urethane (w/v) (5 mL/kg, *i.p.*) [16] and blood samples were collected for haematological and biochemical analysis. The animals were carefully dissected and vital organs including the lungs, spleen, pancreas, brain, heart, liver, kidney and testis were identified, cleared of adherent tissues and harvested. The organs were then rinsed with normal saline, drained on filter paper, carefully examined for gross lesions, and weighed (Mettler-Toledo GmbH digital weighing balance; Type BD202, SNR 06653). Each organ was grossly observed for visible lesions and thereafter standardized for 100 g body weight. Samples were taken from each of the organs for determination of antioxidant indices. The remnants of the organs were preserved in properly labeled containers with 10% formal saline and kept for histopathological assessment [17]. Semen was also obtained for sperm motility, count, and morphology analysis [18]. Mortality in each treatment group was recorded in the course of the experiment.

2.6.2. Sub-chronic Toxicity Reversibility Test

Six rats from each group were reserved for the reversibility study in which treatment was discontinued and the animals were allowed free access to food and water only for 30 days. Samples were collected from animals after 30 days of cessation of extract administration for analysis as done in the main study.

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2.6.3. Haematological assessment

Erythrocyte (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW), red cell distribution width (RDW), and total and differential leukocyte (WBC) were determined using automated haematology analyzer [19].

2.6.4. Biochemical Assessment

Serum levels of urea, albumin, bilirubin, creatinine, total protein, triglycerides and cholesterol were determined as previously reported [19]. Biochemical analysis for ALT, AST and ALP was carried out using standard techniques [20].

2.6.5. Antioxidant indices assessment

The levels of reduced glutathione (GSH; non-enzymatic antioxidant), superoxide dismutase (SOD; enzymatic antioxidant), catalase (CAT; enzymatic antioxidant), and malondialdehye (MDA; marker of lipid peroxidation) in the liver, kidney and brain of the rats were determined spectrometrically using standard techniques [21].

2.6.6. Histopathological Assessment

Post-mortem examination involving gross and microscopic examination of the selected organs of the albino rats was also carried out. The various tissues obtained from experimental animals fixed in 10% formal saline were dehydrated in graded alcohol, embedded in paraffin, and cut into 4-5 µm thick sections. Hematoxylin-eosin was used to stain the sections for photomicroscopic assessment using a Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany). Slides were examined using the × 40, × 100, and × 400 objectives [16].

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2.6.7. Sperm Analysis

Seminal fluid obtained from male animals across the different treatment groups was analyzed to determine sperm motility, count, and morphology using the methods of Cheesbrough [22] and Ogli *et al.* [18].

2.7. Statistical Analysis

Results obtained in this study are expressed as mean ± S.E.M. Data were analyzed statistically using one-way ANOVA and Student's *t*-test for both the main and reversibility studies using GraphPad Prism 6 software (GraphPad Software Inc., CA, USA). Values of $p<0.05$ were taken to imply statistical significance.

3. Results

3.1. Phytochemical Screening

The results of the preliminary phytochemical screening showed the presence of oils, saponins, phlobatannins and tannins in the hydroethanolic leaf extract of *T. occidentalis*.

3.2. Acute Toxicity Test

In the acute toxicity test, no mortality and visible signs of toxicity were observed upon administration of TO orally up to 5 g/kg within14 days of observation. Administered *i.p.*, mortality was 0% at the lowest dose of 1000 mg/kg and 100% at the highest dose of 5000 mg/kg. The LD_{50} of the extract administered *i.p.* was estimated to be 3200 mg/kg. The behavioral manifestations observed after intraperitoneal treatment include reduced locomotion, serenity, writhing and increased respiratory rate.

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3.3. Effect of TO on Body Weight, Food and Water Intake

There were no significant changes ($p < 0.05$) observed in the body weight, food and water intake of rats in all the treatment groups during the 60 days of administration of the extract (Table 1).

3.4. Effect of TO on Weight of Vital Organs (per 100 g body weight)

T. occidentalis extract did not generally produce any significant effect on the weight of vital organs in all the treatment groups when compared with the control group, except in respect of the dose of 80 mg/kg at which there was a significant increase ($p < 0.05$) in the weight of the kidney and the dose of 2000 mg/kg at which a significant reduction ($p < 0.01$) in the weight of the testes relative to the control value was observed. The effects produced in the testes and kidney was reversed upon cessation of treatment for 30 days (Table 2).

3.5. Effect of TO on Haematological Parameters

The extract did not produce any significant effect on haematological parameters after 60 days of administration in all the treatment groups when compared with the control group except in respect of hemoglobin in which there was a significant increase ($p < 0.05$) at doses of 80 mg/kg and 400 mg/kg. There was also a significant increase ($p < 0.05$) in PCV of the groups treated with the extract at doses of 80 mg/kg and 400 mg/kg relative to the control group. TO extract elicited a significant ($p < 0.05$) increase in RDW-CV at the dose of 2000 mg/kg relative to control. The effects *T. occidentalis* on Hb, PVC and RDW-CV were reversed after 30 days of cessation of administration (Table 3).

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3.6. Effect of TO on Serum Biochemical Parameters

There was a significant increase in the concentration of total protein at doses of 80 mg/kg, 400 mg/kg, and 2000 mg/kg, at levels of $p < 0.01$, $p < 0.01$ and $p < 0.05$ respectively, compared to control. There was also a significant increase ($p < 0.05$) in concentration of total bilirubin at doses of 400 mg/kg and 2000 mg/kg relative to control. The concentration of AST decreased at significant levels of $p < 0.01$ and $p < 0.05$ at doses of 400 mg/kg and 2000 mg/kg respectively, relative to the control. There was also a significant reduction in concentration of ALT at doses of 400 mg/kg and 2000 mg/kg at $p < 0.05$ and $p < 0.001$ respectively, compared with control. There was a significant increase ($p < 0.05$) in concentration of ALP at doses of 80 mg/kg, 400 mg/kg and 2000 mg/kg relative to control. There was a significant reduction ($p < 0.05$) in concentration of total cholesterol at the dose of 400 mg/kg and a significant increase ($p < 0.05$) in concentration of HDL at doses of 80 mg/kg, 400 mg/kg, and 2000 mg/kg compared to control value (Table 4). The effects on total bilirubin, total cholesterol, AST, ALT, and ALP were reversed after 30 days of discontinuation of administration of extract. There was significant increase ($p < 0.05$) in total protein at the dose of 2000 mg/kg relative to the control (Table 4). There were no significant effects observed on the level of serum creatinine, urea, and electrolytes in respect of the extract, compared with the control group, in all the treatment groups. However, there was a significant decrease ($p < 0.05$) in the level of urea at the dose of 80 mg/kg compared to the control value. There was also a significant increase ($p < 0.05$) in concentration of PO_4^{2-} at the dose of 400 mg/kg and Ca^{2+} at the dose of 2000 mg/kg relative to the control values. These effects were reversed after 30 days of cessation of administration of *T. occidentalis* (Table 5).

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3.7. Effect of TO on Antioxidant Indices of Rat Kidneys, Testes, Brain and Liver

There was no significant difference in the levels of the various antioxidants (non-enzymatic- GSH; enzymatic- SOD and CAT) and MDA in the kidney (Table 6). In the testes, there was a significant decrease ($p < 0.05$) in the level of SOD at the dose of 2000 mg/kg and a significant increase ($p < 0.01$) in the level of MDA compared to the control value. Also, there was a decrease in the level of MDA at the dose of 400 mg/kg compared with the control. In the brain, there was a significant increase in SOD level at doses of 400 mg/kg and 2000 mg/kg when compared with the control value. Also, there was a significant increase ($p < 0.05$) in GSH level at the dose of 2000 mg/kg relative to control. In respect of the liver, there was a significant increase ($p < 0.05$) in GSH level at doses of 400 mg/kg and 2000 mg/kg relative to control. Also, there was a significant increase ($p < 0.05$) in SOD and decrease in MDA levels at the dose of 400 mg/kg compared to the control (Table 6).

There were no significant differences in the level of antioxidant enzymes in the kidneys across the treatment groups after 30 days discontinuation of therapy. The effects on SOD and MDA in the testes were reversed upon cessation of treatment after 30 days. In respect of the brain, there was a significant increase ($p < 0.05$) in the level of SOD at the dose of 400 mg/kg and also significant increase ($p < 0.05$) in GSH level at the dose of 2000 mg/kg when compared to the control group value in the reversibility study. In the liver, the effects elicited were reversed (Table 6).

3.8. Effect of TO on Sperm Motility, Sperm Count and Morphology (% Abnormality)

The extract did not show any significant effect on sperm morphology in all the treatment groups when compared with the control group. In respect of sperm motility, the extract showed a significant increase ($p < 0.01$) at the dose of 80 mg/kg and a significant decrease ($p < 0.001$) at

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the dose of 2000 mg/kg relative to control. Also, there was a significant increase ($p < 0.05$) in sperm count at the dose of 80 mg/kg and a significant decrease ($p < 0.05$) in sperm count at the dose of 2000 mg/kg compared to the control. However, the reversibility study showed no significant ($p > 0.05$) difference in these parameters between the control and extract treated groups of rats (Table 7).

3.9. Histopathological Assessment of Selected Organs of Treated Rats

Generally, there were no adverse histopathological presentations observed in the treatment groups but cerebral oedema was observed in the 400 mg/kg group (Figure 1). Neuron cell bodies were displayed on a loose fibrillary background.

The heart was normal in all the treatment groups. The cardiac myocytes were arranged in interlacing and parallel array. Their nuclei were spindle shaped and elongated (Figure 2). There were no adverse histopathological presentations in the distilled water and TO at the dose of 80 mg/kg treatment groups.

The lungs appeared normal with alveolar air spaces surrounded by insterstitium containing few blood vessels and inflammatory cells (Figure 3). Interstitial inflammation was observed in the alveolar spaces of the lungs at doses of 400 mg/kg and 2000 mg/kg TO (Figure 3).

There were no adverse histopathological presentations observed in all the treatment groups in respect of the kidney. Normocellular glomerular tufts were displayed on a background containing tubules. No necrosis was observed (Figure 4).

There were no adverse histopathological presentations observed in all the treatment groups in respect of the liver. The liver appeared normal with preserved hepatic architecture, hepatocytes arranged as radial plates, having eosinophilic cytoplasmic and central nuclei. No cytoplasmic inclusions were seen and no portal inflammation (Figure 5).

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The pancreas was normal in all the treatment groups. Closely packed acini, separated by delicate fibrocollagenous stroma that transmits blood vessels were observed (Figure 6).

Histopathological presentations of the spleen in all treatment groups were normal showing lymphoid follicles (Figure 7).

In respect of the testes, normal Histopathological presentations were observed in all the treatment groups except in the group treated with *T. occidentalis* at the dose of 2000 mg/kg which showed mild testicular atrophy, spermatogenic series lining diminished and no luminal spermatozoa (Figure 8).

4. Discussion

Globally, there are diverse medicinal plants and botanical drugs which have been vastly adapted as major therapeutic agents or supplements for treatment of various human diseases [23]. As a result of abundant usage and propensity for prolonged use of the fresh leaves of *T. occidentalis* in traditional medicine, the evaluation of acute and sub-chronic toxicological effects of the hydroethanolic leaf extract of the plant was carried out in this study.

T. occidentalis did not induce mortality in mice when administered orally up to 5 g/kg. Therefore, the herbal preparation can be said to be relatively safe in accordance with the suggestion of Hayes [24] that no dose-related toxicity should be considered above 5 g/kg body weight. There were no visible signs of delayed toxicity. However, the LD_{50} when the extract was administered intraperitoneally was calculated to be 3200 mg/kg.

Administration of the hydroethanolic leaf extract of *T. occidentalis* at the therapeutic dose (400 mg/kg) did not produce any significant effect on the weight of rats and vital organs over the entire duration of administration. Reductions in body weight gain and internal organ weights are

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sensitive indices of toxicity after exposure to toxic substances [25,26]. Also, there were no significant changes in food and water intake.

According to Saeed *et al.* [27], mammalian cells contain antioxidants, including glutathione peroxidase and catalase which can detoxify free radicals by converting them to more stable molecules within the cell. Malondialdehyde, a major breakdown of product of lipid peroxidase, is an index of lipid peroxidation. The overwhelming of antioxidant enzymes by free radicals results in the reduction of the antioxidants defenses and induction of lipid peroxidation evident in elevation of MDA level [28]. In respect of the kidney, TO at the therapeutic dose did not cause significant change in SOD, CAT, GSH and MDA levels. There was a significant increase in SOD level in the brain, with no significant effect on CAT, GSH and MDA levels. Antioxidants and MDA estimation in the liver showed an increase in GSH, SOD, and decrease in MDA levels which suggests enhancement of hepatic *in vivo* antioxidant activity. In respect of the testes, the extract at the therapeutic dose caused significant increase in the level of MDA, although there was no significant change in the levels of the antioxidants assayed. These results suggest that the extract at the therapeutic dose did not change the antioxidant status in the kidney and testes, but enhanced the antioxidant status of the brain and the liver.

T. occidentalis at the therapeutic dose did not produce any significant effect on sperm count, motility and morphology. The haematological analysis showed significant increase in hemoglobin and PCV, with no any significant effect on other haematological parameters at the therapeutic dose. The observed increase in the levels of Hb and PCV possibly suggests that TO stimulated pluripotent red cell lines in the bone marrow. Biochemical estimations showed significant increase in HDL. High concentration of HDL confers protective value against cardiovascular diseases such as ischemic stroke and myocardial infarction from reports of

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epidemiological studies [29]. Low concentration of HDL increases the risk of atherosclerosis and cardiovascular disease [30]. There were significant increases in total protein, total bilirubin, ALP and reduction in total cholesterol, AST and ALT levels. Increased level of total protein supported the non-toxic nature of the extract to the liver. The liver produces protein and low level of protein could show possible impaired synthesis [31]. The high level of ALP possibly reflects impaired excretion and obstruction of bile flow in the biliary tract. However, the relatively high level of ALP may be from extra-hepatic sources because in respect of the major biomarkers for hepatic injury, AST and ALT, significant decreases were observed in this study. On the renal function integrity, there was a significant reduction in the level of creatinine, with no significant effect on the levels of sodium, potassium, chloride, and urea. Renal functions are measured by serum electrolytes, urea and creatinine. Elevation in the serum levels of these parameters show renal dysfunction [31-33]. These results suggest that the extract at the therapeutic dose possess blood boosting anti-anaemic effect, reduced the risk of atherosclerosis and cardiovascular disease, with no deleterious effect on the kidneys and liver. The reversibility studies indicated that the effects induced by *T. occidentalis* at the therapeutic dose in the main study were reversed. This suggests that these effects were not continuous upon cessation of administration.

In respect of the sub-therapeutic dose (80 mg/kg), the hydroethanolic extract of *T. occidentalis* did not produce any significant effect on body weight, food and water intake when compared with the control group at the end of the 60 days treatment period. There was no significant change in the weight of vital organs except significant increase in the weight of the kidneys. Haematological function showed a significant increase in PCV and Hb levels which possibly suggests blood boosting anti-anaemic effect. There was a significant increment in sperm motility and sperm count, with no significant effect on percentage sperm abnormality. This

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suggests that the extract at the sub-therapeutic dose potentially possess male fertility boosting activity. The level of MDA and antioxidants in the kidney, liver, testes and brain were not significantly affected. Biochemical assays showed a significant increase in HDL, TP, and ALP, with no significant effect on other parameters. HDL increment indicates a potential protection against cardiovascular disease like ischemic stroke and myocardial infarction [30]. Increased level of total protein also supports the non-toxic effect of the extract on the liver at the sub-therapeutic dose. The high level of ALP reflects impaired excretion and obstruction of bile flow in the biliary tract. The extract at the sub-therapeutic dose did not significantly alter AST and ALT levels relative to the control group. This suggests that the extract at this dose did not elicit deleterious effect on the liver and the increase in the level of ALP may be from extra-hepatic sources. *T. occidentalis* at this dose caused significant decrease in the level of urea, with no significant effect on other renal parameters. This also suggests that administration of the extract did not cause injury to the kidneys.

T. occidentalis at the supra-therapeutic dose (2000 mg/kg) did not produce any significant effect on body weight, food and water intake in rats relative to control. There were no significant changes in the weight of vital organs except in respect of the testes which showed a significant decrease. There was a significant reduction in sperm mortality and sperm count, with no significant effect on percentage sperm abnormality. This suggests that the extract at the supra-therapeutic dose has the potential to cause male sterility with prolonged treatment. Antioxidants and MDA level estimation in the testes showed a significant decrease in SOD and increase in MDA levels. Superoxide dismutase is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O^{2-}) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Hydrogen peroxidase is a harmful by-product of many normal

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metabolic processes. For prevention of damage, it must be rapidly converted into other less toxic substances. Catalase is used by cells to quickly catalyze the decomposition of hydrogen peroxide into less reactive oxygen and water molecules [34]. A reduction in MDA level also shows ability to mop up dangerous species of free radicals [35]. Increase in MDA and decreased SOD levels of testes show that the extract is toxic to the testes cells at the supra-therapeutic dose. Histopathological assessment of the testes at this dose revealed mild testicular atrophy, diminished spermatogenic series lining and no luminal spermatozoa. In the brain, *T. occidentalis* generally showed no significant effect on antioxidants and MDA level, but there was a significant increase in SOD level. This indicates enhancement of free radical scavenging ability in the brain by the extract. Antioxidants and MDA levels in the kidney and liver did not show any significant change. However, an increase in GSH level was observed in the liver. This shows enhancement of free radical scavenging ability of the extract in the liver. In respect of the kidney function, there were no significant changes in the level of urea and electrolytes generally, except a significant decrease in the level of creatinine. This suggests that the extract at this dose may not be toxic to the kidneys. In respect of biochemical estimation, there was a significant decrease in serum concentration of AST and ALT. The serum levels of these enzymes are raised in acute liver damage. ALT is largely found in the liver and is commonly used as a biomarker for liver problem [36]. The observation at this extract dose in respect of AST and ALT revealed that the extract did not elicit deleterious effect on the liver. There were significant increases in total protein, total bilirubin, ALP and HDL. Increase in HDL suggests potential cardioprotective ability of the extract at the supra-therapeutic dose.

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5. Conclusion

The results obtained in this study suggest that the hydroethanolic leaf extract of *T. occidentalis* is relatively safe after acute and sub-chronic oral administration. The extract demonstrated potential to elicit blood boosting anti-anaemic effect, reduce the risk of atherosclerosis and cardiovascular disease, and enhance antioxidant status in the brain and liver at therapeutic doses. However, the extract could be harmful to the testes on prolong oral exposure at a high dose, possibly predisposing males to sterility, thus caution should be exercised in respect of high dose and long duration of use.

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Author Contributions: A.J.A.- conception, design, interpretation and manuscript writing; J.A.O.- execution, interpretation and manuscript writing; R.A.O.- execution, interpretation and manuscript writing; and D.D.O.- execution and interpretation in respect of histopathological assessments.

Conflicts of Interest: None to declare by authors in respect of this study.

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Table 1. Effect of TO on weekly change in body weight, food and water intake of rats.

Treatment	Body weight (%)	Food intake (g)	Water intake (ml)
Distilled water	54.11±3.24	40.13±2.51	33.25±0.26
TO 80 mg/kg	78.09±0.36	45.50±0.72	34.50±3.12
TO 400 mg/kg	45.45±2.24	44.88±1.27	38.25±5.50
TO 2000 mg/kg	36.93±5.50	34.75±5.29	37.38±0.31

Values are expressed as mean ± S.E.M. (n=5). $p > 0.05$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 2. Effect of TO on vital organs weight (per 100 g body weight).

	Control		TO 80 mg/kg		TO 400 mg/kg		TO 2000 mg/kg	
	Main	**Reversibility**	**Main**	**Reversibility**	**Main**	**Reversibility**	**Main**	**Reversibility**
Testes (g)	1.00 ± 0.20	0.94 ± 0.22	1.27 ± 0.02	1.06 ± 0.12	1.07 ± 0.07	0.92 ± 0.07	0.47 ± 0.23*	0.85 ± 0.32
Heart (g)	0.81 ± 0.07	0.82 ± 0.04	1.00 ± 0.02	0.89 ± 0.05	0.89 ± 0.10	0.84 ± 0.02	0.83 ± 0.14	0.80 ± 0.04
Kidney (g)	0.54 ± 0.08	0.56 ± 0.02	0.62 ± 0.01*	0.61 ± 0.03	0.61 ± 0.08	0.61 ± 0.01	0.57 ± 0.07	0.55 ± 0.04
Liver (g)	5.31 ± 0.42	5.56 ± 0.21	6.59 ± 0.10	6.15 ± 0.12	5.92 ± 0.47	5.40 ± 0.34	5.61 ± 0.61	5.48 ± 0.22
Spleen (g)	0.69 ± 0.06	0.62 ± 0.04	0.66 ± 0.01	0.62 ± 0.03	0.78 ± 0.03	0.71 ± 0.05	0.61 ± 0.03	0.61 ± 0.07
Lung (g)	1.55 ± 0.06	1.54 ± 0.20	1.70 ± 0.03	1.65 ± 0.05	1.58 ± 0.05	1.62 ± 0.01	1.61 ± 0.20	1.58 ± 0.06
Pancreas (g)	0.44 ± 0.02	0.44 ± 0.05	0.49 ± 0.03	0.44 ± 0.06	0.47 ± 0.04	0.45 ± 0.02	0.46 ± 0.04	0.43 ± 0.01
Brain (g)	1.41 ± 0.02	1.48 ± 0.01	1.33 ± 0.05	1.37 ± 0.04	1.41 ± 0.04	1.45 ± 0.07	1.49 ± 0.05	1.50 ± 0.04

Values are expressed as mean ± S.E.M. (n=5 for main study and reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 3. Effect of TO on haematological parameters.

Treatment	WBC (10^9/L)	Hb (g/dL)	RBC (10^{12}/L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW-CV (%)	RDW-SD (fL)	PLT (10^9)	MPV (fL)	PDW (fL)	PCV (%)
Distilled water (Main)	4.78 ± 0.62	9.18 ± 1.99	5.10 ± 0.92	27.90 ± 5.77	54.20 ± 1.36	17.68 ± 0.63	32.70 ± 0.35	15.43 ± 0.90	25.73 ± 3.91	417.75 ± 45.83	7.75 ± 0.35	15.65 ± 0.30	0.32 ± 0.03
Reversibility	6.38 ± 0.43	10.16 ± 1.67	6.21 ± 0.76	31.23 ± 2.43	56.30 ± 0.35	17.48 ± 0.56	32.87 ± 0.55	16.44 ± 0.30	27.63 ± 0.64	647.50 ± 35.84	8.45± 0.36	16.43 ± 0.32	0.35 ± 0.02
TO 80 mg/kg (Main)	8.15 ± 1.83	12.23 ± 0.66*	6.71 ± 0.38	36.88 ± 1.85	55.10 ± 1.50	18.18 ± 0.14	33.08 ± 0.11	14.63 ± 0.42	23.40 ± 6.54	624.25 ± 57.16	7.83± 0.23	15.93 ± 0.17	0.49 ± 0.05*
Reversibility	10.22 ± 0.86	13.45 ± 0.64	7.12 ± 0.23	37.34 ± 1.23	58.10 ± 1.40	18.26 ± 0.16	33.56 ± 0.23	15.83 ± 0.23	26.40 ± 0.59	824.36 ± 22.14	8.86 ± 0.22	18.23 ± 0.14	0.49 ± 0.06
TO 400 mg/kg (Main)	7.53 ± 2.73	11.13 ± 1.00*	6.13 ± 0.51	33.48 ± 2.97	54.63 ± 1.59	18.07 ± 0.60	33.13 ± 0.23	14.88 ± 0.37	29.93 ± 0.59	480.50 ± 152.19	7.50 ± 0.21	15.55 ± 0.24	7.53 ± 2.73
Reversibility	9.83 ± 1.64	12.23 ± 0.12	6.88 ± 0.44	35.47 ± 2.57	55.65 ± 1.86	18.47 ± 0.56	33.45 ± 0.20	15.80 ± 0.17	30.47 ± 0.50	640.50 ± 12.18	8.52 ± 0.51	17.44 ± 0.24	0.46 ± 0.01
TO 2000 mg/kg (Main)	5.53 ± 1.63	9.78 ± 1.71	5.33 ± 0.46	29.23 ± 2.39	54.74 ± 0.52	18.33 ± 0.03	33.38 ± 0.46	13.70 ± 0.46*	29.13 ± 1.07	430.00 ± 30.65	7.68 ± 0.28	5.53 ± 1.63	9.78 ± 1.71
Reversibility	7.56 ± 0.83	10.95 ± 1.46	5.93 ± 0.35	32.42 ± 3.64	5642 ± 0.66	18.63 ± 0.13	33.88 ± 0.26	14.89 ± 0.60	30.23 ± 1.01	643.00 ± 13.44	8.36 ± 0.42	18.65 ± 0.24	0.36 ± 0.02

Values are expressed as mean ± S.E.M. (n=5 for main study and reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 4. Effect of TO on biochemical parameters.

	Control		80 mg/kg TO		400 mg/kg TO		2000 mg/kg TO	
	Main	**Reversibility**	**Main**	**Reversibility**	**Main**	**Reversibility**	**Main**	**Reversibility**
ALP (IU/L)	139.2 0± 0.41	142.22 ± 0.32	190.33 ± 3.31*	139.43 ± 1.24	216.70 ± 18.48*	140.12 ± 4.55	230.75 ± 26.19*	145.00 ± 2.69
AST (IU/L)	130.4 0± 4.23	141.22 ± 2.30	125.00 ± 3.41	131.20 ± 1.28	110.68 ± 2.53***	126.54 ± 2.32	112.86 ± 2.36**	122.43 ± 2.04
ALT (IU/L)	57.35 ± 2.01	62.21 ± 1.22	60.85 ± 0.57	59.40 ± 0.26	48.45 ± 2.27*	53.21 ± 2.34	48.53 ± 2.54***	51.45 ± 1.34
Total Bilirubin (µmol/L)	5.85 ± 0.10	5.10 ± 0.11	6.08 ± 0.10	4.21 ± 0.20	6.38 ± 0.10*	4.28 ± 0.13	6.67 ± 0.08*	4.57 ± 0.27
Albumin (g/L)	40.30 ± 1.76	44.00 ± 0.64	41.08 ± 1.31	45.34 ± 1.23	41.22 ± 2.21	46.12 ± 2.23	40.40 ± 3.71	44.30 ± 2.67
Total Protein (g/L)	78.25 ± 0.63	80.12 ± 0.64	81.93 ± 0.62**	81.41 ± 0.54	82.55 ± 0.24**	81.24 ± 0.12	81.25 ± 0.55*	81.25 ± 0.55*
Cholesterol (mg/dl)	2.03 ± 0.06	2.13 ± 0.10	1.93 ± 0.01	1.95 ± 0.08	1.72 ± 0.05*	1.83 ± 0.12	1.90 ± 0.09	1.92 ± 0.13
TG (mg/dl)	1.00 ± 0.09	1.12 ± 0.07	0.91 ± 0.14	0.96 ± 0.01	0.83 ± 0.05	0.90 ± 0.10	0.75 ± 0.01	0.85 ± 0.05
HDL (mmol/L	1.74 ± 0.05	1.85 ± 0.07	1.99 ± 0.04*	1.94 ± 0.10	1.87 ± 0.03*	1.90 ± 0.01	1.97 ± 0.08*	1.96 ± 0.13
LDL (mmol/L)	0.69 ± 0.01	0.74 ± 0.02	0.67 ± 0.02	0.69 ± 0.04	0.58 ± 0.07	0.68 ± 0.10	0.59 ± 0.03	0.67 ± 0.02

Values are expressed as mean ± S.E.M. (n=5 for main study and n=3 for reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 5. Effect of TO on serum creatinine, urea and electrolytes.

	Control		80 mg/kg TO		400 mg/kg TO		2000 mg/kg TO	
	Main	**Reversibility**	**Main**	**Reversibility**	**Main**	**Reversibility**	**Main**	**Reversibility**
Creatinine (µmol/L)	55.11 ± 0.61	57.31 ± 0.54	52.08 ± 2.51	54.66 ± 0.41	46.92 ± 0.64*	47.89 ± 0.45	48.02 ± 2.14*	50.21 ± 0.67
Urea (mmol/L)	6.10 ± 0.37	6.68 ± 0.56	4.95 ± 0.06*	5.76 ± 0.12	5.95 ± 0.06	6.25 ± 0.05	6.23 ± 0.52	6.44 ± 0.02
Na^+ (mmol/L)	139.00 ± 4.80	136.00 ± 2.05	135.75 ± 4.89	134.70 ± 2.65	136.25 ± 6.06	135.45 ± 3.06	137.00 ± 2.35	134.00 ± 2.30
Ca^{2+} (mg/dl)	2.39 ± 0.05	2.44 ± 0.04	2.54 ± 0.06	2.50 ± 0.05	2.46 ± 0.06	2.44 ± 0.02	2.92 ± 0.23*	2.62 ± 0.23
K^+ (mmol/L)	4.50 ± 0.22	4.30 ± 0.26	4.43 ± 0.31	4.23 ± 0.11	4.50 ± 0.54	4.20 ± 0.21	4.33 ± 0.42	4.13 ± 0.47
HCO_3^{2-} (mmol/L)	19.00 ± 0.92	19.86 ± 0.42	19.50 ± 1.19	19.64 ± 0.66	17.50 ± 0.65	19.50 ± 0.22	18.25 ± 1.38	19.20 ± 1.46
PO_4^{2-} (mmol/L)	0.97 ± 1.07	0.95 ± 1.00	1.07 ± 0.08	1.10 ± 0.02	1.04 ± 0.03*	1.07 ± 0.05	0.96 ± 0.01	1.01 ± 0.02
Cl^- (mmol/L)	105.00 ± 4.92	107.00 ± 0.94	107.00 ± 11.37	106.00 ± 1.23	111.62 ± 5.32	108.62 ± 0.32	116.50 ± 14.16	110.50 ± 1.10

Values are expressed as mean ± S.E.M. (n=5 for main study and n=3 for reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 6a. Effect of TO on kidney and liver antioxidant indices in rats.

	GSH (nm/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nm/mg protein)	Protein (mg)
Kidney					
Control	48.84±2.04	51.55±3.75	620.88±56.68	0.92±0.05	32.23±1.97
Reversibility	50.44±1.42	52.45±2.10	627.00±6.60	0.88±0.12	33.43±1.01
80 mg/kg TO	50.10±4.20	52.32±2.11	625.68±65.02	0.92±0.06	29.34±0.78
Reversibility	51.10±0.20	53.92±0.19	625.73±5.32	0.85±0.54	28.94±0.06
400 mg/kg TO	52.86±5.46	53.22±2.38	633.22±65.48	0.91±0.05	27.63±0.93
Reversibility	52.23±0.09	52.82±1.67	630.27±6.21	0.94±0.04	26.88±0.65
2000 mg/kg TO	51.00±2.30	52.93±0.53	628.98±60.48	0.90±0.06	30.19±0.77
Reversibility	49.60±1.32	50.84±0.06	625.77±0.69	0.91±0.12	29.80±0.40
Liver					
Control	20.59±3.87	57.48±2.09	693.12±15.21	2.35±0.15	32.40±0.88
Reversibility	22.34±2.54	65.68±3.11	700.22±9.23	2.12±0.21	34.23±0.56
80 mg/kg TO	23.90±1.50	69.63±11.55	693.91±8.85	1.95±0.06	28.23±0.64
Reversibility	24.45±1.50	71.03±6.50	695.11±4.09	1.98±0.11	29.23±0.34
400 mg/kg TO	58.49±1.07	110.96±7.40*	705.91±10.61	1.75±0.15	25.18±0.57
Reversibility	45.39±1.12	102.23±4.23**	702.21±1.61	1.87±0.08	28.08±0.04
2000 mg/kg TO	40.07±7.58*	104.13±0.19*	705.78±8.30	1.82±0.40	31.81±0.40
Reversibility	36.26±3.45**	89.12±1.22	703.38±2.40	1.90±0.52	33.22±0.45

Values are expressed as mean ± S.E.M. (n=5 for main study and n=2 for reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 6b: Effect of TO on testes and brain antioxidant indices in rats.

	GSH (nm/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nm/mg protein)	Protein (mg)
Testes					
Control	48.30±8.10	88.87±6.67	599.69±56.29	0.92±0.01	33.09±0.42
Reversibility	51.35±2.21	91.12±12.45	612.24±5.90	0.85±0.03	35.00±0.56
80 mg/kg TO	50.05±4.45	89.85±4.85	587.22±44.01	0.89±0.03	29.22±0.42
Reversibility	53.56±3.76	95.05±0.15	606.10±14.01	0.81±0.12	30.28±0.44
400 mg/kg TO	39.40±12.90	73.42±5.81	577.42±44.12	1.58±0.02**	26.30±0.40
Reversibility	45.68±6.23	81.33±2.43	582.56±3.10	1.01±0.01	27.49±1.12
2000 mg/kg TO	25.55±0.90	59.50±5.50*	440.33±225.10	2.08±0.02**	30.58±1.02
Reversibility	47.69±1.88	79.84±6.42	594.29±15.28	1.13±0.04	31.65±0.92
Brain					
Control	20.59±3.87	57.48±2.09	693.12±15.21	2.35±0.15	32.40±0.88
Reversibility	22.34±2.54	65.68±3.11	700.22±9.23	2.12±0.21	34.23±0.56
80 mg/kg TO	23.90±1.50	69.63±11.55	693.91±8.85	1.95±0.06	28.23±0.64
Reversibility	24.45±1.50	71.03±6.50	695.11±4.09	1.98±0.11	29.23±0.34
400 mg/kg TO	58.49±1.07	110.96±7.40*	705.91±10.61	1.75±0.15	25.18±0.57
Reversibility	45.39±1.12	102.23±4.23**	702.21±1.61	1.87±0.08	28.08±0.04
2000 mg/kg TO	40.07±7.58*	104.13±0.19 *	705.78±8.30	1.82±0.40	31.81±0.40
Reversibility	36.26±3.45**	89.12±1.22	703.38±2.40	1.90±0.52	33.22±0.45

Values are expressed as mean ± S.E.M. (n=5 for main study and n=2 for reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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Table 7. Effect of TO on sperm motility, sperm count and morphology (% abnormality).

Treatment	Sperm motility (%)	Sperm count (million/ml)	Morphology (% Abnormality)
Control	68.50±17.00	26.50± 7.84	11.00± 1.00
Reversibility	63.50±5.60	23.50± 2.56	15.62± 4.20
80 mg/kg TO	86.50± 4.99**	34.00± 2.50*	10.50± 4.33
Reversibility	66.20± 2.24	25.30± 3.24	12.40± 5.46
400 mg/kg TO	68.50± 5.80	24.75±3.30	20.00± 2.04
Reversibility	65.50± 3.20	21.44±1.20	20.00± 2.04
2000 mg/kg TO	26.25± 8.54***	15.00± 4.06*	38.75± 0.6
Reversibility	61.45± 6.53	21.50± 5.02	22.45± 1.46

Values are expressed as mean ± S.E.M. (n=5 for main study and n=3 for reversibility study). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control (one-way ANOVA with Tukey's multiple comparison test).

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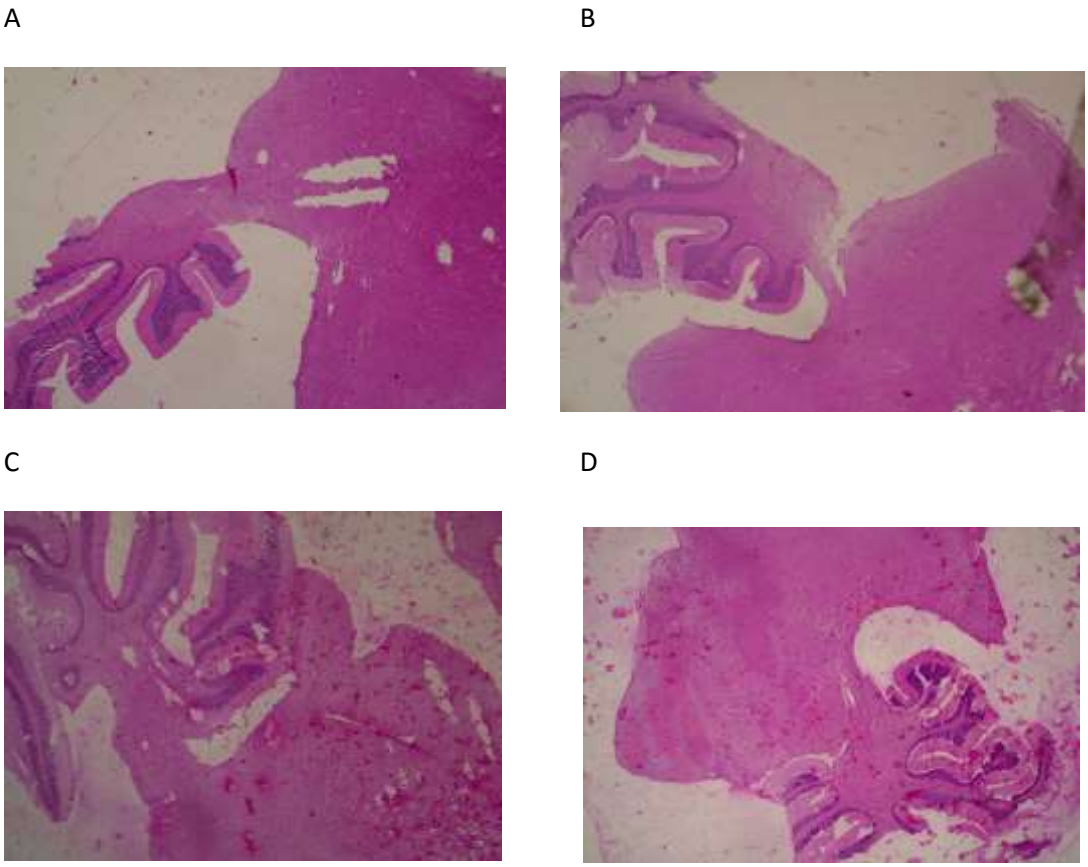

Figure 1. Histopathological presentations of rat brain. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg TO extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (cerebral oedema) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (Normal) (× 400).

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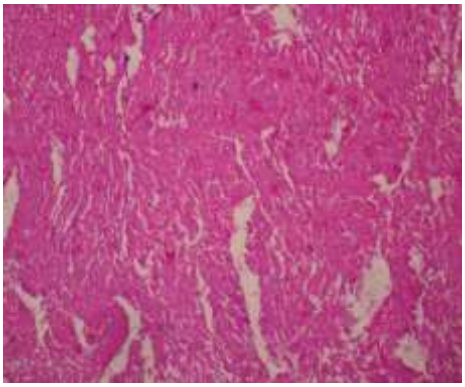
A



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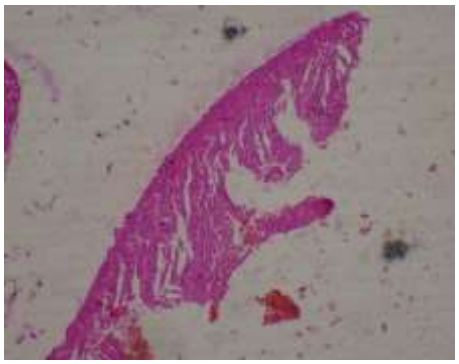

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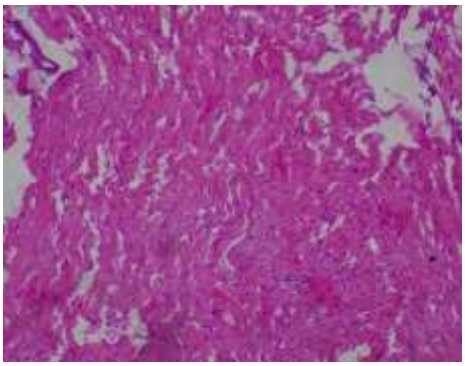

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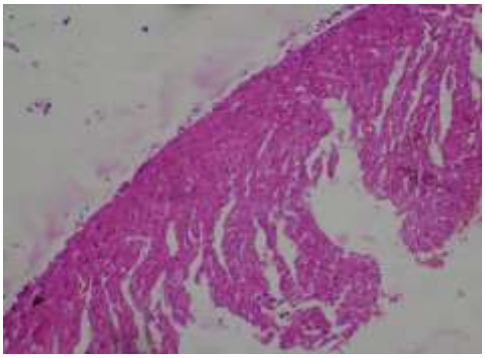

Figure 2. Histopathological presentation of rat heart. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg of *T. occidental* extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (Normal) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (Normal)(× 400).

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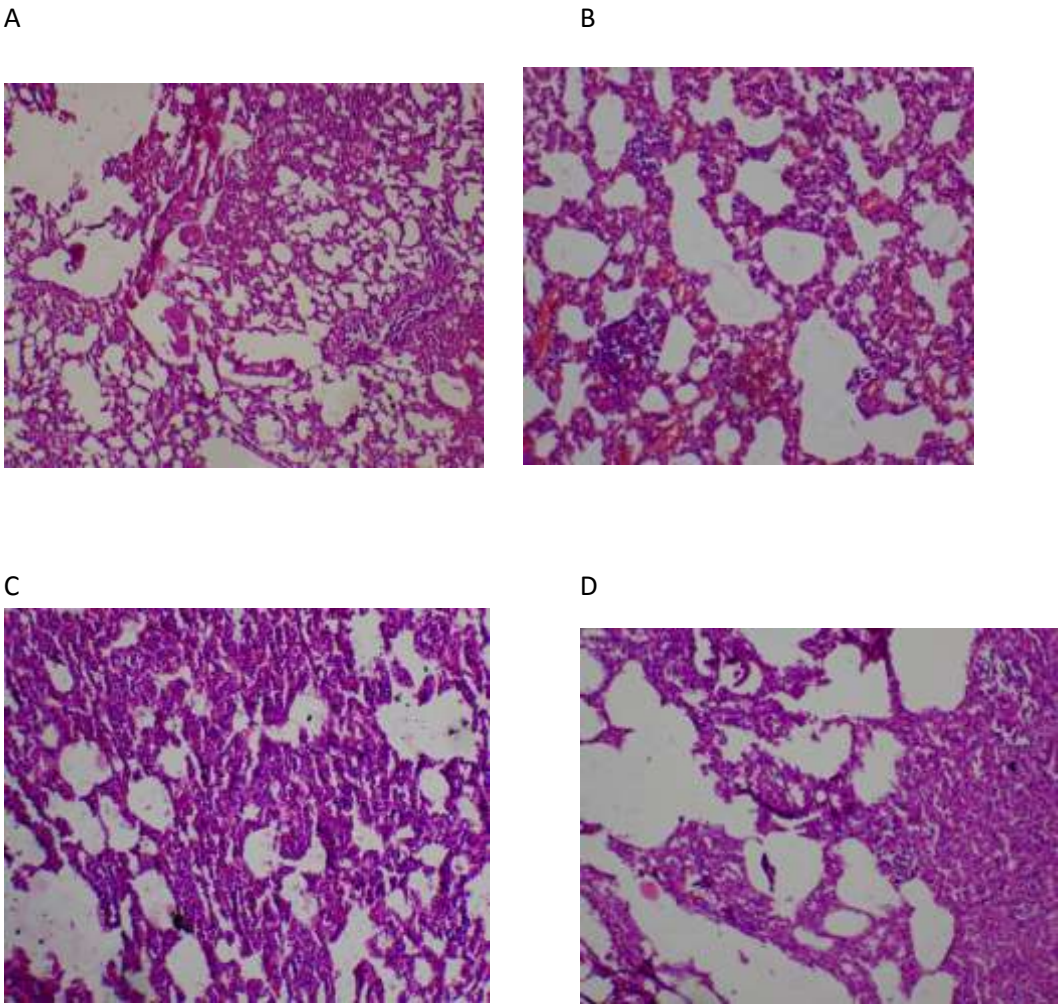

Figure 3. Histopathological presentation of rat lungs. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg *T. occidentalis* extract (interstitial hemorrhage), C represents group treated with 400 mg/kg *T. occidentalis* extract (interstitial inflammation) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (interstitial inflammation) (× 400)

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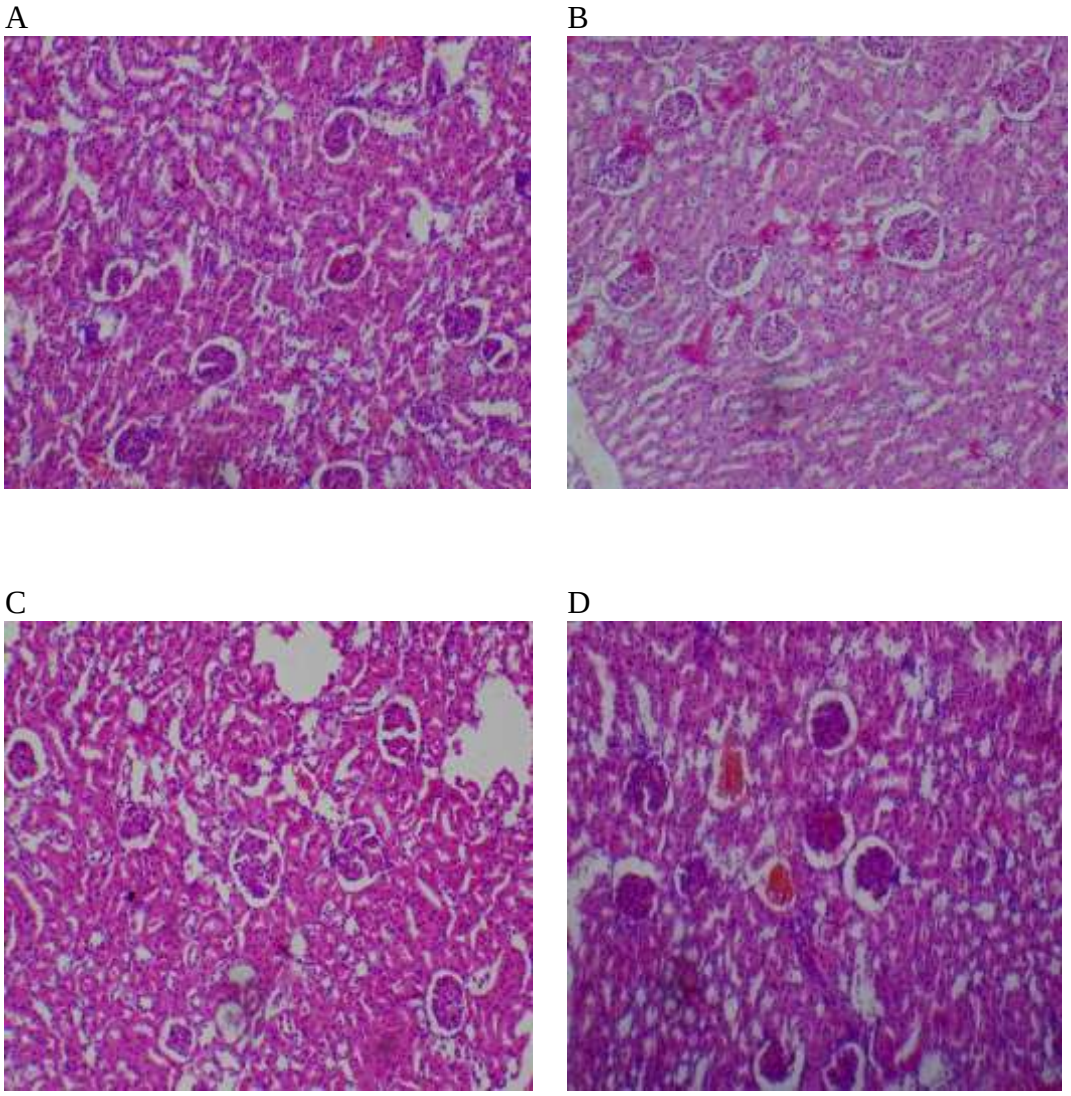

Figure 4. Histopathological presentation of rat kidney. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg *T. occidentalis* extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (Normal) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (Normal) (× 400).

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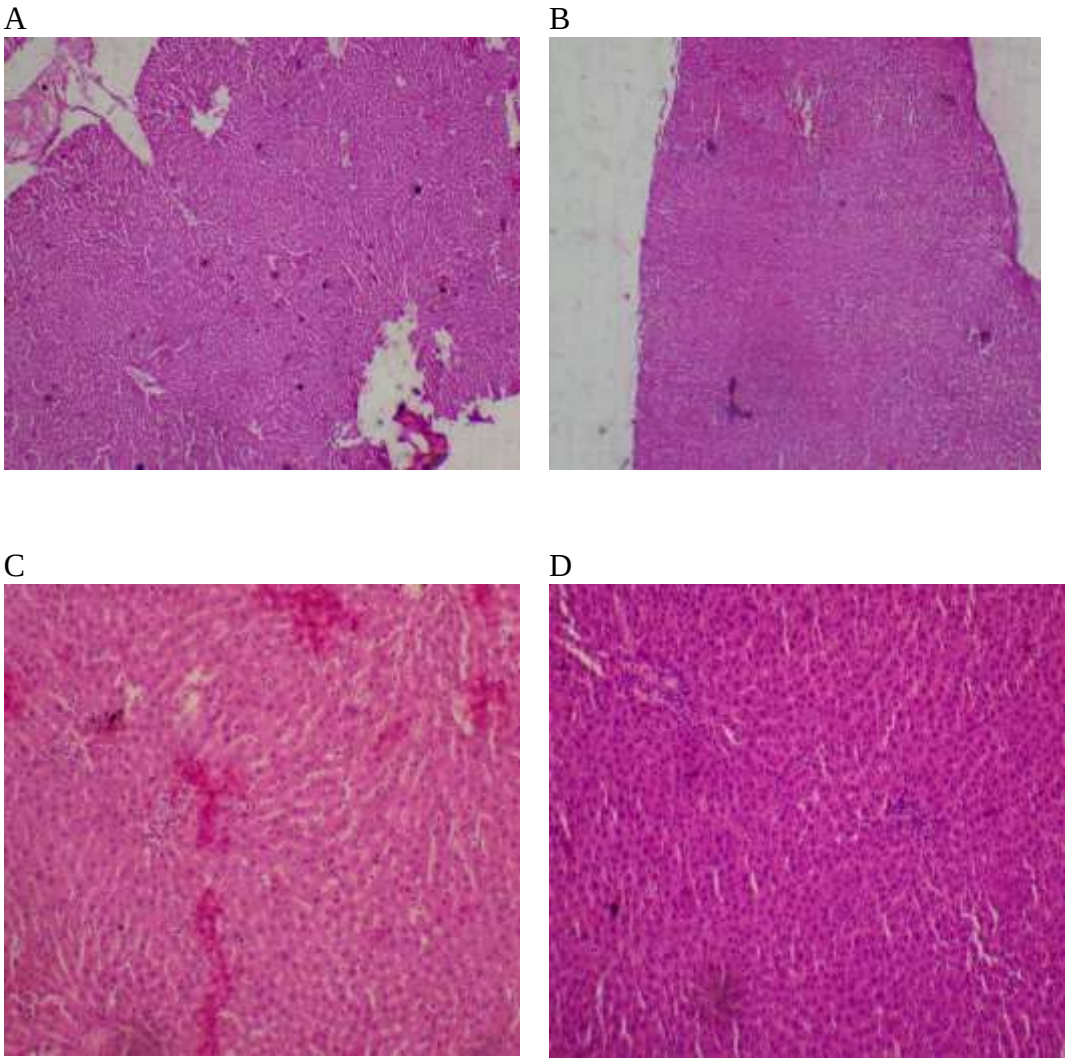

Figure 5. Histopathological presentation of rat liver. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg *T. occidentalis* extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (normal) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (Normal) (× 400).

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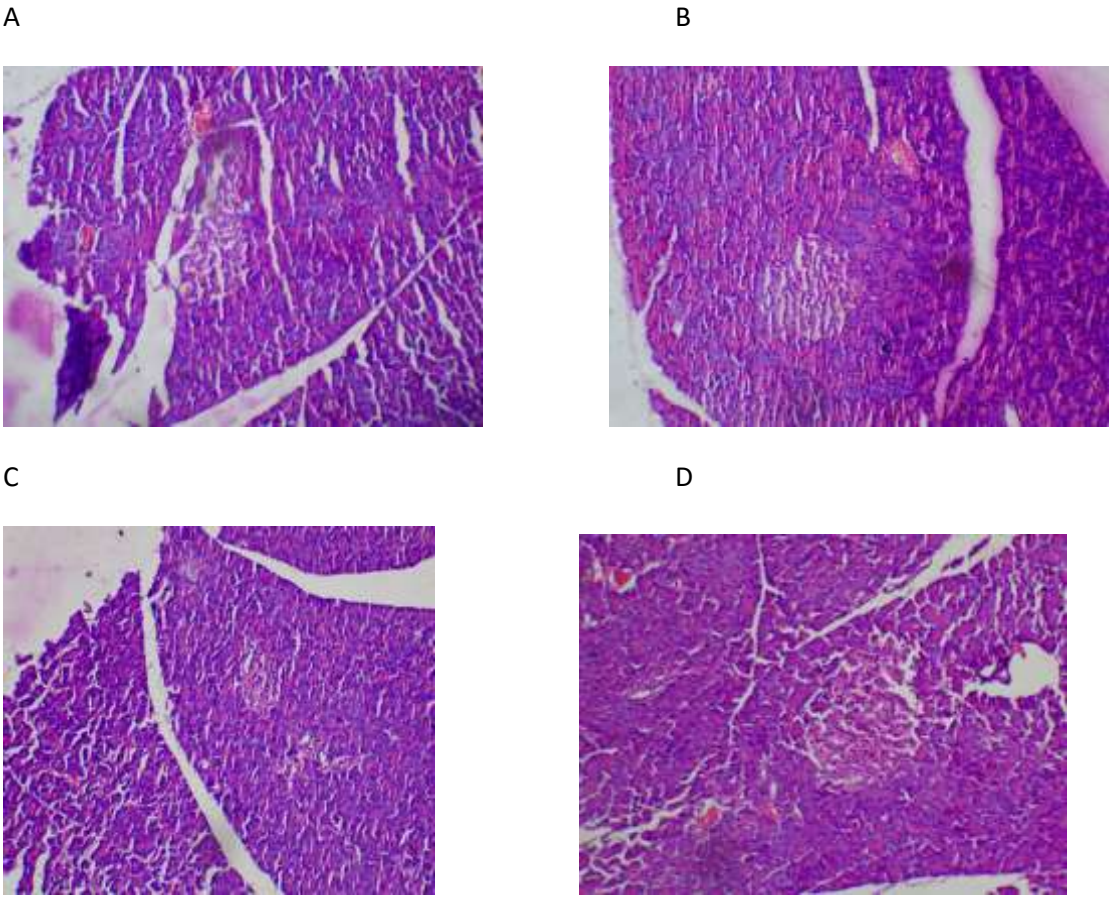

Figure 6. Histopathological presentation of rat pancreas. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg *T. occidentals* extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (Normal) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (Normal) (× 400).

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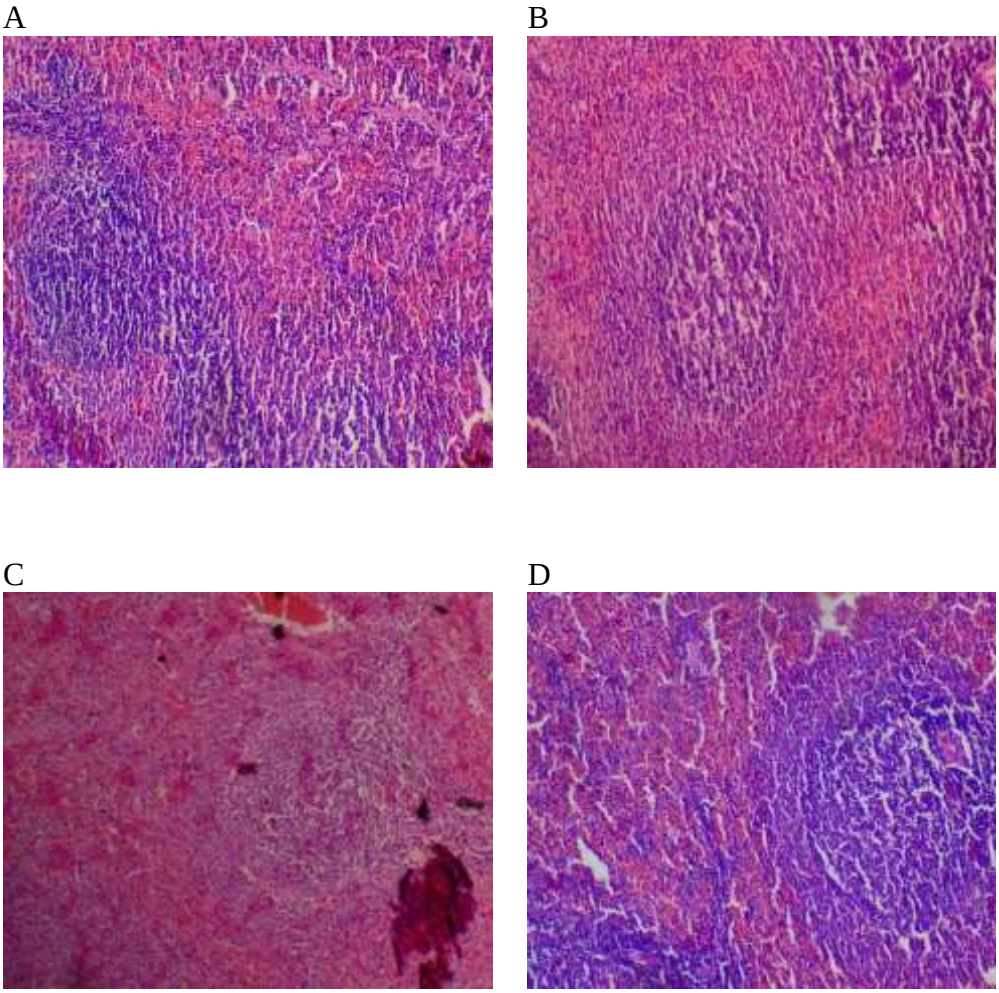

Figure 7. Histopathological presentation of rat spleen. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg *T. occidentalis* extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (Normal) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (Normal) (× 400).

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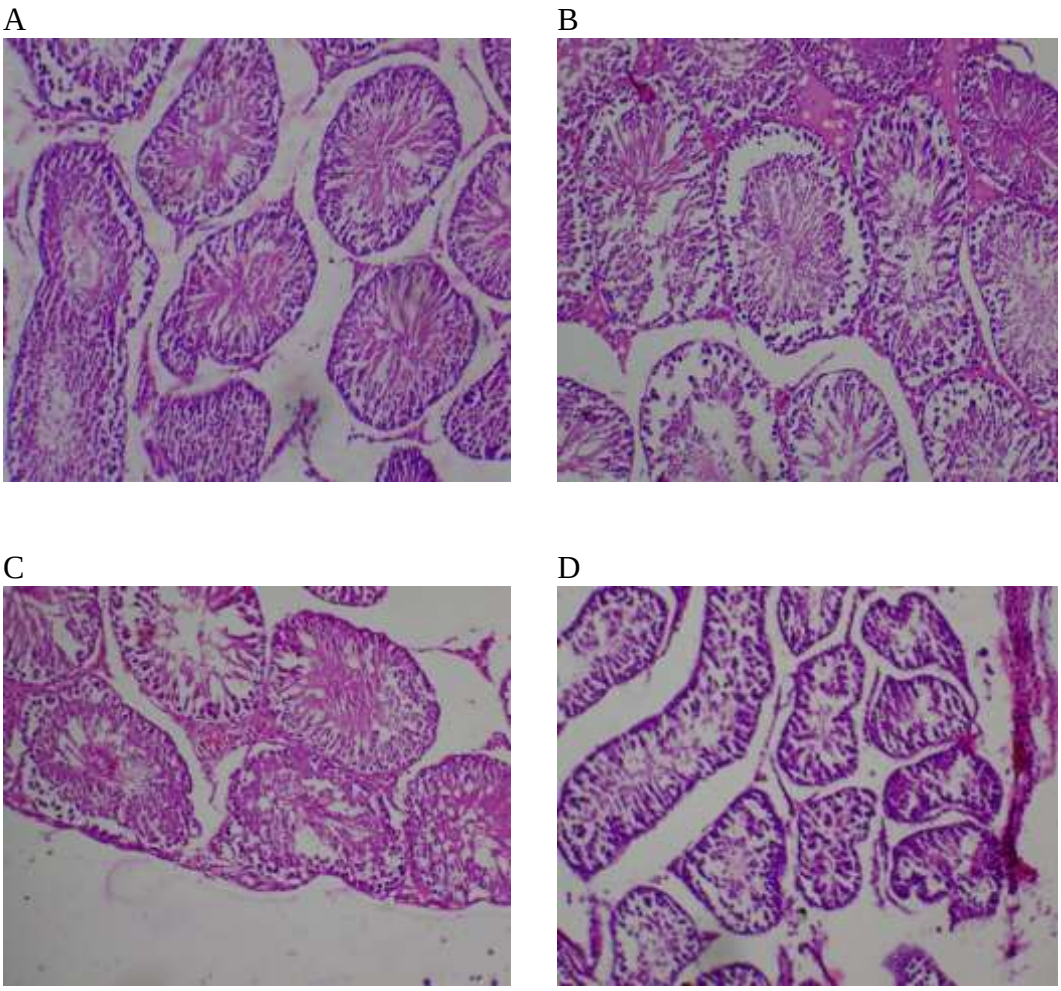

Figure 8. Histopathological presentation of rat testes. A represents control treated with distilled water (Normal), B represents group treated with 80 mg/kg *T. occidentals* extract (Normal), C represents group treated with 400 mg/kg *T. occidentalis* extract (Normal) and D represents group treated with 2000 mg/kg *T. occidentalis* extract (mild testicular atrophy, spermatogenic series lining diminished, no luminal spermatozoa) (× 400).