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The toxicological assessment of ethanolic whole-plant extract of *Eleucine indica* in Wistar albino rats

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ABSTRACT

Introduction: *Eleucine indica* is a medicinal plant used by the Ibibios of Nigeria in the treatment of malaria but its safety with chronic use has not been determined. This study was to evaluate the toxicological effects of the extract in adult albino Wistar rats.
Methods: The rats of both sexes were randomized into 5 groups of 6 animals per group and orally administered with extract (200, 400 and 600 mg/kg) for groups 2–4, respectively. Group 1 received distilled water (10 mL/kg) orally and served as negative control while group 5 was administered with 100 mg/kg of silymarin orally. Drugs were administered on alternate days for 28 days at 09.00 AM. Toxic manifestations and mortality were monitored daily and weight changes of animals were recorded every week. On day 29, after an overnight fast, the animals were weighed, anaesthetized with light chloroform. An autopsy was performed during which any macroscopic abnormalities were noted. The brain, heart, liver, spleen, kidney and lungs were weighed immediately after removal. Samples of these organs were fixed in 10% formalin and kept in that solution for further histopathological examination. Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison post-test.
Results: The results showed that organ weights were not affected but animal weights increased significantly ($P<0.01$-0.001). Relative organ weights were not affected. The extract caused, at low doses, slight inflammation of the liver, spleen, lungs, kidneys and brain. With high dose of the extract, the spleen and lungs showed moderate inflammation. The lungs also showed moderate interstitial fibrosis.
Conclusion: Based on these results, the plant has a potential to damage the lungs when used on the long term. Its use as herbal remedy should be for short periods at a time.

Implication for health policy/practice/research/medical education:
Eleucine indica may lead to inflammation of organs and interstitial fibrosis of the lungs if used on the long term.
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Introduction

Eleucine indica is an annual plant growing to 0.45 m. It is in flower from July to August and the seeds ripen from August to October. The flowers are monaceous (individual flowers are either male or female, but both sexes can be found on the same plant) and are polinated by wind. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well drained soil. The plant prefers acid, neutral and basic (alkaline) soil. It cannot grow in the shade. It requires much soil mostly cultivated beds, for habitats and possible locations (1).

This plant is used for the treatment of malaria among the Ibibios of Southern Nigeria. The whole plant, especially the root, is depurative, diuretic, febrifuge and laxative, and hence is used for the treatment of influenza, hypertension, oliguria and urinary retention (2). It is also used for kidney problems in Trinidad and Tobago (3). The seed is sometimes used as a famine food as well as in the treatment of liver complaints (4). Two main flavonoids have been isolated: schaftoside (6-C-β-glucopyranosyl-8-C-α-arabinopyranosylapigenin) and vitexin (8- C-β-glucopyranosylapigenin) based on ^{1}H and ^{13}C NMR

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spectra and found to have strong anti-inflammatory activities (5). *Eleucine indica* has been reported to have phytochemical content of sterol glucoside forms (6) and C-glycosyl-flavone possessing anti-inflammatory activities (7). *E. indica* leaves are reported to have good bactericidal activity towards methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis*, antioxidant and non-cytotoxic properties (4). It is native in the tropics and subtropical regions (8). It is in flower from July to August and the seeds ripen from August to October (1). The whole plant, especially the root, is depurative, diuretic, febrifuge and laxative. It is used in treatment of influenza, and hypertension, oliguria and urinary retention (2) and kidney problems in Trinidad and Tobago (3). The seed is sometimes used as a famine food and in treatment of liver complaints (4). This plant is used for the treatment of malaria among the Ibibios of Southern Nigeria. It has good bactericidal activity towards methicillin-resistant *staph aureus* (MRSA), *B. Subtilis*, *P. aeruginosa* and *S. choleraesuis*, antioxidant and non-cytotoxic properties (4) and antiplasmodial potentials (9). The phytochemical screening indicated the presence of alkaloids, tannins, flavonoids, cardiac glycosides, terpenes and simple sugar. The median lethal dose (LD_{50}) was determined to be 2090 ± 0.01 mg/kg. It has analgesic and anti-inflammatory activities (10), antipyretic and antioxidant properties (11) and anticonvulsant potentials (12). This study was aimed at evaluating the toxicological effects of the plant on various organs of the body in order to provide information about its safety.

Materials and Methods

Collection and identification of plant sample

Whole plant material of *E. indica* (leaf, stem and root) was collected in 2009 from Uyo, Akwa Ibom State and identified and authenticated by Prof. (Mrs.) Margaret Bassey, Dept. of Botany and Ecological Studies, University of Uyo, where a voucher specimen (UUH 1409) was deposited. Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, granted approval for animal use.

Animal stock

Adult albino rats from the Animal House of the University of Jos were housed in the Animal House, Faculty of Pharmacy, University of Uyo and fed with growers pellet Feed (Bendel Feeds and Flour mills Ltd, Edo State). Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, granted approval for animal use.

Histopathology studies

Using the method of Nongporn et al (13) and Diallo et al, (14), adult albino rats (140-200 g) of both sexes were weighed and randomized into 5 groups of 6 animals per group. Group 1 received 10 mL/kg of distilled water orally, and served as control. Groups 2-4 received the extract at 200-600 mg/kg orally respectively. Group 5 animals received 100 mg/kg of silymarin orally. Drugs were administered on alternate days for 28 days at 09.00 AM. Toxic manifestations and mortality were monitored daily and weight changes of animals were recorded every week. On day 29, after an overnight fast, the animals were weighed, anaesthetized with light chloroform. An autopsy was performed during which any macroscopic abnormalities were noted. The brain, heart, liver, spleen, kidney and lungs were weighed immediately after removal. Samples of these organs were fixed in 10% formalin and kept in that solution for further histopathological examinations by a Consultant Pathologist at the University of Uyo Teaching Hospital.

On the histopathology analysis, the fixed liver, kidney, lungs, brain and spleen and kidney tissues were sectioned (5-micron in thickness) and embedded in paraffin. Sections were then stained with hematoxylin and eosin. This was followed by light microscopic examination of multiple tissue sections from each organ in all groups. Representative images of the typical histological profile were examined. Changes observed were graded as follows: (0) showing no changes, and (1) (2) and (3) indicating mild, moderate and severe changes, respectively, while the grading was determined by percentage as follows: Changes less than 30% (50%) showing severe changes. Changes less than 30%, mild; 30%-50%, moderate; more than 50%; severe (15,16).

Statistical Analysis

Results were expressed as multiple comparisons of mean ± standard error of the mean (SEM). Significance was determined using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison post-test. A probability level of less than 5% was considered significant. GraphPad InStat software was used for all data analysis.

Results

Effect of extract on body weight of animals

Measurements of the body weight of the animals throughout the experimental period showed significant differences between the treated and control groups. The animals all gained weight as the experiment progressed. The increase in weights was reflected in all groups treated with extract relative to control. This increase was statistically significant ($P < 0.01–0.001$) as shown in Table 1.

Effect of *Eleucine indica* extract on relative weight of organs

There were no significant changes in the organ weights of extract-treated groups compared to control (Table 2).

Histopathology

There was only slight inflammation of the liver (Figure 1), spleen (Figure 2), Brain (Figure 3), kidneys (Figure 4) and lungs (Figure 5), with low doses of the extract. However, with high dose of the extract, the spleen and lungs showed moderate inflammation. The lungs also showed mild

Table 1. Effect of subchronic toxicity of *Eleucine indica* on body weights of rats

Dose (mg/kg)	Week				
	0	1	2	3	4
Control	168.67 ± 0.14	184.00 ± 0.10[d]	192.17 ± 0.13[d]	196.17 ± 0.14[d]	199.00 ± 0.50[d]
Extract 200	160.00 ± 0.22[c]	177.83 ± 0.21[c d]	189.33 ± 0.05[c d]	194.83 ± 2.07[d]	197.33 ± 0.06[d]
Extract 400	164.83 ± 0.35[c]	180.00 ± 0.20[c d]	193.17 ± 0.13[d]	199.50 ± 0.22[d]	202.17 ± 1.55[d]
Extract 600	163.83 ± 0.11[c]	180.17 ± 0.11[c d]	188.33 ± 0.15[c d]	190.50 ± 0.30[b d]	196.33 ± 1.05[d]
Silymarin 100	160.67 ± 0.31[c]	180.00 ± 0.04[c d]	193.00 ± 0.59[d]	200.33 ± 0.07[a d]	213.33 ± 0.30[c d]

Values represent Mean ± SEM. Significance relative to control [a] $P < 0.05$; [b] $P < 0.01$, [c] $P < 0.001$; [d] $P < 0.001$ (n = 6).

Table 2. Relative organ weight of rats in subchronic administration of *Eleucine indica* extract

Dose (mg/kg)	Liver	Kidney	Spleen	Lung	Heart	Brain
Control	2.67 ± 0.37	0.27 ± 0.39	0.37 ± 0.03	0.56 ± 0.15	0.34 ± 0.07	0.88 ± 0.03
Extract 200	2.63 ± 0.37	0.29 ± 0.03	0.34 ± 0.05	0.63 ± 0.14	0.34 ± 0.05	0.87 ± 0.05
Extract 400	2.51 ± 0.12	0.25 ± 0.05	0.32 ± 0.05	0.64 ± 0.09	0.32 ± 0.04	0.83 ± 0.03
Extract 600	2.57 ± 0.39	0.27 ± 0.03	0.31 ± 0.05	0.65 ± 0.08	0.32 ± 0.01	0.85 ± 0.05
Silymarin 100	2.58 ± 0.26	0.29 ± 0.03	0.34 ± 0.02	0.70 ± 0.14	0.33 ± 0.07	0.82 ± 0.05

Values represent Mean + SEM; n=6.

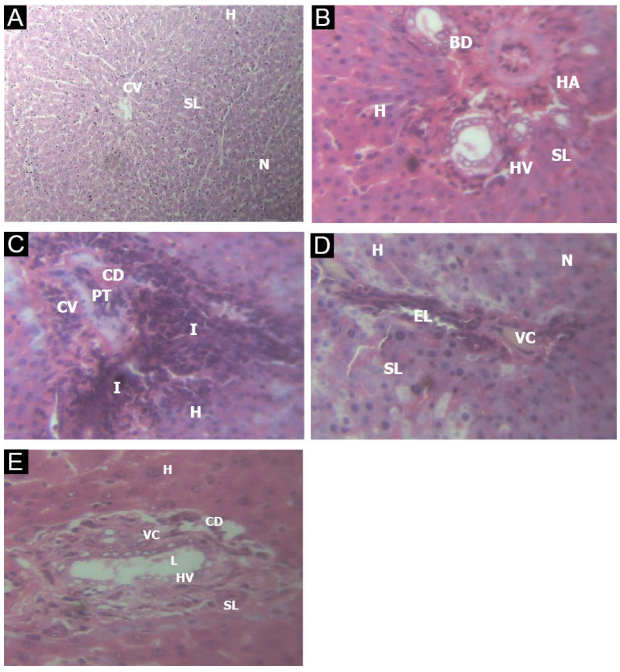

Figure 1. Histopathologic sections of liver (A) Control: Normal; (B) Extract 200 mg/kg x400: Slightly inflamed; (C) Extract 400 mg/kg x400: Slightly inflamed; (D) Extract 600 mg/kg x400: Slightly inflamed; (E) Silymarin 100 mg/kg x400: Slightly inflamed.
CV, central vein; VC, vascular congestion; H, hepatocyte; SL, sinusoidal layer; HA, hepatic artery; HV, hepatic vein; BD, bile-duct; N, nucleus; EL, epithelial lining; L, lumen; PT, portal triad; I, inflammation.

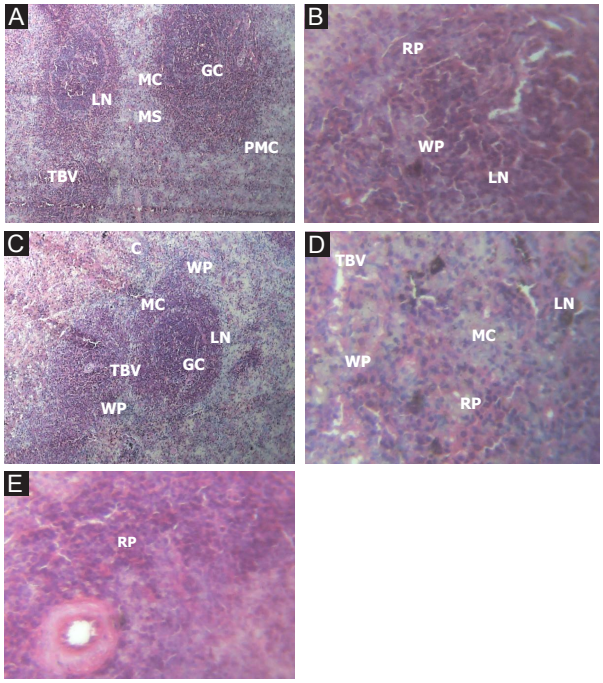

Figure 2. Histopathologic sections of spleen (A) Control: Normal; (B) Extract 200 mg/kg x400: Slightly inflamed; (C) Extract 400 mg/kg x400: Slightly inflamed; (D) Extract 600 mg/kg x400: Moderately inflamed; (E) Silymarin 100 mg/kg x400: Moderately inflamed.
MC, medullary cortex; MS, medullary sinus; LN, lymph node; PMC, polymorphomer cell; N, nucleus; TBV, trabecular blood vessel; WP, white pulp; RP, Red-Pulp; GC, germinal centre; C, cortex.

epithelial degeneration, cellular proliferation and moderate interstitial fibrosis. The heart (Figure 6) was not affected in all groups. All the animals treated with the extract had normal cellular architecture, nucleocytoplasmic ratio and nuclear structure. No necrosis, malignancy, pigment or inclusion bodies were observed.

Discussion

Herbal medicines are generally seen by people as being natural, healthy and without side effects. The chemicals in medicinal plants may be natural to the plant, but foreign to the human body and may be harmful resulting in issues of safety and efficacy (17).

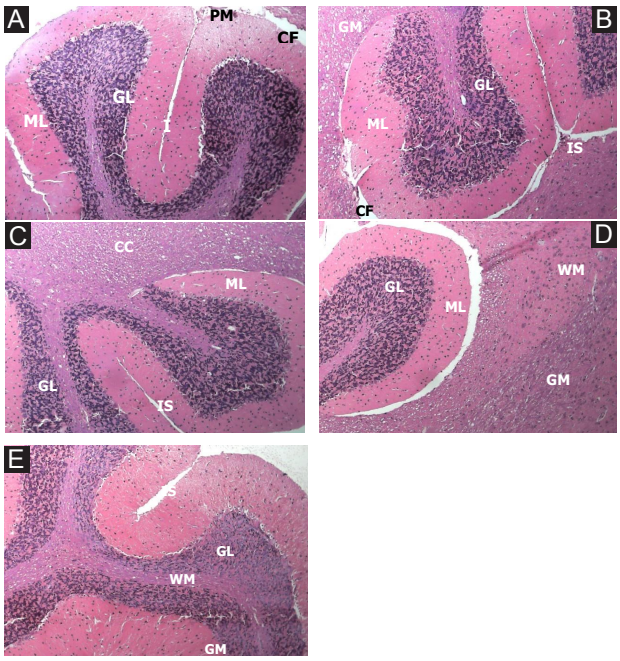

Figure 3. Histopathologic sections of brain (A) Control x100: Normal; (B) Extract 200 mg/kg x100: Slightly inflamed; (C) Extract 400 mg/kg x100: Slightly inflamed; (D) Extract 600 mg/kg x100: Normal architecture; (E) Silymarin 100 mg/kg x100: Slightly inflamed.
ML, molecular layer; GL, granular layer; PC, purkinje cell; BC, basket cells; CC, cerebellar cortex; CF, cerebrospinal folia; IS, interfolia sulci; GM, gray matter; WM, white matter; PM, pia matter.

Drugs and their metabolites can interact with a diverse array of receptors to mediate adverse effects in vivo. Sometimes the parent, unmetabolized drug causes toxicity, but often a metabolite of the drug reacts with proteins, DNA, and oxidative defense molecules (such as glutathione) to cause cellular damage and other adverse reactions. The response to injury after cellular damage is largely determined by the regenerative capacity of the target organ. In organs that are capable of regeneration, such as the liver, repeated insults may be followed by regeneration (18). Over time, however, cellular injury can lead to excessive deposition of collagen and extracellular matrix proteins, causing fibrosis. Organ systems with limited or no regenerative function, such as cardiac and neuronal tissue, lose function as tissue is destroyed. Chronic toxicity in the lungs can be manifested as both loss of function and fibrosis. Liver and kidney are the major organs involved in toxicity (18). The kidney is also susceptible to toxic insult because it concentrates many xenobiotics for excretion. There was only slight inflammation of the liver, spleen, lungs, kidneys and brain with low doses of the extract. However, with high dose of the extract, the spleen and lungs showed moderate inflammation. The lungs also showed mild epithelial degeneration, cellular proliferation and moderate interstitial fibrosis. The heart was not affected in all groups. All the animals treated with the extract had normal cellular architecture, nucleo-

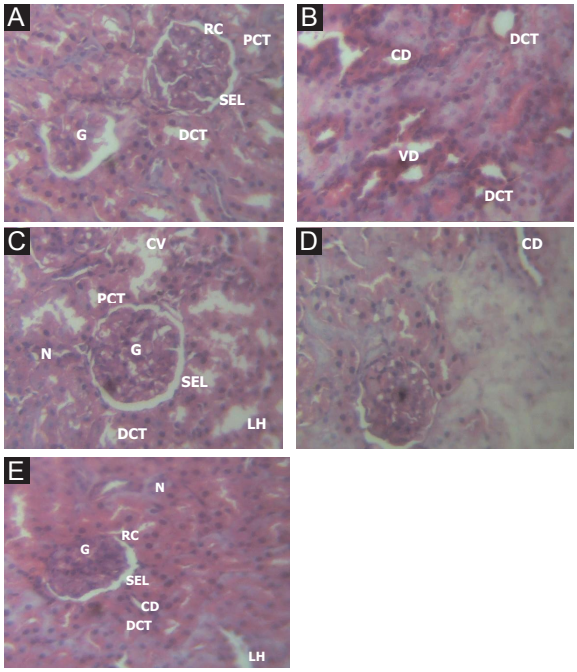

Figure 4. Histopathologic sections of kidneys (A) Control: Normal; (B) Extract 200 mg/kg x400: Slightly inflamed; (C) Extract 400 mg/kg x400: Slightly inflamed; (D) Extract 600 mg/kg x400: Slightly inflamed; (E) Silymarin 100 mg/kg x400: Normal.
DCT, distal convulated tubules; PCT, poximal convulated tubules; G, glomerulus; RC, renal corpuscle; GI, glomerula inflammation; CD, collecting duct; LH, loop of henle; VD, vascular degeneration; SEL, squamous epithelial lining; ELD, epithelial lining degeneration; N, nucleus.

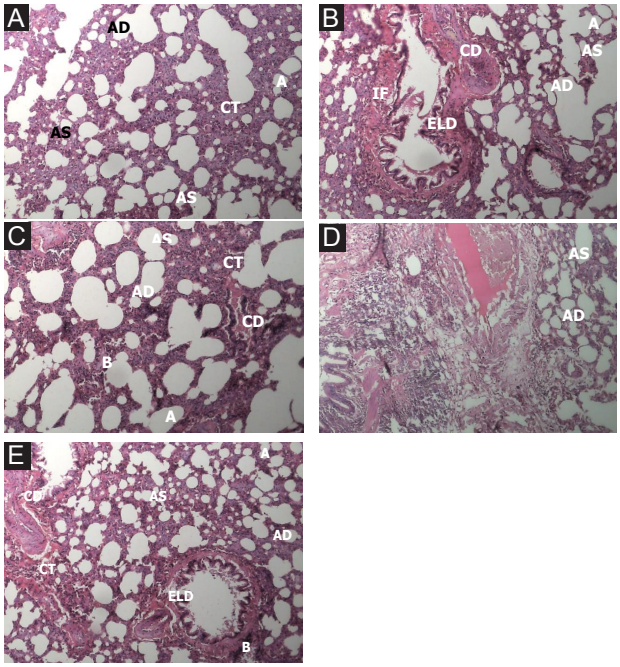

Figure 5. Histopathologic sections of lungs (A) Control: Normal; (B) Extract 200 mg/kg x100: Slight interstitial fibrosis and inflammation; (C) Extract 400 mg/kg x100: Moderate interstitial fibrosis and inflammation; (D) Extract 600 mg/kg x400: Moderate interstitial fibrosis and inflammation (E) Silymarin 100 mg/kg x100: Slight interstitial fibrosis and inflammation
B, bronchus; RB, respiratory bronchiole; AS, air sac; AD, alveolar duct; A, alveolus; ELD, epithelial lining degeneration; VD, vascular degeneration; IF, interstial fibrosis; CD, cellular degeneration; CT, connective tissue.

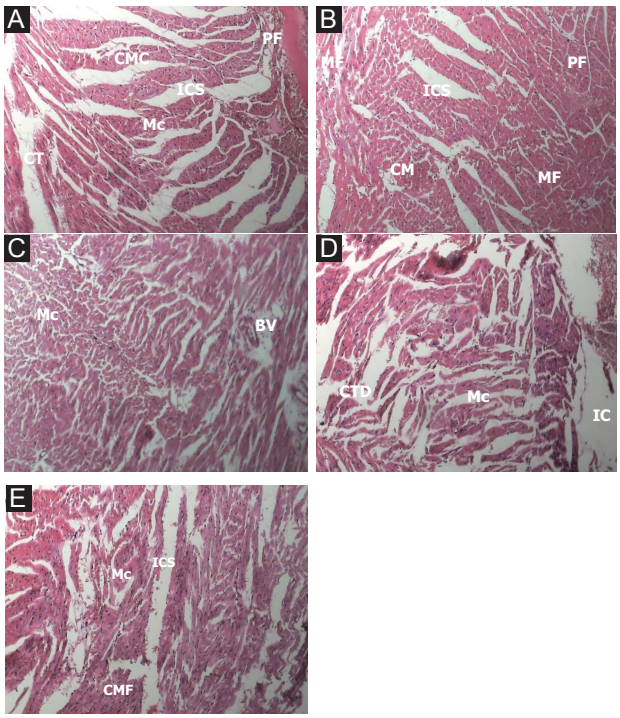

Figure 6. Histopathologic sections of hearts (A) Control: Normal; (B) Extract 200 mg/kg x100: Not affected (Normal architecture); (C) Extract 400 mg/kg x100 Heart: Not affected (Normal architecture); (D) Extract 600 mg/kg x100: (Normal architecture); (E) Silymarin 100 mg/kg x100: Not affected (Normal architecture).
PF, purkinje fibres; ICS, intercelular spaces; CM, cardiac muscle; Mc, myocite; M, myocardium; CMF, cardiac muscle fibre; BV, blood vessel; CT, connective tissue; MF, muscle.

cytoplasmic ratio and nuclear structure. No necrosis, malignancy, pigment or inclusion bodies were observed. The extract caused significant increase in the body weight of the animals compared to control. The increase in body weight is probably due to protein anabolic effect (19,20). Another possible reason of increase in body weight may be the presence of tannins and phenolics in the extract (4,21). It may also be attributed to appetite stimulatory effect of the extract. The regulation of appetite is a complex process involving the gastrointestinal tract, many hormones, aging and both the central and autonomic nervous systems (22). This stimulatory effect may have resulted in increased food and water intake by the extract-treated animals (23). The extract did not cause significant changes in organs of localization such as kidney, liver, spleen, brain and heart which depicted its low toxicity. The liver and kidney which are the most sensitive organs to toxic factors were apparently normal and showed no signs of dysfunction (13). However, the extract caused mild- to- moderate interstitial fibrosis in the lungs. This suggests that the extract should be avoided in patients whose respiratory function is already compromised. The toxicological data obtained from this study are of significance in relation to the consumption of this extract for health benefits especially in the treatment of malaria. Further studies to evaluate the chronic toxicity of this whole-plant extract, is needed to determine its long-term safety.

Conclusion

The extract caused, at low doses, slight inflammation of the liver, spleen, lungs, kidneys and brain. With high dose of the extract, the spleen and lungs showed moderate inflammation. The lungs also showed moderate interstitial fibrosis. The relative organ weights in extract-treated animals were not significantly different from control. It is advisable that those using this plant as herbal remedy should use it for short periods at a time because of its effect on the lungs.

Authors' contributions

EEO did the experiment, analyzed and discussed results, wrote conclusion and prepared the manuscript for publication. NPA designed the work and discussed results. UPE analyzed data and discussed results. UJA discussed the results and helped in data analysis. All read and confirmed the article.

Conflict of interests

We declare that we have no conflict of interest whatsoever.

Ethical considerations

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. All experimental procedures involving animals were conducted in accordance to Organization for Economic Co-operation and Development guidelines and approved by Animal Ethics Committee, Faculty of Pharmacy, University of Uyo.

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