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Histopathological effects of sub-chronic administration of Aqueous and Methanol extracts of Loranthus micranthus leaves parasitic on Parkia biglobosa in rats.

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Abstract

Loranthus micranthus is a plant described as an all-purpose herb because of its rich folkloric uses. It is used indiscriminately in traditional medicine for treatment of life-long illnesses. Therefore, the aim of this study is to investigate the histopathological effects of sub-chronic administration of high doses of aqueous and methanol extracts of *Loranthus micranthus* (African mistletoe) leaves parasitic on *Parkia biglobosa* in male albino rats. Acute toxicity studies were performed according to Lorke´s method. High doses of both extracts (1000 and 2000 mg/kg) were administered daily via the intraperitoneal route to four groups of rats respectively (n=5) for 30 days. The fifth group of rats (n=5) similarly received 5 ml/kg each of normal saline. The liver, kidney and heart of the rats were harvested at the end of the experiment for histological examination. The results of the acute toxicity studies indicated that both extracts are practically safe since the intraperitoneal LD_{50} were greater than 5000 mg/kg in rats. The results revealed mild portal lymphocytic infiltration, mild to moderate portal inflammation and expansion with numerous pyknotic nuclei in the liver of rats treated with the aqueous extract. Both extracts induced cholestatic liver injury in rats. Varying degrees of moderate histopathological findings in the kidney of the treated rats were also seen. There were no morphological changes in the heart tissue of extract treated groups. Though extracts of *L. micranthus* leaves are practically safe under acute administration in rats, caution should be exercised when mistletoes are administered chronically especially at high dose.

Keywords: Loranthus micranthus, histopathology, liver injury, kidney, mistletoe.

Introduction

Medicinal plants constitute an effective source of both traditional and modern medicines and about 80% of rural populations depend on it for their primary health care [1]. Toxicity from botanical compounds has been underestimated due to the perception that drugs made from plants are absolutely safe. However, severe liver

injury has been described after the ingestion of a large variety of different herbal preparations [2]. Determination of efficacy and safety of herbal remedies is necessary as many people use them for self-medication and little data is available about the pharmacology and toxicology for most of the common herbal remedies [3]. Herbal medicine also called botanical medicine or phytomedicine refers to the use of a plant's seed, barriers, roots, leaves, bark or flowers for medicinal purposes [4]. Liver plays an important role in eliminating Gram-positive bacteria like bacillus and their exotoxin from the bloodstream [5] and consequently eliminate risk of bacteria infection. Detoxification of hepatic exotoxin is important and shown by their ability to inhibit hepatic mitochondrial fatty acid oxidation in rat livers and causing liver failure [6]. Although the liver is considered the major organ in drug metabolism and detoxification, the kidneys is also involved in drug elimination through their excretion. Detoxification of drug metabolites can produce different and varying degrees of liver injury, and accumulation of toxin in kidney lead to kidney failure [7. 8].

Loranthus micranthus Linn(family Loranthaceae) known as African mistletoe (Eastern Nigeria specie) is a semi-parasitic evergreen plant which depend on their host tree for minerals and water only but photosynthesize their carbohydrates by means of its green leaves [9]. They grow on a variety of evergreen and deciduous tree all year round, around the branches of the tree. Studies have shown that the composition and activities of the plant is host tree dependent [10, 11]. Leaf extract of Loranthus micranthus linn have been shown to possess anti-inflammatory, anti-diabetic, anti-hypertensive, bactericidal, anti-fungal and anti-cancer effects [12, 13, 14]. Kafaru [15] described mistletoe as "an all-purpose herb" because of its rich folkloric uses. Since it is being consumed commonly, it is necessary to test its safety by examining any possible damage to the important organs following chronic usage. In our previous study on the plant we determined the effect of high doses of the plant extract on the biochemical parameters in rats. We observed that the extracts caused significant biochemical changes which are indicators organ damage [16]. Therefore, the aim of this study is to investigate the histopathological effects of sub-chronic administration of high doses of aqueous and methanol extracts of *Loranthus micranthus* (African mistletoe) leaves in rats.

Materials and Methods

Plant material: Fresh leaves of Loranthus micranthus linn (with African locust bean as host tree) were collected from Agbani, Enugu State, Nigeria and identified by Prof. J. C. Okafor of the department of Botany, Enugu State university of Science and Technology, Enugu. A voucher specimen was deposited at the herbarium of the same department for further reference.

Plant Materials Preparation

Leaves of the plants were dried under shade to constant weight and pulverized with a crush bitter mill, type Ski. One liter of methanol (AR) was used to extract the active ingredients in the fine powder by dispensing the powder in this Solvent for 48hrs with intermittent agitation. After this, the extract were filtered using a whatman No. 12 filter paper and the filtrate exporated to dryness using rotary evaporator. The obtained residue was weighed and stored in the refrigerator until required. Fresh leaves of the plant were also ground into fine paste and powder using a hand manual grinding machine. The content of the fresh leaf paste were extracted through a muslin cloth after the addition of 100mls of clean water. The extract obtained was stored at 4- 8^{o}C until required.

Experimental Animals

Twenty-five albino wistar rats purchased from animal house of University of Nigeria Teachimg Hospital Enugu

were used for the study. They were kept in the animal house of the college of medicine at the University of Nigeria Teaching Hospital Enugu for two weeks to acclimatize before initiating the study. They were maintained at standard laboratory condition of 12 hrs light and 12 hrs dark cycle. They were allowed free access to clean drinking water and were fed on standard pellets ad libitum.

Design of the Experiment

The animals were divided into five (5) groups (A-E) with five (5) animals per group. All the animals were subjected to an overnight fast 8hrs prior to drug administration and given access to food one (1) hr post drug administration. During the period of fast, the animals were allowed free access to clean drinking water only. The animals in groups A and B were given 1000mg and 2000mg/kg body weight of the methanol extract of the Loranthus micranthus linn respectively while those in C and D received 1000mg to 2000mg/kg body weight respectively of the aqueous extract. Group E served as control and received 5ml/kg body weight of normal saline. The plant extract was administered intraperitoneally daily for 30 days. Under ether anaesthesia, the animals were euthanized and the heart, kidney and liver excised and preserved in 10% formol saline for histo- pathological investigation.

Analytical Methods

A. **Acute Toxicity Test (LD50)**

Acute toxicity studies were performed on the aqueous and methanol extract using a variation of the method of Lorke [17] in a two phase process. In the first phase, both extracts of widely differing low doses of 10, 100 and 1000 mg/kg b.w. were administered separately to a group of rats (n= 3). The rats were observed for 24 hrs. Where no death was observed within 24 hrs subsequent high doses of 2000, 3000, 4000, 5000, 6000 and 7000mg/kg b.w were administered to new groups for the second phase. The animals were monitored for 24 hours for changes in behaviour and mortality.

B. **Histopathological Examination**

At the end of the four (4) weeks, the animals were sacrificed and the kidney, liver and heart harvested and fixed in 10% formalin for 24hrs. The tissues were processed in an automatic tissue processor using paraffin wax. Thin sections (about 4-5 microns thick) were made using a rotary microtone and stained by hematoxylin and eosin (H and E) method. These were examined using a light microscope.

Results

Acute Toxicity (LD50) Test

Acute toxicity studies indicated that both extracts had an oral LD_{50} > 5000mg/kg in rats. The result revealed that the extracts were not toxic. There was no visible signs of toxicity in the experimental animals within 24 hours of administration. There was also no physical signs of gross behavioral changes observed in the duration of the study.

Histopathological analysis

Effect of the extracts on the histology of the Liver:

Results of histopathological studies indicate that the liver of the aqueous extract treated groups showed mild portal lymphocytic infiltration, and mild to moderate portal inflammation and expansion with numerous pyknotic nuclei (Fig.1).

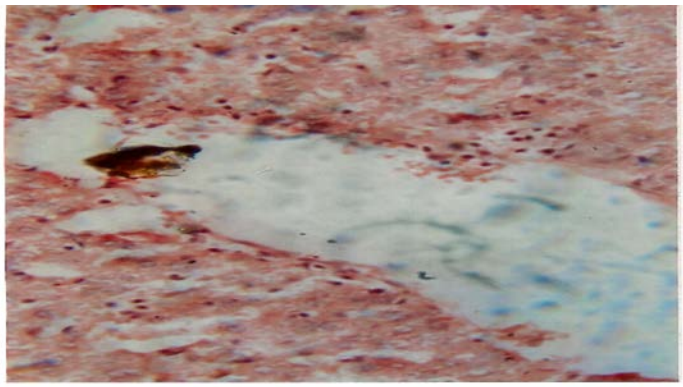

Fig. 1: (H &E x 200): Liver section of the aqueous treated rats showing mild portal lymphocytic infiltration, and mild

to moderate portal inflammation and expansion with numerous pyknotic nuclei.

The liver of the lower dose methanol extract treated groups showed well preserved hepatic architecture, intact portal area, and numerous pyknotic nuclei (fig 2) while the higher dose (2000 mg/kg) revealed normal architecture with mild portal inflammation and fibrosis (Fig. 3).

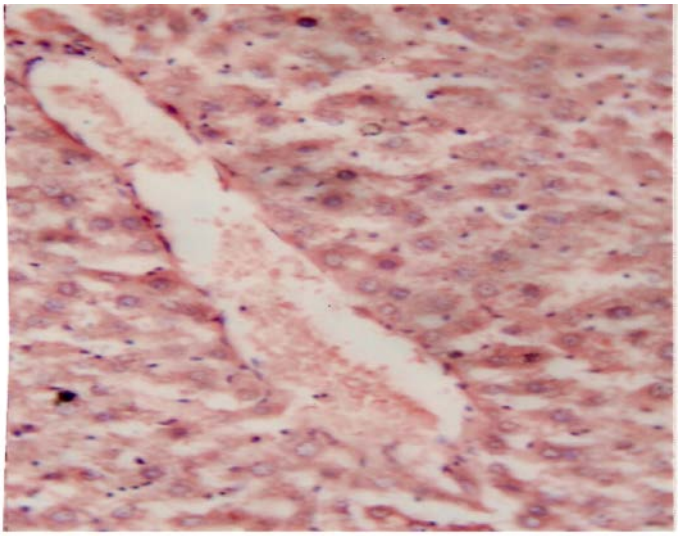

Fig.2: (H & E x 200): Liver section of the methanolic extract (1000mg/kg dose) showing well preserved hepatic architecture, intact portal area and numerous pyknotic nuclei.

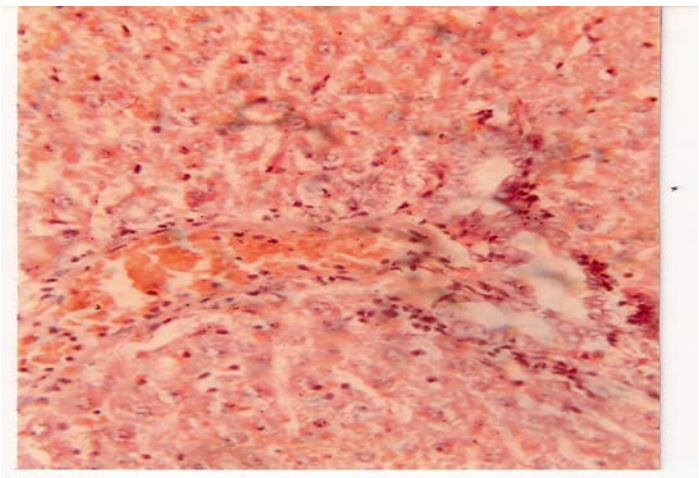

Fig.3 (H & E x 200): Liver section of the methanol extract (2000mg/kg dose) showing normal architecture with mild portal inflammation and fibrosis.

The control group revealed normal hepatic chords (Fig. 4).

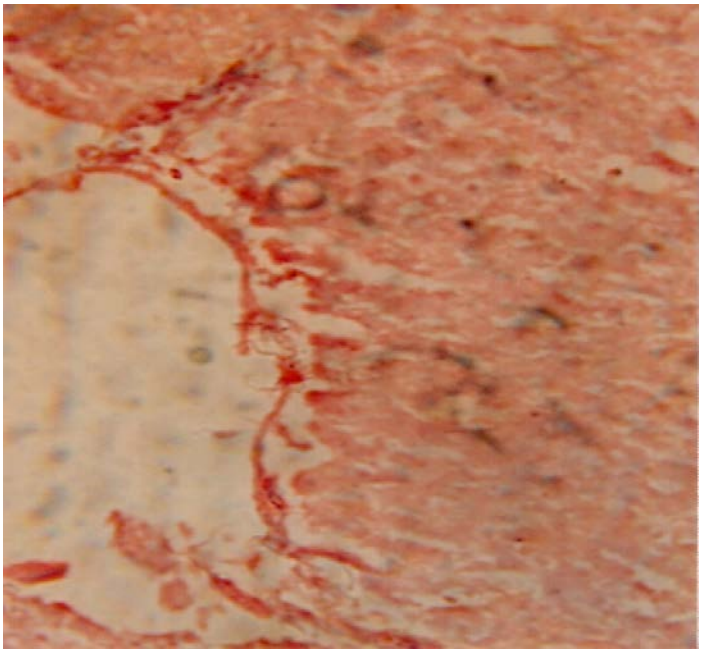

Fig. 4: (H & Ex200): Liver section of the normal control showing normal hepatic chords.

Effect of the extracts on the histology of the Kidney:

There were no morphological changes in the kidneys of the 1000 mg/kg aqueous extract treated group (Fig. 5).

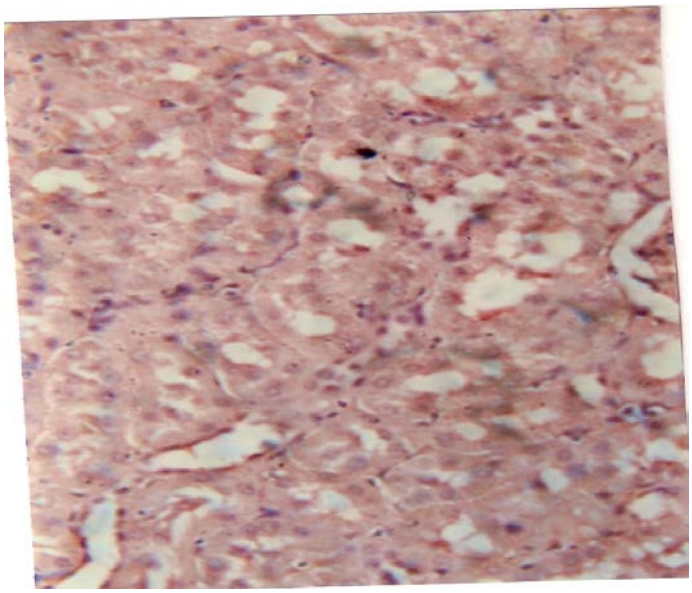

Fig. 5: (H & Ex200): Kidney section of the aqueous treated rats (1000mg/kg dose) showing no morphological changes.

However, the 2000 mg/kg dose of the aqueous extract showed vascular congestion and proliferation, glomerular

hypocellularity and tubular disruption in certain foci (Fig. 6).

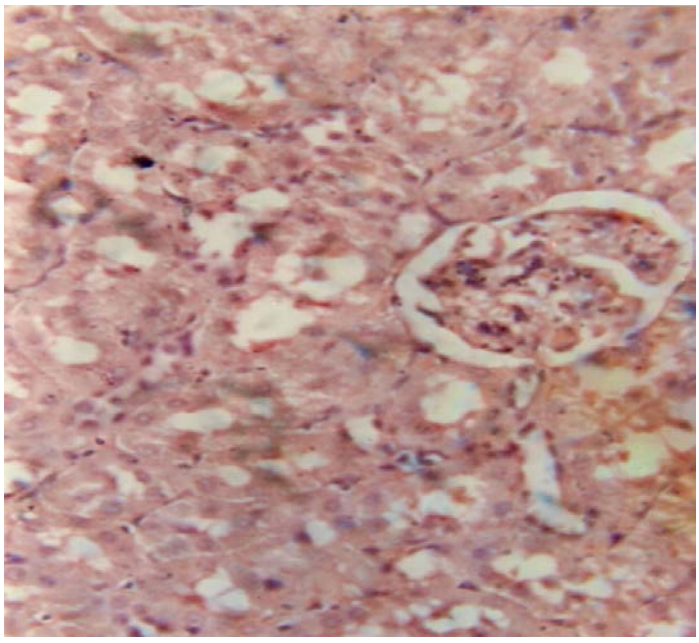

Fig. 6: (H & Ex200): Kidney section of the aqueous treated rats (2000mg/kg dose) showing vascular congestion and proliferation, glomerular hypocellularity and tubular disruption in certain foci.

The kidneys of the methanol extract treated groups showed vascular congestion, disruption of tubule, and glomerular expansion (Fig. 7).

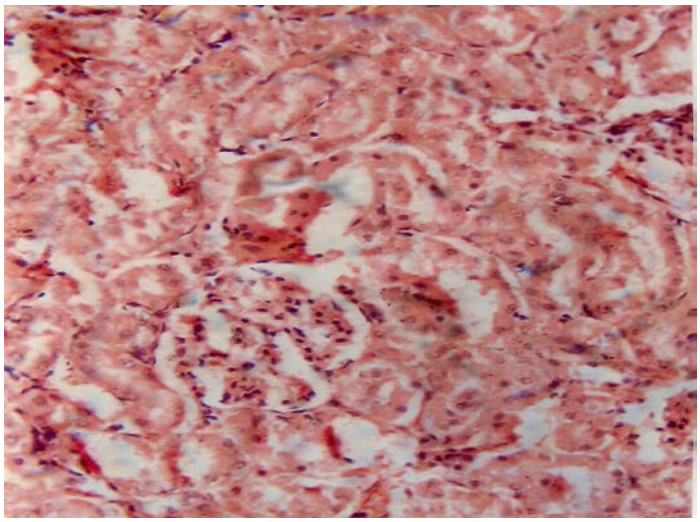

Fig. 7: (H & Ex200): Kidney section of the methanol extract showing vascular congestion, disruption of tubules and glomerular expansion.

The control group showed normal tubular, vascular, and glomerular architecture (Fig. 8).

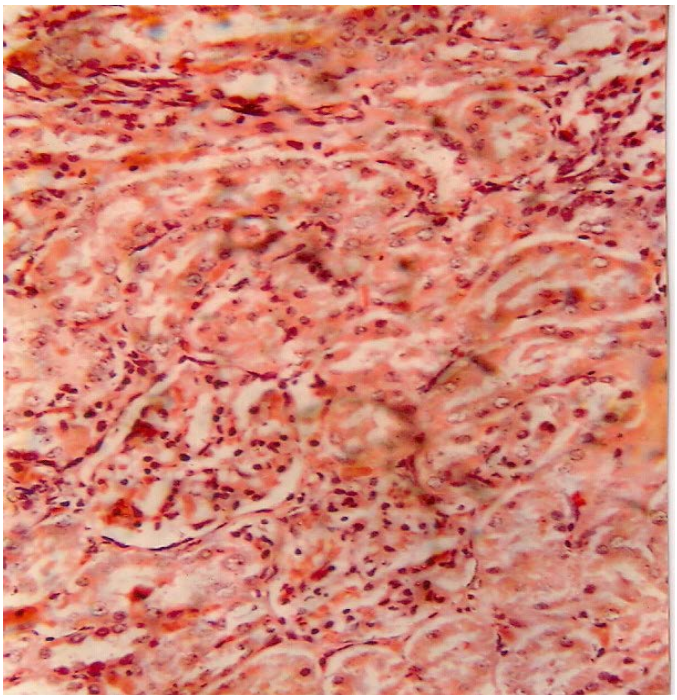

Fig. 8: (H &Ex200): Kidney section of control group showing normal tubular, vascular and glomerular architecture.

Effect of the extracts on the histology of the Heart:

There were no morphological changes in the heart tissue of extract treated groups just as the control (Fig. 9 &10).

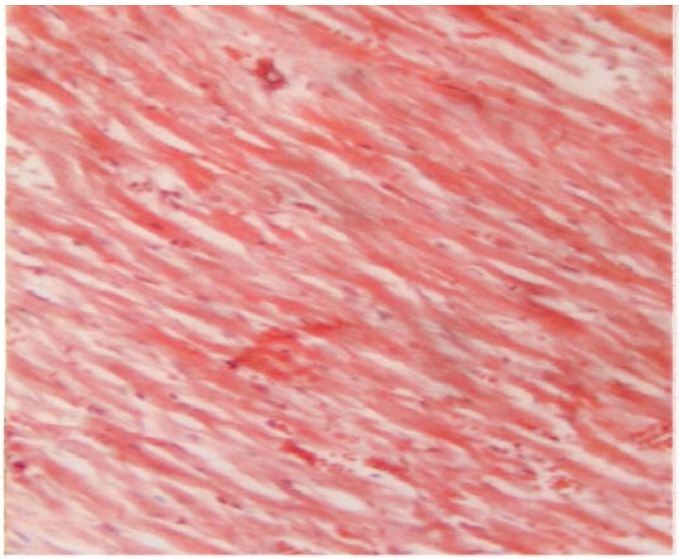

Fig. 9: (H & Ex200): Heart section of the aqueous treated rats showing no morphological changes.

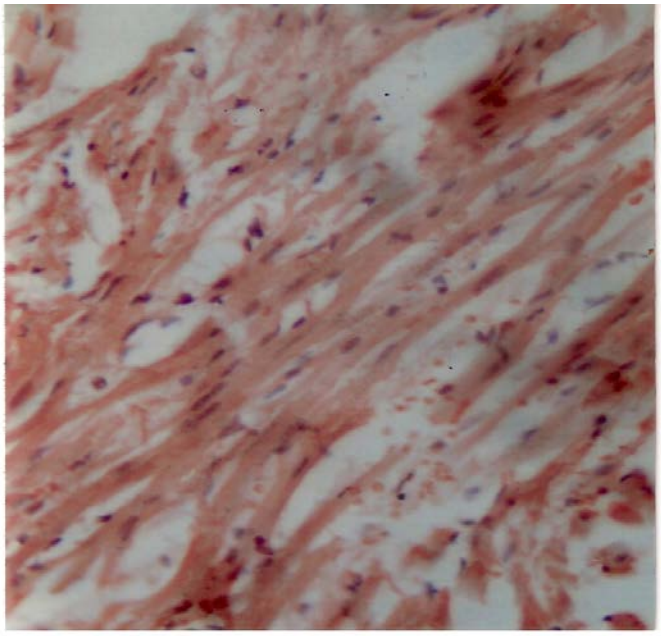

Fig. 10: (H & E x200): Heart section of the control group showing normal architecture.

Discussion

The result of the toxicity test revealed that the plant has LD_{50} is greater than 5000mg/kg. There was no change in behavior observed and no death was recorded. Therefore, the plant has a wide safety margin at acute level. This study was carried out to investigate the effect of administration of aqueous and methanol extracts on the histology of the liver, kidney and heart tissues of Albino Wistar rats. The 1000 and 2000mg/kg doses were selected for this study in order to ascertain the safety of this herb at this dose range since most traditional medical practitioners do not really standardize doses that are given to their patients and in some instances some of the herbal drugs are given for prolonged periods with the belief that they are not harmful. Loranthus micranthus is used in folklore for the treatment of diabetes mellitus and hypertension which are life- long conditions that require long life management. Therefore, long term toxicity could be a problem and consequently 1000 and 2000 mg/kg doses were selected for this sub-chronic study.

Herbal preparations used in therapeutic applications are common across the world [18]. Although these preparations are natural, they can cause deleterious effects such as cancer and malfunctions of important organs like the liver, kidney and the heart leading to death in most cases [19]. This happens when these plant extracts are used in the treatment of chronic illnesses like diabetes mellitus and high blood pressure [20]. It is expedient to examine these vital organs owing to their complex morphology [21] and to avoid cellular shutdown arising from uncontrolled usage of these herbal drugs. The liver is essential in keeping the blood glucose level in a normal concentration and in the metabolism of protein and fat [22]. The kidneys are tubular glands which are highly vascularized functioning to keep body fluids level and composition constant as well as eliminating excretory wastes [23].

In this study, both extracts did not cause much serious deleterious effects on the liver at lower dose. However, at higher dose there was mild portal inflammation and fibrosis. This suggest that these extracts could induce intra-hepatic cholestasis. This is in line with the studies on the effect of the extracts on the liver enzymes where the extract did not alter the levels of transaminases significantly. Histopathological evaluation of the kidneys also revealed vascular congestion with normal tubules and glomeruli in the aqueous extract treated groups. However, since glomerular expansion, tubular disruptions, vascular congestion, and cellular infiltrations were observed in rats that received 2000mg/kg doses of both extracts, it is likely that the increase in blood urea as reported by Ani et al [16] could represent an early warning sign of sub-acute renal toxicity of both extracts. Furthermore, both extracts did not exhibit cardiac toxicity as evident from the normal creatine kinase and phosphocreatine kinase levels in the

previous study as well as the normal cardiac histology in this present study.

Conclusion

In conclusion, the extracts of Loranthus micranthus produced cholestatic injury and minimal renal injury in rats at the dose levels and duration studied. This study calls for caution in the use of the extracts of Loranthus micranthus for long durations. The extracts should be standardized and chronic toxicity studies carried out in order to ascertain safety in long term usage.

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