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HISTOPATHOLOGICAL AND BIOCHEMICAL EFFECTS OF SUB-CHRONIC ADMINISTRATION OF AQUEOUS AND METHANOLIC EXTRACTS OF *LORANTHUS MICRANTHUS* LEAVES (PARASITIC ON *PARKIA BIGLOBOSA) IN RATS*

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Abstract

The histopathological and biochemical effects of sub-chronic administration of high doses of aqueous and methanolic extracts of *Loranthus micranthus* (African mistletoe) leaves parasitic on *Parkia biglobosa* were studied in male albino Wistar 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 (n = 5) respectively for 30 days. The fifth group of rats (n = 5) similarly received 5 ml/kg each of normal saline (negative control). Blood samples were collected from the rats at the end of the experiment by retrorbital puncture for analysis of relevant biochemical parameters using assay kits. The liver, kidney and heart of the rats were also harvested for histopathological 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 mice. Creatine kinase levels were significantly reduced in the treated groups suggesting the stabilization of cardiac musculature by these extracts. Histological results also 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. Even though extracts of *L. micranthus* leaves have been shown to be practically safe under acute administration in rats, caution should be exercised when mistletoes are administered for the treatment of life-long diseases such as diabetes and hypertension.

Keywords: Sub-chronic toxicity, *Loranthus micranthus*, histopathology

INTRODUCTION

Loranthus micranthus Linn (Family Loranthaceae) is the Eastern Nigerian species of African mistletoe. It is a semi-parasitic evergreen plant that depends on its host tree for minerals and water only (Griggs, 1991). They grow on branches of a variety of evergreen and deciduous tree all year round. The host trees of Loranthus include African locust bean, cocoa, coffee, custard, apple, guava, shea butter, citrus fruits, palm tree and breadfruit tree (Obatomi *et al*, 1994; Johnson and Choinski, 1993). *Loranthus micranthus* is also known to parasitize *Persea americana* (avocado pear), *Azadiractha indica (*neem), *Kola acuminata* (kolanut), *Pentacletra macrophylla* (oil bean) and *Baphia nitida* (Osadebe *et al.,* 2004). Studies

have shown that the composition and activities of the plant is host tree dependent (Wagner *et al.,* 1996; Obatomi *et al.,* 1994).

The name African mistletoe has been used for several different plants including *Loranthus bengwensis* Linn (a Northern Nigeria species), *Tapinanthus vittatus* (a Southern African species), *Loranthus micranthus* Linn (an Eastern Nigeria species) and *Erianthemum ulugurense* (a Kenyan species) (Barlow and Wiens, 1975; Gill, 1973)
Several other mistletoes are well known worldwide. These include the European mistletoe (*Viscum album* coloratum), Australian or Argentina mistletoe (*Ligaria cuneifolia*), American mistletoe (*Phoradendron flavescens*), among others.

Leaf extracts of *Loranthus micranthus* Linn have anti-diabetic, anti-hypertensive, anti-inflammatory, bactericidal, anti-fungal and anti-cancer effects (Obatomi, *et al.,* 1994; Obatomi, *et al.,* 1996; Yesilida *et al.,* 1998; Osadebe *et al.,* 2004; Osadebe and Akabogu, 2005; Osadebe and Ukwueze, 2005). Infact, Kafaru (1993) described mistletoe as "an all-purpose herb" because of its rich folkloric uses.

Though the side effects associated with mistletoe formulations are claimed to be minimal and non-life threatening (Kaegi, 1998), ingestion of mistletoe plant and berries has led to seizures, bradycardia, abnormal high and low blood pressures, vomiting and death (Avianweb, 2010). Previous studies by Osadebe *et al.,* 2004 on *Loranthus micranthus* reported the acute toxicity in mice with LD_{50} ranging from 6,500 to 12,500 mg/kg body weight depending on the host tree. Edem and Usoh, (2000) had reported the absence of renal or hepatic toxic effects of the mistletoe extracts at a maximum dose level of 827 mg/kg.

No study has been done on the sub-acute toxicity of *Loranthus micranthus* to our knowledge. With the increased use of herbs in our society today and the traditional use of *Loranthus micranthus* for treatment of life-long conditions such as diabetes mellitus and hypertension, there is a clear need to establish the safety of Loranthus preparations in long-term disease conditions.

Doses of 1000 and 2000 mg/kg were selected for this study in order to ascertain the safety of *L. micranthus* at high dose levels since most traditional medical practitioners rarely standardize patient doses. In some instances, some of the herbal drugs are given for prolonged periods with the belief that they are not harmful. *L. micranthus* is used in folklore for the treatment of diabetes mellitus and hypertension which are life-long conditions that require long-life management.

The study was therefore designed to generate the acute toxicity (LD_{50}) as well as sub-chronic toxicity information (based on biochemical parameters and histopathological effects) on the leaf extracts of *Loranthus micranthus* parasitic on *Parkia biglobosa* in rats.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Loranthus micranthus* Linn, parasitic on *Parkia biglobosa* were collected from Agbani, Enugu State, Nigeria and identified by Prof. J. C. Okafor (Department of Botany) of Enugu State University of Science and Technology, Enugu, Nigeria. A voucher specimen was deposited at the herbarium of the same Department.

The fresh leaves of the plant were dried under shade to constant weight and powdered with a crush

bitter mill (type Ski). Methanolic and aqueous extracts were obtained by maceration for 48 hours in absolute methanol and water respectively. The extracts were filtered using a Whatman No.12 filter paper and the filtrate evaporated to dryness using rotary evaporator.

Methodology

Twenty-five (25) albino wistar rats were used for the study. The animals were acclimatized for two weeks before initiating the study. Animals were divided into five groups (n=5) and allowed free access to clean drinking water and were fed on standard pellets (guinea-feed) *ad libitum*. Prior to drug administration, all the animals were subjected to an overnight fast with free access to clean drinking water. Animals were given access to food one hour post-drug administration. The animals in groups A and B received 1000 and 2000 mg/kg body weight of the methanolic extract of the *L. micranthus* respectively while animals in groups C and D received 1000 and 2000 mg/kg body weight of the aqueous extract respectively. Group E animals (control) received 5 ml/kg body weight of normal saline. Extracts were prepared with normal saline as vehicle. The appropriate doses were administered intraperitoneally daily for 30 days. At the end of 30 days, blood samples were collected through the retrobulbal plexus of the nasal canthus for the estimation of the following biochemical parameters; serum urea, creatinine, alkaline phosphatase, total and conjugated bilirubin, alanine transaminase (ALT) and aspartate transaminase (AST), and creatine kinase. Under ether anaesthesia, the animals were euthanized and the heart, kidney and liver excised and preserved in 10 % formol saline for histo-pathological investigation.

Determination of acute toxicity of *L. micranthus* parasitic on *P. biglobosa*

Acute toxicity studies of the aqueous and methanolic extracts of *L. micranthus* parasitic on *Parkia biglobosa* were performed using the method of Lorke, 1983.

Determination of biochemical parameters of rats

While the serum creatinine was determined using the alkaline picratate method of Henry, (1974), serum urea concentration was determined by the modified diacetylmonoxine method of Tietz, (1976). Serum alkaline phosphatase was determined using the method of Kind and King, 1954. The transaminases (AST and ALT) were determined using the method of Tietz, (1976). Creatine kinase was determined using ultra-violet kinetic method. The Statistical Package for Social Sciences (SPSS) computer software (version 15) was used for data analysis. The student's (t)-test was used to test for levels of significance. $P < 0.001$, $P < 0.01$ and $P < 0.05$ were considered statistically significant.

Histopathological investigations of the liver, kidney and heart of rats

At the end of the experiment, the animals were sacrificed and the liver, kidney and heart harvested. The harvested organs were fixed in 10 % formalin for 24 hours. 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 heamatoxylin and eosin (H and E) method. These were examined using a light microscope by histopathologists.

RESULTS AND DISCUSSIONS

Acute toxicity studies indicated that both extracts of *L. micranthus* parasitic on *Parkia biglobosa* had an intraperitoneal LD_{50} > 5000 mg/kg in rats, indicating high margin of safety according to Lorke, 1983. The levels of the enzymes assayed as well as the concentrations of other biochemical parameters are shown in Table 1.

Though the levels of alanine and aspartate transaminases in the rats were unaffected by the sub-chronic administration of aqueous and methanolic extracts of *L. micranthus*, the plasma levels of alkaline phosphatase and bilirubin showed statistically significant increase in animals treated with both aqueous and methanolic extracts compared to the control. We infer cholestatic injury since the histopathological results showed moderate to marked portal inflammation. Evidently, the aqueous extract was more toxic to the liver than the methanolic extract in this sub-acute toxicity study. As expected, the control rats revealed normal hepatic chords (A_1).

The study assessed the effects of sub-chronic administration of both extracts on the kidney via the urea and creatinine serum levels in addition to the histopathological investigations. Both doses of the aqueous extract and the 2000 mg/kg dose of methanolic extract of *L. micranthus* produced significant elevation in urea levels when compared with the control group. Creatinine levels were unaffected by administration of both extracts.

Elevated blood urea with relatively normal creatinine levels reflects a physiological response to a relative decrease of blood flow to the kidney without indicating any true injury to the kidney (Whitby *et. al.*, 1988). Diuresis, a common consequence of mistletoe infusions used in the treatment of hypertension in folk medicine (Deliorman *et al.*, 2000), may have led to dehydration and subsequent haemoconcentration in the treated rats. The histopathological findings (Fig B_2 and B_3) in the kidney of rats treated with 2000 mg/kg of both extracts and the absence of morphological changes in the kidneys of rats treated with 1000 mg/kg aqueous extract further suggest a physiological response to a relative decrease in blood flow to the kidney. The observed increase in blood urea should however represent an early-warning sign of sub-acute renal toxicity of both extracts.

The sub-chronic toxicities of both the aqueous and methanolic extracts of *L. micranthus* on the cardiac tissues of rats were investigated via the serum creatine kinase levels and histopathological examinations. Both extracts did not exhibit cardiac toxicity as evidence by absence of morphological changes in the heart tissues of the control group and rats treated with both the aqueous and methanolic extracts (Fig C_1 and C_2). Creatine kinase levels of treated rats were decreased significantly ($P < 0.05$) in a non-dose dependent manner when compared with the control. This is suggestive of stabilization of cardiac musculature by these extracts. It is our opinion that the beneficial antihypertensive effects of mistletoes may be accounted for by this stabilization of cardiac musculature. An investigation into the use of mistletoes in congestive heart failures is recommended.

CONCLUSION

The aqueous and methanolic extracts of *Loranthus micranthus* leaves parasitic on *Parkia biglobosa* produced cholestatic injury and minimal renal injury in albino Wistar rats at high dose levels and when administered daily for 30 days. Though these results cannot be extrapolated to humans, there is need for caution in the

Table 1: Result of biochemical parameters of rats (mean value ± sem)

Group	Sample	Dose (/kg)	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)	Aspartate transaminase (iu/L)	Alanine transaminase (iu/L)	Alkaline phosphatase (iu/L)	Urea (mg/dl)	Creatinine (mg/dl)	Creatine kinase (iu/L)
A	Aqueous extract	1000 mg	1.08 ± 0.14*	0.58 ± 0.10	23.00 ± 4.30	27.20 ± 4.72	92.40 ± 6.11***	36.80 ± 4.32*	0.58 ± 0.15	25.60 ± 1.63*
B		2000 mg	1.00 ± 0.10*	0.70 ± 0.11*	22.20 ± 3.30	27.40 ± 3.81	98.00 ± 7.95***	37.75 ± 5.80*	0.66 ± 0.19	26.40 ± 1.46
C	Methanolic extract	1000 mg	0.90 ± 0.13	0.44 ± 0.06	20.80 ± 1.59	17.20 ± 2.31	84.40 ± 7.02**	31.20 ± 4.18	0.32 ± 0.05	23.90 ± 1.50*
D		2000 mg	0.96 ± 0.05**	0.40 ± 0.07	41.80 ± 6.60	19.40 ± 1.88	79.00 ± 6.85**	37.40 ± 3.37**	0.40 ± 0.08	25.00 ± 1.73*
E (Control)	Normal saline	5 ml	0.68 ± 0.05	0.32 ± 0.03	27.40 ± 7.00	22.60 ± 2.48	49.00 ± 3.43	20.20 ± 1.01	0.40 ± 0.13	32.46 ± 2.37

*P < 0.05
**P < 0.01 and
***P < 0.001 were considered significant

Table 2: Photomicrograph of liver ($A_1 – A_3$), kidney ($B_1 – B_3$) and heart ($C_1 – C_2$) of treated rats (H & E x 200)

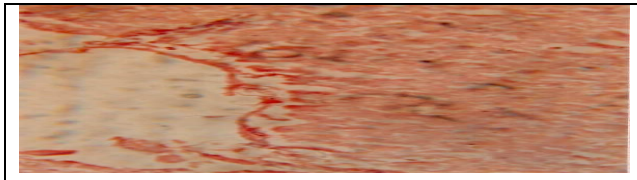	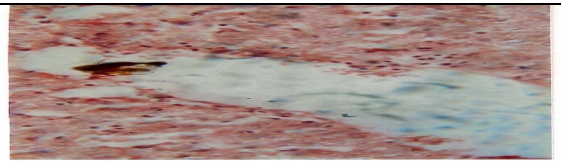	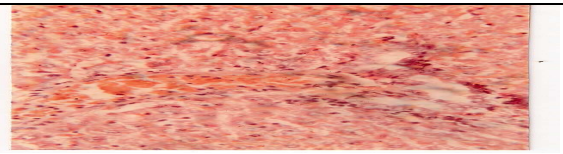
Fig. A_1: Liver section of control rat showing normal hepatic chords.	Fig. A_2: Liver section of the rat treated with aqueous extract (2000 mg/kg) showing mild portal lymphocytic infiltration, mild to moderate portal inflammation and expansion with numerous pyknotic nuclei.	Fig. A_3: Liver section of rat treated with the methanolic extract (2000 mg/kg) showing normal architecture with mild portal inflammation and fibrosis.
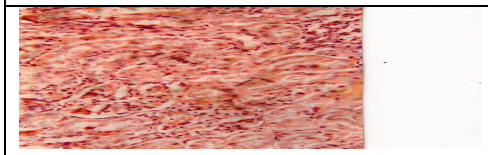	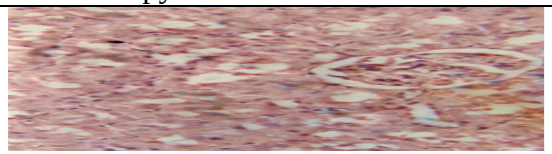	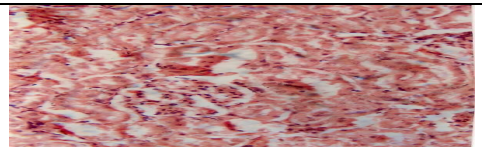
Fig. B_1: Kidney section of control rat showing normal tubular, vascular and glomerular architecture.	Fig. B_2: Kidney section of rat treated with aqueous extract (2000 mg/kg) showing vascular congestion and proliferation, glomerular hypocellularity and tubular disruption in certain foci.	Fig. B_3: Kidney section of rat treated with methanolic extract (2000 mg/kg) showing vascular congestion, disruption of tubules and glomerular expansion.
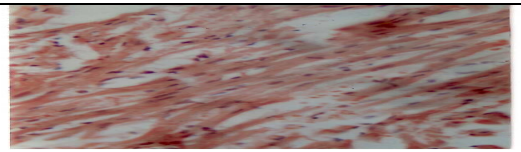		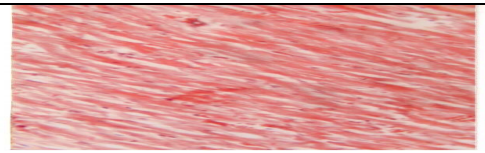
Fig. C_1: Heart section of control rat showing normal architecture.		Fig. C_2: Heart section of rat treated with aqueous extract (2000 mg/kg) showing no morphological changes

use of *Loranthus micranthus* extracts for the treatment of life-long diseases such as diabetes and hypertension as currently practiced by traditional herbalists.

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