

Toxicological Study of leaf extracts of *Loranthus micranthus* Linn Using Albino Wistar Rats

ABSTRACT

This study was aimed to investigate the effect of high sub-chronic doses of the aqueous and methanol leaf extracts of *Loranthus micranthus* on biochemical parameters of albino rats. Acute toxicity studies were performed according to standard methods. The animals were divided into 5 groups (n = 5). Aqueous and methanol extracts of *L. micranthus* leaves were administered in doses of 1000 and 2000 mg/kg body weight to four groups of rats respectively for 30 days through the intraperitoneal route. The fifth group served as control and received saline (5 ml/kg b.w, i.p). Blood samples were collected by retroorbital puncture and analyzed for biochemical and haematological parameters using assay kits. Acute toxicity studies indicated that both extracts had an LD₅₀ > 5000mg/kg. The results indicated significant (p<0.001) increases in alkaline phosphatase serum levels in both extract treated groups. The extracts also produced significant elevation in serum bilirubin levels when compared with normal control (p<0.05). Both extracts did not affect the levels of alanine and aspartate transaminases significantly (p>0.05). There were significant increase in the serum levels of urea in the extracts treated rats (p<0.05; p<0.01). The 2000 mg/kg aqueous extract produced significant increases in mean serum chloride and bicarbonate levels of treated rats when compared with control (p<0.01). The extracts produced significant decrease in the serum creatine kinase levels of treated rats in a non-dose related manner when compared with control (p<0.05). There were no significant changes in sodium and potassium levels of treated rats. The methanol extract had no significant effect on the haematological indices studied. The aqueous extract produced significant reductions in the haemoglobin and PCV of treated rats (p< 0.01). The total and differential leucocyte counts were not affected by extract treatment (p>0.05). From these results, the extracts caused significant biochemical changes but were not cytotoxic to leucocyte cell lines. Therefore, there should be caution in the long term use of these extracts.

Keywords: Toxicity, *Loranthus micranthus*, biochemical parameters, Haematological parameters, Transaminases.

1. INTRODUCTION

The word herb has been defined to mean any plant or part thereof which is valuable for medicinal treatment, nutrition, food seasoning, dyeing and colouring of other materials [1]. Plants have been recognized for long as synthetic medium, capable of making a diversity of organic molecules that have complex chemical structures and a various physical, chemical

and biological properties [2]. Plant will ever remain an inexhaustible source of medicines for the relief of man's many diseases and pains.

Records from ancient Egypt, Assyria, China and India show that the uses of plants for medicinal purposes dates back to the earliest recorded history [3]. Various plants and plant parts such as bark, roots, leaves, flowers and berries from various plants over the years have been used in the treatment of certain disease conditions. For this reason, safety and efficacy of these should be established following scientifically established norms. It is commonly believed that for any disease of man and animal, there are one or more herbal plants in that locality that can bring about its cure.

African mistletoe (Eastern Nigeria specie) known as *Loranthus micranthus* Linn (family Loranthaceae) 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 [4]. They grow on a variety of evergreen and deciduous tree all year round, around the branches of the tree. The name African mistletoe has been used for several different plants including *Loranthus begweensis* linn (a northern Nigeria specie), *Tapinanthus vittatus* (a southern African specie), *Loranthus micranthus* linn (an Eastern Nigeria specie) and *Evianthemum uluguvense* (a Kenyan specie) [5]. Several other mistletoes are well known worldwide. These include the European mistletoe (*Viscum album colovatum*), Australian or Argentina mistletoe (*Ligaria cunenfolia* R.et.T), American mistletoe (*Phoranaendron flavescens*), among others.

Traditional names of *Loranthus micranthus* linn include owube, or awurisi in Ibo language; Afomo onisana in Yoruba, Kauchin in Hausa and children's matches or golden bough in English. The host tree of *Loranthus* include African locust bean, cocoa, coffee, custard, apple, guava, shea butter, citrus fruits, palm tree, breadfruit tree [6, 7].

Several chemical substances have been isolated and identified in *Loranthus* plant. These include MCI, MCII, MCIII and *Viscum album* chitin binding agglutinins, isolable from *V. album* [8]. The complete amino acid sequence of the A. chain of *V. album*'s lectin I has been determined [9]. Leaf extract of *Loranthus micranthus* linn have anti-inflammatory, anti-diabetic, anti-hypertensive, bactericidal, anti-fungal and anti-cancer effects [10, 11, 12]. In fact, Kafaru [13] described mistletoe as "an all-purpose herb" because of its rich folkloric uses.

Studies have shown that the composition and activities of the plant is host tree dependent [6, 14]. Side effects associated with mistletoe formulation are minimal and non-life threatening [15]. Ingestion of mistletoe plants and berries has led to seizures, bradycardia, abnormal high and low blood pressure, vomiting and death [16]. Since its being consumed commonly, it will be good to further investigate its safety by examining any possible effect on biochemical parameters so as to avoid any health hazard. **Therefore, the aim of this study is to determine the biochemical and hematological effects which the sub-chronic administration of this extract can induce.**

2. MATERIALS AND METHODS.

2.1. Plant material collection and identification

Loranthus micranthus linn leaves were collected from farmlands in Enugu State University of science and Technology Agbani, Enugu State. The plant was 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.

2.2 Plant materials extraction procedures:

The plant leaves were shade-dried to constant weight. Dried and fresh leaves were powdered with a crush bitter mill, type Ski and hand manual grinding machine respectively. The dried powder was thereafter sieved with 1mm sieve and kept in air-tight container until time for use. Five hundred grams (500g) of the dried powdered leaf was soaked in 1 liter of methanol for 48hrs with intermittent agitation. After this the extract were filtered using a whatman No. 12 filter paper and the filtrate evaporated to dryness using rotary evaporator. The obtained residues were weighed and stored in the refrigerator until use. Then, 3000g of the fresh leaf paste were extracted through a muslin cloth after the addition of 100mls of clean water. The extract obtained were stored at 4- 8°C until required. The determination of the extractive value (concentration) was done using an aliquot of the water extract.

2.3 Determination of extractive index:

0.1ml of the aqueous extract was placed in a pre-weighed crucible and evaporated to dryness in an oven set at 60°C. After evaporation, the crucible and its contents were allowed to cool and the weight of the crucible with the content determined. The weight of the residue was determined by deducting the weight of the crucible from the weight of the crucible and residue.

2.4. Experimental Animals

Twenty-five (25) male albino wistar rats were used for the experiment. They were kept and maintained in the animal house of the college of medicine at the University of Nigeria Teaching Hospital Enugu. They were kept for two weeks to acclimatize before the study. **The animals were maintained at room temperature, had free access to clean drinking water and were fed on standard pellets (guinea-feed) ad libitum.**

2.4.1 Study Design

The animals were divided into five (5) groups of A-E (n=5). The animals in groups A and B received 1000mg and 2000mg/kg body weight of the methanolic 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. Those in group E served as the control and received 5ml/kg body weight of normal saline. 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 plant extract was administered intraperitoneally daily for 30 days. Blood samples were collected at the end of the fourth week through the retrobulbar plexus of the nasal canthus for the estimation of the biochemical parameters namely serum electrolytes, urea and creatinine, alkaline transaminase, bilirubin, alanine transaminases and aspartate

transaminases, creatinine kinase and creatinine phosphokinase. Some hematological parameters were also carried out like Haemoglobin (HB), Packed Cell Volume (PCV), White Blood Cells (WBC: total and differential), Mean Corpuscular Hemoglobin (MCHC).

2.5 Analytical Methods

2.5.1 Acute Toxicity Test (LD50)

Acute toxicity studies were performed on the aqueous and methanol extract using method of Lorke [17]

2.5.2 Biochemical Assays

Serum creatinine was determined using the alkaline picarate method of Fabing and Ertingshausen [18], while serum urea concentration was determined by the modified diacetylmonoxine method of Wybenga et al [19]. Serum Alkaline phosphatase was determined using the method of Kind and King [20] while the Transaminases, AST and ALT were determined using the Reitman and Frankel method [21]. Sodium (Na⁺) and Potassium (K⁺) were determined using flame photometric method by Barnes *et al* [22]. Chloride and bicarbonate were determined using modified colorimetric method of Henry [23]. Creatinine kinase and creatinine phosphokinase were determined using ultra-violet kinetic method as described by Oliver [24].

2.5.3 Heamatological Assay

Haemoglobin was determined using method of Drabkin and Austin [25]. The improved Neubauer Counting Chamber was used for total white cell count. Microhaematocrit method was used for PCV. WBC differential was done using Leishman's stain [26].

2.6 Statistical Analysis

The statistical package of social sciences (SPSS) computer software and the independent student's (t)-test were used for data analysis. P < 0.01, P < 0.001, P < 0.05 is considered statistically significant.

3. RESULTS

3.1 Acute Toxicity Test:

Acute toxicity studies indicated that both extracts had an oral LD50 > 5000mg/kg. There was no observed physical signs of gross behavioral changes and no death was recorded.

3.2 Analysis of biochemical parameters:

The levels of the enzymes assayed as well as the concentrations of other biochemical parameters are shown in Tables 1a, 1b and 1c.

The results indicated a dose-dependent significant increase in serum levels of alkaline phosphatase in the aqueous extract (AE) treated groups (92.40±6.11 and 98.00±7.95 iu/L respectively) when compared with those of the control (49.00±3.43 iu/L; p<0.001). The

group treated with methanol extract (ME1:1000mg/dl, ME2: 2000mg/dl) also had significant increase in levels of alkaline phosphatase when compared with the control group ($p<0.001$). The extracts of *Loranthus micranthus* also produced significant elevation in serum bilirubin levels ($p<0.05$). The 1000 and 2000 mg/kg doses of the aqueous extract (AE1 and AE2) showed bilirubin levels of 1.08 ± 0.14 and 1.00 ± 0.10 mg/dl respectively and these were significantly higher than the normal control value of 0.68 ± 0.05 mg/dl ($p<0.05$). Both extracts did not affect the levels of alanine and aspartate transaminases.

Table 1a: Mean levels of Bilirubin (Total and conjugated bilirubin), AST, ALT and ALP in control and extract treated groups

Group	TB (mg/dl)	CB (mg/dl)	AST (iu/L)	ALT (iu/L)	ALP (iu/L)
Control	0.68 ± 0.05	0.32 ± 0.03	27.4 ± 7.0	22.6 ± 2.48	49.0 ± 3.43
AE1	$1.08\pm0.14^*$	0.58 ± 0.10	23.0 ± 4.3	27.2 ± 4.72	$92.4\pm6.11^{***}$
AE2	$1.00\pm0.10^*$	$0.70\pm0.11^*$	22.2 ± 3.30	27.4 ± 3.81	$98.0\pm7.95^{***}$
ME1	0.90 ± 0.13	0.44 ± 0.06	20.8 ± 1.59	17.2 ± 2.31	$84.4\pm7.02^{**}$
ME2	$0.96\pm0.05^{**}$	0.40 ± 0.07	41.8 ± 6.60	19.4 ± 1.88	$79.0\pm6.85^{**}$

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 1b shows the serum concentrations of kidney biochemical parameters; urea, creatinine and electrolytes (sodium, chloride, bicarbonate, and potassium) in the experimental animals. There were significant elevations in the serum levels of urea in aqueous and methanol extract treated rats when compared with control ($p<0.05$; $p<0.01$). The 2000 mg/kg aqueous extract (AE2) produced significant increase in mean serum chloride (100.40 ± 7.33 mmol/L) and bicarbonate (26.40 ± 1.46 mmol/L) levels of treated rats when compared with control ($p<0.01$). There were no significant changes in sodium and potassium levels of treated rats when compared with control.

Table 1b: Mean levels of urea, creatinine and the electrolytes in control and extract treated groups.

Group	Urea (mg/dl)	Creatinine (mg/dl)	Na ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO ₃ ⁻ (mmol/L)	K ⁺ (mmol/L)
Control	20.2 ± 1.01	0.4 ± 0.13	118.8 ± 9.201	75.4 ± 4.63	20.2 ± 1.06	4.00 ± 0.00
AE1	$36.8\pm4.32^*$	0.58 ± 0.15	104.2 ± 9.27	91.0 ± 6.46	22.8 ± 1.62	3.84 ± 0.23
AE2	$37.75\pm5.80^*$	0.66 ± 0.19	94.4 ± 5.81	$100.4\pm7.33^*$	$26.4\pm1.46^{**}$	4.58 ± 0.28
ME1	31.2 ± 4.18	0.32 ± 0.05	110.0 ± 10.07	81.4 ± 4.23	22.2 ± 1.28	4.14 ± 0.11
ME2	$37.4\pm3.37^{**}$	0.4 ± 0.08	123.0 ± 5.72	$103\pm5.53^{**}$	21.4 ± 1.32	3.92 ± 0.15

* $p<0.05$; ** $p<0.01$

Table 1c shows the serum concentrations of creatine kinase and phosphocreatine kinase in the experimental animals. The extract produced significant decrease in the serum creatine kinase levels of treated rodents in a non-dose related manner when compared with the control ($p<0.05$). There were no significant changes in phosphocreatine kinase level of treated rats when compared with the control.

Table 1c: Mean levels of creatine kinase and phosphocreatine kinase in control and extract treated groups.

Group	Dose (mg/dl)	PCK (iu/L)	CK (iu/L)
Control		102.00±0.00	32.46±2.37
AE1	1000	96.80±9.70	25.6±1.63*
AE2	2000	88.40±0.00	26.4±1.46
ME1	1000	89.89±9.59	23.9±1.50*
ME2	2000	97.60±7.08	25.0±1.73*

*p<0.05

3.3 Haematological analysis

Table 2 shows the effect of the extracts of *Loranthus micranthus* on some haematological indices. Results showed that the aqueous extract produced significant reductions in the haemoglobin and packed cell volume of treated rats when compared with control (p< 0.01). The total and differential leucocyte counts were unaffected by extract treatment (p> 0.05). The methanol extract had no effect on the haematological indices studied.

Table 2: Haematological indices of extract treated groups with the normal control.

Group	Hb (mg/dl)	PCV (%)	Lymph. (%)	Neut. (%)	Mono (%)	Wbc _{total} (cu.mm)
Control	13.96±0.16	42.8±0.96	77.6±1.43	22.2±1.49	-	2860±265
AE1	13.18±0.13**	39.0±0.54**	71.6±2.54	28.0±2.20*	2.0	2220±276
AE2	12.96±0.16**	37.8±0.66**	72.4±3.05	26.6±2.52	2.50±1.50	2360±188
ME1	13.6±0.17	39.6±1.63	77.2±2.81	22.6±2.78	-	2450±242
ME2	13.6±0.24	40.8±1.06	79.6±3.18	20.0±3.52	-	2560±274

*p<0.05; **p<0.01; ***p<0.001

4. DISCUSSION

From the result of **sub-acute** toxicity (LD₅₀), both extracts had an acute oral LD₅₀ > 5000mg/kg in rats and **indicated** high margin of safety according to Lorke [17]. **There was significant increase in the plasma levels of ALP and bilirubin in animals** treated with both aqueous and ethanolic extracts compared to the control. However, the extracts did not alter the levels of transaminases significantly. Liver enzymes such as ALT, AST and ALP are marker enzymes for liver function and integrity [27, 28]. It has been severally reported that liver enzymes are liberated into the blood whenever liver cells are damaged and enzyme activity in the plasma is increased [29, 30]. Elevation of **liver enzymes is also** associated with cell necrosis of many tissues especially the liver [31]. The fact that the activities of transaminases were not altered after treatment with the extracts indicated that the plant extracts did not have necrotic effect on the liver. Though there are many medicinal plants used in treatment of liver diseases, there are also reports of liver injury after intake of herbal

supplements including those advertised for the treatment of liver diseases [32]. On the other hand, alkaline phosphatase is a membrane bound enzyme and its elevation in serum is indicative of intra- hepatic cholestasis. The rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice [33]. The aqueous and methanol extracts of *Loranthus micranthus* increased ALP and bilirubin levels of treated rats which is indicative of cholestatic injury. This suggests that these extracts induced intra-hepatic cholestasis and consequently jaundice in treated rats. Evidently, the aqueous extract was more toxic to the liver than the methanol extract in this sub-acute toxicity study.

Furthermore, the increase in blood urea by both extracts is indicative of renal impairment. The most common cause of an elevated blood urea, azotemia, is poor kidney function, although a serum creatinine level is a more specific measure of renal function. Impaired renal excretion of urea may be due to temporary conditions such as dehydration or shock, or may be due to either acute or chronic disease of the kidneys themselves. However, an elevated blood urea in the setting of a relatively normal creatinine may reflect a physiological response to a relative decrease of blood flow to the kidney (as seen in heart failure or dehydration) without indicating any true injury to the kidney. It is probable that the extract could have a diuretic effect which could be a setting for dehydration and subsequent haemoconcentration. In addition, an increased diuresis has been recorded as a common consequence of mistletoe infusions used in the treatment of hypertension in folk medicine [34].

Both extracts did not exhibit cardiac toxicity as evident from the normal creatine kinase and phosphocreatine kinase levels. In fact, the CK levels were reduced significantly when compared with control and this is suggestive of stabilization of cardiac musculature by these extracts.

The higher doses of both extracts caused hyperchloremia in treated rodents. Hyperchloremia is sometimes associated with excess fluid loss such as vomiting and diarrhea, diuretic therapy, and kidney disease [35]. It is therefore, likely that the observed effects could be due to diuretic or renal toxic effects or both by the extracts. The aqueous extract reduced the haemoglobin and packed cell volume of rats significantly. This could be due to direct toxic effect to the haemopoietic tissues or due to an indirect action on haemopoiesis by reducing erythropoietin synthesis as a result of decreased renal function. This study does not agree with the work done by Edem and Usoh [36]. Edem and Usoh [36] did not observe any renal or hepatic toxic effects of the extracts at a maximum dose level of 827 mg/kg. The disagreement could be as a result of the higher dose and longer duration used in this study. More so, mistletoe plants contains glycoproteins (viscotoxins and phoratoxins), various alkaloids, a gamma aminobutyric acid, phenols and phenethylamines which are said to be toxicologic substances [37].

5. CONCLUSION

The results from this study indicate that the leaf extracts of *L. micranthus* is safe under acute administration but in prolonged cases could cause some deleterious effects. *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 arising from indiscriminate usage which is evident from the high doses used in this study.

AUTHORS' CONTRIBUTIONS

Author AO conceived the work, wrote the protocol, designed the study and managed the analyses of the study. Author AON managed the literature searches and wrote the first draft of the manuscript. Author OCN performed the statistical analysis. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” were followed and all experiments have been examined and approved by the ethics committee of University of Nigeria, Enugu campus, Nigeria.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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