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Toxicity Studies on Methanolic Leaf Extract of *Rothmannia Longiflora*: Biochemical Effects in Wistar Albino Rats

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Abstract: **Aqueous decoction of leaves of *Rothmannia longiflora* is consumed by many people in the management of pain and inflammation in Nigeria and other African countries without considering its safety. The effects of the extract on functions of the kidney and the liver were investigated in 40 wistar albino rats. The rats were divided into 4 groups of 10 rats per group. The first group was the control and the other 3 groups were the study groups. The oral lethal dose of the extract was determined and was found to be greater than 5000mg/kg indicating its safety. Different doses of 250, 500 and 1000mg/kg were administered daily to the study groups for the periods of 30 days (sub-chronic toxicity studies) and 90 days (chronic toxicity study). Kidney and Liver function tests were assessed using standard techniques. There was no statistically significant change in the hepatic profile with the extract treated groups and control. Similarly, the extract produced no significant change in the kidney function parameters. This result showed that extract did not produce a change in the kidney function following sub-chronic and chronic administration. However, the extract produced significant change in the liver function parameters at high dose after 90 days administration.**

Keywords: **Kidney, lethal dose, liver, *Rothmannnia longiflora*, safety, toxicity.**

1. INTRODUCTION

In the long history of the world, plants have been used medicinally. A large and increasing number of patients use medicinal herbs for the treatment of different ailments. It has been estimated roughly, that more than half of the total population of the world use herbal drugs [3]. Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use [1].

Uncontrolled abuse of analgesics and analgesic combinations may lead to renal damage severe enough to cause end stage renal disease –ESRD- [7] or even the development of urogenital cancer [9]. In United States, the cost of treating ESRD by either dialysis or organ transplantation was $6.6 billion in 1991 [5]. It has been estimated that patients on therapy for ESRD due to analgesic abuse represent about 3% of cases in Queenland Australia [2]. In Nigeria such figures are scarcely available for the consumption of the public and regulatory bodies.

The liver is the major site of metabolism of most substances or compound absorbed through the gastrointestinal tract. Liver function tests are series of tests which are used to assess the efficiency of various liver functions [8].

Rothmannia longiflora Salisb (Family: Rubiaceae) is found in Gambia, Sudan, Kenya, Tanzania, Angola and is also found in Nigeria, Ghana, Sierra Leone, Democratic Republic of Congo, Ivory Coast, Uganda, Liberia and Cameroon.

In Nigeria, the common vernacular names of *Rothmannia longiflora* include: 'Katambiri' (hausa),'Igbo**:**"Uli, Alankita uku, mbembe(Ogwashi)''Yoruba: 'Edo-pata, Kere buje(Ononchie), Osego ikorun(Millson).

Rothmannia longiflora is considered to have febrifugal and analgesic properties, and a decoction of the leaves, twigs, bark and roots is applied internally or externally as lotions, washes and baths [6].

2. MATERIALS AND METHODS

The plant sample was collected in Gargai, Bagauda Road, Kano, Kano State, Nigeria, in October 2010 and was authenticated at the Herbarium Section of Biological Science Department of Ahmadu Bello University (ABU), Zaria by comparing with the existing specimen (Voucher specimen number: 2877).

The leaves were air dried under shade (until constant weight was obtained) and then size-reduced into coarse powder with a pestle and mortar. The powdered leaves (500g) were extracted with 2.5Litres of 100% Methanol for 72 hours using soxhlet extraction apparatus. The solvent was evaporated (at reduced pressure) to give an average yield of 29.47%w/w.

The extract was then stored in the freezer at temperature of 4^{o}C-5^{o}C until needed for the work.

Determination of Sodium and Potassium:

0. lml of the serum was added into universal bottle and 9.9m1 of distilled water added. 5ml of working standard and 5ml of distilled water added to separate universal bottles respectively. The test bottles were capped with parafilm and mixed by inversion.

To determine sodium the following procedure was followed. Sodium filter was adjusted to 590nm and the galvanometer was switched on. The gas supply was fully turned on and the flame ignited. The air pressure was regulated to 101b/sq inch. The gas was adjusted smoothly to obtain discrete cones of flame. The galvanometer reading was then set at zero using the working standard and reset at zero. The test was read, checking the standard after 2-3 test readings. The amount of sodium in mmol/L was calculated as galvanometer reading × 2.

In the determinations of potassium, the potassium light filter was adjusted to 770nm. The galvanometer set with potassium working standard to 70 and the procedure continued as for sodium. The amount of potassium mm/L = galvanometer × 0.1.

Determination of Chloride:

Titration method was used. 0.2rn1 of serum was added to 1 .8m1 of distilled water. 3 drops of diphenylcarbazone indicator was added and mixed. This was titrated with mecuric nitrate to violet-blue coloured end point. The percentage chloride was calculated by comparing the volume of test sample required to volume required of standard sample.

Determination of Urea:

The test sample was prepared by adding 0.1 ml of serum to 1.0mI of distilled water.2ml of mixed colour reagent (0.02g/ml diacetylmonoxirne and 0.005g/ml thio-semicarbazide and of mixed acid(0.02g/ml ferric chloride in 85% phosphoric acid and 0.43%sulphuric acid) each was added. This is was thoroughly mixed and incubated at 100°C for 20 minutes, cooled and the resulting red mixture was read at 520nm.The urea level in mmol/L was calculated by comparing test sample to standard sample.

Determination of Creatinine:

The test sample was prepared by adding 1ml of serum to 3ml of distilled water. 1ml of 10% sodium tungstate and 1 ml 2/3N sulphuric acid were added mixed well and centrifuged for 10 minutes. 3m1 of the supernatant was added to 0.75 N sodium hydroxide, 1ml of picric acid was added mixed and allowed to stand for 1 5minutes (Jaffe's reaction). The resulting red colour was read at 520nm. The creatinine level in µmol/L was calculated by comparing test sample to standard sample.

***Determination of Alkaline Phosphatase* (ALP):**

300µL of reagent volume was added to 6µL of sample volume and was incubated at 37^0C and readings were taken at every 60 seconds.

***Determination of Alanine aminotransferase* (ALT):**

250µL of reagent volume was added to 25µL sample volume and incubated at 37^0C and readings were taken at every 100 seconds.

***Aspartate aminotransferase* (AST):**

250µL of reagent volume was added to 25µL sample volume and incubated at 37^0C and readings were taken at every 100 seconds.

Experimental animals:

Wistar rats (150-250g) and Swiss Albino mice (18-30g) of both sexes were obtained from Animal House, Department of Pharmacology and Therapeutics, A.B.U-Zaria and National Research Institute for Chemical Technology (NARICT), Basawa, Zaria, Kaduna State.

The animals were maintained in a well ventilated room in the laboratory animal house under standard laboratory conditions of temperature (25 ± 2^0C) and light (approximately 12/12h light/dark cycle). They were fed on Vital Grower feeds (Vital feeds, Jos-Plateau State) and watered *adlibitum* and were allowed to acclimatise for three days prior to the experiment. All experimental protocols were approved by the University Animal Ethics Committee.

Acute toxicity study:

LD_{50} determinations were conducted using Lorke's method (1983) for intraperitoneal and oral routes in mice and rats. This method was carried out in two phases. In the initial phase, 3 groups each containing three animals was treated with methanolic leaf extract of the plant at doses of 10,100 and 1000mg/kg body weight i.p/orally and were observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one animal were administered with four more specific doses of the extract 1200mg, 1600mg, 2900mg, and 5000mg/kg body weight based on the result of phase one (initial phase). The LD_{50} value was determined by calculating the geometric mean of the highest non-lethal dose (for which the animal survived) and the lowest dose (for which the animal died).

Sub-Chronic (30 days) Toxicity Studies:

Three test doses of the extract were administered orally to three groups of rats (10 rats per group). Group 1, 2, and 3 received 250mg/kg, 500mg/kg and 1000mg/kg respectively, daily for 30days. The 4^{th} group served as the control was administered Normal saline 1ml/kg. On the 31^{st} day 5 rats were randomly selected from each group, and 4.0ml of blood samples were collected into plain containers by cardiac puncture under anaesthesia from each rat. The blood samples were allowed to clot for two hours, centrifuged at 4000 Revolution per Minutes (rpm) for 10 minutes and serum separated for kidney and liver function tests. The rest of the rats were euthanized by cervical dislocation under the influence of anaesthesia.

Chronic (90 days) Toxicity Studies:

The treatment group 1, 2, and 3 received 250mg/kg, 500mg/kg and 1000mg/kg respectively, daily for 90 days. The control group was administered Normal saline 1ml/kg. On the 91^{st} day, 5 rats were randomly selected from each group, and 4.0ml of blood samples were collected into plain containers by cardiac puncture under anesthesia from each rat. The blood samples were allowed to clot for two hours, centrifuged at 4000 Revolution per Minutes (rpm) for 10 minutes and serum separated for both liver and kidney function tests.

Statistical Analysis:

The data were analysed and expressed as Means ±SEM (Standard Error of Mean)

Difference between the control and treated groups were analysed using one way Analysis of variance (ANOVA) followed by post hoc dunnet t-test for multiple comparism.

$P<0.05$ were considered to be statistically significant.

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3. RESULTS

The median lethal dose values (LD_{50}) of the methanolic leaf extract of *Rothmannia longiflora* in mice and rats were found to be above 5000mg/kg body weight. There were no behavioural signs of toxicity exhibited by any mouse or rat that received the various doses of the extract.

Table1.Median Lethal Dose (LD_{50}) values of the methanolic leaf extract of *R. longiflora*.

Route of administration	Animal species	LD_{50} values mg/kg body weight
Intraperitoneal	Mice	>5000
Oral	Mice	>5000
Oral	Rats	>5000

4. KIDNEY FUNTION TEST

There was no statistically significant difference (P>0.05) in the levels of Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) ions, Urea and Creatinine at all doses tested when compared with that of the control at the end of 30 (sub-chronic) and 90 (chronic) days treatment respectively.

Table 2. Effect of methanolic leaf extract of *R. longiflora* on kidney function in rats following a 30 days sub-chronic treatment

Dose (mg/kg)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Urea (mmol/l)	Creatinine (µmol/l)
Control	138.40±0.20	3.96±0.16	99.60±1.70	3.66±0.32	71.60±3.70
250	138.40±0.51	4.26±0.11	99.20±0.49	4.16±0.37	76.20±2.52
500	138.00±1.52	3.92±0.20	96.40±1.47	4.50±0.15	73.00±2.78
1000	137.40±0.87	4.00±0.16	98.40±0.75	3.98±0.30	72.40±3.31

Values are expressed as Mean±SEM, n=5; K^+ (Potassium), Cl^-(Chloride), Na^+ (Sodium)

Table 3. Effect of methanolic leaf extract of *R. longiflora* on kidney function in rats following a 90 days chronic treatment

Dose (mg/kg)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Urea (mmol/l)	Creatinine (µmol/l)
Control	139.00±0.63	4.10±0.11	99.60±0.75	4.22±0.19	63.60±2.52
250	139.40±1.40	3.94±0.22	99.00±1.41	4.18±0.26	67.20±1.77
500	137.80±1.28	3.88±0.14	98.20±0.80	4.30±0.23	69.60±1.12
1000	137.40±1.33	4.50±0.33	97.02±1.85	5.30±0.50	83.60±6.61

Values are expressed as Mean±SEM, n=5; K^+ (Potassium), Cl^-(Chloride), Na^+ (Sodium)

5. LIVER FUNCTION TEST

There was no statistically significant difference (P>0.05) in the levels of AST, ALT, and ALP enzymes in all the treated groups compared to the control group following a 30 days (sub-chronic) treatment. Similarly, there was no statistically significant difference in the levels of the enzymes in animals treated with 250mg compared to the control group following the 90 days treatment. However, there was statistically significant difference (P<0.05 and P< 0.001) in the groups treated with 500mg/kg and 1000mg/kg extract respectively compared to the control.

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Table 4. Effect of methanolic leaf extract of *R. longiflora* on liver function in rats following a 30 days sub-chronic treatment

Dose (mg/kg)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Control	22.40±1.17	45.00±2.17	72.40±1.96
250	20.20±1.56	41.00±2.56	71.20±2.48
500	21.20±1.36	42.20±1.28	72.40±2.94
1000	22.40±1.33	41.60±3.78	73.20±3.64

Values are expressed as Mean±SEM, n=5; **ALP** (Alkaline phosphatase), **ALT** (Alanine aminotransferase), **AST** (Aspartate aminotransferase)

Table 5. Effect of methanolic leaf extract of *R. longiflora* on liver function in rats following a 90 days chronic treatment

Dose (mg/kg)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Control	53.20±5.95	64.60±5.87	64.80±3.14
250	61.40±8.13	79.00±4.02	68.40±1.25
500	94.40±2.40[b]	105.20±2.31[a]	77.80±4.65[a]
1000	98.40±1.72[b]	128.60±3.47[b]	88.60±7.66[a]

Values are Mean±SEM, n=5. [a]P<0.05; [b]P<0.001

6. DISCUSSION

The present study attempted to evaluate the effects of sub-chronic and chronic treatment of methanol extract of *R. longiflora* on liver function and kidney parameters in laboratory animals. The median lethal dose values (LD_{50}) of the extract were found to be greater than 5000mg/kg body weight indicating that the extract is safe. The chemical labelling and classification of acute systemic toxicity based on LD_{50} values recommended by the Organization for Economic Co-operation Development (OECD, Paris France) [12] are as follows; very toxic, ≤5mg/kg; toxic, >5 ≤50mg/kg; harmful, >50 ≤500mg/kg; and no label, >500 ≤2000mg/kg. Consequently, recognising LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated [13] The electrolytes, urea and creatinine are markers of kidney function, throughout the study the plasma levels of Na^+, K^+, Cl^-, urea and creatinine were not affected by the intake of the extract in both the sub-chronic (30 days) and the chronic (90 days) treatment, this is an indication that the extract is not nephrotoxic.

In medicine, the presence of elevated transaminases, commonly the transaminases alanine transaminase (ALT) and aspartate transaminase (AST), may be an indicator of liver damage.[4] Elevated levels are sensitive for liver injury, meaning that they are likely to be present if there is injury. However, they may also be elevated in other conditions such as thyroid disorders, celiac disease, and muscle disorders [10]. Levels over 1000 can be associated with ischemic hepatitis [11]. The enzymes levels in the rats were not affected by the treatment of the extract in all the doses in the sub-chronic treatment of 30 days and the 250mg/kg in the chronic treatment of 90 days. However, there was significance difference (P<0.05 and P<0.001) in the levels of the enzymes with the 500mg/kg and 1000mg/kg respectively, indicating that the extract is hepatoxic following prolong treatment in higher doses.

7. CONCLUSION

In conclusion, the result obtained from this study showed that the leaf extract of *Rothmannia longiflora* is safe in the kidney but may possess hepatoxic effects following prolong treatment at high doses hence people with liver disease should be cautious in the prolong use of the plant's leaves.

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