

Nephrotoxicity and Hepatotoxicity Evaluation in Wistar Albino Rats Exposed to *Nauclea latifolia* Leaf Extracts

Akinloye, O. A.^{1*} and Olaniyi, M. O.²

¹Department of Biochemistry, College of Natural Sciences, University of Agriculture, P.M.B 2240, Abeokuta, Ogun-State, Nigeria

²Department of Veterinary of Pathology, College of Veterinary Medicine, University of Agriculture, P.M.B 2240, Abeokuta, Ogun-State, Nigeria

ABSTRACT

Consumption of the aqueous leaf extract of *Nauclea latifolia* as anti-malaria concoction without any recourse or regard for its safety is a common practice in the Northern Nigeria. The aim of this study was to evaluate the safety efficacies of the ingestion of the methanolic leaf extract of this plant on the liver and kidney functions in wistar albino rats. Acute toxicity tests were carried out to determine LD₅₀, while sub-chronic toxicity study was carried out by oral administration of graded doses (200, 400, 800, 1600 and 3200mg/ Kg) of the extract to different groups of rats for 30 days. Both the liver and kidney functions assessed biochemically using standard methods revealed the LD₅₀ of *N. latifolia* at 3200mg/Kg body weight as being non-lethal. Meanwhile, biochemical and histological results obtained for the liver and kidney function parameters indicated that ingestion of *N. latifolia* leaf extract has no observable toxic effects on these organs at the tested doses. It was therefore suggested that these results could form the basis for clinical trial in human.

Keywords: Hepatotoxicity, *Nauclea latifolia* Nephrotoxicity, wistar albino rats

INTRODUCTION

Medicinal plants have been known to be useful in the treatment of various diseases all over the world since the time immemorial.

In addition, plants derived products have been used for medicinal purposes for centuries. In fact, it was estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs (Shri, 2003). The uses, modern applications and general therapeutic claims of these herbs receive widespread attention, not only in Nigeria but worldwide day by day (Jyoti *et al.*, 2009). Apart from

ARTICLE INFO

Article history:

Received: 3 January 2011

Accepted: 3 October 2011

E-mail addresses:

oaakin@yahoo.com (Akinloye, O. A), mosh_unaab@yahoo.com (Olaniyi, M. O.)

* Corresponding author

the documented severe toxic reactions arising from the use of herbs, general public and professional traditional medical practitioners/healers sometimes mistakenly think of herbs as natural alternative to drugs, failing to recognize/realize that herbs contain bioactive chemicals, some of which may be toxic (Tyler, 1994). However, many patients are under false assumption that naturally derived herbal medicines are safer with fewer side effects but this is not totally true (Gamaniel, 2000).

Nauclea latifolia (*Rubiaceae*) is a tree species grown in the northern parts of Nigeria, commonly known as “Tuwonbiri” or “Tafashiya” in Hausa, “Ubulumu” in Igbo and “Opepe” in Yoruba, has been claimed to be valuable in a wide spectrum of ailments (Onyeyili *et al.* 2001; Ajagbonna *et al.*, 2002). Nworgu *et al.* (2008) reported blood pressure lowering effect of *N. latifolia* in rats, while potential anti-diabetic properties of the plant were recorded by Gidado *et al.* (2005). Many people in Northern Nigeria treat malaria by drinking aqueous leaf extracts of *N. latifolia*; however, the responses of various organs, especially the liver and kidney (sites of biotransformation) in humans to ingestion of this extract, remain scientifically unknown. More so, there was little or dearth of information on the effects of the extract of this plant at the cellular level. Thus, this study was undertaken to examine to what extent the liver and kidney would be affected in rats exposed to *N. latifolia* leaf extract.

MATERIALS AND METHODS

Plant Materials and Preparation of Plant Extracts

The leaves of *N. latifolia* were collected within Sokoto metropolis and authenticated at the Biological Sciences Department, University of Agriculture, Abeokuta by Dr. Aworinde D.O. (Plant Taxonomist/Anatomist). The leaves were washed with tap water, air-dried and pulverized using a grinding machine. Three hundred grams (300g) of the ground sample was immersed in absolute methanol (1000ml) for 72 hours, under rigorous shaking/mixing to ensure maximum extraction. The extract was filtered through Whatman filter paper No 1, and the decoction was concentrated to dryness in rotary evaporator to obtain the crude methanolic extract, which was stored in a refrigerator until used. The extract yield was 9.8% of the starting materials.

Phytochemical Screening of the Aqueous and Methanolic Extracts of Nauclea latifolia

Phytochemical screening was carried out according to the methods proposed by Trease and Evans (1978), as described by Edeoga *et al.* (2005).

Test for tannins: The dried powdered leaf (0.5g) was boiled in 20ml of water in a test tube and then filtered. Two drops of 0.1% (w/v) ferric chloride reagent were added and observed for brownish-green or brownish-green to indicate the presence of tannins.

Test for saponins: Two (2) grams of powdered leaf was boiled in 20ml of distilled water in water bath and filtered. 10ml of filtrate was mixed with 5 ml of distilled water and shaken vigorously for stable persistence froth. The frothing was mixed with 3 drops of olive oil, before it was shaken vigorously and observed for the formation of emulsion.

Test for flavonoids: Powdered leaf (5mg) was heated in 10ml of ethylacetate over a steam bath for 3 min. The mixture was filtered and 4ml of filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed, indicating a positive test for flavonoid.

Test for steroid: Acetic anhydride (0.2ml) was added to 0.5g methanolic extract of each sample with 2ml H₂SO₄. The colour was expected to change from violet to blue or green.

Test for terpenoids (Salkowski test): The extract (0.5g) was mixed with 2ml chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoids.

Test for cardiac glycosides (Keller Killani test): Methanolic extract (0.5g) was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of conc. H₂SO₄. A brown ring of the interface indicates a deoxy sugar that characterized a cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring was gradually formed throughout thin layer.

Experimental Animals

Thirty six (36) Wistar albino rats (weighing 180-240g) of both sexes, obtained from the Department of Veterinary Anatomy, University of Ibadan, were used for the study. They were housed in well-ventilated rat cages, kept at 27-30°C, with 12 hour natural light and 12 hour darkness, and allowed free access to tap-water and dried rat pellets (Ladokun & Sons Feeds, Ltd). They were also allowed to acclimatize for a week before the commencement of the experiment.

LD₅₀ Determination/Acute Oral Toxicity Study

This was carried out according to the procedure described by Oduola *et al.* (2010). Briefly, graded doses of the extract were administered orally to six (6) groups of rats consisting of six (6) rats per group. Thus, Group 1 served as a control and received normal saline, while Groups 2, 3, 4, 5, and 6 received 200, 400, 800, 1600 and 3200 mg/Kg body weight respectively, with the aid of canula attached to a graduated syringe. All the rats were placed under observation for 24 hours, after which the number of dead rats was recorded and LD₅₀ was calculated using the formula described by Aliu and Nwude (1982).

Sub-chronic Toxicity Study

After 72 hours, none of the rats in oral toxicity study died. Thus, the extract was administered to the animals for 30 more days, at the end of which, the rats were weighed, and their blood samples were

collected through cardiac puncture under chloroform anesthesia into lithium-heparin specimen bottles for biochemical assays. The rats were then sacrificed by cervical dislocation, while liver and kidney collected for function tests and histopathological examinations were carried out using the standard techniques.

All the biochemical parameters were determined using the Chromatest reagents diagnostic kits, except for Glutathione-S-transferase (GST) whose activity was determined using the method of Habig and Jakoby (1980).

Histopathological Studies

The histological examinations of the liver section of the representative samples of these groups of rats were carried out following standard procedures.

Statistical Analysis

The mean, standard deviation and level of significance for the difference between the means of the data generated were computed using student test SPSS 6.

RESULTS

The present study attempted to evaluate the effects of ingestion of the methanolic leaf extract of the plant *Nauclea latifolia* on the liver and kidney functions in wistar albino rats. The results of the phytochemical screening of the aqueous and methanolic extracts of *N. latifolia* are presented in Table 1.

TABLE 1
The results for the Phytochemical constituents

Tests	Results	
	Methanolic extract	Aqueous extract
Cardiac glycosides	++	+
Flavonoids	++	+
Saponins	+	+
Steroids	-	-
Terpenoid	+	+
Tannins	+	+

++ Highly present
+ Present
Absent.

The results of the acute oral toxicity study revealed that there was no record of death even at the highest dose of 3200mg/

TABLE 2
LD₅₀ estimation by arithmetic method adapted by Aliu and Nwude (1982)

Dose (mg/Kg)	No. of rats	Death	Dose Difference	Mean Death	Dose Difference *Mean Death
Saline	6	0	0	0	-
200	6	0	200	0	-
400	6	0	400	0	-
800	6	0	800	0	-
1600	6	0	1600	0	-
3200	6	0	3200	0	-

Kg b.wt, as shown in Table 2. This indicated that the LD₅₀ of the plant was higher than 3200mg/Kg. In fact, all the animals appeared healthy and active throughout the experiment.

Table 3 shows the values of some electrolytes (Na⁺, K⁺ and HCO₃⁻), urea and creatinine levels between the control and the studied groups, as well as between the groups. Table 4 presents the values of total protein and albumin, ALT, AST, ALP and GST obtained for the control and studied groups (B, C, D, E and F). The plant extract over the range of tested doses showed very insignificant changes rather than producing toxicity as compared to normal.

DISCUSSION

Liver and the kidney play important roles in the biotransformation of the ingested. In particular, the liver is much more prone to xenobiotic-induced injury because of its

central role in xenobiotic metabolism, its portal location within the circulation and its anatomic-physiologic structure. The kidney, on the other hand, is highly susceptible to toxicants because of a high volume of blood flows through it and it also filters large number of toxins which can concentrate in the tubules. The leaf extract *N. latifolia* was found to contain high level of cardiac glycoside, moderate levels of flavonoids, saponins, terpenoid and tannins. However, steroids were not present in the tested doses. Judging by the current means of estimating the current LD₅₀ values, based on acute oral toxicity recommended by the Global Harmonised Systems of classification and labelling of chemicals on toxicants (Link/URL, 2010), the LD₅₀ for this particular plant extract would be greater than 3200mg; this suggests or implies that the extract is non-lethal at 3200mg, and it is therefore assumed to be safe for consumption.

TABLE 3
Effects of intake of *Nauclea latifolia* methanolic leaf extract on kidney function

Groups	Group 1 (control)	Group 2 (200mg/Kg)	Group 3 (400mg/Kg)	Group 4 (800mg/Kg)	Group 5 (1600mg/Kg)	Group 6 (3200mg/Kg)
Sodium(mmol/l)	125 ± 3.75	124 ± 4.32	127 ± 5.24	126 ± 3.45	125 ± 5.10	122 ± 4.13
Potassium (mmol/l)	4.58 ± 0.88	4.42 ± 0.76	4.38 ± 0.93	5.43 ± 0.47	5.23 ± 0.81	4.34 ± 0.62
Bicarbonate(mmol/l)	26.17 ± 2.1	24.66 ± 2.81	25.17 ± 1.31	27.17 ± 1.8	25.07 ± 1.10	22.71 ± 3.40
Chloride (mmol/l)	111.83±9.4	109.28 ± 5.68	108.19±9.30	103.50 ± 5.4	105.25 ± 5.40	107.33 ± 6.21
Urea (mmol/l)	6.71 ± 1.50	6.30 ± 1.81	6.32 ± 1.64	6.52 ± 0.99	6.04 ± 0.98	6.21 ± 0.65
Creatinine (mmol/l)	89.97±5.71	90.19 ± 14.33	93.11±10.36	90.34±12.61	92.11 ± 10.30	90.38 ± 8.21

TABLE 4
Effect of intake of *Nauclea latifolia* methanolic extract on liver function profiles

Groups Parameters	Group 1 (control)	Group 2 (200mg/Kg)	Group 3 (400mg/kg)	Group 4 (800mg/kg)	Group 5 (1600mg/kg)	Group 6 (3200mg/kg)
Total Proteins (mg/L)	3.42±0.31	3.85±0.32	3.91±0.29	3.66±0.33	3.67±0.31	3.33±0.40
Albumin (mg/L)	1.30±0.07	1.43±0.07	1.45±0.11	1.34±0.08	1.42±0.07	1.36±0.08
Total Bilirubin (µmol/L)	10.5±2.10	7.65±2.02	8.76±2.03	8.33±2.01	8.45±2.11	8.14±2.30
Conj. Bilirubin (µmol/L)	2.77±0.16	2.63±0.36	2.54±0.16	2.67±0.75	2.70±0.12	2.40±0.34
ALT (1 U/L)	25.32±1.30	26.38±1.28	26.67±1.40	27.14±1.61	27.83±2.01	25.19±2.34
AST (1 U/L)	20.17±1.29	21.88±1.26	21.64±1.70	21.28±2.00	20.71±2.11	20.86±1.44
ALP (1 U/L)	50.34±5.00	51.07±5.10	52.22±4.20	51.98±4.20	53.06±4.46	53.18±4.31
GST(units/mg)	0.96±0.04	0.99±0.03	0.94±0.02	0.90±0.04	0.82±0.03	1.12±0.41

The observed non-toxic effect or the absence of hepatocellular or nephrotoxic damage at these investigated concentrations could be buttressed by the non-significant differences in the liver and the kidney function parameters, which revealed that the conjugating ability of the liver was not compromised, especially from the total and conjugating bilirubin levels obtained. Meanwhile, non-hepatocellular damage as revealed by the ALT and AST values which were further buffered by the histological revelation. The liver sections of the control and the tested groups showed no gross lesion, except for mild hepatic vacuolation which had been observed at 3200mg.

The incidence of nephrotoxicity was also determined as a marker level of the kidney function (electrolytes, urea and creatinine) in all the experimental animals and control within the reference range throughout the period of the study. This is

in agreement with the report of Ajagbonna *et al.* (2002) and also the traditional belief that the consumption of *N. latifolia* aqueous extract, as an anti-malaria agent, seems to be not imposing any serious harmful effect(s). The results are also in agreement with the report of Mesia *et al.* (2005) who stated that the *N. latifolia* extract posed no toxicological threat to the consumer when administered as traditional remedies for malaria.

Meanwhile, the histopathological examinations of the kidney in the control and treated rats showed no visible lesion or necrotic sign. The results of this study suggest that ingestion of *N. latifolia* (at the tested concentration) has no adverse effect on the liver and kidney functions in rats. Therefore, the present study has established that ingestion of *N. latifolia* extract has no observable adverse effect(s) on the liver and kidney of rats and this could form a basis for

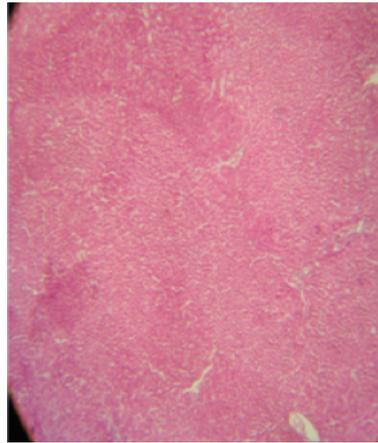


Fig. 1: Section of the liver tissue showing normal hepatocytes (control, X400 magnification)

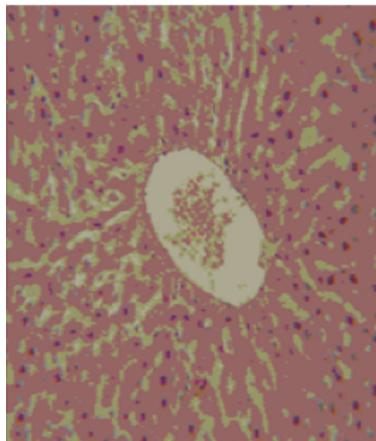


Fig. 2: Liver section showing no visible lesion (Group 2, X400 magnification)

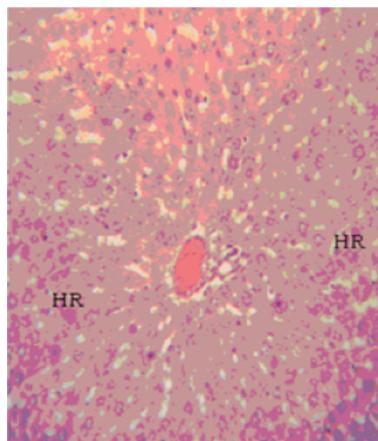


Fig. 3: Liver section showing hepatic regeneration (HR) without visible pathology (Group 3, X100 magnification)

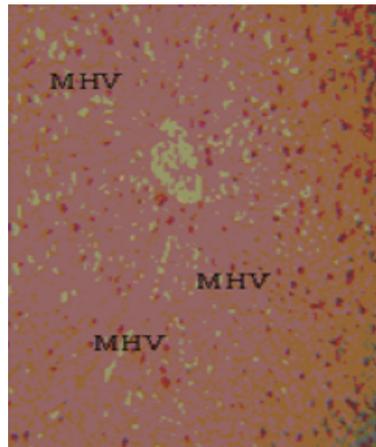


Fig. 4: Liver section showing mild hepatic vacuolation (MHV) (Group 6, X100 magnification)

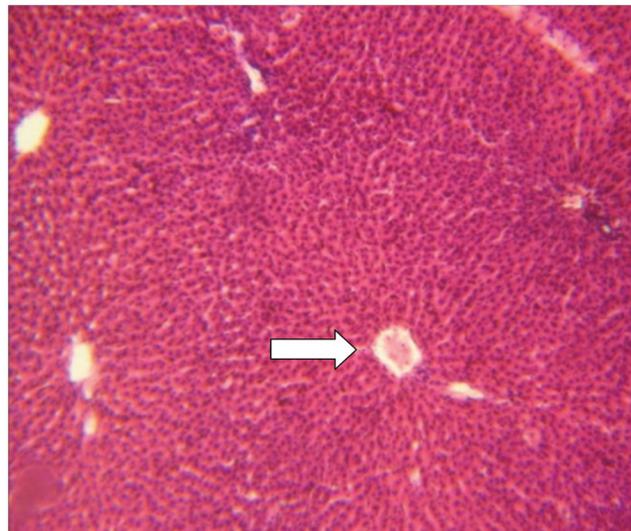


Fig.5: Liver section showing normal portal tract (group 5) x 100

the clinical trial in human. In conclusion, it can be concluded that *N. latifolia* has potential to be used in the management of hepatic and nephritic damages.

REFERENCES

- Ajagbonna, O. P., Esaigun, P. E., Alayande M. O., & Akinloye A. O. (2002). Anti-malaria activity and hematological effect of stem bark water extract of *Nauclea latifolia*. *Bioscience Research Communication*, 14(5), 481-486.
- Aliu, Y. O., & Nwude, N. (1982). *Veterinary Pharmacology and Toxicology Experiments (1st edition)* pp. 104-110. Nigeria: Baraka Press and Publisher Ltd.
- Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). *African Journal of Biotechnology*, 4(9), 685-688.

- Gamani, K. S. (2000). Toxicity from medicinal plants and their products. *Nigerian Journal of Natural Products and Medicine*, 4, 4-8.
- Gidado, A., Amen, D. A., & Atawodi, S. E. (2005). Effect of *Nauclea latifolia* leaves aqueous extract on blood glucose level of normal and alloxan-induced diabetic rats. *African Journal of Biotechnology*, 4(1), 91-93.
- Habig, W. H., & Jakoby, W. R. (1980). Glutathione transferase. In *Enzymatic Basis of Detoxification* Vol. 11 (pp 63-87). New York: Academic Press Inc.
- Jyoti, S., Sushma, S., Shashi, S., & Anjana, T. (2004). Evaluation of Hypoglycemic and antioxidant effect of *Ocimum sanctum*. *Indian J. Clinical Biochemistry*, 19(2), 152-155.
- United Nations, New York and Geneva. (2005). *Globally Harmonised System of classification and labeling of chemicals*. Retrieved from http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev01/English/00e_intro.pdf
- Mesia, G. K., Tona, G. L., & Penge, O. (2005). Anti-malaria activity and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo. *Annual Tropical Medical Parasitology*, 99(4), 345-357.
- Nworgu, Z. A. M., Onwukaeme, D. N., Afolayan, A. J., Ameachina, F. C., & Ayinde, B. A. (2008). Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats. *African Journal of Pharmacology*, 2(2), 37-41.
- Oduola, T., Bello, I., Adeosun, G., Ademosun, A., Raheem, G., & Avivirio, G. (2010). Hepatotoxicity and nephrotoxicity evaluation in wistar albino rats exposed to *Morinda lucida* leaf extract. *North American Journal of Medical Sciences*, 2(5), 230-233.
- Onyeyili, P. A., Nwosu, C. A., Amin, J. D., & Jibike, J. I. (2001). Anti-helminthic activity of crude aqueous extract of *Nauclea latifolia* Stem bark against Orine nematodes. *Fitoterapia*, 72, 12-21.
- Shri, J. N. M. (2003). Ginger: Its role in xenobiotic metabolism. *ICMR Bulletin* 2003: 33(6), 57-63.
- Trease, G. E., & Evans, W. C. (1978). *A textbook on Pharmacognosy* (11th edition). pp.1051. London: Bailliere, Trindal.
- Tyler, V. E. (1994). *Herbs of choice: the therapeutic use of Phytomedicinals*, Binghamton, N.Y: Hartworth Press, Inc.