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Effect of Ethanolic Crude Extracts of *Nauclea latifolia* Smith (*Rubiaceae*) Leaves, Fruits, Stem and Root Barks on the Liver of *Chinchilla* Rabbit

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SIO, AAN and AOO designed the study, wrote the first protocol and wrote the first draft of the manuscript. Authors AOO, RCC and TPPC managed the analysis of the study. Authors ENE and POM did the literature searches. All authors read and approve the final manuscript.

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ABSTRACT

Aims: To determine the histopathological and biochemical effects of ethanolic crude extracts of *Nauclea latifolia* leaves, fruits, stem and root barks on the liver of *Chinchilla* rabbit.
Study Design: This is an experimental study.
Place and Duration of Study: The study, which lasted for 10 weeks, was carried out at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus.
Methodology: Twenty four (24) *Chinchilla* rabbits of both sexes were grouped into 4 (A-D). Group A-C received, orally, 100 mg/kg, 150 mg/kg and 250 mg/kg body weight of the extract respectively per day. Group D (control group) received equal volume of normal saline, in addition to normal diet and water. Blood and tissue specimens were collected under chloroform euthanasia after 60 days.

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Alanine Transaminase (ALT) and Aspartate transaminase (AST) activities were determined using Reitman- Frankel method. Liver samples were processed for Haematoxylin and Eosin staining method.

Results: Decreased mean body weight values of the rabbits treated with 250 mg/kg, for 60 days was observed. The rabbits treated with 250 mg/kg for 20 days, 40 days and 60 days showed significant elevation of AST and ALT activities. However, significant decrease in AST activity was observed when the animals treated for 40 days were compared with those treated for 60 days. There was no significant change in serum ALP in all the groups. Groups B and C treated for 60 days showed hepatic injury.

Conclusion: Crude ethanolic extract of *N. latifolia* fruits, leaf, stem bark and root bark possess the tendency to adversely affect hepatic functions.

Keywords: Nauclea latifolia; Chinchilla rabbit; liver; aspartate aminotransferase; alanine aminotransferase; hypertrophy.

1. INTRODUCTION

The tropical rain forest of West Africa is endowed with enormous natural resources, prominent of amongst them are medicinal plants. Medicinal plants form the basis of medical treatment in many developing countries [1,2]. The increased resistance of parasites and other infectious agents like bacteria, against most drugs has increased the search and use of herbal products, as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases, and preventing the resurgence of older one [3]. World Health Organization [4], actively encouraged governments of their member countries to utilize their traditional systems of medicine with regulations suitable to their national health care systems. In Nigeria, the Federal Government has urged the states to set up traditional Medicine Boards to license and regulate the practice of herbal practitioners, under the supervision of Ministries of Health.

N. latifolia, an evergreen multi-stemmed shrub of the plant family, Rubiaceae, native to tropical Africa and Asia grows up to an altitude of 200 meters [5].

It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa [6]. Three other related species *Nauclea pobeguini*, *N. diderichii*, and *N. vanderguchtii* are forest trees. *N. diderichii* is planted in Omo forest reserve, Nigeria. *N. latifolia* has an open canopy and terminal spherical head lined cymes of white flowers.

The flowers are joined with their calyces. The fruit is syncarp. The tree flowers from April to June, the fruits ripening from July to September. Baboons eat them and disperse the seeds. Livestock eat shoots and leaves. The wood of *N latifolia* (Opepe wood) is termite resistant and is used as lives takes in farms. It bears interesting flowers, large red ball fruit with long projecting stamens. The red fruit is edible but not appealing. It is called *egbesi in Yoruba, Uburu Inu or Mbitinu in Igbo and Marga in Hausa.*

In Nigeria, *N. latifolia* has been widely used either singly or in conjunction with other herbs to treat malaria, trypanosomiasis, stomach upset, Measles, cold cough and general body weakness. Moreover, *N. latifolia* extract is used for the treatment of piles, leprosy, gonorrhea, as antibiotics, and to arrest preterm labour. The efficacy of the plant crude extract in the treatment of the treatment of certain ailments has been reported as follows; malaria [7-9], gastrointestinal tract disorders [10], sleeping sickness [11], prolong menstrual flow [12], hypertension [8]. The stem is also used as a chewing stick [13]. Owing to its wide applications, so many people consume the leaf, root, stem, fruit or their extract on daily basis.

In view of the above observations and sequel to the wide application of *N. latifolia* in the treatment of a wide range of diseases, and the irregularities in doses taken by patients suffering from different diseases, there is need to study the histopathological and biochemical effects of this herb in the liver, since information on these is scarcely available. It is hoped that this study on animal model will provide scientific basis of the safe consumption of this herbal extract by man. The objectives of this study therefore are to determine the onset of toxicity; to establish whether the lesions are both dose and time dependent and to evaluate the relationship between dose and impairment of hepatic functions.

2. MATERIALS AND METHODS

2.1 Collection of Plants Materials

Fresh fruits, leaves, stem bark and root bark of *N. latifolia* were collected from Nnamdi Azikiwe University Awka, Anambra State. The plant was identified at the Botany Department of Nnamdi Azikiwe University, Awka, Anambra state, by a renowned botanist, Mr Gabriel Ogbozobe. A voucher specimen was deposited at the department of Botany, Nnamdi Azikiwe University Awka, Anambra state, for reference purposes (BOT/NAU/NF0121).

2.2 Plant Extraction

The collected plant materials were washed, cut into pieces, air-dried, and then dried in an oven at 40ºC until completely dry. The dried materials were then crushed into powder and the weight determined. Seven hundred grams (700 g) of powdered plant material was macerated in two litters of 70% ethyl alcohol for 48 h, during which the mixture was stirred every 6 h using a sterile glass rod. The extract was filtered and the filtrate evaporated to dryness using rotary evaporator. The percentage yield was calculated to be 6.58%. The concentrated extract was stored in an air tight container, labeled and stored at 2-4ºC until ready for use [14].

2.3 Preparation of Stock Solution of the Extract

On each day of the experiment, 2 g of the plant extract was weighed and dissolved in 5 mL of distilled water. Serial dilutions using distilled water, was made to obtain the different concentrations for the different groups.

2.4 Collection of Animals

Twenty four (24) *Chinchilla* rabbits of both sexes, and varied weights were used for this study. They were purchased from the Animal House of College of Health Sciences, Nnamdi Azikuwe University, Nnewi Campus, Anambra State. The rabbits were allowed two weeks to acclimatize before being subjected to experimental procedures. They were housed in four (4) cages of six (6) rabbits per cage, with a 12 hour light-dark cycle, had access to food (food chow, manufactured by Grand feeds Mills Ltd Jos, Platue State) and water *ad libitum.*

2.5 Experimental Design/ Grouping of Animals

The rabbits were assigned to four (4) groups of six (6) animals on the basis of their weight and each group housed in a different cages.

1. Group A received 100 mg/kg body weight of the extract per day, in addition to normal diet and water.
2. Group B received 150 mg/kg body weight of the extract per day, in addition to normal diet and water.
3. Group C received 250 mg/kg body weight of the extract per day, in addition to normal diet and water.
4. Group D received 1 mL of normal saline per day, in addition to normal diet and water. This served as the control group.

The rabbits were subjected to this procedure for sixty (60) days and the extract was administered orally, using orogastric tube.

2.6 Sample Collection

The rabbits were fasted for 24 h after the last administration of the plant extract, blood and tissue specimens collected under chloroform euthanasia. This procedure was carried out in batches. After the first twenty (20) days of the experimental procedure, two animals, randomly selected from each experimental group were sacrificed. This was repeated after another twenty (20) days and finally after the sixtieth day of the experiment.

2.6.1 Blood samples

The blood samples were collected through the marginal ear vein and put into plain tubes, were allowed to clot and centrifuged for sufficient time to separate the sera for biochemical analysis.

2.6.2 Tissue samples

The rabbits were painlessly sacrificed, under chloroform anesthesia. Liver samples from both test and control groups were excised and examined for signs of lesions. These were later weighed and immersed in jars of 10% Neutral buffered formal saline fixative for at least five days, for histological examination.

2.7 Biochemical Assay

Alanine Transaminase (ALT) and Aspartate transaminase (AST) activities were determined using Reitman- Frankel method [15] while Alkaline Phosphatase (ALP) was determined using standard procedure [16].

2.8 Histological Preparation

2.8.1 Tissue processing

Fixed tissues were processed using Leica Jung Histokinette 2000 tissue processor. The processed tissues were then embedded in paraffin wax and 5 micron (µ) thick sections cut using Leica Rotary microtome according to Okoye et al. [17].

2.9 Staining

The sections were stained using the Haematoxylin and Eosin (H & E) according to Okoye et al. [17].

2.10 Statistical Analysis

Results are expressed as mean and standard deviation of mean (mean±SD). Data collected were analyzed using SPSS windows version 16.0 software. The level of statistical significance of the difference between control and cigarette smoke exposed groups was determined using unpaired Student's *t* test. While for the main effects on treatment groups, One-Way Analysis of Variance (ANOVA) was used for comparisons of multiple group means. Statistical differences were determined at the 5% level ($P<0.05$).

3. RESULTS

The result shows changes in the mean±SD values of the initial and final body weights of the rabbits treated for 20, 40 and 60 days. The mean values of the body weights of the rabbits treated for 20 days and 40 days were found to increase, whereas a decrease in the mean body weight values of the rabbits treated with 250 mg/kg body weight, for 60 days was observed. Therefore, it could be deduced from this analysis that at high dose and prolonged administration, *N. latifolia* extracts caused weight loss in the experimental groups of rabbits, (Table 1) thereby asserting the hypocholesterolemic effect of the plant extracts [5].

ANOVA comparism of serum liver enzyme activities amongst experimental groups, after treatment for 20, 40 and 60 days respectively, shows no significant (P=.05) change in groups A and B. Conversely, a comparism amongst the rabbits treated with 250 mg/kg for 20 and 40 days, and for 20 and 60 days show significant (P=.05) elevation of serum AST and ALT activities.

However, significant (P=.05) decrease in serum AST activity was observed when the animals treated for 40 days were compared with those treated for 60 days. There was no significant change in serum alkaline phosphatase (ALP) in all the groups. From this analysis, it could be inferred that the significant changes in serum aspartate transaminase (AST) and alanine transaminase activities which were both dose and time dependent, is an indication of hepatic injury (Table 2).

Liver sections of animals in Group A, subjected for 20, 40 and 60 days therapy of 100 mg/kg of the extract of *N. latifolia* did not show sign of hepatic injury as well as those in group B subjected for 20 days and 40 days experimentation (Figs. 1A-C). Groups B and C treated for 60 days showed hepatic injury ranging from mild to moderate hypertrophy of the central vein, edema, inflammation, vacuolation, vascular congestion to tissue necrosis (Figs. 1D-F).

4. DISCUSSION

The therapeutic importances of *N. latifolia* fruit, leaf, stem and root bark extract have been documented [2,5,10,14,19,22]. These studies notwithstanding, there is paucity of information regarding the adverse effects or toxicity of the plant extract in spite of its wide spread use in folk medicine in Nigeria. This study investigates the effect of ethanolic crude extracts of fruit, leaf, stem bark and root bark of *N. latifolia* on the liver of *Chinchill*a rabbit.

The rabbits treated for 20 and 40 days showed corresponding increase in size and body weight. On prolonged administration (60 days) of the extract, especially at high dose (250 mg/kg), there was tremendous reduction in both body sizes and weights of the rabbits. There is paucity of information regarding the effects of *N. latifolia* extracts on body weights and organ weights of animal models.

Table 1. Changes in the body weight of rabbits in both experimental and control groups

Duration (days)	Group	Initial body weight (kg)	Final body weight (kg)
20	A	0.35	0.41
	B	0.48	0. 56
	C	0.66	0.73
	D	0.85	0.96
40	A	0.41	0.44
	B	0.56	0.59
	C	0.73	0.80
	D	0.96	1.02
60	A	0.44	0.50
	B	0.59	0.64
	C	0.80	0.84
	D	1.02	1.32

Key: IBWt- Initial body weight, FBWt- Final body weight

Table 2. ANOVA: Comparism of serum liver enzymes concentration amongst experimental groups after treatment for 20, 40 and 60 days respectively

Parameter	Duration (days)	A		B		C	
		Mean value	P- value	Mean value	P-value	Mean value	P-value
AST	20VS40	4.5±1.73	.155	3.50± 1.68	.241	4.50±0.48	.003*
	20VS60	1.50±1.73	.695	0.50± 1.68	.953	-0.50±0.48	.518
	40VS60	-3.00±1.73	.329	-3.00± 1.68	.315	-5.0 ±0.48	.002*
ALT	20 VS 40	2.50±1.29	.274	1.00±1.47	.791	5.00±0.82	.018*
	20 VS 60	0.50±1.29	.932	- 1.50±1.47	.616	-2.00±0.82	.175
	40 VS 60	-2.00±1.29	.388	-2.50±1.47	.339	-7.0±0.82	.007*
ALP	20 VS 40	-42.5±29.9	.378	-25.0±30.5	.719	7.0±11.43	.824
	20 VS 60	18.0±29.9	.796	19.5±30.5	.811	32.0±11.4	.518
	40 VS 60	-60.5±29.9	.208	44.5±30.5	.421	25.0±11.4	.220

** Significant (P=.05)*

The reduction in body weights of rabbits on prolonged administration of ethanolic crude extracts of *N. latifolia* may be attributed to many factors. Omale and Haruna [5] reported that ethanolic extracts of *N. latifolia* has dehydrative effects on albino rats. The weight loss therefore may be as a result of the dehydrative effect. It appears that within 40 days of administration of the extract, the rabbits were able to counteract the dehydrative effect, but on prolonged administration became overwhelmed. Increased thirst, with corresponding water consumption on prolonged extract administration gives further clue on its dehydrative effect. The weight loss may also be attributed to the hypoglycemic activity of *N. latifolia extracts* [18]. The hypocholesterolemic effect of the *N. latifolia"* extracts as reported by Omale and Haruna [5] could also explain the weight loss observed on the rabbits, on prolonged therapy.

It was observed that there was elevated serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activity after the first 20 days of administration of the extracts. Thereafter, the serum AST, ALT and ALP concentrations decreased with increase in the number of days of administration of the extract. However, after 60 days administration of 250 mg/kg of ethanolic *N. latifolia* extracts, elevation of serum AST, and ALT activities were observed in the treated rabbits.

Significant elevation in serum concentrations of AST and ALT in the rabbits on prolonged administration of high dose of the plant extract concurs with the report of some authors [2,17]. Onyeyili et al. [19] in their study of the toxicity and anathematic efficacy of ethanolic stem bark extract of *N. latifolia*, reported elevated serum AST and ALT activities.

The result however, disagrees to an extent with the report of Madubunyi [10], who reported that the elevated Serum AST and ALT induced by CCL4 intoxication in rats was significantly attenuated by administration of *N. latifolia* extract.

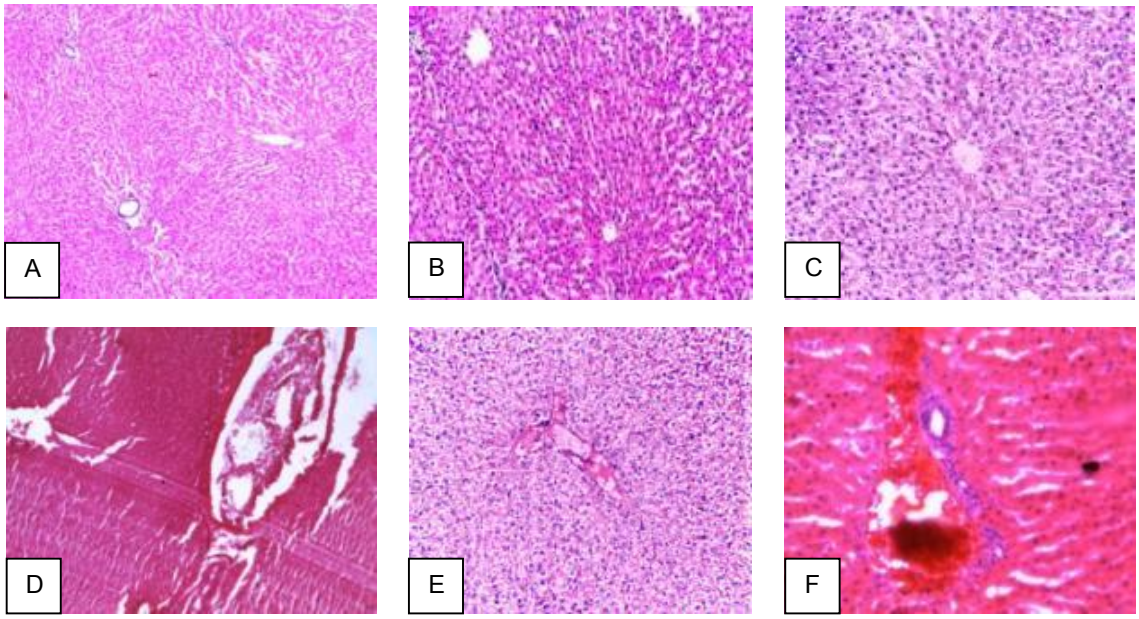

Fig. 1. Photomicrograph of liver sections stained by H&E (x100). (A) Control; showing normal histology. (B) Group A treated for 20 days with 100 mg/kg body weight; showing normal histology. (C) Group A treated for 40 days with 100 mg/kg body weight; showing normal histology. (D) Group B treated for 60 days with 150 mg/kg body weight; showing hypertrophy of central vein with signs of inflammation. (E) Group C treated for 60 days with 250 mg/kg body weight; showing evidence of necrosis. (F) Group C treated for 60 days with 250 mg/kg body weight; showing hypertrophied central vein with sign of edematous infiltrate accumulation within the vein and tissue necrosis

Serum AST and ALT have been used as a marker of liver function, and form major indices to monitor liver pathology [20]. When a cell is damaged, it leaks these enzymes into the blood, where they are measured. ALT and AST rise dramatically in acute liver damage, such as viral hepatitis or paracetamol (acetaminophen) overdose. Elevations are often measured in multiples of the upper limit of normal (ULN). Increased Serum concentration of AST and ALT are indicative of adverse pathological effects on the liver. From this study, the elevated serum AST and ALT, may not indicate serious hepatotoxicity, but suggest the sensitivity of these biochemical liver markers at prolonged administration of high dose of the ethanolic extract of *N. latifolia*.

The signs of hepatic injury observed at prolonged duration of higher dosage of the extract are an indication of hepatotoxic effect of the ethanolic crude extract of *N. latifolia* on prolonged administration. This finding, though not reported by many concurs with the report of Akpanabiatu et al. [21].

5. CONCLUSION

In view of the result of this present study, it could be deduced that at low dosage and short period of administration, ethanolic extracts of *N. latifolia* is relatively non toxic to the liver. However, prolonged period of administration of higher dose may cause various degrees of hepatic injury. That the lesions were both dose and time dependent. Though the extracts of *N. latifola* have been reported to be relatively non toxic to the liver, this study revealed that at high dosage and prolonged therapy, the ethanolic extract of *N. latifolia* fruits, leaf, stem bark and root bark possess the tendency to adversely affect hepatic functions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the ethical committee of Faculty of Health

Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus". All autors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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