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Histopathological Studies of the Effects of Nauclea Latifolia Ethanolic Root Extracts in Rats

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Abstract

Nauclea latifolia (Smith) (Family: *Rubiaceae*) also known as 'Pin Cushion tree' or 'African Peach' is a struggling shrub, native in tropical Africa and Asia where the use of folk medicine is prevalent and the search for herbal cure is but common practise. Extraction of the root of *Nauclea latifolia* with ethanol yielded the ethanol extract. The acute toxicity study on the ethanol extract in mice established an intraperitoneal LD_{50} greater than 4000mg/kg. Thus, the ethanol extract could be generally regarded as safe.. The histopathological sections of the liver and kidney of rats treated with ethanol extract of *Nauclea latifolia* showed that the extract contained some histopatotoxic compounds which might be responsible for the hepatocellular necrosis, inflammation and circulatory disturbances compared to the untreated rats. The present study

established that the continuous administration of the ethanol crude extract of *Nauclea latifolia* is lethal to the hepatocytes and kidneys.

Keywords; Histopathology, *Nauclea latifolia,* Liver, Kidney, Ethanolic extract and toxicity, Hepatocellular necrosis

INTRODUCTION

In recent time, copious attention has been focused on the use of medicinal plants in the management and treatment of ailments such as anemia, diabetes and malaria. Due to the local availability, easy access and relatively low cost, medicinal plants are gaining attentions in health care programmes. Based on estimation by World Health Organization (WHO), larger percentage (between 80 and 90%) of the world's population especially in developing countries depends on traditional system of medicine (Kone *et al.,* 2012; Van Andel and Carvalheiro, 2013). Despite the therapeutic importance of medicinal plants, toxic substances have been shown to be present in large numbers of plants investigated (Mounanga *et al.*, 2015). Contamination of medicinal plants could be as a result of substances such as heavy metals, aflatoxin and pathogenic microbes from the soil and the manner of herbal preparations (Olaniyan *et al.*, 2016). Consumption of medicinal plants without scrutinizing its efficacy and safety can lead to unexpected toxic effects resulting in physiology changes in different body organs. Liver and kidney are the main targets of toxicants because they are respectively involved in biotransformation and excretion of xenobiotics. Hepatic and renal damage have recently been linked with the use of medicinal plants in the treatment of various diseases (Olaniyan *et al.*, 2005; Mapanga and Musabayane, 2010).

Nauclea latifolia is an evergreen multi-stemmed tree that grows to an altitude of 200 meters. It is commonly known as 'Pin cushion tree' and 'African peach' in English and sold by the trade name '*Opepe*' in Nigeria (Udobi and Umoh, 2017). Locally, in South-South region of Nigeria, it is known as '*Itu*' by the Itsekiris in Edo state and '*mbomibong*' by the Ibibios and Efiks in Akwa-Ibom state. In Hausa, Northern Nigeria it is known as '*Tafashiya'* or *'tafiyayaiga* and as '*Uche*' by the Igedes in Benue state, Nigeria. In Ibo land, Southeast Nigeria, it is known as '*Uburuinu*' or '*Ovoroilu*' and '*egbeyesi' or 'egbesi'* in Yoruba, South West

Nigeria (Udobi and Umoh, 2017; Balogun *et al*., 2016). Ethnomedicinal usages includes in treating hypertension and diabetes (Balogun *et al*., 2016). In Northern Nigeria, cold infusion of the stem bark is used as diuretic and anthelmintic. It is used as mouth antiseptic, as chewing stick, as remedy for gastric pain and tuberculosis (Udobi and Umoh, 2017). Usages as antimalarial, antipyretic and aphrodisiac, in wound healing and as a vermifuge have all been reported (Udobre *et al*., 2013). Almost all parts of the plant are useful in disease treatment. Frequency of usage in ethno-medicine is: roots > stem > bark > leaf (Balogun *et al*., 2016). Pharmacological assays have confirmed its effectiveness as anti-infective agent and in malaria treatment (Udobre *et al*., 2013; Iyamah and Idu, 2015). Other properties of *N. latifolia* include its antipyretic, anti-inflammatory, antinociceptive (Abbah *et al*., 2010; Taïwe *et al*., 2014), anthelminthic (Agyare *et al.,* 2014; Tittikpina *et al*., 2016), anticonvulsant and anxiolytic (Bum *et al*., 2009), antidiabetic (Gidado *et al,* 2009), anti-hypertensive (Odey *et al*., 2012); antibacterial (Egwari *et al.,* 2010) and anti-trypanosomal (Ugwu *et al.,* 2016), for wound healing (Udobre *et al*., 2013) and antidiarrheal activities (Owolabi *et al*., 2010).

MATERIALS AND METHODS

Animal material

The experimental animals used for the study were male and female Wister albino rats (weights 103-230g). The rats were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and were kept for seven days to acclimatize and given feed and water *ad libitum*.

Plant material

Nauclea latifolia roots were obtained from a forest in Okpome-Agbada Nenwe, Aninri Local Government Area of Enugu State, Nigeria. The roots were authenticated by Mr. Njokuocha in the Department of Botany, University of Nigeria, Nsukka. The roots were dried at room temperature in the absence of sunlight for one week, cut into bits and were ground to coarse powder using a hammer mill.

Extraction procedure

Preparation of ethanolic extract

About 500g of the powdered root of *Nauclea latifolia* was soaked in 3.4 liters of 70% ethanol. The mixture was left to stand for twenty-four hours with occasional stirring. The

mixture was later extracted using a soxhelet extractor to obtain the ethanol extract. The extract was concentrated over a water bath at a temperature of 25 to 30°C to obtain 46g (9.2%) of the ethanol extract.

Determination of concentration of extract

A crucible was weighed and a known weight of the extract was poured into the crucible and their combined weight determined before heating. The crucible was heated until all its content was charred. After heating, the crucible was re-weighed with its content and the weight recorded. The concentration of the solid extract was then determined from the two weights.

Experimental design

A total of sixty Wister albino rats were used in this study. The rats were randomly divided into four (4) groups of 15 rats each.

Group 1: the control group was administered normal saline

Group 2: was administered 10 mg/kg of the ethanolic extract

Group 3: was administered 50 mg/kg of the ethanolic extract

Group 4: was administered 250 mg/kg of the ethanolic extra

The extracts were administered by dissolving accurately measured doses in water and feeding the rats orally on a daily basis for 42 days. At the end, the rats were sacrificed and some internal organs removed, fixed and later used for histopathological studies.

Acute toxicity test (LD_{50})

The median lethal dose (LD_{50}) of the ethanol extract was determined in rats using the oral route of administration (OECD, 1995). A total of thirty albino Wister rats were randomly divided into six groups of five rats. Each rat was kept in a separate clean stainless cage and provided with water and feed *ad libitum*. The rats in the various groups were injected intraperitoneally with increasing doses of 250, 500, 1000, 2000 and 4000mg/kg body weight of the *Nauclea latifolia* ethanolic extract while the control group received an equal volume of distilled water. The rats were allowed to feed and were closely observed for mortality and toxic signs for 5 days. No death was recorded. Since the extract did not cause any death in the rats at concentrations above 4000mg/kg, the ethanolic extract of *Nauclea latifolia* was considered non toxic (OECD, 1995).

Cytotoxicity test (LC_{50})

The method of Meyer *et al.,* 1982, modified by Mclanghlin *et al*., 1991, was used to study the cytoxicity of *Nauclea latifolia* ethanolic extract. *Artemia salina* eggs obtained from Dr. A.O. Onaga, of the department of veterinary pharmacology and physiology, were incubated in natural sea water (from Bar Beach, Lagos Nigeria) in a dam-well under room condition. Forty-eight hour naupli (10 in each well) were incubated in known concentrations of 10, 100 and 1000ppm of the extract for 24 hours. All tests were conducted in triplicate. The dead nauplii were subtracted from 10 and the data were analysed using profit analysis (Finner Program, DOS) to determine the LC_{50} at 95% confidence interval. Weak nauplii were noted as an indication of central nervous system depressions while the control was incubated in sea water, the incubation medium.

Histopathological Examination (Bigoniya *et al*., 2015)

A. Slicing, fixation and washing.

A thin section of the tissue (about 1 to 2 cm in diameter) was trimmed with a sharp razor blade. Formalin was used as the fixative agent and for the purpose of preservation. The small pieces of the tissues were placed in 10% formalin, the container was shaken gently several times to make sure that the fluid had reached all surfaces and the pieces were not sticking to the bottom. This was incubated for 24 hours, to allow proper fixing. The fixed tissue pieces were washed with running water for 24 hours to free them from excess fixatives (Bigoniya *et al*., 2015)

B. Dehydration

All water was removed from the tissue before embedding them in paraffin. The dehydration was achieved by immersing the thin section of the tissue in automatic tissue processor containing 12 jars. The first three jars contained 70 %, 90% and 90% absolute alcohol respectively. This was done to remove the water content in the tissues. The absolute alcohol reduced the shrinking that occurred in the tissue. The time for each step was 30 minutes. A second change of absolute alcohol was included to ensure complete removal of water. This was achieved in the second three jars of the automatic tissue processor, called a well-refining step. (Bigoniya *et al*., 2015)

C. Clearing

Solution of xylene was used for clearing the tissue sections. This step was achieved in the third three jars of the automatic tissue processor. This was because the alcohol used for dehydration would not dissolve or mix with molten paraffin, the tissue was immersed in xylene solution which was miscible with both alcohol and paraffin before infiltration could take place. Clearing removes opacity from dehydration tissue, making them transparent. A period of 15 minutes was allowed to elapse before the tissue was removed from the solution for infiltration with paraffin. (Bigoniya *et al.*, 2015)

D. Infiltration with Paraffin

Paraffin wax with a melting point of 50^{o}C to 52^{o}C range was used to infiltrate the tissue. The tissue was transferred directly from the clearing to a bath containing melted paraffin. After 30 to 60 minutes incubation in the first bath, the tissue was removed to a fresh dish of paraffin contained in the fourth three jars of the automatic tissue processor for a similar length of time. (Bigoniya *et al.,* 2015).

E. Embedding (Blocking) with Paraffin

As soon as the tissue was thoroughly infiltrated with paraffin, the paraffin was allowed to solidify around and within the tissue. The tissue was then placed in a small container already filled with melted paraffin and the whole content was cooled rapidly with water to embed the tissue sections (Bigoniya *et al.*, 2015).

F. Paraffin Sectioning

The embedded blocks were trimmed into squares and fixed in the microtome knives for sectioning after which the sections were floated on a water bath (Bigoniya *et al.,* 2015).

G. Mounting

Glass slides were thoroughly cleaned and a thin smear of albumen fixative was made on the slides. The albumenized slide was used to collect the required section from the rest of the ribbon in the water. The section on the glass slide was kept moist before staining. (Drury *et al.*; 1967)

H. Staining with Hematoxylin

The slides were passed through a series of jars containing alcohols of decreasing strength and various staining solutions in the following order:

Xylene	3 minutes
Absolute Alcohol	3 minutes
95% Alcohol	2 minutes
70% Alcohol	2 minutes
Lugol's solution	3 minutes
Running water	3 minutes
5% sodium thiosulphate	3 minutes
Running water	5 minutes
Delafield Hematoxylin	5 minutes
Running water	3 minutes
Scott solution	9 minutes
Running water	3 minutes

The counterstaining of the tissue with eosin was followed in the order below:

70% Alcohol	1 dip
95% Alcohol	2 dips
Absolute Alcohol	3 minutes
Absolute Alcohol-Xylene (1:1)	3 minutes
Xylene	3 minutes
Mounting medium	The section was kept with xylene while cover glass was added on the glass slide

I. Microscopic Observation of slide

The slides prepared were mounted on a photomicroscope, one after the other and were then viewed at different magnification powers of the microscope. Photographs of each of the slides were taken.

RESULTS

Extraction

The ethanolic extract obtained weighed 46g, which is 9.2% yield from the 500g of the raw *N. latifolia* root material started with.

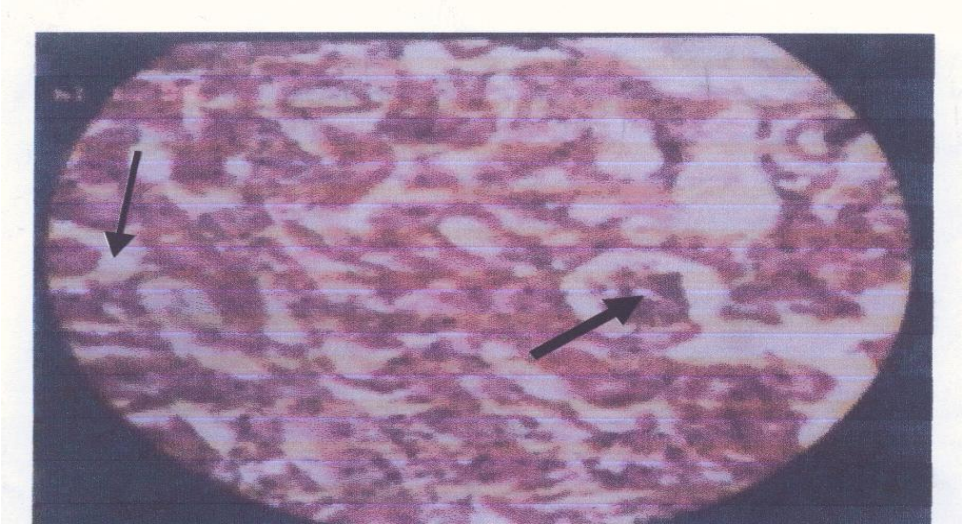
Acute Toxicity test (LD_{50})

Group	Extract	Dose (mg/kg)	Mortality/Group
Group1	Ethanolic	250	0/5
Group2	Ethanolic	500	0/5
Group3	Ethanolic	1000	0/5
Group4	Ethanolic	2000	0/5
Group5	Ethanolic	4000	0/5

In the acute toxicity test, administration of 250, 500, 1000, 2000 and 4000mg/kg doses respectively of the ethanolic extract for 5 days, caused no death in the rats. Therefore, the extract of *Nauclea latifolia* root was considered not toxic even at 4000mg/kg doses.

Histopathological changes

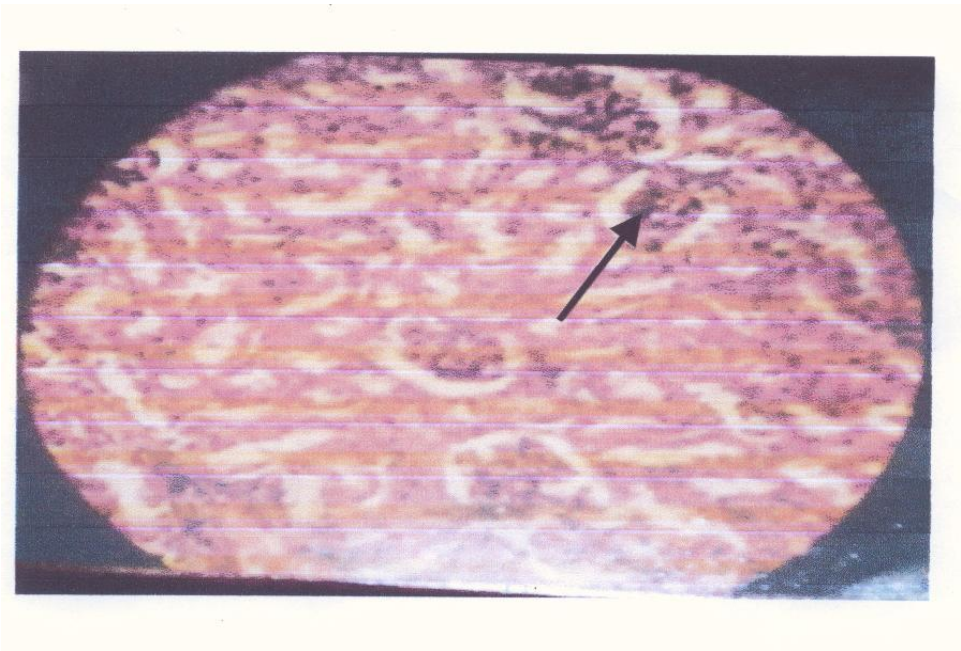
Plate3.10.1: Kidney cortex of rat treated with ethanol extract (Day 28)



H and E stain x 400

The result shows a stained kidney cortex having degeneration of the glomerulus with tubular dilations.

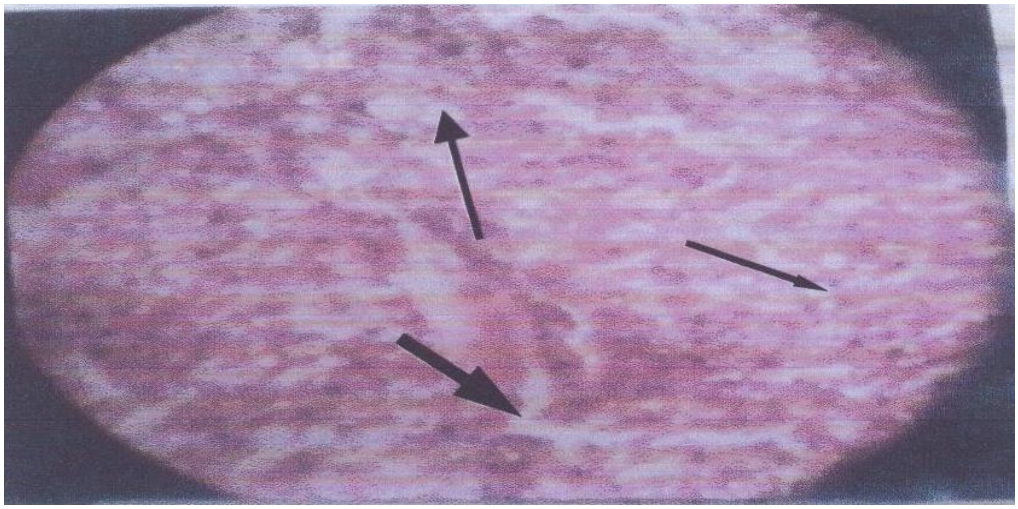
Plate 3.10.2 Kidney cortex section of the control rat



H and E stain x 400

The result shows the normal glomerulus with the proximal and distal convoluted tubules lined by thick cuboidal epithelium.

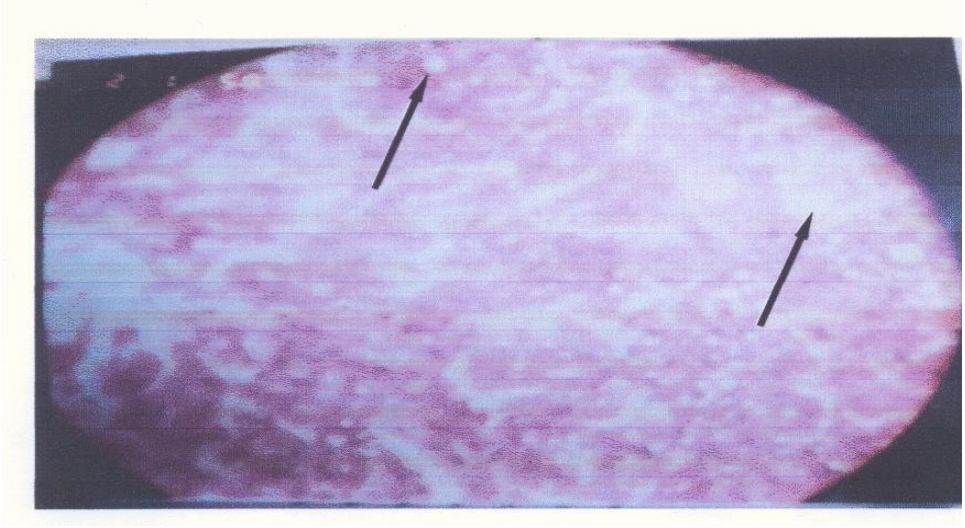
Plates 3.10.3 Liver section of rat treated with ethanol extract (Day 28)



H and E stain x 400

The result showed the haepatocyte degeneration, necrosis, pathological fatty infiltrations and portal mononuclear cell infiltrations seen on the liver section.

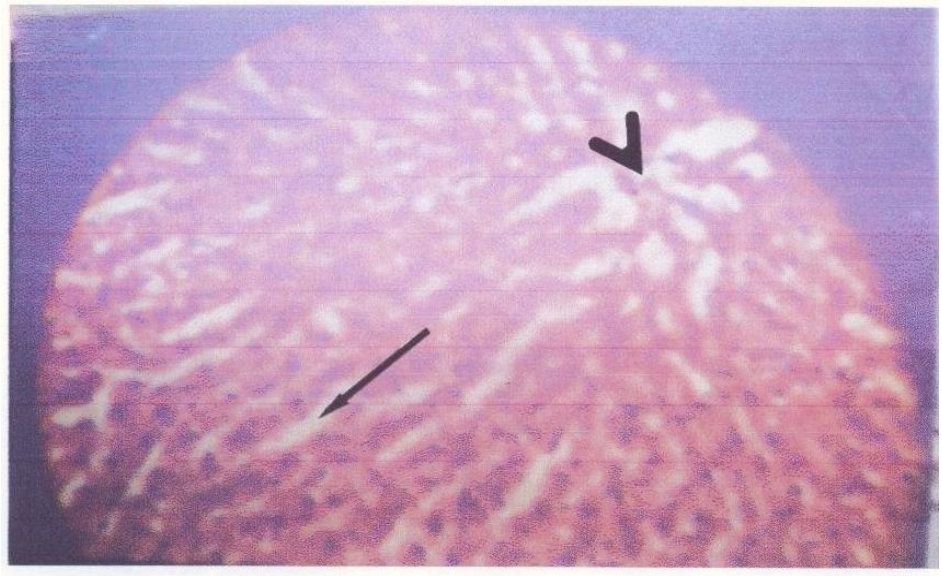
Plate 3.10.4 Liver section of rat treated with ethanol extract (Day 42)



H and E stain x 400

The above result represents a stained liver section with haepatocyte degeneration, necrosis and focal fatty infiltration. The liver section does not show a liver lobule and the central veins are not separated by sinusoids.

Plate 3.10.5 Liver section of the control rat



H and E stain x 400

The result above is the stained liver section of the control rat that shows a healthy liver lobule with centre vein. The hepatocytes are arranged in laminae which radiate from the central vein and are separated by sinusoids.

DISCUSSION

Liver and kidney microscopic pathologies serve as important tools for identifying and characterizing liver and kidney injuries respectively, whether or not biochemical, haematological and macroscopic changes are also identified (Debebe *et al.*, 2017). The acute toxicity study of the ethanol extract in rat established an intraperitoneal LD_{50} greater than 4000mg/kg. Thus, the ethanol extract could be generally regarded as safe (Lorke, 1983). Based on the results obtained, the extract of *Nauclea latifolia* may be slightly toxic. However, since the knowledge of pharmacokinetics of a drug, apart from the dose, is also required in determining the degree of toxicity, toxicological studies on the extract are still being determined. More human and animal experimental studies are still needed to clarify this issue.

The result of this work showed that the histopathological changes in the liver and kidney sections of rats in the different experimental groups were characterized by necrosis, inflammatory and degenerative changes. The liver section of rat treated continuously with the ethanol extract for 42 days showed greater hepatocyte degeneration with fatty infiltrations and the absence of central vein. Liver section of control rats showed a healthy liver lobule having its central vein. The hepatocytes were still arranged in plates, radiating from the central vein and were separated by sinusoids, thus confirmed that the ethanol extract of *Nauclea latifolia* root had some destructive effects on the liver of the treated rats.

The kidney section of rat administered the ethanol extract for 42 days showed mild tubular degeneration having focal mononuclear cell infiltrations. The result indicated that the ethanol extract had a less destructive effect on the kidney than it has on the liver. The liver is a vital organ involved in the detoxification of mostly xenobiotics from the body. Thus, during the process of detoxification and deamination, some non-detoxified compounds in the ethanol extract may have destroyed the hepatocytes which are involved in detoxification than the kidney cells which are mostly involved in excretion.

CONCLUSION

In conclusion, it could be deduced that at low dosage and short period of administration, ethanolic extracts of *N. latifolia* roots is relatively non-toxic to the liver. However, prolonged administration or higher doses may cause various degrees of hepatic and renal injuries as it was seen that the lesions were both dose and time dependent. Hence, this study has revealed that at high dosages and prolonged administration, the ethanolic extract of *N. latifolia* root possesses the properties that can adversely affect hepatic and renal functions.

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