

Acute and Subchronic Evaluation of Aqueous Extracts of *Newbouldia laevis* (Bignoniaceae) and *Nauclea latifolia* (Rubiaceae) Roots used Singly or in Combination in Nigerian Traditional Medicines

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Abstract: This study was aimed at evaluating the acute and subchronic toxicities of aqueous extracts of *Newbouldia laevis* stem bark and *Nauclea latifolia* roots, used extensively, in Nigerian herbal medicine. Acute toxicity study was carried out on Swiss albino mice. The extracts mixed (1:1) in the doses ranging between 1.0 g to 20.0 g/kg body weight (bwt) were administered orally to the mice (22.5±2.5 g) and observed continuously for the first 4 h and hourly for the next 12 h, then 6 hourly for 56 h. In subchronic toxicity study, wistar rats (150±20 g) were fed with 100, 250 and 500 mg/kg bwt daily of the extract mixture (1:1) combination and 500 mg/kg bwt dose of the respective extracts for 30 days. The effects on the biochemical and haematological parameters were evaluated and also the effects on some vital organs were histologically examined. The results showed that all the animals that received 20 g/kg bwt of the extracts (1:1) combination survived beyond 24 h. Significant ($p<0.05$) decrease in the plasma glucose level occurred. Lipid profile study showed insignificant increase in total cholesterol (T Chol), High Density Lipoprotein (HDL) and Triglycerides (TG) levels compared to the control while low density lipoprotein (LDL) level decreased appreciably. Aspartate Aminotransferases (AST), Alanine Aminotransferases (ALT) and creatinine levels decreased significant ($p<0.05$). The photomicrographs of hepatic, kidney and testicular tissues treated with 500 mg/kg bwt of the respective extracts and 500 mg/kg bwt of the combination indicated no abnormalities. The extracts could be considered safe at the doses administered since they did not provoke toxic effect on the key organs examined.

Keywords: Acute, *Newbouldia laevis*, *Nauclea latifolia*, subchronic, toxicity

INTRODUCTION

Plants and their derived products from the outset have served as veritable sources of food for humans and animals. The discovery since prehistoric era that plants products, in addition to their nutritive values, could serve as therapeutic weapons against various human, animal and even plant diseases has made plants a sine qua non to human and animal lives (Ogonnia *et al.*, 2008; 2011a). Plant derived medicines popularly known as “Herbal drug“ or “phytomedicine” is currently renowned and is recognized as the most common form of alternative medicine. It is used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly used (Rickert *et al.*, 1999; Ogonnia *et al.*, 2009).

The use of herbal remedies especially in the form of teas or extracts for the treatment of various diseases

is gaining increasing popularity, making them the main stay of health care system, especially among the rural populace in the developing countries. Their increasing popularity could be attributed to their advantages of being efficacious and a cheap source of medical care. WHO Expert Committee (1985) supports the appropriate use of herbal medicines and encourages the use of herbal drugs that have been proven to be safe and effective. Herbal medicines most often in the developing countries are prepared by the traditional herbalists many without formal education and are simply used on the basis of traditional knowledge and experience to protect, restore or improve health. Only a few of these preparations have been tested and evaluated scientifically for safety. There is therefore the need for a scientific evaluation of herbal medicines’ for safety, in addition to the experience gathered from their traditional use over the years.

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Secondly, there is a growing disillusionment with modern medicine and also misconception that herbal remedy being natural may be devoid of adverse and toxic effects often associated with allopathic medicines (Ogbonnia *et al.*, 2011b). Herbal preparations could be contaminated with microbiological and foreign materials, such as heavy metals, pesticide residues or even aflatoxins. Contaminants when present in an herbal preparation may give it the capacity to produce prominent health defects underscoring the claimed safety. An increase in the morbidity and mortality associated with the use of herbal or the so called traditional medicines has raised universal attention in the last few years (Bandaranayake, 2006; Ogbonnia *et al.*, 2011b). Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations imperative.

Herbal recipes are prepared most often from a combination of two or more plant products which many a time may contain active constituents with multiple physiological activities and could be used in the treatment of a variety of disease conditions (Pieme *et al.*, 2006). Therefore, warning regarding the potential toxicity of herbal therapies employed in the treatment of diseases over a long period of time demands that the practitioners should be kept abreast of the reported incidence of renal and hepatic toxicity associated with the ingestion of medicinal herbs (Tédong *et al.*, 2007). For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine especially for long term treatment, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings (Mythilypriya *et al.*, 2007).

The aim of this study was to evaluate the safety of a *Newbouldia laevis* (*N. laevis*) and *Nauclea latifolia* (*N. latifolia*) roots teas or extracts used singly or in combination by carrying out the acute and subchronic toxicity studies in animal model. Subchronic toxicity evaluation is required to establish potential adverse effects of these highly valuable herbal products that are now widely consumed for their physiological benefits.

MATERIALS AND METHODS

Plant materials: The plant materials, *Newbouldia laevis* (P. Beauv.) Seem. ex Bureau (Fam. Bignoniaceae) root bark and *Nauclea latifolia* Smith (Fam. Rubiaceae) root bark were bought from Mushin market in Lagos suburban and were authenticated by Mr. B.O. Daramola of the Department of Botany and Microbiology, University of Lagos. The voucher specimens LUH 3595 and LUH 3756, respectively were deposited at the Department of Botany Herbarium.

Laboratory animal acquisition and maintenance: Male and female Wistar rats (weighing 150±20 g) and

Swiss albino mice of both sexes (weighing 22.5±2.5 g) were used for this study. These animals were bred and housed in the Animal Facility Centre of the College of Medicine, University of Lagos, Idi-Araba, Lagos and were separated and kept for one week to allow for acclimatization in a cage lined with wooden shaves, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h of light and 12 h of darkness. The animals were fed a certified feed (Feeds Nigeria Limited) and had free access to tap water but fasted overnight prior to oral administration of the extracts.

Preparation of plant extracts: The root barks of the plants were washed to remove foreign materials and then cut into smaller pieces for easy drying and blending. They were dried at an ambient temperature between 45-50°C in an oven for five days and powdered to coarse particles using a commercial blending machine. Four hundred and sixty grams (460 g) of the powdered *N. laevis* root and four hundred and twenty grams (420 g) of the powdered *N. latifolia* roots were, respectively boiled with 2.0 L distilled water for 25-30 min and allowed to decoct. After 24 h they were strained with cotton wool; the liquid extracts were then filtered using a filter paper (What man's no. 4). The resulting filtrates were lyophilized yield 24.8 g (5.4 % yields) and 22.2 g (5.3 % yields) for *N. laevis* and *N. latifolia* extracts, respectively.

For each dose used, the volume administered was calculated using (Tédong *et al.*, 2007) equation as follows: $V \text{ (ML)} = (D \times P) / C$. Where D = dose used (g/kg body weight), P = body weight (g), C = concentration (g/mL) and V = volume.

Acute toxicity study of the extracts mixture: Thirty five male and female Swiss albino mice weighing 22.5±2.5 g were used for the acute toxicity study. They were randomly distributed into one control group and six treated groups, containing five animals per group and were maintained on standard animal diet (Feeds Nigeria Limited) and provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. After fasting the animals' over-night, the control group received 0.4 mL of 2% w/v acacia suspension orally. Then the solution of the extract was prepared by dispersing 4.38 g of the mixture of the dried plant extracts containing an equal amount of each extract with 5 mL of 2% w/v acacia suspension and each treated group received the extract doses as follows: 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 mg/g body weight (bwt) orally. The animals were observed continuously for the first 4 h and for every hour for the next 12 h, then 6 hourly for 56 h after administering to observe any changes in general behaviour or other physiological activities (Shah *et al.*, 1997; Bürger *et al.*, 2005).

Subchronic toxicity study: Male and female Wistar albino rats weighing 150±20 g were used and were allowed to acclimatize to the laboratory conditions for seven days, maintained on standard animal feeds and provided with water *ad libitum*. The animals were weighed and divided into six groups of five animals each. The control group received a dose of 0.5 mL of acacia suspension and other groups received specified doses orally respectively once a day for 30 days (Pieme *et al.*, 2006; Joshi *et al.*, 2007; Mythilypriya *et al.*, 2007).

Treatments were as follows:

Group I : Extract A-*N. laevis*, at a dose of 500 mg/kg bwt.

Group II : Extract B-*N. latifolia*, at a dose of 500 mg/kg bwt.

Group III : (1:1) Mixture at a dose of 500 mg/kg b.wt,

Group IV : (1:1) Mixture at a dose of 250 mg/kg b.wt,

Group V : (1:1) Mixture at a dose of 100 mg/kg b.wt,

Group VI : Normal rats were treated with 0.5 mL 2% w/v acacia suspension (positive control).

Evaluation of biochemical parameters: The initial weights of the animals were recorded. Thereafter, weights were taken at five days intervals from the beginning of the treatment (Ogbonnia *et al.*, 2011a; 2011b). On the 31st day, after overnight fast, the animals were sacrificed under mild diethyl ether anaesthesia and blood was obtained via cardiac puncture into fluoride oxalate, heparinized and EDTA containers. The blood collected with fluoride oxalate tube was centrifuged within 5 min of collection at 4000 g for 10 min and plasma obtained was used to determine the blood glucose level. The Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoprotein (HDL-cholesterol) levels and other biochemical parameters were estimated with heparinized blood using precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan *et al.*, 2001). Low density lipoprotein (LDL-cholesterol) level was calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) and creatinine by standard enzymatic assay method. The Total Plasma Protein (TPP) content was determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). The total bilirubin (T. BIL) was determined using Jandrossik and Grof technique as described by Koch and Doumas (1982). Plasma albumin was determined based on its

reaction with bromocresol green (Binding method) as described by Spencer and Price (1971). Urea was determined according to Urease-Berthelot method as described by Weatherburn (1967). Alkaline Phosphate (ALP) was analyzed according to Kind technique as described by Koch and Doumas (1982).

Haematological study: The haematological parameter was determined using blood collected with EDTA containers (Ogbonnia *et al.*, 2011a; 2011b). Diethyl ether was used to anaesthetize the animals before blood samples were collected through heart puncture into EDTA tubes for the haematological parameters analysis. The blood samples were analyzed for Red Blood Cells (RBC) by haemocytometric method (Dacie and Lewis, 1984); the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984); Packed Cell Volume (PCV) was according to Ekaidem *et al.* (2006) while White Blood Cells (WBC) and its differentials (neutrophil and lymphocyte) were determined as described by Dacie and Lewis (1984).

Tissue histology: The organs were fixed in 10% formal saline for seven days before embedding in paraffin wax. Each organ tissue of heart, kidney, liver and testis was sectioned at 5 µm and stained with Haematoxylin and Eosin (H and E) stain. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

Data and statistical analysis: All data collected were summarized as mean±sem. The data for the subchronic toxicity test were statistically compared by using student t-test.

RESULTS

Acute toxicity test: The acute toxicity study (Table 1) recorded 100% survival beyond 24 h for all the animals that received 20.0 g/kg bwt of the extract. The median acute toxicity (LD₅₀) of the extract should therefore be over 20.0 g/kg bwt.

Variation of weights: The effect of the drug on the body weights of the control and treated animals is shown in Table 2 and the percentage increase in the

Table 1: Acute toxicity of the (1: 1) mixture of the plant extracts in mice

Groups	Doses (g/kg)	Log dose	Number of animals	24 h Mortality	% Mortality	Probit
I	1.00	0.0000	5	0	0	0
II	2.50	0.3940	5	0	0	0
III	5.00	0.6990	5	0	0	0
IV	10.0	1.0000	5	0	0	0
V	15.0	1.1760	5	0	0	0
VI	20.0	1.3010	5	0	0	0

Control received 0.4 mL each of 2% acacia suspension

Table 2: Effect of the extracts on biochemical profile of the rats after 30 days of sub-chronic toxicity study

	GPI	GPII	GPIII	GPIV	GPV	GPVI
ALB (g/L)	44.1±0.7	44.2±2.2	46.1±2.0	47.1±1.9	44.8±2.3	48.1±2.5
TPP (g/L)	71.4±0.7	72.2±0.5	70.2±0.4	68.2±0.4	72.0±0.5	66.8±0.5
HDL (mmol/L)	0.9±0.00	1.20±0.0	1.40±0.2	0.80±0.0	0.90±0.0	0.80±0.3
LDL (mmol/L)	0.03±0.0**	0.01±0.1**	0.02±0.0**	0.10±0.0	0.20±0.2	0.30±0.0
TChol (mmol/L)	2.10±0.1	2.10±0.1	1.40±0.1	1.60±0.0	1.40±0.2	1.40±0.1
TG (mmol/L)	0.60±0.0	0.80±0.2	0.70±0.0	0.90±0.0	0.60±0.1	0.60±0.0
ALP (U/L)	101.7±2.8*	164.9±4.2	140.2±3.5*	128.8±3.3*	133.2±3.4*	162.2±4.5
GLU (Mmol/L)	3.10±0.1*	3.90±0.1**	2.10±0.1*	4.20±0.2	3.60±0.1**	4.80±0.1
AST (U/L)	102.7±6.1*	145.1±8.6*	104.1±6.2*	142.2±8.4*	56.6±3.3*	199.1±8.8
ALT (U/L)	23.1±1.3*	45.0±2.6**	27.0±1.5*	31.4±1.8*	46.5±2.8*	61.3±3.5
T.BIL (Umol/L)	2.30±0.1	1.70±0.0	1.70±0.1	1.80±0.1	1.80±0.1	2.40±0.1
CREA (Umol/L)	24.6±1.2**	20.4±2.5*	23.7±1.2**	21.3±7.6*	24.6±1.3**	34.9±1.7
UREA (Umol/L)	6.00±0.1	7.50±0.2	6.10±0.1	7.10±0.2	6.10±0.2	7.70±0.1

Student t-test *Significant (p<0.05); **Significant (p<0.01); All values are represented as Mean±sem; Group I: *N. laevis*, (500 mg/kg b.wt) Group II: *N. latifolia*, (500 mg/kg b.wt) Group III: Mixture (1:1) (500 mg/kg b.wt), Group IV: Mixture (1:1) (250 mg/kg b.wt), Group V: Mixture (1:1) (100 mg/kg b.wt), Group VI: Control

weight of the treated animals compared to the control is shown in Fig. 1. Generally, there was insignificant (p>0.05) increase in the body weights of the treated animals compared to the control in the early days but from the 20th day a significant increase (p≤0.05) in weight in the group treated with 250 mg and significant decrease in the weight especially in the group treated with the highest dose of the mixture compared to the control was observed.

Biochemical parameters: Table 2 is a summary of the results on the biochemical parameters. The Blood Glucose Level (GLU) showed significant decrease in all the groups with the exception of the group that received the mixture at 250 mg/kg bwt where the decrease was marginal. In the lipid profile study, there was insignificant increase in total cholesterol (T Chol), High Density Lipoprotein (HDL) and Triglycerides (TG) levels in the treated groups compared to the control while Low Density Lipoprotein (LDL) level decreased markedly in all the groups except for the groups that received the extract mixture at doses of 100 and 250 mg/kg bwt respectively where significant decrease (p<0.01) occurred.

There was significant decrease (p<0.05) in ALT, AST and creatinine levels in the treated animals compared to the control. On the other hand, Total Plasma Protein (TPP) showed marginal increase in all the treated animals. There was however insignificant (p>0.05) decrease in the Total Bilirubin (T. BIL), urea and Albumin (ALB) of the treated groups compared to the control while significant decrease in Alkaline Phosphate (ALP) level occurred in all the treated groups with the exception of the group treated with *N. latifolia*.

Haematological study: The effects of the extracts on the blood components and white blood cell differentials were presented in Table 3. Significant increases (p<0.05) were observed in the haemoglobin content and Red Blood Cells (RBC) in the groups that received: *N. laevis* and *N. latifolia* compared to the control while only marginal increase was noticed in the groups that

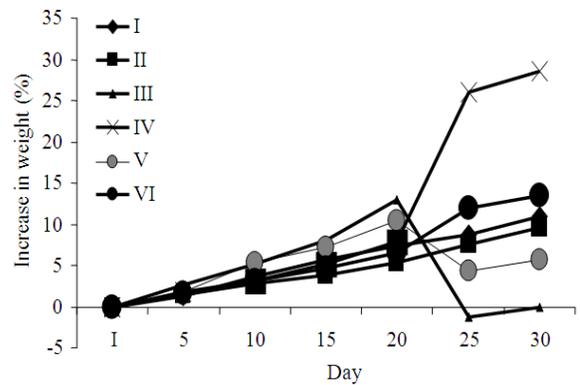


Fig. 1: Increase in weight of the animals treated with the extracts (1:1) mixture and individual extracts
 ♦ Group I : Extract A, *N. laevis*, (500 mg/kg b.wt)
 ■ Group II : Extract B, *N. latifolia*, (500 mg/kg b.wt)
 ▲ Group III: Mixture (1:1) (500 mg/kg b.wt)
 X Group IV: Mixture (1:1) (250 mg/kg b.wt)
 ● Group V : Mixture (1:1) (100 mg/kg b.wt)
 ● Group VI: Control

received the mixtures. Significant increase in Packed Cell Volume (PCV) was noticeable only in the group that received *N. laevis* while marginal increase was observed in the other treated groups. The White Blood Cells (WBC) increased significantly in the group that received *N. laevis* while decreasing marginally in the other treated groups. Significant decrease (p<0.05) in the level of neutrophil was observed in the group that received *N. latifolia* and the mixtures at 250 and 500 mg/kg bwt, respectively while marginal decrease occurred in the other treated groups. On the other hand, lymphocyte level showed marginal decrease in all the treated groups with the exception of the animal group that received the extract mixture at 250 mg/kg bwt where marginal increase was observed. The platelet level increased significantly in all the treated groups with the exception of group that received *N. laevis* where it exhibited marginal decrease.

Histology results: Figure 2a, Normal heart: The photomicrograph of the untreated cardiac muscle in

Table 3: Effect of the individual extracts and their (1: 1) mixture on hematological profile in control and treated rats after 30 days

Groups	WBC×10 ³	RBC×10 ⁶	Hb (g/dL)	PCV (%)	PLTC×10 ³	NEU (%)	LYMP (%)
Group I	16.1±0.7*	7.3±0.1**	13.3±0.2**	41.1±0.5**	53.8±5.6	33.3±1.6	55.8±1.6
I group II	12.7±0.6	7.8±0.1**	13.8±0.1**	34.6±0.4	708±2.2*	21.4±1.0*	59.5±2.3
I group III	10.8±0.4	6.9±0.1	12.9±0.1	35.7±0.4	56.2±2.3*	27.5±1.3**	56.5±1.5
I group IV	9.90±0.2	6.4±0.2	12.5±0.1	35.6±0.4	55.9±3.9**	21.8±1.1*	61.7±1.9
I group V	10.1±0.5	6.3±0.1	11.2±0.1	34.3±0.3	68.9±3.7*	35.5±2.3	51.8±1.1
I group VI	13.0±0.5	6.2±0.2	11.0±0.1	32.5±0.4	54.0±2.3	36.1±1.7	59.9±1.4

Student t-test *Significant (p<0.05); **Significant (p<0.01); All values are represented as Mean±sem; Group I: *N. laevis*, (500 mg/kg b.wt) Group II: *N. latifolia*, (500 mg/kg b.wt) Group III: Mixture (1:1) (500 mg/kg b.wt), Group IV: Mixture (1:1) (250 mg/kg b.wt), Group V: Mixture (1:1) (100 mg/kg b.wt), Group VI: Control

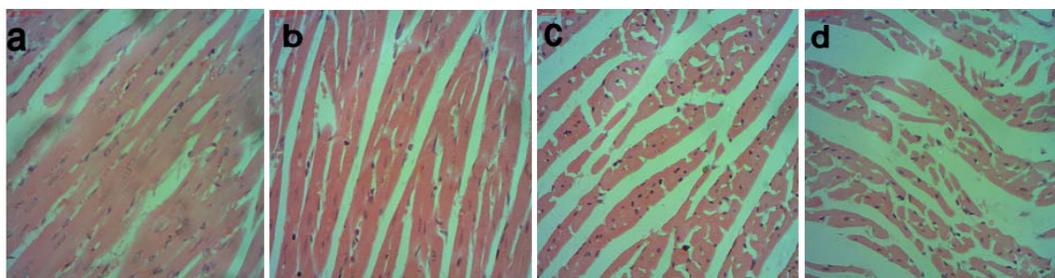


Fig. 2: Normal heart×400 *N. laevis* 500 mg/kg ×400 *N. latifolia* 500 mg/kg×400 (1:1) Mixture 500 mg/kg×400

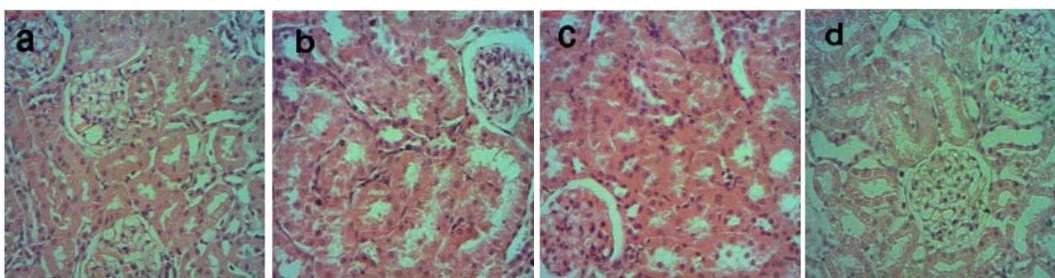


Fig. 3: Normal Kidney×400 *N. laevis* (500 mg/kg)×400 *N. latifolia* (500 mg/kg)×400 Mixture (500 mg/kg)×400

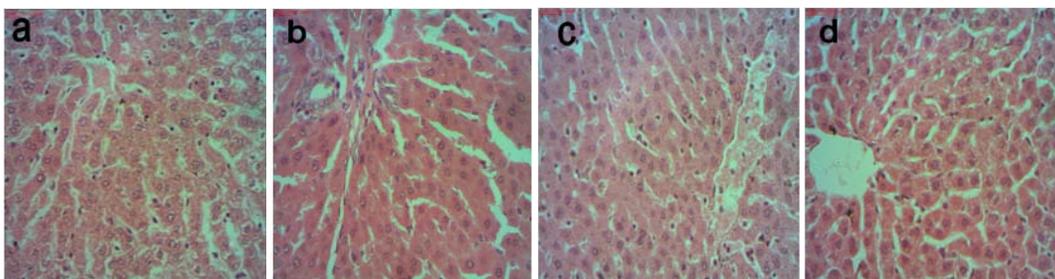


Fig. 4: Normal liver×400 *N. laevis* (500 mg/kg)×400 *N. latifolia* (500 mg/kg)×400 Mixture (1: 1) (500 mg/kg)×400

longitudinal section demonstrated the normal arrangement of muscle fibres which branched to give the appearance of three dimensional networks. The nuclei, deeply stained were seen centrally situated within the muscle fibres. The photomicrographs Fig. 2b to d of cardiac tissues treated with *N. laevis* root extract, *Nauclea latifolia* root extract and the (1:1) mixture (500 mg/kg bwt) respectively showed no abnormal features.

Figure 3a, Normal Kidney: The photomicrograph of the untreated renal tissue showed the cortical area

with the renal corpuscles which appeared as a dense rounded mass separated from surrounding structures by Bowman's space. The photomicrographs Fig. 3b to d of the renal tissue treated with *N. laevis* root extract, *N. latifolia* root extract and the (1:1) mixture (500 mg/kg bwt), respectively showed no abnormal features.

Figure 4a, Normal liver: The photomicrograph showed hepatocytes radially arranged in columns converging from the lobular margins towards the central vein with each column interspaced by hepatic

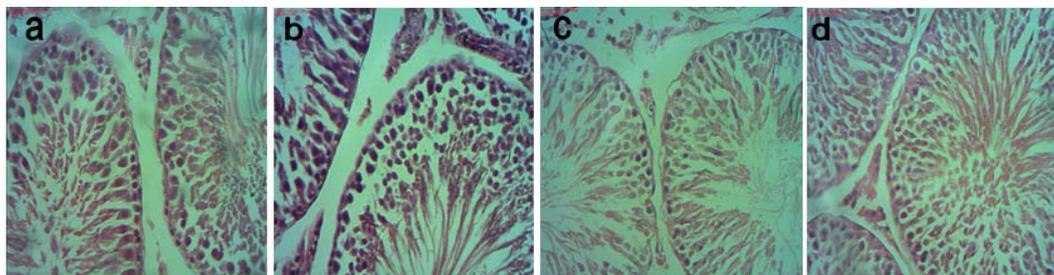


Fig. 5: Normal Testis×400 *N. laevis* (500 mg/kg)×400 *N. latifolia* (500 mg/kg)×400 Mixture (500 mg/kg)×400

sinusoids. The photomicrographs Fig. 4b to d of hepatic tissues treated with *N. laevis* root extract, *N. latifolia* root extract and the (1:1) mixture (500 mg/kg bwt), respectively indicated no lesion.

Figure 5a, Normal Testis: The cyto-architecture of a cross section of normal testicular tissue illustrated the seminiferous tubules in transverse plane with distinct boundary. Close to the germinal epithelium were more primitive spermatogenic cell series actively differentiating while the tails of matured sperm cells show wavy appearance at the lumina. The photomicrographs Fig. 5b to d of hepatic tissues treated with *N. laevis* root extract, *Nauclea latifolia* root extract and the (1:1) mixture (500 mg/kg bwt), respectively showed no abnormal features in the seminiferous tubules as well as cellular interstitium.

DISCUSSION

Herbal medicines are now receiving greater attention as an alternative to clinical therapy leading to increase in their demands (Mythilypriya *et al.*, 2007). In the rural communities of developing countries, the exclusive use of herbal drugs to treat various diseases is still very common and is prepared most often and dispensed by herbalists without formal training. Experimental screening method is therefore important in order to establish the active components present, ascertain the efficacy and safety of the herbal products (Chakarvath, 1993).

In the acute toxicity study of the (1:1) mixture of the extracts, no noticeable changes in the behavior and in the nervous system responses were observed in the treated animals. All the mice that received 20.0 g/kg body weight dose of the mixture survived beyond the 24 hour period of observation, therefore, suggesting the median acute toxicity value (LD_{50}) of the mixture to be above 20.0 g/kg bwt. According to Ghosh (1984) and Klaasen *et al.* (1995), the mixture can be classified as being non toxic, since the LD_{50} by oral route was found to be above 15 g/kg body weight, which was also much higher than WHO toxicity index of 2 g/kg (Ogbonnia *et al.*, 2011a, b). The viscera of the sacrificed animals did not show any macroscopic changes and since none of the survived animals convulsed it could be postulated that the mixture did

not exert some effects on the nervous system (Ogwali-Okeng *et al.*, 2003).

There were no significant ($p \geq 0.05$) changes in the body weight observed in the early days in all the treated animals compared to the control thus signifying that there was no stimulation or depression in the appetite. There was however, a significant decrease in the body weight from the day 20 which might have resulted from the suppression of appetite especially in the group treated with the highest dose of the mixture. A significant increase in weight was observed in the group treated with 250 mg/kg bwt which is attributable to unidentified factors.

The extracts and the mixture exhibited some hypoglycaemic activities having lowered significantly the plasma sugar levels supporting the use of the plants by the local herbalists in the treatment of diabetes. In lipid profile study, though total cholesterol and triglycerides exhibited marginal increase, there were however appreciable decrease in LDL level coupled with an increase in HDL level. Usually, a rise in lipid profile in particular LDL level is known to be predictive for coronary events such as atherosclerosis and coronary heart disease (Blake *et al.*, 2002). In this case, however, where the LDL level decreased considerably, the extract might be said not to be harmful to the cardiovascular system. Moreso, the photomicrograph of cardiac tissue of the treated animals showed normal appearance.

The liver and kidneys have demonstrated to play crucial roles in various metabolic processes and are, therefore, particularly exposed to the toxic effects of exogenous compounds (Bihde and Ghosh, 2004). AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function (Crook, 2006). Therefore an elevation in plasma concentration of ALT is an indication of liver damage (Horton *et al.*, 1996). AST is mostly present in the myocardium, skeletal muscle, brain and kidneys (McIntyre and Rosaki, 1987; Witthawaskul *et al.*, 2003). Thus, the liver and heart release AST and ALT and an elevation in plasma concentration are an indicator of liver and heart damage (Wasan *et al.*, 2001; Crook, 2006). In this study, a significant ($p \leq 0.05$) decrease in both ALT and AST values were observed in the animals treated with

respective single doses of *N. laevis* extract and *N. latifolia* extracts and also in the animals treated with different doses of the mixture. This clearly demonstrated that the extracts and their mixture had neither nephrotoxic nor deleterious effects on the heart. Serum bilirubin levels could be expressed as total bilirubin comprising of conjugated and non conjugated or as direct bilirubin comprising only of the conjugated and an increase in bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis (Tilkian *et al.*, 1979). A decrease in bilirubin level observed in the animals that received *N. latifolia*, *N. laevis* and the different doses of the mixture was confirmatory that the extracts and their mixtures did not have deleterious effect on the liver. The increase in total protein level in all the treated animal groups could be linked to the protective effect of the extracts against oxidative damage to the liver. An increase in total protein level has been reported to have hepato-protective effect (Oyagbemi *et al.*, 2008). Creatinine is excreted by glomerular filtration and the clearance is dependent on the rate at which it is removed from the blood by the kidneys, therefore, an increase in the plasma creatinine level suggests kidney damage specifically renal filtration mechanism (Wasan *et al.*, 2001). In this study, marked decrease in creatinine level occurred in all the treated groups indicating that the extracts might have facilitated renal filtration mechanism.

The RBC, Hb and PCV levels increased considerably particularly in the *N. laevis* extract treatment. An increase in the levels of these haematological parameters was indicative that the extracts have the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis) (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al.*, 2004). Similar observation has been made on a number of plants (Sanchez-Elsner *et al.*, 2004; Mbaka *et al.*, 2010; Mbaka and Owolabi, 2011). The white blood cells serve as scavengers that destroy the micro organisms at infection sites, removing foreign substances and debris that results from dead or injured cells (Guilhermino *et al.*, 1998). Consequently, the level is known to rise as body defence in response to toxic environment (Ngogang, 2005). In this study, WBC count exhibited decrease in all the extract treated groups except in animals' treatment with *N. laevis*. The decrease suggested that most of the extracts did not exhibit toxic effect even at high doses. The lymphocyte, the main effector cells of the immune system (Mc Knight *et al.*, 1999; Tédong *et al.*, 2007) was also observed to decrease appreciably indicating that the extracts did not exert challenge on the immune system of the treated animals. Marked increase in platelet level was however observed in all the treated animals with the exception of *N. laevis* treated. The reason for the increase could not be established.

CONCLUSION

The extracts were relatively safe with LD₅₀ over 20 g/kg bwt. The study showed that the different combinations of the extracts exhibited hypoglycaemic activity. They were also observed not to be harmful to the cardiovascular system due to considerable decrease in LDL level. The study also revealed that the extracts at doses administered did not provoke toxic effects to the animals' liver instead they exhibited hepato-protective effect. There was also no indication of kidney damage which was confirmatory from the renal tissue histology.

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