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Effects of Aqueous and Methanol Extracts of *Newbouldia laevis* Leaves on Some Biochemical Makers of Wistar Albino Rats

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Abstract: *Newbouldia laevis* (P.Beauv) is a medicinal plant traditionally used to treat various diseases across African Countries. In Nigeria, the leaves of the plant are used in the treatment of convulsions, diabetes, and different bacterial infections. In this study, the phytochemical composition of the *Newbouldia laevis* leaves were assessed, and the constituents were found to be alkaloid, flavonoid, tannins, steroid, saponin and cardiac glycoside which could serve as good antioxidants. The effects of the extracts on body weight, hematological, liver and kidney functions and lipid profile of Wistar albino rats were also assessed. The median lethal dose (LD_{50}) of the extract was calculated to be 5400mg/kg in albino rats. In the acute toxicity study, Liver and Kidney functions and hematological parameters assessed in the treated Wistar albino rats were not significantly different ($p \geq 0.05$) from the control except the platelet count that was significantly low ($p<0.05$) at 400mg/kg body weight of methanol extracts administered to the rats. In the case of lipid profile, there were significant reductions ($p<0.05$) in total cholesterol, Low density lipoprotein (LDL) and Triglycerides levels, however; there were much reduction in the groups treated with 400mg/kg body weight of methanol extracts. The results of this study suggest that the leaves of *Newbouldia laevis* could be used as an antioxidant. The results of this study also suggest that the extracts could be good in prevention of atherosclerosis and assist in management of cardiovascular diseases, since they brought about reduction in lipid profile and were observed not to the liver and kidney of the rats used in the study.

Key words: *Newbouldia laevis* · Medicinal Plants · Phytochemical Composition · Hematology · Atherosclerosis and Cardiovascular diseases

INTRODUCTION

One of the basic goals of researchers in their effort to discover new drugs is to develop new products with high therapeutic efficacy and low toxicity profile. To accomplish this, more attention has been given to medicinal plants in the recent years. This is because medicinal plants present a rich source of compound that possesses different therapeutic effects. It is generally believed that medicinal plants and their products are safer than their synthetic equivalents. While in some instances this may be true, a blanket assumption that medicinal plants are free of toxic effects is not correct in its entirety. The fact that medicinal plants are of natural origin does not guarantee for their safety [1].

In recent times, focus on plants research has increased all over the world, with more than thirteen thousand plants studied between 1996 and 2000 [2]. There is sufficient evidence showing immense potential of medicinal plants being used in various traditional systems.

Some medicinal plants that were once considered non-toxic have reported to be hepatotoxic while some have been reported to be responsible for renal impairment [3]. Therefore proper and detailed toxicological assessment should form a critical component of both early and late phase of drug development from medicinal plants to avoid toxicity tragedies after such drugs might have been approved for therapeutic purposes. One way to determine the toxicity profile of herbal preparations is to assess their effects on hematological and biochemical parameters [4].

Newbouldia laevis (P. Beaur) is a medicinal plant that belongs to *Bignoniaceae* Family. It is native to tropical Africa and grows from Guinea Savannah to dense forests. It is found in Nigeria, Senegal, Cameroon, Gabon, Angola

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and some other African countries [5]. Its common names are African Border Tree and Fertility Tree. In Nigeria, it is known by different indigenous names such as 'Aduruku (Hausa), Ogirisi (Igbo) and 'Akoko (Yoruba). It is used by African traditional healers to treat various ailments like diabetes, rheumatism and toothache. Different African countries have different names for *Newbouldia laevis* e.g Togo call it lifui, Ghana call it sesemasa, Senegal call it gimgid, The Gambia call it kallihi, Guinea call it canhom, Urhobo call it Ogiriki, Sierra Leone call it Sherbro, Mali call it kinkin, Edo state call it íkhímì, Tiv call it Kontor, while the Ibibio call it itömö. It is among the most useful plants in Africa and grows up to 10 m height with a cauliflorous habit. It is an ever greenish plant with a height of approximately 7–8 m high in the West Africa and up to 20 m in Nigeria. The plant has a characteristics shiny dark green leaves with large purple flowers. *Newbouldia laevis* is usually grown as an ornamental tree and planted by cuttings. It is a very popular plant in the African continent and is highly valuable due to its numerous immense benefits to human race. Some part of Nigeria commonly regards this tree as the tree of fertility or the tree of life. The wood is pale brown, durable, evenly textured and hard and it tends to remain alive for a long time even after cutting it. This makes it viable for usage as posts, woodworks, yam stakes, house posts, firewood and bridges. *Newbouldia laevis* has different symbols and meanings to different countries for example; some villages in Ivory Coast and Gabon plant the tree near the tombs to act as a protective talisman.

In Nigeria, herbalists use decoction of the bark to treat epilepsy and convulsions in children, elephantiasis, dysentery, rheumatic swelling, syphilis, constipation, piles and as a vermifuge to rounds worms [6]. The root, leaf, stem and fruits have been used variously for febrifuge; wound dressing and stomach ache, including inflamed sores, ulcers and abscesses [7]. The leaves are soaked in methanol for the treatment of diabetes and sickle cell diseases.

In this study, the acute toxicity profile of the methanol and water extracts of the leaves of *Newbouldia laevis* were evaluated on the hematological and some biochemical parameters of Wistar albino rats.

MATERIALS AND METHODS

Plants Collection: Leaves of *Newbouldia laevis* were collected from Awalasi village , Uga , Aguata local Government Area in Anambra State. The leaves were identified at the Botany department, University of Nigeria Nsukka where a voucher specimen was deposited.

Preparation of Plants Extracts: The leaves were thoroughly washed with distilled water to remove soil and other debris that may contaminate the plant sample. The washed sample was then air-dried under shade in the laboratory for 5 days and the dry plant sample was pulverized using an electric grinding machine. The resultant powder sample weighing 500g was then extracted with 70% methanol and water at 70°C by continuous hot percolation using a Soxh- let apparatus. The extraction was carried out for 48h and the resulting methanol and water extracts was concentrated at 40°C in a rotary evaporator. The solid sample obtained weighed 47.5 g (yield = 9.5%). The crude methanol and water extracts was kept in air-tight container and stored in a refrigerator at 4°C until the time of use.

Experimental Animals: Mature male Wistar albino rats weighing 180-200g were obtained from the Animal House of Nnamdi Azikiwe University, Awka (Unizik) Nigeria. The rats were acclimatized for fourteen (14) days before the commencement of the study.All experimental procedures were conducted in accordance with National Institute of Health Guide for the care and Use of Laboratory Animals (National Institute of Health, 1985) as well as Ethical Guidelines for the Use of Laboratory Animals in Unizik, Awka, Nigeria. A maximum of five animals were kept in one cage. The animals were maintained under standard laboratory conditions of temperature (22 ± 2℃), relative humidity (55-65%).During the whole experimental period, animals were fed with a standard balanced commercial pellet diet and portable tab water *ad lilitum.*

Acute Toxicity Study: In this method, six groups each of three animals were fasted prior to dosing (food but not water was withheld overnight for the rats). The fasted body weight was determined for each animal and the dose was then calculated according to the body weight. Food was then further withheld for 3-4 hours in rats and after the extract (methanol and water) had been administered. Six doses were chosen such that smallest dose caused no death while the highest caused 100% mortality. Each of the five doses of the extract was administered by oral gavages to different group of rats. A start dose of 10mg/kg, 100mg/kg and 1000mg/kg were used for each of the six groups of the animal in the first phase. Animals dosed in the first phase were observed for 48 hours after which there was no death and the test proceeded to the second phase. The same procedure was used but at a dose of 1600mg/kg, 2900mg/kg and 5000mg/kg.

Animals were observed individually at least once during the first 30miutes after dosing and periodically during the first 24hours with careful observation during first 4 hours and then daily for 14 days. The parameters observed were grooming, mood, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions.

Sub-Chronic Toxicity Study

Experimental Design: Five groups (A, B, C, D and E) of five rats each were used for the study. Groups A and B were respectively administered 200mg/kg and 400mg/kg body weight of methanol extract orally, C and D were respectively administered 200mg/kg and 400m/kg body weight of water (aqueous) extract orally by gastric gavages once daily for consecutive 28 days, while group E is the control. The doses were chosen based on effective therapeutic doses of methanol and water extracts of the leaves of *N. laevis* in rats as reported earlier [8]. Group E which served as the control was treated with normal saline (0.9% NaCl solution). Before the initiation of dosing, the rats were left for 7 days to acclimatize to laboratory conditions. All animals were monitored for any deviations in normal behavior, fecal discharge, movements and mortality on a daily basis during the 28-day period of study. The body weight (in gram) of each rat was recorded on day 0 and at weekly intervals throughout the course of the study and the average body weight for the groups was calculated. On day 28, the animals received the last treatment dose. Six hours later, each animal was anaesthetized with intraperitoneal injection of sodium pentobarbitone (40mg/kg body weight) and blood samples were collected by cardiac puncture into EDTA anti-coagulated and non anti-coagulated tubes. The heparinzed blood was used for hematological studies while the blood in non anti-coagulated tubes was centrifuged at 1500 rpm for 10 min and the sera collected into clean, dry tubes for biochemical analysis.

Body Weight: Body weight of the rats was measured at weekly intervals for the four weeks of the experiment.

Hematological Analysis: Within ninety minutes after blood collection, the samples were subjected to hematological analysis on Sysmex XE-2100 (Sysmex Corporation, USA), a fully automated analyzer. The following hematological parameters were determined: red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HB), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC), platelet count, lymphocytes, monocytes, neutrophils, basophils and eosinophils.

Biochemical Analysis: Serum aspartate aminotransferase (AST) and alanineamino transferase (ALT) activities were estimated by the method of Reitman and Frankel, [9], using test kit (BioVision Inc. USA). Alkaline phosphatase activity was estimated by the method of Kind and King [10] using ALP test kit (BioVision Inc. USA). Serum creatinine was estimated by a commercial kit (Vitro Scient Co.) based on modified kinetic Jaffe reaction [10], while serum urea was estimated by a method based on modified Urease-Berthelot method [11], using commercial kit (Randox Laboratories Ltd, UK). Total bilirubin was estimated by an assay based on the method of [12]. Total protein and serum albumin were estimated by the method of Tietz and globulin was calculated by subtracting albumin from total protein value.

Statistical Analysis: Data obtained from the experiments were expressed as mean ± standard error of mean (SEM). The data were subjected to one-way analysis of variance (ANOVA) and Student's - Newman-Keul test to determine the statistical significance of differences between groups. Differences were considered to be significant at $P \leq 0.05$. GraphPad Prism version 5.0 for windows was used for these statistical analyses (GraphPad software, San Diego California USA).

RESULTS

The phytochemical constituents of the methanol and water extracts of *Newbouldia laevis* leaves were observed to be flavonoids, tannins, steroids, phenolic Nucleus, Protein, Saponin, glycosides, alkaloids and cardiac glycosides as it was shown in Table 1. Also in Table 1 (triple sign) in methanol extracts compared with other phytochemical constituents of *Newbouldia laevis* leaves.

The results of the effects of the both extracts on body weight gain in Table 2 below revealed that there was significant decrease in body weight gain of all the groups when compared with the control. The results further revealed that the highest decrease in body weight gain was observed in the group treated with 400mg/kg body weight of methanol and water.

Table 1: Phytochemical constituents of the methanol and water extracts of *Newbouldia laevis* leaves.

Parameters	Water Extracts	Methanol Extracts
Alkaloids	+	+
Phenolics	++	++
Flavonoids	+	+++*
Tannins	+	++
Protein	++	+
Saponins	++	+
Steroid	+	++
Cardiac Glycoside	+	++

Lengend: + =Very Low, ++ = Low and +++= High.

Table 2: Effects of *Newbouldia laevis* leaves extracts on the Body weight (g) gain of different groups of rats used in the study

Body Weights (g)					
Dose (mg/kg)	Week 0	Week 1	Week 2	Week 3	Week 4
Control	0.00±0.00	8.10±7.68	10.50±8.42	20.40±9.12	20.40±10.12
Methanol	0.00±0.00	3.00±7.94	7.00±8.17	8.40±9.48	9.20±10.20
200 Water	0.00±0.00	4.00±7.70	8.00±8.25	13.10±9.07	17.10±10.30
200 Methanol	0.00±0.00	3.00±8.67	4.00±9.03	7.30±10.11	12.80±9.42
400 Water	0.00±0.00	5.10±9.05	7.00±9.51	10.20±10.20	14.80±8.53

Value represent mean ± SEM (n=6)n P ≤ 0.05 compared with the control.

Table 3: Effects of *N.laevis* leaf extracts treatment on hematological parameters of different groups of rats used in the study

Treatment groups and doses (mg/kg)					
Parameters	Control	200 methanol	200 water	400 methanol	400 water
HCT (%)	39.20±0.57	35.90±10.61	38.15±2.05	36.80±2.40	31.00±0.00
HGB (g/dl)	13.60±0.14	13.80±3.40	14.05±0.64	14.55±0.92	14.60±0.00
PCT (%)	0.44±0.87	0.46±0.10	0.39±0.04	0.38±0.03	0.07±0.00
PDW	14.65±0.49	14.25±0.07	14.40±0.14	14.60±0.00	15.40±0.00
PLT	520.00±137.18	509.50±126.57	500.50±33.23	480.50±38.89	475.00±0.0
($X10^9$/L) RBC	7.86±0.16	7.92±1.27	8.00±0.10	8.04±0.47	8.19±0.00
($X10^{12}$/L) PCV (%)	14.85±0.92	16.20±3.82	18.90±3.39	19.85±1.34	19.90±0.00
RDW-SD	26.80±0.00	29.85±1.34	29.35±3.61	30.40±2.12	23.80±0.00
(fl) WBC ($X10^9$/L)	12.75±3.89	13.30±11.60	16.55±7.00	16.90±5.51	17.00±0.00

Value represent mean ± SEM (n=6)n P ≤ 0.05 compared with the control.

Table 4: Effects of *N. laevis* leaf extracts treatment on liver Function

Treatment groups and doses (mg/kg)					
Parameters	Control	200 methanol	200 water	400 methaol	400 water
A/G (U/L)	0.82±0.04	1.19±0.30	0.75±0.23	0.95±0.07	1.00±0.17
Albumin (g/dl)	3.30±047	3.77±0.18	2.50±1.30	2.19±0.43	2.71±0.25
ALP (U/L)	116.67±62.42	118.01±68.59	120.33±92.52	122.20±2.12	124.33±31.09
ALT (U/L)	150.00±120.55	149.50±119.50	147.00±25.53	144.00±29.70	142.33±39.68
AST (U/L)	387.00±271.35	386.50±95.87	384.67±55.01	381.50±6.36	380.00±53.78
Conjugated (mg/dl)	0.09±0.07	0.01±0.00	0.15±0.22	0.01±0.00	0.06±0.04
Unconjugated (mg/dl)	0.42±0.36	0.02±0.01	0.22±0.22	0.02±0.01	0.06±0.06
Gamma GT (U/L)	10.33±6.66	8.85±3.18	12.19±9.32	35.50±36.02	8.92±3.50
Globulin (g/dl)	3.10±0.36	3.31±0.61	3.14±1.16	2.28±0.24	0.68±3.33
Total Bilirubin (mg/dl)	0.58±0.39	0.03±0.01	0.43±0.41	0.03±0.01	0.41±0.08
Total protein (g/dl)	7.30±0.83	7.08±0.43	5.65±2.43	4.47±0.67	5.39±0.49

The results were expressed as mean ± SEM (n = 6)n * P ≤ 0.05 compared with the control.

Table 5: Effects of *N. laevis* leaf extract treatment on Kidney Function Test.

Treatment groups and doses (mg/kg)					
Parameters	Control	200 methanol	200 water	400 methanol	400 water
Urea(mg/dl)	62.67±42.19	63.50±0.71	64.73±152.64	66.00±89.10	68.67±48.34
Sodium (mmol/L)	114.67±5.69	116.50±0.71	120.33±2.31	122.00±5.66	122.10±2.65
Creatinne (mg/dl)	2.53±1.21	1.44±0.62	1.40±0.53	1.33±3.43	1.05±1.08
Potassium (mmol/L)	3.40±0.70	3.85±0.07	4.23±0.25	4.80±0.00	4.90±0.26
Chloride (mmol/L)	95.33±6.11	97.50±3.54	98.67±2.31	91.00±1.41	95.67±0.58
Carbonate (mmol/L)	25.00±1.00	22.50±3.54	21.00±1.00	18.00±0.00	21.67±2.52

The results were expressed as mean ± SEM (n = 6)n * P ≤ 0.05 compared with the control.

Table 6: Effects of *N. laevis* leaf extract treatment on Lipid Profile

Treatment groups and doses (mg/kg)					
Parameters	Control	200 methanol	200 water	400 methanol	400 water
Total Cholesterol (mg/dl)	72.33±19.55	70.00±25.46	53.00±15.87	47.00±2.83	50.38±3.21
LDL (mg/dl)	23.33±6.66	16.00±2.83	18.33±3.21	11.50±4.95	14.00±5.00
HDL (mg/dl)	12.67±2.52	10.00±0.00	8.00±2.65	8.00±2.83	7.67±3.79
VLDL	38.00±16.70	44.00±22.63	30.33±11.24	28.50±13.44	28.67±1.15
Triglycerides	189.33±84.06	69.50±115.26	150.33±55.14	140.50±0.71	143.67±6.35

The results were expressed as mean ± SEM (n = 6)n * P ≤ 0.05 compared with the control.

Significant decrease (P≤ 0.05) in the platelet count which was not dose dependent was observed in the groups treated with 400mg/kg body weight of water extracts of *Newbouldia laevis* leaves. All other hematological parameters were insignificantly different (p≥0.05) from the control (Table 3).

The results of the liver and kidney functions respectively indicated that no significant difference (p≥0.05) exists between the groups treated with the extracts and control group (Table 4 and Table 5).

DISCUSSION

Traditionally, herbal products are considered non-toxic and have been used by general public and traditional healers worldwide to treat various ailments. However, the fact that an herbal preparation is of natural origin does not necessarily make it safe. The active ingredients of plant extracts are chemicals like those of synthetic or purified drugs. In low amounts, they may be ineffective, while in the right amounts, they may prove beneficial. When large quantities are used and for a prolonged period, plant extracts may be injurious to health. They have the potential to cause serious toxic effects [3]. Therefore in this study, the acute and sub-chronic toxicity studies of the methanol and water extracts of the leaves of *Newbouldia laevis* were evaluated. These toxicity studies in animals are usually necessary for any drug intended for human consumption. The information obtained from such studies could be used to estimate the therapeutic index of drugs. These studies are also useful in selecting doses for chronic toxicity studies [13].

In the acute toxicity study, adverse reactions such as grooming, mood, hyperactivity and sedation, loss of righting reflex, respiratory rate and convulsions were observed only when the dose of the extract was increased above 5000 mg/kg body weight. The LD_{50} (dose of the extracts that caused 50 % mortality in the animals) was calculated as 5400 mg/kg. This suggests that the extracts were non-toxic according to a toxicity classification [14]. The toxic effects observed at very high doses were likely caused by the chemical constituents of the leaves of N. laevis such as tannins, saponins, terpenes and flavonoids [15].

In the sub-chronic toxicity study, there was no mortality or any toxic manifestations observed at any of the doses selected throughout the study period. Changes in the body are valuable indicators for predicting the toxicity of a compound or plant extract.

The significant reduction in body weight of the animals observed during the period of treatment with the methanol and water extracts of *Newbouldia laevis Leaves* could be normal physiological and adaptational responses mediated by the extracts through suppression of appetite [16].

Estimation of blood parameters is crucial in evaluating the toxicity of drugs as changes in hematological system in animal studies have a high predictive value for human [17]. All blood parameters in hematology estimated in the treated rats, except the platelets count did not show significant difference compared with the control group. The significant decrease observed in platelets count may be due to stimulatory effects of the extracts on the production of hematopoietic

regulatory elements such as thrombopoietin, erythropoietin and colony – stimulating factors by the stromal cells and macrophages in the bone marrow [18].

There was no significant difference ($P \leq 0.05$) in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) between extract-treated and control groups. ALT is a cytoplasmic enzyme found in very high concentration in the liver [19]. An increase in the serum level of this specific enzyme indicates hepatocellular damage. Although AST is less specific than ALT as a marker of liver damage, elevation in the serum levels of the two enzymes is an indicator of tissue damage and altered membrane permeability (Saptal *et al.* 2010), [14,15], while alkaline phosphatase is a marker of obstructive jaundice and intrahepatic cholestasis [20]. Administration of the methanol and water extracts of *Newbouldia laevis* leaves did not cause any significant changes in the levels of these enzymes. This again indicates that the extracts were not toxic to the rats at the doses administered.

The results further suggest that the reduction in Total cholesterol, (LDL) and Triglycerides at 400mg/kg body weight of methanol extracts of *Newbouldia laevis* leaves could probably serve as a new potential natural product for the treatment of hyperlipidemia since high level of cholesterol particularly (LDL) Cholesterol, are mainly responsible for hypercholesterolemia- a risk factor for cardiovascular diseases (CVD) such as atherosclerosis and myocardial infarction, which are common causes of mortality and morbidity [21].

The results also suggest that since there was no significant difference ($p \geq 0.05$) in the liver and kidney function parameters evaluated when compared with the control, that the methanol and water extracts of *Newbouldia laevis* leaves seem not to be toxic to the rats used in the study, since increase in serum Urea and Potassium are indicative of damaged renal function [22].

CONCLUSION

The results of the present study suggest that the methanol and water extracts of *Newbouldia laevis* have low toxicity profile regarding hematological and some biochemical parameters of Wistar albino rats evaluated in this study. Also the results of the study further revealed that the methanol and aqueous (water) extracts of *N. laevis* leaves brought about reduction in the lipid profile of the Wistar albino rats used, especially at higher dose of 400mg/kg body weight. This suggests that the extracts could be useful in prevention and management of cardiovascular diseases.

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