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Effects of the Ethanol Extract of the Stem Bark of *Nauclea latifolia* Smith [Rubiaceae] on Certain Biochemical and Haematological Indices of Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author CEU designed the study and wrote the protocol. Author BEU performed the statistical analysis and managed the analyses of the study. Author EGA managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The ethanol extract of the stem bark of *Nauclea latifolia* was investigated for its effect on certain haematological and biochemical indices of the swiss albino mice. Phytochemical screening of the extract for bioactive compounds indicated the presence of saponins, tannins, terpenes, cardenolide, balsams, alkaloids, flavonoids and cardiac glycosides, phlobatanins, cyanogenic glycosides and anthraquinones. The LD_{50} was determined to be 1414.2 mg/kg body weight. Sub-acute and chronic administration of the extract caused a significant increase of some heamatological indices as the dose administered increased from low to high while WBC level was

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observed to decrease. Biochemical indices such as globulin, urea and AST levels increased significantly as the dose administered increased from low to high, while albumin, total protein, ALT and ALP levels were observed to decrease. Results obtained also suggests certain effects on the heart and kidney with prolonged use. *Nuclea latifolia* stem bark extract should be used with caution.

Keywords: Nuclea latifolia; haematological; bioactive; biochemical; kidney.

1. INTRODUCTION

Herbal medicines are preparations from herbs and plant parts (roots, stems, leaves, barks, or even fruits) which are used to promote and improve health [1]. Preparations from plants known to have medicinal value have been used in Africa by the locals mostly in their crude form for a very long time. In most of the places, these preparations are the only medications known to serve the populace. They meet the medicinal needs of the people by curing a wide range of diseases. This is because plants are known to have the ability to produce and store a wide range of chemical substances, some of which have the ability to defend the plants against microbial attack and are exploited by man for medicinal purposes. The importance of these compounds to man is however undermined by the fact that they exhibit different levels of toxicity [2]. Toxicity study is therefore, paramount in the screening of plants before they can be used by humans.

Toxicity study involves the determination of potential hazards a test substance may likely produce and the characterization of its action. Most of the toxicity testing is carried out on experimental animals [3]. The advantages of using animal models in toxicity testing are enormous. They include the possibility of clearly defined genetic constitution and their amenity to controlled exposure, controlled duration of exposure, and the possibility of detailed examination of all tissues [4].

Nauclea latifolia commonly known as pin cushion tree belongs to the family Rubiaceae. It is a struggling shrub or small tree native to tropical Africa and Asia. In Akwa Ibom State, Nigeria, it is called Mbom-Ibong [5] while the Hausas of the northern part of Nigeria name it Tabashiya [6]. It is known as 'uburuinu' or 'mbitinu' by the Igbos, 'egbesi' by the Yorubas and 'Uche' by the Igbede people all of Nigeria. It is found in the forest and fringe tropical forest [7].

Nuclea latifolia grows up to an altitude of 200 m and is widespread in the humid tropical rainforest zone of West and Central Africa. Three other related species *Nauclea. pobeguini*, *N. diderichii*, and *N. vanderguchtii* are forest trees. *N.diderichii* is planted in Omo forest reserve, Nigeria. In folk medicine, the species *N. diderichii* and *N. orientalis* are used in the same way as *N. latifolia*. *Nauclea latifolia* has an open canopy and terminal spherical head lined cymes of white flowers. The flowers are joined with their calyces and the fruit is syncarp. The fruits ripen between the months of July and September, baboons eat them and disperse the seeds, while livestock eat the shoots and leaves. The wood of *N. latifolia* (Opepe wood) is termite resistant and is used as live stakes in farms [8].

In West Africa, infusions and decoctions of the bark and leaves of N. *latifolia* are used for the treatment of stomach pains, fever, diarrhea, In Kano (Nigeria) it is used as a chewing stick and as a remedy against stomach ache and tuberculosis. In Ivory Coast infusions and decoctions from stems and roots of *N. latifolia* are used against malaria parasites by traditional healers [9]. The plant has been known as the 'African cinchona' or the 'African quinine because of its reported anti-malarial activity. This work is intended to determine the effect of the ethanol extract of the stem bark of *Nuclea latifolia* on certain biochemical and haematological parameters of the Swiss albino mice. The results obtained should be able to help us make a categorical statement as to the safety or otherwise of the extract considering how much it is used in ethno-medicine in Africa.

2. METHODS

2.1 Plant Collection and Authentication

The stem bark of *Nauclea latifolia* was collected from Itak Ikot Akap, Ikono Local Government Area, Akwa Ibom State, Nigeria. It was identified by Prof. Margaret Bassey of the Department of Botany and Ecological studies, of the University of Uyo and a voucher specimen with herbarium No. 57(b) was deposited in the herbarium of the Faculty of Pharmacy, University of Uyo for further references.

2.2 Preparation and Extraction of Plant Material

The stem bark of *Nauclea latifolia* was washed, chopped into pieces and air dried for two weeks after which they were ground into powder. The extract was obtained by soaking 300 g of the dried powdered sample in 2500 ml of 75% ethanol for 48 hours. It was later filtered through Whatman filter paper and the extract was evaporated to dryness in a water bath under 40℃.

2.3 Animal Treatment

Male Swiss albino mice (22-35 g) were used for these experiments. They were kept in cages made with stainless steel wires at the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo where they were fed with standard pellet feeds and clean fresh water in containers to control wastes and spillage. All experimental protocols were in compliance with the Faculty of Pharmacy Ethics on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.4 Phytochemical Screening

Preliminary phytochemical screening was done to determine the chemical constituents of the extract. Standard accepted methods [10,11] were employed.

2.5 Toxicity Studies

2.5.1 Acute toxicity determination

This involves the determination of the concentration of the extract that will kill 50% of the test animal population. The Lorke method [12] was employed. Swiss albino mice (weighing between 22 and 34 g) were used. They were divided into groups, with six animals per group. In the first phase, each group of the animal was administered with either of 1000 mg/kg, 2000 mg/kg, 2500 mg/kg and 3000 mg/kg of the extract respectively. The animals were monitored for 24 hrs and mortalities noted. In the second phase, each group was administered with 1250 mg/kg, 1500 mg/kg and 1750 mg/kg of the extract respectively. They were monitored for 24 hrs and mortalities noted. All extract administrations were done through the intraperitoneal route. The Lethal Dose (LD_{50}) was determined using Highest dose that gave no mortality(D_o) and Lowest dose that produced mortality(D_{100}) [12].

2.6 Sub-acute Toxicity Test

The effect of the extract after 14 days of administration was determined using swiss albino mice. The mice were divided into six groups of six mice each. The LD_{50} was used as baseline dose and used for group one (i.e. low dose) while the middle and the high doses were obtained by doubling and tripling the LD_{50} respectively and administered on the second and third groups. The animals were fed with standard pellets feeds and clean water. Extract concentrations were added to their drinking water avoid injection abscess [13] while the fourth group (control) was fed with standard pellets feed but given only potable drinking water. The doses were administered daily through the same route for 14 days. The animals were then sacrificed and blood collected from the heart of the sacrificed animal were analyzed for the effect of the extract on some hematological and biochemical indices. Mindray 5 part Differential Haematological Auto-analyzer (BC-5300) was used in the analysis.

2.7 Chronic Toxicity Test

The animals used in this study were given the same treatments as in the acute toxicity test but the experiment was run for 28 days before the blood samples were taken for analysis.

2.8 Statistical Analysis

Statistical analysis was done using windows SPSS package (SPSS version 15.0). Data was analyzed using one way ANOVA followed by post hoc t-test least significance difference (LSD). The data was expressed as mean ± standard error (SEM) and values of $p< 0.05$ were considered significant.

3. RESULTS

The result of the phytochemical indicated the presence of saponins, tannins, terpenes, alkaloid, flavonoids and cardiac glycosides and anthraquinones. However carbohydrates and steroidal glycosides were absent. These are is in Table 1.

3.1 Acute Toxicity

Results of the acute toxicity test of the ethanol extract of the stem bark of *Nauclea latifolia* are presented in Table 2. On the administration of

the extract, the behavioral change observed was slight dullness at the onset. The LD_{50} of the extract was determined to be 1414.2 mg/kg which shows that the extract is only slightly toxic [14].

3.2 Sub-acute Toxicity Test

3.2.1 Hematological analysis

The sub-acute toxicity test result showed that the ethanol extract of the stem bark of *Nauclea latifolia* caused an increase in packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Lymphocytes and platelet levels as the dose administered increased from low dose to high dose while the white blood cell (WBC) level was observed to be decreasing as shown in Table 3(a) and 3(b).

3.3 Biochemical Analysis

The sub-acute toxicity test result showed that the ethanol stem bark extract of *Nauclea latifolia* caused an increase in globulin, urea and aspartate amino transferase (AST) levels as the dose administered increased from low dose to high dose while albumin, total protein, Alanine Amino transferase (ALT) and alkaline phosphatase (ALP) level was observed to be decreasing as shown in Table 4.

Table 1. Result of the phytochemical screening of the ethanol extract of the stem bark of *Nauclea latifolia*

Constituents	Indication
Alkaloids	+
Cardiac glycosides	+
Terpenes	+
Carbohydrates	-
Antraquinones	+
Polyphenols	+
Saponins	+
Tannins	+
Flavonoids	+

Key + = present, - = absent

Table 2. Result of the acute toxicity test of the ethanol extract of the stem bark of *N. latifolia*

Phases	Dose (mg/kg)	No. of mice	Mortality	%Mortality	LD_{50}(mg/kg)
1	3000	3	3	100	
	2500	3	3	100	$\sqrt{D_0 X D_{100}}$
	2000	3	3	100	
	1000	3	0	0	=1414.2 mg/kg
2	1750	3	2	66.67	
	1500	3	2	66.67	
	1250	3	1	33.33	

Note: D_0=highest dose that gave no mortality, 1000 mg/kg body weight; D_{100}=lowest dose that produced 100%mortality, 2000 mg/kg

Table 3(a). Result showing the effect of the sub-acute administration of the ethanol extract of the stem bark of *N. latifolia* on red blood cell indices of swiss albino mice

Groups	PCV(%)	Hb(g/dl)	RBC($x10^6$/µl)	MCV($µm^3$)	MCH(pg)	MCHC(g/dl)
Low dose	40.3±0.9*	12±12*	7.8±1.1**	49.3±3.1	15.1±1. 4	31.7±0.4
Middle dose	42.5±3.4*	14.2±1.3**	9.11±0.7	51.3±3.0	1 6.1±1.4**	32.7±0.6**
High dose	43.5±1.9*	14.7±1.2***	9.42±1.6	52.3±2.4	1 6.7±0.9**	31.1±2.0
Control	48.2±1.3	15.7±1.4	9.32±1.1	49.4±1.0	15.8±0 .9	31.2±1.2

*Values presented as Mean ± SEM, (n= 6). Significance relative to control: **p < 0.01, *p < 0.05*

Table 3(b). Result showing the sub-acute effect of the administration of the ethanol extract of the stem bark of *N. latifolia* on total WBC, differential and platelet count of swiss albino mice

Groups	WBC ($x10^3$/µl)	LYM ($x10^3$/µl)	NEU ($x10^3$/µl)	MON ($x10^3$/µl)	EOS ($x10^3$/µl)	Platelet ($x10^3$/µl)
Low dose	11.1±1.1*	7.3±0.8**	0.6±0.3	2.1±0.3*	0.0-0. 0	498±42.0*
Middle dose	9.7±3.6**	8.7±2.2*	1.2±0.3	05±0.2	0.0-0. 0	604±92.2*
High dose	8.5±1.7**	9.7±1.5*	1.4±0.3	0.4±0.3	0.0-0.0	638±103.0*
Control	7.9±1.2	6.3±0.8	0.7±0.1	0.4±0.1	0.03±0.1	5 38±22.1

*Values presented as Mean ± SEM, (n= 6). Significance relative to control: **p < 0.01, *p < 0.05*

Table 4. Result showing the effect of the sub-acute administration of the ethanol extract of the stem bark of *N. latifolia* on biochemical parameters of swiss albino mice

Groups	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Urea (mmol/l)	AST(iu/l)	ALT(iu/l)	ALP(iu/l)
Low dose	68±2.0**	34±1.0**	34.0±2.0	3.6±0.6	97.3±1.2**	33.7±1.5	248±2.6*
Middle dose	65.7±0.6*	31.7±0.6*	34.7±2.1	3.7±0.3**	105.3±9.2*	33±2.0	198.7±2.5*
High dose	65±3.6*	28±2.6*	40±2.6*	4.8±0.3	112.3±8.1*	29±2.0**	191.3±2.5*
Control	71.3±0.6	37±2.0	35.7±1.2	5.7±0.1	94.7±1.5	34.3±2.3	335±1.0

*Note; iu/l (International unit/litre) .Values presented as Mean ± SEM, (n= 6). Significance relative to control: **p < 0.01, *p < 0.05*

Table 5(a). Result showing the effect of the chronic administration of the ethanol extract of the stem bark of *N. latifolia* on red blood cell indices of swiss albino mice

Groups	PCV(%)	Hb(g/dl)	RBC(x10^6/µl)	MCV(µm^3)	MCH(pg)	MCHC(g/dl)
Low dose	43.8±1.4*	16.3±1.9	10.7±1.6	52.3±2.2*	17.9±1.5**	35.7±1.2**
Middle dose	45.1±2.7**	17.3±0.9**	13.3±0.8*	55±2.3*	19.8±0.9*	38.6±1.6*
High dose	47.9±1.96	19.5±1.9*	14.3±2.3*	57.1±1.6*	25.3±2.5*	43.1±1.9*
Control	48.2±1.3	15.7±1.4	9.32±1.1	49.4±1.0	15.8±0.9	31.2±1.2

*Values presented as Mean ± SEM, (n= 6). Significance relative to control: **p < 0.01, *p < 0.05*

Table 5(b). Result showing the effect of the chronic administration of the ethanol extracts of the stem bark of *N. latifolia* on total WBC, differential and platelet count of swiss albino mice

Groups	WBC (x10^3/µl)	LYM (x10^3/µl)	NEU (x10/µl)	MON (x10^3/µl)	EOS (x10^3/µl)	Platelet (x10^3/µl)
Low dose	8.8±0.8*	10.3±0.9*	1.3±0.5	0.9±0.3*	0.0	514±30.4*
Middle dose	5.7±1.2**	12.8±1.9*	1..8±0.2*	0.4±0.3	0.0	614±92.2*
High dose	3.6±0.7*	13.7±1.5*	2.1±0.2*	0.2±0.2	0.0	650±94.3*
Control	7.9±1.2	6.3±0.8	0.7±0.1	0.4±0.1	0.03±0.1	538±22.1

*Values presented as Mean ± SEM, (n= 6). Significance relative to control: **p < 0.01, *p < 0.05*

Table 6. Result showing the effect of the chronic administration of the ethanol extract of the stem bark of *N. latifolia* on biochemical parameters of swiss albino mice

Groups	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Urea (mmol/l)	AST (iu/l)	ALT (iu/l)	ALP (iu/l)
Low dose	65±1.7*	31.7±3.1*	37±1.7**	5.9±0.6	101±1.7*	30.3±2.5	244±4.5*
Middle dose	62.7±1.2*	28.7±1.5*	38.0±2.9*	6.7±1.3*	107±9.6*	29±4.0	196±1.0*
High dose	62±4.3*	23±4.3*	43±2.6*	7.4±1.3*	114.7±10.1*	26±3.0	192.3±2.5*
Control	71.3±0.6	37±2.0	43±2.6	5.7±0.1	94.7±1.5	34.3±2.3	335±1.0

*Note; iu/l (International unit/litre) .Values presented as Mean ± SEM, (n= 6). Significance relative to control: **p < 0.01, *p < 0.05*

3.4 Chronic Toxicity Test

3.4.1 Haematology

The test result showed that the ethanol stem bark extract of *Nauclea latifolia* caused increase in packed cell volume, hemoglobin, red blood cell, mean corpuscular volume, mean corpuscular hemoglobin, Lymphocytes and platelet levels as the dose administered increased from low dose to high dose while the white blood cell level was observed to decrease as shown in Table 5(a) and 5(b).

3.5 Biochemical Analysis

The test result showed that the ethanol stem bark extract of *Nauclea latifolia* caused increase in globulin, urea and aspartate aminotransferase (AST) levels as the dose administered increased from low dose to high dose while albumin, total protein, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) level was observed to decrease as shown in Table 6.

4. DISCUSSION

Plant derivatives and natural products used in folk medicine are of vast medicinal importance. This is because they remain a source of many molecules with pharmacological properties. These products are non-nutritive chemicals constituents of the plant that occur naturally and have protective or disease preventive properties, they are non-essential nutrient meaning they are not required in sustaining life. With the increase in the demand for these natural products in many parts of the world, scientists are becoming more concerned about their safety. This is because it is known that less than 10% of the herbal products in the world market are truly standardized. This along with the lack of available scientific data has made toxicity studies especially of natural products necessary [15].

The ethanol extract of the stem bark of *Nauclea latifolia* was analyzed for the presence of phytochemical compounds which are also responsible for their medicinal use [16]. The study showed that the stem bark of *N. latifolia* contained alkaloid, tannins, resins, balsams, saponins etc (Table 1). The presence of alkaloids, saponins, tanins and flavonoids in *N. latifolia* have been reported to be responsible for the anti-malarial activities of plant, hence, traditional medicine practitioners rely so much on them but not taking cognizance of the adverse effect of their toxicity to the system [17].

All the animals that received doses of *Nauclea latifolia* at 1000 mg/kg survived for 24 hrs but showed a slight dullness in normal activities. This suggests that the LD_{50} is above 1000 mg/kg body weight. The acute toxicity evaluation revealed that the lethal dose (LD_{50}) of the extract was 1414.2 mg/kg body weight (Table 2) suggesting that the extract is only slightly toxic [14].

The ethanol extract of the stem bark of *N. latifolia* was observed to increase the Red blood cell count (RBC), hemoglobin (Hb) and packed cell volume (PCV) of the test animals significantly at both the sub-acute and the chronic test result (Tables 3a and 5a) suggesting that the extract enhanced the production of red blood cell. This supports the findings of Kouadio et al. [18]. Red blood cells, circulates in the blood and carry oxygen throughout the body. The hemoglobin absorbs oxygen in the lungs then travels through blood vessels and brings oxygen to all other cells through the heart. An increase in the levels of these hematological parameters is indicative that the extracts have the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis) [19].

The white blood cell counts of both the sub-acute and chronic tests were decreased significantly (Tables 3b and 5b). White blood cells serve as scavengers that destroy the microorganisms at infection sites, removing foreign substances and debris that results from dead or injured cells. Consequently, the level is known to rise as body defense in response to toxic environment [20]. The decrease suggests that the extracts did not exhibit toxic effect even at high doses. The lymphocyte, the main effector cells of the immune system [21] was observed to increase with higher dose of the extract (Tables 3b and5b) indicating that the extract may have some effect on the immune system.

There was an observed increase in platelets count at the sub-acute and chronic toxicity test results (Tables 3b and 5b). A Sudden increase in platelets can cause blood clot to develop spontaneously. Abnormal clotting can be dangerous as blood clot may block the flow of blood to the brain, liver, heart and other vital organs, it can also lead to other complications such as stroke or heart attack [22a]. This ability of the plant may however prove to be useful in the management of heamorrhage in the case of dengue fever. Dengue fever is caused by a virus, therefore, specific medicines and antibiotics may not be useful for its management. Appropriate concentrations of this extract may prove useful in the treatment of the haemorrhage that will normally follow a severe situation. The root extract of Nuclea latifolia has been reported to have shown some activity against herpes simplex virus (HSV2) [22b] suggesting a possible antiviral potential. More study into the possible use of parts of this plant for the management of dengue fever is suggested.

The liver and kidneys play crucial roles in various metabolic processes and are, therefore, particularly exposed to the toxic effects of exogenous compounds [23]. AST and ALT are common liver enzymes and Liver damage and its recovery is usually assessed by measuring their serum levels [24a,24b]. An elevation in plasma concentration of these enzymes is an indication of liver damage. AST is mostly present in the myocardium, skeletal muscle, brain and kidneys [25]. Thus, the liver and heart release ALT and AST, an elevation in plasma concentration is an indicator of liver and heart damage [24,26]. The

ethanol extract of the stem bark of *N.latifolia* at both sub-acute and chronic toxicity test caused a significant decrease in the ALT level in the blood (Tables 4 and 6). A decrease in the ALT level shows there is no injury or damage to the liver. On the other hand, the extract at both sub-acute and chronic toxicity test caused an increase in the AST level. This elevation may suggest a possible effect on the heart with prolonged usage [24]. A rise in plasma alkaline phosphatase (ALP) level is usually a characteristic finding in cholestatic liver disease [27] the significant reduction in ALP levels by the ethanol stem bark extract of *N.latifolia* at both sub-acute and chronic toxicity test result shows that no possible cholestasis occurred at the dose levels administered (Tables 4 and 6).

The ethanol extract of the stem bark of *N. latifolia* showed a significant decrease in the albumin level at both sub-acute and chronic test results. Albumin is the protein with the highest concentration in plasma. It transports many small molecules in the blood (for example, bilirubin, calcium, progesterone, and drugs). It also prevents the fluid in the blood from leaking out into the tissues [28]. Since albumin is produced in the liver, decreased serum albumin as obtained may arise from liver or kidney disease or function. Other results obtained however, showed the different doses of the extract used had no effect on the liver (Tables 4 and 6). Urea is one of a number of non-protein nitrogenous substances that accumulate in the plasma when renal excretion is reduced. This means that increase in urea observed (Tables 4 and 6) may suggest impaired kidney function [29,30] in support of the same suspicion due to reduced serum albumin.

5. CONCLUSION

Results obtained from this study confirmed that the ethanol extract of the stem bark of Nuclea latifolia was only slightly toxic to the experimental animal. It also showed the potential of the extract to enhance red blood cell (RBC) production. The extract was however observed to have blood clotting ability which means that when used over a long time, it could lead to stroke or heart attack meanwhile, it may be useful if properly studied in the management of heamorrhage due to dengue fever or even the management of dengue fever itself since it's antiviral potential has been reported. It was also observed to have potentials to affect the heart and impair kidney functions on prolonged usage.

Plant natural products may be useful as medicines. They may also have some unknown side effects as observed with *Nuclea latifolia*. They should therefore be used with great caution if they have to be used.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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