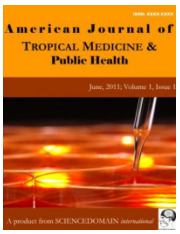

American Journal of TROPICAL MEDICINE & Public Health
1(1): 11-21, 2011

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Hypocholesterolemic Effects of *Nauclea latifolia* (Smith) Fruit Studied in Albino Rats

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Research Article

Received 21st April 2011
Accepted 19th May 2011
Online Ready 2nd June 2011

ABSTRACT

This study was designed to investigate the cholesterol lowering effect of *Nauclea latifolia* fruit as well as its chemical compositions, toxicity, vitamins, and effect on hemoglobin, red and white blood cells count in albino rats. Hypercholesterolemia is a predominant risk for atherosclerosis and associated coronary and cerebrovascular diseases. Control of cholesterol levels through therapeutic drugs, notably statins, have significantly reduced the risk for developing atherosclerosis and associated cardiovascular diseases. However, adverse effects associated with therapeutic drugs warrant to find other alternative approaches for managing hypercholesterolemia which include indigenous medicinal plants. Phytochemical detected in *Nauclea latifolia* increased in the order, cyanide → saponins → phytate → tannin → oxalate (0.09±0.010mg/g, 0.65±0.005%, 2.70±0.010%, 3.27±0.001mg/g and 88.56±0.001mg/100g, respectively). The crude fruit sample lowered plasma cholesterol at 40, 60 and 80% feed supplementation studied and this was dose dependent. All animals fed with the plant sample showed dose dependent increase in white blood cells at 40, 60 and 80% feed supplementation (4.23±0.01, 4.38±0.01, 4.40±0.03 ($X10^9$/L), respectively). Hemoglobin and red blood cell count decreased dose dependently. Vitamin content ranged between 6.42 to 92.72 mg/100g for vitamins E, A and C. The LC_{50} of the plant extract was 1240.73 µg/ml conferring lower toxicity when compared with reference standard, potassium dichromate (LC_{50} = 176.86 mg/ml). In conclusion, this result shows that *Nauclea latifolia* possesses hypocholesterolemic potential (85.20±0.05 mg/dl when compared with control 136.25±0.005 mg/dl) and is relatively non-toxic ($LC_{50=}$1240.73µg/ml. Hypocholesterolemic effect is related to its phytochemical contents.

Keywords: Nauclea latifolia; Hypocholesterolemia; Cholesterol; Phytochemicals; Vitamins;

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1. INTRODUCTION

Nauclea latifolia (Smith) belongs to the family *Rubiaceae.* It is a straggling shrub or small tree, native to tropical Africa and Asia. It bears an interesting flower, large red ball fruit with long projecting stamens. The red fruit is edible but not appealing. *Nauclea latifolia* is an evergreen multi-stemmed shrub or tree. It grows up to an altitude of 200 meters. It is widespread in the humid tropical rain forest zones or in the savanna wood lands of west and central Africa (Burkil, 1985).

The wood of *Nauclea latifolia* is termite resistant and is used as live stakes in farms. All parts of the plant species are rich source of mono-terpene indole alkaloid. The fruits are major source of food for the baboons, livestock, reptiles, birds and man. It is used for the treatment of stomach ache when the decoction of the bark and leaves are infused. It is also used in the treatment of fever, diarrhea and even as anti-parasitic drug. The sticks are used as chewing stick and a remedy against tuberculosis (Burkil, 1985; Esimore et al., 2003.)

Cholesterol is the major sterol in animal tissues and its amphipathic nature enables it to occur in cell membranes (Nelson and Core, 2001). It is abundant in the brain, liver, adrenal glands and nervous system (Osmund, 2001). The maximum dietary cholesterol required in a day by an adult is 300mg. The liver produces sufficient cholesterol (in the absence of enough diary cholesterol) for all normal body functions. Higher cholesterol levels are found in males and older people. It is carried in the blood in the form of lipoproteins (Osmund, 2001).

Maintenance of cholesterol homeostasis is vital for healthy status and achieved through a regulatory network consisting of genes involved in cholesterol synthesis, absorption, metabolism and elimination. Imbalance of cholesterol level as a result of environmental and genetic factors leads to hypercholesterolemia, a predominant risk factor for atherosclerosis and associated coronary and cerebrovascular diseases (Steinberg, 2005; Ballantyne et al., 2005; Meagher, 2004). Cardiovascular diseases including coronary heart disease and stroke are the leading cause of mortality, accounting for nearly 50% of all deaths in the western developed world (Thomas and Rich, 2007; Rich, 2006). If the current trend continues, the future will be even bleaker considering the fact that globalization and wide spread of Western diet to the developing world has been encountered with explosive increase in the rates of obesity and hypercholesterolemia in those regions. Therefore, hypercholesterolemia and its associated cardiovascular diseases represent one of the greatest worldwide economic, social and medical challenges that we are facing now (Olshanky et al., 2005).

In blood plasma, cholesterol is transported by lipoproteins, which can be mainly categorized into tour classes, based on size of cholesterol-lipoproteins complexes: the very-low-density lipoprotein (VLDL), the intermediate-density lipoproteins (IDL), the very-low-density lipoproteins (LDL) and the high-density lipoproteins (HDL), (Steinberg, 2005; Ballantyne et al, 2005). Experimental and chemical studies have shown that the amount of cholesterol transported in the VLDL, IDL and LDL classes of lipoproteins, is a risk factor for the occurrence of cardiovascular disease (Fernandez and Webb, 2008; Nicholls et al., 2007). In contrast, cholesterol transported in HDL particles, known as anti-atherogenic cholesterol, has protective effect on cardiovascular disease (Nichols et al., 2007).

Control of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing atherosclerosis and associated cardiovascular disease (Stacy and Egger, 2006; Ray et al.,2006; Khush and Waters, 2006). Notably, statins, a class of cholesterol-lowering drugs inhibiting cholesterol synthesis, have been most widely prescribed for treating

hypercholesterolemia and reducing cardiovascular diseases (Ray et al., 2006; Khush and Waters, 2006). However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages and potential drug-drug interaction have been reported (Neuvonen et al., 2006; Trifiro, 2006; Kiortsis et al., 2007; Parra and Reddy, 2003). Therefore, development of additional therapies for controlling cholesterol levels is warranted, especially for those with better safety profile.

Herbal remedies or food supplements have increasingly become attractive alternatives to prevent or treat hypercholesterolemia, especially for those with cholesterol at the borderline levels. Excellent safety profile, cost effectiveness and multiple beneficial effects on improving wellbeing all contribute to the emerging trend of increasingly usuage of herbal supplements in Nigeria and other developing countries of the world.

In the light of *Nauclea latifolia* usage as healing agent in Folklore medicine, the present study was undertaken to evaluate its cholesterol lowering potential in experimental rats.

2. MATERIALS AND METHODS

2.1 Animals

Sixteen adult albino rats weighing between 39-40g were purchased from the zoological garden of the University of Nigeria, Nsukka. They were housed in the animal house of the Department of Biochemistry, Kogi State University, Anyigba, Nigeria. They were acclimatized, placed in groups of four animals per cage.

2.2 Collection and Preparation of Plant Sample

The plant sample was collected from Igalaogba, behind school of health technology in Idah Local Government Area of Kogi State, Nigeria, during rainy season. The plant was identified in the Botany unit of the Department of Biological sciences, Kogi State University, Anyigba, Nigeria as *Nauclea latifolia* (Smith). The fruits were washed to remove dirt, and were sliced into pieces, air dried in the laboratory for 170 hours. The sample was then transferred into a hot air drying oven, set at a temperature of 50^0C for 24 hours. The dried sample was pounded into a powdery form using mortar and pestle. The powdered sample was stored in air tight labeled plastic container until used for the experiment.

2.3 Methanol Extraction

Cold extraction method was used for the extraction process. A portion (250g) of the sample was weighed into a 1000ml conical flask, 650ml of pure methanol was added and left for 72 hours. The mixture was then filtered using a vacuum pump and solvent separated using rotary evaporator.

2.4 Animal Grouping and Feeding Experiment

The animals were divided into four (4) groups of four (4) rats each. Therefore there were three (3) test groups and the control was the fourth. Groups 2,3,4 were fed the commercial feed supplemented at 40,60,and 80% with *Nauclea latifolia* fruit while the control group 1 was fed the commercial rat feed alone. They were all fed for four weeks and housed in the departmental metabolic cages group-wise. At the end of the experiment, the animals were

starved overnight so as to prevent the interference of chylomicrons known to carry triglycerides into the circulation immediately after a meal. After the experiment, the rats were dissected under chloroform anesthesia in a desiccators and blood was collected via cardiac puncture into anti coagulated bottle and was subsequently analyzed for the following biochemical parameters: plasma cholesterol, hemoglobin, red and white blood cell counts.

2.5 Analysis of Blood Samples

The heparinized blood sample was spinned in a centrifuge for ten minutes at 3,000 r. p.m to get the plasma which was taken up with a Pasteur pipette into labeled sample tubes that were refrigerated until used.

2.6 Determination of Total Plasma Cholesterol

The method of Zlatkis et al., 1953 was used in the determination of plasma cholesterol. A portion (0.1ml) of plasma was pipette into 3.0ml of glacial acetic acid in a dry test tube. A 2.0ml of colour reagent was added gently down the side of the tube and formed a lower layer to the acetic acid. The two layers were then thoroughly mixed to ensure an even heat distribution after which the tubes were allowed to cool. A portion (0.1ml) of the cholesterol standard (1mg/ml) and 0.1ml of distilled water (blank) were simultaneously treated as above, and absorbance of each was read at 560nm in a spectrophotometer against the blank. The concentration of the cholesterol in the plasma was calculated using the equation:

Conc. of cholesterol of plasma =

$$\frac{\text{O.D. of test}}{\text{O.D. of standard}} \text{ X Conc. of standard}$$

Where O.D. = Optical density.

2.7 Quantitative Determination of Phytochemicals

2.7.1 Saponin determination

The method employed was that of Obadoni and Ochuko (2001). The sample was grounded and 20g was put into a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4h with continuous stirring at 55^0C. The mixture was filtered and the residue re-extracted with 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at 90^0C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation the sample was dried in the oven to a constant weight; the Saponins content was calculated as percentage.

2.7.2 Tannin determination

Tannin determination was done by Van-burden and Robinson (1981) method. A portion (500mg) of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1h on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipette out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M Potassium ferrocyanide. The absorbance was measured at 120nm within 10 min.

2.7.3 Determination of Cyanogenic Glycoside

The extraction was according to Wang and Filled method as described by Onwuka (2005). A portion (5g) of sample was made into a paste and the paste was dissolved into 50ml distilled water. The extract was filtered and the filtrate was used for cyanide determination. To 1ml of the sample filtrate, 4ml of alkaline picrate was added and absorbance was recorded at 500nm and cyanide content was extrapolated from a cyanide standard curve.

$$\text{Cyanide (Mg/g)} = \frac{\text{Absorbance X GF X DF}}{\text{Sample weight}}$$

Where:
GF = Gradient Factor
DF = Dilution Factor.

2.7.4 Determination of Phytic acid

Phytic acid was determined using the procedure described by Lucas and Markakas (1975). A portion (2g) of the sample was weighed into 250ml conical flask; 100ml of 2% concentrated hydrochloric acid was used to soak each sample for 3h. The mixture was filtered, and 50ml of each filtrate was placed in 250ml beaker and 107ml of distilled water was added in each case to give proper acidity. 10ml of 0.3% ammonium thiocyanate solution was added to each solution as indicator and was titrated with standard iron chloride solution which contained 0.00195g iron per ml.

% phytic acid = Y x 1.19x100

Where y = Titre value x 0.00195.

2.7.5 Determination of Oxalate

The determination of oxalate was according to the method described by Odebiyi et al. (2003).

2.8 Hemoglobin Estimation

The acid hematin method as described by Guyton and Hall (2000) was used to estimate the hemoglobin concentration of the blood sample.

2.9 Ascorbic Acid and Vitamin E Determination

The method described by Vogel (1989) was employed for the determination of Vitamin C and E in the plant sample.

2.10 Vitamin A Content Determination

The method described by Asubiojo et al., 1982 was followed in the determination of vitamin A content of the *Nauclea latifolia* fruit.

2.11 Red Blood Cell Count

The method of Tietz (1987) was used for the red blood cell count.

2.12 White Blood Cell Count

The total white blood cell was estimated using the method described by Purves et al., (2005).

2.13 Cytotoxicity Bioassay

Modified method of Solis et al., (1992) was used to determine the inhibitory activity of the extract on *Artemia salina* in vial bottles. Brine Shrimps (*A. Salina*) were hatched using brine shrimp eggs in a plastic vessel (500ml), filled with sterile artificial sea water (prepared using NaCl salt (38g/L) and adjusted to pH 8.5 using 40% NaOH) under constant aeration for 48h. After hatching, active nauplii freed from egg shells were harvested from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 5ml of brine solution. A portion (50ml) of different concentrations of crude methanol extract (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) solution in 0.25% Tween 80 – artificial sea water was added into each well (vial bottles) containing 10 newly hatched brine shrimps and then incubated at room temperature for 24h. This was repeated in two wells to make the overall tested organisms of 20 for each. The living brine shrimps were counted under a hand magnifying lens. Same procedure was followed using potassium dichromate as the reference standard.

Plot of percentage lethality versus log concentration, substituted Y = 50 in the resulted linear equation to obtain the X value. The antilog of X was then the LC_{50} (concentration of 50% lethality) value (Ballantyne et al., 1995).

2.14 Statistical Analysis

Mean and standard deviations were calculated and the effects of each were analyzed by repeated measures of ANOVA (Analysis of variance). Statistical analyses were made by a SPSS for windows Version 13.0 packaged statistics program. All results were represented as mean ± standard deviation (S.D.) of three or four replicate determinations.

3. RESULTS AND DISCUSSION

Table 1 shows some phytochemical contents of *Nauclea latifolia* fruit. The fruit is high in oxalate content and low in both cyanide and saponins.

Table 1. Phytochemical composition of *Nauclea latifolia* fruit

Oxalate (mg/100g)	Saponins (%)	Cyanide (mg/g)	Tannins (Mg/g)	Phylate (%)
8.56±0.001	0.65±0.005	0.09±0.010	3.27±0.001	2.70±0.010

Values are expressed as mean ± S.D. of three determinations.

Some vitamin content of the plant was determined and is as presented on table 2. The plant fruit is richer in vitamin C than any other vitamin determined. Vitamins confer nutritional and medicinal properties on plant products.

Table 2. Some vitamin composition of *Nauclea latifolia* fruit

Vitamin A mg/100g)	Vitamin C mg/100g)	Vitamin E (mg/100g)
12.58±0.005	92.72±0.002	6.42±0.010

Values are expressed as mean ± S.D. of three determinations.

Table 3 represents cytotoxic effect of *Nauclea latifolia* fruit extract. It shows that 1240.73 ug/ml is required to kill 50% of the *Artemia salina* used. This is much lower when compared with the reference standard potassium dichromate used.

Table 3. Inhibitory effect of methanol extract of fruit on brine shrimps

Sample	Concentration (µg/ml)	Log concentration	% Lethality	LC_{50}(µg/ml)
Nauclea latifolia	1000	3.0000	50	1240.75[a]
	500	2.6990	40	
	250	2.3979	30	
	125	2.0969	20	
	62.5	1.7959	20	
	31.25	1.4949	10	
Potassium dichromate	1000	3.0000	70	176.86[b]
	500	2.6990	70	
	250	2.3979	60	
	125	2.0969	60	
	62.5	1.7959	30	
	31.25	1.4949	10	

(a) Linear equation: Y = 25.62 x – 29.26; (b) Linear equation: Y = 39.86 x – 39.59;

Table 4 shows the effect of *Nauclea latifolia* on some biochemical parameters which include hemoglobin, cholesterol, RBC and WBC. The plant's fruit reduced the plasma cholesterol and this was dose dependent. Similarly RBC, and hemoglobin were affected, WBC increased dose dependently when compared with the control.

Table 4. Effects of *Nauclea latifolia* fruit on some biochemical parameters in rats

Sl. No.	Group/Dosage	Hemoglobin (%)	Cholesterol mg/dl)	RBC ($x10^{12}$/L)	WBC ($x10^{9}$/L)
1	Control commercial Feed only	73.75±0.25	136.25±0.05	2.23±0.01	4.15±0.01
2	40% supplementation	70.25±0.01**	100.76±0.01**	3.15±0.01**	4.23±0.01ns
3	60% supplementation	54.25±0.01**	87.11±0.05**	3.08±0.01**	4.38±0.01**
4	80% supplementation	57.75±0.02**	85.20±0.05**	1.07±0.01**	4.40±0.01**

Values are expressed as mean ± S.D. of four replications.
*Values with superscript ** on the same column are significantly (P<0.05) different from the control values.*
ns= non-significant; RBC = Red blood cell; WBC = While blood cell.

This investigation has demonstrated that the fruit of *Nauclea latifolia* (smith) contains phytochemical such as oxalate, saponins, cyanogenic glycosides, tannins and phytates. These phytochemicals may be contributory to the observed biological activity of *Nauclea latifolia* fruit. Oliver and Zahnd (1979) severally implicated saponins and cyanogenic glycosides to be responsible for the hypoglycemic activity in many plants used in ethno medical practice for the management of diabetes.

Oxalate concentration was found to be very high (8.56±0.001 Mg/100g). Oxalate accounts for toxicity of most if not all nutritional food plants. Oxalate can decrease the availability of dietary essential minerals such as calcium and iron. Phytate was present with a percentage of 2.70±0.010 in the fruit sample. Phytate has been reported to decrease the availability of the dietary minerals such as iron and calcium like oxalate. Phytate chelating effect has also been reported to inhibit and even cure some cancers by depriving the cancer cells of calcium and iron they require to grow. This deprivation of mineral elements, calcium and iron serve as broad treatment for cancer (Hunell, 2003). *Nauclea latifolia* may therefore possess anticancer properties.

Phytic acid may also be used as food additives because of their capacity to reduce Iron III to Iron II which facilitates its absorption (Malleshi and Desikacher, 1980).

The plant contains vitamins C, A and E. Vitamins C and E are good antioxidants. This fruit may be useful in combating oxidative stress following its vitamin composition. Analysis of some biochemical parameters revealed that the blood of the rats fed with the plant sample at 40, 60 and 80% supplementation showed reduced plasma cholesterol (Table 4). This reduction was statistically significant (P<0.05) when compared with the control and was also dose dependent. The concentration of cholesterol was reduced significantly (P<0.05) from 136.26 to 100.76 Mg/dl at 40% supplementation with *Nauclea latifolia*. The ability of the fruit

to lower cholesterol level of the blood significantly (P<0.05) therefore implied that it could be used in the management of hypercholesterolemia. The action of the plant in reducing plasma cholesterol concentration could be due to the ability of one or more of the phytochemicals in the plant to activate the functioning enzymes of the rat responsible for cholesterol absorption (Malinon, 1997). Cholesterol lowering is achieved by the binding of bile acids and cholesterol by Saponins thereby forming an insoluble complex with cholesterol. Saponins caused depletion of blood cholesterol thereby preventing its re-absorption thus increasing its excretion (Malinon, 1997). The mechanism could either be by direct inhibition of absorption of cholesterol from the small intestine or indirectly by inhibiting re-absorption of bile acids. If direct inhibition of cholesterol occurs, Saponins prevent absorption not only at high proportion of dietary cholesterol but also at high proportion of cholesterol derived from bile acids of mucosa cells (Malinon, 1997). The presence of Saponins in this plant could be contributory to the cholesterol lowering activity observed in this investigation.

The hematological parameters such as WBC increased significantly (P<0.05) and this increase was dose dependent. There was reduction in RBC and hemoglobin. The presence of some of the phytochemicals such as tannins, oxalates and phytates could be responsible for this reduction as some could pose inhibitory effect on hemoglobin synthesis.

From the results presented on table 3, it is clear that the plant is relatively non-toxic but it appears that high consumption could pose threat to cells.

4. CONCLUSION

Food and food supplements have increasingly become attractive alternatives to prevent or treat hypercholesterolemia and reduce the risk for cardiovascular diseases. The cholesterol – lowering potential of the fruit may be ascribed to the modification of cholesterol uptake from the intestine, conversion of cholesterol to bile acids and increasing excretion of bile acids by the plants phytoconstituents. Our result therefore seems to confirm the anti-hypercholesterolemic potential (85.20±0.05 mg/dl when compared with control (136.25±0.005 mg/dl) of *Nauclea latifolia* which may explain its use in the management of some diseases in ethno medicine.

ACKNOWLEDGMENTS

The authors are grateful Messers Friday Titus Emmanuel, Olusegun Olupinyo, and Gabriel Adah for the technical assistance and advice during the course of this work.

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