

# Hypocholesterolemic Effect of Nauclea Latifolia Fruit on Glucose Changes and Lipid Profile of Alloxan Induced Diabetes in Albino Rats

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## ABSTRACT

*This study is aimed at determining the efficacy of some of the claims and to evaluate the hypocholesterolemic properties of N. latifolia in comparison with metformin in diabetic Albino rats. The objectives are to evaluate the phytochemistry and the effect of N. latifolia on lipid profile in alloxan induced diabetes in comparison with metformin as drug control. Thirty male albino Wister rats weighing 60g-90g were used. The rats were divided into six groups of five animals each. Group 1 (normal control), group 2 (-ve control), group 3 (+ve control), group 4, 5 and 6 were given 10%, 30% and 60% of the fruit as feed supplement as treatment. The abnormal lipid profiles and lipoprotein oxidation especially, Low density lipoprotein cholesterol is more common in diabetics and is aggravated with poor glycaemia control. In this study, hypoglycemic and hypocholesterolemic properties of Nauclea latifolia fruits were investigated. Fruit sample was administered on percentage basis to alloxan - induced diabetic rats once daily for a period of twenty one days. Their blood glucose level was measured every three days while Lipid profile was assayed at the end of the experiment. The fruit sample significantly reduced ( $p < 0.05$ ) the fasting blood glucose levels compared to the diabetic control and was discovered to be dosage dependent. Total Cholesterol, very low density lipoprotein, triglycerides and Low Density Lipoprotein Cholesterol was reduced significantly ( $p < 0.05$ ) by Nauclea latifolia fruit dose - dependently. In diabetics, there is a decrease in the level of electrolytes as a result of osmotic diuresis with subsequent loss of water and electrolytes induced by glycosuria.*

**Key Words:** Photochemistry, hypocholesterolemic, Hypoglycemic, Nauclea latifolia.

## 1. INTRODUCTION

Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account [1]. [2]. The term diabetes mellitus describes a metabolic disorder of multiple etiologies and is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism defects due to absolute or relative lack of insulin produced in the pancreas which aid the transport of glucose from the blood stream to the cells [2]. [3] Some obvious limitations of the drug management methods necessitate a search for alternatives among the arsenal of herbs available in the natural habitat of man. After countless searches the world health assembly in 1989, adopted among its resolutions, the support of national traditional medicine program giving consideration to herbal medicine of being important to the health of individuals in the society [4]

Although the plant *N. latifolia* is mainly cultivated as food, it has continued to be an important medicinal plants, a wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from its possible anti diabetic effect [5], antibacterial activities, ability to ameliorates anemia and osmotic fragility induced by protein energy malnutrition [6], ability to strengthen fingernails, darken gray hair, to lower blood cholesterol, cure alcoholism, scorpion stings, brain and vision improvement [7]. Other studies have also been reported for the potential hepatoprotective, anti-oxidant, hypoglycemic, anti-hyperlipidaemia activities of *Teucrium* [8]. Reports have shown that diabetics are at high risk of developing atherosclerosis through a variety of mechanisms [9]. Atherosclerosis which is often called the "hardening of the arteries" is caused by the reduced arterial blood flow to vital organs. High concentration of blood glucose can irritate the lining

of the arteries that promotes the accumulation of “plaque” [10]. More so the abnormal level of circulating glucose can lead to high concentration of “free radicals” [11].

Nowadays, the frequency of diabetes mellitus is increasing in many folds, Researches have shown that it is the body composition mainly body lipids that are responsible for the increased prevalence of this disease [1]. *Nauclea latifolia* is an evergreen multi-stemmed shrub or a tree; it grows up to an altitude of 200m. The leaves are glabrous, opposite, rounded - ovate, glossy green with tufts of hairs. The plant belongs to the Rubacea family, it is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. Three other related species *Nauclea pobeguini*, *N. diderichii*, and *N. vanderghuchtii* are forest trees. *N. diderichii* is planted in Omo forest reserve, Nigeria. In the 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. The commonly used parts of the plant include the bark, fruit, roots, stem and sap. The tree is flowering from April to June. The fruits are ripening from July to September. Baboons eat them and disperse the seeds. Livestock eat shoots and leaves. The fruits are edible, too. The wood of *N. latifolia* (Opepe wood) is termite resistant and is used as live stakes in farms [12]. The fruits are usually fleshy, shallow-pitched, with numerous embedded seeds surrounded by a pink edible, sweet- sour pulp.. The seeds are usually small, ovoid, numerous and brownish with a pleasant taste but could be emetic if taken in excess [13]. Traditionally, the plant has been reported as an anti-malarial, antibacterial and antiviral. Other potentials of the fruits include, as a laxative and hypocholesterolemic. Phytochemicals such as alkaloids, saponins, tannins, oxalates, phytates and phthalates have been detected and isolated from various parts of the plant [14].

In blood plasma, cholesterol is transported by lipoproteins, which can be mainly categorized into four 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), [15];[16]. 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 [17]. In contrast, cholesterol transported in HDL particles, known as anti-atherogenic cholesterol, has protective effect on cardiovascular disease [17]. Control of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing atherosclerosis and associated cardiovascular disease [18];[19];[20]. Notably, statins, a class of cholesterol-lowering drugs inhibiting cholesterol synthesis, have been most widely prescribed for treating hypercholesterolemia and reducing cardiovascular diseases [19];[20]. However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages and potential drug -drug interaction have been reported [21];[22]. Therefore, development of additional therapies for controlling cholesterol levels is warranted, especially for those with better safety profile.

## 2. Materials and methods

### 2.1 Collection and identification of plant material

The fruits were collected, air dried in a clean environment and pounded into a fine powder using a pestle and mortar. The fruit samples were identified at the herbarium of the department of Biology, Gombe State University.

### 2.2 Animals used

The animals used for this study are albino rats obtained from the small animal house, National Veterinary and Research institute(N.V.R.I), Vom, Plateau state. They were fed with vital feed.

A total number of 30 albino Wister rats were used of specifically male sex weighing about (60-90g) were used in the study. They were allowed to acclimatized and stabilized for a period of 10 days and their health status properly monitored before the experimental study. The albino Wister rats were divided into 6 groups.

- Group1 was the normal control
- Group 2 was the diabetic control
- Group 3 was the drug control treated standard drug, with metformin
- Group 4 was the treatment control which feed was supplemented with 10% of the fruit.
- Group 5 was the treatment control which feed was supplemented with 30% of the fruit.
- Group 6 was the treatment control which feed was supplemented with 60% of the fruit.

### 2.3 Monitoring of the animals

There was constant monitoring of the rats in order to provide conducive living environment for the animals, this was done on a daily basis. The rats were provided with adequate feed and water, good hygiene was ensured by regular cleaning and removal

of faeces along with spilled feeds in their cages. The blood glucose level for each rats were determined on the 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of treatment.

## 2.4 Preliminary Phytochemical Screening Test

The sample was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, saponins, tannins and anthraquinones using standard procedures by coloring and precipitation assays using standard procedures as described by [23];[24].

- Calorimetric determination of vitamin C by the use of **2, 4 DNPH** was done according to the procedure described by Nino and Shah (1986)
- Determination of moisture content in *N. Latifolia* using gravimetric method was done according to the procedure described by Nino and Shah (1986)
- Determination of protein in *N. Latifolia* using formol titration method was done according to the procedure described by Ochei *et al.*,(2008)
- Fat determination in *N. Latifolia* was done according to the procedure described by Ochei *et al.*,(2008)
- Determination of total ash content *N. Latifolia* was done according to the procedure described by Ochei *et al.*,(2008)
- Determination of total carbohydrate (tc) *N. Latifolia* fruit was done by difference method according to the procedure described by Ochei *et al.*,(2008)

## 2.5 Estimation of lipids and lipoprotein

Serum total cholesterol (TC), total triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and phospholipids were analyzed [25].

## 3. 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  $\pm$  standard deviation (S.D.) of three or four replicate determinations.

## 4. RESULTS AND DISCUSSION

**Table 1 Phytochemical Screening *Nauclea latifolia* fruit**

PHYTOCHEMICALS	PRESENCE
Alkaloids	+
Saponins	++
Tanins	-
Flavanoids	+
Anthraquinones	+
Steroids	-
Glycosides	+

**Key;** + Signifies present while,  
- Signifies absent

It was observed from the preliminary screening of the fruit of *Nauclea Latifolia* that it indicated the presence of bioactive component like, flavanoids, alkaloid, saponins, anthraquinones, and glycosides were present while steroids and tannins were absent as shown in table above.

**Table 2: Proximate Composition of *Nauclea latifolia* fruit**

Sample	% Protein	% Fat	% Fiber	% Ash	% Carbohydrate	% Moisture	% vitamin C
Fruits	15.42	1.74	35.88	8.19	49.81	24.72	37.47

The percentage composition of the plant parts in table 1 above shows that the plant fruits contain appreciable amount of the basic food nutrients such as protein, fats, carbohydrates and fibre.

**Table 3. The Body Weight in Gram of Rats Before and After the Experiments are statistically different at 95% level ( $p<0.05$ ).**

Treatment	Day 0		Day 7		Day 14		Day 21	
Normal	71.73	1.70 <sup>a</sup>	74.42	2.30 <sup>b</sup>	78.43	0.99 <sup>c</sup>	84.09	1.29 <sup>d</sup>
Diabetic control	65.4	21.47	63.08	1.23	60.98	4.08	56.74	9.26
Drug control	62.24	2.32	63.94	2.14 <sup>a</sup>	66.36	1.32 <sup>c</sup>	72.41	1.66 <sup>d</sup>
10% <i>N.latifolia</i>	65.58	1.98	65.61	0.21 <sup>ab</sup>	67.94	2.18 <sup>b</sup>	80.45	0.75 <sup>c</sup>
30% <i>N.latifolia</i>	66.35	2.21	66.75	2.41 <sup>ab</sup>	67.78	0.32 <sup>c</sup>	74.96	0.26 <sup>d</sup>
60% <i>N.latifolia</i>	65.01	1.47	67.01	2.08 <sup>a</sup>	71.17	1.32 <sup>c</sup>	76.62	0.88 <sup>d</sup>

Values are means SEM; n = 5

Each day was compared with the normal control, drug control and diabetic control. Same superscript indicates no significant difference while different superscript indicates significant difference. Table 2 shows the body weight of normal control and treated diabetic rats at seven days interval from Day 0 (before commencement of treatment). The result showed that normal control rats had significant ( $p<0.05$ ) weight gain on day 21 (at the end of treatment). Diabetic rats treated with *Nauclea Latifolia* significantly ( $p<0.05$ ) regained their weight on day 21 when compared with day 0. Weight loss due to inducing of alloxan as observed in diabetic in the groups is a prominent symptom of diabetes.

**Table 4; Effect of treatment on blood glucose level of diabetic rats .**

Treatment	Day 0		Day 7		Day 14		Day 21	
Normal	122.50	6.59	103.43	5.45	100.43	5.32	110.00	3.98
Diabetic control	292.00	16.62 <sup>b</sup>	302.43	69.54 <sup>bc</sup>	341.54	76.89 <sup>bc</sup>	370.54	84.65 <sup>b</sup>
Drug control	281.75	29.66 <sup>ab</sup>	260.67	25.68 <sup>a</sup>	198.76	23.89 <sup>a</sup>	132.47	22.67 <sup>a</sup>
10% <i>N.latifolia</i>	300.78	16.61 <sup>bc</sup>	340.41	14.32 <sup>bc</sup>	332.87	46.76 <sup>c</sup>	297.25	37.76 <sup>a</sup>
30% <i>N.latifolia</i>	310.75	20.45 <sup>bc</sup>	299.56	19.53 <sup>a</sup>	261.67	55.65 <sup>a</sup>	235.55	64.98 <sup>a</sup>
60% <i>N.latifolia</i>	305.75	20.35 <sup>ab</sup>	342.56	14.65 <sup>a</sup>	279.67	34.65 <sup>a</sup>	213.56	18.65 <sup>a</sup>

Values are means SEM; n = 5

a= $p<0.05$  indicates a significant difference compared with diabetic control;

b= ( $p<0.05$ ) indicates a significant difference compared with normal control;

c= ( $p<0.05$ ) indicates a significant difference compared with standard agent; metformin.

The effects of the supplement and metformin on serum glucose level in diabetic rats are depicted in table 4. The fall in serum glucose levels of the supplements and metformin treated groups were compared with that of negative control (diabetic untreated) group. The supplementation in different percentages showed significant hypoglycemic effect in comparison with negative control group on 21<sup>st</sup> day.

Table 5; Effect of treatment on blood glucose percentage change of diabetic rats.

Treatment	INITIAL GLUCOS E	FINAL GLUCOS E	% CHANGE
Normal control	122.50 6.59	110.00 3.98	26.4
Diabetic control	292.00 16.62 <sup>b</sup>	370.54 84.65 <sup>b</sup>	33.3
Drug control	281.75 29.66 <sup>ab</sup>	132.47 22.67 <sup>a</sup>	54.3
10% <i>N.latifolia</i>	300.75 20.45 <sup>bc</sup>	297.25 37.76 <sup>c</sup>	32.3
30 % <i>N.latifolia</i>	310.75 20.45 <sup>bc</sup>	235.55 64.98 <sup>a</sup>	38.5
60% <i>N.latifolia</i>	305.75 20.35 <sup>ab</sup>	213.56 18.65 <sup>a</sup>	49.8

Values are means SEM; n = 5

a=<0.05 indicates a significant difference compared with diabetic control;

b= (p<0.05) indicates a significant difference compared with normal control;

c= (p<0.05) indicates a significant difference compared with standard agent; metformin.

Table 6; Result of lipid profile in diabetics Albino rats.

Treatment	HDL-C	LDL-C	VLDL-C	CHOLESTEROL	TRIGLYCERIDES
Normal control	35.09 5.93	24.64 12.70	36.5 11.49	97.97 12.78	181.43 20.71
Diabetic control	41.7 20.61	29.46 79.23 <sup>c</sup>	42.58 10.35	383.76 79.68 <sup>b</sup>	89.24 14.30 <sup>b</sup>
Drug control	17.85 2.89 <sup>ab</sup>	32.06 13.23 <sup>a</sup>	17.85 2.86 <sup>ab</sup>	116.45 0.21 <sup>a</sup>	122.79 21.65 <sup>a</sup>
10% <i>N.latifolia</i>	26.36 0.40 <sup>c</sup>	50.69 14.13 <sup>a</sup>	35.13 2.78 <sup>c</sup>	77.50 10.15 <sup>a</sup>	175.62 13.91 <sup>ac</sup>
30% <i>N.latifolia</i>	31.06 2.67 <sup>c</sup>	42.09 79.23 <sup>a</sup>	33.76 3.17 <sup>c</sup>	108.66 12.77 <sup>a</sup>	166.91 15.44 <sup>ac</sup>
60% <i>N.latifolia</i>	32.78 5.24 <sup>c</sup>	27.93 11.38 <sup>a</sup>	28.43 4.65 <sup>c</sup>	116.86 13.34 <sup>a</sup>	142.16 23.23 <sup>a</sup>

Values are means SEM; n = 5

a=<0.05 indicates a significant difference compared with diabetic control; b=

(p<0.05) indicates a significant difference compared with normal control;

c= (p<0.05) indicates a significant difference compared with standard agent; metformin.

### 3.2 DISCUSSION

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs [26];[27]. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood [28].

The protein content of 15.42% for fruits respectively shows the plant is a rich source of protein supplement for animals fed with the plant fruits. Fruits also are not good sources of fat. The low fat content of 1.74% in fruits is below the range (8.3 – 27.0%) reported for some leafy vegetables consumed in Nigeria. Generally, leafy vegetables are known for their low lipid content [29]. The carbohydrate content 49.81% for the fruit of *Nauclea Latifolia* is also high compared to that reported for some common fruits such as guava and ripe pawpaw with values of 13% and 10% respectively. Carbohydrates are useful as they supply energy to cells such as brain, muscles and blood. They contribute to fat metabolism and spare proteins as energy source and act as mild laxative for human beings. They generally add to the bulk of the diet [30]. High fibre content for the leaves of the plant is usually a major drawback in human nutrition as they cause intestinal irritation and they are low in nutrient availability as humans cannot digest them easily. Dietary fibers are also constituents of many fruits and vegetables. The percentage fibre of 35.88 for the fruits of *N.latifolia* fruit is quite higher than a range of values 0.1% and 6.8% reported for selected fruits on wet basis but corresponds to those reported on dry weight basis 10 – 41% for some fruits. High moisture content of the fruits of *Nauclea latifolia* (24.72%) is a clear indication that the fruit could be a good source of moisture during dryness.

Albino Wister rats were weighed before being induced with alloxan and the weight range was within 47.36 -93.41g. After inducing with alloxan and confirming their diabetic state after 24hours, their weight was taken after every 3 days and it was noticed according to the chart above that the rats began to loose weight. After treatment for 21 days, it was discovered that weight lost was gradually regain although it was dosage dependent. The group administered 60% *N.latifolia* fruit supplement regain as the drug control while 10% and 30% also gain weight but the same as drug control. The diabetic control continued to lose weight even though they ate more. The general regain in body weight of the diabetic rats treated with supplements of *Nauclea Latifolia* (Table 2) suggest that the extract did not induce anorexia, an effect that could have resulted to loss of weight. The lower values of the relative kidney and liver weights of the treated diabetic rats relative to the diabetic control rats may be due to the ability of the heterogeneous phyto constituents in the crude extract to produce synergistic effect on the alloxan mediated free radical activities [31]. [32] Identified flavonoid as one of the bioactive compounds present in *Nauclea Latifolia fruit*. Flavonoid has been associated with antioxidant and free radical scavenging activities [33]. Alloxan causes a massive reduction in insulin release by the destruction of  $\beta$ -cells of the islets of Langerhans and thereby induces hyperglycemia [34]. Daily administration of aqueous *Nauclea Latifolia* for 21 days had resulted in a decrease in blood glucose level in alloxan-induced diabetic rats. The possible hypoglycemic mechanism of *Nauclea Latifolia* may be through potentiation of pancreatic secretion of insulin from  $\beta$ -cell of islets or due to enhanced transport of blood glucose to the peripheral tissues.

The results of this study showed that after induction of diabetes using alloxan, there was a significant increase in the mean of Total Cholesterol(TC), Triglyceride(TG), low density lipoprotein (LDL and very low density lipoprotein (VLDL) significantly increased at  $P<0.05$  when compared with group 1 (control) with exception of HDL which significantly decreased indicating that the rats were diabetic, diabetic-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to underutilization of glucose [35]. The catabolic effects then prevail, resulting in weight loss. High levels of triglycerides, LDL-C, VLDL-C have been associated with heart disease, insulin resistance and diabetic mellitus. The abnormally high concentration of serum lipids in diabetics is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots. In this study, the rise in blood sugar was accompanied by marked increase in cholesterol, triglycerides, LDL-C, VLDL-C and reduction in HDL-C. *N.latifolia* fruit supplement and metformin significantly reduced cholesterol, TG, LDL-C, and VLDL-C and significantly increased the HDL-C level in the diabetic treated rats. *N.latifolia* fruit used as feed supplement have been shown to have hypocholesterolemic effect in diabetic rats. Study of serum of Total Cholesterol levels ; Animals in group 1 (normal control) had a mean serum for total cholesterol (TC) of  $79.97\pm 12.71$ , after induction of diabetes, there was a significant increase in group 2 (alloxan control) with mean of  $383.76\pm 79.68$ . After administration of *N.latifolia* fruit in different doses in Group 4, 5 and 6 when compared to group 2, levels of total cholesterol at  $p<0.05$  were significantly decreased in both *N. Latifolia* and metformin. They had similar effects on total cholesterol but still had more potent effect. HDL-C level decreased in alloxan induced diabetic rats when compared to normal rats whose HDL level is  $35.09 \pm 5.39$ . On administration of the different doses *N.latifolia* fruit and metformin to the diabetic rats, HDL-C level was found to be restored to normal. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency are responsible for the observed accumulation of lipids . About 30% of blood cholesterol is carried in the form of HDL-C, HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease (James *et al* 2010). From this study group 3, 4, 5 and 6 showed significant increase in their means when compared with group 2 (-ve control). Study of serum LDL and VLDL levels; The anti-diabetic and anti-hyperlipidemic effects of *N.latifolia* fruit in this study may be due to the effect of active constituent such as alkaloids, tannins, saponins, flavonoids etc. Therefore on supplementation of *N.latifolia* fruit in their different, it led to a reduction in the concentration of low density lipoprotein (LDL) and very low density lipoprotein concentration in the blood.

#### 4. CONCLUSION

In conclusion, these results confirm the use of *N.latifolia* fruit in traditional medicine for the treatment of diabetes mellitus. The results suggest that *N.latifolia* is capable of normalizing other biochemical and hematological abnormalities associated with diabetes mellitus thus could be prescribed as adjunct to dietary therapy and main therapy for diabetes mellitus. The result of the study carried out reveals that *N.latifolia* fruit has anti-diabetic potentials and could be useful in improving the altered electrolytes that arise from diabetes. The result therefore seems to confirm the anti-hypercholesterolemia potential of *N.Latifolia* fruit which explain its use in the management of diabetes.

#### RECOMMENDATION

Toxicity test should be carried out on the sample and there is a need on enlighten consumers on the consumption of the sample as it is dosage dependent

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