



## PHYTOCHEMICAL COMPOSITION AND EFFECT OF *NAUCLEA LATIFOLIA* AQUEOUS EXTRACTS ON BLOOD GLUCOSE LEVELS OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR ALBINO RATS

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### Abstract

Researches targeted at understanding and controlling abnormally high level of blood glucose in Diabetes mellitus are ongoing. In spite of many available anti-diabetic drugs in the market, safer and cheaper remedies from plant material are being sought for due to the unwanted side effects of these drugs. In this research work, we looked at the beneficial blood glucose lowering effect of the plant *Nauclea latifolia* in streptozotocin-induced diabetic albino Wistar rats. The extracts were first screened for the presence of phytochemicals using standard methods and the result showed the presence of saponins, tannins, reducing sugar, phlobatannins, anthraquinone, flavonoids, steroids and alkaloids. The study design involved 30 male albino Wistar rats which were divided into 6 groups. Groups 1 and 2 represent the Normal Control and Diabetic Control respectively. Groups 3, 4 and 5 were Diabetic rats treated orally with 500mg/kg body weight of aqueous extracts of Stem-bark, Leaves and Root-bark respectively while Group 6 were Diabetic rats treated with 5mg/kg body weight of Glibenclamide. The extracts and Glibenclamide were administered for 28 days. Blood glucose levels were determined on days 0, 7, 14, 21 and 28 by tail tipping method using Glucometer (Accu-Chek, Mannheim, Germany). The extracts and Glibenclamide reduced significantly ( $p < 0.05$ ) the Fasting Blood Glucose levels in the diabetic treated rats compared with the Diabetic Control. Amongst the extracts the root-bark is more efficacious, decreasing the glucose level by 49.80% while the stem-bark and leaves brought about reduction by 28.76% and 12.15% respectively. The findings imply that the extracts of this plant have blood glucose lowering ability and should be further evaluated for its beneficial effect in Diabetes mellitus management.

**Keywords:** Aqueous extracts, Blood glucose, Diabetes mellitus, *Nauclea latifolia*, Phytochemicals

### Introduction

Diabetes mellitus is a diverse and chronic metabolic disease of the endocrine system which causes alteration in glucose, protein and lipid metabolism.<sup>1,2,3</sup> It is linked with abnormality of high blood glucose level due to absence or inadequate insulin secretion with or without deterioration of insulin action.<sup>4,5</sup> The disease is secondary to a reduction or damage to the  $\beta$ -cells of the islets of Langerhans in the pancreas or resistance of the tissues to insulin which aids the entrance of the glucose into the tissues.<sup>3</sup> This precipitates a situation whereby despite ample supply

of glucose, the tissues are not getting it and the body behaves as being starved. This leads to overproduction of glucose through gluconeogenesis in the liver. There is, therefore, abnormally high level of blood glucose (Hyperglycaemia) which may overwhelm the kidneys capacity to reabsorb, with spillover of the excess glucose into the urine (Glucosuria).<sup>2</sup> Chronic hyperglycaemia in Diabetes mellitus is associated with destruction, malfunctioning and failure of the various organs in the body such as kidney, eye, nerve, heart and blood vessels.<sup>6</sup> This ailment has led to many

premature deaths. It has also caused permanent disabilities like blindness and limb amputation.<sup>7, 8, 9</sup>

Diabetes mellitus is rapidly increasing worldwide. The total number of adults between the ages of 20-70 years estimated to have the disease globally by the International Diabetes Federation (IDF) in 2012 was 366 million and led to the death of 4.6 million people.<sup>10, 11</sup> In 2013, IDF put the estimate of people having Diabetes mellitus to be 381 million. This figure is projected to double by 2030.<sup>12</sup>

At the moment the available drugs for the management of diabetes mellitus include insulin and other anti-diabetic drugs which include the glucosidase inhibitors, the biquanides and sulfonyureas. The use of these drugs is associated with some serious side effects like gastrointestinal reactions, hypoglycaemic coma and interference with kidney and liver functions.<sup>13</sup> The drawback of these drugs has increased the use of traditional and complementary medicines. Plant parts with the claim of hypoglycaemic properties for management of Diabetes mellitus have been used traditionally since ancient times around the world.<sup>14, 15</sup>

A number of investigations have shown that secondary metabolites like saponins, flavonoids and tannins that are present in plants possess hypoglycaemic effect on various animal models. The quantity and quality of these metabolites present in plant parts may differ from one part to another.<sup>16, 17</sup> The plant *Nauclea latifolia* (African peach) called *Oya* among the Idoma natives, *kyuraukase* in Tiv,<sup>18</sup> *Tafashiya* among the Hausas,<sup>19</sup> Mbom-Ibom in both Cross River and Akwa Ibom States and Ubuluinu in Igbo land<sup>20</sup> is widely used by traditional healers in Idoma land of Benue State, Nigeria for the management of diabetes mellitus. Although all parts of the plant are used, several Idoma diabetic patients have confirmed the efficacy of the root-bark extract. *Nauclea latifolia* is an evergreen multi-stemmed shrub or a tree. It grows up to a height of between 10 metres to 30 metres. Its natural habitat is the humid tropical rain forest zone or the savannah wood lands of West and Central Africa.<sup>21</sup> See Fig. 1



Fig.1 *Nauclea latifolia* plant.

## Materials and Methods

### Collection and preparation of plant materials

The plant material was harvested from the environs of the Federal University of Agriculture, Makurdi, Benue State, Nigeria. The plant was identified and authenticated by Mr. J. J Azila of the Federal School of Forestry, Jos, Plateau State, Nigeria where the voucher number FHJ 279 was assigned. Samples of the plant were deposited at the school's herbarium.

### Preparation of extracts

The leaves, stem-bark and root-bark were air-dried at room temperature, pulverized using pestle and mortar and stored in air-tight containers until the time of use. One hundred grammes (100g) of the leaves, stem-bark and root-bark powder were separately weighed using an electronic weighing machine (Mettler Toledo). The leaves, stem-bark and root-bark powder were separately soaked in 1000ml of distilled water at a ratio of 1:10 (powder/solvent)<sup>22</sup> These were stirred intermittently for 48 hours at room temperature. The soaked powder was filtered using musclin cloth after which sterile cotton wool and Whatman filter paper No 1 size 110mm were used to obtain a pure filtrate. The filtrates were then separately concentrated to dryness using water bath at temperature of 45°C yielding 13.30g (13.3%), 11.09g (11.09%) and 15.02g (15.02g) for leaves, root-bark and stem-bark respectively. The extracts were then stored in the refrigerator from where aliquots were used for the study.

The percentage yield was calculated by the expression:

$$\% \text{ yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of dry sample (g)}} \times 100$$

### Research design

#### Phytochemical analysis

Qualitative analysis was carried out for each of the extracts. The following phytochemicals: saponins, tannins, reducing sugar, phlobatanins, anthraquinones, flavonoids, steroids and alkaloids were tested for, using the methods of Odebiyi and Sofowora.<sup>23</sup>

#### Animal studies

Male albino Wistar rats weighing between 153.5-177grammes used for the study were obtained from the Animal House, College of Health Sciences, Benue State University, Makurdi, Nigeria. They were kept in

polypropylene cages under room temperature, with 12-hour light and 12-hour dark cycle. They were allowed to acclimatize for two weeks before the commencement of the experiment. The rats were divided into six groups of five rats each. Group A was Normal Control and Group B was streptozotocin-induced Diabetic Control. The controls were administered 1 ml distilled water orally. Groups C, D and E were streptozotocin-induced diabetic rats treated with 500mg/kg body weight of stem-bark, leaves and root-bark extracts of *Nauclea latifolia* respectively for 28 days and group F was streptozotocin-induced diabetic rats treated with 5mg/kg body weight of the standard anti-diabetic drug, Glibenclamide by Sanofi Aventis, Nigeria Limited, daily for 28 days.<sup>3</sup> The extracts and glibenclamide were administered orally through intrapharyngeal feeding canula. The rats were fed ad libitum with pellet diet (Grand Cereals Ltd, Jos, Nigeria) and clean tap water. Good hygiene was maintained by constant cleaning and removal of faeces and spills from cages daily. The experiment was conducted between the hours of 9.00 A.M and 11 A.M. The protocols for these experiments were in accordance with the ethical guidelines on the care and use of laboratory animals.<sup>24</sup>

#### Acute oral toxicity

The acute oral toxicity study was conducted using limit dose test of revised Up- and- Down procedure.<sup>25</sup> Five female rats one at a time were administered 5000mg/kg body weight of *Nauclea latifolia* extracts ( separate administration for leaves, stem-bark and root-bark extracts ) after overnight fast of food.

Each rat was observed each time for the first 5 minutes after dosing for signs of regurgitation and then kept in the cage and watched for every 15 minutes in the first 4 hours and then every 30 minutes for the successive 6 hours. Thereafter, observations were made daily for 14 days in case of delayed toxicity.

#### Induction of Diabetes Mellitus

Diabetes mellitus was induced in overnight fasted rats weighing between 153.5-177.0 grammes by single intra-peritoneal injection of freshly prepared Streptozotocin (Sigma-Aldrich, Germany) 60mg/kg. body weight in 0.1M citrate buffer (pH 4.5).<sup>26, 27, 28</sup> Diabetes was confirmed in the streptozotocin treated rats by measuring fasting blood glucose concentration

using Glucometer (Accu-Chek, Mannheim, Germany) 48 hours after streptozotocin injection. Rats with fasting blood glucose of more than 200mg/dl were considered diabetic and used for the study.

#### Evaluation of the hypoglycaemic activity of the aqueous extracts of *Nauclea latifolia*

The aqueous extracts of the stem-bark, leaves and root-bark were orally administered through intrapharyngeal feeding canula to the diabetic rats at a dose of 500mg/kg body weight after determining their initial fasting blood glucose concentration. The extracts and the standard anti-diabetic drug, glibenclamide, at 5mg/kg body weight were administered daily for a period of twenty-eight days.<sup>5</sup> Blood was collected from the experimental rats for the determination of fasting blood glucose levels on days 0,7,14, 21 and 28 by tail tipping method. The blood sample was taken through a small incision on the tail tip. The blood was dropped on the dextrostix reagent pad which was inserted into the digital glucometer (Accu-Chek, Mannheim, Germany) and the readings were recorded.

#### Statistical analysis

Statistical analysis was done using the statistical package for social sciences (SPSS). The results were expressed as Mean  $\pm$  SEM (Standard error of mean), where n=5, analyzed by One -Way Analysis of Variance (ANOVA) and the level of significance determined by least significant difference (LSD). The p values of 0.05 and less were taken to imply statistical significance between the means.

#### Results

##### Percentage yield of the extracts

The percentage yields of the aqueous extracts of stem-bark, leaves and root-bark of *Nauclea latifolia* are 15.02%, 13.30% and 11.09% respectively (Table 1). The phytochemical constituents of the leaves, stem-bark and root-bark extracts of *Nauclea latifolia* are shown on Table 2. The results show the presence of the active phytochemicals in the leaves, stem-bark and root-bark extracts. The leaves, stem-bark and root-bark of the plant contain saponins, reducing sugar, anthraquinone, flavonoids and alkaloids. Tannin is present in the leaves but absent in the stem-bark and root-bark. Steroid and Phlobatanin are present in both stem-bark and root-bark but absent in the leaves.

**Table 1: Percentage yields of leaves, stem-bark and root-bark Aqueous extracts of *Nauclea latifolia***

Extract	Weight of dry sample(g)	Weight of extract(g)	% Yield of extract
Stem-bark	100	15.02	15.02
Leaves	100	13.30	13.30
Root-bark	100	11.09	11.09

**Table 2: Results of qualitative phytochemical screening of the leaves, stem-bark and root-bark extracts of *Nauclea latifolia***

Phytochemical	Leaves	Stem-bark	Root-bark
Saponins	+ve	+ve	+ve
Tannins	+ve	-ve	-ve
Reducing sugar	+ve	+ve	+ve
Phlobatanins	-ve	+ve	+ve
Antraquinone	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Steroids	-ve	+ve	+ve
Alkaloids	+ve	+ve	+ve

Key: +ve = Present; -ve= Absent

**Table 3: Effects of aqueous extracts of stem-bark, leaves and root- bark of *Nauclea latifolia* and glibenclamide (standard anti-diabetic drug) on Fasting Blood Glucose (FBG) in streptozotocin-induced diabetic Wistar Albino Rat**

Group	Treatment	Day 0 (mg/dl)	Day 7 (mg/dl)	Duration Day 14 (mg/dl)	Day 21 (mg/dl)	Day 28 (mg/dl)
A	Normal control	81.80±19.56	85.80±10.56	89.40±17.16	81.20±9.26	86.00±12.25
B	Diabetic control	356.80±48.62	399.40±40.33**	446.00±47.52**	426.20±29.90**	438.20±49.25**
C	Stem-bark extract	369.20±37.96	336.60±40.66*	343.20±19.06*	323.80±27.45*	263.00±24.33*
D	Leaves extract	388.40±24.40	358.00±21.49*	349.00±20.19*	340.20±23.74 *	341.20±20.24*
E	Root-bark extract	372.00±21.20	223.40±30.02*	193.80±19.77*	168.80±34.10 *	186.80±15.07*
F	Glibenclamide	379.00±36.24	226.80±39.38*	214.60±49.29*	183.40±22.42*	149.00±38.86*

Values are Mean± SEM of 5 determinations.

\*= Statistically significant when compared to diabetic control at (p <0.05)

\*\*= Statistically significant when compared to normal control at (p <0.05)

**Table 4: Percentage (%) reduction of Fasting Blood Glucose levels by aqueous extracts of stem-bark leaves and root-bark of *Nauclea latifolia* and Glibenclamide drug in streptozotocin induced Diabetic Wistar Albino Rats**

Treatment	Day 0 (mg/dl)	Day 28 (mg/dl)	% Reduction of blood glucose
Stem-bark extract	369.20±37.96	263.00±24.33	28.76
Leaves extract	388.40±24.40	341.20±20.24	12.15
Root-bark extract	372.00±21.20	186.80±15.07	49.80
Glibenclamide drug	379.00±36.24	149.00±38.86	60.68

### Results of acute toxicity studies

The results of the acute oral toxicity studies carried out using the aqueous extracts of the leaves, stem-bark and root-bark of *Nauclea latifolia* showed that there was no mortality in the rats when the dose of 5000mg/Kg body weight of extracts were orally administered respectively within the short and long term outcome of the limit dose of the Up- and- Down procedure. The aqueous extracts had no untoward effect on the nervous system since the rats did not convulse. Adverse changes in behaviour, breathing, stool, urine and mucous membrane were not observed within the period.

### Results of Fasting Blood Glucose levels

Table 3 shows significant increase (p <0.05) of the fasting blood glucose levels of the diabetic control rats compared with the normal control rats. The administration of aqueous extracts of leaves, stem-bark and root-bark of *Nauclea latifolia* and standard anti-diabetic drug, glibenclamide, significantly lowered (p <0.05) the fasting blood glucose levels compared with the diabetic control rats. Glibenclamide reduced the fasting blood glucose levels in the diabetic rats by 60.68% while the aqueous extracts of the stem-bark, leaves and the root-bark reduced the fasting blood glucose by 28.76%, 12.15% and 49.80% respectively. The root-bark



extract is more efficacious in lowering blood glucose level compared to the leaves and stem-bark extracts (Table 4).

## Discussion

The detection of the secondary metabolites, saponins, tannins, reducing sugar, phlobatannins, anthraquinones, flavonoids, steroids and alkaloids in the aqueous extracts of *Nauclea latifolia* in the study agrees with the findings of the research work of Yesufu et al, Maitera et al and Arise et al. They found out that *Nauclea latifolia* plant contains these phytochemicals.<sup>29, 30, 31</sup> The presence of flavonoids, saponins, alkaloids and tannins in plant extracts have been reported to be responsible for blood glucose lowering activity.<sup>5, 32, 33, 34, 35</sup>

The lack of mortality of the experimental rats at limit dose of 5000mg/kg body weight implies that the aqueous extracts of this plant have low toxicity when administered orally. This finding concurs with the reports of Assam et al who administered *Nauclea latifolia* ethanolic extract to mice at 8000mg/kg body weight and found no major signs of toxicity within the observation time.<sup>36</sup> The finding of the work done by Effiong and Akpan that *Nauclea latifolia* administered to rats produced no lethality even at doses as high as 8000mg/kg body weight is in consonance with our finding.<sup>37</sup>

Intra-peritoneal streptozotocin injection to the experimental rats causing significant rise in blood glucose level agrees with the reports of research works done by Moore et al, Maiti et al, Beppu et al and Rekha et al in which they independently administered intra-peritoneal streptozotocin injection to experimental rats and found elevated blood glucose levels.<sup>38, 39, 40, 14</sup>

The use of streptozotocin caused damage to the majority of the  $\beta$ - cells of islets of Langerhans leading to pancreatic dysfunction. This causes low insulin with subsequent increase in the blood glucose level.<sup>14</sup> Streptozotocin action on the pancreas causes  $\beta$  - cells destruction by necrosis.<sup>3</sup> The mechanism of action of streptozotocin in causing elevated blood glucose is thought to be through its entry into the  $\beta$ -cells via a glucose transporter (GLUT 2) where it leads to alkylation of DNA molecule and its eventual destruction. DNA destruction induces the activation of poly- ADP- ribosylation. Poly-ADP- ribosylation causes depletion of cellular nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) and adenosine triphosphate

(ATP). Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase leading to the formation of superoxide radicals, hydrogen peroxide radicals and hydroxyl radicals in vivo.<sup>3,41</sup> Streptozotocin also releases toxic amounts of nitric oxide into the body and this prevents aconitase enzyme activity and participates in DNA destruction. Because of the streptozotocin action, the  $\beta$ -cells of islets of Langerhans undergo damage by necrosis.<sup>41</sup>

Our result of significant decrease in the blood glucose levels of the diabetic rats administered aqueous extracts of *Nauclea latifolia* and glibenclamide is similar to earlier reports by Luka and Tijjani and Okonkwo and Okoye. They found out that plant extracts have blood glucose lowering effect on diabetic rats.<sup>42, 43</sup>

The likely mechanism by which the aqueous extracts caused the lowering of the blood glucose in the diabetic treated rats may be by induction of pancreatic insulin secretion from the remaining  $\beta$ -cells of islets of Langerhans or due to improved transport of blood glucose into the peripheral tissues.<sup>44</sup> It is also said that extracts most likely have similar mechanism of action like Glibenclamide in terms of blood glucose lowering ability by inhibiting the sulphonylurea receptor 1 (SUR 1) the regulatory subunit of the ATP- sensitive potassium channels (KATP) in the pancreatic beta cells. This inhibition results in cell membrane depolarization with subsequent opening of the voltage dependent calcium channel. This causes elevation of the intracellular calcium in the  $\beta$ -cells resulting in stimulation of insulin release and subsequent lowering of the blood glucose level.<sup>45</sup>

## Conclusion

This study showed that the phytochemicals, saponins, tannins, reducing sugar, phlobatanins, anthraquinone, flavonoids, steroids and alkaloids are present in the plant *Nauclea latifolia*. This plant has been seen to have blood glucose lowering ability with the root-bark extract being more effective. Furthermore, the aqueous extracts are tolerated and showed no adverse effects. This study validates the claim of the natives regarding the use of the root-bark extract in the management of type 2 diabetes mellitus.

## Recommendations

The blood glucose lowering ability of *Nauclea latifolia* extracts makes it a plant with a potential for diabetes mellitus management. Therefore, further investigation with an aim to identifying its active principles is essential as this will help in probable drug making out of it.

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