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Antidiabetic Effect of *Nauclea latifolia* Leaf Ethanolic Extract in Streptozotocin-induced Diabetic Rats

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ABSTRACT

The antidiabetic and possible toxicity of ethanolic extract of the leaf of *Nauclea* latifolia was studied in streptozotocin-induced diabetic rats. Doses of 100, 200 and 400mg/kg body weight were given orally to the STZ-induced diabetic rats daily for 45 days. All the doses showed significant hypoglycaemic effect. The effect is however not dose dependent. Indices of liver and kidney functions studied were not statistically affected by the extract administration. Ethanolic extract of the leaf of *N. latifolia* thus exhibited antidiabetic action in STZ-induced diabetic rats with minimal toxicity.

Keywords: Antidiabetic activity; Diabetes mellitus; *Nauclea latifolia;* STZ-induced diabetic rats

INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by hyperglycaemia caused by absolute or relative deficiency of insulin. Adult on set (Type 2) diabetes accounts for about 90 percent of cases. Some diabetic patients can be managed on diet alone, most require oral hypoglycaemic drugs and/or insulin. The use of insulin and/or oral hypoglycaemic drugs is not without short comings and side effects (1). These short comings and side effects led to the search for alternative remedies which may produce similar degree of efficacy. The World Health Organisation (WHO) encouraged research on hypoglycaemic agents of plant origin and this has greatly motivated researches in the area. In the last few decades many plants and plant products have been reported to posses' antidiabetic property (2–4).

Nauclea latifolia Sm. (Rubiaceae) commonly known as 'pin cushion tree' is reported to be used in the treatment of malaria (5–7), GIT disorders (8), sleeping sickness (9) and hypertension (6). Recently, we reported the antidiabetic property of the aqueous extract of the leaves of the plant in alloxan diabetic rats (10). In this study different doses of ethanolic extract of the leaves of the plant were orally administered to streptozotocin-induced diabetic rats for 45 days to study the extract antidiabetic property and possible toxicity.

MATERIALS AND METHODS

Plant Material

The leaves of *Nauclea latifolia* were colleted fresh from Ahmadu Bello University main campus in the month of August 2004. It was identified and authenticated at the herbarium unit of Biological Sciences Department, A.B.U. Zaria. It was identical with the voucher specimen (No. 1268) previously deposited at the herbarium. The leaves were dried under the shade and ground into powder.

Extract preparation

The ethanolic extract of *N. latifolia* leaf was prepared by soaking 200g of the powder in 95% ethanol in a glass jar for 2 days at room temperature. The extract was filtered and the process repeated three times. The extract was concentrated to dryness at low temperature under reduced pressure on a rotary evaporator. The percentage yield of the extract was 16.7g w/w.

Animals and induction of diabetes

White albino rats of Wistar strain weighing, 150–200g, obtained from the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, A.B.U. Zaria, were used for the study. They were fed *ad libitum* with pellet diet (Vital feeds, Jos, Nigeria) and water. They were also kept and maintained under laboratory conditions of temperature, humidity and light (24 ± 1, 65% and 12 h light/dark cycle) respectively. The study was design in accordance with the Guide for the Care and Use of Laboratory Animals, NHI Publication No. 86–23.

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) 50mg/kg body weight in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg body weight. Diabetes was confirmed in the STZ-treated rats by measuring fasting blood glucose concentration 48 hours after STZ injection. Rats with fasting blood glucose of more than 200mg/dl were considered diabetic and included in the study after a stabilization period of 7 days.

Experimental Design

The rats were divided into six groups of five rats each. Group I was normal untreated, group 2 STZ-induced diabetic control, groups 3, 4 and 5 were STZ-induced diabetic rats respectively administered 100, 200 and 400mg/kg body weight ethanolic leaf extract of *N. latifolia* for 45 days, and group 6 were STZ-induced diabetic rats given daily glibenclamide (1mg/kg body weight) for the same duration. Extract and glibenclamide were administered by tube feeding (BMI, feeding tube, size 8).

Normal and diabetic controls were administered equivalent volume of distilled water for the 45 days.

Blood glucose was measured weekly through out the experimental period. Twenty four hours after the last treatment the rats were sacrificed and blood collected. Serum harvested from the blood was used for the estimation of alanine amino transferase (ALT), aspartate amino transferase (AST), urea, creatinine, total cholesterol and triacylglycerols.

Analysis

Serum glucose concentration was estimated using glucose oxidase method based on the principle of Trinder, (11). Alanine (ALT) and Aspartate (AST) transaminases were assayed by the method of Reitman and Frankel (12). The diacetylmonoxime and Jaffe's reactions as described by *Kaplan et al*, (13) were used in assaying for urea and creatinine respectively. The methods of Allain et al, (14) and Tietz (15) were respectively used to assay for total cholesterol and triacylglycerols.

Statistical Analysis

The results are presented as Mean + SEM of 5 rats in each group. All the grouped data were statistically analyzed and students t-test used to test differences between untreated and treated groups.

RESULTS

Diabetes induction caused significant ($P<0.001$) hyperglycaemia (Table 1) and insignificant changes in

Table 1: Effect of oral administration of different doses of ethanolic leaf extract of *N. latifolia* on fasting blood glucose (Mean ± SEM) concentration (mg/dl) in streptozotocin-induced diabetic rats (n=5) after 45 days.

Groups	Initial blood glucose (mg/dl)	Final blood glucose (mg/dl)	% change
Normal control	73.16±2.24	75.52±2.10	3.23
Diabetic control	289.17±24.91	310.83±22.18[a]	7.33
Diabetic+100mg/kg	295.63±24.12	137.83±10.23[b]	−53.38
Diabetic+200mg/kg	264.67±16.20	103.84±17.91[b]	−60.77
Diabetic+400mg/kg	262.04±7.50	123.73±11.01[b]	−52.78
Diabetic+1mg/kg Glibenclamide	267.50±14.74	82.50±6.43[b]	−69.16

Diabetic control is compared with normal control.
Experimental groups are compared with diabetic control.
Initial - Values before administration of extract started
Final - Values at the end of experimental period (45 days).
[a]$P<0.001$ Compared with normal control
[b]$P<0.001$ Compared with diabetic control

Table 2: Serum biochemical changes following oral administration of different doses of ethanolic leaf extract of *N. latifolia* in streptozotocin-diabetic rats after 45–days.

Groups	ALT (iu/L)	AST (iu/L)	Urea (mmol/L)	Creatinine (µmol/L)
Normal control	36.13±1.43	173.00±2.46	5.13±0.10	61.75±10.01
Diabetic control	40.63±4.20	184.00±5.65	7.00±1.26	79.25±10.19
Diabetic + 100mg/kg	37.88±3.58	172.50±6.12	7.17±0.60	69.00±0.59
Diabetic + 200mg/kg	41.00±6.89	171.50±7.12	5.88±0.76	63.33±5.94
Diabetic + 400mg/kg	37.50±1.09	169.00±5.41	5.00±0.27	61.00±12.00
Diabetic + 1mg/kg Glibenclamide	38.13±2.46	165.00±8.00	6.17±0.48	63.00±4.10

Values are presented as mean + S.E.M for 5 rats in each group.
Diabetic control is compared with normal control.
Experimental groups are compared with diabetic controls.

Table 3: Effect of oral administration of different doses of leaf ethanolic extract of *N. latifolia* on serum cholesterol and triacylglycerols in streptozotocin-diabetic rats for 45 days.

Groups	Cholesterol (mg/dl)	Triacylglycerols (mg/dl)
Normal control	129.87±2.05	133.33±13.61
Diabetic control	159.59±12.42[a]	162.50±18.48[a]
Diabetic+100mg/kg	150.69±9.55	155.55±19.64
Diabetic+200mg/kg	133.57±6.46	150.00±6.81
Diabetic+400mg/kg	128.09±8.19[b]	137.50±7.98[b]
Diabetic+1mg/kg Glibenclamide	138.36±4.68[b]	141.79±11.79[b]

Results are presented as Mean + SEM of 5 rats in each group.
Diabetic control is compared with normal control.
Experimental groups are compared with diabetic control.
[a]$p<0.05$ compared with normal control
[b]$p<0.05$ compared with diabetes control

serum levels of ALT, AST, urea and creatinine (Table 2). Oral administration of the extract and glibenclamide for 45 days significantly ($P<0.001$) lowered the hyperglycaemia of the experimental groups. The fasting blood glucose of the group treated with 200mg/kg body weight extract lowered the glucose level from 264.67mg/dl to 103.84mg/dl and glibenclamide from 267.50mg/al to 82.50mg/dl representing 60.77% and 69.16% reductions respectively. The effect on the fasting blood glucose is however not dose dependent (Table 1). Serum levels of ALT, AST, urea and creatinine were however, not also affected by the extract treatment (Table 2).

Diabetes induction significantly raised the levels of total cholesterol and triacylglycerols. Administration of the extract at 400mg/kg body weight and glibenclamide brought down the concentrations to near normal values (Table 3).

DISCUSSION

Different parts of *Nauclea latifolia* are prescribed by traditional healers for the treatment of diabetes mellitus. In a previous study we have evaluated this claim and reported the hypoglycaemic activity of the aqueous extract of the leaves of the plant in alloxan-induced diabetic rats (10). Daily oral administration of different doses of ethanolic extract of the leaves for 45 days in this study supports the antidiabetic property of the plant. All the doses administered significantly reduced the fasting blood glucose of the STZ-induced diabetic rats to almost normal values. The effect however is not dose dependent. Diabetic rats given daily oral dose (1mg/kg body weight) of glibenclamide for the same duration also reduced their fasting hyperglycaemia significantly ($P<0.001$).

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are diagnostic enzymes used as sensitive indicators of liver disease, although AST is known to be distributed widely in other tissues like the kidney (13). Oral administration of the different doses of ethanolic extract of *N. latifolia* for the 45 days did not result in a significant serum increase in the concentrations of these enzymes, suggesting that the extract might not be hepatotoxic. Serum urea and creatinine are also used as biochemical indices of renal functions in clinical diagnosis (13). The insignificant changes in the concentrations of these parameters also indicate that the extract might not be nephrotoxic.

During diabetes, the levels of serum and tissue lipids (cholesterol, free fatty acids and phospholipids) are usually elevated (16). The marked hyperlipaemia that

characterizes the diabetic state is a consequence of the uninhibited actions of lypolytic hormones on the fat depots (17). The hypolipidaemic effect of the ethanolic extract of *N. latifolia* can be explained as a consequence of reduction in blood glucose.

A bioassay guided fractionation is being carried out to isolate the hypoglycaemic principle of the extract. Also under study is the extract mechanism of action. However, results of this study further provide a pharmacological basis for the folkloric medicinal application of the plant in the treatment of diabetes mellitus.

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