Original Article

ANTIDIABETIC ACTIVITY AND TOXICOLOGICAL EVALUATION OF THE METHANOL-DICHLOROMETHANE ROOT BARK EXTRACT OF *NAUCLEA DIDERRICHII* (DE WILD) MERR

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ABSTRACT

Objective: In southeastern Nigeria, *Nauclea diderrichii* (De Wild) Merr is used in the treatment of a wide range of ailments including diabetes mellitus (DM). This study evaluates the antidiabetic activity and toxicological profile of the methanol-dichloromethane root bark extract of *N. diderrichii* in normoglycemic and alloxan-induced diabetic models.

Methods: Dried root barks of *N. diderrichii* were extracted using methanol and dichloromethane (1:1) to obtain *N. diderrichii* extract (NDE). The acute and sub-chronic toxicity tests were performed using standard procedures. The effect on alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), packed cell volume (PCV), hemoglobin concentration (HB) and total white blood cell (TWBC) count was determined in the rats after treatment. In order to elucidate its antidiabetic mode of action, an oral glucose tolerance test (OGTT) was performed using glucose (2 g/kg) as substrate and alloxan (100 mg/kg; i. v.) induced diabetic model. Glibenclamide (GLI 0.2 mg/kg) was used as the reference standard drug.

Results: The results indicated that the LD_{50} of the extract is>5000 mg/kg. ALT, AST, ALP PCV, HB and TWBC were insignificantly ($p > 0.05$) different compared with the control. No significant changes were observed in the organ weights compared with the control. In the acute and prolonged antidiabetic study, NDE (100, 200 and 400 mg/kg) significantly reduced the blood glucose level (BGL) by 14.66, 18.9, 25.80% and 75.11, 80.24, 83.74% respectively. In comparison, GLI, when administered, reduced BGL by 38.18 and 92.86% respectively.

Conclusion: *N. diderrichii* possesses antidiabetic activity with good toxicological profile.

Keywords: Antidiabetic, Toxicity study, Biochemical and hematological parameters, *Nauclea diderrichii*

INTRODUCTION

Diabetes mellitus (DM) is one of the major health concerns worldwide. It is characterized by chronic hyperglycemia and associated abnormalities in carbohydrate, fat and protein metabolism, resulting from a deficiency in insulin secretion, action or both [1]. Currently, there are approximately 150 million diabetics worldwide and this number has been predicted to double by the year 2025. Its prevalence, high cost of antidiabetic drugs and the associated side effects of standard agents have necessitated the search for newer molecules from natural sources [2].

The natural environment is endowed with plant materials and fruits which have been found to be of great medicinal and nutritional importance. There are many experimental pieces of evidence indicating the use of plants for medicinal purposes, hence, the plant kingdom has become a target for the search of biologically active lead compounds for the complementary/alternative management of diabetes mellitus [3]. However, plant extracts can elicit different biological effects in the body, some of which may be harmful [4]. Thus, the toxicological evaluation of plant extracts is very necessary as this often helps to assess their safety in humans.

Few of the plants used in traditional medicine for the treatment of diabetes have received little scientific attention and *Nauclea diderrichii* (De Wild) Merr is one of such plants. *Nauclea diderrichii* is an ever green tree belonging to the Rubiaceae family. It is endemic in West and Central Africa and has been extensively studied for its antimalarial [5], antiplasmodial [6], antileishmanial [7] and antitrypanosomal [8] activities. In Nigerian folklore medicine, *N. diderrichii* is used in the management of a wide range of ailments ranging from endocrine disorders, infections, wound healing, antimalarial, anemia, stomach ache, indigestion, and others [9]. The present study was designed to evaluate the antidiabetic activity of *N.* *diderrichii* and its toxicity profile (acute and sub-chronic) in order to validate its folkloric use in the management of diabetes and to ascertain its level of safety.

MATERIALS AND METHODS

Plant material and extraction procedure

Fresh root barks of *N. diderrichii* were collected from Nsukka (6 ° 52' 0" North, 7 ° 23' 0" East), Enugu State, Nigeria in the month of July 2015. The plant sample was identified and authenticated by Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nigeria, where the voucher specimen has been deposited under number Inter CEDD-0715. The fresh root barks were collected, peeled, cut, dried and pulverized into coarse powder. The powdered sample (2 kg) was extracted with a mixture of dichloromethane and methanol in the ratio of 1:1 by cold maceration in closed vessel with intermittent shaking for 48 h. When maceration was completed, the macerated plant material was filtered using Whatman No 1 filter paper and the filtrate concentrated to dryness under reduced pressure and the crude *N. diderrichii* extract (NDE) was obtained.

Animals

Adult albino rats (100-160 g) and mice (15-30 g) of both sexes were obtained from the Animal House facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria. They were maintained under standard laboratory conditions (22±2 °C, 12:12 h dark/light cycle, frequent air change) and had free access to water and standard animal feed *ad libitum*. All procedures were in compliance with the NIH Guidelines for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985), and in accordance with the University Ethical Committee on the use of laboratory animals.

Toxicological evaluation

Acute toxicity test (LD$_{50}$)

The acute toxicity test (LD$_{50}$) of the extract was carried out using Lorke's procedure [10]. Adult albino mice (15-30 g) of both sexes were used. The study was carried out in two stages. In the first phase, 9 mice were randomly divided into three groups (n=3), and were orally administered with the extract at 10, 100, and 1000 mg/kg dose levels, respectively. The animals were allowed free access to food and water; the results were recorded after 24 h which was used as a guide for the second phase of the experiment. In the second phase, three (3) higher doses (1600, 2900 and 5000 mg/kg) of the extract were administered to a fresh batch of animals at one animal per dose. The animals were observed for clinical signs and symptoms of toxicity like behavioural changes and mortality within 24 h. Lethal dose (LD$_{50}$) was then calculated as the geometric mean of the lowest lethal dose and highest non-lethal dose.

Sub-chronic toxicity test

Sub-chronic toxicity test was carried out as per internationally accepted protocol drawn under OECD 407 guidelines. Briefly, rats were randomly divided into four (4) groups of five animals each. The group 1 (control) received daily oral administration of the vehicle (10 ml/kg of distilled water) whereas groups 2, 3, and 4 received 100, 500 and 1000 mg/kg of NDE daily for 28 d, respectively. All animals were observed twice daily for apparent signs of toxicity or behavioural alterations during the experimental period. At the end of the experiment (on the 29th day), blood samples were collected from overnight fasted rats and used for various biochemical estimations. The biochemical parameters: alanine aminotransferase (ALT, EC, and 2.6.1.2), aspartate aminotransferase (AST, EC, and 2.6.1.1), alkaline phosphatase (ALP, EC, 3.1.3.1) and serum total protein (TP) were estimated using standard procedures [11-13]. The hematological parameters: packed cell volume (PCV), hemoglobin concentration (HB) and total white blood cell (TWBC) count were estimated using standard methods [14, 15]. Subsequently, the animals were sacrificed and essential organs (liver, heart, kidney and lungs) were removed and physically examined.

Antidiabetic evaluation

Oral glucose tolerance test (OGTT)

In this method, twenty-five (25) rats were divided into five (5) groups (n=5) and treated as follows: group 1 (control group)

received 10 ml/kg of distilled water; group 2 received a standard, 2.5 mg/kg glibenclamide (GLI) while groups 3, 4 and 5 received 100 mg/kg, 200 mg/kg and 400 mg/kg of NDE, respectively. The baseline blood glucose levels (BGL) were determined prior to oral administration of glucose (2 g/kg) at 0 min and after 30, 60, and 120 min post treatment.

Antidiabetic activity in alloxan-induced diabetic rats

In this method, thirty (30) rats were divided into six groups (n=5). The dosage of extract/standard drug/animal groupings were as described above. The BGL were determined before the induction of diabetes. Diabetes was induced using alloxan monohydrate (Sigma-Aldrich Co., St. Louis, MO, USA). The rats were fasted for 12 h with free access to water prior to the administration of freshly prepared alloxan monohydrate (100 mg/kg; i. v.) dissolved in ice-cold normal saline. After 5 d of stabilization, the fasting BGL was determined by using a One Touch Ultra® glucometer (Lifescan; Johnson and Johnson, Milpitas, CA, USA). Animals having fasting BGL ≥ 200 mg/dl (11.1 mmol/l) were considered diabetic and used for the study. The BGL were monitored at 0 (pre-treatment) and at 1, 2, 3, 4 and 5 h post treatment. Treatment was continued daily for 7 consecutive days per oral. Fasting BGL were monitored on the 3rd and 7th d. The percentage blood glucose reductions (PBGR) were calculated relative to pretreatment values.

Statistical analysis

Results are expressed as mean±SEM. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to compare differences between groups using SPSS statistical software and $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity study (LD$_{50}$)

The NDE gave an estimated LD$_{50}$>5000 mg/kg. Also, no visible sign of toxicity or death was observed in the treated groups.

Effect on biochemical parameters

There were no significant ($p>0.05$) differences in the values of serum biochemical parameters (ALT, AST, ALP and TP) assayed between the treated groups as compared with the control group (table 1).

Table 1: Effect of NDE on biochemical parameters

Treatment	Dose(mg/kg)	biochemical parameters			
		ALT(U/I)	AST(U/I)	ALP(U/I)	TP(mg/dl)
NC	-	20.59±1.30	46.04±2.05	357.37±24.90	6.88±0.18
NDE	100	20.53±2.17	44.59±2.21	409.53±33.02	6.98±0.12
NDE	500	22.96±0.77	50.45±1.99	316.33±28.39	6.85±0.21
NDE	1000	23.02±0.97	49.61±0.97	292.16±6.92	7.05±0.16

Each value represents the mean±SEM, n=5, *$p<0.05$ compared with control (One-way ANOVA; Dunett's post hoc). NC-normal control, NDE-*N. diderrichii* extract.

Effect on hematological parameters

There were no significant ($p>0.05$) differences in the values of the serum hematological parameters (PCV, HB, and TWBC) of the treated groups when compared with the control group. Although, the extract exhibited dose dependent increase in the HB values (table 2).

Effect on organ weight

The effect of the extract on organ weight is shown in table 3. The organ weights of the treated groups were not significantly ($p>0.05$) different from the control group.

Effect in glucose loaded rats

Expectedly, following oral administration of glucose meal, there was significant (p<0.05) increase observed in the BGL of the control group at 30th min post glucose challenge. The oral administration of

the extract (100, 200, 400 mg/kg) and GLI (2.5 mg/kg) suppressed the rise in BGL respectively at the 30th min time compared with the control. The effect was sustained up to 120 min. At this time, relative to the pretreatment values, no significant ($p>0.05$) difference was observed in the BGL of NDE treated rats compared with pre-treatment values (table 4).

Effect in alloxan-induced diabetic rats

The result showed that the extract considerably reduced the fasting BGL to varying degrees in diabetic rats. However, the PBGR was dose related as 400 mg/kg dose exhibited the highest PBGR of 21.46 and 25.80% at the 4th and 5th h respectively, while the 100 mg/kg was the least with 8.33 and 14.66% at the 4th and 5th h of monitoring respectively. The standard agent, GLI (2.5 mg/kg) exhibited the highest PBGR of 28.34% and 38.18% at the 4th and 5th h respectively after administration (table 5).

Table 2: Effect of NDE on hematological parameters

Treatment	Dose (mg/kg) hematological parameters			
		PCV (%)	HB (g/dl)	TWBC (x103/mm3)
NC	-	39.40±1.23	14.40±0.45	16.37±1.25
NDE	100	39.20±0.93	14.53±0.46	14.21±1.16
NDE	500	38.00±1.11	14.56±0.28	14.31±1.57
NDE	1000	39.40±0.29	14.57±0.56	14.73±1.44

Each value represents the mean±SEM, n=5, *$p < 0.05$ compared with control (One-way ANOVA; Dunett's post hoc). NC-normal control, NDE-*N. diderrichii* extract.

Table 3: Effect of NDE on organ weight

Treatment	Dose (mg/kg) organ weight (g)				
		Liver	Heart	Kidney	Lungs
NC	-	3.76±0.29	0.38±0.01	0.39±0.02	1.02±0.13
NDE	100	4.11±0.24	0.42±0.03	0.41±0.03	1.02±0.07
NDE	500	3.52±3.52	0.35±0.35	0.37±0.34	0.97±0.12
NDE	1000	3.99±0.15	0.40±0.02	0.35±0.14	0.98±0.04

Each value represents the mean±SEM, n=5, *$p < 0.05$ compared with control (One-way ANOVA; Dunett's post hoc). NC-normal control, NDE-*N. diderrichii* extract.

Table 4: Effect of NDE in glucose loaded rats

Treatment	Dose (mg/kg) blood glucose (mg/dl)				
		Pre-treatment	30 min	60 min	120 min
Control	-	54.40±1.63	161.60±13.80#	148.20±5.59#	128.2±6.09#
GLI	2.5	43.40±2.99	96.60±7.81*#	48.0±1.81*	47.0±2.21*
NDE	100	55.0±5.54	85.40±10.81*#	65.60±5.08*	57.0±8.33*
NDE	200	49.20±2.96	60.40±6.55*	53.80±5.65*	49.2±3.17*
NDE	400	52.30±3.91	72.67±3.91*	53.0±3.19 *	49.83±3.51*

Each value represents the mean±SEM, n=5, *,#$p < 0.05$ compared with control and pretreatment values respectively (One-way ANOVA; Dunett's post hoc). GLI-glibenclamide, NDE-*N. diderrichii* extract.

Table 5: Effect of NDE in alloxan-induced diabetic rats

Treatment	Dose (mg/kg) blood glucose (mg/dl)					
		Pre-treatment	1 h	2 h	3 h	4 h	5 h
DC	-	482.4±72.07	446.2±57.69	440.6±52.42	436.33±23.25	425.48±10.32	402.32±27.65
GLI	2.5	462.25±81.93	456.51±72.21 (1.22)	451.00±77.29 (2.43)	373.75±102.44*# (19.16)	331.25±104.58*# (28.34)	285.75±108.4*# (38.18)
NDE	100	386.5±58.97	420.50±62.10 (-8.79)	371.66±55.93* (3.84)	367.67±62.17* (4.87)	354.31±59.19* (8.33)	329.83±71.52*# (14.66)
NDEN	200	370.5±70.68	349.25±82.1* (5.74)	342.25±96.52* (7.63)	309.74±53.76 **#(16.4)	303.10±85.69*# (18.2)	300.46±89.77*# (18.9)
DE	400	415.75±106.42	409.25±111.8 (1.56)	369.75±128.9* (11.06)	328.26±133.55*# (21.04)	326.5±136.92*# (21.46)	308.5±136.05*# (25.80)

Each value represents the mean±SEM, n=5, *,# $p < 0.05$ compared with diabetic control and pretreatment values respectively. DC-diabetic control, GLI-glibenclamide, NDE-*N. diderrichii extract.* Values in parenthesis represent reduction (%) in blood glucose level calculated relative to pre-treatment values.

Effect of prolonged administration of NDE in alloxan-induced diabetic rats

Prolonged administration of different doses of the extract to diabetic rats for 7 d reduced the fasting BGL. The 200 mg/kg and 400 mg/kg exhibited the highest percentage blood glucose reduction of 70.94% and 83.74% at days 3 and 7 respectively compared to day 0 values and the diabetic control respectively. The reduction was slightly comparable to that of the reference drug GLI (53.98 and 92.86%) (table 6).

Table 6: Effect of prolonged administration of NDE in alloxan-induced diabetic rats

Treatment	Dose (mg/dl) blood glucose (mg/dl)			
		Day 0	Day 3	Day 7
DC	-	482.4±72.07	254.75±28.11	261.02±22.27
GLI	2.5	462.25±81.93	212.75±93.12#([53]·[98])	33.00±18.00*#([92]·[86])
NDE	100	386.5±58.97	191.3±13.57*#([46]·[53])	88.75±19.12*#([75]·[11])
NDE	200	370.5±70.68 415.8±106.42	107.67±8.57*#([70]·[94])	73.33±11.7*#([80]·[21])
NDE	400		133.75±47.2*#([67]·[83])	67.00±22.8*#([83]·[74])

Each value represents the mean±SEM, n=5, *,# $p < 0.05$ compared with diabetic control and day 0 values respectively (One-way ANOVA; Dunett's post hoc). DC-diabetic control, GLI-glibenclamide, NDE-*N. diderrichii* extract. Values in parenthesis represent reduction (%) in blood glucose level calculated relative to day 0 values.

DISCUSSION

The role of plant extracts in diabetes management has been tried by many researchers. Diabetes mellitus (DM) was induced using intravenous alloxan, a known diabetogen. Alloxan causes an increase in blood glucose levels or hyperglycemia and glucose intolerance or syndromes similar to either type 1 or types 2 DM [16]. Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to the heart, kidney, eyes, nerves, liver, small and large vessels and gastro intestinal system [17]. Thus, the increased blood glucose concentration is as a result of intrinsic oxidative stress in diabetic condition, a result that is consistent with several studies in rats [18, 19].

In the present study, oral administration of NDE exhibited significant hypoglycemic activity in normoglycemic and alloxan-induced diabetic rats. The reduction in blood glucose levels is indicative that the extract has possible hypoglycemic activity. Thus, it is suggestive of the confirmation of its glucose lowering effect. This effect was comparable to that of glibenclamide, the standard drug used in the study. Glibenclamide belongs to the sulfonylurea class of oral drugs that reduce blood glucose levels by stimulating insulin secretion. In the presence of viable pancreatic β cells, sulfonylureas stimulate the release of endogenous insulin from the pancreatic β-cells, thereby reducing blood glucose levels [20]. Plant extracts are well known to favour regeneration of islet beta cells as their primary mechanism of action [21, 22]. Hence, the mechanism through which NDE mediates this hypoglycemic effect could be by stimulating insulin secretion probably, through enhancing the sensitivity of beta cells to glucose, resulting in increased insulin release. Furthermore, bioactive compounds in medicinal plants play an important role in diabetes management [23]. The previous study has shown the presence of alkaloid, flavonoids, saponins and other chemical compounds in the plant extract [24]. Several of these constituents are well known for their antidiabetic activity [25, 26]. This suggests that the antidiabetic activity of the extract may be partly due to the action of these phytoconstituents which could act synergistically or independently in reducing the blood glucose level.

In the normoglycemic rats, the lack of significant alterations observed in the levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), which are good indicators of liver function, suggests that the extract has no harmful effect on the liver. The liver plays an essential role in various metabolic processes and is, therefore, severely affected during exposure to the toxic effects of exogenous compounds [27]. AST, ALT and ALP are important serum biomarker enzymes usually measured to evaluate liver damage or the toxic effect of drugs on liver [28]. Higher values of serum ALT and AST have been implicated in liver necrosis, hepatitis, toxic liver disease and acute myocardial infarction [29]. Similarly, no significant differences were observed in the hematological parameters (PCV, HB, and TWBC) between the treated groups and the control. Analysis of hematological parameters in animal studies is relevant to evaluate the risk of alterations of the hematopoietic system in toxicity studies, for necessary application to humans [30]. The lack of significant changes observed could be attributed to the extract not being toxic to circulating red cells, nor interfered with their production and that of platelets [31].

The relative weight of the organs (heart, lungs, kidney and liver) in the treated groups showed no significant (p<0.05) difference as compared with the control (table 4). These organs are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity [32]. Hence, the non-significant differences observed indicate that, at the oral dose administered, NDE can be considered as non-toxic.

CONCLUSION

In conclusion, the result of the present study has shown that the root bark extract of *N. diderrichii* possesses potent antidiabetic activity with relatively no or minimal toxicity. Further research on the isolation and characterization of the active phytoconstituents is ongoing.

AUTHORS CONTRIBUTION

All authors contributed equally in designing, performing the experiment and writing up of the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest regarding the manuscript

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