



Phytochemical Analysis, Antidiabetic and Toxicity Studies of the Methanolic Leaf Extract of *Detarium Microcarpum* Guill and Perr in Wistar Albino Rats

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ABSTRACT

Diabetes mellitus is a metabolic disorder that impairs glucose homeostasis and can lead to severe complications if poorly managed. Some of these complications may manifest as retinopathy, angiopathy, nephropathy and neuropathy. The methanolic leaf extract of *Detarium microcarpum* was investigated for possible blood glucose lowering activity in alloxan-induced diabetic rats. Diabetes was induced by intra-peritoneal injection of 150 mg/kg of alloxan monohydrate. Body weight and blood glucose levels of control and diabetic rats were monitored. The effect of the extract on internal organs (heart, lung, liver and kidney) of the rat was also determined. The acute toxicity and qualitative phytochemical analysis for the identification of various plants constituents were also carried out using standard methods. The preliminary phytochemical screening of the extract revealed the presence of terpenoids, flavonoids, saponins, tannins and steroids. The methanolic leaf extract of *Detarium microcarpum* at 500, 750, 1000 mg/kg body weight exhibited a dose dependent significant reduction of blood glucose level. A significant increase ($p < 0.05$) in the body weight of diabetic rats treated with *Detarium microcarpum* extract was also observed when compared with the standard drug (Glibenclimide). The result of the acute toxicity studies revealed that none of the organs showed any histopathological presentations within the period of observation. The methanolic leaf extract of *Detarium microcarpum* showed good antidiabetic activity with minimal toxicity and is therefore its ethnomedicinal use in the management of diabetes may be justified.

Keywords: *Detarium microcarpum*; Alloxan monohydrate; Phytomedicines; Acute toxicity; Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a chronic life-long disease, which has been known to mankind for over 2000 years. It requires careful monitoring and control. Currently, diabetes ranks fourth worldwide among the Non-communicable diseases (cardiovascular diseases, cancers, chronic respiratory diseases and diabetes) with prevalence rates of 30%, 13%, 7% and 2%, respectively [1]. It has been projected that by 2025, the number of people in the developing world with diabetes will increase by more than 2.5 fold; from 84 million in 1995 to 228 million in 2025 [2] and that 70% of deaths due to type 2 diabetes will occur in developing countries. The International Diabetes Federation Atlas [3] estimates that 10.8 million people have type 2 diabetes in sub-Saharan Africa in 2006 and this would rise to 18.7 million by 2025, an increase of 80%, as such exceeding the predicted worldwide increase of 55% [4].

National surveys in most parts of Africa indicate that diabetes cases are on the rise due to rapid urbanization as well as fast changing diets which are displacing the traditional ones in favour of Western diets [5]. Nigeria is among the top five countries that have the highest number of people affected by type 2-diabetes in sub-Saharan Africa. Nigeria has about 1.2 million people; South Africa, 841,000; the Democratic Republic of Congo, 552,000; Ethiopia, 550,000 and Tanzania, 380,000 living with diabetes.

Usman and Osuiji [6] had reported that “The use of plants as medicine is an ancient practice common to all societies, especially African society”. *Detarium microcarpum* Guill and Perr has been used for decades in various societies across Africa for the management of various conditions. The stem and root barks have been reported to be used by the Kilba tribe of North East Nigeria as a remedy for infant ailment known as ‘Gedigedi’ or ‘Tando’ (Hausa), stomach aches, wound etc. The seeds and leaves are eaten as a condiment and vegetable [7]. In Burkina Faso, the fruit pulp is used for treating skin infections. Other reported medicinal applications of *D. microcarpum* include the use of its bark, leaves and roots as an astringent and diuretic [8]. Different parts of this plant have been reported to possess anti-rheumatic activity [9] and antiplasmodial activities [10]. Evaluation of the root extract of *D. microcarpum* revealed that it possesses significant antihyperglycemic activity [11]. The phytochemical screening of the various crude extracts of *D. microcarpum* has revealed the abundance of numerous bioactive constituents in the plant. Some of these compound especially flavonoids, tannins, cardiac glycosides and terpenoids have been reported to have antimicrobial and curative properties [12-14]. Saponins, phytates and cyanides are reportedly present as anti-nutrients. Glycosides and alkaloids have also been isolated from the leaves of *D. microcarpum* [15,16]. The fruit is edible and rich in vitamin C [17]. Nutritionally, the seed of *D. microcarpum* which is used as a traditional soup thickener contains carbohydrates (70.38%), crude lipid (7.41%), proteins (12.19%), crude fiber (2.63%), ash (3.51%), and moisture (4.3%) [18].

The antimicrobial principles in the seed coat of *D. microcarpum* might be associated with the steroidal saponins and flavonoids, which may demonstrate synergistic action [19]. Kouyate [20] documented the antimicrobial activity of the ethanolic extract of the bark against *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Listeria monocytogenes*. The extract also showed moderate antitumor activity against breast cancer cells. The methanol extract of *D. microcarpum* has been shown to contain flavanes which show strong inhibitory effects on HIV⁻¹ or HIV⁻² infection and the bark extract exerts significant molluscicidal activity against *Lymnaea natalensis* [20]. Also, the antifungal, acetylcholinesterase inhibitory activities and muco-adhesive properties of various extracts of the plant have been reported [21,22].

This work seeks to investigate the acute toxicity and hypoglycaemic properties of the methanolic leaf extract of *Detarium microcarpum*, and to carry out a preliminary phytochemical screening of the leaf extract.

MATERIALS AND METHODS

Alloxan monohydrate was obtained from Sigma Chemical Co. (St. Louis, M.O., USA) and Glibenclamide tablets were obtained from the Nigerian-German Chemicals PLC, (Ota, Ogun State, Nigeria.). All other reagents used were of analytical grade. All solutions were prepared in distilled water.

Sample Collection

The plant leaves of *D. Microcarpum Guill and Perr* were collected in July, 2015 from Zaria, Nigeria and authenticated by Mallam Namadi Sunusi in the Department of Biological Sciences, Ahmadu Bello University, Zaria; Nigeria. A voucher specimen with No. 901451 was deposited in the herbarium of the Department of Biological Sciences, Ahmadu Bello University; Zaria.

Preparation and Extraction

Leaves of *Detarium microcarpum* plant were air dried for seven days at room temperature. One thousand grams (1000 g) of the pulverized part were extracted exhaustively with 70% methanol at room temperature for 48 hrs. The extract was evaporated to dryness using a rotary evaporator at 40°C and it was stored in a desiccator prior to use [23].

Phytochemical Screening

The crude methanolic extract was evaluated for the presence of bioactive phytochemical constituents using standard procedures [24-26].

Animals

Wistar albino rats (100-200 g) of either sex were obtained from the Animal House of Department of Pharmacology, University of Jos, Jos, Nigeria, and were used for the experiments. Animals were maintained in well ventilated rooms. They were fed with standard feeds and water provided ad libitum. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals. Ethical permission was also sought and obtained from the Institutional Animal Care and Use Committee (IACUC), Department of Pharmacology, University of Jos, Nigeria.

Acute Toxicity Study (LD₅₀)

The animals (n=5 per dose) were fasted 12 h prior to the experiment, up and down procedures were adopted for toxicity studies. Animals were administered with single dose of extract at a dose of 2000 mg/kg and observed for their mortality during 2 and 7 days study period (short term) toxicity and the dose increased up to 5000 mg/kg and the animals observed up to 7 days for their behavioural and neurological changes [27].

Induction of Diabetes

Diabetes was induced by intraperitoneal injection of 120 mg/kg of alloxan monohydrate [28]. The alloxan-induced diabetic rats were kept with free access to food and water. Animals, in which the development of hyperglycaemia was confirmed 72 hours after the administration of alloxan injection (blood glucose level range of 450-500 mg/dL) were used for experiments.

Treatment Protocol

The rats were randomly divided into seven group (n=6/group). Group I received 2 ml/kg of normal saline while Group II, III and IV received 500, 750 and 1000 mg/kg respectively of the methanol extract. Group V received distilled water only while Group VI received 5 mg/kg Glibenclimide. Group VII consisted of normal rats that received 1000 mg/kg of the extract. Blood sample were collected from the tail vein after overnight fast at the interval of 0, 2, 6, 8, 24, 168, 335 and 504 hours of treatment [29].

Group I: (Normal control) - Normoglycemic rats that were orally treated with distilled water.

Group II: (500 mg/kg) – Hyperglycemic rats that were orally gavaged with 500 mg/kg/day of extract.

Group III: (750 mg/kg) - Hyperglycemic rats that were orally gavaged with 750 mg/kg/day of extract.

Group IV: (1000 mg/kg) - Hyperglycemic rats that were orally gavaged with 1000 mg/kg/day extract.

Group V: (Diabetic control) - Hyperglycemic rats that were orally gavaged with distilled water.

Group VI: (Glibenclamide) - Hyperglycemic rats treated with Glibenclimide 5 mg/kg as standard drug.

Group VII: (Normal + 1000 mg/kg) - Normoglycemic rats treated with 1000 mg/kg/day of extracts.

Histopathology Examination

Tissues from the animals (liver, kidney, spleen, heart and lungs) were immersed in 10% buffered formal-saline. These were left for 24 hours for fixation of the organs after which cross-sections of the organs were cut at 3 mm thickness and placed in a processor overnight. In the processor, the tissues were first placed in 70% alcohol for 2 hours, followed by 90% alcohol for another 2 hours, xylol for 4 hours, and finally, in wax for 5 hours. The tissues were removed, embedded in molten fibro wax and allowed to solidify under a running tap. The tissues were brought out and mounted on wooden blocks and then chilled in ice. Sections of the tissue were cut at a thickness between 3 and 5 mm using the rotary microtome and then allowed to float in 20% alcohol, followed by water at 58°C (an incubator), placed on albumized glass slides and dried on a hot plate at 60°C. The slides so prepared were initially placed in xylol and washed with decreasing concentration of absolute alcohol, 90% alcohol, 80% alcohol and finally, 70% alcohol. They were washed in water stained with Cole's haematoxylin, washed again with water, followed by 1% hydrochloric acid, running tap water and rinsed in saturated lithium carbonate. These glass slides were transferred to 1% aqueous solution of eosin for 2 minutes, and washed in a running tap. They were cleaned, mounted on Depex after treatment in absolute alcohol. The slides were finally allowed to dry on the bench at room temperature and then viewed under the microscope [30].

Statistical Analysis

Numerical data obtained from the study were expressed as the mean value \pm standard deviation. Statistical analysis were performed using the analysis of variance (ANOVA) followed by Tukeys post hoc test with the aid of IBM Statistical Package for Social Scientist (SPSS 20) software. Differences were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Table 1: Results of phytochemical screening of *D. microcarpum* methanolic leaf extract

Constituents	Methanol extract
Saponins	+
Tannins	+
Flavonoids	+
Carbohydrate	+
Steroids	+
Terpenoids	+++

Key: + = Present, - = Absent

Table 2: Effect of methanolic extract of *D. microcarpum* leaves on the blood glucose level of alloxan-induced diabetic rats (mg/dL)

Time (hour)	Group I (Normal control)	Group II (500 mg/kg)	Group III (750 mg/kg)	Group IV (1000 mg/kg)	Group V (Diabetic control)	Group VI (Glibenclamide)	Group VII (Normal + 1000 mg/kg)
0	104.50 ± 14.98 ^b	503.25 ± 35.14 ^{b,c,e,a}	393.00 ± 27.36 ^{c,e,a}	125.25 ± 8.62 ^{b,c,e,a}	379.00 ± 177.07 ^a	595.00 ± 8.72	125.25 ± 8.62 ^{b,c,e,a}
2	78.50 ± 9.15 ^b	532.75 ± 50.89 ^{b,e,a}	327.25 ± 56.55 ^{b,c,e,a}	127.75 ± 7.68 ^{b,c,e,a}	451.00 ± 163.99 ^a	528.00 ± 52.29	127.75 ± 7.68 ^{b,c,e,a}
6	78.25 ± 9.53 ^b	494.50 ± 58.41 ^a	307.00 ± 54.79 ^{b,c,e,a}	97.50 ± 17.84 ^{b,c,e,a}	470.00 ± 150.07 ^a	463.25 ± 96.19	97.50 ± 17.84 ^{b,c,e,a}
8	76.25 ± 7.85 ^b	460.50 ± 26.44 ^c	290.00 ± 51.40 ^{b,c}	90.00 ± 20.46 ^{b,c}	494.00 ± 142.33 ^a	391.00 ± 68.74	90.00 ± 20.46 ^{b,c}
24	68.00 ± 4.83 ^b	444.00 ± 25.16 ^{b,c,e,a}	273.00 ± 49.28 ^{b,e,a}	81.00 ± 20.93 ^{b,c}	527.00 ± 103.99 ^a	335.00 ± 55.73	81.00 ± 20.93 ^{b,c}
168 (7 days)	118.00 ± 6.78 ^b	426.50 ± 24.37 ^{b,c,e,a}	253.00 ± 47.04 ^{b,e,a}	76.75 ± 20.98 ^{b,c,e,a}	545.25 ± 95.67 ^a	276.25 ± 64.56	76.75 ± 20.98 ^{b,c,e,a}
336 (14 days)	82.50 ± 7.33 ^b	412.00 ± 23.48 ^{b,c,e,a}	231.75 ± 46.41 ^{b,e,a}	71.25 ± 19.24 ^{b,c}	556.75 ± 88.80 ^a	263.75 ± 67.25	71.25 ± 19.24 ^{b,c}
504 (21 days)	82.50 ± 6.25 ^b	401.00 ± 26.23 ^{b,c,e,a}	211.75 ± 34.16 ^{b,e,a}	65.50 ± 15.72 ^{b,c}	559.33 ± 81.72 ^a	224.50 ± 74.75	65.50 ± 15.72 ^{b,c}

Values are expressed as mean ± standard deviation, n = 6 for each group, ^aSignificant difference as compared with Normal control (p<0.05), ^bSignificant difference as compared with the diabetic control (p<0.05), ^cSignificant difference as compared with the standard drug (Glibenclamide) (p<0.05)

Table 3: Effect of treatment with methanolic extract of *D. microcarpum* leaves on body weight

Treatment Group	Initial weight (g)	Final Weight (g)	Weight Variation (g)	% Variation
Group I (Normal control)	192.55 ± 26.97	211.93 ± 24.19	+19.38 ± 5.79	10.06 ± 5.79
Group II (500 mg/kg)	161.83 ± 14.89 ^{b,a}	149.28 ± 10.15 ^{b,a}	-12.55 ± 6.06	7.76 ± 6.06
Group III (750 mg/kg)	155.58 ± 27.36 ^b	159.88 ± 27.02 ^a	+4.30 ± 4.02	2.76 ± 4.02
Group IV (1000 mg/kg)	167.50 ± 30.95 ^{b,a}	172.50 ± 28.80 ^a	+5.00 ± 3.37	2.99 ± 3.37
Group V (Diabetic control)	200.00 ± 20.00	185.20 ± 6.79	-24.80 ± 6.79	12.40 ± 6.79
Group VI (Glibenclamide)	148.98 ± 7.24 ^{a,b}	161.48 ± 14.01 ^{a,b}	+12.50 ± 18.82	8.39 ± 14.01
Group VII (Normal + 1000 mg/kg)	211.25 ± 21.75	204.30 ± 22.15 ^b	-6.95 ± 10.66	3.29 ± 10.66

Key: (+) = weight gain, (-) = weight loss, Values are expressed as mean ± standard deviation, n = 6 for each group. ^aSignificant difference as compared with Normal control (p<0.05). ^bSignificant difference as compared with the diabetic control (<0.05)

The present study was aimed at evaluating the anti-diabetic properties of the methanolic leaf extract of *D. microcarpum* in alloxan induced diabetic rats. The plant has been used in ethnomedicine as a remedial measure against diabetes mellitus. Alloxan causes a massive decrease in insulin release by the destruction of the β -cells of the islets of Langerhans thereby inducing hyperglycaemia [31]. Medicinal plants are used worldwide to tackle a range of diabetic presentations and systematic scientific evaluation of these plants may therefore help unlock a vast store of active compounds that could be useful in management of the disease. The root and stem bark of *D. microcarpum* have previously been evaluated for antidiabetic activity but there is presently no study on the activity of its leaves and we therefore attempted for the first time, to study the effect of the methanolic leaf extract hyperglycaemic rats. Phytochemical screening revealed the presence of tannins, saponins, flavonoids, terpenoids and steroids (Table 1). Some of these phytochemicals have been shown to possess good hypoglycemic and other activities and this implies that the plant is a potential candidate for new drugs. Reuben and Jada [32] reported high concentrations of terpenoids in the ethyl acetate and methanol extracts of *D. microcarpum* and they linked this to the antidiabetic properties of the plant. The diabetic rats induced by alloxan showed a consistent increase in blood glucose level after

72 hours with the characteristic features of diabetes mellitus been manifested. In the present study, the concentration of fasting blood glucose level was significantly increased in a time dependent manner ($p < 0.05$) in diabetic control group (Table 2), when compared with the normal control group. However, there was dose/time dependent significant reduction ($p < 0.05$) in blood glucose level of diabetic treated groups when extracts of *D. microcarpum* at 500 mg/kg, 750 mg/kg and 1000 mg/kg were administered respectively (Table 2). Also, rats treated with *D. microcarpum* only at 1000 mg/kg and Glibenclimide at 5 mg/kg showed significant decrease ($p < 0.05$) in blood glucose within the period of this study. The hypoglycemic activities of the extracts of *D. microcarpum* may be due to the presence of potent bioactive phytochemical such as terpenoids, tannins, phenolics and flavonoids which appears to be involved in the stimulation of the pancreatic islet beta-cells and the subsequent secretion of preformed insulin [33].

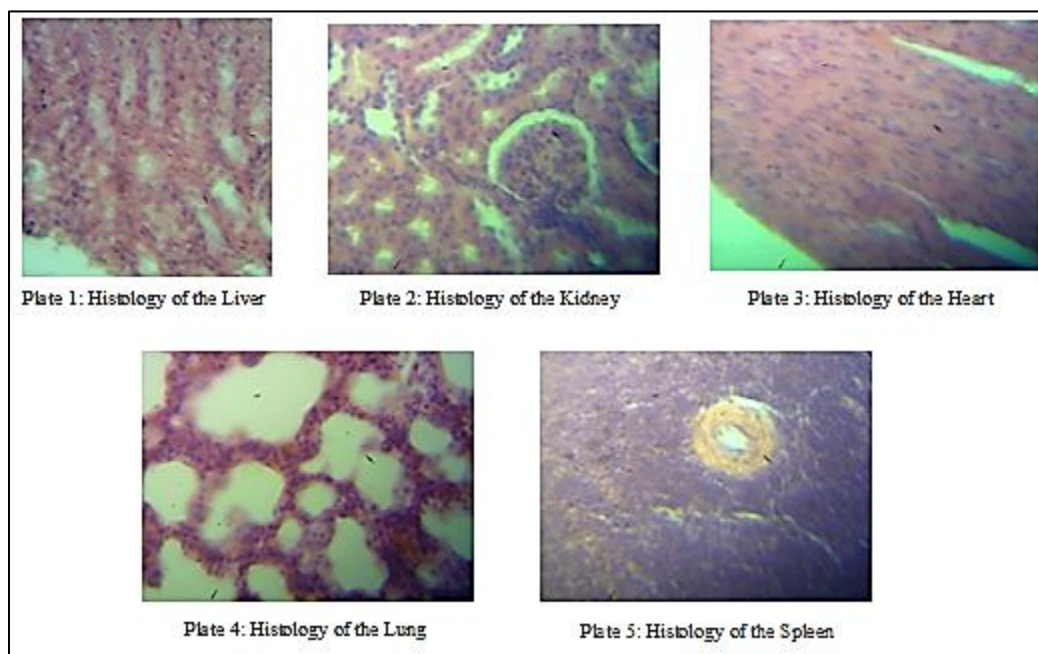


Figure 1: Histology of organs

In this study also, a significant reduction ($p < 0.05$) in body weight was noticed in Alloxan - induced diabetic rats (diabetic control group) by 12.40%, when compared with normal control (Table 3). Alloxan-induced diabetes is characterized by severe loss in body weight of untreated rats which occur as a result of increased muscle wasting of proteins and adipose tissues in diabetes [34]. In addition, when extracts of *D. microcarpum* at (500 mg/kg, 750 mg/kg and 1000 mg/kg) and Glibenclimide at (5 mg/kg) were administered to the diabetic rats for a period of twenty one days, there was dose dependent significant increase ($p < 0.05$) in the weights of the rats when compared with untreated diabetic rats/ diabetic control group (Table 3). These changes in body weight showed that administration of the extracts at varying concentrations have a significant effect in controlling the loss of body weight, which arose during diabetes. The weight gains seem to be as a result of the ability of the extract to reduce hyperglycemia within the period of this study. Histology result of liver, kidney, heart, Lungs and spleen demonstrated that the extract has no toxic effect on the animal's organs. The LD_{50} was also found to be above 5000 mg/kg suggesting that *D. microcarpum* leaf extract is quite safe even at high doses (Figure 1).

CONCLUSION

A conclusion therefore can be drawn from this study that the methanol leaf extracts of *D. microcarpum* possesses antidiabetic effects and is relatively non-toxic at the doses used. The glucose lowering effects of the various concentrations also suggest that the use of the plant as an antidiabetic agent by herbal practitioners may be justified.

ACKNOWLEDGEMENT

This study was supported by The African Centre of Excellence in Phytomedicine, Research & Development (ACEPRD), Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

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