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Antidiabetic effect of methanolic extract of *Piliostigma reticulatum* leaf in streptozotocin-induced diabetic rats

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Abstract
Piliostigma reticulatum (Caesalpiniaceae) is a plant whose leaves are used ethnomedically for the treatment of bacterial infections, wound, injury, diarrhea and dysenteries. The study aims at evaluating the antioxidant and antidiabetic effect of the methanol extract of *Piliostigma reticulatum* leaf on streptozotocin-induced diabetic rats. The powdered leaves were extracted with 70% methanol to afford MeOH extract. The acute toxicity study and phytochemical screening were carried out on the extract followed by the determination of antioxidant property of the extract using DPPH assay. Antidiabetic activity of the extract at the doses of 250mg/kg, 500mg/kg and 1000mg/kg was evaluated on streptozotocin-induced diabetic rats by oral administration. The acute toxicity study showed LD_{50} of 5000mg/kg while the phytochemical screening revealed the presence of flavonoids, saponins and alkaloids majorly. The three doses exhibited significant reduction in blood glucose levels but was more pronounced in 250mg/kg on 4th day when compared with glibenclamide, a well-known antidiabetic drug. The MeOH extract demonstrated significant antioxidant activity at IC_{50} value of 1.96µg/mL compared to standards used ascorbic acid, gallic acid and rutin (IC_{50} 11.8, 47.4 & 75.4µg/mL, respectively). This study showed that methanolic extract of *Piliostigma reticulatum* leaf possesses antidiabetic and antioxidant activities. Therefore, it can be employed as a natural source of antidiabetic agents.

Keywords: Diabetes mellitus, Antioxidant, Streptozotocin, Antidiabetic activity, *Piliostigma reticulatum*

INTRODUCTION

American Diabetes Association defines diabetes mellitus as a group of metabolic disorders characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both [1]. Current treatments of diabetes, in addition to insulin supplement are oral hypoglycemic agents like sulfonylureas, biguanides, thiazolidinediones, D-phenylalanine derivatives meglitinides and α-glucosidase inhibitors along with appropriate diet and exercise. However, none can be termed as an ideal one, due to their toxic side effects and some diminution in response after prolonged use [2]. Therefore, investigations of antidiabetic agents from traditional medicinal plants have become very imperative in providing alternative solution to undesirable side effects encounter with the current antidiabetic agents in commercial use. *Piliostigma reticulatum* is a leguminous plant

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belonging to the family Caesalpiniaceae (common name; Yoruba: 'abafin', Hausa: 'kalgo', Igbo: okpoatu'). It is found in the savannah region of Nigeria and widely distributed in Africa and Asia [3]. Ethnomedically, the leaves are used against cold, eye problems, mumps, cough, headaches, migraines and epilepsy, *P. reticulatum* bark is often prescribed against many diseases such as ulcers, boils, wounds, syphilitic cancer, toothache, gingivitis and diarrhea [4] while leaves and barks are used as antiseptic, wound and injury healing. In addition, barks of *Piliostigma reticulatum* are used against diarrhoea, dysenteries, toothaches, rheumatisms and ulcer while the roots of the same plant are used in the treatment of anxiety, agitation and epilepsy [5-7]. It has been reported experimentally that methanol extracts of P. reticulatum bark and leaves demonstrated stronger antimicrobial activities than standard drugs available in the market [5,6,8-10]. Aqueous extract of *P. reticulatum* demonstrated anthelmintic effects [11] as well as strong anti-trypanocidal activity [12], crude ethanol extract from the stem bark of *P. reticulatum* demonstrated antidarrhoeal activity [13] along with its acute toxicity study that showed that the plant is safe for consumption with the LD_{50} greater 6000 mg/kg. The aqueous extract of *P. reticulatum* stem bark demonstrated lipoxygenase inhibitory and cytotoxic effects against leukemia cancer cell lines [5,14]. Besides, aqueous extracts of *P. reticulatum* demonstrated good anxiolytic and antipyretic activities [15]. The dichloromethane fraction from the ethanolic extract of *P. reticulatum* stem bark possesses spasmolytic activity on isolated rabbit duodenum [10]. The methanolic extract of *P. reticulatum root* showed strong anti-ulcerogenic properties in rats [16]. However, the chronic administration of ethanol extract of the stem bark caused a renal toxicity and a transient disturbance of lipid and carbohydrate metabolism in rats [17]. Various extracts of *P. reticulatum* demonstrated good antioxidant activities, which had led to the isolation of flavonoids [4,6,9,14]. Some essential oils were identified from the leaves, which include: α-pinene, β-caryophyllene, germacrene D, β-pinene, limonene, α-cubebene, α-copaene, trans-α-bergamotene, α-caryophyllene, δ-cadinene, γ-muurolene, tricyclene, o-cymene, terpinen-4-ol, α-terpineol, α-copaene, α-cadinol and farnesol [3,18]. Certain flavonoids were isolated from the leaves of the plant. These include 6-C-methyl-2-p-hydroxyphenyloxy chromonol (piliostigmol), 6,8-di-C-methylquercetin-3,3,7-trimethyl ether, 6,8-di-C-methylquercetin-3,3-dimethyl ether, 3,6,8,-tri-C-methylquercetin-3,7-dimethyl ether, 6-C-methylquercetin-3-methyl ether, 6,8-di-C-methylkaempferol-3-methyl ether, 6-C-methylquercetin-3, 3', 4'-trimethyl ether, 6-C-methylkaempferol-3-methyl ether, 6,8-di-C-methylkaempferol-3,7-dimethyl ether and a 2-phenoxychromone (piliostigmin) were isolated along with quercetin, quercitrin and quercetin-3-O-glucoside [5-6]. No literature was found on antidiabetic activity of *Piliostigma reticulatum* leaves extracts and this study was designed to investigate the antidiabetic activity of the leaf extract of this plant using streptozotocin induced diabetic rats as well as its antioxidant activity using DPPH assay.

EXPERIMENTAL

Collection and preparation of plant materials. The leaves were collected from Kabba Local Government Area, Kogi State in May 2015 and identified by Mr Azila, a taxonomist of the college of forestry Jos. They were dried under shade and pounded.

Extraction. The powdered leaves (160 g) was extracted with 70% methanol for 72 h and dried at reduced temperature to afford methanol extract of *P. reticulatum* leaf, MeOH extract.

Acute toxicity test (LD_{50}). The oral acute toxicity of the methanolic extract of *Piliostigma reticulatum* leaf was determined in rats as described by Lorke [19].

Phytochemical screening. Phytochemical screening was carried out according to the protocols described in Trease and Evans [20].

Experimental animals: Healthy adult male Albino rats (120-150g), in-house bred at the Animal House of Department of Pharmacology and Toxicology, University of Jos were used for the study. Rats were housed in cages lined with husk in standard environmental conditions (temperature 25 ± 2^{o}C, relative humidity 55 ± 10 % and 12:12h light: dark cycle). The rats were fed on a standard pellet diet (Grand Cereal Feeds, Bukuru express, Plateau State, Nigeria) *ad libitum* and had free access to water. The experimental protocol was subjected for ethical review and was approved by the Institutional Animal Ethics Committee, Department of Pharmacology and Toxicology, University of Jos, Jos before the commencement of the study (Reference number F17-00379).

Experimental design. Antidiabetic activity of methanol extract of *Piliostigma reticulatum* leaf was assessed in streptozotocin-induced diabetic rats. In this study, the animals were fasted overnight for 16h with free access to water throughout the duration of the experiment.

Evaluation of MeOH extract in streptozotocin-induced diabetic rats: Experimental diabetes was induced by single intra-peritoneal injection of 65 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5. Control animals received only distilled water. After 5 days of STZ injection, animals with fasting blood glucose above 250 mg/dL were considered as diabetic and included in the study. The animals were randomly assigned into five groups of five animals each and received the following treatments: Group I: Diabetic + distilled water, Group II: Diabetic + MeOH extract (250 mg/kg), Group III: Diabetic + MeOH extract (500 mg/kg), Group IV: Diabetic + MeOH extract (1000 mg/kg) and Group V: Diabetic + glibenclamide (5 mg/kg). The freshly prepared solutions were orally administered daily for 7 days. Body weights and blood glucose level analysis were done on day 1, 4 and 7 on overnight fasted animals [21].

Antioxidant test. The antioxidant activity (free radical scavenging activity) of the methanol extract of *Piliostigma reticulatum* leaves on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the standard method [22]. Briefly, 12.5 mg of the MeOH extract was dissolved in methanol using a 25 ml volumetric flask. The following concentrations of the MeOH extract were prepared 500, 250,125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg/mL. All the solutions were prepared with methanol as solvent. 2 mL of each prepared concentration was mixed with 4 mL of 50 µM DPPH solution in methanol. Experiment was done in triplicate. The mixture was vortexed for 10s to homogenize the mixture and the test tubes were incubated for 30 minutes at room temperature in the dark. After 30 min of incubation, the absorbance was measured at 515 nm on a UV-VIS spectrophotometer (Shimadzu, UV-1620PC, Japan). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C, gallic acid and rutin were used as standards with the following concentration 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195 µM. Blank solution was prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH. The difference in absorbance between the extract and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH

radical was calculated by using the following equation

$$\% \text{ inhibition} = \{(A_{control} - A_{extract})/ A_{control})\} \times 100$$

Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of the plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL).

Statistical analysis. Data generated from the study were analysed with the aid of statistical package for social sciences (SPSS) version 20.0. Mean weight and blood glucose concentration were generated for each study group across study period, also mean change and blood glucose concentration were generated for each group by subtracting the baseline value of each parameter from the specific measuring period. The mean and mean change in weight and blood glucose concentration were compared for statistical significance in relation to normal group values using independent T-test set at $P < 0.05$ for significance. The results were expressed as Mean ± SD.

RESULTS

In the acute toxicity study, MeOH extract treated animals did not show any change in their behavioral pattern within the period of the study, when compared to the vehicle treated group. Thus, it was concluded that MeOH extract was safe at 5000 mg/kg.

The phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, tannins, steroids, cardiac glycosides while the anthraquinones were absent.

The effect of oral administration of MeOH extract on blood glucose levels in STZ-diabetic rats for 7 days is presented in Table 2. MeOH extracts, administered at three different doses of 250 mg/kg, 500 mg/kg, 1000 mg/kg to STZ-treated diabetic rats caused significant ($P < 0.05$) reduction of blood glucose levels, which was dose dependent, related. Maximum reduction was observed on 4th day (63.0%, 28.2% and 13.28%, respectively). MeOH extract, 250mg/kg exhibited maximum glucose lowering effect in diabetic rats compared to the other two doses and the standard employed. Glibenclamide exhibited 36.9% reduction in blood glucose levels at the end of the study when compared to diabetic control. The diabetic control that received distilled water continued to lose weight till the end of the study while MeOH extract at 1000 mg/kg showed significant improvement ($P < 0.05$) in body weight compared to diabetic control as well as the control group that received glibenclamide. Table 1.

From Table 3, MeOH extract due to the presence of some phytochemicals such as flavonoids demonstrated significant antioxidant activity with IC_{50} value of 1.96 µg/ml when compared with the standards used ascorbic acid, gallic acid, rutin (IC_{50} 11.8, 47.4 & 75.4 µg/ml) respectively.

Table 1: Mean weight of animals across study groups

Treatment	Dose mg/kg	Weight (g)			
		Baseline	Day 1	Day 4	Day 7
Extract	250	148.40 ± 6.86	148.53 ± 9.20	144.68 ± 14.28	144.58 ± 15.63
Extract	500	146.70 ± 7.70	132.67 ± 8.95	143.70 ± 12.44	137.27 ± 4.87*
Extract	1000	148.90 ± 7.34	147.98 ± 7.85	130.10 ± 3.54	140.33 ± 6.74*
Glibenclamide	5	125.68 ± 2.41	122.23 ± 8.21	127.03 ± 4.07	129.00 ± 3.11*
Dist. water	0.5 mL	127.75 ± 4.42	127.65 ± 6.56	117.28 ± 7.90	106.00 ± 7.02

*= significant difference ($P < 0.05$) in mean weight of group compared to the negative group

Table 2: Mean blood glucose concentration across study groups compared to control group

Treatment	Dose mg/kg	BGC (mg/dl) mean (±SEM)			
		Baseline	Day 1	Day 4	Day 7
Extract	250	402.00 ± 54.9	358.50 ± 20.04	148.75 ± 38.01*	244.00 ± 84.87
Extract	500	262.00 ± 21.28*	380.33 ± 57.85	188.00 ± 81.07*	199.07 ± 61.22
Extract	1000	401.00 ± 52.22	340.50 ± 31.01	366.75 ± 28.09*	353.00 ± 23.11
Glibenclamide	5	379.50 ± 53.70	382.75 ± 52.14	347.75 ± 39.61*	239.25 ± 16.96*
Dist. water	0.5 mL	395.00 ± 33.29	423.25 ± 22.93	478.25 ± 14.23	500.25 ± 12.11

*Significant difference ($P < 0.05$) in mean blood glucose concentration compared to negative control group

Table 3: Radical scavenging power of *Piliostigma reticulatum*, ascorbic acid, gallic acid and rutin in DPPH

Sample	IC_{50} (μg/ml)
Extract	1.96
Ascorbic acid	11.8
Gallic acid	47.4
Rutin	75.4

DISCUSSION

Diabetes highlights a growing epidemic imposing serious social economic crisis to countries around the world. Despite the scientific breakthroughs, better healthcare facilities, and improved literacy rate, the disease continues to burden several sections, especially middle- and low-income countries. The drugs used for the treatment of type 2 diabetes possess significant side effects [23] not to mention their cost. Though there are various approaches to reduce the ill effects of diabetes, and its secondary complications, herbal formulations are preferred due to lesser side effects and lower cost [24].

According to the WHO, medicinal plants have been found to be relatively safe [25]. In the acute toxicity study, no mortality or behavioural, respiratory patterns or somatomotor activities of toxicity were observed following the oral administration of MeOH extract at 1600mg/kg, 2900mg/kg and 5000mg/kg. Therefore, the LD_{50} of MeOH extract is much higher than 5000mg/kg in rats, which also corroborates the previous findings on *Piliostigma reticulatum* extracts [13,17]. These kinds of extract are considered as practically non-toxic and they are widely used as food and herbs [26].

The phytochemical screening of the MeOH extract revealed the presence of alkaloids, flavonoids and saponins in high amounts. Literature has shown the significant antidiabetic effects of Alkaloids, flavonoids and saponins [27]. The blood glucose lowering activity of the plant might be due to its ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibiting the intestinal absorption of glucose. This is likely due to its phytochemical constituents (flavonoids, alkaloids, glycosides) and these constituents have been frequently implicated for their significant anti-diabetic effect [28]. Flavonoids have been found to cause pancreatic beta cells regranulation and thus enhance insulin release [24].

The mechanism by which the MeOH extract caused a reduction in blood glucose concentration may be that it increases sensitivity to and uptake of glucose by the cells. There was also a significant reduction in weight of the animals. This shows that the extract does not have the ability to increase weight which is the major side effect seen of oral antidiabetics such as the sulfonylureas. The antioxidant activity of MeOH extract is higher than the standards employed in this study, which shows that the MeOH extract has ability to scavenge free radicals than the standard since lower IC_{50} values indicate higher antioxidant activity. This also corroborated the previous findings concerning the significant antioxidant activity of the leaf

extracts of *Piliostigma reticulatum* due to the presence of flavonoids [5-6]. In the progression of diabetes, it is established that tissue damage may be due to free radicals by attacking membranes through peroxidation of unsaturated fatty acids leading to extensive damage and dysfunction of membranes [29]. An improved antioxidant defense mechanism may be a good management to prevent diabetic complications. The presence of antioxidant compound such as flavonoids, saponins in this plant provides further evidence for the beneficial effects of MeOH extract of *Piliostigma reticulatum* leaf on the Streptozotocin-induced diabetic rat. Over the decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some plant as a whole or their identified ingredients with antioxidant properties have substantial protective effects on diabetes [30], cardiovascular and renal disorders [31] and several other human ailments [32].

Streptozotocin, used in induction of diabetes is preferred to alloxan because it has higher inductive rate, lower toxicity, and less mortality rate. STZ causes cellular toxicity and local immune response which leads to hypoinsulinaemia, hyperglycaemia as well as being associated with a characteristic decrease in body weight probably due to muscle wasting.

Conclusion. The results of this study showed that MeOH extract of *Piliostigma reticulatum* leaf possesses significant antioxidant and antidiabetic activities. Moreover, more work is underway to isolate, elucidate and characterise the active constituents from the MeOH extract of this plant.

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