ORIGINAL ARTICLE

Antidiabetic potentials of the methanol leaf extract of Oxytenanthera abyssinica

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Abstract Oxytenathera abyssinica is used for the treatment of diabetes mellitus in folklore medicine in Nigeria. In this study, we evaluated its antidiabetic activity in alloxan-induced diabetic mice. Diabetes was induced in the mice by a single intraperitoneal injection of alloxan monohydrate and the fasting blood glucose (FBG) levels measured with blood from the tail snip at 0, 1, 3 and 6 h. The activity was compared with reference drug, glibenclamide (2 mg/kg) and negative control. Oral glucose tolerance test (OGTT) was evaluated by loading glucose to rats at the dose of 2,000 mg/kg and checking their FBG at 0, 60 and 180 min. Antioxidant activity was evaluated using ferric reducing antioxidant power (FRAP) and 1, 1-diphenyl-2- hydrazyl (DPPH) photometric assay. The extract and the reference drug caused a significant ($P<0.02$–$P<0.002$) time and dose dependent decrease in the FBG levels of the mice when compared to the negative control; the extract (500 mg/kg) reduced FBG by 38.0 % at the 6th hr as against 45.6 % by glibenclamide. In OGTT the extract caused a time dependent decrease in the blood glucose level up to 33.3 % at 180 min at the dose of 300 mg/kg. The extract also caused a concentration dependent increase in antioxidant activity having 91 % increase and 1.95 total antioxidant activities at a concentration of 400 μg/ml. Extract of Oxythenanthera abyssinica showed significant antidiabetic activity.

Keywords Antioxidant · Glibenclamide · Alloxan · Rodent · Mouse-model

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Introduction

Diabetes mellitus [1] is a common metabolic disorder in both developed and developing countries associated with co-morbid conditions, leading to complications [2, 3].

The International Diabetes Federation has predicted that the number of individuals with diabetes will increase from 240 million in 2007 to 380 million in 2025 with 80 % of the disease burden in low and middle income countries [4].

The treatment of diabetes mellitus [5, 6] is based on oral hypoglycemic drugs and intramuscular injection of insulin. Synthetic hypoglycemic agents used in clinical practice have serious side effects which has necessitated the search for more effective and safer antidiabetic drugs as recommended by W.H.O [7].

Compared to synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects [8].

Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [9]. Oxytenantera abyssinica is one such plant.

Oxytenantera abyssinica commonly known as West Afri-can bamboo is a shrubby plant that grows up to 6–10 m high and 4–5 cm in diameter, growing in tuft sand. The leaves are alternate, sessile, oblong-lanceolate, 8–20 cm long and 1–3 cm across, with long acuminate apex, the base tapered to form a false petiole. Flowers are pointed, spindle-shaped spikelets, composed of 2–4 imbricate flowers (the basal ones sterile) and 1.5–2.5 cm long. The fruits are spindle-shaped and pointed at both ends. The flowers do not blossom until it is 20–40 years old. It dies after fruiting and does not grow spontaneously in the same place.

The habitat is mainly Sudanese and Guinea savannahs, often on hills and close to swamps or temporary rivers. It grows on any type of soil except salty and very clayey soils and the distribution is from Senegal to Cameroon, as far as Sudan, tropical Africa and usually gregarious. It is abundant in Nigeria.

The traditional uses include: Seeds as rice substitutes in time of scarcity and leaves in the treatment of diabetes and other disorders.

The stems are widely used in construction to give structure to conical hut roofs and lofts or to reinforce walls and for fencing and baskets [10, 11] .

The present study was undertaken to establish a scientific basis for traditional use of the leaves of O. abyssinica as antidiabetic agent and in diabetic related complications.

Materials and methods

Collection and identification of plant materials

Fresh leaves of Oxytenanthera abyssinica (West African Bamboo) were collected from their natural habitat in Apumiri, Ubakala, Umuahia South LGA, Abia State, Nigeria and was identified by Dr. D.O. Dike of the forestry department of Michael Okpara University of Agriculture, Umudike, Abia State and a voucher specimen No: MOUAU/CVM/VPP/2011021 deposited in the department of Veterinary Physiology and Pharmacology Michael Okpara University of Agriculture, Umudike herbarium.

Extraction of plant material

The leaves of O. abyssinica were cut into pieces, dried under mild sunlight and later pulverized into a coarse powder of about 1 mm in diameter. The pulverized plant material was extracted by cold maceration in 80 % methanol with intermit-tent shaking at 2 h interval for 48 h. The extract was then filtered with Whatman no 1 filter papers and the filtrate concentrated to dryness in an oven (Uniscope SM9023 Lab-oratory Surgifriend medicals, England.) at 40 °C. The percent yield was 11.9 % and was stored as Oxytenanthera abyssinica extract (OAE) in a refrigerator at 4 °C until time of use.

Experimental animals

Adult male Wistar albino rats weighing 80–115 g and mature albino Wistar mice weighing 27–35 g were obtained from the laboratory animal unit of the faculty of Veter-inary Medicine, University of Nigeria. The animals were housed in a cage at room temperature and standard pelleted feed (Vital feed® Nigeria) and clean drinking water provided to them ad libitum. The animals were allowed 2 weeks to acclimatize before use and ethical guidelines governing the conducts of experiments with live animals were strictly followed [12].

Acute toxicity test in mice

Thirty mature mice of mixed sexes were randomly divided into 5 groups of 6 each. O. abyssinica extract at the doses of 100, 500, 1,000, 2,000 and 3,000 mg/kg were administered to mice in groups 1, 2, 3, 4 and 5 respectively. All the mice were given feed and water and were observed for 48 h for signs of toxicity and death [13].

Experimental design

Anti-diabetic study in mice

Adult mice were fasted for about 18 h with clean drinking water provided. Diabetes was induced by single intraperitoneal administration of alloxan monohydrate (Sigma, USA) at the dose of 160 mg/kg body weight [14]. The fasting blood glucose levels (FBG) were checked from the fifth day using auto analyzer (Accu-chek Active®) glucose kit (Mannheim, Germany). By the seventh day, diabetes was established in the mice and the mice having FBG of 200 mg/dl and above were considered diabetic and used for the experiment.

The mice were then randomly divided into 5 groups of 6 mice per group. The blood glucose levels were recorded and regarded as FBG at 0 h.

The mice were then treated as follows: mice in group 1 were given distilled water at 10 ml/kg, group 2 mice were treated with glibenclamide (2 mg/kg), groups 3, 4 and 5 mice were treated with 100, 300 and 500 mg/kg of O. abyssinica extract respectively all by gastric gavage.

The fasting blood glucose levels were measured with blood collected from the tail snip at 1, 3 and 6 h using Accu-chek Active® glucose kit.

Oral glucose tolerance test (OGTT) in rats

Mature albino rats were weighed and randomly divided into 3 groups (1–3) of 6 rats per group and fasted for 18 h.

The fasting blood glucose levels (FBG) of the rats were measured and recorded at 0 h. The rats in the first group received 300 mg/kg of the extract of O. abyssinica while the rats in the second group were treated with distilled water (10 ml/kg) and served as negative control. The rats in the third group were given glibenclamide (2 mg/kg) and served as positive control. 30 min after extract and drug administration, a glucose load (2,000 mg/kg) was given to all the rats. The FBG levels of all the rats were measured at 0, 60 and 180 min using Accu-chek Active® glucose kit [15].

Antioxidant activity

DPPH photometric assay

The method of Mensor et al. [16] was adopted for this study. Two milliliter of O. abyssinica extract at concentrations ranging from 10 μg/ml to 400 μg/ml was each mixed with 1 ml of 0.5 mM DPPH (in methanol). Absorbance at 517 nm was taken after 30 min incubation in the dark at room temperature. The concentrations were prepared in triplicates. The percentage antioxidant activity was calculated as follows:

$$\% \text{ antioxidant activity} = AA\% = 100 - \left(\frac{\text{absorbance of sample} - \text{absorbance of blank}}{\text{Aborbance of control}} \times 100\right)$$

One milliliter of methanol plus 2 ml of the extract was used as blank while 1 ml of 0.5 mM DPPH solution plus 2 ml of methanol was used as control. Ascorbic acid was used as reference standard.

FRAP

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay [17].

Sample (100 μl) of different concentrations of the extract (10, 50, 100, 200 and 400 μg/ml) was mixed with 3 ml of working FRAP reagent. The absorbance was immediately taken at 593 nm at 0 min. Thereafter samples were placed at 37 °C in water bath and absorbance was measured after 4 min with a spectrophotometer. FRAP value of the samples were calculated using the formula below.

$$\text{FRAP value} = \frac{\text{Change in absorbance from 0-4mins} \times \text{FRAP value of standard}}{\text{change in absorbance of standard from 0-4mins}}$$

Data analysis

The results were presented as mean ± SEM and ana-lyzed using one way analysis of variance (ANOVA) and the differences between the means were tested using Post Hoc LSD and values of $P<0.05$ were considered statistically significant.

Results

Acute toxicity test

The administration of Oxytenanthera abyssinica extract (OAE) at different doses to the mice caused no death or signs of toxicity after 48 h.

Antidiabetic study

The result of the antidiabetic study of OAE on alloxan-induced diabetes in mice is presented in Table 1. There was an increase in the mean FBG in the negative control group from 280.6±28.85 to 294.2±22.14 representing 4.8 % at the 6th hr.

The extract at the doses used and the reference drug caused a significant ($P<0.02$ and $P<0.002$) dose and time dependent decrease in the FBG at the 3rd and 6th hr respectively when compared to the negative group. At 1 h the extract at the doses of 300 and 500 mg/kg also caused a significant ($P<0.02$) reduction in the FBG while the 100 mg/kg dose's reduction was not significant. The percent reduction of FBG at the 6th hr by the extract at the doses of 100, 300 and 500 mg/kg were 11.7 %, 25.1 % and 38.0 % respectively as compared to 45.6 % by the reference drug, glibenclamide (2 mg/kg).

Oral glucose tolerance test (OGTT)

Figure 1 shows the effect of Oxytenanthera abyssinica extract on oral glucose tolerance test in normal rats. After glucose load, the mean glucose level of the rats in the negative group

Table 1 Effect of Oxytenathera abyssinica on the fasting blood glucose levels of mice

Fasting blood glucose level (mg/dl)						
Group	Treatment	0 h	1 h	3 h	6 h	% reduction at 6th hr
1	Distilled water (10 ml/kg)	280.6±28.85	305.3±26.33	297.2±26.56	294.2±22.14	–
2	Glibenclamide (2 mg/kg)	253.3±22.11	215.3±13.84*	208.5±12.67*	139.8±9.0**	45.6
3	OAE 100 (mg/kg)	243.3±12.29	239.0±13.11	222.7±13.41*	214.8±11.42**	11.7
4	OAE 300 (mg/kg)	228.0±7.81	199.7±7.5*	200.0±11.52*	170.8±10.33**	25.1
5	OAE (500 mg/kg)	290.5±32.29	230.7±15.31*	208.3±22.53*	180.0±10.80**	38.0

*P<0.02, **P<0.002 when compared to the group 1

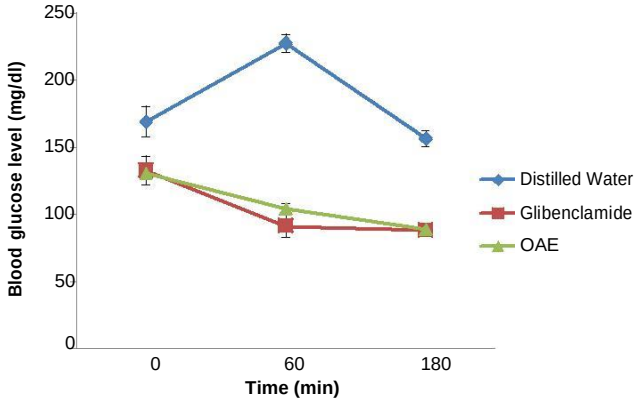

Fig. 1 The effect of OAE on oral glucose tolerance test

increased from 169.0±11.37 to 227.7±6.7 within 60 min representing 24.8 % increase but was slightly reduced to 156.3±6.0 representing 7.5 % decrease at 180 min. OAE extract caused a reduction of the mean blood glucose level of the rats from 132.8±10.71 at zero min to 89.0±1.9 at 180 min representing 33.3 % decrease as against that of glibenclamide treated group which was reduced to 88.3±3.3 at 180 min representing 31.5 % decrease.

Antioxidant assay

DPPH

The percent antioxidant activity of O. abyssinica and ascorbic acid standard is shown in Fig. 2. The result showed that the extract exhibited a very strong and concentration dependent antioxidant activity increasing the percentage antioxidant from 70 % at the concentration of 10 μg/ml to 91 % at the concentration of 400 μg/ml which was higher than the 79 % exhibited by ascorbic acid standard at 400 μg/ml.

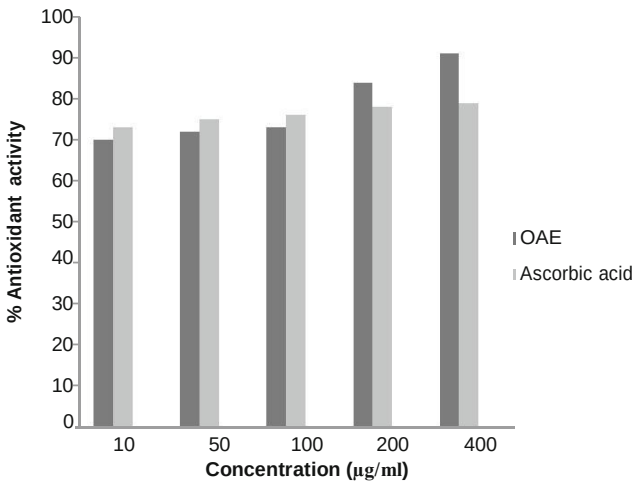

Fig. 2 Percentage antioxidant activity of O. abyssinica

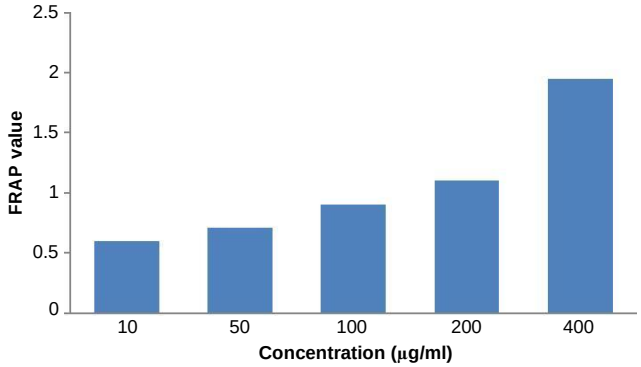

Fig. 3 Total antioxidant activity of O. abyssinica using FRAP

FRAP

The antioxidant activity of O. abyssinica using ferric reducing antioxidant power is presented in Fig. 3. The result showed that the extract had a concentration dependent total antioxidant activity increasing the FRAP value from 0.60 at the concentration of 10 μg/ml to 1.95 μg/ml at the concentration of 400 μg/ml.

Discussion

This study evaluated the antidiabetic activity of Oxrtenanthera abyssinica in alloxan-induced diabetes mouse model.

The acute toxicity test (for 48 h) produced no signs of toxicity or death suggesting the plant extract was safe in this model.

Oxytenanthera abyssinica's antidibetic activity was tested by the ability of the extract to reduce the fasting blood glucose (FBG) levels of alloxan-induced diabetic mice. Fasting plas-ma glucose is measured after overnight fast; in diabetes blood glucose levels are high [18].

Alloxan monohydrate induces diabetes by destruction of the β-cells [19].

In this study, OAE at the doses used like the reference drug caused a significant ($P<0.02$ and $P<0.002$) time and dose dependent reduction in the FBG that lasted up to the 6th hr.

Oral antidiabetic drugs lower glucose levels [20].Similar effect by the plant extract suggests it has potential antidiabetic activity.

The mechanisms proposed include: improving lipid metab-olism, antioxidant status and also by their ability to cause increase in insulin output or insulin release from the β-cells [21]. OAE may suppress hepatic gluconeogenesis, stimulate glycolysis or inhibit glucose absorption [22].

To further demonstrate the efficacy, oral glucose tolerance test (OGTT) was conducted in normal rats. OGTT measures the body's ability to metabolize glucose [23].

OAE (300 mg/kg) reduced the blood glucose level of normal rats by 33.3 %, 180 min after glucose load, the effect

being a little better than glibenclamide (2 mg/kg). This sug-gests that its antidiabetic activity may involve extra pancreatic mechanism or through reduction in the intestinal absorption of glucose or both [24].

Part of the mechanisms by which alloxan monohydrate induces diabetes is by its ability to produce reactive oxygen species (ROS) that have affinity for, and destroy, pancreatic beta cells giving rise to diabetes [25].

OAE caused a concentration dependent increase in percentage and total antioxidant activities. Substances that increase FRAP value and percentage antioxidant activity in DPPH photometric assay is assumed to have antioxidant activity [26]. This antioxidant activity could slow or terminate the production of ROS and this suggests that the plant may be useful in managing and preventing degenerative diseases such as diabetes that are exacerbated by generation of ROS. Although the mechanism by which OAE brought about its action in this study is not clearly established, antioxidation activities which reverses the effect of alloxan were observed.

In conclusion, Oxytenanthera abyssinica has demonstrated significant hypoglycemic activity in alloxan and glucose-induced hyperglycemia in this study. More work is required to isolate and characterize the bioactive principle responsible for antidiabetic activity and to determine its exact mechanism of action.

Conflict of interest The authors declare that there are no conflicts of interest whatsoever.

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Author contribution detail

Maxwell I. Ezeja: Conception, design, experimental studies, data acqui-sition, data analysis, statistical analysis and interpretation and manuscript preparation.

Yusuf S. Omeh: Conception, design, reviewing the article critically for intellectual content, manuscript editing and experimental studies.

Chiamaka Mbagwu: Literature search, experimental studies and data acquisition.