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Research Article

ANTIDIABETIC EFFECT OF *DIOSCOREA BULBIFERA* ON ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT
An antidiabetic effect of ethanolic tuber extract of dioscorea bulbifera l. (aerial yam) on alloxan-induced diabetic rats was studied. The median lethal dose (ld_{50}) of the extract was determined to be 3800.0 mg/kg and a single dose of 380.0, 760.0 and 1140.0 body weight of the extract were intraperitoneally administered as the treatment dose and the blood glucose levels (bgl) examined for 7 hours and 15 hours (prolonged) at 2 and 4 hours intervals respectively. The extract exhibited significant ($p<0.05$and $p<0.01$) reduction in the blood glucose levels of the albino rats. The extract compared favourably with the standard reference drug (metformin) which all gave their maximum bgl reduction at 5 hours duration. The confirmation of antidiabetic potentials of the dioscorea bulbifera tuber has been justified in this study as claimed by traditional medicine practitionersin akwa ibom state.

Key Words: *Dioscorea Bulbifera Tuber, Diabetic Rats, Hypoglycaemic Activity, Ethanolic Extract*

INTRODUCTION
In traditional practices, medicinal plants are used to control diabetes mellitus in many African countries. This has caused awareness in the number of experimental and clinical investigations directed towards the validation of the antidiabetic properties, which are empirically attributed to these remedies. The hypoglycemic effects of some edible plants used as antidiabetic remedies have been reported (Odoemena *et al.,* 2010) which justify their nutraceutical potentials. These types of plants could be developed for diabetic patients in order to improve their diet and control their diseases. With this type of menu, diabetic patients could potentially reduce the dose of their orthodox hypoglycemic drugs.

Diabetes is any disorder characterized by excessive urine excretion. The most common form of Diabetes is Diabetes mellitus, a chronic, progressive, systemic condition of impaired Carbohydrate metabolism (Akah *et al*., 2002). Insulin unavailability may be due to degenerative changes in β-cells in the pancreatic islets, reduced effectiveness of the hormones owing to the formation of anti-insulin antibodies or inactive complexes, immune-mediated islet Cytotoxicity or inappropriate secretion of hormones by neoplasm in other endocrine organs (Sachan *et al*., 2009). Aerial yam, *Dioscorea bulbifera* (Dioscoreaceae) is widely distributed in Asia and Africa in the wild state and widely naturalized elsewhere in the tropics and subtropics, including Central and South America (Overholt *et al*., 2003). Vigorously twining herbaceous vine, with small or absent underground tubers. Leaves long petioled, alternate, flowers rare (in Florida), small, fragrant, male and female arising from leaf axils on separate plants. Fruit a capsule; seeds partially winged (Wagner *et al.*, 1999). Yam leaves and tubers are used to treat a variety of ailments. The leaf of aerial yam is used as a poultice for pimples and tumors and in bath water to soothe skin irritations and stings (Gao *et al*., 2002). Air yam is also used to treat sores, swelling, hemorrhoids, sore throats (Martin, 1974). In northern Bangladesh, air yams are used to treat leprosy and tumors. Researchers have identified antitumor properties in air yams (Czarapata, 2005). Tubers are eaten as vegetable by the ethnic communities of Meghalaya, Kameng district of Arunachal Pradesh and Africans. *Dioscorea bulbifera* tubers contain furanoid norditerpenes, norditerpene glucosides, diosbulbinoside D & F and diosbulbin B and D (Su *et al*., 2003). The purpose of this study is to validate

the anti hyperglycemic effect of *Dioscorea bulbifera* cherished by many Nigerians as a delicacy in food accompliments.

MATERIALS AND METHODS

Plant Materials

Matured tubers of *Dioscorea bulbifera* were collected from local cultivars in Afaha Atan Village, Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria in July, 2012. The plant species was identified and authenticated by a Plant Taxonomists; Dr. (Mrs.) M. E. Bassey in the Department of Botany and Ecological Studies, University of Uyo, Uyo. Herbarium specimen (DBH 555) was deposited at the Department of Botany herbarium. The matured tubers of *Dioscorea bulbifera* sample were shed-dried on a laboratory table for 2 weeks and reduced to powder by pounding the dried sample with mortar and pestle.

Two hundred and fifty grams of the powdered sample was macerated in 70% ethanol (100mL) for 72 hours. The liquid filtrate obtained was concentrated *in-vacuo* at 40^0C. They yield was 34.5%w/w. The extract was stored in a refrigerator at -4^0C until used for this experiment.

Phytochemical Screening

Phytochemicl screening of the extract was carried out according to the standard methods (Trease and Evans, 2009).

Animals

Albino wister rats (120-160 g) and Albino Swiss Mice (22-33g) of both sexes were obtained from the University of Uyo animal house. The animals were maintained on standard animal's pellets and water *ad libitum.*

Determination of Median Lethal Dose (LD_{50})

The median lethal dose (LD_{50}) of the *Dioscorea bulbifera* tuber extract was estimated using albino mice by intraperitoneal (i.p) route administration of different doses of 500-4000 mg/kg to five groups of 5 mice per group respectively after starving the animals for 24 hours according to the method of Lorke (1983). The IP route was adopted because of its sensitivity and rapid results. The animals were observed from possible manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tones, decreased respiration and finally death. Records on the number of deaths observed were taken in each group within 24hours. The LD_{50} was calculated as the geometrical means of the maximum dose producing 0% (a) and minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

Evaluation of Antidiabetic activity of the extract

Induction of Diabetes

Male wister rats were made diabetic by a single dose of intraperitoneal (IP) injection of 150mg/kg body weight of alloxan monohydrate in sterile normal saline. The rats were maintained on 5% glucose solution for next 24 hours to prevent hypoglycaemia. Five days later, blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes (250 mg/dL and above). The diabetic rats were divided into five groups, each containing five animals and treated as follows:

Group I: Diabetic rats administered with 5 mL/kg of saline water as the control

Group II: Diabetic rats treated with *Dioscorea bulbifera* ethanol extract (380.0 mg/kg/day)

Group III: Diabetic rats treated orally with *Dioscorea bulbifera* ethanol extract (760.0 mg/kg/day) in aqueous solution for 15days.

Group IV: Diabetic rats treated orally with mushroom extract (1140.0 mg/kg/day) in aqueous solution

Group V: Diabetic rats given 10 mg/kg of metformin for 15 days.

Blood samples were collected from the tail vein just prior to and 1h, 3h, 5h and 7h after drug administration for acute study. The effect of the *Dioscorea bulbifera* ethanol extract was also tested for a prolonged treatment lasting for 15 days.

The fasting BGL of all the rats were monitored and recorded at regular intervals during the experimental period. For acute study the BGL was monitored after 1, 3, 5, and 7 hour of administration of the extract and at the end of 0, 1, 5, 10 and 15 days for prolonged administration. The blood samples were collected through the tail just prior to and on the time frames after drug treatment. The blood was dropped on the dextrostix reagent pad, which was insert into microprocessor digital blood glucometer and the readings recorded.

Statistical Analysis

The results are expressed as mean S.E.M., the significant of various treatments was calculated (Student's t-test) using SPSS and were considered statistically significant when $p<0.05$ and $p<0.01$.

RESULTS

The results of phytochemical screening of the extract of *Dioscorea bulbifera* showed the presence of alkaloids, carbohydrate, protein, glycosides. The presence of alkaloids, carbohydrates and protein in *Dioscorea bulbifera* indicates the antioxidant activity of that plant. The mice treated intraperitoneally (i.p) with a single dose of 500-4000 mg/kg of *Dioscorea bulbifera* extract after 24-hour starving exhibited physical signs of toxicity in the animals depending on the dose administered with ranged from writhing, respiration distress, decreased limb tone and death within 24 hours post administration of the extract. All the animals given 3500 and 4000 mg/kg doses of the extract died. The LD_{50} of the extract was calculated to be 3800.0 mg/kg.

The antidiabetic activity of the ethanolic *Dioscorea bulbifera* extract gave a dose-dependent reduction in the BGL of the alloxan induced diabetic rats. The significant ($P<0.05$) reduction in the blood glucose level (BGL) of the diabetic rats within the period of acute study was not comparable with that of the control (Table 1). However the highest reduction effect was achieved at 5 hour period with the maximum dose of the extract (1140.0 mg/kg) giving 83.21 ± 5.2mg/dL. However the maximum dose less than that of the standard drug 65 ± 3.07 mg/dL metformin (Table 1).

The result of the prolonged treatment (15-days) of *Dioscorea bulbifera* produced a sustained significant ($p<0.05$) reduction in BGL of the hyperglycemic rats when compared with that of the control (Figure 1). The potent and progressive reduction activity of BGL of the extract with the pretreatment and treatment values confirms the antidiabetic potential of *Dioscorea bulbifera.*

Table I: Phytochemical analysis of *Dioscorea bulbifera* extract

Tests	Inference
Alkaloids	+++
Phlobatannins	-
Carbohydrate	+
Protein	+++
Cardiac glycosides	++
Salkowski	++
Keller Killiani's test	++
Flavonoids	-

Key

+++ = *High concentration*
++ = *Moderate concentration*
\+ = *Trace concentration*
\- = *Absent*

Table 2: Antidiabetic activity of *Pleurotus ostreatus* extract during acute study (BGL, mg/dL)

Treatment Dose (mg/kg)	0h	1h	3h	5h	7h
5mL/kg saline water (control)	152±4.23	163±3.45	176±4.23	170±8.11	172±6.15
380.0	134+3.06	131+7.28	127+5.51*	119+2.31	114+4.21
760.0	130+6.55	126+3.16*	121+7.27	110+4.05*	103+1.73
1140.0	125+5.64	118+9.30*	108+3.54*	83+5.20*	90+3.51**
10 mg/kg/metformin (standard drug)	133+5.12	109+4.21*	93+2.90*	65+3.07*	80+4.51**

Data are expressed as mean ± SEM. of 5 replicates. $p<0.05$ and $p<0.01$ when compared to control.

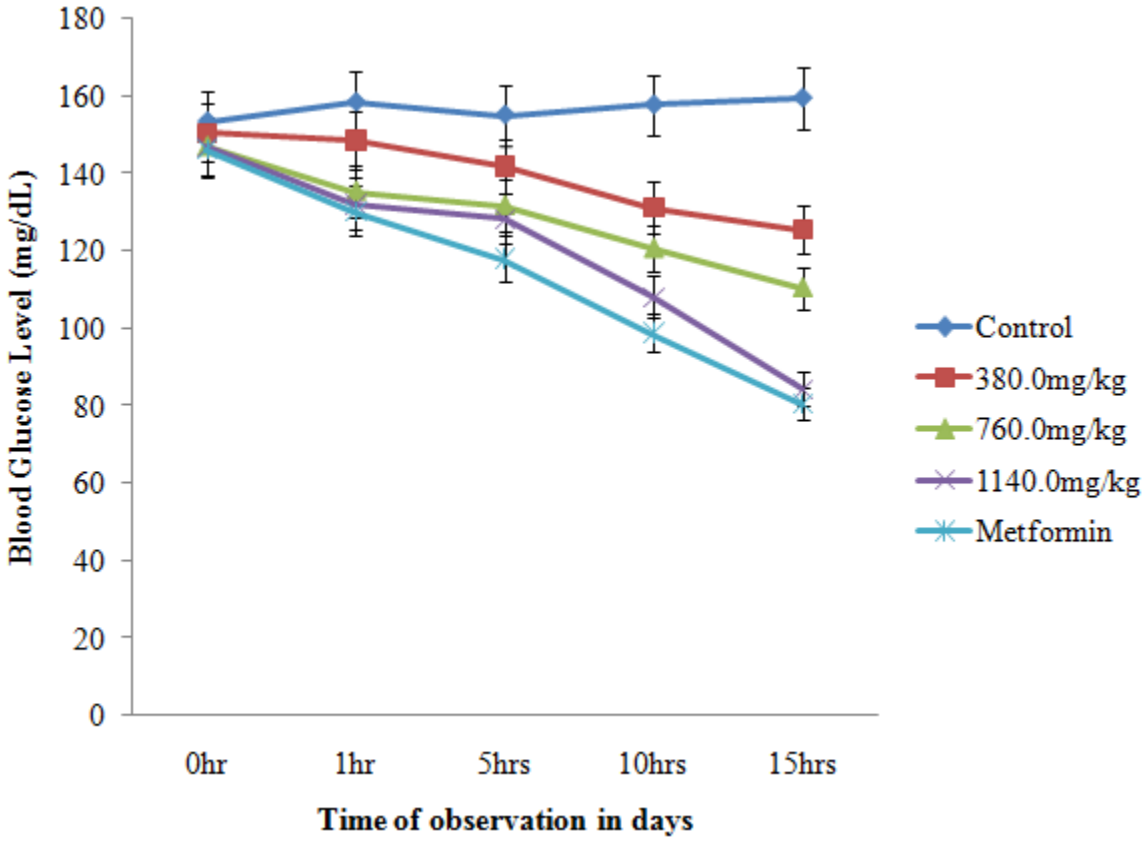

Figure 1: Antidiabetic activity of *Dioscorea bulbifera* during prolong study

DISCUSSION

The need for bioprospecting and development of ethnomedicinal plants as hypoglycemic agents is imperative especially now that most diabetic patients in Nigeria find it increasingly difficult to manage hyperglycemic conditions due to high cost of synthetic antidiabetic drugs with their subsequent side effects. This therefore supports this study.

The evaluation of *Dioscorea bulbifera* tuber extract for its antidiabetic activity in alloxan induced diabetic rats demonstrated a significant ($p<0.05$) reduction in hyperglycaemia. The observed hypoglycaemic effects of the tuber could be implication as a result of the presence of alkaloids, proteins and glycosides present in the extract. This finding corroborates with the earlier reports of Okokon *et al.*, (2009) and

Odoemena *et al.*, (2010) on the roles of some of the phytochemical compounds inherent in such plants. These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. The observed reduction in BGL of the diabetic rats by metformin in this study shows a severe state of diabetes. In this study, continuous administration with the extract for a period of 15 days caused significant decrease in Blood glucose level of the treated rats compared to untreated diabetic ones. The ethanol extract of *Dioscorea bulbifera* reduced the glucose level in the rats to 53.6% in a prolonged treatment study.

Some plants extracts have been reported to exert hypoglycemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin (Shenoy and Ramesh, 2002) or corrections of insulin resistance (Kim *et al*., 2006). Another possible mechanism of glucose reduction utilized by the aerial yam extract may be due to the action of the extract through extra pancreatic mechanism by inhibition of hepatic glucose production (Swantson-Flatt *et al.,* 1999 and Oyedemi *et al*., 2011). Some workers have reported that consumption of fruits and nuts such as apple and walnut, *(Tetracarpidium conophorum)* are associated with a lower risk for diabetes (Odoemena *et al.,* 2010). The result of this study clearly indicates that continuous consumption of *Dioscorea bulbifera,* tubers will always lower the BGL of a diabetic patient and therefore is recommended as a dieting menu for hyperglycemic patients.

Conclusion

The significant lowering of blood glucose level shown in the alloxan-induced diabetic rats in this study is good manifestation to show that *Dioscorea bulbifera* is an effective antidiabetic regimen. This result has given credence to the use of the aerial yam as a menu for diabetic patient under dieting. Further studies are hereby recommended for the isolation and characterization of the active principles responsible for the action as well as the elucidation of the mechanism of the action.

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