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Antidiabetic activities of ethanolic extract and fraction of *Anthocleista djalonensis*

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ABSTRACT

Objective: To evaluate the antidiabetic activities of ethanolic root extract/fraction of *Anthocleista djalonensis* (*A. djalonensis*) in alloxan-induced diabetic rats. **Methods:** *A. djalonensis* root extract/fractions (37-111 mg/kg) were administered to alloxan-induced diabetic rats for 14 days and blood glucose levels (BGLs) of the diabetic rats were monitored at intervals of hours and days throughout the duration of the treatment. **Results:** Treatment of alloxan-induced diabetic rats with the extract/fractions caused a significant ($P<0.001$) reduction in fasting BGLs of the diabetic rats both in acute study and prolonged treatment (2 weeks). The activities of the extract and fractions were more than that of the reference drug, glibenclamide. **Conclusions:** These results suggest that the root extract/fractions of *A. djalonensis* possess antidiabetic effect on alloxan-induced diabetic rats and this justifies its use in ethnomedicine and can be exploited in the management of diabetes.

1. Introduction

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin[1]. Diabetes mellitus is the sixth leading cause of death globally[2]. Several drugs have been used in the management of the disease. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems[3]. Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically[4]. More than 800 plants have been studied for their antidiabetic potentials[3,5] among thousands of plants used in various regions of the world.

Anthocleista djalonensis (*A. djalonensis*) A.Chev (Loganiaceae) is a medium-sized tree of the West tropical Africa, 30-45 feet high with blunt spines on the unbranch, pale grey trunk and widespreading crown[6]. The stem, root, bark and leaves of *A. djalonensis* are used to treat malaria, jaundice, diabetes and abscesses[6]. The Ibibios of Southern Nigeria use the leaves and stembark as malarial remedy[7]. Okorie[8] isolated phthalide and xanthones from *A. djalonensis*. Onocha *et al*[9] isolated monoterpene diol, djalonenol, as well as iridoid glucoside djalonenoside (also sweroside) from *A. djalonensis* and some of these compounds and their semisynthetic derivatives were found to be cytotoxic against the brain tumor transformed fibroblasts[10]. Reports of antibacterial and wound healing activities[11], *in vitro* anthelmintic activity[12] and antiplasmodial activity[7] have been published. Therefore, the present study was aimed to investigate the antidiabetic activity of ethanolic extract of the root and fractions to ascertian their ethnobotanical uses.

2. Materials and methods

2.1. Plant material

Leaves and stembark of *A. djalonensis* (A.Chev)

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(Loganiaceae) were collected in August, 2010 from Nyan forest in Uruan area of Akwa Ibom State and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. A voucher specimen of the plant was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.2. Plant extraction and fractionation

The roots collected were washed with clean water and air-dried for 2 weeks. These dried roots were pulverized (reduced to coarse powder) using pestle and mortar. The powdered root sample (2.0 kg) was divided into two parts; one part was exhaustively macerated in ethanol for 72 h to allow for proper extraction (cold extraction), while the second part was successively and gradiently macerated for 72 h in each of these solvents, *i.e.* chloroform, ethyl acetate and methanol. The mixtures were filtered with filter paper. The liquid filtrate was concentrated and evaporated to dryness in vacuo at 40 ℃ using a rotary evaporator to obtain good yield. The yield of each extract was calculated and recorded. The dry extract/fractions were stored in a refrigerator at 4 ℃ prior to use[13].

2.3. Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures[14], to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides and others.

2.4. Animals

The animals (Swiss albino mice and rats) of both sexes were used for these experiments. They were obtained from University of Uyo Animal House. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water was given *ad libitum*.

2.5. Determination of median lethal dose (LD_{50})

The LD_{50} of the extract was determined using albino mice. The extract was administered intraperitoneally (i.p.) and the method of Miller and Tainter[15] was adopted. This involved the administration of different doses of the extract (100-1 000 mg/kg) to groups of six mice each. The animals were observed for physical manifestation of signs of toxicity. The number of deaths in each group within 24 h was recorded.

2.6. Induction of diabetes

The animals (male rats) were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (130 mg/kg) in ice cold 0.9% saline (NaCl) solution. The animals were given 2 mL of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia. Seventy two hours later, rats with blood glucose levels (BGLs) above 200 mg/dL were considered diabetic and selected for the experiment.

2.7. Evaluation of antidiabetic activity of the extract and fractions

The animals were randomly divided into eight groups with 6 rats in each group and treated as follows:

Group I: Diabetic rats were administered with *A. djalonensis* extract (37 mg/kg/day) orally for 14 days.

Group II: Diabetic rats were given *A. djalonensis* extract (74 mg/kg/day) orally for 14 days.

Group III: Diabetic rats were administered orally with *A. djalonensis* extract (111 mg/kg/day) for 14 days.

Group IV: Diabetic rats were administered orally with chloroform fraction of *A. djalonensis* (74 mg/kg/day) for 14 days.

Group V: Diabetic rats were administered orally with ethyl acetate fraction of *A. djalonensis* (74 mg/kg/day) for 14 days.

Group VI: Diabetic rats were administered orally with methanol fraction of *A. djalonensis* (74 mg/kg/day) for 14 days.

Group VII: Diabetic rats were given glibenclamide (10 mg/kg/day) for 14 days orally.

Group VIII: Diabetic control rats were receiving normal saline (10 mL/kg) for 14 days.

The change in body weight and fasting BGLs of all the rats were recorded at regular intervals during the experimental period. For acute study, the BGLs were monitored after 1, 3, 5 and 7 h of administration of a single dose of the extract and at the end of 1, 3, 5, 7 and 14 days for prolonged treatments. The BGLs were monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were recorded[16].

3. Results

3.1. Phytochemical screening

The *A. djalonensis* root extract was confirmed to contain flavonoids, saponins, tannins, cardiac glycosides and anthraquinones.

3.2. Acute toxicity study

Administration of the ethanolic root extract of *A. djalonensis* (1 000-5 000 mg/kg) after initial body weakness did not produce any mortality in the animals. The LD_{50} was determined to be 5 000 mg/kg.

Table 1
Effect of ethanolic root extract of *A. djalonensis* on body weights of alloxan-induced diabetic rats (mean±SEM) (*n*=6).

Treatments	Dose (mg/kg)	Day 0	Day 15	% Increase/decrease in body weight
Control	10 mL	157.50±16.97	134.50±51.62	−14.60
Extract	37	140.60±18.06	151.60±5.82	7.82
	74	144.48±25.76	157.80±2.41	9.21
	111	131.90±17.98	142.60±15.70	8.11
Chloroform fraction	74	133.80±25.98	150.10±2.60	12.18
Ethyl acetate fraction	74	143.60±23.15	154.80±22.98	7.79
Methanol fraction	74	145.20±14.73	167.70±24.00	15.49
Glibenclamide	10	143.20±27.04	162.60±15.78	13.54

[a]: $P<0.001$ when compared to control.

Table 2
Antidiabetic effect of ethanolic leaf extract and fractions of *A. djalonensis* on blood glucose level of alloxan-induced diabetic rats during acute study (mean±SEM) (*n*=6).

Treatments	Dose (mg/kg)	Blood glucose level (mg/dL) in hours				
		0 h	1 h	3 h	5 h	7 h
Control	10 mL	239.40±8.82	251.00±8.39	255.00±7.24	258.40±6.65	254.20±6.26
Extract	37	230.90±4.34	125.00±5.91	43.00±9.24	79.40±2.19[b]	85.20±2.05[b]
	74	233.40±9.84	136.70±5.61[c]	37.50±13.86[b]	82.60±9.71[c]	86.00±8.54[c]
	111	220.60±10.81	113.30±5.73[a]	36.00±8.08[c]	94.00±5.54[c]	95.40±16.00[c]
Chloroform fraction	74	240.60±19.50	197.60±2.72[a]	34.00±7.37[c]	133.30±2.51[c]	133.60±2.48[c]
Ethyl acetate fraction	74	239.80±19.62	227.30±12.74	38.30±8.73[c]	145.00±9.44[b]	150.30±9.73[c]
Methanol fraction	74	236.40±21.34	204.00±21.60[a]	38.66±16.25[c]	185.30±20.80[c]	184.80±20.70[c]
Glibenclamide	10	242.40±4.61	239.00±6.24	226.60±6.02[c]	207.80±9.25[c]	197.00±5.47[c]

[a]: $P<0.05$, [b]: $P<0.01$, [c]: $P<0.001$ when compared to control.

Table 3
Effect of ethanolic leaf extract and fractions of *A. djalonensis* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment (mean±SEM) (*n*=6).

Treatments	Dose in (mg/kg)	Blood glucose level (mg/dL) in days						
		Day 0	Day 1	Day 3	Day 5	Day 6	Day 7	Day 15
Control	10 mL	239.40±8.82	240.20±3.41	252.40±5.64	258.20±6.01	239.20±7.69	232.60±5.31	224.20±3.56
Extract	37	230.90±4.34	33.20±9.29	125.40±4.00	94.00±17.34[a]	59.40±10.20[a]	61.00±7.93[a]	39.20±10.96[a]
	74	233.40±9.84	40.00±20.80	90.00±13.0	79.30±10.40[a]	64.20±16.01[a]	97.80±3.74[a]	51.30±14.01[a]
	111	220.60±10.81	54.40±23.86	110.60±11.84	67.50±4.16[a]	56.00±2.00[a]	64.40±11.54[a]	32.00±7.21[a]
Chloroform fraction	74	240.60±19.50	204.30±23.10	93.70±10.63	93.00±32.0[a]	57.60±1.52[a]	60.00±14.73[a]	33.20±6.50[a]
Ethyl acetate fraction	74	239.80±19.62	125.60±9.70	172.30±13.55	182.00±19.82[a]	51.70±11.50[a]	58.70±15.37[a]	44.00±12.16[a]
Methanol fraction	74	236.40±21.34	82.33±17.55	213.60±21.76	149.80±10.16[a]	55.00±8.54[a]	69.00±13.45[a]	36.00±6.08[a]
Glibenclamide	10	242.40±4.61	208.30±9.25	176.30±7.86	135.80±11.30[a]	86.00±10.79[a]	73.60±7.36[a]	59.60±6.80[a]

[a]: $P<0.001$ when compared to control.

3.3. Antidiabetic activity

There were observable changes in the body weight of treated and untreated diabetic rats. Treatment of diabetic rats with the root extract/fractions of *A. djalonensis* or glibenclamide improved the weight gain compared to untreated diabetic rats (Table 1).

A dose-dependent reduction in BGLs was observed in alloxan-induced diabetic rats treated with ethanolic root extract of *A. djalonensis*. After a single dose of the extract give to the alloxan-induced diabetic rats, there was a significant ($P<0.01$–0.001) reduction in BGLs of the diabetic rats within the period of acute study compared to control. The maximum effect was observed at 3 h with the various doses of the extract/fractions exerting comparable effect except the chloroform fraction that exerted a more pronounced effect. However, the effects of the extract/fractions were more than that of the standard drug, glibenclamide (Table 2).

During prolonged study (14 days), the extract/fractions produced a sustained significant ($P<0.001$) reduction in BGLs of the diabetic rats compared to control (Table 3). The effects of the highest dose of the extract and fractions were more than that of the standard drug, glibenclamide, 10 mg/kg, on day 15.

4. Discussion

Evaluation of antidiabetic activity of *A. djalonensis* root extract/fractions was carried out in alloxan induced diabetic rats. The extract which showed moderate toxicity was observed to demonstrate significant antidiabetic

activity in alloxan diabetic rats. There are a lot of reports implicating some phytochemical compounds in plants as being responsible for their antidiabetic activities[17-19]. Some of these phytochemical compounds revealed to be present in this extract include terpenes, saponins, tannins and alkaloids. These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. Glibenclamide, like other sulphonylureas, is effective in mild diabetic state and ineffective in severe diabetic animals where pancreatic β-cells are completely destroyed[20]. The observed reduction in BGLs of the diabetic rats by glibenclamide in this study portrays an insevere state of diabetes. In this study, continous treatment with the root extract/fractions of *A. djalonensis* for a period of 2 weeks caused significant decrease in BGLs of treated rats compared to untreated diabetic rats. This was followed by a significant increase in body weight of the treated rats. This is a reverse to diabetic state characterised by a severe loss in body weight due to loss or degradation of structural proteins[21]. This condition was alleviated by the treatment of the diabetic rats with root extract/fractions of *A. djalonensis*. Some plants' extracts are 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[20]. This root extract may have acted through one or more of the above mechanisms resulting in a superior antidiabetic activity than the glibenclamide.

In conclusion, the results of this study show that ethanolic root extract of *A. djalonensis* possesses antidiabetic properties. This confirmation justifies its use in ethnomedical medicine for the treatment of diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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