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Research Article

Antidiabetic activity of methanol root extract of *Aristolochia bracteolata* L. (Aristolochiaceae) on alloxan induced diabetic rats

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ABSTRACT: **This study investigated the hypoglycaemic activity of methanolic root extract of *Aristolochia bracteolata* in alloxan-induced diabetic rats. Thirty male albino rats weighing between 120-150g divided into six groups of five rats each were used for the study. Group 1 served as normal control while Group 2, 3, 4 and 5 were induced with 150mg/kg alloxan monohydrate dissolved in 0.9% sterile saline and group 6 served as the extract control. Group 2, 3 and 4 were treated with 100, 300mg/kg of the plant extract and 10mg/kg of glibenclamide respectively for seven days. Group 5 was not treated post induction and served as the diabetic control group. Induction of alloxan increased blood glucose level, reduced the haematological parameters and increased the biochemical enzymes in the untreated group. However, treatment with the plant extract and glibenclamide caused marked decreased in the blood glucose level and improved the heamatological indices. The results further showed a marked decrease in ALT, AST and ALP activity in the treatment groups when compared with the positive control group. These findings showed that *A. bracteolata* possess potent antidiabetic properties and beneficial in the management of Type 1 diabetes.**

KEYWORDS: ***Aristolochia bracteolata*, Chemical components, Alloxan monohydrate, Hypoglycaemic, Diabetes mellitus.**

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INTRODUCTION

Diabetes mellitus is regarded as a common metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Kameswararao *et al.*, 2003). The disease is characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism (Barcelo and Rajpathak, 2001). Hyperglycemia and glucose intolerance are common manifestations of several types of hormonal disturbances or imbalances, of which the most important is diabetes mellitus (Forster, 1987). Chronic hyperglycemia causes damage to eyes, kidneys nerves, heart and blood vessels and damage in some of these organs can lead to death (Pari and Saravanan, 2004; Mayfield, 1998).

Diabetes mellitus is considered to be at epidemic level (Petal and Rybczynski, 2003). According to the World Health Organisation (WHO) in 2000, at least 171 million people worldwide suffered from diabetes, or 2.8% of the population (Wild *et al.*, 2004). Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double (Wild *et al.*, 2004). The Diabetes Association of Nigeria (DAN) had put the diabetic's population in Nigeria at about 10 Million as at 2004 (Ogbera *et al.*, 2005).

Treatment of diabetes mellitus takes three main forms: diet and exercise, insulin replacement therapy and the use of oral hypoglycemic agents (Meyer *et al.*, 2000). Consequently, the current available synthetic hypoglycemic agents produce serious side effects (Gupta *et al*., 2008). Also, the high cost, availability, uncertainly of use during pregnancy has been some of the factors leading to a strong preference for hypoglycemic drugs of plants origin, which are believed to be effective for chronic treatments with less side effects (Okigbo and Mmeka, 2006).

Medicinal plants are largely used by all division of the population either directly as folk medicine or indirectly in the preparation of recent pharmaceuticals (Pushpangadan, 1995) The primary benefits of using plant derived medicines are that they are relatively safer, offering profound therapeutic benefits and treatment (Iwu *et al.,* 1999). The use of herbs for the treatment of diabetes mellitus has a long folkloric history (Sharma, *et al.*, 2007). Several plants and isolated compounds have been demonstrated to have anti-diabetic potentials (Grover *et al*., 2000; Li *et al*., 2004). Herbal formulation alone or in combination with oral hypoglycaemic agents sometimes produced good therapeutic responses in some resistant cases where modern medicines alone have failed (Anturlikar *et al.*, 1995; Ghosh *et al.*, 2004).

Aristolochia bracteolata is a plant that has been used traditionally for the treatment of several diseases including diabetes. It is a shrub belonging to the family Aristolochiaceae (MacMillan, 2008). It is commonly called worm killer in English and Akogun in Yoruba. They are commonly found and widespread across tropical Asia, Africa and South America (MacMillan, 2008). Some of the pharmacological use of *A. bracteolate* includes; anti-snakebite (Meenatchi *et al.*, 2009), anti-inflammation and as a purgative (Negi *et al*., 2003), antidermatitis, antileprosy and antijaundice (Ratna *et al*., 2011), antiulcer, antiamenorrhoea and antihelmintic agents (Seliya and Patel, 2009). However, there is dearth of information on the antidiabetic activity of this plant and the toxicological or otherwise effects of its use. This study therefore investigated the hypoglycemic activity of the root extract of *A. bracteolate* in alloxan induced diabetic rats and its effects on some aspects of the physiology of treated rats.

MATERIALS AND METHODS

Collection of plant materials

Whole plants of *Aristolochia bracteolata* were collected from the nursery of the Department of Botany, University of Ibadan, Nigeria. The plant was identified and authenticated at the University of Ibadan Herbarium (UIH). The roots were then cut-off from the samples for further studies.

Preparation of the extract

The dried roots were pulverized into a coarse powder using electric grinder and the powder was stored in an air-tight bottle at 4°C prior to experiments. 400g of the pulverized root of *A. bracteolata* was soaked in 300ml of methanol for 72hours. Cold maceration method as described by WHO (1998) was employed in the extraction of the plant material. The content was filtered through a Whatman filter paper 1 into a conical flask. The filtrate was concentrated using rotary evaporator at 40^{0}C and further concentrated into dryness using a vacuum oven set at 40ºC and a pressure of 600mmHg. The yield of the crude extract was 14.17g. The concentrated extract was stored in a properly labelled air- tight bottle at 4^0 C.

Phytochemical screening

Qualitative Phytochemical Screening, Proximate and Mineral determination

Qualitative phytochemical screening of the methanolic root extract of *A. bracteolata* was carried out using standard methods as described by Sofowora, (1993), Harbone (1998) and Trease and Evans (2006) for the detection of active components: saponins, tannins, alkaloids, flavonoids, terpenoids, anthraquinone, Phlobatannins, Phenols**,** Steroids, Cardenolides, Chalcones and glycosides.The powdered of *A. bracteolata* root was analysed for the presence of proximate contents: fats, proteins, fibre, moisture and ash, using standard methods as described by Association of Official Analytical Chemist[31]. The extract was thereafter digested at the Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria. The digested sample was analysed for the level of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) were analysed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bausch scientific AAS). The phosphorous content was determined using Molybdate (yellow method). The percentage transmittance was determined at 400nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

Experimental set-up

Animals: Thirty adult male Albino rats weighing between (120g-150g) were purchased from the animal house of the Department of Anatomy, University of Ibadan, Nigeria. The rats were acclimatized for two weeks in the well ventilated, pathogen free cages at room temperature (25 – 27°C) in the animal house of the Department of Zoology, University of Ibadan. They were feed with grower pelletized feed and given water *ad libitum*. All experiment was conducted according to the National Institute of Health guidelines of care and use of laboratory animals[33].

Chemicals

Alloxan used in carrying out this study was obtained from Sigma-Aldrich (Chemie Gmbh, Steinheim, Denmark). The standard drug used in the animal experiment was glibenclamide. The chemical and drug used were of analytical grade.

Induction of diabetes mellitus

Diabetes was induced in the rats after an overnight fast by a single intraperitoneal injection of 150mg/kg of alloxan monohydrate dissolved in 0.9% sterile saline per body weight. After a period of 48hours, the blood glucose concentrations were checked using the Accu-chek active glucometer. Rats having blood glucose level > 200 mg/dl were considered diabetic and used for this study. The extract was dissolved in distilled water and administered orally.

Experimental design

The rats were divided into six groups of five rats each.

Group 1: normal control

Group 2: alloxan-induced rats treated with 100mg/kg of the extract

Group 3: alloxan-induced rats treated with 300mg/kg of the extract

Group 4: alloxan-induced rats treated with 10mg/kg of glibenclamide

Group 5 untreated alloxan-induced rats (positive control)

Group 6: administered with 200mg/kg of the extract only (extract control)

Periodic weighing of rats and blood glucose determination

Throughout the course of experiment, the body weight of each rat was measured daily and recorded as mean weight per group. Mortality was also recorded daily. A drop of blood was collected by snip cutting the tail tip of the conscious rats daily before treatment with the extract. The blood glucose concentration was then determined by placing a drop of blood on the strip of the digital Accu-check glucometer (Roche diagnostics, Mannheim, Germany). Blood glucose level was checked an hour post induction and after treatment. At the end of the experiment, blood samples were collected from each surviving rat by ocular puncture into heparinised bottles for biochemical analysis.

Measurement of haematological indices

Packed Cell Volume (PCV) was estimated using microhaematocrit method, White Blood Cell (WBC), Red Blood Cell (RBC) were determined using microscopy and hemoglobin level was determined using cyanomethaemoglobin method as described by Baker and Silverton (1985).

Biochemical analysis

Estimation of liver enzymes

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were determined by method described by Reitman and Franked[35]. Alkaline Phosphatase (ALP) activities were measured using standard enzymatic methods as described by Schmidt and Schmidt (1963).

Statistical analysis

Data were expressed as mean ± standard error. Significant differences between groups were analysed using ANOVA of the SPSS computer software, version 16.0. Values were considered significant at $P<0.05$.

RESULTS

Qualitative phytochemical analysis of methanol root extract of *A. bracteolata*

A. bracteolata root contained high content of alkaloids, saponins, tannins, phlobatannins, phenols and glycosides, whereas anthraquinones, terpenes, steroids, chalcones, flavonoids were slightly present. Cardenolides were absent in sample (Table 1).

Proximate and mineral constituents of methanolic root extract of *A. bracteolata*

The proximate content of *A. bracteolata* is presented in Table 2. The sample was rich in fibre (26.80%) and protein (11.33%) contents (Table 2). *A. bracteolata* had high contents of iron (56.00 mg/kg) and zinc (45.70 mg/kg) (Table 3).

Mean body weight and percentage mortality of all the experimental groups

The mean weights of the rats before the induction of alloxan ranged between 150-170g (Table 3). However, induction of diabetes caused marked decrease of 40.6, 46.1, 29.8 and 32.8g in the mean body weight of groups 2, 3, 4, 5 respectively. Treatment with the extract resulted in increase in the body weight of treated rats. No mortality was recorded in all the groups prior to induction. At the end of the experiment, group 2 and 3 recorded 40% mortalities each while group 4 and 5 recorded 60% mortality respectively however, no mortality was recorded in group 1 and 6.

Table 1: Qualitative phytochemical analysis of methanolic root extract of *A. bracteolata*

Parameters	Inference
Alkaloids	+++
Saponins	+++
Tannins	+++
Phlobatannins	+++
Phenols	+++
Glycosides	+++
Anthraquinones	++
Terpenes	+
Steroids	+
Chalcones	+
Flavonoids	+

+ =slightly present, + + = moderately present, + + + = highly present, – = absent

Table 2: Proximate and mineral constituents of methanolic root extract of *A. bracteolata*

Proximate Constituents	Quantity
Protein (%)	11.33±1.00
Fat (%)	5.41±1.10
Fibre (%)	26.80±2.18
Ash (%)	6.22±1.27
Moisture (%)	7.71±1.11
Sodium (%)	0.02
Calcium (%)	0.06
Potasium (%)	0.12
Phosphorus (%)	0.36
Copper (mg/Kg)	7.65
Zinc (mg/Kg)	45.70
Iron (mg/Kg)	56.00

*Values are mean of two determination ± S.E

Table 3: Mean body weight (g) of all the experimental groups

Experimental animals	Pre-Induction Weight (g)	Induction Weight (g)	Post Treatment Weight (g)
Group 1 Control	150.4± 7.5^{a}	155.3±4.7^{b}	159.3±4.1^{c}
Group 2 (100 mg/kg)	173.3±5.3^{b}	132.7±3.9^{ab}	133.9±3.7^{b}
Group 3 (300 mg/kg)	175.6±4.7^{b}	129.5±6.3^{a}	137.4±4.9^{b}
Group 4 (10 mg/kg) Glibenclamide	151.4±3.9^{a}	121.6±6.1^{a}	123.5±7.1^{ab}
Group 5 Diabetic control	160.2±5.4^{a}	127.4±3.9^{a}	117.3±4.0^{a}

Values are mean ± S.E, (n≤5). Values within the same column with different superscript are significantly different at p≤0.05

Blood glucose concentration of alloxan-induced diabetic rats treated with methanol root extract of *A. bracteolata*

The glucose concentration (Table 4) prior to induction ranged between 97.33 and 108.67 mg/dL which are within the normal range of fasting blood sugar (FBS) level in rat. Intraperitoneal injection of 150mg/kg of alloxan monohydrate geometrically increased the glucose level of all induced rats. The FBS of the rats post-induction ranged from 244.67 to 413.33 mg/dL. Treatment with various doses of the extract caused a dose-dependent reduction of FBS in all treated rats. At the end of treatment, all rats treated with various doses of the extract had significantly ($p<0.05$) lower FBS (which were less than 200 mg/dL) compared to rats treated with glibenclamide. The FBS of the diabetic control rats were higher than 200mg/dL throughout the experiment.

Table 4: Mean glucose concentration of alloxan-induced diabetic rats treated with methanolic root extract of *A. bracteolata*

Experimental animals	Pre-induction (mg/dl)	Post-induction (mg/dl)	TREATMENT DAYS Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Group 1 Control	103.67 ±6.69^{a}	101.12 ±6.69^{a}	105.00 ±5.77^{a}	103.17 ±6.13^{a}	103.19 ±3.69^{b}	100.00 ±1.15^{ab}	98.33 ±2.72^{a}	103.00 ±1.81^{ab}	107.33 ±84.93^{a}
Group 2 (100 mg/kg)	108.67 ±11.46^{a}	244.67 ±61.55^{b}	177.33 ±71.75^{ab}	385.00 ±66.03^{b}	194.33 ±60.07^{ab}	143.67 ±36.43^{b}	229.67 ±86.25^{ab}	259.33± 68.81^{c}	199. 00±8.83^{c}
Group 3 (300 mg/kg)	97.33 ±8.41^{a}	340.67 ±66.02^{c}	246.33 ±15.16^{b}	400.67 ±98.10^{c}	133.33 ±46.72^{b}	136.33 ±49.69^{b}	288.00 ±39.00^{c}	246.50 ±66.50^{c}	151.00 ±11.00^{b}
Group 4 (10 mg/kg) Glibenclamide	106.00 ±2.64^{a}	391.67 ±53.44^{c}	366.33 ±51.77^{c}	585.33 ±2.18^{e}	205.67 ±44.93^{b}	196.00 ±25.32^{c}	226.67 ±114.89^{ab}	191.00 ±54.00^{b}	210.00 ±10.00^{c}
Group 5 Diabetic control	106.00 ±2.62^{a}	413.33 ±58.47^{d}	246.67 ±38.74^{b}	477.67 ±37.56^{d}	310.67 ±42.33^{c}	281.67 ±36.19^{d}	254.33 ±75.45^{b}	241.27 ±26.21^{c}	225.61 ±15.33^{d}
Group 6 Extract only	102.67 ±4.17^{a}	102.67 ±4.17^{a}	88.33 ±4.80^{a}	93.33 ±13.38^{a}	68.33 ±9.24^{a}	77.33 ±18.98^{a}	95.33 ±2.18^{a}	79.67 ±10.08^{a}	91.33 ±17.63^{a}

Values are mean ± S.E, ($n \leq 5$). Values within the same column with different superscript are significantly different at $p \leq 0.05$

Haematology of alloxan-induced diabetic rats treated with methanol extract of *A. bracteolata*

Untreated diabetes mellitus caused marked decrease in the PVC, Hb and RBC counts in the positive control (group 5) compared to other groups treated with various doses of the extract and glibenclamide. However, rats treated with the extract (group 2 and 3) had significantly higher ($p<0.05$) PVC, Hb and RBC values compared to group 4 treated with 10mg/kg of glibenclamide. Group 6 that was treated with the extract alone had the highest erythropoetic indices compared to the treated and control groups. The highest WBC and platelet counts were recorded in group 2 and 3 treated with 100 and 300mg/kg of the extract respectively while diabetic and normal control groups had the lowest WBC and platelet counts.

Table 5: Haematological analysis of alloxan-induced diabetic rats treated with methanolic root extract of *A. bracteolata*

Groups	PCV (%)	Hb (g/dl)	RBC (×10³µl)	WBC (×10³µl)	Platelets (×10³µl)
Group 1	41.33±1.66^{b}	13.26±0.43^{b}	4.04±0.35^{a}	6583.33±2560.97^{c}	125000.00±47606.02^{b}
Group 2	44.33±1.20^{c}	14.20±0.40^{bc}	7.20±0.17^{d}	9050.00±946.48^{d}	160000.00±20784.61^{c}
Group 3	42.00±3.00^{b}	14.00±0.80^{bc}	6.86±0.35^{c}	3625.00±1625.00^{ab}	80000.00±18000.00^{ab}
Group 4	37.50±0.50^{ab}	12.55±0.05^{ab}	6.47±0.09^{c}	3475.00±1475.00^{a}	71500.00±1500.00^{a}
Group 5	31.50±3.00^{a}	10.21±2.11^{a}	5.98±2.07^{b}	3211.76±1314.14^{a}	69300.00±1100.00^{a}
Group 6	44.00±2.30^{c}	14.36±1.13^{bc}	7.56±0.38^{d}	4966.67±704.94^{b}	77666.67±9061.51^{ab}

Values are mean ± S.E, (n≤5). Values within the same column with different superscript are significantly different at p≤0.05. Group 1 (Control), Group 2 (100mg/kg extract), Group 3 (300mg/kg extract), Group 4 (10mg/kg glibenclamide), Group 5 (Diabetic control), Group 6 (200mg/kg, extract only)

Table 6: Liver enzymes activities in serum of alloxan-induced diabetic rats treated with methanolic root extract of *A. bracteolate*

Groups	AST	ALT	ALP
Group 1	41.33±0.33^{a}	29.67±0.88^{a}	73.67±2.02^{a}
Group 2	46.33±0.33^{ab}	32.33±1.20^{b}	78.00±4.16^{ab}
Group 3	44.00±3.00^{ab}	32.50±0.50^{b}	85.50±3.50^{c}
Group 4	46.00±3.00^{ab}	35.50±1.50^{b}	78.50±3.50^{ab}
Group 5	49.50±3.00^{b}	39.20±2.20^{c}	89.50±1.50^{d}
Group 6	45.33±1.20^{ab}	33.00±1.15^{b}	82.00±2.08^{b}

Values are mean ± S.E, (n≤5). Values within the same column with different superscript are significantly different at p≤0.05. Group 1 (Control), Group 2 (100 mg/kg extract), Group 3 (300 mg/kg extract), Group 4 (10mg/kg glibenclamide), Group 5 (Diabetic control), Group 6 (200 mg/kg, extract only)

Liver enzymes activities of alloxan-induced diabetic rats treated with methanolic root extract of *A. bracteolata*

The highest activities of AST, ALT and ALP were recorded in the serum of diabetic control rats compared to all the other experimental groups. However, rats treated with 300mg/kg of the extract recorded a marked decrease in the AST level compared to those treated with 100mg/kg of the extract and 10mg/kg of glibenclamide. Also, there was a decrease in the ALT levels of rats in groups 2 and 3 compared to group 4 while rats in group 3 recorded a higher ALP level compare to groups 2 and 4.

DISCUSSION

Diabetes mellitus is a chronic disease characterized by hyperglycemia caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin (Matsui *et al.,* 2007). However, numerous plants have being reported to show antidiabetic effects because they possess potent hyperglycaemic properties that can aid increase in insulin production through the pancreatic tissues (Mitra *et al.*, 1996; Shukla *et al.,* 2000; Bhattaram *et al.*, 2002).

Alloxan is regarded as a diabetes chemical with a cytotoxic action which is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration leading to a rapid destruction of beta cells (Jadhav, 2009). In this study, alloxan-induced groups showed significant elevated levels of blood glucose post induction which is expected and this might be due to pancreatic β-cells necrosis mediated by alloxan which enhances ATP dephosphorylation resulting in the generation of free radicals into the blood circulation or simply due to decrease in serum insulin or an integration of both processes.

However, treatment with different dosage of the methanolic root extract of *A. bracteolate* restores the high blood glucose to normal an indication that the plant has a potent hypoglycemic activity. Although, the exact mechanism in restoring a normal blood glucose level is not well understood but the probable cause might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor or *A. bracteolate* could play a role in repairing the damage of pancreatic beta cells and promoting insulin synthesis thereby lowering the level of plasma glucose. Documented reports have shown that species of Aristolochia posses hypoglycermic properties (Goverdhan *et al.,* 2008; Marlin and Rajeshkumar, 2012; Ramachandran and Divya, 2013).

There was a decrease in PCV, Hb and RBC of the positive control which may indicate that high blood glucose level can cause haematological alterations, however, the plant extract was able to reverse this condition with a marked increase in the haematological indices which implies that the plant may serve as a blood boosting factor.

In diabetic patients, altered enzymatic activities of AST, ALT and ALP are physiologically and clinically important. These enzymes are located in the cytosol or mitochondria of a variety of tissues both in the liver and other organs. Any perturbations to these cells or tissues as a result of the presence of a xenobiotic substance or free radical may affect the overall biochemical processes in experimental animals and may result in increase or decrease in the activity of such enzymes (Edet *et al.,* 2011). In this study, higher enzyme activity of AST, ALP and ALT levels recorded in the untreated alloxan induced rats is an indication of hepatic damage or tissue injury which occurs normally in diabetes. However, treatment with the plant extract decreased the AST, ALT and ALP activities an indication of

the possible antihepatotoxic effect. According to Varadarayan *et al*. (2008), phytochemicals and other chemical constituents of plants account for their medicinal value. The observed activity could be due to the phytochemical, proximate and mineral constituents' resident in the plant. Studies have reported different activities of plant constituents on various ailments including diabetes (Muzychkina, 1998; Norberg *et al*., 2004; Okwu and Okwu, 2004; Stanley *et al*. 2004; Miura *et al*., 2005; Manikandan *et al*., 2006; Mankil *et al*., 2006; Raghavan and Krishnakumari, 2006; Singh, 2010). The present study confirmed the antidiabetic activity of methanol root extract of *A. braceteolata*. However, further studies to isolate/identify the lead active principles of the plant as well as to elucidate their mechanism(s) of action is suggested while more detailed investigation is needed to ascertain dose and safety.

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