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Anti-Diabetic Effect of *Anthocleista vogelii* Ethanolic Root Extract in Alloxan-Induced Diabetic Rats

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ABSTRACT

This study was carried out to evaluate the potential anti-diabetic effect of *Anthocleista vogelii* ethanolic root extract in alloxan-induced diabetic rats. Albino rats of both sexes were randomly divided into five groups with five rats each. Group 1 (control; 10 mL kg^{-1} distilled water), group 2-4 (100, 200 and 400 mg kg^{-1} *A. vogelii* ethanolic root extract) and group 5 (5 mg kg^{-1} glibenclamide). Diabetes was induced physiologically using 10 g kg^{-1} glucose p.o. and chemically using 150 mg kg^{-1} alloxan i.p. Fasting blood glucose levels of the diabetic rats were determined at intervals of 30, 60, 120 and 240 min in glucose loaded rats and on days 4, 7, 10 and 14 in alloxan-induced diabetic rats. After two weeks, the levels of serum cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, alanine aminotransferase, aspartate amino transferase and creatinine of all the groups were analyzed. The LD_{50} of *A. vogelii* ethanolic root extract was ≥5000 mg kg^{-1} (p.o.). The extract exerted a significant ($p<0.05$) reduction in Fasting blood glucose levels, serum cholesterol, triglyceride, low density lipoprotein, creatinine, alanine aminotransferase and aspartate aminotransferase levels and an increase in serum high density lipoprotein levels when compared to the control. The extract also elicited a significant decrease in body weight, food and water intake in diabetic treated rats. The results show that *A. vogelii* ethanolic root extract have anti-diabetic and anti-hyperlipideamic effect when administered for 14 days in alloxan-induced diabetic rats.

Key words: Diabetes, *Anthocleista vogelii*, alloxan, glibenclamide, root

INTRODUCTION

Medicinal use of plants in the prevention, treatment and management of diseases in recent years are on the increase and studies concerning the biological effect of medicinal plants used for the treatment of various diseases are also on the increase. One of such ailments that herbal remedies have been widely used to manage is that of diabetes (Lu and Foo, 1995; Katerere and Eloff, 2005; Balde *et al*., 2006). Diabetes mellitus is a metabolic disorder, characterized by high fasting blood glucose level (hyperglyceamia) due to lack or insufficiency of insulin production by the beta cells of the islet of langerhans found in the pancreas or because the cells in the body are not sensitive to the insulin that is being produced by the beta cells (Murray *et al*., 2003). This metabolic disorder is also characterized by hypercholesterolemia (high serum concentration of cholesterol, triglyceride and low density lipoprotein and low serum concentration of functional high density

lipoprotein) (Nathan *et al*., 2005). In ethno-medicine, different parts of *A. vogelii* have been reported in literature to be used for the management and treatment of various diseases. For instance, the decoction of *A. vogelii* root is reported to be commonly taken to treat constipation and to regulate menstruation. In Sierra Leone, the decoction of the root is taken to alleviate chest pain and for the treatment of hepatitis when taken with lemon. In Ghana also, it has been reported that a root decoction of *A. vogelii* and *Combretum mucronatum* with pepper and ashes is taken to treat chest pain (Jegede *et al*., 2011). In South western part of Nigeria, traditional medicine practitioners use the leaves and root of *Anthocleista vogelii* for the treatment of stomach ache, pile, hepatitis and diabetes. Hence, this study evaluates the anti-diabetic effect of the ethanolic root extract of *Anthocleista vogelii* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

The animal experiments were performed according to the approved guidelines of Obafemi Awolowo University research ethics committee.

Plant collection and extraction: *Anthocleista vogelii* root was collected from the premises of National Biotechnology Development Agency, Bioresources centre, Ogbomosho, Oyo state and it was authenticated by Mr. G. Ibhanesebhor, officer in charge of Ife-Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. A voucher specimen (No. 17399) was deposited at Ife-Herbarium. The 1.0 kg of *A. vogelii* root was washed, air dried, pulverized and macerated in 4.0 L of 70% ethanol for 72 h before undergoing filtration using muslin cloth and cotton wool in funnel. The filtrate was then concentrated into a solid paste *in vacuo* at 45°C using a rotary evaporator (Mogale *et al*., 2012) and was then freeze dried using a freeze drier. The dried extract was then stored in a refrigerator at 4°C prior to use.

Animals: Albino rats (both sexes) weighing between 150-200 g were obtained from Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State. They were kept in well ventilated aluminium cages and fed with Vita feed and were given water *ad libitum*. The rats were allowed to acclimatize with the environment at ambient temperature under natural day light/night conditions for two weeks before the start of the experiment.

Preparation of extract for administration: The dried extract was reconstituted in distilled water (40 mg extract mL^{-1} of distilled water) and was administered to the animals orally using feeding cannula.

Acute toxicity studies (Median lethal dose [LD_{50}] determination): The (LD_{50}) of the root extract was determined in Albino rats through oral route (p.o.) using the method of Lorke (1983). The study was carried out in two phases:

- **Phase 1:** In the first phase, nine Albino rats were randomly divided into three groups of three rats each. Each group (1-3) was given 10, 100 and 1000 mg extract kg^{-1} b.wt., (p.o.). The rats were kept under laboratory ambient conditions and observed for signs of toxicity which include but not limited to stretching, respiratory distress, change in body weight and mortality for the

first critical 4 h and there after daily for 14 days. The result obtained from this test was used as basis for selecting the subsequent doses in the phase two test

- **Phase 2:** In the second phase of the study, higher doses were administered to the animals because 1000 mg kg^{-1} from phase 1 caused no death. To another fresh set of three groups of a rat each, 1600, 2900 and 5000 mg kg^{-1} b.wt., (p.o.) of the extract was administered. The animals were examined at 10, 30, 60 and 120 min and at 4, 6 and 24 h for gross behaviourial changes as under phase 1 and subsequently for mortality

Glucose loading: The 10 g kg^{-1} b.wt., of glucose was administered orally (p.o.) to Albino rats that were fasted overnight (for 12 h). After 30 min of glucose administration, the blood glucose level was checked using glucometre and glucose strip (Etuk, 2010). Rats with blood glucose level above 7.0 mmol L^{-1} were taken for the test.

Induction of diabetes using alloxan monohydrate: The animals (rats) were fasted overnight (for 12 h) and diabetes was induced by a single intraperitoneal injection (i.p.) of freshly prepared solution of alloxan monohydrate (150 mg kg^{-1}) in ice cold distilled water. The 72 h later rats with Fasting Blood Glucose Levels (FBGL) above 11.1 mmol L^{-1} (200 mg dL^{-1}) were considered diabetic and selected for the experiment (Lenzen and Munday, 1991; Okokon and Nwafor, 2009).

Administration of doses: Diabetic rats were randomly sorted into five groups of five rats per group. Groups 1-5 received 10 mL kg^{-1} distilled water, 100, 200 and 400 mg kg^{-1} *A. vogelii* ethanolic root extract and 5 mg kg^{-1} glibenclamide respectively (p.o.) for 14 days.

Reagents: Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRT), cholesterol (CHOL), triglyceride (TRIG) and High Density Lipoprotein (HDL) concentration were purchased from Randox Laboratories Limited, U.K. Determination of Biochemical Parameters: The FBGL was measured at 0, 30, 60, 120 and 240 min and also on day 1, 4, 7, 10 and 14 (Adebajo *et al*., 2007; Okokon and Nwafor, 2009) using glucometer and glucose strips (Accu-Check Active Glucometer, model: GC0088, Mannheim Germany). The animals were fasted overnight on the 14th day before they were sacrificed on the 15th day. On the 15th day, the rats one at a time were euthanized in an air tight glass chamber saturated with diethyl ether, they were dissected and blood samples were collected by cardiac puncture into plain bottles. Diethyl ether was used because it is a slow onset and a long acting anesthesia; it takes a longer time for animals to become anesthetized and hence increases the margin of safety in place of chloroform that is a potent hepatotoxin and a suspected carcinogen (ATSDR., 1997). Blood samples in plain bottles were centrifuged at 2500 rpm for 25 min and the serum was used for biochemical analysis these include; CHOL (Abbel *et al*., 1952, Richmond, 1973; Roeschlau *et al*., 1974; Trinder, 1969), TRIG (Tietz, 1990; Trinder, 1969; Koditschek and Umbreit, 1969), HDL, LDL (Jacobs *et al*., 1990), CRT (Bartels *et al*., 1972; Schirmeister *et al*., 1964), AST and ALT (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963).

Body weight: The body weight was taken daily and the dose of the extract administered was adjusted accordingly. The percentage change in body weight was calculated and used for statistical analysis.

Food and water intake: The weight of food and volume of water consumed by rats in each group were measured daily as the difference between the quantity of food and water supplied and the amount remaining after 24 h, respectively. The weekly percentage food intake/body weight and weekly percentage water intake/body weight was calculated and used for statistical analysis.

Statistical analysis: All quantitative data were expressed as the mean±standard error of mean (Okokon and Nwafor, 2009; Osadebe *et al*., 2014). Statistical analysis was carried out using one way analysis of variance and significant difference between means was assessed using Bonferroni t-test at 95% level of significance using Primer (version 3.01).

RESULTS

Acute toxicity studies of *A. vogelii* aqueous root extract: The animals showed no changes in general appearance during the 14 days period. There was also no mortality or morbidity observed in the rats throughout the fourteen days period following single oral administration of all selected doses of *A. vogelii* ethanolic extract. The LD_{50} ≥5000 mg kg^{-1} b.wt., for *A. vogelii* ethanolic root extract (p.o.).

Effect of *A. vogelii* ethanolic root extract on percentage FBGL: The ethanolic extract and glibenclamide exerted a significant decrease in FBGL in both glucose loaded (Table 1) and alloxan-induced diabetic rats (Table 2) when compared with the control.

Effect of ethanolic root extract of *A. vogelii* on lipid profile in alloxan-induced diabetic rats: The ethanolic extract and glibenclamide elicited a significant ($p<0.05$) decrease in CHOL, TRIG and LDL levels and an increase in HDL level when compared with the control (Fig. 1).

Effect of ethanolic root extract of *A. vogelii* on biochemical parameters in alloxan-induced diabetic rats: The ethanolic extract and glibenclamide also elicited a significant ($p<0.05$) decrease in ALT, AST and CRT levels when compared with the control (Fig. 2).

Table 1: Effect of *A. vogelii* ethanolic root extract on percentage FBGL in glucose loaded rats

		Time (min)				
Groups	Does	0	30	60	120	240
Control	10 mL kg^{-1} distilled water	100.0±1.0	100.0±0.6	92.5±0.58	88.3±0.7	76.1±0.3
Ethanolic extract	100 mg kg^{-1}	100.0±1.1	88.0±0.8*#	82.6±0.6*#	68.5±0.9*#	59.4±0.4#
Ethanolic extract	200 mg kg^{-1}	100.0±1.8	95.7±1.5*#	80.0±1.9*	67.0±1.2*	44.3±0.3*#
Ethanolic extract	400 mg kg^{-1}	100.0±0.8	91.7±0.3*	81.2±0.3*#	74.0±0.4*#	59.4±0.4#*
Glibenclamide	5 mg kg^{-1}	100.0±0.4	93.3±0.2*	77.1±0.4*	662.8±0.3*	50.0±0.2*

Values are given as Mean±SEM, n: 5, *$p<0.05$ comparison of values vs that of control at Tt, #$p<0.05$ comparison of values vs that of glibenclamide at Tt, Tt: Percentage FBGL at 30, 60, 120 and 240 min

Table 2: Effect of *A. vogelii* ethanolic root extract on percentage FBGL in alloxan-induced diabetic rats

		Days				
Groups	Does	1	4	7	10	14
Control		100.0±3.2	101.1±2.8	102.4±3.1	103.2±3.0	102.9±3.2
Ethanolic extract	100 mg kg^{-1}	100.0±0.7	81.3±0.6*	39.30±0.6*#	24.80±0.5*#	19.90±0.3#
Ethanolic extract	200 mg kg^{-1}	100.0±1.6	75.3±1.6*#	28.60±0.8*#	22.30±0.6*#	16.80±0.5*#
Ethanolic extract	400 mg kg^{-1}	100.0±1.1	70.2±1.6*#	35.40±0.8*#	24.20±0.8*#	19.70±0.5#
Glibenclamide	5 mg kg^{-1}	100.0±1.0	77.0±1.2*	53.00±0.1*	39.80±0.5*	23.20±0.4*

Values are given as Mean±SEM, n: 5, *$p<0.05$ comparison of values vs that of control at D_d, #$p<0.05$ comparison of values vs that of glibenclamide at D_d, D_d: Percentage FBGL on day 4, 7, 10 and 14

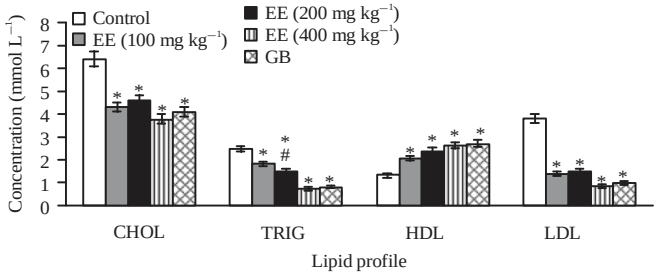

Fig. 1: Effect of *A. vogelii* ethanolic root extract on lipid profile (serum CHOL, TRIG, HDL and LDL) in alloxan-induced diabetic rats, Control: 10 mL kg^{-1} distilled water, EE: Ethanolic extract, GB: Glibenclamide, Values are given as Mean±SEM, n = 5, *Significantly different from control at $p<0.05$, #Significantly different from glibenclamide at $p<0.05$, CHOL: cholesterol, TRIG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein

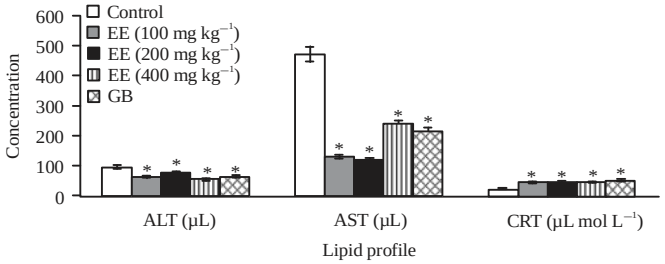

Fig. 2: Effect of *A. vogelii* ethanolic root extract on serum liver and kidney enzymes (ALT, AST and CRT) in alloxan-induced diabetic rate, Control: 10 mL kg^{-1} distilled water, EE: Ethanolic extract, GB: Glibenclamide, Values are given as Mean±SEM, n = 5, *Significantly different from control at $p<0.05$, #Significantly different from glibenclamide at $p<0.05$, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRT: Creatinine

Effect of *A. vogelii* ethanolic root extract on percentage in body weight in alloxan-induced diabetic rats: The ethanolic extract at 100 and 200 mg kg^{-1}, the extract exerted a significant ($p<0.05$) decrease in body weight in week 2 when compared with week 0 and decrease in body weight in week 1 ($p<0.05$) and 2 ($p<0.05$) when compared with glibenclamide (Fig. 3). At 400 mg kg^{-1}, the extract exerted a significant ($p<0.05$) increase in body weight in week 2 when compared with the control.

Effect of *A. vogelii* ethanolic root extract on weekly percentage food intake per body weight in alloxan-induced diabetic rats: The ethanolic extract and glibenclamide elicited a significant ($p<0.05$) decrease in food intake in week 2 when compared with week 1 (Fig. 4). The ethanolic extract (100 and 200 mg kg^{-1}) exerted a significant ($p<0.05$) decrease in food intake in week 2 when compared with glibenclamide (Fig. 4).

Effect of *A. vogelii* ethanolic root extract on weekly percentage water intake per body weight in alloxan-induced diabetic rats: The ethanolic extract elicited a significant ($p<0.05$) decrease in water intake in week 2 when compared with the control, a significant ($p<0.05$) decrease

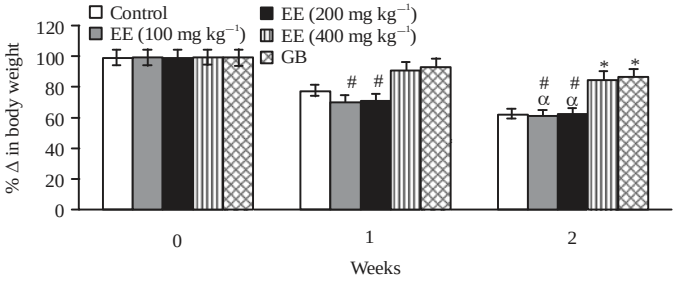

Fig. 3: Effect of *A. vogelii* ethanolic root extract on percentage in body weight in alloxan-induced diabetic rats. Control 10 mL kg^{-1} distilled water, EE: Ethanolic extract, GB: Glibenclamde, Values are given as Mean±SEM, n = 7, *p<0.05 comparison of values vs that of control, #p<0.05 comparison of values vs that of glibenclamide, a: p<0.05 comparison of week 0 values vs week 2 within the group

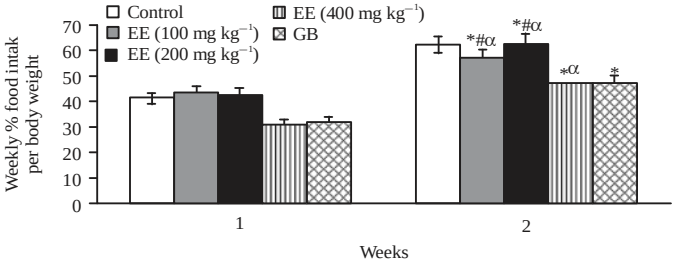

Fig. 4: Effect of *A. vogelii* ethanolic root extract on weekly percentage food intake per body weight in alloxan-induced diabetic rats, Control: Distilled water, EE: Ethanolic extract, GB: Glibenclamide; Values are given as Mean±SEM, n = 7, *p<0.05 comparison of values vs that of control, #p<0.05 comparison of values vs that of glibenclamide, a: p<0.05 comparison of values in week 1 vs week 2 within the group

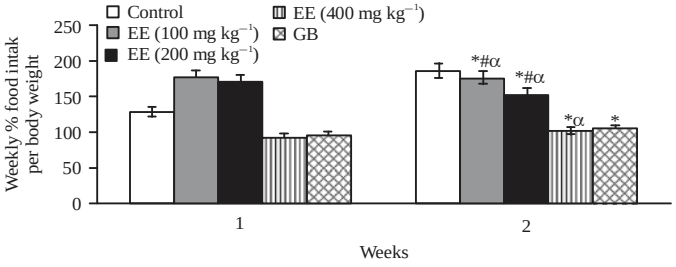

Fig. 5: Effect of *A. vogelii* ethanolic root extract on weekly percentage water intake per body weight in alloxan-induced diabetic rats, Control: 10 mL kg^{-1} distilled water, EE: Ethanolic extract, GB: Glibenclamide, Values are given as Mean±SEM, n = 7, *p<0.05 comparison of values vs that of control, #p<0.05 comparison of values vs that of glibenclamide, a: p<0.05 comparison of values in week 1 vs week 2 within the group

in water intake in week 2 when compared with week 1 and a significant (p<0.05) increase in water intake in week 2 when compared with glibenclamide (Fig. 5).

DISCUSSION

The acute toxicity result shows that *A. vogelii* ethanolic root extract are safe following oral administration in Albino rats. Diabetes mellitus arises from a deficient production of insulin by the beta cells of the pancreatic islets which lead to the complete or relative insufficiency of insulin secretion and or insulin action (Balkau *et al*., 2000). Over the years, several animal models have been developed for testing anti-diabetic agents. Two models were employed in the induction of hyperglyceamia; these models include oral glucose loading (physiological induction of diabetes mellitus) and alloxan-induced diabetes model (chemical induction of diabetes mellitus) (Etuk, 2010). Oral glucose loading model has to do with the induction of hyperglycaemia in the presence of intact pancreas while alloxan is a diabetogenic agent that selectively destroys the insulin secreting pancreatic beta cells when administered intraperitoneally to an experimental animal. Alloxan induce a multiphasic blood glucose response, which is accompanied with corresponding inverse changes in the plasma insulin concentration as well as sequential ultrastructural beta cell changes finally leading to necrotic beta cell death (Mythili *et al*., 2004). Alloxan not only destroys the pancreatic beta cells, it also causes kidney damage, which is however reversible (Gupta *et al*., 2005). Glibenclamide was used as the standard drug in the present study. Glibenclamide belongs to the class of sulphonylureas (anti-diabetic drug) and it has been widely accepted as a standard drug in diabetic animal experiments associated with mild or moderate hyperglycaemia (Owolabi *et al*., 2011). It has been proposed that sulphonylureas produce anti-diabetic effects through secretion of insulin (Jackson and Bressler, 1981). The FBGL of *A. vogelii* treated diabetic rats was reduced significantly at 30 min in glucose loaded rats and on the 4th day onwards all through the period of the experiment in alloxan-induced diabetic rats. The 200 mg kg^{-1} ethanolic extract elicited a more reduction in FBGL than the other ethanolic extract doses. Osadebe *et al.* (2014), reported that the stem bark of *A. vogelii* possesses hypoglycemic activity in both normoglyceamic and alloxan-induced diabetic animals. Phytochemical studies carried out on *A. vogelii* ethanolic root bark extract showed that the extract contained alkaloid, saponin, tannin, steroid and cardiac glycosides (Anyanwu *et al*., 2013) while, the powdered leaves and stem bark revealed the presence of carbohydrates, saponins, flavonoids, terpenes, sterols and phenols (Jegede *et al*., 2011). Studies have shown that the presence of flavonoids in plants helps in the reduction of fasting blood glucose levels since flavonoids have been found to stimulate the secretion of insulin (Owolabi *et al*., 2011). The possible mechanism of action in relation to reduction of FBGL might be that it; stimulates the pancreatic beta cells to secrete insulin, improves insulin sensitivity (Bosenberg and van Zyl, 2008), slows down absorption of carbohydrate and hence slows down glucose production (Kruger and Gloster, 2004) or it slows down gastric emptying and increase satiety (VanDeKoppel *et al*., 2008). Diabetes mellitus is usually associated with high levels of serum lipids and such an increase causes a risk factor for coronary heart disease (Nathan *et al*., 2005). The result of this study reveals that the administration of *A. vogelii* ethanolic root extract not only lowered serum cholesterol, triglyceride and low density lipoprotein level but also enhanced serum high density lipoprotein level in alloxan-induced diabetic treated rats. In diabetic state, insulin deficiency also contributes to derangements of various metabolic and regulatory mechanisms in the body (Nathan *et al*., 2005). *Anthocleista vogelii* ethanolic root extract exerted a significant decrease in alanine aminotransaminase, aspartate aminotransaminase and creatinine level in alloxan-induced diabetic treated rats. The result of serum liver and kidney enzymes suggests that the extracts have the potential of reducing the serum levels of these enzymes in diabetic conditions. *Anthocleista vogelii* ethanolic root extract exerted this effect may be by virtue of the phytochemicals

found present in the plant. High blood sugar levels in diabetic state also leads to increased thirst (polydipsia), increased hunger (polyphagia) and at times slight increase or decrease in body weight (Zhang, 2000). *Anthocleista vogelii* ethanolic root extract elicited a significant decrease in body weight in alloxan-induced diabetic treated rats. The decrease in body weight could be due to dehydration and the catabolism of fats and proteins in the setting of diabetes mellitus and due to unavailability of carbohydrate for utilization as energy source (Nabeel *et al*., 2010). The ethanolic extract also elicited a significant decrease in food and water intake in alloxan-induced diabetic treated rats.

CONCLUSION

The study concluded that *A. vogelii* ethanolic root extract is safe when administered acutely (p.o.), it has anti-diabetic and anti-hyperlipideamic effect when administered for fourteen days in alloxan induced diabetic rats. This justifies the use of the plant roots in ethno-medicine for the management of diabetes.

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