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Full Length Research Article

EFFECT OF AQUEOUS EXTRACT OF *ANISOPUS MANNI* STEM IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT
This study examined the effect of aqueous extract of *Anisopus manni* stem in alloxan-induced diabetic rats. Fifteen male rats were randomly distributed into three groups of five rats each. Normal and diabetic control groups were given distilled water. Diabetic treated group, received 400 mg/kg body weight of aqueous extract of *Anisopus manni*. All Administrations (oral) were carried out daily for 28 days using gavage. Fasting blood glucose and body weight were recorded at intervals of 7 days. Significant (P<0.05) increases in TG, Total cholesterol, LDL-C with significant (P<0.05) decrease in HDL-C levels were observed in diabetic control compared to normal control. Significant increase was also observed in the fasting blood glucose of diabetic control rats relative to normal control. The plant extract reverses the effects of alloxan on lipid profile levels and fasting blood glucose of diabetic treated animals. The extract exerted significant (P< 0.05) reduction in fasting blood glucose level in diabetic treated rat from week one to week three compared to diabetic control. No significant (P>0.05) effect was observed in the fourth week. The findings indicate that *Anisopus mannii* possess hypolipidemic and antihyperglycemic potentials.

Keywords: Alloxan, *Anisopus manni*, antihyperglycemic, lipid profile, rats.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia (Tietz, 2008). The disease ranks highly among the top ten disorders which cause mortality and lower the life expectancy throughout the world (Adeneye & Agbaje, 2008). The increase in prevalence of the disease is a global phenomenon and this has led to the reason why the disease is being described as one of the main threats to human health in the twenty-first century (Tietz, 2008). Hyperglycemia and dyslipidemia, among other disorders, are metabolic syndromes associated with the disease (Mohini *et al.,* 2012; Mamun *et al.,* 2013). Ideal oral treatment and management of diabetes would be a drug that controls the glycemic level, as well as preventing the development of complications of diabetes (Sampanis, 2008). In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al*., 2002; Peter *et al.,* 2016). Many traditional plant treatments for diabetes are used throughout the world. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important (WHO, 1980). The attributed antihyperglycemic effects of these plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or decrease in the intestinal absorption of glucose (Ali *et al.,* 2009; Kujur *et al.,* 2010). Hence treatment with herbal drugs has an effect on protecting β -cells and smoothing out fluctuation in glucose levels. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects (Loew & Kazkin, 2002; Sabu & Ramadasan, 2004).
Sani *et al.* (2009) have reported the presence of such important phytochemicals in *Anisopus mannii.* The plant (*Anisopus mannii*) belongs to the family of Asclepiadaceae. It is known as 'Sakayau' or 'Kashezaki' (meaning sweet killer) among the Hausas of the Northern Nigeria, where a cold decoction of the stem is traditionally used as hypoglycaemic agent (Sani *et al*., 2009). Despite the widespread use of this plant, there is little information in scientific literature regarding its anti-diabetic potentials. This study is therefore aimed at determining the anti-diabetic effect of aqueous extract of *Anisopus mannii* stem by measurement of body weight and assessment of some biochemical parameters such as blood glucose and lipid profile.

MATERIALS AND METHODS

Experimental animals

Male rats (Wistar strain) obtained from Department of Veterinary Pathology, Ahmadu Bello University Zaria, Kaduna State, Nigeria were used for this study. The rats (adults) were maintained under standard animal house condition and were allowed free access to food (growers mash) and water for two weeks to acclimatize to the new environment. All animals were handled with proper care and humanely treated according to the internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH, 1992).

Medicinal plant

Stems of *Anisopus mannii*were collected from Gidandawa Local Government area of Katsina State, Nigeria. They were identified in the Department of Biological Sciences, Federal University Dutsinma.

Reagents and chemicals

All reagents used were of analytical grade.

Preparation and extraction of plant material
Preparation and extraction of the plant material was carried out according to the method described by Osibemhe & Onoagbe (2015) with little modifications. The stems of the plant were thoroughly washed with clean water and the barks were peeled off by incision. They were then dried under shade for two weeks and then pulverized into fine powder with the aid of a mortar and pestle. Measured quantity of the powdered sample was extracted in aqueous by maceration for 72 hrs followed by periodic stirring and it was kept in a refrigerator to avoid any microbial growth. The extract was filtered using cheese-cloth and the filtrate re-filtered using Whatman No. 42 (125 mm) filter paper. 1 ml of the filtrate was measured into a previously weighed Watch glass and was then evaporated to dryness using oven at 50 °C. This was to determine the mg/ml of *Anisopus manni* using the following formula:
mg/ml = (Weight of watch glass + 1 ml sample) (after drying) – Initial weight of watch glass.

Alloxan induction
Diabetes was induced by intraperitoneal administration of 150 mg/kg body weight of freshly prepared alloxan in normal saline to overnight fasted rats, using insulin syringe. To prevent initial alloxan-induced hypoglycemia, the rats were given glucose (5 ml per kg body weight of 5 % solution) orally by gavage. 72 hrs post administration of alloxan, diabetes was confirmed in alloxan treated rats with a fasting blood glucose concentration ≥ 200 mg/dl using the glucometer (On-Call Plus).

Experimental design
A total of 15 male rats were used in this experiment. The rats were randomly distributed into 3 groups of five rats each and were kept in standard cages. Normal and diabetic control rats were given distilled water. Diabetic treated group received 400 mg/kg of aqueous extract of the plant for 28days. All administrations were carried out orally using gavage. At the end of 28 days of extract administration, the animals were sacrificed and blood was collected for analysis.

Blood collection
Blood samples were collected into heparinized containers through the abdominal aorta from the rats under chloroform anesthesia using a 5 ml syringe. The blood sample was centrifuged at 3000 rpm for 15 mins. After centrifugation, the plasma was aspirated into clean plain sample bottles for the analyses of plasma lipid profile (total cholesterol, triglyceride, HDL- cholesterol and LDL-cholesterol) following the methods described in Randox Laboratory kits.

Determination of blood glucose
Fasting blood glucose was determined by pricking the tail of the rats with a needle after massaging. Glucose concentration was determined using On-Call Plus glucometer on weekly basis for four weeks. The weight of the rats was also noted.

Statistical analysis
Data are presented as mean ± S.E.M of five independent determinations. One-Way Analysis of Variance (ANOVA) was used in comparing the means using statistical package for Social Sciences (SPSS) version 16.0, followed by Duncan Post Hoc Multiple Comparisons. Values lower than 0.05 were taken as statistically significant.

RESULTS AND DISCUSSION
One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan (Etuk, 2010). It is a well-known diabetogenic agent that is used to induce Type I diabetes in experimental animals (Viana *et al.,* 2004). The mechanism of action of alloxan is selective destruction of the beta cells of the pancreas. The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Szkudelki, 2001; Iranloye *et al.,* 2011). This study examined the effect of aqueous extract of *Anisopus mannii* in alloxan-induced diabetic rats. The results indicated that administration of 150 mg/kg body weight of alloxan altered the lipid profile levels and fasting blood glucose concentrations significantly ($P<0.05$) (Table 1). To be specific, significant ($P<0.05$) elevation was observed in the levels of total cholesterol (TCHOL), triglycerides (TG), and low density lipoprotein cholesterol (LDL-c) in diabetic rats when compared with normal control whereas it lowered the level of high density lipoprotein cholesterol (HDL-C) (Table 1).

Table 1: Effect of 28 days administration of aqueous extract of *Anisopus mannii* on plasma lipid profile in alloxan- induced diabetic rats.

Treatment	Lipid profile levels (mg/dl)			
	TCHOL	HDL	TG	LDL
Normal Control	48.80±0.92^a	32.80±1.83^a	15.51±1.10^a	12.91±1.05^a
Diabetes Control	80.97±1.79^b	17.27±1.32^b	26.46±1.22^b	58.50±3.21^b
Diabetes Treated	54.72±1.87^c	25.65±1.72^c	18.26±0.63^a	26.42±2.08^c

Values are expressed as plasma lipid profile (mg/dl) and mean ± SEM (n =5). Values in the same column with different superscript represent significant ($P<0.05$) difference from control.

Table 2: Effect of administration of aqueous extract of *Anisopus mannii* on fasting blood glucose concentration in alloxan- induced diabetic rats for four weeks

Treatment	Blood glucose concentration (mmol/L) on weekly basis				
	Basal	Week One	Week Two	Week Three	Week Four
Normal Control	3.90±0.18^a	4.18±0.12^a	4.22±0.14^a	4.06±0.16^a	3.94±0.20^a
Diabetes Control	19.42±0.59^b	27.68±0.89^b	22.86±2.03^b	17.50±1.02^b	11.10±1.11^b
Diabetes Treated	19.62±0.89^b	14.42±0.44^a	10.22±0.68^c	11.90±0.57^c	9.76±1.10^b

Values are expressed as fasting blood glucose concentration (mmol/L) and are mean ± SEM (n =5). Values in the same column with different superscript represent significant ($P<0.05$) difference.

Table 3: Effect of administration of aqueous extract of *Anisopus mannii* on body weight (g) of alloxan- induced diabetic rats for four weeks.

Treatment	Body Weight (g) on weekly basis				
	Basal	Week One	Week Two	Week Three	Week Four
Normal Control	139.5±1.29[a]	148.9±0.51[b]	156.6±2.38[bc]	160.6±4.52[c]	165.7±4.58[c]
Diabetes Control	143.6±1.89[a]	139.0±1.50[a]	125.8±1.82[b]	120.4±1.18[c]	125.2±1.61[b]
Diabetes Treated	132.1±1.75[a]	130.6±1.06[a]	134.4±1.68[ab]	133.9±1.42[ab]	136.9±1.45[b]

Values are expressed as body weight (g) and are mean ± SEM (n =5). Values in the same row with different superscript represent significant (P<0.05) difference compared to basal values.

Altered lipid profile levels in the serum of diabetic patients had been reported by Orchard (1990) and Betteridge (1994). Chronic hyperglycemia is known to precipitate complications in diabetes via formation of advanced glycosylation end products and this eventually tend to lower HDL- cholesterol levels, the protective molecule against atherosclerosis (Robinson & Johnson, 1997) probably due to glycation of the protein portion of the molecule. Administration of 400 mg/kg body weight of aqueous extract of *Anisopus mannii* reverses the effects of alloxan on lipid profile in diabetic treated animals (Table 1). Total cholesterol (TCHOL), TG, LDL-C concentrations were lowered by the extract in diabetic treated rats compared with diabetic control whereas the level of HDL-C was elevated. The effect of the extract on lipid profile levels was not brought to the level of the normal control except for the level of TG. The decrease in TG, TCHOL, and LDL-C levels as well as the increase in HDL-C (Table 1) exhibited by aqueous extract of *Anisopus mannii* in the diabetic treated rats may be an indication of progressive metabolic control of the plant extract on the mechanisms involved in the elimination of lipids from the body. It may also be as a result of important bioactive compound present in the plant. Sani*et al.* (2009) have reported the presence of important phytochemicals such as alkaloids, flavonoids, saponins etc in *Anisopus mannii.* Disarrangement in serum lipid profile engendered by lipemia in experimental animals have been successfully readjusted and brought to normalcy by the actions of bioactive principles of plant origins as previously demonstrated elsewhere (Ojiako *et al.,* 2013; Toma *et al.,* 2014). Lowered lipid profile of rats treated with medicinal plant extracts had also been reported by a number of authors (Anusha *et al.,* 2017; Freshet *et al.* 2017). Alloxan also exerted significant (P<0.05) elevation of fasting blood glucose in the rats when compared with normal control (Table 2). Aqueous extract of *Anisopus mannii* significantly (P < 0.05) lowered fasting blood glucose concentration of diabetic treated rats from week one to week three when compared with diabetic control. No significant effect was however observed in the fourth week (Table 2).The extract may have achieved this hypoglycaemic property via increased insulin secretion, increased peripheral utilization of glucose, inhibition of endogenous glucose production or by inhibition of intestinal glucose absorption as reported in existing literatures (Eddouks *et al.,* 2003; Bakirel *et al.*, 2007). Significant (P<0.05) and non-significant (P>0.05) progressive increase in body weight was observed in normal control and diabetic treated rats respectively when compared with their basal values, whereas the diabetic control group recorded significant (P<0.05) progressive decrease in bodyweight (Table 3).

Conclusion

The importance of continuing surveys for medicinally important plants with acclaimed hypoglycemic and anti-diabetic properties cannot be overemphasized. This study has shown that aqueous extract of *Anisopus manni* has hypolipidemic properties. It has also shown that the plant extract has the potential to reduce diabetes. Hence could be used for the control of glycemic levels.

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