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ANTI-DIABETIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES AND STEM OF *ANISOPUS MANNII* IN ALLOXAN-INDUCED DIABETIC RATS.

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ABSTRACT

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Diabetes mellitus is a metabolic disorder in which carbohydrate, protein and lipid metabolism is not properly regulated due to defects in insulin secretion, action or both. Many indigenous medicinal plants have been successfully used to manage diabetes. However, the use of *Anisopus mannii* is growing rapidly in the rural areas. Thus, the aim of this study is to investigate the effect of the aqueous extract of *Anisopus mannii* on hyperglycemia, lipid profile, hepatic and renal damages in alloxan-induced diabetic rats. These rats were treated with different concentrations of the extract at 100 mg/kg, 200 mg/kg and 400 mg/kg orally for 28 days. Fasting blood glucose, lipid profiles, serum urea, creatinine, bilirubin, activities of alanine transaminase and aspartate transaminase were significantly lower ($p < 0.05$) in the test groups than in untreated diabetic group. The aqueous extracts (200 mg/kg) of this plant have significantly lowered LDL and total cholesterol. While triglycerides and HDL levels were significantly higher ($p < 0.05$) in the test groups. Thus, it can be concluded that the extract has anti-hyperglycemic, nephroprotective, hepatoprotective, and hypolipidaemic properties.

INTRODUCTION

The need for traditional medicine in modern drug development cannot be overemphasized. However, to reach its optimum potential, it must go through a series of processes before it is ultimately deployed in the clinic. These processes include discovery, isolation and mechanistic studies among others (Corson and Crews, 2007). Most of these plant-derived drugs were originally discovered through herbal cures and folk knowledge of traditional people and some of these could not be substituted despite

advancement in synthetic chemistry (Gilani, 2005). Quite a number of medicinal plants discovered by herbal practitioners have proved to be important sources of potential therapeutic agents e.g. quinine (antimalarial agent) from *Cinchona* bark, digoxin and digitoxin (cardiac activity) from *Digitalis purpurea* Linnaeus zingiberone (antihelminthic) from *Zingiber officinalis* Rosc., vincristine and vinblastine alkaloids (antileukaemic agents) from *Catharanthus roseus* Linnaeus and bourvadine from *Bourbadia ternifolia* Cav (Clark, 1996).

Medicinal plants are used based on the evidences of phytochemical constituents available in them. The multiple roles of 'wild' traditional fruits as both food and medicinal sources have widely been documented (Flyman and Afolayan, 2006; Smith and Eyzaguirre, 2007). Recent studies have shown that increasing daily intake of green leafy vegetables could significantly reduce the risk of type 2 diabetes (Bazzano et al., 2008; Carter et al., 2010; Liu et al., 2004). Some of these wild edible fruits such as *Detarium microcarpum*, *Adansonia digitata*, *Ziziphus mauritania*, *Ximenia americana* and *Lannea microcarpa* have been reported to contain comparatively high amounts of vitamin A and C and other antioxidant micronutrients (Lamien-Meda et al., 2008). It is believed that these bioactive compounds promote good health by assisting in preventing cancer and high blood pressure, stimulating the immune system improving drug metabolism and tissue regeneration (Flyman and Afolayan, 2006; Kris-Etherton et al., 2004).

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide; thus the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder (Association, 2010). It was reported that the world prevalence of diabetes among adults (aged 20–79 years) is 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030 (Shaw et al., 2010). The search for compounds with novel properties to deal with this menace is still in progress (Etuk et al., 2010). Recently, there has been a resurgent interest in the herbal treatments of diabetes. The apparent reversal of trend from Western to herbal medicine is partly due to undesirable side effects associated with synthetic medicines and partly because synthetic medicines cause adverse reactions more than herbal medicine. This has led to the belief that natural products are safe because they are more harmonious with biological systems. Additionally the cost of administering modern antidiabetic drugs is beyond the reach of people in the low income group and those living in the rural areas. A good number of these plants used traditionally have been proven beyond doubt to have antidiabetic activity. These include *Allium sativum* L., *Gymnema sylvestre* Gurmar.,

Citrullus colocynthis L., *Trigonella foenum greacum*, *Momordica charantia* Linn.etc (Patel et al., 2012). Very little scientific research has been carried out on the plant, *Anisopus mannii*. This study is designed to explore the folkloric claim that *Anisopus mannii* is useful in the treatment of diabetes mellitus (Mohammed et al., 2014; Sani et al., 2009a). The plant is known as Sakayau or kashe-zaki (meaning sweet killer) among the Hausas of northern Nigeria, where a cold decoction of stem/leave is traditionally used as remedy for hyperglycemia. The aim of this study is to investigate the antidiabetic activity of the aqueous extract of the combined leaves and stem of *Anisopus mannii* plant in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The whole plant *Anisopus mannii* was collected in August, 2016 within the main campus of Umaru Musa Yar'adua University, Katsina, and was identified and authenticated at the herbarium section in the Biology Department, Umaru Musa Yar'adua University, Katsina, Nigeria where a voucher specimen was deposited for reference with specimen no. V1078. The stems and leaves were sorted, cleaned and air dried at room temperature for 2 weeks and then pulverized to coarse powder using pestle and mortar. A hundred gram (100) of the powdered samples was mixed with 1 litre of distilled water, in a beaker. The mixture was homogenously mixed and left for 24 hours before decanting, and the supernatant was filtered using muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using oven preset at 50°C and was later stored at 4°C. Half a liter of distilled water was added to the residue and allowed to stay overnight and above procedure was repeated. The dried extract was stored in a refrigerator until needed. The extract was reconstituted freshly for each study.

Acclimatization of the Experimental Rats

Wister albino rats of both sexes weighing 170-250g were obtained for the study from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

The animals were maintained in a well-ventilated room, fed on standard rodent feed (Grand cereals Ltd, Jos) and granted access to water *ad-libitum*. They were kept in clean cages under approximately 12/12 hours normal light / dark cycle and allowed to acclimatize to the laboratory environment for a period of two weeks before commencement of the experiments (Sani *et al.*, 2009a).

Experimental Design

Thirty three (33) albino wistar rats were used for the study. The animals were divided in to seven groups (Lawal *et al.*, 2008).

Group I is a Normal Control (were normal rats that received no treatment). Group II are Extract control (were normal rats treated with extract at a dose of 200 mg/kg). Group III were Diabetic Control (diabetic rats that received no treatment). Group IV were Diabetic animals treated with glibenclamide at a dose of 5mg/kg. Group V were Diabetic animals treated with extract at a dose of 100 mg/kg. Group VI were Diabetic animals treated with extract at a dose of 200 mg/kg. Group VII were Diabetic animals treated with extract at a dose of 400 mg/kg.

Each rat was treated orally for 28 days. Fasting blood glucose levels was determined on weekly basis for four weeks. They were then slaughtered by decapitation and the blood, kidneys, liver, and spleen were collected. The blood was centrifuged at 4000rpm for 15 min to obtain the serum, which was used for the biochemical analysis. The fasting blood glucose levels of all the rats were recorded at weekly intervals during the experimental period. The fasting blood glucose was measured from blood drawn by tail snipping using a Glucometer and its corresponding test strips. Major organs including liver, kidney, and spleen were collected after sacrifice and weighed to get the relative organ body weight (ROW). The organs were then refrigerated before further experimental analysis at about 4°C.

Induction of Diabetes

Hyperglycemia was induced by using alloxan as described by (Katsumata *et al.*, 1993). Twenty-five overnight fasted rats were intraperitoneally administered with alloxan monohydrate (dissolved in 0.9 % normal saline) at a dose of 150 mg/kg body weight.

Blood samples were withdrawn at the time of injection to determine the initial fasting blood glucose concentration (FBGC) by pricking the tail tip arteries of the rats. Food and water containing 10% glucose was then immediately returned to them. The glucose solution was allowed to stay with the rats for only 4 hours to protect against massive hypoglycemia that will be seen as a result of excessive depletion of pancreatic beta cells containing insulin. The rats were then observed over a period of 72 hours for signs of hyperglycemia. The fasting blood glucose was measured using test strips which follow the glucose oxidase principles (Trinder, 1969).

Determination of the Lipid Profile

The collected serum was investigated to determine any effect on the lipid profile using enzymatic methods. Total cholesterol was determined as described (Allain *et al.*, 1974), while HDL- cholesterol (Burstein *et al.*, 1970) and triglycerides (Fossati and Prencipe, 1982) and LDL- cholesterol was calculated using Friedewald formula (Friedewald *et al.*, 1972).

Determination of Biochemical Markers for Liver and Kidney Functions

The collected blood samples were investigated for the activity/concentration of the following biochemical parameters: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST) (Bergmeyer *et al.*, 1978), bilirubin (Malloy and Evelyn, 1937), Serum urea and creatinine (Levey *et al.*, 1999) as contained in the manual of Aggape diagnostics, Switzerland.

Statistical Analysis

Statistical analysis of data was carried out using SPSS Version 19. Analysis of variance (ANOVA) using Duncan post hoc test was carried out. Results were expressed as Means \pm Standard Deviation (SD). Differences in means were considered to be significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

The results showed that there was a statistically significant ($p \leq 0.05$) decrease in blood glucose levels during the first week of the treatment for all groups with the exception of diabetic and normal control groups as indicated in Table 1. The pattern was maintained up to the fourth week.

The diabetic control group showed a significant ($p \leq 0.05$) increase in blood glucose level throughout the period of the experiment. Glibenclamide was observed to have lowered blood glucose level drastically, whereas, the extract showed a slight initial rise in blood glucose level during the first three hours before significantly ($p \leq 0.05$) reducing it at both doses of 200 mg/kg and 400 mg/kg.

The observed hypoglycaemic effect of *A. mannii* in this study is an indication that the plant contains active constituents with potent hypoglycaemic property which could be acting via increased peripheral glucose utilization. Recent studies have revealed that the aqueous stem extract of *Anisopus mannii* contains various pharmacologically active compounds like saponins, flavonoids, alkaloids, glycosides, terpenes and steroids (Sani *et al.*, 2009b). Literature has shown the biological activities of alkaloids and flavonoids to include hypoglycaemia (Jadhav and Puchchakayala, 2012; Sharma *et al.*, 2008; Zhou *et al.*, 2009), hypolipidemia (Chen and Li, 2007; Feng *et al.*, 2011; Sharma *et al.*, 2008; Sudheesh *et al.*, 1997; Zhou *et al.*, 2009), hypotension (Kitic *et al.*, 2012; Majewska-Wierzbicka and Czczot, 2012) and other biological effects. Alkaloids have been reported to produce hypoglycaemic effect in mice (Patel and Mishra, 2011; Sharma *et al.*, 2010). Purified form of alkaloids has also been implicated in hypoglycemic effect in human subjects with type II diabetes (Baldeón *et al.*, 2012). Saponins have also been reported to produce hypoglycaemic effect in mice (Deng *et al.*, 2012; Elekofehinti *et al.*, 2013; Yoshikawa *et al.*, 2008). Since these biologically active compounds are present in *Anisopus mannii* stem extract, these could be responsible for the oral hypoglycemic effect observed in this study. Although few anti-diabetic studies were conducted using this plant, similar results was obtained (Manosroi *et al.*, 2011; Sani *et al.*, 2009a; Zaruwa *et al.*, 2012).

After 28 days of treatment with aqueous extract of *A. mannii* on diabetic rats, the result of lipid profile shows a significant increase ($p < 0.05$) in triglycerides, cholesterol, and LDL-cholesterol and significant decrease in HDL-cholesterol as depicted in Table 2. Conversely, administration of aqueous extract of *A. mannii*

on non-diabetic rats caused significant decreases ($p < 0.05$) in triglyceride, cholesterol and LDL-cholesterol and significant increase in HDL-cholesterol. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolysis of triglycerides (Wang and Eckel, 2009). In uncontrolled type-2 DM, an increase in triglycerides, total cholesterol and LDL-C with decrease in HDL-C termed dyslipidaemia occurs which contribute to coronary artery disease (Arvind *et al.*, 2002; Assmann and Schulte, 1992; Assmann *et al.*, 1998). The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots. Since insulin inhibits the hormone sensitive lipase (Saltiel and Kahn, 2001). The marked hyperlipidaemia that characterize the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. Although dyslipidaemia results in the untreated diabetic group in this study, administration of both glibenclamide and aqueous extract of *A. mannii* was seen to have brought these abnormalities to normal level. Similar hypolipidaemic effect of *A. mannii* has been reported by (Sani *et al.*, 2009a).

Induction of diabetes with alloxan caused significant ($P < 0.05$) increase in the level of serum urea of the rats not treated with the extract or standard drug (Table 3). However, the diabetic rats treated with the extract recorded urea levels significantly ($P < 0.05$) lower than the levels in the Diabetic Control group. Treatment with the standard drug also had a similar effect on urea levels. The concentration of serum creatinine (Table 3) was not significantly affected by either the disease or the extract. On the other hand, effect of the extract on some biochemical indices of liver function of all groups of rats showed that the levels of AST, ALT, ALP, direct and total bilirubin were all significantly ($P < 0.05$) higher in the Diabetic Control group as compared to the Normal Control group. Treatment with the extract caused significant ($P < 0.05$) decreases in the levels of AST, ALT and total bilirubin from diabetic levels after four weeks of treatment in a manner comparable with the effect of the standard drug used in this work.

Direct bilirubin and ALP levels were not affected by treatment of diabetic rats with the extract. Thus, the extract of this plant has hepatoprotective and nephroprotective

properties in addition to the afore-mention properties. Similar results with different plant extracts were reported (Ahmed et al., 2010; Elekofehinti et al., 2013; Kareru et al., 2013)

Table 1: Effect of treatment of alloxan-induced hyperglycaemic albino rats with aqueous extract of *anisopus manni* on blood glucose levels (mg/dl).

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	121.0 ± 3.6 ^a	124.6 ± 2.0 ^a	126.2 ± 2.9 ^a	125.8 ± 11.3 ^a	125.6 ± 6.9 ^a
Extract Control	136.8 ± 1.6 ^a	84.4 ± 3.4 ^a	79.0 ± 4.1 ^a	75.4 ± 3.9 ^a	74.0 ± 3.7 ^a
Diabetic Control	322.0 ± 63.5 ^b	346.0 ± 61.4 ^b	347.0 ± 50.5 ^b	357.0 ± 82.8 ^c	286.6 ± 58.9 ^b
Diabetic + Glibenclamide	421.0 ± 11.3 ^c	353.8 ± 13.3 ^c	287.2 ± 8.6 ^b	201.6 ± 10.11 ^b	126.4 ± 16.5 ^a
Diabetic + Extract (100 mg/Kg)	458.0 ± 19.6 ^c	438.6 ± 14.0 ^d	423.8 ± 11.6 ^c	411.4 ± 24.9 ^c	221.8 ± 21.2 ^b
Diabetic + Extract (200 mg/Kg)	391.0 ± 5.6 ^c	352.6 ± 13.8 ^c	312.8 ± 26.3 ^b	234.8 ± 21.6 ^b	132.2 ± 11.8 ^a
Diabetic + Extract (400 mg/Kg)	473.0 ± 19.4 ^c	431.4 ± 22.1 ^c	400.4 ± 20.4 ^c	385.8 ± 22.7 ^c	270.0 ± 14.4 ^b

Values are Means ± SD of five determinations; those with different superscripts along the rows are statistically different (P < 0.05).

Table 2: Effect of aqueous extract of *anisopus manni* on some biochemical indices of kidney and liver functions in normal and alloxan – induced diabetic rats.

Parameters	Normal Control	Extract Control	Diabetic Control	Diabetic + Glibenclamide	Diabetic + Extract (100mg/Kg)	Diabetic + Extract (200mg/Kg)	Diabetic + Extract (400mg/Kg)
AST (i.u/ml)	39.03 ± 2.38 ^{ab}	32.54 ± 1.37 ^a	79.85 ± 5.40 ^d	45.69 ± 2.16 ^{bc}	54.20 ± 3.37 ^c	46.19 ± 3.15 ^{bc}	43.50 ± 1.71 ^b
ALP (i.u/ml)	14.21 ± 1.71 ^b	9.38 ± 0.98 ^a	17.67 ± 0.58 ^c	14.00 ± 0.35 ^b	17.78 ± 0.61 ^c	16.88 ± 1.12 ^c	14.63 ± 0.60 ^b
ALT (i.u/ml)	18.82 ± 1.82 ^b	12.02 ± 1.17 ^a	29.17 ± 2.97 ^d	24.92 ± 1.76 ^c	24.50 ± 3.08 ^c	19.10 ± 1.32 ^b	22.53 ± 2.60 ^{b,c}
BD (mg/dL)	0.34 ± 0.05 ^a	0.32 ± 0.11 ^a	1.39 ± 0.12 ^e	0.93 ± 0.12 ^b	1.31 ± 0.08 ^{de}	1.18 ± 0.06 ^{cd}	1.25 ± 0.06 ^{cde}
BT(mg/dL)	0.91 ± 0.07 ^b	0.77 ± 0.07 ^a	1.93 ± 0.07 ^e	1.49 ± 0.03 ^c	1.68 ± 0.01 ^d	1.55 ± 0.10 ^c	1.51 ± 0.02 ^c
Urea (mg/dL)	36.76 ± 7.57 ^{ab}	38.21 ± 6.57 ^{ab}	52.25 ± 14.48 ^b	47.84 ± 4.23 ^{ab}	33.65 ± 1.90 ^a	49.29 ± 5.46 ^{ab}	49.15 ± 5.34 ^{a,b}
Creatinine (mg/dL)	2.10 ± 0.50 ^{cd}	1.00 ± 0.20 ^a	2.70 ± 0.68 ^d	2.40 ± 0.33 ^{cd}	2.03 ± 0.50 ^{cd}	1.20 ± 0.33 ^{ab}	1.80 ± 0.52 ^{b,c}

Values are Means ± SD of five determinations; those with different superscripts along the rows are statistically different (P < 0.05).

Key: AST = aspartate transaminase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, BD = Bilirubin (Direct), BT = Bilirubin (Total)

Table 2: Effect of treatment of alloxan-induced diabetic rats with aqueous extract of *A. mannii* on triglyceride, cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) levels.

Treatment	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal Control	42.3 ± 1.9 ^a	30.1 ± 2.6 ^a	69.7 ± 10.8 ^b	87.7 ± 13.6 ^b
Extract Control	39.3 ± 3.7 ^a	39.5 ± 4.1 ^b	61.0 ± 9.6 ^b	68.3 ± 9.2 ^c
Diabetic Control	117.9 ± 6.7 ^d	42.9 ± 1.5 ^b	48.3 ± 3.4 ^a	91.6 ± 5.2 ^d
Diabetic + Glibenclamide	69.0 ± 2.6 ^c	37.1 ± 2.9 ^b	206.3 ± 50.3 ^c	58.0 ± 4.9 ^b
Diabetic + Extract (100 mg/Kg)	78.3 ± 8.7 ^c	38.1 ± 0.9 ^b	129.7 ± 26.0 ^b	80.9 ± 3.1 ^d
Diabetic + Extract (200 mg/Kg)	64.3 ± 9.5 ^c	23.2 ± 6.1 ^a	228.6 ± 20.6 ^c	59.2 ± 2.8 ^b
Diabetic + Extract (400 mg/Kg)	50.8 ± 10.9 ^b	18.8 ± 6.8 ^a	347.8 ± 20.3 ^d	43.3 ± 1.5 ^a

Data are presented, as Mean ± SD. Differences between values signed with same superscripts along the row are not statistically significant: (n) =5 (P < 0.05).

Key: TG = Triglycerides, TC = Total Cholesterol, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein

CONCLUSION

The aqueous extract of *A. mannii* showed a significant hypoglycaemic properties in both alloxan induced hyperglycaemic wistar rats and normal control group. The extract also shows hypolipidaemic activity. Additionally, it

has hepatoprotective and nephroprotective ability on alloxan-induced diabetes. It may therefore be concluded that the use of *A. mannii* in traditional medicine to manage diabetes mellitus is justifiable.

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