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Phytochemical Composition and Evaluation of The Anti- Hyperglycemic Activity of The Ethanol Extract of *Anisopus Mannii(Sakayau)* Stem And Leaves.

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Abstract
Diabetes is a serious metabolic disorder prevailing among people with ageing and sedentary lifestyle associated with rapidly growing urbanization and industrialization. Medical plants used by folklore have been a source of relief in contorting different types of diabetes all over the world. At this present time, the uses of these herbal drugs are growing at high pace because of their cost effectiveness and less side effects over synthetic hypoglycemic drugs. This study has identified the phytochemical constituents of *Anisopus mannii* stem and leaves powdered and ethanol extract samples. The following phytochemicals are present anthraquinones, flavonoids, cardiac glycosides, tannins, saponins and alkaloids but alkaloids were absent in the ethanol extract sample and steroids were absent in both samples. Thin layer chromatography (TLC) and column chromatography (CC) were performed on the ethanol extract. Three fractions were obtained from the thin layer chromatography and column chromatography of the ethanol extract of the plant. The anti-hyperglycemic activity was investigated by studying the effect of the crude ethanol extract of the plant in alloxan-induced diabetic rats. Fifteen male rats were randomly distributed into three groups of five rats each. Normal and diabetes control (groups A and B), which were given distilled water. While diabetes treated (group C) were given 400 mg/kg body weight of the ethanol extract of the plant. All administrations (oral) were carried out daily for 28 days using gavage. Fasting blood glucose and body weight were also recorded at intervals of 7 days. A significant difference was observed on body weight and fasting blood glucose concentration of diabetes treated rats compared to diabetes control rats. The ethanol extract has reverse the effect of alloxan on body weight and fasting blood glucose concentration of the diabetes treated rats.

Keywords: phytochemicals constituents, alloxan-induced, anti-hyperglycemic activity.

Introduction

Background of the Study

Diabetes mellitus has been identified as the most common endocrine disorder that currently affects 200 million people of the world's population (Wais *et al.,* 2012). It is projected to rise to over 366 million in the year 2030 (Wild *et al.,* 2004). Diabetes mellitus is characterized by hyperglycaemia with alteration of carbohydrates, protein and fat metabolism, resulting from defects in insulin secretion or sensitivity of insulin to body cells or both (Hovens *et al.,* 2005). Such alterations result in elevated blood glucose concentration. It causes acute complications like hyperglycaemia and hypoglycaemia and long-term complications in many organs. This may lead to increase the risk of atherosclerosis, renal failure, nerve damage, coronary heart disease and blindness resulting in increasing disability (ADA, 2009). Diabetes mellitus is associated with reduced quality of life and increased risk factors for mortality and morbidity (Upendra-Rao *et al.,* 2000).

Traditional medicine products are playing greater roles in the lives of the people across the world in the face of the global upsurge of drug resistance, toxicity, adverse effects and increasing costs of synthetic products (Abubakar *et al.,* 2017). In Nigeria, several thousands of plants have been claimed to possess medicinal properties and are employed in the treatment of many ailments (Iweala and Oludare, 2011). Many of these indigenous medicinal plants are used as spices and food plants and for medicinal purposes (Nwaogu *et al.,* 2007). However,

the knowledge of medicinal uses of plants in the management of diabetes mellitus is still intact with the traditional medicine practitioners, and this knowledge is either lost or passed to their children by the word of mouth. Therefore, there is need to collect and document this knowledge before such rich heritages are lost (Abubakar et al., 2017).

The current economic recession, low per capita income, and poorly developed healthcare infrastructure may worsen diabetic condition since the conventional drugs are expensive and often unaffordable by the poor population (Sunday *et al.,* 2010). Interestingly, Nigeria is a country endowed with biodiversity of medicinal plants that are now gaining relevance in traditional medicine for the management of diverse human diseases including Diabetes. Presently; there are significant number of both the public citizens and health practitioners depending on herbal drugs compared to scientifically validate proved therapies. These herbal drugs may serve as a potential source of novel molecules for the treatment of diabetes that can represent a more cost-effective treatment, with new prospect of fewer side effects (Sunday *et al.,* 2017).

However, many reasons, complementary medicine has developed in popularity in current years. Several native medicinal plants have been found to be helpful to deal with diabetes, some of them have been tested, and their active ingredients isolated. The beneficial uses of medicinal plants in traditional system of medicine of many cultures are extensively documented. Numerous plants have been used as nutritional adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents. Many recent scientific investigations have also confirmed the efficacy of plant preparations, only some of these are extremely efficient (Li *et al.,* 2012).

The aim is to study the phytochemical constituents of the whole stem and leaves of Anisopus mannii and establish a scientific bases for the traditional use in treating diabetes.The objectives ininclude Identification of the phytochemical constituents, present in Anisopus mannii samples and evaluation of the anti-hyperglycaemic activity of the crude extract of Anisopus mannii.

Significance of the Study

Hyperglycaemic conditions can cause decrease in the production of insulin in the β-cells of the islets of langerhans or increase insulin resistance. And most synthetic drugs may be either toxic or have some side effects as well as being generally very expensive to the poor population. The provision of scientific basis for the use of plants is necessary in order to offer them a place in our health care system as alternative drug therapy. Diabetes mellitus is a chronic disease that requires long term therapy, often with multiple drug administration (polypharmacy), the likelihood of side effects might hinder the use of synthetic drugs. The cost of long term treatment of diabetes mellitus in our country is a cause for concern and the need to integrate our resources (herbs) into our healthcare system will reduce the suffering of the patients. The choice of using these resources (plants) and their evaluation for therapeutic activity lies in the hands of our scientists. So, there is need for a natural source of remedy that is effective, and natural products from medicinal plants pave a way to this. However, this study will focus on the extraction of natural products from Anisopus mannii stem and leaves, which may serve as an effective remedy to hyperglycaemic conditions and would be affordable and less toxic.

Plant Description

A. mannii (Asclepiadaceae) is a glabrous twinning shrub with leaves petiole, elliptic, ovate and shortly cuspidate at apex upto 15 cm or more and 12 cm broad. The stem twines to a height of 3.7 – 4.6 m. It is a perennial herb with leaves spread and petiole 1.3 - 2 cm long, glabrous twining shrub, strong climber with greenish flower in globose, lateral umbelliform cymes and horizontally opposite follicle 6-8 inches long and about half inch thick, tapering to a slightly hooked point at the apex (Osuntokun et al.,2016). It belongs to the family Asclepiadaceae and a genus of Anisopus with specific epithet called mannii (Aluka, 2009). The family consists of 130 genera and 2,000 species (Evans, 2004); they are found often in the tropics and subtropical regions. Similar species to A. mannii are Anisopus batesii S.Moore, Anisopus bicornatus (K.Schum.) N.E. Br, Anisopus bicoronata (K. Schum.) N.E. Br, Anisopus rostriferus (N.E. Br.) Bullock, Marsdenia batesii (S.Moore) S.Moore, Marsdenia bicoronata K. Schum, Marsdenia rhynchogyna K. Schum, Marsdenia rostrifera N.E.Br. and so on. A. mannii is native to the tropical Americas, including the West Indies. It is rarely cultivated. A. mannii is a herb that grows in Northern and Southern parts of Northern Nigeria. It is popularly known as sakayau, Kashe Zaki, Kar zaki (which means sweet killer) or Yado in Hausa language. It is commonly used in folk medicine (Mohammed, 2011).

Literature review

The proximate composition, mineral elements and anti-nutritional factors of A. mannii was reported and it showed the presence of crude fiber (89.64), moisture (8.41), soluble carbohydrate (7.94), fats (8.67), crude protein (8.4), soluble oxalates (0.34), tannins (10.55) and free cyanides (6.5) in the plant. It contains other minerals such as potassium, calcium, iron, zinc, copper and manganese (Aliyu et al., 2009). The phytochemical screening and antimicrobial activity of the stem aqueous extract of A. mannii was reported by Sani et al. (2009) as well as the isolation of chemical constituents such as 1, 7-naphthyridine alkaloid- named anisopusin, 5-hydroxy-lup-20(29)-en-3-yl eicosanoate, [6]-gingerdione,[6]-dehydrogingerdione and ferulic acid from acetone extract of the stem bark have been reported by Tsopmo et al. (2009). Plants belonging to Asclepiadaceae family were found to be rich in cardinolides (Warashina and Noro, 1994) and saponin glycosides (Ye et al., 2000). Saponins chemically consist of fat-soluble nucleus (aglycone) that is either a triterpenoid (C-30) or steroid (C-27) attached with one or more sugar side chains (glycone) at different carbon sites of the aglycone. Saponins have characteristic surface-active properties and form foamy solutions in water (Aliyu et al., 2011). The toxicological studies of the Ethanol Extract of Anisopus mannii was carried out by sani et al. (2010).The analgesic and anti-inflammatory studies of methanol leaf extract of Anisopus mannii was evaluated by Musa et al. (2009). The Chemical composition and antimicrobial activity of hexane leaf extract of (Asclepiadaceae) was investigated and 32 compounds were identified by Aliyu et al. (2015). Activity of saponin fraction of Anisopus mannii against some pathogenic microorganisms was carried out by Aliyu et al. (2011) and was found effective. A cold decoction of the stem and leaves

is traditionally used as remedy for hyperglycemia. It is a familiar herb in the traditional medicinal preparations in northern Nigeria, where a decoction of the whole plant is used as a remedy for diabetes, diarrhea, impotent in men and pile (Osuntokun et al., 2016). The stem is used as chewing stick. It is also used by herbalist to treat malaria fever, pneumonia and body pains.

Materials and Methods

Chemicals and reagents

The chemicals and reagents used throughout the research work include; Alloxan Monohydrate, sulphuric acid, hydrochloric acid, distilled water, ethanol, hexane, glacial acetic acid, benzene, and ammonia (Sigma chemical Co, St Louis, MO, USA). While Dragendoff's reagent, anhydrous ferric chloride (BDH Chemical Laboratory, England, UK), 5% dextrose (unique pharmaceuticals) and all other chemicals were of analytical grades.

Sample collection and preparation of plant materials

The leaves and stem of the selected plants (Anisopus mannii) was obtained from a location in the north eastern part of Nigeria, in the forest of Bauchi, Bauchi state Nigeria. The leaves and stem were washed thoroughly with distilled water, air dried at room temperature for about two weeks to ensure that the samples lose most of their moisture content. The dried stem and leaves were grounded into powder, stored in air tight containers and kept at room temperature prior to use (Osuntokun *et al.*, 2016).

Extraction of plant metabolites.

The method used by Osuntokun *et al.* (2016) was followed with some modifications. The extraction was carried out by cold maceration using Ethanol as solvent of extraction. 300 g of the powdered plant material was weighted separately and put into 2000 cm^3 beaker. Then, 1500 ml of ethanol was poured to the powdered plant. The mixture was initially shaken rigorously, then covered using aluminium foil to avoid evaporation of the solvent. The sample mixture was left for 5 days. It was shaken every day for at least 2 hours using mechanical shaker. After 5 days, the mixture was filtered using sterile Whatman filter paper and the filtrate was collected directly into sterile conical flask. The filtrate obtained was introduced into clean beaker and heated continuously in water bath/rotary evaporator at a temperature of 78ºC until it is concentrated and dried to solid. The solid extract obtained was kept at room temperature for further investigations (Osuntokun *et al.*, 2016).

Determination of percentage yield

The percentage yield of the extract was calculated using the following formulation;

$$\text{The percentage yield (\%)} = \frac{\text{weight of the extract(g)}}{\text{weight of the powdered sample(g)}} \times 100 \quad \ldots \text{Equation (1)}$$

to know the weight of the extract, the weight of the beaker must be measured before using it to collect the extract, and the weight of the beaker and extract was also measured using beam balance. Then, subtract the weight of the beaker from the weight of the beaker and extract.

$$\text{weight of the extract(g)} = \text{(weight of the beaker)} - \text{(weight of the beaker)} + \text{weight of extract} \quad \ldots \text{Equation (2)}$$

Phytochemical screening of Anisopus mannii

Test for Alkaloids

Measured 0.2 gram of the solid extract obtained was warmed with 2% of H_2SO_4 for two minutes in a test tube. It was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the present of Alkaloids (Osuntokun et al., 2016).

Test for Tannins

One milliliter of the filtrate was mixed with 2 ml of $FeC1_3$ in a test tube, A dark green colour will indicates a positive test for the tannins (Edeoga, 2005).

Test for Saponins

One milliliter of the plant filtrate was diluted with 3 ml of distilled water in a test tube. The mixture was vigorously shaken and left to stand for 10 minutes, during this time, the development of foam on the surface of the mixture that last for more than 10 minutes, indicates the presence of Saponins (Edeoga, 2005).

Test for Anthraquinones

One milliliter of the plant filtrate was shaken with 10 ml of benzene in a test tube. The mixture was filtered and 5 ml of 10 % (v/v) ammonia was added, then shaken and observed. A pinkish solution indicates a positive test (Edeoga, 2005).

Test for Flavonoids

Add 1% NH_3 solution to 5 ml of the ethanol extract in a test tube. A positive test result was confirmed by the formation of a yellow colouration or turbidity (Ekpo and Etim, 2009).

Test for Cardiac Glycoside

Measured 5 ml of the ethanol extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution in a test tube. To this, 1 ml of concentrated sulphuric acid was slowly poured on the test tube to reach the sample mixture. A positive test result was confirmed by the presence of a brown ring at the interface (Ekpo and Etim, 2009).

Test for steroids

Ten (10) ml of the ethanol extract put in a test tube was evaporated to dryness over a steam bath and cooled to room temperature. It was then defatted repeatedly with hexane. The defatted layer was then warmed over a steam bath to remove the residual hexane. To this, 3 ml of 1% $FeCl_3$ reagent was added and 1 ml of concentrated sulfuric acid was slowly added. A positive test was evident when a reddish-brown colouration occurred. (Guevarra, 2005).

Experimental animals

Adult Male rats (Wistar strain) obtained from Department of Veterinary Pathology, Ahmadu Bello University Zaria, Kaduna State, were used for this study. The rats were maintained under standard animal house condition and allowed free access to food (growers mash) and water for two weeks to acclimatize to the new environment. The rats 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).

Alloxan induction to the rat samples

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. After 72 hours 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) (Osibemhe et al., 2017).

Experimental design for evaluation of anti-hyperglycemic activity

A total of 15 male rats were used in this experiment. These 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 were receiving 400 mg/kg of ethanol extract of the plant for 28 days. All administrations were carried out orally using gavage (Osibemhe et al., 2017).

Determination of blood glucose concentration of the rats

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 (Osibemhe et al., 2017).

Determination of body weight of the rats

The weight of the rats was also measured using a beam balance. The rats were weighted before the administration of alloxan, then on weekly basis for four weeks. The weight of the rats was measured five times during the research period (Osibemhe et al., 2017).

Blood collection from the rats for lipid profile analysis

After 28 days the rats were sacrificed and blood samples were collected into heparinized containers through the abdominal aorta from the rats under chloroform anesthesia using a 5 ml syringe. The blood samples were 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 (TC), triglyceride (TG), HDL-cholesterol (HDL) and LDL-cholesterol (LDL)) following the methods described in Randox Laboratory kits (Osibemhe et al., 2017).

Statistical Analysis

Data were 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 (Osibemhe et al., 2017).

RESULTS

Table 1: Phytochemical -constituents in the sample of the stem and leaves of Anisopus mannii.

Secondary metabolites	Qualitative analysis on powdered sample	Qualitative analysis on Ethanol sample
Alkaloids	+	-
Cardiac glycoside	+	+
Steroids	-	-
Anthraquinones	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+

NOTE: From table 1 above (–) symbol shows absent of the secondary metabolites while (+) symbol shows the present of the secondary metabolites.

Table 2: Results for the Extraction of the stem and leaves sample of A.mannii.

Sample	Initial weight(g)	Solvents of extraction	volume(L)	Final weight(g)	Percentage (%) yield
Stem and leaves	600	Ethanol	3	59	9.83

The yield was low because the plat stem and leaves were not finely powdered, so there is need for more than one experiment to have the required yield for the research. The colour of the extract obtained was black and the texture is gummy solid.

Evaluation of antihyperglycemic activity

The results indicated that administration of 400 mg/kg body weight of alloxan altered the levels of fasting blood glucose concentrations significantly (P<0.05) (Table III).

Table 3: Effect of administration of ethanolic extract of Anisopus mannii on fasting blood glucose concentration in alloxan- induced diabetic rats for four weeks.

Fasting Blood Glucose Concentration of the Rats (mmol/L)					
Treatments	0 day	7 Days	14 Days	21 Days	28 Days
Normal Control	03.97 ± 0.02^{a}	04.06 ± 0.07^{a}	04.06 ± 0.05^{a}	04.01 ± 0.05^{a}	04.04 ± 0.06^{a}
Diabetes Control	19.36 ± 0.28^{b}	24.33 ± 0.76^{b}	22.66 ± 0.54^{b}	21.10 ± 0.45^{b}	21.40 ± 0.42^{b}
Diabetes Treated	19.20 ± 0.26^{b}	14.23 ± 0.58^{c}	10.44 ± 0.24^{c}	09.70 ± 0.11^{c}	08.83 ± 0.16^{c}

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.

Before Alloxan administration, the fasting blood glucose level did not differ significantly ($p < 0.05$) between the three groups of experimental animals.72 hours after administration of Alloxan, the blood glucose level was

significantly ($p < 0.05$) higher in groups B and C animals. The blood glucose level of animals in group C gradually decreased on treatment with extracts of A. mannii over the period of four weeks (Table III). Control rats treated with distilled water were euglycemic throughout the period of experiment. At the end of the experiment, there was a Significant ($p < 0.05$) reduction in the blood glucose level of groups C rats compared to that of group B rats. In addition, there exist no significant ($p < 0.05$) difference between blood glucose level of rats in groups A and C (Table III) This shows that extracts of A. mannii demonstrate a potent antihyperglycemic activity.

Table 4: Effect of administration of ethanolic extract of Anisopus mannii on body weight (g) of alloxan- induced diabetic rats for four weeks.

Body Weight of the Rats (g)					
Treatments	0day	7 Days	14 Days	21 Days	28 Days
Normal Control	155.14±1.12[a]	165.62±1.13[a]	170.28±1.16[a]	176.56±1.12[a]	179.95±1.21[a]
Diabetes Control	151.71±1.70[a]	140.59±0.87[b]	135.59±1.22[b]	128.77±0.74[b]	127.40±1.91[b]
Diabetes Treated	153.92±1.74[a]	148.35±2.21[c]	151.79±2.06[c]	159.94±2.80[c]	169.94±1.77[c]

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.

Before Alloxan administration, there was no significant ($p < 0.05$) difference in the average weights of the three groups of experimental animals. By the end of the first week after diabetes mellitus was experimentally induced, the weights of Groups B and C animals was significantly ($p < 0.05$) reduced despite the increase in food and fluid intake in these animals. This weight loss continued for four weeks after Alloxan administration (Table IV) in Group B. However, the weight of animals in group C gradually increased on treatment with extracts of A. mannii over the period of four weeks as shown in (Table IV). At the end of the experiment, there was a significant ($p < 0.05$) difference in the weights of groups B and C animals while there was no statistically significant ($p < 0.05$) difference between weights of animals in groups A and C (Table IV).

Table 5: Effect of 28 days administration of ethanolic extract of Anisopus mannii on plasma lipid profile in alloxan- induced diabetic rats.

Lipid Profile Levels of the Rats (mg/dl)				
Treatments	T C	HDL	TG	LDL
Normal Control	150.80±0.92[a]	65.80±1.83[a]	126.51±1.10[a]	31.56±1.05[a]
Diabetes Control	280.97±1.79[b]	33.27±1.32[b]	228.76±1.22[b]	128.50±3.21[b]
Diabetes Treated	153.72±1.87[a]	64.65±1.72[a]	125.26±0.63[a]	36.42±2.08[c]

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

Before the alloxan administration, the plasma lipid profile level did not differ significantly ($p < 0.05$) between the three groups of experimental animals. 24 hours after administration of Alloxan, the plasma lipid profile level was altered significantly ($p < 0.05$) in groups B and C animals. The plasma lipid profile level of animals in groups B and C gradually elevated in TC, TG and LDL but decreases gradually in HDL. On treatment with extracts of A. mannii over the period of four weeks (Table V), Group C animals' plasma lipid profile was almost restored to normal. Control rats treated with distilled water were having normal plasma lipid profile throughout the period of experiment. At the end of the experiment, there was a significant ($p < 0.05$) difference in the plasma lipid profile level of groups C rats compared to that of group B rats. In addition, there exist no significant ($p < 0.05$) difference between plasma lipid profile level of rats in groups A and C (Table V). This shows that extracts of A. mannii demonstrate a potent antihyperglycemic activity and normalized the plasma lipid profile level.

Discussion

Many traditional plant treatments for diabetes mellitus are used throughout the world, and Management of diabetes without any side effect is still a challenge to the medical system. This has led to an increasing demand for natural products with anti-diabetic activity and fewer side effects. Many herbs and plant products have been shown to have hypoglycemic action. Various morphological parts of plants have been reported to be useful as effective remedies against diabetes. The unprecedented increase in diabetes continues to attract wider interest in the quest for more efficient management of diabetes mellitus (Jebur et al., 2016).This study investigated the anti-hyperglycemic potential of A.mannii stem and leaves using normoglycemic (normal control), oral glucose - loaded and alloxan induced (diabetes control and diabetes treated) hyperglycemic rat models. In the normoglycemic model, normal healthy animals were used in testing A.mannii as a potential oral hypoglycemic agent. Alloxan-induced hyperglycemia is a valid screening method that tests the effect of drugs in animals with an intact pancreatic activity (Williamson et al., 2016). Alloxan-induced hyperglycemia is widely accepted as a screening method for the study of antidiabetic agents (Etuk, 2010; Okoye et al., 2012). Alloxan monohydrate induces diabetes by selectively destroying the β-cells of the islets of Langerhans due to its selective accumulation through the glucose transporter 2 (GLUT2) and hence, minimizes the release of insulin and glucose uptake by peripheral tissues (Tafesse et al., 2017). The beta cytotoxic action of alloxan resulted in a sudden release of insulin, leading to severe hypoglycaemia and even mortality if glucose therapy is not given. It has been reported that insulin deficiency in animals, leads to the development of various metabolic aberrations (Gupta and Sharma, 2017).

Since the A.mannii ethanolic extract at low dosage prove to be antihyperglycemic. The oral glucose tolerance test (OGTT) is a research model used to verify the anti-

hyperglycemic actions of medicinal drugs (Ernsberger and Koletsky, 2015). As A.mannii ethanolic extract suppressed the rise in blood glucose concentration (BGC) following a glucose load, it suggests that the extract may be effective in controlling the overt postprandial rise in blood glucose that increases the risk of chronic hyperglycemia in diabetes. This may be as aresult of the reduced rate at which glucose is absorbed from the intestine; increased peripheral glucose utilization through a variety of metabolic pathways (Patel *et al.,* 2011); or a possible incretin mimetic effect. Thus, postprandial hyperglycemia control in diabetes is considered beneficial in reducing the risk of micro and macrovascular complications (Balkau, 2000; Ceriello, 2000).

The observed increase of BGC is in conformity with previous reports documenting elevated BGC in alloxan-induced hyperglycemic rats (Omonije *et al.,* 2019). Treatment with *A.mannii* ethanol extract even at the lowest dose of 400 mg/kg caused a marked reduction of BGC in this study, an indication of higher potency and a possible non-pancreatic mechanism of anti-hyperglycemic action (Patel *et al.,* 2011; Saxena andArgal, 2018). The reduction in BGC correlates with that of the normoglycemic model, which showed that *A.mannii* ethanol extract does not precipitate hyperglycemia in diabetic animals, this is an indication of its anti-hyperglycemic actions.

The significant loss of body weight observed from alloxan induced diabetes rats occur due to degradation of structural proteins responsible for the rigidity, consistency and elasticity of the tissues and also due to the increase in lipolysis. As insulin is responsible for regulating carbohydrate metabolism, protein synthesis, RNA synthesis and storage of cellular proteins, muscular glycogen and triglycerides in adipocyte. It also increases proteins catabolism and lipolysis which contributes to reduction in body weight of the rats (Klockner *et al.,* 2021). The significant increase in body weight after administration of *Anisopus mannii* show its ability to restore insulin in the body.

Diabetes is also associated with a variable lipid profile (Virdi *et al.,* 2003), which can be further linked to obesity and renal impairment (Al-Shamaony *et al.,* 1994). The marked hyperlipidemia that characterizes the diabetic state may be a consequence of the abnormal function of lipolytic hormones on the fat depots (Ayeleso *et al.,* 2012). Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Ayeleso *et al.,* 2012). The results of this study and previous investigations show that anti-hyperglycemic phytoconstituents produced a significant decrease in TC, TG and LDL and a significant increase in HDL of diabetes treated rats as compared to diabetic control. This improvement in the lipid profile status of alloxan-treated rats may revealed the anti-hyperlipidemic properties of *A.mannii* stem and leaves crude ethanol extract. It has also been previously reported that about 30% of blood cholesterol is circulated in the form of HDL-cholesterol (HDL). HDL removes cholesterol atheroma from arteries and transports it to the liver for excretion or re-utilization, which is a beneficial approach in cardiovascular diseases (Owolabi *et al.,* 2010). Therefore, the increase in the serum HDL level in hyperglycemic rats indicates that *A.mannii* stem and leaves crude ethanol extract may augment HDL effects (Osibemhe *et al.,* 2017).

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