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ANTIDIABETIC EFFECT OF FERMENTED *Pennisetum glaucum* (MILLET) SUPPLEMENT IN ALLOXAN INDUCED HYPERGLYCEMIC WISTAR RATS

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ABSTRACT
Diabetes mellitus is a debilitating disease that is characterized clinically by hyperglycemia due to chronic and/or relative insulin insufficiency. The disease is associated with disturbance in lipid and protein metabolism. Pennisetum glaucum (PG) has been recommended for several therapeutic purposes as it has been shown to have high amount of magnesium which helps to increase the levels of adiponectin hormone but the hypoglycemic effect of millet as a supplement is yet to be fully elucidated. Therefore this research was designed to determine the effect of Pennisetum glaucum supplement on blood glucose level and serum lipid profile. Diabetes mellitus was induced by single intraperitoneal (i.p) injection of alloxan dissolved in 0.1ml fresh cold citrate buffer pH 4.5 at a dose of 150 mg/kg body weight, after which the rats were randomly divided into 5 groups. Group 1 normoglycemic, Group 2 Diabetic untreated, Group 3 Diabetic treated with 1 mg/kg of Glibenclamide (GB). Group 4 Diabetic treated with 33%w/v P.G. supplement, Group 5 Diabetic treated with 66% P.G. supplement. When compared with the diabetic control, the study revealed a significant decrease (p>0.05) in blood glucose level at both 33%w/v and 66% w/v P.G. supplementation. It also showed that P.G. at both 33% and 66% supplement was able to lower Triglycerides and LDL serum levels as well increase HDL serum levels although not statistically significant, but significantly restored HDL/Cholesterol and Triglycerides/HDL ratios to normal physiological range. Pennisetum glaucum supplementation showed high hypoglycemic effect and also hypolipidemic property in alloxan induced hyperglycemic wistar rats.
Keywords: Pennisetum glaucum, hypolipidemia, hyperglycemia

INTRODUCTION

Diabetes is a serious health hazard currently affecting more than 220 million people worldwide and expected to afflict 400 million by 2030 (Wild, 2004; Shaw *et al*., 2010). The greatest increase in prevalence in the near future, however, is expected to occur in Asia, the Middle East, and Africa, where it is likely that there will be a 50% increase in diabetes in these parts of the world (Shaw *et al*., 2010). This is of public health concern due to its social and economic burdens. Eventhough diabetes has no known cause, complex interplay of several factors including genetic, social, and environmental factors are implicated in its etiology. It is a metabolic disorder characterized by insufficient secretion of insulin or resistance to the action of insulin or both. The major clinical manifestation of type 1 diabetes state is hyperglycemia and hypoinsulinemia however, insulin deficiency and/or insulin resistance also are associated with disturbance in lipid and protein metabolism (Mutaa and Omar, 2013). In type 1 diabetes, hyperglycemia occurs as a result of a complex disease process where genetic and environmental factors lead to an autoimmune response that remains to be fully elucidated. During this process, there is the destruction of the pancreatic-cells within the islets of Langerhans resulting in individuals with this condition relying essential on exogenous insulin administration for survival, although a subgroup has significant residual C-peptide production (Keenan *et al*., 2010). So far, the management of this disorder entails increased physical activity, healthy eating or diet and administration of anti-diabetic drugs and/or insulin. However, the currently available anti-diabetic drugs are have numerous side effects. Besides, these agents are costly and, in some cases, not readily available (Omotayo, 2014).

Pennisetum glaucum, also known as Pearl Millet is a cereal crop grown in tropical semi-arid regions of the world primarily in Africa and Asia. Pearl millet is rich in several nutrients as well as non-nutrients such as phenols. It has high energy, less starch, high fiber, low glycemic index and gluten free. The protein content ranges from 8 to 19% and it is low in lysine, high in tryptophan, threonine and the sulfur-containing amino acids. Pearl millet is rich in fat content (NIN 2003) such as Omega 3, linolenic acid (C18:3n-3) (LNA) comprises 4% (Burton *et al,* 1972). It contains various essential micro nutrients needed by the body. Its mineral content are B-vitamins, potassium, phosphorous, magnesium, iron, zinc, copper and manganese (NIN, 2003).

It has been recommended for several therapeutic purposes, as it has been found to inhibit tumor development (Lee, 2010), control blood pressure and plasma low density lipoprotein cholesterol levels (Hosoda, 2012), and possesses anti-allergenic characteristics.

The aim of the study was to evaluate the effect of the *Pennisetum Glaucum* supplement on blood glucose level and lipid profile in alloxan induced hyperglycemic wistar rats.

MATERIALS AND METHODS

Experimental Animals

A total of 25 wistar rats of both sexes weighing 160g-200g were purchased at the experimental animal house of the Department of Human Physiology, Ahmadu Bello University Zaria. Animals were maintained under normal laboratory conditions. The animals were fed on *pellets* of growers mash and allowed access to water *ad libitum.* The study was conducted in accordance with the guidelines of Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally (National Institute of Health Guide for Care and Use of Laboratory Animals)

Collection, Identification and Preparation of Supplement

Pennisetum Glaucum was purchased from Institute of Agricultural Research Zaria, Kaduna State of Northern Nigeria. It was identified at the Herbarium unit of the department of Biological Sciences, Ahmadu Bello University, Zaria and a specimen voucher with the number 27111 was deposited. Three (3) kg of the pearl millet (*Pennisetum Glaucum*) was soaked in water for 72 hours. Thereafter, it was air dried under the sun for seven days. Later it was grounded to powder using grinding machine.

Drugs Used

Alloxan was purchased from (Sigma chemical company St. Louis U.S.A), glibenclamide and all other chemicals and reagents that were used were of analytical grade.

Induction of Diabetes Mellitus

After 16-18 hours of fasting, hyperglycemia was induced by a single intraperitoneal injection of alloxan monohydrate (Sigma St. Louis, M.S., U.S.A.) at a dose of 150mg/kg body weight (dissolved in 0.9% NaCl cold normal saline solution) (Katsumata *et al*., 1999). Rats were treated with 20% glucose solution orally for 6hrs due to the ability of alloxan to produce fatal hypoglycemia as a result of massive pancreatic insulin release. They were placed on 5% glucose solution for 24 hrs to prevent hypoglycemia (Dhandapani *et al*., 2002). Blood was collected from the tail vein of the rats after 72hrs of alloxan injection. Rats having fasting blood glucose level greater than 200mg/dl were selected for the study (Matteucci and Giampietro, 2008).

Experimental Design and Treatment

Wistar rats were weighed and randomly assigned into 5 groups, with 5 animals (n=5), in each. Group 1 (normal control) - Normogycemic wistar rats fed with pellets grower mash only , Group II (diabetic control)- Alloxan induced hyperglycemic wistar rats +normal saline (0.2ml) orally fed with, Group III- Alloxan induced hypeglycemic wistar rats + 33% millet supplement (Goodarzi, *et al*., 2011), Group IV- Alloxan induced hyperglycemic wistar rats + 66% millet supplement (Goodarzi, *et al*., 2011), Group V- Alloxan induced hyperglycemic wistar rats + glibenclamide (5mg/kg) orally

Blood Glucose Determination

Fasting blood glucose levels was determined at day 0, 4, 8, 12 and 14 respectively for 2weeks using the glucose oxidase principle (Beach and Turner, 1958) using one touch digital glucometer

Sample Collection

At the end of the experiment, after overnight fasting, the animals were anaesthesized using chloroform and dissected, blood was collected through cardiac puncture, centrifuged and serum was used for assessment of lipid profile.

Lipid Profile Assay

The method of Roeschlau and Gruber was applied using assay kits (Randox Laboratories Ltd., UK) to determine the total serum cholesterol spectrophotometrically at 546 nm following enzymatic hydrolysis and oxidation (Allain et al., 1974). The HDL-cholesterol was determined using assay kits (Randox Laboratories Ltd., UK) after low density lipoprotein (LDL and VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction in the supernatant was assayed colorimetrically at 540 nm (Allain et al., 1974).

Statistical Analysis

Data collected were expressed as mean $\pm$ SEM. They were analyzed by one way analysis of variance, ANOVA, and *Tukey's post-hoc* test was used to compare the level of significance between the control and treatment groups, using SPSS version 20.0. Values of $p<0.05$ were considered significant.

RESULTS

Effect of *Pennisetum Glaucum* (PG) supplement on blood glucose level: The results of the present study showed that blood plasma glucose level in the diabetic control group was significantly higher($p<0.05$) when compared with the normal control group in the entire 16 days of the study. The effect of P.G. on blood plasma glucose level was determined by comparing the values in the diabetic control group (i.e. Group 2) with the values in Group 4 (i.e. 33% P.G.) and in Group 5 (i.e. 66% P.G.). These results showed that P.G. supplement was lowered the blood Glucose Level ($p<0.05$) within sixteen days of its administration dose dependently on Day 12 and 16 respectively, when compared with the Diabetic Control. Its effect appeared to be even more potent and significant than that of the standard drug glibenclamide (Table 1)

Table 1: Effect of Sixteen Days Administration of *Pennisetum glaucum* (PG) Supplement on Blood Glucose Levels (mg/dl) in Alloxan Induced Hyperglycemic Wistar Rats

Group/Treatment	Day 0	Day 4	Day 8	Day 12	Day 16
Normal control	68.00 ± 6.25	50.40 ± 2.75	70.60 ± 7.09	63.80 ± 3.87	70.40 ± 3.54
Diabetic control	430.60 ± 56.14	414.60 ± 80.37	326.40 ± 31.04	324.20 ± 23.06	292.40 ± 25.13
33% PG	434.60 ± 53.22	456.80 ± 88.31	315.20 ± 58.20	253.00 ± 70.79	128.80 ± 34.50*
66%PG	434.20 ± 69.98	418.00 ± 74.35	246.40 ± 50.70	143.60 ± 26.26*	106.40 ± 15.48*
Glibenclimide	430.00 ± 74.65	332.40 ± 72.29	181.80 ± 38.94	120.20 ± 27.43*	85.40 ± 19.03*

Group 1: Normoglycemic Rats; Group 2: Hyperglycemic Rats (Diabetic Control); Group 3: Diabetic Rats + 2mg/kg Glibenclamide (Positive Control); Group 4: Diabetic Rats Treated with 33% of P.G. Supplement; Group 5: Diabetic Rats Treated with 66% of P.G. Supplement.

* Statistically Significant at p< 0.05 when compared with Untreated Diabetic Control

Effect of *Pennisetum Glaucum* Supplement on Serum Lipid Profile: These results of the present study revealed that P.G. at 33% and 66% supplement was able to lower cholesterol levels dose dependently and significantly when compared to the diabetic untreated group more than the standard drug glibenclamide. It also showed that P.G. at both 33% and 66% supplement lowered the Triglycerides and LDL serum levels(p<0.05) as well increase HDL serum levels although not statistically significant (Table 2).

Table 2: Effect of Sixteen Days Administration of *Pennisetum glaucum* (PG) Supplement on Lipid Profile in Alloxan Induced hyperglycemic Wistar Rats

Group/Treatment	Total Cholesterol(mmol/L)	Triglycerides(mmol/L)	HDL(mmol/L)	LDL(mmol/L)
Normal control	1.16 ± 0.10	1.11 ± 0.10	0.51 ± 0.09	0.14 ± 0.04
Diabetic control	2.64 ± 0.08	1.42 ± 0.12	0.33 ± 0.07	1.46 ± 0.14
33% PG	1.72 ± 0.26	0.81 ± 0.01	1.85 ± 0.81	0.10 ± 0.99
66%PG	1.60 ± 0.35*	1.39 ± 0.14	0.60 ± 0.21	0.43 ± 0.26
Glibenclimide	1.43 ± 0.25*	1.21 ± 0.25	0.65 ± 0.14	0.23 ± 0.08

Group 1: Normoglycemic Rats; Group 2: Hyperglycemic Rats (Diabetic Control); Group 3: Diabetic Rats + 2mg/kg Glibenclamide (Positive Control); Group 4: Diabetic Rats Treated with 33% of P.G. Supplement; Group 5: Diabetic Rats Treated with 66% of P.G. Supplement.

* Statistically Significant at p< 0.05 when compared with Untreated Diabetic Control

DISCUSSION

The results of the present study showed that blood plasma glucose level in the diabetic control group was significantly higher(p<0.05) when compared with the normal control group in the entire 16 days of the study. The effect of P.G. on blood plasma glucose level was determined by comparing the values in the diabetic control group (i.e. Group 2) with the values in Group 4 (i.e. 33% P.G.) and in Group 5 (i.e. 66% P.G.). These results showed that P.G. supplement was able to lower blood glucose Level within sixteen days of its administration dose dependently and significantly particularly on Day 12 and Day 16 when compared with the Diabetic Control. Its effect appeared to be even more potent and significant than that of the standard drug glibenclamide. Glibenclamide, a well-known oral hypoglycaemic drug belongs to the family of the sulfonylureas a first generation anti-diabetic drug; elicits its hypoglycaemic effect mainly by acting on ATP-sensitive potassium channel in pancreatic beta-cells. The blood glucose lowering effect of P.G. and the possible mechanism involved in the hypoglycemic action of P.G. may be stimulation of insulin secretion by the pancreas or/and enhance insulin sensitivity in various organs due to its high amount of magnesium and selenium. Magnesium helps to improve the ability of cells to respond to insulin, by increasing the levels of adiponectin hormone. Adiponectin prevents the accumulation of glucose in the blood by increasing fat metabolism in tissues (Nishizara, 2009). Selenium-yeast has shown to possess hypoglycaemic property that is comparable to the oral hypoglycaemic drug, glibenclamide as described by Mohammed *et al.* (2015). Results of lipid profile are considered as good indicators of whether someone is prone to develop stroke or heart attack, caused by atherosclerosis. Hyperlipidaemia, a risk factor of cardiovascular diseases is frequently seen among diabetic patients. Serum lipid levels are commonly increased in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease (Daniel *et al*., 2011). The results of the present study revealed that P.G. at 33% and 66% supplement was able to lower cholesterol levels dose dependently and significantly when compared to the diabetic untreated group more than the standard drug glibenclamide. It also showed that P.G. at both 33% and 66% supplement was able to lower Triglycerides and LDL serum levels as well increase HDL serum levels although not statistically significant, but significantly restored HDL/Cholesterol and Triglycerides/HDL ratios to normal physiological range. The findings of the present study agrees with the findings of Lee(2010) who reported that the levels of triglycerides, low density lipoprotein decreased while high density lipoprotein levels increased significantly in diabetic mice fed P.G. supplement (Lee, 2010). Medugu *et al*., (2010) showed that abdominal fat of broilers on millet-based diets were significantly lower than those on maize and high-tannin sorghum based diets.

These indicate that millet and sorghum can be well-utilized to produce broiler chickens with superior carcass quality compared to maize. Nishizara also showed that dietry Japanese millet protein ameliorated plasma levels of lipids in type 2 diabetic mice and also increased the levels of high density lipoprotein (Nishizara, 2009).

The ability of P.G. supplement to reduce plasma cholesterol and triglycerides in diabetic animals could be explained by its reported high content of magnesium. Magnesium helps to improve the ability of cells to respond to insulin, by increasing the levels of adiponectin hormone. Adiponectin is produced in fat tissues, which is a beneficial hormone that helps in energy metabolism, it helps cells burn fats for energy and improve cardiovascular health (Nishizara, 2009). Since insulin is required for the inhibition of hormone-sensitive lipase and hence increases the utilization of glucose and thereby decreasing the mobilization of free fatty acids from the fat depots. The decreased level of FFA is also associated with decreased actions of lipolytic hormones, which, in turn, decreased the activity of hormone sensitive lipases on fat deposits (Daniel *et al*., 2015). The cholesterol lowering effect of P.G. may be due to the availability of flavonoids in it, Flavonoids as antioxidants may inhibit the oxidation of LDL cholesterol (prevention of atherosclerotic plaque formation) (Middleton, *et al.,* 2000; Duarte-Alameida, *et al*., 2007).

CONCLUSION

The study confirmed the association between of hyperglycemia and abnormal lipid profile. Administration of *Pennisetum glaucum* supplement at different percentages lowered blood glucose level which was even more effective than the standard drug glibenclamide. *Pennisetum glaucum* lower the cholesterol levels more than the standard drug glibenclamide. Its lower triglyceride levels, increased serum HDL levels although not statistically significant, but significantly restored HDL/Cholesterol and Triglycerides/HDL ratios to normal physiological range. Hence, it can be concluded that supplementation with *Pennisetum Glaucum* has high hypoglycemic effect and also hypolipidemic property in alloxan induced hyperglycemic wistar rats.

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