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Effect of soya beans supplement on blood glucose levels and haematological indices on alloxan induced diabetic wistar rats

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ABSTRACT

The aim of this study is to evaluate the effects of soya bean supplement blood glucose levels and haematological parameters on alloxan-induced diabetes in Wistar rats. The animals were grouped into four groups of five rats in each group. Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate at a dose of 150mg/kg body weight. The animals were fed with 25% and 50% of the soya beans supplement for two weeks. Glibenclamide was used as a standard anti-diabetic drug and was given orally to the positive control diabetic group. The fasting blood glucose levels were determined at intervals of 0, 1, 3, 6, 9 and 12 days respectively. The animals were sacrificed and blood samples were taken from all the groups for the determination of haematological parameters. As regard to the blood glucose levels there was a significant decrease ($p<0.05$) when compared with the control non supplemented group. The highest activity resides at the group supplemented with 25% soya beans. In relation to the haematological parameters there was a significant increase in the white blood cell count, while packed cell volume, Red blood cell count diferential count and haemoglobin level there was no significant different between the control and soya beans supplemented groups.

Keywords: Soya beans; Glibenclimide; Alloxan monohydrateBlood glucose, haematological indices.

INTRODUCTION

Uncontrolled diabetes leads to several micro-vascular (neuropathy, nephropathy, retinopathy) and macro-vascular (atheroma) complications that affect many organs of the body. Diet plays an important role in the management of diabetes mellitus and the health- beneficial effects of dietary fibers and antioxidants derived from plant food sources have been extensively studied [1,2,3,4].

Diabetes is a chronic disease, which occurs when the pancrease does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia) [5]. This chronic hyperglycemic condition is associated with long term damage, dysfunction and failure of various organs especially eyes, kidney, nerves, heart and blood vessels [6,7].

The incidence of diabetes mellitus in the human population has reached epidemic proportions worldwide, and it is increasing at a rapid rate. World Health Organization (WHO) estimated that approximately 120 to 140 million people were globally affected by diabetes mellitus in 1999 [8]. In 2000, this figure increased to more than 177 million [9] and is projected to increase to 221 million by 2010 [10] and to double by the year 2025[11,9]

Soya beans (*Glycine max L*) belong to the legume family and have been shown to have numerous health promoting effects mostly attributed to their high nutrient[12] and phytochemical (isoflavone) content[13]. Soya protein is considered a complete protein in that it contains most of the essential amino acids that are found in animal proteins. The nutritional value of soy protein is roughly equivalent to that of animal protein of high biological value [14] . Soya bean contains complex carbohydrates, protein, dietary fiber, oligosaccharides, phytosterol, saponin, lecithin, isoflavone, phytic acid, trypsin inhibitor, and minerals. Complex carbohydrates and dietary fiber contents contribute to low glycemic indexes, which benefit diabetic individuals [15] and reduce the risk of developing diabetes.

MATERIALS AND METHODS

Animals

A total of twenty Wistar rats of both sexes, weighed between 120 to 150 g were used for the study. The animals were housed in the animal house, Department of Human Physiology, ABU, Zaria. The animals were randomized into experimental and control groups and were kept in polypropylene cages. The animals were maintained on standard animal feeds and drinking water *ad libitum*.

Chemicals and drugs

All chemicals and drugs used were of analytical grade. Alloxan was purchased from (Sigma chemical Company St. Louis U.S.A.). A digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) was used for the determination of the blood glucose levels of the animals.

Collection of soya beans seed

Soya beans seed were purchased from Samaru market, Zaria, Kaduna State in the month of October 2012. It was identified and authenticated in herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria. Nigeria.

Induction of experimental diabetes mellitus

The animals were fasted for 16–18 hours with free access to water prior to the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9%$^{v/v}$ cold normal saline solution at a dose of 150 mg/kg body weight [16]. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution intraperitoneally after 6h. The rats were then kept for the next 24h on 5 % glucose solution bottles in their cages to prevent hypoglycemia [17]. The diabetes was assessed in alloxan-induced rats by determining the blood glucose concentration 72 hours after injection of alloxan. The rats with blood glucose level above 200mg/dl were then selected for the study.

Experimental design

After the induction of diabetes mellitus in the Wistar rats, the animals were randomly divided into experimental and control groups. All animals were fasted for 16-18 hours before treatment. Fasting blood glucose levels of each group was determined at 0, 1, 3, 6, 9, and 12days. All the animals were sacrificed at the end of the two weeks after fasting them for 16-18 hours. The rats were anaesthetized at the time of sacrifice by being placed in sealed cotton wool soaked chloroform inhalation jar. Blood was collected via cardiac puncture from each animal for determination of haematological parameters

Group 1: Diabetic negative control, were administered to distilled water orally daily.
Group 2: Diabetic positive control, administered to 5mg/kg b/w of glibenclamide orally daily.
Group 3: Diabetic rats were fed with 25% soya beans for two weeks
Group 4: Diabetic rats were fed with 50% soya beans for two weeks.

Determination of blood glucose levels

Blood samples were collected from the tail vein of the rats at intervals of 0, 1, 3, 6, 9, and 12 days respectively. Blood glucose levels were determined using the glucose oxidase method [18] using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) and the results were expressed in the unit of *mg/dl* [19].

Determination of haematological parameters

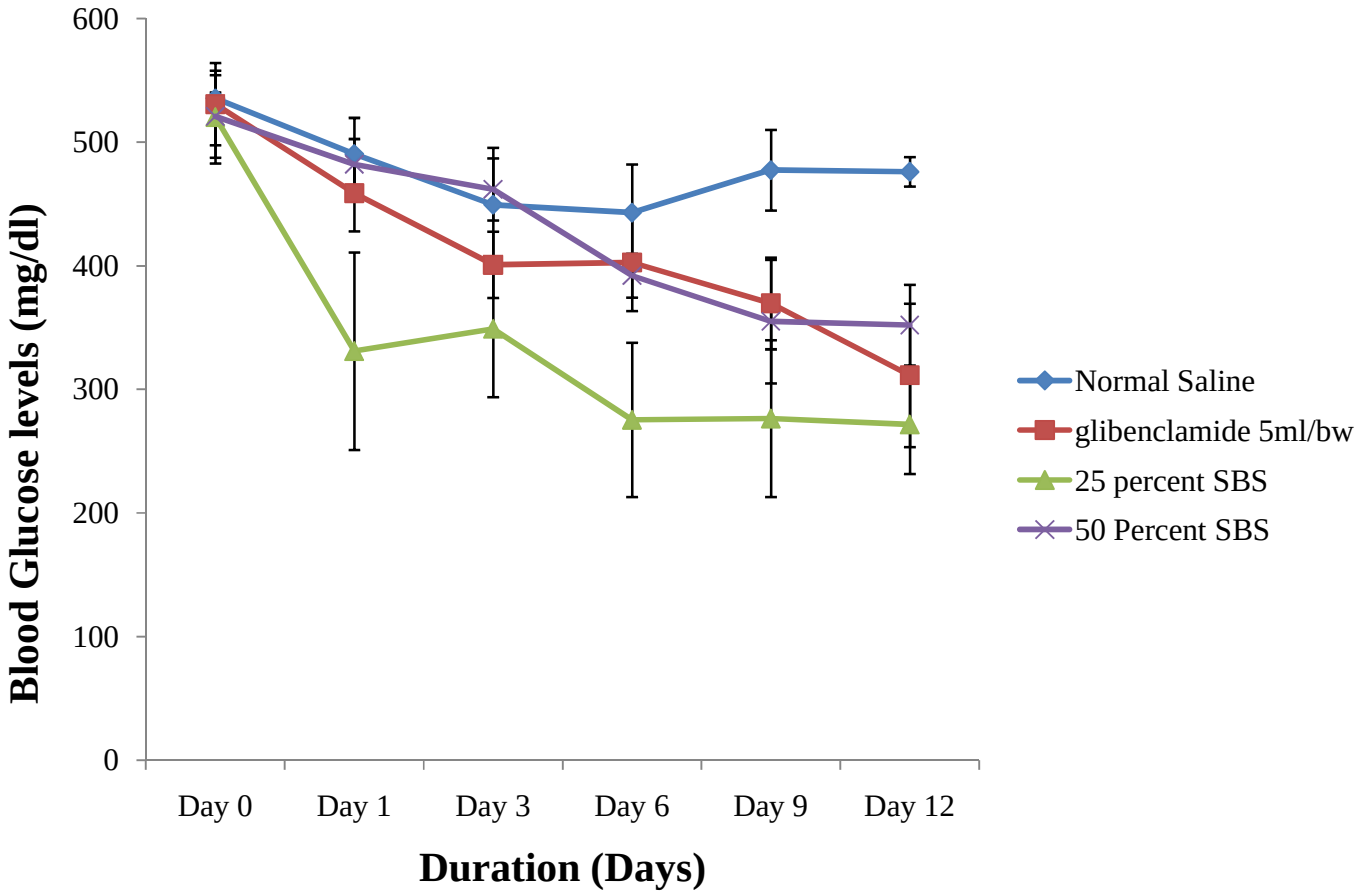
Red blood cells (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell count (WBC) and differential counts (DC) using the method of [20] .

Statistical Analysis

All the data are expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests [21]. The results were considered statistically significant if the P values were 0.05 or less.

RESULTS

Fig 1: Effect of Soya beans supplement on blood glucose levels



There was a significant reduction in the blood glucose levels in the groups supplemented with soya beans ($p<0.05$) when compared to the control group. The highest activity reside at the group fed with 25% soya beans as showed in fig 1 above.

Table 1: Showing erythrocytes indices levels in Alloxan-induced diabetic wistar rats over 2 weeks treated with soya beans supplement

Group	RBC10^{12}/L	PCV%	Hb g/dl
Normal saline	7.50 ± 0.29	30.6 ± 2.58	10.2 ± 0.36
Positive control	8.0 ± 0.52 ^{ns}	33.6 ± 1.16 ^{ns}	11.6 ± 0.33 ^{ns}
25% SBS	8.28 ± 0.53 ^{ns}	38.0 ± 1.35 ^{ns}	11.2 ± 0.56 ^{ns}
50% SBS	8.44 ± 0.25^{ns}	36.1 ± 1.18 ^{ns}	11.0 ± 0.44 ^{ns}

Values are expressed as mean ±SEM; n = 5. Value considered statistically significant when compared with control group: a = p<0.05 significant and ns = not significant.

The above table shows the results of the effect of 25%, 50% soya beans supplement, glibenclamide on red cell indices on Alloxan-induced Wistar rats. There was no statistically significant difference in all the tested parameters i.e RBC, PCV and HB levels as compared with the untreated group.

Table 2: Showing leucocytes levels in Alloxan-induced diabetic Wistar rats after 2 weeks of treatment with soya beans supplement.

Group	WBCx10^9/L	Neutrophil %	Lymphocyte %	Monocyte %	Eosinophils %	Basophils %
Glibenclamide	5.02 ± 0.98^{a}	21.2 ± 1.15^{ns}	74.2 ± 1.31^{ns}	0.32 ± 0.37^{ns}	1.4 ± 0.24^{ns}	0.2 ± 0.2
Normal saline	3.98 ± 0.17	17.0 ± 1.7	65 ± 1.32	0.19 ± 0.58	1.4 ± 0.24	0.0 ± 0.0^{ns}
25% SBS	5.36 ± 0.58^{a}	19.4 ± 1.72^{ns}	73.8 ± 2.08^{ns}	0.26 ± 0.61^{ns}	1.4 ± 0.4^{ns}	0.0 ± 0.0^{ns}
50% SBS	5.06 ± 0.42^{a}	2.02 ± 1.15^{ns}	76.6 ± 1.2^{ns}	0.20 ± 0.84^{ns}	1.2 ± 0.2^{ns}	$0.0. \pm 0.0^{ns}$

Values are expressed as mean ±SEM; n = 5. Value co nsidered statistically significant when compared with control group: a = p<0.05 significant and ns = not significant.

There was no statistically significant difference in Neutrophil, Basophil, Eosinophil, Monocytes and Lymphocyte levels in the treated groups as compared with the untreated group, while there was a statistical significant increase in the WBC count.

DISCUSSION

Alloxan acts as a cytotoxin for beta-cells of the islet of langerhans, causing diabetes by inducing cell necrosis [22,23]. The reactive oxygen species mediates the cytotoxic action with the increase in cytosolic calcium concentration, leading to rapid beta-cells destruction[24]. This results in decreased insulin secretion and elevated blood glucose levels [25]. The alloxan- treated rats, therefore, appear to represent a good laboratory model for type 1 diabetes mellitus experimental diabetes state, with residual or remnant insulin production by the pancreatic beta - cells. There is possibility for the survival of a few beta-cells and this has been proved by several workers who observed antihyperglycemic activity with oral hypoglycemic agents like glibenclamide, tolbutamide etc. in alloxan-induced diabetic rats[26,27,28].

[29] had reported that soya fiber supplementation in the diets of patients with IGT (Impaired glucose tolerance) significantly reduced fasting blood glucose level and the level of insulin was significantly increased. The results of this study indicate that the supplement caused a significant ($p<0.05$) decrease on the blood glucose levels in the Alloxan-induced diabetic rats compared to the negative control group. The highest activity was seen with the lowest dose of the supplement, which is 25%. The effect of improving glucose levels may be explained by the function of various components of the soya beans. The soya bean fiber contains pectins, galactomannans and arabinogalactans with high viscosity. These substances delay gastric emptying and glucose absorption[30]. The effect of soya bean fiber may also be attributed to reduced glucose absorption caused by slowing gastric emptying and by limiting the rate of intraluminal diffusion of glucose to the absorption surface[31]. Consumption of high-isoflavone soy protein diet has also been shown to improve glucose tolerance, insulin resistance, and hepatic cholesterol and triglyceride concentrations in obese Zucker rats[32]. In a study by[33], it was indicated that the soya bean extracts (chloroform and alcohol) possess significant antidiabetic activity along with antihypercholesterolemic activity[33]. The possible mechanism by which the soya beans supplement reduced blood glucose concentration of the diabetic rats maybe either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhan's or by its release from bound insulin [34]. This mechanism of action has been suggested for other plants that have been studied [35,36,37].
As regards to the haematological indices there was a significant increase ($p<0.05$) in the WBC count while there was no significant change in the counts of RBC,DC ,PCV and Hb, although there was a slightly increase but it is not statistical significant when compared with the control untreated group. Anaemia occurs frequently in adult patients with type 1 diabetes [38,39]. Diabetes has been associated with several complications occasioned by oxidative stress[40]. Oxidative stress has been implicated in the major complications of DM, including accelerated coronary artery disease [41] and increased erythrocyte osmotic fragility. Erythrocyte lifespan was shortened because of increased mechanical fragility of the cell membrane[42]. Because RBCs are oxygen carriers with high polyunsaturated fatty acid (PUFA) content on their membranes and a high concentration of cellular Hb, they are therefore particularly exposed to oxidative damage[43]. The oxidative stress in patients with type 1 diabetes caused Hb glycation [44], glutathione oxidation [45], erythrocyte fragility and an increase in lipid peroxidation [32] Hb values were ubiquitous in clinical practice for the diagnosis of type 1 diabetes and also important for evaluating long-term glycaemic control in patients with type 2 diabetes [46]. Extracorpuscular Hb is increased in diabetes due

to increased RBC fragility resulting in a shorter RBC half-time [47]. More specifically, Hb derived iron might contribute to the pathogenesis of type 1 diabetes by inducing oxidative stress [48].

In conclusion, the results of the present study clearly demonstrated that the soya beans supplement possessed a remarkable blood-glucose lowering potential and increase in the white blood cell count (WBC) on alloxan induced diabetic Wistar rats.

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