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Modulatory role of soya beans supplement on lipid profiles and liver enzymes on alloxan-induced diabetic wistar rats

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ABSTRACT

This study evaluates the modulatory role of soya beans supplement on lipid profiles and liver enzymes on alloxan-induced diabetic Wistar rats. Twenty Wistar Rats of both sexes weighed between 120-150 grams were used for the study. Induction of diabetes was done by single intraperitoneal injection of Alloxan monohydrate at a dose of 150mg/kg body weight. The animals were fed with 25% and 50% soya bean as supplements for a period two weeks. Glibenclamide was used as a standard anti-diabetic drug and was given orally. At the end of the two weeks of treatment, the animals were sacrificed and blood serum samples were taken from all the groups for the determinations of lipid profiles and liver enzymes. As regards to the lipid profiles there was a significant decrease ($P<0.05$) in the serum levels of TG and TC in the soya beans supplemented groups when compared with the control unsupplemented group, while there was a significant increase in the serum level of HDL . In relation to the liver enzymes there was a significant decrease ($p<0.05$) in serum levels of AST,ALP and ALT in the soya beans fed groups when compared to the control group.

Keywords: Soya bean, Alloxan, Glibenclimide, Liver enzymes, Lipid profiles.

INTRODUCTION

Diabetes mellitus is a metabolic disorder with different etiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism, caused by the complete or relative insufficiency of insulin secretion and action[1]. This chronic hyperglycemic condition is associated with long term damage, dysfunction and failure of various organs especially eyes, kidney, nerves, heart and blood vessels[2,3] .

It is the fourth or fifth leading cause of death in most high-income countries and there is substantial evidence that it is epidemic in many economically developing and newly industrialized nations[4] .

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 [5]. In 2000, this figure increased to more than 177 million [6] and is projected to increase to 221 million by 2010 [7] and to double by the year 2025[8,6] . All eukaryotic organisms and even a few prokaryotes have the ability to synthesize triacylglycerols, and the process has been studied intensively in plants and animals especially [9]. The lipid serves as a store of energy, which can be released rapidly on demand, and as a reserve of essential fatty acids and precursors for eicosanoids. However, lipid

droplets may also serve as a protective agency to remove any excess of biologically active and potentially harmful lipids such as free fatty acids, diacylglycerols, cholesterol (as cholesterol esters), retinol esters and coenzyme A esters [10].

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[11] and phytochemical (isoflavone) content [12]. 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 soya protein is roughly equivalent to that of animal protein of high biological value[13]. 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

[14] and reduce the risk of developing diabetes.
The aim of this research is to determine modulatory role of soya bean supplement on Lipid profiles and Liver enzymes on alloxan -induced diabetes Wistar rats

MATERIALS AND METHODS

Animals

Twenty albino Wistar rats of both sexes between the ages of 8 to 12 weeks old and weighing 120-150grams were used for this 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. Standard animal feeds were used during the experimental period. The control and experimental animals were provided with commercially prepared feeds and drinking water *ad libitum*. The "Principle of laboratory animal care " (NIH publication No 85- 23) guideline and procedures were followed in this study (NIH publication reserved 1985).

Chemicals and drugs

All chemicals and drugs used were of analytical grade.

Collection of soya beans seed

Soya bean seeds 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.

Experimental Induction of Diabetes mellitus

The animals were fasted for 16 to 18 h with free access to water prior to the induction of diabetes. Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate (Sigma St. Louis, M.S., U.S.A.) at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline solution[15]. Since Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia[16]. After 72 hours of alloxan treatment, blood was collected from tail vein of the rats. Rats having fasting blood glucose level greater than 200 mg/dl were considered as diabetic and selected for the study

Experimental design

After the induction of diabetes mellitus in the rats, the animals were randomly divided into experimental and control groups. All the animals were sacrificed at the end of the two weeks after fasting them for 12 to 16 h. 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 lipid profiles and liver enzymes. The Wistar rats were subdivided as follows;

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

Preparation of Serum Samples
After two weeks treatment period, the rats were fasted for 12 – 16 hours and then anaesthetized at the time of sacrifice by being placed in sealed cotton wool soaked chloroform inhalation jar. Blood samples were collected from all the animals through cardiac puncture into plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 r p m for 10 minutes and the supernatant (serum) collected was used for the determination of lipid profiles and Liver enzymes analysis.

Determination of Serum Total Cholesterol
The serum level of total cholesterol (TC) was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of [17]. 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value of TC present in serum was expressed in the unit of mg/dl.

$$TC\ concentration = A\ sample\ /A\ standard\ x\ 196.86\ mg/dl$$

Determination of Serum Triglyceride
The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of [18] . 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value of triglyceride present in the serum was expressed in the unit of mg/dl.

$$TGL\ concentration = A\ sample/A\ standard\ x\ 194.0\ mg/dl$$

Determination of Serum High-Density Lipoprotein Cholesterol
The serum level of HDL-C was measured by the method of [19] . Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphaturic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dl.

Determination of Serum Low-Density Lipoprotein Cholesterol
The serum level of (LDL-C) was measured according to protocol of [20] using the equation below:

$$LDL\text{-}C = TC - (TGL/5 + HDL\text{-}C)$$

The value was expressed in the unit of mg/dl.

Determination of Liver Enzyme assay
Blood sample were collected via cardiac puncture, which were centrifuge to get serum for liver enzymes assay. This include; Alkaline phosphatase, Alanine aminotranferase and Aspartate aminotranferase using the method of [21] .

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[22]. The results were considered statistically significant if the p values were 0.05 or less.

RESULTS

Table 1: Effect of soya beans supplement on Serum Lipid profiles on Alloxan-induced diabetic Wistar rats

Group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	High density lipoproteins (mg/dl)	Low density lipoproteins (mg/dl)
Negative control(Distilled water)	113.6 ± 14.50	121.2 ± 5.10^{ns}	18.5 ± 2.92	20.4 ± 2.55
Positive control(Glibenclimide)	99.4 ± 9.61^{ns}	103.9 ± 2.53^{a}	22.7 ± 2.21^{a}	17.3 ± 2.01^{ns}
25% Soya beans supplement	74.0 ± 4.37^{a}	95.9 ± 1.20^{a}	37.3 ± 2.50^{a}	11.6 ± 1.76^{a}
50% Soya beans supplement	84.6 ± 2.46^{a}	104.4 ± 4.20^{a}	26.1 ± 1.63^{a}	13.9 ± 1.52^{a}

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

Table 2: Effect of soya beans supplement on serum Liver enzymes on Alloxan-induced diabetic Wistar rats

Group	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)
Negative control (5ml/kg)	184.8 ± 5.13	76.7 ± 4.79	62.8 ± 2.25
Positive control glibenclamide (5mg/kg)	156.4 ± 4.35 ^{a}	52.7 ± 3.30 ^{a}	52.7 ± 3.42 ^{a}
25% Soya beans	156.4 ± 4.35 ^{a}	51.2 ±3.29 ^{a}	41.9 ±2.25 ^{a}
50% Soya beans	143.5 ± 5.13 ^{a}	56.0 ± 4.33 ^{a}	42.8 ±2.68^{a}

Values are expressed as means ±SEM; n=5. Value considered statistically significant when compared with control group: = p<0.05 as significant.

DISCUSSION

Oxidative stress, altered lipid levels, and disturbances in glucose metabolism are important risk factors for diabetes, cardiovascular, oncologic and many other diseases. Diet undoubtedly plays a key role as chemopreventive agent against various diseases and optimizing the diet in both quality and quantity, has a preventive function. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids such as triglyceride and total cholesterol in diabetic patients [23]. Diabetes is associated with hyperlipidemia [24]. Diabetic-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to under utilization of glucose[25,26] . Soya protein is associated with fatty acids, saponins, isoflavones and phospholipids [27] . Many nutritional factors such as isoflavone, saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia [26,28] . This study showed a decrease in the concentrations of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in the diabetic rats. These effects might be due to low activity of cholesterol biosynthesis enzymes or low level of lipolysis that are under the control of insulin [29] . Phyhtosterols, another component of soya beans have structural similarity to cholesterol. This enables them to compete with cholesterol for incorporation into micelles, which are translocated over the brush border membrane, from the gut into the plasma, *via* intestinal cholesterol transporters, known as NPC1L1 and SR-BI [30,31] . This leads to a reduction in the concentration of low density lipoprotein (LDL) and total cholesterol concentration in the blood.

After soya beans supplementation, compared to the control group, levels of triglycerides ($p<0.05$) were significantly lower in the soya bean group. The study of [32] observed that soya bean fiber significantly decreased the level of triglycerides in diabetic rats. It appears that soya bean fiber delayed the absorption of glucose and fatty acids from the upper small intestine, thus providing less substrate for triglycerides synthesis. [33] also reported that soy polysaccharide significantly reduced the rise of postprandial plasma triglyceride levels. About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease[34,35]

Damage of cellular components may play an important role in death of liver cells [36,37,38], hence Alanine aminotransferase (ALT), Aspartate aminotransferase(AST) and Alkaline phosphatase (ALP) may be released to plasma, and serum levels of these enzymes would increase. High serum level of AST and ALT are usually indicative of liver damage in animals [39] and humans [40]. A study conducted by [41]. suggested that liver enzymes levels

change considerably even in alloxan-induced diabetes, and that using onion or garlic extract can be helpful in reducing these enzymes[41]. There was a significant ($p<0.05$) decrease in the level of these enzymes in the treated groups when compared with the control group after treatment in the diabetic groups. The high level of liver enzymes, Alanine aminotransferase (ALT), Aspartate aminotransferase(AST) and Alkaline phosphatase (ALP) in the control group as compared to the treated group could be revealing acute related hepatocytes damages, probably caused by the effect of alloxan on the liver, since these enzymes are hepatocytes enzymes and can be released due to hepatocytes injury [42].

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