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Research Article

Effects of fermented soya bean supplements on lipid profile and oxidative stress biomarkers in high fat diet-induced Type 2 diabetes mellitus in rabbits

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ABSTRACT

Background: High fat diets are known to increase body weight and fat mass, induce alterations in carbohydrate and lipid metabolism, leading to insulin resistance and diabetes mellitus. The aim of this study was to evaluate the effects of fermented soya bean supplements on lipid profile and oxidative stress biomarkers in high fat diet-induced type 2 diabetes mellitus in rabbits. **Methods:** Twenty rabbits weighing between 1kg – 2kg were used for the study. Type 2 diabetes mellitus was induced by feeding the animals with high fat diet (2% cholesterol, 20% groundnut meal, 10% groundnut oil) for eight weeks. Rabbits having fasting blood glucose levels of 7.2 mmol/L (130mg/dL) and above after the induction period were selected for the study. The animals were grouped into four groups of five rabbits each: Group 1 (diabetic control), received distilled water *ad libitum* for six weeks; groups 2, 3 and 4 (diabetic rabbits) were fed with 12.5%, 25% and 50% fermented soya bean supplements respectively for six weeks. Thereafter, the rabbits were sacrificed and blood samples obtained for analyses. Data were analysed using analysis of variance (ANOVA). **Results:** There was a significant decrease ($p \leq 0.05$) in triglyceride and LDL concentrations in the group fed 50% fermented soya bean supplements and a significant increase in HDL levels ($p \leq 0.05$) in all the fermented soya bean supplemented groups when compared with the diabetic control group. The result also showed a significant decrease ($p \leq 0.05$) in malondialdehyde concentration in all the treated groups when compared with the control group. Superoxide dismutase was significantly decreased in the group fed 12.5% and 25% fermented soya bean supplements while catalase was significantly decreased in the group fed 12.5% fermented soya bean supplement when compared with the control group. **Conclusion:** Fermented soya bean supplements decreased lipid profile and improved antioxidant activities in diabetic rabbits and may prove beneficial in the management of hyperlipidaemia and oxidative stress in diabetic patients.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. This is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. (ADA, 2006). The prevalence of diabetes mellitus has been steadily increasing for the past 3 decades and is growing most rapidly in low- and middle-income countries. Associated risk factors such as being overweight or obese are also increasing (WHO, 2016).

Diabetes-related changes in serum lipid levels are among the key factors that increase the risk of cardiovascular related diseases (CVDs) and the high mortality rate associated with CVDs in patients with diabetes mellitus (Chen and Tseng, 2013; Firdous, 2014). The lipid changes associated with diabetes mellitus is characterized by high plasma triglyceride (TG) concentration, low HDL cholesterol concentration and increased concentration of LDL-cholesterol particles, which has been attributed to increased free fatty acid flux (lipogenesis) that is secondary to insulin deficiency and insulin resistance (Chen and Tseng, 2013; Firdous, 2014).

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Hyperglycaemia in diabetes mellitus is one of the most important factors responsible for the development of oxidative stress, which underlies the major complications in diabetes mellitus patients (Vicentini *et al.,* 2011; Aly and Mantawy, 2012). In the hyperglycaemic state, sugars react with lipids and proteins which results in the generation of reactive oxygen species (ROS) (Palmeira *et al.,* 2001). The ROS enhance lipid peroxidation (Griesmacher *et al.,* 1995) and causes damage to cells, tissues and biomolecules, thus contributing to diabetic complications (Mahmoud, 2015). Advanced glycation end products (AGEs) are a heterogeneous group of molecules formed from non-enzymatic reaction of reducing sugars (monosaccharides) with amino group of proteins, lipids, and nucleic acids. The AGEs interact with their cellular receptor "receptor for AGEs" (RAGE) and activates nuclear factor kappa-B (NF-κβ), generating ROS. NF-κβ increases the gene expression of cytokines, known to activate NADPH-oxidase, which generates ROS. These cytokines include interleukins (IL-1, IL-6, IL-8) and tumor necrosis factor-α). NF-κβ also increase the gene expression of adhesion molecules, including vascular cell adhesion molecules-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Glycation and oxidative stress are closely linked and are often referred to as "glycoxidation" processes. (Bucala and Cerami, 1992; Yan et al., 1994; Kailash and Indu 2014).

Soya beans belongs to the legume family and represents an excellent source of high quality protein, great amount of fibers, phytosterols including isoflavones, with a low content in saturated fat. Its isoflavone content makes it singular among other legumes (Hamada *et al.,* 2002; Ren *et al.,* 2006; Asif and Acharya, 2013). Soya bean have received increased attention as a functional food due to their beneficial physiological effects in controlling and preventing a wide variety of chronic and degenerative diseases (Sada *et al.,* 2013; Ahmad *et al.,* 2014). Hence, this research study was aimed at evaluating the effects of fermented soya beans supplements on lipid profile and oxidative stress biomarkers in high fat diet-induced diabetes in rabbits.

MATERIALS AND METHODS:

All chemicals used were of analytical grade: (Cholesterol: Kem Light Laboratories Pvt Ltd., Mumbai, India), digital glucometer and strips (Accucheck advantage, Boche Diagnostic, Company).

Experimental Animals Twenty (20) rabbits of both sexes weighing between 1kg – 2kg were used for the study. The rabbits were handled in accordance with the principles guiding the use and handling of experimental animals, ABU, Zaria. Ethical approval was obtained, with Approval No: ABUCAUC/2017/035. The animals were fasted from feeds overnight for 12 hours prior to commencement of the experiment but allowed water ad libitum.

Experimental Induction and Determination of Diabetes Mellitus

Diabetes was induced by feeding the animals with a high fat diet (2% cholesterol, 20% groundnut meal 10% groundnut oil) for eight weeks. After eight weeks of feeding with the high fat diet, blood samples were collected from the rabbits and tested for hyperglycaemia using the glucose oxidase method (Beach and Turner, 1958), and by means of a digital glucometer. Rabbits having fasting blood glucose levels of 7.2 mmol/L and above were selected for the study.

Experimental design

The animals were grouped into four groups of five rabbits each as follows: Group 1 (diabetic control), received distilled water orally for six weeks; Group 2 (diabetic rabbits) were fed with 12.5% fermented soya beans supplement for six weeks; Group 3 (diabetic rabbits) were fed with 25% fermented soya beans supplement for six weeks; Group 4 (diabetic rabbits) were fed with 50% fermented soya beans supplement for six weeks.

Collection and Preparation of Fermented Soya Bean Supplements

The soya bean (variety, TGX-1448-2E) was purchased from Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. The seeds were washed and soaked in a plastic container for forty-eight hours, with the water unchanged (fixed fermentation) (Uyoh *et al.,* 2009). After fermentation, the seeds were drained, air dried, ground into fine granules and constituted into the 12.5%, 25% and 50% supplements with the normal animal feed (Grower's mash from Vital Feeds Company Plc, Jos).

Blood Sample Collection and Serum Preparation

At the end of the six weeks treatment period, the rabbits were sacrificed by cervical dislocation and blood samples were collected from the animals through cardiac puncture for the determination of lipid profile. The blood was allowed to clot and the serum separated by centrifugation.

Biochemical Estimations

The sera were then used for the determination of total cholesterol by the method of Stein (1987), triglycerides

Table 1: Effect of fermented soya bean supplements on lipid profile in high fat diet– induced type 2 diabetes mellitus in rabbits

Group	Total Cholesterol (mg/dL)	Triacylglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Untreated Diabetic	192.85 ± 9.76	132.33 ± 7.40	8.59 ± 2.03	66.48 ± 10.45
DM + 12.5% SY	221.64 ± 6.33[a]	118.62 ± 1.91[a]	19.99 ± 0.16[a]	23.66 ± 6.44
DM + 25% SY	180.88 ± 13.02	139.65 ± 8.50	20.63 ± 1.36[a]	19.50 ± 6.79
DM + 50% SY	169.50 ± 17.12	68.12 ± 8.30[a]	12.76 ± 1.14[a]	25.90 ± 2.92[a]

Values are expressed as mean ± SEM; n = 5. a: Value considered statistically significant when compared with control group ($P \leq 0.05$). SEM = Standard error of mean; HDL – High density lipoprotein, LDL - Low density lipoprotein, SY – Fermented soya bean supplement; n = Number of animals

Table 2: Effect of fermented soya bean supplements on malondialdehyde and antioxidant biomarkers in high fat diet– induced type 2 diabetes mellitus in rabbits

Group	MDA (mmol/L)	SOD (IU/mL)	CAT (IU/mL)	GPx (IU/mL)
Untreated Diabetic	1.68 ± 0.02	1.74 ± 0.04	39.60 ± 0.40	44.40 ± 0.40
DM + 12.5% SY	1.18 ± 0.05[a]	1.22 ± 0.02[a]	30.40 ± 1.17[a]	43.20 ± 0.20
DM +25%SY	1.26 ± 0.06[a]	1.26 ± 0.04[a]	39.40 ± 0.24	42.20 ± 0.20
DM + 50%SY	1.02 ± 0.02[a]	1.84 ± 0.04	40.60 ± 0.60	41.80 ± 0.80

Values are expressed as mean ± SEM; n = 5. a: Value considered statistically significant when compared with control group ($P \leq 0.05$); SEM = Standard error of mean; MDA – Malondialdehyde; SOD – Superoxide dismutase; CAT – Catalase; GPx – Glutathione peroxidase; SY – Fermented soya bean supplement; n = Number of animals.

by the method of Tietz (1990) and high-density lipoprotein (HDL) by the method of Wacnic and Albers (1978). These were determined spectrophotometrically, using enzymatic colometric assay kits. The serum level of low density lipoprotein (LDL) was measured according to the protocol of Friedewald *et al.,* (1972) using the following equation: LDL-C = TC - (TG/5 + HDL-C). Glutathione peroxidase (GPx) was estimated by the method of Avissar *et al.*, (1994), superoxide dismutase (SOD) by the method of Martin *et al.*, (1987), catalase (CAT) by the method of Beers and Sizer (1952) and malondialdehyde (MDA) according to the method described by Janero (1990).

Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. Statistical comparisons were performed by one-way analysis of variance (ANOVA). The results were considered statistically significant if $P \leq 0.05$.

DISCUSSION

Dyslipidaemia is common in both insulin deficiency and insulin resistance, which affects enzymes and pathways of lipid metabolism. It is a major risk factor for cardiovascular diseases which is currently a leading cause of morbidity and mortality worldwide (Yadav *et al.*, 2007). The result of the present study showed a significant increase in total cholesterol concentration in the group fed 12.5% fermented soya bean supplement when compared with the diabetic untreated control group. Though a decrease was observed in the groups fed 25% and 50% fermented soya bean supplements, the decrease was not statistically significant. However, long-term supplementation with 25% and 50% may ensure that the decrease in total cholesterol is biologically relevant and hence clinically useful to the diabetic patient.

The result also showed a significant decrease in LDL concentration in the group fed 50% fermented soya bean supplement when compared with the untreated diabetic control group. Many nutritional factors such as isoflavones, saponins and tannins have been reported to contribute to the ability of plant derived medicines to improve dyslipidaemia (Nimenibo-Uadia, 2003; Rotimi *et al.*, 2011). Soya bean proteins especially glycinin and β-conglycinin may be responsible for the decrease in cholesterol concentration by causing an increase in bile acid production and excretion via faeces (Kim *et al.*, 1980; Beynen, 1990; Ferreira *et al.*, 2010), leading to a shift in hepatic cholesterol system to provide more cholesterol for the bile acid deficit; and at the same time, causing increased LDL receptor activity; with the whole mechanism leading to a reduction in total blood cholesterol concentration (Tanaka *et al.,* 1984). Peptides from protein hydrolysates (due to their hydrophobicity) have high binding ability with bile acid and greatly reduce the cholesterol concentration by restricting the reabsorption of the bile acid in the ileum (Iwami *et al.*, 1986) and the Jejunum (Nagaoka *et al.*, 1997); inhibiting the micelles solubility of cholesterol and ultimately causing the reduction in the cholesterol absorption. This is achieved by the direct interaction of the cholesterol mixed micelles and soya bean protein peptic hydrolysates with the bound phospholipids in the jejunal epithelia (Nagaoka *et al.*, 1997). Soya proteins also have a direct effect on the hepatic metabolism of cholesterol (Nagaoka *et al.,* 1997). Besides soya proteins, soya isoflavones were also reported to decrease the plasma cholesterol by arresting the hepatic assembly as well as by inhibiting the secretion of apo B- containing lipoproteins (Borradaile *et al.,* 2002). The increase in total cholesterol observed in the group fed 12.5% fermented soya bean supplement could be due to the lower percentage supplementation and may probably suggest that higher amount of the soya bean supplementation provides a better cholesterol lowering effect as seen by the dose dependent decrease in the 25% and 50% supplements.

Low serum triacylglycerides (TGs) has been reported to reduce the risk of CVDs, while high serum level of TGs is classified as an independent predictor of CVDs (Chen and Tseng, 2013). The result of the present study showed a significant decrease in the triacylglyceride concentration in the groups fed 12.5% and 50% fermented soya bean supplements. The decrease in the triacylglyceride concentration was more with the 50% supplement, indicating a better therapeutic benefit with the consumption of higher percentage of the fermented soya beans. About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high levels of HDL-C protect against cardiovascular diseases (Kwiterovich, 2000; James *et al.,* 2010). The result of this study showed a statistically significant increase in HDL-C level in all the treated groups compared with the untreated diabetic control group, which confirms the protective effect of soya beans against hyperlipidaemia. These findings are in agreement with the result of Sartang *et al.,* (2015) who reported that fermented soy milk decreased total cholesterol and triacylglyceride concentrations, and increased HDL concentration in diabetic rats.

The present study showed a significant decrease in MDA levels in the fermented soya bean supplemented groups when compared with the untreated diabetic control group, indicating reduced lipid peroxidation in the treated groups. The antioxidant enzymes (SOD and CAT) were significantly decreased with the 12.5% and 25% supplementation when compared with the untreated diabetic control group. Their levels were however, increased in the 50% supplemented group, though not statistically significant. GPx showed no significant difference between all the groups, although slight decreases were observed in all the treated groups when compared with the untreated diabetic control group.

The antioxidant enzymes (SOD, CAT and GPx) form the first line of defence against ROS in an organism. They play an important role in scavenging the toxic intermediates of incomplete oxidation. The SOD catalyses the dismutation of superoxide anion into hydrogen peroxide (H_2O_2), which is then degraded to water (H_2O) by CAT or GPx. The decrease in the antioxidant enzyme levels seen in this study might therefore be due to the enzymes utilization in alleviating the oxidative stress, seen as decrease in the level of MDA. The antioxidant activities of soymilk and fermented soymilk have been observed in other studies (Seo *et al.,* 2009 and Sartang *et al.,* 2015), which were attributed to soya isoflavones, soya protein, and saponins (Esaki *et al.*, 1998). The increase in the antioxidant enzymes SOD and CAT in the groups fed 50% fermented soya bean supplements may be due to upregulation of the endogenous antioxidant enzymes activities by fermented soya bean supplements. This imply that higher percentage of fermented soya bean supplementation is able to stimulate increased SOD and CAT levels, and hence scavenging capacity for ROS; thus providing higher protection against oxidative damage induced by diabetes.

CONCLUSION

The results of this study show that fermented soya bean supplementation decreases serum cholesterol, serum triglyceride and serum LDL concentrations, and increase serum HDL concentration, when compared with the diabetic control.

This shows the efficacy of fermented soya bean supplement as an anti-lipidaemic and antioxidant food that abates diabetes related cardiovascular complications caused by hyperlipidaemia and oxidative stress.

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