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Research Article

Effects of Fermented Soya Bean Supplements on Serum Insulin and Leptin Levels of High Fat Diet-induced Type 2 Diabetes Mellitus in Rabbits

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ABSTRACT

Background: Increase in body fat and obesity are the main risk factors for type 2 diabetes mellitus which leads to several complications that affect many organs of the body when poorly controlled. Plant food sources rich in fibre and antioxidants play an important role in the management of diabetes mellitus. This study aimed to evaluate the effects of fermented soya bean supplements on serum insulin and leptin levels of high fat diet-induced type 2 diabetes mellitus in rabbits. **Methods:** Twenty rabbits of both sexes weighing, 1–2kg were used. Type 2 diabetes was induced by feeding the animals with a high fat diet for eight weeks. Blood glucose levels were determined after the induction period and rabbits having 130 mg/dL and above were selected for the study. The animals were grouped into four groups with five (n=5) rabbits in each group: 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 a period of six weeks. Fasting blood glucose levels were determined at weekly interval during the treatment period. At the end of the experiment, the rabbits were euthanized by cervical dislocation and blood samples were collected for the determination of insulin, and leptin levels. Data obtained were analysed using ANOVA. **Results:** The result showed a significant decrease ($P \leq 0.05$) in leptin levels (ng/mL) in groups 2, 3 and 4 (11.30 ± 0.20, 9.20 ± 2.06 and 6.40 ± 1.36, respectively) as compared with the control (18.8 ± 1.59). Insulin levels were also decreased in all the treated groups when compared with the control, though not statistically significant, it may be biologically significant. **Conclusion:** The results of the study show that fermented soya bean supplementation possesses anti-diabetic properties and may help in the control of hyperleptinaemia.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder, associated with a high disease burden in developing countries, including Nigeria. Type 2 diabetes mellitus (type 2 DM) is the most common documented form of diabetes mellitus in most clinics and it accounts for about 90%-95% of all cases of diabetes mellitus (Ogbera and Ekpebegh, 2014). The prevalence of type 2 diabetes mellitus is increasing worldwide (Shaw *et al*., 2010; Unwin *et al.*, 2010). This increase is strongly associated with obesity and insulin resistance (Yoon *et al*., 2006; Guilherme *et al.*, 2008), as well as defects in pancreatic β- cell function and mass (Bell and Polonsky, 2001; Butler *et al.*, 2003). Development of type 2 diabetes mellitus usually starts with obesity, associated with reduced insulin sensitivity, along with an activated β-cell compensatory mechanism, such as excess basal insulin secretion (hyperinsulinemia) and hyperproinsulinaemia (Kahn and Halban, 1997). These pathological conditions occur early in the disease progression (Tabak*et al.,* 2009), before the β-cells severely fail in later stage of manifest type 2 diabetes mellitus (Prentki and Nolan, 2006).

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MATERIALS AND METHODS

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

Collection and Preparation of Fermented Soya Bean Supplements

Soya bean of the variety, TGX-1448-2E was purchased from Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria (ABU). 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 forty-eight hours, the seeds were drained, air dried and ground into fine granules. The fermented soya beans were then constituted into 12.5%, 25% and 50% supplements by combining the appropriate percentage by weight with the animal feed (The 12.5% contained, 12.5% fermented soya bean and 87.5% animal feed; 25% contained, 25% fermented soya bean and 75% animal feed; 50% contained 50% fermented soya bean and 50% animal feed).

Experimental Animals

Twenty (20) rabbits of both sexes weighing between 1kg – 2kg of about five to eight weeks of age were used for the study. The animals were obtained from the animal house, Faculty of Veterinary Medicine, ABU, Zaria. They were kept in well aerated laboratory cages in the animal house, Department of Human Physiology, ABU, Zaria and left to acclimatize for two weeks before the commencement of the experiment. They were fed with Grower's mash from Vital Feeds Company Plc. Jos and water was provided *ad libitum*.

Experimental Induction and Determination of Diabetes Mellitus

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 for 12-14 hours prior to commencement of the experiment, but allowed water *ad libitum*. Type 2 diabetes mellitus was induced by feeding the animals with a high fat diet (2% cholesterol, 20% groundnut mill and 10% groundnut oil) as reported by Kolawole *et al.*(2012) for eight weeks. Fasting blood glucose levels were determined by using the glucose oxidase method (Trinder, 1969) with ONE TOUCH BASIC® Glucometer (LIFESCAN, Inc 2001 Milpitas, CA 95035, USA) and results were reported as mg/dl (Rheney and Kirk, 2000).

Experimental design

After the induction of type 2 diabetes mellitus, rabbits having fasting blood glucose levels of 130mg/dL (Sharma *et al*., 2003; Diez *et al.*, 2013) and above were selected for the study. The animals were randomly assigned into experimental and control group of five (5) rabbits each, as follows;

Group 1: Diabetic positive control, were allowed access to distilled water *adlibitum* for six weeks.

Group 2: Diabetic rabbits were fed on 12.5% fermented soya bean supplement for six weeks.

Group 3: Diabetic rabbits were fed on 25% fermented soya bean supplement for six weeks.

Group 4: Diabetic rabbits were fed on 50% fermented soya bean supplement for six weeks.

Blood Sample Collection and Serum Preparation

At the end of the six weeks treatment period, the rabbits were euthanized by cervical dislocation and blood samples were collected from the animals through cardiac puncture. About 5 mL of blood were collected into specimen bottles and allowed to clot and separated by centrifugation at 3,000 g for 10 minutes using Centrifuge Hitachi (Universal 32). The supernatant obtained were used for the determination of insulin, and leptin concentrations.

Biochemical Estimations

The sera were used for the determination of serum insulin and leptin levels, using rabbit ultra-sensitive enzyme-linked immunosorbent assay (ELISA) kits (GenAsia Biotech, Co., Ltd. Shanghai, China), with catalogue numbers, GA-E0015RB (insulin) and GA-E0017RB (leptin) according to the manufacturer's instructions.

The principles were based on biotin double antibody sandwich technology (Schmidt *et al.*, 2012).

Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA), followed by Tukey's post hoc test. The results were considered statistically significant if the $P \leq 0.05$.

RESULTS

The result showed a statistically significant decrease ($P \leq 0.05$) in serum leptin levels in the groups fed on 12.5%, 25% and 50% supplement (11.30 ± 0.20, 9.20 ± 2.06 and 6.40 ± 1.36 ng/mL) respectively, as compared to control group (18.8 ± 1.59 ng/mL). Serum insulin levels were also decreased, though not statistically significant, in all the treated groups, when compared with the control group as shown in table 1

DISCUSSION

Chronic excess energy consumption promotes hyperinsulinaemia and insulin resistance through stimulation of insulin secretion, triacylglyceride synthesis and fat accumulation with down-regulation of insulin receptors and post receptor signalling (Kahn *et al.*, 1997).

Table 1: Effect of fermented Soya bean supplement on Serum Insulin and Leptin levels of High Fat Diet–Induced diabetic rabbits.

Group/Treatment	Leptin (ng/mL)	Insulin (ng/mL)
Diabetic+ Untreated	18.8 ± 1.59	26.60 ± 2.27
Diabetic + 12.5% Soya beans	11.30 ± 0.20 [a]	16.80 ± 3.32
Diabetic + 25% Soya bean	9.20 ± 2.06 [a]	19.20 ± 0.73
Diabetic + 50% Soya bean	6.40 ± 1.36 [a]	24.40 ± 6.12

Values are expressed as mean ± SEM; n = 5.
[a]Statistically significant when compared with control group ($P \leq 0.05$).

Insulin resistance is associated with increased level of insulin along with high level of fasting blood glucose level, as seen in type-2 diabetic condition. This is because the adipose tissue, liver and muscles fail to absorb glucose in response to increasing serum insulin. In the present results, animals fed with the fermented soya bean supplements showed lower insulin level concentration, though not statistically significant when compared with the diabetic untreated control group. This suggests that there was an improvement in the hyperinsulinaemia. Soya beans are a significant source of phytochemicals such as isoflavones, phytosterols and lecithins, as well as soluble fibres, saponins, proteins and polysaccharides, which may act collectively or through independent mechanisms to confer unique health benefits (McCue and Kalidas, 2004; Manach *et al.*, 2004). The decreasing effect may have been as a result of the soya isoflavone ability to activate AMP-activated protein kinase (AMPK), which is an energy sensor that promotes glucose uptake, fatty acid oxidation, mitochondrial biogenesis, and insulin sensitivity (Cederroth *et al.*, 2008; O'Neill, 2013).

Soya bean proteins are involved in the improvement of peripheral insulin sensitivity and glucose tolerance probably due to the glycine and arginine ability to stimulate pancreatic secretion (Lavigne *et al.*, 2000). Resistant starch, like dietary fibre, which is not digested in the small intestine but passes to the large bowel acts as a substrate for colonic fermentation and has been reported to decrease postprandial glucose and insulin responses, improve whole body insulin sensitivity, lower triacylglycerides and HDL, increase satiety and reduce fat storage (Higgin *et al.*, 2004). The lower insulin level may therefore be as a result of the activities of soya bean proteins and soya fibre, along with naturally occurring isoflavones, which are phytoestrogen in nature and are structurally and functionally similar to oestradiol (Knight and Eden, 1996). The result is in agreement with the work of Choi *et al.*, (2016), which showed that insulin levels were significantly lower in animals supplemented with fermented soya bean paste than in the non-supplemented group in diet induced obese (C57BL/6J) mice.

Serum leptin levels were significantly decreased in this study in all the groups treated with the fermented soya bean supplements, when compared to the untreated diabetic control group. Soya bean is rich in isoflavones, protein, fatty acids, saponins and phospholipids which were shown to have beneficial effects on serum leptin level, and other hormonal, cellular, or molecular mechanisms associated with adiposity (Manzoni *et al.*, 2005). Soya protein was also shown to stimulate satiety and then prevent weight gain (Aoyama *et al.*, 2000; Nagasawa *et al.*, 2002), hence inhibiting body fat induced leptin secretion. Similarly, an anti-obesity effect on rats was also reported from soya peptide consumption by activating the leptin-like signalling and AMP-activated protein kinase (Jang *et al.*, 2008), which in turn regulates leptin secretion. This work is consistent with the works of Choi *et al.* (2016) which showed that leptin levels were significantly decreased in animals supplemented with fermented soya bean paste than in the non-supplemented group in diet induced obese (C57BL/6J) mice and that of König *et al.*, (2008), who reported that meal replacement among humans with soya bean products twice daily, significantly reduced serum leptin levels as compared to the control diet. Other studies like that of Chen *et al.* (2006), indicated that soya isoflavones decrease body weight of rats and leptin mRNA, increase serum leptin levels, and ameliorate leptin and insulin sensitivities. In contrast, Azadbakht and Esmaillzadeh (2010), showed that soya beans products with the natural levels of isoflavone did not change the serum leptin level in postmenopausal women with metabolic syndrome. Inconsistent results in different studies might be due to the type of model used, method of processing the soya bean adopted, doses of isoflavone intake and the duration of study and also gender of the animals used. Further studies are therefore needed to clarify the effect of soya beans products consumption on serum leptin levels.

CONCLUSION

The results of the current study show that fermented soya bean supplementation decreased serum insulin and leptin levels. This shows the efficacy of fermented soya bean supplement as anti-diabetic food that mitigates hyperleptinaemia.

REFERENCES

Aoyama, T., Fukui, K., Nakamori, T., Hashimoto, Y., Yamamoto, T., Takamatsu, K. and Sugano, M. (2000). Effect of soy and milk whey protein isolates and their hydrolysates on weight reduction in genetically obese mice. *Bioscience, Biotechnology and Biochemistry, 64 (12),* 2594–2600.

Azadbakht, L. and Esmaillzadeh, A. (2010). A cross-over trial on soy intake and serum leptin levels in women with metabolic syndrome. *Iran Journal of Research in Medical Sciences*, *15* (6), 317–323.

Bell, G. I. and Polonsky, K. S. (2001). Diabetes mellitus and genetically programmed defects in beta-cell function. *Nature, 414,* 788–791.

Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A. and Butler, P. C. (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes,* 52, 102-110.

Cederroth, C. R., Vinciguerra, M., Gjinovci, A., Kühne, F., Klein, M., Cederroth, M., Caille, D., …. and Nef, S. (2008). Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes*, *57*, 1176–1185.

Chen, S. W., Zhang, L. S., Zhang, H. M., Feng, X. F. and Peng, X. L. (2006). Effect of soy isoflavone on gene expression of leptin and insulin sensibility in insulin-resistant rats. *Beijing Da Xue Bao*, *38* (2), 197–200.

Choi, J. H., Pichiah, P. B. T., Kim, M. and Cha Y.J. (2016). Cheonggukjang, a soybean paste fermented with B. licheniformis_67 prevents weight gain and improves glycaemic control in high fat diet induced obese mice. *Journal of Clinical Biochemistry and Nutrition*, *59*, 1.

Diez, M. J., Sierra, M., Sahagún, A. M., Calle, Á. P., et al. (2013). Hypoglycemic and hypolipidemic potential of a high fiber diet in healthy versus diabetic rabbits. BioMed Research International, Article ID 960568, 8 pages.

Guilherme, A., Virbasius, J. and Vishwajeet, P. (2008). Adipocyte dysfunction linking obesity to insulin resistance and type 2 diabetes. Nature Reviews Molecular Cell Biology, 9, 367–377.

Higgins, J. A., HigbeeD. R., DonahooW. T., BrownI. L., BellM. L. and BessesenD. H. (2004).. Resistant starch consumption promotes lipid oxidation. *Nutrition and Metabolism (London), 1,* 8.

Jang, E. H., Moon, J. S., Ko, J. H., Ahn, C. W., Lee, H. H., Shin, J. K., Park, C. S. and Kang J. H. (2008). Novel black soy peptides with antiobesity effects: activation of leptin-like signalling and AMP-activated protein kinase. *International Journal of Obesity (London), 32* (7), 1161–70.

Kahn, S. E. and Halban, P. A. (1997). Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. *Diabetes, 46,* 1725–1732.

Kahn, S. E., McCulloch, D. K. and Porte, D. (1997). *Insulin secretion in the normal and diabetic human*. In: Alberti KGMM, Zimmet P, Defronzo RA, editors and Keen H, (hon) editor. International Textbook of Diabetes Mellitus. (2nd ed.) John Wiley and Sons, New York, pp. 337-54.

Knight, D. C. and Eden, J. A. (1996). A review of the clinical effects of phytoestrogens. *Obstetetrics and Gynecology*, *87*, (5 Pt 2), 897–904.

Kolawole OT, Kolawole SO, Ayankunle AA, Olaniran IO. Methanol leaf extract of Persea Americana protects rats against cholesterol-induced hyperlipidaemia. *British Journal of Medicine and Medical Research*. 2012; 2 (2): 235-242.

König, D., Deibert, P., Frey, I., Landmann, U. and Berg, A. (2008). Effect of meal replacement on metabolic risk factors in overweight and obese subjects. *Annals of Nutrition and Metabolism, 52* (1), 74–8.

Lavigne, C., Marette, A. and Jacques, H. (2000). Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. *American Journal of Physiology Endocrinology and Metabolism, 278*, 491–500.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L. (2004). Polyphenols:Food sources and bioavailability. *American Journal of Clinical Nutrition, 79,* 727–747.Manzoni, M. S., Rossi, E. A., Carlos, I. Z., Vendramini, R. C., Duarte, A. C. and Dâmaso, A. R. (2005). Fermented soy product supplemented with isoflavones affected fat depots in juvenile rats. *Nutrition*, *21* (10), 1018–24.

McCue, P. and Kalidas, S. (2004). Health benefits of soya isoflavonoids and strategies for enhancement: A review. *Critical Reviews in Food Science and Nutrition*, *44*, 361–367.

Nagasawa, A., Fukui, K., Funahashi, T., Maeda, N., Shimomura, I., Kihara, S., Waki, M., Takamatsu, K. and Matsuzawa, Y. (2002). Effects of soy protein diet on the expression of adipose genes and plasma adiponectin. *Hormone and Metabolic Research, 34* (11-12), 635–639.

Ogbera, A. O and Ekpebegh, C. (2014). Diabetes mellitus in Nigeria: The past, present and future. *World Journal of Diabetes; 5* (6), 905-911.

O'Neill, H. K. (2013). AMPK and exercise: glucose uptake and insulin sensitivity. *Diabetes and Metabolism Journal*, *37*, 1–21.

Prentki, M. and Nolan, C. J. (2006). Islet beta cell failure in type 2 diabetes. *Journal of Clinical Investigation 116*, 1802–1812.

Rheney, C. C. and Kirk, K. K. (2000). Performance of three blood glucose meters. *Annals of Pharmacotherapy*, 34(3):317-21.

Schmidt, S. D., Mazzella, M. J., Nixon, R. A. and Mathews, P. M. (2012). Aβ measurement by enzyme-linked immunosorbent assay. *Methods in Molecular Biology*, 849: 507–27.

Sharma, S. B, Nasir, A., Prabhu, K. M., Murthy, P. S., Dev, G. (2003). Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxaninduced diabetic rabbits. Journal of Ethno pharmacology, 85(2-3):201–206. 26.

Shaw, J. E., Sicree, R. A. and Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87, 4-14.

Tabak, A. G., Jokela, M. and Akbaraly, T. N. (2009). Trajectories of glycemia, insulin sensitivity and insulin secretion preceeding the diagnosis of type 2 diabetes: The Whitehall II Study. *Lancet, 373*, 2215–2221.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical. Biochemistry, *6:* 24-27.

Unwin, N., Gan, D. and Whiting, D. (2010). The IDF Diabetes Atlas: providing evidence, raising awareness and promoting action. *Diabetes Research and Clinical Practice, 87*: 2–3.

Uyoh EA, Ntui VO, Udoma NN. Effect of local cassava fermentation methods on some physiochemical and sensory properties of fufu. *Pakistan Journal of Nutrition*.2009; 8(8): 1123-1125.

Yoon, K. H., Lee, J. H and Kim, J. W. (2006). Epidemic obesity and type 2 diabetes in Asia. *Lancet, 368,* 1681–1688.