

SJMLS - 4 (3) - 009

**Effects of Fermented Soya Bean Supplements on Liver Enzymes of High Fat Diet-induced Type 2 Diabetes Mellitus in Rabbits**Sada N.M.\*<sup>1</sup>, Tanko, Y.<sup>2</sup>, Abdulrazak, A.<sup>1</sup>, Tukur, M.A.<sup>3</sup>, Mohammed, A.<sup>2</sup>

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Kaduna State University, Kaduna State, Nigeria<sup>1</sup>, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria<sup>2</sup>, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Maiduguri, Borno State, Nigeria<sup>1</sup>.

Author for Correspondence: maryamnaiya@gmail.com/+234-803-365-5327

**Abstract**

Type 2 diabetes mellitus consists of an array of dysfunctions characterized by hyperglycaemia, resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion. Hyperglycaemia and concomitant biochemical abnormalities (liver abnormalities, dyslipidaemia and oxidative stress) and other clinical complications are common in poorly managed diabetic patients. This study aimed to evaluate the effects of fermented soya bean supplements on liver enzymes activity of high fat diet-induced type 2 diabetes mellitus in rabbits. Twenty rabbits of both gender weighing, 1–2kg were used. Type 2 diabetes mellitus 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 of five (n=5) 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 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 liver enzyme activity. Data obtained were analysed using ANOVA. The result showed a significant decrease ( $p \leq 0.05$ ) in aspartate amino transferase (IU/L), ( $25.20 \pm 1.36$ ,  $24.00 \pm 1.00$  and  $20.20 \pm 0.20$ ) in groups 2, 3 and 4

respectively when compared with the control ( $32.00 \pm 2.03$ ). Alanine amino transferase concentrations (IU/L) were also significantly decreased ( $p \leq 0.05$ ) in groups 2, 3 and 4 ( $9.00 \pm 0.89$ ,  $11.20 \pm 1.66$  and  $15.80 \pm 2.20$  respectively) when compared with the control ( $47.40 \pm 3.49$ ), so also alkaline phosphatase (IU/L), which was significantly decreased ( $p \leq 0.05$ ) in groups 2, 3 and 4 ( $2.06 \pm 0.21$ ,  $2.10 \pm 0.15$  and  $1.16 \pm 0.09$ ), when compared with the control ( $4.73 \pm 0.22$ ). The results of the study show that fermented soya bean supplementation exhibit hepatoprotective properties in diabetic rats.

**Introduction**

**Diabetes mellitus is one of the largest global health emergencies of this century.** Globally, more than 415 million people had diabetes in 2015, with more than 14 million people in the African continent. This figure is projected to rise to more than double by the year 2040 (IDF, 2015). The management of diabetes and its complications places an enormous financial burden on millions of individuals and its families, due to the cost of essential medicines and technologies. Despite substantial progress made in elucidating the insulin signalling pathways following the Banting group's discovery of insulin, diabetes mellitus continues to be a global problem, and has been variously described as an epidemic or even as the plague of the 21st century (Pournaras and le Roux, 2015). Ectopic lipid accumulation impairs insulin signalling in type 2 diabetes mellitus. With accumulation of intra-myocellular lipid, insulin mediated skeletal muscle glucose uptake is impaired. As a result, glucose is diverted to the

liver. In the liver, increased liver lipid also impairs the ability of insulin to regulate gluconeogenesis and activate glycogen synthesis (Samuel and Shulman, 2012). Liver enzymes are indices of liver dysfunction which assist in diagnosis and patient follow-up and response to treatment. They reflect inflammatory activity in the parenchyma of the liver (Villela-Nogueira *et al.*, 2005; Kim *et al.*, 2012; Whitfield, 2001).

Soya bean belongs to the family Leguminosae and the cultivar *Glycine max*. It represents an excellent source of high-quality protein, with a low content in saturated fat, containing a great amount of dietary fibre. Its isoflavone content makes it singular among other legumes (Asif and Acharya, 2013). Whole grains and cereals that serve as the source of energy in Asian foods have been suggested as potential anti-diabetic food due to their low-glycaemic indices. Nutritional quality and safety of food is enhanced by fermentation, which improves the digestibility and beneficial components of the food. The vitamin, essential amino acids, and bioactive compounds of fermented foods are enriched compared to their initial contents (Thapa and Tamang, 2015).

The formulation of good affordable anti-diabetic diet from soya beans, which is a common local food rich in protein, fibre and phytochemical nutrients in our environment has not been considered. This study is therefore aimed at evaluating the effects of fermented soya bean supplements on liver enzymes activity of high fat diet-induced type 2 diabetes mellitus in rabbits.

### 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 gender 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. 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 (Diez *et al.*, 2013; Sharma *et al.*, 2003) 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 3,000g for 10 minutes using Centrifuge Hitachi (Universal 32). The supernatant obtained were used for the determination of liver enzyme.

### Biochemical Estimations

Activities of plasma alanine amino transaminase (ALT) was estimated by method adopted by Tietz (1995), aspartate amino transaminase (AST) was determined by method of Henderson and Moss (2001), while alkaline phosphatase (ALP) was determined according to the method of Scherwin (2003). All tests were carried out using ELITECH clinical system kits (United Kingdom).

### Statistical Analysis

Data obtained from the study were expressed as mean  $\pm$  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 when  $p \leq 0.05$ .

### Result

**Table 1: Effect of fermented soya bean supplements on liver enzymes of high fat diet– induced diabetic rabbits.**

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Untreated Diabetic	32.00 $\pm$ 2.03	47.40 $\pm$ 3.49	4.73 $\pm$ 0.22
DM + 12.5% SY	25.20 $\pm$ 1.36a	19.00 $\pm$ 0.89a	2.06 $\pm$ 0.21a
DM + 25% SY	24.00 $\pm$ 1.00a	11.20 $\pm$ 1.66a	2.10 $\pm$ 0.15a
DM + 50% SY	20.20 $\pm$ 0.20a	15.80 $\pm$ 2.20a	1.16 $\pm$ 0.09a

### Key:

Values are expressed as mean  $\pm$  SEM; n = 5.

a: Value considered statistically significant when compared with control group ( $p \leq 0.05$ ).

SEM = Standard error of mean.

AST – Aspartate amino transferase

ALT – Alanine amino transferase

ALP – Alkaline phosphatase

SY – Fermented soya bean supplement

n = Number of animals

The result in table 1 shows a significant decrease ( $p \leq 0.05$ ) in aspartate amino transferase (AST) (25.20  $\pm$  1.36 IU/L, 24.00  $\pm$  1.00 IU/L and 20.20  $\pm$  0.20 IU/L) in the groups fed with 12.5%, 25% and 50% supplements respectively when compared with the control group (32.00  $\pm$  2.03 IU/L). Alanine amino transferase (ALT) concentrations were also significantly decreased ( $p \leq 0.05$ ) in the groups fed with 12.5%, 25% and 50% supplements (9.00  $\pm$  0.89 IU/L, 11.20  $\pm$

1.66 IU/L and 15.80  $\pm$  2.20 IU/L respectively) when compared with the control group (47.40  $\pm$  3.49 IU/L). Alkaline phosphatase (ALP) concentrations were significantly decreased ( $p \leq 0.05$ ) in the groups fed 12.5%, 25% and 50% supplements (2.06  $\pm$  0.21 IU/L, 2.10  $\pm$  0.15 IU/L and 1.16  $\pm$  0.09 IU/L respectively), when compared with the control group (4.73  $\pm$  0.22 IU/L).

## Discussion

Diabetic liver complications (fatty liver, glycogen deposition, cirrhosis and biliary disease) are associated with changes in serum levels of the liver enzymes, and it is commonly used to predict pathologies of the liver. The enzymes include among others alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (Singh *et al.*, 2011; Harris, 2005). The results of the present study showed that there was a significant decrease in AST, ALT and ALP in all the treated groups, when compared with the untreated diabetic control group. The decrease in ALT, ALP and AST activities recorded in the group treated with the fermented soya bean supplements show that the fermented soya bean supplements offer protection against increase in the activities of these enzymes, and may be beneficial in improving symptoms of liver dysfunction in diabetics. The protective effect of the fermented soya beans on the liver may be attributed to the soya beans protein, which has been shown to alleviate stress on fatty liver, by partially restoring the Wnt/ $\beta$ -catenin signalling pathway, which plays a crucial role in fat metabolism (Zhou *et al.*, 2014). Saponins and isoflavones, due to their antioxidant activities may have contributed to these effects. Genistein, a major soy isoflavone, which represses adipogenic differentiation via activating Wnt/ $\beta$ -catenin signalling pathway (Kim *et al.*, 2010), may also be involved in the protection of the liver. This result is in agreement with the work of Yusuf *et al.* (2013) who reported the hepatoprotective effect of fermented soya bean in normalizing liver enzyme levels.

## Conclusion

The results of current study show that fermented soya bean supplementation decreases serum liver enzymes activities in the fermented soya bean supplemented groups, when compared with the diabetic control. This finding shows the hepatoprotective properties of fermented soya bean supplement and can therefore be considered as a promising alternative therapeutic agent in the management of diabetes related liver complications.

## References

- Asif, M. and Acharya, M. (2013). Phytochemicals and nutritional health benefits of soy plant. *International Journal of Nutrition, Pharmacology and Neurological Diseases*; 3: 64-69.
- Harris, E.H. (2005). Elevated Liver Function Tests in Type 2 Diabetes. *Clinical Diabetes*; 2 (3): 115-119.
- Henderson, A. R. and Moss. D. W. (2001). *Enzymes, Tietz fundamentals of clinical chemistry*, 5th edition, Buritus C. A. and Ashwood E. R., (W.B. Saunders eds. Philadelphia USA): 352.
- International Diabetes Federation (IDF) (2015). *IDF Diabetes Atlas. 7th edition*, Brussels, Belgium: International Diabetes Federation, 2015.
- Kim, M. H., Park, J. S., Seo, M. S., Jung, J. W., Lee, Y. S. and Kang, K. S. (2010). Genistein and daidzein repress adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells via Wnt/ $\beta$ -catenin signalling or lipolysis. *Cell Proliferation*; 43: 594–605.
- Kim, Y.J., Jang, B.K., Kim, E.S., Park, K.S., Cho, K.B., Chung, W.J. and Hwang, J.S. (2012). Rapid normalization of alanine aminotransferase predicts viral response during combined peg interferon and ribavirin treatment in chronic hepatitis C patients. *The Korean Journal of Hepatology*; 18 (1): 41-47.
- Kolawole, O.T., Kolawole, S.O., Ayankunle, A.A. and Olaniran, I.O. (2012). Methanol leaf extract of *Persea Americana* protects rats against cholesterol-induced hyperlipidaemia. *British Journal of Medicine and Medical Research*; 2 (2): 235-242.
- Pournaras, D.J. and le Roux, C.W. (2015). Type 2 diabetes: Multimodal treatment of a complex disease. *Lancet*; 386: 936–937.
- Rheney, C.C. and Kirk, K.K. (2000). Performance of three blood glucose meters. *Annals of Pharmacotherapy*; 34(3):317-321.
- Samuel, V.T. and Shulman, G.I. (2012). Integrating Mechanisms for Insulin Resistance: Common Threads and Missing Links. *Cell*; 148 (5): 852–871.
- Scherwin, J.E. (2003). *Liver function. Clinical*

- chemistry; Theory analysis correction*, 4th edition, Kaplan I.A., Pesce A.J. and Kazmierczak S.C., (Mosby Inc. eds. St. Louis USA): 492.
- Singh, A., Bhat, T.K. and Sharma, O.P. (2011). *Clinical Biochemistry of Hepatotoxicity. Journal of Clinical Toxicology*; S4: 001.
- Thapa, N. and Tamang, J.P. (2015). *Functionality and therapeutic values of fermented foods*, in; *Health Benefits of Fermented Foods*, ed. Tamang, J.P. (New York: CRC Press):111–168.
- Tietz, N. W. (1995). *Clinical guide to Laboratory Tests*. 3rd edition, W.B. Saunders Company, Philadelphia, USA.
- Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry*; 6: 24-27.
- Uyoh EA, Ntui VO, Udoma NN. (2009). Effect of local cassava fermentation methods on some physiochemical and sensory properties of fufu. *Pakistan Journal of Nutrition*;8(8): 1123-1125.
- Villela-Nogueira, C.A., Perez, R.M., de SegadasSoares, J.A. and Coelho, H.S.M. (2005). Gamma-glutamyl transferase (GGT) as an independent predictive factor of sustained virologic response in patients with hepatitis C treated with interferon-alpha and ribavirin. *Journal of Clinical Gastroenterology*;39(8):728-730.
- Whitfield, J.B. (2001). Gamma glutamyl transferase. *Critical Reviews in Clinical Laboratory Sciences*;38(4): 263-355.
- Yusof, H.M., Ali, N.M., Yeap, S.K., Ho, W.Y., Beh, B.K., Koh, S.P., Long, K., Abdulaziz, S and Alitheen, N.B. (2013). Hepatoprotective effect of fermented soybean (nutrient enriched tempeh) against alcohol-induced liver damage in mice. *Hindawi: Evidence-Based Complementary and Alternative Medicine*;2013:274274.
- Zhou, D., Lezmi, S., Wang, H., Davis, J., Banz, W. And Chen, H. (2014). Fat accumulation in the liver of obese rats is alleviated by soy protein isolate through  $\beta$ -catenin signalling. *Obesity (Silver Spring)* ;22(1): 151–158.

**Citation:** Sada N.M., Tanko, Y., Abdulrazak, A., Tukur, M.A., Mohammed, A. Effects of Fermented Soya Bean Supplements on Liver Enzymes of High Fat Diet-induced Type 2 Diabetes Mellitus in Rabbits. *Sokoto Journal of Medical Laboratory Science*;4(3): 72 - 76.

**Copyright.** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.