

Effect of Fermented Cabbage (*Brassica Oleraceae*) Supplementation on Liver Enzymes Level in Streptozotocin-Nicotinamide Induced Type 2 Diabetic Male Wistar Rats

Muhammad A,^{1*} Mohammed A,¹ Kawu MU,² Tanko Y.¹

¹Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

²Department of Veterinary Physiology and Pharmacology, Ahmadu, Bello University, Zaria, Nigeria.

Article Metrics

Date submitted: 22/03/2021

Date Accepted: 26/05/2021

Date Published: 30/07/2021



ABSTRACT

Background: Diabetes mellitus is a global health problem with evidence suggesting lifestyle modification in form of diet playing an integral part of management for type 2 diabetes mellitus and reduce the risk of complications. Despite significant progress in the treatment of diabetes mellitus using conventional medicines, research in relation to plants in form of fermented cabbage supplementation have not been studied to assess its impact on treatment. The aim of this study was to evaluate the effect of fermented cabbage supplementation on liver enzymes on streptozotocin-nicotinamide induced diabetic male Wistar rats.

Methodology: This study was carried out on thirty healthy male Wistar rats weighing 150 to 300g (8-10 weeks old). They were randomly divided into six groups of five rats each. Groups I and II were normal control and diabetic control rats that received distill water 1 ml/Kg orally. Groups III, IV and V were diabetic rats that received 12.5%, 25%, and 50% fermented cabbage supplementation mixed with 87.5%, 75% and 50% of vital feed. Group VI were diabetic control rats treated with metformin that received 500 mg/kg/day of metformin orally via gavage. All treatments lasted for four (4) weeks.

Results: Fermented cabbage supplementation significantly decreased ($P < 0.05$) serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities when compared with animals in the diabetic control untreated groups. In addition, it also showed a significant ($P < 0.05$) increase in serum total proteins when compared with animals in the diabetic control untreated groups after four weeks of treatment.

Conclusion: Fermented cabbage supplementation may have significant protective properties on the liver due to its ability to keep the serum liver enzymes within normal range.

Keywords: Fermented Cabbage, Liver enzymes, Streptozotocin and Nicotinamide.

*Correspondence: Dr. Abdulmalik Muhammad.

Email: drmodee8@gmail.com Tel: +2348036053628

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website: <http://www.jmbsr.com.ng>

DOI: <https://doi.org/10.46912/jmbsr.37>

How to cite this article

Muhammad A, Mohammed A, Kawu MU, Tanko Y. Effect of Fermented Cabbage (*Brassica Oleraceae*) Supplementation on Liver Enzymes Level in Streptozotocin-Nicotinamide Induced Type 2 Diabetic Male Wistar Rats. J Med Bas Sci Res 2021;2(1):55-63.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by devastating chronic disease that affect the body glucose utilization with ensuing insulin inadequacy or irregularities in peripheral insulin uptake due to resistance to insulin activity¹. As much as 422 million people were estimated worldwide to be diabetic, and majority are living in low and middle income countries, and it account for 1.6 million deaths each year. Both the number of cases and the prevalence of DM have been steadily rising over the past few decades.² It is among the top 10 causes of death in adults, and was estimated to have caused four million deaths globally in 2017 with Type 2 Diabetes Mellitus (T2DM) accounting for approximately 90% of the total prevalence.³ Lifestyle modification play an important role in the development of diabetes mellitus in Nigeria, with prevalence higher in urban areas compared to rural areas.⁴

The liver is well known to play an important role in glucose homeostasis by storing (glycogenesis) or releasing (glycogenolysis/gluconeogenesis) glucose upon interaction with insulin and glucagon, respectively.⁹ The biomarkers of liver injury, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are probably the most commonly used in both clinical diagnosis and research involving liver damage.

Studies have shown that individuals with T2DM are highly prone to liver function test derangement than healthy nondiabetic individuals. Liver diseases such as nonalcoholic fatty liver disease (NAFLD), liver cirrhosis and hepatocellular carcinoma are considered as a significant contributor to death in patients with T2DM.¹⁰

Dietary management in the form of nutrition plays an integral part of management for type 2 DM to improve glycaemic control and reduce the risk of

complications.⁵ Diabetes mellitus is a global health problem with evidence suggesting lifestyle modification in form of diet playing a key role in its management.⁶ Despite significant progress in the treatment of DM using conventional medicines, research in relation to plants in form of fermented cabbage supplementation have not been studied to assess its impact on treatment of DM.^{7,8}

Presently to the best of our knowledge, there is no study exploring the therapeutic efficacy of cabbage supplementation in the management of DM. It is therefore necessary to evaluate the therapeutic efficacy of fermented cabbage supplementation to discover its effects against type 2 DM.

METHODOLOGY

Chemicals/ Reagents

All chemicals used and drugs (streptozotocin, nicotinamide and metformin) were of analytical grade and purchased from Sigma Chemical Company St, Louis USA.

Plant material

Fresh cabbage was purchased in August 2018 from Kubani farm Zaria, Kaduna State. Confirmation was done by a Taxonomist in the Herbarium section of Department of Botany, Ahmadu Bello University, Zaria and a voucher number (43382) was given for future reference

Animals

A total of thirty (30) male Wistar rats weighing 150 to 300g (8-10 weeks old) were used for the study. The animals were obtained from the animal house, Department of Human Physiology, Ahmadu Bello University, Zaria. They were kept in well aerated laboratory cages, under room temperature and

lightning. Feed compounded from grower's mash (Vital Feeds® Company Plc. Jos) and drinking water were provided ad libitum. Ethical clearance was obtained from the Animal Use and Care Committee, Ahmadu Bello University, Zaria and approval number (ABUCAUC/ 2018/ 068) was given for future reference

Preparation of fermented cabbage supplement

Fresh cabbage was washed and trimmed and the outer, dirty leaves were removed. For every 1 kg of cabbage, 30 g of salt was sprinkled in layers and a clean cloth placed above the cabbage with a weight to compress it and fermentation was allowed for 5 to 7 days.^{16,17} The addition of salt during fermentation restricts the growth of gram-negative bacteria and enhances the growth of lactic acid bacteria (LAB). The LAB tolerate high salt concentrations, which give them an advantage over other less salt-tolerant species and allows the LAB to produce acid that inhibits the growth of undesirable microorganisms and for this reason, initiates the majority of lactic acid fermentations.¹⁴ After fermentation the cabbage was then shade dried, weighed and added (molded) to the animal feed (growers mash) at 12.5 %, 25 % and 50 % proportions made up to 1 g of feed.⁹

Induction of diabetes mellitus

Diabetes Mellitus was induced in overnight fasted male Wistar rats (6-8 hours) by administering a single dose of freshly prepared sodium citrate buffer solution of streptozotocin (STZ), 65 mg/Kg intraperitoneal (IP) in 50 mM cold citrate buffer (pH 4.5), 15 minutes after IP administration of 120 mg/Kg Nicotinamide (NA)¹⁵. The animals were continued to be fed and maintained with 10% glucose solution after 6 hours of STZ/ NA injection for the next 24 h to prevent hypoglycemia.¹⁶ On day 7 after STZ /NA treatment, venous blood was collected from tail vein

blood sample to determine the fasting blood glucose level of the animals using one touch digital glucometer (Accu-check advantage®, Boche Diagnostic Company) device. Animals having fasting blood glucose level > 150 mg/dl were used for the study.^{17,18}

Experimental Design

The study was carried out on STZ/ NA induced Wistar rats. The animals were fasted for 6-8 hours with free access to water prior to the induction of diabetes. After induction of DM, the animals were randomly divided into 6 groups of 5 animals each as follows:

Group 1: Normal control (NC) rats that received distill water 1 ml/Kg orally daily as for four weeks.

Group 2: Diabetic control untreated (DCUT) rats that received distill water 1 ml/Kg daily for four weeks.

Group 3: Diabetic rats that were fed with 12.5 % fermented cabbage supplementation daily for four weeks.

Group 4: Diabetic rats that were fed with 25 % fermented cabbage supplementation daily for four weeks.

Group 5: Diabetic rats that were fed with 50 % fermented cabbage supplementation daily for four weeks.¹⁹

Group 6: Diabetic control rats treated with metformin (DCTM) that received 500 mg/kg/day of metformin orally as gavage for four weeks.²⁰

Determination serum for liver enzymes

At the fourth week of the experiment, all animals were euthanized using chloroform. Prior to exposure, chloroform was soaked in cotton wool and placed in a desiccator for 5 minutes to ensure circulation. After 30-35 seconds, the chloroform exposed rats were knocked off.³¹ All chemicals used were manufactured by SIGMA-ADRIK, INC., USA. Blood samples

were drawn from the heart of each animal from all groups by cardiac puncture. The blood samples were collected in Eppendorf tubes, allowed to clot and the serum separated by centrifuging using Denley BS400 centrifuge (England) at 3000 g for 10 minutes. The supernatant (serum) collected was then used for the analysis of liver enzymes as follows:

Determination of Serum Alanine aminotransferase (ALT)

Alanine aminotransferase was estimated by the method described by Bergmeyer and Welefeld.²¹ 1000 μ L of the reagent was added to 100 μ L of the sample, mixed and incubated at 37°C for one minute. The change in absorbance of the sample was measured using spectrophotometer per minute at a wavelength of 590 nm as follows:

Total proteins activity (μ L) = AB/min x 1768.

Determination of Serum Aspartate aminotransferase (AST)

Aspartate aminotransferase was estimated by the method described by Bergmeyer and Welefeld.²¹ 1000 μ L of the reagent was added to 100 μ L of the sample, mixed and incubated at 37°C for one minute. The change in absorbance of the sample was measured using per minute at a wavelength of 590 nm as follows:

Total proteins activity (μ L) = AB/min x 1768

Determination of Serum Alkaline phosphatase (ALP)

Alkaline phosphatase was estimated by method described by Bowers and Mc Comb.²² 0.5 mL of the reagent was added to 0.05 mL (50 μ L) of the sample mixed and incubated at 37°C for 10 minutes. The change in absorbance of the sample was measured per minute spectrophotometrically at wavelength of 590nm as follows:

Absorbance of sample/Absorbance standard \times Value of standard (μ L).

Determination of Serum Total Proteins (Albumin/Globulin)

The serum total proteins were determined by Biuret method of Reinhold²³ using a commercial kit (Randox Laboratories Ltd, United Kingdom). The serum albumin which quantitatively binds bromocresol green (BCG) to form an albumin-BCG complex was measured as an endpoint reaction at 596 nm according to the method of Doumas.²⁴ Globulin was obtained by subtracting the albumin from the total protein.

Ethical Approval

Abiding to the international standard and Ahmadu Bello University standard guidelines, ethical approval has been taken from the Animal Ethic committee to carry out this study.

Statistical Analysis

Result obtained from each group was expressed as mean \pm SEM. The data was statically analyzed using ANOVA and post-hoc test was used to compare the level of significance between the control and experimental groups. All statistical analyses were evaluated using SPSS version 21.0 software. The value of $p < 0.05$ was considered significant.

RESULTS

Figure 1 shows that the diabetic untreated rats had a significant ($P < 0.05$) increase in serum alanine aminotransferase activity (74.8 ± 1.7 IU/L), when compared with the animals in the normal control group (59.5 ± 0.3 IU/L). The results of the various percentages (12.5%, 25% and 50%) of fermented cabbage supplementation and Metformin (500 mg/kg) produced a significant ($P < 0.05$) decrease

(58.3 ± 2.8 , 60.3 ± 2.5 , 58.5 ± 1.1 IU/L) and (60.12 ± 0.2 IU/L) respectively in serum alanine aminotransferase activity when compared with animals in the diabetic control untreated groups (74.8 ± 0.2) after four weeks of treatment expressed as mean \pm SEM.

In addition, it shows results obtained from diabetic untreated rats had a significant ($P < 0.05$) increase in serum aspartate aminotransferase activity (177.5 ± 3.8 IU/L), when compared with the animals in the normal control group (134.8 ± 1.3 IU/L). The results of various percentages (12.5%, 25% and 50%)

of fermented cabbage supplementation and oral administration of Metformin (500 mg/kg) produced a significant ($P < 0.05$) decrease (132.3 ± 4.7 , 133 ± 2.1 , 134 ± 1.9 IU/L) and (137.2 ± 1.5 IU/L) respectively in serum aspartate aminotransferase activity when compared with animals in the diabetic control untreated group (177.5 ± 3.8) after four weeks of treatment expressed as mean \pm SEM.

Finally, the results obtained from diabetic untreated rats had a significant ($P < 0.05$) increase in serum alkaline phosphatase activity (82.8 ± 2.4 IU/L), when compared with the animals in the normal control

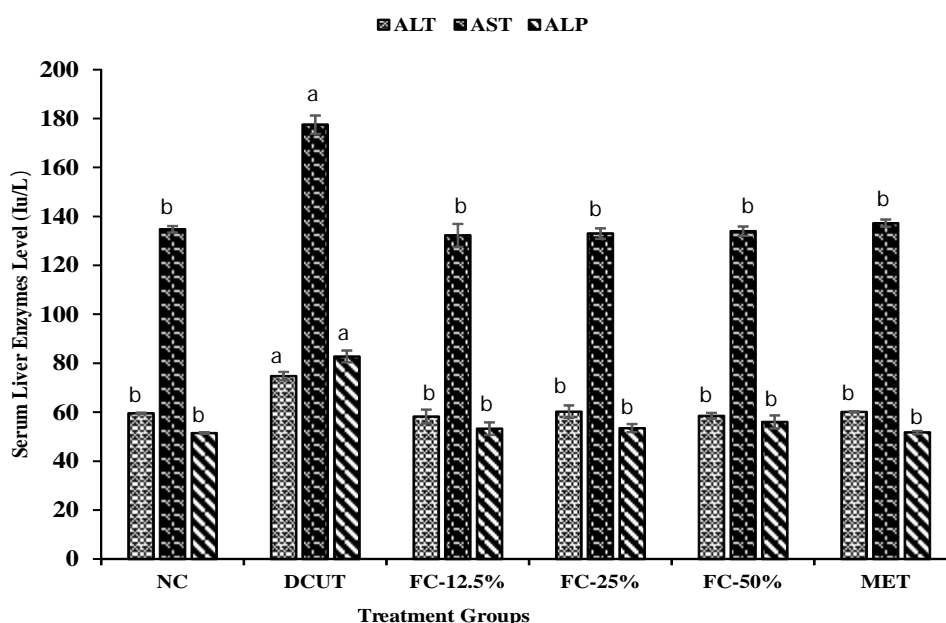


Figure 1: Effect of Fermented Cabbage Supplementation on Serum Aspartate Aminotransferase (AST) Activity in Streptozotocin/ Nicotinamide induced Diabetic Male Wistar Rats.

Bars with different superscript letters (a and b) differ significantly ($P < 0.05$) compared with normal control and diabetic control untreated respectively.

NC= Normal control, DCUT= Diabetic control untreated, FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage, FC-25%= Diabetic rats treated with 25% of fermented cabbage, FC-50%= Diabetic rats treated with 50% of fermented cabbage, MET= Diabetic rats treated with 500 mg/kg of metformin.

group (51.5 ± 0.3 IU/L). The results of the various percentages (12.5% 25% and 50%) of fermented cabbage supplementation and Metformin (500 mg/kg) produced a significant ($P < 0.05$) decrease (53.3 ± 2.6 , 53.5 ± 1.7 , 56 ± 2.6 IU/L) and (51.70 ± 0.6 IU/L) respectively in serum alkaline phosphatase activity when compared with animals in the diabetic control untreated groups (82.8 ± 2.8) after four weeks of treatment expressed as mean \pm SEM.

Result of serum total protein showed that the diabetic untreated rats had a significant ($P < 0.05$) decrease in

serum total protein (49.2 ± 1.5 g/L), when compared with the animals in the normal control group (61.8 ± 0.6 g/L). Fermented cabbage supplement (12.5% 25% and 50%) and Metformin (100 mg/kg) produced a significant ($P < 0.05$) increase (64.2 ± 1.7 , 69.1 ± 1.4 , 64.5 ± 2.1 g/L) and (61.67 ± 1.1 g/L) respectively in serum total protein when compared with animals in the diabetic control untreated groups (49.2 ± 1.5) after four weeks of treatment expressed as mean \pm SEM.

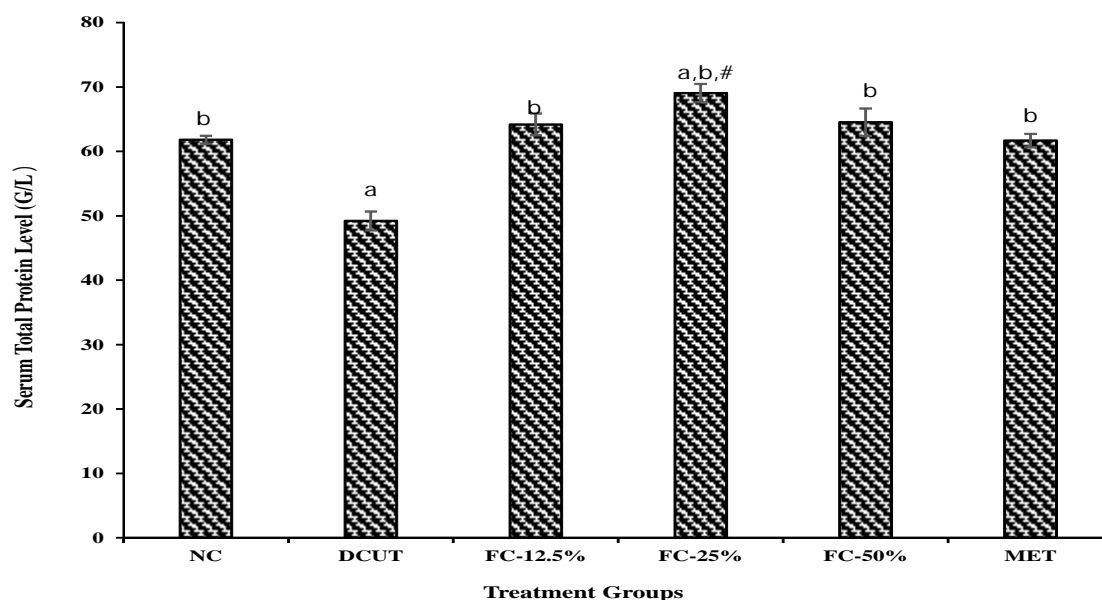


Figure 2: Effect of Fermented Cabbage Supplementation on Serum Total Protein in Streptozotocin/Nicotinamide induced Diabetic Male Wistar Rats.

Bars with different superscript letters (a and b) differ significantly ($P < 0.05$) compared with normal control and diabetic control untreated respectively.

NC= Normal control, DCUT= Diabetic control untreated, FC-12.5%= Diabetic rats treated with 12.5% of fermented cabbage, FC-25%= Diabetic rats treated with 25% of fermented cabbage, FC-50%= Diabetic rats treated with 50% of fermented cabbage, MET= Diabetic rats treated with 500 mg/kg of metformin.

DISCUSSION

The Liver is an efficient organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. High concentrations of ALT, AST and ALP with structural variations are typical indications of liver dysfunction and are observed more regularly in experimental diabetic rats. Serum enzyme measurements are valuable tools in clinical diagnosis that provides information on the effect and nature of pathological damage to any tissue.²⁵ AST, ALT and ALP are biomarkers of damage to the plasma membrane and endoplasmic reticulum and are often used to assess the integrity of the plasma membrane and tissues after being exposed to certain pharmacological agents.²⁶

Result obtained in the present study showed that the activities of serum liver enzymes; AST, ALT and ALP were significantly increased in the diabetic untreated animals in comparison with non-diabetic untreated rats. This is consistent with the studies of Daisy and Saipriya²⁵, who reported increased transaminase levels in streptozotocin-induced diabetes.

Abolfathi²⁷ reported that the elevation in markers of liver injury such as ALT, AST and ALP indicate hepatocyte damage in experimental diabetes. Treatment with fermented cabbage decreased the activities of these elevated enzymes when compared with the diabetic untreated group caused by NA/STZ-induced diabetes. This may suggest hepato-cellular protective function.

The result on serum total protein (TP) shows a significant decrease in the diabetic control untreated group when compared with normal control. This is in consonant with the work of Mohammed et al., Indira et al, and Zaidi et al.^{28,29,30} who found out that diabetic patients suffers from significant decrease in serum TP which was as a result of chronically elevated blood glucose that leads to the formation of advanced

glycosylated products with subsequent hyper filtration [a potential increase of 5%-10% in glomerular filtration rate (GFR)] and glomerular hypertrophy or decrease in production due to defect in insulin secretion or action or both. After treatment with graded doses of fermented cabbage, there was a significant increase in serum total protein with the group given 25% FC having the highest increase. This increase could be due to the anti-hyperglycemic effect of fermented cabbage that reduce the formation of glycosylated products and thereby subsequently decreasing hyper filtration or due to the ability of fermented cabbage to increase insulin level in the treated group which will in turn increase protein production.²⁸

CONCLUSION

The results of the present study demonstrated that fermented cabbage supplementation lowered serum liver enzymes levels and increases serum total proteins levels when compared with the diabetic control untreated.

The results obtained from the study demonstrate that fermented cabbage supplementation may have some protective properties on the liver.

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