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Evaluation of the Markers of Oxidative Stress and Hepatocellular Injury in Diabetic Wistar rats fed on Black Finger Millet (*Eleusine coracana*) Seed Coat Matter.

Okoyomoh Kingsley[1], Ewa Ogbonnaya[2, 3*], Ibezim Ebere Otuomasirichi[4], Elizabeth Obioma Nweke[5], Nweke Toochukwu Miracle[5], Elendu Melford Uche[6], Ude Ugochinyere Treasure[7], Wassagwa John[8]

[1]Department of Science Laboratory Technology,
Federal Polytechnic Nasarawa,
Nigeria.

[2]Department of Medical Biochemistry,
David Nweze Umahi Federal University of Health Sciences,
Uburu,
Ebonyi State.

[3]International Institute for Toxicology,
Environmental and Occupational Health,
David Umahi Federal University of Health Science,
Uburu Ebonyi State,
Nigeria.

[4]Department of Surgery,
Faculty of Clinical Medicine,
College of Medicine,
Owerri Nigeria.

[5]Department of Human Anatomy,
Chukwuemeka Odumegwu Ojukwu University,
Nigeria.
[6]Department of Human Physiology,
David Nweze Umahi Federal University of Health Sciences,
Uburu,
Ebonyi State

[7]Department of Medical Laboratory Science,
Faculty of Health Science and Technology,
David Umahi Federal University of Health Sciences,
Uburu, Ebonyi State,
Nigeria.

[8]UNESCO International Centre for Biotechnology,
Nsukka 410001,
Enugu State,
Nigeria.

Email: ewahalfred@gmail.com

Author for Correspondence

Abstract

Excessive generation of reactive oxygen species (ROS) can cause diseases by damaging cellular integrity. Antioxidant-rich foods may help prevent the development of diabetes mellitus (DM) by counteracting these harmful effects. The study aimed to assess oxidative stress markers and hepatocellular injury in diabetes mellitus rats on a Finger millet seed coat (FMSC) diet. Precisely, 20% and 40% of FMSCM were formulated by blending 400 g and 300 g of rat chow with 100 g and 200 g of SCM, respectively. Exactly 10 mL and 15 mL of clean water were introduced into the mixture to make a paste. Five groups of five male rats were used in this study. The 2nd-5th groups were injected with 60 mg/kg b.w of streptozotocin to trigger diabetes. The 1st group was fed with 100% rat chow, while the 2nd group was not treated. The 3rd-4th groups were fed with 20% and 40% of FMSC, respectively, and the 5th group received standard medication. The rats were fed for six weeks and then sacrificed, and the blood sample was collected. Biochemical analyses were performed using standard procedures. Results from the study revealed that ingestion of seed coat matter (SCM) markedly reduced the levels of the aforementioned enzymes. Observations showed that while MDA levels were substantially lowered in groups fed with SCM, CAT and SOD activities were markedly raised. This study has ascertained that black finger millet SC can counteract oxidative stress and its attendant consequences.

Keywords: Diabetes, Hepatocellular, Injury, Oxidative, Streptozotocin, Stress

Introduction

Characteristically, reactive oxygen species wield the potential to damage macromolecules, notably deoxyribonucleic acid (DNA), proteins, and lipids (Azzi, 2022). Oxidative stress is initiated when the rate at which reactive oxygen species (ROS) are synthesized outweighs clearance, leading to tissue damage and cellular dysfunction in the form of disease conditions (Gonzalez *et al*., 2023; Bhatti *et al*., 2024), which underscores its indispensability to the functionalities of life (Evans *et al*., 2022). Unfortunately, acute exposure to high levels of ROS constitutes the basis for the pathogenesis of numerous liver disorders (Apostolova *et al*., 2011).

The liver is the second most massive organ of the body saddled with the responsibility of executing a plethora of life dependent functions some of which are xenobiotics metabolism, blood clotting, among others (Sivakrishnan, 2019). Diseases affecting the liver are responsible for an estimated two million deaths yearly and have been implicated in about 4% of all deaths globally translating to1 out of every 25 deaths. In recent times, it is established that liver diseases are the leading cause of death in the world (Griffin *et al*., 2021).

Substances which wield the potential to impede oxidation of the components of the cell are referred to as antioxidants. The systems that make up the antioxidant defense system are a composite of the innate and diet derived antioxidants. It is an established fact that intake of diets rich in antioxidants shields the body against cellular oxidation, inflammation, and other disease-related processes (Mari and Alicja, 2023). Mechanisms explored by antioxidants to deliver their antioxidative roles include transforming reactive oxygen species into their non-radical counterparts halting the destructive chain reaction initiated by reactive oxygen species (Oroian and Eseriche, 2015).

Recently, emphasis on relying on food-derived antioxidants to boost the body's innate antioxidant defense system has grown stronger owing to the emergence of study outcomes

that implicate certain synthetic antioxidants with potential deleterious side effects (Sofia *et al*., 2019).

Finger millet botanically known as *Eleusine coracana* is of the genus *Eleusine* and a member of the *Poaceae* family. It is a minor cereal and an important source of several phytochemicals, notably polyphenols, which are abundantly localized in the seed coat, harboring more than 70% of the millet polyphenols (Banerjee *et al*., 2012).

MATERIALS AND METHODS

Collection of samples

One kilogram (1kg) of seeds of *E. coracana* (black finger millet) was sourced from the seed production unit of the Institute for Agricultural Research (IAR), Faculty of Agriculture, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The identification was carried out at the Department of Biological Sciences' herbarium unit at Ahmadu Bello University, Zaria, and was subsequently given the voucher number 2843.

Seed Coat Matter Preparation

The seed coat of the millet was processed into a diet following the description provided by Chethan and Malleshi (2007). To prepare a 20% SCM based diet, a mixture of 400 g of rat chow and 100 g of SCM was blended with 30 mL of water, dried, and then formulated into a 40% SCM diet by combining 300 g of rat chow and 200 g of SCM with 10 mL of water.

Animal Housing and Care

The weights of the animals used in this study ranged from 150 to 250 g. The rats were sourced from commercial breeders in Zaria. The animals were housed in adequately aerated cages at ambient temperature and relative humidity. Light and dark cycles were maintained at 12 hours each. They were fed rat chow and had unrestricted access to water. The animals were acclimatized prior to the start of the study.

Induction of Diabetes

Intraperitoneal injection of 60 mg/kg body weight of streptozotocin dissolved in 0.1 mol/L fresh cold citrate buffer (pH 4.5) into 24-hour fasted rats (Burcelin *et al.*, 1995) triggered diabetes in the animals that had been denied access to food for 24 hours. A glucometer was used to determine the blood sugar levels 3 days later, and a blood glucose level of 126 mg/dL was considered diabetic.

Animal Grouping

Five groups of five rats per group were involved in the study:

1st Group (Normal control): Rats fed only on rat feed (rat chow).
2nd Group (Negative control): Untreated diabetic rats.
3rd Group: Diabetic rats fed on 20% FMSCM.
4th Group: Diabetic rats fed on 40% FMSCM.
5th Group: Diabetic rats received 2.5 mg/kg body weight of 2.5 mg/kg metformin daily.

The animals were fed daily for six weeks and were fasted overnight before sacrifice, followed by the collection of blood samples.

Sample preparation

Plasma used for this study was obtained by centrifuging blood sample at 4000 rpm for 15 min

Assessment of the activity of liver enzymes

Five milliliters (5mL) of working solutions containing AST and ALT were introduced into test tubes before heating in a waterbath for 5 minutes at 37°C to assess the activity of serum AST and ALT. Subsequently, 0.1 mL of serum was added to the test tubes, and the mixture was incubated for 60 minutes at 37°C. After that, 0.5 mL of 1 mmol 2,4-dinitrophenylhydrazine was added, and the mixture was allowed to stand for 20 minutes at 25°C. Following this, 5 mL of 0.4 N sodium hydroxide was added, and the AST and ALT activity were spectrophotometrically measured at 505 nm (Reitman and Frankel, 1957).
For the evaluation of ALP activity, 2 mL of substrate buffer was placed into a test tube and heated for 5 minutes at 37°C. Then, 0.1 mL of serum was added to the test tube, and the resulting mixture was incubated for 15 minutes at 37°C. Subsequently, 0.8 mL of 0.5 N sodium hydroxide, 1.2 mL of 0.5 M sodium carbonate, 1.0 mL of 0.6% 4-aminoantipyrene, and 1.0 mL of 2.4% potassium ferricyanide were added. The ALP activity was assessed with the aid of a spectrophotometer at a wavelength of 520 nm (King and King, 1954).

Determination of Antioxidant Status

Determination of Catalase Activity and Malondialdehyde

A cuvette holding 1000 µL of sample dilution was then placed in the reference cuvette holder and incubated for 25 minutes at 28°C. The wavelength was set at 240 nm, and the spectrophotometer was zeroed. Diluted standard (50 µL) or sample was introduced into the cuvette, and the solution was mixed vigorously with the same pipette tip. The absorbance was immediately recorded at 240 nm every 2 seconds for 15 seconds. To determine MDA level, 0.5 ml of saline and 1.0 ml of 10% TCA were introduced into 0.5 ml of serum sample. Then, 0.25 mL of 0.1 M TBA was added to the mixture. The mixture was incubated for 1 hour at 95°C, cooled, and centrifuged at 3000 rpm for 20 minutes, and absorbance read at 535 nm (Aebi, 1984).

Analysis of Data

The results were presented as mean ± standard deviation. Analysis of variance (ANOVA) was used to test the differences between treated and untreated groups. Comparison of mean values was performed with the aid of the Tukey's post hoc test. Test. $p < 0.05$ was considered statistically significant.

Results

Antioxidant enzyme levels were displayed in Figure 1, indicating that catalase activity reported for the group induced with diabetes and not treated was significantly ($p<0.05$) lower than that reported for other groups. However, no significant difference in catalase activity was reported for the normal control group (1st) and the group fed a 40% FMSCM diet (4th), which was markedly higher compared to the group fed a 20% FMSCM diet (3rd) but significantly ($p<0.05$) lower than that reported for the Wistar rats administered the standard drug (metformin).

Similarly, superoxide dismutase levels reported for the normal control group were significantly lower compared to those reported for the third and fourth groups, but not significantly different from the fifth group which received the standard drug. The concentration of MDA recorded in the untreated diabetic group was significantly higher ($p<0.05$) compared to the other groups. However, there was no significant difference ($p>0.05$) in MDA levels between the third group fed a 20% FMSMC diet and the fifth group

administered standard drugs. A similar observation was made between the group fed a 40% FMSMC diet and the normal control group.

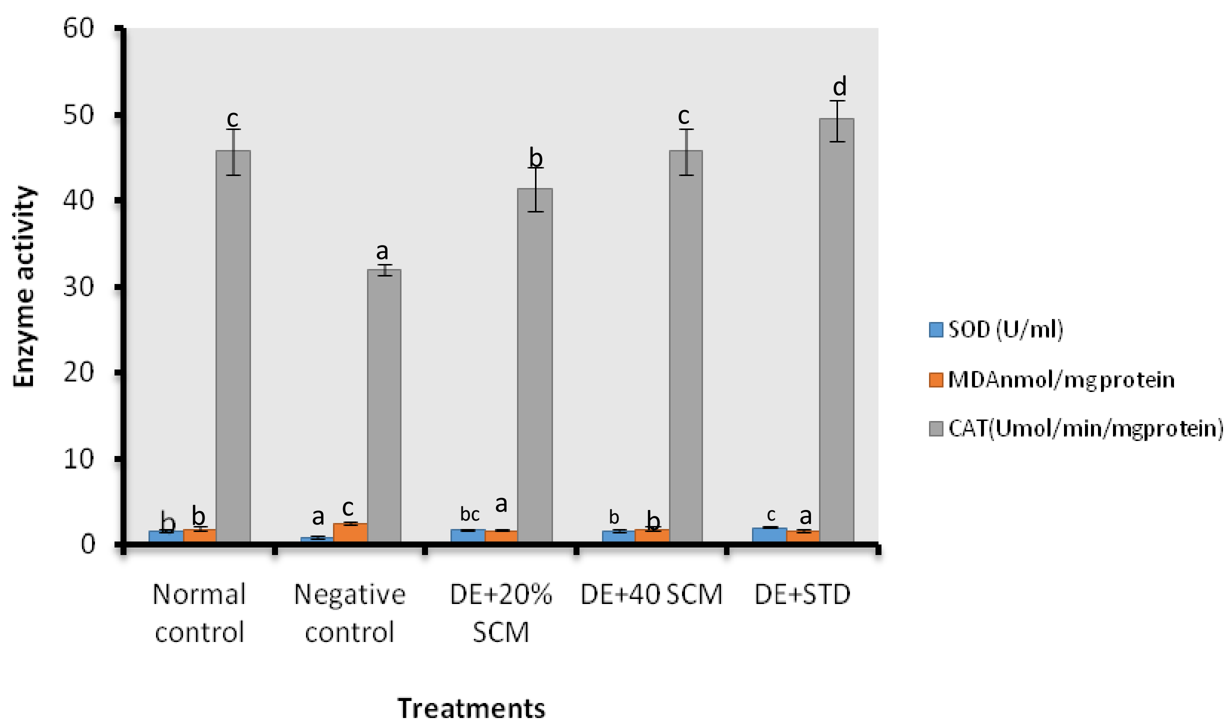

Figure 1: Antioxidant status of diabetic Wistar rats fed on black finger millet seed coat matter (*E. coracana*)
DE [Experimentally induced diabetes], FM**SCM** [Finger Millet Seed Coat Matter]

The activities of liver enzymes in rats induced with diabetes and fed on FMSCM-based diet are displayed in Figure 2, indicating a markedly high serum AST in the untreated diabetic rats compared to their counterparts fed 20% and 40% SCM diet. However, AST levels for the 3rd, 4th, and 5th groups did not significantly differ from that of the SCM fed on 20% (3rd group) and 40% (3rd group) FMSCM diet and 2.5 mg/kg b.w of metformin, respectively, but were significantly higher than those recorded for animals fed rat chow only. The ALT activity reported for the untreated diabetic group was significantly higher than that reported for groups fed 20% and 40% FMSCM diets, which, however, did not significantly differ from each other but were significantly higher than that reported for the rats administered metformin (standard drug). The activity of ALP reported for in the negative control was significantly higher than that reported for rats induced with diabetes and subsequently treated with the standard drug, which in turn was significantly higher than that reported for groups III and IV fed 20% and 40% FMSCM diet, respectively. Meanwhile, the activity of the enzyme recorded in groups II-V was significantly higher than that recorded in the first group.

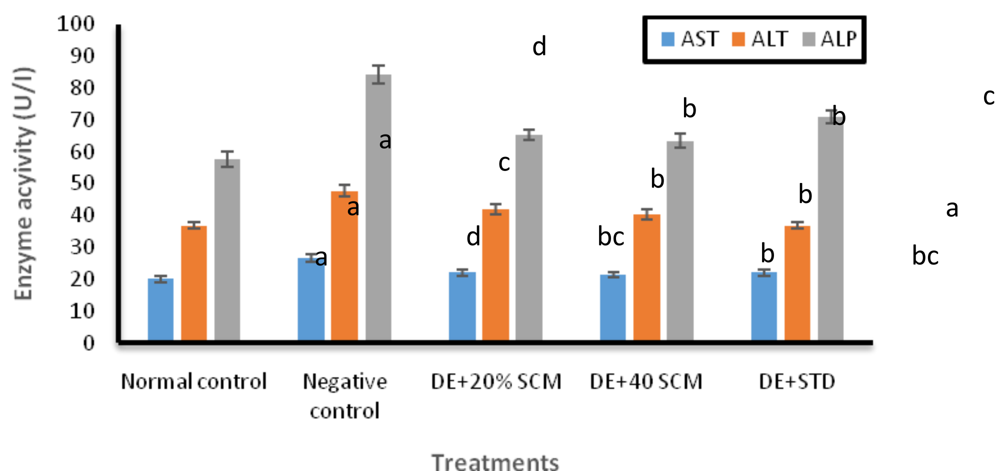

Figure 2: Liver Enzyme activity in Wistar rats fed FMSC based diet
DE: Experimentally induced diabetes; SCM: Seed Coat Matter

Discussion

Diabetes is a chronic hyperglycemic metabolic illness defined by abnormalities in carbohydrate, protein, and lipid metabolism caused by absolute or relative insulin insufficiency along with organ system failure. During diabetes, the redox balance of the antioxidant systems might be disrupted, resulting in deleterious repercussions (Ozougwu, 2016). Low levels of antioxidant enzymes can increase the risk of diabetes mellitus; on the other hand, an antioxidant-rich diet may reduce the risk of diabetes and other oxidative stress-related disorders. The presence of phenols and flavonoids in finger millet seed coat matter could explain the lower MDA and enhanced activity of antioxidant enzymes found in these groups. This is similar to the findings of Bannerjee *et al.* (2012), who concluded that finger millet phenols could function as natural antioxidants, particularly for reducing the risk of oxidative degradation.

Liver cells, known as hepatocytes, contain AST, ALP and ALT, which are commonly measured in clinical settings to assess liver health. These enzymes are released into the bloodstream following liver injury (Taiwo *et al*., 2024). The decreased activities of these liver enzymes might be due to the therapeutic properties of certain phytochemicals inherent in FMSCM. This observation aligns with the study of Muhamad *et al*. (2021), which highlighted the hepatoprotective effects of flavonoids from propolis against doxorubicin-induced hepatotoxicity. The outcome of this study also aligns with the finding made by Alzahrani *et al*. (2022), which affirmed the hepatoprotective activity of *Pennisetum glaucum* (pearl millet).

Conclusion

This study assesses the effects of finger millet seed coat matter on hepatic health and diabetes showing that seed coat matter (SCM) derived from *Eleusine coracana* (finger millet) possesses both antioxidant and hepatoprotective effect.

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