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Nutraceutical Potential of Fermented *Hunteria umbellata* Seeds-Cassava in Reducing Hyperglycemia-Related Oxidative Stress in Diabetic Rats

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While there are many pharmacological treatments for diabetes, recent evidence shows a troubling rise in diabetes-related complications, highlighting the need for supplementary strategies beyond existing medications. This study aimed to investigate the nutraceutical properties of fermented *Hunteria umbellata* seeds-cassava (HUSCA) on glucose transport proteins and hyperglycemia-related oxidative stress in diabetic rats. Portions of cassava flour were replaced with *H. umbellata* seeds at ratios of 70:30 and 80:20, both for unfermented (UF73 & UF82) and fermented (F73 & F82) for 36 hours. Forty-nine albino rats were divided into seven groups, with groups B-G induced with diabetes via alloxan injection (150 mg/kg body weight). Group A served as a normal control, while groups B and C were used as diabetic and drug control (5 mg/kg bw metformin) groups, respectively. Groups D-G received the respective test diets for 21 days. Proximate composition analysis revealed that fermentation significantly increased the protein composition of *H. umbellata* seeds (10.25–14.29 g/100g d.w) and decreased carbohydrate levels (64.86–58.98 g/100g). The test diets, particularly F82, significantly reduced elevated serum glucose levels (230.0–109.0 mg/dl) and malondialdehyde (2.78–1.42 mg/dl), while enhancing glutathione (2.52–3.59 mg/dl), catalase (1.35–2.96 U/mg), and glucose transporters compared with the diabetic group. No significant change was observed in superoxide dismutase levels ($P > 0.05$). Histological analysis of the pancreas supported these findings. Overall, these results suggest that fermented HUSCA may offer a promising nutraceutical approach for managing diabetes and its related oxidative stress complications.

Keywords: Diabetes, fermentation, *Hunteria umbellata* seed, oxidative stress, cassava.

Introduction

Diabetes affects people of every age, gender, and geographic location, making it one of the most widespread causes of mortality and morbidity. Recent reports reveal that more than 537 million people were living with diabetes in 2021, a figure anticipated to reach 783 million by 2045, making up to 12.2% of the global population (Hossain et al., 2024; Ong et al., 2023). Diabetes mellitus (DM) is a state of carbohydrate, protein, and lipid metabolic disequilibrium characterized by sustained hyperglycemia. During DM, glucose homeostasis is lost due to dysfunction of pancreatic β-cells or defects in tissue-insulin receptors (Dilworth et al., 2021; Onyeabo et al., 2022). This leads to multiple metabolic disturbances, including heightened hepatic glucose production via glycogenesis and gluconeogenesis, impaired lipid metabolism (Anter et al., 2023; Anyiam et al., 2024; Jiang et al., 2020), and alterations in protein metabolism characterized by increased proteolysis and muscle loss (Dilworth et al., 2021; Nwuke et al., 2024). Recent studies have established a strong link between hyperglycemia and elevated levels of reactive oxygen species (ROS) (Fiorentino et al., 2013; Gonzalez et al., 2023; Bhatti et al., 2022; Binjawhar et al., 2023; Anyiam et al., 2024). There is growing evidence that uncontrolled production of ROS due to hyperglycemia causes oxidative stress in a variety of cells and tissues (Bhatti et al., 2022; Chawlar et al., 2016; Fiorentino

et al., 2013). Recent studies have shown that chronic oxidative stress causes the onset of many complications associated with diabetes, such as nephropathy, neuropathy, loss of vision, and cardiovascular disease, among others (Chawlar et al., 2016; Bhatti et al., 2022; Caturano et al., 2023; Anyiam et al., 2024). Despite the many pharmacological treatments for diabetes, such as hypoglycemic agents and insulin therapy, recent studies show a troubling rise in complications among individuals with diabetes (Harding et al., 2019; Ali et al., 2022). The limitations and side effects of current medical treatments for diabetes underscore the necessity for additional complementary and alternative strategies. The use of functional foods could be one of these new approaches to managing the complications arising from diabetes. Functional foods have the potential benefits to promote health and reduce the risk of chronic diseases, beyond the basic nutritional functions offered by food (Mirmiran et al., 2014). Recent studies have indicated that functional foods can significantly aid in the management of diabetes mellitus (Carvalho et al., 2023; Kayode et al., 2023). Hence, a food-based approach may be a missing step in enhancing the dietary management of diabetes complications, but this aspect has not received sufficient attention by researchers.

Africa is blessed with many plants for ethnomedicinal use. *Hunteria umbellata* is one such medicinal plant that is being used to treat various ailments. *H. umbellata* has been used in Africa as an ancient folk medicine for treating a range of diseases. The fruits, seeds, leaves, stems, bark, and roots of *H. umbellata* have medicinal value (Momodu et al., 2016). In Nigeria, water decoctions of *Hunteria umbellata* seeds are highly valued by traditional healers in the traditional management of diabetes mellitus and hyperlipidemia. Previous studies have reported various biological activities of *H. umbellata* leaves and seeds extract, such as antioxidant (Adeneye et al., 2014; Ajiboye et al., 2017), anti-inflammatory (Ajiboye et al., 2017; Ogunlana et al., 2021), antidiabetic (Bature et al., 2023; Ejelonu, 2019), anticancer (Kumar et al., 2023), cardioprotective, and hypocholesterolemic (Adeneye and Crooks, 2015). These pharmacological properties were attributed to the bioactive compounds present in the seed extracts, such as glucosinolates, isothiocyanates, hexadecanoic acid, quercetin, and phenolic glycosides (Ejelonu, 2019; Bature et al., 2023), which have nutraceutical properties. Among the several nutrients found in the seed, proteins (10–25%) are the most abundant after carbohydrates (Onawumi et al., 2017; Anani et al., 2024; Ajayi and Ojelere, 2013). Despite these advantages, *H. umbellata* seed is underutilized, with no studies exploring its potential in functional food development for diabetes management. This may be attributed to its poor sensory qualities due to the presence of antinutritional factors (Anani et al., 2024).

Cassava (*Manihot esculenta*) is the cheapest food and one of the most utilized staple food crops in developing countries in Africa after maize. The major problem associated with cassava in terms of nutritional quality is low protein content (0.8–3 g/100g dw) and the presence of anti-nutritional factors like cyanide and phytate (Boyiako et al., 2020; Anyiam et al., 2023). However, fermentation has been found adequate for detoxifying cassava of cyanide and reducing the content of anti-nutritional factors. Fermentation is a beneficial biochemical modification of the primary food matrix, driven by microorganisms, primarily lactic acid bacteria (LAB), and their enzymes (Adebo et al., 2022; Anyiam et al., 2022a). Fermented foods have been shown to benefit health by lowering blood cholesterol levels (Anyiam et al., 2024), enhancing immunity through probiotics (Anyiam et al., 2020; Jeong et al., 2021), and combating carcinogenesis (Adebo et al., 2022), osteoporosis, and diabetes (Jeong et al., 2021). This is attributed to the metabolic activities of LAB during fermentation. LAB break down plant cell walls, which helps to liberate or synthesize various antioxidant compounds (de Marco-Castro et al., 2019; Zhao et al., 2021). Additionally, LAB proteases produce bioactive peptides through peptide bond hydrolysis, offering additional health benefits (Antony and Vijayan, 2021; Zhao et al., 2021). For instance, bioactive peptides have been linked to the antioxidant and antidiabetic properties of fermented milk (Zhao et al., 2021; Kinariwala et al., 2020). Fermented foods also typically have a lower glycemic index, resulting in a slower rise in blood sugar levels compared to unfermented options. Studies in the literature have focused solely on the pharmacological properties of crude extracts of *H. umbellata* seed, both in in-vivo and in-vitro studies (Oseyomon and Ilodigew, 2021; Ajiboye et al., 2017; Ogunlana et al., 2021; Adeneye et al., 2015). There is a scarcity of information on the potential of fermented *H. umbellata* seed as a functional food for managing metabolic complications associated with diabetes mellitus. Therefore, this study aims to assess the impact of fermented *H. umbellata* seed-cassava (HUSCA) inclusion on glucose-induced oxidative stress in diabetic rats. Enhancing commonly consumed foods like cassava with nutraceutical sources such as *H. umbellata* seeds could boost their utilization in treating diabetes and related complications.

Materials and Methods

Sample Collection

The matured seed pods of *H. umbellata* (Figure 1) were collected from Okija in Ihiala Local Government Area of Anambra state, Nigeria. An expert taxonomist at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, identified and authenticated the seed. About 5 kg of fresh matured *H. umbellata* seeds were collected. The seeds were removed from the pods, washed to remove impurities, and stored at room temperature before being transported to the Department of Biochemistry laboratory for processing. Cassava roots were sourced from the National Root Crops Research Institute, Umudike, Nigeria, and transported in a container to the laboratory for processing.

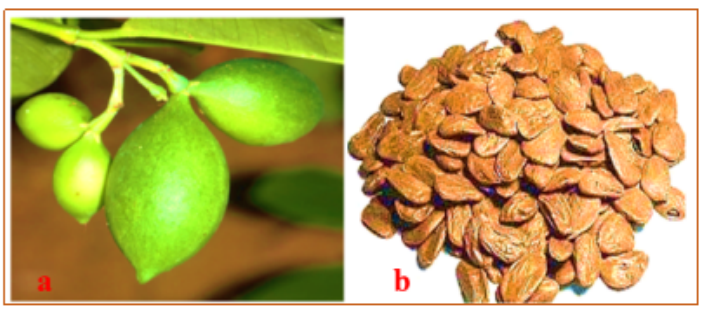

Figure 1 *Hunteria umbellata*: fruits (a) and seeds (b).

Sample Processing

H. umbellata seeds were removed from the seed pods, dehulled, and then oven-dried at 45 °C for 48 hours. The dried seeds were then divided into two portions (A and B). Portion A (50 g) was soaked in 250 mL of water, kept in a container with a cover in a dark place, and allowed to ferment for 3 days at room temperature (27 ± 2 °C) conditions. The fermented seeds were oven-dried (45 °C) for 24 hours and milled (Model QBL-18L40), alongside the already dried raw seeds, into powder, sieved through a 2 mm sieve to obtain fine powder. The powder was then labelled and stored at 4 °C in air-tight containers for further analysis. Portion B was not fermented and was used to monitor the effect of fermentation on the seed's nutritional value and pharmacological properties. The collected cassava roots were cleaned, peeled, and washed again with tap water to remove sand particles. They were cut into smaller pieces and oven-dried at 50 °C for 24 hours until constant weight before being subjected to milling. The milled white powder was sieved to produce smooth, fine dried flour, which was stored in an airtight container until used.

Proximate Evaluation

All analyses for proximate compositions in both fermented and raw *H. umbellata* seeds were carried out by the method described by AOAC (2012) as follows: The thermal drying method was used in the determination of the moisture content of the samples after measuring the loss in weight of the sample after drying in an oven at 105 °C until a constant weight was obtained. The ash content was determined using the ignition method by burning the sample in a muffle furnace at 550 °C for 6 hours until the samples turned grey. The crude protein content in raw and fermented *H. umbellata* seed flour was determined using the Kjeldahl method with a conversion factor of 6.25; the Soxhlet method was used for the measurement of fat with petroleum ether, while the crude fiber was evaluated following digestion with hot sulfuric acid (1.25% w/v) and hot sodium hydroxide (1.25% w/v). The residue obtained was oven-dried (105 °C) for 2 hours, weighed, and ashed in a muffle furnace. The fiber composition was calculated by weight difference. The total carbohydrate content was determined by subtracting the sum of the percentage moisture, ash, fat, crude protein, and crude fiber from 100% using Equation 1.

$$\text{Carbohydrates} = 100 - (\%\text{moisture} + \%\text{ash} + \%\text{fat} + \%\text{crude protein} + \%\text{crude fiber}) \quad (1)$$

Composite Flour Formulation

The milled *H. umbellata* seed powder and cassava flour were thoroughly mixed to make 100% at different ratios (Table 1). This enabled the evaluation of the dose-dependent effect of *H. umbellata* seed inclusion on each formulation. The choice of ratios between cassava and *H. umbellata* was based on their nutrient profiles and sensory attributes as reported previously (Onawumi et al., 2017; Anani et al., 2024), in order to achieve desired fermentation outcomes, maximize nutritional benefits, and ensure feed consumption by the experimental animals.

Note: HUS = *Hunteria umbellata* seed.

Table 1 Proportion of composite formulation.

Sample	Label	Cassava (%)	HUS (%)	Treatment
A	UF82	80	20	Unfermented (80:20)
B	F82	80	20	Fermented (80:20)
C	UF73	70	30	Unfermented (70:30)
D	F73	70	30	Fermented (70:30)

Note: HUS = *Hunteria umbellata* seed.

Preparation of Fermented Hunteria umbellata Seed-Cassava Blend

The composite flour of *H. umbellata* and cassava (F73 and F82) (Table 1) was fermented using the method described by Boyiako et al. (2020) with modifications. *Procedure:* 100 g of each sample was dissolved in 50 mL of boiled distilled water and mixed thoroughly. The mixture was boiled for a minute and then cooled to room temperature before the addition of 2 g of commercial starter culture containing lactic acid bacteria (as inoculum) and allowed to ferment for 36 hours in an enclosed vessel at room temperature while monitoring the pH using a pH meter. A pH of 4.5 was obtained after 36 hours of fermentation, which marked the end of fermentation. The fermented gruels were dried to obtain the enriched samples in powdered form before pelleting into animal feed. Samples UF73 and UF82 did not undergo fermentation and were directly pelleted into animal feed. Salt (0.5%) was added during pelleting to mask the mildly bitter taste of *H. umbellata* seed in the feed. The pelletized rat feeds were oven-dried at 45 °C for 8 hours and used as the experimental diet. Standard feed was used as a control diet.

Experimental Animals

All animal experiments were carried out following the guidelines of animal experiments at the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike. Forty-nine (49) male albino rats of the Wistar strain, weighing between 80–120 g, obtained from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. The animals were housed in metabolic cages in a well-ventilated experimental room with 12-hour light/dark cycles. They were allowed to acclimatize for 14 days to their new environment before the commencement of the experiment. The rats had free access to their diets and water ad libitum.

Ethical Approval

This study was performed according to the guidelines suggested by the Declaration of Helsinki. The study protocol was approved by the ethical committee at the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike (Reference number: CREC/009/24).

Induction of Diabetes

This was done by following the method described by Anyiam et al. (2024). A freshly prepared solution of alloxan (250 mg dissolved in 40 mL of freshly prepared sodium citrate buffer 0.1 M, pH 4.5) was injected intraperitoneally into the experimental rats at a dosage of 150 mg/kg body weight in a fasting state. Blood was collected from the tail vein, and blood glucose concentration

was analyzed using an Accu-Check Active glucometer and test strips (Roche Diagnostics GmbH, Germany). Rats with blood glucose > 200 mg/dL were considered diabetic and were used for the study (Onyeabo et al., 2022). The study commenced a week after the alloxan injection. The blood glucose levels of all experimental rats were checked every week throughout the study.

Experimental Design

A completely randomized experimental design comprising seven (7) treatment groups, replicated thrice, was used for the study. Alloxan-induced diabetic rats with stable diabetic conditions were divided into 6 subgroups (groups B to G) with seven animals per group, while the non-diabetic rats formed the first group (Group A). The diabetic rats were treated with different proportions of fermented and unfermented *H. umbellata* seed-cassava pellets. Metformin was used as a standard drug. The groups were as follows (Table 2): The rats were administered the prepared diet twice daily for three consecutive weeks (i.e., 21 days).

Table 2 Experimental design and animal grouping.

Group	Label	Treatment
A	Normal control	Received the standard feed and water
B	Diabetic control	Diabetic control but not treated. Received standard diet
C	Drug control	Received standard drug (Metformin; 5 mg/kg bw)
D	UF73	Received unfermented HU seed-based diet (30%)
E	UF82	Received unfermented HU seed-based diet (20%)
F	F73	Received fermented HU seed-based diet (30%)
G	F82	Received fermented HU seed-based diet (20%)

Percentage Change in Body Weight

All diets were administered in a total amount of 50 g per day. The rats were weighed using a digital compact scale (Model: ML204-01 Switz). The initial weights of rats were taken, and their weights were monitored every week. Percentage body weight gain/loss was determined using the formula (Equation 2).

$$\% \text{ Body weight gain} = \frac{W_2 - W_1}{W_1} \times 100 \quad (2)$$

where W_2 = final weight, W_1 = initial weight.

Blood Collection and Serum Preparation

At the end of the feeding experiment, which lasted for 21 days, the animals were fasted overnight, weighed, and then anesthetized and euthanized by cervical dislocation. The blood from the rats was rapidly collected by direct heart puncture into plain sample bottles for biochemical assay. The blood was allowed to stand for 30 minutes to clot and subsequently centrifuged at 3000 $\times$ g for 10 minutes at room temperature to separate the serum (Onyeabo et al., 2022). The serum samples were collected by aspiration using a Pasteur pipette into sterile bijou bottles and stored frozen until required for biochemical analysis, which was performed within 48 hours. Each rat's carcass was promptly dissected, and the tissues were collected and rinsed in saline buffer for histological examination.

Biochemical Evaluation

Determination of Fasting Blood Glucose

Fasting blood glucose was tested after overnight fasting using an Accu-Check Active glucometer and test strips (Roche Diagnostics GmbH, Germany). The principle was based on the reaction of glucose in the blood (from the tail end of the rats) with the glucose dehydrogenase enzyme (on the test strip), resulting in a color change. The intensity of this color gives the blood glucose concentrations (mg/dL) as converted by the glucometer.

Determination of Glucose Transport Proteins (GLUT)

The GLUT 1 and GLUT 4 Enzyme-Linked Immunosorbent Assay (ELISA) kits were used for the assay of whole blood GLUT 1 and muscle homogenate GLUT 4 using the methods described by Li and McNeil (1997). Changes in the intensity of the color, which was measured at 450 nm and 440 nm respectively, were determined using a microplate reader. The GLUT 1 and GLUT 4 levels were determined by comparing the optical density of each sample to the standard curve.

Oxidative Stress Biomarkers

Superoxide Dismutase Determination

Superoxide dismutase (SOD) activity in the serum was determined as described by Misra and Fridovich (1972). The assay mixture consisted of a blood serum sample, 0.05 M carbonate buffer (pH 10.2), and 0.3 mM of freshly prepared epinephrine. The increase in absorbance was monitored at 480 nm every 30 seconds for 150 seconds. One unit of enzyme activity is defined as 50% inhibition of the rate of autooxidation of epinephrine, as determined by the change in absorbance per minute at 480 nm.

Catalase Evaluation

Catalase (CAT) activity was determined as described by Aebi (1974). The reaction mixture (3.0 mL) consisted of the sample, phosphate buffer (pH 7.0), and 30 mM hydrogen peroxide. Absorbance was read at 240 nm using a spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1}, was used to calculate the CAT activity.

Reduced Glutathione (GSH)

The level of GSH was determined using the procedure described by Ellman (1959). Briefly, the sample (1.0 mL) was added to 0.1 mL of 25% trichloroacetic acid (TCA), and the precipitate was removed by centrifugation at 5000 $\times$ g for 10 minutes. The supernatant (0.1 mL) was added to 2 mL of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer (pH 8.0). The absorbance was read at 412 nm.

Malondialdehyde (MDA)

The determination of MDA followed the procedure described by Ohkawa et al. (1979). Briefly, the sample (1 mL) was mixed with

TBA/HCl/TCA (15%, 0.2 N, 0.37%) at a reagent/sample ratio of 2:1 (v/v), placed in a boiling water bath for 15 minutes, cooled to room temperature, and centrifuged at 1000 × g for 10 minutes at room temperature. The absorbance of the solution was read at 535 nm against the blank (containing all reagents except the sample). MDA content was determined using the extinction coefficient of 1.56×10^6 and expressed as mg/dL.

Histopathological Examination

Pancreatic tissues were collected, fixed in 10% formalin for 48 hours, dehydrated through different grades of ethanol and xylene, and embedded in paraffin. Sections (4–5 μm) of the tissues were stained with hematoxylin and eosin stains for microscopic investigation. The fixed slides were viewed under a light microscope, and photomicrographs were captured (400×). Photomicrographs were taken with a computer having Microscopic Analysis Software (ScopeImage-9.0) connected to an Olympus digital light microscope (Olympus UK Ltd., Essex, UK) (Onyeabo et al., 2022).

Statistical Analysis

All values were expressed as mean ± SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SPSS program 20.0. A probability (p-value) of < 0.05 was considered statistically significant. Charts were plotted using Microsoft Excel 2013.

Result

Effect of Fermentation on Proximate Composition of H. umbellata Seed

The result on the proximate composition (Table 3) revealed that *H. umbellata* seed contains a considerable amount of protein (10.25 g/100g), lipids (2.53 g/100g), and fiber (2.96 g/100g), but is high in carbohydrate (64.86 g/100g). Fermentation improved ($P < 0.05$) the protein composition by 39% and significantly decreased ($P < 0.05$) the ash, fiber, and carbohydrate content compared with the raw seeds. There was a gradual increase in moisture content (albeit $P > 0.05$) following fermentation.

Table 3 Proximate composition of fermented and raw *H. umbellata* seed (g/100g) dry weight.

Composition	Raw Seed	Fermented Seed
Moisture	18.75 ± 0.32^a	19.06 ± 0.08^a
Protein	10.25 ± 0.0^b	14.29 ± 0.06^a
Ash	4.34 ± 0.14^b	2.98 ± 0.04^a
Fiber	2.96 ± 0.01^a	2.61 ± 0.13^a
Fat	2.53 ± 0.13^a	2.12 ± 0.02^b
Carbohydrate	64.86 ± 1.69^a	58.98 ± 2.85^a

Note: Means with different superscripts (abc) along each row are significantly different at ($P < 0.05$).

Body Weight Gain of Animals Before and After Treatment

Results in Table 4 showed the percentage body weight gain of animals fed with fermented and unfermented *H. umbellata* seed supplementation. The highest percentage gain in body weight (40.55%) was recorded in the normal control group without diabetes, while the lowest gain in body weight (23.98%) was recorded in the diabetic control group, which was not treated. Administration of fermented *H. umbellata* seed-cassava diet at the ratio used in this study resulted in significant improvement ($P < 0.05$) in body weight gain when compared with the diabetic control group. Fermented *H. umbellata* seed-cassava (80:20) resulted in better improvement in body weight gain (35.14%), while the fermented ratio of 70:30 recorded a 28.57% increase in body weight, which is significantly lower ($P < 0.05$) than the normal control (group 1).

Effect of Fermented H. umbellata Seed-Cassava on Organ Weight

The results in Table 5 represent the organ weights of animals fed fermented and unfermented *H. umbellata* seed-cassava supplementation in different ratios. No significant difference was recorded ($P > 0.05$) for liver and kidney weights across all treated groups compared with normal and positive controls. Diabetic rats in group 2 (untreated) showed a significant ($P < 0.05$) increase in the weight of the heart (0.57 g) compared with the normal control (0.40 g). Consumption of both fermented and unfermented *H. umbellata*-cassava feed reduced the increased heart weight (0.57–0.42 g) close to normal (0.40 g). Similar effects ($P > 0.05$) were recorded in all the treated groups with HUSCA. The influence of HUSCA on spleen and pancreas followed a similar pattern to the heart.

Effects of HUSCA on Fasting Blood Glucose of Normal and Diabetic Albino Rats

Table 6 shows that groups administered fermented *H. umbellata* seed-cassava (HUSCA) feed (groups D–G) were able to reverse their elevated blood glucose to near normal at the end of the experiment, which lasted for three weeks. The animals fed with unfermented HUSCA (20% and 30%) were almost still diabetic (182 and 147 mg/dL) at the end of the 21 days of feeding. Though a slight reduction ($P > 0.05$) in fasting blood sugar was seen in these groups compared with the diabetic untreated group, consumption of fermented HUSCA (30% and 20%) resulted in the highest lowering effect of fasting blood glucose in diabetic rats (109 and 118 mg/dL) compared to the diabetic control and other treatments.

Effects of H. umbellata-Cassava on Glucose Transport Proteins

Induction of diabetes had no significant effect on GLUT 1 and GLUT 4 ($P > 0.05$) compared to the normal control group (Figure 2). Consumption of fermented *H. umbellata* seed-cassava feed also had no significant effect on GLUT 1 and GLUT 4 at 30% incorporation, but significantly increased the level of both transporters at 20% incorporation ($P < 0.05$).

Effect of Fermented HUSCA on Oxidative Stress Markers

Table 7 represents the antioxidant effect of unfermented and fermented *H. umbellata* seed-cassava consumption on alloxan-induced diabetes in rats. A significant reduction ($P < 0.05$)

Table 4 Percentage increase in body weight of rats fed *H. umbellata*-cassava.

Group	Treatment	Initial wt (g)	Final wt (g)	% Body Weight Gain
A	Normal	89.66 ± 2.08	126.0 ± 5.29	40.53 ± 1.87^{a}
B	Diabetes	124.67 ± 3.00	164.0 ± 4.00	23.98 ± 0.35^{e}
C	Drug control	120.0 ± 1.73	184.0 ± 3.01	34.17 ± 3.84^{b}
D	UF73	115.67 ± 2.51	169.0 ± 5.36	31.44 ± 2.78^{c}
E	UF82	110.0 ± 2.00	165.0 ± 4.02	32.32 ± 4.05^{b}
F	F73	112.0 ± 1.37	154.0 ± 6.02	28.57 ± 1.12^{d}
G	F82	114.0 ± 1.02	168.0 ± 2.02	35.14 ± 0.59^{c}

Note: Means with different superscripts (abc) are significantly different at ($P < 0.05$) down the columns. UF73 = unfermented 70:30, UF82 = unfermented 80:20, F73 = fermented 70:30, F80:20 = fermented 80:20 ratio.

Table 5 Organ weight distribution of animals fed fermented cassava-based *H. umbellata* diet.

Group	Treatment	Liver (g)	Heart (g)	Kidney (g)	Spleen (g)	Pancreas (g)
A	Normal	4.57 ± 0.26^{a}	0.40 ± 0.06^{b}	0.93 ± 0.11^{a}	0.84 ± 0.08^{ab}	0.59 ± 0.07^{c}
B	Diabetes	5.96 ± 0.96^{a}	0.57 ± 0.07^{a}	1.08 ± 0.12^{a}	0.96 ± 0.23^{a}	0.90 ± 0.12^{a}
C	Drug control	5.22 ± 2.00^{a}	0.52 ± 0.09^{ab}	1.03 ± 0.19^{a}	0.87 ± 0.12^{ab}	0.47 ± 0.11^{cd}
D	UF73	4.87 ± 1.04^{a}	0.43 ± 0.08^{b}	1.00 ± 0.27^{a}	0.55 ± 0.24^{ab}	0.45 ± 0.17^{cd}
E	UF82	4.48 ± 0.05^{a}	0.42 ± 0.04^{b}	0.83 ± 0.19^{a}	0.62 ± 0.30^{ab}	0.53 ± 0.14^{d}
F	F73	5.48 ± 1.37^{a}	0.42 ± 0.14^{b}	0.90 ± 0.08^{a}	0.93 ± 0.43^{ab}	0.72 ± 0.04^{b}
G	F82	5.65 ± 0.82^{a}	0.46 ± 0.04^{ab}	0.90 ± 0.03^{a}	0.51 ± 0.07^{b}	0.60 ± 0.06^{bc}

Note: Means with different superscripts (abc) are significantly different at ($P < 0.05$) down the columns. UF73 = unfermented 70:30, UF82 = unfermented 80:20, F73 = fermented 70:30, F82 = fermented 80:20 ratio.

Table 6 FBG levels (mg/dL) of diabetic rats fed fermented *H. umbellata* seed supplementation.

Group	Treatment	Pre-induction	After-induction	Week 1	Week 2	Week 3
A	Normal	90.66 ± 7.37^{a}	NI	84.66 ± 3.05^{d}	88.66 ± 2.08^{f}	87.33 ± 2.51^{d}
B	Diabetes	88.33 ± 3.21^{ab}	337.33 ± 10.97^{a}	282.67 ± 22.30^{a}	279.33 ± 13.31^{a}	230.0 ± 16.37^{a}
C	Drug control	83.00 ± 5.01^{ab}	315.67 ± 18.77^{a}	211.04 ± 8.88^{c}	114.62 ± 3.05^{e}	98.66 ± 3.51^{d}
D	UF73	83.02 ± 4.58^{ab}	306.33 ± 6.65^{a}	245.17 ± 8.54^{b}	213.33 ± 2.51^{c}	182.0 ± 10.44^{b}
E	UF82	80.06 ± 2.00^{b}	311.33 ± 19.73^{a}	233.71 ± 18.71^{b}	218.00 ± 2.00^{b}	147.17 ± 12.09^{b}
F	F73	84.66 ± 6.11^{ab}	312.0 ± 19.73^{a}	276.0 ± 9.16^{a}	206.33 ± 2.08^{c}	109.0 ± 7.93^{c}
G	F82	82.00 ± 2.64^{b}	309.10 ± 8.10^{a}	288.16 ± 5.03^{a}	195.16 ± 7.57^{d}	118.00 ± 8.54^{c}

Note: Means with different superscripts (abc) are significantly different at ($P < 0.05$) down the columns. UF73 = unfermented 70:30, UF82 = unfermented 80:20, F73 = fermented 70:30, F80:20 = fermented 80:20 ratio. NI = No induction.

in MDA concentration (2.78 to 1.49 mg/dL) was recorded in treated animals compared with the positive control, which was untreated. No significant increase in superoxide dismutase (SOD) ($P > 0.05$) was recorded in test groups (groups D–G) compared with the diabetic group. On the contrary, intake of fermented *H. umbellata* seed-cassava significantly improved ($P < 0.05$) the level of glutathione (GSH), especially in the group fed with 20% fermented *H. umbellata* seed supplementation, which was reduced in the diabetic group.

Organ Histology

Figure 3 displays photomicrographs of the pancreas from experimental animals fed fermented and unfermented *H. umbellata* seed-cassava feed. Plate 1 shows a normal control rat's pancreas (NHN) with intact islets of Langerhans. Plate 2 depicts the diabetic control group (NHD), where beta cells are partially destroyed, leading to necrosis and vacuolation. Plate 3 (NHM) illustrates the drug control group with regenerated beta cells and no necrosis. Plate 4 indicates that UF73 feed did not restore beta cells, while Plate 5 reveals shrunken islets with minor effects from UF82. Plates 6 and 7 show that diabetic rats treated with F73 and F82 exhibit improved histoarchitecture and nearly restored islet beta cells, respectively.

Discussion

This study evaluated the nutraceutical potential of fermented and unfermented *H. umbellata* seed in cassava-based supplementation on diabetic rats. In the first part of the study, the effect of

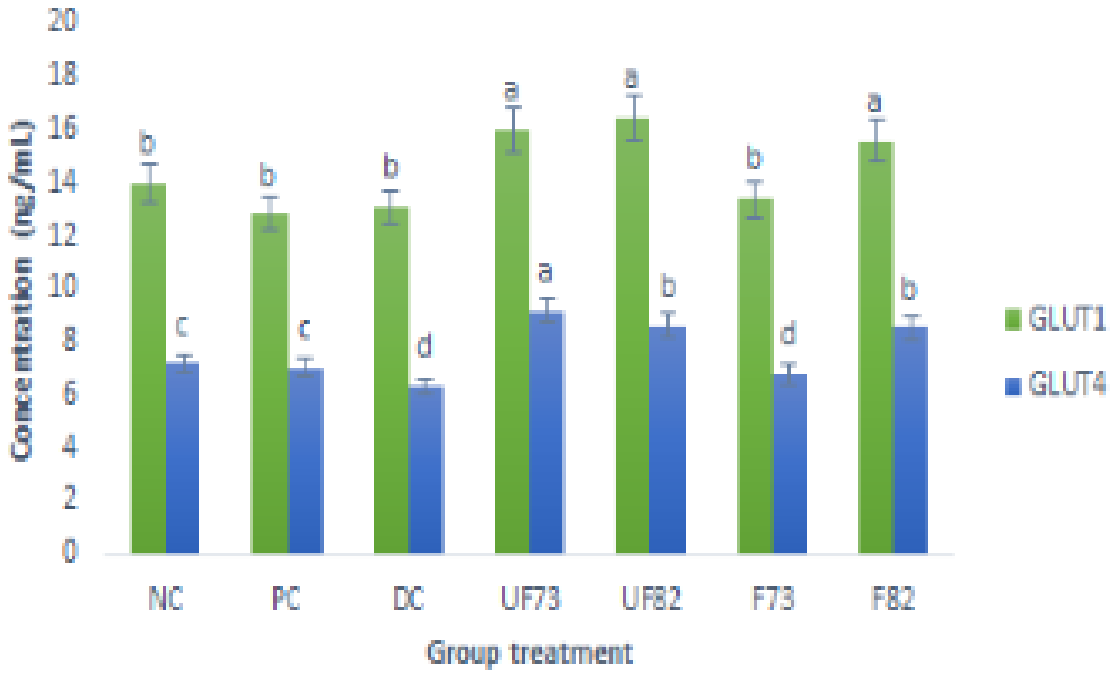

Figure 2 Effect of *H. umbellata* seed-cassava on glucose transporters in rats.

Table 7 Antioxidant effect of fermented *H. umbellata* seed-cassava on diabetic rats.

Group	Treatment	MDA (mg/dL)	SOD (U/mg)	Catalase (U/mg)	GSH (mg/dL)
A	Normal	1.34 ± 0.29^{b}	11.09 ± 0.06^{a}	2.39 ± 0.99^{a}	4.48 ± 0.20^{a}
B	Diabetic	2.78 ± 0.22^{a}	11.07 ± 0.05^{a}	1.35 ± 0.04^{c}	2.52 ± 0.19^{d}
C	Drug control	2.34 ± 0.14^{a}	11.01 ± 0.06^{a}	1.38 ± 0.17^{c}	2.63 ± 0.21^{d}
D	UF72	1.42 ± 0.26^{b}	11.00 ± 0.10^{a}	1.59 ± 0.22^{b}	2.88 ± 0.26^{cd}
E	UF82	1.43 ± 0.21^{b}	11.15 ± 0.24^{a}	1.68 ± 0.26^{b}	3.27 ± 0.32^{c}
F	F72	1.53 ± 0.35^{b}	11.36 ± 0.11^{a}	2.96 ± 0.20^{a}	2.49 ± 0.28^{d}
G	F82	1.49 ± 0.39^{b}	11.17 ± 0.27^{a}	2.57 ± 0.43^{a}	3.56 ± 0.11^{b}

Note: Means with different superscripts along each column are significantly different at $P < 0.05$. MDA = Malondialdehyde, SOD = superoxide dismutase, CAT = catalase, GSH = glutathione.

fermentation on the nutritional value (proximate) of *H. umbellata* seed was evaluated. Proximate composition of food is an important criterion in the food industry for evaluating the nutritional quality of a nutrient source (Anyiam et al., 2024). The proximate assay results showed a slight increase in moisture content after fermentation (from 18.75% to 19.05%), although this change was not statistically significant ($P > 0.05$). This minor increase may be attributed to the metabolic activities of the fermenting microorganisms, which enhance growth and fermentation performance. The finding supports the previous reports of Anyiam et al. (2023), Ojokoh et al. (2015), and Adejuwon et al. (2021), who separately observed increased moisture content during fermentation of cassava, millet, and sorghum-soybean-sweet potato, respectively. In contrast, previous studies have found that the moisture content of unripe plantain (Desta et al., 2021) and soymilk-nono (Obadina et al., 2013) decreases during fermentation. This discrepancy may be attributed to variations in experimental design, fermentation duration, and the raw materials used. According to Kumolu-Joh and Ndimele (2011), dry foods with water content exceeding 14% are more susceptible to bacterial and fungal growth. The moisture content obtained in this study is slightly above this range ($\geq 14\%$), which means the flour of *H. umbellata* requires a proper storage system to minimize the proliferation of spoilage microorganisms due to elevated water activity.

Insufficient protein intake has been identified as a contributing factor to malnutrition, particularly in schoolchildren (Anyiam et al., 2022b). The crude protein of *H. umbellata* increased ($P < 0.05$) from 10.25% to 14.29% after fermentation. This indicates that the fermented *H. umbellata* seed is a better source of protein than the unfermented seed. This is in a similar range to the protein content of 9.17%, 13.92%, and 12.25% reported by Morakinyo et al. (2020), Anani et al. (2024), and Udinyiwe and Aghedo (2022), respectively. However, our result is lower than

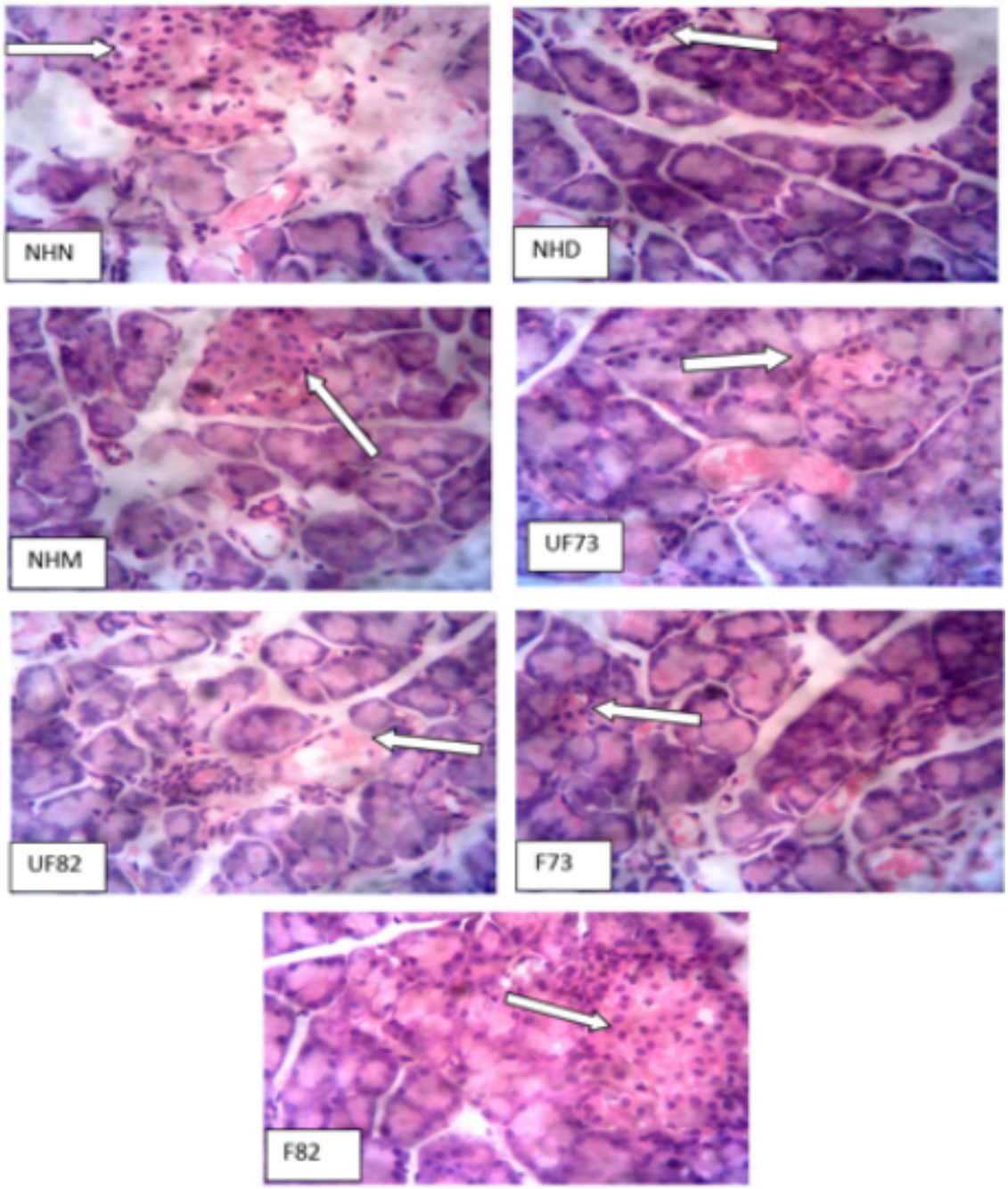

Figure 3 Histoarchitecture of the pancreas of rats following 3 weeks consumption of HUSCA (×400). (NHN): control, (NHD): diabetic group, (NHM): Metformin-drug group, (UF73): received unfermented HUSCA 70:30; (UF82): received unfermented HUSCA 80:20; (F73): fermented HUSCA 70:30; (F82): fermented HUSCA 80:20.

the 21.31% reported by Ajayi and Ojelere (2013). The difference could be due to variations in samples, geographical location, and/or analytical method of analysis. Previous studies have also reported that microbial fermentation enhanced the protein content of various food materials (Obadina et al., 2013; Adebo et al., 2022; Adejuwon et al., 2021; Anyiam et al., 2023). For instance, fermenting Bambara nut into dawadawa (a fermented condiment) increased the protein content by about 18% (Adebiyi et al., 2019). This enhancement is attributed to the release of proteins that were previously bound to antinutritional factors during fermentation. Similarly, studies by Jude-Ojei et al. (2017) and Anyiam et al. (2024) found that adding fermented moringa seed flour to maize ogi and cassava increased protein content. This increase was attributed to microbial proliferation and the secretion of protein-rich extracellular enzymes during fermentation to obtain energy (Anyiam et al., 2024). In contrast, some studies

(Ejiqui et al., 2005; Nnam and Obiakor, 2003) reported a decrease in protein content during fermentation, linking this to the leaching of soluble proteins and amino acids into the fermentation medium.

Plant-based fats are recognized as significant sources of mono- and polyunsaturated fatty acids (Risso et al., 2023; Saini et al., 2021), which are vital for human health. During fermentation, a reduction ($P < 0.05$) in fat content was observed compared with the unfermented seed sample. This could be due to the activation of lipases from the fermenting microorganisms (Anyiam et al., 2023), which hydrolyzed fat into fatty acids and glycerol as a source of energy. This finding is similar to the 3.53 g/100g, 2.59 g/100g, and 2.00 g/100g fat content reported by Morakinyo et al. (2020), Anani et al. (2024), and Udinyiwe and Aghedo (2022), respectively, but lower than the 17.60% reported by Ajayi and Ojelere (2013). Various authors (Chinma et al., 2020; Granito et al., 2002; Farinde et al., 2018) have reported reductions in fat concentration in African bean, cowpea, African yam bean, common bean, and Lima bean during fermentation. These reductions have been attributed to microbial metabolism in the fermentation medium, lipid breakdown by lipase, the utilization of lipids as a food source by fermenting organisms, and the loss of total solids and fat-related components into the soaking water. For instance, in a study conducted by Onwurafor et al. (2014), fermenting mung bean seed using spontaneous and back-slopping methods for 72 hours reduced the fat content by 38%. This was attributed to the activities of the lipolytic enzymes during fermentation. A similar mechanism for the decrease in fat content was also reported by Adebowale and Maliki (2011) in fermented pigeon peas. However, contradictory findings were reported in fermented chickpea (1.8% increase) (Xiao et al., 2015) and fermented Bambara nut (2% increase). This could likely be due to differences in sample type and duration of fermentation. The decrease in fat composition observed in this study benefits the final flour product by extending its shelf life, as it lowers the risk of rancidity caused by lipid oxidation during storage.

Fiber is a non-digestible carbohydrate that helps slow gastric emptying and promotes the excretion of fecal cholesterol (Grube et al., 2013; Anyiam et al., 2024), thereby lowering the risk of chronic diseases. From the result obtained, the fiber content of *H. umbellata* seed (2.96%) reduced (albeit, $P > 0.05$) to 2.61% after fermentation. This could be a result of increased activity of hydrolyzing enzymes such as cellulase and α-galactosidase (Sharma et al., 2020; Anyiam et al., 2023), which hydrolyze the dietary fiber constituents as a source of energy. Anyiam et al. (2023), Chinma et al. (2020), and Granito et al. (2002) reported a decrease in crude fiber content during the fermentation of cassava flour and pigeon peas, respectively. This reduction was attributed to the fermentation microorganisms utilizing cellulose and arabinose, which they break down using enzymes such as cellulase, xylanase, and hemicellulase (Adebo et al., 2022). A moderate level of fiber in functional foods is helpful because it reduces bulkiness and encourages gradual absorption of essential nutrients and bioactive compounds (Anyiam et al., 2024). The level of ash in food reflects the total available minerals (Anyiam et al., 2023). In this present study, a reduction in ash content (4.34–2.98%) was observed following fermentation of *H. umbellata* seed. The ash content reported in this study is in line with the reports of Ajayi and Ojelere (2013) and Udinyiwe and Aghedo (2022), who separately reported ash content of 5.56% and 3.20% in raw seed of *H. umbellata*. It is slightly higher than the 2.53 g/100g reported by Anani et al. (2024) on ethanol extract of *H. umbellata* seed. This reduction in ash content following fermentation could be due to the leaching of the minerals into the discarded water. Different studies have also shown a decrease in ash content during fermentation of food materials. For instance, a study by Adebiyi et al. (2019) and Granito et al. (2002) reported a decrease in total ash and mineral content during natural fermentation of pearl millet (1.86 to 1.36%) and *Vigna sinensis* (4–2%) for 3 days. This was attributed to the utilization of mineral elements for the proper growth of microorganisms during fermentation. Conflicting results were observed in the fermentation of mung beans (Onwurafor et al., 2014) and tamarind seeds (Olagunju et al., 2018), where an increase in ash content was reported. The authors attributed this rise in ash to the breakdown of complex-chelated compounds by fermenting microorganisms, which enhances mineral synthesis, depending on the specific fermentation conditions used.

The carbohydrate level in *H. umbellata* (64.86%) reduced to 58.98% after fermentation. This was attributed to the use of carbohydrates as a major energy source for the fermenting microorganisms (Anyiam et al., 2023; Xiao et al., 2015). This result is consistent with trends in the literature (Chinma et al., 2020; Asensio-Grau et al., 2020; Olaleye et al., 2020; Anyiam et al., 2024) showing a reduction in carbohydrate after fermentation of different seeds. This result supports previous findings (Ajayi and Ojelere, 2013; Morakinyo et al., 2020), which indicate that *H. umbellata* seed is rich in carbohydrate, seconded by protein. According to Anyiam et al. (2023), fermenting microorganisms are significant producers of enzymes like maltase, α-amylase, glucosidase, fructofuranosidase, and lactanase. These enzymes break down various carbohydrate components for energy during fermentation, resulting in their reduction and the production of lactic acid, which lowers the pH of fermented foods and helps extend their shelf life. In contrast to our findings, increases in carbohydrate levels were reported for fermented cowpea (5%) (Prinyawiwatkul et al., 1996) and fermented Bambara nut (3%) (Ijarotimi and Esho, 2009), attributed to the conversion of resistant starches into available starches by microbial enzymes. Reducing carbohydrate intake while increasing protein in diabetes can enhance satiety and lower overall calorie consumption, helping stabilize blood sugar levels and reducing glycemic load. This may improve insulin sensitivity and support better glucose management (Layman et al., 2005).

The effect of HUSCA consumption on the body weight of animals was reported. The body weight changes serve as a sensitive indicator of the general health status of the experimental animals throughout the experiment. The result of the percentage body weight gain showed a reduction in percentage body weight gain in disease control rats (untreated) when compared with the normal rats. Treatment with the standard drug and HUSCA improved the body weight of rats close to normal, compared with the diabetes control (untreated). This result agrees with the study of Onyeabo et al. (2022) and Anyiam et al. (2024), who reported a reduction in body weight in the presence of experimentally induced diabetes in rats. The lower percentage gain in body weight seen in the disease group may be attributable to loss of appetite as a result of discomfort caused by alloxan-induced diabetes condition. No significant differences ($P > 0.05$) were observed in the weights of all organs in the treated groups compared to the disease control, except for the heart and pancreas, which increased ($P < 0.05$) in the diabetic group compared with the negative control. Oxidative stress can lead to organ damage and inflammation, which may cause changes in organ weight, often resulting in increased weight due to swelling or hypertrophy (Naomi et al., 2023). Studies have shown an association between hyperglycemia and increased relative organ weights of diabetic animals (Zafar and Naqui, 2010; Eleazu et al., 2013).

This increase in heart and pancreas weight was nearly reversed by the consumption of HUSCA when compared with the negative control. This result indicates a potential protective effect of HUSCA against the oxidative damage induced by diabetes, as supported by the pancreas histology. This implies that HUSCA may help manage oxidative stress in the pancreas, which is crucial for maintaining insulin production and overall metabolic health in diabetic conditions.

Accumulating evidence suggests that the health benefits associated with fermented foods are often attributed to the bioactive compounds (such as bioactive peptides) that are synthesized by LAB during fermentation (Anyiam et al., 2020; Sanlier et al., 2019; Jeong et al., 2021). In the present study, alloxan injection was associated with significant and sustained hyperglycemia, which indicated that alloxan reliably induced hyperglycemia in the treated rats. Alloxan is known to cause direct and selective cytotoxicity to the pancreatic β-cells by causing cell membrane disruption after its intracellular accumulation (Adeneye et al., 2014), resulting in a decrease in endogenous insulin secretion and release, which leads to decreased glucose utilization by the tissues. A significant ($P < 0.05$) reduction in blood glucose level was observed in diabetic rats fed with *H. umbellata* seed-cassava-based diet (HUSCA) (at 70:30%) when compared with the diabetic control. The study showed the ability of HUSCA to reduce blood glucose levels, which was also confirmed by the histological findings of the pancreas. Recent studies have shown that *H. umbellata* seed crude extracts exhibited anti-hyperglycemic and anti-diabetic effects in alloxan- and streptozotocin-induced diabetes in rats, respectively (Nwaogwugwu et al., 2022; Fadahunsi et al., 2021; Bature et al., 2023), which is in line with the present observation. The upregulation of glucose transporters (GLUT1 and GLUT4) recorded in this study was an indication that HUSCA could have mediated its hypoglycemic effect via increased peripheral glucose uptake via the transporters (GLUT1), since alloxan selectively destroys the insulin-producing pancreatic β-cells, causing insulin deficiency. GLUT1 is typically expressed at a constant level and does not undergo significant regulation in response to changes in blood glucose levels or insulin secretion, while GLUT4 plays a critical role in insulin-mediated glucose uptake into tissues, primarily muscle tissues (Adeneye et al., 2014). Nevertheless, the observed hypoglycemic effect of the metformin standard drug may indicate that there is some residual beta cell function, thus not entirely excluding the possibility that stimulation of insulin secretion may contribute to the activity of the HUSCA diet.

Hyperglycemia has been reported to increase oxidative stress during diabetes, which is associated with a high risk of diabetic complications (Fiorentino et al., 2013; Jiang et al., 2020; Bhatti et al., 2022; Binjawhar et al., 2023). Oxidative stress is caused by an imbalance between antioxidants and reactive oxygen species, which increases the active radicals, thus decreasing the efficient functioning of the body's immune system. In the present study, oxidative stress was observed in the diseased group (without treatment), evidenced by the increased level of MDA and decreased level of antioxidant enzymes (such as superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT)) activities compared with that of the negative control. The declined antioxidant enzyme activities are responsible for the increased lipid peroxidation, which could result in loss of cell function. Diabetic animals fed with the HUSCA diet for 21 days showed improvement in antioxidant parameters and nearly restored them to their normal levels compared with the untreated group. Glutathione is an antioxidant compound that protects the cell by reducing the level of free radicals, while superoxide dismutase (SOD) is an enzyme that also exhibits an antioxidant role. Therefore, by lowering the level of these enzymes during diabetes, the body cells are more likely to be damaged by active radicals. Studies have shown that phenolic glycosides, which are widely distributed in *H. umbellata*, have a well-characterized antioxidant property both in vivo and in vitro (Wei et al., 2023), which can significantly improve glucose-induced oxidative stress. In addition to phenolic compounds, bioactive peptides derived from fermented foods have been shown to possess antioxidant and antidiabetic properties (Sanjukta and Rai, 2016). These peptides are inactive fragments of proteins that become active through in vitro hydrolysis or microbial fermentation. Although the exact mechanisms of their antioxidant effects are not fully understood, it is suggested that aromatic amino acid residues in their side chains help stabilize electron-deficient radicals by donating protons, thus acting as radical scavengers (Sanjukta and Rai, 2016). Therefore, it is reasonable to attribute this antioxidant effect of HUSCA to the active constituents present in *H. umbellata* seed, including the production of bioactive peptides during fermentation, that have a scavenging effect on the free radicals. The lack of significant effect of HUSCA on superoxide dismutase (SOD) activity in treated animals compared to controls may be due to insufficient dosage or duration of study, which could prevent the elicitation of a measurable response. Additionally, HUSCA may not have sufficiently targeted the pathways that stimulate SOD expression or activity, limiting its potential effects. Furthermore, compensatory mechanisms involving other antioxidants could have mitigated the need for a substantial change in SOD, resulting in stable activity despite the treatment. Further studies are needed to explore this in greater depth. Meanwhile, the slight increase in SOD observed (albeit $P > 0.05$) is still beneficial because even slight enhancements in antioxidant enzyme activity can improve the organism's ability to manage oxidative stress, thereby reducing cellular damage. This increase can contribute to overall better health outcomes by supporting cellular functions and potentially enhancing resilience against complications linked to oxidative stress in diabetic conditions.

Conclusion

Conclusively, this study has demonstrated that supplementing cassava with *H. umbellata* seeds and fermentation significantly enhances protein quality, presenting an effective strategy for mitigating protein malnutrition in developing countries. Additionally, the results indicate that fermented *H. umbellata* seed-cassava combination may help reduce the risks of hyperglycemia and protect against glucose-induced oxidative stress and pancreatic damage by modulating glucose metabolism. Thus, integrating fermented *H. umbellata* seeds into dietary practices could serve as a viable approach to managing diabetes-related complications. Further research is needed to identify the active components responsible for these effects and to elucidate their mechanisms of action. Moreover, additional studies are warranted to assess the toxicity and long-term safety of incorporating *H. umbellata* seeds into human food formulations.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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