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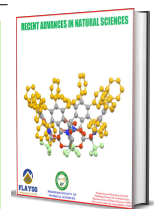

Fermented *Moringa oleifera* seed-cassava inclusion improves protein and selected biochemical indices in alloxan-induced diabetes in animal model

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ABSTRACT

Despite available diabetes medications, rising complications suggest the need for complementary approaches. This study evaluated the nutraceutical properties of fermented *Moringa oleifera* seed-Cassava (FMOC) inclusion on selected biochemical parameters in diabetic rats. Cassava was replaced with moringa seed at 80:20 (unfermented:T1), 80:20 (fermented:T2) and 70:30% (fermented:T3). Fermentation was achieved using a starter culture for 36 h. 36 male albino rats were randomly divided into six groups. Groups B-F were rendered diabetic using alloxan (150 mg/kg. bw; IP) while group A served as the normal control. The effects of FMOC on proximate and selected biochemical indices were evaluated. Results showed protein increased ($p<0.05$) (2.43±0.1 to 20.44 ± 02 g/100g) with fermentation compared to control. All test diets (especially T2) lowered ($P<0.05$) the elevated serum glucose level (249.0-105.6 mg/dl), Cholesterol (6.73 to 4.13mmol/L), TAG (2.43 to 1.99mmo/L), LDL (3.72 to 2.66mmol/L), Urea (35.3 to 23.2mmol/L) and MDA (2.34 to 1.29mg/dl) compared with diabetic group. An improvement in ($P<0.05$) glutathione (2.69-3.76mg/dl) and catalase activity (1.52-2.90 U/mg) was recorded. No significant effect was recorded for HDL ($P>0.05$). Histological outcomes of pancreas supported these findings. Incorporating fermented *M. oleifera* seed in food-based approach may effectively manage diabetes-related complications.

1. INTRODUCTION

Prevention and management of diabetes mellitus (DM) is one of the public health priorities across the world, due to the rising prevalence and burden of the disease. According to recent reports, over 529 million people suffered from diabetes in 2023, which is expected to double to 1.31 bilion by 2050 [1, 2]. During DM, glucose homeostasis is lost due to dysfunction of β-cells or impaired insulin function which results in various metabolic derangements. This includes an increased hepatic glucose production (hyperglycemia) via glycogenolysis and gluconeogenesis, oxidative stress, impaired lipid and protein metabolism which involves increased proteolysis and muscle loss [3–5]. These multiple metabolic derangements are the leading causes of long-term undesirable complications seen in diabetes such as nephropathy,

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neuropathy, retinopathy, cognitive impairment [6, 7], decreased quality of life and mental health [8]. There is a greater risk of developing dyslipidemia, atherosclerosis, hypertension, and other cardiovascular diseases in patients with DM than in other individuals without DM.

Despite the availability of many pharmacological interventions, including oral hypoglycemic agents and insulin therapy for diabetes management, current evidence shows an alarming rising trend in the occurrence of many undesirable complications among diabetic patients [3, 9]. The ineffectiveness of the current medical treatments for diabetes and associated side effects [10], confirm that other complementary and unconventional approaches are required. 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 [11–13]. Copious shreds of evidence have shown that functional foods may play a vital role in the management of DM in both animal and human experiments [14–17]. Hence, food-base approach utilizing functional foods and nutraceuticals may be a missing step in enhancing the dietary management of diabetes complications.

Medicinal plants have posed as natural sources of bioactive compounds with nutraceutical properties. *Moringa oleifera* (family: *Moringacea*) is an edible tropical plant that is widely cultivated in Africa and other parts of the world. It has gained a lot of interest for its diverse biological properties. Different parts *M. oleifera* (leave, stem, root, pod and seed) have been traditionally used in traditional medicine to treat diseases such as diarrhea, paralysis, sores, hyperglycemia, skin infections, and fever. Several scientific reports have also shown that different extracts of *M. oleifera* seed possess different pharmacological potentials such as anti-inflammatory [18, 19], immune-regulation effects [20, 21], anti-tumor and cancer properties [22, 23], in-vivo and in-vitro antioxidant properties [24–26], cholesterol-lowering effects [27–29] and anti-diabetic properties [30–32]. A more recent study by Oyeleye *et al.* [33] revealed that *M. oleifera* seeds modulated the activities of enzymes linked to hypertension and lipid metabolites in high-fat-fed rats. These pharmacological properties were attributed to the bioactive compounds present in moringa seed. *M. oleifera* seed contains three classes of bioactive constituents that is; flavonoids (e.g quercetin and kaempferol), phenolic compounds (such as niaziri and chlorogenic acid) and glucosinolates (e.g isothiocyanates) among others [18, 30].

Moringa oleifera is also a nutritious plant and almost all parts of the plant are edible [34]. Among the several nutrients found in *M. oleifera* seed, proteins are the most abundant (10.74-51.8 g/100g) [35, 36], accounting for more than 25% of dry weight. At least, 7 essential amino acids have been identified including *methionine and lysine* [37], which are lacking in soybean and maize respectively. The total content of essential amino acids (EAA) in Moringa seed (0.824g/100g) accounts for 25.65% of the total amino acid content (3.22g/100g) [37], which is close to 28.8 % of EAA in peanuts [38]. Furthermore, *M. oleifera* seed is an excellent source of essential minerals (such as iron, zinc and calcium) and vitamins (beta-carotene vitamins E, C, B1) were identified [39]. Lipids are also abundant in the seeds, representing about 30% of dry weight, among which α-linolenic acid (44.57%) and oleic acid (75-77%) are the principal fatty acids [40]. Despite these nutritional advantages, the utilization of moringa seed in food processing is still very low. This may be attributed to presence of unwanted antinutritional factors and poor sensory properties of the seed.

Cassava (*Manihot esculenta*), belonging to the family *Euphoriaceae,* is the second most utilized staple food crops in many developing countries after maize. The major problem associated with cassava is low protein content [41] and the presence of antinutritional factors like cyanide and phytate [42, 43]. Fermentation is an inexpensive traditional food processing technology that utilizes microbial activity and has been found adequate for reducing anti-nutritional factors and improving nutrient modifications in food [44]. This involves several mechanisms such as the release of nutrients initially bound to antinutrients, de novo synthesis of vitamins, minerals, and proteins by microorganisms (especially *Lactic acid bacteria*), degradation of antinutrients and complex compounds and improved nutrient bioavailability through reduced antinutritional factors via enzyme activation (e.g., phytase, tannase) [43, 44]. Fermented foods offer several health benefits which include the reduction of blood cholesterol levels [45, 46], antihypertensive effect by inhibiting angiotensin-converting enzyme [46, 47], stimulating immunity through probiotics [48, 49], fighting carcinogenesis [50], and anti-diabetic properties [45, 51], as well as alleviating the symptoms of lactose intolerance [52, 53]. These therapeutic effect of fermented food are largely attributed to the production of bioactive compounds including bioactive peptides, during fermentation [47, 48].

Despite these advantages of *M. oleifera* seed, only a few studies bring an approach to its potential use in food applications as a functional food ingredient for potential management of metabolic disorders. This study was designed to evaluate the effect of fermented *Moringa oleifera* seed-cassava (FMOC) inclusion on fasting blood glucose, oxidative stress, lipid metabolism, and renal function analytes of rats induced with diabetes. This approach could enhance the sufficient utilization of the plant to address nutrition problems, as well as add substantial health benefits.

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION

The matured seed pods of *Moringa oleifera* were collected from Isiukwuato community. The identification and authenticity of the plant seeds were done on the same day of purchase by an expert taxonomist at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. Freshly harvested cassava roots were collected from the National Root Crops Research Institute, Umudike, and transported to the laboratory for processing.

2.2. SAMPLE PROCESSING

M. oliefera seeds were manually removed from the seed pods and de-husked. The white kernels were oven-dried at 45 ^{o}C for 48 hours. The dried seeds were then milled into a fine powder, sieved through a 2mm sieve to get the fine powder, and then stored in an air-tight container. The cassava was thor-

Table 1. Proportion of composite formulation.

Portion	Label	Cassava Flour (%)	Moringa seed flour (%)	Treatment
A	MOS	0	100	*Raw moringa seed*
B	CF	100	0	Cassava flour
C	T1	80	20	Unfermented sample
D	T2	80	20	Fermented (36 h)
E	T3	70	30	Fermented (36h)

Note. Portion C (T1) was left unfermented. This is to control the effect of fermentation on other samples. MOS= moringa seed, CF= Cassava flour.

oughly washed with tap water to remove sand particles, peeled and the husks were removed and washed again before cutting into smaller pieces and dried at 50 ^{o}C to constant weight using a hot air oven. Dried samples were then milled into flour and sieved to produce fine powder which was preserved in an airtight container. All chemicals and reagents used in this study were of analytical grade.

2.3. COMPOSITE FLOUR FORMULATION

The milled moringa seed and cassava were thoroughly mixed together to make 100% at different ratio (Table 1), this is to enable the evaluation of the dose-dependent effect of Moringa seed inclusion on each formulation. The choice of ratios between cassava and *M. oleifera* in the fermentation process was based on their nutrient profiles, in order to achieve desired fermentation outcomes while maximizing nutritional benefits.

2.4. FERMENTATION PROCESS

Fermentation of the composite flour of cassava and *M. oleifera* seed was done for 36 hours following the method described by Anyiam *et al.* [43] with little modifications. Portion C was not fermented to monitor the effect of fermentation on the nutraceutical properties of other treatments. The fermented gruels were dried and manually pelletized into rat feed. Salt (1.5%) was added during pelleting to mask the mildly bitter taste of *Moringa* seed in the feed. The pelletized rat feeds were oven-dried at 45 ^{o}C and used as the experimental diet. Standard feed was used as a control diet.

2.5. PROXIMATE EVALUATION

All analyses for proximate compositions in control and Moringa seed-cassava inclusion were carried out in triplicates by the method described by AOAC [54]. The Kjeldahl method was used for protein determination in both standard and test diets, dry ashing was employed for ash determination, soxhlet extraction method with petroleum ether for fat determination, hot air oven method for moisture, and acid hydrolysis for crude fiber. Carbohydrate content was determined by difference (Eq. (1)), while the Atwater conversion factor was used in estimating the energy value of the fermented foods by multiplying carbohydrate, protein, and fat contents by 4, 4 and 9 respectively (Eq. (2)).

$$Carbohydrates = 100\% - (\%Protein + \%moisture + \%fat + \%ash + \%Fiber) \quad (1)$$

$$\text{Total Energy value}(Kcal) = [(4.0\ x\ \%Protein) + (9.0x\ \%Fat) + (9\ x\ \%Carbohydrate)] \quad (2)$$

2.6. EXPERIMENTAL STUDY

All animal experiments were carried out under the protocols for animal experiments at the College of Natural Science, the Michael Okpara University of Agriculture, Umudike, **while adhering to the RANS ethics policy for research involving human participants and animals.** Thirty-six (36) 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 12h 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*.

2.7. INDUCTION OF DIABETES

A freshly prepared solution of alloxan (250 mg dissolved in 40 ml of freshly prepared Sodium Citrate buffer 0.1M, pH 4.5) was injected intraperitoneally into the experimental rats at a dosage of 150 mg/kg body weight at 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 as being diabetic and were used for the study [55]. The study commenced a week after the alloxan injection. The blood glucose levels of all experimental rats were checked every week throughout the study.

2.8. EXPERIMENTAL DESIGN

A complete randomized experimental design comprising six treatment groups (with 6 animals per group), replicated thrice was used for the study. Diabetic rats with stable diabetic conditions were divided into 5 subgroups (groups B to F) while the non-diabetic rats formed the first group (i.e, normal control). The groups were as follows (Table 2): Group A was the normal control (without diabetes) which received control feed (devoid of *Moringa seed*). Group B was diabetic animals without treatment (Positive group). Group C: Diabetic and received 5mg/kg. b.wt of metformin (Drug control); Group D: Diabetic, fed unfermented moringa seed-cassava feed (80:20%; T1), Group E: Diabetic, fed fermented *Moringa* seed-cassava (80:20% T2) and Group F was diabetic rats fed fermented *Moringa* seed-cassava feed (70:30%; T3). The rats were administered the prepared diet twice daily. The experiment lasted for 21 days.

Table 2. Experimental design and animal grouping.

Group	Label	Status	Treatment
A	Normal control	No diabetes	Received the standard feed and *water ad libitum*
B	Positive control	Diabetic control	Diabetic control, not treated.
C	Drug control	Diabetic, received drug	Received Standard drug (Metformin; 5mg/kg bwt)
D	Test group 1	Diabetes, Test group 1	Received Unfermented *Moringa seed*-cassava (80:20%)
E	Test group 2	Diabetes, Test group 2	Received Fermented *Moringa seed*-cassava diet (80:20)
F	Test group 3	Diabetic, Test group 3	Received fermented *Moringa* seed-cassava diet (70:30%)

Table 3. Proximate composition and energy value of *Moringa oleifera* seed-cassava cake.

Sample	Moisture(g/100g)	Protein(g/100g)	Ash(g/100g)	Fiber(g/100g)	Fat (g/100g)	CHO(g/100g)	Energy value (Kcal/100g)
MOS	8.24 ± 1.5^{a}	31.43 ± 1.8^{a}	5.11 ± 0.89^{a}	9.33 ± 1.01^{a}	24.81 ± 2.5^{a}	21.62 ± 5.3^{c}	430.54 ± 8.6^{a}
CF	9.50 ± 6.6^{a}	2.43 ± 0.4^{e}	1.28 ± 0.23^{d}	2.09 ± 0.17^{c}	1.79 ± 0.5^{d}	81.01 ± 3.7^{a}	357.43 ± 4.3^{c}
T1	10.94 ± 1.1^{a}	11.37 ± 2.6^{d}	3.93 ± 0.35^{b}	4.87 ± 3.88^{b}	16.11 ± 2.9^{b}	58.31 ± 8.5^{b}	423.78 ± 5.5^{a}
T2	8.36 ± 1.3^{a}	17.44 ± 1.3^{c}	2.56 ± 0.52^{c}	4.92 ± 0.54^{b}	11.29 ± 1.7^{c}	52.42 ± 4.1^{b}	393.08 ± 3.1^{b}
T3	8.44 ± 1.9^{a}	20.44 ± 1.2^{b}	2.09 ± 0.32^{c}	2.26 ± 1.34^{c}	8.89 ± 0.5^{c}	42.4 ± 1.0^{b}	391.48 ± 3.4^{b}

Mean with different superscript abc are significantly different at ($P<0.05$) along the columns. MOS= *Moringa oleifera seed flour*, CF= control cassava flour T1=unfermented moringa-cassava (8:2), T2=fermented Moringa – cassava (8:2), T3=fermented Moringa-cassava diet at 70:30 ratio.

Table 4. Percentage increase in body weight of rats fed fermented *Moringa oleifera* seed-diet.

Group	Treatment	Initial wt (g)	Final wt (g)	% Weight gain	% Feed intake
A	Normal	92.01±8.01	230.0±7.21	48.70 ± 4.9^{a}	86.22 ± 5.20^{d}
B	Diabetes	122.33±1.53	160.33±13.77	23.46 ± 5.32^{d}	66.41 ± 7.16^{b}
C	Drug control	128.00±2.02	204.00±10.58	37.16 ± 2.66^{b}	72.12 ± 4.31^{c}
D	T1	117.67±1.53	160.36±8.50	26.47 ± 3.94^{cd}	62.08±4.01a
E	T2	108.0±2.3	165.33±9.07	34.58 ± 2.57^{b}	65.53 ± 3.12^{b}
F	T3	105.33±7.02	154.33±13.27	31.64 ± 2.62^{bc}	64.11 ± 6.03^{ab}

Mean with different superscript (abc) are significantly different at ($P<0.05$) down the columns. T1=unfermented 8:2, T2=fermented 8:2, T3= fermented 7:3 ratio.

2.9. DETERMINATION OF DAILY FEED INTAKE AND BODY WEIGHT GAIN

The daily feed intake was determined by weighing the remnants of the feed and subtracting it from the amount of feed (in grams) given to the rats daily. The percentage feed intake was then calculated using Eq. (3). For body weight, the rats were weighed using digital compact scale (Model: ML204-01 Switz). The initial weights of rats were taken and their weights monitored every week. Body weight gain was determined using the formula (Eq. (4))

$$\%Feed\ intake = \frac{Feed\ administered - Residue}{Feed\ administered} X\ 100 \quad (3)$$

$$\%Body\ weight\ gain = \frac{W2 - W1}{W2}\ x\ 100, \quad (4)$$

where W_2= final weight, W_1 = initial weight.

2.10. BLOOD SAMPLING AND SERUM PREPARATION

At the end of the 21-day feeding experiment, the animals were fasted overnight, and the final weights of the rats were taken. Each rat was anesthetized with dichloromethane vapour. Blood samples were quickly collected by cardiac puncture for biochemical analysis. The blood was allowed to stand for 30 min to clot and subsequently centrifuged at 3000 × g for 10 min at 4 ^{o}C to obtain the serum [55]. Each rat's carcass was promptly dissected, and the pancreas tissues were collected and rinsed in saline buffer for histological examination.

2.11. BIOCHEMICAL ASSAY

Determination of fasting blood glucose

Fasting blood glucose was tested after overnight fasting using 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 glucose dehydrogenase enzyme (on the test strip) resulting in a colour change. The intensity of this colour gives the blood glucose concentrations (mg/dl) as converted by the glucometer.

Lipid Profile Assay

Determination of serum total cholesterol (TC) and high-density lipoprotein (HDL) were carried out by following the enzymatic-colorimetric method of Allain *et al.* [56]. The triacylglycerol (TAG) concentration was determined using the method described in Albers *et al.* [57]. Also, the low-density lipoprotein (LDL) concentration was evaluated using the method of Friedelwald *et al.* [58]. From the values of lipid profile obtained, the positive and negative atherogenic risk predictor index were calculated as ratio of HDL to total serum cholesterol (HDL/TC), ratio of LDL to HDL (LDL/HDL) respectively [59].

Determination of Lipid Peroxide and Antioxidant enzymes activity

The activities of superoxide dismutase (SOD) and catalase were determined by the method of Kakkar *et al.* [60] and Aebi [61] respectively expressed in unit of enzyme/mg protein. Glutathione (GSH) level was determined according to the method of Ella-

Table 5. FBG levels (mg/dl) of diabetic rats fed fermented *M. oleifera* seed-improved diet.

Group	Pre-induction	After-induction	Week 1	Week 2	Week 3
A	85.00± 7.91	NA	84.00± 3.39	82.00± 7.21	80.20± 1.58^{e}
B	87.00 ±10.36	325.20±97.66	324.60±130.43	288.20± 47.72	239.0±44.4^{a}
C	84.00± 8.3	336.40±70.36	254.20± 42.43	143.20± 46.64	92.20± 6.64^{d}
D	76.60± 6.84	333.00±108.0	339.40± 62.12	231.20 ±40.81	201.00±18.2^{b}
E	75.40±12.42	359.40±136.6	318.8 ±57.91	215.20± 34.75	188.0±37.3^{c}
F	86.80±13.66	307.60±76.42	301.60± 58.69	184.60 ±63.34	105.60±22.8^{f}

Means with different superscripts along each column are significantly different at P< 0.05. NI=No induction.

Table 6. Lipid profile of normal and diabetic rats fed fermented moringa seed-cassava cake.

Group	TC (mmol/L)	TAG (mmol/L)	LDL(mmol/L)	HDL (mmol/L)	HDL/TC	LDL/HDL
A	4.56±0.15^{d}	2.03±0.21^{ab}	1.99±0.22^{c}	2.83±0.64^{b}	0.62±0.01^{a}	0.70±0.22c
B	6.73±0.25^{a}	2.43±0.15^{a}	3.72±0.84 ^{a}	1.90±0.10^{a}	0.28±0.02^{c}	1.96±0.49^{a}
C	5.02±0.26^{c}	2.06±0.15^{ab}	2.45±0.27^{c}	2.80±0.60^{b}	0.54±0.13^{ab}	0.92±0.33^{bc}
D	6.53±0.40^{a}	2.13±0.15^{b}	2.66±0.31^{bc}	2.23±0.55^{a}	0.34±0.09^{b}	1.22±0.23^{bc}
E	5.50±1.90^{bc}	1.99±0.17^{b}	3.08±0.45^{ab}	2.03±0.66^{a}	0.37±0.12^{bc}	1.64±0.58^{ab}
F	5.13±0.40^{b}	2.06±0.05^{ab}	2.87±0.52^{ab}	2.20±0.72^{a}	0.36±0.10^{bc}	1.39±0.47^{bc}

Means with different superscripts along each column are significantly different at P< 0.05. NA=No induction. TC= Total cholesterol, TAG =Triglycerides, LDL = Low density lipoproteins, HDL=high density lipoproteins.

Table 7. Antioxidant effect of fermented *Moringa oleifera* seed-cassava on diabetic rats.

Group	MDA (mg/dl)	SOD (U/mg)	CAT (U/mg)	GSH (mg/dl)
A	1.41±0.31^{c}	11.49±0.06a	2.32±1.06^{a}	3.58±0.93^{ab}
B	2.34±0.14^{a}	11.29±0.05^{a}	1.52±0.19^{ab}	2.69±0.26^{bc}
C	1.86±0.17^{b}	11.25±0.02^{a}	1.44±0.66^{ab}	1.08±0.60^{a}
D	1.44±0.26^{c}	11.40±0.08a	1.65±0.29^{ab}	2.87±0.71b^{c}
E	1.29±0.12^{d}	11.68±0.08a	1.90±0.15^{b}	3.76±0.57b^{c}
F	1.33±0.11^{dc}	11.46±0.11a	1.63±0.58^{c}	2.49±0.28^{c}

Mean with different superscripts along each column are significantly different at P< 0.05. MDA=Malondialdehyde. SOD=superoxide dismutase, CAT=catalase, GSH=glutathione, VIT.E=Vitamin E.

man [62], while lipid peroxide was estimated by measuring malondialdehyde (MDA) spectrophotometrically at 535nm and expressed as mg/dl following the method described by Ohkawa *et al*. [63].

2.12. DETERMINATION OF RENAL FUNCTION BIOMARKERS AND ELECTROLYTES

The method of Fawcett and Scott [64] was used to determine the urea concentration with the Randox commercial kit following the manufacturer's instruction. This method is based on the principle that urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically at 546 nm. Likewise, creatinine concentration was measured by following the method described by Henry *et al*. [65]. This method was based on the principle that creatinine reacts with picric acid in alkaline conditions to form a colour complex which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine concentration in the sample. Serum electrolytes (sodium, potassium, chloride and bicarbonate) were quantified by following the methods described in Onyeabor *et al*. [66].

Histopathological Examination

Pancreatic tissues were collected after animal sacrifice, fixed in 10% formalin, processed routinely, and embedded in paraffin. 5 μm thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation [67]. The fixed slides were viewed under light microscope and photomicrographs were captured (400x). Photomicrographs were taken with a computer having a Microscopic Analysis Software (Scopelmage-9.O) connected to an Olympus digital light microscope (Olympus UK Ltd. Essex, UK).

Statistical analysis

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

Table 8. Effect of fermented *M. oleifera* seed-cassava on Serum electrolytes of diabetic rats.

Group	Treatment	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	HCO^3 (mmol/L)
A	Normal	127.33 ± 9.1^{cd}	4.60 ± 0.17^{a}	95.66 ± 0.57^{a}	27.33 ± 0.58^{ab}
B	Diabetes	124.0 ± 2.0^{d}	4.46 ± 0.76^{a}	94.0 ± 1.02^{b}	26.67 ± 0.57^{ab}
C	Drug	116.33 ± 3.0^{e}	4.73 ± 0.23^{a}	94.33 ± 0.57^{ab}	27.66 ± 5.5^{b}
D	T1	142.33 ± 7.5^{a}	4.03 ± 0.30^{a}	94.66 ± 1.15^{ab}	27.66 ± 0.57^{a}
E	T2	128.0 ± 8.0^{c}	4.40 ± 0.36^{a}	95.33 ± 0.57^{ab}	26.5 ± 7.81^{b}
F	T3	136.6 ± 9.1^{b}	4.46 ± 0.47^{a}	95.33 ± 0.57^{ab}	27.33 ± 0.57^{ab}

Mean with different superscripts along each column are significantly different at P< 0.05.

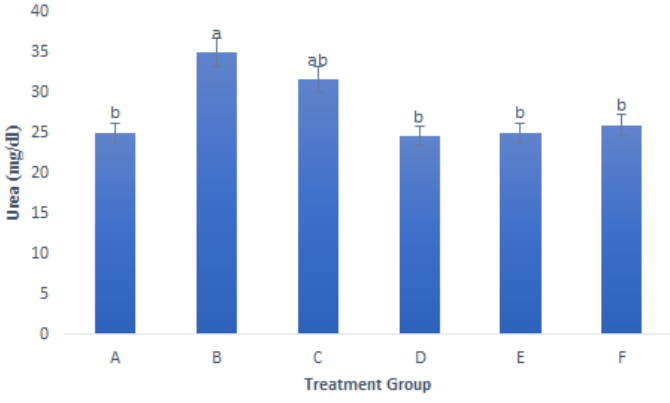

Figure 1. Urea (mg/dl) concentration of diabetic rats treated with FMOC.

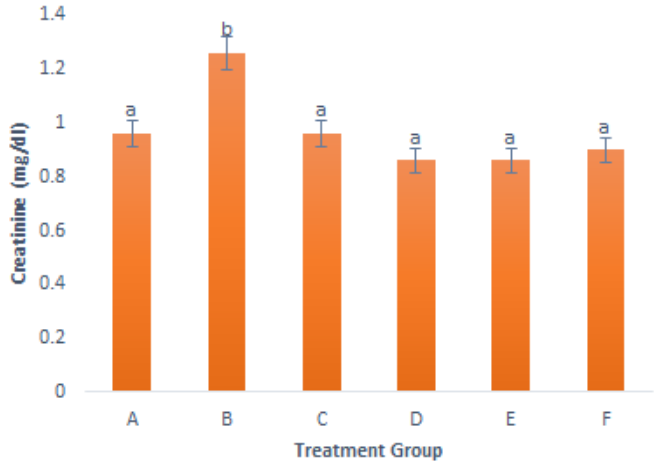

Figure 2. Creatinine (mg/dl) conc. of diabetic rats treated with FMOC.

3. RESULT

3.1. PROXIMATE AND ENERGY VALUE

The result on the proximate composition (Table 3) revealed that *Moringa olefiera* seed contains considerably amount of protein (31.43 g/100g), fiber (9.33 g/100g), ash (5.11 g/100g) and fat (24.81g/100g dwt). Inclusion of *Moringa oleifera* seed (20%) to cassava significantly (P<0.05) improved the protein content of the cake from 2.43 to 11.37 g/100g and 20.44 g/100g before and after fermentation respectively. The highest increase in protein content (20.44g/100g) was noted following addition of 30% of *Moringa* seed followed by fermentation for 36 hours. Fermentation improved the protein content and significantly decreased (P<0.05) the ash, fiber and carbohydrate content compared with the control sample (devoid of *Moringa* seed). There was gradual decrease in moisture content (albeit P>0.05) with fermentation. *Moringa oleifera* seed showed the highest energy value (430.54 kcal/100g) compared to other samples. A reduction in energy value was recorded (391.48-393.08 Kcal/100g) after the fermentation process.

3.2. BODY WEIGHT GAIN AND FEED INTAKE MEASUREMENT

The highest percentage gain in body weight (48.70%) was seen in the normal control group without diabetes (Table 4), while the lowest gain in body weight (23.46%) was recorded in diabetic control group which was not treated. Administration of fermented Moringa 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. Fermented moringa seed-cassava (80:20) resulted in better improvement in body weight gain. Feed intake consumption rate did not differ significantly (P>0.05) across the experimental groups (T_1-T_3). However, the normal and drug control groups consumed more feed (P<0.05) than the experimental groups (Figure 1).

3.3. RESULT FOR BIOCHEMICAL ASSAY

3.3.1. Effects of FMOC on fasting blood glucose (FBG)

Table 5 shows that groups administered fermented *Moringa oleifera* seed-cassava (FMOC) feed (T_2 and T_3) were able to reverse their elevated blood glucose to near normal at the end of the experiment (WK3). The animals fed with unfermented *M. oleifera* seed-cassava (20%) (T_1) were almost still diabetic (195mg/dl) at the end of the 21 days of feeding. Though a slight reduction (P>0.05) in fasting blood sugar was seen in this group, however, the effect was still above 200 mg/dl, which was the benchmark for diabetic condition in the animals.

3.4. EFFECT OF FMOC ON LIPID PROFILE AND ATHEROGENIC RISK PREDICTOR INDEX

The lipid profile of diabetic rats fed FMOC was shown in Table 6. A significant reduction (P<0.05) in total cholesterol (TC), triaglycerides (TAG) and low-density lipoprotein (LDL) was recorded in diabetic rats fed with fermented moringa seed-cassava cake at 20 and 30% inclusions respectively, when compared with the diseased group. Fermented cake at 30% inclusion was more effective in lipid profile improvement than the unfermented seed and other samples. No significant effect (P>0.05) was observed on high density lipoprotein (HDL) across all samples. In terms of atherogenic risk predictor index, a significant

improvement in good (HDL/TC) and a reduction in bad atherogenic index (LDL/HDL) was recorded following the consumption of fermented FMOC for 21 days, when compared with the diabetic group.

3.5. EFFECT OF FMOC ON OXIDATIVE STRESS MARKERS

Table 7 represents the antioxidant effect of unfermented and fermented Moringa seed-cassava consumption on alloxan induced diabetes in rats. An increase in superoxide dismutase (SOD) (albeit P>0.05), catalase activity and glutathione (GSH) was recorded in tests groups (T_1-T_3) compared with the diabetic group. On the contrary, intake of fermented *M. oleifera* seed-cassava significantly reduced (P<0.05) the level of malondialdehyde (MDA) which was increased in diabetic group. No significant effect (P>0.05) was recorded for vitamin E in all the treated groups compared with the disease and normal control.

3.6. EFFECT OF FERMENTED *MORINGA* SEED-CASSAVA ON KIDNEY FUNCTION ANALYTES

The effect of fermented and unfermented *M. oleifera* seed- cassava on serum electrolytes is presented in Table 8. An increase (P<0.05) in sodium concentration was recorded in diabetic rats treated with fermented *M. oleifera* seed-cassava diet compared with the diabetes and normal control. However, no significant effect (P>0.05) was recorded on potassium, chlorine and bicarbonate in all the test groups when compared with control. An improvement in bicarbonate values was observed in rats fed with fermented moringa seed-cassava (80:20) when compared with the control.

In Figure 1, the diabetic group of animals recorded the highest value of urea compared with the normal control. Following the intake of diet containing fermented *M. oleifera* seed in cassava, a significant reduction (P<0.05) in urea concentration was noted. There was no difference between the urea concentrations among the rats in the treated groups (T_1-T_3). Similarly, the increased level of creatinine recorded in diabetic control group of rats was significantly reduced (P<0.05) by the intake of fermented *Moringa* seed- cassava diet after 21 days when compared with the disease and normal control (Figure 2). Again, no significant difference (P>0.05) was recorded among the test samples (T_1-T_3).

3.7. HISTOLOGY OF THE PANCREAS

The photomicrographs of pancreas of experimental animals fed with FMOC pellet is presented in Figure 3. Plate 1 represents pancreas of normal control rat showing normal islets of Langerhans interspersed among the acini as compact spherical mass (Arrow) without necrosis. Plate 2: represents pancreas section of diabeteic control group. Beta cells were partially destroyed resulting to necrosis and vacuolations of pancreatic acini and shrunken Langerhans islets cells (arrow). Plate 3: Drug control group; showing partial restoration of pancreatic histology. Beta cells of the pancreas regenerated (arrow), there was no necrosis and vacuolations of pancreatic acini and Langerhans islets cells. Plate 4: T_1 fed group; Beta cells had necrosis and vacuolations of pancreatic acini and Langerhans islets cells. Result showed that T_1 feed was unable to restore or regenerate the β-cells of the pancreas. Plate 5: T2 fed group, showing shrunken islets of Langerhans, displaying degenerative and necrotic changes. Result showed minor effects of T2 on the Pancreas. Plate 6: experimental animal administered T3 diet. Treated diabetic rat showed improvement of histoarchitecture, reduction in vacuolization concomitantly with an increase in size of islets. The islet beta cells of the pancreas have nearly been restored showing no necrosis and vacuolations of pancreatic acini and Langerhans islets cell.

4. DISCUSSION

Providing information on the nutraceutical properties of underutilized medicinal plants, such as *Moringa oleifera* seed, could enhance their utilization as functional food ingredients. Proximate composition of food is an important criterion in the food industry for evaluating the nutritional quality of food source [68]. From the result obtained on the proximate assay, a reduction in moisture content was observed at fermentation (10.94-8.36%). The highest moisture content was observed in T_1 diet (10.94%). Decrease in moisture content could be due to increase in dry matter during fermentation as a result of proliferation of microbial cells. Previous studies have also shown that moisture content of food decreases during fermentation [69–71]. However, contradicting results were reported by Anyiam *et al.* [43], Ojokoh *et al.* [72] and Adejuwon *et al.* [73], who reported an increase in moisture content during fermentation of cassava, millet and sorghum-soybean-sweet potato respectively. The difference could be likely due to different experimental designs, fermentation durations and variations in raw material. Water content in excess of 14% in dry food has greater susceptibility to bacteria and fungi growth Kumolu-joh and Ndimele [74]. The lower moisture content obtained in this study (<14%) means extended shelf-life due to lower water activity, as this condition does not favor the proliferations of food spoilage microorganisms.

Adequate protein intakes supports proper growth and strong immunity especially in children. The crude protein of cassava increased (P<0.05) from 2.34 to 11.37% following the inclusion of fermented moringa seed at 20% before fermentation. This indicates that the moringa seed is a good source of protein as shown in Table 3 (31.43%) which is line with previous report [35–37]. Following fermentation for 36 hr, a 34.9 % increase in protein content was observed in FMOC. Previous studies have also reported that microbial fermentation enhanced the protein composition of various food materials [43, 44, 70, 73]. However, different study durations, designs, and raw materials yield mixed results. While most studies reported increased protein levels [43, 44], some showed decreases after fermentation. For example, the fermentation of Bambara nut into a *dawadawa* (a fermented condiment) increased the protein composition by approximately 18% [75]. This was attributed to the release of proteins which were initially bound to the anti-nutritional factors during fermentation process. Similarly, a study carried out by Jude-Ojei *et al.* [76] reported that the addition of fermented moringa seed flour to maize ogi, increased the protein content which was attributed to microbial mass proliferation and/or possible secretion of some proteinous extracellular enzymes during fermentation in order to obtain energy. A study by Anyiam *et al.* [43] found a 21.2% increase in protein composition in a fermented insect-based cassava beverage after 42 hours of fermentation. This increase is likely due to enzyme synthesis during carbohydrate breakdown, increased mi-

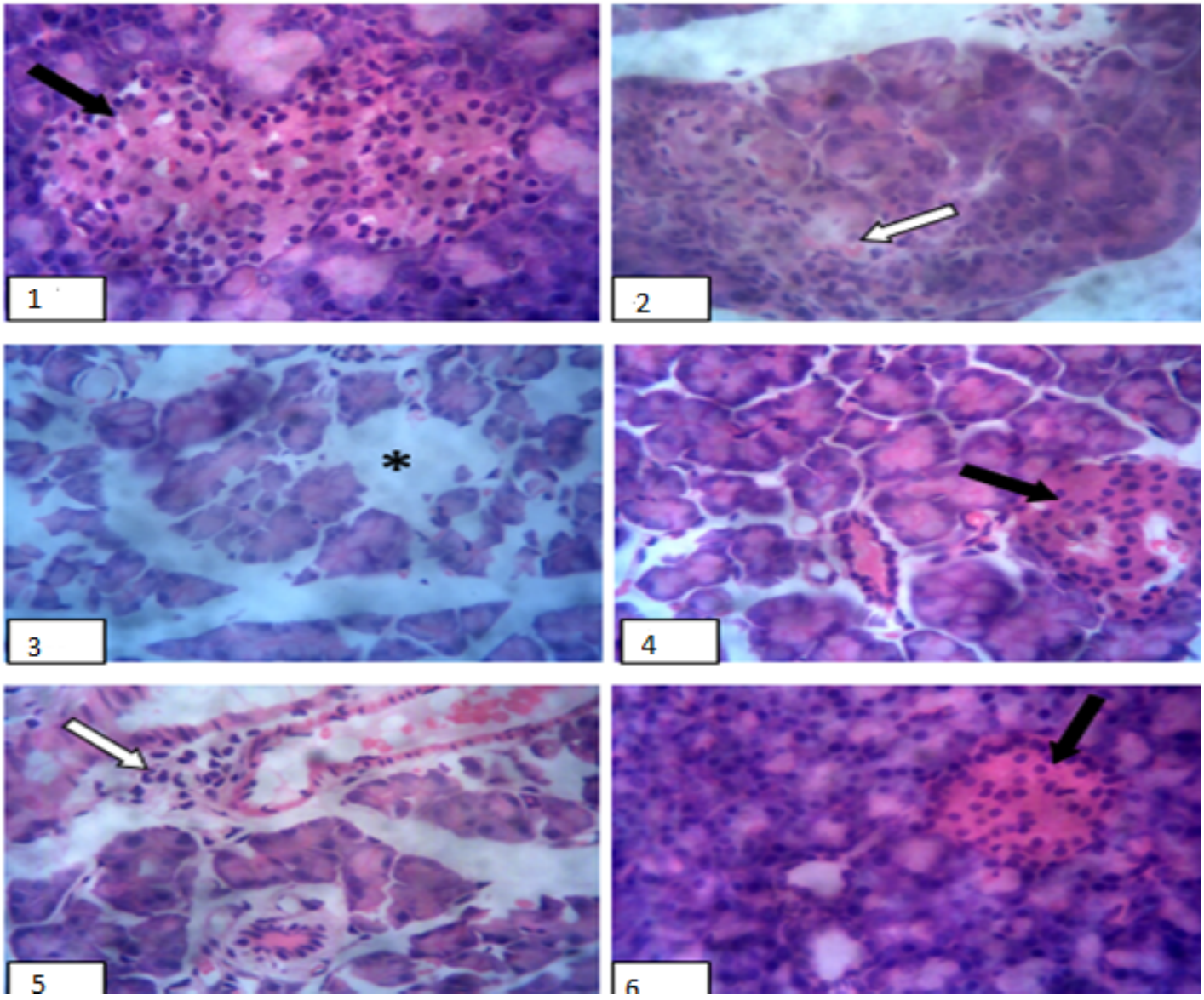

Figure 3. Photomicrograph of the pancreas histology.

crobial mass, degradation of storage proteins, and de-novo protein synthesis by fermenting microorganims. On the contrary to this study, few studies [77, 78], have observed that the fementation process decreases the protein contents of certain food. The authors linked this observation to the leaching of more soluble proteins and amino acids into the fermentation medium. When digestibility and limiting sulphur-containing amino acid factors and are not considered, consuming, 100 g (dw) of FMOC per day, has the potential to deliver about 52% of the total recommended daily protein requirement for school children (34 g/day) between the ages of 9–14 years old. However, it is important to remind that the proteins in plant-based materials are very different to animal-based in terms of amino acid compositions and digestibility, due to the presence of antinutritional factors which reduces protein bioavailability [43].

The low crude fat composition of cassava (1.79%) was improved (16.11%) following the inclusion of moringa seed. Fats from plant-based sources have been reported as leading sources of mono-and polyunsaturated fatty acids [79, 80], that play an important role in human health. During fermentation, a reduction ($p<0.05$) in fat content from 16.11 to 8.89 g/100g (44.81%) was observed compared with unfermented sample. This could be due to the activation of lipases from the fermenting microorganisms, which hydrolysed fat into fatty acid and glycerol (18-52) as a source of energy. Different authors [81–84] have all equally reported reductions in fat concentration of African bean, cowpea, African yam bean, common bean and Lima bean respectively during fermentation. Some of the authors attributed these reductions in fat to the metabolism of microorganisms in the fermentation medium, the breakdown of lipids by lipase, the use of lipids as the food source by fermenting organisms and the loss of total solids and fat-related components into the processing water during soaking. For instance, in a study conducted by Onwurafor *et al.* [85], fermenting mung bean seed using spontaneous and back-slopping methods for 72 h, 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 contents was also reported by Adebowale and Maliki [86] in fermented pigeon peas. However, contradicting findings was reported in fermented Chickpea (1.8% increase) [87] , fermented Bambara nut (2%) and fermented African yam bean [81]. This could be likely due to differences in sample type and fermentation time. The reduction in fat content in the present study is beneficial in terms of shelf-life elongation of the final product due to reduced chances of rancidity.

Fiber is a non-digestible carbohydrate which helps to delay gastric emptying and enhances the excretion of fecal cholesterol [88], thereby reducing the risk of risk of chronic diseases. From the result obtained, the inclusion of moringa seed flour improved the fiber content of cassava by 57.08%. Following fermentation, a reduction ($P<0.05$) in the fiber content was observed from 4.87 to 2.26%. This could be as a result of increased activity of hydrolyzing enzymes such as *cellulase* and *??-galactosidase* which hydrolyze the dietary fiber constituents as source of energy [46, 89]. Chinma et al. [82], Onoja and Obizoba [90] and Granito et al. [83] also reported the reduction in crude fiber content in African yam beans, lima beans and pigeon pean respectively during fermentation, which was also attributed to the use of cellulose and arabinose by the fermenting microorganisms that secrets cellulase, xylanase and hemicellulase during [44]. Additionally, a study on soybeans curd wastes using two types of yeast showed a decrease in fiber (7.4-4.6%), which was an indication of the secretion of cellulase and hemicellulose-degrading enzyme during fermentation [91]. Moderate level of fiber in complementary foods is helpful because it reduces bulkiness and encourages high digestibility and absorption of essential nutrients.

Ash content in food is regarded as a reflection of the total available minerals present in the food product [43]. In this present study, a reduction in ash content (3.93-2.09%) was observed following fermentation of moringa seed-cassava supplementation.This reduction in ash content could be due to the leaching of the minerals into the discarded fermentation water. Different studies have also shown a decrease in ash content during fermentation of food materilas. For instance, a study by Adebiyi *et al.* [75] and Granito *et al.* [83] 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. Contradicting results were reported for fermented mung beans [85] and tamarind seeds [92] after fermentation, showing increase in ash content. The authors attributed this increase in ash to the breakdown of complex-chelated compounds by the fermenting microorganisms leading to an improved synthesis of minerals, which also depends on the fermentation conditions employed. The carbohydrate level in FMOC reduced by 47.6% after 36 hours of fermentation in T_3 when compared with the unfermented control. This was due to the utilization of carbohydrate as an major energy source of the fermenting miroorganims [43, 87]. This result is consistent with the trends in literature [82, 93, 94]. According to a study by Olapunju *et al.* [92], fermenting microorganism are notable producers of enzymes such as maltase, α-amylase, glucosidase, fructofuranosidase and lactanase which could break down different components of carbohydrate, leading to their reduction.

On the contrary, an increase in carbohydrate levels of fermented cowpea (5%) [95] and fermented Bambara nut (3%) [96], were reported, which was linked to the conversion of resistant starches to available starches by activities of microbial enzymes, subsequently increasing the carbohydrate contents. The energy value of FMOC reduced by 7.6% during fermentation. Different studies have reported conflicting results in the energy content of food materials during fermentation. For example, a decrease in energy contents of fermented pigeon pea flour (3%) [86], and fermented African oil bean (26%) [81] was reported which supports the present study. However, an increase in the energy value of fermented pigeon pea flour (50.6–57.4 %) [97], and fermented lentil flour (15%) [98], were also reported. Whereas these studies did not describe the possible mechanisms of such modifications in the energy values from the seeds, Adebowale and Maliki [86] linked the decrease in energy value of fermented pigeon pea flour to the decrease in fat values and nitrogen-free extract of the samples during fermentation.

Accumulating evidence suggested that the health benefits associated with fermented foods are often attributed to the bioactive compounds (such as bioactive peptides) that are synthesized during fermentation [45, 51, 99]. A significant ($P<0.05$) reduction in blood glucose level was observed in diabetic rats fed with FMOC (at 70:30) when compared with the diabetic control. The study showed the ability of FMOC to reduce blood glucose level which was also confirmed by the histological findings of the pancreas (Figure 4). Previous studies have shown that moringa seed crude extracts exhibited anti-hyperglycemic and anti-diabetic effects in alloxan and streptozotocin-induced diabetes in rats, respectively [32, 100], which is in line with the present observation. Thus, it is reasonable to attribute this pharmacological activities of FMOC to the presence of bioactive compounds in moringa seed. For instance, Naizirin (a phenolic glycoside) and quercetin (bio-flavonoid) isolated from moringa seed, was found to improve glucose uptake and enhances insulin sensitivity through the activation of adenosinemonophosphate-activated protein kinase (AMPK)-signaling pathway in hepatic cells [101–103]. In another study, quercetin showed a high suppression of α-glucosidase and α-amylase (enyzmes responsible for release of glucose), stimulates insulin secretion by increasing Ca^{2+} influx [104, 105] and increases hepatic glucokinase thereby improving postprandial hyperglycemia and insulin sensitivity. Similarly, a study by Jaja-Chimedza *et al.* [106] showed that a dietary isothiocyanate-enriched Moringa seed supplementation improves glucose tolerance and modulates gut microbiome in diabetic mice models. Therefore, it could be possible that the anti-hyperglycemic properties of FMOC reported in this study followed similar mechanimsms which could be helpful in the management of diabetes complications.

The body weight changes serve as a sensitive indicator of the general health status of the experimental animals throughout the experiement. The result of the percentage body weight gain showed a reduction (20.9%) in percentage body weight gain in disease control rats when compared with the normal rats. Treatement with standard drug and FMOC diet 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. [66] and Pournaghi *et al*. [107] who reported a reduction in body weight in the presence of diabetes in rats. The lower percentage gain in body weight seen in disease group and FMOC fed group may be attributable to loss of appetite as a result of discomfort caused by alloxan-induced diabetes condition and/or due to the poor sensory quality of the treatment diet. Hypercholesteromia and hypertriglyceridemia have been reported to occur in diabetes condition through the elevation of free fatty acid flux [6, 8]. The significantly elevated serum TAG, Cholesterol and low-density lipoprotein (LDL-C) levels in the diabetes control group relative to the normal control showed that dyslipidemia might be implicated in the pathogenesis of diabetes, which aligns with these previous reports. This study showed a significant ($P<0.05$) decrease in LDL-C, triglycerides and total cholesterol in diabetic rats fed with FMOC for 3 weeks. However, there was no significant effect ($P>0.05$) in HDL in the test groups when compared with the diabetes group (without treatment). This is in agreement with the findings of Setyawati *et al.* [108], Aborhyem *et al.* [109] and Ajayi *et al*. [28] that reported significant reductions in LDL-C, triglycerides and cholesterol in rats treated with moringa seed extract. Elevated low-density-lipoprotein-cholesterol is a major risk factor for atherosclerosis and myocardial infarction, so reducing the level of LDL-C lowers the risk of these metabolic disorders. On the contrary, HDL-C is protective cholesterol and is responsible for transportation of unhealthy cholesterol from peripheral tissues to the liver for excretion and other tissues thereby preventing cardiovascular diseases [110]. The minor increase in HDL levels from FMOC ($P>0.05$) may be due to factors like the study duration. Fermentation might not substantially alter HDL-modifying compounds in FMOC. Nevertheless, even small increases in HDL are beneficial, as HDL helps remove excess cholesterol, improving cardiovascular health and potentially reducing heart disease risk. Over time, these small increases could lead to significant health benefits and a better lipid profile. This means that maintaining a healthy lipid profile through a food-based approach could play a critical role in managing diabetes progression and associated micro/macrovascular complications. Kobori *et al*. [110], reported that quercetin isolated from moringa seed, decreased expression of peroxisome proliferator-activated receptor and sterol regulatory element-binding protein in the liver of mice fed a western diet, resulting in decreased synthesis of triglycerides. In similar study, quercetin was found to reduce *de novo* synthesis of fatty acid and triglycerides synthesis and inhibit acetyl-CoA carboxylase activity in rat hepatocytes [111]. These were suggested as the possible mechanisms of action contributing to the hypolipidemic effect of FMOC in this study. Thus, FMOC can be assumed a potential hypolipidemic functional food agent, which could be helpful in preventing atherosclerosis and other complications associated with diabetes.

Normal cells produce low levels of reactive oxygen species (ROS) and free radicals for cell signaling and homeostasis, with intrinsic antioxidants managing these levels [47, 112]. Oxidative stress, is caused by an imbalance between antioxidants and reactive oxygen species which increases the active radicals thus decreasing the efficient functioning of body's immune system, leading to cell death and tissue damage. Hyperglycemia increases oxidative stress which is associated with a high risk of diabetic complications [3, 5]. Antioxidants combat excess free radicals by scavenging them directly and regulating oxidative enzyme activity. Given the toxicity of synthetic antioxidants, there is growing interest in natural antioxidants from food sources, which are safer and more compatible with tissues. In the present study, oxidative stress was observed in the diseased group (without treatment), evidenced by the decreased level of superoxide dismutase (SOD), Glutathione (GSH) and catalase (CAT) activities compared with that of the negative control (albeit $P>0.05$). The declined antioxidant enzymes activities is responsible for the increased lipid peroxidation which could result in loss of cell function [24]. This result is consistent with previous investigations on *Moringa oleifera* extracts [24, 25, 113]. 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 exhibit antioxidant role. Therefore, by lowering the level of these enzymes, the body cells are more likely to be damaged by active radicals. Diabetic animals fed with FMOC for 21 days showed improvement in these antioxidant enzymes and nearly restored them to their normal levels when compared with the normal control. The curative effect of FMOC observed in this study could be due to the bioactive constituents present in *M. oleifera* seed. For example, studies have shown that niazirin (a phenolic glycoside) widely distributed in moringa seed has a well-characterized antioxidant properties both in-vivo and in-vitro [114] and can significantly improve glucose-induced oxidative stress. These significant effects of niazirin were mediated by the reduction of gluconeogenesis and lipid accumulation, as well as by the improvement of glycolysis and lipid oxidation. During fermentation, proteins in the raw material are broken down by microbial enzymes into smaller peptides. In addition to phenolic compounds, bioactive peptides isolated from fermented foods have been reported to have antioxidant and antidiabetic properties [47, 112]. These peptides are protein fragments that are inactive in their precursor molecules but can be activated through in-vitro hydrolysis or microbial fermentation. Though the mechanisms of their antioxidant properties are not yet clear, it was suggested that aromatic amino acid residues found on the side chain groups contribute the radical scavenging properties by donating protons to electron-deficient radicals and stabilizing them [112]. Therefore, it is reasonable to attribute this antioxidant effect of FMOC to the active constituents present in moringa seed, including the production of bioactive peptides during fermentation, that have scavenging effect on the free radicals.

The renal function analytes in this study (i.e, urea, creatinine and sodium) were increased as a result of diabetic nephropathy which is considered a major complication [115]. This finding agrees with the earlier report of Xie *et al.* [116] that diabetes causes significant elevation of the serum urea concentration. Urea is a key metabolic product of biological pathways comprising of ammonia, which is a toxic waste product to the body. Therefore, transportation of urea plays a vital role in nitrogen removal and osmotic homeostasis. The elevation in urea and creatinine concentration caused by diabetes was significantly ($P<0.05$) lowered by the standard drug and FMOC diet, when compared to the normal control. This is in agreement with the report of Wen *et al*. [117] who reported a reduction in urea and creatinine levels in diabetes-induced rats treated with *Moringa*

oleifera seed crude extract. The ability of FMOC to reverse the alterations in the excretory function of the kidney, which was impaired by the diabetes condition, shows that the fermented diet seems to be effective in ameliorating renal impairment in diabetes.

Clinically, electrolytes such as sodium, chloride and bicarbonate ions are among the parameters that are useful in the determination of kidney function. For instance, bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered [66], thus any reduction in serum bicarbonates implies a reduction in blood pH. From the results obtained, no significant (P>0.05) variations in different electrolyte concentrations were observed among the groups except for serum sodium level which was observed to increase following the consumption of FMOC compared to the normal control. This may be due to the addition of 1.5% salt to the FMOC during pelleting to mask its slightly bitter taste for rat consumption. However, no alteration of bicarbonate, serum potassium and chloride concentration was recorded, indicating that the induction of diabetes using alloxan in this study and consumption of FMOC did not alter the pH haemostasis of the animals. Including fermented moringa seeds in the diet could effectively boost intake of beneficial compounds for long-term disease prevention, such as diabetes and cardiovascular diseases. This study highlights the potential health benefits of fermented moringa seed-cassava for diabetes in animal models, however, assessing its safety and long-term effects in humans is crucial.

5. CONCLUSION

In conclusion, the study has revealed that supplementing cassava food with moringa seed and fermentation improved the protein composition which could be considered an efficient means of reducing the menace of protein malnutrition in developing countries. On the other hand, the findings from this study showed that fermented *M. oleifera* seed-cassava supplementation has the potential of averting the risks of hyperlipidaemia, protecting the host against glucose-induced oxidative stress and renal damage by regulating the metabolism of glucose. Therefore, it seems that a food–based approach incorporating fermented *M. oleifera* seed could be considered an effective strategy to manage the complications of diabetes. Further study is necessary to identify the active components that mediated these effects and to understand the actual underlying mechanisms of action. Also, more new studies on the toxicity and safety of using Moringa seed directly in human food formulations need to be carried out.

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