

THE EFFECT OF FERMENTED AND UNFERMENTED *MORINGA OLEIFERA* SEEDS-CASSAVA SUPPLEMENTATION ON HAEMATOLOGICAL INDICES OF DIABETIC RATS

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ABSTRACT

This study aimed to determine the effect of fermented and unfermented *Moringa* seed supplementation on haematological indices of diabetic rats. Thirty-six male albino rats of the wistar strain weighing between 80-120 g were used for the study. Alloxan- induced diabetic rats with stable diabetic condition were divided into 5 subgroups (groups 2 to 6) with six animals per group while the non-diabetic rats formed the first group. The rats were administered the prepared diet twice daily for three consecutive weeks. Addition of *Moringa oleifera* seed flour, followed by fermentation, significantly ($P < 0.05$) improved the protein (20.44 ± 1.3 g/100g), fiber (2.26 ± 1.34 g/100g), ash (2.09 ± 0.32 g/100g) and fat content (8.89 ± 0.5 g/100g) of cassava *Mahewu*. Fermented *Moringa* seed-cassava *Mahewu* (80:20) resulted in improvement in body weight gain than other fermented samples. The hematological parameters of alloxan-induced diabetic rats fed with fermented and unfermented *Moringa oleifera* seed-cassava showed that white blood cells (WBCs) and packed cell volume (PCV), HB RBC and platelets increased significantly. The data obtained from this study suggest that *Moringa oleifera* seed-improved cassava diet possess medicinal effects on the hematological indices and can be used as nutritional supplement in diabetes. This was indicated by the effect of the extract on RBC, PCV, HB platelets and WBC of the albino rats.

Keywords: Diabetes, Cassava supplement, Haematological indices, *Moringa oleifera* seed

INTRODUCTION

The study of traditional medicine has evolved over the millennia of human existence. *Moringa oleifera* is a plant with numerous uses and adaptability. It contains nutrients, as well as secondary metabolites that have health benefits [1]. In many developing countries, *Moringa* is used as an ingredient in traditional medicine [2]. *Moringa* is a tropical plant that is rich in bioactive compounds. It has pharmacological activities such as anticancer, antidiabetic [3], anti-inflammatory and antioxidant [4]. The pharmacological properties of *Moringa* are adequately related to the presence of its bioactive compounds [5]. *In vitro* and *in vivo* studies confirmed various biological activities such as antioxidant, anti-inflammatory, antidiabetic, anticancer [6], cardioprotective [7], hypocholesterolaemia [8], hepatoprotective, antihypertensive and antibacterial. *M. oleifera* leaves were also found to contain substantial amounts of total phenol, protein, calcium, potassium, magnesium, iron, manganese and copper [9].

Diabetes mellitus (DM) is clinically referred to a large group of diseases resulting in hyperglycaemia [10] and related metabolic disorders elicited by overwhelming oxidative stress. The Type-1-DM is elicited by insulin insufficiency in plasma, whereas Type-2-DM is as a result of peripheral tissue resistance to insulin action [11].

More than 400 herbal plants have been shown to possess antidiabetic activities, suggesting their significance for treating and managing diabetes [3]. Herbal plant secondary metabolites, including alkaloids, polyphenols, flavonoids, saponins, tannins, and terpenoids, are responsible for the antihyperglycemic effect [5]. The reduction in glucose levels is mediated through different mechanisms, including restoring the function of pancreatic tissues by protecting the intact functional β -cells from further deterioration or regenerating destroyed β -cells, stimulating insulin secretion, inhibiting intestinal absorption of glucose, increasing insulin-induced signaling in various tissues, and decreasing oxidative stress [4].

Therefore, the present study was undertaken to perform an experimental validation of the antidiabetic roles of fermented and unfermented *M. oleifera* seed extract. The objectives of this investigation are: to study the effect of fermented and unfermented *Moringa* seed supplementation on haematological indices of diabetic rats; determine the fermented *Moringa* seed supplementation on haematological indices of diabetic rats and to determine the unfermented *Moringa* seed supplementation on haematological indices of diabetic rats.

EXPERIMENTAL

Sample collection

The matured seed pods of *Moringa oleifera* were collected at Isiukwuato, in Abia State, Nigeria. The identification and authentication of the plant seeds were done same day of purchase by a taxonomist at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. About five (5 kg) of fresh matured *M. oleifera* seeds were collected; the seeds were removed from the pods and stored at room temperature before being transported to the Department of Biochemistry laboratory for processing. Cassava roots were sourced from the Natural Root Crops Research Institute, Umudike, Nigeria, and transported in a container to the laboratory for processing. The ethical guidelines provided by the ethical committee of College of Natural Science, Michael Okpara University of Agriculture, Umudike, were strictly followed.

Sample processing

M. oleifera seeds were removed from the seed pods, dehusked and the white kernels were oven-dried at 40 °C for 48 hours. The dried seeds were then milled into fine powder using electric blender (model QBL-18L40), then sieved through a 2 mm sieve to get the fine powder. The powder was then stored at 4 °C in air-tight containers for further analysis. The 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 to constant weight before subjecting to milling. The milled white powder was sieved to produce smooth fine dried flour which was stored in an airtight container until used.

Composition test diet formulation

Experimental diets (Four) were formulated (Table 1). Standard rat feed was reduced to fine powder with the aid of mortar and pestle. The milled *Moringa oleifera* seed flour and standard rat feed flour were thoroughly mixed together at different proportions shown in Table 1, with the aid of a laboratory sized mixer to make 100 g at different ratios and stored in well labeled plastic containers prior to fermentation. The formulation was designed in different proportions to obtain the effective product that has the high pharmacological effect on diabetic rats. All the prepared formulations were mixed thoroughly with a laboratory mixer before been manually pelletized and oven dried at 40 °C [12].

Table 1: Ratio of composite blend formulation

Sample	Mo Flour (%)	Standard feed (%)	Treatment label
A	0	100	Control diet
B	20	80	Unfermented diet
C	20	80	Fermented diet
D	30	70	Fermented diet

Mo = *Moringa oleifera*

Fermentation procedure

Each formula in Table 1 was fermented under controlled conditions using the method of Singh *et al.*, [12] with little modifications. The fermented gruels were freeze dried at -20 °C for 24 h to obtain the enriched samples in powdered form prior to biochemical analysis.

Experimental animals

Male albino rats of the wistar strain (thirty-six) 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 wistar rats were housed in animal cages in a well-ventilated experimental room with 12 h light/dark cycles. The rats were allowed to acclimatize for a period of 14 days to their new environment before the commencement of treatments. The rats had free access to standard feed (Guinea Feeds, Nigeria) and clean water *ad libitum*.

Induction of diabetes

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 to the experimental rats at a dosage of 50 mg/kg body weight at fasting state. Blood was collected from the tail vein and blood glucose concentration was analyzed prior to the commencement of the oral administration using a blood glucose meter (Double G glucometer, USA). The alloxan-treated rats with fasting blood glucose levels > 200 mg/dL after seven days of induction were considered to be diabetic and were used for the study. The blood glucose levels of all experimental rats were checked every week throughout the duration of the experiments.

Experimental procedure

A complete randomized experimental design comprising of six treatment groups replicated thrice was used for the study. Alloxan- induced diabetic rats with stable diabetic condition were divided into 5 subgroups (groups 2 to 6) with six animals per group while the non-diabetic rats formed the first group. The diabetic rats were treated with different proportions of fermented and unfermented *M. oleifera* seed-supplement and Metformin as standard drug. The groups were as follows (Table 2): Group 1: Normal rats received only water (Normal control); Group 2: Diabetic induced rats received only the vehicle,(non-treated).Group 3: Diabetic induced rats received 5 mg/kg bwt of metformin (drug control); Group 4: Diabetic induced rats fed with unfermented *Moringa* seed-supplementation (80:20%) diet, Group 5: Diabetic induced rats fed fermented *Moringa* seed-diet (80:20%) and Group 6; Diabetic induced rats a fed 70:30% fermented *Moringa* seed diet. The experiment lasted for 21 days.

Table 2: Experimental design and animal grouping

Group	Label	Treatment
A	Negative control	Received the standard feed and water
B	Disease control	Diabetic control but not treated. Received standard diet
C	Drug control	Received Standard drug (Metformin)
D	Test group 1	Received Unfermented <i>Moringa</i> seed diet (80:20%)
E	Test group 2	Received Fermented <i>Moringa seed</i> diet (80:20)
F	Test group 3	Received fermented <i>Moringa</i> seed diet (70:30%)

Percentage feed intake and change in body weight

All diets were administered in measured amount of 50 g per day and the left over was measured the next day to ascertain the rate of feed intake in all groups. The animals were weighed prior to the commencement of the experiment and subsequently every day till the end of the experiment. The treatment was daily and lasted for 21 days (3 weeks).

Blood collection and serum preparation

At the end of the feeding experiment which lasted for 21 days, the animals were fasted overnight, weighed before being anesthetized and euthanized by cervical dislocation. The blood from the rats was rapidly collected by direct heart puncture into plain sample bottles for serum lipid profile assays.

Biochemical assay

Hematological parameters assay

The concentrations of hematological parameters such as red blood cell (RBC), packed cell volume (PCV), hemoglobin (Hb), White blood cell (WBC), Platelets, Mean Corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were obtained for each blood sample using an automated hematology analyzer.

Histological studies

The method used by Alaebo *et al.* [13] was used for liver tissue preparation. Liver tissue was dissected out and immediately fixed in 10% formal saline for histological studies. The liver was fixed in 10% formal saline fixative for 24 hours. They were then dehydrated in grades of ethanol, cleared in xylene then infiltrated with and embedded in paraffin. Each was sectioned at 5 μ m and stained with hematoxylin and eosin. The fixed slides were viewed under light microscope and photomicrographs were captured (400x). Photomicrographs were taken with a computer having a microscopic analysis software (Scope Image-9.0) connected to an Olympus digital light microscope (Olympus UK, Essex, UK).

Statistical analysis

Results are presented as means $\pm$ standard deviation of triplicate independent determinations and analyzed for statistical significance by one-way ANOVA followed by Duncan multiple range tests for multiple comparisons. Values were considered to be statistically significant at $P < 0.05$. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 16.

RESULT AND DISCUSSION

The results in Table 3 showed that there was a significant ($p < 0.05$) increase in pancrease weight in diabetic control group when compared with other groups and the normal control. But when fed with fermented *Moringa oleifera* seed-cassava diet at different proportions for 3 weeks, there was a significant decrease in pancrease when compared to disease control.

Table 3: Effect of fermentation time on proximate and energy value of *Moringa* seed-Cassava diet

Sample	Moisture (g/100g)	Protein (g/100g)	Ash (g/100g)	Fiber (g/100g)	Fat (g/100g)	CHO (g/100g)	Energy (Kcal)
MS	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 ^c	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
UF0h	8.94±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
F24h	8.36±1.3 ^a	20.44±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
F36h	9.57±1.9 ^a	25.73±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 (a-b-c) are significantly different at ($P < 0.05$) along the columns.

MS= *Moringa* seed, CF=cassava flour, UF0h=unfermented, F24h=fermented for 24h, F36h=fermented for 36 h

The results in Table 3 revealed that *Moringa* seed is a good source of crude protein, ash and dietary fiber than cassava. *Moringa oleifera* seed contains higher amount of protein (31.43 g/100 g), fiber (9.33 g/100g), ash (5.11 g/100g) and fat (24.81g/100g) respectively (dry weight) than cassava. However, cassava has highest carbohydrate content of approximately 80%. Addition of *Moringa oleifera* seed flour followed by fermentation significantly ($P < 0.05$) improved the protein content of cassava *Mahewu* (Figure 1). The highest increase in protein content was noted after 36 h of fermentation compared to the raw cassava flour (CF). On the other hand, a significant decrease ($P < 0.05$) in ash, fiber and carbohydrate was recorded as fermentation time was increased from 24 to 36 h compared to unfermented cassava *Mahewu*. There was gradual increase in moisture content (though not significant) as fermentation time proceeds.

Also, a reduction in energy value was recorded with raw and unfermented *Moringa* seed having the highest energy value compared to other samples as a result of high fat composition.

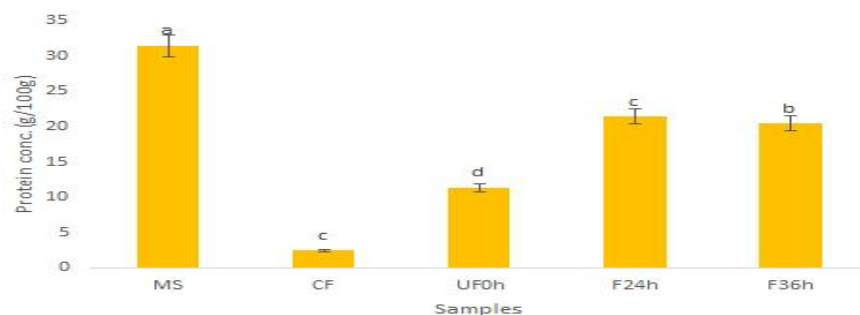


Figure 1: Effect of fermentation on the protein content of *M. oleifera* seed-improved cassava

Table 4: Percentage increase in body weight of rats fed fermented *Moringa* seed-cassava pellet

Treatment	Initial wt (g)	Final wt (g)	% Change weight	% feed intake
Normal control	92.01±8.01	230.0±7.21	48.70±4.9 ^a	86
Diabetic control	122.33±1.53	160.33±13.77	23.46±5.32 ^d	72
Drug control	128.0±2.02	204.0±10.58	37.16±2.66 ^b	82
Unfermented 80:20	117.67±1.53	160.36±8.50	26.47±3.94 ^{cd}	65
Fermented 80:20	108.0±2.3	165.33±9.07	34.58±2.57 ^b	68
Fermented 70:30	105.33±7.02	154.33±13.27	31.64±2.62 ^{bc}	61

*Note: Mean with different superscript (a-b-c) are significantly different at (P<0.05) along the columns. T1=unfermented 8:2, T2=fermented 8:2, T3= fermented 7:3

Table 4 shows the percentage change in body weight of the diabetic rats fed with fermented *Moringa oleifera* seed-cassava *Mahewu*. The maximum percentage gain in body weight was seen in the normal control group without diabetes while the lowest gain in body weight was recorded in diabetic control group which was not treated. Administration of *Moringa* seed-cassava *Mahewu* at different proportion resulted in significant improvement in body weight gain when compared with the diabetic control. However, the drug control group recorded more efficient improvement in body weight gain compared to the test samples.

Fermented *Moringa* seed-cassava *Mahewu* (80:20) resulted in better improvement in body weight gain than other fermented samples (Figure 2).

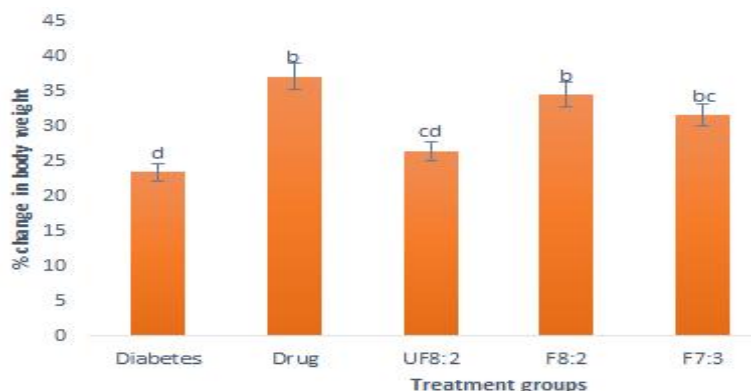


Figure 2: Change in body weight of rats fed fermented *M. oleifera* seed-improved cassava

Table 5: Relative organ weight of rats fed with fermented *Moringa oleifera* seed-Cassava *Mahewu*

Treatment	Liver	Heart	Kidney	Lungs	Pancreas
Normal Control	6.83±0.74 ^b	0.56±0.07 ^a	1.02±0.04 ^b	1.22±0.19 ^a	0.69±0.07 ^b
Diabetic	6.01±0.25 ^{abc}	0.60±0.03 ^a	1.18±0.05 ^{ab}	1.52±0.35 ^a	0.84±0.12 ^a
Drug control	4.94±6.54 ^c	0.51±0.05 ^a	1.02±0.18 ^b	1.20±0.24 ^a	0.52±0.06 ^c
Unfermented 80:20	5.34±0.30 ^{bc}	0.52±0.04 ^a	1.04±0.07 ^b	1.46±0.07 ^a	0.73±0.03 ^{ab}
Fermented 80:20	6.12±1.36 ^a	0.58±0.12 ^a	1.20±0.18 ^{ab}	1.29±0.02 ^a	0.69±0.01 ^b
Fermented 70:30	5.29±0.78 ^{bc}	0.52±0.11 ^a	1.26±0.14 ^a	1.40±0.04 ^a	0.60±0.08 ^{bc}

Note: Mean with different superscript (a-b-c) are significantly different at (P<0.05) along the columns.

The result in Table 5 showed that there was a significant (p<0.05) increase in pancreas weight in diabetic control group when compared with other groups and the normal control. But when fed

with fermented *Moringa oleifera* seed-cassava diet at different proportions for 3 weeks, there was a significant decrease in pancreas when compared to disease control. There was no significant difference in heart, kidney and lungs when compared with disease and normal control, but in liver there was significant decrease in disease control when compared to normal control.

Table 6: FBG levels (mg/dl) of Diabetic rats fed fermented *M. oleifera* seed-improved cassava diet.

Group	Pre-induction	After-induction	WK 1	WK 2	WK 3	WK 4
A	85.00± 7.91	NI	84.00± 3.39	82.00± 7.21	83.00± 1.58	80.20 ±2.28 ^a
B	87.00 ±10.36	425.20±97.66	414.60±130.43	303.20±47.72	308.80±44.4	239.0± 17.31 ^b
C	84.00± 8.3	436.40±70.36	254.20± 42.43	143.20±46.64	89.20± 6.64	92.20 ±4.21 ^c
D	76.60± 6.84	423.00±108.0	289.40± 62.12	231.20±40.81	205.00±18.2	201.0± 23.78 ^{cd}
E	75.40±12.42	399.40±136.6	280.8 ±57.91	215.20±34.75	186.40±37.3	188.0± 22.93 ^d
F	76.80±13.66	347.60±76.42	251.60± 58.69	164.60±63.34	107.60±22.8	105.6 ±14.97 ^e

^{a-e}Mean with different superscripts along each column are significantly different at P< 0.05.

NI=No induction.

The biological effects of fermented *M. oleifera* seed-improved cassava *Mahewu* on fasting blood glucose in Table 6 showed that groups fed fermented *Moringa* seed-improved cassava *Mahewu* were able to reverse their blood glucose to near normal at the end of the treatment while unfermented sample showed no such significant effect compared with the disease group. The animals treated with unfermented *M. oleifera* seed-improved were still diabetic at the end of the 21 days treatment. Though a slight reduction (P>0.05) in fasting blood sugar was seen in this group, however, the effect was not statistically significant when compared with disease control (P>0.05).

Table 7: Effect of fermented *M. oleifera* seed-cassava on hematology parameters of diabetic rats

Group	PCV%	HB (g/dl)	WBC *10 ¹² /L	RBC*10 ¹² /L	Platelet*10 ⁹ /L
Normal	51.66±2.51 ^{ab}	13.43±0.49 ^c	3.50±0.10 ^{ab}	4.27±0.25 ^{bc}	158.33±5.22 ^a
Diabetes	45.33±1.15 ^b	10.56±0.66 ^d	4.10±0.43 ^a	3.73±0.20 ^c	178.33±7.63 ^a
Drug	54.00±2.0 ^a	15.46±0.50 ^{ab}	3.70±0.30 ^{ab}	8.50±1.00 ^a	146.67±4.43 ^a
UF8:2	53.33±2.5 ^a	17.16±1.01 ^a	3.53±0.23 ^{ab}	7.00±1.80 ^{ab}	151.67±8.25 ^a
F8:2	54.0±4.0 ^a	16.13±0.23 ^a	3.56±0.28 ^{ab}	6.0±0.20 ^{bc}	163.33±6.04 ^a
F7:3	49.33±1.52 ^{ab}	14.13±0.83 ^{bc}	3.33±0.15 ^b	6.50±1.80 ^{bc}	160.0±6.46 ^a

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

The hematological parameters of alloxan-induced diabetic rats fed with fermented and unfermented *Moringa oleifera* seed-cassava was represented in Table 7. A significant reduction (P<0.05) in packing cell volume (PCV) was seen in diabetic group which received no treatment when compared with the normal control. Following the administration of the feed, a significant improvement (P<0.05) in PCV was noted in all the test samples. The highest improvement in PCV was observed for diabetic animals fed fermented *Moringa* seed-cassava at 80:20 ratio. Similarly, a reduction in hemoglobin level (Hb) was recorded in diabetic control group when compared with the normal control. Fermented and unfermented *Moringa* seed-cassava intake significantly improved the Hb levels in the treated rats. The highest effect was observed for the unfermented test diet in group 4.

Though, a slight increase in WBC was recorded in the diabetic rats which was modulated by the intake of the test diets at different proportions, however, the effect was not significant (p>0.05).

Also, no significant difference was observed (P>0.05) in the platelet concentration in both control and treated rats.

Red blood cell indices are used to evaluate erythropoiesis. Alterations in the process may be in the direction of a decrease, indicated by the addition of *Moringa* seed-cassava as observed in this study. The lowered RBC in diabetic rats could be accounted for by the destruction of mature RBC leading to decreased hemoglobin concentration that is usually accompanied by lowered PCV [14]. Akindele *et al.* [15] and Kumar *et al.* [16] reported that anemia is a common

pathophysiology condition associated with diabetes mellitus. These effects were mitigated by administration of fermented *Moringa* seed-cassava to diabetic rats confirming the antianemic activity. Findings of reduced RBC indices in diabetic control rats compared to the normal group thus suggests anaemia, consistent with previous reports [17,18]. The observed increase red cell indices in diabetic rats following treatment with the fermented *Moringa* seed-cassava also suggests enhanced erythropoiesis.

Elevated WBC count is a classical marker of inflammation and is associated with diabetes mellitus as well as other diseases [19]. The significant reduction of WBC after administration of fermented *Moringa* seed-cassava to diabetic rats indicates the ability of the plant to protect against diabetes-induced elevation of total white blood cell counts. The other effects of chronic hyperglycemia mentioned above may have been countered by the hypoglycemic effect of the fermented *Moringa* seed-cassava.

The study also showed decreased PCV in the fermented *Moringa* seed-cassava group when compared with diabetic rats. This study yielded higher platelet indices for diabetic controls compared to fermented *Moringa* seed-cassava fed rats. The significant ($P<0.05$) increase in count is in accord with earlier findings [20], and may have resulted from activation of the megakaryocyte-platelet system which occurs in diabetes resulting in increased turnover of platelets [21].

The fermented *Moringa* seeds-cassava flour contain a low-fat content ($14.93\pm0.06\%$), which is desirable. Moreover, *Moringa* contains more dietary polyunsaturated fatty acids (PUFAs) than saturated fatty acids (SFAs). A higher content of PUFAs and a lower amount of SFAs is desirable [22] as such, the inclusion PUFAs in the diet is recommended, as they can prevent the occurrence of diseases, thereby promoting good health.

The fermented *Moringa oleifera* seed-improved cassava diet is rich in carbohydrates and has great caloric value that can contribute to the caloric requirements of the body. Carbohydrates are an essential part of a healthy diet and should make up 50% of our daily calorie intake. The low moisture content of the fermented *Moringa* seeds cassava flour is an attribute of a very high shelf life.

Ash in food contributes to the residue remaining after all the moisture has been removed and after the organic materials (fat, protein, carbohydrates, vitamins and organic acid) have been incinerated [23].

The crude fiber content of fermented *Moringa*-cassava diet (9.33 g/100g) obtained in this study was considered to be at an acceptable level, making *Moringa* seeds a promising ingredient for human diets.

CONCLUSIONS

Data obtained from this study suggest that *Moringa oleifera* seed-improved cassava diet possess medicinal effects on the hematological indices and can be used as nutritional supplement in diabetes. This was indicated in this study by the effect of the extract on RBC, PCV, HB platelets and WBC of the albino rats. It also indicate the ability of *Moringa oleifera* seed-improved cassava diet to cause shedding of weight. The research work was limited to hematological indices due to funding.

More researches are recommended to investigate the synergistic effect of *Moringa oleifera* seeds-cassava diet on histological and hematological parameters especially using graded level to ascertain the exact bioactive compounds that make it healthy for consumption to promote desired performance characteristics.

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