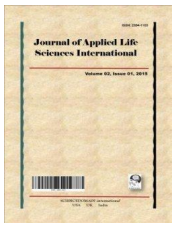

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In vivo Evaluation of Antidiabetic Properties of Seed Oil of *Moringa oleifera* Lam

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MBB, HLM, EOO and AYK designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors MBB, SS and RSY managed the literature searches, analyses of the study performed the spectroscopy analysis and authors MBB and SS managed the experimental process and author HLM identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the antidiabetic properties of seed oil of *Moringa oleifera* Lam. Extracted with petroleum ether and dichloromethane and compared with glibenclimide.
Study Design: Experimental study design was adopted.
Methodology: The 2.0 ml/kg body weight (kg.bw) of the oil, 500 µg/kg.bw of glibenclimide and 2.0 ml/kg.bw of DMSO (Diabetic control group) were given orally to rats in their respective groups after induction with 130 mg/kg.bw of alloxan monohydrate.
Results: At 21st day, the glibenclimide treated group and oil extract of petroleum ether and

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dichloromethane treated groups showed significant reduction (P<0.05) in blood glucose level at 78.00, 51.16 and 77.31% respectively when compared with control that showed 3.25% increase. There was also significant (P<0.05) gain in body weight for both oil treated and glibenclimide groups as compared with diabetic control group. The cholesterol, triglyceride and low density lipoprotein were significantly (P<0.05) reduced in the treated group with high density lipoprotein as compared with diabetic control group. Serum total bilirubin, total protein urea and creatinine of the oil treated groups and glibenclimide were significantly reduced (P<0.05) as compared with diabetic control group. Significant (P<0.05) high activities of liver enzymes AST (57.40±2.53) and ALT (68.94±0.90) with low mean percentage ratio of fresh organs to whole body weight (liver, kidney and spleen).

Conclusion: The seed oil of *Moringa oleifera* Lam. at 2 ml/kg.bw has significant antidiabetic properties on the diabetic rats in addition to improving the biochemical abnormalities arising from the disease.

Keywords: Moringa oleifera; glibenclimide; antidiabetic; biochemical; diabetes.

1. INTRODUCTION

Diabetes mellitus is defined as metabolic abnormalities characterized by continuous increase in blood glucose with disturbances in metabolism of protein, fat and carbohydrate which is caused by the abnormal secretion of insulin, insulin action, or both [1]. Insulin is known to be a hormone produced by beta cells of pancreas located in the Islet of Langerhans that enables the cells of the body to take in glucose for metabolic process. However, when the body cells fail to absorb glucose, accumulation occurs in the blood and results in many abnormalities [2,3]. The prolong effects of diabetes mellitus are progressive development on various complications such as retinopathy, neuropathy, and / or nephropathy [4]. Cardiovascular disease is one of the risks of People with diabetes [5]. Many studies have shown that oxidative stress, caused mainly by hyperglycemia-induced free radical generation leads to the growth and progression of diabetes as well as its complications [6,7]. When the free radicals are abnormally high, it results in membrane damage due to protein glycation, membrane lipid peroxidation, followed by the simultaneous diminish of antioxidant defense mechanisms [8].

According to World Health Organization, 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly and it is estimated that by 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially Type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 [9]. Ninety-ninety five percent of diabetes patients have non-insulin dependent or type 2 diabetes (T2D). T2D contributes to combination of an adequate compensatory insulin secretory response and insulin resistance [10]. Oral antidiabetic drug such as sulfonylureas, thiazolidinediones and biguanides are sold for type 2 diabetes treatments, but these drugs have serious adverse effects and are not active against some complications of long-term diabetes [10].

Herbs are alternative medicines for diabetes treatment due to their perceived acceptability, effectiveness, affordability and safety with lesser side effects in clinical experience and coupled with low cost [10]. As such the World Health Organization recommends the use of traditional and plant based medicines for the management of diabetes mellitus [11].

Moringa oleifera also known as the “horse-radish tree” is the most popular and widely naturalized among the 13 species of moringa and is said to have originated from sub-Himalayan tracts, part of northwestern India. All parts of moringa are used in the traditional systems of human medicine for the treatment of several ailments [12]. The leaves, flowers, and roots are used to treat ailments such as ascites, venomous bites, rheumatism and can be used as circulatory stimulants and cardiac in folk remedies [13].

Recently, the use of oil has increased for preventing or treating many chronic diseases such as diabetes. Oil *Nigella sativa* [14], walnut oil [15], coconut oil [16], *Picralima nitida* [17], garlic oil [18] have been reported to possess hypoglycemic effects. The hypoglycemic activities of *Moringa oleifera* in normoglyceamic

rats was also reported by [19]. It is therefore worthwhile to evaluate the hypoglycemic properties of seed oil in alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

The dried pods of *M. oleifera* were collected from Bosso estate at Bosso Local Government Area, Niger State, Nigeria, in June 2013. The plant material was authenticated at the herbarium of Department of Biological Sciences, Federal University of Technology Minna, Niger State. The authentication was done using a taxonomic aid provided by [20] as well as [21]. The seeds were threshed from the pods, air dried, pulverized into powdered form, using a rotary blender, and till ready for use.

2.1.2 Experimental animals

Wistar rats of same sex weighing between 120-145g used for the experiment were obtained from Biochemistry and Chemotherapy section of the National Institute for Trypanosomiasis and Onchocerciasis Research (NITR) Vom, Plateau State, Nigeria. The animals were allowed to acclimatize in the Department of Biochemistry laboratory, Federal University of Technology, Minna, for two (2) weeks. All experiments involving the animals were conducted in compliance with the internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care guidelines on animal use protocol review (1997) and as also described by [22].

2.2 Methods

2.2.1 Extraction of *Moringa oleifera* oil

Fifty grammes (50 g) of *M. Oleifera* seeds powder seeds were placed into a cellulose paper cone and fed to a soxhlet extractor fitted with a 500ml round-bottom flask and a condenser. The extraction was carried for about 8 hours with 300 ml of petroleum ether. Same process was adopted for dichloromethane extraction. The extracts were condensed using rotary evaporator as reported by [22].

2.2.2 Determination of physicochemical parameters

The refractive index and density were determined according to [23], viscosity was determined as described by [24], saponification value and free fatty acid were determined as described by [25] while peroxide value and iodine value were determined as reported by [26].

2.2.3 Animal grouping

2.2.3.1 Effects of PEEMO and DCMMO on alloxan induced diabetic rats

The wister albino rats were divided into 5 groups of 3 animals per group.

NRML: Normoglycemic rats received 2.0 ml/kg dimethyl sulphoxide orally.
CNTRL: Diabetic rat received 2.0 ml/kg dimethyl sulphoxide orally.
STND: Diabetic rats received 500µg/kg of standard drug glibenclimide orally.
DCMMO: Diabetic rats received 2.0 ml/kg of *M. oleifera* seed oil extract of dichloromethane orally.
PEEMO: Diabetic rats received 2.0 ml/kg of *M. oleifera* seed oil extracted with petroleum ether orally.

2.2.4 Treatment of diabetic rats

The serum glucose concentrations of the rats were checked after 72 hours of induction with achu-check glucometer and allowed stabilizing for seven days before the commencement of treatment. The standard drug of 500µg/kg bodyweight (bw) and various doses of the extracts used were based on design protocol as derived from the pilot studies conducted prior to the experiment which established the LD_{50} (LD_{50}>5 ml/kgbw) and hypoglyceamic effect of *Moringa oleifera* seed oil in normoglyceamic rats [19]. The treatment went on daily for twenty one days and the experimental animals were allowed to have access to food and water after fifteen minutes of treatment.

2.2.5 Total body weight

The weights of the experimental rats were determined with weighing balance (Adventurer/UK) before, during and after the period of treatment on selected day interval.

Overnight fasting was ensured on the animal with free access to water before the weighing.

The percentage weight loss or gain was calculated from the result obtained.

2.2.6 Organ-body weight ratio

At the conclusion of the experiment, the experimental animals were sacrificed under mild anaesthetization. The organs; liver, kidneys, lungs, liver and pancreas were excised from each animal, weighed and percentage ratios of organ – body weight was calculated.

2.2.7 Biochemical analysis

The activities of serum aspartate aminotransferase (AST), alanine aminoransferase (ALT), total cholesterol, Serum Triglyceride (TG), low density lipoproteins (LDL), high density lipoprotein were determined using commercial kits (AGAPE, Switzerland) while urea, creatinine serum concentration were determined with randox commercial kits and bilirubin serum concentration was determine using biosystems diagnostic kit (Barcelona Spain). The analyses were carried out according to individual kits manual.

2.3 Statistical Analysis

Data obtained were expressed as mean ± SEM. The analysis was carried by using student T-test or analysis of variance (ANOVA) followed by Post hoc Duncan multiple comparisons test, using SPSS 16.00 version. The tests results with *P* values < 0.05 or < 0.01 were taken to be significant different for ANOVA and student T-test respectively.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of *Moringa oleifera* Seed Oil

The Physicochemical characteristics of the *Moringa oleifera* Seed oil are shown in Table 1. There was no significant (P≥0.01) difference in the specific gravity, free fatty acid, saponification value and iodine value of both oil extracts while there was significant (P≤0.01) difference in their refractive index, viscosity, and peroxide value.

3.2 Hypoglycemic Effect of *Moringa oleifera* Seed Oil in Alloxan Induced Diabetic Rats

The hypoglycemic effect of the oil extract in alloxan induced diabetic rat based on the long term treatment model was shown in Fig. 1. Significant reduction (P≤0.05) in glucose level was observed in STND and DCMMO group on the seventh day of treatment compared to CNTRL control group. The percentage glucose reduction of STND and DCMMO are 42.36% and 7.56% respectively compared to the CNTRL which showed 5.29% glucose increase. At day 14, both PEEMO and DCMMO significantly reduced the blood glucose by 39.34% and 57.00% while 51.16% and 77.31% of glucose reduction was observed in day 21 respectively. There was no significant difference (P≥0.05) between DCMMO and STND at day 14 and 21 and no significant difference in the blood glucose of NRML and that of DCMMO at 21st day.

Table 1. Physicochemical characteristics of *Moringa oleifera* seed oil

Properties PEMMO	DCMMO	FAO/WHO	
Density at (mg/ml at 27.5)	0.91±0.02^{a}	0.91±0.01^{a}	
Refractive index (nD at 27.5)	1.46±0.01^{a}	1.47±0.00^{b}	
Visicosity (mPas)	45.16±0.59^{a}	50.06±0.03^{b}	
Free fatty acid (%)	1.22±0.06^{a}	1.39±0.05^{a}	5.78-7.38
Saponification value (mgKOH/g)	190.37±1.16^{a}	186.15±1.06^{a}	181.4-260.0
Iodine value (g/100 g)	65.82±0.14^{a}	66.02±0.36^{a}	0 80-106
Peroxide value(meqO_2/kg of oil)	1.90±0.04^{a}	1.32±0.06^{b}	≤10

**FAO/WHO standard for consumable oil. Values are means ± Standard Error Mean (SEM); n=3*
Values with different superscripts on the same row are significantly different at (P≤0.01); using T-Test 2 tailed; SPSS Software (Version 16).; PEEMO: Moringa oleifera seed oil extract of petroleum ether
DCMMO: Moringa oleifera seed oil extract of dichloromethane

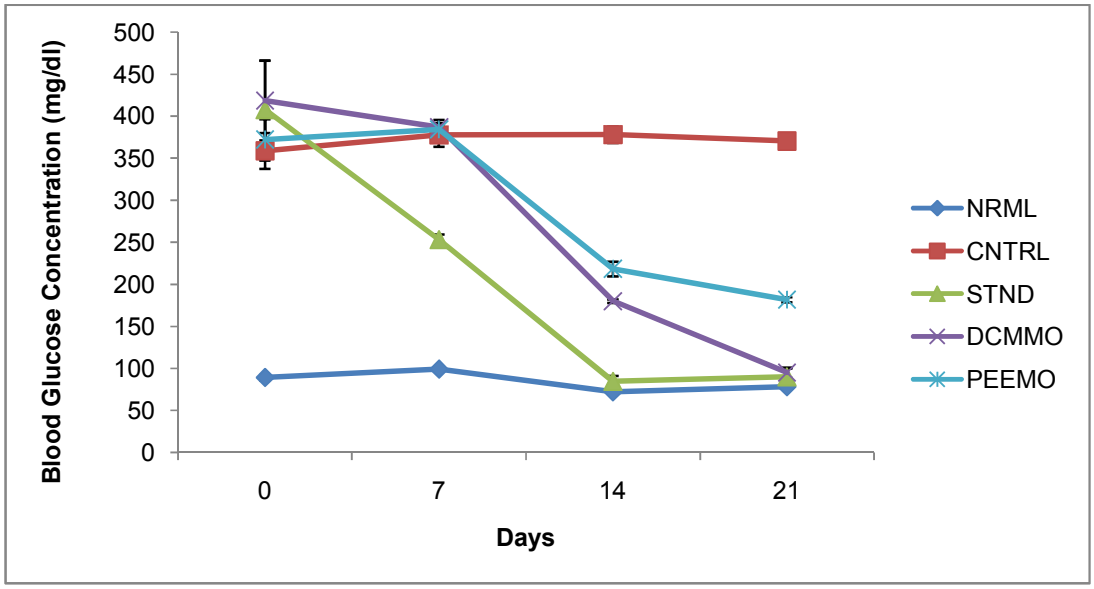

Fig. 1. Hypoglycemic effect of *Moringa oleifera* seed oil in treated and diabetic control rats

NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0 ml/kg.bw dimethyl sulphoxide orally.STND: Diabetic rats received 500µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0ml/kg.bw of M. oleifera seed oil extract of dichloromethane.PEEMO: Diabetic rats received 2.0 ml/kg.bwof M. oleifera seed oil extract of petroleum ether

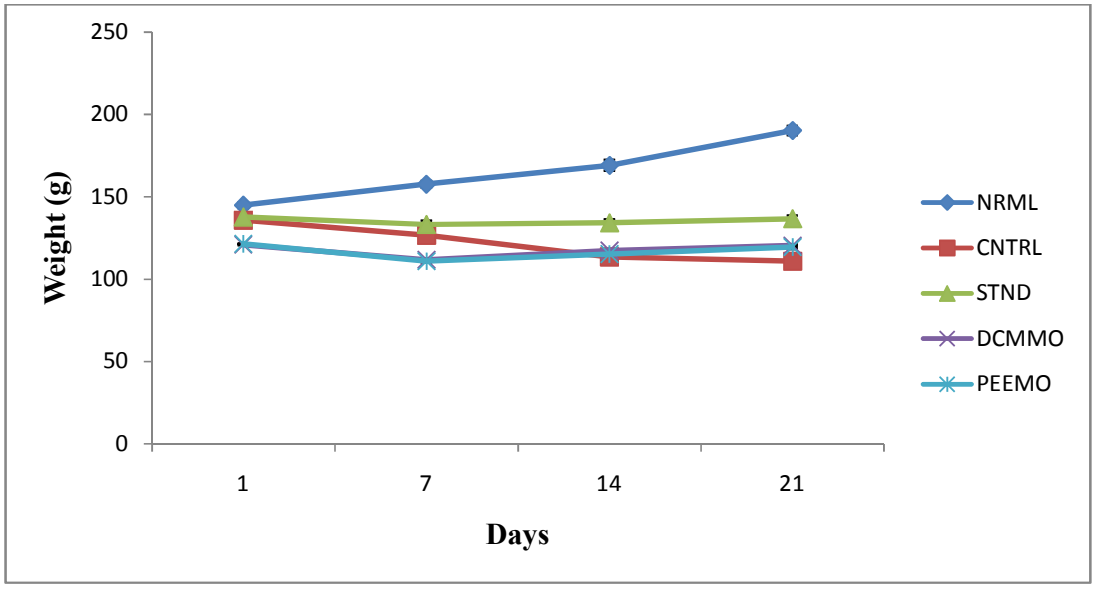

Fig. 2. Effect of *M. oleifera* seed oil on bodyweight of the treated and diabetic control rats

NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0 ml/kg.bw dimethyl sulphoxide orally.STND: Diabetic rats received 500 µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane. PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

3.3 Average Body Weight of Rats

Fig. 2 represents variation in whole bodyweight of rats in the various experimental groups. The NRML, which is the non-diabetic group shows significant increase (P≤0.05) in the body weight (31.28% of the initial weight gain) while PEEMO, DCMMO, STND and CNTRL showed no significant increase (P≥0.05) in the bodyweight throughout the experimental period.

3.4 Effect on Organ-body Weight Ratios

The mean percentage ratios of fresh organs (Liver, Heart, Lungs Kidneys, and spleen) to the whole body weight of the experimental rats on day 21 are presented in Table 2. Significant (P≤0.05) high value was observed in the liver, spleen, kidney and heart of diabetic control group as compared with oil treated and standard drug treated groups. No significant (P≥0.05) difference between the percentage heart ratio to the body weight was observed in the entire treated group.

3.5 Analysis of Biochemical Parameters

3.5.1 Estimation of serum cholesterol and triglyceride

Fig. 3 showed the different serum concentration of total cholesterol and tryglyceride between the various groups. Only CNTRL group showed significant (P≤0.05) higher concentration of serum cholesterol (142.45±3.05) and triglyceride (188.36±3.60) when compared with other groups.

3.5.2 Estimation of serum high density lipoprotein (HDL) and low density lipoprotein (LDL)

The results of HDL and LDL are represented in Fig. 4. Only CNTRL showed significant (P≤0.05) lower concentration (20.29±1.42) of HDL when compared with other treated groups. The LDL of the CNTL group was significantly (P≥0.05) higher (84.95±3.08) compared to other groups.

3.5.3 Estimation of total protein

The total protein concentration was presented in Fig. 5.The STND, NRML, CNTRL and DCMMO group showed significant lower concentration of total protein (P≤0.05) compared to CNTRL group.

3.5.4 Estimation of total bilirubin

The total bilirubin concentration was presented in Fig. 6. The CNTRL group showed significant high concentration of total bilirubin at P≤0.05 (2.81.±0.05) compared to other groups. No significant difference (P≥0.05) between the bilirubin concentration of STND and PEEMO group.

3.5.5 Estimation of urea

The urea concentration was presented in Fig. 7. The CNTRL and PEEMO group showed significant high concentration of urea at P≤0.05 (65.46±1.14 and 62.36±0.71) while NRML group was significantly lower (P≥0.05) compared to other groups.

Table 2. Effect of *M. oleifera* seed oil on the mean percentage ratios of fresh organs to whole bodyweight of the treated and diabetic control rats

	Percentage organs-body weight (g)				
Group	**Spleen**	**Liver**	**Kidney**	**Heart**	**Lung**
NRML	0.50±0.01^{a}	3.72±0.07^{a}	0.75±0.14^{a}	0.38±0.01^{a}	0.59±0.05^{a}
CNTRL	0.76±0.02^{c}	4.68±0.01^{c}	0.96±0.01^{d}	0.40±0.01^{b}	0.58±0.01^{a}
STND	0.62±0.07^{a,b}	4.23±0.04^{b}	0.91±0.09^{c}	0.36±0.09^{a}	0.56±0.09^{a}
DCMMO	0.48±0.01^{a}	3.78±0.04^{a}	0.77±0.09^{a}	0.38±0.01^{a}	0.58±0.02^{a}
PEEMO	0.61±0.09^{b}	4.36±0.17^{b}	0.81±0.06^{b}	0.36±0.01^{a}	0.57±0.02^{a}

Values are mean ± standard error mean (SEM); n=3.
Values with different superscripts on the same row are significantly different at p ≤ 0.05, using ANOVA SPSS software (Version 16)
NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0 ml/kg.bw dimethyl sulphoxide orally. STND: Diabetic rats received 500 µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane. PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

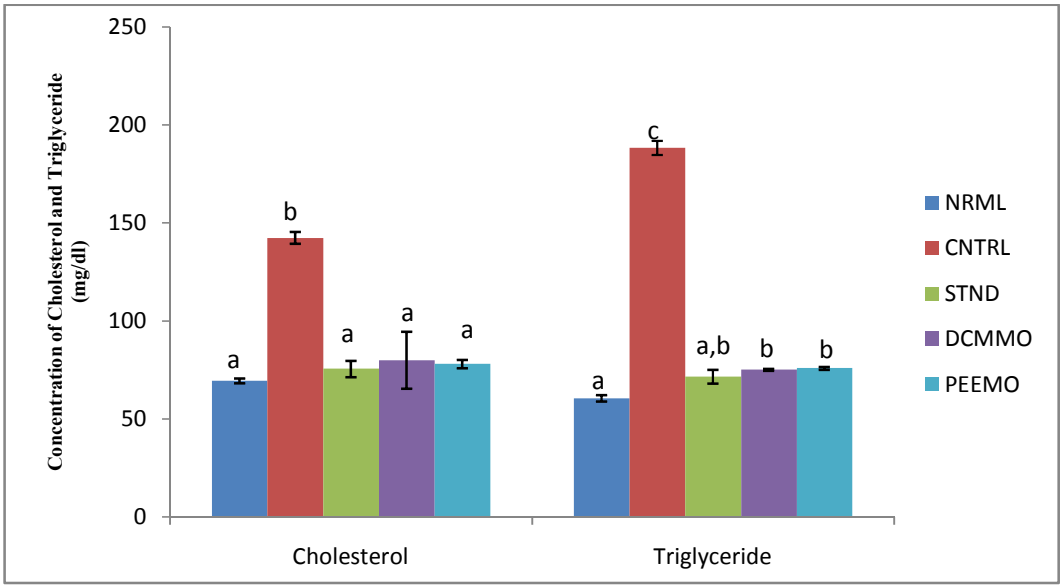

Fig. 3. Effect of *M. oleifera* seed oil on serum total cholesterol and triglyceride of the treated and diabetic control rats

Values are mean ± standard error mean (SEM), n=3.; Values with different alphabet on the chart are significantly different at p ≤ 0.05; using ANOVA SPSS software (Version 16).; NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0ml/kg.bw dimethyl sulphoxide orally.; STND: Diabetic rats received 500µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane.PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

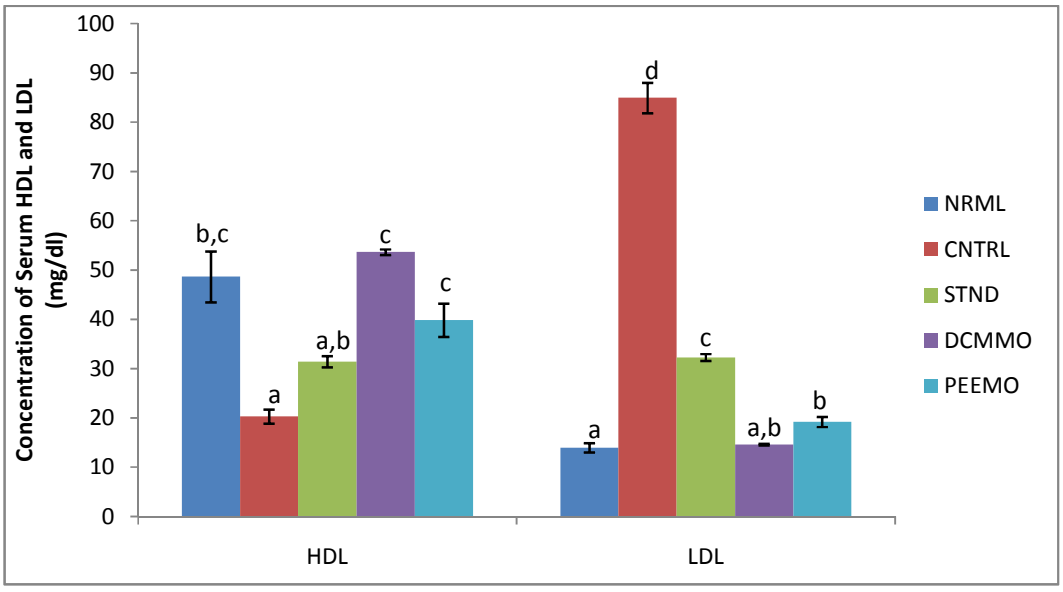

Fig. 4. Effect of *M. oleifera* seed oil on serum HDL and LDL of the treated and diabetic control rats

Values are mean ± standard error mean (SEM), n=3.; Values with different alphabet on the chart are significantly different at p ≤ 0.05, using ANOVA SPSS software (Version 16).; NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0ml/kg.bw dimethyl sulphoxide orally.STND: Diabetic rats received 500 µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane. PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

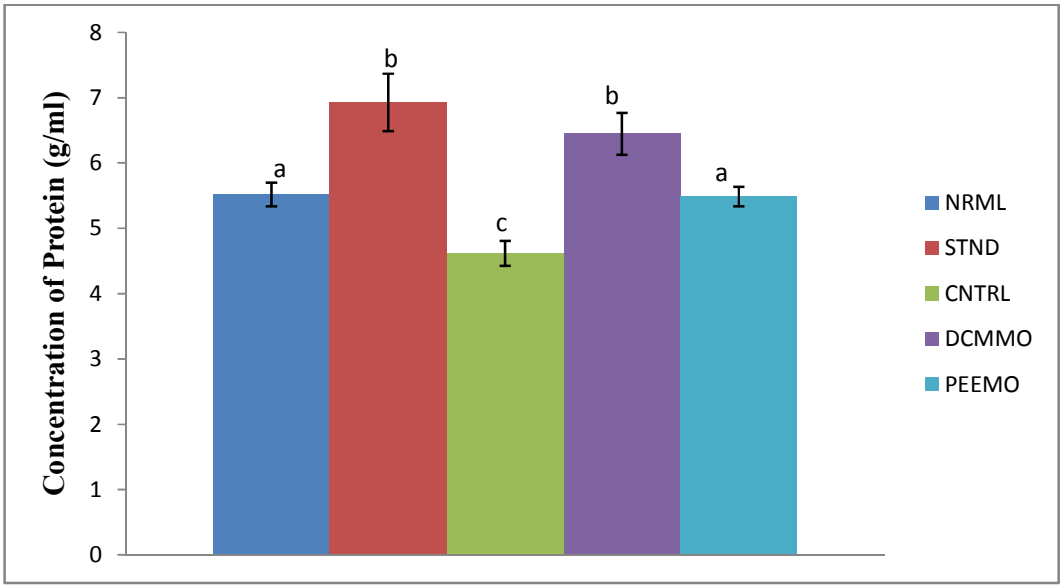

Fig. 5. Effect of *M. oleifera* seed oil on serum total protein of the treated and diabetic control rats

Values are mean ± standard error mean (SEM); n=3.; Values with different alphabet on the chart are significantly different at p ≤ 0.05; using ANOVA SPSS software (Version 16).; NRML: Normoglycemic rats received 2.0ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0 ml/kg.bw dimethyl sulphoxide orally.STND: Diabetic rats received 500µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane.; PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

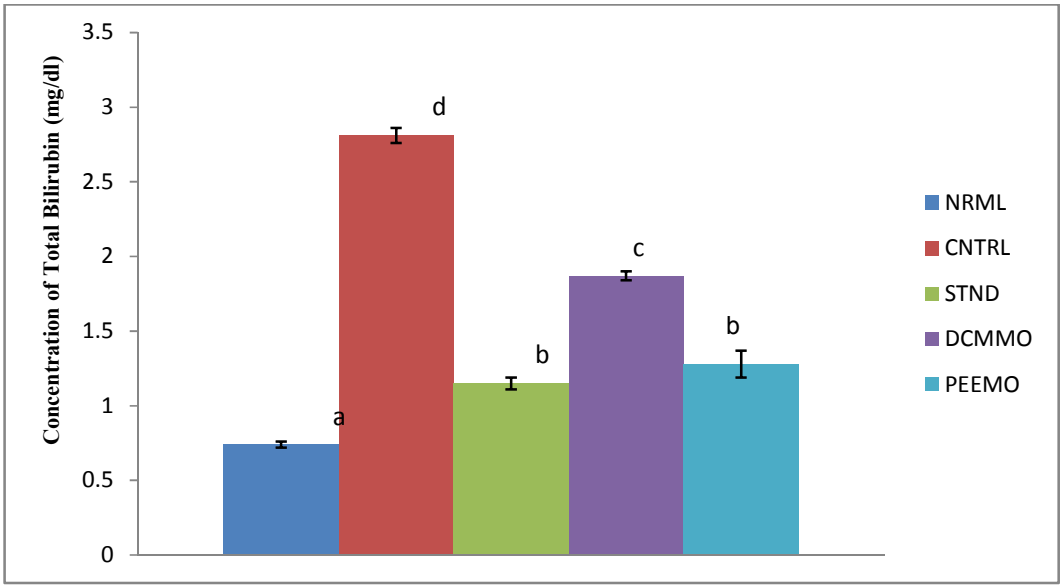

Fig. 6. Effect of *M. oleifera* seed oil on serum total bilirubin of the treated and diabetic control rat

Values are mean ± standard error mean (SEM), n=3.; Values with different alphabet on the chart are significantly different at p ≤ 0.05; using ANOVA SPSS software (Version 16).;NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0 ml/kg.bw dimethyl sulphoxide orally.STND: Diabetic rats received 500 µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane.PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

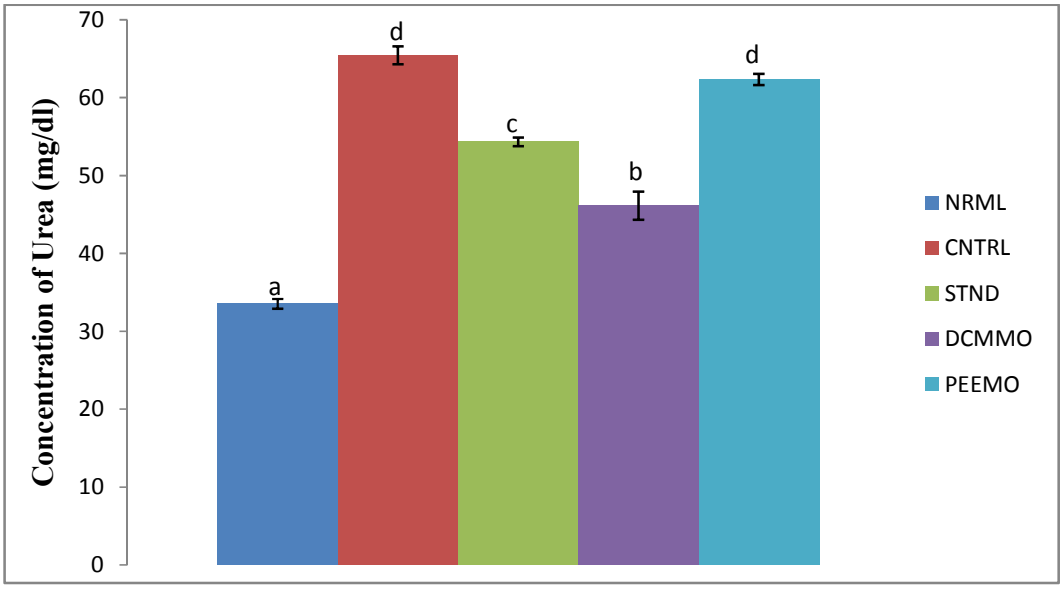

Fig. 7. Effect of *M. oleifera* seed oil on serum urea of the treated and dibetic control rats

Values are mean ± standard error mean (SEM); n=3.; Values with different alphabet on the chart are significantly different at p ≤ 0.05, using ANOVA SPSS software (Version 16).; NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0ml/kg.bw dimethyl sulphoxide orally.STND: Diabetic rats received 500 µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane.; PEEMO: Diabetic rats received 2.0ml/kg.bw of M. oleifera seed oil extract of petroleum ether

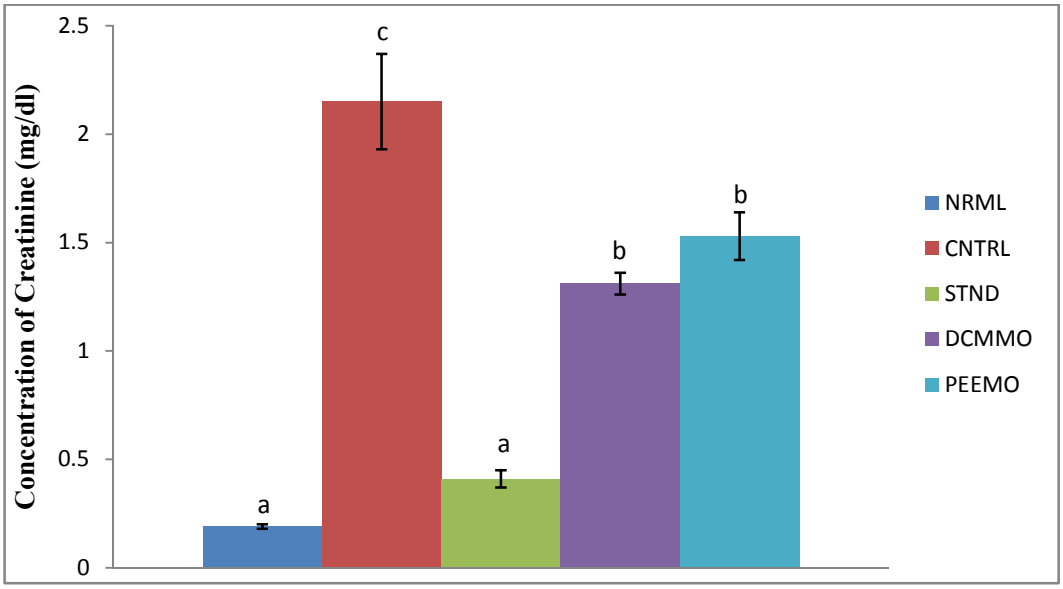

Fig. 8. Effect of *M. oleifera* seed oil on serum creatinine of the treated and diabetic control rats

Values are mean ± standard error mean (SEM), n=3.; Values with different alphabet on the chart are significantly different at p ≤ 0.05; using ANOVA SPSS software (Version 16).; NRML: Normoglycemic rats received 2.0 ml/kg.bw dimethyl sulphoxide orally. CNTRL: Diabetic rat received 2.0 ml/kg.bw dimethyl sulphoxide orally. STND: Diabetic rats received 500 µg/kg.bw of standard drug glibenclimide orally. DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of dichloromethane.
PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seed oil extract of petroleum ether

3.5.6 Estimation of creatinine

The concentration of creatinine was shown in Fig. 8. The CNTRL group showed significant high concentration (2.15±0.22) and NRML group was significantly lower (P≤0.05) compared to other groups. However, no significant difference was observed between creatinine concentration of PEEMO and DCMMO treated groups.

3.5.7 Estimation of alanine transaminase (ALT) and aspartate transaminase (AST) enzymes

The serum concentration of ALT and AST is shown in Fig. 9. There was significant (P≤0.05) high concentration of ALT (68.94±0.90) in CNTRL group as compared with other groups. No significant difference was observed between ALT concentration of DCMMO (36.34±0.98) and NRML (37.81±1.40) group.

Significant high concentration of serum AST (57.40±2.53) was observed in CNTRL group and the opposite was observed in NRML (16.80±0.51) group as compared with other groups at P≥0.05. No significant difference between AST concentration of PEEMO (26.18±0.34), DCMMO (31.64±2.61) and STND (27.63±1.50).

4. DISCUSSION

The quest for novel anti-diabetic medication from natural vegetation is still important since they have substances that possess substitute and possibly safer effects on diabetes mellitus [27]. Herbal preparation usage is a widespread practice in various countries, especially in Asia [28] and Africa [29]. Alloxan, acytotoxin of pancreatic beta cells, stimulate diabetes in a broad variety of animal species by destroying the beta cell of the pancreas that secretes insulin resulting in a decline of endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [30]. Diabetes is associated with lots of biochemical abnormalities which in turn affects the physiological conditions of the system [31,32]. The most prominent biochemical abnormality in diabetes is hyperglycemia, that is, uncontrolled elevation of blood glucose [33].

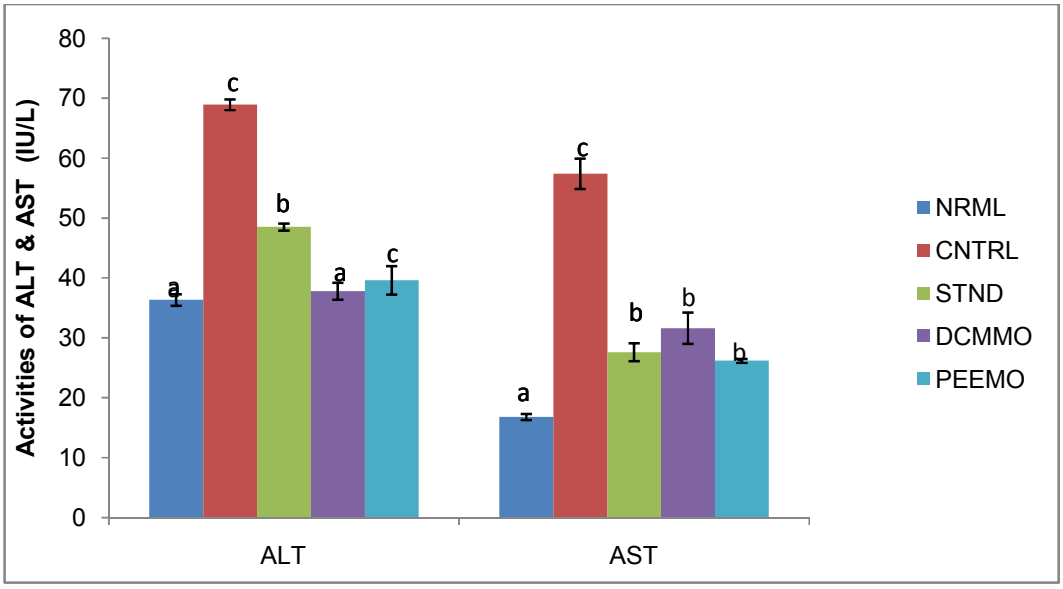

Fig. 9. Effect of *M. oleifera* seed oil on serum ALT and AST of the treated and diabetic control rats

Values are mean ± standard error mean (SEM), n=3; Values with different alphabet on the chart are significantly different at p ≤ 0.05, using ANOVA SPSS software (Version 16).; NRML: Normoglycemic Rats Received 2.0 ml/kg.bw Dimethyl Sulphoxide Orally.
CNTRL: Diabetic Rat Received 2.0 ml/kg.bw Dimethyl Sulphoxide Orally.
STND: Diabetic Rats Received 500 µg/kg.bw of Standard Drug Glibenclimide Orally.
DCMMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seeds Oil extracted with Dichloromethane.PEEMO: Diabetic rats received 2.0 ml/kg.bw of M. oleifera seeds Oil extracted with Petroleum ether

The differences in refractive index and viscosity(1.47±0.01 and 50.06±0.03) of DCMMO compared to that of PEEMO (1.46±0.01 and 45.16±0.59) as shown in Table 1.can also be attributed to the polarity of the solvents used for the extraction as different factors such as environmental condition can also lead to same effect [34]. There was no significant difference in average value obtained for PEEMO and DCMMO as regard percentage free fatty acid (1.22±0.06% and 1.39±0.05%) and iodine value (65.82 ±0.14 g/100 g and 66.02 g/100 g) respectively. However, PEEMO shows significant higher peroxide value (1.90±0.04 meqO_2/kg) and saponification value (190.37±1.16 mgKOH/g) when compared with DCMMO (1.32±0.06 meqO_2/kg and186.15±1.06mgKOH/g of oil). The peroxide value of PEEMO is very close to that obtained by [35] while that of DCMMO is closer to that of [25]. Since, *Moringa oleifera* seed used *for this r*esearch were of the same source, other factors such as geographical zone, climatic change and nature of soil might have led to such differences as observed by [25] are not included. Therefore, the most likely factor that might have caused the disparity in all these physicochemical parameters is the polarity of solvents used for the extraction.

Alloxan monohydrate, an analogue of glucose induces chemical diabetes in many animal species by its toxic action onpancreatic beta cells involved in oxidation of essential sulphydryl (-SH) groups, inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis [36]. The underlying mechanism involves the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic beta-cells [37]. In addition, glibenclimide, a standard drug used in hypoglycemic study of various compound elicit its hypoglycemic effect via various mechanisms such as binding to ATP-K^+ channel, hence reduce glucose level, suppression of glucose production from hepatic region, enhancing insulin sensitivity of the pancreas tissues and oxidation of fatty acid, enhancement of glucose uptake by peripheral tissues, decreasing of hepatic gluconeogenesis and glycogenolysis and reduction of glucose absorption from gas to intestinal tract [38].

There was significant ($P<0.05$) reduction in blood glucose level between the seventh to twenty oneth days of treatment. However, the blood glucose reduction is more pronounced at fourteenth and twenty one day in the entire treated group. At fourteenth day, the percentage reduction in glucose level of PEEMO, DCMMO and STND are 39.34%, 57.00% and 79.31% respectively while that of twenty oneth day are 51.16%,77.31% and 78.00% respectively as compared to their base line blood glucose levels. The hypoglyceamic activity of the *Moringa oleifera* oil as this study revealed could be due to the presence of fatty acids in the oil. Monounsaturated fatty acid was reported to have tendency of improving ß-cell secretory function by preventing ß-cell apoptosis, reducing impairment of ß-cell activities and ß-cell proliferation [39].It was also reported by Horváth [40], that vitamin E regulate the immunoresponse of the cell and restores damaged liver function via their antioxidant activities as Moringa oil contain reasonable quantity of vitamin [41]. Antioxidant compounds either naturally or syhthetic provide protective effect on various diseases including diabetes mellitus [7,42].

Diabetic inductions do cause great body weight loss as this is also part of the clinical symptom of diabetes according [31,43]. Thus, the gradual body weight gains as observed in oil treated groups as compared with CNTRL (Fig. 2.) signifies the therapeutic effects of the oil on the hyperglycemic rats which may be as a result of glucose utilization during treatment. Organ-body weight ratio can be used to indicate organ swelling, atrophy, or hypertrophy as presented in Table 2. The hepatomegaly, splenomegaly and high weight ratio of kidney that occurred in CNTRL groups might be as result of oedema that was developed from tissues damage of these vital organs. Damage in organs-tissue is associated with diabetic individual which in turn affects their weight [32]. Therefore, regeneration of the organs tissues might have been achieved through the antioxidant activities of the oil as explained earlier.

Hyperproteinamia, hyperalbuminemia, hyperuremia and hypercreatininemia have been reported to occur in alloxan induced diabetic rat [44]. This results as shown in Figs. 5-8 respectively in the present study also indicate the high significant ($P<0.05$) values of the aforementioned parameters in CNTRL groups as compared to DCMMO and PEEMO. This confirmed the possibility of the *M. oleifera* seed oil to have protected the vital organs such as liver, kidney and pancreas, thereby reducing the complication associated with diabetes in the oil treated diabetic animals.

Elevated oxidative stress and hyperlipidemia as a result of diabetes can result to cardiac complications in diabetic patients. In this study, tryglyceride, total cholesterol, and low density lipoprotein are significantly ($P<0.05$) reduced in PEEMO and DCMMO treated groups as compared with diabetic control group as shown in Figs. 3-4. DCMMO and PEEMO extracts which were rich in medium chain fatty acid such as oleic acid has been reported to metabolize fast and inhibit accumulation of fat [45,46]. It was also reported that antioxidants can reduce triglyceride [47]. Thus, vital antioxidant vitamins in these oil might have contributed in lipid reduction [45] since antioxidants can regulate cholesterol synthesis by regulating HMG- CoA reductase activity [48]. This cholesterol synthesis regulation may have resulted into activation of LDL receptors in hepatocyte which is responsible for uptake of LDL into the liver and reduce the serum LDL level [49]. Likewise, the lipid reduction can also be attributed to improvements in insulin levels [49].

Significant rise in serum AST and ALT levels in diabetic rats was as a result of massive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores and such increase has been reported by Hassan [50]. ALT is considered to be more specific and sensitive indicator of hepatocellular injury than AST in the damaged liver tissues. The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury because of the longer half-life of AST and the greater proportion of ALT that is bound to mitochondria [51]. Increased serum ALT activity, with or without increased AST activity, include hepatocellular necrosis, or regenerative/reparative activity [52, 53]. AST and ALT levels were significantly ($P<0.05$) reduced (Fig. 9.) in oil treated groups as compared with control group and this signifies the restoration of the damaged hepatocytes hence, amelioration of the liver necrosis which was likely to have occurred in diabetic rats.

In summary, oral administration of *Moringa oleifera* seed oil extracts have demonstrated antidiabetic properties in alloxan induced diabetic wistar rats with positive effect on their bodyweight gain, organ to body weight ratio coupled with better improvement in some of their biochemical parameters and these antidiabetic properties is more pronounced in the moringa seed oil extracted with dichloromethane.

5. CONCLUSION

Conclusively, *Moringa oleifera* seed oil extract can serve as a source of potential antidiabetic agents which can serve as oral hypoglycemic agent or adjuvant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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