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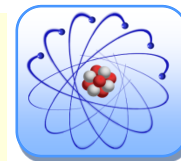

ANTIHYPERGLYCEMIC AND ANTIDISLIPIDEMIC ACTIVITIES OF ETHANOLIC ROOT BARK EXTRACT OF *Moringa oleifera* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Abstract

The aim of this study is to evaluate the antihyperglycemic and antidyslipidemic activities of ethanolic root bark extract of Moringa oleifera in streptozotocin-induced diabetic rats. Thirty (30) albino rats were randomised into six groups of five animals each. Diabetes was induced in groups II-VI with single intraperitoineal (i.p) injection of Streptozotocin (STZ) that was freshly dissolved in citrate buffer (0.1M, pH 4.5). Diabetes was confirmed in rats after 48hours using Acucek glucometer. Control group I and diabetic untreated group VI were administered 1 ml of distilled water while 1 ml of 100, 200, and 400 mg/Kg body weight of ethanolic root bark extract of M. oleifera was administered to groups II-IV respectively. 1 ml of 14.2 mg/Kg of Standard drug, Met formin was given to the remaining group V. The treatment lasted for 21days. Result showed that there was a significant (P<0.05) reduction in fasting blood glucose of the diabetic rats following the administration of 100, 200, and 400mg/Kg of the extract when compared with the diabetic untreated rats. Also, the extract reduced significantly (P<0.05), the concentrations of serum cholesterol, triglyceride and low density lipoprotein, LDL-c while the serum high density lipoprotein, HDL-c concentration was significantly (P<0.05) increased compared with diabetic untreated group. Overall, the ethanolic root bark extract of M. oleifera can be used in the management of diabetes mellitus and its complications (hyperglycemic and dyslipidemic).

1.0 Introduction

One of the serious major causes of health problems worldwide nowadays is diabetes mellitus. It is a serious complex chronic metabolic disorder that is characterized by hyperglycemia and disturbances of carbohydrate, protein, and lipid metabolisms due to an absolute or relative lack of the hormone, insulin. Diabetes may be due to absolute lack of insulin orinsulin resistance/action or combination of both factors [1]. Apart from hyperglycemia, hyperlipidemia or dyslipidemia are other complications of diabetes. These complications lead to morbidity and death [2, 3] has reported that there were about 150 million patients of diabetes worldwide, and according to him, the numbers are likely to jump to about 300 million by the 2025.This prediction in the rise may be attributed to obesity, consumption of energy-rich diet, increased life span and sedentary lifestyle of people [4]. This disease is now very common among Nigerians in West Africa [4]. Dislipidemia refers to the abnormal concentration of lipids in the blood. Dislipidemia is characterised by hyperlipidemia and hypolipidemia. These involved triglycerides, cholesterol and/ or fat phospholipids. Due to relatively absent or deficient of insulin in diabetic patients, metabolic regulation of lipid is altered. Triacylglycerols, the main bulk of ingested fatty food can only be absorbed as chylomicrons into the lymph and then enter the blood mainly through the thoracic duct. The removal of chylomicrons from the circulation is mainly carried out by the adipose tissue. However,other tissues such as intestine and the liver may take up a small proportion of it [5]. Plant products are now undergoing exploitation to treat diabetes mellitus as some plants are now known to contain many bioactive substances with therapeutic potential. The efficacy of several medicinal plants has been confirmed for the treatment of diabetes mellitus. However, there are still several other medicinal plants that are yet to be tested for their antidiabetic effects [6]. One of such is *M. oleifera. M. oleifera* is a miracle tree that grows widely in many tropical and subtropical countries such as India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia. It is also called Drumstick tree due to the appearance of its immature seed pods and the Horseradish tree based on the taste of ground root preparations. It is grown commercially. *M. oleifera* is believed to be very nutritious. Its immature seed pods are eaten, while the leaves are widely used as a basic food [7, 8, 9]. Seeds, leaves, oil, sap, bark, roots, and flowers are widely used in traditional medicine. Furthermore, the leaves of *M. oleifera* contain antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids [10, 11].

Figure 1: *Moringa oleifera* Leaves ant Root

Due to the increase in the mortality rate as a result of the complications from diabetes mellitus and the harsh side

effect of synthetic drugs, there is need for the development of alternatives that are less expensive and have minimal side effects. Thus, ethanolic extract of root bark of *M. oliefera* is used in this study to treat insulin dependent diabetes.

2.0 Materials and Methods

The root bark of *M. oliefera* used in this study was collected in Malete, Kwara state.

2.1 Experimental Animals

Albino rats with average weight of 140g used for this experiment were obtained from the Animal House of the Department of Biochemistry, Kwara State University, Malete, Kwara State. They were provided with rat feed and water *ad-libitum.* Theywere acclimatized at room temperature for 1 week before the commencement of the experiments.

2.2 Chemicals and Reagents.

HDL, G6PDH and Triglycerideskits were obtained from Randox Laboratory Ltd. All other chemicals and reagents were of analytical grade, prepared in glass wares.

2.3 Preparation of Extract

The root bark was air dried at room temperature until constant weight was obtained and grinded to powder using an electric blender (Philip comfort blender, mode HR1727, Holland). Four hundred grammes (400 g) of the powdered root bark of*M. oleifera* was weighed and soaked with 4000ml of ethanol for 48hours with intermittent shaking. The mixture was filtered using micelles cloth, oven-dried at 40°C to give a yield of 50g (25%). The dried extract was stored in air tight container and kept in refrigerator at 4°C. A calculated amount of residue was weighed and constituted in distilled water to give the required doses of 100, 200, and 400mg/Kg body weight.

2.4 Induction of Diabetes Mellitus

Thirty rats were used for the study. Experimental diabetes mellitus was induced in twenty five rats (groups II-VI) by a single intraperitoneal (i.p) injections of STZ (60 mg/Kg body weight) dissolved in 0.1 citrate buffer of pH 4.5. 48 hours after STZ administration, blood samples were obtained from tips of tail of the rats and glucose level were determined to confirm experimental induced diabetes mellitus. Rats with blood glucose level above 180 mg/dl was recruited as been diabetic and used for the study.

2.5 Animal Grouping and Extract Administration.

A total number of thirty (30) albino rats were completely randomized into six groups of five rats each.

Group 1: Control (Non- diabetic) orally received 1 ml of distilled water.

Group 2: Diabetic, orally received 1 ml of 100mg/Kg body weight of the extract.

Group 3: Diabetic, orally received 1 ml of 200mg/Kg body weight of the extract.

Group 4: Diabetic, orally received 1 ml of 400mg/Kg body weight of the extract.

Group 5: Diabetic, orally received 1 ml of 14.2mg/kg body weight of Metformin.

Group 6: Diabetic, orally received 1 ml of distilled water.

2.6 Collection of Blood and Preparation of serum and Tissue Homogenates (liver and kidney).

After 21 days, the animals were decapitated after an overnight fast. Under ether anesthesia, the neck area of the animals was quickly cleared of fur on the skin to expose the jugular veins. The animals were then made to bleed

through their cut jugular veins into a clean, dry sample bottle which then allowed toclot for 30minutes. The blood samples were centrifuge for 10minutes at 3000g using Uniscope laboratory centrifuge (Model SM800B, Surgifriend medicals, Essex, England). The serum was later separated with Pasteur pipette into clean, dry sample bottle and kept frozen overnight before being used for the assay. The animals were also dissected and the liver and kidney were removed. The organs were weighed. Small portions of the liver and kidney were homogenized separately with 5ml ice-cold 0.25M sucrose solution (pH 7.0). The homogenate was centrifuge at 12,500 rpm for 15minutes under 4°C using an Eppendorf refrigerated centrifuge. The homogenates were immediately transferred into separate sample bottle and kept frozen for further analyses.

2.7 Lipid Profile Analyses

2.7.1 Determination of Total Cholesterol concentration

The concentration of total cholesterol in the serum of animals was carried out using the CHOD-PAP reaction[12].

Briefly, 2 ml of the reagent was added to 0.02 ml of sample. The standard was constituted by adding 2 ml of the reagent to 0.02 ml of standard reagent. The blank was constituted by replacing the sample with distilled water. The reaction constituents were thoroughly mixed and incubated at 37°C for 5 minutes. The absorbance was read against the blank at 546 nm.

2.7.2 Determination of Triacylglycerol Concentration

Serum triglyceride determination was done using the method of Randox laboratories U.K with Randox kits[13].

Briefly, it involved setting test tubes according to Reagent Blank (RB), Standard (STD), and Sample (SAM). Ten micro-litres (10µl) each of the sample and standard were pipette into test tubes labelled SAM and STD, respectively. Then, 1000µl of the reagent was pipette into all the test tubes (Reagent Blank, Standard, and Sample). The test tube contents were mixed thoroughly and incubated for 10 minutes at 25°C. The absorbance of the Sample (Asample) and Standard (Astandard) were measured against the reagent blank at 500nm wavelength. Finally, the concentration of triacylglycerol in the serum was determined.

Calculation:

Concentration of triglycerides in sample = $\frac{\Delta A\ \text{sample} \times \text{Concentration of standard (mg/dl)}}{\Delta A\ \text{standard}}$

2.7.3 Determination of High Density Lipoprotein (HDL)-Cholesterol Concentration

This was also carried out using the method of Randox laboratories U.K with Randox kits [13].

The procedure involved two steps(Precipitation Step and Cholesterol CHOD-PAP Assay).Briefly, the first step involved pipetting 500µl of sample into a test tube. About 1000µl of the precipitant was also pipette into the test tube. They were mixed properly and allowed to stand for 10 minutes at room temperature. The mixtures were centrifuged for 10 minutes at 4,000rpm. After centrifugation, the clear supernatant was separated and used for the next step. Second step involved pipetting 100µl of distilled water into test tube labelled RB (Reagent Blank) only. This was followed by the addition of 100µl of the standard into the second

test tube labeled STD (Standard). Then, 100µl of the supernatant was also pipetted into test tubes labelled SNT (Supernatant). Finally, 1000µl of the reagent was pipetted into all the test tubes (Reagent Blank, Standard, and Supernatant). They were mixed thoroughly and incubated for 10 minutes at 25°C. After the incubation, the absorbance of the sample (Asample) and standard (Astandard) were measured against the reagent blank at 500nm wavelength. The HDL concentration in the supernatant was then calculated thus: Conc. of HDL-Cholesterol in supernatant = ΔA sample x Concentration of standard (mg/dl)

ΔA standard

Low density lipoproteins (VLDL and LDL) cholesterol were calculated as per Friedewald's equationthus:

VLDL Cholesterol = 1/5 of Triglyceride; and

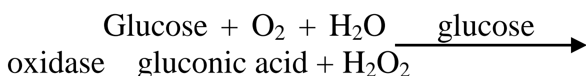
LDL Cholesterol= Total Cholesterol – (VLDL-C + HDL-C)

2.7.4 Estimation of Hepatic Glucose and Glycogen Concentrations

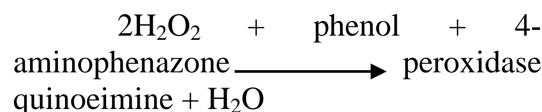
2.7.4.1 Estimation of Hepatic Glucose concentration

Hepatic glucose is determined in diluted hepatic homogenate after enzymatic oxidation in the presence of glucose oxidase[14]. Glucose is oxidised enzymatically to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoeimine dye as an indicator.

Glucose + O_2 + H_2O glucose oxidase gluconic acid + H_2O_2



$2H_2O_2$ + phenol + 4-aminophenazone peroxidase quinoeimine + H_2O



The absorbance of the red-violet indictor from standard and samples were then measured against reagent blank at 500 nm within 60minutes.

Calculation:

Concentration of glucose in sample = ΔA sample x Concentration of standard (mg/dl)

ΔA standard

2.7.4.2 Estimation of Hepatic Glycogen Concentration

Briefly, 100 µl of diluted hepatic homogenate was hydrolysed with 2M HCl and then heated for 2hours at 95°C, followed by neutralization with 2M NaOH. The sample wasanalyzed and result expressed as glucose equivalent by determining glucosyl units using commercial kit for glucose medical diagnosis using Randox kit[15].

2.7.4.3 Determination of Glucose-6-phosphate dehydrogenase (G6PDH) Activity.

The enzyme activity was determined by measurement of the rate of absorbance change at 340nm due to the reduction of $NADP^+$[16].

G-6-P + $NADP^+$ G-6-P-D gluconate -6- p + NADPH + H^+

Statistical Analysis

The results were expressed as mean ± S.E.M (standard error of mean): analyzed by one-way analysis of variance (ANOVA). Means found to be significantly different at $P<0.05$ were separated by Duncan multiple range test.

The statistical evaluation was carried out using graph pad Data Edition version 16.0.

3.0 Results and Discussion

Results

3.1 Effect of Ethanolic Root Bark Extract of *M. oleifera* on Fasting Blood Glucose Level of Diabetic Rats:

All treatment doses of ethanolic root bark extract of*M. oleifera* showed significant (P<0.05) reduction in elevated blood glucose level of streptozotocin-induced diabetic rats. However, oral treatment with 400mg/Kg body weight of ethanolic root bark extract of *M. oleifera* produced a normal glycemic condition after the tenth day of treatment and the effect was maintained until the end of the experiment (Fig.2).

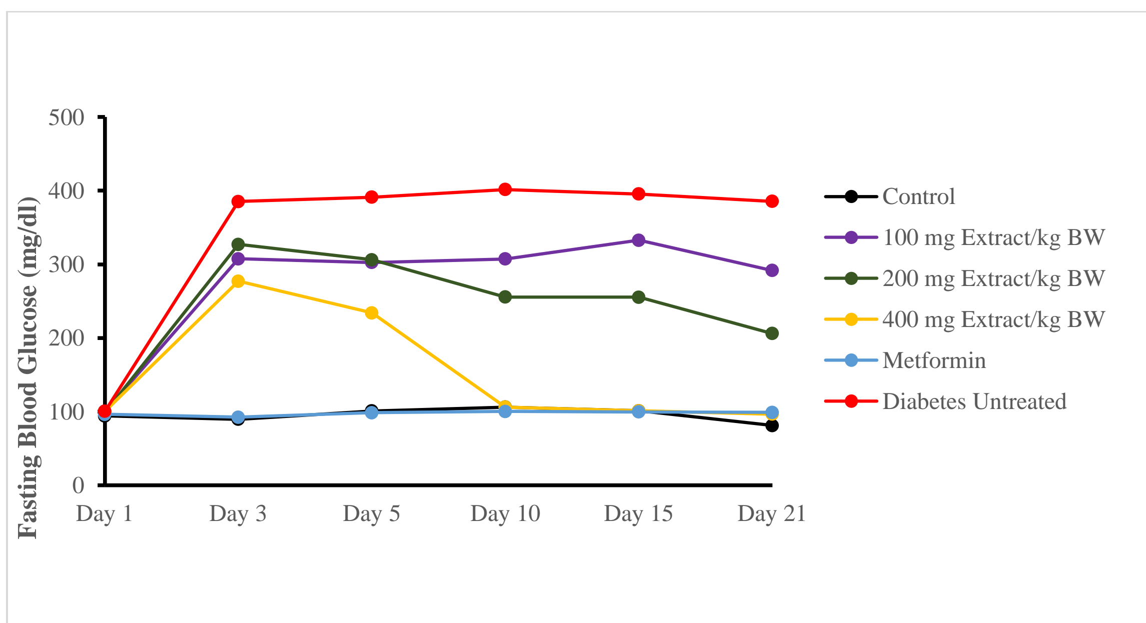


Figure 2**:** Effect of Ethanolic Root Bark Extract of *M. oleifera* on Fasting Blood Glucose Concentration of Streptozotocin - induced Diabetes in Rats

3.2 Effect of Ethanolic Root Bark Extract of *M. oleifera* on Hepatic Glucose of Diabetic Rats

There was significant (P<0.05) reduction in the glucose concentrations in extract- and metformin-treated groups compared with diabetes untreated group V. However, the reduction is concentration dependent with highest extract concentration (400 mg/Kg body weight) having the highest significant reduction (Fig. 3).

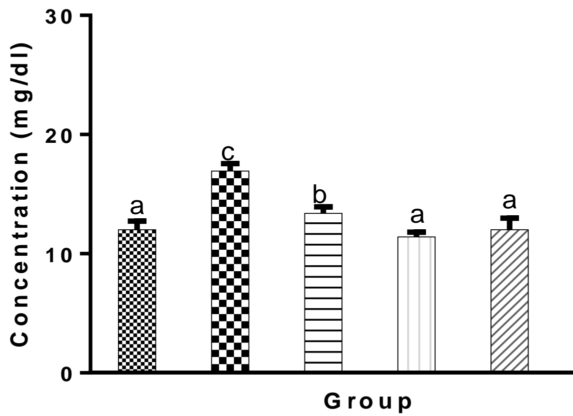


Figure 3**:** Effect of Ethanolic Root Bark Extract of *M. oleifera* on Hepatic Glucose Concentration of Streptozotocin - induced Diabetes in Rats

3.3 Effect of Ethanolic Root Bark Extract of *M. oleifera* on Hepatic Glycogen of Diabetic Rats:

The ethanolic rootbark extract of *M. oleifera*-treated diabetic rats (groups II-IV) showed significant (P<0.05) increased glycogen concentration compared to diabetic untreated groups. Furthermore, the significant increase in glycogen concentrations were concentration dependent among the extract concentrations with the highest extract concentration producing the highest concentration of glycogen. Standard drug, Metformin produced the highest glycogen concentration compared with all other groups except normal control group I. Also, there was a significant (p<0.05) reduction in the glycogen concentration in diabetic untreated group VI compared with normal control group I (Fig.4).

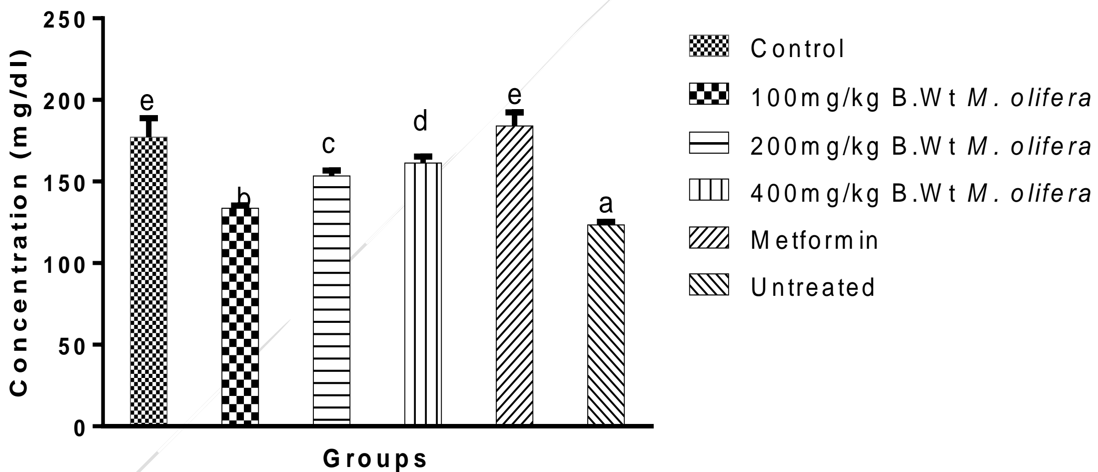


Figure 4: Effect of Ethanolic Root Bark Extract of *M. oleifera* on Hepatic Glycogen Concentration of Streptozotocin - induced Diabetes in Rats

3.4 Effect of Ethanolic Root Bark Extract of *M. oleifera* on Serum Total Cholesterol of Diabetic Rats

The level of total cholesterol were significantly (P<0.05) increased in the diabetic untreated group VI compared withall other groups. All the extract-treated groups II-IV showed significant (P<0.05) decreased serum level of total cholesterol with maximum reduction observed in the 400 mg/Kg body weight of extract-treated group IV. However, metformin-treated group V has the highest significant (P<0.05) reduction

compared with all other groups except the diabetic untreated group VI (Fig.5).

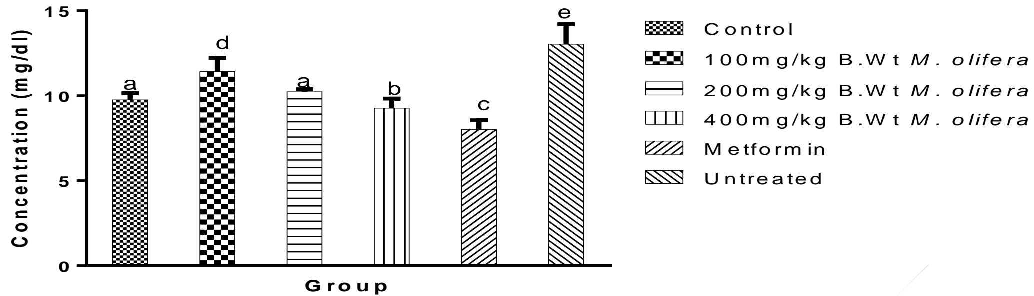


Figure. 5: Effect of Ethanolic Root Bark Extract of *M. oleifera* on Serum Total Cholesterol Concentration of Streptozotocin - induced Diabetes in Rats

3.5 Effect of Ethanolic Rootbark Extract of *M. oleifera* on Serum Triglycerides of Diabetic Rats

There was a statistically significant (P<0.05) elevation in the level of serum triglyceride in the diabetic control group VI when compared with all other groups I-V. Meanwhile there was no statistically significant difference in serum triglyceride of rats treated with 100 and 200 mg/Kg body weight of the extract when compared with each other. However, group IV rats that were treated with 400mg/Kg body weightof the extract showed significant (P<0.05) reduction in their serum triglyceride level compared with other extract-treated groups II and III. The standard drug, Metformin-treated group V reduced the serum triglyceride to normal when compared with normal control group I (Fig.6).

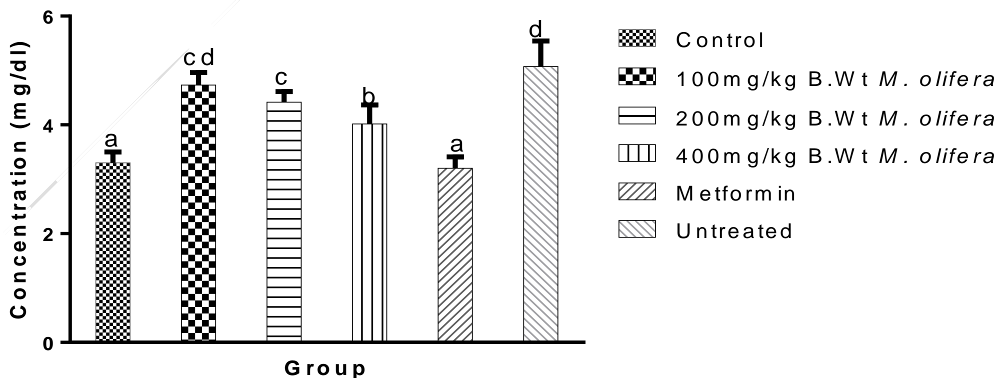


Figure 7: Effect of Ethanolic Root Bark Extract of *M. oleifera* on Serum Triglycerides Concentration of Streptozotocin - induced Diabetes in Rats

3.6 Effect of Ethanolic Root Bark Extract of *M. oleifera* on HDL-c of Diabetic Rats:

HDL-c level was significantly (P<0.05) reduced in the diabetic untreated group VI when compared with all other groups I-V. However, all other treated groups showed statistically significant (P<0.05) increase when compared with the normal control except in 100ml/Kg body weight group II, which showed no difference. The reduction ability of the extract here is concentration dependent with 400 mg/Kg body weight-treated group IV showing highest significant (P<0.05) increase compared with other extract-treated groups II and III (Fig.7).

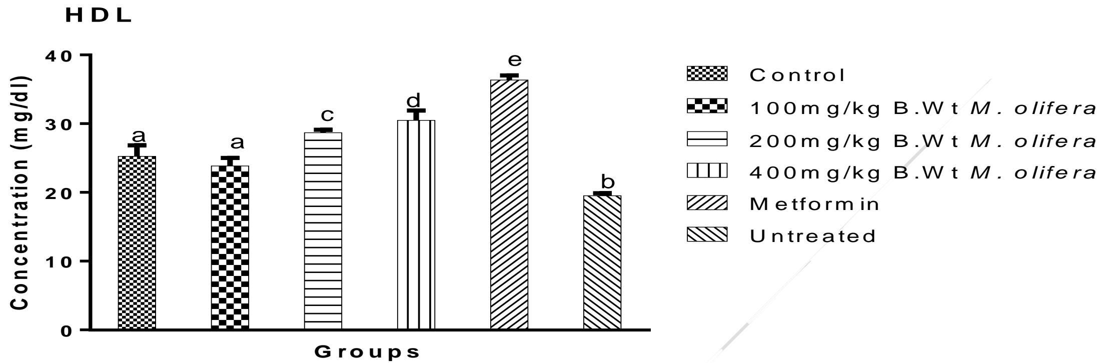


Figure 7: Effect of Ethanolic Root Bark Extract of *M. oleifera* on HDL-c Concentration of Streptozotocin - induced Diabetic Rats

3.7 Effect of Ethanolic Root Bark Extract of *M. oleifera* on the Activity of Glucose-6-phosphate dehydrogenase of Diabetic Rats

There was a significant (P<0.05) reduction in the activity of G-6-P-dehydrogenase (G6PDH) in diabetic untreated group VI compared with all other groups. The effect of ethanolic root bark extract of *M. oleifera* on G6PDH is concentration dependent with 400 mg/Kg body weight having the highest activity of the enzyme. Furthermore, there was no significant difference in the activity of G6PDHbetween the highest extract concentration (group IV) and Metformin-treated group V (Fig.8).

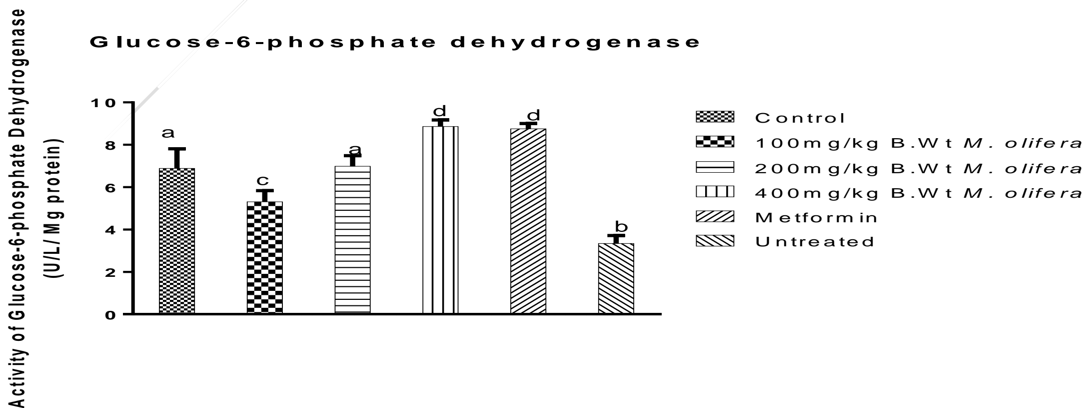


Figure 8: Effect of Ethanolic Root Bark Extract of *M. oleifera* on Activity ofG-6-P-dehydrogenaseinStreptozotocin - induced Diabetes Rats

3.8 Effect of Ethanolic Root Bark Extract of *M. oleifera* on Atherogenic Index:

The result showed that the levels of atherogenic index were significantly (P<0.05) higher in the diabetic untreated control group VI compared with all other groups. However, the effect of the extract on atherogenic index in the extract-treated rats is concentration dependent with the 100 mg/Kg body weight having the highest level. Furthermore, there was no significant difference in atherogenic index level between rats in normal control group I and those treated with 400 mg/Kg body weight group IV. Metformin-treated group V has the least level of atherogenic index compared with all other groups (Fig.9).

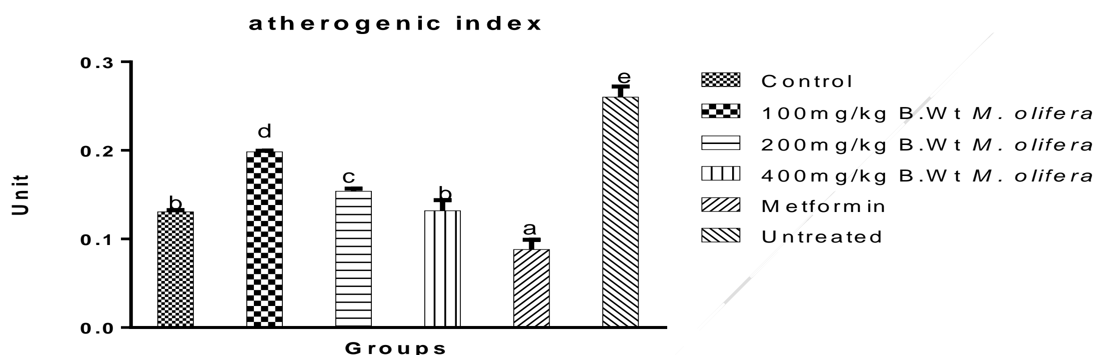


Figure 9**:** Effect of Ethanolic Root Bark Extract of *M. oleifera* on Atherogenic Index of Streptozotocin - induced Diabetes in Rats

3.0 Discussion

The present study evaluated the effect of ethanolic root bark extract of *M. oleifera* on fasting blood glucose and serum lipid profile in STZ-induced diabetic rats. Insulin is an hormone responsible for conversion of excess glucose in the blood to glycogen and then stored in the liver or muscle. The production of insulin is carried out in pancreatic β-cells. STZ can completely destroy the pancreatic β-cells thereby inducing diabetes due to the presence of excess glucose in the blood. Hyperglycemia results from deficiency or reduction in insulin production. The obtained significant (P<0.05) reduction in fasting blood glucose, hepatic glucose concentration, serum total cholesterol, serumtotal triglycerides and atherogenic index concentration in diabetic rats after the administration of ethanolic root bark extract of *M. oleifera* may be associated with phytochemicals in the extract, which might have been responsible for decreased glucogenic activity. This decrease may lead to a reduction in urea excretion. It may also cause inhibition of glycogenolysis due to an observed increase in liver glycogen[17].Similar reports have been obtained after the administration of medicinal plants by earlier researchers [18, 19, 20]. G6PDH is an enzyme located in the pentose phosphate pathway. This pathway supplies and maintains nicotinamide adenine dinucleotide phosphate (NADPH), a reducing energy in cells. The observed significant reduction in the activity of G6PDH in diabetic untreated group is due to the reduction of this

reducing equivalent[21, 22, 23]and this leads to an increase in oxidation stress[24]. This then aggravates to complications in diabetes [22]. The administration of ethanolic root bark extract of *M. oleifera*, boosted the activity of G6DPH in extract-treated groups, probably, by increasing the inflow of glucose into pathways that reduce, use up or store the glucose as glycogen. For examples, such pathways include Glycolytic pathway and hexose monophosphateshunt, the alternative pathways of glucose metabolism toreduce the levels of glucoseinblood. This observed increased activity of G6DPH in ethanolic root bark extract of *M. oleifera* may have led to an increased production of reducing agent NADPH, and a concomitant decrease in oxidative stress [24].This observation is further supported by the observed decreased in hepatic glucose level in the extract-treated groups compared with the untreated diabetic control rats. One of the common complications in diabetes patients is abnormalities in lipid metabolism [25, 26]. This include variations in the levels of serum lipids and different lipoproteins, which are associated with atherosclerosis [27]. Furthermore, some of the major important features of diabetic dyslipidemia are low HDL cholesterol with corresponding highserum TG and LDL cholesterol concentration [28].The observed high concentrations of triglyceride, total cholesterol, and decrease in high density lipoprotein in diabetes untreated rats compared to normal rats group in this study, agreed with earlier reports [29], which suggests that as the glucose level is elevated (which indicates the absence or inadequacy of insulin), the serum lipid profile of the diabetic rats is also compromised, leading to dyslipidemia. Dyslipidemia is a factor of cardiovascular diseases associated with diabetes mellitus. It is often characterized by elevated cholesterol, triglycerides, LDL-c and reduced HDL-c [30]. There is therefore a need for therapeutic agents that can revert these anomalies in lipid metabolism. The ethanolic root bark extract of *M. oleifera* may serve these benefits. The administration of the standard drug and this extract led to a drop in TC,TG, LDL and VLDL with a corresponding increase in HDL. Therefore, the ethanolic root bark extract of *M. oleifera* shows that it may regulate lipid metabolism and prevent the upsurge of atherosclerosis and coronary artery disease in diabetic rats. This dyslipidemic effect of *M. oleifera* confirmed the report of Lin *et al*. [31] and is also in accordance with findings of Chen *et al*. [32]. Atherogenic index has been shown to be useful in monitoring atherosclerosis [33]. Among the important findings of this present study is the significant reduction in atherogenic ratio, confirming the statement of Orwa *et al.* [33] that is useful in studying atherosclerosis. High levels of triglycerides and more importantly LDL cholesterol are major coronary risk factors because it carries cholesterol to tissues, including the heart arteries. The result of this study indicates that diabetes predispose to increase coronary risk in diabetic untreated group whereas the administration of ethanolic root bark extract of *M. oleifera,* most especially at 400 mg/Kg body weight lowers the risk.

4.0 Conclusion

Result obtained in this study indicates that ethanolic root bark of *M. oleifera*

contains numerous medicinal properties that may serve as an alternative to the treatment of hyperglycemia and dyslipidemia in diabetes patients.

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