Acute Effect of Moringa Oleifera Raw Seeds on Blood Glucose, Serum Lipid and Liver Enzyme Profiles in Alloxan–Induced Diabetic Albino Wister Rats

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ABSTRACT

Background and Objective: Moringa oleifera has multiple uses and almost all parts of the plant form part of the traditional diet in many Tropical and Sub-tropical countries. It is not clear how the dried raw seed was eaten affects diabetes. Research has shown that the raw seed has a wide range of medical properties and nutritive values. Most of the plants used for diabetes mellitus are not edible therefore studying a plant with hypoglycemic activity would have to amerce value in the management of the disease.

Material and Method: The diabetic state was confirmed after 72 hours of induction of diabetes with 150mg/kg of alloxan. The animals were separated into 5 groups (n=5). Group1 and 2 served as normal control (NC) and Diabetic non Treated (DNT) feed with normal chow and water ad libitum. Group 3 received metformin hydrochloride orally. Group 4 and 5 received 450mg and 350mg of raw Moringa oleifera powder orally. A weekly analysis of blood glucose from the tail vein was done. Animals were sacrificed at the end of 14 days and serum was collected for determination of serum lipids and enzymes profile.

Result: Total cholesterol (TC), Triglyceride (TG), Low-density lipoprotein (LDL), Very low-density lipoprotein (VLDL) were seen to be reduced while High-density lipoprotein (HDL) was seen to be significantly increased at the end of two weeks. ALT, AST and ALP in the treatment groups at the end of the period were seen to be decreased significantly.

Conclusion: Moringa oleifera Raw seeds given at doses 350mg, and 450mg/day showed anti hyperglycemic and hepatoprotective activity against alloxan induced diabetes in the rat.

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Keywords: Moringa Oleifera Raw Seed, Diabetes, Enzymes, Lipids, Rats.

1. Introduction:

Moringa oleifera leaves, seeds, bark, roots, sap, and flowers are widely used in traditional medicine, and the leaves and immature seed pods are used as food products in human nutrition. Several safety studies in animal involving aqueous, ethanolic and methanolic extracts indicate a high degree of safety. Moringa oleifera leaves have been found to contain a desirable nutritional balance, containing vitamins, minerals amino acids, and fatty acids (Moyo et al., 2011; Teixeira et al., 2014; Abdull et al., 2014). According to various commentaries (Anwar et al., 2007; Mbikay, 2012; Abdull et al., 2014), various preparations of Moringa oleifera are used for their anti-inflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, anti-neoplastic, antipyretic, antiulcer, cardio protectant and hepato protectant activities. The therapeutic potential of Moringa oleifera in treating hyperglycemia and dyslipidemia has been reviewed by Mbikay, (2012).

Several animal studies have assessed the toxicity of different preparations on Moringa oleifera. The safety of an aqueous leave extract was given orally to rats at doses

of 400, 800, 1600, and 2000mg /kg body weight has been examined. Toxicity study in mice by Araújo et al., (2013), following a 14days of extract administration (500 and 2000mg/kg) showed no signs of systemic toxicity, with the survival of all the animals, no change in organ indices between test and control groups. Doses of 4000 and 5000mg/kg methanolic seed extract of Moringa oleifera used for acute toxicity studies revealed signs of toxicity at 4000mg/kg and mortality at 5000mg/kg (Ajibade et al., 2013).

The seeds also have antimicrobial activity and are utilized for waste water treatment. In some developing countries, the powdered seeds of Moringa oleifera are traditionally utilized as natural coagulating properties for sedimentation of suspended undesired particles (Kalogo et al., 2000; Anwar et al., 2007). One of the major characteristics of diabetes mellitus (a chronic metabolic disorder) is the persistent (hyperglycemia) and other associated clinical complications such as glycosuria, increased total cholesterol level, and keto acidosis. This disorder which leads to chronic hyperglycemia and glucose tolerance impairment is as a result of a metabolic disorder of the endocrine system that precipitates disturbance in glucose, lipid and protein homeostasis (Tiwari & Rao 2002; Van den Berghe et al., 2006; Sangeeta et al., 2010). Furthermore, oxidative stress concurrently occurs with hyperglycemia (Vijayalingam et al., 1996) and causes pathogenesis, in many organs, such as vasculopathy, neuropathies, nephropathies and opthalamopathies (Atkinson & Maclaren, 1994). Thus patients suffering from this condition normally use synthetic drugs to control their blood glucose level and to improve blood glucose tolerance (Nath et al., 1992; Singh & Kumar, 1999; Gupta & Mishra 2002) and other clinical complications associated with the disease.

The incessant rate of diabetes mellitus and its clinical complications have become a source of worry in Nigeria particularly in the Northern Senatorial District of Cross River State. Most patients in these areas are poor and cannot afford the hospital bills for the treatment of this disease and its associated clinical complications. Worst still is the long distances and bad access roads to the urban towns. Most often, therefore, they eat raw seeds of Moringa oleifera as a common means of ameliorating diabetes mellitus. Studies that reveal the effects of Moringa oleifera raw seeds on blood glucose level, total lipid profile and enzyme profile level in alloxan induced diabetes mellitus are still rare. Therefore it becomes necessary to investigate the effect of Moringa oleifera Raw seed on blood glucose, lipid and enzyme profile in alloxan induced diabetes mellitus in the rat.

2. Materials and Methods:

2.1. Preparation of Moringa oleifera Raw Seeds

The dry seeds of Moringa oleifera were collected from a home garden at Okuku in Yala Local Government of Cross River State, Nigeria (June, 2015).They were sun dried and stored in air tight container before use.

2.2. Experimental Animals

Twenty-five (25) albino Wister rats weighing between 180-230g were obtained from the Animal House of the Department of Human Physiology, Cross River University of Technology, Okuku. The animals were acclimatized for one week and their weight noted before the commencement of experimental treatment. The animals were housed in stainless steel cages at a temperature (28± 2C) and had 12 hours light-dark cycle. They were fed and allowed access to water ad libitum. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily and treated for 14 days. The rats were randomly assigned into five (5) groups of five rats as follows:

Group 1: Normal control: (non-diabetic) fed with normal feeds and water

Group 2: Diabetes non treated; fed with normal feeds and water

Group 3: Diabetes test group: treated with 0.4ml of metformin hydrochloride orally

Group 4: Diabetes test group: treated with 0.9ml of Moringa oleifera Raw seeds orally

Group 5: Diabetes test group: treated with 0.7ml of Moringa oleifera Raw seeds orally.

2.3. Induction of Diabetes Mellitus

The animals were fasted 12 hours prior to the induction of diabetes mellitus, their body weight was also noted and the freshly prepared alloxan (Sigma) was injected 150/kg dissolve in normal saline intraperitoneally (Szkudelski et al., 2001). After 2hours there were allowed to eat and drink ad libitum. Blood samples were collected from the tail vein after 72 hours to confirm the diabetic state using glucometer (Accu-Chek sensor from Roche Diagnostic Corporation) and results were expressed as mg/dl (Sangeeta et al., 2010). The test was done early hours of each test day at 9 to 10.00 o'clock in the morning. However, all experiments on the animals were carried out in absolute compliance with ethical guide line for research, care, and use of laboratory animals.

Administration of Metformin Hydrochloride Moringa oleifera Raw Seeds Metformin hydrochloride was given 100mg/kg was given orally to each experimental rat in the group for 14 days. Dried Moringa oleifera seed powder 350mg (0.7ml) and 450mg (0.9ml) were given an orally as low dose and high dose respectively to test groups for 14 days of treatment.

2.4. Collection of Blood and Serum Samples

After fourteen days of treatment, the rats were subjected to an overnight fast and were sacrificed and blood is withdrawn by cardiac puncture using a 10ml syringe into properly labeled nonheparinized tubes. The sample bottles were allowed to stand for one hour after

which they were spun at a speed of 3000 revolutions per minute (rpm) for five minutes using a centrifuge. At the end of the spinning process, the sample bottles were removed from the centrifuge and the uppermost straw-colored serum was drawn out using syringes. The serum samples were emptied into different sample bottles properly labeled for the determination of serum lipid and enzyme profile.

2.5. Analysis of result

2.5.1. Blood Glucose Level

Blood glucose level was done weekly at 9 - 10 am using glucometer Accu-Check sensor from Roche Diagnostic Corporation as described by Sangeeta et al. (2010). Standard methods were employed for the assay of serum lipids and enzymes: Radoxdiagnostic assay kits were used for lipids Total cholesterol (TC), Triglyceride (TG), High-density lipoprotein (HDL) (Hiller 1987).

Low-density lipoprotein (LDL), (Burtis & Ashwood, 1994), Aspartate aminotransferases (AST), Alanine aminotransferase (ALT) (Reitman & Frankel, 1957), Alkaline phosphatase (ALP) (Wright et al, 1972).

2.5.2. Statistical Analysis

Data are expressed as mean ± SEM (standard error of the mean). Data were analyzed using one way ANOVA (analysis of variance), followed by a post hoc (LSD) test for significant value. P –values of less than 0.05 were considered statistically significant.

3. Results:

3.1. Blood Glucose Level (BGL)

Figure 1 showed that in the first week, blood glucose levels in treatment groups was significantly high (P< 0.05) compared with normal control group, But there was no significant difference when compared with the non-treated group. In the second week, the treatments groups had significantly decreased levels of glucose (P< 0.05) compared with DNT.

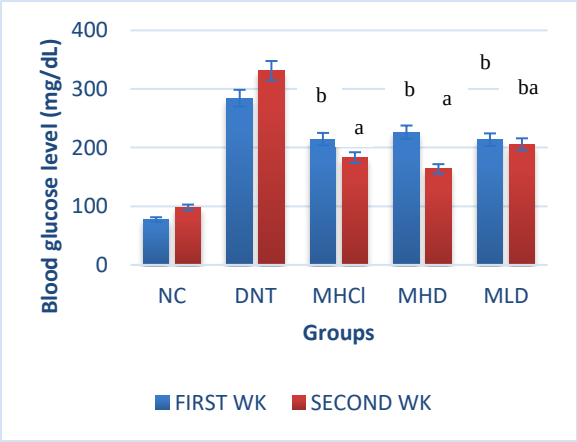

Figure 1. Comparison of blood glucose levels in different experimental rats, n= 5.

[a] significantly different from DNT (P <0.05) [b] significantly different from NC (P< 0.05).

3.2. Lipid Profile

3.2.1. Total Cholesterol (TC)

Results for Total cholesterol (Figure. 2) showed a significant decrease (P < 0.05) in treatment groups compared with DNT but there were no significant differences between the Moringa oleifera groups and normal control. However, the total cholesterol level in MHCl decreased significantly (P<0.05) compared with MHD and NC.

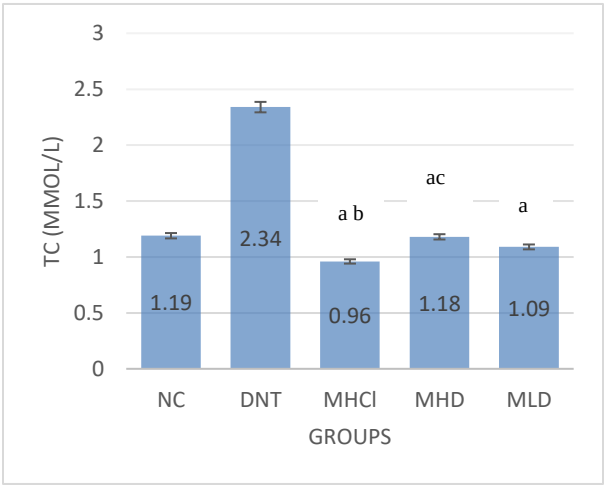

Figure 2. Comparison of total cholesterol levels in different experimental rats, n= 5.

[a]significantly different from DNT (P <0.05), [b] significantly different from NC (P< 0.05), [c] significantly different from MHCl (P< 0.05).

3.2.2. Triglyceride (TG)

Figure. 3 showed that Triglyceride levels in treatment groups significantly decreased compared with NC and DNT (P< 0.05)

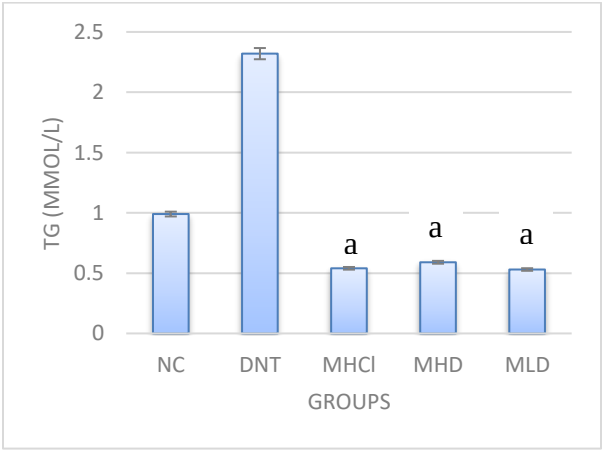

Figure 3. Comparison of triglyceride levels in different experimental rats, n= 5.

[a] significantly different from NC and DNT (P< 0.05)

3.2.3. High-Density Lipoprotein (HDL)

The results for High-Density Lipoprotein (Figure 4) showed that the levels of HDL in treatment groups significantly decreased (P< 0.05) compared with DNT but there were no significant differences between the Moringa groups and normal control. However, the HDL level in MHCl decreased significantly (P<0.05) compared with Moringa groups and NC.

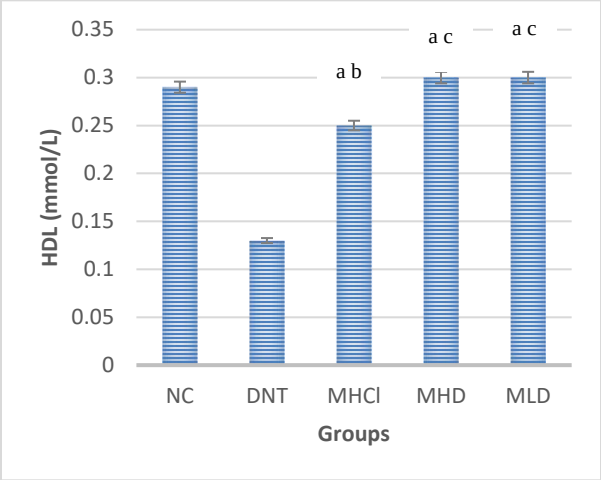

Figure. 4 Comparison of high-density lipoprotein in different experimental rats, n= 5.

[a]significantly different from DNT (P <0.05), [b] significantly different from NC (P< 0.05), [c] significantly different from MHCl (P< 0.05).

3.2.4. Low-Density Lipoprotein (LDL)

The result obtains for low-density lipoprotein (Figure.5) showed that LDL levels in treatment groups significantly decreased (P< 0.05) compared with DNT, but there were no significant differences between treatment groups and NC.

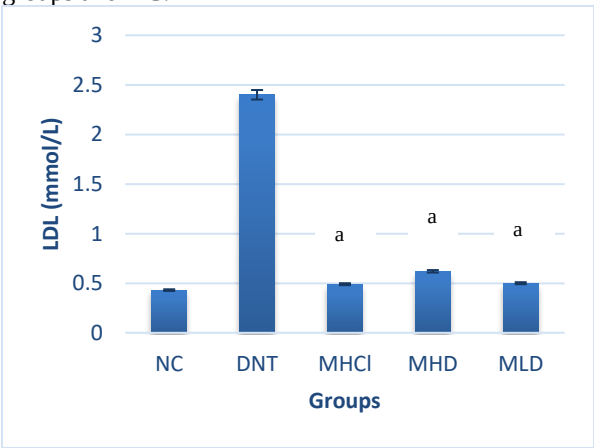

Figure 5. Comparison of low-density levels in different experimental rats, n= 5.

[a]significantly different from DNT (P <0.05)

3.2.5. Very Low-Density Lipoprotein (VLDL)

Figure. 6 shows the result of very low-density lipoprotein (VLDL) levels in treatment groups significantly decreased compared with NC and DNT (P< 0.05).

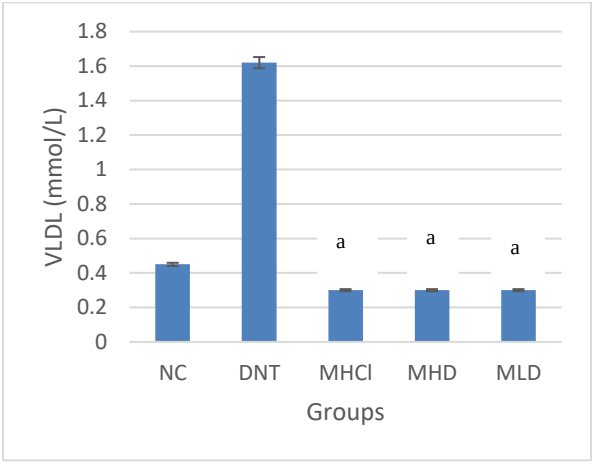

Figure 6. Comparison of very low-density levels in different experimental rats, n= 5.

[a] significantly different from NC and DNT (P< 0.05)

3.3. Liver Enzymes

3.3.1. Alanine Amino Transferase (ALT)

The result of Alanine amino transferase (Figure 7) showed that ALT in the treatment groups decreased significantly (P< 0.05) compared with DNT. However, in Moringa groups, ALT levels decreased significantly (P< 0.05) compared with MHCl and NC groups.

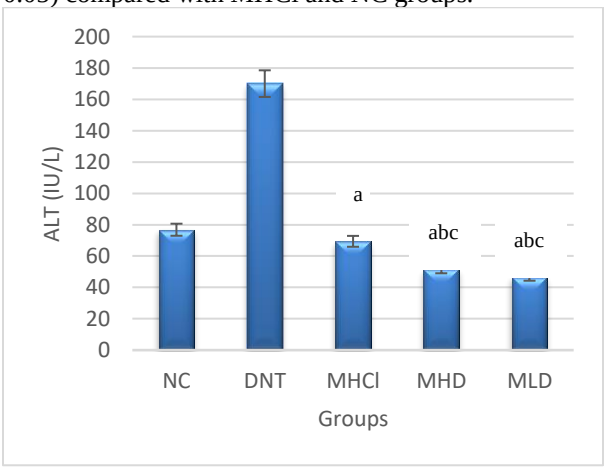

Figure 7. Comparison of alanine aminotransferase levels in different experimental rats.

[a]significantly different from DNT at P< 0.05, [b] significantly different from NC at P< 0.05, [c] significantly different from MHCl at P< 0.05

3.3.2. Aspartate Amino Transferase (AST)

Figure 8 showed that AST levels in treatment groups significantly decreased (P< 0.05) compared with DNT. The levels in MHCl and MHD did not show significant differences compared with NC. But the levels of AST in MLD decreased significantly (P< 0.05) compared with MHCl and NC.

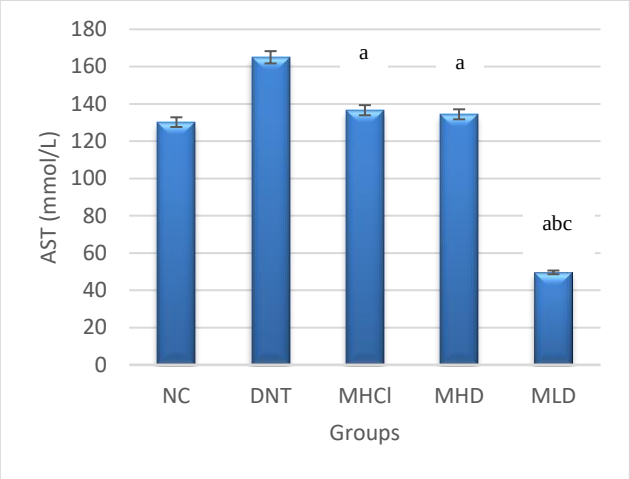

Figure 8. Comparison of aspirate aminotransferase levels in different experimental rats, n= 5

[a] significantly different at P< 0.05 from DNT,
[b] significantly different at P < 0.05 from NC,
[c] significantly different at P< 0.05 from MHCl

3.3.3. ALT/AST Ratio (Figure. 9)

The ratio in MHD and MHCl significantly decreases (P<0.05) compared with DNT and MLD.

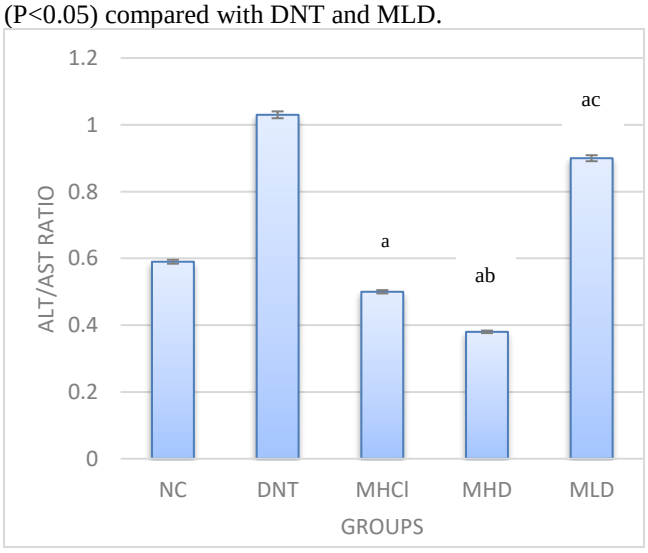

Figure 9. Comparison of ALT/AST ratio in different experimental rats, n= 5

[a] significantly different from DNT at P< 0.05,
[b] significantly different from NC at P < 0.05,
[c] significantly different from MHD and MHCl at P < 0.05.

3.3.4. Alkaline Phosphatase (ALP)

The result of Alkaline Phosphatase (Figure 10) showed that the levels of ALP in treatment groups decreased significantly (P< 0.05) compared with DNT. The Moringa groups showed a significant decrease compared with NC and MHD (P<0.05).

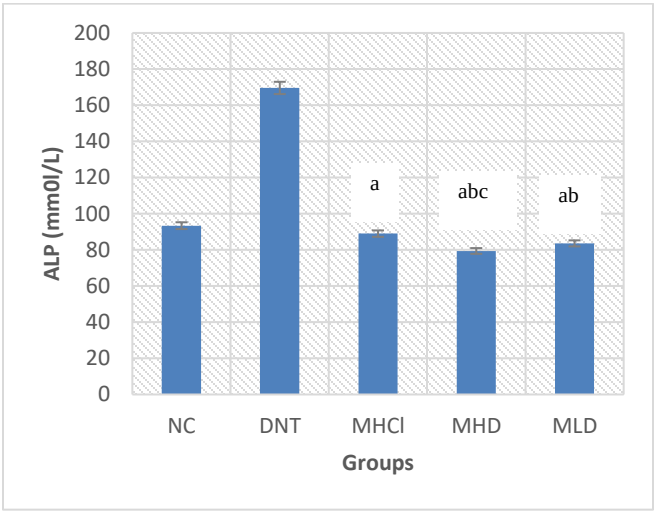

Figure 10. Comparison of alkaline phosphate levels in different experimental rats, n= 5

[a] significantly different from DNT at P< 0.05,
[b] significantly different from NC at P < 0.05,
[c] significantly different from MHCl at P < 0.05

4. Discussion:

This study focus on the effects of Moringa oleifera raw seeds on blood glucose level, lipid and liver enzymes profile in comparison with Metformin hydrochloride in alloxan induced diabetic albino Wistar rats. In the first week, blood glucose levels in treatment groups were significantly higher compared with the normal control. The treatment groups showed a percentage decrease in blood glucose levels compared with diabetic non treated group. Though the decrease was not statistically significant at the end of the second week, the levels of blood glucose in treatment groups significantly decreased compared with diabetic non treated group. From the result obtained, the levels of blood glucose in Moringa oleifera high dose group and MHCl groups were not significantly different from NC, but Moringa oleifera high low group showed a significant increase compared with normal control. This may be as a result of dose differences.

One of the major characteristics of diabetes mellitus is the persistently high level of blood glucose known as hyperglycemia. This disorder which leads to chronic hyperglycemia and glucose tolerance impairment is as a result of a metabolic disorder of the endocrine system that precipitates disturbance in glucose (Tiwari & Rao 2002; Van den Berghe et al., 2006; Sangeeta et al., 2010). Sustained higher levels of blood sugar cause damage to the

blood vessels and to the organs they supply, leading to the complications of diabetes (Giugliano et al., 1997). Induction of diabetes using alloxan has been described as a useful experimental model for studying the effects of hypoglycemic agents (Szkudelski et al., 2001). Alloxan and the products of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals.

These radicals undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration, resulting in the destruction of pancreatic beta cells and severe hyperglycemia (Szkudelski et al., 2001). Alloxan induces a multiphasic blood glucose response when injected into to an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultra-structural beta cell changes ultimately leading to necrotic cell death (Goldner & Gomori, 1944; Ajibola et al., 2014)

Chemical compounds isolated from Moringa oleifera have been shown to contain useful pharmacological properties with prospective medicinal applications (Rockwood et al., 2013). A list of possible medical applications conferred by Moringa oleifera plant parts includes, but is not limited to, hypoglycemia capabilities, (USDA, 2003; Ghebremichael et al., 2005; Katayon et al., 2006; Anwar & Rashid, 2007). It has reported that hypoglycemic activity of Moringa oleifera, with significant blood glucose lowering activities, has been confirmed. The methanol extract of its dried fruit powder produced N-Benzyl trio-carbonate, N-benzyl carbonates, benzyl nitrites and a benzyl; which prove to trigger insulin release significantly from the rodent pancreatic beta cells and have cyclooxygenase enzyme and lipid peroxidation inhibitory activities (Francis et al., 2004). Moringa oleifera seed has been reported to reverse the inhibition of insulin secretion from the pancreatic beta cells (Ajibola et al., 2014) indicating that it can be used as a curative for the ameliorating of hyperglycemia and diabetes as well.

Taken together lipid profile showed that TC, TG, LDL, and VLDL levels in treatment groups decreased significantly when compared with diabetic non treated (DNT) The decrease in TG, LDL, and VLDL in the treatment groups indicates a decreased risk of atherosclerosis. Mehta et al. (2003), have shown that the administration for 120 days of Moringa oleifera fruit, like lovastatin, lowered the serum TC, phospholipids, TG, LDL, VLDL cholesterol ratio and atherogenic index. The observed reduction of TC TG VLDL and LDL in Moringa groups suggests that Moringa oleifera Raw seeds possess cholesterol lowering ability (Mehta et al., 2003). Mehta et al. (2003), observed that Moringa oleifera treated rabbits had higher levels of cholesterol in their feces, suggesting that Moringa oleifera promotes gastrointestinal excretion of cholesterol.

HDL levels increased significantly in treatment groups compared with DNT. Elevated levels of HDL suggest a decreased risk of atherosclerosis. HDL has anti-atherogenic properties since it transports cholesterol from peripheral tissue to the liver thereby reducing the amount stored in the tissues and the possibility of developing atherosclerotic plaque, Physiologically increase HDL in the rat is beneficial to its health. It has been shown that increase in HDL reduces the high risk of coronary heart disease (CHD). Several authors have reported on the composition of Moringa seeds agents responsible for lowering cholesterol. Mehta et al. (2003), reported that the administration for 120 days of Moringa oleifera fruit, like lovastatin, increase the high-density lipoprotein (HDL). It has also been reported that it contains cyclooxygenase enzyme and lipid peroxidation inhibitory activities (Francis et al., 2004). It can be inferred that the increased serum HDL in treatment groups suggest a reduced risk factor for atherosclerosis and cardiovascular related disorders which can be beneficial to man.

Liver enzyme profile result showed a significant decrease in ALT, AST and ALP activity in treatment groups compared with DNT. Increase ALT activity is known to indicate liver disease and it is used as a tool to measure hepatic necrosis (Bush 1991; Duncan et al., 1994) while increased in AST activity indicates acute liver damage. Increased ALT and AST activities have been reported by several authors to be indicators of calculated risk of cardiovascular diseases. According to Pincus and Schaffner (1995), ALT and AST are released into the blood when there is a severe hepatocellular injury. In normal individuals, ALT value is higher than AST value and so their ratio ALT/AST is more than 1. Reverse ratio means that AST value becomes greater than ALT value and so AST/ALT>1. It was discovered that the Moringa oleifera seed extract exhibited anti-fibrotic effects on liver fibrosis in rats (Hamza, 2010) and showed significant protective effects against CCl4-induced liver fibrosis in rats which were confirmed by histological findings as well as biochemical analysis of a maker of collagen deposition in the liver known as hydroxyproline. In the work of Hamza (2010), treatment with Moringa was found to stimulate hepatoprotective effects against hepatocellular injury by blocking the increase of two enzymes, ALT, and AST, which are indicators of liver health conditions. The hepatoprotective properties of Moringa seed extract which was discovered from an anti-fibrotic study by Hamza (2010), indicate that the Moringa oleifera also possessed anti-inflammatory properties against CCl4-induced liver damage and fibrosis. ALP is reported as a marker for plasma membrane and endoplasmic reticulum and is found in cells lining the biliary ducts, liver, etc (Dasofunjo et al., 2013) an increase in ALP is a sensitive indicator of cholestasis.

The observed increase in serum ALP in DNT may suggest biliary duct obstruction, intrahepatic cholestasis, and infiltrative disease of the liver while the decrease in

serum ALP in the treatment groups may be beneficial in the metabolic activity of the liver.

5. Conclusion:

Moringa oleifera Raw seeds given at a dose of 350mg and 450mg/day (2 to 3 seeds respectively) showed anti-hyperglycemic and hepatoprotective activity against alloxan induced diabetes mellitus in the rats. The increased ALT and AST activity and ratio in DNT indicate damage to the heart and liver.

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