EFFECT OF AQUEOUS EXTRACT OF MORINGA OLEIFERA SEED ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT
The objective of this research work is to investigate some biochemical parameters in the serum of normal and alloxan-induced diabetic rats treated with Moringa oleifera seeds aqueous extract. Alloxan-induced diabetic rats (150mg/kg) were administered orally with Moringa oleifera seed aqueous extract (200 mg/kg) for twenty eight days after which some biochemical parameters in the serum was measured and compared with the control. The results showed that the level of urea, creatinine, uric acid, PO43- enzymes markers (ALT, AST, ALP) of the diabetic group were significantly (p<0.05) high when compared with the control and extract treated groups while significant (p<0.05) reduction were observed in the serum level of Ca2+ and Mg2+ of the diabetic untreated group when compared to both extract treated and control group. The administration of aqueous seed extract of Moringa oleifera to the diabetic rats showed significant (P<0.05) reduction in the level of urea, creatinine, uric acid, PO43-, enzymes markers (ALT, AST, ALP) and a significant (P<0.05) increase in the level of Ca2+ and Mg2+. Therefore, It can be concluded that administration of aqueous seed extract of Moringa oleifera to diabetic rats ameliorate the adverse effects of diabetes complications.

Keywords: Enzymes, seeds, Diabetes, Alloxan monohydrate, electrolytes Moringa oleifera

INTRODUCTION
Diabetes mellitus is a disease of worldwide significance and increasing prevalence. It is a multifactorial disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system (Subbiah et al., 2006; Amos et al., 1997). According to World
Health Organization, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million people living with diabetes mellitus and the numbers is likely to rise to 300 million or more by the year 2025 (Jadhav et al., 2009). Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both (Kangralkar et al., 2010). Beside hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis (Saikat et al., 2008). Insulin affects many sites of mammalian lipid metabolism. It stimulates synthesis of fatty acid in liver adipose tissue and in the intestine (Suryawanshi et al., 2006). It has been shown that pathogenic course of both Type 1 and Type 2 DM involves alterations in the structures, organization and protein functions of membranes of cells and tissues (e.g. retina, glomerulli, erythrocyte, nerve), culminating in diabetic complications such as retinopathy, nephropathy and peripheral neuropathy (Budak et al., 2004; Carneiro, 2004; Zaman, 2006).

Hyperglycemia is clinical hallmark of DM but etiology of this heterogeneous disorder likely involves multiple genetic and environmental interactions that ultimately result in alterations in insulin secretion, insulin action or both. Several approaches like lifestyle changes, food intake modifications: lowering the fat content (Van Dam et al., 2002) or enhancing the fiber and magnesium content of the diet (Lopez-Ridaura et al., 2004) and/or physical activity promoting weight loss, smoking status (Tuomilehto, 2005), moderate coffee (Van Dam and Hu, 2005), moderate alcohol consumption (Conigrave et al., 2001) and finally bariatric gastric surgery have been tried for the prevention of DM (Gruber et al., 2006). A wide range of medicinal plants have been used by various cultures to treat diabetes mellitus because of their hypoglycaemic properties (Adesokan et al., 2009).

*Moringa oleifera* (family *Moringaceae*) is commonly known as Drumstick tree. *Moringa* leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (Tesfay et al., 2011). It has great use medicinally both as preventative and treatment. Its bark, sap, roots, leaves, seeds, oil, and flowers are used in traditional medicine in several countries. A folk remedy for stomach complaints, catarrh, cancer (Murakami et al., 1998), gastric ulcers, skin diseases, lowering blood sugar, increasing bone density, nervous conditions, diabetes, fatigue, increase lactation, hay fever, impotence, edema, cramps, hemorrhoids,
headaches, sore gums; to strengthen the eyes, the brain, liver (Farooq et al., 2007), hepatotoxicity (Ruckmani et al., 1998), rheumatism, venomous bites and also for cardiac stimulation (Chaudhary and Chopra, 1996). The present study was therefore undertaken to investigate the phytochemical constituents and to assess the effect of aqueous seed extract of *Moringa oleifera* on some biochemical parameters in normal and alloxan - induced diabetic rats.

**MATERIALS AND METHODS**

**Plant Material**
The seeds of *Moringa oleifera* were obtained from Federal College of Forestry, Jos, Nigeria and were authenticated at the Herbarium of the Department of Plant Biology, University of Jos, Nigeria, where a voucher specimen was deposited at the Herbarium of the Institute.

**Experimental Animals**
Wister male and female adult albino rats (16) weighing between 180-360g were obtained from National Veterinary Research Institute, Vom, Jos, Nigeria. The animals were housed in aluminum cages under standard conditions. They were maintained on standard animal pellets (purchased from Grand Cereal and Oil mills limited Jos, Nigeria) and water *ad libitum*. The animals were acclimatized for two weeks before the commencement of the experiment.

**Preparation of Plant Extract**
The plants seed was collected and air dried at room temperature under the shade. The plant was then pounded to powdery form using local pestle and mortar. The powdery form was stored in air-tight plastic container until required for use. The preparation of the plant extract was prepared using hot water, 100g of fine powder was boiled in one (1) liter of distilled water for 15 minutes (to ensure maximum extractions of phytochemicals) using hot plate. The mixture was allowed to stand for 30 minutes before filtering using white cloth and Whatman filter paper No. 1 to remove all un-extractable matter; the filtrate was then fed 0.5ml to the induced and non-induced alloxan diabetic rats to control their diabetic level.

**Phytochemical Screening**
The extracts of *Moringa oleifera* was screened for some phytochemical constituents using standard qualitative Procedure (Trease and Evans, 2002; Sofowora 1993)
Induction of Diabetes
Diabetes mellitus was induced to rats of group B and C (as diabetic control and diabetic treated rats respectively) by a single intraperitoneal infection of Alloxan at 150mg/kg. Diabetes was confirmed from the fasting blood glucose after 24 hours using one touch glucometer. Prior to each study the animals were made to fast for 14 hours but had free access to water (Ragavan and krishnakumari, 2006).

Experimental Design
The animals grouping were done as follows:
GROUP A: Normal control of four rats on normal diet for 28days.
GROUP B: Diabetic control of four rats fed with normal diet for 28days.
GROUP C: Diabetic treated of four rats fed with normal diet-plus extract for 28days.
GROUP D: Normal treated of four rats fed with normal diet-plus extract for 28days.
Groups C and D rats were given 400mg/kg of the Moringa oleifera leaf extract orally.

Collection of Blood Sample and Serum preparation
The methods described by Yakubu et al. (2005) were used for the collection of blood sample and preparation of serum. The animal under ether anesthesia, the neck area was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade. Blood was collected into EDTA sample bottles for haematological assay and also collected into clean sterile sample bottles which were allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Uniscope Laboratory Centrifuge (model SM800B). The sera were aspirated with Pasteur pipettes and stored frozen overnight at -20°C before being used for the biochemical analyses.

Enzymes Assay Determination and Measurement of some Serum Metabolites
The protein content of serum was determined using the Biuret method of Gornal et al. (1949). Specific activities of aspartate transaminase (AST) (E.C.2.6.1.1), alanine transaminase (ALT) (2E.C.2.6.1.2) in serum were determined based on the method described by Reitman and Frankel (1957), specific activity of alkaline phosphatase (ALP) (E.C.3.1.3.1) in the serum was determined as described by Wright et al. (1972). Creatinine, Urea and Uric acid were
determined as described by Tietz et al. (1994), Hare (1950) and Morin and Prox (1973) respectively. Also, all measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY). While serum magnesium, phosphate and calcium ions were determined by flame photometry using the Jenway Clinical PFP7 Flame Photometer.

**Statistical Analysis**
All data are expressed as mean ± standard deviation (SD). Comparison of the data from test control groups of rats were analyzed by One Way Analysis of Variance (ANOVA) at the confidence limit of 95% and where applicable, least significant difference (LSD) was used to determine significant results; differences between groups were considered statistically significant at P<0.05.

**RESULTS**

**TABLE 1: RESULT OF PHYTOCHEMICAL SCREENING OF THE PLANT EXTRACT (MORINGA OLEIFERA SEED) USING DIFFERENT SOLVENT (WATER, ALCOHOL (ETHANOL), ACETONE (PROPANONE))**

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL</th>
<th>REAGENT</th>
<th>WATER</th>
<th>ETHANOL</th>
<th>ACETONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALKALOIDS</td>
<td>Dragendorff</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FLAVONOIDS</td>
<td>5% lead acetate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TANNIN</td>
<td>10% fecl₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAPONIN</td>
<td>Distilled water</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TERPENES AND STEROIDS</td>
<td>Concentrated H₂SO₄</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CARDIAC GLYCOSIDES</td>
<td>Salkowski's test, chloroform and conc. H₂SO₄</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BALSAM</td>
<td>10% FeCl₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CARBONHYDRATE</td>
<td>Benedict's Reagent</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PHENOL</td>
<td>10% FeCl₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RESINS</td>
<td>Acetic anhydride + conc. H₂SO₄</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

KEY = + POSITIVE - NEGATIVE
### TABLE 2: EFFECT OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* SEED ON TISSUE MARKER ENZYMES OF BOTH NORMAL AND ALLOXAN INDUCED DIABETIC RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>14.00±0.81</td>
<td>17.00±1.41</td>
<td>109.00±1.41</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>20.00±1.41a</td>
<td>20.00±1.41a</td>
<td>134.00±0.73a</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic treated</td>
<td>17.00±0.82ab</td>
<td>18.00±0.82b</td>
<td>124.00±0.71ab</td>
</tr>
<tr>
<td>D</td>
<td>Normal treated</td>
<td>10.00±0.82ab</td>
<td>14.00±1.41ab</td>
<td>86.00±0.70ab</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 4 for each group

*a* values are significantly different from normal control (p<0.05)

*b* values are significantly different from the diabetic control group (p<0.05)

### TABLE 3: EFFECT OF AQUEOUS EXTRACT OF *MORINGA* SEED ON UREA, CREATININE AND URIC ACID

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Uric Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>4.60±0.08</td>
<td>108.00±1.41</td>
<td>201.00±1.41</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>9.80±0.08a</td>
<td>132.00±0.82a</td>
<td>443.00±0.82a</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic treated</td>
<td>5.20±0.08ab</td>
<td>109.00±1.41b</td>
<td>396.00±0.82ab</td>
</tr>
<tr>
<td>D</td>
<td>Normal treated</td>
<td>3.20±0.08ab</td>
<td>92.00±0.82ab</td>
<td>194.00±0.82ab</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 4 for each group

*a* values are significantly different from normal control (p<0.05)

*b* values are significantly different from the diabetic control group (p<0.05)

### TABLE 4: EFFECT OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA* SEED ON ELECTROLYTE OF BOTH NORMAL AND ALLOXAN INDUCED DIABETIC RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mg$^{2+}$</th>
<th>PO$_4^{3-}$</th>
<th>Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>1.06±0.03</td>
<td>1.20±0.08</td>
<td>2.30±0.16</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>0.84±0.04</td>
<td>1.50±0.14a</td>
<td>2.00±0.22</td>
</tr>
<tr>
<td>C</td>
<td>Diabetic treated</td>
<td>1.00±0.22</td>
<td>1.30±0.08</td>
<td>2.18±0.08</td>
</tr>
<tr>
<td>D</td>
<td>Normal treated</td>
<td>1.14±0.01b</td>
<td>2.30±0.14ab</td>
<td>2.20±0.08</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 4 for each group

*a* values are significantly different from normal control (p<0.05)

*b* values are significantly different from the diabetic control group (p<0.05)
DISCUSSION

Diabetes mellitus (DM) is a metabolic disorder which occurs when the pancreas produces insufficient amounts of insulin, or in when individual’s system fail to respond appropriately to insulin (due to defects in reactive oxygen species scavenging enzymes and high oxidative stress impairing pancreatic beta cells) (Ajiboye et al., 2014). DM is characterized with increases in glucose levels build up in the blood and urine, causing excessive urination, thirst, hunger and problems with carbohydrate, fat and protein metabolism (Kathleen, 1996; Adesokan et al., 2009). Hyperglycemia leads to long-term tissue damages and complications, such as liver-kidney dysfunctions, often associated with serious diseases (Takeda et al., 2011; Kokil et al., 2010). Alloxan also acts by destroying the beta cells (Adebayo et al., 2009). Intraperitoneal administration of alloxan to rats in this study led to chemical induced diabetes, a result that is consistent with several studies in rats (Mohammed et al., 2010; Sanjay et al., 2010; Prem et al., 2012).

The effects of aqueous seed extracts of *Moringa oleifera* on liver enzymes of alloxan-induced diabetic rats are as presented in table 2. The levels of AST, ALT and ALP have been reported to increase in alloxan-induced diabetic rats (Gonzalez and Fevery, 1992; Nwanjo, 2007), as they were also reported in this study. There was a significant reduction (p<0.05) in the serum level of AST and ALT by each plant extract, when compared with the alloxan-induced diabetic control. This suggests that aqueous extracts may have ameliorated the drug-induced damage to the liver cells as observed in diabetic untreated rats. Measurement of the activities of “marker” enzymes or biomarkers in body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues (Malomo, 2000; Yakubu et al., 2003). Such measurements can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques (Akanji, 1986).

Renal disease is one of the most common and severe complications of diabetes. Insulin is a physiological factor, which plays an important role in the maintenance of protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation (Pathak and Dhawan, 1988). The level of renal function markers (serum creatinine, urea and uric acid) was significantly increased in diabetic rats compared with the control and other treated groups. These results agreed with Verma and Bordia (1998).
who indicated that increased kidney functions are signs of kidney dysfunctions in the diabetic disease compared to control. These results confirmed by Uladimir (2003) who revealed that hyperglycemia are associated with long-term damage, dysfunction and failure of various organs, especially kidneys. Recently, Jarald et al. (2008) showed that diabetic rats had a significant increase in creatinine and urea levels as compared to the normal animals. Kidney dysfunctions in the diabetic rats may be related to the generation of reactive oxygen species and lipid peroxidation which are associated with tissue injury following ischemic insult. In addition, Shah et al. (2007) reported that increased oxidative stress and reduce antioxidative ability in diabetes results in renal tubular injury, proteinuria and leads to gradual loss of renal function (Elgazar et al., 2013). Another characteristic feature of severe diabetic is an elevated excretion of urea whose concentration may be five times higher than the normal value (Lehninger, 1998). The increase in the level of urea in diabetic rats may be attributed to enhanced catabolism of both liver and plasma proteins that accompany glyconeogenesis (khushk et al., 2010). Moringa oleifera seed extract improved renal function by reversing these effects.

Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction and blood clotting and muscle contraction. Electrolytes imbalance resulting from kidney failure, dehydration and fever and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders (Rao, 1992). Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes (Tanko et al., 2013). From this study, the level of serum phosphate ion (PO$_4^{3-}$) increased significantly while the level of magnesium and calcium ion (Mg$^{2+}$ and Ca$^{2+}$) reduced significantly in the diabetic rats when compared with the control.

The increase in the level of PO$_4^{3-}$ may be due to renal dysfunction, phosphate excretion is further reduced to cause an even greater increase of serum phosphate concentration (Popovtzer, 2003). Also, the observed decrease in the serum Mg$^{2+}$ of alloxan induced diabetic rats in this study may be due to hyperesmolar non-ketosis while the significant decrease observed in the level of Ca$^{2+}$ may be due to decreased extra skeletal inability to absorb dietary calcium (Luka et al., 2012). Oral administration of diabetic rats with the seed extracts
reduced the serum $\text{PO}_4^{3-}$ and increased the serum $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$ (Table 4). The significant increase of $\text{Mg}^{2+}$ in diabetic rats administered with aqueous extract of M. oleifera seed suggest that *Moringa oleifera* enhances transfer of intracellular $\text{Mg}^{2+}$ to extracellular space there by reducing or preventing hyperosmotic non-ketotic state and the significant increase in calcium ions may be due to increase in extra skeletal ability to absorb dietary calcium. Hence, the administration of aqueous seed extract of *M. oleifera* at the dose of 200 mg/kg body weight ameliorates this condition electrolyte changes in alloxan-induced diabetic rats.

The performance of *Moringa oleifera* seed extract in reversing the negative effects of alloxan on diabetic rats may due to the present of phytochemicals shown on table 1. Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease (Krishnaiah *et al.*, 2009). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Okwu, 2001). Flavonoids of different plant origin showed a promising anti-diabetic activity, as demonstrated in diabetic animal models (Zarzuelo *et al.*, 1996; Nojima *et al.*, 1998; Kim *et al.*, 2004). Saponins are glycosides of triterpenes, steroids or alkaloids. Previous researchers have demonstrated the anti-diabetic activity of triterpenoid and glycosides (Reher *et al.*, 1991; Kako *et al.*, 1997). In this study, the phytochemical investigation of *Moringa oleifera* seed indicates the presence of alkaloids, saponin, terpenes and steroids, Resins, cardiac glycoside and carbohydrate. Thus the phytochemical constituents indicate that the aqueous seed extract of *Moringa oleifera* could have potentials to be an anti-diabetic agent.

**CONCLUSION**

The results from this study showed that aqueous seed extract of *Moringa oleifera* ameliorate the adverse diabetic condition in alloxan-induced diabetic rats. Further studies need to be carried out, however, to isolate and identify the active principle(s) in the extract as well as elucidate its mode of action.

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