



## Evaluation of Hypoglycaemic and Antioxidant Activity of *Moringa oleifera* Root in Normal and Alloxan-Induced Diabetic Rats

Sanusi A. Umar<sup>1\*</sup>, Zainab Mohammed<sup>1</sup>, Aliyu Nuhu<sup>1</sup>, Kabir Y. Musa<sup>1</sup>, Yusuf Tanko<sup>2</sup>

<sup>1</sup>Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria-Nigeria.

<sup>2</sup>Department of Human Physiology, Ahmadu Bello University, Zaria-Nigeria.

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### ABSTRACT

*Moringa oleifera* is traditionally used for treatment of diarrhea, diabetes mellitus, rheumatism, venomous bites, for cardiac stimulation and as a diuretic. The aim of this study was to investigate the hypoglycaemic and antioxidant activity of *M. oleifera* root in normal and alloxan-induced diabetic rats. The root was extracted with n-hexane, ethyl acetate and methanol successively using Soxhlet apparatus. All the extracts were subjected to phytochemical screening and toxicity study. The powder and methanol extracts were subjected to anti-diabetic evaluation using alloxan-induced diabetes in rats for 28 days. Glibenclamide was used as standard drug. The anti-diabetic effect of the extract was evaluated by measuring changes in blood glucose level, haematological indices, biochemical parameters, oxidative stress biomarkers and pancreatic tissue. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, carbohydrate, cardiac glycosides and saponins. LD<sub>50</sub> was above 5000 mg/kg and did not cause mortality in all the tested rats. Both the methanol extract and powdered root showed significant reduction ( $p < 0.05$ ) in blood glucose level after treatment. Also, the concentrations of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) increased significantly ( $p < 0.05$ ) as compared with the diabetic control. Histopathological examination of the pancreas from diabetic rats showed degenerative changes in  $\beta$ -cells. In the treatment groups, there was a significant reversal to the damage done to the islet of Langerhans.

This study has shown that *M. oleifera* root possesses hypoglycaemic effect and ameliorates other adverse diabetic conditions imposed by alloxan-induced diabetes on the experimental rats.

**Keywords:** *Moringa oleifera*, Hypoglycaemic, Alloxan, Oxidative stress, histopathology.

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### Introduction

Diabetes mellitus is now recognized as a metabolic disorder of multiple etiology which is characterized by chronic hyperglycaemia resulting from absolute or relative deficiency in insulin secretion/insulin action or both.<sup>1</sup> The number of diabetics was 171 million in 2000, which might increase to 360 million in the year 2030.<sup>2</sup> As the number of people with Diabetes mellitus (DM) multiplies worldwide, national and international health care budget increases.<sup>3</sup> The prevalence of diabetes is on the increase globally and in African communities due to the ageing of the population and drastic lifestyle changes accompanying urbanization and westernization.<sup>4</sup> The prevalence of diabetes mellitus in Nigeria is 1.9% with more than 1.5 million cases.<sup>5</sup> Hence, it represents a growing burden on health care systems of African countries, most of which already face difficult economic conditions. The disease remains incurable and can only be controlled with drugs; hence, a scrupulous control is needed to help reduce hyperglycaemia and the risk of long-term complications, which are known to be the major causes of morbidity and mortality.<sup>6</sup> Despite considerable progress

in therapies using expensive synthetic drugs, the search for herbal remedies is growing which can be accounted for the effectiveness, minimal side effects in clinical experience and relatively low cost of the herbal drugs.<sup>7</sup> Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown.<sup>8</sup>

*Moringa oleifera* (Linn) is a medicinally important plant, belonging to family *Moringaceae*. It is a highly valued plant, distributed in many countries of the tropics and subtropics.<sup>9</sup> It is a tropical tree whose numerous economic applications and facility of propagation are arousing growing international interest. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals and a good source of protein, vitamins,  $\beta$ -carotene, amino acids and various phenolic.<sup>10</sup> The root of *M. oleifera* were shown to possess antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic affections; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, anti-inflammatory articular pains, lower back or kidney pain and constipation.<sup>11</sup> Different parts of moringa plant contains 46 antioxidants which help cells to neutralize free radicals. It is traditionally used for relieving spasm, for treatment of diarrhea, diuretic and stimulant in paralytic affliction, epilepsy and hysteria<sup>12</sup> and treatment of diabetes mellitus,<sup>13</sup> hepatotoxicity,<sup>14</sup> rheumatism, venomous bites and also for cardiac stimulation.<sup>15</sup> Despite the wide spread use of *M. oleifera* root powder in the treatment of diabetes in Northern Nigeria, there is no scientific report for the use of its roots powder in treatment of diabetes. It becomes extremely important to make an effort towards establish scientific evidence of its traditional use as antidiabetic's agent. Therefore, this research was carried to investigate the hypoglycaemic and antioxidant activity of *M. oleifera* root powder and its methanol extract in normal and alloxan-induced diabetic rats.

\*Corresponding author. E mail: [sanusidudu@gmail.com](mailto:sanusidudu@gmail.com)  
Tel: +2348032707288

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## Materials and Methods

### Chemicals / drugs used

All chemicals and drugs used were of analytical grade. Glibenclamide (Aventis Pharm LTD, France), Alloxan monohydrate (Sigma, St. Louis, USA), Methanol, Ethyl acetate and Hexane (JHD, AR; Lobal Chem, India).

### Experimental animals

Seventy-eight (78) Wistar rats of both sexes weighing 120 - 150 g and were separated from each other. The animal was obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained under standard conditions (12 hours light /12 hours dark cycle, temperature of about  $37 \pm 2^\circ\text{C}$ , 35 - 60% humidity). The rats were fed with standard (grower) mash (Vital feed, Jos, Nigeria) and water ad-libitum. The handling of the animals was in accordance with the principles of laboratory animal care<sup>16</sup> and with approval of Animal Ethics and Care Committee, Ahmadu Bello University, Nigeria (ABUCAUC/2016/018).

### Collection, identification and preparation of the plant material

*Moringa oleifera* plant was first identified in the field using its morphological features. Sample of the plant was then collected from Kachako, Takai Local government of Kano, State of Nigeria, in the month of August 2015 and was identified by Mal. Namadi Sunusi of the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria where a specimen (voucher number 2582) was deposited. The root air-dried and comminuted to powder form, stored in an air-tight container for subsequent use.

### Extract preparation

Extraction of the *M. oleifera* root was done following previously described technique.<sup>17</sup> Briefly, One thousand grams (1 kg) of the pulverized plant sample was extracted with n-Hexane, Ethyl acetate and Methanol successively in a Soxhlet apparatus at  $50^\circ\text{C}$ . The filtrate was further concentrated via rotary evaporator to recover some solvent and final evaporation to dryness of the extracts were done via the water bath after which it was stored in desiccators for subsequent use. The extracts MOHE (M. oleifera Hexane Extract), MOEE (M. oleifera Ethyl acetate Extract) and MOME (M. oleifera Methanol Extract) were stored in refrigerator before use.

### Phytochemical screening

A preliminary phytochemical screening of the root extracts of *M. oleifera* was also done using the standard phytochemical procedures.<sup>18,19</sup>

### Acute toxicity study

The lethal dose was determined by Lorke's method. Phase I: Nine Wistar rats were used. They were divided into three groups of three animals each. Each groups of animals were administered different doses (10, 100, and 1000 mg/kg) of the extracts and then observed for 24 hours to monitor their behavior as well as mortality. Phase II: Three animals were used. They were divided into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg of the extracts and observed for behavior as well as mortality. The oral median lethal dose ( $\text{LD}_{50}$ ) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose.<sup>20</sup>

### Induction of Diabetes

Wistar rats were fasted for about 12 - 18 hours, after which diabetes mellitus was induced by a single intraperitoneal injection of Alloxan monohydrate dissolved in 0.9% cold normal saline solution at a dose of 150 mg/kg body weight.<sup>21</sup> Alloxan produces fatal hypoglycaemia<sup>22</sup> and to prevent this, the rats were treated with 20% glucose solution orally for 6 hours. After which they were placed on 5% glucose solution for 24 hours.<sup>23</sup> Blood was collected from the prominent jugular vein of the rats after 72 hours of Alloxan injection and blood glucose was measured using ACCU-CHECK ACTIVE (Roche Diagnostics, Germany). The rats having fasting blood glucose level greater than or equal to 200 mg/dL were selected for the study.

### Experimental Design

Rats are divided into seven groups as follows:

Group I: Control rats received normal saline 5 mL/kg orally for 4 weeks. Group II: Diabetic groups received / administered normal saline 5 mL/kg orally for 4 weeks. Group III: Diabetic rats treated with glibenclamide (5 mg/ kg body weight in aqueous solution) orally for 4 weeks.<sup>24</sup>

Group IV: Diabetic rats treated with *M. oleifera* roots powder (15gm/kg BW/day, orally 16% in diet) for 4 weeks. Group V: Diabetic rats treated with methanolic extract of *M. oleifera* roots (1 g/kg BW/day, intraperitoneal injection (I.P.) for 4 weeks. Group VI: Normal rats treated with *M. oleifera* roots powder (15gm/kg BW/day, orally 16% in diet) for 4 weeks. Group VII: Normal rats treated with methanolic extract of *M. oleifera* roots (1 g/kg BW/day, by intraperitoneal injection (I. P.) for 4 weeks.

### Blood sample collection and serum preparation

At the end of 28 days treatment period, the animals were fasted overnight and sacrificed using chloroform as an anesthetic agent. Blood sample (5 mL) were collected into ethylene diamine tetra acetic acid (EDTA) sample bottles and allowed to clot then separated by centrifugation at 3,000 rpm for 10 minutes using Centrifuge Hitachi (Universal 32, Made in Germany). The supernatant obtained were used for the determination of biochemical parameters and oxidative stress biomarkers.

### Determination of Blood glucose level

Determination of blood glucose level was conducted by collecting blood samples from the animal tail vein on a seven days interval (starting with day 0 then 7, 14, 21 and 28) for a period of 28 days. A digital ACCU-CHECK ACTIVE (Roche Diagnostics, Germany) was used to monitor blood glucose levels throughout the experiment and the results were expressed in the unit of mg/dL.<sup>25</sup>

### Estimation of insulin and glycosylated haemoglobin

Insulin level was determined by using a rat-specific insulin-Ak ELISA according to Anderson and co-worker.<sup>26</sup> Glycosylated haemoglobin (HbA1c) assay was determined using Human kits.<sup>27</sup>

### Haematological analysis

Blood samples were collected into ethylene diamine tetra acetic acid (EDTA) bottles for analyses of blood indices including white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), platelet (PLT), packed cell volume (PCV), using automated haematology analyzer (Mindray BC-2300, Guangzhou Medical Equipment Co., Ltd, China).

### Estimation of serum biochemical parameters

The activities of serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and that of Alkaline Phosphatase (ALP) were assayed using colorimetric assay kits (Bio-diagnostic kits, Cairo, Egypt) according to manufacturer's instruction and values were expressed in IU/L.<sup>28</sup> The total protein content of the serum was determined using the Biuret method.<sup>29</sup> Albumin (ALB) level was determined as described by Grant and Kacchman.<sup>30</sup>

### Determination of oxidative stress biomarkers

Serum malondialdehyde (MDA) levels was measured by the double heating method of Draper and HadLey<sup>31</sup> using Malondialdehyde Assay kits (Bio-diagnostic kits, Cairo, Egypt) Superoxide dismutase activities (SOD) was measured by the method of Misra and Fridovich.<sup>32</sup> Catalase (CAT) activities were assayed by the method of Sinha.<sup>33</sup> The Glutathione peroxidase (GPx). Assay kit (Bio-diagnostic kits, Cairo, Egypt) was determined by the method of Paglia and Valentine.<sup>34</sup>

### Histological studies

The pancreatic tissues was harvested from the sacrificed rats after dissection and wash with saline, cut into small pieces and then specimens was fixed in 10% formalin solution for sectioning (5 mL). The fixed specimens were sliced, processed, and embedded into paraffin blocks. The blocks were cut into 5µm thick, paraffin sections by rotary microtome. The sections were stained with haematoxylin and eosin H and E for histological observations.<sup>35</sup>

### Statistical analysis

The results were expressed as mean  $\pm$  standard errors of the mean (SEM) for all values. The data was statistically analyzed using one-way ANOVA followed by Tukey's post-hoc test to compare the levels of significant between the control and experimental groups using SPSS version 20.0 software. Results were considered to be significant when P values are less than 0.05 ( $p < 0.05$ ).

## Results and Discussion

### Phytochemical screening

The preliminary phytochemical screening of the root extracts revealed the presence alkaloids, tannins, flavonoids, cardiac glycosides, saponins, triterpenes and steroids as shown in Table 1. Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will help the future investigators regarding the selection of the particular extract for further investigation or isolating the active principle.<sup>36</sup> Phytochemical screening of the root powder extracts had revealed the presence of some secondary metabolites namely alkaloids, tannins, flavonoids, cardiac glycosides, saponins, triterpenes and steroids. This result is in agreement with the finding of Choudhary and co-worker.<sup>37</sup>

### Acute toxicity study

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental animals using Lorke's method to predict toxicity and to provide guidelines for selecting a "safe" dose in animals and also used to estimate the therapeutic index ( $LD_{50}/ED_{50}$ ) of drugs.<sup>38,39</sup> In this study, median lethal dose ( $LD_{50}$ ) of the extracts (hexane, ethyl acetate and methanol) of the *M. oleifera* root powder was carried out orally in rats. The  $LD_{50}$  was found to be greater than 5000 mg/kg when administered orally in rats (Table 2) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extracts of *M. oleifera* root are practically non-toxic when administered using the oral route. This is based on the toxicity classification which states that substances with  $LD_{50}$  values of 5000 to 15,000 mg/kg body weight are practically non-toxic.<sup>40</sup>

### Effect of *M. oleifera* root powder and its methanol extract on blood glucose level

The effect of *M. oleifera* root powder and its methanol extract on blood glucose level were shown in Table 3. The blood glucose level of untreated diabetic group was significantly increased ( $p < 0.05$ ) than normal control group while there was decrease significantly ( $p < 0.05$ ) in all treated groups when compared with untreated diabetic group and this may be due to insulin deficiency or resistance state in diabetic group. This represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic  $\beta$ -cells in alloxan-induced diabetic rats.<sup>41</sup>

### Effect of *M. oleifera* root powder and its methanol extract on insulin and glycosylated haemoglobin (GHBA1c) level

The insulin level was significantly decreased in diabetic group when compared with normal group. Treatment with *M. oleifera* root powder and its methanol extract and glibenclamide significantly increased the level of insulin when compared with untreated diabetic group. Normal group treated *M. oleifera* root powder 15 gm/kg (16% in diet) showed the highest insulin level ( $21.72 \pm 3.43 \mu\text{u/ml}$ ). The level of GHBA1c in the entire treated groups showed significant decrease ( $p < 0.05$ ) when compared with the diabetic group while that there was significant ( $p < 0.05$ ) increase in the untreated diabetic group when compared with normal control (figure 1). In diabetes, HbA1c is considered as a diagnostic marker and helps to know about degree of protein glycation, long term blood sugar level and correlation of diabetes associated complications.<sup>42,43</sup> HbA1c has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin.<sup>44</sup>

**Table 1:** Preliminary phytochemical screenings of extracts of *M. oleifera* root.

Phytoconstituents	MOHE	MOEE	MOME
Saponin	-	-	+
Steroids/Triterpenes	+	+	+
Flavonoids	-	-	+
Tannins	-	-	+
Alkaloids	-	-	+
Cardiac glycosides	-	+	+

Key: Present (+); Absent (-); MOHE: *M. oleifera* Hexane Extract; MOEE: *M. oleifera* Ethyl acetate Extract; MOME: *M. oleifera* Methanol Extract

**Table 2:** Acute toxicity effect of hexane, ethyl acetate and methanol root extracts of *M. oleifera* when administered orally to Wistar Rats

Experiment	Dose (mg/kg)	Number of dead rats after 24 hours		
		MOHE	MOEE	MOME
Phase 1	10	0/3	0/3	0/3
	100	0/3	0/3	0/3
	1000	0/3	0/3	0/3
Phase 2	1600	0/1	0/1	0/1
	2900	0/1	0/1	0/1
	5000	0/1	0/1	0/1

MOHE: *M. oleifera* Hexane Extract; MOEE: *M. oleifera* Ethyl acetate Extract; MOME: *M. oleifera* Methanol Extract

### Effect of *M. oleifera* root powder and its methanol extract on haematological parameters

Assessment of haematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal.<sup>45</sup> There were significantly increased ( $p < 0.05$ ) variations observed in all the haematological indices of the treated groups compared to untreated diabetic group while the untreated diabetic group show significantly ( $p < 0.05$ ) decrease when compared with normal control. It revealed that untreated diabetic group showed some abnormalities in the haematological parameters (PCV, HB, RBC and WBC) when compared to normal control group (Figure 2). The group treated with *M. oleifera* root powder and its methanol extract showed positive change in the haematological parameters suggesting that it might not contribute further diabetic complications to haematological parameters. Some of these abnormalities might be due to destruction of mature red blood cells, leading to the low HB counts accompanied by the fall in the RBC and PCV.<sup>45,46</sup>

### Effect of *M. oleifera* root powder and its methanol extract on serum biochemical parameters

The level of total protein, albumin, and liver marker enzymes such as ALT, AST and ALP in the serum are presented in Table 4. The level of ALT, AST and ALP levels were elevated significantly ( $p < 0.05$ ) in untreated diabetic group compared to normal control group. Both the powder root of *Moringa* and its methanol extract doses and glibenclamide treatment significantly ( $p < 0.05$ ) reduced the above parameters when compared to untreated diabetic group. The increase in the activities of ALT, AST and ALP in the serum of untreated diabetic group may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream<sup>47</sup>, which gives an indication of the hepatotoxic effect of alloxan. Significant reductions in serum protein and albumin were observed in untreated diabetic group when compared with the control and the treated groups. Also, on administration of *M. oleifera* root powder and its methanol extract, the total protein and

**Table 3:** Effect of *M. oleifera* Root Powder and its Methanol Extract on Blood Glucose Level on Normal and Alloxan-Induced Diabetic Wistar Rats.

Groups/Treatment	Glucose Level (mg/dL)				
	Initial	Week 1	Week 2	Week 3	Week 4
NR + Normal saline	104.33 ± 3.57	97.50 ± 1.20	96.33 ± 0.61	94.17 ± 0.70	95.50 ± 0.43
DR + Normal saline	320.0 ± 1.51 <sup>#</sup>	352.0 ± 0.82 <sup>#</sup>	367.50 ± 0.99 <sup>#</sup>	383.50 ± 0.76 <sup>#</sup>	389.33 ± 0.21 <sup>#</sup>
DR + GBC 5 mg/kg	321.83 ± 2.89	218.83 ± 0.54*	178.50 ± 0.50*	145.67 ± 0.33*	120.83 ± 0.31*
DR + MOR Powder 15 g/kg (16% in diet)	316.17 ± 6.37	221.33 ± 0.42*	181.33 ± 0.21*	151.17 ± 0.31*	130.67 ± 0.56*
DR + MEMOR 1 g/kg	320.33 ± 1.86	217.50 ± 0.56*	177.17 ± 0.48*	148.17 ± 0.48*	118.0 ± 0.45*
NR + MOR Powder 15 g/kg (16% in diet)	99.67 ± 2.70	95.33 ± 0.21*	86.67 ± 0.49*	75.83 ± 0.31*	63.17 ± 0.67*
NR + MEMOR 1 g/kg	101.50 ± 1.60	91.83 ± 0.40*	82.63 ± 0.92*	71.83 ± 0.54*	58.33 ± 0.33*

NR: Normal Rats + Normal Saline (Negative Control); DR: Diabetic Rats + Normal Saline (Untreated Diabetic Control); DR: Diabetic Rats + Glibenclamide 5mg/kg (Positive Control); MOR: *Moringa oleifera* Root; MEMOR: metabolic Extract of *Moringa oleifera* Root.

n = 6; values expressed as mean ± SEM.; data analyzed using one-way ANOVA followed by Turkey's post hoc test; Statistically Significant at p < 0.05, \* when compared between treated groups and untreated diabetic control, <sup>#</sup> when compared between normal control and untreated diabetic Control.

**Table 4:** Effect of *M. oleifera* Root Powder and Its Methanol Extract on Serum Biochemical parameters of Normal and Alloxan-Induced Diabetic Wistar Rats.

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (IU/L)	ALB (IU/L)
NR + Normal saline	12.53 ± 0.04	16.50 ± 0.50	75.40 ± 4.69	5.96 ± 0.69	2.89 ± 0.10
DR + Normal saline	37.17 ± 0.48 <sup>#</sup>	42.17 ± 1.05 <sup>#</sup>	126.39 ± 1.12 <sup>#</sup>	3.67 ± 0.08 <sup>#</sup>	1.97 ± 0.67 <sup>#</sup>
DR + GBC 5 mg/kg	13.77 ± 0.05*	17.16 ± 0.47*	77.53 ± 0.73*	7.12 ± 0.11*	2.84 ± 0.12*
DR + MOR Powder 15 g/kg	14.65 ± 0.12*	19.83 ± 0.75*	80.35 ± 0.17*	7.49 ± 0.42*	3.16 ± 0.15*
DR + MEMOR 1 g/kg	14.28 ± 0.43*	18.32 ± 0.49*	78.50 ± 0.27*	7.84 ± 0.71*	2.86 ± 0.18*
NR + MOR Powder 15 g/kg	13.18 ± 0.73*	17.75 ± 0.58*	76.73 ± 1.07*	7.86 ± 0.49*	3.09 ± 0.12*
NR + MEMOR 1 g/kg	12.87 ± 1.02*	17.43 ± 0.83*	74.90 ± 0.71*	8.01 ± 0.51*	3.26 ± 0.07*

NR: Normal Rats + Normal Saline (Negative Control); DR: Diabetic Rats + Normal Saline (Untreated Diabetic Control); DR: Diabetic Rats + Glibenclamide 5mg/kg (Positive Control); MOR: *Moringa oleifera* Root; MEMOR: Methanolic Extract of *Moringa oleifera* Root. n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; Statistically Significant at p < 0.05, \* when compared between treated groups and untreated diabetic control, <sup>#</sup> when compared between normal control and untreated diabetic Control. ALT: Alanine Aminotransferases, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, TP: Total Proteins, ALB: Albumin.

albumin levels were significantly (p < 0.05) increased when compared with the untreated diabetic group. This observation may be attributed to numerous effects of hyperglycaemia in alloxan-induced diabetes. Hyperglycaemia increases gluconeogenesis and as such leads to excess protein breakdown as well as excess loss of nitrogen resulting in negative nitrogen balance.<sup>48</sup> A decline in the total protein level in untreated diabetic group has been attributed to inhibition of oxidative phosphorylation, which leads to decrease in protein synthesis, increase in catabolic processes and reduction in protein absorption.<sup>49</sup>

#### Effect of *M. oleifera* root powder and its methanol extract on oxidative stress biomarker

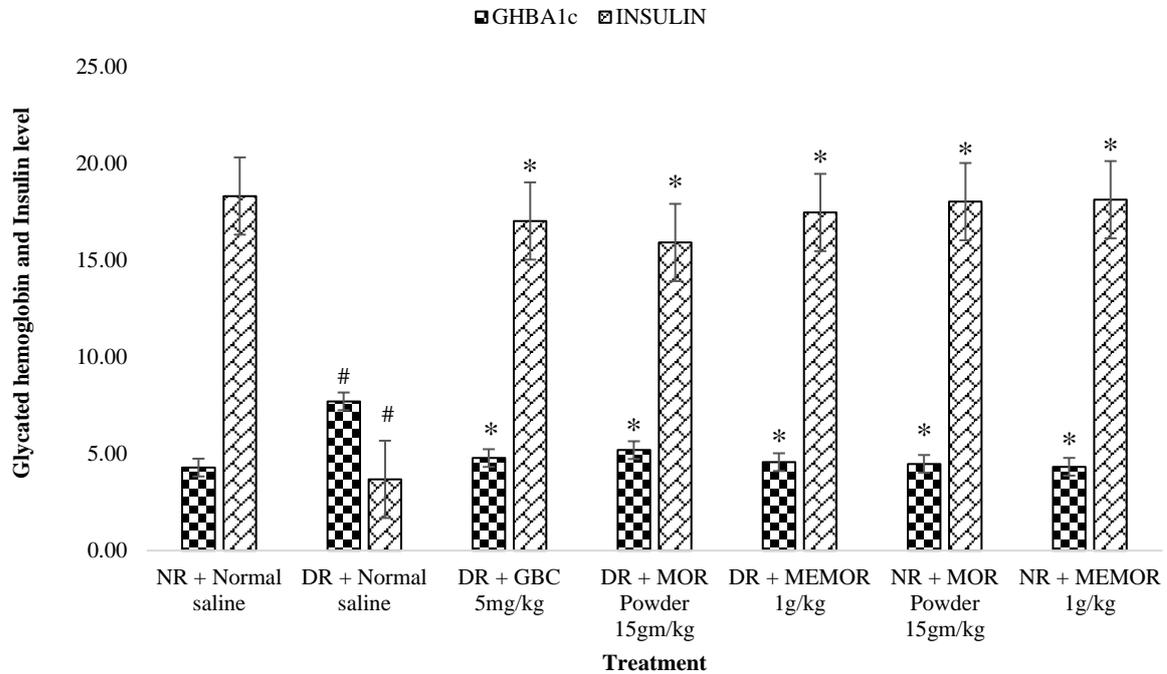
When compared with the untreated diabetic group, there was significant (p < 0.05) increased in the oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) level in all the groups administered with *M. oleifera* root powder and its methanol extract while that of untreated diabetic group showed significant (p > 0.05) decreased when compared with normal control (figure 3). However, the increased in SOD, CAT and GPx activity shows that the *M. oleifera* root powder and its methanol extract can reduce reactive free radicals that might reduce oxidative damage, and this might be due to rich presence of flavonoids which

have antioxidant property. Superoxide dismutase (SOD) serves as the first line of defence against the deleterious effect of ROS. The function of intracellular SOD is to scavenge superoxide (O<sub>2</sub><sup>-</sup>) produced by cellular metabolism, and catalyse dismutation of superoxide to oxygen (O<sub>2</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>50,51</sup> The increased dismutation of O<sub>2</sub><sup>-</sup> by SOD leads to increase in production of H<sub>2</sub>O<sub>2</sub>, which is further detoxified by CAT and GPx to H<sub>2</sub>O and O<sub>2</sub>.<sup>51</sup> *Moringa oleifera* is a rich source of antioxidant<sup>52,53</sup> such as quercetin and kaempferol (major bioactive compounds of phenolics) and are responsible for antioxidant activity.<sup>54,55</sup> Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation.<sup>56</sup> The antioxidant property also can be due to the presence of carotenoids, alkaloids, proanthocyanidins in this plant<sup>56</sup> or to the high content of flavonoids such as kaempferol, presence of other polyphenols, carotenoids and cinnamic acid derivatives.<sup>54,55</sup> Furthermore, the malondialdehyde (MDA) concentration showed decreased in the treated groups when compared to untreated diabetic control group (figure 3). This is associated with an increased activity of SOD, CAT and GPx respectively. This means that the *M. oleifera* root powder and its methanol extract can reduce reactive oxygen free radicals and improve the activities of the antioxidant enzymes.

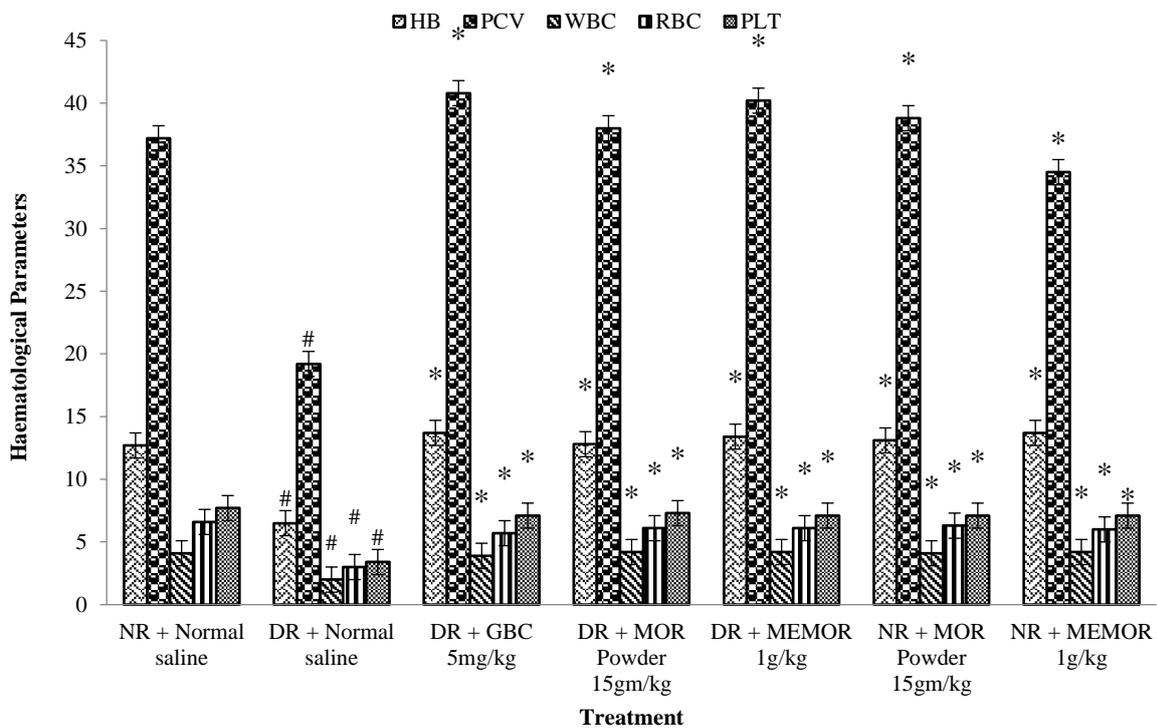
*Effect of M. oleifera root powder and its methanol extract on pancreas histopathology*

Histological sections of endocrine regions of pancreas of untreated diabetic group revealed a significant reduction in the size of the islets of langerhans when compared to that of normal groups. Also, the study

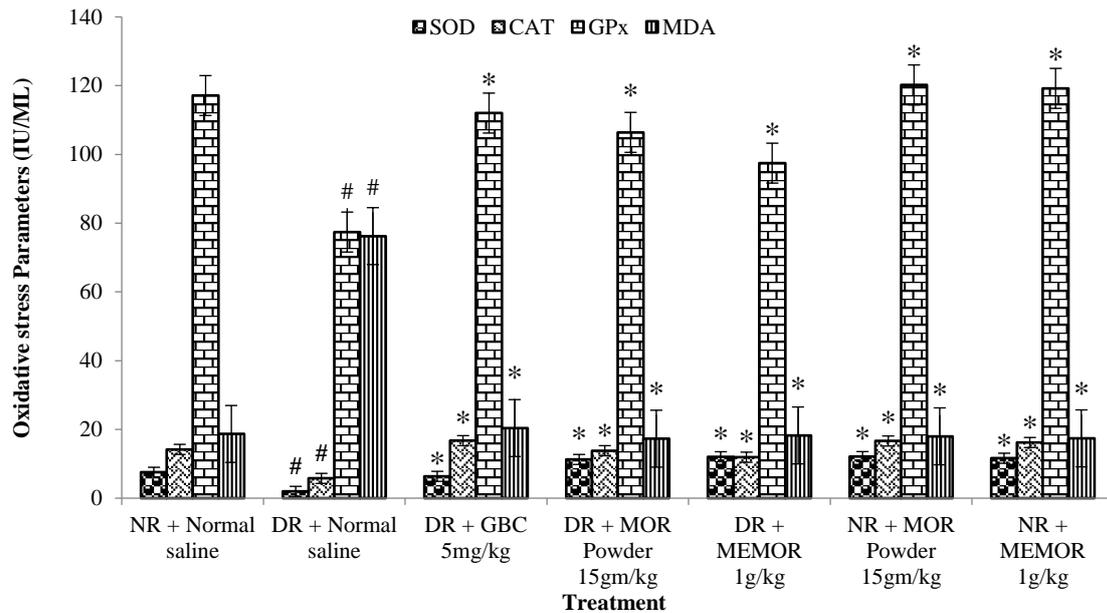
revealed the presence of damaged  $\beta$ -cell population. This damage of the  $\beta$ -cells is due to Alloxan induction and reduction in  $\beta$ -cell number can be as low as 50% in diabetes.<sup>57</sup> The restoration of  $\beta$ -cells was very good in all the treated groups.



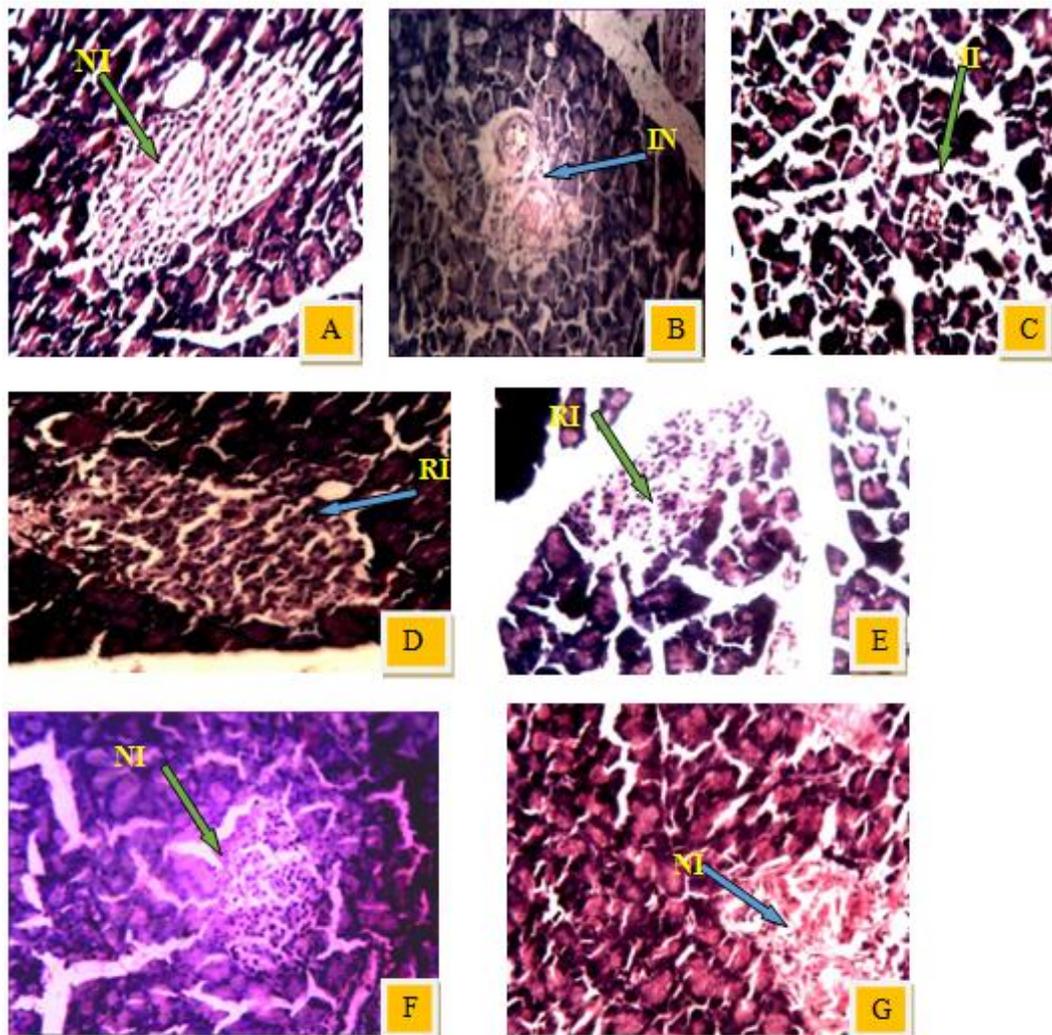
**Figure 1:** Effect of *M. oleifera* Root Powder and its Methanol Extract on Insulin and Glycated hemoglobin (GHBA1c) level on Normal and Alloxan-Induced Diabetic Wistar Rats.



**Figure 2:** Effect of *Moringa oleifera* Root Powder and its Methanol Extract on Haematological parameters of normal and Alloxan-Induced Diabetic Wistar Rats.



**Figure 3:** Effect of *M. oleifera* Root Powder and its Methanol Extract on oxidative stress biomarkers of normal and Alloxan-Induced Diabetic Wistar Rats.



**Figure 4:** Photomicrograph of Effect of *M. oleifera* root powder and its methanolic extract on histopathological damages in the Pancreas. (A) Normal control group showing normal islet (NI), (B) Diabetic group (DG) showing intense islet necrosis (IN), (C) DG treated with Glibenclimide showing intense islet cells, (D) DG + MOR Powder 15gm/kg (16% in diet) showing moderate restoration of islet cells (RI), (E) DG + MEMOR 1g/kg showing moderately restored islet cells (RI), (F) NR + MOR Powder 15gm/kg (16% in diet) showing normal islet (NI), (G) NR + MEMOR 1g/kg showing normal islet (NI). (Hematoxylin and eosin stain; original magnification X400).

## Conclusion

This study has shown that the *M. oleifera* root powder and its methanol possesses hypoglycaemic effects and ameliorates other adverse diabetic condition caused by alloxan-induced diabetes on the experimental rats as indicated by the haematological and liver functional indices. The experimental findings clearly indicate an exciting opportunity to develop a potent anti-diabetic drug from this plant. There is need for further investigation for the isolation of active principles responsible for the anti-diabetic activity.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

- American Diabetes Association (ADA). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 2012; 35(Suppl 1):S64–S71.
- World Health Organisation (WHO). WHO Expert Committee on Diabetes Mellitus. Second Report Geneva. World Health Organisation. 2000.
- Sai MD, Ramesh B, Sarala KD. Evaluation of Antidiabetic and Antihyperlipidemic Potential of Aqueous Extract of *Moringa Oleifera* In Fructose Fed Insulin Resistant and STZ Induced Diabetic Wistar Rats: A Comparative Study. *Asian J Pharm Clin Res*. 2012; 5(1):67-72.
- Sobngwi, E, Mauvais-Jarvis F, Vexiau P and Gautier JF). Diabetes in Africans: epidemiology and clinical specificities. *Diabetes Metabol*. 2001; 27:628-634.
- International Diabetes Federation (IDF). *Diabetes Atlas*. (4th ed.). The economic impact of diabetes. 2015; <http://www.diabetesatlas.org/content/economic-impact-of-diabetes>. Accessed on September 10, 2016.
- Rotimi SO, Olayiwola I, Ademuyiwa O, Adamson I. Inability of legumes to reverse diabetic-induced nephropathy in rats despite improvement in blood glucose and antioxidant status. *J Med Food*. 2010; 13:163-9.
- Daryoush M, Bahram AT, Yousef D, Mehrdad N. *Brassica rapa* L. extract alleviate early hepatic injury in alloxan-induced diabetic rats. *J Med Plants Res*. 2011; 5(31):6813-6821.
- Valiathan MS. Healing plants. *Cur Sci*. 1998; 75:112-126.
- Rebecca HSU, Sharon M, Arbainsyah A, Lucienne D. *Moringa oleifera*, medicinal and socio-economic uses. International Course on Economic Botany. National Herbarium Leiden, Netherlands; 2006. 2-6 p.
- Farooq A, Sajid L, Muhammad A, Anwarul HG. *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *Phytother Res*. 2007; 21:17–25.
- Padmarao P, Acharya BM, Dennis TJ. Pharmacognostic study on stem bark of *Moringa oleifera* Lam. *Bull Medico-Ethno-Botanical Res*. 1996; 7:141–151.
- Quisumbing E. 1978. *Moringa oleifera* Lam, Medicinal plants of the Philippines. Katha Publication Company, Inc. 1978. 346-349 p.
- Babu R, Chaudhuri M. Home water treatment by direct filtration with natural Coagulant. *J Water Health*. 2005; 3:27–30.
- Ruckmani K, Davimani S, Jayakar B, Anandan R. Anti-ulcer activity of the alkali preparation of the root and fresh leaf juice of *Moringa oleifera* Lam. *Anc Sci Life*. 1998; 17(3): 220-223.
- Chaudhary RD, Chopra RD. (1996). *Herbal Drug Industry: A Practical Approach to Industrial Pharmacognosy*. New Delhi: Eastern Publishers; 1996. 58 p.
- NIH, Guidelines for the care and use of laboratory animals. NIH Revised 1985. publication No. 85-23.
- Kokate CK. *Practical Pharmacognosy*. New Delhi, India: Vallabh Prakashan; 2003. 107-127 p.
- Sofowora A. *Medical plants and traditional medicine in Africa*. (Rep. ed.). Ibadan, Nigeria: Spectrum books LTD; 2006. 150-153 p.
- Evans WC. *Trease and Evans pharmacognosy*. (15th ed.). London: W.B. Saunders Ltd; 2002. 191-393 p.
- Lorke D. A New Approach to Practical Acute Toxicity Testing. *Arch Toxicol*. 1983; 5:275-287.
- Katsumata KY, Katsumata TO, Katsumata T. Potentiating effects of diabetes combined usage of three sulfonylurea drugs on the occurrence of alloxan-induced in rats. *Horm Metab Res*. 1999; 25:125-126.
- Tanko Y, Jimoh A, Sulaiman I, Mohammed K, Salisu AI, Yusuf, R. and Musa, S.A. (2017). The Effect of Rutin on Lipid Profile and Liver Function Enzymes on Alloxan Induced Hyperglycaemic Wistar Rats. *Bayero J Pure Appl Sci*. 2017; 10(1):210-214.
- Dhandapani S, Ramasamy, SV, Rajagopal, S, Namasivayam, N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol Res*. 2002; 46(3):251-255.
- Ghada ZAS. Anti-Diabetic Activity of Dried *Moringa Oleifera* Leaves in Normal and Streptozotocin (Stz)-Induced Diabetic Male Rats. *Indian J Appl Res*. 2013; 3(9):18-23.
- Rheney CC, Kirk KK. Performance of three blood glucose meters. *Ann Pharmacother*. 2000; 34 (3):317-321.
- Anderson L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME. Enzyme immune assay for intact human insulin in serum or plasma. *Clin Chem*; 1993; 39:578-582.
- Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Chin Chem*. 2001; 47(2):153 – 163.
- Scherwin JE. *Liver function. Clinical Chemistry: Theory Analysis Correction*. (4th ed.). St Louis, USA: Mosby Inc. Eds; 2003. 492 p.
- Henry RJ, Cannon DC, Winkelman JW. *Clinical Chemistry, Principles and Techniques*. (2nd ed.). Harper and Row; 1974.
- Grant GH, Kacchman JF. *Fundamental of Clinical Chemistry*. (3rd ed.). Philadelphia: W.B. Saunders Company; 1987. 298-320 p.
- Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzym*. 1990; 186:421–431.
- Misra HP, Fridovich I. The role of the superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J Biol Chem*. 1972; 247:3170-3175.
- Sinha KA. Colorimetric assay of catalase. *Anal Biochem*. 1972; 47:389-394.
- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase. *J Lab Clin Med*. 1976; 70:158-169.
- Bancroft JD, Gamble M. *Theory & practice of histological technique*. (5th ed.). N.Y: Churdhill Livingstone; 2002.
- Mishra SB, Mukerjee A, Vijayakumar M. Pharmacognostical and Phytochemical Evaluation of Leaves Extract of *Jatropha curcas* Linn. *J Pharmacog*. 2010; 2:9-14.
- Choudhary MK, Bodakhe SH, Gupta SK. Assessment of the Antulcer Potential of *Moringa Oleifera* Root-Bark extract in Rats. *J Acupunct Meridian stud*. 2013; 6(4):214-220.
- Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Deun KV, Smith P, Berger B, Heller A. (). Concordance of Toxicity of Pharmaceuticals in Humans and in Animals. *Regul Toxicol Pharmacol*. 2000; 32:56-67.

39. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's pharmacology. (7th ed.). London: Churchill Livingstone; 2012. 377 p.
40. Loomis TA, Hayes AW. Loomis Essentials of Toxicology. (4th ed.). California, U.S.A: Academic Press; 1996. 208-245 p.
41. Sezik E, Aslan M, Yesilada E, Ito S. Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. Life Sci. 2005; 76:1223-1238.
42. Deguchi Y, Miyazaki K. Anti-hyperglycemic and antihyperlipidemic effects of guava leaf extract. Nutr Metab. 2010; 7(9):1-10.
43. Lanjhiyana S, Garabadu D, Ahirwar D, Bigoniya P, Chandrana A, Chandrapatra K et al. Antidiabetic activity of methanolic extract of stem bark of *Elaeodendron glaucum* Pers. in alloxanized rat model. Adv Appl Sci Res. 2011; 2:47-62.
44. Alagammal M, Nishanthini A, Mohan VR. Antihyperglycemic and antihyperlipidaemic effect of *Polygala rosmarinifolia* Wright & Arn on alloxan induced diabetic rats. J Appl Pharm Sci. 2012; 2: 143-148.
45. Muhammad NO, Oloyede OB. Haematological Parameters of Broiler Chicks Fed Aspergillus niger-fermented *Terminalia catappa* seed Meal-Based Diet. Glob J Biotech Biochem. 2009; 4:179-183.
46. Moss PP. Blood Banking: Concepts and Application. Philadelphia: P.A Saunder; 1999. 12-34 p.
47. Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM. Free radicals scavenger and antihepatotoxic activity of *Rosmarinus*. Plant Med. 1993; 59:312-314.
48. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Biochemistry. (25th ed.). Philadelphia: Appleton and Lange, 2000. 179-203, 621-630 p.
49. Yassin MM, Ashour ARA, Elyazji NR. Alterations in Body Weight, Protein Profile, Non-Protein Nitrogen Constituents and Kidney Structure in Diabetic Rats under Glibenclamide Treatment. J Islamic Univ Gaza. 2004; 12(1):37-54.
50. Bernabucci U, Ronchi B, Lacetera N, Nardone A. Markers of Oxidative Status in Plasma and Erythrocytes of Transition Dairy Cows during Hot Season. J Dairy Sci. 2002; 85:2173-2179.
51. Das A. Heat stress-induced hepatotoxicity and its prevention by resveratrol in rats. Toxicol Mech Meth. 2011; 2(5):393-399.
52. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales PN, Phivthong-ngam, L, Ratanachamnong P, Srisawat S, Pongrapeeporn KS. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of the water extract of *Moringa oleifera* Lam. leaves. J Ethnopharmacol. 2008; 116:439-446
53. Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G, Singh HB. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. Food Chem Toxicol. 2009; 47:1109-1116
54. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agricul Food Chem. 2003; 51:2144-2155.
55. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sci Nutr. 2005; 56: 287-291.
56. Lukacinova A, Mojziz J, Benacka R, Keller J, Maguth T, Kurila P. Preventive Effects of Flavonoids on Alloxan-Induced Diabetes Mellitus in Rats. Acta Vet. 2008; 77:175-182.
57. Sharma VK, Kumar S, Patel HJ, Hugar S. Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. Int J Pharm Sci Rev Res. 2010; 1(2):18-22.