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**Original Article**

# Protective effects of Moringa Oleifera leaves against oxidative stress in diabetic rats

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## ABSTRACT

Free radicals have been reported in various diabetic complications, therefore this study was undertaken to evaluate the protective effect of *Moringa oleifera* against oxidative stress in alloxan-induced diabetic rats. Induction of diabetes in rats increases serum glucose, cholesterol and triacylglycerol while it reduced superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) content and increased malondialdehyde (MDA) level in hepatic and renal tissues. Treatment of diabetic rats with 50 mg/kg and 100 mg/kg of *Moringa oleifera* for 7 days significantly lowered serum glucose, cholesterol and triacylglycerol levels. It also increases SOD, CAT activities and GSH level while MDA level was significantly reduced in hepatic and renal tissues when compared with the control diabetic rats. In a separate experiment, *Moringa oleifera* extract was able to scavenge the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 2,-azinobis (3-ethylbenzoline-6-sulphonic acid (ABTS) radicals and these radicals scavenging abilities were found to be dose-dependent. The present results indicate that *Moringa oleifera* possesses antioxidant, hypoglycemic, hypocholesterolemic and hypolipidemic potential. *Moringa oleifera* was found to contain a high level of total phenolic content (65.90 ± 1.85 mg/g in GAE/g dried weight) which may be responsible for the effectiveness of the plant in the management of diabetic complications.

***Keywords***: *Moringa oleifera*, Free radicals, Malondialdehyde, Hyperglycaemia

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## INTRODUCTION

Antioxidants are micronutrients that neutralize the actions of free radicals [1]. Under normal circumstances, endogenous antioxidants defence system detoxified free radicals, however, when free radicals are overproduced endogenous antioxidant defence system may be overwhelmed resulting in oxidative stress [2]. Free radicals possess a strong oxidizing effect and induce damage to biological molecules, including proteins, lipids and deoxyribonucleic acid, with concomitant changes in their structure and function [3, 4]. This oxidative damage is a crucial etiological factor implicated in several chronic diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process [5].

Diabetes mellitus (DM) is a prevalent chronic disease in many countries [6]. It is estimated that the number of diabetic patients worldwide would increase to 366 million by 2030 [7]. Although the aetiology of this disease is not well defined, viral infection, an autoimmune disorder, and environmental factors have been implicated [8]. Increased oxidative stress, impaired antioxidant defence systems and consequently lipid peroxidation are major participants in the development and progression of DM and its complications [9]. Many herbal medicines and foodstuff are believed to have preventive effects on chronic diseases due to their radical scavenging or antioxidant properties [10]. Phenolic compounds present in plants have been shown to be effective antioxidant constituents and play a supportive role to antioxidant defence systems in-vivo. Studies have shown that phenolic compounds inhibit lipid peroxidation and scavenge reactive oxygen species (ROS), such as hydroxyl, superoxide and peroxynitrite radicals [11].

*Moringa oleifera* Lam. (*M. oleifera*) is one plant that has been shown in many studies to be a natural source of antioxidants such as phenolic compounds, vitamin A, C and E, ascorbic acid oxidase, polyphenol oxidase and catalase [12, 13]. *M. oleifera* belongs to the Moringaceae family [14], it is a multipurpose tree widely distributed in Asia, Africa and tropical areas of the world [15]. The leaves are a rich source of essential amino acids such as methionine, cysteine, tryptophan, lysine, vitamins and minerals [16]. Moringa leaves have been reported to act as a hypocholesterolemic agent, thyroid hormone regulator, an antidiabetic agent, antitumor agent and hypotensive agent [17, 18].

Therefore the present study was aimed to investigate the in-vitro and in-vivo antioxidant potential of *M. oleifera* extract and to determine the efficacy of the extract in lowering blood sugar and lipids levels in alloxan-induced diabetic rats.

## MATERIALS AND METHODS

**Reagents:** 6-Hydroxy- 2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ABTS, Gallic acid, thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) were obtained from Sigma–Aldrich Chemical Co. Ltd. (England) Nitrobluetetrazolium (NBT), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was obtained from Fluka (Buchs, Switzerland). All other chemicals used were analytical grade.

**Plant material (*Moringa oleifera*):** Fresh leaves of *M. oleifera* were collected from the premises of the research farm, faculty of Agriculture Science, Ladoke Akintola University of Technology Ogbomosho, Oyo state. The plant was authenticated by Prof. A.J. Ogunkunle of Department of Pure and Applied Biology of the institution. The plant was air dried and ground to a coarse powder.

**Preparation of *Moringa oleifera* Extract:** The coarse powder of *M. oleifera* (200 g) was soaked in 600 mL of methanol for 72 hours. The extract was filtered and the solvent was removed from the extract with a vacuum rotary evaporator at 45°C. The concentrated dried methanolic extract was then stored at -20°C before use.

**Determination of total phenolic compounds and in-vitro antioxidant potential of *Moringa oleifera***
The content of total phenolic compounds in *M. oleifera* was determined by Folin–Ciocalteu method as described by Miliauskas *et al.*, [19], while in-vitro antioxidant potential of *M. oleifera* was determined by following established procedures for Trolox equivalent antioxidant capacity (TEAC) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity assays as previously described in our earlier studies [20, 21].

**Animals and induction of diabetes:** Twenty-four Wistar albino rats (180-220 g) were obtained from the animal house at LAUTECH Agricultural Department, Ogbomoso, Oyo state and they were maintained under standard environmental conditions and had free access to feed and water. Animal studies were approved by the Committee for Ethical Animal Care and Alternatives to Animal Use in Research, Testing, and Education of 1986. The rats were acclimatized in the animal house of

the department for two weeks prior to the commencement of the experiment. For the induction of diabetes, rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan tetrahydrate (150 mg/kg body weight). Blood glucose concentration was checked by Glucometer after 4 days of alloxan injection. The animals with glucose concentration exceeding 230 mg/dl were considered diabetic.

**Grouping of animals:** The rats were divided into four groups, six rats in each group and treated as follows:

Group1. Normal Control: This group of rats were not injected with alloxan and served as non-diabetic control animals.

Group 2. Diabetic control (Alloxan 150 mg/kg body weight): Alloxan was given intraperitoneally for the induction of diabetes to this group.

Group 3. Diabetic plus *M. oleifera* treatment: diabetic rats treated with a methanolic extract of *M. oleifera* (50mg/kg body weight) for a period of 7 days.

Group 4. Diabetic plus *M. oleifera* treatment: diabetic rats treated with a methanolic extract of *M. oleifera* (100mg/kg body weight) for a period of 7 days.

After completion of 7 days of treatment, the animals were sacrificed by diethyl ether anaesthesia. Blood samples of each rat were collected by heart puncture and were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 4000 × g for 5 min and stored at -20°C for biochemical analysis. Liver and kidney tissues were excised at -4°C. The tissues were washed with ice-cold saline and immediately stored at -20°C for further biochemical analysis.

**Preparation of liver and kidney homogenates:** Prior to biochemical analyses, the liver and kidney samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10 % (w/v) liver and kidney homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for the assay of superoxide dismutase, catalase, reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

**Biochemical analysis:** Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (CHO), triglyceride (TAG) and high-density lipoprotein-cholesterol (HDL-c) in serum were determined using enzymatic kits (Labkit, Spain) according to the manufacturer's instructions.

**Determination of hepatic and renal antioxidant enzyme activities and MDA levels:** Hepatic and renal superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of Kakkar, *et al.* [22] at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of nitrobluetetrazolium reduction/mg protein. Catalase (CAT) activity was determined at room temperature by using the method of Aebi [23] and the absorbance of the sample was measured at 240 nm in a UV spectrophotometer. The concentration of reduced glutathione (GSH) in liver and kidney homogenates was measured, as described by Jollow et al. [24]. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid-reactive product malondialdehyde (MDA), using the method of Draper and Hadley, [25]. All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry *et al.*, [26], using bovine serum albumin (BSA) as a standard.

**Statistical Analysis:** Results are expressed as means ± SEM. Statistical analyses were performed using one-way analysis of variance followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and $p < 0.05$ was considered statistically significant.

## RESULTS

**The total phenolic compounds and in-vitro antioxidant potential of *Moringa oleifera:*** The *M. oleifera* demonstrated a concentration and time dependent scavenging activity by quenching DPPH radicals (Figure 1) and was compared with gallic acid, as a positive control. The IC50 values (defined as the concentration of test compound required to produce 50% inhibition) for DPPH scavenging by *M. oleifera* and gallic acid were 98.45 ± 3.47 µg/dL and 16.33 ± 1.50 µg/dL respectively (Table 1). In TEAC assay, the TEAC value of Trolox is 1.00. Gallic acid responded as the strongest with TEAC value of 4.25 ± 0.12 while *M. oleifera* responded lowest with TEAC value of 0.56 ± 0.07 (Table 1 & Figure 2). The phenolic content of *M. oleifera* was determined using Folin-Ciocalteu assay and was found to be 65.90 ± 1.85 mg/g in Gallic acid equivalent (Table 1)

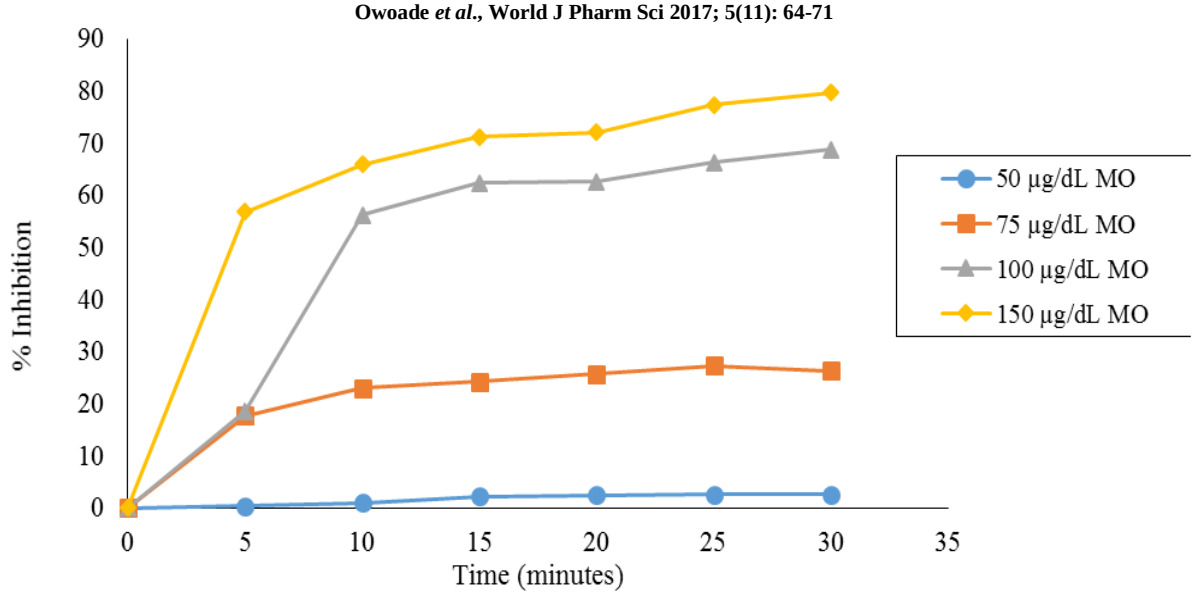


**Figure 1.** The effects of time on different concentration of methanolic extract of *M. oleifera* on inhibition of DPPH radical

**Table 1. Total phenolic content, DPPH radical scavenging value and Trolox equivalent antioxidant capacity (TEAC) of *Moringa oleifera***

| Sample | Total phenol[a] | DPPH scavenging activity (IC 50)[b] | Trolox equivalent antioxidant capacities (TEAC)[c] |
|---|---|---|---|
| Trolox | ND | ND | 1.00 |
| Gallic | ND | 16.32 ± 1.50 | 4.25 ± 0.12 |
| *M. oleifera* | 65.90 ± 1.85 | 98.45 ± 3.47 | 0.56 ± 0.07 |

Each value represents the mean ± SEM (*n*=3).
a Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.
b Expressed as µg/mL
c Expressed as mmol/L

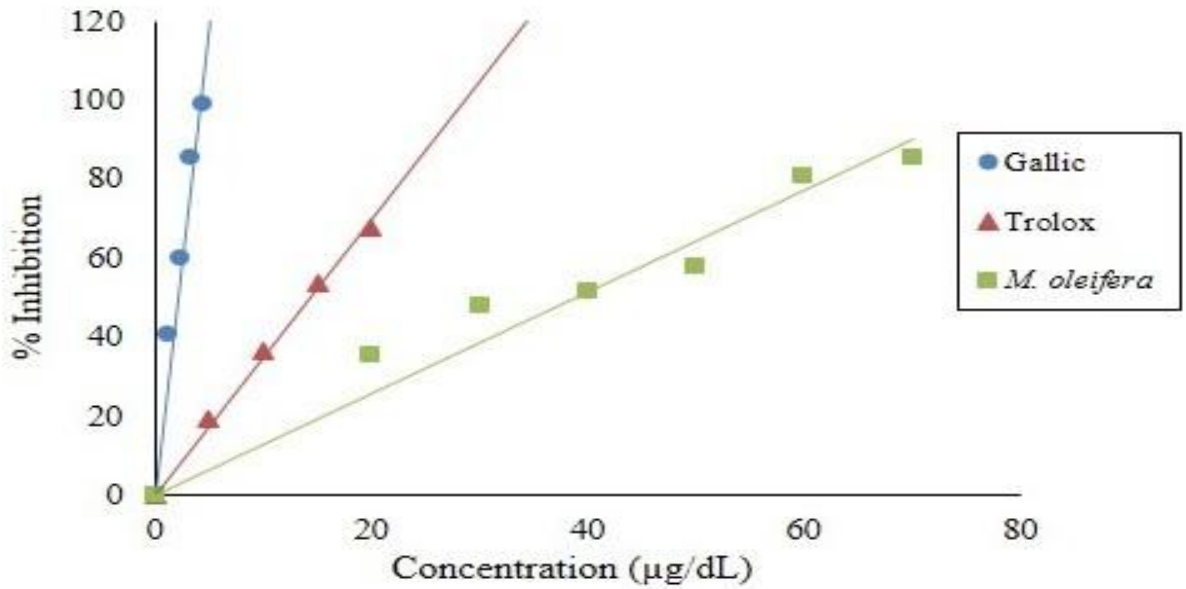


**Figure 2.** The effects of different concentrations of Gallic, Trolox and *M. oleifera* on the inhibition of the ABTS radical. Values are the means of three experiments ± SEM.

**Effect of *Moringa oleifera* extract on the blood glucose levels:** The results obtained in this study showed a significant (P< 0.05) increase in blood glucose levels in diabetic rats. However, treatment of diabetic rats with *M. oleifera* extract significantly decreased the blood glucose levels when compared to the diabetic control rats (Tables 2).

**Effect of *Moringa oleifera* extract on the levels of AST, ALT and ALP activities:** Induction of diabetes with alloxan resulted in significant ($p < 0.05$) rise in the levels of AST, ALT and ALP when compared to the normal rats. Oral administrations of *M. oleifera* extract at two different doses (50mg/kg and 100mg/kg) to diabetic rats for 7 days lower the levels of these marker enzymes, namely, AST, ALT and ALP significantly ($p < 0.05$) (Table 3).

**Effect of *Moringa oleifera* on hepatic antioxidant enzyme activities and MDA levels:** There were significant ($p < 0.05$) decreases in hepatic SOD, CAT, and GSH levels and a high hepatic level of MDA were observed in the diabetic control rats compared with normal control rats. Diabetic rats treated with *M. oleifera* showed significantly ($p < 0.05$) increases in hepatic SOD, CAT activities and GSH level, and a decrease in hepatic MDA level, which reflects the restoration of the antioxidant enzyme systems to near-normal values (Table 4).

**Effect of *Moringa oleifera* on renal antioxidant enzyme activities and MDA levels:** Renal SOD, CAT, and GSH levels were significant ($p < 0.05$) decreased while renal MDA level was significant ($p < 0.05$) increased in diabetic control rats as compared with normal control rats. However, treatment of diabetic rats with *M. oleifera* significant ($p < 0.05$) increases renal SOD, CAT activities and GSH level, and decrease renal MDA level, which reflects the restoration of the antioxidant enzyme systems (Table 5).

**Table 2.** Effects of *M. oleifera* treatments on blood glucose level in alloxan-induced diabetic rats

| Parameter | Normal rats | Diabetic control | Diabetic + 50mg/kg *M. oleifera* | Diabetic + 100mg/kg *M. oleifera* |
|---|---|---|---|---|
| DAY 1 | 75.44 ± 1.68 | 239.25 ± 6.83* | 241.53 ± 5.74 | 239.48 ± 5.29 |
| DAY 4 | 76.62 ± 1.73 | 240.42 ± 7.83* | 185.47 ± 3.28 | 157.38 ± 3.57** |
| DAY 7 | 75.48 ± 1.20 | 238.37 ± 6.52* | 146.17 ± 2.83** | 112.55 ± 2.32** |

Values are expressed as mean ± SEM of six rats.
*Significant at *P<0.05* when diabetic control group was compared with normal rats group.
**Significant at *P<0.05* when *M. oleifera* treated rats group was compared with diabetic control rats group

**Table 3**. Effect of *M. oleifera* extract on serum levels of AST (IU/L), ALT (IU/L) and ALP (IU/L) in alloxan induced diabetic rats

| Parameter | Normal rats | Diabetic control | Diabetic + 50mg/kg *M. oleifera* | Diabetic + 100mg/kg *M. oleifera* |
|---|---|---|---|---|
| ALP | 88.18 ± 1.58 | 156.60 ± 2.49* | 111.80 ± 1.67** | 93.83 ± 1.71** |
| AST | 117.30 ± 2.01 | 235.70 ± 6.00* | 168.70 ± 2.77** | 142.00 ± 3.44** |
| ALT | 49.38 ± 3.60 | 111.60 ± 2.88* | 82.55 ± 1.85** | 65.58 ± 1.22** |

Values are expressed as mean ± SEM of six rats.
*Significant at *P<0.05* when diabetic control group was compared with normal rats group.
**Significant at *P<0.05* when *M. oleifera* treated rats group was compared with diabetic control rats group

**Table 4**. Effect of *M. oleifera* extract on hepatic antioxidant enzyme activities and MDA levels in alloxan-induced diabetic rats

| Parameter | Normal rats | Diabetic control | Diabetic + 50mg/kg *M. oleifera* | Diabetic + 100mg/kg *M. oleifera* |
|---|---|---|---|---|
| SOD | 14.20 ± 1.11 | 9.56 ± 0.42* | 12.50 ± 0.85 | 14.10 ± 0.85** |
| CAT | 0.59 ± 0.02 | 0.22 ± 0.02* | 0.39 ± 0.20** | 0.47 ± 0.02** |
| GSH | 5.07 ± 0.19 | 3.20 ± 0.21* | 4.83 ± 0.18** | 5.94 ± 0.32** |
| MDA | 2.29 ± 0.23 | 4.03 ± 0.23* | 2.40 ± 0.45** | 2.15 ± 0.26** |

Values are expressed as mean ± SEM of six rats.
*Significant at *P<0.05* when diabetic control group was compared with normal rats group.
**Significant at *P<0.05* when *M. oleifera* treated rats group was compared with diabetic control rats group

**Table 5**. Effect of *M. oleifera* extract on renal antioxidant enzyme activities and MDA levels in alloxan-induced diabetic rats

| Parameter | Normal rats | Diabetic control | Diabetic + 50mg/kg *M. oleifera* | Diabetic + 100mg/kg *M. oleifera* |
|---|---|---|---|---|
| SOD | 11.70 ± 0.78 | 5.87 ± 0.27* | 9.46 ± 0.29** | 10.70 ± 0.55** |
| CAT | 0.51 ± 0.02 | 0.22 ± 0.01* | 0.35 ± 0.02** | 0.47 ± 0.02** |
| GSH | 6.40 ± 0.18 | 3.20 ± 0.26* | 5.66 ± 0.24** | 6.05 ± 0.23** |
| MDA | 3.58 ± 0.03 | 5.34 ± 0.16* | 3.78 ± 0.27** | 3.52 ± 0.14** |

Values are expressed as mean ± SEM of six rats.
*Significant at *P<0.05* when diabetic control group was compared with normal rats group.
**Significant at *P<0.05* when *M. oleifera* treated rats group was compared with diabetic control rats group

**Effect of *Moringa oleifera* extract on the serum levels of TAG, CHO and HDL-C in alloxan-induced diabetic rats:** As presented in Table 6 injection of alloxan increased serum CHO and TAG levels of control diabetic rats significantly ($p < 0.05$) above the normal levels while it reduced HDL-C below the normal level. Treatment of diabetic rats with 50mg/kg and 100mg/kg *M. oleifera* extract significantly ($p < 0.05$) decreased serum triglyceride and cholesterol while it increased serum HDL-C when compared with diabetic control rats.

**Table 6**. Effect of *M. oleifera* extract on serum levels of CHO (mg/dL), CAT (mg/dL) and HDL-C (mg/dL) in alloxan induced diabetic rats

| Parameter | Normal rats | Diabetic control | Diabetic + 50mg/kg *M. oleifera* | Diabetic + 100mg/kg *M. oleifera* |
|---|---|---|---|---|
| TAG | 102.80 ± 6.36 | 135.30 ± 6.50* | 101.20 ± 4.40** | 80.35 ± 4.39** |
| CHO | 74.48 ± 1.94 | 115.30 ± 3.28* | 74.38 ± 2.15** | 59.27 ± 2.33** |
| HDL-C | 55.62 ± 1.15 | 33.67 ± 0.99* | 43.14 ± 1.58 | 52.15 ± 1.54** |

Values are expressed as mean ± SEM of six rats.
*Significant at *P<0.05* when diabetic control group was compared with normal rats group.
**Significant at *P<0.05* when *M. oleifera* treated rats group was compared with diabetic control rats group

## DISCUSSION

The present study evaluated the effect of methanolic extract of *M. oleifera* on biomarkers of oxidative stress, in serum, liver and kidney of alloxan-induced diabetic rats. The results of the *in-vitro* investigation revealed that *M. oleifera* extract has antioxidant potential against $ABTS^{\bullet+}$ and DPPH radicals. The extract showed significant $ABTS^{\bullet+}$ and DPPH radicals scavenging activity in a concentration-dependent manner, which can be attributed to hydrogen donating ability of the extract. In this study, diabetes was induced in experimental rats by intraperitoneal injection of alloxan. Alloxan is known for its selective pancreatic islet β – cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals [27, 28]. Diabetic rats had their blood glucose level increased 3 fold when compared with the normal rats, however, treatment with 50 mg/kg and 100 mg/kg methanolic extract of *M. oleifera* leaves reduces the blood glucose level in diabetic rats by 22.86 % and 34.53 % respectively after 4 days of administration and 38.70 % and 52.78 % respectively after 7 days of administration. The hypoglycemic effect of *M. oleifera* obtained in this study could be that the extract increased tissue utilization of glucose [29] either by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues [30].

ALP, AST and ALT are the major serum hepatic enzymes used for a liver function test. The elevated activities of these enzymes in serum are an indication of liver damage [31]. In this study, we found that diabetic rats had elevated activities of serum ALP together with elevated activities of serum AST and ALT, which are more specific to liver damage [28]. Administration of 50 mg/kg and 100 mg/kg methanolic extract of *M. oleifera* for 7 days significantly reduced ALP activity by 29 % and 40 % respectively, AST activity by 28 % and 40 % respectively and ALT activity by 26 % and 41 % respectively. These results indicated that *M. oleifera* leaves had a protective effect on diabetic-induced liver injury.

In the present study, the activities of SOD and CAT as well as the level of GSH in the liver, and kidney tissues of diabetic rats were significantly reduced. Treatment of diabetic rats with 50 mg/kg and 100 mg/kg methanolic extract of *M. oleifera* for 7 days significantly regenerate hepatic SOD activity by 31 % and 47 % respectively, hepatic CAT activity by 77 % and 113 % respectively and hepatic GSH level by 51 % and 85 % respectively. It also increases renal SOD activity by 61 % and 82 % respectively, renal CAT activity by 59 % and 114 % respectively and renal GSH level by 76 % and 89 % respectively. Decreased levels of SOD, CAT and GSH in the diabetic state may be due to inactivation caused by free radicals [32]. The stabilization of these enzymes by the extract is an indication of the improvement of the functional status of the liver and kidney. This can probably indicate that the *M. oleifera* extract either increase the biosynthesis of SOD, catalase and GSH or reduce the extent of oxidative stress leading to less degradation, or it may have both effects.

Alloxan injection into rats significantly increased the levels of serum cholesterol and triglyceride, however, administration of methanolic extract of *M. oleifera* to diabetic rats for 7 days significantly reduced the levels of cholesterol and triglycerides in the rats when compared to diabetic control rats. These changes are beneficial in preventing diabetic complications as well as in improving lipid metabolism in diabetics [33]. Lipid peroxidation is the most extensively investigated process in antioxidant defence mechanism as this is induced by the extensive generation of free radicals. The abundant presence of lipids at sites where radicals are formed, render them easily accessible as endogenous targets. Lipid peroxide mediated tissue damage has been observed in the development of both type 1 and 2 diabetes mellitus. An increase in TBARS in diabetic control rats, as a marker of lipid peroxidation in diabetes has been observed in our study. However, administration of 50 mg/kg and 100 mg/kg methanolic extract of *M. oleifera* decreases TBARS in the liver of diabetic rats by 40 % and 47 % respectively and decreases TBARS in the kidney of diabetic rats by 29 % and 34 % respectively. Hence, the present study confirms that the *M. oleifera* possess an ability to inhibit the lipid peroxidation in diabetes.

Plant phenolics are commonly found in both edible and non-edible plants and their antioxidant activities mainly due to their redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quencher [34, 35]. In this study, phenolic compounds in *M. oleifera* was found to be 66 mg/g in Gallic acid equivalent, we, therefore, speculate that the presence of these phenolic compounds may account for the ability of methanolic extract of *M. oleifera* to protect the rats against alloxan-induced liver and kidney damaged. It may, however, be possible that *M. oleifera* contains other natural antioxidants such as β- carotene, vitamin C and others which were not determined in this study that may be working synergistically with the phenolic compounds in the extract to produce the observed effects.

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