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# ASSESSMENT OF THE ANTI-HYPERGLYCEMIC AND ANTIOXIDANT ACTIVITIES OF THE METHANOLIC EXTRACT OF *Moringa oleifera* IN ALLOXAN-INDUCED DIABETIC RATS

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Key words : Diabetes, *Moringa oleifera*, Methanolic Extract, Alloxan, Antioxidants activities

The aim of the study was to assess the antidiabetic and antioxidant properties of *Moringa oleifera* leaf on blood glucose level, body weight and kidneys weight as well as to determine serum biochemical makers of kidneys and antioxidants such as SOD (superoxide dismutase), CAT (Catalase) in the kidneys. It has been reported earlier that the leaves of this plant possess high antioxidant and medicinal properties which may be helpful in the treatment of diabetes related complications. It offers protective action against alloxan induced renal damage and reactive oxygen species (ROS). As observed, 400 mg/kgb.wt. of *Moringa oleifera* extract (MOF) may be useful for the improvement of the complication related to diabetes.

## INTRODUCTION

Diabetes mellitus (DM) is long-term complications which include kidney failure, heart disease and damage to the eyes (Alberti *et al.* 1998; WHO, 2014). It is either due to body cells responding inproperly to the insulin or due to pancreas not producing enough insulin (Gardner and Dolores *et al.* 2011). It is also characterized by three "polyps" implying inability to reabsorb water, resulting in excessive thirst (Polydipsia), emptying more urine (Polyuria) and excessive eating (Polyphagia). Mellitus is a heterogeneous group of disease which leads to an elevation of blood glucose level. In diabetic patients, it is observed that free radical production is increased due to increased oxidative stress (Giugliano *et al.,* 1996). Vascular disease associated with oxidative damage is due to free radicals in diabetic patients (Oberley, 1988; Grididhari *et al.* 2011). Susceptibility of liver and kidney increases in diabetic animals which may be due to increased oxidative stress and lipid peroxidation.

*Moringa oleifera* commonly known as drumstick belongs to the Moringaceae family. It is widely distributed in the tropics and subtropics and extensively cultivated in Africa, Mexico, Indonesia, Malaysia, Central and South America, Philippines and India (Fuglie *et al.* 1999). Various parts of this plant contain important minerals such as Fe, Ca, P,K and various phenolics, viz., quercetin, β-sitosterol, Kaempferol and caffeoylquinic acid and are good source of proteins, amino acids, vitamins and β-carotene (Anwar *et al*., 2006). It is rich in four natural dietary antioxidants, Vitamins A, E, C and phenolics (Gowrishankar *et al*.,2010) and also carbohydrates, proteins, fats, minerals, moisture, ash contents and crude fibre (Oluduro *et al.* 2012).

*Moringa oleifera* has medicinal and industrial value (Wadhwa *et al*., 2013). In ethno-medicine it has been used in the treatment of various complications such as stomach ulcer, gastric discomfort, dysentery, diarrhoea, diabetes and skin infections (Ndong *et al.* 2007; Jaiswal *et al.* 2009; Kasolo *et al.* 2010). Ethanolic and aqueous extracts of *Moringa oleifera* contain alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, anthraquinone and anthocyanin (Azubuogu and C. U., 2012; Nweze and Nwafor, 2014). Presence of tannins and flavonoids possess antidiabetic activity (Sharma *et al.* 2010)

The leaves possess antipyretic, antiepileptic, anti-inflammatory, antitumor, anti-ulcer, anti-oxidant and anti-hypertensive properties (Bukar *et al.* 2010) Leaves of *Moringa oleifera* exhibit lipid lowering activity which may be due to the presence of β-sitosterol (Ghasi *et al.* 2010), enhance spermatogenesis process in mice (Lilibeth and Glorina, 2010) and hepatoprotective activity (Fakurazi *et al.*2008). In diabetic rats aqueous extract of leaves of *Moringa oleifera* has shown to decrease the blood glucose level (Ndong *et al.* 2007; Jaiswal *et al.* 2009). The fresh leaf juice was found to inhibit the growth of microorganisms which are pathogenic to humans like *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Caceres *et al.* 1991)

## MATERIALS AND METHODS

**Plant collection, identification and preparation of extract** : Leaves of *Moringa oleifera* were collected manually form the campus of Patna University. Plants were taxonomically indentified and authenticated by the Department of Botany, Patna University, Patna. Leaves were washed with distilled water, dried and then crushed into coarse powder with the help of blender. The powder (100g) was suspended in absolute methanol and allowed to stand in a shaker at room temperature for 72 hours. It was then filtered with the help of Whatmann filter paper and dried in rotatory evaporator at temperature 50-55°C. A crystalline brownish residue weighing 9 gm. was obtained. This extract was preserved in air tight bottle in a refrigerator until used.

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**STUDY DESIGN**

**Experimental animals :** For this study experimental rats were adult male Wister rats weighing about 150-180 gm. Rats were taken from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Reg-No. 1129/ bc/07/CPCSEA). The rats were maintained under standard laboratory conditions at temperature of 22±2°C and a normal period (12h light/12h dark). Rats were housed in Polypropylene cages. Experiments on these rats were performed with approval by the IAEC (Institutional Animal Ethics Committee) with IAEC No. 2017/1E-10/08/17.

**Induction of Diabetes**

Rats were fasted for 24 hours before the intraperitoneal induction of alloxan. Stock solution of alloxan was prepared by dissolving 120 mg/kg b. wt. alloxan monohydrate in 0.9% NaCl solution (0.9 gm. NaCl dissolved in 100 ml distilled water). After one week of alloxan induction, when rats were stabilized in diabetic condition their blood glucose level was measured by using glucometer from the tail vein of overnight fasted rats. The rats having blood glucose level more than 200 mg/dl were considered to be diabetic. The process was repeated after 10-15 days.

**Treatment**

According to LD50, 400 mg/kg body weight is suitable dose of *Moringa oleifera*. Normal saline was used as the diluents and administered via oral gavages for 30 days. For our experiments 30 rats were taken and divided into groups and given the following treatments.

Group I: Normal Control Groups (received normal saline).

Group II: Diabetic Control Groups (received 400mg/kg MOE).

Group III: 10 days Treated Groups (received 400mg/kg MOE).

Group IV: 20 days Treated Groups (received 400mg/kg MOE).

Group V: 30 days Treated Groups (received 400mg/kg MOE).

On 30th days animals were anaesthetized and decapitated after overnight fasting with free access to water. Fasting blood sample drawn for biochemical test and kidney were rapidly excised, rinsed with cold normal saline to eliminate blood contamination, dried by blotting with filter paper and weighed. Until analysis tissues were kept in deep freeze-80°C

**Blood and Homogenate Preparation**

Blood samples were drawn from the heart via cardiac puncture and collection in RIA tubes. Samples were allowed to clot for 1 hour and the serum separated by centrifugation at 3000 rpm for 15 min.

Kidneys were excised, washed with cold normal saline, homogenized on ice in PBS buffer and centrifuged at 15000 rpm for 10 min at 4°C to remove all cell debris and nuclei.

Supernatants were used to assay biochemical parameters.

**OBSERVATIONS**

**Effect of *Moringa oleifera* on body weight, Plasma glucose levels and Kidney weight of rats (Table I)**

The body weight and kidney weight of diabetic control rats were significantly decreased and increased respectively. Increased kidney size in diabetic rats is a sign of acute inflammation. Alloxan induced diabetic rats showed elevated plasma glucose levels, which shows hyperglycemia; other authors have also reported identical results (Yassa *et al.* 2014; Toma *et al.* 2012). Treatment with *Moringa oleifera* showed a significant decrease in weight of kidney and blood glucose. After administration of methanolic extract of *Moringa oleifera* body weight of diabetic rats were significantly increased.

**TABLE-I**

**Body weight, Glucose level and Kidney weight in normal control, diabetic control and treated groups**

| Parameter | Normal Control | Diabetic Control | 10 days treated | 20 days treated | 30 days treated |
|---|---|---|---|---|---|
| Body Weight (g) | 181.7±1.05 | 145.0±7.52 | 150.8±3.74 | 160.8±4.36 | 176.7±4.40 |
| Blood Glucose (mg/dl) | 112.0±13.48 | 275.0±5.16 | 235.0±12.78 | 198.3±19.00 | 127.8±32.93 |
| Kidney Weight (g) | 1.175±0.15 | 2.25±0.07 | 2.21±0.10 | 2.15±0.06 | 2.08±0.02 |

**Biochemical parameters of kidney after treatment with *Moringa oleifera* (Table II)**

In diabetic condition serum urea (fig-1) uric acid (fig-2) and creatinine (fig-3) levels were significantly increased as compared to normal control rats. After 30 days of treatment with MOE these levels had reduced.

**TABLE-II**

| **Estimation of Kidney Function Test in normal control, diabetic control and treated groups** | | | | | |
|---|---|---|---|---|---|
| Parameter | Normal Control | Diabetic Control | 10 days treated | 20 days treated | 30 days treated |
| Urea (mg/dl) | 20.33±0.80 | 39.24±1.02 | 37.14±0.77 | 27.00±0.73 | 24.83±1.62 |
| Uric acid (mg/dl) | 4.90+0.21 | 8.73±0.07 | 8.05±0.07 | 7.13±0.27 | 6.73±0.06 |
| Creatinine (mg/dl) | 0.73+0.04 | 0.89+0.02 | 0.81±0.02 | 0.77±0.02 | 0.71±0.01 |

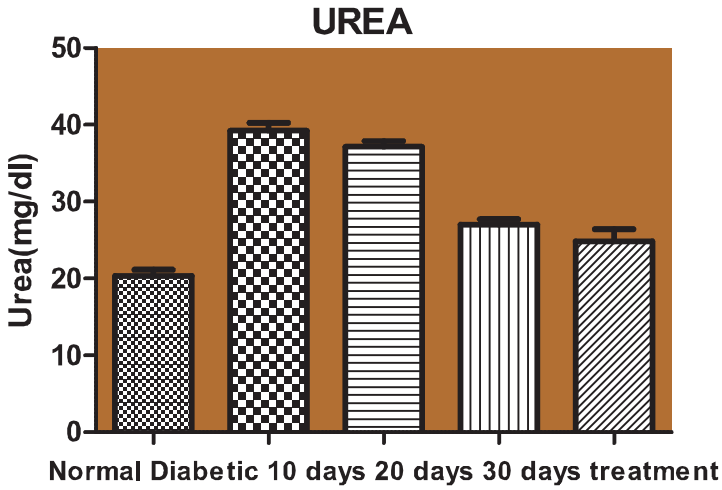



**Figure-1 :** Effect of MOE on Urea levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control.

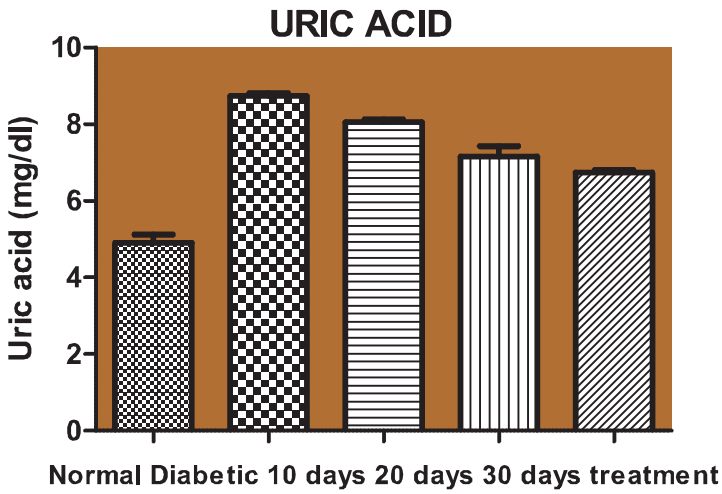



**Figure-2 :** Effect of MOE on Uric acid levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control.

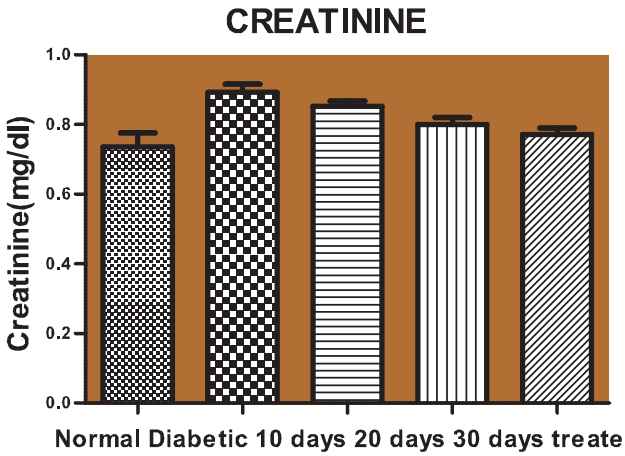



**Figure-3 :** Effect of MOE on Creatinine in control and experimental rats. Values are given as mean ± SE, n = 6, p < 0.05 compared to normal control and diabetic control.

**Activities of Antioxidant Enzymes in the kidneys and effect of Lipid Peroxidation after *Moringa oleifera* administration (Table III)**

Diabetic rats showed significantly (P < 0.05) decreased SOD (fig-4) and catalase (fig-6) levels when compared to normal control rats. SOD and CAT levels increased upon *Moringa oleifera* administration when compared to diabetic controls rats. In the present study, significantly increased LPO (fig-5) levels in the kidney of diabatic rats were found. Afater treatment with *Moringa oleifera,* LPO (fig-5) levels reduced. Similarly, other workers' observations show agreement with our results (Verma *et al.* 2009).

**TABLE-III**

**Estimation of Antioxidant activities in normal control, diabetic control and treated groups**

| Parameter | Normal Control | Diabetic Control | 10 days treated | 20 days treated | 30 days treated |
|---|---|---|---|---|---|
| SOD (U/mg) | 1.48±0.14 | 0.64±0.02 | 0.65±0.02 | 0.78±0.02 | 1.04±0.07 |
| LPO (nmole/g) | 4.09±0.20 | 7.90±0.19 | 6.15±0.04 | 5.00±0.33 | 4.36±0.16 |
| Catalase (U/mg) | 1.38±0.13 | 0.93±0.02 | 0.94±0.02 | 1.01±0.04 | 1.04±0.06 |

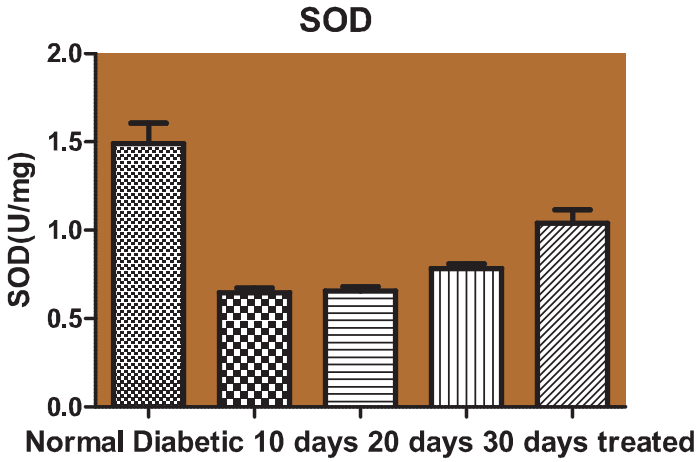



**Figure-4 :** Effect of MOE on SOD (Superoxide dismutase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, p < 0.05 compared to normal control and diabetic control.

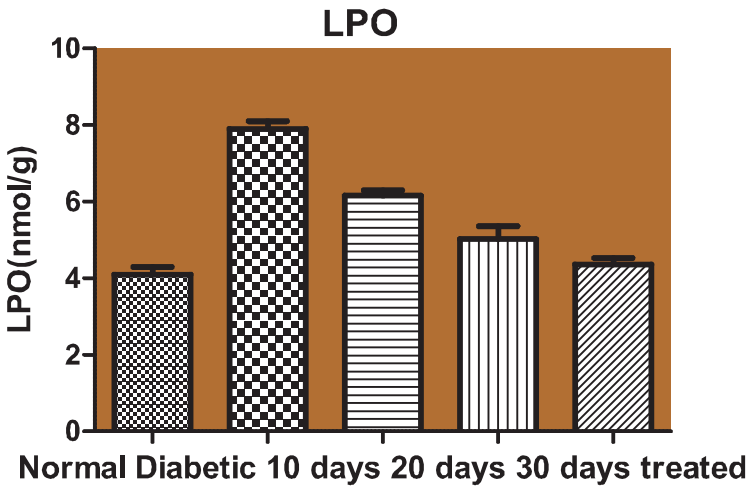



**Figure-5 :** Effect of MOE on LPO (Lipid Peroxidase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, p < 0.05 compared to normal control and diabetic control.

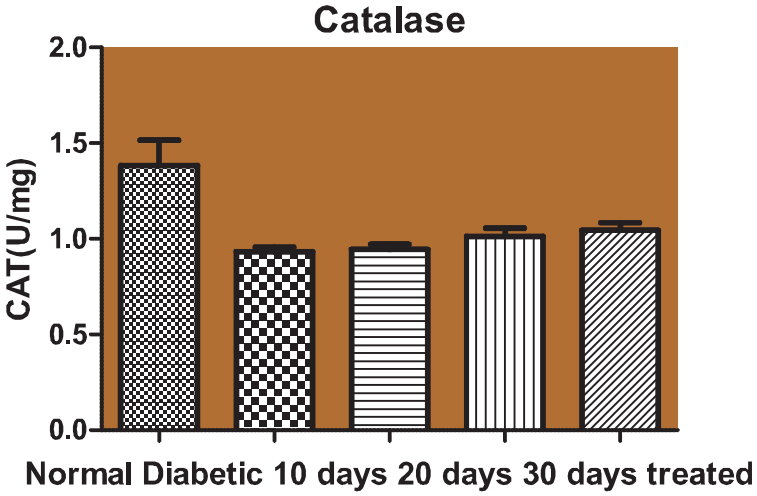


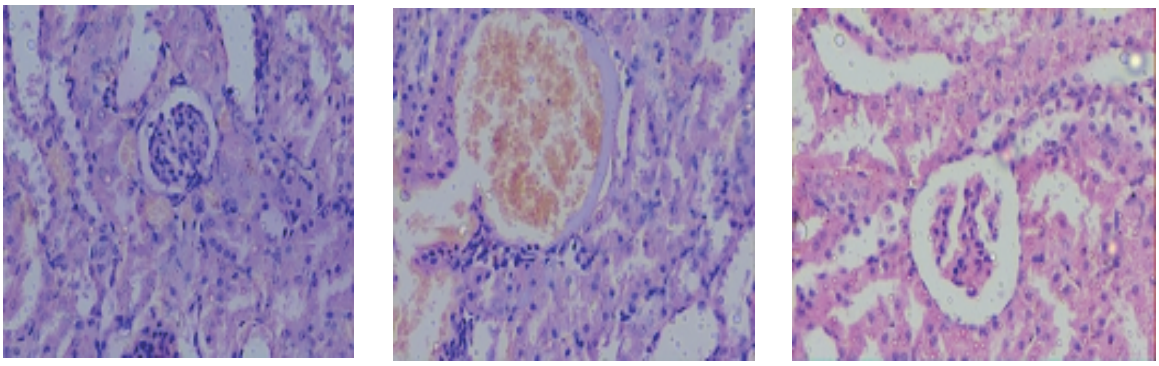

**Figure-6 :** Effect of MOE on CAT (Catalase) levels in control and experimental rats. Value are given as mean ± SE, n = 6, P < 0.05 compared to normal control and diabetic control.

**Annotations on effect of *Moringa oleifera* on Kidney Histopathology :**

The protective effect of *Moringa oleifera* on kidney section of normal, diabetic and MOE treated groups was seen. Normal architecture of kidney in normal control rats (A) was observed. Diabetic rats showed several renal damages showing hemorrhage and accumulation of glomerulus (B). Adminstration of *Moringa oleifera* showed appreciable improvements in the renal cells with mild vascular accumulation of the glomerulus (C). This study is relevant with other findings which show significant changes in the histopatholoy of kidney of diabetic rats after treatment (Donath *et al.*, 2011; Kandasamy *et al.* 2013)

**Histological Examination :**

Kidney tissues were dissected out and washed thoroughly in normal saline (0.9%) and fixed in formalin (10%) and then dehydrated in ethanol; after that it was embedded in paraffin wax at 60°C with the help of hot air oven and blocks were made with the help of L-shaped mould. The tissue blocks were cut and fixed on Mayer's albumin rubbed glass slides. For histological examination slides were stained in hematoxylin and eosin. The slides were examined under a light microscope at a magnification 40X.



(A) Normal Control (B) Diabetic Control (C) MOE treated group

**Figure : Microphotographs of kidney histology of different groups after treatments of MOE (H&E Staining, 40 X)**

## STATISTICAL ANALYSIS

Results were analyzed using Prism Graph Pad 5.0 (Graph Pad Software, San Diego, CA, USA) to calculate mean ±SE (Standard Error) and total variation present in a set of data was analyzed through one-way analysis of variance (ANOVA). Differences in multiple groups have been analyzed with Dunnett's t-test. The criteria for statistically significant between control and test means was at $P < 0.05$.

## DISCUSSION

In Diabetes Mellitus severe renal damage is observed due to elevated glucose level, abnormal glucose regulation and glycosylated protein tissue levels, increased oxidative stress and hemodynamic changes within the kidney tissue (Aurell and Bjorck, 1992). Alloxan monohydrate destroys the pancreatic β-cells (Lenzen and Panten, 1988; Oberley and L. W, 1988) while methanolic leaf of *Moringa oleifera* plays to counteract action by the regeneration of β-cells to release insulin (Amira *et al.* 2014; Jadhav *et al.* 2009) thereby normalizes the increased serum level of glucose (Amira *et al.* 2014). Previous worker reported that kidney enlargement may be due to hypertrophy (enlargements of cell components) and hyperplasia (rapid production of the cell) of the kidney (Vlassara *et.al.*, 2002; Thomas *et al.*, 2008; Rodriguez-perez *et al.*, 2015). After 30 days of treatment with *Moringa oleifera* the level of creatinine had reduced. Our results are in agreement with the result from leaf extract of *Sphagneticola trilobata* (Kade *et al.*, 2010), from *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Manikandan and Doss, 2010).

Enzymatic antioxidants (SOD, CAT) prevent ROS (Reactive Oxygen Species) induced oxidative stress, delay or prevent the oxidation of substrates (Naugler *et al.*,2008). SOD and CAT are two major radical scavenging enzymes. SOD is the main enzymatic defense against ROS. It converts superoxide radical into hydrogen peroxide and molecular oxygen CAT is a heme protein that catalyses the reduction of hydrogen peroxide into water. In this way, two toxic species superoxide radical and hydrogen peroxide are converted into water and oxygen (Navarro *et al.*1993). In diabetic condition activities of SOD and CAT decreases in both liver and kidney may be due to over-production of ROS (Kaleem *et al.* 2006). The phytochemicals present in *Moringa oleifera* may either be reducing oxidative stress by decreasing blood glucose or may be scavenging the alloxan metabolites. With *Capparis aphylla* activity of SOD, incrases in liver and kidney (Dangi and Mishra, 2011) have been reported earlier. In diabetic rats, generation of ROS increased which may be due to the damage of cell membrane lipids and thus MDA level increases (Farombi *et al.* 2015).

## CONCLUSION

In our study, *Moringa oleifera* was able to enhance antioxidant activity and reduce lipid peroxidation as well as it has ability to protect against oxidative damage due to its phenol content. So, results suggest that *Moringa oleifera* is the potential antidiabetic agent in the management and treatment of diabetes .

Further studies are recommended for structural activity of *Moringa oleifera* and their mechanism of action.

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