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EFFECT OF *Moringa oleifera* lEAF EXTRACT ON LIVER MARKERS AND THEIR ANATOMIC RECOVERY IN ALLOXAN INDUCED DIABETIC RATS.

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Key words : Diabetes, *Moringa oleifera, methanolic extract, antioxidant activities* and *histopathological study*.

The aim of the preset study was to evaluate the ameliorating effect of *Moringa oleifera* extract (MOE) by evaluation of comparative hepatic biochemical parameters (ALP, AST, ALT and bilirubin) and histopathological slides of liver of normal, control and treated rats. Results have shown significant changes ($p<0.05$) in biochemical parameters as well as histology of liver. The results have suggested that *Morngа oleifera* (400 mg/kg b.wt) can be used in the treatment of diabetes.

INTRODUCTION

Diabetes mellitus is the largest endocrine disorder of the world (Pattanayak *et. al.,* 2009). It is a metabolic disorder of carbohydrate, lipid and protein metabolism which is improperly regulated by insulin; therefore fasting as well as post prandial blood glucose level increases (Dewanjee *et al*. 2008). Hyperglycemia increases production of reactive oxygen species via at least seven pathways namely increased intracellular activation of sorbitol pathway, increased glycolysis, and disruption of polyol pathway, glycosylation decreased antioxidant defenses and altered eicosanoid metabolism (Hakim *et al.* 2008).

Diabetes Mellitus was earlier classified as rich man's disease; now it has spread worldwide (Sachan *et al.* 2009). Chronic hyperglycemia leads to long term damage, dysfunction and failure of organs such as liver, kidneys, eyes, heart, nerves, and blood vessels. Nevertheless, for the treatment of diabetes neither insulin nor oral hypoglycemic drugs are available because of their side effects and they have to be given throughout the life (Halim, 2003)

The medicinal plants might prove as a boon for the development of pharmaceutical entities or as a dietary assistant to existing therapies (Upwar *et. al*, 2011). Many medicinal plants have been reported effective for treating diabetes, although their mechanism of action is not known. Some of them have mimetic property with insulin, some may inhibit insulinase activity and some others may increase β-cells in pancreas. The fiber present in the plant may interfere with carbohydrate absorption, thereby affecting blood glucose (Jelodar *et. al*, 2005).

MATERIALS AND METHODS

Extract Preparation

Fresh leaves of *Moringa oleifera* were taken from the campus of Patna University, rinsed with distilled water, dried and later ground with the aid of an electrical grinder. Plants were identified by the Department of Botany, Patna University. *Moringa oleifera* leaf powder was suspended in absolute methanol and allowed to stand for 72 hours in a shaker at room temperature. The extract was filtered with the help of whatmann no. 1 filter paper. The filtrate was preserved in a refrigerator.

Experimental Animals

About 150-180 gm male Wister rats were used in these experiments. Rats were housed in cages at an ambient temperature of 22 ± 2°C with 12 hours each of dark and light cycle. Rats were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Reg-No.1129/bc/07/CPCSEA). Animals were fed special diet and water ad libitum throughout the period of experiments. Our experiments were approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. 2017/IE – 10/08/17.

Induction of Diabetes

Diabetes was induced in rats by intraperitoneal infusion (120 mg/kg b.wt. of alloxan monohydrate in 0.9% Nacl solution (0.9 gm NaCl dissolved in 100 ml distilled water). After 15 days of induction, blood glucose level was checked using glucometer.

Experimental Design

For this study 30 rats were taken and divided into groups and given the following treatments.

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

Group II: Diabetic Control Groups (given normal saline).

Group III: 10 days Treated Groups (given 400 mg/kg b.wt MOE).

Group IV: 20 days Treated Groups (given 400mg/kg b.wt MOE).

Group V: 30 days Treated Groups (given 400 mg/kg b.wt MOE)

Analysis of Biochemical Parameters

On the 30th day of the experiments all the animals were sacrificed and their blood samples were collected into specimen bottle. Blood samples were centrifuged at 3000 rpm for 15 minutes and serum was separated into new specimen bottle and stored at –20°C. Liver was rapidly excised, rinsed with distilled water and stored in refrigerator at –80°C for estimation of antioxidant activity.

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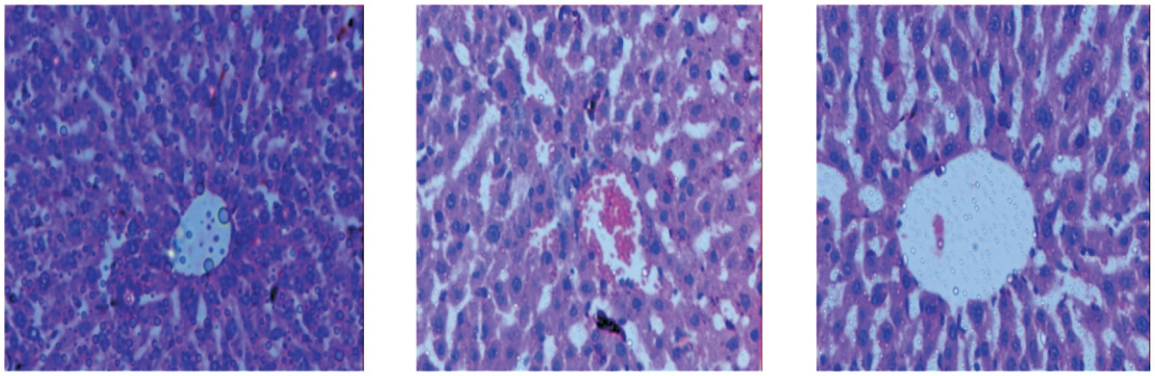
HISTOPATHOLOGICAL EXAMINATION

The liver tissues from each animal were removed after sacrificing and then washed with normal saline (0.9%) and fixed in formalin (10%) solution. After 24 hours liver tissues were embedded in molten paraffin wax with the help of L-shaped metallic blocks and kept under freezing plates to allow to solidify. The cross sections of fixed tissues were cut using microtome. The sections were then deparaffinized in xylene and hydrated in a series of alcohol gradients (100, 90, 70, 50 and 30% aqueous alcohol) for 2 minutes each and stained with hematoxylin (0.5%) for 1 min, differentiated in 1% acid alcohol and then eosin for 30s. At last section were mounted with DPX and observed under light microscope.

OBSERVATION

MORPHOLOGICAL FINDINGS OF LIGHT MICROSCOPY

Microscopic examination showed normal architecture of liver of normal control including hepatic lobules with normal hepatocytes surrounded by sinusoids and distributed towards the central veins (Fig A). In contrast, diabetic control showed progressive loss of general organ structure including degenerated central veins and abnormal cellular hemorrhages (Fig B). After treatment with MOE, liver showed changes in their morphological structure (Fig C).



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

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

MEASUREMENT OF TISSUE ENZYMES

Assay of antioxidant enzymes (Table I)

Antioxidative enzymes like SOD (Fig-1) and CAT (Fig-2) reduces in oxidative stress (Snehal *et al.* 2009). Antioxidants protect the human body against damage by reactive oxygen species (ROS) and two scavenging enzymes such as superoxide dismutase (SOD) and catalase (CAT). SOD scavenges superoxide ions by catalyzing its dismutation and catalase removes hydrogen peroxide (Lee *et al.* 2001). Catalase destroys hydrogen peroxide into oxygen and water by catalyzing its two-electron dismutation (Ali *et al.* 2008). SOD destroys superoxide into hydrogen peroxide and water by catalyzing its one-electron dismutation. The decreased activities of SOD and CAT showed increased production of oxygen and hydrogen peroxide by non enzymatic glycation and auto oxidation of glucose (Lubec *et al.* 1996). Lipid Peroxidase (Fig-3) levels reduce after treatment with medicinal plants.

Table I

Estimation of Antioxidant enzymes activity in normal, diabetic and treated groups					
Parameter	Normal Control	Diabetic Control	10 days treated	20 days treated	30 days treated
SOD (U/mg)	1.28±0.02	0.59±0.01	0.68±0.01	0.80±0.02	1.26±0.24
CAT (U/mg)	3.16±0.47	1.79±0.32	1.82±0.23	1.93±0.03	2.14±0.09
LPO (nmol/gm)	6.07±0.14	11.01±0.09	10.02±0.14	8.01±0.11	6.58±0.05

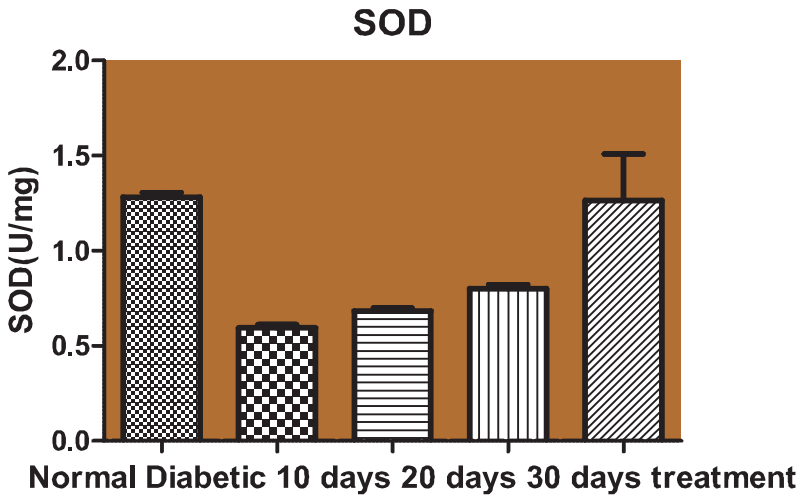


Figure 1 : Effect of MOE on SOD (Superoxide dismutase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control.

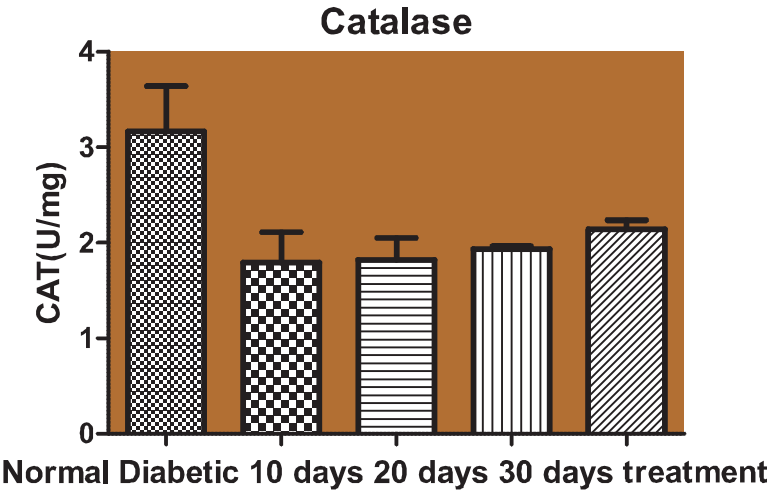


Figure 2 : Effect of MOE on CAT (Catalase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control.

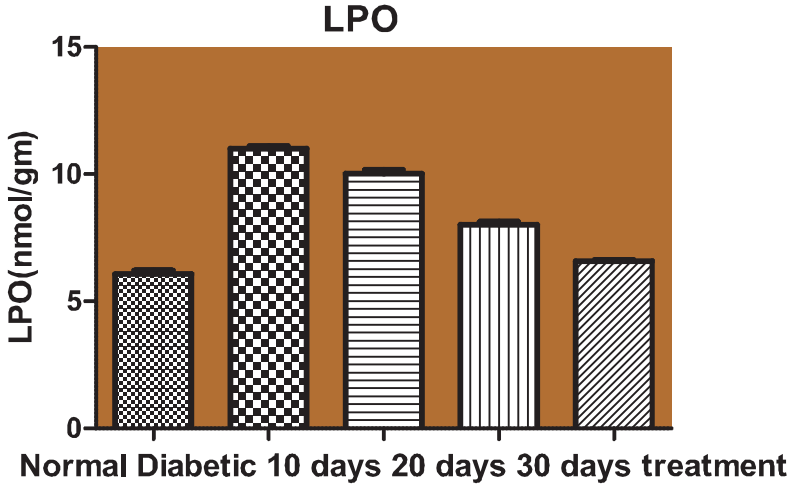


Figure 3 : Effect of MOE on LPO (Lipid Peroxidase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p< 0.05$ compared to normal control and diabetic control.

Estimation of biochemical parameters (Table II)

Moringa oleifera ameliorates hepatic toxicity as well as diabetes correction. A significant difference was observed in their biochemical parameters like ALT (fig-4), AST (fig-5), ALP (fig-6) and Bilirubin (fig-7) when compared to diabetic group.

Table II

Estimation of liver function test in normal, diabetic and extract treated groups					
Parameter	Normal Control	Diabetic Control	10 days treated	20 days treated	30 days treated
ALT (U/l)	38.50±1.62	81.52±1.76	67.58±1.79	57.28±1.24	38.30±1.50
AST (U/l)	40.68±1.65	100.8±2.30	76.93±2.21	60.95±1.56	55.73±1.70
ALP (U/l)	57.13±1.59	106.2±3.75	90.08±1.79	71.52±1.49	61.03±0.99
Bilirubin (mg/dl)	0.43±0.02	0.83±0.01	0.76±0.02	0.64±0.01	0.51±0.01

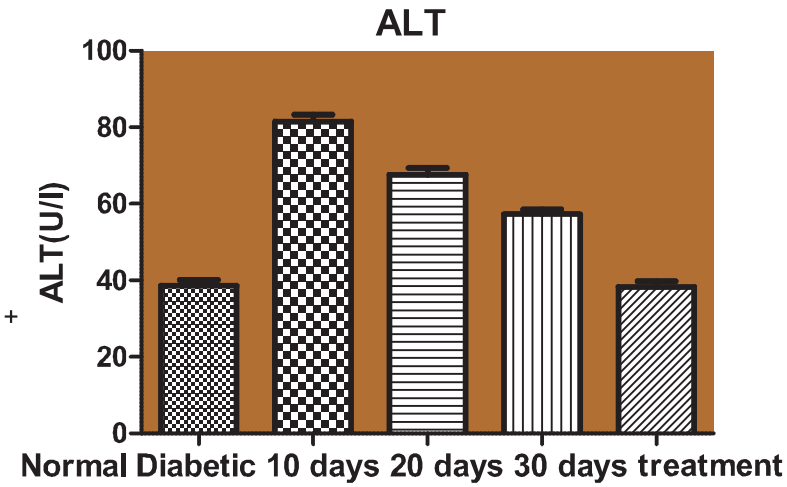


Figure 4 : Effect of MOE on ALT (Alkaline Transferase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control.

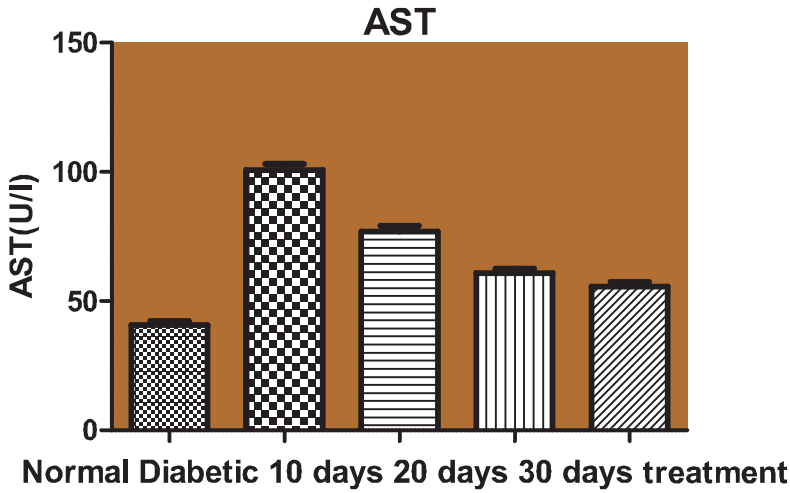


Figure 5 : Effect of MOE on AST (Aspartate Transferase) levels in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control

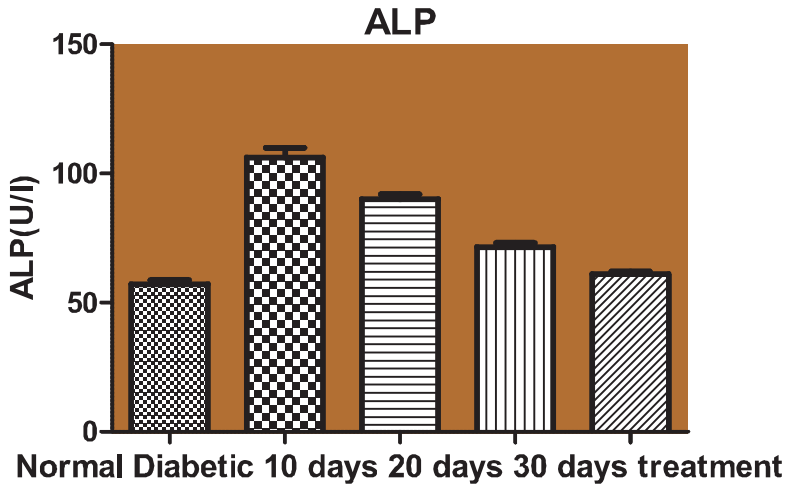


Figure 6 : Effect of MOE on ALP (Alkaline Phosphatase) level in control and experimental rats. Values are given as mean ± SE, n = 6, $p < 0.05$ compared to normal control and diabetic control.

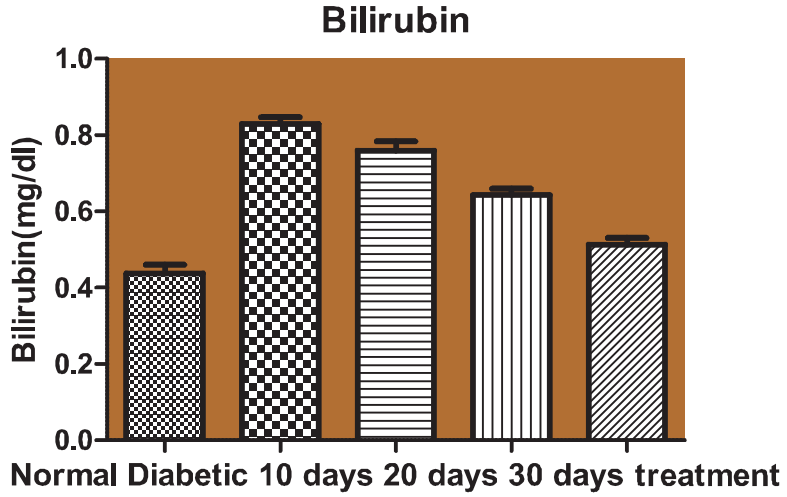


Figure 7 : Effect of MOE on Bilirubin levels in control and experimental rats. Values are given as mean ± SE n = 6, $p < 0.05$ compared to normal control and diabetic control.

STATISTICAL ANALYSIS

Results were presented in mean ± SE (Standard Error). The data were statistically analyzed using Graph Pad 5.0 (Graph Pad Software, San Diego, CA, USA) and total variation present in a set of data was analyzed through one way analysis of variance (ANOVA). Biochemical parameters between the test and control groups were compared using Dunnett's t-test. $P<0.05$ was considered to be significant.

DISCUSSION

The enhanced profile of plasma ALP, ALT and AST shows that diabetes may induce hepatic malfunctioning. Our findings are in agreement with those of other workers (Larcan *et al.* 1979) that liver was necrotized in diabetic patients. The increment in plasma markers ALP, AST and ALP may be because of the leakage of these enzymes form liver cytosol into the blood stream (Garcia *et al.* 2003). Nevertheless, treatment with *Moringa oleifera* initiated impoverishment in the profile of plasma markers. Our findings are supported by other workers' observation in rats (Ohaeri, 2001)

The findings showed that the plasma bilirubin was also enhanced in diabetic condition. However, treatment with *Moringa oleifera* induced decrease in plasma bilirubin. The increment in plasma bilirubin may be because of increment of bilirubin formation, lack of liver uptake and conjugation (Rana *et al.* 1996).

In diabetes, oxidative stress increases because of increment in oxygen free radical formation as well as compromise of antioxidant enzymes (Baynes *et al.* 1999).

The abatement of free radical levels by oral treatment of *Moringa oleifera* are in agreement with the findings by Baynes and Thorpe (1999), Kumari and Augusti (2002), Anwar and Meki (2003), Shewieta *et al.* (2002) and Campos *et al.*(2003) It was also noted that single treatment of alloxan processed a reduction in the action of the liver SOD (Chaudhary *et al.* 2007). Liver antioxidant enzymes such as CAT and SOD were recuperated with *Moringa oleifera* treatment in the liver tissues.

Single intraperioneal infusions of alloxans (120 mg/kg b.wt.) showed over-production (excessive hepatic gluconeogenesis and glyconeogenesis) and decreased utilization of glucose by tissue (Latner 1958). It has also shown to induce free radical production and cause tissue injury (Halliwell *et al.* 1985; Etuk, 2010) as well as periportal vacuolization with central putrefaction in rat's liver. After administration of *Moringa oleifera* hepatic cells were recovered.

CONCLUSION

Conclusively, these research findings showed that methanolic extracts of *Moringa oleifera* possess hypoglycemic effects and are capable of restoring liver tissue damage.

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