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EFFECT OF MANGIFERA INDICA LEAVES EXTRACT ON ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

The aim of the present study was to investigate the effects of *Mangifera indica* leaves extract on the kidney function parameters in alloxan induced diabetic Swiss albino mice. Forty five albino mice (weighing 28 to 32 g) were randomly divided into control, alloxan treated and *Mangifera indica* treated mice group. Diabetes was induced in mice by injecting intraperitoneally alloxan monohydrate at dose of 150 mg/kg body weight. Ethanolic extracts of *Mangifera indica* leaves at dose of 80 mg/kg body weight were given orally in diabetic mice daily for four weeks after established LD_{50} value. Results and discussion: In diabetic mice, serum urea, uric acid, creatinine and glucose levels were significantly increased but level of serum albumin was decreased in comparison with the control group. Diabetic mice group, treated with ethanolic extract of *Mangifera indica* leaves (80 mg/kg b.w), on comparison with diabetic group showed a significant decrease in these biochemical parameters such as urea, uric acid creatinine and glucose level. However, the serum albumin level got increased as compared to diabetic mice. The results suggested that ethanolic extract of *Mangifera indica* leaves possesses protective effect against alloxan induced diabetic mice.

KEYWORDS: **Alloxanized Diabetes, Urea, Uric acid, Creatinine and Albumin**

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INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that affects more than 100 million people worldwide. Diabetes is a heterogeneous primary disorder of carbohydrate metabolism and it has affected around 171 million people worldwide in 2000 which may increase to 366 million by 2030 [1]. Major limitations of the oral anti-diabetic agents are their side effects and cost of the therapy. The high cost of modern treatment of diabetes indicates a great need for the development of alternative strategies for prevention and treatment of diabetes. Origin and natural products plays an important role in drug development programs in the pharmaceutical industry and plants are the basic source of knowledge for modern medicine[2].There have been many studies on hypoglycaemic plants and a great variety of compounds have been isolated, but the main bottleneck is the further development of such leads into clinically useful medicines and especially phyto-medicines are adequate nutritional supplements, which would be of direct benefits to patients. *Mangifera indica* commonly known as mango is large evergreen tree of tropical and subtropical region and has been used for traditional medicine by a number of peoples for centuries. In Ayurvedic literature of India, different parts of this plant have been recommended as a remedy having anti-diabetic, anti-oxidant, antiviral, hypolipidemic effects. The extract showed a powerful scavenging activity of hydroxy radicals and acted as a chelator of iron. It also showed a significant inhibitory effect on the peroxidation of rat brain phospholipid and prevented DNA damage caused by bleomycin or copper-phenenthroline systems[3]. The aqueous extracts of the leaves of *M. indica* possess hypoglycaemic activity[4]. Alloxan is a toxic glucose analogue, which selectively destroys insulin producing β-cells in the pancreas .When administered to rodents and many other animal species, it causes insulin dependent Diabetes mellitus (called “Alloxan Diabetes”) in these animals, with characteristics similar to type 1 Diabetes in humans. Alloxan is selectively toxic to insulin producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The β-cell toxic action of alloxan is initiated by free radicals in this redox reaction [5]. *Mangifera indica* has been reported to have hypoglycemic effect in both laboratories animals[4,6-8] and human diabetic subjects[9] The purpose of this work was to evaluate the hypo- and anti-hyperglycemic effects of *M. indica* in normal and both type of diabetic model rats and to find out their possible mode(s) of anti-diabetic action. The purpose of the present study was to experimentally assess the anti-diabetic effect of the alcoholic extract of the leaves of *Mangifera indica* in alloxan induced diabetic mice.

MATERIALS AND METHODS

Experimental animals

The research work was approved by the IAEC (Institutional Animal Ethics Committee) with no. IAEC/2011/12/01 A total of forty five Swiss albino mice (28g to 32 g) were maintained on food and water ad libitum (prepared mixed formulated feed by the laboratory itself). Animals were maintained in colony rooms with 12 h light/dark cycle at 22 ± 2ºC.

Chemicals

Alloxan was purchased from the Loba Chemie (Batch no-G204207), Mumbai, India. Commercially available kits for chemical analyses such as urea, uric acid, creatinine and albumin were used with crest coral clinical

system, Goa, India. Analytical grade ethanol was purchased from Merck Company.

Induction of diabetes in Swiss albino mice

Overnight fasted Swiss albino mice were induced diabetic by injecting alloxan monohydrate (in the distilled water, intraperitoneally) at a dose of 150mg/kg body weight. Diabetes was confirmed in alloxan injected mice by measuring the fasting blood glucose concentration, 72 hr after the alloxanization. Mice with blood glucose level above 200 mg/dl were considered to be diabetic and were used in this study.

Collection & Preparation of Mangifera indica extract

In the present study fresh leaves of *Mangifera indica* were collected from the local garden and confirmed by a botanist of the Institute. The collected fresh leaves of *Mangifera indica* were thoroughly cleaned with distilled water, dried well and powdered for extraction. It was soaked in 70% ethanol for 48 hrs, finally extracted with 5% absolute ethanol using soxhlet apparatus for 6 -8 hr and the residue was concentrated and dried at 37ºC. The dose was calculated after LD_{50} estimation and finally made to 80mg/kg b.w.

Experimental design

During the present study, 45 mice were taken and divided into groups - control, alloxan treated and thereafter *Mangifera indica* treated. Alloxan at the rate of 150 mg /kg body weight were administered *intra peritoneally* (i. p.) for making the alloxan induced diabetic mice. To this alloxan treated group *Mangifera indica* at the rate of 80 mg / kg body weight was administered for four weeks. After the completion of the experiment**,** blood samples were collected by orbital sinus puncture method and then serum was extracted.

Biochemical estimation

After the entire treatment protocol the experimental animals were sacrificed. Blood was collected by orbital sinus puncture method [10] for the determination of Serum Glucose [11], Urea[12], uric acid[13], Creatinine[14] and Albumin [15].

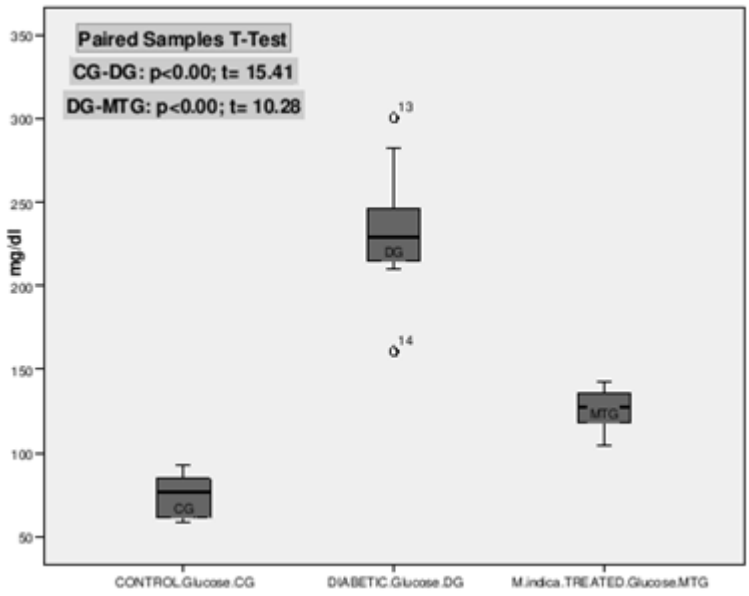
STATISTICAL ANALYSIS

Data from the experiments were presented as mean ± Standard deviation. Statistical analysis was done by using the Statistical Package for Social Science (SPSS) software for windows version 15 (SPSS Inc., Chicago, Illinois, USA). Paired T-test was done to see any difference between the paired groups. The level of significance was set at $p \leq 0.05$.

RESULTS & DISCUSSION

The mean level of glucose in the control group of mice was evaluated to be 75.00 ± 12.42 mg/dl (range 58.59 to 92.99) whereas it was 233.84 ± 35.35 mg/dl (range values 160.47 to 300.24) in alloxanised group. After the treatment of mice with the extract of *M. indica* the glucose level decreased down to 125 ± 12.31 mg/dl having a range of 104.25 - 142.47 mg/dl. These variations in glucose concentrations are evident from Figure 1.

Figure 1
Glucose Concentration in Control, Diabetic and M. indica treated mice.



The concentrations of glucose, albumin, urea, uric acid and creatinine in Control, Diabetics and *M. indica Treated groups* were evaluated and are shown in Table 1.

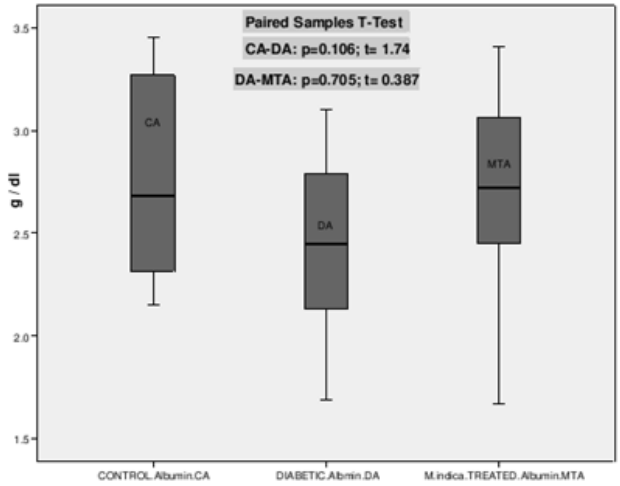
Table 1
Concentrations of albumin, urea, uric acid & creatinine in Control, Diabetics and M. indica treated mice.

Parameters	Control	Diabetics	M. indica Treated	*p*-value (t*-value)*	*P** (t**-value)*
Glucose mg/dl	75.00 ± 12.42	233.84 ± 35.35	125 ± 12.31	>0.00 (-15.41)	>0.00 (11.08)
Albumin g/dl	2.78 ± 0.48	2.45 ± 0.44	2.70 ± 0.5	0.106 (1.74)	0.15 (-1.5)
Urea mg/dl	16.08 ± 3.09	30.11 ± 3.18	20.77 ± 4.13	>0.00 (-10.46)	>0.00 (6.26)
Uric Acid mg/dl	4.4 ± 0.88	7.8 ± 2.22	5.27 ± 1.00	>0.00 (-5.51)	0.001 (4.03)
Creatinine mg/dl	0.55 ± 0.11	1.00 ± 0.40	0.60 ± 0.10	0.001 (-4.20)	0.001 (4.06)

p*= Comparison between Control and Diabetics p**= Comparison between Diabetic & M. indica Treated

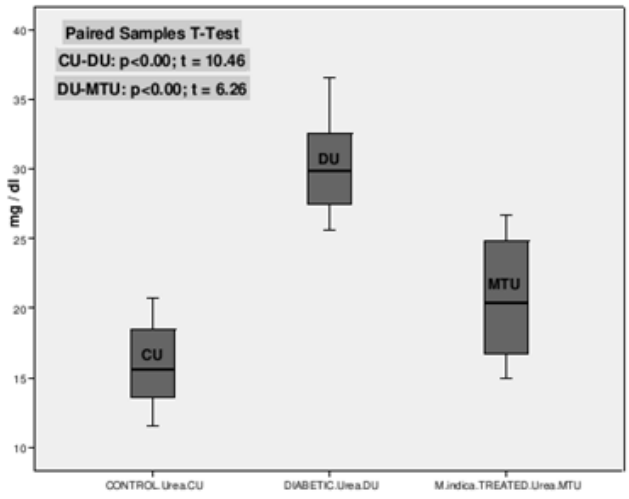
The Control was found to have mean albumin concentrations as 2.78 ± 0.48 g/dl with a range of 2.15 to 3.45 g/dl which decreased to 2.45 ± 0.44 g/dl (range 1.69 to 3.10) showing 11.87% decline in blood. The level was found to increase (2.70 ± 0.5 g/dl with the range: 1.67 to 3.41) in the group of mice treated with *M. indica*. The albumin concentrations in various groups are shown in Figure 2.

Figure 2
Albumin Concentration in Control, Diabetic and M. indica treated mice.



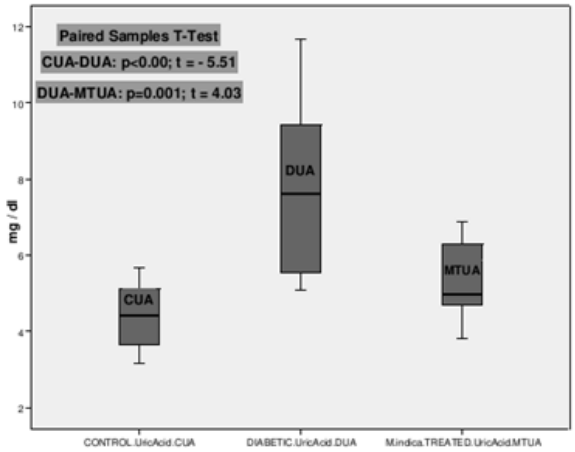
The mean concentration of urea in control was found to be 16.08 ± 3.09 mg/dl with the range of 11. 56 to 20.78 which got increased to 30.11 ± 3.18 mg/dl (ranging from 25.58- 36.55) on alloxanisation showing an increase by 87.25%. However, the level came down to 20.77 ± 4.13 mg/dl (range: 14.98 to 26.66) showing a decline of 31.0% after the treatment with *M. indica* extract. These variations in urea concentrations are evident from Figure 3.

Figure 3
Urea Concentration in Control, Diabetic and M. indica treated mice.



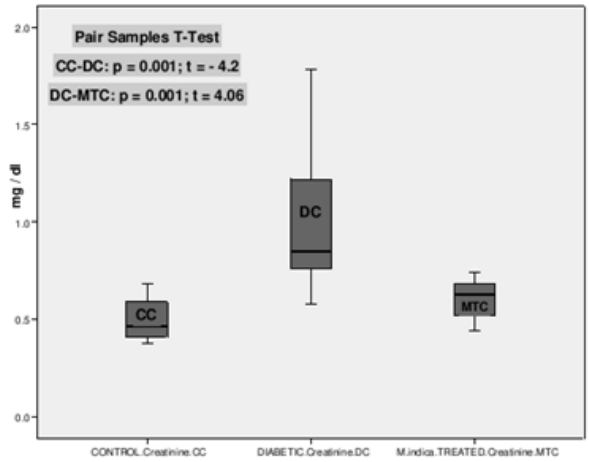
The level of uric acid ranged from 3.18 to 5.66 mg/dl having the mean value of 4.4 ± 0.88 mg/dl. It increased to 7.8 ± 2.22 mg/dl in diabetics with the range from 5.09 to 11.66 showing an increase of 77.27% as compared to Control. However, its value declined to 5.27 ± 1.00 mg/dl (32.44%) with the range of 3.83 to 6.88 on treatment with *M. indica* extract. The uric acid contents in Control, Diabetics and *M. indica* treated groups are represented by Figure 4.

Figure 4
Uric Acid Concentration in Control, Diabetic and M. indica treated in mice.



The creatinine content in Control group was 0.55 ± 0.11 mg/dl with the range of 0.38 to 0.68 mg/dl which increased to 1.00 ± 0.40 mg/dl in diabetics (81.82% increase) and its values ranged from 0.58 to 1.78 mg/dl. But in the group of mice treated with *M. indica* the level was found to be 0.60 ± 0, a decline of 40%. The *M. indica* treated group had levels ranging from 0.44 to 0.74 mg/dl. Their creatinine levels in various groups are shown in Figure 5.

Figure 5
Creatinine Concentration in Control, Diabetic and M. indica treated mice



During the present investigation, alloxan (150 mg/kg *i. p*.) was used to induce diabetes in mice and their serum glucose levels were found to be significantly elevated as compared to normal mice. The increased levels of serum glucose may be due to the partial damage of the pancreatic β-cells. Alloxan, a β-cytotoxin, induces "chemical *Diabetes*" in a wide variety of animal species including rats by damaging the insulin secreting β-cells [16, 17]. Similar results reported by Vuksan & Sievenpiper [18] show that the administration of alloxan significantly increases the level of glucose when compared to control, which might account for the cytotoxic effect of alloxan on beta cells. Alloxan is relatively toxic to insulin producing pancreatic β-

cells because it preferentially accumulates in β-cells through uptake via the GLUT-2 glucose transporter. This cytotoxic action is mediated by ROS source of generation of ROS is dialuric acid, a reduction product of alloxan. These radicals undergo dismutation to H_2O_2. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells, thereby decreasing the secretion of insulin, which in turn increases the blood glucose level. Another result of alloxan, a β-cytotoxin, was preferred to produce the diabetic state in mice as it induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cell resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [19].

On the other hand the treatment of ethanolic leaf extracts of *Mangifera indica* for four weeks on diabetic mice leads to significantly reduction in the serum glucose levels in alloxan induced diabetic Swiss albino mice. This suggests that the extracts may possess insulin like effect on peripheral tissues by either promoting glucose uptake or metabolism by inhibiting hepatic gluconeogenesis [6, 4]. In agreement with our results, several studies have shown the hypoglycaemic effect of several medicinal plants inducing insulin release from pancreatic cells of diabetic [20-25]. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in patients with diabetes. Thus, sustained reduction in hyperglycaemia will decrease the risk of developing micro-vascular complications and most likely reduce the risk of macro-vascular complications [20]. On this basis we have selected the glucose induced hyperglycaemic model to screen the anti-hyperglycaemic activity of the plant extracts.

Our results showed that alloxanisation caused significant increase in serum uric acid, urea, creatinine and decrease the level of albumin in diabetics when compared with non-diabetic control. This may be due to the protein glycation in diabetes which may lead to muscle wasting and increased release of purine, the main source of uric acid, as well as increased activity of xanthine oxidase. Our results are consistent with those reported by others [26-28] who showed that serum uric acid, urea, and creatinine levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels. Similar results were reported [29] showing the increased concentrations of urea, creatinine due to excessive lipolysis in severe diabetes *mellitus* leading to ketosis and later acidosis.

Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine. During renal diseases, the concentration of these metabolites increases in blood [30]. In this regard, the decrease of serum albumin in diabetic animals was restored to control rate by insulin treatment, which accelerates amino acid transport through cells and stimulates the protein manufacturing machinery of the cell [31]. Furthermore, the treatment of ethanolic extract of *Mangifera indica* leaves extract at the rate of 80 mg/kg b.w. for four weeks and the elevated levels were decreased after treatment with extracts. Our results are consistent with those reported by others [32, 33]. Reduction in plasma albumin was observed in alloxan induced diabetic rats which may be due to microproteinuria and albuminuria , which is an important clinical marker of diabetic nephropathy [34-36] and / or may be due to increased protein catabolism [37]. Lack of insulin also reduces RNA and mRNA, which is another factor for the reduction of total protein [38]. Our results also correlate with the above findings. Serum albumins, markers of liver synthetic ability assessed during this study, showed increase in albumin level of mice treated with ethanolic extract of *Mangifera indica* extract is in agreement with other report [39, 40] which support the hepatoprotective action of the extract.

CONCLUSION

Ethanolic extracts of *Mangifera indica* leaves exhibited significant anti-hyperglycemic activities in alloxan induced diabetic mice. These extracts showed improvement in parameters like kidney function test as well as control in serum glucose level showing its significance in diabetes treatment.

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