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Antihyperglycemic activity of *Mangifera indica Linn.* in alloxan induced diabetic rats

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ABSTRACT

Present investigation was undertaken to evaluate antihyperglycemic, activity of ethanolic extract of Mangifera indica leaves in alloxan induced diabetic rats. Alloxan produced a significant increase in serum glucose, creatinine, urea, uric acid, ALT, AST levels. Treatment with Mangifera indica extract produced decrease in alloxan induced glucose, urea, uric acid, and creatinine levels. There was a significant decrease in total protein, haemoglobin, body weight, albumin and globulin. Administration of Mangifera indica to diabetic rats reduced the effect of alloxan and increased the levels of above parameters. The results suggest Mangifera indica to be beneficial for the treatment of diabetes mellitus.

Key words: Ethanolic extract, Alloxan, Diabetes mellitus, Hypoglycemic effect, *Mangifera indica.*

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that affects more than 100 million people worldwide. It is a heterogenous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism [1]. Over time, uncontrolled diabetes can lead to serious damage to the various body systems [2]. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. According to WHO Survey, India will be the world's diabetes capital. It has been estimated that 2.4% of rural population and 8.4% of urban population is affected by diabetes [3]. More than 16 million people in the U.S have diabetes mellitus [4].

This disorder results from relative or absolute deficiency of insulin due to impairment of insulin action and or moderate to gross inadequacy of insulin secretion [5]. Hence, the syndrome of

diabetes mellitus is characterized by chronic hyperglycemia with glucosuria and a tendency to develop ketoacidosis [6].

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 beta cell toxic action of alloxan is initiated by free radicals in this redox reaction [7].

Mangifera indica (Anacardiaceae) is a tree, distributed in rural and semi urban parts of the India. It is one of the most important tropical plants marketed in the world [8] . It is grown widely in different parts of Africa, especially in the southern part of Nigeria, where it is valued for its edible fruit [9]. There are traditional medicinal uses for the bark, roots and leaves of *M.indica* throughout globe. *Mangifera indica* is used medicinally to treat ailments such as asthma, cough, diarrhoea, dysentery, leucorrhoea, jaundice, pains, malaria [10] and diabetes [11].

Phytochemical research from different parts of *M. indica* has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols [12-16]. This species is purposed to possess numerous therapeutic uses including analgesic, anti inflammatory [17], immunostimulant [18] antioxidant [19,20], spasmolytic, anti diarrhea [21], antilipidemic [22] ,antidiabetic [23], antiamebic [24] anthelminthic, antiallergic [25] and anti bacterial applications.

Althrough *M. indica* has been investigated for its various medicinal properties, detailed studies on its anti diabetic potential is still lacking. Keeping in view of the above, the present study was designed to determine the antidiabetic activity of *M. indica* leaves in alloxan induced albino rats.

EXPEIMENTAL SECTION

Collection of plant materials - Fresh leaves of *Mangifera indica* were collected during March-April 2011, from Mahadhevapattinam, Thiruvarur District, Tamil nadu, India and botanically identified. The leaves were washed with distilled water, shade dried, powdered, and stored in an air tight container until future use.

Preparation of ethanolic extract- Preparation of plant extract was done according to the previously described procedure (Reka and Varga, 2002). The collected fresh leaves were thoroughly cleaned with distilled water, dried well and powdered. It was soaked in absolute ethanol in cold (72 hrs). After three days, the extract was filtered, and then it was evaporated at 40^0C in cylindrical water bath for the elimination of solvent. A semisolid extract (40g) was obtained after complete elimination of alcohol under reduced pressure. It was stored in refrigerator until used.

Experimental animals- In the present study, healthy, pathogen free, albino rats (both sexes) of Swiss strain weighing 150 -200g were purchased from Rainbow institute, Bangalore, Karnataka, India, and housed under, standard husbandry conditions (30^0C $\pm$ 2^0, 60- 70% relative humidity and 12h : 12h day- night cycle), supplied with standard rat feed (Sai Durga feed and food, Bangalore) and water *ad libitum.*

Glass wares and chemicals- All the glasswares used for analytical purpose were Borosil make, and ethanol used for the extraction of plant leaves was obtained from Scientific chemicals, Chennai. Alloxan was purchased from Pvt. Ltd., Cochin. All other chemicals and reagents used in this study were procured from Qualigen and Ranbaxy fine chemicals Pvt. Ltd., Mumbai. All chemicals used were of analytical grade.

Induction of diabetes mellitus in rats- Diabetes was developed by injecting alloxan at a dose of 2mg/ 100 g b.wt., in distilled water intraperitoneally.

Experimental design- Animals were divided into five groups of six animals each. Group I served as control which received standard feed and water. GroupII disease induced by intraperitoneal injection of alloxan (2mg /100g bodyweight) daily for 20 days.Group III co treated with alloxan (2mg/100g b.w., intraperitoneally) and ethanolic extract of *Mangifera indica* (300mg/kg b.w.,) orally. Group – IV received herbal extract in a dosage of (300mg / kg b. w.,) orally.Group – V received standard drug Glibenclamide in a dosage of **(**100mg/ kg b.w.,) orally.

Study protocol-The test formulations were administered for 20 days, once in a day. At the end of experiment rats were scarified by cervical decapitation. Blood was collected and centrifuged for serum separation. The tissues were dissected out, weighed and washed using ice cold saline solution and dried between the folds of filter papers, weighed and homogenized using standard phosphate buffer in glass homogenizer with teflon pestle. The homogenate was then centrifuged at 1000 rpm for 5 minutes and the supernatant was used for various biochemical assay.

Statistical Analysis- The values are expressed as mean ± S.D. The statistical comparison was performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT), using SPSS version 12 for windows (SPSS Inc. Chicago; http: www.spss.com). The values are considered statistically significant if the p value was less than 0.05.

RESULTS AND DISSCUSSION

Plants may act on blood glucose through different mechanisms[26]. Anti diabetic herbs stimulates beta cell in the pancreas by activating regeneration of pancreatic cells [27,28]. Fiber of plant also interferes with carbohydrate absorption, affecting blood glucose level. Alloxan induced diabetic rats exhibited loss of body weight (Table 1) which is one of the threats associated with DM. Treatment with *Mangifera indica* extract showed signs of recovery as comparable with the standard drug glibenclamide . Qualitative analysis of urine sample indicated the presence of glucose and albumin (Table 2) in group 2 which was a characteristic feature of diabetes. Treatment with herbal extract arrested excretion of glucose and protein in urine.

Administration of alloxan significantly increased the level of glucose when compared to control rats, which might account for the cytotoxic effect of alloxan on beta cells. Alloxan is relatively toxic to insulin producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the $GLUT_2$ glucose transporter [6]. 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[29] there by decreasing the secretion of insulin, which in turn increase the blood glucose level. In the present study there is increase in blood glucose (Table ,3) in alloxan induced rats when compared to normal group,

which account for the cytotoxic action of alloxan. Administration of *Mangifera indica* extract remarkably reduced the altered sugar level.

The decresed level of haemoglobin observed in diabetic rats might be due to increased formation of glycosylated haemoglobin. It has been reported that in diabetic subjects the total haemoglobin levels are much lower than the normal level [30], and increased levels of HbA_1C increases [31]. Earlier reports state that during diabetes mellitus excess of blood glucose react with haemoglobin leading to the formation of HbA_1C [32]. The level of HbA_1C is always monitored as a reliable index of glycemic control in diabetes. Reduced level of haemoglobin (Table ,3) observed in the present study indicates that diabetic animals had prior high blood glucose level. Administration of *Mangifera indica (*300mg/ kg b. wt) ameliorated the above effect.

Reduction in plasma total protein , albumin and globulin levels were observed in alloxan induced rats (Table, 4) and this is consistent with previous results obtained The decrease in protein may be due to microproteinuria and albuminuria , which is an important clinical marker of diabetic nephropathy [33-35] and / or may be due to increased protein catabolism [36] Lack of insulin also reduces RNA and mRNA, which is another factor for the reduction of total protein[37]. Our results also correlates with the above findings.

Significant increase in renal parameters like urea, uric acid and creatinine (Table,5) in alloxan induced group indicates impaired renal function i.e due to decreased excretion of urea, uric acid and creatinine in the urine, which in turn may be due to the basement membrane injury. Treatment with *Mangifera indica* leaf extract produced significant improvement in the levels of urea, uric acid and creatinine.

ALT and AST are the specific markers to assess hepatocellular damage leading to liver cell necrosis[38]. In present study ALT and AST activities were assessed as it is the more specific index of liver cell damage in humans[39] and in experimental animals[40]. The mass spectrometry (MS) techniques available today have opened possibilities for clinical research, e.g., the use of tandem MS for analysis of drug metabolites in human plasma [41-43]. Thus lowering of these enzymes content in serum is a definite indication of hepatoprotective action of a drug. High level of AST indicates hepato cellular damage.

Activity of AST in serum was increased in alloxan intoxication (Table,6) *Mangifera indica* extract afforded a significant protection against alloxan induced increase in the serum enzyme level. Ethanolic extract of *Mangifera indica* may induce accelerated regeneration of liver cells by reducing the leakage of AST in to blood there by lowering its value to normal levels.

Table 1: The effect of *Mangifera indica* on body weight

Groups	Treatment	Body weight(gms)
Group I	Control	168±4.0
Group II	Alloxan alone	$150\pm2.5^{+}$
Group III	Alloxan+ MIELEt(300mg/kg b.wt)	$158\pm2.0^{*}$
Group IV	MIELEt (300mg/ kg b.wt)	$170\pm3.5^{*}$
Group V	Glibenclamide	$169\pm3.7^{*}$

Values are mean ±SD (n=6)

MIELEt- Mangifera indica ethanolic leaves extract, p^{+} <0.05 compared with normal control rats, $P^{*} < 0.05$ compared with diabetic control rats.

Table 2: The effect of *Mangifera indica* on qualitative analysis of urine sugar and urine albumin

Groups	Treatment	Urine sugar	Urine albumin
Group I	Control	-	-
Group II	Alloxan alone	++++	++++
Group III	Alloxan +MIELEt(300mg/kg b .wt)	-	-
Group IV	MIELEt (300mg/ kg b.wt)	-	-
Group V	Glibenclamide	-	-

(-) indicates the absence of sugar and albumin,
(+) indicates the presence of sugar and albumin

Table 3: The effect of *Mangifera indica* on blood sugar and haemoglobin

Groups	Treatment	Blood sugar (mg/dl)	Haemoglobin
Group I	Control	130±1.8	14.05±0.5
Group II	Alloxan alone	270±2.1	10.0±1.0^{+}
Group III	Alloxan +MIELEt (300mg/ kg b .wt)	200±1.5	12.04±1.1*
Group IV	MIELEt (300mg/ kg b.wt)	140±2.1	15.03±0.9*
Group V	Glibenclamide	142±1.5	14.50±1.4*

Values are mean ±SD (n=6)
MIELEt- Mangifera indica ethanolic leaves extract,
p^{+} <0.05 compared with normal control rats,
P^{*} < 0.05 compared with diabetic control rats.

Table 4: The effect of *Mangifera indica* on Total protein , albumin and globulin

Groups	Treatment	Total protein (g/dL)	Albumin(g/dL)	Globulin(g/dl)
Group I	Control	6.92±1.80	3.66±0.62	3.25±0.61
Group II	Alloxan alone	5.22±0.89^{+}	3.48±0.51^{+}	2.73±0.48^{+}
Group III	Alloxan + MIELEt (300mg/ kg b.wt)	6.23±0.92*	3.40±0.62*	2.85±0.35*
Group IV	MIELEt (300mg/ kg b.wt)	7.02±0.96*	3.66±0.25*	3.36±0.5*
Group V	Glibenclamide	6.98±1.6*	3.53±0.62*	3.24±0.4*

Values are mean ±SD (n=6)
MIELEt- Mangifera indica ethanolic leaves extract,
p^{+} <0.05 compared with normal control rats,
P^{*} < 0.05 compared with diabetic control rats.

Table 5: The effect of Mangifera indica on urea, uric acid and creatinine levels

Groups	Treatment	Urea(mg/ dL)	Uric acid (mg/ dL)	Creatinine(mg/dL)
Group I	Control	30±1.8	4.0±0.2	1.2±0.06
Group II	Alloxan alone	55±2.0^{+}	8.0±1.5^{+}	1.8±0.04^{+}
Group III	Alloxan +MIELEt (300mg/ kg b. wt)	40±1.9*	5.0±1.8*	1.5±0.02*
Group IV	MIELEt (300mg/ kg b.wt)	28±1.5*	3.4±2.5*	1.0±0.03*
Group V	Glibenclamide	29±2.0*	3.8±2.2*	1.1±0.04*

Values are mean ±SD (n=6)
MIELEt- Mangifera indica ethanolic leaves extract,
p^{+} <0.05 compared with normal control rats,
P^{*} < 0.05 compared with diabetic control rats.

ALT is more specific to the liver and a better parameter for detecting liver damage [44]. In the present study alloxan induced ALT level was brought back to normal by the administration of *Mangifera indica*. Several studies on different parts of *M.indica* have demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterols and polyphenols[12-15], which are

known to possess medicinal properties [18,19]. The data of our studies suggest that *Mangifera indica* is more beneficial in diabetes and its associated complications, holding hope of the new generation antihyperglycemic drug.

Table 6: The effect of *Mangifera indica* on ALT and AST levels

Groups	Treatment	AST(U/L)	ALT(U/L)
Group I	Control	86.18±1.5	84.06±2.0
Group II	Alloxan alone	95±1.0^{+}	96.43±2.5^{+}
Group III	Alloxan+MIELEt (300mg/kg b.wt)	90.4±1.6*	89.1±3.5*
Group IV	MIELEt(300mg/kg b.wt)	84±0.9*	83.21±2.4*
Group V	Glibenclamide	85±1.0*	86.04±1.9*

Values are mean ±SD (n=6)
MIELEt- Mangifera indica ethanolic leaves extract,
p^{+} <0.05 compared with normal control rats,
P^{*} < 0.05 compared with diabetic control rats.

REFERENCES

[1] CS Paulose; A Das; PS Padayatti. *Indian J Experimental Biology.*, **1996,** 35, 1141 -1145
[2] Rambhade S; Chakraborty AK; Patil UK; Rambhade A. *J. Chem. Pharm. Res.,* **2010**, 2(6), 7-25.
[3] CK Sreeja; C Elizabeth Samul; Kesavachanran; Shankar Shasidhar. *Indian J Physiology Pharmacol.,* **2001**, 47, 87 -93.
[4] R Chakrabarti; R Rojagobalan. *Current Sci.,* **2002**, 83, 1533-1538.
[5] P Kamtchouing; SM Kahpui; PD Djomeni Dzeufiet; L T' edong . *J Ethnopharmacol.*, **2006**, 104 , 306-309.
[6] V Vuksan; JL Sievenpiper. *Nutr Metab Cardiovasc Dis.*, **2005**, 3, 149-160.
[7] S Lenzen. *Diabetologia.,* **2008**, 51, 216-266.
[8] IA Ross. *Human Press Inc., New Jersey USA,* **1999,** 199-202.
[9] Nwinuka; M Nwibani; O Monanu Michacl; L Nwiloh Barine. *Pakistan J of Nutrition.*, **2008**, 7 , 663 -666.
[10] BE Madunagu; RUB Ebana; ED Ekpe. *West Aft J Biol Appl Chemi.,* **1990,** 35, 25-30.
[11] K Muruganandan; S Srinivasan; JL Gupta. *J Ethnopharmacol.,* **2005,** 93, 497-501.
[12] UP Singh; DP Singh; M Singh; S Maurya; JS Srinivatava;RB Singh; SP Singh. *Int J Food Science Nutrition* ., **2004,** 55, 163-169.
[13] NAJ Selles; HTV Castro; Aguero-Aguero; J Gonzalez; F Nadeo; F De Simone; L Rastelli. *J Agric Food Chem. ,* **2002,** 50 , 762-766.
[14] V Anjaneyulu; IS Babu; JD Connollu. *Phytochemistry.*, **1994**, 35, 1301-1303.
[15] MA Kharn; SS Nizami; MNI Kharn; SW Azeem; Z Ahamed. *J Nat Prod.*, **1994**, 57, 988-991.
[16] NA Saleh; M Ei-Ansari. *Planta Med.*, **1975**, 28 , 124-130.
[17] GO Garrido; C Gonzalez; N Delporte; AJ Backhouse; Quintero; Nunez- Selles; MA Morales. *Phytother Res.*, **2001,** 15, 18-21.
[18] N Makare; S Bodhankar; V Rangari. *J Ethnopharmacol.,* **2001,** 78, 133-137.
[19] G Martinez; R Delgado; G Perez; AJ Garrido; OS Nunez selles; OS Leon. *Phytother Res.,* **2000,** 14, 424-427.
[20] GM Sanchez; A Giuliani A; AJ Nunes- selles; GP Leon-Fernandez; L Re Davison. *J Pharmacol Res.,* **2000**, 42, 565-573.
[21] K Sairam; S Hemalatha; A Kumar; T Srinivasan; J Ganesh; M Shankar; S Venkatraman. *J Ethnopharmacol.*, **2003**, 84, 11-15.
[22] L Anila; NR Vijayalakshmi. *J Ethnopharmacol.,* **2002,** 79 , 81-87.

[23] AO Aderibigbe; TS Emudianughe; BA Lawal. *Phytother Res.*, **1999,** 13, 504- 507.
[24] L Tona; N Kambu; K Ngimbi; Cimanga; AJ Vlietinck. *J Ethnopharmacol.*, **1998,** 61 , 57-65.
[25] D Garcia; M Escalante; R Delgado; FM Ubeira; J Leiro. *Phytother Res.*, **2003,** 17, 1203-1208.
[26] B Chakravarthy; S Gupta; SS Gambhir; KD Gode. *Indian J of Pharmacology.*, **1980**, 12, 123-128.
[27] KN Boppanna; J Kanna; S Godgil; R Balaraman; SP Rathode. *Indian J Pharmacol.*, **1997**, 29 , 162- 172.
[28] V Chorvathova; P Bobek; E Ginter; J Klavanova*Physiology Research.*, **1993**, 42, 175-179.
[29] T Szudelski. *Physiology Research .*, **2001**, 50, 537-546.
[30] HB Chandalia; PR Krishnaswamy; *Curr Sci.*, **2002,** 83, 1522-1615.
[31] EP Paulsen. *Metabolism.*, **1973**, 22, 269- 271.
[32] RL Koening; CM Peterson; RL Jones; Saudesk. *New Eng J Med.*, **1976**, 295 417- 420.
[33] GI Bakris. *Post grad med.*, **1997** ,93, 89-94.
[34] T Tuvemo; V Ewald; M Kobbon; LA Proos. *Acta paldiatr Suppl.*, **1997**, 418, 7-10.
[35] N Makare; S Bodhankar; V Rangari. *J Ethnopharmacol.*, **2001**, 78 , 133-137
[36] JP Almdal; H Vilstrup. *Diabetologia.*, **1988**, 31, 114-118.
[37] A Benn; M Zinic; R AI-Rikabi. *Croattan med J.*, **2005**, 46, 303-307.
[38] DE Amacher. *Regul Toxicol pharmacol.*, **1998**, 27, 119-130.
[39] R Clark; RP Thompson; V Borirachanyarat; B Widoop; AR Davidson; R Goulding; R Williams. *Lancet.*, **1973** ,1 , 66-90.
[40] Mitchell; SS Thoegersson. *J, Clin Pharmacol and therep.*, **1974,** 16, 676-684.
[41] Reddy SR; Chandiran IS; Jayaveera KN; and Divi KR. *J. Chem. Pharm. Res.*, **2010**, 2(3),59-69
[42] Ganesan M; Nanjundan S; Gomathi M; and Muralidharan S**.** *J. Chem. Pharm. Res.*, **2010**,2(4), 740-746.
[43] Chandiran IS; Jayaveera KN; and Reddy SR. *J. Chem. Pharm. Res.*, **2011**, 3(2), 341-353.
[44] A Willianson Warnholtz; H Mollnall; M Oeleze; M Wentdt; T Munzel. *Current Hypertention Reports.*, **1996**, 3, 53-60.