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ANTIDIABETIC EFFICACY OF *MANGIFERA INDICA* SEED KERNELS IN RATS: A COMPARATIVE STUDY WITH GLIBENCLAMIDE

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Key words: antioxidant, insulin, Mangifera indica*, mangiferin*

SUMMARY

The present study was conducted to examine the hypoglycemic potency of seed kernels of Mangifera indica *ethanol extract (MIEtE) in streptozotocin diabetic rats. Remarkable abnormalities were observed in serum and tissue parameters in hyperglycemic rats after streptozotocin administration. Administration of MIEtE, 300 mg/kg b. w./day for 14 and 21 days resulted in their normalization. Data were in parallel analyzed with a standard drug, glibenclamide, to compare plant drug efficacy. The findings of the present study demonstrated* M. indica *to possess a potent hypoglycemic activity.*

INTRODUCTION

Mangifera (M.) indica (family *Anacardiaceae*) is indigenous to the Indian subcontinent and prescribed for strengthening the nervous and blood systems, removal of body toxins, treating anemia, dysentery, diarrhea and urinary tract inflammation. It is reported to be rich in prebiotic dietary fibers, vitamins (A, B_6, C, D, E and K), carotenoids, essential elements (potassium and copper) and amino acids (1). Peel and pulp contain antioxidants, carotenoids, polyphenols, omega-3 and omega-6 polyunsaturated fatty acids (2), provitamin A, carotene (a and b), lutein (3), polyphenols (quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins and mangiferin), which counteract free radicals in various disease (4).

Available literature shows that antidiabetic efficacy of *M. indica* seed kernel has not been investigated to date. Therefore, the present study was undertaken to evaluate the antidiabetic as well as antioxidant efficacy of *M. indica* ethanolic extract (MIEtE) in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Experimental animals

The study was approved by the Ethics Committee of the Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India. The Indian National Science Academy (INSA), New Delhi guidelines were followed for maintenance and use of experimental animals. Colony bred, adult,

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Wistar albino rats (200±30 g) were housed in polypropylene cages and had free access to food and drinking water until 30 min before sampling.

Plant material and extraction

Fresh *M. indica* fruits were collected from Hanumangarh district, Rajasthan, India, and authenticated by Dr. M.S. Dashora, Department of Dravya Guna, National Institute of Ayurveda, Jaipur, India. Pulp was removed from fruit and seeds were selectively separated and washed with distilled water to remove pulp traces. Seed kernels were separated from the seed coat, shed dried, powdered in an electrical grinder and subjected to extraction with ethanol (100%) for 72 hours. This material was dried in oven for 48 hours at 35±2 °c. This final extract was employed to study the antidiabetic activity of *M. indica*.

Plant extract and standard drug administration

Plant extract was reconstituted with distilled water (serving as a vehicle) and administered orally at a dose of 300 mg/kg b. w./day. Glibenclamide (reference standard drug) was administered at a dose of 0.3 mg/kg b. w./day. Control animals received distilled water (placebo).

Experimental protocol

In overnight fasted rats, diabetes was induced by freshly prepared 0.2 mL solution of streptozotocin, Himedia Laboratory Limited, Mumbai, India (50 mg/kg b. w. dissolved in 0.1 mM sodium citrate buffer) and pH was adjusted to 4.5. Streptozotocin treated animals were considered diabetic when the fasting glucose levels exceeded 250 mg/dL.

Control rats were injected with 0.1 mM sodium citrate buffer alone. Diabetic animals were allowed to drink 2% glucose solution overnight to overcome the drug induced hypoglycemic shock. Rats were randomly divided into six groups of 7 rats and duration of the experiment was set to 14 and 21 days. The experiments were designed as described below:

- group I: control group
- group II: diabetic group
- group III: diabetic rats orally administered MIEtE (300 mg/kg b. w./day) for 14 days
- group IV: diabetic rats orally administered MIEtE (300 mg/kg b. w./day) for 21 days

Table 1. **Effect of ethanolic extract of *M. indica* (MIEtE) on serum biochemical parameters in streptozotocin-diabetic rats[a]**

Treatment		Group I Control (vehicle treated)	Group II Diabetic control	Group III Diabetic + M. indica (300mg/kg b.w./day)	Group VI Diabetic + Glibenclamide (0.3mg/kg b.w./day)
Serum glucose (mg/dL)	14 days	80.83 ± 17.80	257.54** ± 13.42	197.34^{*a} ± 9.38	187.25^{*a+} ± 17.34
	21 days	85.32 ± 12.31	273.55** ± 12.49	162.23^{*a+} ± 17.26	138.73^{*a+} ± 13.83
Insulin (µU/mL)	14 days	18.29 ± 1.11	9.12** ± 0.78	12.12^{*ns} ± 1.12	15.26^{nsa} ± 1.45
	21 days	18.43 ± 0.77	9.68** ± 0.26	13.11^{*a}± 0.86	16.25^{nsa+} ± 0.90
Total protein (mg/dL)	14 days	6.43 ± 0.43	3.52* ± 0.13	6.11^{nsa+} ± 0.29	5.89^{nsa} ± 0.52
	21 days	6.77 ± 0.12	3.46** ± 0.18	4.42^{nsa+} ± 0.97	5.83^{nsa} ± 0.62
Albumin (mg/100 mL)	14 days	3.48 ± 0.14	2.25* ± 0.24	3.39^{nsa} ± 0.09	3.19^{nsa} ± 0.13
	21 days	3.61 ± 0.19	1.36** ± 0.21	3.54^{nsa+} ± 0.11	3.73^{nsa} ± 0.34
Triglycerides (mg/dL)	14 days	101.34 ± 5.23	152.41* ± 6.83	80.33^{*a+} ± 2.44	80.47^{*a+} ± 4.13
	21 days	092.28 ± 6.46	140.91** ± 4.58	100.13^{nsa+} ± 2.26	102.77^{nsa+} ± 2.53
Phospholipids (mg/100 mL)	14 days	158.87 ± 3.13	235.21** ± 3.87	136.53^{*a+} ± 5.39	131.49^{*a+} ± 8.12
	21 days	165.39 ± 5.83	247.38** ± 8.89	147.14^{nsa+} ± 6.12	152.21^{nsa+} ± 6.43
Glycated hemoglobin (%)	14 days	6.11 ± 0.51	12.27** ± 0.53	9.72^{ns} ± 1.47	9.01^{ns} ± 1.61
	21 days	5.87 ± 0.46	12.51** ± 0.92	9.09^{ns} ± 1.81	8.31^{ns} ± 1.59

[a]Values are given as mean ± SEM, 7 rats *per* group; diabetic group compared with normal group; experimental groups compared with normal and diabetic group; values are statistically significant at *P<0.05; **P<0.001 as compared with the normal control ; ^{a}P<0.05; ^{a+}P<0.001 as compared with diabetic control; ^{ns}nonsignificant.

- group V: diabetic rats orally administered glibenclamide (0.3 mg/kg b. w./day) dissolved in 0.5 mL distilled water for 14 days
- group VI: diabetic rats orally administered glibenclamide (0.3 mg/kg b. w./day) dissolved in 0.5 mL distilled water for 21 days

Serum and tissue biochemistry

At experiment termination, blood of overnight fasted rats was collected by cardiac puncture under mild ether anesthesia and analyzed for glycated hemoglobin. Serum was separated by centrifugation to analyze insulin, total protein (5), albumin (6), phospholipid (7) and triglycerides (8). Pancreas was dissected and kept at 4 °C for biochemical estimations, i.e. protein (5), lipid peroxidation (9), superoxide dismutase (10) and reduced glutathione (11).

Statistical analysis

All group data were statistically evaluated with the Stat Plus/4.7.5.0 software. The hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Tukey's test to analyze difference. Statistical significance was set at $P<0.05$.

RESULTS

Effect of MIEtE on serum glucose and glycated hemoglobin

Streptozotocin administration to normoglycemic rats caused significant elevation ($P\leq0.001$) in serum glucose level. MIEtE as well as glibenclamide administration to diabetic rats reduced serum glucose level (MIEtE, $P<0.05$ at 14 and 21 days; glibenclamide, $P\leq0.001$ at 14 and 21 days) (Table 1).

The time course changes in the glycated hemoglobin (HbA_{1c}) measure are shown in Table 1. HbA_{1c} level of diabetic rats was found to be elevated significantly (more than double). Oral administration of MIEtE brought the HbA_{1c} level back to the normal range (20.78% and 27.33% after 14 and 21 days, respectively). Furthermore, glibenclamide treated diabetic group showed a noteworthy decline in HbA_{1c} level (26.56% and 33.57% after 14 and 21 days, respectively).

Effect of MIEtE on biochemical parameters

Diabetic rats demonstrated significant reduction in serum insulin, protein ($P\leq0.001$) and albumin ($P<0.05$) levels when compared with control rats at both 14 and 21 days. There were also significant ($P<0.05$ and $P\leq0.001$, respectively) increases in serum levels of serum triglycerides (TG) and phospholipids. MIEtE feeding to diabetic rats caused significant to highly significant elevations in serum insulin, protein and albumin levels (insulin, $P<0.05$; protein, $P\leq0.001$; albumin, $P<0.05$ at 14 days; and insulin, $P<0.05$; protein and albumin, $P\leq0.001$ at 21 days). Significant decreases ($P\leq0.001$) in serum TG and phospholipid measures were noticed when MIEtE was orally given to hyperglycemic rats (Table 1).

Total protein, lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH) remained unchanged throughout the experimentation in control group. Significant decline in pancreatic protein, SOD and GSH as well as elevation in LPO levels were observed after streptozotocin diabetes induction. MIEtE administration was found to play a significant role in normalizing disturbed tissue biochemical parameters. Total pancreatic protein, SOD and GSH contents were also found to be elevated markedly ($P\leq0.001$), while pancreatic LPO was significantly decreased ($P\leq0.05$ at 14 and 21 days) after oral MIEtE administration (Table 2).

DISCUSSION

Streptozotocin administration causes reduction in the number of β-cells and induces hyperglycemia (12). In our study, MIEtE was observed to decrease serum glucose level and increase serum insulin concentration in treated rats. The possible mechanism by which MIEtE exerts its hypoglycemic action may be through potentiating the plasma insulin effect by increasing either pancreatic secretion of insulin from regenerated

Table 2. **Effect of ethanolic extract of *M. indica* (MIEtE) on pancreatic tissue biochemical parameters in streptozotocin-diabetic rats[a]**

Treatment		Group I Control (vehicle treated)	Group II Diabetic control	Group III Diabetic + M. indica (300 mg/kg b.w./day)	Group VI Diabetic + glibenclamide (0.3 mg/kg b.w./day)
Total protein (mg/g)	14 days	47.93 ± 3.42	18.35** ± 1.38	27.84^{*a} ± 2.02	34.45^{*a+} ± 2.26
	21 days	51.42 ± 3.18	17.64** ± 4.45	34.21^{*a+} ± 4.32	48.47^{nsa+} ± 0.56
Lipid peroxidation (n mole MDA/mg protein)	14 days	2.02 ± 0.63	8.13** ± 0.42	6.89^{*a} ± 1.26	5.41^{*a} ± 1.23
	21 days	2.13 ± 0.81	8.87** ± 0.53	5.12^{nsa} ± 1.43	4.30^{nsa} ± 0.52
Superoxide dismutase (µmole/mg protein)	14 days	3.93 ± 0.22	1.58** ± 0.28	2.36^{*a+} ± 0.23	3.21^{nsa} ± 0.23
	21 days	3.41 ± 0.32	1.17** ± 0.43	2.38^{nsa} ± 0.41	3.44^{nsa+} ± 0.11
Reduced glutathione (n mole/g tissue)	14 days	2.47 ± 0.21	1.26** ± 0.12	1.75^{*a+} ± 0.21	2.22^{nsa} ± 0.14
	21 days	2.35 ± 0.11	1.32** ± 0.13	2.14^{nsa} ± 0.31	2.22^{nsa+} ± 0.10

[a] Values are given as mean ± SEM, 7 rats *per* group; diabetic group compared with normal group; experimental groups compared with normal and diabetic group; values are statistically significant at *P<0.05; **P<0.001 as compared with normal control; ^{a}P<0.05; ^{a+}P<0.001 as compared with diabetic control; ^{ns}nonsignificant.

β-cells or its discharge from bound insulin. In this context, other plants have also been observed previously to have hypoglycemic effects (13).

Streptozotocin-diabetes is due to the excess production of reactive oxygen species (ROS) leading to cytotoxicity in β-cells, thus decreasing the synthesis and release of insulin (14) and also affecting the pancreas (15). Increased levels of the pancreas thiobarbituric acid reactive substances (TBARS) could be due to the increase in free radicals and decrease in nonenzymatic antioxidants (16). Diminution of SOD and GSH in streptozotocin induced diabetes has also been noticed by Kakkar *et al*. (17). Decreased SOD and GSH activity observed in the pancreas with progression of diabetes may be due to nonenzymatic glycosylation of the enzyme, which occurs in diabetic state (18). Continuous MIEtE administration to diabetic rats caused a significant decline in free radical generation, thus showing normalized SOD, GSH and lipid peroxidation levels depending on administration duration.

In diabetes, excess glucose reacts with hemoglobin to form glycated hemoglobin, thus elevating the glycated hemoglobin level in blood. MIEtE administration prevented significantly glycated hemoglobin elevation. This could be due to the improved glycemic control produced by *M. indica* ethanol extract.

Protein metabolism in diabetes is abnormal due to insulin secretion defect, leading to various metabolic disorders (19). Reduction in total protein level may be due to the increased protein catabolism caused by streptozotocin (20). MIEtE administration caused normalized serum protein level, possibly through the increase in insulin-mediated amino acid uptake, enhancement of protein synthesis and inhibition of protein degradation (21).

Increased permeability to albumin is a well-known feature of diabetic microvasculature and a negative prognostic factor of vascular complications. Serum albumin level of diabetic rats was found to be reduced, possibly by extravasation into urine (22). Oral plant extract treatment elevated serum albumin level, perhaps as a consequence of reduced membrane permeability caused by regular MIEtE administration.

Serum phospholipid content was increased in rats after diabetes induction. This may take place as a result of damaged bilayer plasma membrane consisting of phospholipids, deranged metabolic control, and increased serum concentration of cephalin and lecithin fractions (23). MIEtE oral gavage results in normalized phospholipid concentration, possibly due to refurbished plasma membrane integrity with regular metabolism.

Future work directed at purification and characterization of active components may reveal new agents for diabetes therapy.

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