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Hypoglycemic activity studies on root extracts of *Murraya koenigii* root in Alloxan-induced diabetic rats

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ABSTRACT

Aim of the study: Murraya koenigii (MK), an aromatic perennial shrub known as 'Curry patta' in India. Literature review suggests that root of this plant is used as purgative, stimulant and for general body aches apart from various medicinal uses of leaves of this plant. In this context, the standardized methanol, aqueous and petroleum ether extracts of roots of MK to total phenolic content is hypothesized for the evaluation of the hypoglycemic activity. Material and Methods: Inbreed adult male Charles-Foster (CF) albino rats were used in the experiment. The suspension of standardized methanol (ME), aqueous (AE) and petroleum ether (PEE) to total phenolic content were treated for hypoglycemic activity in oral glucose tolerance test (OGTT) method, normoglycemic and alloxan-induced diabetic rats. Results: The ME (200 mg/kg and 400 mg/kg) showed potential hypoglycemic activity in normoglycemic and diabetic rats, and further in OGTT method. Furthermore, ME showed significant (P<0.05) increase in final body weight, total hemoglobin, insulin, albumin and high density lipoprotein levels, however, decrease in fluid intake, glycosylated hemoglobin, urea, creatinine, total cholesterol, triglyceride and low density lipoprotein levels. Moreover, it mitigated the liver function in terms of reducing serum glutamate oxaloacetate transaminases, serum glutamate pyruvate transaminases and alkaline phosphatase activity. Additionally, it improved oxidative stress in terms of reducing lipid peroxidase and superoxide dismutase, and elevating catalase activity. Conclusions: These findings suggest that the ME found to be potential hypoglycemic compared to AE and PEE, and would be a promising candidate for the treatment of diabetes.
Keywords: *Murraya koenigii*, diabetes, hyperglycemia, dyslipidemia and oxidative stress.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with aberration in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It has been reported that the chronic hyperglycemia of diabetes is associated with complications like renal failure, coronary artery disorder, neurological complications, cerebro-vascular disease, blindness, limb amputation, long term dysfunctions and failure of various organs and eventually premature death [1]. It has been predicted by world health organization that Indian people are more genetically susceptible to diabetes accounting about 30 to 33 millions and would go up to 40 millions by the end of 2010 which further will reach to maximum of 74 millions by 2025 [2]. Management of this disease may include lifestyle modifications, diet, exercise, long – term use of oral hypoglycemic agents or insulin therapy [3]. Recently, phytomedicines gains greater importance compared to synthetic antidiabetic drugs due to several adverse effects of the synthetic drugs. It has been assumed that plant based drugs are considered to be less toxic and free from adverse effects in comparison to modern allopathic medicines [4]. Ethnobotanical information suggests that about 800 medicinal plants possess hypoglycemic or antidiabetic potential [5] and the bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids and floavonoids are effective antidiabetic drugs both in preclinical and clinical studies [6,7].

Murraya koenigii (L.) Spreng (family: Rutaceae; MK) is an aromatic pubescent perennial shrub or small tree commonly known as 'Curry patta' in India. The plants originated in Tarai regions of Uttar Pradesh, India and now widely found in all parts of India. It grows in every house yard of Southern India and is also cultivated in Sri Lanka, China, Australia and the Pacific islands [8]. Literature review suggests that the plant is used in Indian system of medicine to treat various disorders [9-11]. It has been documented that the essential oil of the leaves of MK is reported to possess antimicrobial [12], antifungal [13] and pesticidal [14] anti-amoebiasis, antidiabetic and anti-hepatitis [15-17] hepatoprotective [18], acetylcholinesterase inhibitory potential [19], pancreatic lipase inhibitory potential [20], antiamnesic [21], immunomodulatory [22] and antioxidative [23] activity. The roots are slightly anti-diarrhoeal activity [24] purgative, stimulant and used for general body aches [25].

Therefore, in the present experiment we have extended our research work to standardize to total phenolic content, and to evaluate the antidiabetic potential of methanolic (ME), aqueous (AE) and petroleum ether (PEE) extracts of roots of MK in normal and alloxan induced diabetic rats. Further, an attempt has been undertaken to establish a possible mechanism of action of root extracts of MK.

MATERIALS AND METHODS

1.1. Chemicals and reagents

Alloxan monohydrate was obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Tolbutamide (TBM) was provided as gift sample by Hoechst Pharmaceuticals, Mumbai, India. All other reagents and chemicals used were of analytical grade procured from Hi-media Labs, Mumbai, India.

1.2. Plant material and extraction

The fresh root of *Murraya koenigii* was harvested from local horticulture garden of Bilaspur (Chattissgarh), India in the month of July-August 2008. The plant was identified and authenticated by an expert Dr N. Shiddamallayya, Regional Research Institute (Ay.), Bangalore, India and specimen sample (Voucher Specimen No. RRCBI/Mus/3) were deposited in their herbarium. Plant material was carefully washed with tap water and left to dryness in dark at room temperature and finally stored in well-closed cellophane bags. The shade-dried and coarse powdered root (2 kg) was subjected to defat with petroleum-ether (bp 40-60°C) using Soxhlet extraction apparatus (Quickfit, England). The defatted sample was air dried in order to remove solvent residue. Extract was prepared by extracting the defatted powder with methanol solvent (80% v/v) for period of 48 h, which was then concentrated to a semisolid mass under reduced pressure (Buchi Rotavor R-200, Switzerland) for 20 min at 70°C (yield: 12.6% w/w). Another batch was subjected to extract with water (yield: 14.8% w/w) and petroleum ether (yield: 8.5% w/w) solvents. Further, there was prepared a fresh suspension of 0.3% v/v carboxymethylcellulose (CMC) in distilled water. Thereafter, the methanol, aqueous and petroleum ether extracts were mixed with 0.3% CMC suspension separately to form methanol (ME), aqueous (AE) and petroleum ether (PEE) suspension respectively before administration to rats.

1.3. Preliminary phytochemical screening and standardization to total phenolic content

Each plant extracts were subjected to identification tests for alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols [26]. According to Velioglu et al., 1998 [27] total phenolic content in each extract was estimated using Folin-ciocalteu reagent with minor modification. Briefly, 100 µl of plant extract (1 mg/ml) were mixed with 0.75 ml of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to stand for 5min at room temperature. Thereafter, 0.75 ml of Na_2CO_3 solution (60 g/l) was added to mixture and allowed it to stand at room temperature (25±1°C) for 2 h intervals. Absorbance was measured against blank using Double-Beam UV-Vis Spectrophotometer at λmax 725 nm respectively. The total phenolic content was expressed as milligrams of gallic acid equivalent/ g extract.

1.4. Animals

Inbreed adult male Charles-Foster (CF) albino rats (150-200 g), 2-3 months old, were obtained from School of Pharmacy, Chouksey Engineering College, Bilaspur (India) and were used in the study. The animals were grouped and housed in Poly-acrylic cages lined with husk under standard condition (24 ± 2°C temperature, 45-55% relative humidity and 12 h light: 12 h dark cycle) respectively. Animals were allowed to freely feed their standard pellet diet (Lipton India, Ltd., Mumbai) and water *ad libitum*. Fasted animals were deprived with their food for 16-18 h before experimentations but allowed to water *ad libitum*. All the experimental procedures utilized were performed in accordance with the approval of the Institutional Animal Ethics Committee (1169/ac/08/CPCSEA) under strict compliance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the experimental studies.

1.5. Oral toxicity studies

An acute oral toxicity study was performed according to "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral

administration of methanolic, aqueous and petroleum ether extracts (suspended in 5% tween 80 solution). Albino male rats (N=6, 150-200 g), overnight fasted for 18 h were used for the study. Each extract in increasing dose of 5, 50, 300 and maximum dose up to 2000 mg/kg was found tolerable in the experimental animals. Median dose: 200 mg/kg (One-tenth of the maximum lethal dose) and high dose: 400 mg/kg (Twice that of one-tenth dose) were selected for assessment of antidiabetic activity. Thereafter, the rats were continuously observed for first 3 h for any gross behavioral, neurological or autonomic toxic effects and for any lethality after 24 to 72 h till 14 days.

1.6. Induction of diabetes in rats

Diabetes was induced by a single dose subcutaneous injection of freshly prepared alloxan monohydrate (120 mg/kg, Sigma chemicals, USA) dissolved in normal saline (0.9% w/v NaCl in distilled water) to overnight fasted male CF albino rats [28]. Blood glucose level was measured by using one-touch glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats with fasting blood glucose level more than 250 mg/dl were considered to be diabetic and were selected for studies.

1.7. Experimental Design

1.7.1. Study on Oral Glucose Tolerance Test (OGTT)

Initially, hypoglycemic activity of plant extracts was carried out in overnight fasted normal rats, which were equally divided into eight groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and standard group received 1 ml of reference drug TBM suspended in the vehicle (250 mg/kg, p.o.), while group from third to eight were administered with 1 ml of ME, AE and PEE (200 and 400 mg/kg, p.o.) respectively. Following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diabnostics, GmbH, Germany).

1.7.2. Study on normoglycemic rats

Normoglycemic studies were carried out in overnight fasted normal rats, which were equally divided into eight groups of six rats each. Normal control group received only vehicle (1 ml of 0.3% CMC; p.o.) and 1 ml of standard group received reference drug TBM suspended in the vehicle (250 mg/kg, p.o.), while group from third to eight were administered with 1 ml of ME, AE and PEE (200 and 400 mg/kg, p.o.) respectively. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level.

1.7.3. Study on Alloxan-induced diabetic rats

In the experiment, diabetic rats were selected after alloxan induction and were equally divided into eight groups of six rats each. Diabetic control group received only vehicle (1 ml of 0.3% CMC; p.o.) and standard diabetic group received 1 ml of reference drug TBM suspended in the vehicle (250 mg/kg, p.o.), while group from third to eight were administered with 1 ml of ME, AE and PEE (200 and 400 mg/kg, p.o.) respectively once a day regularly up to 21th day. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 7, 14 and 21 respectively and subjected to fasting blood glucose level. The body weight, food

and fluid intake of all groups of animals were monitored on a daily basis for 21 days at regular time. Fixed amount of rat chow and fluid was given to each rat and replenished the next day. For experimental purposes, only the weights before treatment and at the end of the study were used for analysis. At the end of 21th day, all the rats were euthanized by pentobarbitone sodium (60 mg/kg) and sacrificed by cervical dislocation. Blood sample was withdrawn from abdominal aorta into fresh centrifuge tubes and centrifuged at 2,500 rpm for 15 min to obtain serum and plasma. Serum samples were stored at -20°C until utilized for further biochemical estimations.

1.8. Biochemical estimations

Plasma insulin was assayed by using commercial enzyme-linked immuno sorbent assay kit (ELISA, Boerhringer Mannheim, Germany). Serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), urea, creatinine, protein and albumin were estimated in a Hitachi Auto analyzer using commercial kit (Ecoline, E-Merck, India; Roche Diagnostics, GmbH, Germany) following standard procedures. The level of hemoglobin (Hb) and glycosylated hemoglobin ($HbA1_C$) was estimated using Drabkin reagent.

1.9. Estimation of lipid peroxidase

Plasma malondialdehyde (MDA) content was estimated using the method described by Uchiyama and Mihara, 1978 [29] with minor modification by Sunderman et al., 1985 [30] based on the thiobarbituric acid reaction (TBAR) test. Plasma samples were added (1/10, v/v) to ice-cold 1.15% KCl solution and mixed with 0.1 ml of 8.1% sodium dodecyl sulfate (SDS), 0.75 ml of 20% acetic acid, and 0.75 ml of 0.8% TBA solution. The mixture was made up to 2.0 ml with distilled water and heated at 95^0C for 60 min. After cooling with tap water, 0.5 ml of distilled water and 2.5 ml of *n*-butanol/pyridine mixture (15:1, v/v) were added and the mixture shaken vigorously. The mixture was centrifuged at 4000×*g* for 10 min and the absorbance of the organic layer (upper layer) was measured at 532 nm. The TBAR reaction was standardized by the analysis of tetraethoxypropane standard solutions, which yield MDA, mole for mole, under the described reaction conditions.

1.10. Estimation of superoxide dismutase (SOD)

The SOD activity was estimated by the method described by Ukeda et al., 1997 [31]. Into 2.6 ml of 50 mmol/L sodium citrate buffer (pH 9.4) were added 0.1 ml each of 30 mmol/l xanthine (dissolved in 1 mol/L NaOH), 3 mmol/l EDTA, 0.8 mmol/L XTT (3-*{*1-[(phenylamino)-carbonyl]-3,4-tetrazolium*}*- bis(4-methoxy-6-nitro)-benzenesulfonic acid hydrate) dissolved in buffer at 50^0C and 0.05 ml of sample solution containing SOD or water. The reaction consists of the addition of 0.02 ml of xanthine oxidase solution (500 mU/ ml) with the formation of a red formazan dye. The SOD activity was monitored for 30 s at 470 nm (25^0C) by the degree of inhibition of this reaction.

1.11. Estimation of catalase (CAT)

Catalase activity was measured by the method of Aebi, 1984 [32]. The homogenate was prepared with 50 mmol/l phosphate buffer, pH 7.0, with a drop of TritonX100 and centrifuged at 15,000×*g* for 15 min at 4^0C. To 3.0 ml of phosphate buffer, 0.05 ml of 90 mmol/l hydrogen

peroxide solutions and 0.02 ml of extract or water were added. The absorbance was read at 240 nm for 30 s.

1.12. Statistical Analysis

The results are expressed as mean±S.E.M. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by *Post-hoc* Student Newman Keuls test. $P < 0.05$ was considered to be statistically significant.

RESULTS

1.13. Effect on oral glucose tolerance test (OGTT) and plasma glucose level in normoglycemic and diabetic rats:

Table-1 illustrates the effect of root extracts of MK on OGTT at different time points. Statistical analysis by One-way ANOVA showed that there was no significant difference among the groups at 0 min [F (7, 40) = 0.18, P>0.05]. Similarly, statistical analysis at 30 min showed that there was significant difference among the groups [F (7, 40) = 6.405, P<0.05]. Post-hoc test revealed that TBM (250 mg/kg) and ME (200 and 400 mg/kg) showed significant attenuation in the plasma sugar level compared to control. Further, statistical analysis at 60 min showed that there was significant difference among the groups [F (7, 40) = 9.142, P<0.05]. Post-hoc by Student Newmann Keuls test revealed that TBM (250 mg/kg) and ME (200 and 400 mg/kg) showed significant decrease in the plasma sugar level compared to control. Furthermore, ME (200 mg/kg) showed significant increase in the sugar level (P<0.05) compared to TBM (250 mg/kg) and this trend was similar at 90 min [F (7, 40) = 5.62, P<0.05] and 120 min [F (7, 40) = 21.54, P<0.05].

Table-1: Hypoglycemic effect in oral glucose tolerance test (OGTT)

Groups	0 min	30 min	60 min	90 min	120 min
Control	87.4±2.51	146.6±4.52	138.3±3.23	108.2±1.94	101.5±2.65
TBM(250 mg/kg)	83.5±1.75	120.2±3.45[a]	102.9±2.3[a]	81.6±1.75[a]	68.6±3.15[a]
ME(200 mg/kg)	85.3±1.54	125.4±3.45[a]	115.1±2.66[a,c]	92.4±3.45[a,c]	77.3±1.76[a,c]
ME(400 mg/kg)	84.6±2.71	120.6±2.75[a]	110.7±3.45[a]	87.3±4.72[a]	74.6±2.10[a]
AE(200 mg/kg)	85.1±4.62	131.2±2.46	120.3±5.25	98.3±4.21	84.6±1.52
AE(400 mg/kg)	84.7±2.78	138.4±3.64	116.2±4.28	95.6±1.53	81.9±2.36
PEE(200 mg/kg)	86.3±1.72	136.6±2.64	125.1±3.78	102.3±5.61	92.8±1.77
PEE(400 mg/kg)	86.5±4.31	135.2±5.36	123.7±1.78	101.7±3.71	90.7±2.68

All values are Mean±SEM. [a]P<0.05 compared to control, [c]P<0.05 compared to TBM (One-way ANOVA followed by Student Newmann keuls test).

Time dependant effect on the level of plasma glucose level in fasted normoglycemic rats is depicted in Table-2. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [F (7, 40) = 0.0976, P>0.05]. Similarly, statistical analysis at 7 day showed that there was significant difference among the groups [F (7, 40) = 4.275, P<0.05]. Post-hoc test revealed that TBM (250 mg/kg) and ME (200 and 400 mg/kg) showed significant decrease in the plasma sugar level compared to control. However, there was no significant difference between TBM (250 mg/kg), ME (200 mg/kg) and ME (400

mg/kg) groups. Further, the similar trend like at 7 day was observed at 14 day [F (7, 40) = 7.16, P<0.05] and 21 day [F (7, 40) = 5.11, P<0.05].

Table-2 showed time dependant effect on the level of plasma glucose level in alloxan treated rats. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups at 0 day [F (7, 40) = 2.14, P>0.05]. Further, statistical analysis at 7 day showed that there was significant difference among the groups [F (7, 40) = 3.37, P<0.05]. Post-hoc test revealed that TBM (250 mg/kg) and ME (200 and 400 mg/kg) showed significant reduce in the plasma sugar level compared to diabetic control. Similarly, statistical analysis at 14 day showed that there was significant difference among the groups [F (7, 40) = 5.26, P<0.05]. Post-hoc test revealed that TBM (250 mg/kg) and ME (200 and 400 mg/kg) showed significant reduce in the plasma sugar level compared to diabetic control. Further, it has been observed that ME (200 and 400 mg/kg) showed significant increase in sugar level compared to TBM (250 mg/kg). Furthermore, the similar trend was observed at 21 day [F (7, 40) = 28.81, P<0.05] like at 14 day, however, there was significant decrease in sugar level in ME (400 mg/kg) compared to ME (200 mg/kg).

Table 2: Time dependant effect on the level of plasma glucose in fasted normoglycemic and Alloxan treated animals.

	Fasted normoglycemic				Alloxan induced			
Groups	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
Control/Diabetic	75.6±2.73	78.3±3.64	73.7±3.32	71.2±2.52	358.1±2.64	392.6±4.16	381.3±10.45	365.3±10.23
TBM (250 mg/kg)	73.1±4.32	59.5±1.35[a]	51.8±1.74[a]	52.8±1.52[a]	316.8±1.45	263.2±8.87[b]	191.6±13.52[b]	93.6±14.56[b]
ME (200 mg/kg)	73.8±1.75	65.1±2.71[a]	58.5±2.62[a]	59.5±2.32[a]	325.7±5.35	263.5±12.65[b]	243.5±12.72[b,c]	163.5±11.52[b,c]
ME (400 mg/kg)	73.2±2.31	60.7±2.79[a]	52.9±4.35[a]	51.4±3.45[a]	323.5±5.49	253.7±10.52[b]	236.6±11.73[b,c]	121.6±11.53[b,c,d]
AE (200 mg/kg)	75.7±3.45	70.4±3.78	67.2±1.28	65.7±1.36	329.3±1.78	288.1±12.15	275.7±15.29	273.7±16.23
AE (400 mg/kg)	75.4±1.62	69.7±1.78	65.5±3.71	64.8±2.51	327.6±2.34	280.3±9.78	266.2±12.36	266.2±15.26
PEE (200 mg/kg)	74.7±5.35	71.1±2.13	69.3±2.65	68.9±2.55	326.9±3.42	296.7±4.29	266.1±12.13	256.1±16.19
PEE (400 mg/kg)	74.6±3.45	70.3±4.27	68.8±3.29	67.7±3.89	337.4±4.56	291.6±7.73	258.2±11.78	248.2±13.73

All values are Mean±SEM [a]P<0.05 compared to control, [b]P<0.05 compared to Diabetic, [c]P<0.05 compared to TBM and [d]P<0.05 compared to ME-200 mg/kg (One-way ANOVA followed by Student Newmann keuls test).

1.14. Effect on body weight, food and fluid intake:

The effect of methanolic, aqueous and petroleum ether extract of root of MK on initial and final body weight is illustrated in Table-3. Statistical analysis by One-way ANOVA revealed that there was no significant difference among the groups during initial body weight estimation [F (8, 45) = 0.405, P>0.05]. Further, statistical analysis revealed that there was significant difference among the groups during final body weight estimation [F (8, 45) = 0.98, P<0.05]. Post-hoc test revealed that Diabetic, AE (200 and 400 mg/kg) and PEE (200 and 400 mg/kg) showed significant decrease, and ME (400 mg/kg) showed significant increase, however, TBM (250 mg/kg) and ME (200 mg/kg) showed no change in body weight compared to control. Furthermore, there was significant increase in body weight in TBM (250 mg/kg) and ME (200 and 400 mg/kg) compared to diabetic group.

The effect of different extracts of root of MK on food intake is illustrated in Table-3. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 3.94, P<0.05]. Post-hoc test revealed that all the groups showed significant increase in food intake compared to control. However, TBM (250 mg/kg) showed significant reduce in food intake compared to diabetic group. Table-3 illustrates the effect of MK on fluid intake.

Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 121.40, P<0.05]. Post-hoc test revealed that all the groups showed significant increase in food intake compared to control. However, all the treated groups showed significant decrease in fluid intake compared to diabetic group. Moreover, ME (200 and 400 mg/kg) showed no significant difference in fluid intake compared to TBM (250 mg/kg).

Table 3: Effects of ME, AE and PEE on Body weight, food and fluid intake

Groups	Body weight (in gm)		Food intake (g/day)	Fluid intake (ml/day)
	Initial	Final		
Control	175±7.65	188±8.35	51.4±4.48	198.2±3.76
Diabetic	176±8.35	135±8.42[a]	75.9±5.61[a]	345.6±2.61[a]
TBM(250 mg/kg)	184±9.35	196±7.45[b]	63.3±3.91[a,b]	293.4±2.17[a,b]
ME (200 mg/kg)	187±7.34	209±5.81[b]	76.8±4.29[a]	292.3±1.75[a,b]
ME (400 mg/kg)	186±7.28	215±8.31[a,b]	75.2±5.35[a]	289.3±4.64[a,b]
AE (200 mg/kg)	180±6.12	147±8.65[a]	78.5±5.91[a]	315.2±2.45[a,b,c]
AE (400 mg/kg)	182±9.83	150±10.5[a]	78.3±3.65[a]	310.6±2.51[a,b,c]
PEE (200 mg/kg)	173±10.61	156±9.35[a]	76.1±4.35[a]	320.3±5.11[a,b,c]
PEE (400 mg/kg)	174±8.34	167±7.65[a]	79.4±4.25[a]	318.9±6.22[a,b,c]

All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to Diabetic and [c]P<0.05 compared to TBM (One-way ANOVA followed by Student Newmann keuls test)

1.15. Effect on Total Hb, Glycosylated Hb, plasma insulin, urea, creatinine and albumin:

The effect of methanolic, aqueous and petroleum ether extract of root of MK on total Hb is illustrated in Table-4. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 2.71, P<0.05]. Post-hoc test revealed that diabetic, AE (200, 400 mg/kg) and PEE (200, 400 mg/kg) showed significant decrease in total Hb level, however, there was no change in total Hb level in TBM (250 mg/kg) and ME (200, 400 mg/kg) group compared to control. Further, there was significant increase in total Hb level in TBM (250 mg/kg) and ME (200, 400 mg/kg) groups compared to diabetic group. The similar trend was observed in Glycosylated Hb [F (8, 45) = 3.88, P<0.05], plasma insulin [F (8, 45) = 4.98, P<0.05], Creatinine [F (8, 45) = 32.83, P<0.05] and albumin [F (8, 45) = 3.59, P<0.05].

Table-4 showed the effect of methanolic, aqueous and petroleum ether extract of root of MK on urea level. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 12.79, P<0.05]. Post-hoc test revealed that diabetic, TBM (250 mg/kg), ME (200, 400 mg/kg), AE (200, 400 mg/kg) and PEE (200, 400 mg/kg) groups showed significant increase in urea level compared to control. However, there was significant decrease in urea level in TBM (250 mg/kg) and ME (200, 400 mg/kg) groups compared to diabetic group.

Table 4: Effects of ME, AE and PEE on Total Hb, Glycosylated Hb, plasma insulin, urea, creatinine and albumin

Groups	Total Hb (mg/dl)	Glycosylated Hb (HbA1c)	Plasma Insulin(μU/ml)	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)
Control	15.7±1.46	3.4±0.75	16.45±1.6	28.5±3.11	0.82±0.07	4.25±0.25
Diabetic	7.2±1.64^{a}	11.7±1.64^{a}	5.31±0.55^{a}	66.8±2.75^{a}	1.42±0.04^{a}	2.63±0.25^{a}
TBM(250mg/kg)	14.9±1.25^{b}	4.2±0.35^{b}	15.3±2.75^{b}	33.15±2.9^{a,b}	0.71±0.02^{b}	4.19±0.15^{b}
ME(200 mg/kg)	15.3±1.70^{b}	6.8±0.91^{b}	13.2±1.25^{b}	35.19±1.6^{a,b}	0.78±0.04^{b}	4.01±0.21^{b}
ME(400 mg/kg)	15.5±1.70^{b}	5.7±0.55^{b}	14.9±1.76^{b}	32.28±3.0^{a,b}	0.72±0.01^{b}	4.38±0.15^{b}
AE(200mg/kg)	13.2±1.75^{a}	8.9±1.24^{a}	10.6±1.58^{a}	42.35±1.1^{a}	0.90±0.03^{a}	3.77±0.58
AE(400 mg/kg)	13.8±1.65^{a}	8.6±0.85^{a}	11.4±1.83^{a}	41.69±2.8^{a}	0.83±0.05^{a}	3.81±0.43
PEE(200mg/kg)	11.8±1.81^{a}	9.5±2.75^{a}	7.7±1.17^{a}	47.6±5.34^{a}	1.15±0.05^{a}	3.25±0.17
PEE(400 mg/kg)	12.3±1.68^{a}	9.1±1.65^{a}	8.7±1.91^{a}	43.2±4.13^{a}	0.95±0.01^{a}	3.36±0.20

All values are Mean±SEM. ^{a}P<0.05 compared to control and ^{b}P<0.05 compared to Diabetic group (One-way ANOVA followed by Student Newmann keuls test)

1.16. Effect on plasma lipid profile:

The effect of methanolic, aqueous and petroleum ether extract of root of MK on TC is illustrated in Table-5. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 7.66, P<0.05]. Post-hoc test revealed that the result was similar to that of urea level. Further, statistical analysis by One-way ANOVA [F (8, 45) = 13.20, P<0.05] followed by post-hoc test revealed that the result observed in TG level was similar to that of total Hb level. Furthermore, statistical analysis by One-way ANOVA followed by post-hoc test revealed that the result observed in both LDL [F (8, 45) = 35.34, P<0.05] and HDL [F (8, 45) = 27.26, P<0.05] level was similar to that of TG level, however, there was significant increase in both LDL and HDL level in ME (200 mg/kg) compared to TBM (250 mg/kg).

Table 5: Effects of ME, AE and PEE on plasma lipid profile

Groups	(TC mg/dl)	(TG mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control	65.7±1.35	82.4±3.06	52.7±2.48	35.2±5.35
Diabetic	98.4±2.15^{a}	119.6±2.35^{a}	116.1±1.9^{a}	19.7±3.2^{a}
TBM(250mg/kg)	70.1±3.40^{a,b}	81.7±2.95^{b}	49.3±3.7^{b}	38.7±2.7^{b}
ME (200 mg/kg)	68.7±3.62^{a,b}	88.7±5.25^{b}	65.0±4.1^{b,c}	34.7±2.9^{b,c}
ME (400 mg/kg)	63.9±5.02^{a,b}	86.2±4.52^{b}	61.5±5.0^{b}	37.1±5.0^{b}
AE (200mg/kg)	79.5±4.66^{a}	97.6±4.17^{a}	87.2±1.7^{a}	25.7±1.0^{a}
AE (400 mg/kg)	77.3±5.67^{a}	95.4±1.47^{a}	78.5±5.7^{a}	28.6±2.3^{a}
PEE (200mg/kg)	84.9±4.42^{a}	111.2±3.02^{a}	98.4±2.1^{a}	23.7±5.8^{a}
PEE (400 mg/kg)	83.1±3.76^{a}	109.3±5.25^{a}	94.1±5.1^{a}	24.5±4.9^{a}

All values are Mean±SEM. ^{a}P<0.05 compared to control, ^{b}P<0.05 compared to Diabetic and ^{c}P<0.05 compared to TBM (One-way ANOVA followed by Student Newmann keuls test)

1.17. Effect on liver function and plasma antioxidant profile:

The effect of methanolic, aqueous and petroleum ether extract of root of MK on SGOT is illustrated in Table-6. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 21.95, P<0.05]. Post-hoc test revealed that diabetic, TBM (250 mg/kg), ME (200, 400 mg/kg), AE (200, 400 mg/kg) and PEE (200, 400 mg/kg) groups showed significant increase in SGOT level compared to control. Further, there

was significant decrease in SGOT level in TBM (250 mg/kg) and ME (200, 400 mg/kg) groups compared to diabetic group. Furthermore, there was significant increase in SGOT level in ME (400 mg/kg) compared to ME (200 mg/kg) group. The similar trend was observed in SGPT [F (8, 45) = 30.93, P<0.05] level.

The effect of methanolic, aqueous and petroleum ether extract of root of MK on ALP is illustrated in Table-6. Similarly, statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 28.68, P<0.05]. Post-hoc test revealed that diabetic, TBM (250 mg/kg), ME (200, 400 mg/kg), AE (200, 400 mg/kg) and PEE (200, 400 mg/kg) groups showed significant increase in ALP level compared to control. Further, there was significant decrease in ALP level in TBM (250 mg/kg) and ME (200, 400 mg/kg) groups compared to diabetic group. Furthermore, there was significant increase in ALP level in ME (200 mg/kg) compared to TBM (250 mg/kg) group.

Table 6: Effects of ME, AE and PEE on liver function and plasma antioxidant profile

Groups	SGOT (mg/dl)	SGPT (mg/dl)	ALP (U/L)	LPO (g/dl)	SOD (Units/ mg protein)	CAT (Units/ mg protein)	Total protein (g/dl)
Control	56.7±2.14	43.7±2.71	135.2±2.6	0.15±0.01	92.2±4.10	35.3±3.1	7.03±0.53
Diabetic	105.4±3.6[a]	107.3±5.2[a]	213.7±4.7[a]	0.33±0.02[a]	210.3±12.4[a]	10.8±3.4[a]	4.21±0.65[a]
TBM(250mg/kg)	65.4±4.27[a,b]	59.6±5.35[a,b]	151.7±6.7[a,b]	0.18±0.01[b]	123.5±9.20[a,b]	27.2±4.2[b]	6.61±0.52
ME(200 mg/kg)	69.9±3.24[a,b]	64.9±1.37[a,b]	170.5±6.0[a,b,c]	0.20±0.03[b]	145.6±10.2[a,b]	29.1±6.2[b]	6.21±0.82
ME(400 mg/kg)	72.5±5.51[a,b,d]	67.8±1.62[a,b,d]	162.3±2.7[a,b]	0.17±0.03[b]	131.4±8.70[a,b]	27.5±4.1[b]	6.43±0.76
AE (200mg/kg)	98.2±6.34[a]	86.5±2.51[a]	180.7±3.1[a]	0.34±0.03[a]	198.8±9.20[a,c]	18.6±3.3[a]	5.77±0.35
AE (400 mg/kg)	95.6±2.71[a]	83.7±4.72[a]	184.7±1.9[a]	0.32±0.02[a]	203.1±10.8[a,c]	12.4±3.6[a]	5.81±0.75
PEE (200mg/kg)	109.7±5.4[a]	95.3±2.76[a]	198.2±4.6[a]	0.33±0.02[a]	207.3±9.80[a,c]	13.7±1.9[a]	5.26±0.12
PEE(400 mg/kg)	105.7±3.9[a]	90.7±3.63[a]	190.3±5.7[a]	0.34±0.02[a]	204.1±11.6[a,c]	11.1±2.1[a]	5.38±0.26

All values are Mean±SEM. [a]P<0.05 compared to control, [b]P<0.05 compared to Diabetic, [c]P<0.05 compared to TBM and [d]P<0.05 compared to ME-200 mg/kg (One-way ANOVA followed by Student Newmann keuls test)

The effect of methanolic, aqueous and petroleum ether extract of root of MK on LPO and CAT is illustrated in Table-6. Statistical analysis by One-way ANOVA followed by post-hoc test revealed that the result observed in both LPO [F (8, 45) = 14.09, P<0.05] and CAT [F (8, 45) = 6.12, P<0.05] level was similar to that of total Hb level.

Table-6 showed the effect of methanolic, aqueous and petroleum ether extract of root of MK on SOD level. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 21.26, P<0.05]. Post-hoc test revealed that diabetic, TBM (250 mg/kg), ME (200, 400 mg/kg), AE (200, 400 mg/kg) and PEE (200, 400 mg/kg) groups showed significant increase in SOD level compared to control. Further, there was significant decrease in SOD level in TBM (250 mg/kg) and ME (200, 400 mg/kg) groups compared to diabetic group. Furthermore, there was significant increase in SOD level in AE (200, 400 mg/kg) and PEE (200, 400 mg/kg) groups compared to TBM (250 mg/kg) group.

Similarly, Table-6 showed the effect of methanolic, aqueous and petroleum ether extract of root of MK on total protein level. Statistical analysis by One-way ANOVA revealed that there was significant difference among the groups [F (8, 45) = 2.14, P<0.05]. Post-hoc test revealed that only diabetic group showed significant decrease in total protein level compared to control.

DISCUSSION

In the present study, we for the first time have reported that the routine post-treatment of ME for 21 days showed potential hypoglycemic activity in OGTT and normoglycemic rats and antihyperglycemic activity in alloxan-induced rats compared to AE and PEE.

Preliminary phytochemical screening revealed that all the extracts of roots of MK showed positive response to alkaloids, saponins and triterpenes, tannins, flavonoids, carbohydrates and sterols. Further, the ME showed highest total phenolic content (262.8 mg/g) compared to AE (102.2 mg/g) and PEE (98.6 mg/g). It has been reported that MK leaf extracts possesses several carbazole alkaloids, viz. mahanimbine, koenimbine, O-methylmurrayamine-A and murrayazolinine [33], Copolin-α-glucoside and free glucose [34]. Further, it has been investigated that certain alkaloids are responsible for antidiabetic activity in experimental animals [35]. It has been suggested that diabetes is of two categories out of which one is genetically based and other as a result of dietary indiscretion [36]. In the present experiment we have found that only the ME significantly reduced the blood sugar level in hyperglycemic rats in dose-dependent manner. However, all the doses of ME showed similar significant lowering of blood glucose level in OGTT and normoglycemic rats. Therefore, the present study is indicating that the ME would be a potential candidate to treat both categories of diabetes which could be due to presence of carbazole alkaloids.

In the present study we have found that the final body weight decreased in spite of no change in initial body weight and increased food and fluid intake in diabetic animals. Further, there was significant decrease in total Hb, plasma insulin and albumin levels, however there was significant increase in HbA1c, urea and creatinine levels. The above findings are found to be similar with the previous published results [37]. It has been reported that protein glycation during hyperglycemia mostly leads to production of HbA1c. Hence, HbA1c level is used as the most reliable indicator for assessment of retrospective glycemic control in the management of diabetes [38]. The reduction of HbA1c levels in diabetic animals indicated that there was less protein glycation, possibly resulting from the decrease in blood glucose levels observed in these animals. In consistency with the findings observed in methanolic leaf extract of MK, the ME showed significant mitigation effect in the HbA1c levels.

It has been suggested that the levels of serum lipids are usually elevated in diabetes mellitus, representing a high risk factor for coronary heart disease [39]. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. It has been observed earlier that the hypercholesterolemia and hypertriglyceridemia occurs in diabetic animals. Under normal conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides [40]. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Since lipid abnormalities accompanying with premature atherosclerosis, is the major cause of cardiovascular diseases in diabetic patient therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. It has been systematically reported that cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes [41].

The ME (200 mg/kg and 400 mg/kg) not only lowered the TC, TG and LDL levels but also enhanced the cardio protective lipid HDL in diabetic rats, respectively. It has been evidenced that an increase in HDL levels is associated with a decrease in coronary risk [42]. In the present study the ME not only decreases the total cholesterol but also enhances the HDL levels significantly. High levels of TC and more importantly LDL levels are major coronary risk factors [43]. In the present study it has been found that the ME mitigated the elevated TC and LDL levels in diabetic rats. Further, it has been suggested that TG itself is independently related to coronary heart disease [44] and in the present investigation the ME lowered TG levels in diabetic animals.

Routine post-treatment of the ME for 21 days to diabetic animals improved the liver function by decreasing the serum SGOT, SGPT and ALP levels. It is reported that the increase in levels of SGOT and SGPT will increase the incidence of heart and liver diseases. SGOT is an enzyme found primarily in the cells of the liver, heart, skeletal muscles, kidneys, and pancreas and to a lesser extent in red blood cells. Its serum concentration is directly proportional to the amount of cellular leakage or damage. It is released into serum in larger quantities when any one of these tissues is damaged. The increased levels of these enzymes are usually associated with heart attacks or liver disease. The ME decreased the SGOT level, which is an indication of the protective effect on liver and heart. It is known that SGPT, an enzyme found primarily in the liver, the enhanced release of which into the bloodstream is the result of liver aberration. It therefore serves as a fairly specific indicator of liver status and its elevated levels in serum indicate liver damage. It has been reported that the increased levels of ALP indicates bone disease, liver disease or bile tract blockage. The ME reduced the ALP levels, indicating its protective effect on liver function.

It has been established that oxidative stress plays a central role in the pathogenesis of diabetes [45]. Oxidative stress is the result of excessive free radical production and lipid peroxidation due to aberrant protection by the enzymatic and nonenzymatic anti-oxidative defense system. Oxidative stress is thought to be a direct concequence of increased production of reactive oxygen species (ROS). Degradation of lipid peroxides leads to the accumulation of toxic aldehydes, one of the most toxic being malondialdehyde (MDA), as an index of lipid peroxidation. In the present study we measured MDA because this parameter is now considered to be one of the indicative of *in vivo* lipid peroxidation [46]. Further, it has been studied that lipid peroxidation is augmented in diabetes [47]. Oxidative stress, arising from an increase in ROS coupled with impaired antioxidant protection, has been suggested to be an important etiological factor for diabetes [48]. During stress LPO and SOD were significantly increased and CAT level was significantly decreased. The increase in SOD was due to increased ROS generation. This led to increased generation of $H_2O_2^-$ and its accumulation due to decreased CAT level [49]. In the present study it has been found that LPO and SOD levels were increased, and CAT levels were decreased in diabetic rats compared to control rats and was reversed by the ME significantly, indicating improved in oxidative damage and antioxidant profile.

CONCLUSION

The ME found to be potential antidiabetic extract compared to AE and PEE in alloxan-induced diabetic animals. Further, isolation and establishment of exact mechanism of action of specific

compound from ME is to be carried out in the future. Moreover, a comparative study between root and leaf extract has to be carried out to maintain the biodiversity.

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