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Full Length Research Paper

Evaluation of anti-hyperglycemic and free radical scavenging activity of *Melothria maderaspatana* Linn. in streptozotocin-induced diabetic rats

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The anti-hyperglycemic activity of aqueous extract of *Melothria maderaspatana* Linn. was evaluted in streptozotocin-induced diabetic rats. Free radical scavenging activity of aqueous extract of *M. maderaspatana* was assessed *in vitro* using 1, 1-diphenyl-2-picryhydrazyl (DPPH). Oral administration of aqueous extract of *M. maderaspatana* significantly decreased the blood glucose level in normal and streptozotocin-induced diabetic rats. This extract also lowered the serum cholesterol, lipid peroxidation (LPO) and hepatic tissue LPO levels in streptozotocin-induced diabetic rats. *M. maderaspatana* showed free radical scavenging activity when assayed in an *in vitro* system using 1, 1-diphenyl-2-picryhydrazyl (DPPH) stable free radical. Pretreatment of *M. maderaspatana* failed to show any protective action against streptozotocin-induced diabetes. Aqueous extract of *M. maderaspatana* (2 g/kg p.o.) showed significant antihyperglycemic, hypocholesterolemic and free radical scavenging activities in streptozotocin-induced diabetic rats.

Key words: Antihyperglycermic activity, DPPH, free radical scavenging activity, LPO, *Melothria maderaspatana*, streptozotocin.

INTRODUCTION

Oxidative stress has been shown to play a very crucial role in some disease state like liver cirrhosis, atherosclerosis, cancer, diabetes, etc. Free radical that generate inside, the body is responsible for oxidative stress and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Wilson, 1988). Antioxidants have ability to protect the human body against damage by reactive oxygen species (Lollinger, 1981). Long term hyperglycemia is associated with several complications such as atherosclerosis, myocardial infarction, neuropathy, nephropathy etc. These complications is mediated and complicated through oxidative stress (Singer, 1992). *Melothria maderaspatana* Linn belonging to family Cucurbitaceae, is comprised mainly of slender scadentor prostrate annual herbs. It is reported as expectorant and has been used traditionally from a long time for a number of ailments. The tender shoots and bitter leaves are used as a gentle aperient and prescribed in vertigo and biliousness (The Wealth of India, 1969; Kirthikar and Basu, 1933). This plant has a unique place in the Siddha system of medicine (Krishna and Suresh, 1995). *M. maderaspatana* has been shown to exert hepatoprotective (Thabrew et al., 1988) antioxidant (Jayatilaka et al., 1990), anti-inflammatory (Sinha et al., 1997) and antiarthiritic activities (Ramakrishnamacharya et al., 1995). The immunomodulatory activity of *M. maderaspatana* was established by Thabrew et al. (1991). Anti microbial activity of methanolic and petroleum extract was evaluated by Hemamalini et al. (2007). The ethyl acetate (100, 200, 400 g/ml), hexane and methanol extracts of *M. maderaspatana* were reported to have antiplatelet activity in *in vitro* (Iman et al., 2006).

The consumption of *M. maderaspatana* leaf tea decreased the blood pressure and showed beneficial effects on lipid profile, fibrinogen, bilirubin and body mass

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index in human volunteer (Raja et al., 2007). *M. maderaspatana* is reported to contain sugar, amino acids, flavanoids (Retnam and De Britto, 1998) and columbin (Rastogi, 1979). A number of plants have been screened for anti hyperglycemic activity based on different system of medicines and still few medicinal plants are yet to be evaluated to validate their use in traditional medicine. Some tribes of India (Orissa) also use this herb for the treatment of diabetes mellitus (Sinha, 1996). Since the antidiabetic activity of this plant has so far not been scientifically investigated, therefore, the present work is an attempt to evaluate the anti-hyperglycemic activity of an aqueous extract of *M. maderaspatana* in streptozotocin-induced diabetic albino rats.

MATERIALS AND METHODS

Plant material

The whole dried plant was procured from a herbal market and authenticated by Chief Botanist Mr. D. Narayanappa, TAMPCOL Aurmbakkam, Chennai, India the voucher specimen (SH/MM/02) has been deposited in our department.

Extraction procedure

The dried coarse powder of *M. maderaspatana* was extracted by decoction method with distilled water (6 h, 80°C). The aqueous extract was concentrated in vacuum in a rotary evaporator to provide dry extract (yield 15.6% w/w) . Preliminary phytochemical analysis of the aqueous extract of the drug revealed the presence of flavanoids and tannins. The dried aqueous extract was stored in a desiccator.

Animals

The albino rats (Male, Charles foster strain) were bought from Central Animal House (542/02/ab/CPCSEA) Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The body weight of these animals ranged between 150 - 180 g. The rats were housed in polypropylene cages three in each cage at an ambient temperature of 25 ± 2°C and 55-60% relative humidity. The animals were given commercially available rat feed (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The experiment was performed in accordance with CPCSEA (as per animal ethical committee) guidelines for care and use of laboratory animals.

Experimental induction of diabetes in rats

The rats were administrated freshly prepared solution of streptozotocin (Sigma USA) dissolved in citrate buffer pH 4.5 at a dose of 70 mg/kg, ip. After 72 h of streptozotocin injection the blood glucose level of each rat was assayed. The rats with fasting blood glucose level above 200 mg/dl were used in this investigation.

Effect of aqueous extract of *M. maderaspatana* in streptozotocin-induced diabetes

The diabetic animals were divided in 6 groups with 6 rats in each group. The animals of group I and II served as normal and diabetic control and those groups III, IV and V received aqueous of *M. maderaspatana* in dose of 500 mg/kg, 1 g/kg, 2 g/kg body weight respectively once daily orally for 7 days. The animals in the group VI received glibenclamide (10 mg/kg) and the animals in the normal group were administered with normal saline. After 7 days of treatment blood from each rat was collected through retrorbital plexus of eye under light anesthesia and blood glucose was determined by glucose oxidase method. Briefly glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. Generated hydrogen peroxide catalyzed reaction and liberated oxygen is accepted by the chromogen system to give a red coloured quinoneamine compound. The red colour so developed is measured at 505 nm and is directly proportional to glucose concentration (Tietz, 1994). The effective dose was found and the serum was used for the determination of serum cholesterol (Parekh and Jung, 1970) and serum lipid peroxidation (LPO) (Okhawa et al., 1979). The liver tissue from each rat was dissected out cleaned with cold KCl (1.15%) and stored at -20°C pending biochemical analysis. A small piece of liver was weighed and homogenized in cold KCl. The homogenate was used for the assay of hepatic LPO. The pancreas of each rat was dissected out and processed for histopathalogical evaluation.

Protective effect of aqueous extract of *M. maderaspatana* (2 g/kg, po) in streptozotocin-induced diabetes

A group of rats were administered aqueous extract of *M. maderaspatana* (2 g/kg, po) for 5 days and then injected with streptozotocin (70 mg/kg, i.p.). Blood glucose level in these animals was estimated before and after 72 h of streptozotocin injection. Another group of streptozotocin injected rats were maintained as diabetic control and these rats did not receive the drug. Similarly a group of animals were maintained for normal control without any treatment (Kamtchouing et al., 1998).

Acute toxicity studies

Different doses of aqueous extract of *M. maderaspatana* were administered to different groups of rats and mortality rate was recorded (Bruce, 1995).

Free radical scavenging activity of *M. maderaspatana* by DPPH

To determine the free radical scavenging activity, a method based on the reduction of a methanolic solution of the coloured free radical DPPH was used. The decrease in absorption of DPPH at its absorption maximum of 517 nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution. This activity was expressed as the effective concentration at 50% (EC 50) that is, concentration of the test solution (methanolic solution of aqueous extract of *M. maderaspatana*) required to give a 50% reduction in absorbance of the test solution as compared to that of a blank solution (Kato et al., 1988).

Statistical analysis

Students 't' test and ANOVA were used for the statistical analysis of data.

RESULTS

Intraperitoneal injection of streptozotocin significantly

Table 1. Effect of different doses of aqueous extract of *M. maderaspatana* on blood glucose levels in streptozotocin-induced diabetic rats.

Groups	Blood glucose levels in mg/dl	
	1st day	7th day
Normal rats (vehicle)	42.37 ± 2.7	46.51 ± 2.4
Diabetic rats (vehicle)	269.60 ± 3.2	259.90 ± 4.2ns
M maderaspatna (500 mg/kg)	240.30 ± 2.9	230.40 ± 1.6ns
M. maderaspatna (1 g/kg)	233.04 ± 1.6	223.12 ± 0.7ns
M. maderaspatna (2 g/kg)	259.90 ± 10.4	59.82 ± 11.7*#
Glibenclamide (10 mg/kg)	245.50 ± 1.4	90.15 ± 10.27*#

Mean ± SEM (n = 6). *p < 0.001 compared with diabetic control; ns - not significant # p < 0.05 (ANOVA).

Table 2. Effect of aqueous extract of Melothria maderaspatana (2 g/kg, po body weight) on cholesterol and lipid peroxidation.

Group	Serum cholestrol (mg/dl)	Serum LPO (nmoles/ml)	Liver LPO (nmoles/gm of wet tissue)
Normal control	87.67 ± 3.03	22.57 ± 1.17	344.79 ± 5.0
Diabetic control	200.71 ± 3.40	43.94 ± 2.22	479.98 ± 5.5
M. maderaspatana (2 g/kg)	84.62 ± 11.07*#	24.64 ± 1.40*#	378.02 ± 4.7*#

Mean ± SEM (n = 6). *p < 0.001 compared with diabetic group. # p < 0.05 (ANOVA).

Table 3. Protective effect of aqueous extract of *M. maderaspatana* (2 g/kg, po) in streptozotocin-induced diabetes.

Group	Blood glucose level (mg/dl)		
	0 day	5th day	8th day
Normal control	49.52 ± 3.20	50.10 ± 1.50	48.75 ± 4.10
Diabetic control	46.97 ± 5.60	48.08 ± 4.60	475.33 ± 10.30
M. maderaspatana	47.53 ± 1.33	26.41 ± 2.10*	434.47 ± 9.50ns

Mean ± SEM (n = 6). * P < 0.001 compared with diabetic control (5th day). ns- not significant compared with diabetic control (8th day).

enhanced the fasting blood glucose level as compared to normal euglycemic rats. Administration of aqueous extract of *M. maderaspatana* to diabetic rats at a dose of 500 mg/kg and 1 g/kg body weight did not significantly alter the blood glucose level in comparison to diabetic control (p > 0.05). On the contrary, aqueous extract of *M. maderaspatana* at a dose of 2 g/kg, significantly decreased blood glucose level in diabetic rats (p < 0.001) (Table 1). The serum cholesterol, LPO and hepatic LPO levels were increased following streptozotocin injection. Administration of aqueous extract of *M. maderaspatana* 2 g/kg p.o. significantly lowered serum cholesterol, LPO and hepatic LPO in diabetic rats (Table 2). Administration of aqueous extract of *M. maderaspatana* to normal rats for 5 days significantly lowered the fasting blood glucose levels compared to untreated normal rats (p < 0.001). However, pretreatment with this extract did not showed any protective action against the streptozotocin-induced hyperglycemia as the blood glucose level between the untreated and pretreated rats did not differ significantly (p > 0.05) (Table 3). Aqueous extract of *M. maderaspatana* at the dose of 20 g/kg; p.o. did not produce mortality to rats. *M. maderaspatana* (2 mg/ml) exhibited free radicals scavenging activity in an in vitro system using DPPH (Table 4). The histopathalogical assessment of pancreas of *M. maderaspatana* treated rats showed some protection against the streptozotocin induced cell damage (Figure 1).

DISCUSSION

It is now well established that streptozotocin selectively destroys the pancreatic cells and produces hyperglycemia. The hyperglycemia induced by streptozotocin mimics insulin dependent diabetes mellitus (IDDM) in adult animals and also induces a variety of metabolic abnormalities (Soling et al., 1976). In view of

Table 4. Free radical scavenging activity of aqueous extract of Melothria maderaspatana by DPPH method.

Sample	Scavenging activity Ec 50		r*
Aqueous extract of *M.maderaspatana* (2 mg/ml)	673.60 µl	1347.20 µg	0.980
Ascorbic acid 0.5 mg/ml	25.50 µl	11.25 µg	0.996

*Correlation coefficient.

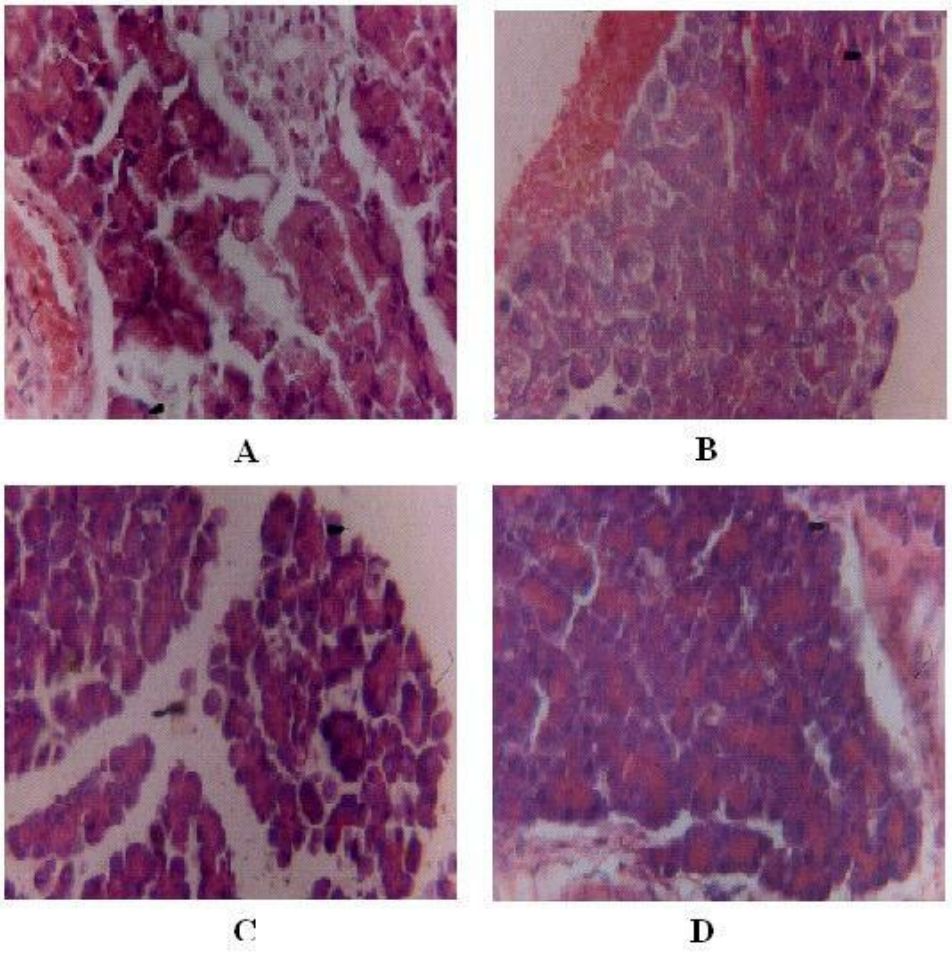

Figure 1. Photomicrograph of pancreas of diabetic rats on treatment with *M. maderaspatana*. Where A: Pancreas of normal control rats, B: Pancreas of diabetic control rats, C: Pancreas of diabetic rats treated with glibenclamide and D: Pancreas of diabetic rats treated with *M. maderaspatana*.

this, streptozotocin induced diabetic model has been ideal for screening of antihyperglycaemic/antidiabetic activity of various phytochemicals and synthetic products. In the present study the antihyperglycemic activity of different doses of aqueous extract of *M. maderaspatana* was assessed in streptozotocin-induced diabetic rats. Administration of aqueous extract of *M. maderaspatana* (2 g/kg; p.o.) exhibited significant lowering of blood glucose levels at the end of 7 days ($p<0.001$). Similarly the administration of aqueous extract of *M. maderaspatana* (2 g/kg; p.o.) to normal rats also significantly ($p<0.001$) reduced the blood glucose levels. The observations tend to suggest that possibly the action of aqueous extract of *M. maderaspatana* may be similar to insulin. Further, there is also possibility that this extract might increase the uptake of glucose by peripheral tissue and thereby presumably decreased the blood glucose level. However, pretreatment with this extract did not showed any protection against the streptozotocin-induced hyperglycemia as the blood glucose level between the

untreated and pretreated rats did not differ significantly ($p>0.05$).

It is now well documented that LPO mediated tissue damage plays an important role in the development of type I and type II diabetes (Metz, 1984: Walsh and Pek, 1984). In the present study administration of aqueous extract of *M. maderaspatana* (2 g/kg; p.o.) to diabetic rats significantly lowered the liver and serum LPO level. The results suggested that possibly the aqueous extract of *M. maderaspatana* may prevent the lipid peroxidation and in turn protect the tissue from free radicals. In an *in vitro* system, the aqueous extract of *M. maderaspatana* also showed free radical scavenging activity. Thus, the presence of free radical scavenging activity and lipid peroxidation lowering activity in aqueous extract of *M. maderaspatana* might have helped in providing protection to some degree against oxidative damage to beta cells of pancreas. Further, the free radical scavenging potential could also help in reducing the known complication of diabetes mellitus.

Medicinal plants belonging to Cucubitaceae family are well known for their hypoglycemic activity like *Cirtrullus colocynthis* (Nmila et al., 2000), *Coccinia indica* (Dhanapal et al., 2003), *Luffa aegyptiaca* (El-Fiky et al., 1996), *Momordica charantia* (Sreejayan and Rao, 1990) and *M. cymbalaria* (Geetha and Shyamala, 2003) have shown antidiabetic effect in experimental rats. The observation recorded in the present study tends to suggest that aqueous extract of *M. maderaspatana* contains some potent anti-hyperglycemic phytoconstituent which can give us a first report and new ray of hope in the treatment of diabetes. In the view of the earlier report the plant is also used in the treatment of hypertension which may reduces the secondary complications in the diabetes.

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REFERENCES

Anonymous (1969). The Wealth of India. C.S.I.R. Publications, New Delhi, p. 336.

Bruce RD (1985). An up and down procedure for acute toxicity. Funda. Appl. Toxicol., 5: 151-157.

Dhanapal SP, Kokate CK, Patil MB, Elango K, Suresh B (2003). National Convention On Current Trends in Herbal Drugs and Annual Conference in Indian Society of Pharmacognosy, Gandhi Nagar, A (1): 33.

EL-Fiky FK, Abou-Karam MA, Afify EA (1996). Effect of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) extracts on blood glucose level of normal and streptozotocin diabetic rats J. Ethnopharmacol., 50(1): 43-47.

Geetha G, Shyamala G (2003). National Convention on Current Trends in Herbal Drugs and Annual Conference in Indian Society of Pharmacognosy, Gandhi Nagar, A (2): 33.

Hemamalini K, Varma VK (2007). Antimicrobial activity of methanolic leaves extract of *Melothria maderaspatana* Linn. Pharmacologyonline, 3: 323-326.

Iman RA, Lakshmi Priya B, Chithra R, Shalini K, Sharon V, Chamundeeswari D, Vasantha J (2006). *In vitro* antiplatelet activity-guided fractionation of aerial parts of *Melothria maderaspatana*. Indian J. Pharm. Sci., 68(5): 668-670.

Jayatilaka KAPW, Thabrew AI, Perera DJB (1990). Effects of *Melothria maderaspatana* on CCl4-induced changes in rats' hepatic microsomal drugmetabolism enzyme activity. J. Ethnopharmacol., 30: 97-105.

Kamtchouing P, Sokeng SD, Moundipa PE, Watcho P, Jatsa HB, Lontsi D (1998). Protective role of *Anacardium occidentale* extract against streptozotocin –induced diabetes in rats. J. Ethnopharmacol., 62(2): 95-99.

Kato K, Terao S, Shimamoto N, Hirata M (1988). Studies of active oxygen species. Synthesis and biological activity of 2-o-alkyl ascorbic acid. J. Med. Chem., 31: 793-798.

Kirthikar KR, Basu BD (1933). Krishna. Indian Med. Plants, III: 1160-1160.

Kumar PR, Suresh K (1995). Conservations of plants from Siddha system. Industry meet cum-seminar in Biodiversity and informations on Medicinal and Aromatic Plants, New Delhi, 53: 15-17.

Lollinger J (1981). Free Radicals and Food Additives. Ed. by Taylor and Francis, London, p. 121.

Metz SA (1984). Oxygenation products of arachidonic acid: Third messengers for insulin release. Prostaglandins, 27: 147-151.

Nimla R, Gross R, Rchid H, Roye M, Manteghetti M, Petit P, Tijane M, Ribes G, Sauvarie Y (2000). Insulinotropic effect of *Citrullus colocynthis* fruit extracts. Planta. Med., 66(5): 418-423.

Okhawa H, Ohishi N, Yagi K (1979). Assay of lipid peroxides in animal tissuses by Thiobarbituric acid reactions. Anal. Biochem., 95: 351-358.

Parekh AC, Jung DH (1970). Cholesterol determination with ferric acetate-uranium acetate and sulphuric acid ferrous sulphate reagents. Anal. Chem., 42: 1423-450.

Raja B, Kaviarasan K, Arjunan MM, Pugalendi K.V (2007). Effect of *Melothria Maderaspatana* leaf-tea consumption on blood pressure, lipid profile, anthropometry, ibrinogen, bilirubin and albumin levels in patients with hypertension. J. Altern. Complement Med., 13(3): 349-354.

Ramakrishnam A, Krishnaswamy MK, Rao RR, Vishwanathan S (1995). Clinical Studies on anti rhuematic activity of *Melothria maderaspatana*. Seminar on research in Ayurveda and Siddha, CCRAS, New Delhi, 36.

Rastogi RP (1979). Compendium Indian Med. Plants, Central Drug Research Institute and New Delhi, Publication, Lucknow, 2: 472.

Retnam KR, De Britto AJ (1998). Preliminary phytochemical screening of three medicinal plants of Tirunelveli hills. J. Econo. Taxon. Bot., 22(2): 677-681.

Singer DE, Nathan DM, Anderson KM, Wilson PW, Evans JC (1992). Association of HbA1c with prevalent cardio vascular disease in the original cohort of the Framingham Heart Study. Diabetes, 41: 202-208.

Sinha BN, Sasnal D, Basu SP (1997). Pharmacological studies on Melothria maderaspatana. Fitoterapia, 68: 75-78.

Soling D, Klenlneke J (1976). In: Hanson RW, Mehlman MA Gluconeogenesis (Eds). John wiley, New York pp. 369-462.

Sreejayan Rao MNA (1990). Nitric oxide scavenging by curcuminoids. Proceedings of 42nd IPC Manipal Dec. 28-30, AP 03: 16.

Thabrew MI, De Silva KTD, Labadio RP, De Bio PAF, Vander Berg B (1991). Immunomodulatory activity of three SriLankan medicinal plants used in hepatic disorders. J. Ethanopharmacol., 33(1-2): 63-66.

Thabrew MIRA, Jayatilaka PAPW, Perera DJB (1988). Evaluation of efficacy of *Melothira madaraspatama* in the alleviation of carbon tetrachloride induced liver dysfunction. J. Ethnopharmacol. 23: 305-312.

Tietz NW (1994). Clinical Guide to Laboratory tests, 2nd Edn. W.B.

Saunders: Philadelphia, pp. 1073-1091.
Walsh MF, Pek SB (1984). Possible role of endogenous arachidonic acid metabolites in stimulated release of insulin and glucagons from the isolated, perfused rat pancreas. Diabetes, 33(10): 929-936.
Wilson RL (1998). Free radicals and tissue damage, mechanistic evidence from Radiation studies. In: Biochemical Mechanisms of Liver Injury. Academic Press, New York pp. 12.