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Antidiabetic Activity of *Trapa natans* Fruit Peel Extract Against Streptozotocin Induced Diabetic Rats

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Abstract: *Trapa natans* L. (Lythraceae), commonly known as Water Chestnut in English, is an annual aquatic floating herb occurring throughout the Indian subcontinent and used traditionally for several medicinal purposes. The present study was aimed to evaluate antidiabetic activity of methanol extract of *T. napans* fruit peels (METN) in Wistar rats. The effect of METN on oral glucose tolerance and its effect on normoglycemic rats were studied. Diabetes mellitus was induced in rats by single intraperitonial injection of streptozotocin (STZ, 65 mg/kg body weight). Three days after STZ induction, the hyperglycemic rats were treated with METN orally at the dose of 100 and 200 mg/kg body weight daily for 15 days. Glibenclamide (0.5 mg/kg b.wt., orally) was used as reference drug. The fasting blood glucose levels were measured on every 5th day during the 15 days treatment. METN at the dose of 100 and 200 mg/kg orally significantly ($p < 0.001$) and dose dependently improved oral glucose tolerance, exhibited hypoglycaemic effect in normal rats and antidiabetic activity in STZ-induced diabetic rats by reducing and normalizing the elevated fasting blood glucose levels as compared to those of STZ control group. The present study concludes that *T. natans* fruit peel demonstrated promising antidiabetic activity in STZ-induced diabetic Wistar rats.

Key words: Antidiabetic · Hypoglycaemic · *Trapa Natans* · Streptozotocin · Glucose Tolerance

INTRODUCTION

Diabetes mellitus or simply diabetes is a chronic metabolic disorder of carbohydrate, lipid and protein metabolism characterized by hyperglycemia, glycosurea, hyperlipemia, negative nitrogen balance and sometimes ketonemia due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action. Diabetes is still not completely curable by the present antidiabetic drugs. Insulin therapy is the only satisfactory approach in diabetes mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment [1]. Treatment of Type 2 diabetes mellitus patients with oral hypoglycemic agents like sulphonylureas and biguanides is always associated with several adverse effects [2]. Therefore, herbal drugs are gradually gaining popularity in the treatment of diabetes mellitus. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Trapa natans L. (Lythraceae), commonly known as Water Chestnut in English, *Paniphal* in Bengali is an annual aquatic floating herb occurring in ponds and lakes throughout the Indian subcontinent [3]. It is commercially cultivated across different parts of India for its consumable seasonal fruits. Traditionally the plant has been used in India for several important medicinal purposes. It has been used as nutritive, appetizer, astringent, diuretic, aphrodisiac, cooling, antidiarrhoeal and tonic, it is also useful in lumbago, sore throat, bilious affections, bronchitis, fatigues and inflammation. Its fruits are also used in making liniments for the cure of rheumatism, sores and sunburn. Its stem is used in the form of juice in eye disorders [3-5]. The dried kernels of its fruits are recommended for use in bleeding disorders, threatened abortion, dysuria, polyuria and oedema [6]. Previous researchers have reported analgesic and psycopharmacological activities of its roots [7, 8], antibacterial and antifungal activity of its fruit peel [9, 10]. Despite several important traditional medicinal usage the reports on the experimental pharmacological studies on

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this plant are comparatively scanty. Present study was therefore aimed to investigate the possible antidiabetic effects of methanol extract of *T. napans* fruit peel (METN) against streptozotocin (STZ)-induced diabetic Wistar albino rats.

MATERIALS AND METHODS

Plant Material: The mature fruits of *Trapa natans* L. (Lythraceae) were collected during September-October 2010 from Nadia, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/I-I/(81)/ 2010/Tech.II/350] was maintained in our research laboratory for future reference. The peels from the fruits were removed by hand and the peels were shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40 and stored in an airtight container for use in the study.

Drugs and Chemicals: Streptozotocin (STZ) from SISCO Research Laboratory, Mumbai, India; Glibenclamide from Hoechst, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially.

Preparation of Extract: The powdered plant material (450 g) was macerated at room temperature (24-26°C) with methanol (750 ml) for 4 days with occasional shaking, followed by re-maceration with the same solvent similarly for 3 days. The macerates were combined, filtered and evaporated to dryness *in vacuo* (at 35°C and 0.8 MPa) in a Buchi evaporator, R-114. The dry extract (METN, yield: 6.35 % w/w) was kept in a vacuum desiccator until use. Preliminary phytochemical analysis performed on METN revealed the presence of true alkaloids, triterpenoids, steroids, polyphenols and carbohydrates [11].

Experimental Animals: Adult male Wistar albino rats weighing 170-200 g, procured from registered breeders (Rita Ghosh & Co., Kolkata, India) and were housed in a clean polypropylene cage with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 10 days prior to experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Acute Toxicity: The acute oral toxicity of METN in male Swiss albino mice was studied as per reported method [12]. METN was given to four groups (n = 6) of animals at 100, 1000, 2000 and 3500 mg/kg body weight (b.w.) *per os* (p.o.). The treated animals were kept under observation for 3 days, for mortality and general behaviour. No death was observed till the end of the study.

Effect on Oral Glucose Tolerance in Rats: The oral glucose tolerance test (OGTT) was performed in overnight fasted normal rats. Rats were divided into three groups (n = 6). Group I served as normal control and received distilled water (5 ml/kg b.w., p.o.) and groups II and III, received METN at the doses of 100 and 200 mg/kg b.w., p.o., respectively. After these treatments all groups received glucose (5 g/kg b.w.) orally. Blood was withdrawn from the tail vein just prior to and 30, 60, 120 and 240 min after the oral glucose administration [13]. Blood glucose levels were measured using single touch glucometer (Accu-check, Roche Diagnostics, USA).

Oral Hypoglycaemic Activity in Normal Rats: The rats used for the study were fasted for twelve hours. The animals were classified into four groups (n = 6). Group I served as normal saline control (5 ml/kg b.w. p. o.); Groups II and III were treated with METN at dose levels 100 and 200 mg/kg b.w. p.o. Group IV was treated with glibenclamide (0.5 mg kg^{-1} p. o.). Blood samples were collected from the tail tip at 0 (before oral administration) and 2 hours 30 minutes after the treatments. Blood glucose levels were measured by using single touch glucometer (Accu-check).

Induction of Diabetes: Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of streptozotocin (65 mg/kg body weight) [14]. After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level ≥ 225 mg/dl were used for the present investigation [15].

Treatment of Diabetic Rats and Estimation of Blood Glucose Level: The rats were divided into five groups (n = 6). Except group I which served as normal non-diabetic control all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III and IV, received METN (100 and 200 mg/kg b.w., p.o., respectively) and group V received reference drug glibenclamide (0.5 mg/kg b.w., p.o.) daily for 15 days. Fasting blood glucose (FBG) was measured on 0, 5^{th}, 10^{th} and 15^{th} day by using a one touch glucometer (Accu-check®) [16].

Body Weight: The body weights of rats of each group were measured on 1st, 7th and 15th days of treatment.

Statistical Analysis: The experimental data were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test of significance. *P* values of < 0.01 were considered as statistically significant.

RESULTS

The METN was found to be safe up to the dose of 3500 mg/kg body wt. in Swiss mice when administered orally.

Glucose loading to normal rats (OGTT) increased serum glucose levels from 62.78 ± 2.4 to 185.54 ± 4.5 at 60 min and returned to normal at 240 min. METN administration improved glucose tolerance significantly (p<0.001) in a concentration dependent manner at 60 min (Table 1). The effect of METN on glucose tolerance remained statistically significant ($p < 0.001$) at 120th min for its higher dose (200 mg/kg b.wt.).

The effects of METN in lowering of blood sugar levels of normal rats are summarized in Table 2. The MECL treated groups show significant ($p < 0.001$) reduction in the blood sugar levels as compared to the reference drug in a dose dependant manner.

The fasting blood glucose levels of normal, diabetic and treated rats are summarized in Table 3. STZ at the dose of 65 mg/kg produced marked hyperglycemia as evident from significant ($p < 0.001$) elevation in FBG level in STZ control group as compared to normal control group. Administration of METN in STZ-induced diabetic rats at the doses of 100 and 200 mg/kg produced significant (p<0.001) and dose dependent fall in blood glucose levels when compared with the STZ-control group. The FBG reducing effect by METN at the dose of 200 mg/kg was found to be comparable to that of the reference drug glibenclamide (0.5 mg/kg).

Vehicle control treated animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 15 days (Table 4). Streptozotocin caused body weight reduction, which was significantly reversed by METN after 7 days of treatment.

Table 1: Effects METN on oral glucose tolerance in rats

		Blood glucose concentration (mg/dl)				
Group	Dose	0 min	30 min	60 min	120 min	240 min
I. (Distilled water)	5 ml/kg p.o.	62.78±2.4	110.33±4.3	185.54±4.5	128.87±3.3	70.46±2.7
II. (METN)	100 mg/kg	60.51±3.7	109.08±2.5	164.83±2.3*	104.04±3.8	64.75±4.4
III. (METN)	200 mg/kg	61.66±4.0	91.15±4.3	107.64±3.1*	76.16±2.7¶	67.23±3.4

Data are expressed as mean ± SEM (n = 6); *$p < 0.001$, compared to normal control at 60 min and ¶$p < 0.001$ compared to normal control at 120 min

Table 2: Hypoglycemic activity of METN in normal rats

		Blood glucose level mg/dl	
Group	Dose	0th hour	2 hour 30 min
I. (Normal saline)	5 ml/kg (p.o.)	78.62±4.3	77.86±1.5
II. (METN)	100 mg/kg (p.o.)	95.1±1.2*	86.7±2.3*
III. (METN)	200 mg/kg (p.o.)	96.3±1.1*	64.7±4.2*
IV. (Glibenclamide)	0.5 mg /kg (p.o.)	81.5±1.4*	76.83±2.4*

Values are expressed as mean ± SEM (n = 6); *$p < 0.001$ compared with saline control group

Table 3: Effects of METN on fasting blood glucose levels in STZ-induced diabetic rats

		Fasting blood glucose level (mg/dl)			
Group	Dose	Day 0	Day 5	Day 10	Day 15
I (Normal saline)	5 ml/kg	76.85±2.1	75.32±4.2	74.92±3.6	74.72±2.9
II (STZ)	65 mg/kg	278.54±9.2*	280.75±10.5*	285.73±11.4*	292.72±9.8*
III (STZ+METN)	100 mg/kg	265.36±16.5	110.65±7.8**	91.45±1.6**	87.73±1.8**
IV (STZ+METN)	200 mg/kg	261.76±9.9	90.59±5.4**	82.58±3.2**	73.91±2.9**
V (STZ+Gliben.)	0.5 mg/kg	278.53±17.3	95.45±3.8**	81.69±1.5**	71.61±5.4**

Data are expressed as mean ± SEM (n = 6); *$p < 0.001$ compared with normal saline control and **$p < 0.001$ compared with STZ control group. Gliben: Glibenclamide. STZ: Streptozotocin

Table 4: Effects of METN on body weight in STZ-induced diabetic rats

		Mean body weight (g)		
Group	Dose	Day 1	Day 7	Day 15
I (Normal saline)	5 ml/kg	179.42±7.5	180.04±5.2	180.44±5.5
II (STZ)	65 mg/kg	180.50±8.5	158.54±4.5*	137.33±5.8*
III (STZ+METN)	100 mg/kg	171.50±5.1	164.16±5.3	147.72±2.9¶
IV (STZ+METN)	200 mg/kg	185.16±8.8	172.83±6.3¶	159.84±1.8**
V (STZ+Gliben.)	0.5 mg/kg	184.33±9.5	175.50±6.4**	164.76±3.3**

Data are expressed as mean ± SEM ($n = 6$); *$p < 0.001$ compared with normal saline control on corresponding day; **$p < 0.001$ and ¶$p < 0.01$ compared to STZ control group on corresponding day. Gliben: Glibenclamide. STZ: Streptozotocin

DISCUSSION

The present work was aimed to study the antidiabetic activity of methanol extract of *T. natans* fruit peel (METN) in STZ-induced diabetic rats. The results of this study revealed that METN at the doses of 100 and 200 mg/kg body weight orally, dose dependently demonstrated effective hypoglycemic and antihyperglycemic activity in normoglycemic, glucose overloaded as well as STZ induced diabetic rats; and restored body weight towards normal.

Streptozotocin (STZ) is an antibiotic obtained from *Streptomyces achromogenes*. STZ enters the pancreatic β cells via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA) leading to pancreatic damage. Its toxicity depends upon the potent alkylating properties combined with the synergistic action of nitric oxide and reactive oxygen species that continue to DNA fragmentation. As a result of STZ action, pancreatic β cells are destroyed by necrosis [17]. STZ is not only damaging to the pancreatic β cells but also to hepatocytes, nephrons and cardiomyocytes [18].

In the present study, hyperglycemia was observed in rats after 3 days of STZ-induction. Treatment with METN in STZ-induced diabetic rats, dose dependently started reducing fasting blood glucose levels after 5 days and made them completely normoglycemic after 15 days. The antidiabetic effect of METN at 200 mg/kg dose was found to be comparable to that the effect exerted by the reference drug, glibenclamide at the dose of 0.5 mg/kg.

Induction of diabetes with STZ had been associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins [19]. Diabetic rats treated with the METN showed significant improvement in body weight as compared to the STZ control animals; hence METN exhibited marked effect in controlling the loss of body weights of diabetic rats. The results of the present study are in agreement with those of previous workers [20].

Preliminary phytochemical studies showed the presence of alkaloids, triterpenes, steroids, polyphenols and carbohydrates in METN. Among them polyphenolics are the most reported phtoconstituents showing a wide range of pharmacological effects including antidiabetic activity [21, 22]. The presence of polyphenols or other putative constituents may be responsible for the promising antidiabetic activity of METN.

In the present investigation, oral administration of METN to glucose overloaded rats exhibited significantly improved oral glucose tolerance, normoglycemic rats showed marked hypoglycaemic effect and on 15 days continuous treatment STZ-induced diabetic rats demonstrated prominent reduction and normalization of elevated blood sugar levels i.e. antihyperglycemic or antidiabetic effect, comparing to respective control rats. Therefore, it can be concluded that the methanol extract of *Trapa napans* fruit peel possessed remarkably effective antidiabetic potential against streptozotocin-induced diabetes in Wistar rats. The findings of the present study can substantiate the traditional uses of this plant in India. Furthermore, outcome of the present study is encouraging enough to warrant further studies on this pant in pursuit of a new oral hypoglycaemic agent.

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REFERENCES

1. Piedrola, G., E. Novo, F. Escober and R. Garcia-Robles, 2001. White blood cell count and insulin resistance in patients with coronary artery disease. Ann. Endocrinol., 62: 7-10.

2. Weidmann, P., L.M. Boehlen and M. Courten, 1993. Pathogenesis and treatment of hypertension associated with diabetes mellitus. American Heart J., 125: 1498-1513.
3. Anonymous, 2003. The Ayurvedic Pharmacopoeia of India. 1st ed. Part 1, Vol. IV. New Delhi, India: Government of India, Ministry of Health and Family Welfare, pp: 452.
4. Anonymous, 1976. The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products. Vol. X. New Delhi, India: Council of Scientific and Industrial Research, pp: 197.
5. Anjaria, J., M. Parabia and S. Dwivedi, 2002. Ethnoveterinary Heritage Indian Ethnoveterinary Medicine - an Overview. Ahmedabad, India: Pathik Enterprise, pp: 223.
6. Khare, C.P., 2007. Indian Medicinal Plants: an Illustrated Dictionary. Berlin, Heidelberg: Springer, pp: 667.
7. Agrahari, A.K., M. Khaliquzzama and S.K. Panda, 2010. Evaluation of analgesic activity of methanolic extract of *Trapa natans* L. var. *Bispinosa* Roxb. roots. J. Curr. Pharm. Res., 1: 8-11.
8. Panda, S.K., K. Khaliquzzama and A.K. Agrahari, 2010. Evaluation of psycopharmacological activity of methanolic extract of *Trapa natans* L. var. *Bispinosa* Roxb. roots. Adv. Pharmacol. Toxicol., 11: 71-76.
9. Parekh, J. and S. Chanda, 2007. *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. African J. Biotechnol., 6: 766-770.
10. Parekh, J. and S. Chanda, 2008. *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. African J. Biotechnol., 7: 4349-4353.
11. Harborne, J.B., 1998. Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis. New Delhi, India: Springer (India) Pvt. Ltd.
12. Anonymous, 2008. OECD Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Paris: OECD Publishing.
13. Soltani, N., M. Keshavarz and A.R. Dehpour, 2007. Effect of oral magnesium sulfate administration on blood pressure and lipid profile in streptozotocin diabetic rat. Eur. J. Pharmacol., 560: 201-205.
14. Ravi, K., B. Ramachandran and S. Subramanian, 2004. Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. Biol. Pharm. Bull., 27: 1212-1217.
15. Ewart, R.B.L., S. Kornfeld and D.M. Kipnis, 1975. Effect of lectins on hormone release from isolated rat islets of langerhans. Diabetes, 24: 705-714.
16. Biswas, M., B. Kar, S. Bhattacharya, R.B.S. Kumar, A.K. Ghosh and P.K. Haldar, 2011. Antihyperglycemic activity and antioxidant role of *Terminalia arjuna* leaf in streptozotocin-induced diabetic rats. Pharm. Biol., 49: 335-340.
17. Mythili, M.D., R. Vyas, G. Akila and S. Gunasekharan, 2004. Effect of streptozotocin on the ultrstructure of rat pancreatic islets. Microscopy Res. Tech., 63: 274-281.
18. Frode, T.S. and Y.S. Medeiros, 2008. Animal models to test drugs with potential antidiabetic activity. J. Ethnopharmacol., 115: 173-183.
19. Swanston-Flat, S.K., C. Day, C.J. Bailey and P.R. Flatt, 1990. Traditional plant treatments for diabetes: Studies in normal and streptozotocin diabetic mice. Diabetol., 33: 462-464.
20. Chakraborty, U. and H. Das, 2010. Antidiabetic and antioxidant activities of *Cinnamomum tamala* leaf extracts in STZ-treated diabetic rats. Global J. Biotechnol. Biochem., 5: 12-18.
21. Saraf, S., M.S. Ashawat and S. Saraf, 2007. Flavonoids: a nutritional protection against oxidative and UV induced cellular damages. Phcog. Rev., 1: 30-40.
22. Bhattacharya, S., 2011. Are we in the polyphenols era? Phcog. Res., 3: 147.