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Preventive effects of *Trichosanthes dioica* in STZ-Nicotinamide induced type (II) diabetes

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ABSTRACT

The present study was aimed to scientifically validate the use of *Trichosanthes dioica* fruits in prevention of diabetes by evaluating their antihyperglycemic and antilipidemic potential. The effect was observed on fasting blood glucose (FBG) levels of STZ-nicotinamide (45, 110 mg/kg BW, respectively, i.p) induced diabetic rats after seven days daily administration of chloroform extract rich in moderately polar components (250 mg/Kg BW, p.o). FBG was significantly reduced ($p < 0.05$ %), when estimated on seventh day of treatment with fruit extract compared to diabetic control. Pre-treatment with fruit extract for seven days also normalized the blood glucose to basal level by the third hour of oral glucose challenge (2g/Kg BW, p.o) in diabetic rats. Moreover, the treatment with fruit extract resulted in significant reduction in the levels of total cholesterol and triglycerides comparable to metformin. Results of the present study showed that chloroform extract of *T. dioica* possesses antihyperglycemic properties and beneficial effects on diabetic hyperlipidemia. All these effects could be due to the bioactive components revealed in the *T. dioica* fruits such as cucurbitacins, which could justify their ethnomedical use.

Keywords: Diabetes, *Trichosanthes dioica*, STZ-nicotinamide, cucurbitacins, metformin

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INTRODUCTION

The richness of nature's flora has been complaisant to scientific community in search of answers on most abstruse queries. The verdant flora full of sustenance can prove to be a major breakthrough in many lifestyle associated disorders. The importance of lifestyle in both primary and secondary prevention of type II diabetes has been well documented[1]. Reports bolstering the hypothesis that type II diabetes is preventable include clinical trials of diet, exercise, or both in persons at high risk for the disease[2,3]. Phytotherapeutic agents including dietary supplements with their multitarget modes of action and generally favorable safety profiles are an interesting approach in integrative diabetes management including diabetes prevention and glycemic control. Dietary supplements in edible fruits and vegetables of which phytochemicals play an important part help in reducing the risk of type II diabetes mellitus by modifying imbalanced lipid and glucose homeostasis[4,5]. In our effort to scientifically validate the beneficial effect of fruits of *Trichosanthes dioica*, we utilized STZ-nicotinamide (NA) induced diabetes model. The low dose STZ-NA induced diabetes model is ideal for the screening of dietary herbs and parts thereof against moderate hyperglycemia[6,7].

Trichosanthes dioica Roxb. (Cucurbitaceae), fruits referred as pointed gourd are also known by the common name of *Parwal* (Hindi)[8]. In India, it is cultivated as a vegetable mainly in the plains of northern to eastern regions. It is one of the most nutritive cucurbit vegetable and holds coveted position in the Indian diet. The plant has been known as a rich source of vitamins, proteins, pepetides, tannins and minerals[9]. Reports on phytochemical composition of its fruits and seeds reveal the presence of phytosterols[10] and flavonoids[11]. Its seeds have been reported to contain a carbohydrate binding protein named lectin which is homologous to Type-II ribosome inhibitory proteins (Type-II RIP) that possess significant role in controlling and managing diabetes by altering carbohydrate metabolism as well as insulin release[12]. Pharmacologically the plant has been investigated for its hypoglycemic[13,14], wound healing[15] and immuno-modulatory[16] activities. The focus of our study was to screen the chloroform extract rich in moderately polar components from the fruits of *T. dioica* in type II diabetes mellitus by evaluating its anti-hyperglycemic and anti-lipidemic potential in STZ-NA induced diabetic rats.

MATERIALS AND METHODS

Chemicals

STZ was obtained from Sigma-Aldrich chemicals (St Louis, MO, USA). NA was obtained from Central Drug House (P) Ltd., New Delhi (India) and metformin was obtained from Franco-

Indian Pharmaceuticals Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Raw material

Fresh fruits of *T. dioica* were obtained from local vegetable market in New Delhi, India. The fruit samples were authenticated by Dr. H.B. Singh, Taxanomist, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. The reference number of authentication letter received for the plant samples is NISCAIR/RHMD/Consult/-2011-12/1746. Voucher specimens have been deposited in NISCAIR, Delhi. Fruits were sliced, shade dried and grounded to yield the coarse powder.

Preparation of extract

Dried fruit powder was extracted with hot chloroform for 6 h. Chloroform extract was partitioned with hexane to exude out the non-polar components. The chloroform fractions were pooled together, dried and the residue was used for biological investigation.

Animals

Male albino Wistar rats (3 months old, 150-200 g BW), raised in the animal house of Jamia Hamdard, New Delhi, were used. They were housed in the colony cages (5 rats per cage), at ambient temperature and allowed free access to water. Animals were fed with standard rodent diet. Experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Induction of diabetes

Diabetes was induced by STZ-NA combination in overnight fasted rats. STZ was dissolved in 50 mM citric acid buffer (pH 4.5) for intraperitonial administration (45 mg/kg BW). NA was dissolved in physiological saline and intraperitoneally administered (110 mg/kg BW), 20 min before STZ administration. One week later, characteristics of diabetic rats were investigated. Mildly diabetic rats with fasting blood glucose (FBG) levels between 150-200 mg/dl were selected for further study.

Sub-chronic antidiabetic activity

The rats were randomized into three groups comprising of five animals each. Diabetic control rats, received orally 1 ml/kg BW vehicle (0.3 % CMC in distilled water). Metformin treated diabetic rats, received metformin (15 mg/kg BW p.o) in vehicle 1 week after STZ-NA treatment and continued for seven days. *T. dioica* treated diabetic rats, received chloroform extracts of *T. dioica* fruits (250 mg/kg BW, p.o.) in vehicle, 1 week after STZ-NA treatment and continued for seven days. Blood was withdrawn from tail vein after 0, 3 and 7 days and FBG was measured.

Oral glucose tolerance test

Oral glucose tolerance test was performed after overnight fasting of rats on seventh day of treatment. Vehicle (0.3% CMC in distilled water), extract of *T. dioica* fruits in vehicle (250 mg/kg BW, p.o), and metformin (15 mg/kg BW) were administered orally to respective groups (n = 5). Glucose (2g/kg BW, p.o) was fed to all groups after 60 minutes of treatment. Blood was withdrawn from the tail vein at 0, 30, 60, 90, 120 and 180 min and blood glucose was measured.

Estimation of blood glucose

Blood glucose concentration was estimated using an Accu-Check active (Roche Diagnostic GmbH, Germany), based on the glucose oxidase method.

2.9. Biochemical estimations

At the end of the experimental period, the overnight fasted animals were sacrificed. Blood samples were collected by orbital sinus puncture under mild ether anaesthesia in Eppendroff's tubes (1 ml) and the serum obtained by centrifugation (3000 x g for 10 min) was stored at -20 °C until analysis. The serum was analysed for biochemical parameters as per the methods specified by the manufacturers of diagnostic kits.

Statistical analysis

Observed data are represented as mean ± S.D. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparison. A difference in the mean values of $p < 0.05$ was considered significant statistically.

RESULTS AND DISCUSSION

STZ is well known diabetogenic agent exerting cytotoxic action on pancreatic β-cells, whereas NA partially protects these cells thereby mimicking a type II diabetic response[17,18]. It successfully induced type II diabetes marked by high FBG of the experimental animals. The results of sub-chronic treatment for seven days with chloroform extract of fruits of *T. dioica* on the blood glucose of diabetic rats are shown in Table 1. Diabetic control showed a consistent increase in FBG when estimated over a period of a week. *T. dioica* fruit extract (250 mg/kg BW, p.o) given daily to diabetic animals for a week showed a gradual decrease in FBG and was significantly reduced to 155.4 ± 8.55 on the seventh day of the treatment ($p < 0.05\%$ *vs* diabetic control). The treatment with metformin (15 mg/kg BW, p.o) daily for seven days significantly decreased FBG ($p < 0.01\%$ *vs* diabetic control) when estimated on the seventh day of the treatment. It is noteworthy to mention that the fruit extract not only prevented the progression of

hyperglycemia but also reduced the elevated FBG of diabetic rats comparable to metformin. However, there was a steep and more significant decrease in FBG with metformin. Figure 1 represents the effect of oral glucose challenge (2g/kg BM, p.o) in diabetic rats pre-treated with *T. dioica* fruit extract (250 mg/kg BW, p.o) and metformin (15 mg/Kg BW, p.o) daily for seven days separately.

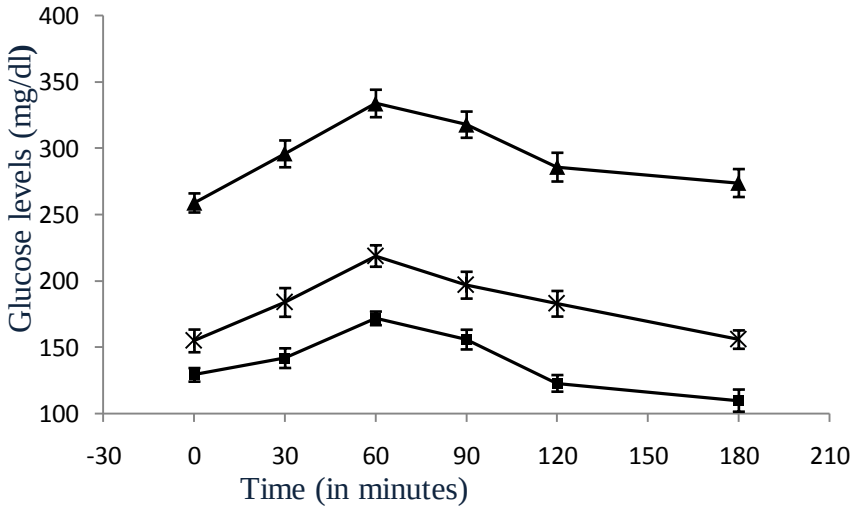

Figure 1: Effect on the glucose tolerance in mild diabetic rats after oral glucose overload (2g/Kg BW). (▲) Diabetic control (1 mg/Kg BW of 0.3 % CMC in distilled water, p.o); (■) pre-treated with metformin (15 mg/kg BW, p.o) for seven days; (×) pre-treated with chloroform extract of *Trichosanthes dioica* fruits (250 mg/kg BW) for seven days. Values are expressed as of mean ± S.D.; n = 5 in each group.

Table 1. Effect of chloroform extract of fruits of *Trichosanthes dioica* on fasting blood glucose (FBG) in diabetic rats

Treatment	Dose	Fasting Blood Glucose FBG (mg/dl)		
		T0	T3	T7
Vehicle	1 mg/kg BW of 0.3% CMC in distilled water, p.o	176.2 ± 9.14	209.4 ± 11.90	259.2 ± 7.19
Metformin	15 mg/kg BW, p.o	161.8 ± 6.01	150.6 ± 6.69	129.4 ± 5.12**
T. dioica fruit extract	250 mg/kg BW, p.o	171.8 ± 9.01	166.0 ± 7.10	155.4 ± 8.55*

T0 = basal glucose level, T3 = FBG after 3 days of treatment, T7 = FBG after 7 days of treatment. Values are expressed as mean ± S.D.; n = 5 in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, significant compared to diabetic control

The fruit extract blunted the effect of glucose challenge as signified by about 60 mg/dl increase in peak blood glucose level after 1 hour of the glucose overload, compared to about 75 mg/dl increment in diabetic control animals at the same time level. Further, both the fruit extract and

metformin normalized blood glucose to basal level by the third hour of glucose challenge. Figure 2 presents an easy visual comparison of the effect of fruit extract and metformin with respect to area under the curve (AUC) in glucose intake induced hyperglyceamia. It is clear that the fruit extract and metformin produced a significant reduction in AUC compared to diabetic control ($p < 0.01\%$). As expected in STZ-NA induced diabetic rats, elevation in cholesterol and triglyceride levels was observed. The treatment with fruit extract resulted in significant reduction in the levels of cholesterol and triglyceride comparable to metformin (Table 2). Thus the reduction of blood glucose levels of animals by *T. dioica* fruit extract is also associated with a reduction of total cholesterol and triglyceride levels. The study implies that the inclusion of *T. dioica* fruits in food can be useful for newly diagnosed diabetic patients or high risk population for glycemic control. The phytochemical investigation carried out in past with *T. dioica* reports a number of moderately polar phytosterols especially cucurbitacins from its fruits and seeds[10] that could be responsible for these pharmacological effects. An astute investigation with focus to isolate and characterize these leads as probable molecular targets is required for more prolific outcomes. There is a need of expound research in this area, with a focus to examine the mechanism involved in the anti-diabetic effects of *T. dioica* fruits and their use as dietary supplement in the control of diabetes mellitus.

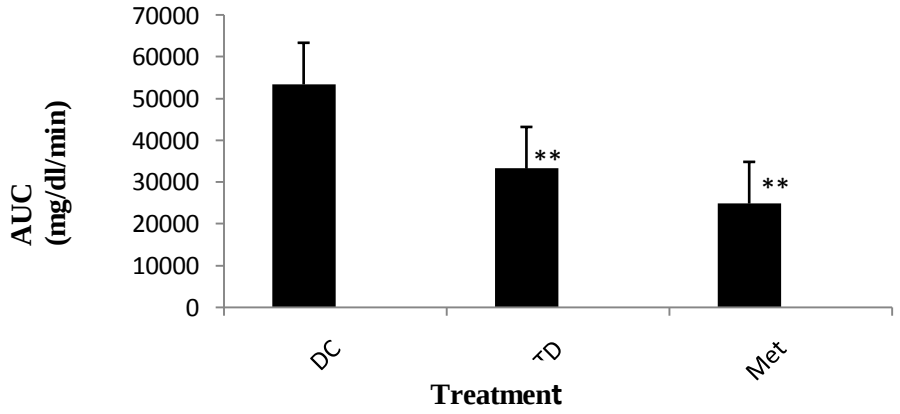

Figure 2: Representation of area under the curve (AUC) of blood glucose levels after OGTT (2 g/Kg BW) in STZ-NA induced mild diabetic rats. (DC) Diabetic control (1 mg/Kg BW of 0.3 % CMC in distilled water, p.o); (MF) pre-treated with metformin (15 mg/kg BW, p.o) for seven days; (TD) pre-treated with chloroform extract of *Trichosanthes dioica* fruits (250 mg/kg BW) for seven days. Values are expressed as of mean ± S.D.; n = 5 in each group. **p < 0.01 *vs* DC.

Table 2. Effect of chloroform extract of fruits of *Trichosanthes dioica* on total cholesterol and triglyceride level in diabetic rats after seven days of treatment

Treatment	Dose	Total cholesterol (mg/dl)	Triglyceride level (mg/dl)
Vehicle	1 mg/kg BW of 0.3% CMC in distilled water, p.o	153.2 ± 9.88	141.2 ± 8.31
Metformin	15 mg/kg BW, p.o	90.8 ± 3.34[b]	89.2 ± 10.52[*]
T. dioica fruit extract	250 mg/kg BW, p.o	106.8 ± 7.39[a]	103 ± 11.48[**]

Values are expressed as mean ± S.D.; n = 5 in each group. [*]$p < 0.01$, [**]$p < 0.05$, significant compared to diabetic control

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REFERENCES

1. Tuomilehto J, Lindstrom J, Eriksson JG. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Eng J Med 2001; 344:1343-50.
2. Knowler WC, Barrett-Connor E, Fowler SE. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin diabetes prevention program research group. N Eng J Med 2002; 346:393-403.
3. Kosaka K, Noda M, Kuzuya T. Prevention of type 2 diabetes by lifestyle intervention: a Japanese trial in IGT males. Diab Res Clin Pract 2005; 67:152-62.
4. Kiec-Wilk B, Malecki M, Gruca A, Dembinska-Kiec A. Dietary supplements and herbs in diabetes and its prevention. In: Watsan RR, Preedy ER, editors. Bioactive foods as dietary interventions for diabetes. USA: Elsevier Inc.; 2013, p.121-138.
5. Vennos C, Schwabl H, Bommeli C, 2013. Phytotherapeutics in treating diabetes. In: Watsan RR, Preedy ER, editors. Bioactive foods as dietary interventions for diabetes. USA: Elsevier Inc.; 2013, p.139-144.
6. Ananda PK, Kumarappan CT, Christudas S, Kalaichelvan VK. Effect of *Biophytum sensitivium* on streptozotocin and nicotinamide induced diabetic rats. Asian Pac J Biomed 2012; 2(1): 31-35.
7. Pierre W, Gildas AJH, Ulrich MC, Modeste W, Benoit NT, Albert K. Hypoglycemic and hypolipidemic effects of Bersama engleriana in nicotinamide/streptozotocin-induced type 2 diabetic rats. BMC complem Alterm M 2012; 12: 264.

8. Chakravarthy HM. Fascicles of flora of India-11 Cucurbitaceae, Calcutta: Botanical Survey of India, 1982, p. 136
9. Nadkarni KM. Nadkarni's Indian Materia Medica, 3rd ed. Mumbai: Popular Prakashan 1982, p. 1236-37.
10. Akihisa T, Shimizu N, Ghosh P, Thakur S, Rosentein FU, Tamura T, Matsumoto T. Sterols of Cucurbitaceae. Phytochem 1987; 26(6): 1693-700.
11. Rai PK, Shukla S, Mehta S, Rai NK, Rai AK, Watal G. Therapeutic phytoelemental profile of *Trichosanthes dioica*. Adv Mat Lett 2010; 1(3): 210-16.
12. Sultan NAM, Kenoth R, Swamy MJ. Purification, physicochemical characterization, saccharide specificity and chemical modification of Gal/GalNAc specific lecithin from the seeds of *Trichosanthes dioica*. Arch Biochem Biophy 2004; 432: 212-21.
13. Sharma G, Pant MC. Effect of feeding *Trichosanthes dioica* (Parwal) on blood glucose, serum triglyceride, phospholipid, cholesterol and high density lipo-protein-cholesterol levels in normal albino rabbits. Curr Sci 1988; 1085-7.
14. Rai PK, Jaiswal D, Rai DK, Sharma B, Watal G. Effect of water extract of *Trichosanthes dioica* fruits in STZ-induced diabetic rats. Indian J Clin Biochem 2008; 23(4): 387-90.
15. Shivhare Y, Singour PK, Patil UK, Pawar RS. Wound healing potential of methanolic extract of *Trichosanthes dioica* Roxb. (fruits) in rats. J Ethanopharmacol 2010; 127(3): 614-9.
16. Bhadoriyal SS, Mandoriya N. Immunomodulatory effect of *Trichosanthes dioica* Roxb. Asian Pac J Trop Biomed 2012; 2(2): 905-7.
17. Atsuo T, Akiko MY, Ryosuke N, Yuka S, Masayuki S. Hypoglycemic effects of antidiabetic drugs in streptozotocin-nicotinamide-induced mildly diabetic and streptozotocin-induced severely diabetic rats. Basic Clin Pharmacol Toxicol 2008; 103:560-68.
18. Tomasz S. Streptozotocin-nicotinamide-induced diabetes in the rat-characteristics of experimental model. Exp Biol Med 2012; 237: 481-90.