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Anti-diabetic effect of a novel N-Trisaccharide isolated from *Cucumis prophetarum* on streptozotocin–nicotinamide induced type 2 diabetic rats

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ABSTRACT

Ethnopharmacological relevance: Cucumis prophetarum (L.) is used in traditional Indian medicine for the treatment of inflammation related problems.
Aim of the study: The present investigation was designed to study the effect of N-Trisaccharide (a new compound isolated from the fruit of *C. prophetarum* (L.)) on hyperglycemia in streptozotocin (STZ)–nicotinamide (NA) induced type 2 diabetic rats.
Materials and methods: Different doses of N-Trisaccharide (25 and 50 mg/kg b.w.) were administered once daily for 28 days to STZ–NA induced diabetic rats. Plasma insulin and glycogen levels were measured. The activities of hexokinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase and glycogen phosphorylase were measured. Further, histological studies on pancreas were also carried out.
Results: The active compound at doses of 25 and 50 mg/kg b.w. given orally for 14 days showed 47.7% and 69.3% antihyperglycemic activity, respectively. Treatment at the same doses for 28 days provided complete protection against STZ–NA challenge (65 and 230 mg/kg b.w., respectively), intraperitoneally. N-Trisaccharide significantly ($p \leq 0.05$) increased the plasma insulin and liver glycogen levels in diabetic rats. The altered enzyme activities of carbohydrate metabolism in the liver and kidney of the diabetic rats were significantly ($p \leq 0.05$) improved. Additionally, N-Trisaccharide increased glycogen synthase and decreased glycogen phosphorylase activity in diabetic rats. Histological studies confirmed an increase in insulin level is due to stimulation of injured pancreatic β-cells.
Conclusion: The results of the study suggested that N-Trisaccharide possesses propitious effect on STZ–NA induced type 2 diabetes, indicating its usefulness in diabetes management.

Introduction

Diabetes is a leading cause of morbidity and mortality for the world's growing population. The International Diabetes Federation has predicted a worldwide increase from 8.3% to 9.9% by the year 2030, with China and India projected to have the largest number of diabetic cases (IDF 2012). Diabetes is a chronic condition characterized by major derangements in glucose metabolism and disturbances in fat and protein metabolism. Type 2 diabetes is a chronic and progressive illness that particularly targets 80% of the cases. It is one of the primary threats to human health due to increased prevalence and associated disabling complications. At present, the treatment mainly involves a sustained reduction in hyperglycemia using oral hypoglycemic agents besides injectable insulin. However, prominent side-effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side-effects, hence the demand has arisen for using a more benign drug (UK Prospective diabetes study group 1995 and Moller 2001).

A number of plants originating from different parts of the world possessing antidiabetic and related beneficial effects have been documented (Kavishankar et al. 2011). Such plants with potential medicinal property are known to stimulate insulin production, secretion, and, enhancement of glucose uptake by adipose or muscle tissues, inhibition of glucose absorption from intestine and glucose production from liver (Hui et al. 2009). Scientific data is lacking on safety and efficacy of these plants and their use in modern medical practice. WHO Expert Committee on diabetes recommends further investigation in this area.

Cucumis prophetarum (L.) (CP) commonly known as Globe cucumber or Wild cucumber is native to Asia and Africa belongs to *Cucurbitaceae* family, which is mostly a prostate or climbing,

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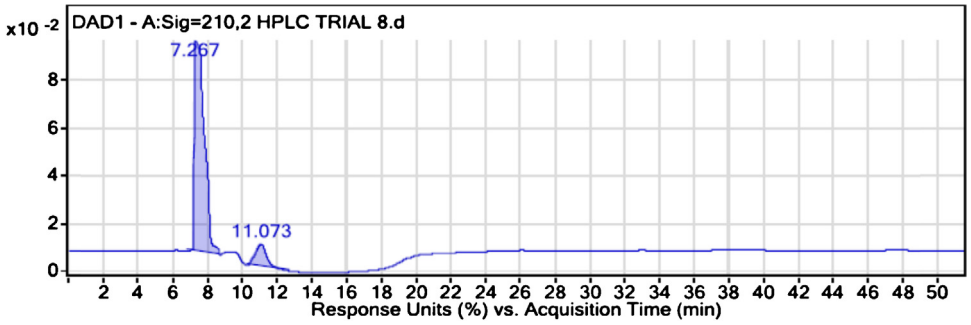

Fig. 1. HPLC of N-Trisaccharide.

monoecious herb. The fruits are ellipsoid, echinate and green with white strips. It is distributed geographically in the Western Ghats of Coorg region, Karnataka, India (Saldanha and Ramesh 1984). Earlier work in our laboratory has shown that, the newly isolated compound N-Trisaccharide, has significant *in vitro* antidiabetic and antioxidant activities (unpublished data). However, no studies on *in vivo* antidiabetic activity of this plant were previously reported. Hence, in the present study, efforts were made to evaluate the antidiabetic activity of active compound from aqueous extract of this fruit.

Materials and methods

Plant material

The fruits of *C. prophetarum* (L.) were collected during July and August 2011 from Western Ghats of Coorg region (Karnataka, India) and were authenticated by taxonomist. The herbarium with voucher no KU/BL/MK/105 has been deposited in Applied Botany Department, Kuvempu University, Shimoga, India.

Extract preparation

Fresh fruits were washed, cleaned, and air dried. Whole fruits were homogenized without water in a commercial blender. The fresh juice was filtered using muslin cloth and filtrate was centrifuge at 23,000 × *g* for 10 min, the supernatant was lyophilized and stored at −20 °C until use.

Isolation of active compound

The active compound was isolated *via* a novel one-step precipitation process. The compound was precipitated with the addition of methanol:water (4:1) to the crude extract, centrifuge at 8500 × *g* for 5 min to separate the supernatant layer. The white precipitate was washed twice with methanol and dried using lyophilizer. The purity of the compound was determined by HPLC-DAD experiment showing RT value of 7.26 min (Fig. 1). The structure was elucidated using NMR, 2D NMR, LC–MS/MS and IR data (Fig. 2).

HPLC and LC–MS/MS analysis

The active compound was analyzed using an Agilent HPLC series 6400 equipped with diode array detector (DAD). The separation was carried out in Zorbax C18 column. The column oven temperature was set at 40 °C with 10 μl of sample injection volume using mobile phase of ultra-pure water (A) (10%) containing 10% formic acid and acetonitrile (B) (90%). The flow rate was 0.5 ml/min with a linear gradient of 3 min run time. A triple quadrupole mass spectrometer equipped with an ESI interface and a Q-array-Octapole mass analyzer was used for LC–MS/MS analysis. Mass spectra were

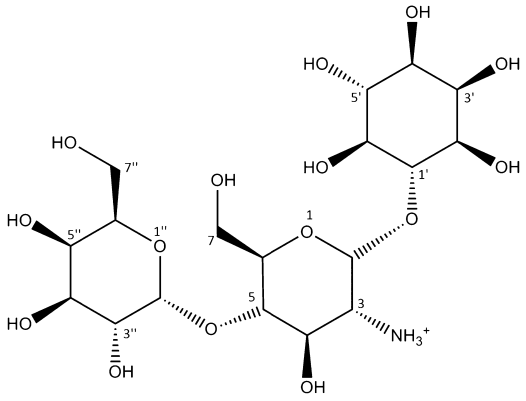

Fig. 2. Structure of N-Trisaccharide.

recorded in the range of *m*/*z* 0–1000. ^{13}C NMR (400 MH$_Z$ D_2O): 98.4 (C2), 54.8 (C3), 70.1 (C4), 78.3 (C5), 75.2 (C6), 60.7 (C7), 83.8 (C1′), 73.9 (C2′), 72.6 (C3′), 71.5 (C4′), 72.8 (C5′), 74.2 (C6′), 100.2 (C2″), 69.1 (C3″), 71.3 (C4″), 70.7 (C5″), 72.3 (C6″), 61.7 (C7″). ^{1}H NMR (400 MH$_Z$ D_2O): δ 5.3 (H, C2), 4.1 (H, C3), 7.0 (C-NH_3^+), 4.2 (H, C4), 3.4 (9H,S-OH,2′,3′,4′,5′,6′,3″,4″,5″), 3.02 (H, C5), 4.02 (H, C6), 3.7 (2H, dd, C7a, C7a), 3.5 (2H, dd, C7b, C7b), 2.75 (H, C1′), 3.3 (H, C2′), 3.23 (H, C3′), 3.23 (H, C4′), 3.23 (H, C5′), 3.57 (H, C6′), 5.03 (H, C2″), 3.5 (H, C3″), 3.25 (H, C4″), 3.10 (H, C5″), 3.5 (H, C6″); LC–MS/MS: *m*/*z* 504.19 for [M2+ + H]. The molecular formula of the compound is $C_{12}H_{33}O_{15}N_1$.

Experimental animals and induction of diabetes

Albino wistar rats of either sex, weighing 150–180 g were used. Animals were maintained at 22 ± 2 °C with 12 h light and dark cycle, fed on standard pellet diet supplied by Lipton India Ltd. Animals had free access to diet and water. All animal studies conducted were approved by the Institutional Animal Ethics Committee, University of Mysore (Approval No. UOM/IAEC/4/2012), Mysore, as stated by prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

After initial determination of 12 h fasting blood glucose levels (end tail puncture), development of experimental type 2 diabetes was achieved by intraperitoneal administration of Nicotinamide (NA) at 230 mg/kg b.w. dissolved in saline 15 min before Streptozotocin (STZ) at 65 mg/kg dissolved in 0.1 M citrated buffer (pH 4.5) immediately before use. Eight hours after STZ–NA administration, the rats were kept for next 24 h on 15% glucose solution bottles in their cages to prevent hypoglycemia (Masiello et al. 1998).