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Original article

Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus

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ABSTRACT

Background: Plant-derived polysaccharides such as galactomannans (GAL) have very interesting and useful applications in the biomedical and biopharmaceutical field. GAL from fenugreek (*Trigonella foenum graecum* L.) seeds has been shown to have promising but inconsistent anti-hyperglycemic activity due to variable composition.
Aim: We have isolated and characterized low molecular weight GAL fraction from fenugreek seeds (LMWGAL-TF) and evaluated its anti-hyperglycemic potential.
Materials and methods: LMWGAL-TF was isolated, well characterized (HPLC and LC-MS) and evaluated for anti-hyperglycemic activity after acute and subacute oral treatment in doses of 50, 100 and 200 mg/kg in alloxan-induced hyperglycemia in mice.
Results: The isolated LMWGAL-TF was of 91.5% purity with low molecular weight. LMWGAL-TF showed dose-dependent anti-hyperglycemic activity against alloxan-induced hyperglycemia without body weight gain, and protected mice pancreas from alloxan-induced histological changes.
Conclusions: LMWGAL-TF showed promising and dose-dependent anti-hyperglycemic effects in animal model of DM.

1. Introduction

The chronic metabolic disorder diabetes mellitus (DM) is a fast-growing global problem with huge social, health, and economic consequences. DM is a major endocrine metabolic disorder characterized by increased blood glucose, due to insulin production deficiency by pancreatic β-cells or by the ineffectiveness of the endogenous insulin [1]. It is estimated that globally 285 million people (approximately 6.4% of the adult population) are suffering from this disease and the number is estimated to increase to 430 million in the absence of better control or cure [2].

Around 90% of all cases are associated with type 2 DM (noninsulin-dependent diabetes mellitus, NIDDM). Type 2 DM (T2DM) mainly associated with dysfunction of pancreatic β-cell as well as insulin resistance in skeletal muscle, liver and fat cells leading to hyperglycemia and complications such as neuropathy, nephropathy, retinopathy and cardiomyopathy [3]. Therefore, effective control over the elevated blood glucose (glycemic control) in diabetic patients is the main objective for reversing DM, preventing complications and improving quality of life [4,5]. Beside glycemic control, continuous medical care along with patient self-management is required for prevention of acute as well as long-term complications.

An increased prevalence of DM and its related complications lead many researchers to for search hypoglycemic agents with better efficacy. Current pharmacotherapy for the management of type I DM includes exogenous insulin whereas agents like biguanides, thiazolidinediones, sulphonylureas, D-phenylalanine derivatives, meglitinides, α-glucosidase inhibitors, etc. are used for T2DM [6]. However, the unwanted side effects of oral hypoglycemic agents limit their utility.

In recent years, many new compounds from natural origin demonstrated the potential for treatment of DM and its complications. Fenugreek (*Trigonella foenum graecum* L., family: Fabaceae) seeds are extensively used for treatment of metabolic disorders like DM and hypercholesterolemia [7,8]. The antidiabetic potential of the defatted fraction of fenugreek seeds has been proven in animal models [9,10] and patients [11–15] of DM.

The endosperm of the fenugreek seed is a rich source of fiber (20%) and gum (32.4%) [12,15] which include galactomannans (GAL). GAL is a group of storage polysaccharides from plant seeds that reserve energy for germination in the endosperm [16]. GAL are heterogeneous polysaccharides composed by a

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β-(1-4)-D-mannane backbone with a single D-galactose branch linked α-(1-6). They differ from each other by the mannose/galactose (M/G) ratio. Fenugreek GAL is a group of polysaccharides composed of mannose as backbone with galactose as side groups in the ratio of 1:1. Fenugreek GAL has the highest galactose (~48%; M/G, 1.02:1) in its molecule, and its linear mannan backbone has α, 1→6 linked single galactose grafts on nearly all the mannose groups of the main chain.

Fenugreek GAL has been shown to reduce postprandial blood glucose and improve insulin sensitivity in both non-diabetic [17–19] and diabetic subjects [19–21]. Feeding guar-GAL fibre has also been shown to reduce both total and LDL cholesterol levels in healthy animals [22] and type 2 diabetic (T2D) human subjects [23,24]. However, there are many inconsistencies among the results of the studies of anti-hyperglycemic activities of GAL or GAL-containing diet. For example, many studies did not show an improvement in glycemic control in T2D subjects [25,26] or devoid of direct effect on cholesterol absorption [27].

The chemical structure and composition of GAL was suggested to be the major reason for the discrepancies in glycemic responses to GAL [28]. Interestingly, the chemical structure has influenced the magnitude of the effect such as presence of small amount of sugars other than mannose and galactose [27]. Variation in genotype and environmental conditions under which plants are cultivated further contributes to inconsistency in structure, biochemical composition and subsequently biological activity profile [29].

The composition and proportion of water-soluble and water-insoluble fraction of GAL in evaluated compounds is also a major reason of inconstancies found in reported activity profile of fenugreek GAL. The less efficient methods of purification, contamination and the different analytical method employed for analysis are primary reasons for variable compositions of GAL [16]. Fenugreek GAL is extracted from the endosperm or ground whole seed with water or dilute alkali, and the yield varies from 13.6 to 38%, depending on the variety/cultivar and extraction methods [16]. In some commercial products, the GAL compositions from fenugreek seeds are claimed to be 80% GAL (with unknown proportion of water-soluble and -insoluble fraction).

Interestingly, only water-soluble fractions of dietary fiber have been convincingly shown to lower plasma cholesterol concentrations [27,30]. However, purification/standardization and anti-hyperglycemic activity evaluation of water-soluble low molecular weight fraction from GAL from fenugreek seeds (LMWGAL-TF) is not yet reported. Therefore, present study was undertaken with an objective of isolation and characterization of LMWGAL-TF and its evaluation for anti-hyperglycemic effects on experimental diabetes in laboratory mice.

2. Materials and methods

2.1. Animals

Swiss albino mice (25–30 g) were purchased from National Toxicology Centre, Pune, India, and used for the study. They were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12-h light: 12-h dark cycle with free access to food pellets (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. All experiments were carried out between 09:00 and 17:00 h. Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India, approved the experimental protocol. The experiments were performed in accordance with the guidelines on animal experimentation recommended by Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

2.2. Drugs and chemicals

Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem, India), glucose estimation kit (glucose oxidase/peroxidase GOD-POD kit) (Accurex Biomedical Pvt. Ltd., India), D-glucose (S.D. Fine-Chem. Ltd., India), Tween-80 (Research-Lab, India) were purchased from respective vendors.

2.3. Preparation of LMWGAL-TF

Fenugreek seeds (1000 g) having a moisture content less than 7% were flaked in a roller flaker to a thickness of 5 mm. The flaked material was stalked in a column having a bed height of 300 mm and passed through 5 L of hexane. The elluent was collected from the bottom and recycled for a period of 10 h at 35 °C. After 10 h, the fenugreek layer was drained free of hexane and then solvent mixture (8 L) comprising of ethyl alcohol and water in the ratio of 4:1 was passed through the layer for a period of 8 h at 35 °C by recycling the elluent. After 8 h, the fenugreek extract bed was drained free of solvent and concentrated to semisolid mass under vacuum at 50 °C. The concentrated mass was re-dissolved in 5 L of demonized water to get a clear solution. The clear aqueous solution was passed through a column containing 500 mL of macroporous and strong acid cation exchange resin slowly over a period of 2 h. The column elluent were checked for the presence of alkaloids and nitrogenous substances like amino acids by TLC system (butanol, acetic acid, water, in the ratio 12:8:2) with ninhydrin reagent as detecting solution. After completion of the column, the outlet liquid was passed through 75 mL of resin bed containing Amberlite XAD-761 over a period of 3 h. The column remainder was collected, concentrated and spray dried under following conditions: inlet temperature: 160 °C, outlet temperature: 80 °C, at 12,000 rpm to obtain LMWGAL-TF (yield 32 g, 3.2%).

2.4. Characterization of LMWGAL-TF

The concentration of marker compound LMWGAL-TF was detected by high performance liquid chromatography (HPLC) using reverse phase column and evaporative light-scattering detector (ELSD) detector as follows: 100 mg of LMWGAL-TF powder was dissolved in 50 mL methanol with stirring. The solution was filtered through Whatman filter paper (#41) and injected in HPLC system. The HPLC conditions were as follows: Model: JASCO LC 2000, Column: reverse phase C-18 L column (250 mm × 4.6 mm, particle size of 5 μ); Column temperature of 30 °C, Method time: 25 min, Mobile phase: gradient elution method using water (solvent A) and acetonitrile (solvent B), and eluted by the following program at the flow rate of 1 mL/min: 0–20 min (A:B is 75:25 to 65:35), 20–25 min (A:B is 65–35 to 75–25). Detector: Alltech ELSD 2000, the drift tube temperature: 42 °C, the helium flow: 1.3 L/min. The area under the curve (AUC) was recorded from the resultant chromatogram and content of marker compound LMWGAL-TF calculated.

Characterization of sample of LMWGAL-TF obtained from HPLC was carried out by liquid chromatography-mass spectrometry (LC-MS). The molecular mass was determined in negative mode. The main working parameters for the MS were the following: ionization mode, negative electrospray (ESI−); capillary (kV), 4.01; cone voltage, 90–91.21 V; extractor (V), 2.32; RF lens (V), 0.3; source temperature (°C), 99 °C; desolvation temperature (°C), 148 °C; cone gas flow (L/h), 49; desolvation gas flow (L/h), 695; multiplier (V), 650; gas cell Pirani pressure (mbar), 1×10^{-4}.

2.5. Preparation of test compound solutions

LMWGAL-TF, being water soluble, was freshly prepared daily as 1% aqueous solution of distilled water in three different dosages