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Original article

Antidiabetic and antidyslipidemic nature of trigonelline, a major alkaloid of fenugreek seeds studied in high-fat-fed and low-dose streptozotocin-induced experimental diabetic rats

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ABSTRACT

Fenugreek (*Trigonella foenum graecum*) is one of the most widely used medicinal plants in folk medicine. It is known to have diuretic, cardio tonic, hypotensive, hypoglycemic and hypolipidemic effect. Trigonelline, a major alkaloid component of fenugreek, is reported to be responsible for most of its pharmacological activities. The present study was designed to investigate the effect of trigonelline on blood glucose, glycosylated hemoglobin and plasma insulin levels in high-fat-fed (HFD)/streptozotocin (STZ)-induced type 2 diabetic rats. Diabetes was induced by high-fat diet and low-dose STZ (35 mg/kg.b.wt). Diabetic rats were treated with trigonelline (150 mg/kg b.wt) for 30 days. The toxicological as well as biochemical parameters such as blood glucose, HbA1C, insulin, insulin resistance (HOMA-IR) and lipid profile were measured. The activities of serum AST, ALT and ALP were also assayed. Trigonelline supplementation attenuated the elevated levels of glucose, glycosylated hemoglobin, AST, ALT and ALP. The insulin level was improved with an improvement in hepatic and muscle glycogen content of insulin resistant diabetic rats. Trigonelline effectively normalized the status of lipid profile. These results showed that trigonelline have potential anti-hyperglycemic and antidyslipidemic effects in HFD/STZ-induced type 2 diabetic rats.

1. Introduction

Diabetes mellitus is a metabolic disorder which arises from complex interactions between multiple genetic and environmental or lifestyle factors. DM is characterized by the presence of persistent hyperglycemia due to defective insulin secretion, insulin action, or both. Long-term diabetes is associated with several co-morbidities, such as erectile dysfunction, blindness, poor wound healing, kidney failure, heart disease, etc; as a result of considerable damage, dysfunction, and failure of various organs that develop as the disease progresses [1]. The two major forms of the diabetes result from either lack of the regulatory hormone, insulin (type 1 diabetes, T1D), or because body tissues fail to respond to the hormone (type 2 diabetes, T2D). The majority or 90% of patients with diabetes have T2DM. As insulin is crucial for the maintenance of life, T1DM patients depend on externally administered insulin, while for T2DM patients who do not respond to diet and exercise regimes, oral anti-diabetes drugs (OADs) and sometimes external insulin are administered to maintain their blood glucose as normal as possible [2].

The global pandemic of type 2 diabetes mellitus (T2DM) places an incalculable burden on health care systems. Currently available therapeutic options for T2DM, such as dietary modification, oral hypoglycemics, and insulin, have limitations of their own. Many natural products and herbal medicines have been recommended for the treatment of DM. Amelioration of T2DM risk usually targets lifestyle and diet, primarily with the aim of reducing obesity, the foremost risk factor in the development of insulin resistance and ultimately T2DM. However, particular dietary components, such as flavonoids, alkaloids may assist in T2DM prevention in ways other than those already followed by the currently available therapeutic approaches.

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family *Leguminosae* widely grown in India, Pakistan, Egypt, and Middle Eastern countries [3]. Fenugreek (*Trigonella foenum graecum*) is one of the most widely used medicinal plants in folk medicine. Due to its strong flavor and aroma, fenugreek is one of such plants whose leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine. It is rich source of calcium, iron, β carotene and other vitamins [4].

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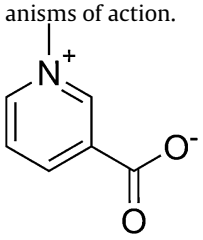
The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels [5–7].

Trigonelline is the major component of alkaloids in fenugreek. Earlier reports indicate that trigonelline reduces blood glucose concentrations in rats [8,9] and in human [10,11]. Trigonelline protects β-cells of the pancreas and increases insulin sensitivity index as well as insulin content [12]. However, only few reports were available on the antidiabetic and antilipidemic actions of trigonelline. Hence, the present study was conducted to evaluate the efficacy of trigonelline on the impaired glucose tolerance, insulin resistance and some biochemical parameters of high-fat diet/streptozotocin-induced diabetic albino rats and to suggest their probable anti-hyperglycemic and anti-hyperlipidemic mechanisms of action.



Liver has been shown to play a central role in the maintenance of glucose homeostasis with a minor contribution to kidney [13]. Hyperglycemia in type 2 diabetes is characterized by enhanced glucose production in the liver and kidney. Fasting blood glucose is determined by de novo glucose production and glucose deposition in peripheral tissues. In the presence of insulin resistance, enhanced glucose output by liver contributes to hyperglycemia together with reduced glucose deposition in skeletal muscle, heart and adipose tissue.

Although there are numerous animal models (natural as well as developed) available for the study of type 2 diabetes, the pattern of disease establishment and progress in most of them did not appear to be similar to the clinical situation in humans. Thus, there is a continued quest among the investigators with respect to the establishment of better animal model for type 2 diabetes by adjusting the existing methods, developing new methodologies, or a combination of both.

It is suggested that the HFD might be a better way to initiate the insulin resistance, which is one of the important features of type 2 diabetes. At the same time, streptozotocin (STZ) is widely used to reproducibly induce both insulin-dependent and non-insulin-dependent diabetes mellitus presently by inducing β cell death through alkylation of DNA [14]. Although high-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of insulin secretion, which is similar to the feature of the later stage of type 2 diabetes. Therefore, investigators have started to develop a rat model by feeding the animal with high-fat-diet following low-dose STZ that would closely mimic the natural history of the disease events (from insulin resistance to β cell dysfunction) as well as metabolic characteristics of human type 2 diabetes [15–17]. Hence in the present study, HFD-STZ-induced animal model was used to assess the antidiabetic efficacy of trigonelline.

2. Materials and methods

2.1. Chemicals

Trigonelline, streptozotocin, were procured from Sigma Aldrich, stored at 2–4 °C and protected from light. All other chemicals used were purchased from standard commercial suppliers and were of analytical grade quality.

2.2. Experimental animals

Male albino wistar rats weighing 150–170 g were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in polypropylene cages lined with husk. They were maintained at an ambient temperature of 25 ± 2 °C and 12/12 h of light/dark cycle. Animals were fed with standard commercial rat chow (Hindustan Lever Ltd) and water *ad libitum* and housed under standard environmental conditions throughout the study. The experiments were strictly conducted according to the ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines [IAEC NO: 01/079/09].

2.3. High-fat diet fed streptozotocin-induced diabetes

The rats were divided into two dietary regimens by feeding either normal or high-fat-diet (HFD) for the initial period of two weeks [16]. The ingredients and chemical composition of the HFD was followed as before reported. After two weeks of dietary manipulation, the groups of rats fed with HFD was injected intraperitoneally (IP) with a low-dose of STZ (35 mg/kg b.w) dissolved in 0.1 M cold citrate buffer, pH 4.5. One week after STZ injection, the rats were screened for blood glucose level. Rats having fasting blood glucose (FBG) > 250 mg/dl that exhibited random hyperglycaemia and glycosuria were selected for the experiment. The rats were allowed to continue to feed on their respective diets until the end of the experiments.

2.4. Toxicity and dosage fixation studies

The acute toxicity of trigonelline was studied in the control rats according to OECD guideline 423. Different doses of trigonelline dissolved in water were given orally and the animals were observed continuously for the first 2 hours followed by every hour up to 6 hours and daily thereafter for fourteen days for any signs of morbidity, mortality and behavioural toxicity. Trigonelline was found to be non-toxic up to 2 g/kg b.w.

Graded doses of trigonelline (100, 150, 200 mg/kg b.w) was administered to HFD + STZ-induced diabetic rats for various periods of treatment. From the data obtained, the optimum dosage was fixed as 150 mg/kg b.w for 30 days. The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

- group 1 – control rats;
- group 2 – HFD + STZ (i.p. 35 mg/kg b.w.) induced rats;
- group 3 – trigonelline (150 mg/kg b.w.) treated diabetic rats;
- group 4 – diabetic rats treated with metformin (200 mg/kg b.w/day) in aqueous solution orally for 30 days.

At the end of the treatment period, the rats were fasted overnight, anesthetized and sacrificed by cervical decapitation. The blood was collected with and without anticoagulants for plasma and serum separation, respectively.

2.5. Oral glucose tolerance test

On the day prior to sacrifice, oral glucose tolerance test (OGTT) test was performed in all the groups. Blood samples were obtained from the lateral tail vein of rats deprived of food overnight.