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Short-term effect of G-400, polyherbal formulation in the management of hyperglycemia and hyperlipidemia conditions in patients with type 2 diabetes mellitus

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ABSTRACT

Objective: *Salacia oblonga*, *Tinospora cordifolia*, *Emblica offinalis Gaertn*, *Curcuma longa* and *Gymnema sylvestre* are Ayurvedic medicinal plants reported to lower plasma glucose levels in animal models. To our knowledge, however, no clinical validations of those extracts for efficacy have been. The aim of this study was to evaluate the effect of polyherbal combination in patients with type 2 diabetes mellitus.

Methods: We screened 250 patients enrolled in a diabetes mellitus screening camp held at District Ayurvedic Hospital, Kottayam, Kerala, India. Of these, 89 patients diagnosed with type 2 diabetes mellitus and 50 healthy volunteers of similar age group were included in the study. Patients were treated with a polyherbal combination drug namely G-400 (1000 mg/d) for 8 wk with a follow-up of 2wk interval.

Results: Fasting and postprandial blood glucose levels measured after 8 wk of G-400 treatment in patients were significantly lower. Indeed diabetic rats showed similar protection with G-400 administration. Furthermore, glycosylated hemoglobin, serum total cholesterol, both high- and low-density lipoprotein cholesterol, and triglycerides showed a significant improvement in G-400–administered patients. Toxicologic profile of the drug was assessed by analyzing the enzyme activities of alkaline phosphatase and alanine aminotransferase along with the concentration of blood urea nitrogen and creatinine in blood and found insignificant change compared with control.

Conclusion: Short-term supplementation of G-400 not only attenuates the hyperglycemia, but also acts as hypolipidemic agent in patients with diabetes. Further study should be done for the long-term effect of the drug in larger populations.

Introduction

Unprecedented increases in the rates of diabetes and prediabetes place the disease as a public health concern that imparts financial burden to society. According to the International Diabetes Federation, 366 million individuals had diabetes in 2011 and by 2030 this number is estimated to increase to 552 million. It has been suggested that there will be a 69% increase in the numbers of adults with diabetes in developing countries and a 20% increase in developed countries between 2010 and 2030 [1]. India registered the largest increase during the past few years. The prevalence of type 2 diabetes mellitus (T2DM) is about 2.4% in rural populations and 11.6% in urban populations. It has been proposed that by 2025, approximately 57 million Indians will be affected by T2DM [1].

There are several classes of antidiabetic drugs on the market, but achieving and maintaining long-term glycemic control often is challenging. In fact, many current agents have treatment-limiting side effects. So, a significant need for novel antidiabetic drugs

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exists [2]. The progressive nature of T2DM eventually requires insulin use to control glucose level. To delay the onset of T2Dm, patients usually require a combination of at least two oral agents in the long term. As the risk for long-term complications in patients with diabetes can be dramatically reduced with appropriate glycemic control, food ingredients that can attenuate postprandial glucose in individuals with diabetes would be useful [3].

Some ingredients that can attenuate postprandial glucose include *Salacia oblonga* root, *Tinospora cordifolia* stem, *Emblica officinalis Gaertn*, *Curcuma longa*, and *Gymnema Sylvestre*. The therapeutic efficacy of these individual plant extracts has been reported in *streptozotocin*-induced diabetic rats. *Salacia oblonga* is known to prevent the breakdown of oligosaccharides and polysaccharides into monosaccharides by competitive inhibition of α-glucosidase activity within the lumen of the intestinal tract [4]. The antidiabetic activity of *Tinospora cordifolia* is mediated through an insulin-dependent pathway [5] and also by up-regulating Glut-4 and peroxisome proliferator activated receptor alpha (PPAR-α) expression. *Emblica officinalis* stimulates basal insulin output and potentiates glucose-stimulated insulin secretion [6]. *Curcuma longa* activates PPAR-γ to improve insulin resistance [7] and ameliorates T2DM by the same biological mechanism as thiazolidinedione derivatives [8]. *Gymnema Sylvestre* promotes regeneration of islet cells thereby increasing secretion of insulin [9]. It also causes inhibition of glucose absorption from the intestine [10]. Traditionally, these plants have been used as part of the Ayurvedic system of Indian medicine to treat diseases such as diabetes. Currently, extracts of these plants are consumed as food supplements in India for the treatment of diabetes and hypercholesterolemia.

The safety of *Salacia oblonga* root, *Tinospora cordifolia* stem, *Emblica offinalis Gaertn*, *Curcuma longa*, and *Gymnema Sylvestre* were evaluated in different scientific groups in a series of tests such as acute toxicity, chromosomal aberration, and micro-nuclei.

In this study, we have formulated a polyherbal drug comprised of *Salacia oblonga* root, *Tinospora cordifolia* stem, *Emblica offinalis Gaertn*, *Curcuma longa*, and *Gymnema Sylvestre* that may possess α-glucosidase inhibitor action, stimulating insulin secretion from β cells, regulating glucose transporter 4 transporters, reducing hyperlipidemia and promoting β-cell regeneration. The formulation was prepared according to the guidelines of the Indian traditional medical system to treat diabetes and was based on the principle that it might exert synergistic properties and potentiate antidiabetic, hypolipidemic, and antioxidant activities. An extensive range of oral antidiabetic drugs for T2DM are available to clinicians, however, a majority of patients have poor glycemic control. In fact, the focus has shifted to traditional medicines and herbal formulations, which are preferred because of fewer side effects and lower cost. In this study, we include both preclinical and clinical trials to evaluate the antidiabetic efficacy of the herbal formulation G-400.

Methods

Plant material and drug preparation: polyherbal drug G-400

Plants were collected from the botanic garden of the Kerala Ayurvedic Pharmaceutical Company, Department of Science and Technology; government of India-approved company and processed in their research division. The polyherb named G-400 contains a mixture of the following herbs expressed as percentage in dry weight: 30% *Salacia Oblonga* leaves, 10% *Tinospora Cordifolia*, 10% *Emblica officinalis*, 10% *Curcuma longa*, and 40% *Gymnema sylvestre*. In-house formulation of G-400 was prepared as per the Ayurvedic Formulary of India. The dynamic nature of diabetes medication prompted us to design a polyherbal combination drug that has the composition characteristics of the α-glucosidase inhibitor (*Salacia Oblonga*), sulfonylureas (*Emblica officinalis*), thiazolidinediones (*Tinospora Cordifolia, Curcuma longa*), and regeneration of β cells (*Gymnema Sylvestre).*

Preclinical trials: animals

To demonstrate the antidiabetic property of polyherbal combination G-400 and its effect on blood glucose levels, male albino Wistar rats ages 7 to 8wk (180–200g) bred in the animal division of the King's Institute, Chennai were used. Animals were kept in the animal house at an ambient temperature of 25°C to 30°C and 45% to 55% relative humidity with 12 h each of a dark and light cycle. Animals were fed with a pellet diet (Sai Durga Feeds and Foods, Bangalore, India.) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee and by the regulatory body of the government (Reg. No 817/AC/*CPCSEA*, dated 6/8/04).

Induction of experimental diabetes

The animals were fasted overnight, and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (55 mg/kg body weight [all doses in kg body weight]) in 0.1 M citrate buffer (pH 4.5) [11]. Control rats were injected with citrate buffer alone. On the third day of STZ injection, the rats were fasted for 6 h and blood was taken from the tail artery. Rats with moderate diabetes having hyperglycemia (i.e., blood glucose of 250–400 mg/dL) were taken for the experiment. The rats were kept for 15 d to stabilize the diabetic condition.

In the experiment, 24 rats (18 diabetic surviving rats and 6 normal rats) were used. The rats were divided into 4 groups with 6 animals in each group as follows:

Group I: normal untreated rats.
Group II: diabetic control rats.
Group III: diabetic rats were given G-400 (100 mg/kg) daily using an intragastric tube for 4 wk. A pilot study on oral glucose tolerance test (OGTT) changes in diabetic rat with three different doses of G-400, namely 50, 100, and 200 mg/kg were conducted to finalize the dosage of the G400.
Group IV: Diabetic rats were given glibenclamide (0.025 mg/kg) daily using an intragastric tube for 4 wk.

At the end of 4 wk, the animals were fasted overnight and sacrificed. Blood samples were collected in two different tubes, one with anticoagulant, potassium oxalate, and sodium fluoride for plasma and another without anticoagulant for serum separation. The blood was then centrifuged at 3000*g* for 20 min using a refrigerated centrifuge at 4°C to separate the plasma and serum, used for different biochemical studies.

Measurement of blood glucose, OGTT, and insulin levels

Fasting blood glucose was measured by the oxidase/peroxidise method [12]. An OGTT was performed 4 wk after treatment by feeding glucose 2 g/kg through gavages. Blood samples were collected at 0, 30, 60, 120, and 180 min. Serum samples were analyzed for glucose concentration. After data were collected, the area under curve was calculated using the trapezoidal method