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Pre-treatment of Syndrex® protects mice from becoming diabetic after streptozotocin injection

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to either insufficiency of insulin or inability of cells to respond to insulin. Many clinical and experimental evidence have suggested the strong association between hyperglycemia, oxidative stress and diabetic complications. Therefore, the antidiabetic drugs with antioxidant potential would have a higher therapeutic value. To check its antidiabetic and antioxidant properties *in vivo*, experiments were done wherein mice were fed with Syndrex® in different schedules and/or made diabetic by intraperitoneal injection of streptozotocin. Animals fed with Syndrex® prior to the induction of diabetes by streptozotocin injection showed resistance to an increase in blood glucose levels. This treatment increased the activities of antioxidant enzymes namely, catalase, glutathione reductase and superoxide dismutase and reduced serum triglyceride and cholesterol levels as compared to those found in uncontrolled diabetic mice. Among the three different schedules used for Syndrex® treatment, the best effect was seen in the case of mice pretreated with Syndrex® prior to STZ injection. In our opinion, Syndrex® given along with insulin may reduce the amount of insulin dose required and because of its strong antioxidant activity would certainly help to reduce the development of diabetic complications.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to either insufficiency of insulin or inability of cells to respond to insulin. Type 1 diabetes is a case of β cell death due to their autoimmune destruction, whereas type 2 is a syndrome of β cell dysfunction involving relative insulin deficiency associated with insulin resistance [1]. Many clinical and experimental evidence have suggested the strong association between hyperglycemia, oxidative stress and diabetic complications. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins [2]. Abnormally high level of free radicals and the simultaneous decline of antioxidant defense mechanisms lead to damage of cellular organelles and enzymes. Oxidative stress is shown to be a major cause of development of diabetic complications such as retinopathy, neuropathy and nephropathy [3,4]. Therefore, the antioxidant compounds, which can either scavenge free radicals or inhibit formation of free radicals, are beneficial and antidiabetic drugs with antioxidant potential would have a higher therapeutic value. *Trigonella foenum-graecum* Linn. commonly known as fenugreek is an Indian spice extensively used in Indian food from ancient times; aqueous extracts of seeds and plants of fenugreek have been shown to possess antidiabetic properties and are nontoxic [5]. Fenugreek seed extracts have shown to lower blood glucose, reduce levels of glycated haemoglobin and reduce lipidemia in STZ-induced diabetic rats in a dose dependent manner [6]. Soluble dietary fibre fractions of fenugreek have shown improvement in the glucose homeostasis in animal models of types 1 and 2 diabetes and enhanced insulin action [7]. Fenugreek leaf extracts showed hypolipidaemic and antihyperglycemic

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effects in diabetic rats [8]. Vijaykumar et al [9] demonstrated activation of the insulin signaling pathway in adipocytes and liver cells *in vivo* by fenugreek seed extract. In the present study we have used Syndrex®, a formulated preparation, manufactured by Plethico Laboratories, Indore, India, containing powder of germinated fenugreek (*T. foenum-graecum*) seeds. In our earlier studies [10] Syndrex® showed a significant antioxidant capacity measured in terms of radical scavenging, inhibition of radical formation and protected islets from a streptozotocin-induced oxidative damage. It also improved viability as well as functionality of cultured mouse pancreatic islets. To check its antidiabetic and antioxidant properties *in vivo*, experiments were done wherein mice were fed with Syndrex® in different schedules and/or made diabetic by intraperitoneal injection of streptozotocin. Those fed with Syndrex® prior to the induction of diabetes by streptozotocin injection showed resistance to an increase in blood glucose levels.

2. Materials and methods

2.1. Materials

All the chemicals required for the experiments were purchased either from Sigma; (U.S.A.), Sisco Research Laboratories; (India), or Merck; Germany, Hi-media; India. Syndrex® capsules manufactured by Plethico Laboratories, Indore, India, of the same lot were purchased in bulk from a medical distributor (Shree Sai Medicals, Pune). Kits for the measurement of triglyceride and cholesterol from serum were purchased from Accurex Biomedical Pvt. Ltd. Mumbai, India.

2.2. Biologicals

8–10 week old Swiss albino mice of either sex weighing about 18–20 g were used for the experiments. These animals were inbred in the animal house facility at the Agharkar Research Institute, Pune, for several generations. They were housed in polypropylene cages in air-conditioned area at 25 ± 2 °C with 12:12 h light and dark cycle. They were given animal feed and water *ad libitum*. Prior approval was obtained from the Institutional Animal Ethical Committee for the protocols used involving animals. The doses of Syndrex® were decided on the basis of a pilot experiment carried out using 3 animals for each treatment and final experiments were carried out using at least 6 animals.

2.3. Preparation of Syndrex®

Syndrex® was prepared in 1% carboxymethylcellulose (W/V). The required quantity of Syndrex® was taken in a mortar. 1% carboxymethylcellulose was added to it drop-by-drop and mixed properly with pestle.

2.4. Glucose tolerance test

Mice (18–20 g body weight) were fasted overnight. Next day small amount of blood (approximately 10 µl) was collected from tail vein and 0 h sugar was estimated using glucometer (Roche, Germany). Mice were then divided into different groups and fed with two doses of Syndrex® namely, 125 and 250 mg/kg body weight. After 30 min mice were given a glucose load of 1 g/kg body weight. Blood samples were collected from tail vein at various time points (0–120 min) for the measurement of blood glucose. Chlorpropamide was used as a positive control [11].

2.5. Insulin tolerance test

Mice (18–20 g body weight) were fasted overnight and next day onwards were administered Syndrex® either 125 or 250 mg/kg body weight for 7 days. On the 6th day, mice were again kept for fasting overnight. Next day 0 h blood sugar was measured. After 30 min insulin was prepared in normal saline (2 U/kg body weight) and injected intraperitonealy. Small amount of blood (approximately 10 µl) was collected at different time intervals between 0 and 120 min from the tail vein and sugar was estimated using a glucometer.

2.6. Experimental design

For all further experiments 8–10 weeks old Swiss albino mice of either sex, (18–20 g body weight) were divided into 6 different groups. Each group contained at least 6 animals.

- Group A: Animals from the control group were given animal feed.
- Group B: After an overnight fast, mice were made diabetic by injecting streptozotocin (200 mg/kg body weight) intraperitonealy.
- Group C: Mice were fed with either 125 or 250 mg/kg body weight of Syndrex® prepared in 1% carboxymethylcellulose alone for 7 days.
- Group D: The mice from this group were fed with either 125 or 250 mg/kg body weight Syndrex® for 7 days and then made diabetic by injecting 200 mg/kg body weight streptozotocin intraperitonealy. Mice were fasted for 18 h before injection. 72 h after STZ injection, blood glucose of mice was checked.
- Group E: The mice from this group were treated with Syndrex® after the induction of diabetes. After an 18 h fasting, mice were made diabetic by intraperitoneal streptozotocin (200 mg/kg body weight) injection. Mice with confirmed diabetes (blood glucose ≥ 200 mg/dl) were fed orally once a day with either 125 or 250 mg/kg body weight Syndrex® for 3 weeks. One group of animals was given insulin (2 U/kg body weight) every day intraperitonealy for 3 weeks. Blood sugar of these mice was checked intermittently till 3rd week.

2.7. Tissue isolation

On the day of sacrifice, animals were exsanguinated under anesthesia and blood was collected by giving a cut to the jugular vein to separate the serum for measuring triglyceride and cholesterol. Different organs of the mouse were perfused with 0.1 M potassium phosphate buffer, pH 7.2, through an abdominal aorta to remove contaminating blood. Individual organs were perfused with the same buffer. All the tissues namely, liver, pancreas, kidney, lungs, spleen, heart and skeletal muscle, were removed, washed with 0.1 M potassium phosphate buffer, pH 7.2, weighed and then homogenized in