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A Novel Approach for Oral Delivery of Insulin via *Desmodium gangeticum* Aqueous Root Extract

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ABSTRACT

Many challenges are associated with the oral delivery of insulin, relating to the physical and chemical stability of the hormone, and its absorption and metabolism in the human body. The present study aims to demonstrate the oral delivery of insulin in both normal and steptozotocin (STZ)-induced diabetic rats with the help of the aqueous extract of *Desmodium gangeticum* (DG) root. Human insulin was mixed with the aqueous extract of DG root (0.1 mg/ml) with human insulin (40 IU/ml) in ratio 1:1(v/v), to prepare oral insulin drug. Decreased plasma glucose level and increased plasma insulin in normal and STZ-induced diabetic rat suggested the probable absorption of insulin through GI tract when insulin was administered by mixing with DG extract. Indeed, insulin mixed DG potentially stimulates the release of insulin in STZ-induced diabetic rat rather than in normal animal. *In vivo* insulin secretaguage action of oral insulin drug was determined by isolated rat heart model and the results showed a significant cardio protection in STZ rat. The finding of this study suggests that insulin mixed with DG extract can be a promising vehicle for oral delivery of insulin. However, further studies are required to explore the exact compound(s) responsible for the protective delivery of insulin orally. Increased plasma insulin level by insulin mixed DG extract administration in STZ-treated diabetic rat indicates not only insulin secretaguage action of the mixture but also a probable altered insulin release mechanism in diabetic condition.

Key words: *Desmodium gangeticum*, glucose tolerance test, oral insulin, streptozotocin-induced diabetes

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INTRODUCTION

Insulin is a natural hormone which controls the level of blood glucose and are limited in tissues of diabetes mellitus (DM)-induced rats. Patients with Type 2 DM are insulin resistant, have relatively low insulin production, or both. Some patients with Type 2 diabetes may eventually require insulin when other medications fail to control blood glucose levels adequately.

Many new pharmacological agents have been added to our armamentarium of treatments for DM in the last decade. The goal of all treatments is the same irrespective of the cause of the DM, namely to normalize blood glucose. Insulin therapy affords effective glycemic control, yet its short comings such as ineffectiveness on oral administration, short shelf life, and requirement of constant refrigeration.[1] Treatment with sulfonylureas and biguanides is also associated with side effects.[2] However, recently numerous researches are being carried out to reduce the limitations of insulin therapy.

Researchers are trying to find various alternatives to deliver

insulin via noninvasive routes, such as nasal,[3] rectal,[4] pulmonary,[5] and ocular deliveries.[6] However, among all alternative routes for the administration of insulin, the oral route is the most convenient. In addition, because orally administered insulin undergoes a first hepatic pass, it will produce a similar effect as pancreas-secreted insulin by inhibiting the hepatic gluconeogenesis and suppressing the hepatic glucose production.[7] Unfortunately, oral delivery of peptides or proteins such as insulin poses unique problems of instability, susceptibility to proteolysis, and inability to traverse membranes and biological barriers due to their large molecular size.[8] As a result, the absolute amount of intact protein reaching the target site is too small to be of pharmacological benefit. To overcome these major problems, it was suggested to increase the oral absorption of insulin to circumvent the digestion of this polypeptide in the gastrointestinal (GI) tract by entrapping insulin in polymeric microspheres[9] or by coating with polymer films.[10] However, no study has been done to transport insulin with the help of an herbal extract as a vehicle.

Desmodium gangeticum (DG) (Leguminosae) is a shrub common on the lower hills and plains throughout India. DG is widely used in the indigenous system of medicine in India and is reported to contain flavone and isoflavonoid glycoside.[11] It forms the ingredient of many Ayurvedic formulations used for diabetes. Moreover, it strengthens the nervous system, improves digestion, and secretion of digestive enzymes.[11] It also acts as diuretics. In this study we used insulin mixed with an extract (aqueous) obtained from DG root as a vehicle to transport insulin across the GI tract.

MATERIALS AND METHODS

Preparation of aqueous extract of the roots of *Desmodium gangeticum*

The plant after collection from the herbal garden maintained in the department was washed and cleaned. The plant material was taxonomically identified and the voucher specimen A/C no. 3908 was retained in our laboratory for future reference.

One kilogram (1 kg) of fresh secondary roots of DG were sliced and air-dried at room temperature. The sliced, air-dried roots of the plant were milled into fine powder. Soxhlet extraction with 2.5 l of distilled water at room temperature for 24 h with shaking was used to obtain the extracts. The aqueous extracts were filtered and concentrated to dryness under reduced pressure at 30±1 °C. The resulting aqueous extract was freeze-dried, finally giving 18.66 g [i. e., 1.866% yield]. Aliquot portions of the crude root aqueous extract residue were weighed and dissolved in distilled water for the experiment.

Animals

Male wistar rats (170-190 g) were procured from King Institute of Preventive Medicine, Chennai, India, and used for the investigation of oral delivery of insulin via DG. These studies were approved by the Institutional animal ethical committee (IAEC). The animals were housed under standard conditions of temperature (22±3° C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet (Amrit Feeds Ltd, Bangalore.) and water ad libitum. Animals were fasted for 18–24 h and streptozotocin (STZ; 65 mg/kg) in 0.02 M citrate saline buffer was administered intraperitoneally, as described previously.[12] A blood glucose level exceeding 200 mg/dl was considered diabetic.

Preparation of DG-mixed insulin

DG-mixed insulin mixtures were prepared by mixing the aqueous extract of DG (1 mg/ml) with human insulin (40 IU/ml) in ratio 1:1(v/v).

Oral glucose tolerance test

Oral glucose tolerance test was performed in normal and diabetic rats after an overnight fasted (18 h). Animals were randomized into following groups: (1) normal control; (ii) diabetic control; and (iii)drug group. In fact drug groups were again divided into two subgroups: (a) effect of drug on OGTT in normal rat and (b) effect of drug on OGTT in diabetic rats. Animals were fasted overnight and were placed in restraining cages for 30 min in the morning before commencing the study. Glucose was given orally by an esophageal tube as a 40% solution (1 g/kg body wt). The drugs were administered both intraperitoneal and oral routes. Blood was withdrawn from the orbital plexus of each animal at 0, 30, 60, 90,120, and 180 min after administration of the oral load. The fasting blood glucose levels were estimated by using standard kits from Qualigens India. Plasma insulin was analyzed by the IRMA method.

Isolated heart preparation and ischemia-reperfusion experiment for insulin secretogenic action

Rats were heparinized (500 IU/kg, i.p.) and anesthetized with pentobarbital (50 mg/kg, i.p.), and the heart was isolated and perfused retrogradely using the Langendorff method with Krebs-Henseleit buffer (pH 7.4, in mM)