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Original Article

Protective effect and potential mechanisms of propolis on streptozotocin-induced diabetic rats

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الملخص

أهداف البحث: تهدف هذه الدراسة إلى معرفة التأثيرات الوقائية لمكملات العكبر ضد التأثير السام لمادة الاستربتوزوتوسين على خلايا بيتا في البنكرياس ومعرفة آلية العمل الممكنة من جراء هذا التأثير.

طرق البحث: تم تقسيم ٤٥ جرذا تقسيما عشوائيا إلى ٣ مجموعات، في كل مجموعة ١٥جرذا. المجموعة الأولى: الجرذان العادية التي تتغذى على الغذاء العادي تشاو. والمجموعة الثانية: الجرذان المصابة بداء السكري الناجم عن الاستربتوزوتوسين. والمجموعة الثالثة: الجرذان المعالجة بالعكبر ٠.٣ غرام /كغ/ يوميا لمدة أسبوعين قبل الإصابة بالسكري الناجم عن الاستربتوزوتوسين. في نهاية الفترة التجريبية، جمعت عينات الدم الصائم لقياس مستويات الجلوكوز ونشاط بيروكسيد الدهون. وتم تجهيز أنسجة البنكرياس للجرذان المشرحة لغرض الدراسات المورفولوجية والمناعية.

النتائج: أظهرت نتائج الدراسة الحالية أن معالجة الجرذان بالعكبر قبل الإصابة بداء السكري، قد قلل بشكل ملحوظ من قياس الجلوكوز الصائم مقارنة مع الجرذان المصابة بداء السكري. وزادت منتجات بيروكسيد الدهون بشكل كبير في المجموعة الثانية مقارنة بالأولى. ولكن، هذه المنتجات قلت بشكل ملحوظ في المجموعة الثالثة مقارنة بالثانية. أظهر التشريح المرضي والتحليل المناعي لأنسجة البنكرياس زيادة في حجم جزر لانجرهانس وزيادة في صبغة خلايا بيتا في البنكرياس مع خلايا بيتا من الأجسام المضادة في المجموعة الثالثة مقارنة مع الثانية.

الاستنتاجات: أظهر العكبر تأثيرا وقائيا، وحافظ على سلامة خلايا بيتا في البنكرياس لدى الجرذان المصابة بداء السكري الناجم عن الاستربتوزوتوسين. هذه الدراسة تحث على ضرورة إجراء مزيد من البحوث لتوضيح الآلية الدقيقة لهذا التأثير المتغير.

الكلمات المفتاحية: العكبر؛ الاستربتوزوتوسين داء السكري؛ بيروكسيد الدهون؛ التشريح المرضي؛ الجرذان المصابة بداء السكري.

Abstract

Objectives: The objective of this study was to determine the protective effects of propolis supplementation against the toxic effect of streptozotocin (STZ) on pancreatic beta-cells and to identify the possible mechanism of actions underlying this effect.

Methods: Forty-five rats were randomly divided into three groups, with each group containing 15 rats. Group I consisted of normal rats fed a normal chow diet. Group II included diabetic rats induced with STZ. Group III consisted of rats treated with 0.3 g/Kg/day propolis for 2 weeks before the induction of diabetes by STZ. At the end of experimental period, blood samples were collected for the measurement of fasting blood glucose (FBG) and lipid peroxidation activity. Pancreatic tissues from the dissected rats were processed for morphological and immunohistochemical studies.

Results: The findings of this work showed that the treatment of rats with propolis before the induction of diabetes mellitus is associated with significantly decreased FBG levels compared to the diabetic rats. The lipid peroxidation products were significantly increased in group II compared to group I. However, these products were significantly decreased in group III compared to group II. The histopathological and

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immunohistochemical analysis of the pancreatic tissue showed an increase in the size of the islets of Langerhans and the enhanced staining of beta-cells of the pancreas with beta-cell antibodies in group III compared to group II.

Conclusion: Propolis showed protective and preservative effects on pancreatic beta-cell integrity in STZ-induced diabetic rats. This study suggests the need for further research to elucidate the exact mechanism of this effect.

Keywords: Diabetes mellitus; Diabetic rats; Histopathology; Lipid peroxidation; Propolis; Streptozotocin

Introduction

Diabetes mellitus is a common chronic disorder characterized by hyperglycaemia due to inadequate insulin secretion or function and under-utilization of glucose in peripheral tissues that results in the development of diabetes, a specific microvascular pathology in the peripheral nerve, retina and glomerulus. As a consequence, it is a leading cause of many debilitating neuropathies, blindness and end-stage renal disease.[1]

According to the International Diabetes Federation (IDF), diabetes is one of the most challenging health problems of the 21st century. Additionally, there are numerous health problems and issues associated with diabetes, which ultimately reduces the quality of life. Another tragic factor associated with diabetes is that most people are unable to afford proper treatment because it is a costly illness, particularly because it is long term. Furthermore, KSA was estimated to be among the top 10 countries with a higher prevalence of diabetes (23.9%).[2]

Maintaining the balance between oxidative stress and antioxidants is an important mechanism for preventing damage from oxidative stress. Therefore, supplementation with flavonoids has been used to prevent oxidative stress induced by STZ in a diabetes model.[3]

Propolis is a resinous hive product collected by bees from various plant sources. More than 300 components have been identified in propolis, mainly phenolic compounds (e.g., flavonoids and aromatic compounds), terpenes and essential oils.

Propolis possesses various pharmacological properties, such antibiotic, anti-inflammatory, anti-cancer, antioxidant, and anti-hepatotoxic activities.[4]

The effect of propolis against the toxicity of STZ in rats was evaluated. It was reported that propolis may prevent beta-cell destruction via the free radical scavenging activity of propolis.[5]

Thus, we hypothesized that propolis ameliorates the toxic effects of STZ on pancreatic beta-cells in diabetic rats and glycaemic function via its antioxidant effect.

The objectives of this study were to determine whether propolis supplementation prevents the toxic effect of STZ on pancreatic beta-cells and to identify possible mechanisms of action underlying this effect.

Materials and Methods

Experimental designs

The study was conducted on (45) normal male Wister albino rats weighing 150−250 gm obtained from the animal house. All of the rats were housed under standard environmental conditions (temperature 25−29° C, 12 h light and 12 h darkness cycles). The animals were allowed free access to pelleted standard rat diet and water. The Scientific Research Ethics Committee of Dammam University approved this study, in accordance with the ethics standards of "Principles of Laboratory Animal Care".

The rats were randomly divided into three groups, with 15 rats in each group (n = 15) as follows:

Group I: normal rats fed with normal chow diet;

Group II: diabetic rats induced by STZ single dose (60 mg/kg BW) IP;[6]

Group III: rats treated with propolis (0.3 g/Kg/day) for 2 weeks before the induction of diabetes by STZ. Propolis was obtained from Dosic Import and Export Co., Ltd., China. The best purity (70%) and freshness were guaranteed, and the propolis was supplied in powder form. Each day, freshly ground propolis was weighed and dissolved in distilled water for a final concentration of 300 mg/ml. The selected dose of propolis was administered orally using an orogastric feeding needle. This dose was selected on the basis of a previous study.[7]

Diabetes in experimental rats was induced by a single intraperitoneal injection of 60 mg/kg body weight of STZ (Sigma−Aldrich). Three days after drug-injections, urine strips (Medi-Test Combi 10; Macherey−Nagel GmbH & Co, Düren, Germany) were used to detect glucosuria (a dark-green colour indicates blood glucose $\geq$ 500 mg/dl). These rats were selected as diabetic rats for the experiment.[8]

Blood sampling

At the end of the experimental period, food was stopped 12 h before the rats were sacrificed. Animals were weighed and then anesthetized with ketamine 50 mg/kg BW intraperitoneal. Blood was collected from the abdominal aorta through a midline incision. A plain tube was used for the separation of serum to determine the glucose level and lipid peroxidation products, malondialdehyde (MDA). Blood glucose was measured using a glucometer (Accu-Chek Go, Roche Diagnostics GmbH, Indianapolis, IN), and thiobarbituric acid reactive substance (TBARS) was used (BioAssay Systems, Hayward, CA, USA) to measure the product of the reaction