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Antioxidant protection of Malaysian tualang honey in pancreas of normal and streptozotocin-induced diabetic rats

Action protectrice anti-oxydante du miel malésien Tualang sur le pancréas de rats normaux ou diabétiques induits par la streptozotocine

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Résumé

La glucotoxicité contribue à la dysfonction β cellulaire par le biais du stress oxydatif. Notre étude précédente avait démontré que le miel Tualang améliorait le stress oxydatif rénal et induisait un effet hypoglycémiant chez des rats présentant un diabète induit par la streptozotocine (STZ). La présente étude avait pour but d'évaluer si l'effet hypoglycémiant du miel Tualang pouvait en partie être lié à son action protectrice anti-oxydante sur le pancréas. Un diabète a été induit par une seule dose de STZ (60 mg/kg ; voie intrapéritonéale). Les rats diabétiques étaient randomisés en deux groupes dont l'un recevait de l'eau distillée 0,5 ml/j et l'autre du miel Tualang (1 g/kg par jour). En parallèle, deux groupes de rats non diabétiques recevaient de l'eau distillée (0,5 ml/j) ou du miel Tualang (1 g/kg par jour). Les animaux étaient traités oralement pendant 28 jours. À la fin de la période de traitement, les rats traités par le miel avaient une glycémie significativement plus basse ($p < 0{,}05$) lorsqu'ils étaient comparés aux rats témoins diabétiques [8,8 (5,8) mmol/L versus 17,9 (2,6) mmol/L ; médiane (interquartile)]. Le pancréas des rats diabétiques témoins contenait des niveaux significativement plus élevés de malondialdéhyde (MDA) ainsi qu'une ascension de l'activité superoxyde dismutase (SOD) et glutathion péroxidase (GPx). L'activité Catalase (CAT) était significativement réduite tandis que la glutathion-S-transférase (GST) et la glutathion réductase (GR) étaient inchangées dans le pancréas des rats diabétiques. Le miel Tualang réduisait significativement les niveaux élevés de MDA ($p < 0{,}05$). Le traitement par le miel restaurait également des activités SOD et CAT. Ces résultats suggèrent que l'effet hypoglycémiant du miel Tualang peut être attribué à ses effets anti-oxydants sur le pancréas.

Mots clés : Stress oxydatif ; Diabète sucré ; Glucotoxicité ; Streptozotocine ; Miel Tualang ; Pancréas

Abstract

Glucotoxicity contributes to β-cell dysfunction through oxidative stress. Our previous study demonstrated that tualang honey ameliorated renal oxidative stress and produced hypoglycemic effect in streptozotocin (STZ)-induced diabetic rats. This present study investigated the hypothesis that hypoglycemic effect of tualang honey might partly be due to protection of pancreas against oxidative stress. Diabetes was induced by a single dose of STZ (60 mg/kg; ip). Diabetic rats were randomly divided into two groups and administered distilled water (0.5 ml/d) and tualang honey (1.0 g/kg/d). Similarly, two groups of non-diabetic rats received distilled water (0.5 ml/d) and tualang honey (1.0 g/kg/d). The animals were treated orally for 28 days. At the end of the treatment period, the honey-treated diabetic rats had significantly ($p < 0.05$) reduced blood glucose levels [8.8 (5.8) mmol/L; median (interquartile range)] compared with the diabetic control rats [17.9 (2.6) mmol/L]. The pancreas of diabetic control rats showed significantly increased levels of malondialdehyde (MDA) and up-regulation of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Catalase

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(CAT) activity was significantly reduced while glutathione-S-transferase (GST) and glutathione reductase (GR) activities remained unchanged in the pancreas of diabetic rats. Tualang honey significantly ($p < 0.05$) reduced elevated MDA levels. Honey treatment also restored SOD and CAT activities. These results suggest that hypoglycemic effect of tualang honey might be attributed to its antioxidative effect on the pancreas.

Keywords: Oxidative stress; Diabetes mellitus; Glucotoxicity; Streptozotocin; Tualang honey; Pancreas

1. Introduction

Reactive oxygen species (ROS) is implicated in the etiology of diabetes induced by chemical agents such as alloxan and streptozotocin (STZ) in experimental animals [1]. Besides the pathogenesis of diabetes, ROS is associated with diabetic status and this condition has been proposed as one of the pathogenic mechanisms of diabetic complications [2]. Chronic hyperglycemia impairs β-cell function and insulin sensitivity, a phenomenon known as glucotoxicity [3]. Glucotoxicity is believed to contribute to β-cell dysfunction through oxidative stress, a consequence of increased mitochondrial generation of ROS that follows excessive glucose metabolism [4]. The pancreatic β-cells are highly susceptible to oxidative stress because they have very low expressions and activities of anti-oxidative enzymes [5]. The role of oxidative stress on pancreatic β-cells is further reinforced by studies which showed that alloxan generates ROS in the pancreas and that antioxidant drug, n-acetylcysteine (NAC) inhibits NF-κB activation and reduces hyperglycemia [6]. Studies have shown that over-expression of antioxidant enzymes protects against increased levels of free radicals in β-cells and its micro milieu [7,8].

Honey is a supersaturated sugar solution of which fructose and glucose are the predominant constituents [9]. In addition to carbohydrates, honey contains protein including enzymes, amino acids, vitamins and minerals, antioxidants such as catalase, peroxidase, alkaloids, polyphenols and flavonoids [10–14]. Generally, honey consists of variable compositions. These differences depend on floral sources, geographical origin, total phenolic content, water proportion and color [15,16]. These compositional variations have been reported to influence the antioxidant properties and other therapeutic effects of honey in both in vitro and in vivo studies [17,18]. Tualang honey is produced by *Apis dorsata*, the bees which build their hives on tualang tree (*Koompasia excelsa*). In our previous study, we have reported that tualang honey reduced hyperglycemia and ameliorated oxidative stress in kidney of STZ-induced diabetic rats [19]. Based on our results, we have hypothesized that hypoglycemic effect of tualang honey might have been mediated partly through ameliorating oxidative stress in the pancreas. In the literature, interest on the role and use of natural antioxidants for prevention of oxidative stress and free radical damage in diabetes has recently increased. So far, there are no available data about the effect of honey on pancreas in STZ-induced diabetic animals. Therefore, this study was carried out to investigate the effect of chronic hyperglycemia on lipid peroxidation and free radical scavenging enzymes in pancreas of normal and diabetic rats supplemented and not supplemented with tualang honey (AgroMas®, Malaysia).

2. Materials and methods

2.1. Chemicals

STZ, tris(hydroxymethyl)aminomethane-HCl (Tris-HCl), thiobarbituric acid (TBA), reduced glutathione (GSH), oxidized glutathione (GSSG) and glutathione reductase (GR) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) assay kits were purchased from Cayman (MI, USA). Bio-Rad protein assay kit was purchased from Bio-Rad (USA). All other chemicals used were of analytical grade.

2.2. Composition and preparation of tualang honey

Tualang honey (AgroMas®, Malaysia) was supplied by Federal Agricultural Marketing Authority (FAMA), Kedah, Malaysia. The composition of tualang honey is presented as follows: total reducing sugar (67.5%) [fructose (29.6%), glucose (30.0%), maltose (7.9%); fructose/glucose ratio (0.99)], sucrose (0.6%) and water (20.0%). It was diluted with 0.5 mL of distilled water and prepared freshly each time it was administered.

2.3. Experimental animals

Twenty-four male Sprague-Dawley rats weighing 250–300 g, housed in a well ventilated animal room at ambient temperature ($25 \pm 2\,^{\circ}C$) with 12-h light and dark cycles, were used in this study. The animals were bred in Laboratory Animal Research Unit of Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia. An ethical approval was obtained from the Animal Ethics Committee of Universiti Sains Malaysia, Malaysia. The care and handling of our animals followed the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes from Helsinki Declaration. Rats had free access to standard chow and drinking water *ad libitum*, unless otherwise stated.

2.4. Induction of diabetes and treatment

After an overnight fast, diabetes was induced by a single dose of STZ (60 mg/kg body weight) administered intraperitoneally in citrate buffer (0.1 mol/L, pH 4.5). Control rats received citrate buffer alone without STZ. Two days after STZ injection, fasting blood samples were collected from the tail vein and used for the estimation of blood glucose concentrations using an Accu-Chek Glucometer (Roche, Germany). Animals with blood glucose concentrations equal to 12 mmol/L or greater with symptoms of diabetes mellitus such as polyuria, polydipsia, polyphagia and