Pathophysiology 22 (2015) 189–194

Contents lists available at ScienceDirect

Pathophysiology

journal homepage: www.elsevier.com/locate/pathophys

Gum Arabic extracts protect against hepatic oxidative stress in alloxan induced diabetes in rats

Abdelkareem A. Ahmed[a,*], Jaafar S. Fedail[b,c], Hassan H. Musa[d], Asghar Ali Kamboh[e], Amal Z. Sifaldin[a], Taha H. Musa[f]

[a] Department of Physiology and Biochemistry, Faculty of Veterinary Science, University of Nyala, P.O Box 155, Sudan
[b] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[c] Department of Biology, Faculty of Education, University of Nyala, Nyala, Sudan
[d] Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan,
[e] Department of Veterinary Microbiology Faculty of Animal Husbandry and Veterinary Science Sindh Agriculture University, 70060 Tandojam, Pakistan
[f] Key Laboratory of Environmental Medicine, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, Jiangsu, China

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 20 August 2015
Accepted 21 August 2015

Keywords:
Gum Arabic
Diabetes type I
Oxidative stress
Rat

ABSTRACT

Gum Arabic (GA) from *Acacia seyal* and *Acacia senegal* is a branched-chain polysaccharide which has strong antioxidant properties, and has been used to reduce the experimental toxicity. Yet, the effects of GA on oxidative stress in type I diabetic rats have not been reported. The aim of the study was to investigate the effects of GA on oxidative stress in Alloxan induced diabetes in rats. The rats were divided into 3 groups ($n = 20$ of each): control group, diabetic group injected with allaoxan, and diabetic group given 15% GA in drinking water for 8 weeks. Oxidative damage to liver tissue was evaluated by measurement of key hepatic enzymes, lipid peroxidation, antioxidant enzymes and expression of oxidative stress genes. Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP*x*) were significantly ($P < 0.05$) increased in GA group compared to diabetic and control groups. Treatment of GA decreased liver malondialdehyde (MDA), and increased glutathione (GSH). In addition, GA was significantly ($P < 0.05$) reduced the activities of key liver enzymes, including alanine transaminase (ALT) and aspartate transaminase (AST). SOD, GP*x* and heat shock protein 70 (HSP70) mRNA were significantly increased in GA group compared to control and diabetic groups. Liver of all diabetic rats showed marked degeneration whereas slight degeneration was observed in GA treated rats compared to control. The results suggest that GA may protect liver by modulating the expression of oxidative stress genes, and thus can improve antioxidant status.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Activity of key antioxidant enzymes plays a critical role in the induction of hyperglycemia-related tissue damage [1]. Oxidative stress induced by the imbalance of oxidants/antioxidants damage of biological macromolecules, including carbohydrates, proteins, lipids, and nucleic acids, cause disturbances in cellular homeostasis and production of other reactive molecules that cause more damage [2]. The importance of oxidative stress and its relationship with the pathology of diabetes mellitus (DM) along with associated complications have been extensively investigated [1,3]. Previous studies reported that the production of reactive oxygen species (ROS) in diabetes initiate the development of chronic diabetic lesions on blood vessels [4], retina [5], kidneys [6], and neurodegenerative diseases [7].

DM is a chronic and most common metabolic disorder that has become epidemic in the twenty-first century [8]. About 347 million people were affected with DM in 2011 worldwide [9]. The World Health Organization predicts that diabetes will be the seventh leading cause of death in 2030. Oxidative stress in DM causes several adverse effects on the cellular physiology [10]. It decreased glutathione (GSH) level diabetes [11], decreased catalase activity [12], downregulated renal SOD [13] and increased heat shock protein 70 (HSP70) level in patients with type 2 [14]. Oxidative stress is has been reported as a key factor in the onset of pathogenesis and diabetic complications [15]. Clinical and experimental studies pro-

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; GA, gum arabic; GP*x*, glutathione peroxidase; SOD, superoxide dismutase.

* Corresponding author. Fax: +249 711833123.
E-mail addresses: kareemo151@gmail.com, kareemo151@vet.nyalau.edu.sd (A.A. Ahmed).

http://dx.doi.org/10.1016/j.pathophys.2015.08.002
0928-4680/© 2015 Elsevier B.V. All rights reserved.

posed that the liver may influenced by DM in the long-term [16–18]. Histological features of fatty liver disease induced by DM and non-alcoholic fatty liver disease (NAFLD) can not be distinguished from ethanol-induced hepatic steatosis [18].

Gum Arabic (GA) is an edible, dried sticky exudate from *Acacia seyal* and *Acacia senegal* is rich with non-viscous soluble fiber. It is commonly used in food industry and pharmaceutical field as an emulsifier and preservative [19]. In North Africa and Middle East, it used as an oral hygiene agent by various communities for several centuries [20]. GA is used in Arabic folk medicine to decrease both frequency and need of hemodialysis in chronic renal failure patients [21]. It has strong antioxidant properties, and used to reduce the experimental nephrotoxicity against gentamicin [21], cisplatin [22] and to ameliorate cardiotoxicity [23]. However, the effects of GA on oxidative stress in liver of type I diabetic rats have not been reported. Whether GA can change oxidative related genes expression in liver of type I diabetic rat remains less clear.

Therefore, in the present study, we used type I diabetic rat model to investigate our hypothesis that supplementation of GA in drinking water may protect liver by reducing oxidative damage, and the reduction of oxidative damage may be associated with modulation of liver oxidative related genes expression.

2. Materials and methods

2.1. Animals and experimental protocol

Male Sprague-Dawley (SD) rats weighing 200 ± 10 g were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Science (SLAC, CAS) and housed in a controlled environment with a 12 h light–dark cycle. Animals were acclimatized for one week before the study and had free access to water and standard rat chow throughout the experimental period. The rats were divided into 3 groups: control group (n = 20) given standard animal pellet and water *ad labitum*; diabetic group (n = 20); and diabetic group (n = 20) given 15% Gum Arabic (GA) in drinking water for 8 weeks. The GA was purchased from Sudanese Company for Gum Arabic (Khartoum, Sudan). The dose of GA and the time duration was chosen according to our previous trials (unpublished data). Type I diabetes mellitus (DM) was induced as described by [24]. Briefly, Alloxan monohydrate was purchased from Sigma–Aldrich China (Shanghai, China), and type I DM was induced by single intraperitoneal injection of 150 mg/kg of Alloxan monohydrate dissolved in normal saline after an overnight fasting. Surviving rats after 3 days with blood glucose concentration more than 200 mg/dL of blood were considered as type I diabetic rat models and used for further investigations. On day 56, the animals were fasted overnight, urine and blood samples were collected prior to euthanasia. Body weights and organ weights were recorded; blood and tissue samples were collected and stored at −80 °C until analyzed.

2.2. Liver functions tests and blood glucose

Serum samples were obtained from blood by centrifugation (at 3000 rpm for 15 min) and stored at −20 °C until analyzed. The hepatic function was evaluated by the measurement of key hepatic enzymes activities. Serum alanine transaminase (ALT), aspartate transaminase (AST), total protein, albumin, globulin (GLB) and fasting blood glucose were measured using assay kits (Nanjing Jiancheng Bioengineering Company, Nanjing, China), according to the manufacturers' instructions. All above assays were measured using automatic analyzer (Hitachi 777) at Nanjing Military Hospital, Nanjing, China.

2.3. Assessment of hepatic lipid peroxidation

Liver lipid peroxidation was evaluated by measuring the amount of malondialdehyde (MDA) using commercial MDA kit from (Nanjing Jiancheng Bioengineering Company, Nanjing, China) as described by [25]. The absorbance of spectrophotometry was assessed at 532 nm. Briefly, approximately, 0.5 g liver samples were homogenized in 4.5 mL of ice-cold PBS buffer for preparing liver homogenate, the homogenates were then centrifuged for 10 min at 3000 rpm and the supernatant was stored at −20 °C until analyzed. Total protein concentration was determined as described by [26] using bovine serum albumin as the standard. The results were expressed as nmol MDA per mg protein.

2.4. Evaluation of hepatic antioxidant enzyme activity

The Superoxide dismutase (SOD), glutathione peroxidase (GP*x*), catalase (CAT) and glutathione (GSH) commercial reagents were purchased from (Nanjing Jiancheng Bioengineering Company, Nanjing, China). Liver tissues (1 g) were cut into small pieces and homogenized in ice-cold saline buffer (0.85%, pH 7.4) (1:9, wt/v) with an Ultra-Turrax (T8, IKA-labortechnik Staufen, Germany). Liver homogenates were centrifuged at 1000 × g for 15 min at 4 °C, and the supernatants were collected. The supernatants were used for the assays of SOD, GP*x*, CAT and GSH. The antioxidative status of liver was estimated by measuring the level of different antioxidants in the liver. The activity of SOD was measured according to the method of [27]. The activities of GP*x* and CAT were measured by the methods described by [28], and [29] respectively. All assays were measured with the clinical chemistry assay kits according to the manufacturer's recommended procedure.

2.5. Histopathology examinations

Livers were fixed in paraformaldehyde solution and embedded in paraffin, sectioned serially at 4 μm and stained with hematoxylin and eosin (H&E) to investigate the morphological changes in control, diabetic and diabetic rats treated with GA. Slides at every time-point were stained with H&E and observed under a light microscope (Nikon, Tokyo, Japan).

2.6. RNA extraction and real-time PCR

About 100 mg of liver was ground in liquid N_2, and a portion of about 50 mg was used for RNA extraction using TRIzol total RNA kit (Invitrogen, Biotechnology Co., Ltd., Carlsbad, CA, USA) according to the manufacturer's instruction. Two approaches were taken to ensure that all the total RNA preparations are free of genomic DNA contamination. First, total RNAs were treated with 10 U DNase I (RNase Free, D2215, Takara, Japan) for 30 min at 37 °C, and purified according to the manufacturer's protocol. Second, the primers for the reference gene (β-actin) were designed to span an intron, so any genomic DNA contamination could be reported easily with an extra product in the melting curves for real-time PCR. Real-time PCR was performed in Mx3000P (Stratagene, USA) according to our previous publications [30,31] Primers specific for SOD, CAT, GP-*x* and HSP70 (Table 5) was synthesized by Geneary (Shanghai, China), and rat β-actin was used as a reference gene for normalization purpose. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data [32]. The mRNA abundances were presented as the fold change relative to the average level of the control group.

2.7. Statistical analysis

Descriptive statistics was performed to check the normality and homogeneity of variances before using parametric analyses.

Download English Version:

https://daneshyari.com/en/article/4136962

Download Persian Version:

https://daneshyari.com/article/4136962

Daneshyari.com