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

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\section*{ABSTRACT}

Gum Arabic (GA) from \textit{Acacia seyal} and \textit{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 1 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 alloxan, 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 (GPx) 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, GPx 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.

\section*{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-

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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 libitum; 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 1 diabetes mellitus (DM) was induced as described by [24]. Briefly, Alloxan monohydrate was purchased from Sigma–Aldrich China (Shanghai, China), and type 1 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

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 (GPx), 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, GPx, 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 GPx 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 N2, 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 Genery (Shanghai, China), and rat β–actin was used as a reference gene for normalization purpose. The method of 2−ΔΔct 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.
Table 1
Effect of GA on organs weight. Data were expressed as means ± S.E.M. of 10/group. Different letters in the rows indicate significantly different mean values at P<0.05.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver weight (g)</th>
<th>Kidney weight (g)</th>
<th>Spleen (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.5 ± 0.34a</td>
<td>1.3 ± 0.04a</td>
<td>2.4 ± 0.2a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>14.5 ± 0.8a</td>
<td>1.7 ± 0.08a</td>
<td>0.9 ± 0.8a</td>
</tr>
<tr>
<td>D + GA</td>
<td>11.7 ± 1a</td>
<td>1.4 ± 0.11a</td>
<td>0.8 ± 0.8a</td>
</tr>
</tbody>
</table>

Table 2
Effect of GA on liver key enzymes activities. Data were expressed as means ± S.E.M. of 10/group. Different letters in the rows indicate significantly different mean values at P<0.05.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Albumin (g/L)</th>
<th>Globulin (g/L)</th>
<th>Total Protein (g/L)</th>
<th>Blood Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.6 ± 3.0a</td>
<td>160.8 ± 15.7a</td>
<td>39.68 ± 2.1a</td>
<td>36.6 ± 2.8a</td>
<td>75.18 ± 4.1a</td>
<td>2.52 ± 0.52a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>76 ± 5.7a</td>
<td>220.25 ± 9.3a</td>
<td>37.3 ± 0.50a</td>
<td>32.23 ± 0.60a</td>
<td>69.53 ± 1.1a</td>
<td>4.63 ± 0.71a</td>
</tr>
<tr>
<td>D + GA</td>
<td>54.6 ± 9.7a</td>
<td>168.8 ± 24.4a</td>
<td>35.92 ± 1.2a</td>
<td>31.46 ± 1.5a</td>
<td>67.38 ± 1.1a</td>
<td>4.28 ± 0.27a</td>
</tr>
</tbody>
</table>

Table 3
Effect of GA on hepatic antioxidant enzymes activities. Data were expressed as means ± S.E.M. of 10/group. Different letters in the rows indicate significantly different mean values at P<0.05.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (U/g)</th>
<th>CAT (μmol/g)</th>
<th>GP-x (μmol/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.56 ± 0.33a</td>
<td>78.47 ± 2.02a</td>
<td>10.72 ± 0.29a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>7.11 ± 0.31a</td>
<td>46.97 ± 0.89a</td>
<td>6.90 ± 0.30a</td>
</tr>
<tr>
<td>D + GA</td>
<td>10.77 ± 0.20a</td>
<td>75.59 ± 6.1a</td>
<td>9.13 ± 0.81a</td>
</tr>
</tbody>
</table>

Body weight, hepatic key enzymes activities, oxidative enzymes activities, lipid peroxidation parameters, as well as the relative quantitative data of gene expression were analyzed by one-way ANOVA using SPSS 16.0 for Windows, followed by a least-significant difference (LSD) test for individual comparisons. A P-value ≤ 0.05 was considered significant.

3. Results

3.1. Effect of GA on body weight and organs weight

Diabetic rat groups that treated with GA showed less body weight during the experimental period compared to the control and diabetic groups (Fig. 1). No significant differences were observed in liver weight and kidney weight between all groups. However, spleen weight for both diabetic rat group and diabetic rat group treated with GA was significantly (P<0.05) decreased compared to the control group (Table 1).

3.2. Effect of GA on liver key enzymes activities

Blood ALT and AST activities are frequently used to evaluate the liver damage. In the present study, diabetic rat group showed significant increases in the activities of liver key enzymes including ALT and AST. The treatment of GA significantly (P<0.05) decreased the activities of ALT and AST when compared to that of diabetic group (Table 2).

3.3. Effect of GA on hepatic antioxidant enzymes activities

Key antioxidant enzymes including SOD, CAT and GPx of rat liver were measured. Diabetic rat group showed significant decreases in all antioxidant enzymes activities when compared to the control. However, the treatment of GA significantly (P<0.05) increased the antioxidant enzymes activities including SOD, CAT and GPx compared to that of control and diabetic groups (Table 3).

3.4. Effect of GA on hepatic lipid peroxidation

Malondialdehyde (MDA) as a biomarker of oxidative stress is routinely used to evaluate the extent of lipid peroxidation. In the
Table 4
Effect of GA on hepatic lipid peroxidation. Data were expressed as means ± S.E.M. of 10/group. Different letters in the rows indicate significantly different mean values at *P* < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>MDA (nmol/g wet tissue)</th>
<th>GSH (mg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.5 ± 4.57a</td>
<td>126.2 ± 3.147a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>97.20 ± 8.72c</td>
<td>95.34 ± 8.92b</td>
</tr>
<tr>
<td>D + GA</td>
<td>32.7 ± 9.41a</td>
<td>120.1 ± 1.62a</td>
</tr>
</tbody>
</table>

3.5. Histopathological changes

Histology of the liver (n = 10) of the control group showed normal hepatocytes morphology with no visible fatty liver changes (Fig. 3A). However, liver (n = 10) of diabetic rats revealed marked degeneration in liver cells, with severe vacuolations (Fig. 3B). The treatment of GA significantly protected the liver (n = 10) of diabetic rats compared with control and untreated diabetic rats (Fig. 3C).

3.6. Effect of GA on hepatic antioxidant genes expression

Q-PCR was used to measure antioxidant enzymes mRNA expression in the liver. Alloxan induced diabetes caused significant decreases in liver SOD and GPx mRNA expression. However, the treatment of GA significantly increased mRNA expression of hepatic SOD (Fig. 2A) and GPx (Fig. 2C) compared to diabetic group. Alloxan induced diabetes caused significant increases in HSP70 mRNA expression compared to the control. But the treatment of GA did not change the mRNA expression of CAT (Fig. 2B) or HSP70 (Fig. 2D).

4. Discussion

A large body of evidence proposes a major role of oxidative stress in development and progression of diabetic complications. Liver function tests are frequently used in clinical practice to monitor liver disorders and to check the progression of known diseases [33]. In the present study, Gum Arabic (GA) reduced body weight and spleen weight. Our results were agreed with earlier report that GA ingestion decreased body weight in human [34]. The reduction of body weight by GA may be due to the fact that high intake of dietary fiber including GA, was associated with beneficial effects on fat metabolism [19,35] or lowering caloric density of food [36]. Unfortunately we did not measure the effect of GA on water/food intake in the present study. In addition, it’s difficult to control the dose of GA extract administration by drinking water.

In the present study, the GA treatment significantly reduced liver alanine transaminase (ALT) and aspartate transaminase (AST) activity. The reduction of ALT and AST activity following GA treatment may be due to the protection of intracellular enzyme leakage, resulting in cell membrane stability or cellular regeneration as indicated in antioxidant plants [37]. Efficient control of albumin and bilirubin may indicate improvement in secretory and functional mechanism of hepatic cells.

Superoxide dismutase (SOD) [38], catalase (CAT) [39], and glutathione peroxidase (GPx) [40] are considered the most important defense mechanisms against reactive oxygen metabolites which implicated in the oxidative damage [10,41]. In the present study, the treatment of Alloxan caused significant decreases in activities of SOD, CAT, and GPx, associated with significant increases in malondialdehyde (MDA) production could be reflects oxidative stress; which may be mirror a decreased antioxidant defense potential [10,42]. However, the treatment of GA significantly increased activity of SOD, CAT and GPx in the liver. GA may act either directly by scavenging the reactive oxygen metabolites, because of the presence of various antioxidant compounds [19,43,44], or via increasing the synthesis of antioxidant molecules. The mechanism of action by which GA improves antioxidant capacity could be due to the fact that GA contains amino acid residues such as lysine, tyrosine and histidine, which are generally considered as antioxidants molecules [45,46]. Moreover, the antioxidant prosperities of GA in biological systems require a more direct knowledge of the antioxidant capacity [19,47].

In the present study, Alloxan induced diabetes significantly increased MDA whereas decreased GSH concentration. Elevation of MDA level together with reduction of glutathione (GSH) in diabetic rats suggests that peroxidative injury may be involved in the development of diabetic complications [48], due to free radical damage which is one of the possible mechanisms in the progress of diabetic hepatopathy [49]. Administration of GA significantly decreased MDA levels which indicate that the GA treatment could improve the antioxidant capacity. Increased oxidative stress in diabetic rats was also reported as a contributory factor in the development of diabetic complications [50,51]. Moreover, the administration of GA significantly protected the degeneration of liver cell.

Increased oxidative stress with impaired antioxidant defense system is thought to be the vital factors leading to the pathogenesis and diabetic complications. In the present study, we used Q-PCR to find out whether steady-state transcription levels were altered. The treatment of GA significantly increased mRNA expression of SOD and GPx but not CAT. The increase in GPx and SOD mRNA together with the increase in their activities may suggest the involvement of post-translational modification in altering the activities of these enzymes [52]. However, the mismatch in CAT activity and mRNA expression in this study may indicates the presence of very complex mechanisms regulating the activity of CAT in order to prevent the effects of oxidative damage [53]. Heat-shock protein 70 (HSP70), a stress-induced protein is proposed to play a protective role against oxidative stress. Here we reported for the first time that GA treatment significantly reduced HSP70 mRNA expression in the liver of diabetic rat. Unfortunately, we did not measure the blood HSP70 level due to the unavailability of kit, since we are very interesting to continue in this direction in the future.

Table 5
Real-time PCR primers.

<table>
<thead>
<tr>
<th>Target genes</th>
<th>PCR products (bp)</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>103</td>
<td>F: 5′-ACACACAGCTGCTACCACTCC-3′</td>
</tr>
<tr>
<td>CAT</td>
<td>115</td>
<td>F: 5′-TGCGTCGACCCATTCCAC-3′</td>
</tr>
<tr>
<td>GP-x</td>
<td>218</td>
<td>F: 5′-GTCACCTGGTATCGCTTCC-3′</td>
</tr>
<tr>
<td>HSP70</td>
<td>241</td>
<td>F: 5′-ATCTCTCTCTCTGTCGCTTAAC-3′</td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td>F: 5′-ATATCGGCAATGAGGGTTECC-3′</td>
</tr>
</tbody>
</table>
Fig. 2. Effect of GA on hepatic mRNA expression of SOD (A), CAT (B), GPx (C) and HSP70 (D). The values are the means ± SEM, n = 10/group. Bars with different letters are significantly different at P < 0.05.

Fig. 3. Effect of GA on liver histopathology control (A), diabetic (B), and diabetic treated with GA (C), n = 10/group. Scale bar 50 μm.

5. Conclusion

We concluded that the GA treatment reduced lipid peroxidation, improved the activities of antioxidant enzymes and their mRNA expression in the liver of diabetic rats. Thus, GA may be useful in reducing oxidative stress. Further investigations are required to find out the effects of GA in diabetic model.

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