Research Article

Protective Effect of Gum Arabic on Liver Oxidative Stress, Inflammation and Apoptosis Induced by CCl₄ in vivo

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Abstract: Gum Arabic (GA) is known exudative polysaccharide from gum acacia trees and has been used as an antioxidant, to protect the liver, kidney and heart against different toxicants. The current study was undertaken to estimate the effect of GA on the inflammatory factors, hepatocytes apoptosis and antioxidant status, of rats suffering from hepatotoxicity caused by Carbon Tetrachloride (CCl₄). Four experimental groups (n=8) were established. Group A used as a control and received basal diets only. Groups (B & D) received basal diets containing GA (5%). Groups (C & D) were injected with CCl₄, to induce hepatotoxicity in rats twice a week, for 3 weeks. GA is found to improve the amount of total protein (TP) in the serum, and the activities of glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC) and superoxide dismutase (SOD). In addition, hepatic expression of glutathione peroxidase 1 (GPX1) and superoxide dismutase 1 (SOD1), were also increased, while effectively decreased the tissue levels of lipid peroxidation (MDA), serum amount of alanine amino transferase (ALT) and aspartate amino transferase (AST). Moreover, GA down-regulate the expression levels of hepatic caspase3, IL-β, IL-6 and TNF-α genes that were increasing in CCl₄ only treated group. Histopathological evaluation of the liver in rats treated only by CCl₄ revealed cellular necrosis, fatty degeneration with inflammatory changes, however, GA revealed a noticeable amelioration of the severity of these changes. Dietary administration of GA has a beneficial result on the hepatic apoptosis, oxidative stress and inflammatory response in experimentally- induced hepatotoxicity in rats.

Keywords: Gum Arabic, CCl₄, oxidative stress, apoptosis, rats, liver.

INTRODUCTION

The liver has a noticeable function in the control of biological processes and homeostasis of the body. It participates in a set of vital functions such as metabolism, storage, secretion, reproduction, and most biochemical pathways. Additionally, detoxification of multiple xenobiotic, chemicals and drugs [1, 2]. Liver disorders are a part of the main threats to public health worldwide [3]. They can be divided into acute or chronic inflammatory conditions and non-inflammatory conditions such as fibrosis, cirrhosis, and apoptosis [4, 3]. The liver may be adversely affected by drugs like sulphonamides, tetracycline, and chemicals such as CCl₄. CCl₄ is utilized as hepatotoxin in various researches to investigate the hepato-protective effects of natural products and plants [5, 6]. Various experimental studies have shown that administration of CCl₄ lead to hepatic damage as well as protecting the lipid peroxidation and hepatocytes apoptosis associated with free radical production [5, 7].

Many natural products are known to show vital functions in the control and management of liver conditions. They act as a natural antioxidant with higher efficiency and lower toxicity than synthetics antioxidants in the prevention of liver disorders [8, 6].

Gum Arabic(GA) is the oldest and well-known dried gummy exudate and branched-chain, a complex polysaccharide from the branches and stems of Acacia senegal and A. seyal. These trees develop largely across sub-Saharan Africa, especially in Sudan [9-12]. According to FAO/WHO Joint Expert Committee; dried exudation of GA was approved for use as hygienic substance and food additives in many food industries [9, 10, 12, 13]. GA is reported to has robust anti-oxidant effects; it has been able to

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amelirote the cardiac, renal and hepatic lipid peroxidation and toxicity, besides its anti-inflammatory, antimicrobial, antidiarrheal, anti-obesity and antihypertensive effects [9, 14, 15]. Moreover, GA has potential hepatoprotective effect, it has been applied to mitigate the acetaminophen-intoxication by reduction of hepatic oxidative stress, blocking of liver macrophage function and nitric oxide scavenging and may prevent liver by improvement of antioxidant status [16-18]. It has been declared to be utilized for the prevention of liver function, histological changes, fatty liver and antioxidant status encouraged by high fat diet, such as trichloroacetate or CCl<sub>4</sub> [19, 10, 20, 18, 21]. Since the effect of GA on CCl<sub>4</sub> induced hepatocytes apoptosis and inflammation still not well understood. Thus, the current study was carryout to detect whether GA mixed diet prevents the liver from CCl<sub>4</sub> induced apoptosis and inflammation in Wistar rats.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

The ALT, AST, TP, SOD, MDA, T-AOC, and GSH-Px, kits, were available from Jiancheng Biotechnology Institute (Nanjing, China). CCl<sub>4</sub> was acquired from Shoude Institute (Nanjing, China). GA (SUPERGUM™ EM 10) was obtained from San-Ei Gen FFI Inc, Japan. Primary monoclonal antibodies for caspase3 and IL-β1 were obtained from Abcam (Cambridge, U.K.).

**Experimental Animals and Treatment**

Thirty two Wistar strain rats weighing between 200 – 210g and 10 to 12 week’s age were available from the Centre of Laboratory Animals, Yangzhou University (Yangzhou, China). The rats were distributed equally into four groups; A, B, C & D (n=8). All rats were housed under controlled light, humidity and temperature. All rats were received a normal diet (ECWA feed, Jos) and water and acclimatized for 1 week prior to use. The dose of GA was mixed with a balanced diet to improve a novel experimental diet. Rats in group A administered olive oil (2 ml kg<sup>-1</sup>, i.p.) twice weekly for 3 weeks. B and D groups were subjected GA (SUPERGUM™ EM 10) at a concentration of 5% of the normal diet [11]. Groups C and D were administered CCl<sub>4</sub> (2 ml kg<sup>-1</sup>, i.p.) after mixed with Olive oil (1:1) twice weekly for 3 weeks [22]. After 3 weeks, rats were kept for 12 h fasting and anesthetized randomly. Blood samples were collected for serum biochemistry and liver specimens were harvested and stored at –80 °C for molecular, antioxidant and histopathological analysis.

**Biochemical Analysis**

The concentrations of AST, ALT and TP were estimated using an automated chemistry analyser (BS-300, Mindray Medical International Limited), according to the guidelines of commercial kits. The MDA, SOD, GSH-Px, and T-AOC, activities were detected from 200 mL homogenized liver tissue after processed according to the advices of the commercial kits.

**Histopathology Examination**

Liver specimens immediately fixedin 10% formaldehyde solution and were processed and cut into 4 μm thickness, then stained with haematoxylin and eosin (H&E) as described in [23].

**Immunohistochemistry Analysis**

Immunohistochemical examination were conducted on Paraffin embedding sections of liver by using mouse monoclonal antibodies against caspase3 as described previously [24].

**mRNA isolation and quantitative real-time PCR determination**

Total RNA was isolated from 50 mg liver tissue by TRizol (Invitrogen, Carlsbad, CA, USA) exaction kit, following the kit’s instructions. Complementary DNA (cDNA) was performed using Reverse transcription (RT) with PrimeScript RT Master Mix Perfect Real Time (Takara Co., Otsu, Japan), according to manufacturer’s instruction. The RT-PCR analysis was performed with ABI Prism 7300 Detection System (Applied Biosystems, USA). SYBR Green (Takara Co., Otsu, Japan) was used for amplification of all cDNA samples. The primer sequences were designed online by Primer-Blast of NCBI and listed in Table-1. mRNA was performed for GPX1, SOD1, IL-β1, IL-6 and TNF-α by using the ΔΔ<sup>CT</sup> method. GAPDH is used as housekeeping gene. All samples were analysed in triplicate.

**Table-1: Primers for Quantitative real-time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer(5′→3′)</th>
<th>Antisense primer(5′→3′)</th>
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<tbody>
<tr>
<td>GPX1</td>
<td>ACAGGCCGAATATTTTCTGGG</td>
<td>ATTCTCTCTCCTGCTGCTGCTG</td>
</tr>
<tr>
<td>SOD1</td>
<td>TGGAGATGACCAACACAAACGG</td>
<td>AAGACCTCTTITGACACCTG</td>
</tr>
<tr>
<td>TNF-α</td>
<td>AGCGGATCTAGGCGGGGAAGA</td>
<td>TGTCCTGCTCCTTGTGTCG</td>
</tr>
<tr>
<td>IL-6</td>
<td>CTCTCTGCGGGATCGCTTGT</td>
<td>CTCCCCAGFTGCGCTTGTGAT</td>
</tr>
<tr>
<td>IL-β1</td>
<td>CACGGATCTAGGCGCTGCC</td>
<td>AGATCCTGAGCCTGATG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GAAGGTGCAAGAAGGAT</td>
<td>CCCGCTCTCAGGCCATGAGT</td>
</tr>
</tbody>
</table>
Statistical Analysis

Data analyses were performed by using SPSS Statistics 19 for Windows. Each group consisted of 8 animals. The statistical significance and differences between groups were determined by using analysis of variance (ANOVA) and Duncan’s contrasts that expressed as means ± SEM.

Ethical Approval

The animals’ experiment in this study was approved by the Animal Care Committee for laboratory animal experiments of Nanjing Agricultural University (Certification No.: SYXX (Su) 2011-0036). And with “Principles of Laboratory Animal Care and Use in Research” State Council of China, 1988.

RESULTS

Effect of GA on Serum TP and Liver Markers

The activities of AST and ALT are used as enzymatic markers for assessment of liver injury. The serum activity of AST was substantially (P=0.000) elevated in the rat treated only by CCl₄ treatment group opposed to the control rats. Gum Arabic supplementation during CCl₄ treatment suggestively lowered (P=0.000) the serum level of AST as compared with those of CCl₄-treated animals (Fig-1B). Serum activity of ALT substantially (P=0.000) elevated in the CCl₄ treatment animals opposed to the control animals. Gum Arabic administration during CCl₄-treatment significantly lowered (P=0.000) the serum activity of ALT opposed to those of CCl₄-treated animals (Fig-1A). The serum amount of TP was considerably (P=0.009) declined in the CCl₄- treatment rats opposed to those of control rats. GA plus CCl₄ administration obviously increased (P=.031) the amount of TP as compared with rats treated with CCl₄ alone (Fig-1C).

Effect of Gum Arabic on histological assessment of hepatic necrosis and inflammation

Histopathological examination of the liver revealed severe cellular necrosis, with extensive inflammatory cells infiltration and hepatocyte vacuolization or fatty degeneration in the rats treated only by CCl₄ (Fig-2C) opposed to the GA or normal rat liver tissue section (Fig 2A & B). Rats treated with GA plus CCl₄ lowered this morphological damage (Fig-2D).

Fig-1: Effect of Gum Arabic on CCl₄ caused alterations in the serum total protein and liver enzymes of different groups. (A) alanine aminotransferase; (B) aspartate aminotransferase; and (C) total protein. Data were presented as mean ± SEM (n=8). Columns with different letters differ significantly.
Fig-2: Haematoxylin and eosin stained sections of liver (Scale bar = 50 μm). (A) control group, (B) Gum Arabic group, (C) CCl4 group, (D) CCl4+ Gum Arabic group

Effect of Gum Arabic on T-AOC, lipid peroxidation and antioxidant enzymes

Total antioxidant capacity and enzymes play an essential role in the defence system against free radicals. As shown in Fig. 3A, levels of T-AOC significantly (P=0.000) lowered in the rats only treated with CCl4 opposed to the control rats, while GA + CCl4, increased (P=0.033) the T-AOC production opposed to the rats treated only by CCl4 (Fig 3A a). The levels of SOD significantly (P=0.002) lower in the rats treated only by CCl4 opposed to the control rats, while GA plus CCl4, increased (P=0.003) the SOD activity opposed to rat treated only by CCl4 (Fig. 3A c). The levels of GSH-Px significantly (P=0.000) reduced in the rats treated only by CCl4 when compared to the control rats, while GA plus CCl4, increased (P=0.002) the GSH-Px activity opposed to CCl4 alone treated rats (Fig 3A b). Rat treated with CCl4 alone significantly augmented (P=0.000) MDA opposed to control, while GA + CCl4, decreased (P=0.008) in MDA activity opposed to CCl4 alone treated rats (Fig 3A d). This result was supported by normalized mRNA expression of GPX and SOD1 (Fig-3B). The CCl4 alone treated rats, considerably (P=0.000) decreased the expression levels of GPX opposed to controls. While the expression levels of this gene in the GA + CCl4 rats were substantially increased (P=0.000). The CCl4–treated group, significantly (P=0.000) decreased the expression levels of SOD1 opposed to controls. While the expression levels of this gene in the GA + CCl4 rats were substantially increased (P=0.000).
Effects of Gum Arabic on CCl₄-induced hepatic inflammation

To evaluate whether GA could prevent inflammation caused by CCl₄, we investigated the normalized mRNA expression levels of some inflammatory factors such as; TNF-α, IL-β1 and IL-6 genes in the hepatic tissue of rats as shown in Fig-4A. Carbon tetrachloride alone treated rats have higher (P=0.000) expression level of IL-β1 gene opposed to the control rats. In contrast, the expression levels of this gene in the GA plus CCl₄ group was substantially (P=0.003) decreased opposed to CCl₄ alone treated rats. The group treated only by CCl₄ has higher (P=0.000) expression level of IL-6 gene opposed to the control rats. Meanwhile, the expression level of this gene in the GA + CCl₄ group were substantially (P=0.000) decreased opposed to the group treated only by CCl₄. Rats treated only by CCl₄ has higher (P=0.000) expression levels of TNF-α gene opposed to the control rats, while the expression levels of this gene in the GA plus CCl₄ group was considerably (P=0.011) decreased opposed to the group treated only by CCl₄. This result was further supported by immunofluorescence staining of IL-β1 as shown in Figure 4B. Immunofluorescence examination showed red signals indicating IL-β1 was existing obviously in the cytoplasm with a little amount in the nuclei of hepatic cells in rats only treated by CCl₄ (Figure 4B; c) opposed to control rats (Figure 4B; a). Gum Arabic and GA+CCl₄ groups exhibited a moderate red staining of IL-β1 (Figure 4B; b, d).

Effects of Gum Arabic on CCl₄-induced hepatocytes apoptosis

To evaluate whether GA could prevent apoptosis caused by CCl₄, we measured the normalized mRNA expression levels of hepatic caspase3 as shown in Fig-5A. Carbon tetrachloride alone treated rats have higher (P=0.000) expression levels of caspase3 gene opposed to the control rats. In contrast, the expression levels of this gene in the GA plus CCl₄ group was substantially (P=0.049) decreased opposed to the group treated only by CCl₄ alone. This result was confirmed by immunohistochemistry staining of caspase3 as shown in Figure 5B. The group only treated by CCl₄ showed extensive expression of caspase3 (Figure 5B; c), compared to group treated with GA or control rats (Figure 5B; a & b), while the GA plus CCl₄ group effectively mitigated the expression of caspase3 near to control levels.

DISCUSSION

Recent studies indicated that administration of carbon tetrachloride (CCl₄) is effectively induces oxidative damage, increasing inflammatory cytokines and apoptosis in experimental rats due to the production of reactive oxygen species [25-27]. Gum Arabic (GA) has strong antioxidant properties; therefore it could be...
one of the mechanisms of hepatoprotective [28, 10, 21]. Liver enzymes such as ALT and AST are commonly utilized as essential indicators for evaluation of hepatic damage. Carbon tetrachloride may increase the amount of ALT and AST enzymes in the serum due to increased membranous permeability and hepatocellular necrosis [28, 29]. The administration of GA may increase the ability of hepatocytes to defend against the harmful action of CCl₄ due to its antioxidant effects. Moreover, CCl₄ administration effectively reduced total serum protein, due to less numbers of hepatocytes able to protein synthesis after hepatic necrosis [30, 31]. In the present study, GA efficiently restored the total serum protein. Furthermore, the histopathological results of the liver from different groups of rats revealed that, various pathological changes such as severe cellular necrosis, cytoplasmic vacuolization and fatty degeneration with extensive infiltration of inflammatory cells due to application of CCl₄ while CCl₄ plus GA supplementation mitigated all of these abnormalities and confirmed its hepatoprotective and anti-inflammatory efficacy.

The antioxidant enzymes are considered the best essential defence system against free radicals which involved in all types of oxidative injuries [32, 33]. A relationship between hepatotoxicity and oxidative stress has been studied in numerous experimental animals. In our study, intra peritoneal administration of CCl₄ induced substantial reductions in the amount of SOD, GSH-Px and T-AOC, accompanied with considerable increases in MDA activities that indicated lipid peroxidation and oxidative stress injury in hepatocytes; which may decrease the antioxidant resistance potential [10, 14, 32]. Conversely, the administration of GA and GA plus CCl₄ significantly elevated the levels of hepatic TAOC, SOD and GSH-Px and decreased the stages of lipid peroxidation (MDA). Gum Arabic may support these actions by elevating the synthesis of antioxidant compounds or by scavenging the free radicals, then occurrence of numerous antioxidant molecules [34, 9, 35]. In this study, we utilized qRT-PCR to detect whether steady-state transcription activities were changed. Supplementation of GA plus CCl₄ had substantially improved mRNA expression levels of SOD1 and GPX1. The elevation in SOD1 and GPX1 mRNA levels with the elevation in their protein levels may suggest the participation of post-translational alteration in modifying the levels of these antioxidants enzymes [28, 36].

The level of pro-inflammatory mediators; such as TNF-α, IL-β1and IL-6, play vital function in liver injury and inflammation. They are produced in great amounts by the hepatocytes endothelial cells and Kupffer cells in response to inflammation and liver damage caused by several chemicals including trichloroacetate and CCl₄ [37, 10, 38]. Thus, in this study, to detect the inflammatory status of the liver, some pro-inflammatory parameters were confirmed through measurements the expression stages of TNF-α, IL-β1 and IL-6 by qRT-PCR or expression of IL-β1 by immunofluorescence staining, and we found that CCl₄ elevated the expression of these genes while GA supplementation to CCl₄-treated rats considerably inhibited the up-regulation of these genes. The inhibition of these pro-inflammatory cytokines and free radicals together could suggest that GA has anti-inflammatory properties [34, 20, 39].

The initiation of specific proteases including caspases3 has been suggested to be an important mechanism of apoptosis [27]. Carbon tetrachloride could activate the hepatocyte apoptosis through mitochondria release of pro-apoptotic proteins in caspase-independent cell death [40, 26, 27]. Our results indicate that CCl₄ elevated the expression of caspase 3 while GA supplementation to CCl₄-treated rats considerably inhibited the up-regulation of this gene. This may reflects the anti-apoptotic effect of GA [41, 42].

Limitations and Future Perspectives
Future work is required. That includes the in vitro experiments to evaluate the effect of GA on the CCl₄-induced apoptosis in the individual hepatocytes and the associated mechanisms.

IN CONCLUSION
Our results demonstrate that, GA has anti-hepatotoxic effects, and this protective action may be attributed to its ability to dropping the amount of free radicals that decreasing lipid peroxidation and block the oxidative stress, and its action on the extent of hepatocellular necrosis, inflammation and hepatocytes apoptosis. Therefore, GA could be potential hepatoprotective dietary agent against CCl₄-induced liver inflammation and apoptosis in vivo.

ACKNOWLEDGMENTS
The authors are extremely thankful to Professor Kehe Huang and Mrs Dandan Liu at Nanjing Agricultural University, China for their provision to carry out this research.

Conflict of interest: The authors declare that they have no competing interests.

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