Protective effect of arabic gum against acetaminophen-induced hepatotoxicity in mice

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Abstract

Overdose of acetaminophen, a widely used analgesic drug, can result in severe hepatotoxicity and is often fatal. This study was undertaken to examine the effects of arabic gum (AG), which is commonly used in processed foods, on acetaminophen-induced hepatotoxicity in mice. Mice were given arabic gum orally (100 g l\(^{-1}\)) 5 days before a hepatotoxic dose of acetaminophen (500 mg kg\(^{-1}\)) intraperitoneally. Arabic gum administration dramatically reduced acetaminophen-induced hepatotoxicity as evidenced by reduced serum alanine (ALT) and aspartate aminotransferase (AST) activities. Acetaminophen-induced hepatic lipid peroxidation was reduced significantly by arabic gum pretreatment. The protection offered by arabic gum does not appear to be caused by a decrease in the formation of toxic acetaminophen metabolites, which consumes glutathione, because arabic gum did not alter acetaminophen-induced hepatic glutathione depletion. Acetaminophen increased nitric oxide synthesis as measured by serum nitrate plus nitrite at 4 and 6 h after administration and arabic gum pretreatment significantly reduced their formation. In conclusion, arabic gum is effective in protecting mice against acetaminophen-induced hepatotoxicity. This protection may involve the reduction of oxidative stress.

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1. Introduction

An overdose of the widely used analgesic drug acetaminophen causes hepatotoxicity [1,2]. Although a large dose of acetaminophen is directly conjugated with glucuronic acid or sulfate and excreted, a significant amount of acetaminophen is metabolized by the cytochrome P450 system [3]. This leads to the formation of a reactive metabolite, presumably N-acetyl-p-benzoquinoneimine (NAPQI), which reacts rapidly with glutathione [3]. Thus, acetaminophen causes dramatic depletion of cellular glutathione levels in the liver [4]. If the formation of NAPQI exceeds the capacity of hepatocellular GSH, it will covalently bind to cellular proteins leading to hepatocellular injury [5].

The mechanism of hepatocellular injury after the initial NAPQI formation, glutathione depletion and covalent binding to proteins is still unclear. However, recent studies have suggested that macrophages and formation of reactive oxygen and nitrogen species are involved in the development of toxicity of acetaminophen. Pretreatment of mice with gadolinium chloride or dextran sulfate to inactivate macrophages have been shown to dramatically decrease acetaminophen toxicity in mice [6–8]. In the acetaminophen-treated mice there was a direct correlation between nitric oxide synthesis, as measured by serum levels of nitrate plus nitrite, and hepatotoxicity [9]. It was also reported that toxic doses of acetaminophen lead to induction of the inducible nitric oxide synthase and that pretreatment of mice with a nitric oxide synthase inhibitor, aminoguanidine, decreases the toxicity [10].

Arabic gum (AG) is a naturally occurring compound consisting of calcium, magnesium, and potassium salts of the polysaccharide of arabic gum acid [11,12]. Ingested orally, arabic gum is non-toxic. It is used extensively in pharmaceutical preparations and in most categories of processed foods, to as high as 45% in candy products [13]. In folk medicine, arabic gum is used internally in inflammation of intestinal mucosa, and externally to cover inflamed surfaces.
It has been reported that arabic gum has nitric oxide scavenging properties [14]. Moreover, arabic gum was found to block the hepatic macrophage function [15–17]. Since, nitric oxide and hepatic macrophages are important mediators of acetaminophen-induced hepatotoxicity [6–10]. The present study was undertaken to test whether oral administration of arabic gum could protect mice from acetaminophen-induced hepatotoxicity.

2. Materials and methods

2.1. Chemicals

Acetaminophen was purchased from Merck (Germany). Arabic gum was purchased from Riedel-deHaen, D3010 Seelze, Germany. Thiobarbituric acid was a product of Fluka (Buchs, Switzerland). N-(1-Naphthyl) ethylenediamine dihydrochloride, sulfanilamide, vanadium trichloride, sodium nitrate and absolute methanol were all purchased from Sigma-Aldrich and were used without further purification. Vanadium trichloride was stored in the dark under vacuum. All other chemicals were of the highest analytical grade and obtained from commercial suppliers. Acetaminophen was dissolved in saline just before use. Arabic gum was dissolved in drinking water.

2.2. Animals

Male Swiss albino mice weighing 25–30 g were used in all experiments. They were obtained from the Experimental Animal Care Center, King Saud University, Riyadh, Saudi Arabia. They were housed under conventional laboratory conditions in a room temperature maintained at 25 ± 1 °C and a relative humidity range of 40–75% with a regular 12 h light:12 h dark cycle. The mice were fed a standard animal pellet diet and allowed free access to water unless otherwise indicated. Experiments were approved by a local ethical committee of college of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.3. Experimental protocol

In this experiment, mice were randomly allocated into 10 groups, each consisting of eight animals. All animals were fasted 24 h before the experiment [18]. The first group, the control group (C), received saline. The second group, the arabic gum group, received AG 100 g l−1 in drinking water which is equivalent to 25 g kg−1 per day p.o. [19]. The 3rd, 4th, 5th and 6th groups, acetaminophen groups, were treated with a single dose of acetaminophen (500 mg kg−1 i.p.) and killed after 2, 4, 6 and 24 h, respectively. The 7th, 8th, 9th and 10th groups (AG + acetaminophen) were given AG (25 g kg−1 per day p.o.) for 5 days then given a single injection of acetaminophen (500 mg kg−1 i.p.) and killed after 2, 4, 6 and 24 h, respectively.

2.4. Biochemical assessment of hepatotoxicity

Before sacrificing the animals, blood samples were collected from the orbital venous plexus, under light ether anaesthesia, into non-heparinized capillary tubes. Serum was separated by centrifugation for 5 min at 1000 × g and stored at −20 °C until analysis. Animals were sacrificed by cervical dislocation and the livers were quickly isolated, washed with saline, plotted dry on a filter paper and weighed. Homogenization was carried out in ice-cold KCl (1.15%, pH 7.4) to yield a 10% (w/v) tissue homogenates using Glas-Col homogenizer (USA) and the following biochemical parameters were assessed.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined according to the methods of Bergmeyer et al. [20]. Tissue lipid peroxides (LP) level was determined as thiobarbituric acid-reactive substances, measured as malondialdehyde (MDA) [21]. The absorbance was measured photometrically at 532 nm and the concentrations are expressed as nmol MDA g−1 liver tissue.

Tissue levels of acid soluble thiols, mainly glutathione, were determined colourimetrically at 412 nm [22]. Homogenates were precipitated with 5% perchloric acid and after centrifugation at 1000 × g for 5 min, supernatants were used for the estimation of GSH level. The concentration of GSH is expressed as μmol g−1 liver tissue.

The serum level of total nitrate/nitrite (nmol·ml−1) was determined by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride. The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfanilamide and N-(1-naphthyl) ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm. Prior to the Griess reaction all nitrate is converted to nitrite using vanadium trichloride as described in [23]. Serum proteins were precipitated by absolute methanol and after centrifugation, supernatants were used for estimation of nitrate/nitrite level.

2.5. Statistical analysis

Data are expressed as means ± S.E.M. Statistical comparison between different groups were done using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Significance was accepted at P < 0.05.

3. Results

In agreement with previous studies, a dose of 500 mg kg−1 i.p., of acetaminophen caused significant liver injury in mice at 24 h after dosing, as indicated by the substantial increase in serum ALT and AST activities. Pretreatment with arabic gum was protective, as indicated by significant reduction of ALT and AST activities (Table 1
Acetaminophen was administered at 500 mg kg\(^{-1}\) i.p. and arabic gum was administered p.o. in the drinking water (100 g l\(^{-1}\)) 5 days before acetaminophen. Serum ALT and AST activities were measured 24 h after acetaminophen treatment. Results are expressed as mean ± S.E.M. of eight mice and data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparisons test.

Acetaminophen treatment caused depletion of hepatic glutathione levels at 2 h after acetaminophen intoxication. The glutathione levels recovered to values 80 and 90% of untreated controls at 4 and 6 h, respectively (Fig. 2). At 24 h after acetaminophen treatment, glutathione content was significantly increased (Table 1) which indicated an intracellular oxidant stress. Arabic gum did not alter acetaminophen-induced hepatic glutathione depletion (Fig. 2).

Serum levels of nitrate plus nitrite were determined in the acetaminophen group. Fig. 3 shows that serum levels of nitrate plus nitrite were significantly increased by 4 and 6 h. Pretreatment of mice with arabic gum significantly decreased the serum levels of nitrate plus nitrite (Fig. 3).

4. Discussion

The results of the study demonstrated that arabic gum protects mice from acetaminophen-induced liver injury as
It has been reported that arabic gum has nitric oxide scavenging properties and macrophage inhibition functions. It has been shown that arabic gum has been added to the drinking water (100 g l⁻¹) 5 days before acetaminophen treatment. Serum nitrate plus nitrite was measured at 2, 4 and 6 h after acetaminophen treatment. Results are expressed as mean±S.E.M. of eight mice and data were analyzed by one-way ANOVA, followed by Tukey-Kramer multiple comparisons test. *Significant increase (P<0.01) compared to acetaminophen-s arabic gum. **Significant increase (P<0.001) compared to acetaminophen-s arabic gum.

The biochemical mechanism involved in the development of acetaminophen-induced hepatotoxicity is well documented [6–9]. Metabolism of acetaminophen to reactive metabolite N-acetyl-p-benzoquinoneimine and its detoxification by glutathione are critical determinants in acetaminophen toxicity [3,4]. We examined glutathione depletion in presence and absence of arabic gum. The protection afforded by arabic gum does not appear to be caused by a decrease in the formation of toxic acetaminophen metabolites which consumes glutathione, because arabic gum did not alter acetaminophen-induced hepatic glutathione depletion (Fig. 2).

Under conditions of glutathione depletion reactive nitrogen and oxygen species may both mediate cytotoxicity [6–9]. To better understand the protective effect of arabic gum in acetaminophen-induced hepatotoxicity, serum levels of nitrate plus nitrite at 2, 4 and 6 h after administration in agreement with other study [9]. Pretreatment of mice with arabic gum were found to produce significantly less nitric oxide (nitrate + nitrite) than acetaminophen-treated mice. It has been reported that arabic gum has nitric oxide scavenging properties [14] and macrophage inhibition functions [15–17], for example when Arabic gum was added to the medium of rat hepatic macrophages cultured with normal rat sera, their ability to produce super oxide anions was reduced in a dose-related manner. This may account for its hepatoprotective effect against acetaminophen-induced hepatotoxicity. The results are in agreement with previous observations that arabic gum protects against doxorubicin-induced cardiotoxicity [19] and gentamycin-induced nephrotoxicity [24,25], where oxidative stress is a common denominator of these models of toxicity.

In summary, oral administration of arabic gum protected mice from acetaminophen-induced hepatotoxicity. The protection is not through the change in metabolism of acetaminophen but may be due to reduction of oxidative stress. These observations suggest that arabic gum may find clinical application in a variety of conditions where cellular damage is a consequence of oxidative stress.

References
