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

Ayman M. Gamal el-din, Adel M. Mostafa, Othman A. Al-Shabanah,
Abdullah M. Al-Bekairi, Mahmoud N. Nagi*

Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

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.

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.

* Corresponding author. Fax: +9661-4677200.
  E-mail address: mnagi@ksu.edu.sa (M.N. Nagi).

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 $25 \pm 1\,^{\circ}\text{C}$ 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 $100\,\text{g}\,\text{l}^{-1}$ in drinking water which is equivalent to $25\,\text{g}\,\text{kg}^{-1}$ per day p.o. [19]. The 3rd, 4th, 5th and 6th groups, acetaminophen groups, were treated with a single dose of acetaminophen ($500\,\text{mg}\,\text{kg}^{-1}$ i.p.) and killed after 2, 4, 6 and 24 h, respectively. The 7th, 8th, 9th and 10th groups (AG + acetaminophen) were given AG ($25\,\text{g}\,\text{kg}^{-1}$ per day p.o.) for 5 days then given a single injection of acetaminophen ($500\,\text{mg}\,\text{kg}^{-1}$ 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 $1000 \times g$ and stored at $-20\,^{\circ}\text{C}$ 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 malondialdhyde (MDA) [21]. The absorbance was measured photometrically at 532 nm and the concentrations are expressed as $\text{nmol}\,\text{MDA}\,\text{g}^{-1}$ 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 $1000 \times g$ for 5 min, supernatants were used for the estimation of GSH level. The concentration of GSH is expressed as $\mu\text{mol}\,\text{g}^{-1}$ liver tissue.

The serum level of total nitrate/nitrite ($\text{nmol}\,\text{ml}^{-1}$) 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, sulfonamide 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 $500\,\text{mg}\,\text{kg}^{-1}$ 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

Table 1

Effect of acetaminophen administration on serum alanine and aspartate aminotransferase activities in mice

Treatments	ALT (U l^{-1})	AST (U l^{-1})
Control	60 ± 11	321 ± 19
Arabic gum	49 ± 2	298 ± 14
Acetaminophen	4065 ± 447*	2111 ± 141*
Arabic gum + acetaminophen	92 ± 12**	331 ± 19**

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.

  * Significant increase ($P < 0.001$) compared to control.
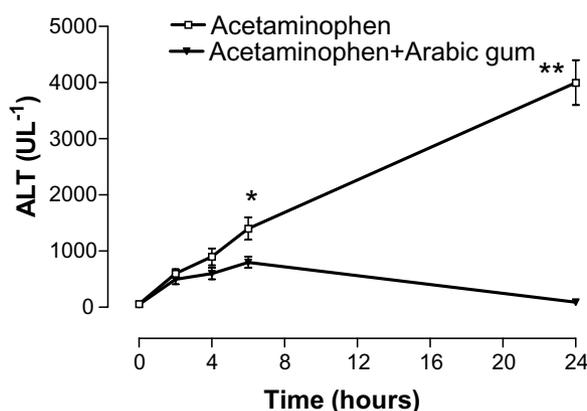 ** Significant decrease ($P < 0.001$) compared to acetaminophen.

Fig. 1. Time course for appearance of serum ALT activity following toxic dose of acetaminophen in mice and in mice pretreated with arabic gum. 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 was measured at 2, 4, 6, and 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. *Significant increase ($P < 0.01$) compared to acetaminophen + arabic gum; **Significant increase ($P < 0.001$) compared to acetaminophen + arabic gum.

and Fig. 1). Similarly, hepatic lipid peroxides level was significantly decreased in arabic gum-treated group compared to mice treated with acetaminophen alone (Table 2).
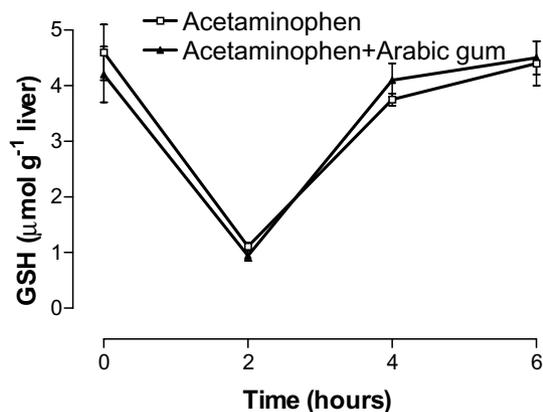

Fig. 2. Time course for depletion of hepatic glutathione following toxic dose of acetaminophen in mice and in mice pretreated with arabic gum. Acetaminophen was administered at 500 mg kg^{-1} i.p. and arabic gum was administered p.o. in the drinking water (100 g l^{-1}) 5 days before acetaminophen. Liver glutathione 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. No significant difference between the two groups.

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

Table 2

Effect of acetaminophen administration on hepatic lipid peroxides, glutathione and serum nitrate plus nitrite levels in mice

Treatments	Hepatic MDA (nmol g^{-1})	Hepatic GSH (µmol g^{-1})	Serum nitrate + nitrite (nmol ml^{-1})
Control	464 ± 44	4.6 ± 0.5	42.1 ± 0.2
Arabic gum	435 ± 30	4.1 ± 0.8	29.2 ± 0.8
Acetaminophen	2038 ± 208*	5.9 ± 1.1**	43.8 ± 5.2
Arabic gum + acetaminophen	512 ± 21***	5.1 ± 0.3	35.7 ± 3

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 nitrate plus nitrite, liver glutathione and lipid peroxides 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.

  * Significant increase ($P < 0.001$) compared to control.
  ** Significant increase ($P < 0.05$) compared to control.
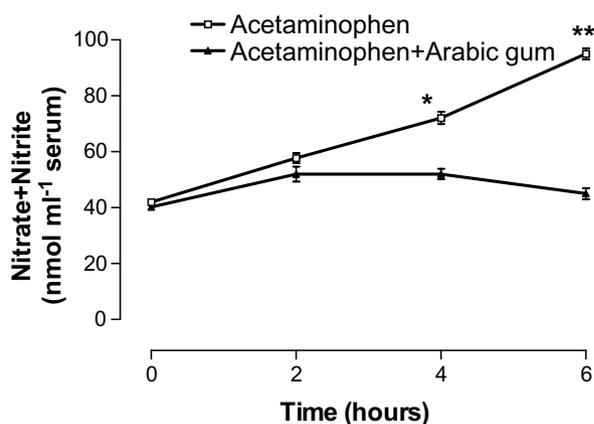 *** Significant decrease ($P < 0.001$) compared to acetaminophen.

Fig. 3. Time course for appearance of serum nitrate plus nitrite following toxic dose of acetaminophen in mice and in mice pretreated with arabic gum. 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 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+arabic gum; **Significant increase ($P < 0.001$) compared to acetaminophen + arabic gum.

evident from the significant decrease in serum ALT and AST and hepatic lipid peroxides.

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 were determined. These levels can be utilized as a biomonitor of nitric oxide synthesis. We found that acetaminophen caused a significant increase in nitrate plus nitrite at 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 hepatotox-

icity. 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

[1] Boyd ED, Bereczky GM. Liver necrosis from paracetamol. Br J Pharmacol 1966;26:606–14.

[2] Boyer TD, Rouff SL. Acetaminophen-induced hepatic necrosis and renal failure 1971;218:440–1.

[3] Nelson SD. Molecular mechanisms of hepatotoxicity caused by acetaminophen. Semin Liver Dis 1990;10:267–78.

[4] Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodi BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 1973;187:211–7.

[5] Jollow DJ, Michell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis 2. Role of covalent binding in vivo 1973;187:195–202.

[6] Goldin RD, Ratnayaka ID, Breach CS, Brown IN, Wichramasingfe SN. Role of macrophages in acetaminophen (paracetamol)-induced hepatotoxicity. J Pathol 1996;179:432–5.

[7] Michael SL, Pumford NR, Mayeux PR, Niesman MR, Hinson JA. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatology 1999;30:186–95.

[8] Laskin DL, Gardner CR, Price VF, Jollow DJ. Modulation of macrophage functioning abrogates the acute hepatotoxicity of acetaminophen. Hepatology 1995;21:1045–50.

[9] Michael SL, Mayeux PR, Bucci TJ, Warbrtton AR, Irwin LK, Pumford NR, et al. Acetaminophen-induced hepatotoxicity in mice lacking inducible nitric oxide synthase activity. Nitric Oxide 2002; 6:160–7.

[10] Gardner CR, Heck DE, Yang CS, Thomas PE, Zhang XJ, de George GL, et al. Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat. Hepatology 1998;26:748–54.

[11] Phillps GO. Acacia gum (gum Arabic): a nutritional fiber metabolism and calorific value. Food Addit Contam 1998;15:251–64.

[12] Bliss DZ, Stein TP, Schleifer CR, Settle RG. Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. Am J Clin Nut 1995;63:392–8.

[13] Leung AY. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. New York: Wiley; 1980.

[14] Rehman K, Wingertzahn MA, Harper RG, Wapnir RA. Proabsorptive action of gum arabic: regulation of nitric oxide metabolism in the basolateral potassium channel of the small intestine. J Pediatr Gastroenterol Nutr 2001;35:529–33.

[15] Mochida S, Ogata I, Hirata K, Ohata Y, Yamada S, Fujiwara K. Provocation of massive hepatic necrosis by endotoxin after partial hepatectomy in rats. Gastroenterology 1990;99:771–7.

[16] Fujiwara K, Ogata I, Mochida S, Yamada S, Hirata K, Tomiya T, et al. Activated Kupffer cells as a factor of massive hepatic necrosis after liver resection. Hepato-Gastroenterology 1990;37:194–7.

[17] Fujiwara K, Mochida S, Nagoshi S, Iijima O, Matsuzaki Y, Takeda S, et al. Regulation of hepatic macrophage function by oral administration of Xiao-Chai-Hu-Tang (Sho-Saika-to, TJ-9) in rats. J Ethnopharm 1995;46:107–14.

[18] Walker RM, Massey TE, McElligott TF, Racz WJ. Acetaminophen toxicity in fed and fasted mice. Can J Physiol Pharmacol 1982;60:399–404.

[19] Abd-Allah AR, Al-Majed AA, Mostafa AM, Al-Shabanah OA, Gamal El-Din A, Nagi MN. Protective effect of arabic gum against cardiotoxicity induced by doxorubicin in mice: a possible mechanism of protection. J Biochem Mol Toxicol 2002;16(5):254–9.

[20] Bergmeyer HU, Schiebe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem 1978;24:58–73.

[21] Ohkawa H, Ohish N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid. Anal Biochem 1997;95:351–8.

[22] Ellman G. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70.

[23] Miranda K, Espey M, Wink D. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide: Biology and Chemistry 2001;5(1):62–71.

[24] Ali BH, Al-Qarawi AA, Haroun EM, Mousa HM. The effect of treatment with gum arabic on gentamicin nephrotoxicity in rats: a preliminary study. Ren Fail 2003;25(1):15–20.

[25] Al-Majed AA, Mostafa AM, Al-Rikabi AC, Al-Shabanah OA. Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. Pharmacol Res 2002;4(5):445–51.