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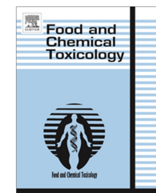

Hepatoprotective activity of puerarin against carbon tetrachloride -induced injuries in rats: A randomized controlled trial

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The protective effects of puerarin on liver damage were evaluated by carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Male rats were orally treated with puerarin daily, and received CCl_4 intraperitoneally twice a week for 4 weeks. Our results showed that puerarin at doses of 50, 100, and 200 mg/kg b.w. significantly reduced the elevated activities of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase at least 15%, 17%, 14% and 18%, respectively. In addition, puerarin at different doses significantly decreased ($p < 0.05$) the level of hepatic thiobarbituric acid reactive substances compared to the CCl_4-treated group. Furthermore, the treatment of puerarin was also found to significantly increase the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, and glutathione content at least 40%, 12%, 25%, 52%, 17% and 44% in the liver of CCl_4-treated rats, respectively. Liver histopathology also showed that puerarin reduced the incidence of liver lesions induced by CCl_4. The results suggest that puerarin exhibits potent hepatoprotective effects on CCl_4-induced liver damages in rats, and that the hepatoprotective effects of puerarin may be due to both the inhibition of lipid peroxidation and to increase of antioxidant enzymes activity.

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1. Introduction

Hepatotoxicity is the most widespread pathology worldwide, representing up to 83% of all cases (Khan et al., 2012). More than 600 chemicals can cause injury in liver, such as carbon tetrachloride (CCl_4), ethanol, and acetaminophen (Cengiz et al., 2013; Hsu et al., 2009). CCl_4 is a xenobiotic that produces hepatotoxicity in human as well as in various experimental animals (Pinto et al., 2012; Rudnicki et al., 2007). During phase I of hepatotoxicity, cytochrome P450 metabolises CCl_4 to trichloromethyl radical ($^{\cdot}CCl_3$) and trichloromethyl peroxy radical ($^{\cdot}OOCCl_3$) that are assumed to initiate free radical-mediated lipid peroxidation (Khan et al., 2012). In phase II, CCl_4 is known to reduce antioxidant enzymes and substrates (such as superoxide dismutase, catalase, glutathione peroxidase, lipid peroxidase, and glutathione) and induce oxidative stress, which is an important factor in acute and chronic injury in liver tissue, and finally induce corresponding health problems (Preethi and Kuttan, 2009; Szymonik-Lesiuk et al., 2003).

Drugs used for the treatment of liver diseases are occasionally inadequate and can have serious adverse effects (Cengiz et al., 2013). The general strategy for prevention and treatment of liver damage includes reducing the production of reactive metabolites by using antioxidants (Bansal et al., 2005).

Many edible and medicinal plants have been tested and found to contain active antioxidant substances with curative properties for a variety of diseases (Kim et al., 2010; Dean, 2005; Lewis, 1977). *Pueraria lobata* (Willd.) Ohwi, a plant native to southeast Asia that belongs to the Leguminosae family, has been used as food, fodder, and medicine for thousands of years (Xiong et al., 2010; Yao et al., 2010). In traditional Chinese medicine, *P. lobata* has been used in therapy to counteract the problems associated with liver injury (Zhang et al., 2009). The major active antioxidant ingredient extracted from *P. lobata* is puerarin (7-hydroxy-3-(4-hydroxyphenyl)-1-benzopyran-4-one 8-(β-D-glucopyranoside, Fig. 1), which is an isoflavone glycoside (Teng et al., 2009; Zheng et al., 2009; Wang et al., 2008). Puerarin has a variety of biological

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione-S-transferase; LDH, lactate dehydrogenase; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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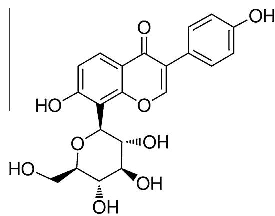

Fig. 1. Puerarin structure.

actions in cardiovascular diseases, gynecology disease, osteoporosis, cognitive capability, diabetic nephropathy (Hwang et al., 2011; Liu et al., 2011; Benlhabib et al., 2004). In addition, many reports have demonstrated that puerarin possesses strong antioxidative activity (Guerra et al., 2000; Teng et al., 2009; Cherdshewasart and Sutjit, 2007). Increasing evidence shows that puerarin can protect the liver from injury induced by hepatotoxin (Liu et al., 2012; Zhao et al., 2010). Although the protective effects of puerarin (intraperitoneally) on acute hepatotoxicity (3 days) caused by CCl_4 in mice was investigated (Hwang et al., 2007), the scientific studies of puerarin (orally) usefulness with respect to relative long time liver injuries (4 weeks) induced by CCl_4 in rats are lacking. Therefore, the present study attempted to estimate the hepatoprotective activity of puerarin supplementation against CCl_4-induced hepatic injuries in Sprague–Dawley male rats.

2. Materials and methods

2.1. Chemicals

Puerarin and CCl_4 were obtained from the Sigma–Aldrich (St. Louis, MO, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents used were obtained from local sources and were of the highest grade commercially available.

2.2. Animals and treatment

Sixty male Sprague–Dawley rats (190 ± 10 g) were obtained from SLAC Laboratory Animal Co., Ltd. (Shanghai). Rats were acclimated to the experimental facility for 1 week and housed in stainless steel cages in a room with a 12 h dark/light cycle, ambient temperature of 23 ± 1 °C and relative humidity of 55 ± 5%. Rats were allowed to standard laboratory feed and water (Xia et al., 2010). Our University Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the ethical guidelines of Zhejiang University.

The animals were randomly divided into six groups with each consisting of 10 rats. Group I received only vehicles olive oil (3 mL/kg b.w.) and 20% DMSO (3 mL/kg b.w.). Animals of group II, III, IV and V received CCl_4 3 mL/kg b.w. (30% in olive oil; v/v) intraperitoneally (i.p.) twice a week for 4 weeks. Group II was treated with CCl_4 only, while groups III, IV and V were treated with 3 mL/kg b.w. of puerarin dissolved in 20% DMSO at dose levels of 50, 100 and 200 mg/kg b.w. by oral gavage, respectively, per day for 28 days. Animals of group VI were only given puerarin (200 mg/kg b.w.) daily by oral gavage. At the end of the experimentation period (i.e. day 28), after 24 h of the last treatment, all the animals were anesthetized with CO_2. The animals were weighted and sacrificed. Blood samples were collected from all animals from retro-orbital venous plexus for biochemical variables analysis. Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible, and immediately stored at -70 °C until analysis. An extra sample of liver was excised and fixed in 10% formalin solution for histopathologic analysis. Sections (5 μm thick) were cut and stained with hematoxylin and eosin (H&E) for the histological examination.

2.3. Hepatic homogenate preparation

The liver was homogenized in a solution of 10 mM KCl, 1 mM EDTA and 100 mM phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 g for 15 min. The supernatant was used as a source to assay enzymatic markers of oxidative stress, including GPx, GST, GR, SOD and CAT activities. Also were determined GSH, TBARS and the total protein content.

2.4. Measurement of serum ALT, AST, ALP, and LDH activities

Liver damage was assessed by the estimation of serum activities of ALT, AST, ALP and LDH using commercially available test kits from by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The results were expressed as units/liter (U/L).

2.5. Measurement of GSH level

The level of GSH in hepatic supernatant was determined according to the method of Ellman (1959). Briefly, 0.02 mL of the supernatant was added to 9 mL of distilled water. Then 1 mL of phosphate buffer (pH 8.0) was added. Subsequently, 0.02 mL 5,5′-dithiobis(2-nitrobenzoic acid) was added to 3.0 mL this solution. The absorbance was determined at 420 nm. The results were expressed as the contents (nmol GSH) per mg protein.

2.6. Measurement of the level of lipid peroxidation

Hepatic tissue lipid peroxidation was measured by shaking the 2 mL of liver homogenate (5%, w/v) in 150 mM KCl, 0.025 M Tris–HCl buffer (pH 7.5) for 30 min at 37 °C and measuring the malondialdehyde formed with the thiobarbituric acid reaction by the method of of Saxena and Flora (2004). The amount of TBARS was calculated using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.7. Measurement of SOD activity

SOD activity in hepatic supernatant was measured using the method described by Marklund and Marklund (1974). The reaction mixture was composed of supernatant with 0.2 M pyrogallol, 1 mM EDTA and 50 mM Tris–HCl (pH 8.2), in a final volume of 1 mL. The enzymatic activity was measured by monitoring the absorbance at 420 nm. The results were expressed as units/min/mg protein (U/min/mg protein).

2.8. Measurement of CAT activity

CAT activity in hepatic supernatant was assayed according to the method described by Aebi (1974). The reaction mixture contained supernatant with 10 mM H_2O_2 and 50 mM phosphate buffer (pH 7.0), in a final volume of 1 mL. The rate of decomposition of H_2O_2 was measured.

2.9. Measurement of GPx, GR and GST activities

The antioxidant enzymes activities of GPx, GR and GST in hepatic supernatant were assayed using commercially available test kits from by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The results were expressed as units/mg protein (U/mg protein) for GPx and GST, and as units/g protein (U/g protein) for GR.

2.10. Statistical analysis

The data for the rats were expressed as means ± S.E.M. All statistical analyses were performed using SPSS 13.0 statistical software. Significant differences among the treatment means were determined using analysis of variance (ANOVA) and Duncan's multiple range test. Results were considered to be statistically significant at p values less than 0.05.

3. Results

3.1. Effect of puerarin on liver weight

The relative liver weights of each group of rats are shown in Table 1. The results showed a significant increase ($p < 0.05$) of relative weight, by nearly 42%, for CCl_4-treated rats when compared with the normal control group. In contrast, rats received the indicated dose of puerarin showed significant decrease ($p < 0.05$) in liver weight compared to the CCl_4-treated group.

3.2. Effect of puerarin on the levels of serum ALT, AST, ALP and LDH activities

Results in Table 2 revealed a significant elevation of serum ALT, AST, ALP and LDH activities in CCl_4-treated group as compared to normal controls ($p < 0.05$), indicating that CCl_4 induced obvious damage to the hepatic cells. The treatment of rats with puerarin at 50, 100 and 200 mg/kg b.w. markedly lowered ($p < 0.05$) serum

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