African Journal of Pharmacy and Pharmacology Vol. 6(37), pp. 2686-2691, 8 October, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.689
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Polyporus umbellatus polysaccharides ameliorates carbon tetrachloride-induced hepatic injury in mice

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Accepted 10 September, 2012

The purpose of this study was to investigate the hepatic protective function and potential mechanism of *Polyporus umbellatus* polysaccharides (PPS) to hepatic injury. Previous study has revealed that PPS possesses antitumor and immunomodulatory activities. In this study, we further demonstrate the protective function of PPS using carbon tetrachloride (CCl_4)-induced hepatic injury in mouse model. The effects of PPS were evaluated by biochemical values and histopathological examinations; mRNA expression was measured by real-time polymerase chain reaction (PCR). We found PPS dose-dependently alleviated hepatic injury manifested by the recovery of lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) levels. Histopathological examination also confirmed the alleviation of hepatic injury. Meanwhile, the suppressed mRNA expression of GPx, CAT and SOD by CCl_4 were restored by PPS treatment. These data indicated that PPS are protective against CCl_4-induced hepatic injury, and the mechanism involves the upregulation of GPx, CAT and SOD expression.

Key words: *Polyporus umbellatus* polysaccharides, carbon tetrachloride (CCl_4), liver.

INTRODUCTION

Hepatic injury is a common disease state that may lead the liver gradually developed into fibrosis, cirrhosis, and life-threatening liver failure. The pathophysiological mechanisms of hepatic injury are very complex (Sherman et al., 1994). Oxidative stress, energetic production malfunction, and inflammatory response etc, have been shown to play important role in the pathogenesis of hepatic injury (Poli, 1993). Interestingly, a number of herb originated agents demonstrated promising activity against hepatic injury, representing an important source of hepatoprotective drug development (Stickel et al., 2007).

Polyporus umbellatus polysaccharides (PPS) is a principle component extracted from traditional chinese medicine, *P. umbellatus*, which has been used as a major composition of traditional Chinese medicine recipes in treating liver diseases with a long history. PPS has been demonstrated to possess antitumor and immunomodulatory activities. PPS carried by liposome effectively inhibited the liver metastases of melanoma in mice (Zhang et al., 1999). Zhang et al. (2011) showed that PPS enhanced the expression of glutathione s-transferase pi isoform (GSTP1) and thus, inhibited the growth of bladder tumor. Li et al. (2010, 2011) showed that PPS modulate immune function via toll-like receptor 4 pathway. Guo et al. (1999) demonstrated that PPS inhibited hepatitis B virus (HBV) surface protein antigens (HBsAg) expression in hepatitis B virus (HBV) transgenic mice.

Based on its immune modulatory effects, PPS has been widely used to treat hepatitis B or C together with antivirus drugs in the form of injections or tablets in

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China. However, the role of PPS on hepatic injury remains unclear. Therefore, we established carbon tetrachloride (CCl_4)-induced hepatic injury model in mice to test the effect of PPS on hepatotoxicity.

MATERIALS AND METHODS

Animals

Adult Balb/C mice (22 to 25 g) used in the current study were kept under standard animal room conditions (temperature 21 ± 1°C, humidity 55 to 60%, 12 h light period) with food and water *ad libitum* for 1 week before experiment. All experimental procedures were in accordance to the Institutional Animal Care and Use Committee of Harbin Medical University, P.R. China.

Induction of hepatotoxicity and drug treatment

The animals were randomly assigned into 5 groups (control, CCl_4, CCl_4 + 10 mg/kg PPS, CCl_4 + 30 mg/kg PPS, and CCl_4 + 100 mg/kg PPS), with 10 animals in each group. The low dose of PPS (10 mg/kg) is set equivalent to the clinically used dosage. PPS was orally administered to experimental animals once a day for 14 consecutive days.

Animals of the control group were given the same volume of physiological solution. After 14 days of PPS treatment, hepatic injury was induced by intraperitoneal administration of CCl_4 at a dose of 0.5 ml/kg body weight dissolved in olive oil (1:1, v/v). Twenty-four hours (24 h) later, blood samples were obtained with heparinized syringes from the abdominal aorta under anesthesia with pentobarbital, and then the livers were collected after perfusion with ice-cold physiological saline. PPS was purchased from DaTang Biopharmaceutical Company, China.

Serum biochemical assays

Enzyme activities of lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in blood serum were evaluated by an auto biochemistry analyzer (BIOBASE-CRYSTAL, China).

Measurement of malondialdehyde (MDA), reduced glutathione (GSH reduced glutathione, glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT)

The weighed frozen liver tissue was homogenized on ice with 50 mM phosphate buffer (pH 7.4). The homogenates were then centrifuged at 10000 × g for 15 min at 4°C and the supernatant was used for further measurement. MDA were determined to evaluate for lipid peroxidation, and GSH for antioxidant state. The activities of antioxidant enzymes including GPx, CAT and SOD were also determined. All the measurement procedures were performed according to the manufacturer's instructions of the kits (Nanjing jiancheng, China).

Histological examination

Liver tissues were fixed in 10% formalin, dehydrated in gradual ethanol (50 to 100%), cleared in xylene, and embedded in paraffin. After that, the embedded tissue were cut into 7 µm thick sections and stained with hematoxylin and eosin (H&E) dye for histopathological observation.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA from mice liver was isolated using Trizol reagent (Invitrogen, USA) according to manufacturer's protocol. Total RNA (0.5 µg) was then reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) to obtain cDNA. The RNA levels of GSTP1 were determined using SYBR Green I incorporation method on LightCycler® 2.0 PCR System (Roche, Swiss), with β-actin as an internal control. The sequences of primers were as follows:

β-actin
Forward: 5'- AGCTCCTTCGTTGCCGGTCC -3'
Reverse: 5'- GCTTTGCACATGCCGGAGCC -3'

CAT
Forward: 5'- TGCCTTCTCCGGGTGGAGACC-3'
Reverse: 5'- TCATCTGGTCGCTGGCTGGGT-3'

SOD
Forward: 5'- TTCCGTCCGTCGGCTTCTCG -3'
Reverse: 5'- ACGCACACCGCTTTCATCGC -3'

GPx
Forward: 5'- GCCGGATAAGGCGGGACCCT -3'
Reverse: 5'- TCCGTACTAGCGCTCACAGGGC -3'

Statistical analysis

Data are presented as mean ± standard deviation (SD). The difference among groups was analyzed by one-way analysis of variance followed by Tukey's test. Differences were considered as statistically significant when P values were less than 0.05.

RESULTS

Effects of PPS treatment on serum LDH, AST and ALT activities in CCl_4-induced hepatotoxicity in mice

Treatment of mice with CCl_4-induced apparent liver injury, as evidenced by increased serum LDH, AST and ALT activities. Concomitant administration of PPS significantly alleviated CCl_4-induced serum LDH, AST and ALT enhancement in a dose-dependent manner. At the dose of 100 mg/kg, PPS nearly completely diminished the increase of AST and ALT (Figure 1).

Effects of PPS treatment on hepatic MDA and GSH concentration and GPx, CAT and SOD activities in CCl_4-induced hepatotoxicity in mice

MDA is one of the end products and also an important marker of lipid peroxidation. The level of MDA significantly increased after CCl_4 treatment, which were inhibited dose-dependently by PPS (Figure 2A). Then, the level of GSH, a key intracellular antioxidant, was measured. Application of PPS markedly rescued the depletion of GSH by CCl_4 at the doses of 100 mg/kg. At the dose of 10 and 30 mg/kg, GSH levels were not

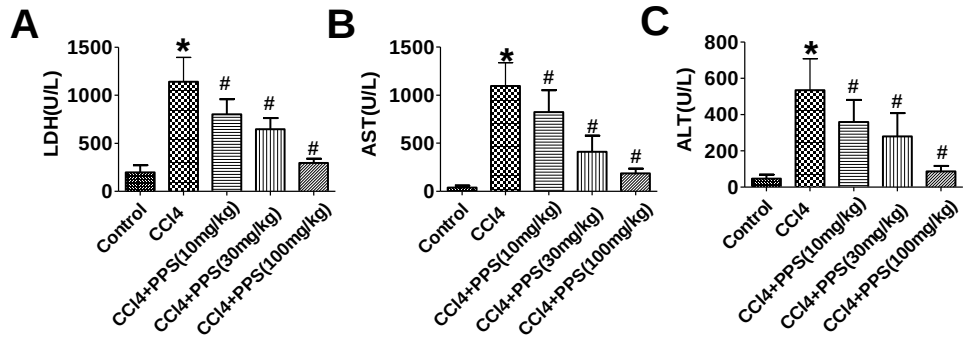

Figure 1. Effect of PPS on serum levels of LDH, AST and ALT in CCl_4 treated mice. Data are expressed as mean ± SD, n = 9. *, P < 0.05 versus Control; #, P < 0.05 versus CCl_4.

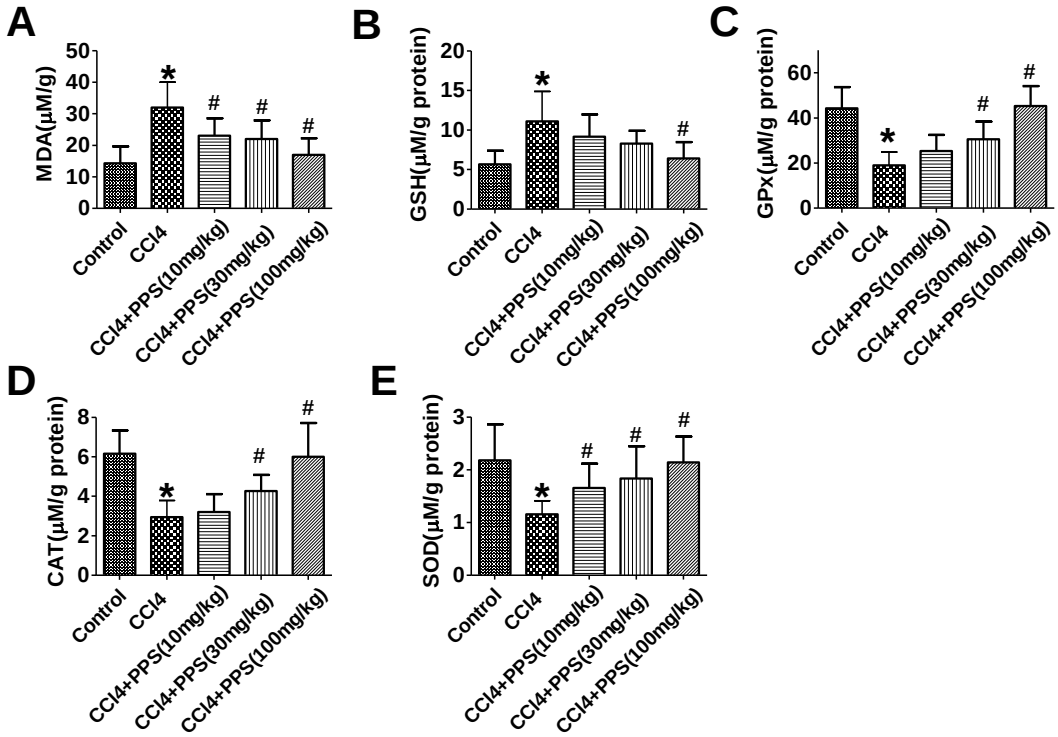

Figure 2. Effect of PPS on hepatic levels of MDA, GSH and activities of GPx, CAT and SOD in CCl_4 treated mice. A to E, MDA, GSH, GPx, CAT, SOD. Data are expressed as mean ± SD, n = 9. *, P<0.05 versus Control; #, P < 0.05 versus CCl_4.

affected (Figure 2B). These results imply that both abnormal lipid peroxidation and antioxidant dysregulation were relieved by PPS. The activities of antioxidants were also measured. PPS significantly increased the activity of GPx, CAT and SOD repressed by CCl_4 (Figure 2C to E). However, at the dose of 10 mg/kg, PPS produced no significant influence on GPx and CAT activities.

Histological examination

To further confirm the hepatoprotective effect of PPS on CCl_4-induced liver injury, we performed morphological evaluation of liver sections after H&E staining. CCl_4 administration caused significant damage to the liver, as manifested by severe fatty changes, ballooning degeneration, scattered necrosis, infiltration of inflammatory cells and the loss of cellular boundaries (Figure 3A and B). These pathological changes were alleviated by PPS treatments (Figure 3C to E). At the dose of 100 mg/kg, the histological architecture of the liver was greatly improved, and only mild fatty deposition with slight inflammatory reaction was observed (Figure 3E).

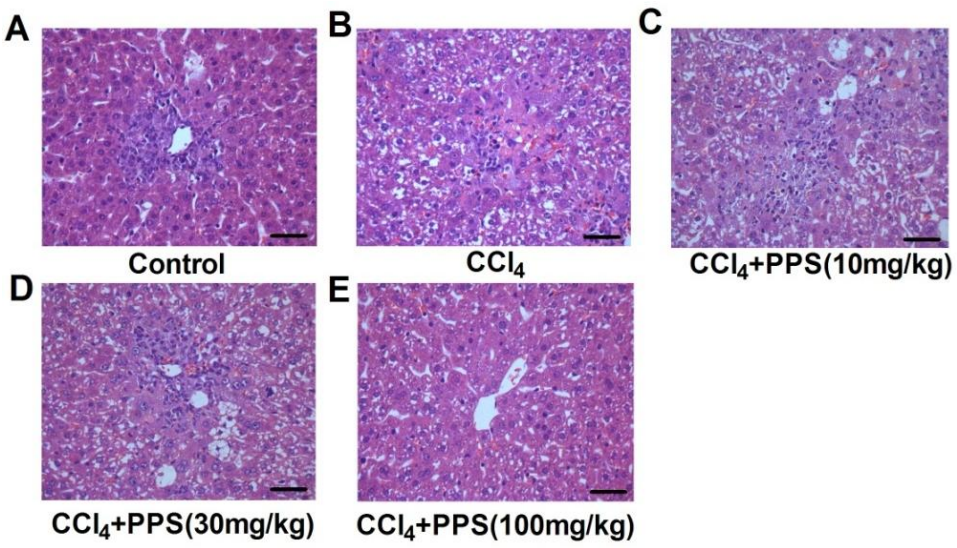

Figure 3. Effect of PPS on hepatic histological alterations in CCl_4 treated mice. Scale bar = 50 μm.

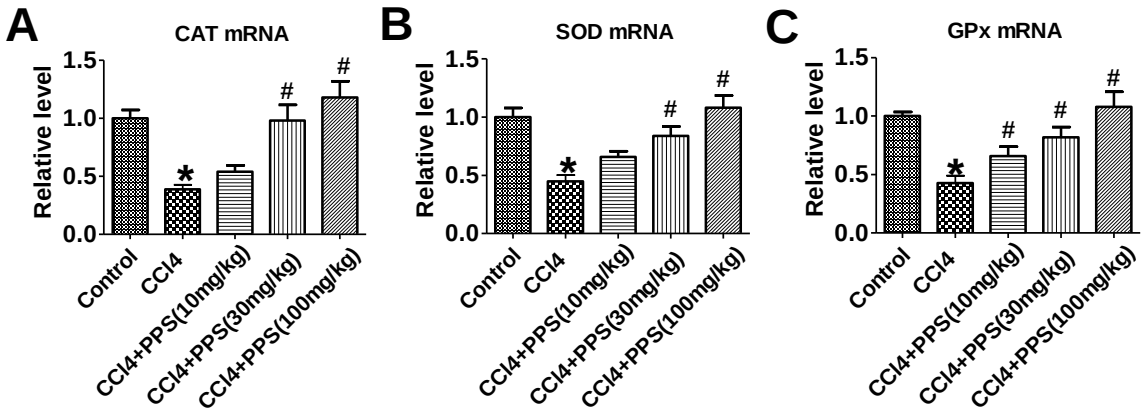

Figure 4. Effect of PPS on mRNA expression of CAT (A), SOD (B) and GPx (C) in the liver of CCl_4 treated mice. CAT, Catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase. Data are expressed as mean ± SD, n = 5. *, P<0.05 versus Control. #, P < 0.05 versus CCl_4.

Effects of PPS on the mRNA expression of GPx, CAT and SOD

The mRNA expression of GPx, CAT and SOD was measured by real-time PCR. In CCl_4 treated mice, the mRNA levels of GPx, CAT and SOD were decreased, which were abrogated by PPS pretreatment (Figure 4). GPx and CAT mRNA levels did not change when treated with 10 mg/kg PPS (Figure 4A and B).

DISCUSSION

In this study, we found PPS pretreatment produced significant hepatoprotective activity in CCl_4-induced hepatotoxicity in mice, as evidenced by alleviated oxidative stress and improved hepatic function. As liver damage caused by CCl_4 is close to human hepatotoxicity, CCl_4-induced mouse hepatic injury model was used in this study. CCl_4 is metabolized by cytochrome P450 2E1 to the trichloromethyl radical ($CCl_3^{\cdot}$), which is subsequently converted into a peroxyl radical ($\cdot OOCCl_3$) in the presence of oxygen. Oxygen free radicals are the causal factors in the pathogenesis of degenerative diseases, including some hepatopathy (Poli, 1993). The reactive free radical metabolites of CCl_4 initiate free radical-mediated lipid peroxidation and lead to the accumulation of lipid peroxidation products that cause hepatic injury (Recknagel et al., 1983; Recknagel et al., 1989; Goeptar et al., 1995). The increase in plasma

transaminase activities, for example, AST and ALT is known as a sign of CCl_4-induced hepatotoxicity (Sheweita et al., 2001; Sotelo-Felix et al., 2002; Ritter et al., 2004). LDH is an enzyme found in many body tissues, including the liver. Elevated serum levels of LDH may indicate liver damage. In this study, we observed apparent increase of serum LDH, AST and ALT after CCl_4 treatment, indicating the successful establishment of hepatic injury model. The hepatic injury was further confirmed by immunohistological examination. The morphological manifestations include the characterized acute liver injury changes such as severe fatty changes, ballooning degeneration, scattered necrosis, infiltration of inflammatory cells and the loss of cellular boundaries. Application of PPS significantly decreased the increase of serum LDH, AST and ALT, and alleviated the pathological changes, showing that PPS is protective against CCl_4-hepatic injury.

Reactive oxygen species degrade polyunsaturated lipids to form MDA. MDA is a major reactive aldehyde that is formed in the degradation of polyunsaturated lipids catalyzed by reactive oxygen species (Vaca et al., 1988). Increment of MDA is used as an indicator of lipid peroxidation state (Ohkawa et al., 1979). We found PPS inhibited CCl_4-induced increase of hepatic MDA, indicating that PPS treatment was able to suppress hepatic lipid peroxidation.

GSH is an endogenous non-enzymatic antioxidant that prevents damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella et al., 2003). In this study, PPS increased the hepatic level of GSH that is suppressed by CCl_4, supporting that PPS has antioxidant activity.

Antioxidant enzymes catalyze decomposition of reactive oxygen species and maintain the normal physiological state of cells. SOD, GPx and CAT are three major antioxidant oxidants, and they differ from each other in structure, tissue distribution and cofactor requirement (Moreno et al., 2005). Upon hepatic injury, SOD, GPx and CAT activities were reported to be reduced (Shahjahan et al., 2005). Li et al. (2007) showed that a polysaccharide-peptide complex (F22) from mushroom (*Pleurotus abalonus*)-fruiting bodies exerts its hepatic protective property by upregulating the mRNA expression of SOD, GPx and CAT. Consistently, we found PPS was able to increase the activities of hepatic SOD, GPx and CAT in CCl_4 treated mice together with the upregulation of their mRNA expression. These data imply that antioxidant activity is a critical mechanism for the protective effects of PPS on CCl_4-induced hepatic injury.

Polysaccharides are major components existing in both plants and animals. More and more polysaccharides extracted from plants were demonstrated to have a wide range of biological activities, such as antitumor (Li et al., 2011), anti-inflammatory (Siqueira et al., 2011), anti-coagulant (Pawlaczyk et al., 2011), antibacterial (Naqash et al., 2011), and antioxidant (Kardosova et al., 2006).

Some polysaccharides with antioxidant activity has been shown to inhibit hepatic oxidative injury (Jin et al., 2011). *Atractylodes macrocephala* polysaccharide alleviated liver ischemia-reperfusion injury (Jin et al., 2011). *Lycium barbarum* polysaccharide significantly ameliorated the progression of alcohol-induced fatty liver and improved hepatic function by increasing SOD, CAT and GSH-Px activities in rats (Cheng et al., 2011). These studies strongly indicate that plant polysaccharides are a new source for antioxidant agent development. Our observation that PPS possesses antioxidant activity hints at the potential of PPS in the treatment of oxidative stress related diseases.

ACKNOWLEDGEMENT

This work was supported by Scientific Research Foundation of the Education Department of Heilongjiang Province (12511290) to Peng Kang.

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