

Preventive activity of banana peel polyphenols on CCl₄-induced experimental hepatic injury in Kunming mice

RUI WANG^{1,2*}, XIA FENG^{2*}, KAI ZHU^{1,2}, XIN ZHAO^{1,2} and HUAYI SUO³

¹Chongqing Collaborative Innovation Center of Functional Food;

²Department of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067;

³College of Food Science, Southwest University, Chongqing 400715, P.R. China

Received August 27, 2014; Accepted July 13, 2015

DOI: 10.3892/etm.2016.3155

Abstract. The aim of the present study was to evaluate the preventive effects of banana peel polyphenols (BPPs) against hepatic injury. Mice were divided into normal, control, 100 mg/kg and 200 mg/kg banana peel polyphenol and silymarin groups. All the mice except normal mice were induced with hepatic damage using CCl₄. The serum and tissue levels of mice were determined by a kit and the tissues were further examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis. BPPs reduced the serum levels of aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase in a CCl₄-induced mouse model of hepatic injury. Furthermore, BPPs reduced the levels of malondialdehyde and triglyceride, while increasing glutathione levels in the serum and liver tissues of mice. In addition, the effects of 200 mg/kg treatment were more evident, and these effects were comparable to those of the drug silymarin. Serum levels of the cytokines, interleukin (IL)-6, IL-12, tumor necrosis factor (TNF)- α and interferon- γ , were reduced in the mice treated with BPPs compared with injury control group mice, and these levels were comparable to those of the normal and silymarin-treated groups. Histopathological examination indicated that BPPs were able to reduce the extent of CCl₄-induced liver tissue injury and protect the liver cells. Furthermore, the mRNA and protein expression levels of the inflammation-associated

factors cyclooxygenase-2, nitric oxide synthase, TNF- α and IL-1 β were reduced in mice treated with BPPs compared with the control group mice. Mice that received 200 mg/kg BPP exhibited reduced expression levels of these factors compared with mice that received 100 mg/kg BPP. In conclusion, the results of the present study suggested that BPPs exert a good preventive effect against hepatic injury.

Introduction

Banana peel has been widely used in traditional Chinese medicine for the treatment of inflammation of the oral cavity and to promote bowel movements (1). Furthermore, as banana peel contains large amounts of vitamins and minerals, it may be used to produce cosmetics as industrial products. In addition to vitamins and minerals, banana peel contains a certain quantity of polyphenols (2). Polyphenols, which are also present in tea and pomegranate, have been demonstrated to protect the liver against alcohol-associated damages (3). Polyphenols exert a marked antioxidative effect, and the most evident manifestation of liver damage is oxidative damage of liver cells (4). As a result, polyphenols may be able to protect liver cells against oxidative damage and promote the repair of oxidatively-damaged cells, in order to maintain health (5). Polyphenols present in banana peel may function in a similar manner.

Oxidative stress and inflammation may result in liver disease, cardiovascular disease and cancer (6). CCl₄ produces reactive-free radicals if metabolized, and thus had been used to induce hepatic damage in animal models (7). Furthermore, CCl₄ is able to increase lipid peroxidation on the cell membrane and alter enzyme activity, thereby inducing hepatic injury and necrosis (8).

In the present study, the preventive activity of banana peel polyphenols (BPPs) on CCl₄-induced experimental hepatic damage was determined. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), malondialdehyde (MDA), glutathione (GSH) and triglyceride (TG) were evaluated, and in addition tissue levels of MDA, GSH and TG were determined. Cytokine levels of interleukin (IL)-6, IL-12, tumor necrosis factor (TNF)- α and interferon (IFN)- γ in the serum were also measured. In addition, liver tissue was subjected to histological

Correspondence to: Professor Xin Zhao, Department of Biological and Chemical Engineering, Chongqing University of Education, 9 Xuefu Street, Nan'an, Chongqing 400067, P.R. China
E-mail: foods@live.cn

Professor Huayi Suo, College of Food Science, Southwest University, 2 Tiansheng Road, Beibei, Chongqing 400715, P.R. China
E-mail: birget@swu.edu.cn

*Contributed equally

Key words: banana peel, polyphenol, hepatic damage, cytokine, expression

examination and the expression levels of inflammation-associated genes were determined in Kunming mice.

Materials and methods

Preparation of BPPs. Bananas were purchased from a local market (Chongqing, China), and were native to Hainan, China. The banana pulp was removed and the peel was washed and stored at 20°C. Subsequently, 1 kg banana peel was blanched at 95°C for 3 min to remove the polyphenol oxidase. The banana peel was incubated in 40% ethanol solution (5 liters), and then ultrasonic-assisted extraction (Ultrasonic extractor, THC 300, Jining Tianhua Ultrasonic Electronic Instrument Co. Ltd., Shandong, China) was performed at 50°C for 1 h. The extract was passed through an AB-8 macroporous resin (Donghong Chemical Co., Ltd., Zhongshan, Guangdong, China) and the BPPs were adsorbed. The adsorbed BPPs were washed with 80% ethanol solution, and the eluent was evaporated and concentrated using an N-1100 rotary evaporator (Eyela; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) (9).

Inducing hepatic injury. Male ICR mice (n=50; age, 7 weeks) were purchased from the Experimental Animal Center of Chongqing University of Education (Chongqing, China). The mice were allocated at random into five groups (n=10 mice per group): Normal; control; 100 mg/kg banana peel polyphenol; 200 mg/kg banana peel polyphenol; and silymarin (Shanghai Yuanye Bio Technology Co., Ltd., Shanghai, China) groups. The normal and control group mice were administered 0.2 ml physiological saline everyday for 14 days. The two banana peel polyphenol group mice received 100 or 200 mg/kg banana peel polyphenol solution in 0.2 ml doses by oral gavage for 14 days. Silymarin was used for drug control (10), at a concentration of 100 mg/kg, and the mice were administered 0.2 ml silymarin solution for 14 days. At day 14, the control, 100 and 200 mg/kg banana peel polyphenol and silymarin group mice received abdominal subcutaneous injections of CCl₄ solution (0.2 ml/kg dissolved in olive oil, 1:1 v/v) to induce hepatic damage. After 24 h, the mice were sacrificed using CO₂. Blood and liver tissues were collected and preserved at -70°C until required for the biological assays. Experimental protocols were approved by the Animal Ethics Committee of Chongqing University of Education (11).

Levels of AST, ALT, LDH, MDA, GSH and TG. The blood of the mice was centrifuged at 1,795 x g for 10 min. After centrifugation, the serum supernatant was collected, the serum (0.1 ml) and kit reagents were mixed and then the levels determined according to the instructions described in the kits at 532 nm using a UV-2600 spectrophotometer (Shimadzu, Tokyo, Japan). A total of 0.1 g mice tissues were added into 0.5 ml sucrose buffer (0.25 mol/l sucrose, 10 mmol/l HEPES, 1 mmol/l EDTA at pH 7.4) and then this mixture was homogenized by a High speed tissue homogenate machine (T10, IKA, Staufenim Breisgau, Germany). The homogenized tissues were centrifuged at 1,795 x g, 10 min, the supernatant fluid was collected and was determined as the serum test method.

Serum levels of AST, ALT, LDH, MDA, GSH and TG were determined using AST (no. C010), ALT (no. C009), LDH (no. A020), MDA (no. A003), reduced GSH (no. A006) and

TG (no. F001) assay kits, respectively (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). In addition, the tissue levels of MDA, GSH and TG were determined using the kits from Nanjing Jiancheng Bioengineering Institute.

Serum cytokine levels determined using an enzyme-linked immunosorbent assay (ELISA). Mouse blood from the inferior vena cava was collected in a tube and centrifuged at 1,795 x g for 10 min at 4°C. The serum (0.1 ml) and kit reagents were mixed and the concentrations of the proinflammatory-associated cytokines, IL-6, IL-12, TNF- α and IFN- γ , were determined using ELISA, according to the manufacturer's instructions (BioLegend ELISA MAX™ Deluxe kit; BioLegend, San Diego, CA, USA) (11) and using a UV-2600 spectrophotometer at a wavelength of 450 nm (Shimadzu, Tokyo, Japan).

Histological examination of hematoxylin and eosin (H&E) stained sections. The liver tissues of mice were collected and washed. Then the liver tissues were fixed in 10% (v/v) buffered formalin for 24 h and they were score cut and embedded into paraffin. The paraffin was cut 4- μ m thick and stained by a H&E kit (Beijing Nobleryder Technology Co., Ltd., Beijing, China). The stained sections were then observed by a microscope (BX41, Olympus, Tokyo, Japan) (12).

Western blot analysis of protein expression levels in liver tissue. Total protein was obtained from the mice liver tissue samples using a radioimmunoprecipitation assay buffer as previously described (12). Protein concentrations were determined using the RC DC protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Nitrocellulose membranes (Schleicher and Schuell BioScience, Inc., Keene, NH, USA) were then subjected to immunoblot analysis and proteins were visualized using an enhanced chemiluminescence (ECL) method (GE Healthcare Life Sciences) (7). Liver tissue cell lysates were separated using 12% SDS-PAGE, transferred onto a polyvinylidene fluoride membrane (GE Healthcare Life Sciences), blocked with 5% skimmed milk and hybridized with primary antibodies (dilution, 1:1,000). The following primary antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA): monoclonal mouse anti-human cyclooxygenase-2 (COX-2; cat. no. sc-514489), monoclonal mouse anti-human nitric oxide synthase (iNOS; cat. no. sc-7271), monoclonal mouse anti-human TNF- α (cat. no. sc-48418) and monoclonal mouse anti-human IL-1 β (cat. no. sc-52013). The membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Blots were washed three times with phosphate-buffered saline with Tween-20 and developed using an ECL reagent (Amersham Life Science, Arlington Heights, IL, USA), and the protein expressions were also quantitative analyzed using ImageJ software (version 1.44).

Statistical analysis. Data are presented as the mean \pm standard deviation. Differences between the mean values for individual groups were assessed using one-way analysis of variance with Duncan's multiple range test. P<0.05 was considered to indicate a statistically significant difference. SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

Table I. Sequences of reverse transcription-polymerase chain reaction primers.

| Gene | Sequence |
|---------------|--|
| COX-2 | Forward: 5'-TTAAAATGAGATTGTCCGAA-3' Reverse: 5'-AGATCACCTCTGCCTGAGTA-3' |
| iNOS | Forward: 5'-AGAGAGATCGGGTTCACA-3' Reverse: 5'-CACAGAACTGAGGGTACA-3' |
| TNF- α | Forward: 5'-GACCCTCAGACTCAGATCATCCTTCT-3' Reverse: 5'-ACGCTGGCTCAGCCACTC-3' |
| IL-1 β | Forward: 5'-CTCCATGAGCTTTGTACAAGG-3' Reverse: 5'-TGCTGATGTACCAGTTGGGG-3' |
| GAPDH | Forward: 5'-CGGAGTCAACGGATTTGGTC-3' Reverse: 5'-AGCCTTCTCCATGGTCGTGA-3' |

COX-2, cyclooxygenase-2; iNOS, nitric oxide synthase; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table II. Serum levels of AST, ALT and LDH in mice following CCl₄-induced hepatic damage.

| Group | AST (IU/l) | ALT (IU/l) | AST/ALT | LDH (IU/l) |
|---------------------------------|--------------------------------|---------------------------------|------------------------------|---------------------------------|
| Normal | 207.3 \pm 23.6 ^a | 209.3 \pm 25.6 ^a | 0.99 \pm 0.02 ^a | 1243.6 \pm 178.2 ^b |
| Control | 2571.6 \pm 97.6 ^c | 1511.3 \pm 122.9 ^c | 1.70 \pm 0.11 ^c | 6512.3 \pm 202.6 ^c |
| Banana peel polyphenols (mg/kg) | | | | |
| 100 | 1874.3 \pm 65.9 ^d | 1207.1 \pm 112.3 ^d | 1.55 \pm 0.08 ^d | 4876.3 \pm 152.4 ^d |
| 200 | 746.2 \pm 62.3 ^e | 605.3 \pm 58.9 ^e | 1.23 \pm 0.09 ^e | 3103.2 \pm 121.5 ^e |
| Silymarin | 712.0 \pm 68.3 ^e | 602.3 \pm 49.8 ^e | 1.18 \pm 0.10 ^e | 2801.3 \pm 107.6 ^a |

^{a-c}Mean values with different letters in the same column are significantly different ($P < 0.05$) according to Duncan's multiple-range test. AST, ALT, AST/ALT: ^a $P < 0.05$ vs. normal, ^c $P < 0.05$ vs. control, ^d $P < 0.05$ vs. 100 mg/kg BPP and ^e $P < 0.05$ vs. 200 mg/kg BPP and silymarin; LDH: ^a $P < 0.05$ vs. silymarin, ^b $P < 0.05$ vs. normal, ^c $P < 0.05$ vs. control, ^d $P < 0.05$ vs. 100 mg/kg BPP, ^e $P < 0.05$ vs. 200 mg/kg BPP. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; BPP, banana peel polyphenol.

Results

Serum levels of AST, ALT and LDH. The levels of AST, ALT and LDH in the serum of the normal group mice were reduced compared with the other groups (Table II). Control group mice were subjected to hepatic injury but received no further treatment, and thus exhibited markedly increased serum levels of AST, ALT and LDH. Treatment with silymarin (100 mg/kg) appeared to significantly ($P < 0.05$) reduce the serum levels of these proteins compared with the control mice, as the serum levels of AST, ALT and LDH in the silymarin-treated mice were the most comparable to the normal group mice. Treatment with BPPs appeared to induce a statistically significant reduction in the serum levels of AST, ALT and LDH compared with the control group mice ($P < 0.05$). These levels were increased in the 100 mg/kg banana peel polyphenol group mice compared with the normal and silymarin group mice, but reduced compared with control mice. Furthermore, the 200 mg/kg BPP group mice exhibited lower levels of AST, ALT and LDH compared with the 100 mg/kg dose group. In addition, the AST/ALT ratio was used as a key index of hepatic injury. The high AST/ALT ratio meant severe hepatic injury (13). The AST/ALT ratio in

the normal group mice was ~1, and the control mice had the highest ratio. The 100 and 200 mg/kg BPP and silymarin groups (1.55, 1.23 and 1.18, respectively) had reduced ratios compared with the control mice, but remained >1 . Therefore, the results of the present study indicated that BPPs are able to significantly reduce the levels of a number of key markers of hepatic injury, and these effects were comparable to those of the drug of silymarin.

MDA, GSH and TG levels in serum and liver tissues. The MDA, GSH and TG levels in the serum and liver tissues of the mice were determined using a variety of testing kits. The MDA and TG levels in the serum and liver tissues significantly decreased ($P < 0.05$) as a result of the CCl₄-induced hepatic injury. Control mice exhibited the highest levels of MDA and TG, while the banana peel polyphenol and silymarin groups exhibited reduced levels of these analytes ($P < 0.05$). The levels of MDA and TG in the 200 mg/kg banana peel polyphenol and silymarin group mice were comparable to those in the normal group mice (Tables III and IV; $P < 0.05$). However, the differences ($P < 0.05$) between groups in GSH levels followed a different trend from that of MDA and TG. The control mice exhibited the lowest levels

Table III. Serum levels of MDA, GSH and TG in mice following CCl₄-induced hepatic damage.

| Group | MDA (nmol/ml) | GSH (mg/l) | TG (mmol/l) |
|---------------------------------|-------------------------|---------------------------|------------------------|
| Normal | 5.48±0.62 ^a | 339.45±42.18 ^b | 0.92±0.10 ^c |
| Control | 14.05±0.82 ^b | 141.08±20.36 ^d | 1.32±0.11 ^b |
| Banana peel polyphenols (mg/kg) | | | |
| 100 | 10.12±0.59 ^c | 207.36±16.87 ^c | 1.19±0.08 ^c |
| 200 | 7.31±0.41 ^c | 255.21±19.87 ^e | 0.95±0.06 ^c |
| Silymarin | 6.65±0.32 ^d | 265.32±18.33 ^e | 0.94±0.08 ^c |

^{a-e}Mean values with different letters in the same column are significantly different (P<0.05) according to Duncan's multiple-range test. MDA: ^aP<0.05 vs. normal, ^bP<0.05 vs. control, ^cP<0.05 vs. 200 mg/kg BPP, ^dP<0.05 vs. silymarin and ^eP<0.05 vs. 100 mg/kg BPP; GSH: ^bP<0.05 vs. normal, ^cP<0.05 vs. 100 mg/kg BPP, ^dP<0.05 vs. control and ^eP<0.05 vs. 200 mg/kg BPP and silymarin; TG: ^bP<0.05 vs. control, ^cP<0.05 vs. 200 mg/kg BPP, silymarin and normal, ^eP<0.05 vs. 100 mg/kg BPP. MDA, malondialdehyde; GSH, glutathione; TG, triglyceride.

Table IV. Hepatic tissues of MDA, GSH and TG in mice following CCl₄-induced hepatic damage.

| Group | MDA (nmol/ml) | GSH (mg/l) | TG (mmol/l) |
|---------------------------------|------------------------|-------------------------|--------------------------|
| Normal | 2.10±0.31 ^a | 25.32±2.69 ^b | 0.022±0.003 ^c |
| Control | 7.87±0.71 ^b | 5.56±0.42 ^a | 0.047±0.004 ^b |
| Banana peel polyphenols (mg/kg) | | | |
| 100 | 5.97±0.62 ^d | 13.58±1.69 ^c | 0.040±0.002 ^d |
| 200 | 3.32±0.30 ^e | 17.69±1.48 ^d | 0.031±0.004 ^e |
| Silymarin | 3.23±0.32 ^e | 18.21±1.71 ^d | 0.026±0.002 ^a |

^{a-e}Mean values with different letters in the same column are significantly different (P<0.05) according to Duncan's multiple-range test. MDA, malondialdehyde; GSH, glutathione; TG, triglyceride. MDA: ^aP<0.05 vs. normal, ^bP<0.05 vs. control, ^dP<0.05 vs. 100 mg/kg BPP and ^eP<0.05 vs. 200 mg/kg BPP and silymarin. GSH: ^aP<0.05 vs. control, ^bP<0.05 vs. normal, ^dP<0.05 vs. 200 mg/kg BPP and silymarin, ^eP<0.05 vs. 100 mg/kg BPP. TG: ^aP<0.05 vs. silymarin, ^bP<0.05 vs. control, ^cP<0.05 vs. normal, ^dP<0.05 vs. 100 mg/kg BPP and ^eP<0.05 vs. 200 mg/kg BPP.

Table V. Cytokine levels of IL-6, IL-12, TNF- α and IFN- γ in mice following following CCl₄ induced hepatic damage.

| Group | IL-6 (pg/ml) | IL-12 (pg/ml) | TNF- α (pg/ml) | IFN- γ (pg/ml) |
|---------------------------------|-------------------------|-------------------------|-----------------------|-----------------------|
| Normal | 43.6±4.9 ^a | 211.3±15.3 ^a | 22.6±4.8 ^a | 19.6±2.5 ^b |
| Control | 285.2±26.5 ^c | 795.3±26.9 ^c | 89.9±6.8 ^c | 75.3±4.9 ^c |
| Banana peel polyphenols (mg/kg) | | | | |
| 100 | 212.6±22.9 ^d | 572.6±29.3 ^d | 68.1±4.2 ^d | 55.1±2.6 ^d |
| 200 | 152.6±19.2 ^e | 415.8±21.2 ^e | 47.2±3.2 ^e | 32.9±1.8 ^e |
| Silymarin | 125.3±12.3 ^b | 390.6±24.6 ^b | 39.2±2.6 ^b | 31.9±3.7 ^e |

^{a-e}Mean values with different letters in the same column are significantly different (P<0.05) according to Duncan's multiple-range test. IL, interleukin; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ . IL-6, IL-12, TNF- α : ^aP<0.05 vs. normal, ^bP<0.05 vs. silymarin, ^cP<0.05 vs. control, ^dP<0.05 vs. 100 mg/kg BPP and ^eP<0.05 vs. 200 mg/kg BPP; IFN- γ : ^bP<0.05 vs. normal, ^cP<0.05 vs. control, ^dP<0.05 vs. 100 mg/kg BPP and ^eP<0.05 vs. 200 mg/kg BPP and silymarin.

of GSH, while the BPPs and silymarin group mice expressed increased levels of GSH compared with the control group (P<0.05). However, no statistically significant difference was identified in the levels of GSH between the 200 mg/kg BPP and silymarin group mice (P>0.05), and these levels were moderately reduced compared with the normal group mice (P<0.05).

Serum cytokine levels. IL-6, IL-12, TNF- α and IFN- γ are proinflammatory cytokines. The control group mice exhibited the highest cytokine levels (P<0.05), while normal group mice had the lowest cytokine levels (Table V). BPPs and silymarin group mice presented with significantly decreased cytokine levels (P<0.05) compared with the control group mice. The

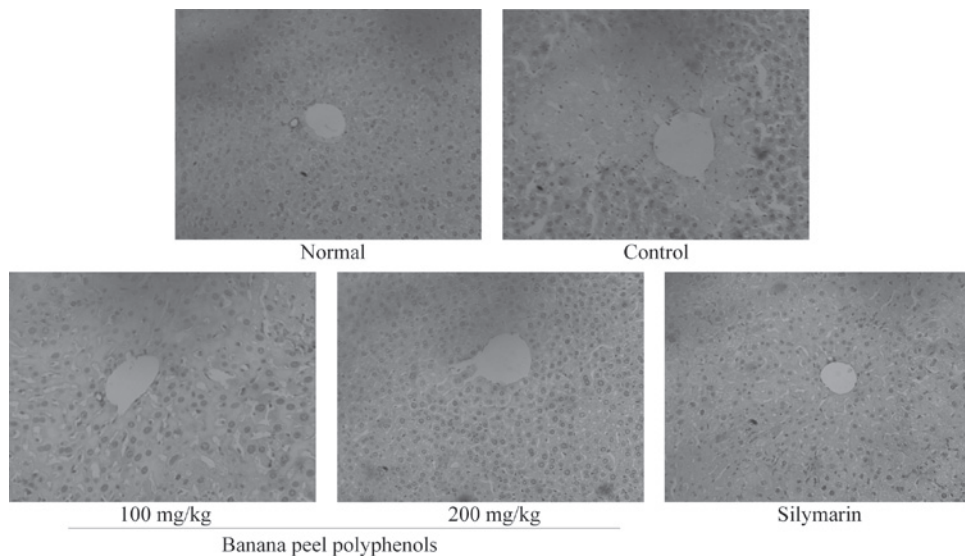


Figure 1. Histological images of the liver tissue of mice with CCl₄-induced hepatic damage (magnification, x200).

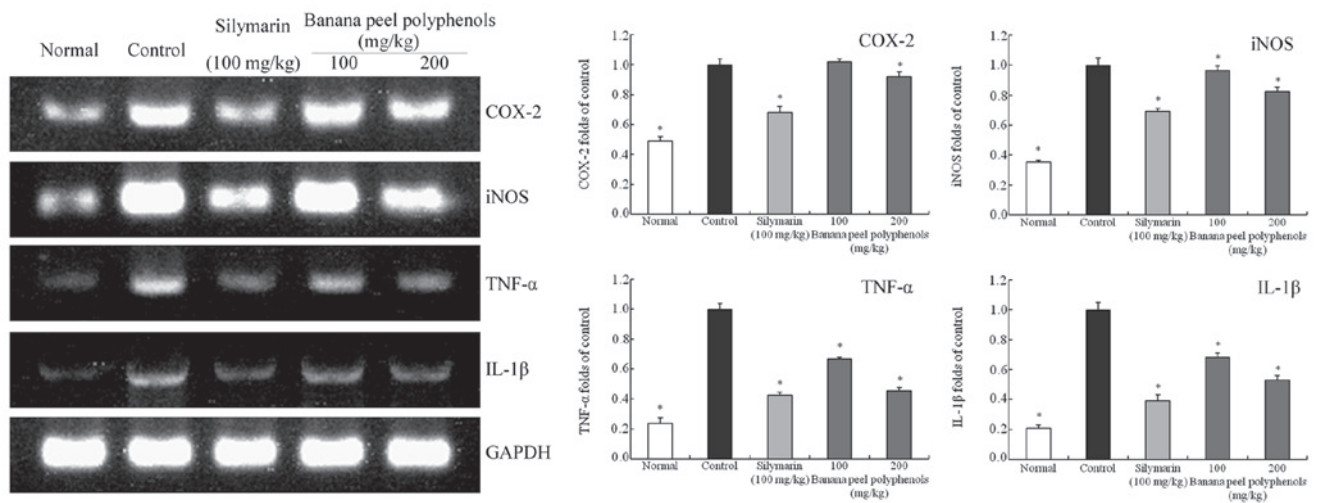


Figure 2. mRNA expression levels of COX-2, iNOS, TNF-α and IL-1β in hepatic tissue of mice. *P<0.05, compared with the control, according to Duncan's multiple range test. COX-2, cyclooxygenase-2; iNOS, nitric oxide synthase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

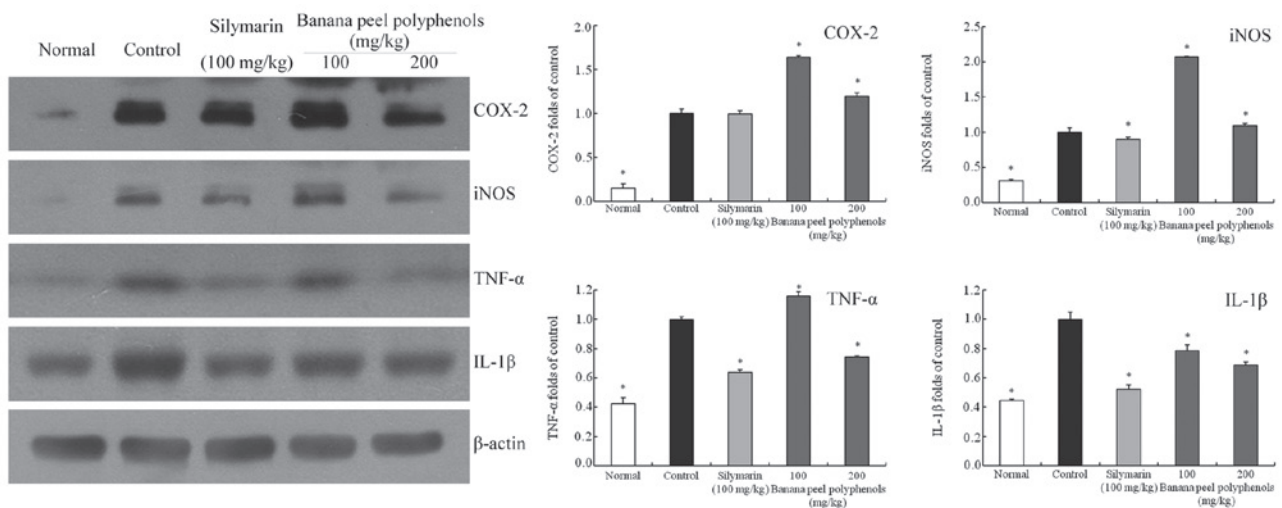


Figure 3. Protein expression levels of COX-2, iNOS, TNF-α and IL-1β in hepatic tissue of mice. *P<0.05, compared with the control, according to Duncan's multiple range test. COX-2, cyclooxygenase-2; iNOS, nitric oxide synthase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β.

levels of IL-6, IL-12 and TNF- α in the 200 mg/kg BPP group mice were moderately increased compared with the silymarin group mice; however, no statistically significant difference was detected in IFN- γ levels between the 200 mg/kg BPP and silymarin group mice ($P < 0.05$).

Histopathological examination of liver tissues. Observation of the H&E-stained sections revealed that the structure of hepatic lobules in the tissues of the normal group mice was complete with a clear boundary (Fig. 1). Normal group liver cells exhibited funicular radiation, central veins showed no expansion, and the size of liver cells was normal. Control group liver cells exhibited widespread CCl₄-induced liver cell necrosis and tissue damage, including collapse of the hepatic lobule mesh stent and a disorderly tissue structure. In the 100 mg/kg BPP group, the hepatic lobules were damaged and the central areas were partially necrotic; however, these necrotic regions were reduced in size compared with those observed in the control group mice. The 200 mg/kg BPP group exhibited a markedly reduced degree of liver damage, with complete hepatic lobules and no necrosis in the central region of the liver. The condition of the silymarin group livers was comparable to that of the 200 mg/kg BPP group, with no large areas of necrosis, and complete and normal liver tissue structure.

Inflammation-associated gene expression in the liver tissues. The mRNA and protein expression levels of COX-2, iNOS, TNF- α and IL-1 β in mice liver tissues were determined using RT-qPCR and western blot assays (Figs. 2 and 3). The liver tissue mRNA levels of COX-2 (0.49-fold of control), iNOS (0.35-fold), TNF- α (0.23-fold) and IL-1 β (0.21-fold) in the normal group mice were at reduced levels compared with the control group, as the CCl₄-induced hepatic injury caused these expression levels to increase in the control group mice. The protein expression levels of COX-2 (0.15-fold of control), iNOS (0.31-fold), TNF- α (0.43-fold) and IL-1 β (0.45-fold) in liver tissues were also reduced compared with the control. Banana peel polyphenol treatment significantly reduced the expression levels of these factors compared with those of the control mice ($P < 0.05$). The mRNA expression levels of COX-2, iNOS, TNF- α and IL-1 β in the silymarin group mice (0.68-, 0.69-, 0.42- and 0.39-fold of control levels, respectively) were comparable to those in the 200 mg/kg BPP group mice (0.92-, 0.82-, 0.46- and 0.53-fold of control levels, respectively), and lower compared with those of the 100 mg/kg BPP group mice (1.02-, 0.97-, 0.67- and 0.68-fold of control levels, respectively). However, the 200 mg/kg BPP group mice also showed reduced protein expression levels of TNF- α (0.74-fold of control) and IL-1 β (0.69-fold of control) compared with the 100 mg/kg BPP group and control mice, however, the expression of COX-2 (1.20-fold of control) and iNOS (1.10-fold of control) in the 200 mg/kg BPP group mice was not significantly reduced compared with the control group mice ($P < 0.05$).

Discussion

ALT and AST are primarily expressed in liver cells, and liver cell necrosis will elevate the content of ALT and AST. This elevation is consistent with the degree of liver cell damage, and is thus the most commonly-used indicator of liver function (14).

The distribution of the two enzymes in liver cells differs: ALT is predominantly distributed in the cytoplasm, while AST is located in the cytoplasm and the mitochondria (15). In liver function examinations, ALT levels indicate liver cell damage and AST is a marker of liver cell necrosis; thus, the two enzymes are accurate indicators of liver cirrhosis, fibrosis and cancer (16). The elevation of ALT and AST expression levels and the AST/ALT ratio may differ between patients with various different types of hepatitis. In cases of mild hepatitis, although liver cells may be damaged, the mitochondria in the liver cells remain intact. As a result, only the ALT from the cytoplasm of liver cells is released into the blood, so liver function examinations indicate elevated levels of ALT and an AST/ALT ratio of < 1 . However, in cases of fulminant hepatitis and severe liver damage, including cirrhosis and liver cancer, mitochondria in liver cells may be severely damaged (17). As a result, AST is released from the mitochondria and cytoplasm and AST levels are evidently increased, resulting in an AST/ALT ratio of > 1 (18). LDH, a glycolytic enzyme, is a key analyte in liver function examinations, which indicates the degree of liver damage. If the liver is damaged, the activity of LDH is expected to increase significantly (19).

CCl₄ is generated through hepatic P-450 enzyme metabolism. CCl₄ is able to induce lipid peroxidation of the liver cell membrane, which is a crucial factor in liver cell damage (20). MDA is a key index of lipid peroxidation. By detecting the activity of MDA, lipid peroxidation of liver tissues may be detected, indicating the influence of CCl₄ on liver health (21). GSH is a crucial antioxidant and free radical scavenger, which is able to combine with free radicals and heavy metals to transform harmful toxins into harmless substances for excretion (22). Furthermore, GSH combines with harmful substances produced by CCl₄ in the liver and reduces liver damage in order to protect the liver. Previous studies have indicated that GSH may be used to detect the liver damage caused by CCl₄ (23,24). Elevation of TG levels indicated higher fatty acid content, while in a clinical context very high levels of TG are usually associated with liver disease (25). Experimental results have indicated that liver damage induced by CCl₄ may result in increased levels of MDA and TG and reduced levels of GSH (26,27). In the present study, BPPs appeared to mitigate these changes, reducing MDA and TG levels, and elevating GSH levels.

Cytokines are a class of micromolecule polypeptide secreted by various cells, which are able to regulate cell growth and differentiation, immune function, inflammation and wound healing (28). Confirmed proinflammatory cytokines include TNF- α , IL-1 β and IL-6, which serve key functions in the pathogenesis and development of biological damage (29). As key inflammatory mediators, cytokines such as IL-6 and TNF- α are crucially involved in the inflammatory response. Under pathological conditions, the serum levels of TNF- α and IL-6 may increase, resulting in the release of marked quantities of various inflammatory factors, which may in turn lead to inflammation and damages of liver tissue (30). IL-6 may induce increased levels of INF- γ , the primary function of which is to activate non-specific effector cells and mediate the cellular immune process (31). Under laboratory and clinical conditions, the levels of TNF- α , IL-1 β , IL-6 and INF- γ may be used as markers of the degree of liver damage. The higher

the levels of these markers, the faster the liver cell injury will be and the more severe the resulting liver injury. COX-2 is an inducible enzyme that is expressed more markedly in liver cells that are stimulated to exhibit inflammation. The expression of COX-2 increases significantly in liver tissue that has undergone various types of damage, so COX-2 has been regarded as a therapeutic target for the treatment of liver damage (32). In a number of previous studies, the expression levels of iNOS and COX-2 were found to be comparable and positively correlated, and thus the expression of iNOS is increased in liver tissue that exhibits lesions (33-35).

In the present study, BPPs appeared to significantly reduce the serum levels of AST, ALT and LDH in a CCl₄-induced mouse model of hepatic damage. Furthermore, BPPs were able to regulate proinflammatory cytokine levels, resulting in the CCl₄-induced alterations in serum cytokine levels being comparable to those of normal mice. By detecting liver tissues, it was observed that BPPs were able to reduce liver damage. By evaluating the levels of MDA, GSH and TG in the mouse serum and tissues, BPPs were found to reduce the CCl₄-induced lipid peroxidation of the liver by increasing the levels of GSH and reducing the levels of MDA and TG. Furthermore, molecular experiments demonstrated that BPPs were able to reduce the mRNA and protein expression levels of COX-2, iNOS, TNF- α and IL-1 β in the hepatic injury model mice, thus mitigating liver damage. Thus, BPPs may serve a preventive role in hepatic injury by reducing the expression levels of COX-2, iNOS, TNF- α and IL-1 β . In addition, the examination of H&E liver tissue sections indicated that BPPs were able to reduce the manifestations of liver injury.

In conclusion, the results of the present study suggest that BPPs are able to significantly improve a number of the symptoms of CCl₄-induced liver damage in mice, and that the effect is more marked with an increased treatment dose. In future, banana peel could be used in waste utilization or may be used as medicine or a functional compound.

Acknowledgements

This study was supported by the Scientific and Technological Research Program of Chongqing Municipal Education Commission (grant no. KJ1501415) and the Program for Innovative Research Team in Chongqing University of Education (grant no. KYC-extd03-20141002).

References

- Sang LW, Li L and Zheng FC: Study on the comprehensive utilization of banana by-product. *Hei Long Jiang Nong Ye Ke Xue* 4: 96-98, 2006 (In Chinese).
- Liu S, Tang YF, Zhao QL, Li Y, Chen J, Li M, Zhao H and Zhou Y: Extraction of polyphenols from banana peel and anti-fungal effect research. *Hu Nan Sheng Chang Sha Shi Xian Jia Hu Hu Nan Shi Fan Da Xue Yi Xue* 6: 12-14, 2009 (In Chinese).
- Kaviarasan S and Anuradha CV: Fenugreek (*Trigonella foenum-graecum*) seed polyphenols protect liver from alcohol toxicity: a role on hepatic detoxification system and apoptosis. *Pharmazie* 62: 299-304, 2007.
- Kang MC, Kang SM, Ahn G, Kim KN, Kang N, Samarakoon KW, Oh MC, Lee JS and Jeon YJ: Protective effect of a marine polyphenol, dieckol against carbon tetrachloride-induced acute liver damage in mouse. *Environ Toxicol Pharmacol* 35: 517-523, 2013.
- Sebai H, Sani M, Yacoubi MT, Aouani E, Ghanem-Boughanmi N and Ben-Attia M: Resveratrol, a red wine polyphenol, attenuates lipopolysaccharide-induced oxidative stress in rat liver. *Ecotoxicol Environ Saf* 73: 1078-1083, 2010.
- Cauwels A and Brouckaert P: Survival of TNF toxicity: dependence on caspases and NO. *Arch Biochem Biophys* 462: 132-139, 2007.
- Zhao X: Hawk tea (*Litsea coreana* Levl. var. *lanuginosa*) attenuates CCl₄-induced hepatic damage in Sprague-Dawley rats. *Exp Ther Med* 5: 555-560, 2013.
- Weber LW, Boll M and Stampfl A: Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 33: 105-136, 2003.
- Shang D, Xu XQ and Wu WJ: Study of extraction of polyphenols from different states banana peels. *Food Sci Technol* 35: 204-208, 2010 (In Chinese).
- Jahan S, Khan M, Imran S and Sair M: The hepatoprotective role of Silymarin in isoniazid induced liver damage of rabbits. *J Pak Med Assoc* 65: 620-622, 2015.
- Li GJ, Sun P, Wang Q, Qian Y, Zhu K and Zhao X: *Dendrobium candidum* Wall. ex Lindl. attenuates CCl₄-induced hepatic damage in imprinting control region mice. *Exp Ther Med* 8: 1015-1021, 2014.
- Melgar S, Karlsson L, Rehnström E, Karlsson A, Utkovic H, Jansson L and Michaëlsson E: Validation of murine dextran sulfate sodium-induced colitis using four therapeutic agents for human inflammatory bowel disease. *Int Immunopharmacol* 8: 836-844, 2008.
- Chen SS, Zhu K, Wang R and Zhao X: Preventive effect of polysaccharides from the large yellow croaker swim bladder on HCl/ethanol induced gastric injury in mice. *Exp Ther Med* 8: 316-322, 2014.
- Liu D, He H, Yin D, Que A, Tang L, Liao Z, Huang Q and He M: Mechanism of chronic dietary iron overload-induced liver damage in mice. *Mol Med Rep* 7: 1173-1179, 2013.
- Liu H, Qi X, Cao S and Li P: Protective effect of flavonoid extract from Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) fruit on alcoholic liver oxidative injury in mice. *J Nat Med* 68: 521-529, 2014.
- Poortahmasebi V, Alavian SM, Keyvani H, Norouzi M, Mahmoodi M and Jazayeri SM: Hepatic steatosis: prevalence and host/viral risk factors in Iranian patients with chronic hepatitis B infection. *Asian Pac J Cancer Prev* 15: 3879-3884, 2014.
- Nyblom H, Berggren U, Balldin J and Olsson R: High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol Alcohol* 39: 336-9, 2004.
- Mabrouk M, El-Raziky M, Zayed N, Salama R, El-Akel W, Awad T, El Beshlawy M and Esmat G: Clinical, biochemical and pathological profiles of 5464 Egyptian chronic hepatitis C-infected patients. *Hepatogastroenterology* 60: 1731-1735, 2013.
- Dickens H, Ullrich A, Runge D, Mueller B, Olszewski U and Hamilton G: Anticancer drug cis-4-hydroxy-L-proline: Correlation of preclinical toxicology with clinical parameters of liver function. *Mol Med Rep* 1: 459-464, 2008.
- Srivastava SP, Chen NQ and Holtzman JL: The in vitro NADPH-dependent inhibition by CCl₄ of the ATP-dependent calcium uptake of hepatic microsomes from male rats. Studies on the mechanism of the inactivation of the hepatic microsomal calcium pump by the CCl₃ radical. *J Biol Chem* 265: 8392-8399, 1990.
- Liu L, Fan H, Qi P, Mei Y, Zhou L, Cai L, Lin X and Lin J: Synthesis and hepatoprotective properties of *Acanthus ilicifolius* alkaloid A and its derivatives. *Exp Ther Med* 6: 796-802, 2013.
- Karabulut AB, Kafkas ME, Kafkas AS, Onal Y and Kiran TR: The effect of regular exercise and massage on oxidant and anti-oxidant parameters. *Indian J Physiol Pharmacol* 57: 378-383, 2013.
- Yin L, Wei L, Fu R, Ding L, Guo Y, Tang L and Chen F: Antioxidant and hepatoprotective activity of *Veronica ciliata* Fisch. extracts against carbon tetrachloride-induced liver injury in mice. *Molecules* 19: 7223-7236, 2014.
- Ranjbar A, Sharifzadeh M, Karimi J, Tavilani H, Baeri M, Heidary Shayesteh T and Abdollahi M: Propofol attenuates toxic oxidative stress by CCl₄ in liver mitochondria and blood in rat. *Iran J Pharm Res* 13: 253-262, 2014.
- Tomizawa M, Kawanabe Y, Shinozaki F, Sato S, Motoyoshi Y, Sugiyama T, Yamamoto S and Sueishi M: Elevated levels of alanine transaminase and triglycerides within normal limits are associated with fatty liver. *Exp Ther Med* 8: 759-762, 2014.

26. Song HY, Mao ZM, Yang LL, Liu T, Li DF, Zhang L, Ge YL, Zheng PY, Liu P, Zhang XQ and Ji G: Dangfei liganning capsules attenuate the susceptibility of rat nonalcoholic fatty liver to carbon tetrachloride toxicity. *J Tradit Chin Med* 31: 327-333, 2011.
27. Sun Y, Yang X, Lu X, Wang D and Zhao Y: Protective effects of Keemun black tea polysaccharides on acute carbon tetrachloride-caused oxidative hepatotoxicity in mice. *Food Chem Toxicol* 58: 184-192, 2013.
28. Schett G, Elewaut D, McInnes IB, Dayer JM and Neurath MF: How cytokine networks fuel inflammation: Toward a cytokine-based disease taxonomy. *Nat Med* 19: 822-824, 2013.
29. Bae UJ, Yang JD, Ka SO, Koo JH, Woo SJ, Lee YR, Yu HC, Cho BH, Zhao HY, Ryu JH, Lee SM, *et al*: SPA0355 attenuates ischemia/reperfusion-induced liver injury in mice. *Exp Mol Med* 46: e109, 2014.
30. Yang Y, Yang J and Jiang Q: The protective effect of huperzine A against hepatic ischemia reperfusion injury in mice. *Transplant Proc* 46: 1573-1577, 2014.
31. Bodienkova GM, Alekseev R, Boklazhenko E and Kurchevenko S: Inflammation mediators in employees in chronic exposure to neurotoxicants. *Int J Occup Med Environ Health* 27: 619-626, 2014.
32. Malekinejad H, Rezabakhsh A, Rahmani F and Razi M: Paraquat exposure up-regulates cyclooxygenase-2 in the lungs, liver and kidneys in rats. *Iran J Pharm Res* 12: 887-896, 2013.
33. Ibrahim MA, Abdel-Gaber SA, Amin EF, Ibrahim SA, Mohammed RK and Abdelrahman AM: Molecular mechanisms contributing to the protective effect of levosimendan in liver ischemia-reperfusion injury. *Eur J Pharmacol* 741: 64-73, 2014.
34. Dong M, Hong T, Liu S, Zhao J, Meng Y and Mu J: Hepatoprotective effect of the flavonoid fraction isolated from the flower of *Inula britannica* against D-Galactosamine-induced hepatic injury. *Mol Med Rep* 7: 1919-1923, 2013.
35. Huang GJ, Deng JS, Huang SS, Lee CY, Hou WC, Wang SY, Sung PJ and Kuo YH: Hepatoprotective effects of eburicoic acid and dehydroeburicoic acid from *Antrodia camphorata* in a mouse model of acute hepatic injury. *Food Chem* 141: 3020-3027, 2013.