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Full Length Research Paper

Hepatoprotective effect of *Lysimachia paridiformis* Franch. var. *stenophylla* Franch. on CCl_4-induced acute liver injury in mice

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As Chinese folklore medicine, *Lysimachia paridiformis* Franch. var. *stenophylla* Franch. (LPF) has been used for treatment of various diseases such as rheumatism and hemiplegia. Our previous studies have shown that LPF have strong antioxidant activity *in vitro*. Nevertheless, there have been no reports on the hepatoprotective effect of the extracts of LPF in animals. The aim of this study is to investigate the hepatoprotective effect of the extracts of LPF (LPFPE, LPFEA and LPFBU) on CCl_4-induced acute liver injury mice. Intragastric administration of LPFPE (1000, 500 and 250 mg/kg, respectively), LPFEA (800, 400 and 200 mg/kg, respectively) and LPFBU (600, 300 and 150 mg/kg, respectively) were carried out on mice for 8 days. 2 h after the final administration except for the normal control group, the other groups were intraperitoneally injected with CCl_4 diluted in olive oil at the dose of 0.05 ml/kg, and normal control group was injected with an equivalent volume of olive oil alone. The level of glutamic-oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in each treatment group significantly decreased ($p < 0.01$ and $p < 0.05$, respectively). The level of malondialdehyde (MDA) in liver for each treatment group significantly decreased ($p < 0.001$), and the level of SOD in liver for each treatment group significantly increased ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively). It was demonstrated that three extracts of LPF had good hepatoprotective effect for CCl_4-induced liver injury mice, which may be attributable to its antioxidant activity.

Key words: Antioxidant activity, hepatoprotective effect, *Lysimachia paridiformis* Franch. var. *stenophylla* Franch.

INTRODUCTION

CCl_4 is widely used to induce liver damage in animal models and to investigate the role of lipid peroxidation as a mediator of hepatic injury (Brattin et al., 1985). It is now generally accepted that CCl_4 toxicity results from its bio-activation to the trichloromethyl free radical ($CCl_3^{\cdot}$) by cytochrome P450 isozymes (P450s) (Raucy et al., 1993), which reacts rapidly with molecular oxygen to produce the trichloromethyl peroxy radical ($CCl_3O_2\cdot$). These radicals initiate lipid peroxidation by withdrawing allylic hydrogens from polyunsaturated fatty acids (Slater, 1984; Recknagel et al., 1989). When the amount of reactive oxygen species production exceeds the capacity of the endogenous cellular antioxidant system, significant liver injury can occur (Williams et al., 1990). Hepatic damage induced by CCl_4 resulted in an increase in the level of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in serum (Berry et al., 1992; Romero et al., 1998). Lipid peroxidation can produce some free radicals including the malondialdehyde content increased (MDA). SOD was responsible for the detoxification of deleterious oxygen radicals (Sandesh et al., 2010). Many studies have reported that herbal medicines play an important role in the treatment of hepatic disorders for their good antioxidants (Park et al.,

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2000; Tang et al., 2006; Yue and Kang, 2011; Gong et al., 2012).

Lysimachia paridiformis Franch. var. *stenophylla* Franch. (LPF), belongs to Primulaceae family. It is distributed in the southwest region of China. The roots or whole plant of LPF are used as a traditional Chinese herbal medicine for rheumatism, limb spasm, hemiplegia, convulsions in children, and fracture (State Administration of Traditional Chinese Medicine, 1997). Phytochemical research showed that flavonoids and volatile oil were main compounds in LPF (Zhou et al., 2002; Zhang et al., 2010). Pharmacological research showed that total flavonoids of LPF have anti-rheumatism activity (Qi et al., 2010), but there is no research on LPF about hepatoprotective effect.

MATERIALS AND METHODS

Plant material and extract preparation

Air-dried plant of LPF was collected from Duyun in the province of Guizhou, China, in August 2007 and identified by Professor Fan Liu (Guiyang College of Traditional Chinese Medicine). The specimen was deposited in the Institute of Chinese Materia Medica, Henan University, model codes 20070823. The air dried LPF were extracted three times with methanol for 3 days at room temperature, respectively. After evaporation of solvent in a vacuum pump, the concentrated extract was suspended in water and extracted with petroleum ether, EtOAC (ethyl acetate) and *n*-BuOH (*n*-butanol), respectively. The solution was concentrated under reduced pressure to yield petroleum ether extract (LPFPE), EtOAC extract (LPFEA) and *n*-BuOH extract (LPFBU), respectively.

Materials and animals in experiments

CCl_4 were purchased from Sigma Chemical Co. Bifendate pills were purchased from Zhejiang pharmaceutical Co., Ltd. (No: 090205). Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), maleicdialdehyde (MDA) and superoxide dismutase (SOD) were purchased from the Nanjing Jianchen Bioengineering Institute (Jiangsu, China). Coomassie brilliant blue G-250 (packing plant of Chemical Reagent Co. Shanghai, Batch No: 20050115), and bovine serum albumin were obtained from Beijing AoBoxing research bio-tech co., Ltd (Beijing, China). Other chemicals and reagents used in these experiments were of analytical grade and were purchased from commercial sources.

Animals

Male Kunming normal mice weighing 20 ± 2 g was obtained from the Experimental Animal Center of Henan Province (Zhengzhou, Hennan, China) and maintained in a temperature (23 ± 2°C), humidity (55 to 60%) controlled room with a 12-h light–dark cycle. Mice were housed in plastic cages with free access to food and water. All animal procedures were approved by the ethical committee in accordance with the 'Institute ethical committee guidelines' for Animal Experimentation and Care (HNPR-2009-05003).

Experimental design and treatment schedule

Mice were randomly divided into twelve groups with 10 mice per group. Group 1 (normal control) was treated with distilled water. Group 2 (liver injury model control) were treated with distilled water. Group 3 was given bifendate (70 mg/kg) as positive control. Group 4, 5 and 6 were given 1000, 500 and 250 mg/kg of LPFPE, respectively. Group 7 to 9 received 800, 400, and 200 mg/kg of LPFEA, respectively. Group 10 to 12 received 600, 300, and 150 mg/kg of LPFBU, respectively. The duration of treatment was 8 days for mice by intragastric administration. After 8 days, at 2 h after the final administration except for the Group 1, the mice were intraperitoneally injected with CCl_4 diluted in olive oil at the dose of 0.05 ml/kg bodyweight, and the mice of Group 1 were injected with an equivalent volume of olive oil alone (Chen et al., 2004). Fasting for 16 h after the CCl_4 injection, mice was weighed and then killed for blood collection via puncture of the retro-orbital venous plexus for determination of plasma GPT and GOT activities. Following collection of blood sample, the liver was rapidly removed, and weighed. Blood samples were centrifuged (3000 rpm for 15 min at 4°C) for separating the serum. The liver homogenate solution of 10 and 1% for determining the level of MDA and SOD was processed at 0 to 3°C by tissue homogenizer respectively.

Biochemical analyses

The protein content in homogenates was assayed by the method of Lowry et al. (1951) using bovine plasma albumin as a standard. The level of GOT, GPT, SOD and MDA were measured following the commercial kit's instructions.

Statistical analysis

Statistical analyses were carried out using SPSS 17.0 software. The overall significance of the results was examined using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. $p < 0.05$ was considered statistically significant. All values were expressed as mean values ± standard deviation (SD).

RESULTS

Effect of LPFPE, LPFEA and LPFBU on GPT and GOT in serum

Figure 1 showed the level of GOT and GPT in normal and acute liver injured rats. The level of GOT and GPT was significantly increased in acute liver injury control mice compared with normal mice ($p < 0.01$ and $p < 0.05$, respectively). Compared with liver injury control, the level of GPT and GOT were significantly decreased ($p < 0.01$ and $p < 0.05$, respectively) in administration of each dose group of LPFPE, LPFEA, LPFBU and bifendate (70 mg/kg).

Effect of LPFPE, LPFEA and LPFBU on MDA and SOD in liver

As shown in Figures 2 and 3, the level of MDA in liver was significantly increased in liver injury control mice ($p < 0.001$) and the level of SOD in liver was significantly decreased ($p < 0.001$) compared with normal control. It

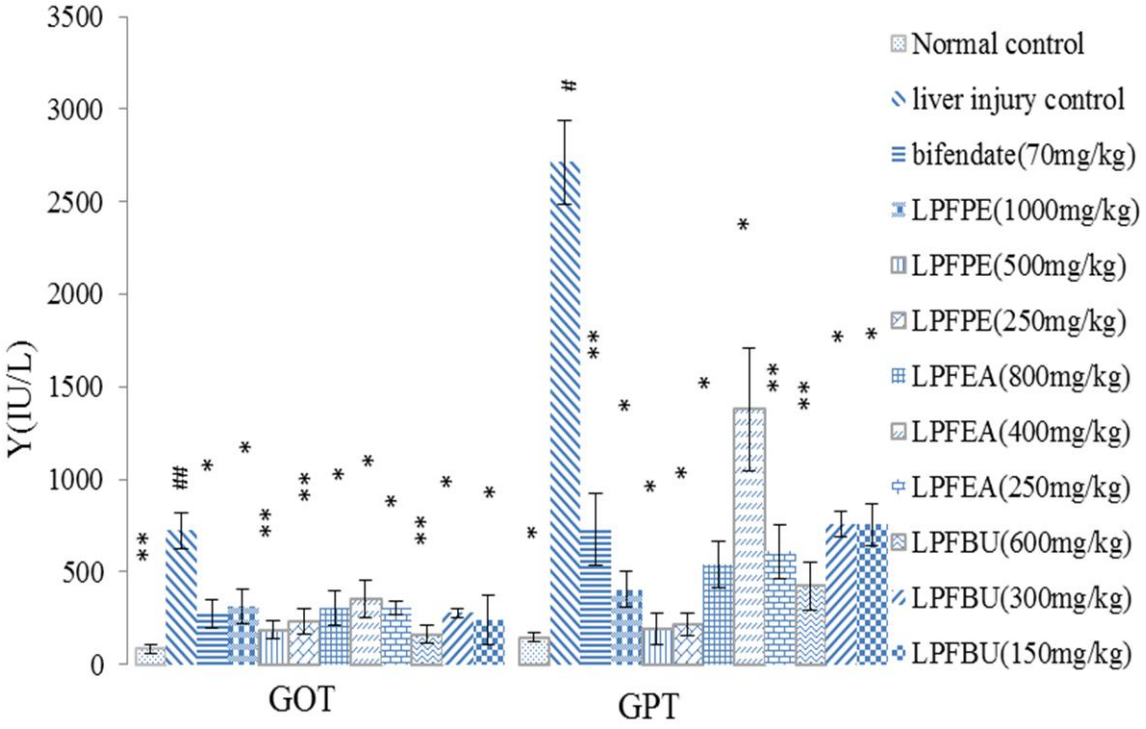

Figure 1. Effects of LPEPE, LPFEA and LPFBU on GPT and GOT in acute liver injury mice. Data expressed as mean ± SD (n = 10). Bifendate was used as the positive control drug. $^{\#}$, $p < 0.05$, $^{\#\#}$ p <0.01, $^{\#\#\#}$ $p < 0.001$ Normal group compared CCL_4-induced acute liver injury. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ treated group compared with CCL_4-induced acute liver injury.

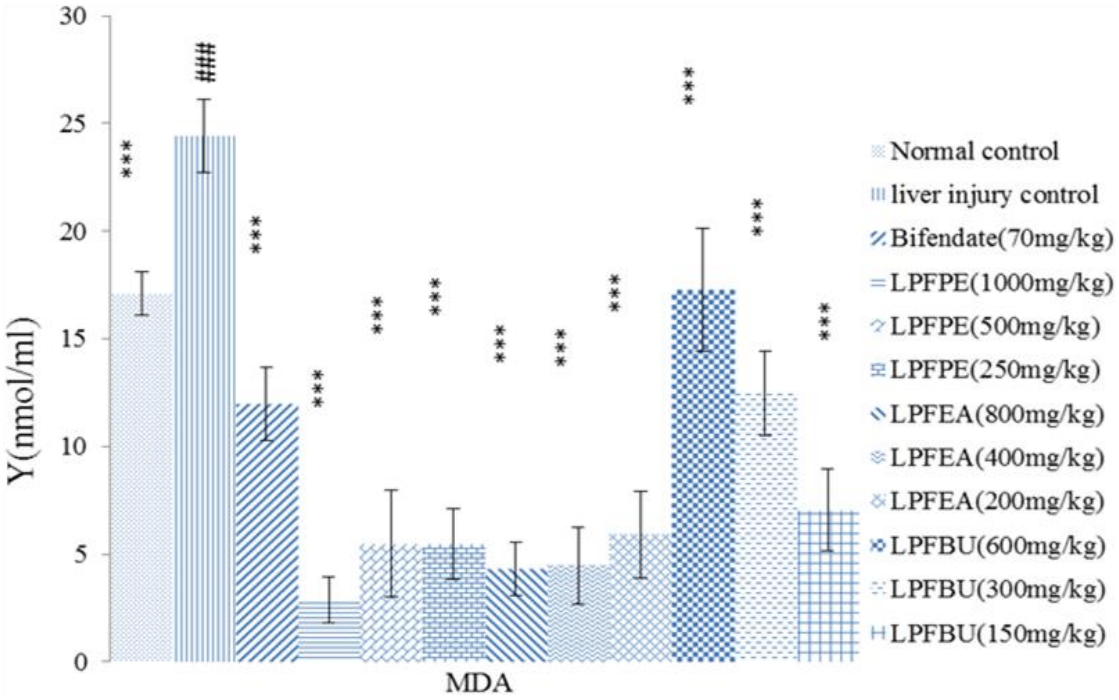

Figure 2. Effects of LPEPE, LPFEA and LPFBU on liver tissue MDA in acute liver injury mice. Data expressed as mean ± SD (n = 10). Bifendate was used as the positive control drug. $^{\#}$, $p < 0.05$, $^{\#\#}$, $p < 0.01$, $^{\#\#\#}$, $p < 0.001$ normal group compared CCL_4-induced acute liver injury. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ treated group compared CCL_4-induced acute liver injury.

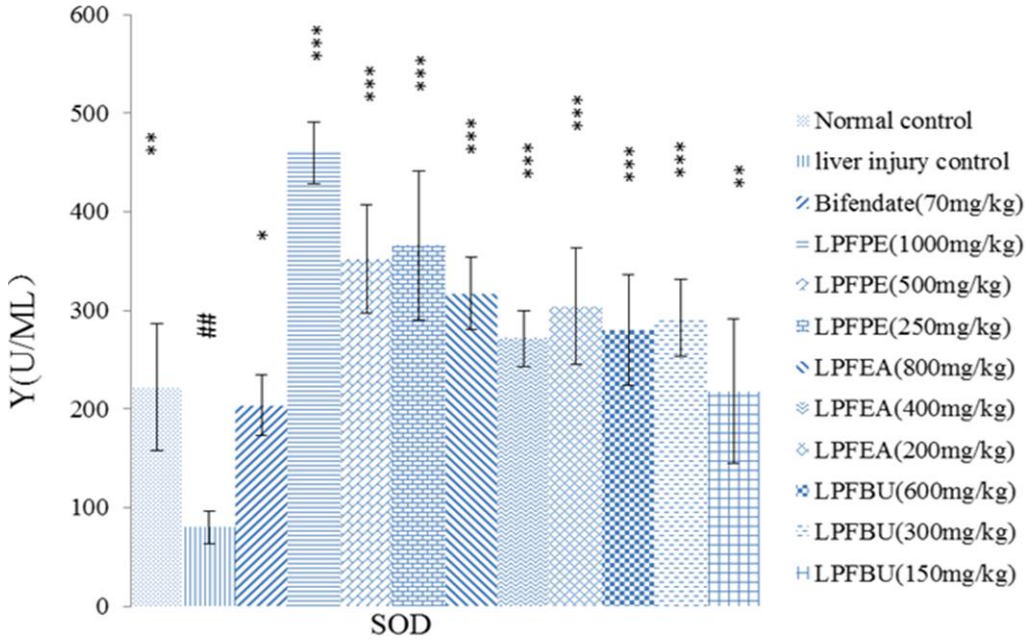

Figure 3. Effects of LPEPE, LPFEA and LPFBU on liver tissue SOD in acute liver injury mice. Data expressed as mean ± SD (n = 10). Bifendate was used as the positive control drug. $^{\#}$, $p < 0.05$, $^{\#\#}$, $p < 0.01$, $^{\#\#\#}$, $p < 0.001$ Normal group compared CCL_4-induced acute liver injury. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ treated group compared CCL_4-induced acute liver injury.

indicated the model of CCL4-induced liver injury control mice was established. On intragastric administration of LPFPE, LPFEA, LPFBU and bifendate to mice for 8 days, the level of MDA was decreased significantly ($p < 0.001$). The level of SOD in liver was significantly increased ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively).

DISCUSSION

Free radicals can lead to oxidative stress and cell injury due to damage to cellular proteins, lipids, and carbohydrates (Yu, 1994). According to the free radical theory, one of the strategies in preventing oxidative stress-induced hepatotoxicity is to block or retard the chain reaction of oxidation (Lee et al., 2007). CCl_4, a hepatotoxin for evaluating hepatoprotective agents, is commonly used to induce liver damage by producing free radical intermediates (malondialdehyde and 4-hydroxy-2-nonenal). Hepatoprotective studies showed that plants have active ingredients that are capable of free radical scavenging in living systems (Mitra et al., 1998). A major defense mechanism involves the antioxidant enzymes, including SOD, catalase and glutathione peroxidase (GPx), and is well known to protect liver cells against oxidative damage through chemical or enzymatic reactions. Scavenging of free radicals is one of the major mechanisms to inhibit the chain reaction of lipid peroxidation (Merlin and Parthasarathy, 2011). In this study, the free radical scavenging activity of extracts of LPF was evaluated by 3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP assay in *vitro* and antioxidant activity was assayed by estimation of MDA and SOD levels in *vivo*. The result showed that *n*-butanol and ethyl acetate extracts showed higher antioxidant activity than and ethyl acetate extracts and the petroleum ether extract. The level of MDA was decreased significantly ($p<0.001$). The level of SOD in liver was increased significantly ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively). Reducing the level of MDA and increasing the level of SOD, decrease lipid peroxidation and/or decrease utilization, enhance antioxidant capability and protect the body to further oxidative damage from free radicals (Sandesh et al., 2010; Hu et al., 2008).

CCl_4 is known to cause hepatic damage, with a marked elevation in the serum levels of the aminotransferases enzymes GOT and GPT, because these enzymes are cytoplasmatic and are released into the blood after cellular damage (Recknagel et al., 1989). The results showed that the level of GPT and GOT were significantly decreased ($p < 0.01$ and $p < 0.05$, respectively) in administration of each dose group of LPFPE, LPFEA, LPFBU and bifendate (70 mg/kg), compared with liver injury control.

In conclusion, the study indicates that LPF has a remarkable protective effect against CCl_4- induced liver injury in mice and its mechanism is related, at least in part, to its free radical scavenging and antioxidant activity.

However, further studies are in progress for better understanding of the mechanism of action and its hepatoprotective activity.

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