Full Length Research Paper

Hepatoprotective effect of *Mitragyna rotundifolia* Kuntze on CCl$_4$-induced acute liver injury in mice

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In the previous studies, it was revealed that *Mitragyna rotundifolia* Kuntze (MR) had strong antioxidant activity *in vitro*. Oxidative stress is considered to play a prominent causative role in the development of various hepatic disorders. *n*-Butanol extract from *M. rotundifolia* barks (MRBBU) and leaves (MRLBU) were investigated, and it played a protective role against carbon tetrachloride (CCl$_4$)-induced acute liver injury in mice. The degree of protection has been measured by using biochemical parameters, such as serum glutamic pyruvic transaminase (GPT), serum glutamic oxaloacetic transaminase (GOT), malondialdehyde (MDA) and superoxide dismutase (SOD) in liver tissue homogenate. The results of the intragastric administration of MRBBU (300, 150 and 75 mg/kg body weight per day, respectively) and MRLBU (300, 150 and 75 mg/kg body weight per day, respectively) on CCL$_4$-induced acute liver injury in mice for 8 days demonstrated that the level of GPT and GOT in each treatment group decreased significantly (P < 0.001). It was observed that the level of liver MDA in MRLBU (75 mg/kg) group did not significantly decreased (P > 0.05), but that in the other treatment groups significantly decreased (P < 0.01 and P < 0.05, respectively). With the exception of MRLBU group (150 and 75 mg/kg) (P > 0.05), the level of SOD in other treatment groups significantly increased (P < 0.001, P < 0.01 and P < 0.05, respectively). Results indicated that MR has hepatoprotective effect against CCl$_4$-induced oxidative damage in mice, and the hepatoprotective effect may be correlated with its antioxidant effects.

Key words: *Mitragyna rotundifolia* Kuntze, antioxidant activity, carbon tetrachloride, hepatoprotective effect.

INTRODUCTION

Liver injury can be induced by various factors, and hepatotoxins, such as CCl$_4$, ethanol and acetaminophen which are metabolized by cytochrome P450 2E1 (CYP2E1) (Sun et al., 2001). CCl$_4$, the classic heaptotoxin, 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). The mechanism of CCl$_4$-induced acute liver injury is accepted widely that CCl$_4$ was metabolized to a highly reactive trichloromethyl radical (CCl$_3^-$) by cytochrome P450 in liver. CCl$_3^-$ in liver can induce lipid peroxidation and leads to hepatocellular membrane damage (Ohta et al., 1998; Drill, 1952). Natural antioxidants could prevent the deleterious effects of toxic agents by scavenging free radicals and other reactive oxygen species (Domitrovi´c

et al., 2011). Many herbs, which have been reported to counteract the oxidative damage may directly interact with the reactive radicals or increase the capacity of endogenous antioxidant defense. The defensive provided by antioxidant systems is therefore crucial in liver injury and disease.

Mitragyna rotundifolia Kuntze (MR), belongs to Rubiaceae family, is widely found in Africa and Asia. It has been used in various folk medications for the treatment of fever, hernia, muscle pain and antimalarial (Shellard and Phillipson, 1964). Phytochemical research showed that alkaloids and triterpenoids were main compounds in MR stems, and flavonoids were main compounds in leaves (Kang and Hao, 2006; Kang et al., 2007). Pharmacological research showed that it has antitumor, cardiovascular disease and antibacterial activity (Shellard et al., 1978, 1967, Shellard and Hought 1974). In our previous studies, it was suggested that ethyl acetate and *n*-butanol extract from *M. rotundifolia* barks and leaves, caffeic acid, catechin and *epi*-catechin in

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MRS have good antioxidant effects (Kang and Li, 2009, Kang et al., 2010; Song and Kang, 2009). There is no research of MR about hepatoprotective effect. In order to investigate the protective effect of MR in treating liver injury mice, MR extracts were assayed using the CCl_4-induced liver injury mice model. The parameters studied include the serum enzyme level of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) (Berry et al., 1992; Romero et al., 1998). Furthermore, markers of hepatic oxidative damage were the measure-ment of level of malondialdehyde (MDA) and superoxide dismutase (SOD).

MATERIALS AND METHODS

CCl_4 were purchased from Sigma Chemical Co. Bifendate pills (Zhejiang pharmaceutical Co., Ltd., No: 090205). GOT, GPT, MDA and SOD were from the Nanjing Jianchen Bioengineering Institute (Jiangsu, China). Coomassie brilliant blue G-250 (packing plant of Chemical Reagent Co. Shanghai, Batch No: 20050115), bovine serum albumin 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.

Plant and extract preparation

Dried barks and leaves of MR were collected from Xishuangbanna regions in Yunnan province, China, in October 2006 and were identified by Professor Jing-yun Cui. The specimen was deposited in Institute of Chinese Materia Medica, Henan University.

Air-dried barks and leaves of MR were extracted three times with 70% acetone water for 7 days at room temperature, respectively. After evaporation of solvent in a vacuum, the concentrated extract was suspended in water and was extracted with petroleum ether, ethyl acetate and n-butanol, respectively. Solution was concentrated under reduced pressure to yield petroleum ether of barks and leaves of MR (MRBPE and MRLPE), ethyl acetate of barks and leaves of MR (MRBEA and MRLEA) and n-butanol of barks and leaves of MR extract (MRBBU and MRLBU), respectively.

Animals

Male Kunming normal mice that weighted 20 ± 2 g was obtained from the Experimental Animal Center of Henan province. (Zhengzhou, Hennan, China) and was maintained in a temperature ($23 \pm 2°C$) and humidity (55 to 60%) controlled room with a 12 h light-dark cycle. Animals 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 nine groups with 10 mice per group: normal control group, CCl_4 model group, bifendate (70 mg/kg) group, MRBBU (300, 150 and 75 mg/kg) groups, and the

MRLBU (300, 150 and 75 mg/kg) groups, respectively. Mice were administered orally by gastric gavage with different doses of MRBBU, MRBBU and bifendate at a volume of 10 ml/kg once a day for 8 days. The normal control group and the CCl_4 model group were administered with an equivalent volume of distilled water. On day 8, at 2 h after the final administration of MRBBU, MRBBU and bifendate, the mice were intraperitoneally injected with CCl_4 diluted in olive oil at the dose of 0.05 ml/kg body weight, and the normal control group was injected with an equivalent volume of olive oil alone (Chen et al., 2004). At 16 h after the CCl_4 injection, each mouse was weighed and then killed under light ether anesthesia for blood collection via puncture of the retro-orbital venous plexus. All mice sacrificed comply with the international guides of animal sacrifice. Serum was obtained from the collected blood by centrifugation immediately after death; livers were isolated and cut into small pieces and homogenized with Tris/HCl (5 mmol/L containing 2 mmol/L ethylenediaminetetraacetic acid (EDTA), pH 7.4). The homogenates were centrifuged at 3000 g for 10 min at 4°C and clear supernatants were used immediately for assessment of MDA and SOD.

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 levels 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 $\pm$ standard deviation (SD).

RESULTS

Effect of MRBBU and MRLBU on GPT and GOT in serum

The level of GPT and GOT in normal and acute liver injury mice is as shown in Table 1. The levels of hepatic GPT and GOT in CCl_4-treated rats increased significantly when compared with the normal control ($P < 0.001$). The level of GPT and GOT significantly decreased ($P < 0.001$) in administration of each dose group of MRBBU, MRLBU and bifendate (70 mg/kg), and tends to bring the level to near normal. Compared with positive control of bifendate (70 mg/kg), intragastric administration of MRBBU and MRLBU has no significantly difference (Table 1 and Figure 1).

### Effect of MRBBU and MRLBU on MDA and SOD in liver

The effect of different doses of MRBBU and MRLB on the level of SOD and MDA in normal and CCl_4-induced liver injury mice was given in Table 2 and Figures 2 and 3.

The level of MDA in liver significantly increased in liver

Table 1. Effect of *M. rotundifolia* Kuntze on GPT and GOT in acute liver injury mice.

Group	Dose (mg/kg)	GPT (IU/L)	GOT (IU/L)
Normal control	/	330.66 ± 68.72***	170.94 ± 44.74***
Liver injury control	/	1437.15 ± 158.88###	576.15 ± 93.99###
Bifendate	70	845.36 ± 79.03***	321.21 ± 31.69***
MRBBU	300	783.13 ± 65.37***	388.27 ± 66.55***
MRBBU	150	725.15 ± 31.15***	237.27 ± 20.73***
MRBBU	75	765.91 ± 40.36***	310.214 ± 53.00***
MRLBU	300	881.91 ± 78.80***	331.7 ± 57.67***
MRLBU	150	765.92 ± 61.42***	197.76 ± 46.18***
MRLBU	75	774.75 ± 104.89***	255.66 ± 19.12***

Data expressed as means ± SD (n = 10). Bifendate was as the positive control drug. P < 0.05, ##P < 0.01 and ###P < 0.001, normal group as compared with CCL_4-induced acute liver injury. *P < 0.05, **P < 0.01, and ***P < 0.001, treated group as compared with CCL_4-induced acute liver injury.
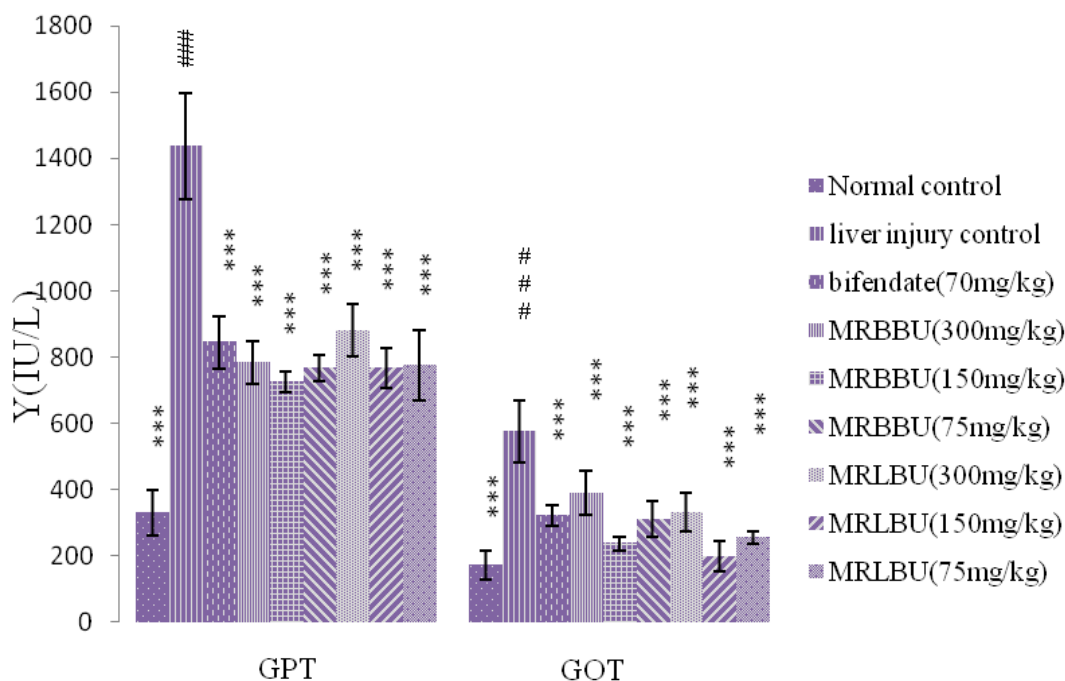
Figure 1. Effect of *M. rotundifolia* Kuntze on GPT and GOT in serum.

injury control mice (P < 0.05) and the level of SOD in liver significantly decreased (P < 0.01), when compared with normal mice. It suggested that CCl_4-induced liver injury control mice had been established successfully. The level of MDA decreased significantly (P < 0.01 and P < 0.05, respectively) except for the MRLBU group (75 mg/kg) (P > 0.05). The level of SOD in liver only in administration of MRLBU (150 and 75 mg/kg) had no significant increase (P > 0.05), the other treatment groups had significant increase (P < 0.001 and P < 0.05, respectively), but did not present dose-dependent manner.

DISCUSSION

Acute and chronic liver diseases constitute a global concern, and it is mostly induced by viral hepatitis, alcoholism, iron overload or drug toxicity. Among these types of liver injuries, there is consistent evidence of enhanced production of free radicals and/or a significant decrease in antioxidant defence mechanisms (Hoek and Pastorino, 2002). Many drugs have been used in the treatment of liver damage. However, the medical treatments for acute and chronic liver diseases are often

Table 2. Effect of *M. rotundifolia* Kuntze on SOD and MDA in liver.

Group	Dose (mg/kg)	MDA (nmol/ml)	SOD (U/ml)
Normal control	/	8.02±0.43*	334.85±49.65**
liver injury control	/	22.03±1.77[#]	115.91±16.70[##]
Bifendate	70	9.43±1.44**	199.19±28.23
MRBBU	300	11.27±0.80*	212.49±39.45*
MRBBU	150	10.66±0.81*	290.21±52.49***
MRBBU	75	6.53±2.87**	243.51±58.51***
MRLBU	300	9.52±1.56**	206.49±25.96*
MRLBU	150	7.18±1. 31**	181.65±27.95
MRLBU	75	10.54±0.52	186.98±27.06

Data expressed as means ± SD (n = 10). Bifendate was as the positive control drug. $P < 0.05$, [##]$P < 0.01$, [###]$P < 0.001$, normal group as compared with CCL_4-induced acute liver injury. *$P < 0.05$, "$P < 0.01$, ***$P < 0.001$, treated group as compared with CCL_4-induced acute liver injury.
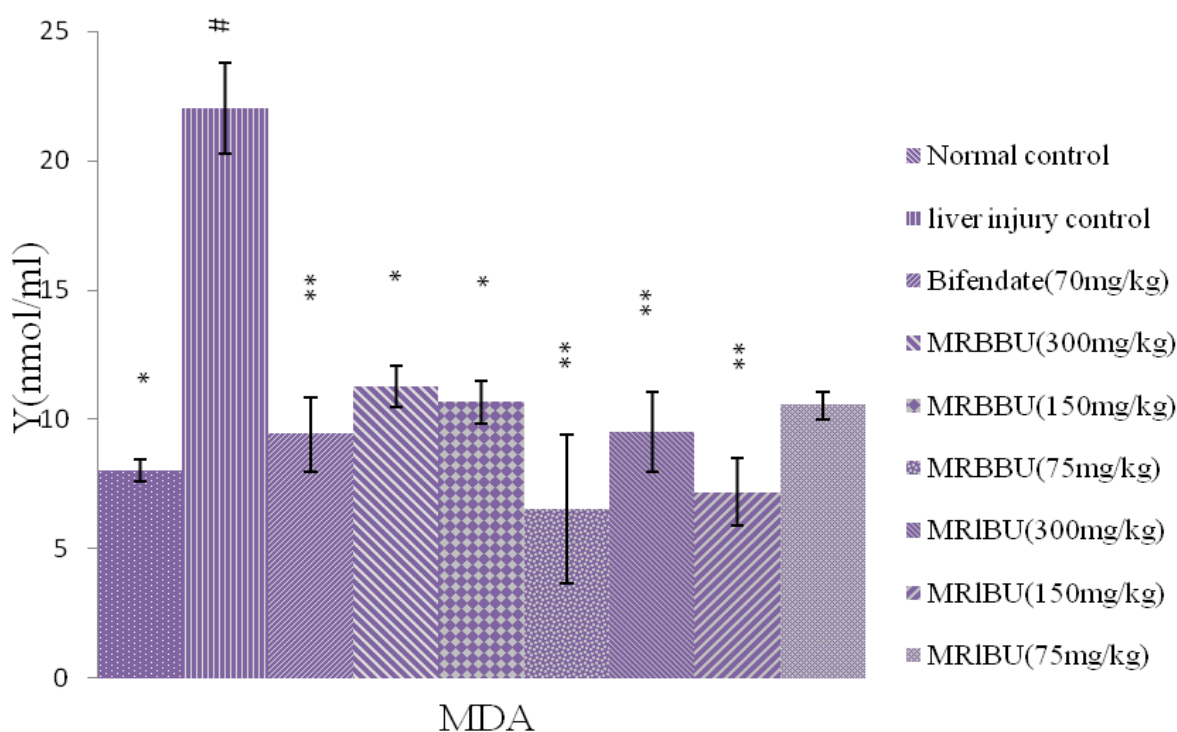
Figure 2. Effect of *M. rotundifolia* Kuntze on MDA in liver.

difficult to handle and have limited efficacy. In recent years, the therapeutic benefits of traditional Chinese medicine have been recognized and widely accepted. Hepatoprotective effects of MR in the CCl_4-induced liver injury model in mice are reported for the first time.

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). Results showed that a significant increase in the level of GOT and GPT in CCl_4-treated mice ($P < 0.001$). The level of GPT and GOT decreased significantly ($P < 0.001$) in administration of each dose group of MRBBU, MRLBU and bifendate (70 mg/kg), tend to bring the level to near normal and is not dose-dependent. These results indicate that MR can exert a hepatoprotective effect on CCl_4-induced acute liver injury in mice.

In the liver, CCl_4 is responsible for oxidative stress and lipid peroxidation through the cytochrome P450-mediated generation of the highly reactive $CCl_3\cdot$, leading to eventual cellular damage characterized by producing free
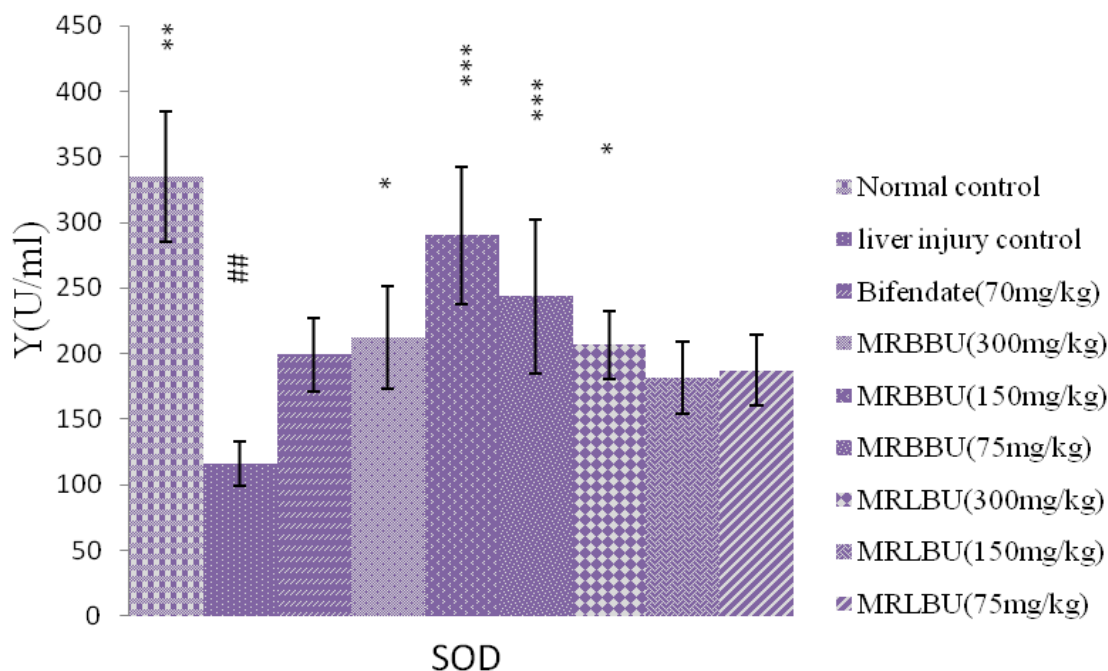
Figure 3. Effect of *M. rotundifolia* Kuntze on SOD in liver.

radical intermediates (malondialdehyde and 4-hydroxy-2-nonenal) (Taieb et al., 2005). SOD was responsible for the detoxification of deleterious oxygen radicals (Sandesh et al., 2010). Lipid peroxidation is thought to be one of the major pathways of disease initiation and proliferation. Increased lipid peroxidation and impaired antioxidant enzyme function in the liver tissue are characteristic observations in CCl_4-induced mice (Weber et al., 2003; Recknagel et al., 1989). In a previous study, the free radical scavenging activity of extracts of *M. rotundifolia* Kuntz was evaluated by 1,1-diphenyl-2-picryhidrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assay *in vitro*. Result showed that *n*-butanol extract of MR barks and leaves of MR, and leaves showed higher antioxidant activity than ethyl acetate extracts, and the petroleum ether extracts and the leaves and bark extract of the same solvent had similar antioxidant activity (Kang et al., 2010). Antioxidant is one of the hepatoprotective mechanisms to decrease lipid peroxidation and oxidant stress. Therefore, protective effect of *n*-butanol extracts for CCl_4-induced liver injury in mice was investigated in this paper. The level of MDA decreased significantly ($P < 0.01$ and $P < 0.05$, respectively), except for the group of MRLBU (75 mg/kg) ($P > 0.05$). The level of SOD in liver only in administration of MRLBU (150 and 75 mg/kg) had no significant increase ($P > 0.05$), the other treatment groups had significantly increase ($P < 0.001$ and $P < 0.05$, respectively). The level of glutamic pyruvic transaminase

(GPT) and glutamic oxaloacetic transaminase (GOT) in each treatment group significantly decreased ($P < 0.001$). The result indicates that MRBBU and MRLBU had very good hepatoprotective activity. Further work is necessary to isolate active ingredients and elucidate the actual mechanism involved in the hepatoprotective and antioxidant activity of this plant.

Conclusion

Conclusively, results indicate that the MRBBU and MRLBU extract of *M. rotundifolia* Kuntze possesses potential hepatoprotective activity against CCl_4-induced acute liver injury and this may be attributed to its free radical scavenging potential.

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