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Radical Scavenging Effects and Protective Effect of *Spatholobus suberectus* against CCl_4-induced Liver Damage in Rats

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Abstract – In the present study, we obtained an extract from the dried stem of *Spatholobus suberectus* Dunn by using 85% methanol (MeOH extract of *S. suberectus*; MSS) and investigated its radical scavenging effects *in vitro* and its protective effect against CCl_4-induced liver damage in rats. MSS scavenged the 1,1-diphenyl-2-picrylhydrazyl radical almost completely and inhibited $FeSO_4$-induced lipid peroxidation (LPO) in the liver homogenate. Oral administration of MSS significantly reduced the serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase and inhibited LPO in the liver tissue after CCl_4 treatment in rats. These results suggest that *S. suberectus* could be a candidate agent for the treatment of diseases related to oxidative stress.
Keywords – *Spatholobus suberectus*, carbon tetrachloride, free radical, lipid peroxidation, antioxidant effect

Introduction

The stem of *Spatholobus suberectus* Dunn has been used to treat menstrual abnormalities, inflammation of the peripheral blood vessels and arthritis in Asia (Wu, 2005). It has been reported to have an anti-inflammatory activity (Li *et al*., 2003), a hypocholesterolemic effect (Wang *et al*., 1991) and a radical scavenging effect in an *in vitro* system (Cha *et al*., 2005). However, the effects of *S. suberectus* against free radical-related organ damage in an *in vivo* system have not been studied.

In the investigation for antioxidant agents, pathological models and free radical-inducing agents have been used (Sanchez-Moreno, 2002). Carbon tetrachloride (CCl_4) is one of the agents used to induce free radical toxicity and has been widely used in animal models of free radical-related organ injury (Weber *et al*., 2003). Lipid peroxidation (LPO) is the major phenomenon underlying CCl_4-induced tissue injury and is the result of damage to the cell membrane by free radicals (Janero, 1990). Besides the induction of LPO, there is an increase in the serum levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (ALP) after CCl_4 treatment (Reddrop *et al*., 1983).

We therefore designed the present study to investigate the protective effect of *S. suberectus* against free radical-related tissue damage in an *in vivo* system. For this purpose, we confirmed the scavenging effect of *S. suberectus* against the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), its inhibition of LPO induced by $FeSO_4$ in liver homogenate and its protective effect against CCl_4-induced liver damage in rats by measuring LPO and serum GOT, GPT and ALP levels.

Experimental

Plant material and preparation of extracts – A dried stem of *S. suberectus* was purchased from Dongwoodang Pharmacy Co., Ltd. (Youngchun, Kyungbuk, Korea) in 2006 and was identified by Prof. H. Kim, a professor at the College of Oriental Medicine, Kyung Hee University. A voucher specimen (dried drug, WH044; extract, WME044) has been deposited at the Department of Oriental Pharmacy, College of Pharmacy, Woosuk University. An extract was obtained twice from the dried sample (200 g) with 3,000 mL of 85% MeOH under ultrasonification for 2 h. It was evaporated and lyophilized to yield an MeOH extract of *S. suberectus* (MSS, 23.2 g),

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which was then stored at –20 °C until use.

DPPH radical scavenging assay – The radical scavenging activity of the samples against the DPPH radical was measured using the method reported by (Chu *et al*., 2000), with minor changes. We added 100 μL of the sample solution (10, 100 and 1,000 μg/mL in MeOH) to 900 μL of DPPH solution (1 mg DPPH in 14.28 mL MeOH) and measured the absorbance at 517 nm after a 30 min incubation in the dark.

Animals – All procedures were conducted according to the animal welfare guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Male Sprague-Dawley rats (200 - 240 g) were housed under controlled temperature (20 ± 2 °C) and lighting (07:00 - 19:00) conditions, with food and water made available ad libitum.

Estimation of LPO-induced by $FeSO_4$ in liver homogenates – The rats were killed by an overdose injection of chloral hydrate (400 mg/kg, ip) and the liver tissues were isolated. Rat liver tissue homogenates were prepared by homogenization with ice-cold 50 mM Tris-HCl buffer (pH 7.4) and the concentration of the homogenate was set at 0.1 g/mL. Different concentration of extracts (1, 10, 100 and 1,000 μg/mL) 100 μL were added to the liver homogenate 500 μL. The degree of LPO was assayed by the measurement of malondialdehyde (MDA) by following a previously described method (Asakawa *et al*., 1979).

Sample treatment and induction of liver damage by CCl_4 in rats – The rats were administered MSS at concentrations of 30, 100 and 300 mg/kg by total 10 times (3 times per d for 3 d and one more time at 4 d) before CCl_4 treatment. CCl_4 (0.6 mg/kg) was dissolved in corn oil and injected intra-peritoneally to induce liver damage immediately after the 10th treatment with MSS.

Serum GOT, GPT and ALP measurement – At 24 h after CCl_4 injection, the rats were anesthetized with chloral hydrate (200 mg/kg, i.p.) and blood samples were collected from the femoral artery. After collecting the blood samples, the serum was used for the measurement of GOT, GPT and ALP. All the procedures were carried out using standard kits (Fuji DRI-CHEM slides, namely, GOT-ASTPIII, GPT-SLT-PIII and ALP-PIII Fuji Photofilm Co., Ltd., Japan) and measured by an auto-analyzer (Fuji DRI-CHEM3500i, Fuji Photofilm Co., Ltd., Japan).

Estimation of LPO in the liver after CCl_4 treatment in rats – Liver tissues were isolated immediately after blood collection. The methods of homogenization of liver tissue and the LPO assay were the same as the abovementioned method with minor changes. We used butylhydroxytoluene (BHT) to inhibit LPO after liver isolation and $FeSO_4$ was not used for LPO induction.

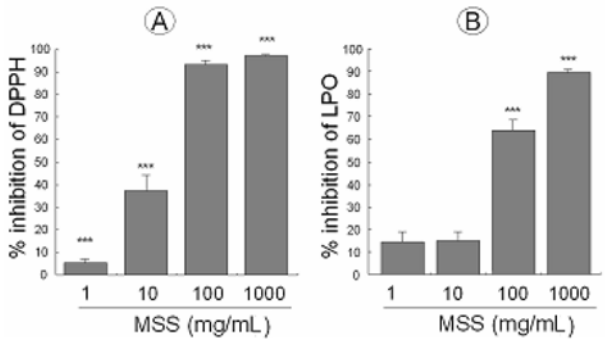

Fig. 1. DPPH radical scavenging effect of MSS (A) and inhibitory effect of MSS against $FeSO_4$-induced LPO in liver tissue homogenates (B). All experiments were performed in triplicate. The values are mean ± SD of the triplicates. * represents the statistical significance. *** $p < 0.001$ vs. the vehicle-treated group using the Student's *t*–test.

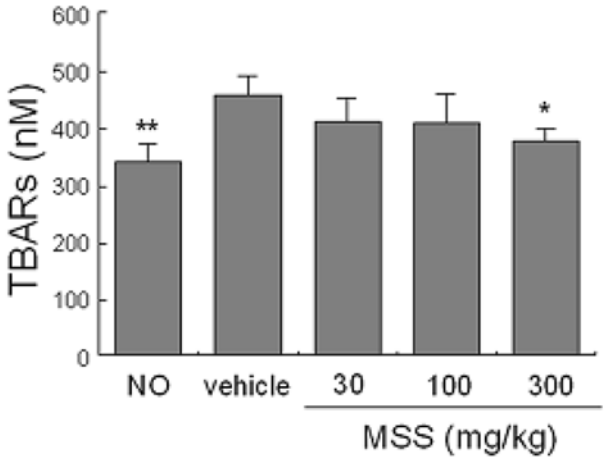

Fig. 2. Inhibitory effect of MSS on TBARs formation after CCl_4-induced liver damage in rats. NO is normal group. The values are expressed as the mean ± SEM of 5 to 8 rats. * represents statistically different from vehicle treated group *$p < 0.05$, ** $p < 0.01$ vs. the vehicle-treated group using the Student's *t*-test.

Statistics – All measurements were expressed as mean ± SD for *in vitro* and mean ± SEM for *in vivo* study. Data of the groups were compared by a paired Student's *t*-test and *p*-values less than or equal to 0.05 were considered significant.

Results and Discussion

MSS concentrations of 1, 10, 100 and 1,000 μg/mL exhibited dose-dependent radical scavenging effects that peaked at 1,000 μg/mL. A nearly complete scavenging effect was observed at the concentrations of 100 and 1000 μg/mL (Fig. 1A).

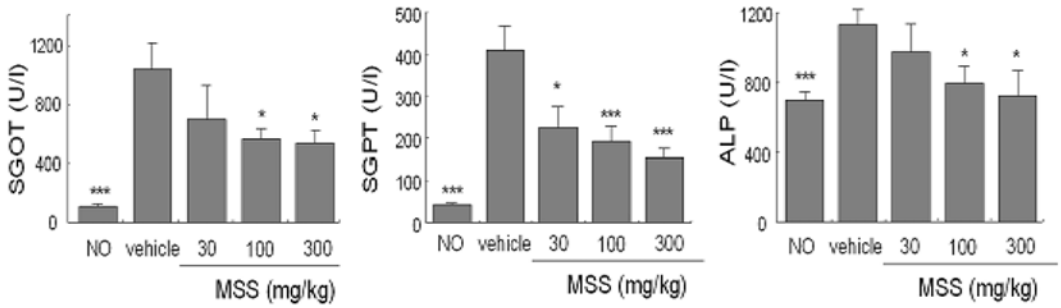

Fig. 3. Inhibitory effects of MSS against LPO and effects on serum GOT, GPT, and ALP after CCl_4-induced liver damage in rats. NO is the normal group. The values are expressed as the mean ± SEM. of 5 to 8 rats. * represents the statistical significance. * $p < 0.05$, ***$p < 0.001$ vs. the vehicle-treated group using the Student's *t*-test.

MSS exhibited inhibitory effects against LPO in the liver homogenate after $FeSO_4$ treatment in a dose-dependent manner these peaked at the concentration of 1,000 μg/mL. It showed the similar effect with 10 μg/mL of BHT (92 ± 1.2%, data not shown) in the dosage 1,000 μg/mL. The effect demonstrated by 1,000 μg/mL of MSS was similar to that demonstrated by 10 μg/mL of BHT (92 ± 1.2%, data not shown) (Fig. 1B).

The vehicle-treated group exhibited an MDA concentration of 455 ± 33.0 nM, while the normal group exhibited 337.4 ± 33 nM of MDA (Fig. 2). The group treated with 30, 100 and 300 mg/kg MSS exhibited MDA concentrations of 409.2 ± 41.4, 407 ± 50.2 and 373 ± 25.0 nM, respectively (Fig. 2). The group treated with 300 mg/kg MSS exhibited 70% LPO inhibition after CCl_4 administration as compared to the vehicle-treated group.

Significant elevations in the serum GOT, GPT and ALP levels were observed in the vehicle-treated group as compared to the normal group. MSS concentrations of 30, 100 and 300 mg/kg inhibited GPT release by 16.6, 44.4 and 69.5% GOT release by 16.6, 43.2 and 53.6% and ALP release by 37.4, 78.0 and 95.5%, as compared with the vehicle-treated group (Fig. 3).

In the present study, MSS exhibited scavenging effects against the DPPH radicals and an inhibitory effect against $FeSO_4$-induced LPO in *in vitro* studies it also protected the liver against radical-related liver damage induced by CCl_4. MSS concentrations of 100 and 1,000 μg/mL exhibited 86.7 and 91.2% scavenging of DPPH radicals, respectively. The DPPH radical assay is an easy and accurate method for measuring the electron donation (Sanchez-Moreno, 2002). However, it is difficult to extrapolate the results to the physiological environment of the human body because this assay is carried out at non-physiological pH values (Sanchez-Moreno, 2002). Therefore, other assays must be applied to investigate the exact radical scavenging effects.

The MSS concentration of 1,000 μg/mL concentration showed 83% inhibition of LPO induced by $FeSO_4$ in liver homogenates. LPO results in cell membrane damage that is induced by the hydroxyl radical MDA is the product of LPO and reacts with thiobarbituric acid (TBA) and forms TBA-reactive substances (TBARs) (Janero, 1990). In this study, we used $FeSO_4$ to induce LPO (Reif, 1992) and quantified the formation of TBARs for LPO measurement (Asakawa *et al*., 1979).

Oral administration of MSS at concentrations of 30, 100 and 300 mg/kg for 3 d before CCl_4 treatment inhibited LPO to normal levels. CCl_4 is one of the most well-known hepatotoxicants and is used to develop animal models of organ injury (Weber *et al*., 2003). One of the major mechanisms underlying liver damage is free radical generation by CCl_4 and LPO is a principal phenomenon causing the injury. This fact is supported by the studies on various kinds of radical scavengers that exhibit inhibitory effects against LPO in CCl_4-induced liver injury (Chen *et al*., 2004; Taira *et al*., 2004). MSS also inhibited the serum levels of GOT, GPT and ALP. These enzymes are released easily due to the alteration in the permeability of the membrane caused by LPO, subsequently resulting in increased serum levels (Reddrop *et al*., 1983). Therefore, the effects of MSS on these enzymes exhibited its ability to protect against CCl_4-induced liver damage and substantiated its inhibitory effects against LPO.

In conclusion, an 85% MeOH extract of *Spatholobus suberectus* confers a potent radical scavenging effect *in vitro* and oral administration of the extract for 3 d before CCl_4 treatment confers protection against CCl_4-induced free radical-related liver damage.

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