

Hepatoprotective Properties of *Allium tuberosum* in CCl₄ intoxicated Rats

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CCl₄ 투여 흰쥐에서 부추의 간보호 작용

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요 약

대표적 간장해 유발 물질인 사염화탄소로 유도된 급성 간독성에 미치는 한국산 부추(*Allium tuberosum*) 에탄올 추출물의 효과를 관찰하였다. 사염화탄소 투여로 혈청 AST (aspartate transaminase), ALT (alanine transaminase)와 ALP (alkaline phosphatase) 활성이 현저히 증가하였으며, 부추 에탄올 추출물의 투여 (50, 100, 200 mg/kg)로 특히 CCl₄에 의해 유도된 AST와 ALT의 활성이 농도 의존적으로 회복되었다. 사염화탄소 투여로 감소된 SOD (superoxide dismutase)와 catalase 활성은 부추의 에탄올 추출물 투여로 유의성 있는 활성 증가를 나타내어 간장해로 인한 산화적 스트레스가 부추의 항산화작용에 의해 완화되었음을 시사하였다. 결과적으로 부추 ethanol 추출물의 간보호 및 항산화작용으로 사염화탄소로부터의 간장해를 개선시키는 것으로 사료된다.

Key words : *Allium tuberosum*, oxidative stress, liver damage, CCl₄

INTRODUCTION

Allium is a genus of some 500 species belonging to the family Liliaceae. However, some of these are important as food and medicinal plants. *Allium* vegetables including garlics, onions, leeks, chives, shallots and shallots, are rich in flavonols and organosul-

fur compounds, which have tumor inhibitory properties (Fenwick and Hanley, 1985). *Allium tuberosum* is a perennial herb which is cultivated widely and the leaves are used for food (Shanghai Science & Technical Publisher, 1985). Movahedian *et al.* (2004) presented antihyperlipidemic effect of this plant. Until now there have been no studies about liver protective properties of this medicinal plant. So we investigated protective effects of ethanol extract of *Allium tuberosum* (EEAT) on oxidative stress in acute liver damage induced by CCl₄.

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MATERIALS AND METHODS

Animals

Sprague–Dawley rats weighing 200 ± 20 g (4 weeks old) were purchased from Daehan Biolink and used in this experiment. Animals were caged at $20 \pm 2^\circ\text{C}$ with a 12 h light and dark cycle and allowed free access to food and water.

Preparation of plant extract

Three kilograms of *Allium tuberosum* were dried in the shade thoroughly and roughly powdered. The coarse powder was subjected to continuous hot extraction in a soxhlet by using ethanol (95% v/v). The ethanol was removed by distillation under reduced pressure. This extract was lyophilized and used in the present experiment.

Experimental design

The animals were divided into 5 groups of 8 animals and treated as follows: Group I served as received olive oil (1.0 mL/kg i.p.). Group II got 30% CCl_4 suspended in olive oil 1.0 mL/kg, i.p. every 48 h for 2 week. Group III, IV, V received a various dose of plant extracts (50, 100, 200 mg/kg, p.o.) daily for 2 weeks and CCl_4 was injected as Group II. On the last day of experiment, CCl_4 was administered 30 min after the last dose to all rats except rats in Group I. After 24 h, all the rats were sacrificed under light ether anesthesia and blood was collected in sterile centrifuge tube and allowed to clot. Serum was separated by centrifuging at 3,000 rpm for 15 min.

Enzyme assay

In serum, AST and ALT were determined based on the method for Reitman and Frankel (1957). ALP was determined by using method of Kind and King (1954).

Liver samples were homogenized on ice in a teflon–glass homogenizer containing a phosphate

homogenization buffer. Homogenized samples were centrifuged at 10,000 rpm for 45 min and the supernatant was collected for analysis of SOD and catalase activities. The activity of SOD was determined based on the method for Marklund and Marklund (1974). No distinction was made between the Cu–Zn and Mn–isozymes of SOD. The method involves spectrophotometric monitoring of the autooxidation of pyrogallol (10 mM) at 420 nm. One unit of enzyme activity is defined as the amount of enzyme necessary to inhibit pyrogallol autooxidation by 50%. Each sample was measured twice, in triplicate, using semimicro (1.5 mL) cuvettes. Catalase activity was determined using the method of Cohen *et al.* (1970), which involves measuring hydrogen peroxide (6 mM) titration with permanganate, following reaction between catalase and H_2O_2 . Spectrophotometric monitoring was conducted at 480 nm. Catalase activity is defined as the amount of catalase required to decrease permanganate color change by 50%. Protein determinations were made using the method of Lowry (1951).

Statistical analysis

Student's t test was used for statistical significance between groups.

RESULTS AND DISCUSSIONS

As shown in Table 1, activities of serum enzymes such as AST, ALT and ALP were increased remarkably and significantly ($p < 0.05$) in toxicant group after CCl_4 treatment, as compared to normal group, indicating that CCl_4 induced damage to the liver.

A significant reduction was observed in AST, ALT and ALP in the group treated with EEAT in comparison with those observing CCl_4 treated group. The enzyme levels were almost restored to the normal level in dose dependent manner.

CCl_4 has been widely used for inducing experimental hepatic damage due to free radical formation during its metabolism by hepatic microsomes (Sher-

Table 1. Changes in serum AST, ALT and ALP levels in CCl₄ intoxicated rats treated with EtOH extract of *Allium tuberosum* (EEAT)

Groups	Treatment	AST ¹⁾	ALT ¹⁾	ALP ²⁾
I.	Normal	34.7±3.6	40.4±5.2	70.8±5.6
II.	CCl ₄	106.4±7.6*	159.6±9.8*	115.6±8.8*
III.	CCl ₄ +EEAT 50 mg/kg	75.6±5.8**	59.6±7.7**	80.5±6.9**
IV.	CCl ₄ +EEAT 100 mg/kg	45.6±6.9**	55.2±6.1**	78.4±7.4**
V.	CCl ₄ +EEAT 200 mg/kg	43.5±5.5**	42.7±5.7**	61.5±4.8**

Results are expressed as mean±S.D. of 8 rats. ¹⁾IU/L ²⁾King-Armstrong unit. *significantly different from Group I, **significantly different from Group II (p<0.05)

lock, 1981), which causes the lipid peroxidation of cellular membrane leading to the necrosis of hepatocytes. The increased activities of liver maker enzymes such as ALT, AST and ALP in the serum of CCl₄ exposed rats indicate damage to hepatic cells (Wolf, 1999).

The efficacy of any liver protective drug is essentially dependent on its ability in reducing the harmful effects of maintaining the normal hepatic physiology that has been disturbed by a hepatotoxicant such as CCl₄. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with CCl₄ (Recknagel *et al.*, 1989). CCl₄ and its metabolites such as trichloromethyl peroxy radical are known to be involved in the pathogenesis of liver injury (Mehendale *et al.*, 1986). Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl₄ and is responsible for cell membrane alterations (Bandyopadhyay *et al.*, 1999).

And CCl₄-mediated acute toxicity increased permeability of the hepatocyte membrane and cellular leakage (Paduraru *et al.*, 1996). The EEAT mediated suppression of the increased AST, ALT and ALP activities suggested the possibility of the extract to give protection against liver injury on CCl₄ induction.

As shown in Table 2, significant decrease in the activities of SOD and catalase in CCl₄ administered rats (Group II) and recovery to near normal level in Groups III, IV and V revealed that oxidative stress elicited by CCl₄ intoxication has been lessened by the antioxidant effect of EEAT. Ohta *et al.* (1995) have

Table 2. Changes in hepatic SOD and Catalase levels in CCl₄ intoxicated rats treated with EtOH extract of *Allium tuberosum* (EEAT)

Groups	Treatment	SOD ¹⁾	Catalase ¹⁾
I.	Normal	40.6±3.9	0.62±0.04
II.	CCl ₄	19.6±3.1*	0.34±0.05*
III.	CCl ₄ +EEAT 50 mg/kg	38.4±5.7**	0.55±0.06**
IV.	CCl ₄ +EEAT 100 mg/kg	43.5±7.2**	0.61±0.04**
V.	CCl ₄ +EEAT 200 mg/kg	46.9±5.1**	0.65±0.05**

Results are expressed as mean±S.D. of 8 rats. ¹⁾U/mg protein. *significantly different from Group I, **significantly different from Group II (p<0.05)

reported the decreased activities of SOD and catalase after the administration of single dose of CCl₄.

SOD plays an important role in the prevention of cell death and apoptosis. The decrease in Mn-SOD activity is associated with increased mitochondrial oxidative damage, as demonstrated by a decrease in the activities of iron sulfhydryl proteins sensitive to oxidative stress (Van Remmen *et al.*, 2004).

Catalase is a hemoprotein which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (Chance *et al.*, 1952). Szymonik-Lesuk *et al.* (2003) have also shown that CCl₄ intoxication can lead to alteration in gene expression and depletion of SOD and catalase level in kidney and heart.

In conclusion, the present findings demonstrated the hepatoprotective and antioxidant activities of

EEAT in the experimental rat model. Further works are needed to isolate and purify the active principle involved in the hepatoprotective activity of this plant.

REFERENCES

- Bandyopadhyay U, Das D and Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis, *Curr Sci* 1999; 77: 658–665.
- Chance B, Greenstein DS, and Roughton RJW. The mechanism of catalase action—steady state analysis, *Arch Biochem Biophys* 1952; 37: 301–339
- Cohen G, Demblich D and Marcus J. Measurement of catalase activity in tissue extracts, *Anal Biochem* 1970; 34: 30–38.
- Fenwick GR and Hanley AB. The genus *Allium*—Part 1, *Cri Rev Food Sci Nutr* 1985; 22: 199–271.
- Kind PRM and King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine, *J Clin Pathol* 1954; 7: 322–326.
- Lowry OH, Rosebrough HJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent, 1951; 193: 265–275.
- Marklund S and Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for SOD, *Eur J Biochem* 1974; 47: 469–474.
- Mehendale HM, Roth RA, Gandolfi AJ, Kraunig JE, Lemasters JJ and Curtis LR. Novel mechanism in chemically induced hepatotoxicity, *FASEB J* 1986; 104: 826–829.
- Movahedian A, Ghannadi A, Sadeghi H, Gharavi M and Azarpajhoh S. Study of Leek extract on cholesterol plasma levels in hyperlipidemic animals, *Asia Pac J Clin Nut* 2004; 13(suppl): S103.
- Ohta Y, Sasaki E, Nishida K, Kobayashi T, Nagata M and Ishiguro I. Preventive effect of dia-saiko-to (da-chai-hu-tang) extract on disrupted hepatic active oxygen metabolism in rats with carbon tetrachloride induced liver injury, *Am J Chin Med* 1995; 23: 53–64.
- Paduraru I, Saramet A, Danila GH, Nichifor M, Jerca L, Iacobovici A, Ungureanu D and Filip M. Antioxidant action of a new flavonic derivative in acute carbon tetrachloride intoxication, *Eur J Drug Metab Pharmacokinet* 1996; 21: 1–6.
- Recknagel RO, Glende EA Jr, Dolak JA and Walker RL. Mechanisms of carbon tetrachloride toxicity, *Pharmacol Ther* 1989; 43: 139–154.
- Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetate and serum glutamate pyruvate transaminase, *Am J Clin Pathol* 1957; 28: 56–75.
- Shanghai Science & Technical Publisher, *The Dictionary of Chinese Drugs Shougakukan*, Tokyo, 1985, 449.
- Sherlock S. *Biochemical assessment of liver function*, Blackwell Scientific Publication, Oxford, 1981, 14.
- Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Slomka M, Madro A, Celinski K and Wielosz. Catalase, superoxide dismutase and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication, *J Hepatobiliary Pancreat Surg* 2003; 10: 309–315.
- Van Remmen H, Williams MD, Guo Z, Estack L, Yang H, Carlson EJ, Epstein CJ, Huang TT and Richardson A. Knockout mice heterozygous for *Sod2* show alterations in cardiac mitochondrial function and apoptosis, *Am J Physiol Heart Circ Physiol* 2004; 281: H1422–1432.
- Wolf PL. *Biochemical diagnosis of liver disease*, *Indian J Clin Biochem* 1999; 14: 59–65.