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Effects of Medicinal Plant Extracts on Antioxidant System in Ethanol-intoxicated Rats

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ABSTRACT : Four medicinal plants selected from preliminary screening study were evaluated in the aspects of their antioxidant activities in alcohol-intoxicated rats. Rats fed 1% α-tocopherol-supplemented diet as positive control and ones done α-tocopherol-deficient diet as negative control were compared with ones done the plant extract-supplemented diet (n=8). After the administration of the experimental diets for 4 weeks, typical increments in activities of manganese-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GSH-px) indicated in alcohol-intoxicated rats, were not observed in ones fed *Lagerstroemia* and *Ulmus* extract-supplemented diet. The content of thiobarbituric acid reactive substance (TBARS), the product of lipid peroxidation, did not increased in rats fed plant extracts-supplemented diet except for *Terminalia*. From the results, it is concluded that *Lagerstroemia* and *Ulmus* have physiologically efficient antioxidant activities.

Key words : medicinal plants, peroxidation, antioxidant, alcohol, superoxide dismutase, glutathione peroxidase, catalase

INTRODUCTION

For a healthy life and long life span, human beings have been using many medicinal plants as therapeutic drugs for a long time. Recently many researches have tried to find new physiologically effective materials from the medicinal plants for preventing and curing diseases.

Antioxidants are components having inhibitory activities on many oxidation reactions induced by free radicals and transition metal ions. Lipid oxidation in an aerobic cell initiated by oxidation initiators such as free radicals and metal ions can make tissue injuries (Halliwell & Gutteridge, 1985). Free radical components are correlated with evoking diseases such as cancer, atherosclerosis, rheumatoid arthritis, inflammation, neurological disorders and aging (Kehrer & Smith, 1994). Tissue injuries originated from oxidative stress could be prevented by antioxidants including compounds and enzymes (Evans, 1993). And now, antioxidants were believed to play roles in prevention and treatment of such degenerative diseases and aging (Vinson *et al.*, 1995; Masuda & Jitoe, 1994; Draper & Bird, 1997).

Antioxidant activity of plants have been screened by many researchers (Choi *et al.*, 1992; Moon *et al.*, 1994; Chevolleau *et al.*, 1992). Many phenolic antioxidants have been identified from plant resources (Azuma *et al.*, 1999; Lee *et al.*, 1994). We have reported the screening results on antioxidant activities of medicinal plants on DPPH radical and linoleic acid peroxidation (Lee *et al.*, 2002; Lee *et al.*, 2003). This study was conducted to evaluate antioxidant effects of the plant extracts selected from the previous studies in rat liver.

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MATERIALS AND METHODS

Materials

Four week-old Sprague Dawely male rats with 110 ~120 g body weight were randomly departed into 6 groups (n=8) and administered with experimental diets for 4 weeks (as shown at Table 1). All of the groups were intoxicated with 10% alcohol for experimental periods through the water bottle.

Medicinal plants used for this study were *Lagerstroemia indica* L. (leaves), *Terminalia chebula* R. (fruits), *Ulmus davidiana* Planchol var. *japonica* N. (bark), *Zelkova serrata* M. (leaves), which were obtained from Younggwang (Korea), Keongdong Market (originated from China and Korea) or National Institute of Crop Science (NICS, Korea), respectively. These plants were used for extract preparation with aqueous ethanol or absolute ethanol.

Reagents used for analysis of enzyme activities and content of thiobarbituric acid substance (TBARS) and glutathione, were products made by Sigma Co. (USA). Ethanol for extract preparation from plants was guaranteed grade.

Table 1. Composition of experimental diet.

Ingredients	Experimental groups (%)		
	Cont[§]	Toc[¶]	Plant ext.
Corn starch	39.75	39.75	39.75
Casein	20	20	20
Dextrin	13.2	13.2	13.2
Sucrose	10	10	10
Soybean oil	7	7	7
Fiber	5	4	4
AIN-mineral mixture[†]	3.5	3.5	3.5
AIN-vitamin mixture[†]	1	1	1
L-cystine	0.3	0.3	0.3
Choline bitartrate	0.25	0.25	0.25
Extract (or α-tocopherol)	0	1	1

† Composition of mineral mixture (g/kg) : calcium carbonate 357 g, potassium phosphate monobasic 196 g, potassium citrate 70.78 g, sodium chloride 74 g, magnesium oxide 24 g, ferric citrate 6.06 g, zinc carbonate 1.65 g, manganese carbonate 0.63 g, cupric carbonate 0.3 g, sodium meta-silicate 1.45 g, chromonic potassium sulfate 0.275 g, lithium chloride 0.174 g, sodium fluoride 0.0635 g, boric acid 0.0815 g, nickel carbonate 0.0318 g, ammonium vanadate 0.0066 g, sodium selenate 0.01025 g, ammonium paramolybdate 0.00795 g, potassium iodate 0.01 g, sucrose 221. 26 g.

‡ Composition of vitamin mixture (g/kg) : thiamin-HCl 0.6 g, riboflavin 0.6 g, pyridoxin-HCl 0.7 g, nicotinic acid 3 g, calcium panthothenate 1.6 g, folic acid 0.2 g, phylloquinone 0.075 g, D-biotin 0.02 g, cyanocobalamin 0.0025 g, cellulose 2.4975 g, retinyl palmitate 0.8 g, cholecalciferol 0.25 g, sucrose 974.655 g (1500 mg of tocopheryl acetate was excluded).

§ Cont (α-tocopherol deficient) group.

¶ Toc (α-tocopherol added) group.

Methods

Rat livers were weighed, minced, and homogenated in 0.1 M phosphate buffer (pH 7.4) and used to analyze the content of TBARS and glutathione. Liver homogenates were centrifuged at 3,000 rpm for 15 min. and the supernatant was centrifuged at 10,000 rpm for 20 min. The pellet was used for analysis of catalase activity and the supernatant was again centrifuged at 35,000 rpm for 60 min., and the supernatant, cytosol fraction, was used for the analysis of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) activities.

The contents of liver lipid peroxidation product (TBARS, ㎎/㎎ protein) and antioxidant (glutathione, ㎍) was analyzed by the methods of Botsoglou *et al.* (1994) and Ellman (1959), respectively. The analyses of activities of catalase (k/㎎ protein), SOD (U/㎎ protein) and glutathione peroxidase (U/㎎ protein) were performed according to the method of Abei (1984), Flohé (1984, 1989), respectively. The contents of protein in homogenate, mitochondrial and cytosol fraction were determined by the method of Bradford (1976) using bovine serum albumin as standard compound for calibration curve. The results was exhibited as mean±standard deviation, and statistical analysis for the significance was verified by the one way ANOVA and Duncan's multiple range test at the level of $p<0.05$.

RESULTS AND DISCUSSION

The results on SOD activity of liver of rat fed the experimental diets were shown in Fig. 1. The activity of manganese-superoxide dismutase (Mn-SOD) of *Lagerstroemia* and *Ulmus* extract-fed groups were

significantly lower than that of −tocopherol−deficient group (control group), but a little higher than that of α−tocopherol−fed group at the level of $p<0.05$. Cu,Zn−SOD activities in all of the experimented rats were relatively lower than Mn−SOD activities, but those in rats fed *Lagerstroemia* extract showed the lowest activity among the experimented groups at $p<0.05$. The activity of Mn−SOD is characteristically enhanced after alcohol intoxication in animals (Chen *et al.*, 1996). This phenomenon was explained by the fact that the free radicals produced by cytochrome P4502e1 of microsomal ethanol−oxidizing system (MEOS) or other ethanol metabolizing pathways can play as signals inducing the enhanced expression of mRNA of antioxidant enzyme (Oneta *et al.*, 2002; Koch *et al.*, 2002). Therefore, increase in activity of Mn−SOD indicates that free radicals made from ethanol−breakdown were not scavenged by antioxidants, but reduction in the activity of this enzyme indicate that free radicals were scavenged appropriately.

As shown in Fig. 2, the activities of glutathione−peroxidase (GSH−px) in *Lagerstroemia* and *Ulmus* extracts−fed rats were significantly lower than that of tocopherol−deficient rats at the level of $p<0.05$. The activity of the enzyme in *Terminalia* extract−fed rats was much higher than that in control rats. It is the same result that the activity of GSH−px also increased in the case of alcohol−intoxicated rat like that of Mn−SOD, and antioxidants should recover the activity of the enzyme into the normal level (Lee *et al.*, 2001). From the data, it is suggested that *Lagerstroemia* and *Ulmus* extracts have more effective antioxidant activity than *Terminalia* and *Zelkova* extract.

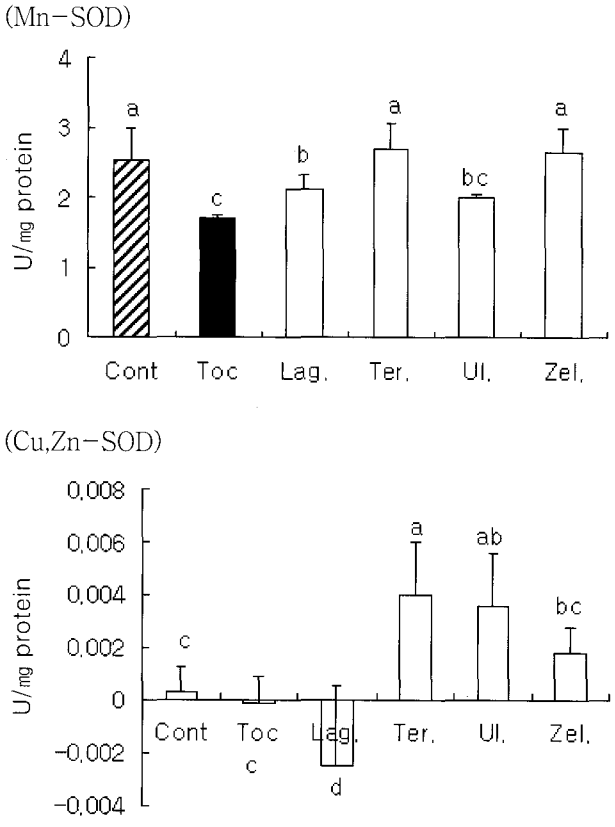

Fig. 1. Activities of superoxide diamutase (SOD) determined in medicinal plant extracts and alcohol-administrated rat liver (experimental groups including Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

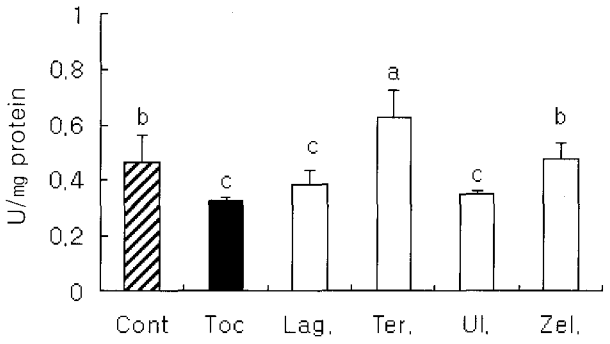

Fig. 2. Activities of liver glutathione peroxidase (GSH-px) determined in medicinal plant extracts and alcohol-administrated rat (experimental groups including Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

Activity of catalase in rat fed the plant extract and tocopherol was significantly higher than that of rat fed tocopherol−excluded diet at the level of $p<0.05$ (Fig. 3).

Therefore, it is supposed that catalase could act two roles as antioxidant on free radicals and alcohol-breakdown enzyme. This result was coincident with the report of Cho *et al.* (2001)

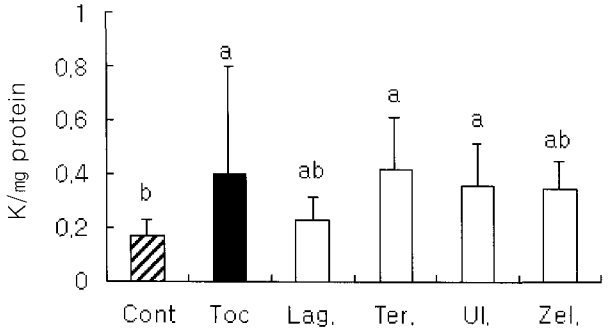

Fig. 3. Activities of liver catalase determined in medicinal plant extracts and alcohol-treated rat (experimental groups including Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

The products of peroxidation, TBARS, in rats fed the plant extracts and tocopherol were not significantly lower than those of rat fed tocopherol-deficient diet fed group at $p<0.05$. The contents of glutathione in *Ulmus* and *Zelkova* extract fed groups were significantly higher than those in rat fed the other experimental diet at $p<0.05$. Many researches showed that increase in TBARS content and reduction in antioxidants such as tocopherol and glutathione were observed in the tissues of chronic ethanol fed rats and antioxidants-supplement can attenuate the increase of peroxidation product (TBARS) and reduction of antioxidants (Coudray *et al.*, 1993; Norman *et al.*, 1990). These data indicates that *Ulmus* and *Zelkova* extracts partly acted as antioxidants on alcohol-induced free radicals and lipid oxidation in rat liver (Lieber, 1997).

According to all of the results, it can be suggested that *Lagerstroemia* and *Ulmus* extracts could play roles as efficient antioxidants.

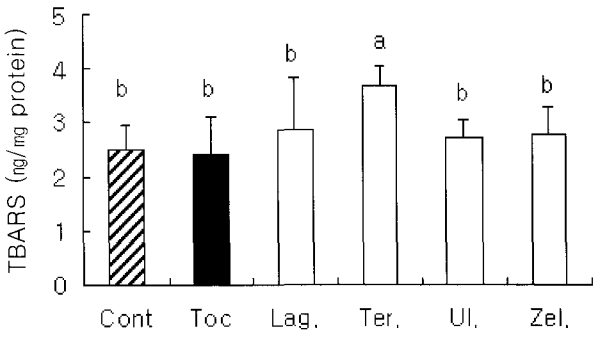

Fig. 4. Content of lipid-oxidation product (TBARS) determined in medicinal plant extracts and alcohol-administrated rat liver (experimental groups including Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean± standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

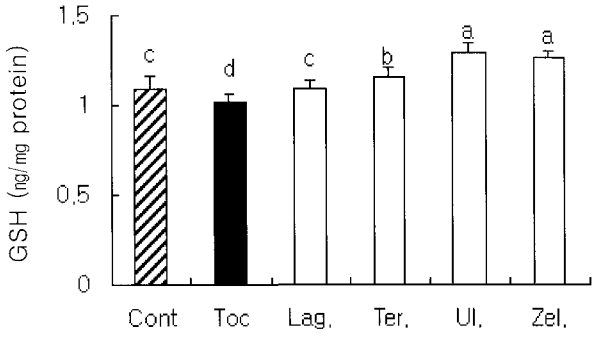

Fig. 5. Content of glutathione (GSH) determined in medicinal plant extracts and alcohol-administrated rat liver (experimental groups including Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

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