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Effect of Herbal Extracts Mixtures on Antioxidant System in Chronic Enthanol-treated Rats

Mok-Kyung KIM, Eun-kyung WON and Se-Young CHOUNG*

Department of Hygienic Chemistry and Kyung Hee East-West Pharmaceutical Research Institute, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea

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Abstract – Disturbance of antioxidant system is very common in chronic alcoholics and herbal or natural products with antioxidant activity have been used for its treatment. This study was to investigate the effect of *Vitis vinifera* extract (V), *Schisandra chinensis* extract (S), *Taraxacum officinale* extract (T), Gardenia jasminoides extract (G), *Angelica acutiloba* extract (A) and *Paeonia japonica* extract (P), and their combinations on the antioxidant and ethanol oxidation system. Male Sprague-Dawley rats were subjected to Lieber-DeCarli ethanol liquid diet(ED) and were then given different herbal extract mixtures for 6 weeks including VST (V 100 + S 150 + T 150 mg/kg/day), VSG (V 100 + S 150 + G 150 mg/kg/day), VTG (V 100 + T 150 + G 150 mg/kg/day), and VAP (V 100 + A 150 + P 150 mg/kg/day). When the activity of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) were compared between ED only group and herbal extracts treatment group, the differences were statistically significant. Phase I and II (glutathione-S-transferase, phenol sulfatransferase) enzyme activities were found to be significantly higher in the VAT treatment group compared to the ED group. Herbal extracts not only repressed the ethanol-induced elevation of malondialdehyde level, but also protected against ethanol-induced decrease in glutathione content, glutathione reductase, glutathione peroxidase, catalase and superoxide dismutase activities. The administration of the herbal extracts was found to be effective in eliminating lipid-peroxides induced by long-term consumption of alcohol by activating various enzyme systems and physiological active compound formation system. After a chronic consumption of alcohol, Angelica Radix protected the liver *via* activating the ethanol-metabolism enzyme system, and Paeoniae Radix *via* activating the ethanol-metabolism enzyme and the phase I, II-metabolism enzyme system. Taraxaci Herba was also effective in liver protection *via* activating the ethanol-metabolism enzyme system and the phase I, II-metabolism enzyme system, Gardeniae Fructus *via* activating the phase II-metabolism enzyme system and the anti-oxidation system enzyme, and Schisandra Fructus and a grapestone *via* activating the anti-oxidation system. Our data suggest that these herbal extracts may be useful as a health functional food or new drug candidate for fatty liver and hepatotoxicity induced by chronic alcohol consumption.

Keywords □ *Vitis vinifera, Schisandra chinensis, Taraxacum officinale, Gardenia jasminoides, Angelica acutiloba, Paeonia japonica*, ethanol liquid diet, antioxidant

INTRODUCTION

Herbal and natural products with antioxidant capacity have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown a great interest in this field as they recognize the true health benefits of these remedies. We studied the effects on alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) after herbal extracts administer for 6 weeks to rats induced by Lieber-DeCarli ethanol liquid diet. Antioxidant system disturbance is a common diagnosis shown in the long-term alcoholics. The reactive oxygen species, generated from ethanol metabolism, have been indicated to play on a set of liver obstructions in ethanol-intoxicated animals. The oxidant balance for the liver plays a very important role in protecting the liver and allowing normal liver function. The reduction of liver anti-oxidative capacity and morphological changes have been observed in rats chronically intoxicated with ethanol. Antioxidants may play a protective role in these conditions.

Through various kinds of references and the survey of informations, our researchers have utilized the mixtures of the natu-

*Corresponding author
Tel: +82-2-961-0372, Fax: +82-2-961-0372
E-mail: sychoung@khu.ac.kr

ral products, which are concluded with the *Vitis vinisfera* extract, *Schisandra chinensis* extract, *Taraxacum officinale* extract, *Gardenia jasminoides* extract, *Angelica, acutilica acutiloba* extract, *Paeonia japonica* extract, those the protective effects of liver recognized to be excellent, and through ethanol enzyme system and antioxidant enzyme system, they have closely investigated the blood serum and the lipid amount induced from the rats, and the metabolism effected on the chronic liver toxicity.

MATERIALS AND METHODS

Sample preparation

Vitis vinifera, Schisandra chinensis, Taraxacum officinale, Gardenia jasminoides, Angelica acutiloba, Paeonia japonica were purchased from the Kyung-Dong (Seoul, Korea) market and distilled water was added at 10-fold the volume to each. G was boiled at 60°C for 5 hr, while the other natural materials were extracted twice at 90°C for 4 hr. They were filtered, concentrated, and then freeze dried. The yield of S and T was 40%, G 29%, A 54%, and P 34%. Grapeseed (*Vitis vinifera,* catechine 25%, V) extracts were purchased from Herb Valley Co., Ltd.

Experimental animals and experimental diets

Male SD white rats, 5 weeks old, weighing 140~150 g were purchased from Orient Inc. (Seoul, Korea) and used as an experimental animal model rats were adapted to solid feed for 1 week in the laboratory environment where the temperature was kept constant at 24±2°C and the humidity at 60% using a climate control system. The 6 experimental groups (n = 8) each were fed an experimental diet for 6 weeks. The group with hepatotoxicity was orally administered prepared samples, and the control group was fed as a diet without alcohol while simultaneously receiving the equivalent amount of distilled water. The experimental groups were designated the normal diet (ND), ethanol diet (ED), ED + V 100 + S 150 + T 150 mg/kg/day (ED + VST), ED + V 100 + G 150 + S 150 mg/kg/day (ED + VGS), ED + V 100 + G 150 + T 150 mg/kg/day (ED + VGT), ED + V 100 + A 150 + P 150 mg/kg/day (ED + VAP) groups. (Kim *et al.,* 2006)

The experimental diet was prepared according to the Lieber DeCarli rat liquid diet method,(Lieber *et al.,* 1982) and was prepared fresh every day prior to use. Table I shows the compositions of the diets, which were designed to provide 1 kcal/ml. The ED consisted of 35% fatty acids, 11% carbohydrate, 18% protein, and 36% ethanol, while the ND consisted of 35% fatty acids, 47% carbohydrate, and 18% protein. In the ED group, 36% of total calories was obtained from ethanol, while maltose dextrin corresponding to the amount of the above calories replaced ethanol in the ND group. This experimental design is clearly demonstrated in Table II. The total experimental duration was 10 weeks. The 5 ED groups were adapted to increasing amounts of ethanol for the first 4 days and then received ethanol (36% of total calories) for the next 5 days to 6 weeks. The amount of diet and weight were observed at a constant interval twice a week.

Table I. Composition of Lieber-DeCarli liquid diet

Ingredients	Normal diet (g/l)	Ethanol diet (g/l§)
Casein	41.4	41.4
L-cystein	0.50	0.50
DL-methionine	0.30	0.30
Corn oil	8.50	8.50
Olive oil	31.10	31.10
Safflower oil	2,70	2.70
Dextrin maltose	115.20	25.60
Cellulose	10.00	10.00
Choline bitartrate	0.53	0.53
Xantan gum	3.00	3.00
Vitamin mix[a)]	2.55	2.55
Mineral mix[b)]	8.75	8.75
Ethanol		54.51

[a)]AIN-76A Vitamin mix provided the following g/kg mix: thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; niacin, 3; calcium pantothenate, 1.6; folic acid, 0.2; biotin, 0.02, Vitamin B12 (0.1%), 1; Vitamin A palmitate(500000IU/g), 0.8; Vitamin D3 (400000IU/g), 0.25; Vitamin E acetate(500IU/g), 10; menadione sodium sulfite, 0.08; sucrose, finely powdered, 981.15; Dyets, Bethlchem, Pennsylvania, U.S.A.

[b)]AIN-76 Mineral mix provided the following g/kg mix : calcium phosphate, dibasic, 500; codium chloride, 74; potassium citrate·H_2O, 220; potassium sulfate, 52; magnesium oxide, 24; manganous carbonate, 3.5; ferric citrate. 6; zinc carbonate. 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium K sulfate, 12·H_2O, 0.5; sucrose, finely powdered, 118.03; Dyets, Bethlchem, Pennsylvania, U.S.A.

Statistical analysis

The results are presented as mean±standard deviation (SD). Data were analyzed by using the Statistical Analysis System (SAS) program. Differences between the control and ED groups were analyzed with Duncan's multiple range tests. A value of $P < 0.05$ was considered to represent a statistically significant difference.

RESULTS

Change in body and liver weights

Table II. Experimental design

Group	Treatment and duration	
	1 - 6 weeks	6 - 10 weeks
1 ND	Normal diet (fat 35%, glucose 47%, protein 18%)	
2 ED	Ethanol diet (fat 35%, glucose 11%, protein 18%, ethanol 36%)	
3 ED+VST	Ethanol diet	Ethanol diet + V100 + S150 + T150(mg/kg/day)
4 ED+VSG	Ethanol diet	Ethanol diet + V100 + S150 + G150(mg/kg/day)
5 ED+VTG	Ethanol diet	Ethanol diet + V100 + T150 + G150(mg/kg/day)
6 ED+VAP	Ethanol diet	Ethanol diet + V100 + A150 + P150(mg/kg/day)

Normal liquid diet(ND)
Ethanol liquid diet(ED)
VST: *Vitis vinifera+Schisandra* chinensis+Taraxacum officinale
VSG: *Vitis vinifera+Schisandra* chinensis+Gardenia jasminoides
VTG: *Vitis vinifera+Taraxacum* officinale+Gardenia jasminoides
VAP: *Vitis vinifera+Angelica* acutiloba+Paeonia japonica

Table III. The effect of herbal extracts mixtures on body weight and liver index in ethanol-administered rat

Group	Body weight(g)	Liver index(g/B.W. 100 g)
ND	324.0±31.4^{a}	2.77±0.22^{b,c}
ED	264.0±25.5^{b}	3.27±0.35^{a}
ED + VST	276.9±51.8^{b}	2.68±0.12^{c}
ED + VGS	281.0±35.6^{b}	2.95±0.21^{b,c}
ED + VGT	296.9±24.5^{a,b}	2.86±0.26^{b,c}
ED + VAP	304.3±27.2^{a,b}	3.08±0.40^{a,b}

We studied the effects of administering herbal extract mixtures (HEM) for 6 weeks on rat body and liver weights (Table III). Body weights were significantly lower in the ED group than in the ND group.

HEM treated groups were not significantly different from those in the ND group. But HEM treated groups reduced body weights versus the ED group. It is considered that the reasons for this decrease in body weight gain are a result of the increased oxygen uptake due to alcohol consumption, an increase in metabolic rates, and a reductions in the production of ATP by means of oxidizing alcohol in microsomes.

With regard to liver index, the ED group showed a 15% increase as compared to that of the ND group. HEM treated groups reduced liver weights versus the ED group.?The ED+VST, ED+VGS and ED+VGT groups had similar values to the ND group, because their liver weights were significantly reduced by the alcohol.

Gastric alcohol enzyme activity

The result measuring the gastric ADH activity is shown on Fig. 1. As compared the ND group with ED group has a tendency to increase. ADH activity increased by putting in ethanol in HEM-treated groups has a tendency to increase more than that of ED group. Particularly the VTG group showed significantly a higher increase on the gastric ADH activity levels as compared with the ED group. ALDH levels showed a similar tendency to ADH levels, while the ED group showed 17% increase as compared with that of ND group. HEM-treated groups had higher levels as compared with those of the ED group where ALDH levels had significantly an increase like the ED+VSG group.

Hepatic alcohol enzyme activity

In the liver tissue, the change of ADH activity is shown on Fig 3. The ED group had 12% increase in ADH levels compared with the ND group. In case the ED group had a tendency to increase 44% as compared with ND group, while all HEM-treated groups had significantly a tendency to decrease in their ALDH values as compared with those in the ED group(Fig. 4). (Kim et al., 2006)

Phase I, II in the liver enzyme activity

2EI activity in enzyme P450 induced by alcohol had about 43% increase by supply of ethanol as compared with that in ND group, and the activity had more increase than ED group by the mixed supply of HEM-treated groups(Fig 5). Especially the HEM-treated groups had statistically more increase than that of the ED group($P<0.05$).

GTS activity, to exclude lipid peroxidation combined to the membrane, and to protect the living membrane by help of the long supply of ethanol, had about 25% decrease as compared with that of ND group(Fig. 6). By the supply of HEM-treated groups, the GTS activity decreased by the chronic supply of ethanol had an increase, especially VTS group showed statisti-

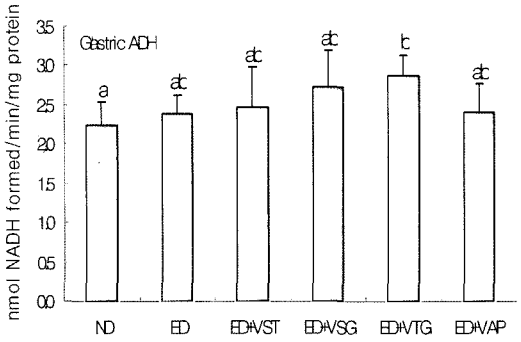

Fig. 1. Effect of herbal extracts on gastric ADH activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different ($p< 0.05$) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+ Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+ Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+ Paeonia japonica

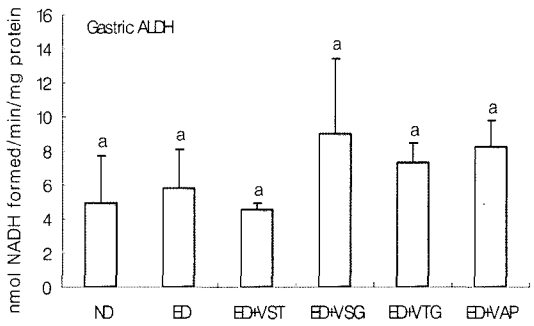

Fig. 2. Effect of herbal extracts on gastric ALDH activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different ($p< 0.05$) among the groups by Duncan's multiple range test. ND: normal diet ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+Paeonia japonica

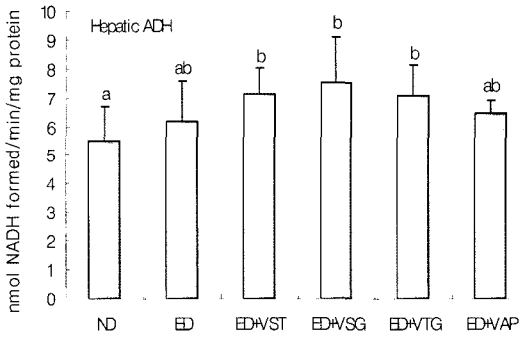

Fig. 3. Effect of herbal extracts on hepatic ADH activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different ($p< 0.05$) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+Paeonia japonica

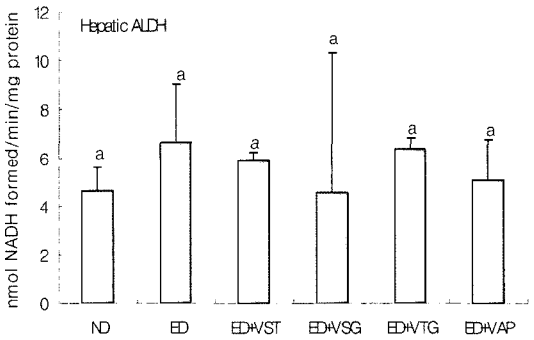

Fig. 4. Effect of herbal extracts on hepatic ALDH activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different ($p<0.05$) among the groups by Duncan's multiple range test. ND: normal diet ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+Gardenia jasminoides VAP: Vitis vinifera+Angelica acutiloba+Paeonia japonica

cally the significant increase($P<0.05$).

In the activity of PST catalyzed sodium Sulfate, the ED group had the significant increase($P<0.05$). The supply of HEM-treated had not any influence on the PST activity(Fig. 7).

In the UDP-GT activity catalyzed Glucuronidation, the ED group showed about 25% increase compared with the ND group. But all the groups supplied by HEM-treated showed lower activities than the ED group(Fig. 8). Especially VSG, VTG, VAP groups decreased significantly more than the ED group($P<0.05$).

In the liver, GSH/GSSG contents

In order to know the effect of eliminating the oxygen free radical made by putting alcohol of many kinds of natural extracts, after putting the white rat for 6 weeks in the alcohol and the natural extracts, the influence on GSH contents in tissues of the liver was shown in Table IV.

The GSH contents of the ED group by putting ethanol showed about 60% decrease as compared with those of ND group. The GSH contents decreased by supply of the etnanol, in HEM-treated groups, showed statistically the significant

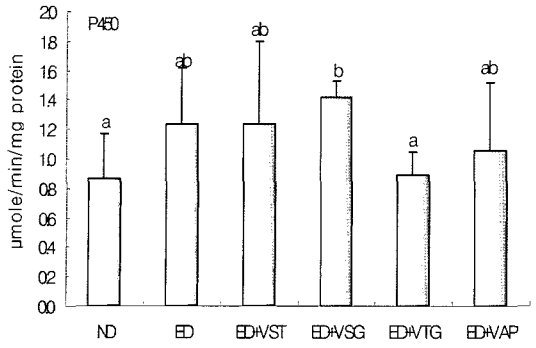

Fig. 5. Effect of herbal extracts on hepatic *p*-nitrophenol hydroxylase activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different (p<0.05) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+ Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+ Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+ Paeonia japonica

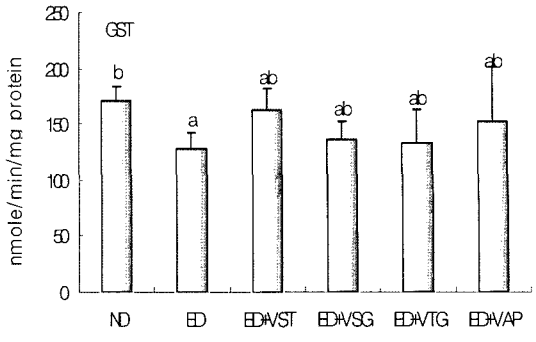

Fig. 6. Effect of herbal extracts on hepatic GST activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different(p<0.05) among the groups by Duncan's multiple range test. ND: normal diet ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+Paeonia japonica

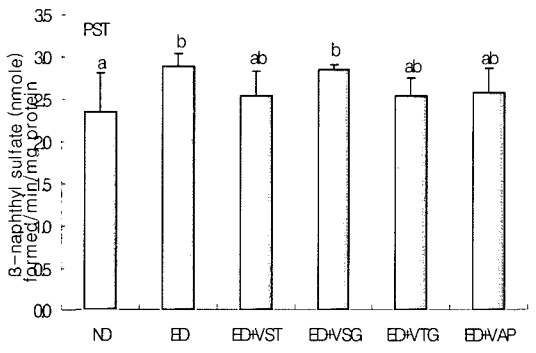

Fig. 7. Effect of herbal extracts on hepatic PST activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different(p<0.05) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+Paeonia japonica

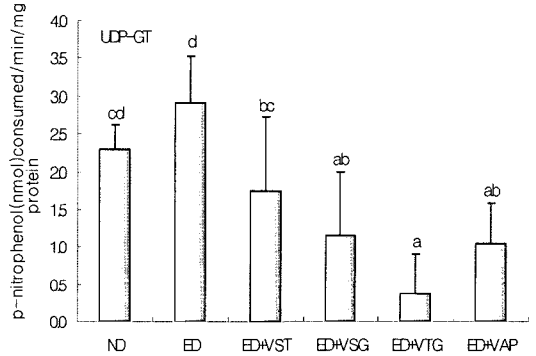

Fig. 8. Effect of herbal extracts on hepatic UDP-GT activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different (p< 0.05) among the groups by Duncan's multiple range test. ND: normal diet ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+ Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+ Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+ Paeonia japonica

increases (P<0.05).

In case of GSSG the liver, the ND group and HEM-treated group had the high tendency to increase, but it is difficult to judge the effect by only quantitative changes, we made the ratio of glutathione in reduction form and oxidation form. In according to the GSH/GSSG contents, the ED group showed about 34% decrease, and the glutathione content by supply of ethanol in reduction form was changed as the oxidation form. The ratio of glutathione of reduction form and oxidation form showed an increase in the HEM-treated groups(P<0.05)

In the liver GR activity

In the liver glutathione levels of GR activity are shown on Fig. 9. As compared with the ND group there was a decrease in the ED group after feeding alcohol in diet, and the activity of GR catalyzed glutathione in the lever is same on Fig. 9. In it's activity the ED group supplied only by the ethanol was lower level than that of ND group and the GR activity shown low level by the chronic supply of ethanol in the mixed supply of HEM-treated groups showed a tendency to increase, especially did in the VSG group(P<0.05).

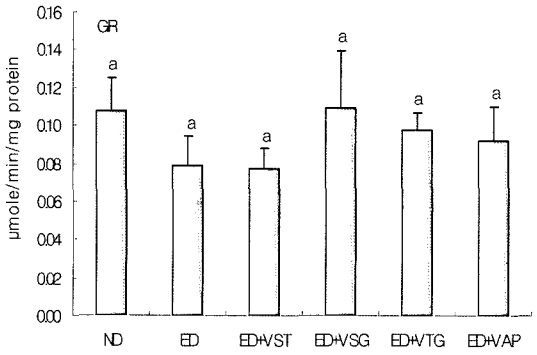

Fig. 9. Effect of herbal extracts on hepatic GR activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different(p<0.05) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+ Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+ Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+ Paeonia japonica

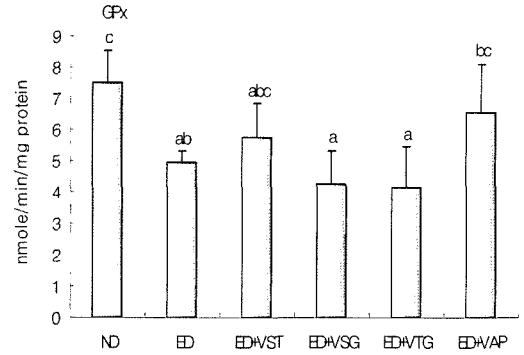

Fig. 10. Effect of herbal extracts on hepatic GPx activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different (p<0.05) among the groups by Duncan's multiple range test. ND: normal diet ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+Paeonia japonica

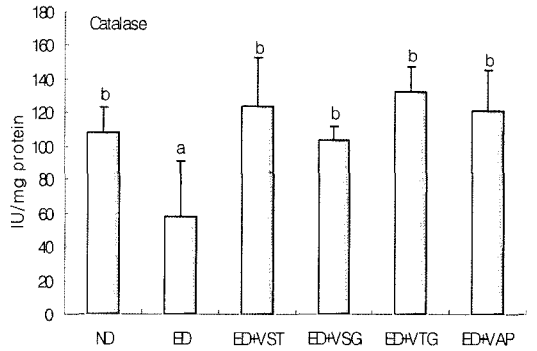

Fig. 11. Effect of herbal extracts on hepatic CAT activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different (p< 0.05) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+ Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+ Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+ Paeonia japonica

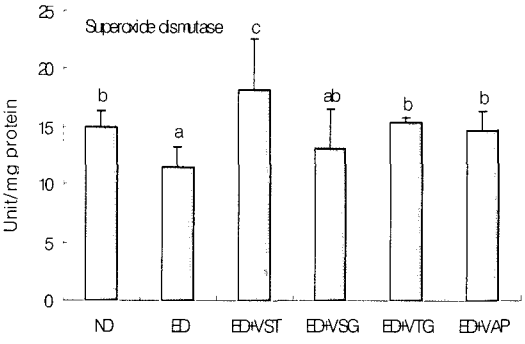

Fig. 12. Effect of herbal extracts on hepatic SOD activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different (p< 0.05) among the groups by Duncan's multiple range test. ND: normal diet ED: ethanol diet, VST:Vitis vinifera+Schisandra chinensis+ Taraxacum officinale VSG:Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides VTG:Vitis vinifera+Taraxacum officinale+ Gardenia jasminoides VAP:Vitis vinifera+Angelica acutiloba+ Paeonia japonica

Before the H2O2 damaged the cell membrane, in its activity of GPx disposing it. The ND group showed a significant decrease compared with the ED group(P<0.05, Fig. 10). Compared with the ED group, the VST, VAP groups showed a tendency to increase, especially did a significant increase in the VAP group(P<0.05)

In CAT the ED group had a significant decrease compared with the ND group. The mixed supply of HEM-treated group creased CAT activity and all mixed supplies of HEM-treated groups also showed statistically a significant increase(P<0.05, Fig. 11)

In case of SOD, the ED group showed significantly a low level compared with the ND group, the SOD activity, decreased by the ethanol when supplied the HEM-treated group, showed an increase(Fig. 12), especially did statistically a significant increase in VST group(P<0.05).

Contents of lipid peroxidation in the liver

In the ED group, contents of lipid peroxidaion had about 72% increase as compared with the ND group by the chronic

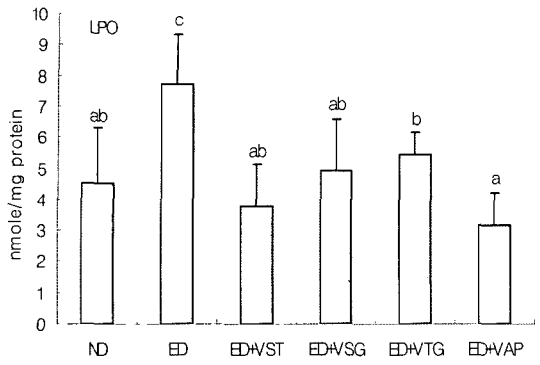

Fig. 13. Effect of herbal extracts on hepatic LPO activity in ethanol-administered rats. values are mean±SD. Letters with different superscripts in a column are significantly different($p<0.05$) among the groups by Duncan's multiple range test. ND: normal diet, ED: ethanol diet, VST:*Vitis vinifera+Schisandra chinensis+ Taraxacum officinale* VSG:*Vitis vinifera+Schisandra chinensis+ Gardenia jasminoides* VTG:*Vitis vinifera+ Taraxacum officinale+ Gardenia jasminoides* VAP:*Vitis vinifera+Angelica acutiloba+ Paeonia japonica*

Table IV. Effects of herbal extracts on hepatic GSH and GSSG levels in ethanol-administered rats.

	GSH	GSSG	GSH/GSSG
	μmol/mg protein		
ND	6.68±1.10^{b}	0.32±0.09^{a}	21.71±4.88^{a}
ED	2.69±0.99^{a}	0.21±0.07^{a}	14.32±8.83^{a}
ED+VST	7.98±1.92^{bc}	0.36±0.16^{ab}	23.73±7.17^{a}
ED+VSG	10.05±1.33^{cd}	0.58±0.26^{bc}	20.98±11.35^{a}
ED+VTG	11.28±1.85^{d}	0.72±0.10^{c}	15.79±1.64^{a}
ED+VAP	9.58±1.77^{cd}	0.39±0.08^{ab}	24.97±1.91^{a}

supply of ethanol(Fig. 13). The increase of lipid peroxidation induced by supply of ethanol, showed statistically the significant decrease by the mixed supply of HEM-treated groups.

DISCUSSION

In this study, we've induced fatty liver and hepatotoxicity in rats fed with a chronic alcohol-containing diet, and at the same time we've studied the mechanisms of anti-oxidation system and ethanol oxidation system in various herbal extracts. In case of ADH and ALDH measured in the gastric mucous membrane and liver, a trend of increased activity has been observed in the alcohol-administered group as compared with the normal group. And neither statistically significant increase nor decrease has been observed in a group administered herbal extracts. Since there is no significant change in levels of ADH and ALDH, it is thought that the other enzymes were activated.

Phase I reaction is the elimination of compounds inactivated or detoxified by the oxidase system in smooth endoplasmic reticulum of the hepatic cell while phase II reaction is composed of conjugation reaction. Among the phase I enzymes, the activation of P450 2E1, which is induced representatively by ethanol, has been increased by the administration of ethanol, noticeably by the administration of herbal extracts. The result was in agreement with the report (Lieber *et al.,* 1968) that the increase of the ethanol metabolism was caused by increased activities in the other alcohol metabolism enzymes that included MEOS except ADH in chronic ethanol consumer(Khan *et al.,* 1992). The phase I reaction is the conjugation of GSH, glucuronic acid or sulfate with endogenic and exogenous substances or medicines metabolized through the phase II reaction. There are GST, PST, UDP-GT and others as catalysts for the phase II reaction. GST is an endogenous anti-oxidation enzyme that catalyzes the conjugation of GSH with free radicals generated by the ethanol inducement. This result means that the Phase II metabolism enzymes such as GST, PST and UDP-GT are complementary in the liver. Also agreeing with the report by Jung(2000), the activity is increased in PST but decreased in UDP-GT by administering Luecocyanidian extracted in a grapestone to a mouse which had a hepatotoxicity induced by acetaminophen. Ethanol primarily makes free radicals in mitochondrion, resulting in a unique toxicity. The exhaustion of GSH stored in mitochondrion can lead to a decrease of viability of the cell resulting from the toxicity of ethanol(Lieber *et al.,* 1968). Rosenblum(1987) has reported that when a fluid-diet composed of 36% alcohol out of the total diet calorie is fed to a rat, the content of GSH was decreased with an increase of lipid-peroxide in the rat's testis. The interaction between ethanol and GSH led to a decrease of the content of GSH through these reactions: direct reaction between acetaldehyde and GSH, indirect combination between acetaldehyde and cysteine or lysine which are procompounds of GSH, and reaction between acetaldehyde and lipid-peroxide generated from oxidation of ethanol(Lieber *et al.,* 1968: Vina *et al.,* 1980). In this study, although the content of GSH was decreased in the ED group administered only ethanol, it increased by administering the herbal extracts. GR has a job to reduce oxidized GSSG, which is produced by glutathione peroxidase, to GSH by consuming NADPH(Recknagal *et al.,* 1977). It was shown that the activity of GR in the ED group administered ethanol-only was lower than that in the ND group. And the big activity of GR was shown in most of the groups administered the herbal extracts. Therefore, it is considered that

hepatotoxicity was decreased due to the decreased activity of GR; and on the contrary, due to the increased activity of GR in the group administered the herbal extracts, there was a decrease in the content of GSH which conjugates with xenobiotics. The report has said that the activity of GPx in serum is decreased in both the group of rats administered 5g of ethanol per body weight (kg) and all other groups administered chronically 5% ethanol-fluid diet(Aykac *et al.,* 1985). In this study, we've found that the decreased activities of GPx and CAT by administering ethanol in the ED group is increased with the administration of the herbal extracts, which is especially effective for increasing the activity of CAT. It has been considered that the increased activity of GPx in a group administered herbal extracts is a hemostasis action to reduce the oxides generated from the oxidation of alcohol. SOD, as metalloenzyme, inhibits the accumulation of oxygen radical generated from the ethanol metabolism with converting O_2^- to H_2O_2. In this study, the low activities have been shown in the group administered a high concentration of lipid-peroxide(Schisler *et al.,* 1989). This result has been agreed with the report that the activities are decreased due to the consuming SOD for metabolizing O_2^- generated as time goes by when administered ethanol lipid diet. In this study, as well, we also have found that the long-administration of ethanol leads to a generation of lipid-peroxide and to the effective elimination of lipid-peroxide, generated from administering the herbal extracts, by activating the various enzyme systems and the physiological active compound formation system. Consequently, such herbal extracts that we used in this study, as Angelicae gigantis Radix, Paeoniae Radix and etc, have been effective in protecting the liver. After a chronic consumption of alcohol, the liver has been protected by activating the ethanol-metabolism enzyme system from Angelicae gigantis Radix and by activating the ethanol-metabolism enzyme and the phase I, II-metabolism enzyme system from Paeoniae Radix. It is considered that the liver is protected by activating the ethanol-metabolism enzyme system and the phase II-metabolism enzyme system from Taraxaci Herba, by activating the phase II-metabolism enzyme system and the anti-oxidation system enzyme from Gardeniae Fructus, and by activating the anti-oxidation system from Schizandrae Fructus and a grapestone.

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