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Effects of an Ethylacetate Fraction of Chrysanthemi Flos on the Antioxidative System and Lipid Profile in Rats with Ethanol-Induced Liver Damage

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Abstract

To investigate the antioxidative effects of an ethylacetate fraction extracted from the flowers of *Chrysanthemum indicum* L. (Chrysanthemi Flos) on the antioxidative system and lipid profiles of rats with ethanol induced hepatotoxicity. Sprague-Dawley rats weighing 100~150 g were divided into 5 groups: normal group (NOR), Chrysanthemi Flos EtOAC fraction (200 mg/kg) treated group (S1), 35% ethanol (10 mL/kg) treated group (S2), Chrysanthemi Flos EtOAC fraction (200 mg/kg) and ethanol concomitantly treated group (S3) and Chrysanthemi Flos EtOAC fraction (400 mg/kg) and ethanol concomitantly treated group (S4), respectively. The antioxidative activity of each fraction was decreased in order of EtOAC, n-hexane, n-BuOH, water and chloroform. The growth rates and feed efficiency ratios were decreased by ethanol treatment, but were gradually restored to similar levels as in the NOR group by administering Chrysanthemi Flos EtOAC fraction. The whole blood concentrations of total cholesterol and LDL-cholesterol, and the activities of ALT and AST that were elevated by ethanol were significantly decreased in the Chrysanthemi Flos EtOAC fraction treated groups. It was also observed that the activities of SOD, catalase, xanthine oxidase and GSH-Px elevated by ethanol in rat liver were markedly decreased in the Chrysanthemi Flos EtOAC fraction treated group as compared to S2. These results suggest that Chrysanthemi Flos EtOAC fraction has possible protective effects against ethanol induced hepatotoxicity in rat liver.

Key words: *Chrysanthemum indicum* L., glutathione peroxidase, ethanol, protective effect

INTRODUCTION

Alcoholic hepatic diseases are frequently associated with hepatocyte damage caused by the increases in both the amount and frequency of alcohol consumption that are inevitably induced by the increases in environmental pollution and various stresses resulting from rapid industrialization (1,2). These diseases are usually caused by direct damage to hepatocytes, immunologic response, and biochemical reactions by either alcohol itself or its intermediate product acetaldehyde, or other foreign substances (3). Moreover, alcohol consumption increases oxygen free radical production, and lipid peroxides produced by these free radicals induce hepatocytic destruction and fibrosis, which can participate in the hepatic damage. Consequently, sufficient amounts of antioxidative enzymes and antioxidative substances are essential to effectively remove these various free radicals.

Chrysanthemum indicum L. is a perennial plant, which belongs to the genus Chrysanthemum, and is used for antipyretic, anti-inflammatory, antihypertensive, and to treat neurasthenic headache in oriental medicine (4). Its major components are flavonoid compounds such as luteolin, apigenin, apigenin 7-O-β-D-glucoside, luteolin 7-O-β-D-glucoside, acacetin and its glycosides (5,6), sesquiterpene lactone compounds such as cumambrin A, cumambrin B, arteglastin A, angeloyliadin, and acacetin 7-O-β-D-galactopyranoside (7,8), essential oils such as camphor, p-cynene, Δ-selinene, $C_{15}H_{22}$, etc. (9), and 35 kinds of volatile compounds such as epi-bicyclophellandren, camphor, 1,8-cineol, etc. (10). Additionally, Jung et al. (11) have recently reported on 1-octen-3-ol-3-O-β-D-xylopyranosyl (1→6)-β-D-glucopyranoside, a new alkyl alcohol glycoside in Chrysanthemi Flos. Moreover, numerous of pharmacological effects of this flower have been reported, including: antispasmolysis and anti-inflammatory (12) and anticancer properties of luteolin (13); inhibition of aldose reductase (14), electron donor and sensory properties of flower ethanol extracts (15), antimicrobial action of sesquiterpene lactones (16), antihypertension by inhibition of MAO (monoamine oxidase) by components such as acacetin, diosmetin and 5,7-dihydroxy chromone (17), and antihypertension in

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rats with spontaneous hypertension (18).

This study was designed to examine the effects of Chrysanthemum flower on ethanol-induced oxidative damage to hepatocytes caused by oxygen free radical metabolism and blood lipid metabolism. Hence, we bred rats for 6 weeks and measured their body weight increase ratio, feed efficiency ratio (FER), blood concentrations of total cholesterol, HDL-cholesterol level and LDL-cholesterol, and serum alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) activities. Meanwhile, we examined the hepatic damage caused by oxygen free radicals by reciprocally comparing the activities of xanthine oxidase (XO), an enzyme producing free radicals, of superoxide dismutase (SOD), an enzyme detoxifying the body from free radicals, of catalase, and of glutathione peroxidase (GSH-Px), and the contents of lipid peroxides like thiobarbituric acid reactive substances (TBARS) and glutathione (GSH).

MATERIALS AND METHODS

Materials

Chrysanthemum indicum L. (Chrysanthemi Flos; hereafter, CF) was cultivated and purchased from the Chrysanthemum Floriculture land of the Agricultural Technology and Extension Center in Gwangju megalopolis, Korea, and then, was dried in the shade. A 100 g sample of the dried flowers and ethanol (80:20 v/v) were mixed and ground in a blender (Braun, MR350, CA, USA). With the aid of a reflux condenser, this mixture was extracted at 65°C for 12 h twice and was filtrated through millipore filter (type FH 0.5 μM). The filtrate was concentrated, and then, phylogenically fractionated and separated into n-hexane, chloroform, EtOAC and n-BuOH fractions. After removing the solvent from each fraction using a rotary evaporator at 45°C, the fraction was vacuum concentrated, dried, and the antioxidative effects evaluated using a Rancimat 676 (Metrohm, Switzerland) for the reciprocal comparison (Table 1). To determine the antioxidant index (AI), the induction period for the experimental group where each fraction was added was divided by the induction period for the control group. The EtOAC fraction having the strongest antioxidative effect among all fractions was used as the samples.

Test animals and preparation

Male Sprague Dawley rats weighing 100~150 g were adapted to solid assorted feed (Samyang Feed Ltd., Korea) for 1 week. According to the randomized complete block design, the rats were divided into 5 groups of 6 rats each, as shown in Table 2: a normal control group (NOR); a group (S1) where 200 mg/kg body weight of the CF EtOAC fraction was administered; a group (S2) administered only ethanol; a group (S3) administered a combination of ethanol and 200 mg/kg body weight of the CF EtOAC fraction; and a group (S4) administered ethanol and 400 mg/body weight (bw) kg of the CF EtOAC fraction. Each of the rats was raised in a cage for 6 weeks. For each rat receiving ethanol, 35% ethanol 10 mL/kg bw/day, was administered according to the method of Fujji et al. (19). The CF EtOAC fraction was dissolved to make 200 mg or 400 mg/bw kg in saline solution, and was sterilized through suspension in 0.5% CMC, which was orally administered for 6 weeks. The body weights were measured once a week and food intakes every 2 days, and the data used to calculate the feed efficiency ratio (FER) of each experimental group by dividing the body weight increase ratio for a given period by the food intake for the same period. Prior to treatment the animals were fasted for 16 h and blood collected from the carotid artery under diethyl ether anesthesia. After the liver was extracted, the residual blood and other alien substances on the liver were removed with 0.9% saline solution. Then, the liver was weighed and stored in a cryogenic freezer at -70°C to be used for measuring enzyme activities. The rats were given free access to solid feed and water throughout the experimental period.

Table 1. Compositions of experimental diet

Groups	Diet composition	
NOR	Basal diet[1)]	-
S1	Basal diet + CF200[2)]	-
S2	Basal diet	+ EtOH[3)]
S3	Basal diet + CF200	+ EtOH
S4	Basal diet + CF400[4)]	+ EtOH

[1)]AIN-76 diet.
[2)]200 mg of *Chrysanthemum indicum* L. flowers ethanol extract /kg of body weight/day.
[3)]10 mL of 35% ethanol/kg of body weight/day.
[4)]400 mg of *Chrysanthemum indicum* L. flowers ethanol extract /kg of body weight/day.

Table 2. Antioxidative activity of each fraction of *Chrysanthemum indicum* L. ethanol extract on soybean oil

Fraction	IP[1)]	AI[2)]
Control	7.83 h	1.00
n-Hexane	9.17 h	1.17
Chloroform	7.82 h	0.99
Ethylacetate	13.63 h	1.74
n-Butanol	8.82 h	1.12
Water	8.07 h	1.02

[1)]Induction period of oil was determined by Rancimat test at 110°C.
[2)]Antioxidant index was expressed as IP of oil containing various fraction/IP of control oil.

Preparation of enzyme source

Four volumes of 0.25 M sucrose buffer (pH 7.5) to 1 g of liver and homogenized in an ultra turax homogenizer (10,000×g, 2 min) under glacial conditions. A part of the homogenate solution was used to measure TBARS, and the rest was centrifuged at 4°C and 600 ×g for 10 min to eliminate nucleus and unpulverized part. The supernatant was centrifuged at 15,000×g for 20 min to obtain and separate mitochondrial fraction. This supernatant was ultracentrifuged at 15,000×g for 1 h to obtain the cytosol fraction. The mitochondrial fraction was used to measure the catalase activity, and the cytosol fraction was used to measure SOD, XO and GSH-Px.

The carotid artery was incised to collect the blood in a 35 μL capillary tube for measuring the levels of total cholesterol, HDL-cholesterol and LDL-cholesterol using Choestech LDX (British England). Additionally, the collected blood was left and then centrifuged at 3,000 rpm for 15 min to separate serum for measuring ALT and AST activities.

Estimation of enzyme activity

The enzyme activities were measured as follows: the method of Downry et al. (20) for XO activity, Crapo et al. (21) for SOD activity, Aebi (22) for catalase activity, and Flohe et al. (23) for GSH-Px activity in the hepatic tissue. The content of lipid peroxide was measured by the method of Buege and Aust (24) to colorimetrically quantify the amount of malondialdehyde with thiobarbituric acid, and the content of GSH was estimated by the method of Tietze (25). The activities of serum ALT and AST were estimated using a kit (Asan Pharmaceutical Co., Ltd., Korea) manufactured according to Reitman Frankel's method (26).

Protein quantification and statistics for test results

Protein was quantified by measuring bovine serum albumin (Sigma Fr.v) as a standard based on the method of Lowry et al. (27). The experimental results were expressed as mean±standard error (S.E) using SPSS software, and the statistical significance was tested through ANOVA (one way analysis of variance) and then was reciprocally tested through t-test at $p<0.05$.

RESULTS AND DISCUSSION

Antioxidative effects of each fraction

Table 2 illustrates the antioxidative effects obtained by measuring each fraction of ethanol induced extract of Chrysanthemi Flos by the Rancimat method. The AI for each fraction shown to be 1.74 for EtOAC, 1.17 for n hexane, 1.12 for n-BuOH, 1.02 for water, and 0.99 for chloroform, which means that the antioxidative substances contained in Chrysanthemi Flos were massively extracted in EtOAC fraction.

Body weight increase ratio and feed efficiency ratio (FER)

Table 3 shows the body weight increase ratios and FER for 6 weeks. Rats in the ETH group (S2), where only ethanol was administered, gained significantly less body weight each week, compared with the control (NOR) group ($p<0.05$). In particular, at the 6th week, the body weight increased ratio of the S2 group was approximately 69% of that of the NOR group. However, compared with that in the S2 group, the body weight increase ratios in the groups administered ethanol combined with CF EtOAC fractions (S3 200 mg/kg or S4 400 mg/kg) were not significantly different from the ethanol group for the first 5 weeks, but then significantly accelerated by the 6th week. The FER of the S2 group was much lower, 39~45% of the other groups, but was increased by the combined administration with the CF EtOAC fraction. Especially, the S4 group demonstrated a similar FER to that of the NOR group. In this study, ethanol administration resulted in significant decreases in body weight increase ratios and FER. The causes of these results have

Table 3. The growth rate and feed efficiency ratio (FER) of rats treated with alcohol and/or ethanol extract of flowers of *Chrysanthemum indicum* L.

Groups[1] \ Weeks	Growth rate[2]						FER[3]
	1	2	3	4	5	6	
NOR	1.141±0.007[4)a5)]	1.242±0.007	1.374±0.006	1.544±0.016[a]	1.666±0.006[a]	1.834±0.012[a]	0.137±0.005[a5)]
S1	1.159±0.024[a]	1.281±0.015[a]	1.397±0.022[a]	1.526±0.069[a]	1.624±0.095[a]	1.706±0.123[ad]	0.138±0.008[a]
S2	1.042±0.023[b]	1.134±0.032[b]	1.202±0.043[b]	1.174±0.048[b]	1.249±0.056[b]	1.265±0.065[b]	0.074±0.018[b]
S3	1.072±0.015[b]	1.147±0.029[b]	1.204±0.039[b]	1.162±0.018[bc]	1.248±0.027[b]	1.471±0.021[c]	0.122±0.005[a]
S4	1.100±0.021[c]	1.168±0.04	1.246±0.046	1.251±0.094[c]	1.324±0.120[b]	1.546±0.127[d]	0.134±0.014[a]

[1)]Abbreviation: See the Table 1.
[2)]Ratio of the body weight (W_1) to initial body weight (W_0).
[3)]The total amount of weight increased/the total intake of food.
[4)]Values are mean±SE of 6 rats per each group.
[5)]Values with different superscripts in the same column are significantly different ($p<0.05$) between groups by Tukey (T) test.

been explained differently by various researchers. Shaw and Lieber (28) stated that the excessive intake of ethanol supplies a high caloric which reduces the appetite and leads to malnutrition. Halsted (29) explained that chronic alcoholism damages the digestive tract impairing nutrient absorption and finally leading to malnutrition. Mitchell and Herlong (30) suggested that ethanol consumption reduces energy efficiency because of the increase in oxygen consumption and the use of ethanol for oxidative energy for the thermal production. The reason for the differences in these reports is that the nutritional disorder caused by excessive ethanol intake can induce various pathological states caused by the varied and complicated reactions. In the present study, combining the CF EtOAC fraction with ethanol increased the body weight increase ratio and the FER to nearly those of the NOR group. This demonstrates that bioactive components of Chrysanthemi Flos can gradually relieve different toxic symptoms caused by ethanol. However, further experiments are needed to determine the effects of ethanol when dietary intake is adequate, to separate the toxic effects of ethanol itself from the effects of malnutrition.

Serum ALT and AST activities

Ethanol and CF EtOAC fraction (200 mg/kg or 400 mg/kg, bw/day) were orally administered to rats for 6 weeks, and then, serum ALT and AST activities known as one of the indices for hepatic damage level were measured and shown in Table 4. The ALT activity was increased by over 85% due to ethanol administration, in comparison with that of the NOR group ($p<0.05$); However, the elevated ALT was decreased by about 31% and 33% each in EtOAC fraction combined groups (S3 and S4) below the activity in the S2 group. The AST activity in the ETH group was increased by over 49%, compared with that in the NOR group, but the elevated AST was decreased by over 29% in both S3 and S4 groups. In this study, the S1 group where only the EtOAC fraction, without ethanol, was administered did not show any special changes in the serum ALT and AST activities compared with the NOR group. Therefore, the CF EtOAC fraction appeared to be safe and was not associated with hepatic toxicity at the dosage and duration used in this study. The fact that the ethanol administration apparently increased the serum ALT and AST activities was consistent with the reports of Lieber (31) and Shaw and Lieber (28) that the acute administration of ethanol increases ethanol oxidation by the MEOS (microsomal ethanol oxidizing system) to produce oxygen radicals such as O^{-}_{2}, H_2O_2. These free radicals continuously produce lipid peroxides that damage hepatocytes and hepatic tissue, and the hepatocytic damage in turn increases the serum ALT and AST activities. Serum ALT and AST that were elevated in the ETH group were significantly reduced by the combined administration with CF EtOAC fraction as were hepatic TBARS concentrations as shown in Table 7; therefore CF EtOAC fraction can normalize the functions of the hepatocytes damaged by ethanol. One study (32), however, reported that the acute administration of ethanol did not change the serum ALT and AST activities. Based on the hypothesis that ethanol toxicity can be affected by the dosage and the administration period, further precise experiments in characterizing the effects of duration of administration on the etiology of liver pathology are needed before concluding that CF EtOAC fraction prevents ethanol induced liver damages from only the results of this study.

Enzyme activities to produce oxygen free radicals in the liver

Table 5 shows the XO activities in the liver of rats that were administered with CF EtOAC fraction for 6 weeks. The XO activity was not notably changed in the S1 group compared with the NOR group, but was meaningfully increased in the S2 group. The ethanol induced increase in XO activity was reduced in the S3 and S4 groups. Because XO participates in purine metabolism *in vivo* or forms uric acid from xanthine or hypoxanthine, the accumulation of XO in the articulations induces gout and causes severe pain when plasmic uric acids massively increase (33,34). The inhibition of this enzyme has significant health implications as does the inhibition of free radical production. The result of this experiment that ethanol administration increased the XO activity is consistent with previous reports (35,36) that chronic administration of ethanol to rats increases XO activity.

Table 4. The activities of ALT and AST in serum of rats treated with alcohol and/or ethanol extract of flowers of *Chrysanthemum indicum* L.

Enzyme activities (karmen unit/mL)	Groups[1)]				
	NOR	S1	S2	S3	S4
ALT	32.91 ± 1.78^{a}[2)]	25.71 ± 3.05^{a}	40.88 ± 0.70^{b}	32.13 ± 0.88^{a}	30.88 ± 1.31
AST	139.82 ± 12.48^{a}	133.17 ± 12.59^{a}	168.86 ± 8.97^{b}	148.92 ± 8.32	145.41 ± 9.82

[1)]Abbreviation: See the Table 1.
[2)]Values are mean ± SE of 6 rats per each group and different superscripts indicate significance at $p<0.05$ between groups.

Table 5. The activities of XO, catalase, SOD and GSH-Px of rats treated with alcohol and/or ethanol extract of flowers of *Chrysanthemum indicum* L.

Enzyme activity	Groups[1)]				
	NOR	S1	S2	S3	S4
XO[3)]	24.14±1.34[ac2)]	22.83±0.30[a]	43.85±2.70[bc]	35.36±4.32[c]	34.36±4.32[c]
Catalase[4)]	5389.3±660.5	4216.2±478.8[a]	6978.8±635.5[b]	5995.1±456.1	5718.0±716.0
SOD[5)]	19.53±2.02[ac]	14.90±0.51[a]	30.86±0.99[bc]	26.82±1.28[c]	24.70±3.39[c]
GSH-Px[6)]	345.52±29.11[a]	302.49±23.43[a]	592.71±21.49[b]	438.27±58.25	408.56±55.28[c]

[1)]Abbreviation: See the Table 1.
[2)]Values are mean±SE of 6 rats per each group and different superscripts indicate significance at $p<0.05$ between groups.
[3)]mU/g protein.
[4)]Decreased H_2O_2 μmol/min/mg protein.
[5)]μmol/min/mg protein.
[6)]Decreased NADPH μmol/min/mg protein.

Only the groups (S3 and S4) that were administered the CF EtOAC fraction combination showed relatively lower enzyme activities than the S2 group. According to this result, the CF EtOAC fraction can inhibit the production of oxygen free radicals that are increased by ethanol administration.

Antioxidant enzyme activities in the liver

Catalase activity was increased in the S2 group above that in the NOR group, and was decreased in the S3 and S4 groups, compared with the S2 group, but without any significance. Catalase reduces H_2O_2 into nontoxic H_2O in peroxisomes (37) existing in most organs to protect against tissue damage caused by increases in H_2O_2 (38). This catalase has a relatively higher Km value than does GSH-Px has, and naturally, and is usually elevated with high concentration of H_2O_2 (39,40). DeMaster et al. (41) and Schisler and Singh (42) reported that the chronic intake of ethanol decreases catalase activity. However, this study had similar results to the reports of Oh et al. (43), Antonenkov (44) and Kino (45) that ethanol administration increases the activity of this catalase enzyme.

The SOD activity was elevated in the S2 group above that of the NOR group but was significantly decreased in the S3 and S4 groups, compared with the S2 group. SOD as a metalloenzyme, which can be classified according to the associated metal ion types such as Cu, Zn, Mn and Fe, reduces unstable superoxide anion ($O_2^{\cdot}$) into hydrogen peroxide (H_2O_2) because $O_2^{\cdot}$ accepts one electron (46,47). The increase in the SOD activity by ethanol administration in this study can be associated with the induction of the SOD enzyme by the increase of oxygen free radical production, and the combined administration with CF EtOAC fraction can somewhat inhibit the production of oxygen free radicals.

The GSH-Px activity was significantly increased in the S2 group in comparison with the CON group, and the increased GSH-Px activity by ethanol was considerably decreased in the S3 and S4 groups. GSH-Px can protect against peroxide induced damage of tissue (49) by catalyzing the reaction to produce oxidized glutathione (GSSG) and water from H_2O_2 and GSH, and the reaction to produce GSSG, alcohol (ROH) and water from peroxides and GSH (48).

Generally, the activities of these antioxidative enzymes are reduced in the existence of antioxidative substances such as vitamin E, β-carotene, and flavonoids because these substances are substituted for the functions of the enzymes (50). In particular, flavonoids exert antioxidative effects by the formation of stable metal ion complexes with Cu and Fe ions in a free state (51), or by the direct scavenging roles of oxygen free radicals such as superoxide anion (52), hydroxyl radical (53), and peroxy radical (54). Therefore, flavonoid itself acts as an antioxidative substance like vitamin E or β-carotene to inhibit lipid peroxidation, instead of directly affecting the antioxidative activity. That is, flavonoids inhibit the peroxidized lipid accumulation in the tissue rather than restoring the reduced antioxidative enzyme activities by ethanol.

Contents of lipid peroxide in the liver

Table 6 shows the liver concentrations of peroxidized lipids of rats administered ethanol and CF EtOAC fraction for 6 weeks. The lipid peroxides were lower in the S1 group than the NOR group, but were significantly increased in the S2 group. The contents of peroxidized lipid in the S3 and S4 groups were higher than that in the NOR group but were significantly lower than in the S2 group. The increase in oxygen free radicals with increased ethanol intake is caused by the oxidation of ethanol by cytochrome P-450 mixed function oxidase (MFO) of the microsomal fraction, especially, of the cytochrome P-450 II E1, or is caused by the use of the mitochondrial respiratory chain (55). Lipid peroxidation is the most important mechanism of the hepatic damage by various toxic compounds or drugs (56), which is in-

Table 6. The contents of thiobarbituric acid reactants (TBARS) in liver of rats treated with alcohol and/or ethanol extract of flowers of *Chrysanthemum indicum* L.

Content	Groups[1)]				
	NOR	S1	S2	S3	S4
TBARS (μm/g liver)	6.49±0.37[a2)]	5.90±0.11[a]	9.78±0.24[b]	7.98±0.24[cd]	7.73±0.21[d]

[1)]Abbreviation: See the Table 1.
[2)]Values are mean±SE of 6 rats per each group and different superscripts indicate significance at $p<0.05$ between groups.

duced by the increase in intracellular oxidative stress, that is, the increase of free radical production, and by the decrease in antioxidative capacity.

In this study we found that ethanol administration notably increased the hepatic peroxidized lipid content, which is in accordance with other reports (57,58) that free radicals produced in the metabolism of xenobiotics increase lipid peroxidation. Because acetaldehyde produced after the excessive intake of ethanol (59,60) reacts with tubulin to inhibit polymerization and intracellular protein transfer, the hepatic damage by ethanol leads to the accumulation of abnormal intracellular proteins and to hepatocyte damage and necrosis (61). In particular, if acetaldehyde is oxidized by low Km acetaldehyde dehydrogenase, lipid peroxidation is promoted by producing more reactive substances containing oxygen free radicals (62,63). Based on the finding of this experiment that the contents of lipid peroxide were not different between the CF EtOAC fraction administered groups and the control group, the dosage and administering period of the CF EtOAC fraction used in this experiment was insufficient to induce toxicity to the hepatic tissue cells. In contrast, ethanol administration significantly increased the peroxidized lipids in the liver, compared with the normal control group; however, it was considerably decreased it by administering the CF EtOAC fraction. Therefore, CF EtOAC fraction can effectively protect the liver from ethanol induced damage.

Contents of GSH in the liver

Table 7 shows the content of GSH in the liver of rats that were administered ethanol and CF EtOAC fraction for 6 weeks. The GSH content in the S2 group was significantly decreased from that of the NOR group, but the contents in the S3 and S4 groups were close to that of the NOR group. GSH reduces the reduction speed of cytochrome C to exhibit an O_2 scavenging effect, and removes hydroperoxide produced during the conjugation with other alien substances and the formation of peroxidized lipid, in addition it eliminates free radicals by producing relatively stable thiol radicals (64). That is, GSH holds most parts of nonprotein thiols in the animal tissue, and plays an important role as an intracellular antioxidative substance through its role as a substrate of GSH-Px to metabolize free radical scavengers, H_2O_2 and lipid peroxide. GSH in the liver directly participates in a variety of biologically important reactions such as diverse detoxification, reduction of peroxidized lipid caused by GSH-Px, protein or DNA synthesis, amino acid transfer, thiol radical storage, etc. Accordingly, if the remnants of GSH are reduced even in the presence of sufficient GSH-Px enzyme activity, the intracellular hydroperoxide is not completely scavenged, and therefore, a small amount of hydroperoxide is available to participate in several reactions (65). In this study, administration of ethanol significantly reduced the intrahepatic GSH content in comparison with that of the normal group. The decrease in the GSH content is due to its consumption by increased GSH-Px activity, removing hydroperoxide by using GSH as the substrate. Moreover, the finding that the GSH content was increased by the combined administration of the CF EtOAC fraction with ethanol can be attributed to the sparing of GSH by the decreased need for GSH-Px action due scavenging of the free radicals like H_2O_2 produced by ethanol by the extract.

Levels of total cholesterol, HDL-cholesterol and LDL-cholesterol in blood

Table 8 shows the concentrations of total cholesterol, HDL-cholesterol and LDL-cholesterol in blood of rats administered and/or CF EtOAC fraction for 6 weeks. The

Table 7. The contents of GSH in liver of rats treated with alcohol and/or ethanol extract of flowers of *Chrysanthemum indicum* L.

Content	Groups[1)]				
	NOR	S1	S2	S3	S4
GSH (mg/g liver)	52.97±2.51[a2)]	53.21±3.23[a]	39.81±2.56[b]	45.50±2.22[a]	47.2±1.34[a]

[1)]Abbreviation: See the Table 1.
[2)]Values are mean±SE of 6 rats per each group and different superscripts indicate significance at $p<0.05$ between groups.

Table 8. Contents of total cholesterol, HDL-cholesterol and LDL cholesterol in blood of rats treated with alcohol and/or ethanol extract of flowers of *Chrysanthemum indicum* L.

Content	Groups[1)]				
	NOR[1)]	S1	S2	S3	S4
Total cholesterol (mg/dL)	105.83±12.81[a2)]	103.07±13.00[a]	130.86±11.89[b]	107.40±14.28[a]	103.03±13.00[a]
HDL-cholesterol (mg/dL)	27.17±2.26[a]	36.50±2.90[b]	19.50±1.19[a]	28.00±1.92[ab]	23.75±1.44[a]
LDL-cholesterol (mg/dL)	69.67±3.18[a]	57.50±5.52[a]	102.3±2.14[b]	70.80±3.54[a]	70.75±4.80[a]

[1)]Abbreviation: See the Table 1.
[2)]Values are mean±SE of 6 rats per each group and different superscripts indicate significance at $p<0.05$ between groups.

blood total cholesterol level was significantly increased in the ETH group than the S1 group, however, it was meaningfully decreased in the S3 and S4 groups. When ethanol is chronically administered to the rat, the levels of serum triglyceride and total cholesterol are appreciably increased in comparison with those in the normal rats (66). The increases of serum triglyceride and total cholesterol levels in chronic alcoholic patients were reported to be caused by increased esterification of free fatty acids by the ethanol, a result of increasing the $NADH/NAD^+$ ratio according to *in vivo* ethanol metabolism, by the inhibition of metabolism or decomposition of hepatic chylomicron remnants, or by the increased production or decreased utilization of serum triglyceride (67,68). The HDL-cholesterol level was increased in the S1 group above that of the NOR group, but was decreased in the S2 group while the S3 and S4 groupss had relatively increased levels in comparison with that in the S2 group. In particular, the S3 group, where the small dosage of the CF EtOAC fraction was administered, increased the level radically, which was even higher than the normal level. HDL-cholesterol plays a part in lowering the total cholesterol level by transferring cholesterol from several organs into the liver and by promoting oxidation, decomposition and excretion (69). In this experiment, the administration of the CF EtOAC fraction increased the HDL-cholesterol level that was decreased by the ethanol administration, to almost normal levels. Accordingly, the CF EtOAC fraction can effectively prevent hyperlipidemia. The LDL-cholesterol level was significantly increased in the S2 group compared to the NOR group, but was notably decreased in the S3 and S4 groups in compared with the S2 group. LDL-cholesterol, as a main carrier of serum cholesterol, accumulates in the arterial wall to cause atherosclerosis, and causes cardiac and circulatory system diseases (70). In reviewing the present experimental results that the EtOAC fraction of Chrysanthemi Flos significantly reduced the level of blood LDL-cholesterol that was increased by ethanol administration, the CF EtOAC fraction can be effective for reducing the cholesterol levels in hypercholesterolemia.

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