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Effect of Herb Distillate on Hepatic Xanthine Oxidase Activity and Serum Lipid Profiles in Carbon Tetrachloride-Administered Rats

Bum Ho Park[1], Sang Il Lee[2] and Soon Dong Kim[1†]

[1]*Department of Food Science and Technology, Catholic University of Daegu, Gyeongsan 712-702, Korea*
[2]*Department of Food Nutrition and Cookery, Keimyung College, Daegu 704-703, Korea*

Abstract

In order to evaluate the hepatoprotective effect of an herb distillate, ie., a mixture of 28 traditional Korean herbs, germanium, tormarine and Gijangsoo (Gijang water), CCl_4 was intraperitoneally administered to rats before or after supplementation of the diluted herb distillate (HD) for 2 weeks. Then hepatic xanthine oxidase activity and serum lipid profiles were determined. The experimental groups had higher feed intake than the normal control (NC), but had lower weight gain. Water intake and the amount of feces were not significantly different, but urine was excreted in lower amounts in all the experimental groups compared to the NC. Liver weights in the HD-supplemented groups were lower than that of the distilled water-supplemented groups (DW-groups) after CCl_4-administration. Serum ALT activities in all the experimental groups were higher than that of the NC-group. However, the increasing activity of serum ALT in the HD-supplemented groups (HD-groups) was lower than that of the DW-groups. Total serum and LDL-cholesterol levels were higher in all the CCl_4-administered groups than in the NC-groups, and serum HDL-cholesterol levels were lower in all the experimental groups compared with the NC-groups. Meanwhile, the increasing rate of total serum and LDL-cholesterol levels and the decreasing rate of HDL-cholesterol in the HD-groups were lower than that of the DW-groups. But, levels of serum TG were similar among all the experimental groups. The activities of hepatic xanthine oxidase (XOD) type O of the CCl_4-administered rats showed a significant increase in and an increasing rate of XOD in the HD-groups, which was lower than that of the DW-groups. On the other hand, GST activities in all the experimental groups were significantly decreased, and the decreasing rate was lower in the HD-groups than in the DW-groups. The hepatic contents of GSH and LPO in all the rats were not changed by CCl_4 administration. These results suggest that the decreased liver damage in the HD-supplemented groups was due to the inhibition of XOD-type O activity by constituents of HD, as well as by a prevention/inhibition of serum lipid profile changes in CCl_4-treated rats. However, further detailed studies are needed to support this hypothesis.

Key words: herb distillate, carbon tetrachloride, xanthine oxidase activity, hepatic lipid profiles

INTRODUCTION

It is well-known that water serves as the universal solvent in which a variety of solutes are dissolved. Water in the body is related to nutrient absorption, transport, and metabolism, as well as excretion of waste products, maintenance of osmolarity, pH, BP and temperature control. Water intake is strongly influenced by habit or health. Healthy people have a remarkable ability to maintain the tonicity of their body fluid. Water balance is regulated by hypothalamic ADH and the kidneys by the osmolarity of body fluid. But, diseases, such as diabetes mellitus, and renal disease alter water balance (1). Water quality is critical for maintaining human health. However, with worldwide industrial development leading to water pollution, water quality has become too low and insufficient for maintaining health.

Germanium, tourmaline, and Gijangsoo (made from yellow earth: loess) have been known to irradiate in the far-infrared portion of the light spectrum, which activates anti-oxidant activity in the herbal mixture by affecting heat transport (2). Also, bioactive water made by treatment of far-infrared light produced from ceramic stone has a protective effect on alcohol-induced hepatic injury in pigs (3). It is also well-known that water is converted from higher to relatively lower molecular mass clusters by ceramics, jade, yellow soil or granite porphyry (mackban-stone), which emit far infrared rays (4-6). However, low molecular mass water clusters have only been studied a little in relation to their effect on human health.

The vapor of specific medicines during distillation

†Corresponding author. E-mail: kimsd@cu.ac.kr
Phone: +82-53-850-3216, Fax: +82-53-850-3216

results in what is referred to as herb distillate. Medicines contain various volatiles which have been researched for use in promoting health, treatment of disease, and for beauty care products (7-9). In the present study, we examined the detoxification effect of an herb distillate made from 28 traditional Korean herbs, as well as ceramic mixtures of germanium, tourmaline, and Gijangsoo, which is traditionally used in Korean medicine for preventing hepatic damage and cancer (4-6), on CCL_4-induced toxicity in rats. CCl_4 was intraperitoneally injected into rats before or after supplementation of a diluted herb distillate for 2 weeks. Body weight, feed intake, water intake, organ weight, serum lipid profiles, content of hepatic glutathione, lipid peroxide content, and xanthine oxidase, glutathione S-transferase and serum alanine aminotransferase activity were determined to investigate liver damage in the CCl_4-administered rats.

MATERIALS AND METHODS

Materials

Various herbs (Table 1) were purchased from a commercial firm in Daegu, Korea and stored at 4°C until used. Tourmaline, germanium (38 microliters per liter) was obtained from the Uncheon mine in Gyeongsansi, Korea. Gijangsoo (Gijang water) was obtained from the Haksan mine, Gyeongsansi, Korea.

Preparation of the herb distillate

One hundred g of herbs were added to 4 L of distilled water and distilled at 120°C for 5 hours, as shown in Table 1. The diluted herb distillate (HD) was prepared by adding herb distillate to a final concentration of 2% into ozone-treated (0.5 microliters per liter) water.

Animals, experimental plots and preparation of diets

Male Sprague-Dawley rats with a mean mass of 195 ±5 g were purchased from Hyochang Science (Daegu, Korea). The experimental animals were divided into five groups (10 rats/group), as shown in Fig. 1. The following notafious were indicated: Normal (designated NC), distilled water (DW) supplement after CCl_4 injection (CCl_4-DW), DW supplement for 2 weeks and CCl_4 injection before 2 days of sacrifice (DW-CCl_4), diluted herb distillate (HD) supplement after CCl_4 injection (CCl_4-HD), and HD supplement for 2 weeks and CCl_4 injection before 2 days of sacrifice (HD-CCl_4). All animals were fed a prepared basal diet according to the AIN-76A diet (Teklad, Indiana, USA), as shown in Table 2. The ratio of carbohydrate, protein and lipid content in the basal diet was adjusted to 60:20:15.

Rats were individually housed in stainless steel cages with wire bottoms in a room maintained at 20±2°C and 60±5% relative humidity. The room was exposed to alternative 12-hours of light and dark. Rats were fed an animal diet (Purina Co., Seoul, Korea) during an initial 1-week acclimation period. All rats were allowed to eat their respective diets and drink their water freely.

Table 1. Materials for preparation of herb distillate

No	Korean name	Scientific name	Ratio (%)
1	Danggui	*Angelica gigas*	2.2
2	Gamcho	*Glycyrrhizae glabra*	2.2
3	Sasam	*Codonopis lanceolata*	2.2
4	Sanyak	*Disocorea japonica*	2.2
5	Hwangkee	*Astragalus membranaceus*	2.2
6	Sukjihwayang	*Rehmanniae* radix	1.0
7	Hasuo	*Pleuropterus multflorus*	2.5
8	Gungang	*Zingiber officinale*	2.5
9	Yukgae	*Cinnamomum loureirii*	2.5
10	Hwacho	*Zanthoxylum piperitum*	2.5
11	Dansam	*Salvia miltiorrhiza*	2.5
12	Bongsun	*Impatiens balsamina*	3.0
13	Hongwha	*Carthamus tinctorius*	3.0
14	Dokhwal	*Aralia contientalis*	3.0
15	Mahwang	*Ephedra sinica*	3.0
16	Galgun	*Pueraria thunbergiana* radix	4.5
17	Woosul	*Achyranthes japonica* radix	3.0
18	Mokhwa	*Chaenomeles sinensis*	3.0
19	Haesong	*Pinusdensiflora* neeles	3.0
20	Jermagun	*Boehmera niver* radix	3.0
21	Suk	*Artemisia princeps* var. orientalis	4.0
22	Yongsullan	*Agave americana*	2.0
23	Hwanggum	*Scutellaria baicalensis*	2.5
24	Hwangyeun	*Coptis chinensis*	2.5
25	Hwangback	*Phellodendron amurense*	2.5
26	Hetgae	*Hovenia dulcis*	1.5
27	Orkbun	Corn powder	12.0
28	Germanium	Germanium	5.0
29	Tourmaline	Tourmaline	7.0
30	Gijangsoo	Gijang water made from yellow earth	8.0

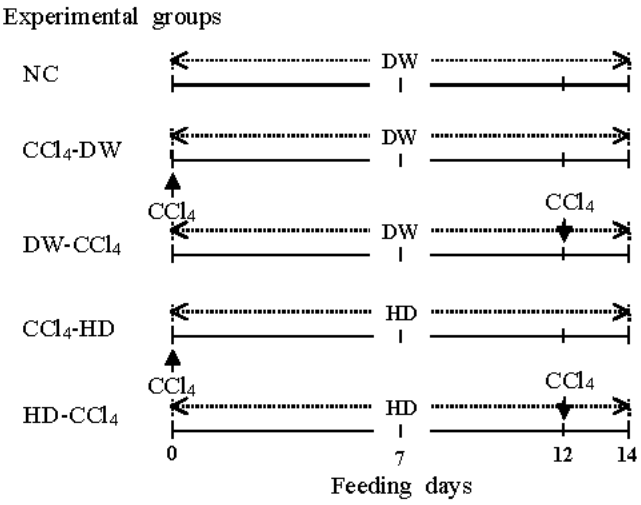

Fig. 1. Experimental design for control and treatment groups. NC, normal control; DW, distilled water; HD, herb distillate.

Table 2. Compositions of basic diets (g/kg diet)

Ingredients	Content
Corn starch	150
Casein	200
Corn oil	50
Sucrose	500
Cellulose	50
AIN mineral mixture[1)]	35
AIN vitamin mixture[2)]	10
DL-Methionine	3
Choline bitartrate	2
Total	1,000

[1)]AIN mineral mixture (g/kg): calcium lactate 620.0, sodium chloride 74.0, potassium phosphate di-basic 220.0, potassium sulfate 52.0, magnesium oxide 23.0, manganous carbonate 3.3, ferric citrate 6.0, zinc carbonate 1.0, cupric carbonate 0.2, potassium iodate 0.01, sodium selenite 0.01, chromium potassium sulfate 0.5, finely powdered to make 1,000 g.

[2)]AIN vitamin mixture (mg/kg): thiamin-HCl, 600; riboflavin, 600; pyridoxine-HCl, 700; nicotinic acid, 3,000; D-calcium pantothenate, 1,600; folic acid, 200; D-biotin, 20; vitamin B12, 2.5; vitamin A, 400,000 IU; vitamin D3, 100,000 IU; vitamin E, 7,500 IU; vitamin K 75, finely powdered to make 1,000 g.

Induction of hepatic damage

Fifty percent CCl_4 (Sigma Chem. Co., St. Louis, MO, USA) mixed with olive oil (1:1; v/v) was administered intraperitoneally at 0.1 mL of 50% the CCl_4 per 100 g body weight once daily for 2 days to CCl_4-DW and CCl_4-HD groups before supplementation of HD or DW. The DW-CCl_4 and HD-CCl_4 groups were administered the CCl_4 solution 1 and 2 days before sacrifice; The NC group was administered olive oil.

Weight gain, feed and water intake, and feed efficiency ratio

Body weight, feed and water intake were measured daily. The feed efficiency ratio (FER) was calculated by dividing the weight gains into the feed intake weekly.

Preparation of analytical samples

After the treatment of the experimental groups for 2 weeks, rats were fasted for 24 hours and anesthetized with ethylether; and blood was collected from the abdominal aorta. The collected blood was centrifuged at 3,000 rpm ($\times g$) for 10 minutes at room temperature and the serum separated was kept at -70°C. The internal organs, such as the liver were exhaustively perfused with cold physiological saline solution though the portal vein and quickly removed. The liver was homogenized with 4 volumes of 0.25 M sucrose, centrifuged at 1,000$\times g$ for 10 minutes and the supernatant was recentrifuged at 10,000$\times g$ for 20 minutes. The pellet was resuspended with 0.25 M sucrose for use as a mitochondrial fraction and the supernatant was used as a post-mitochondrial fraction (PMF). The collected urine and feces were measured everyday.

Analysis of blood and urine sugar, and serum lipids

The contents of triglyceride, total cholesterol and HDL-cholesterol in the serum were measured using kit reagents from AM 157S-K, Asan Pharm Co., Seoul, Korea; AM 202-K, Asan Pharm Co., Seoul, Korea, and AM 203-K, Asan Pharm Co., Seoul, Korea; respectively. The content of LDL-cholesterol was calculated using the method of Friedewald et al. (10).

Measurement of hepatic xanthine oxidase (XOD), glutathione S-transferase (GST) activities and serum alanine aminotransferase (ALT) activities

Xanthine oxidase (11) and glutathione S-transferase (12) activities in the liver were determined and expressed as uric acid nmole per minute per mg of PMF protein and thioether nmole per minute per mg of PMF protein, respectively. Alanine aminotransferase (ALT) activity in the serum was determined by using a kit reagent (Asan Pharm. Co., Seoul, Korea) and expressed as a Karmen unit.

Determination of glutathione, lipid peroxide and protein content

The hepatic glutathione content and lipid peroxide content were determined by the method of Ellman (13) and Satho (14), respectively. The protein content was determined by the Lowry (15) method with bovine serum albumin as a standard.

Statistical analysis

All experiments were carried out with 10 replicates and the means ± standard errors are reported. The means of the main effects were separated by Duncan's multiple range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Weight gain, feed intake, feed efficiency ratio and water intake

The results of body weight, weight gain, feed intake, feed efficiency ratio, water intake, excreted amounts of feces and urine are shown in Table 3. There was no significantly different body weight (mean 221 g) among the experimental groups. Weight gains of the CCl_4-DW and CCl_4-HD groups compared with the NC group were 42.7% and 47.8%, but those of the DW-CCl_4 and HD-CCl_4 groups were 53.9% and 48.5%, respectively.

The feed intake in all the experimental groups were

Table 3. Weight gain, feed intakes, FER, water intakes, amounts of feces and urine on rats supplemented with diluted herb distillate for 2 weeks after or before carbon tetrachloride administration

Measurements	NC[1)]	CCl_4-DW[2)]	DW-CCl_4[3)]	CCl_4-HD[4)]	HD-CCl_4[5)]
Initial body weight (g)	207.9±6.23[b7)]	224.3±8.21[a]	219.0±8.45[a]	226.5±5.37[a]	229.4±4.63[a]
Final body weight (g)	266.5±8.50[a]	249.3±7.00[b]	250.6±4.90[b]	254.5±5.00[ab]	257.8±6.20[ab]
Weight gain (g/week)	29.3±3.80[a]	12.5±8.90[b]	15.8±6.60[b]	14.0±4.10[b]	14.2±4.50[b]
Feed intakes (g/week)	195.3±8.00[b]	210.6±4.50[a]	210.0±4.60[a]	203.8±6.00[ab]	203.1±9.70[ab]
FER[6)]	0.15±0.02[a]	0.06±0.02[b]	0.08±0.03[b]	0.07±0.02[b]	0.04±0.01[b]
Water intakes (mL/week)	215.3±12.3[b]	253.5±14.4[a]	229.8±27.0[ab]	259.5±15.9[a]	255.8±14.9[a]
Feces amounts (g/day)	2.4±0.03[a]	2.1±0.05[b]	1.9±0.04[c]	2.1±0.04[b]	1.9±0.06[c]
Urine amounts (mL/day)	8.7±0.14[a]	8.0±0.12[c]	7.8±0.10[d]	8.3±0.13[b]	8.0±0.08[c]

[1~5)]See Fig. 1. [6)]FER (feed efficiency ratio): weight gain/feed intakes.
[7)]Values are means±SE of 10 rats, different superscripts within a row (a~c) indicate significant difference ($p<0.05$).

significantly higher than that of the NC group, but the feed efficiency ratios were significantly lower than that of the NC group, showing no significant difference between each experimental group. Water intake in all the groups were similar, but the amount of feces in the DW-CCl_4 and HD-CCl_4 groups were significantly lower compared with the NC, CCl_4-DW and CCl_4-HD groups. The amount of urine in all the other groups were significantly lower than that of the NC group.

Organ weights

Table 4 shows the weight of organs and their relative % of total body weight. The liver and kidney weight per body weight in all the experimental groups increased to a greater extent compared with the NC group. Also, the CCl_4-HD and HD-CCl_4 groups were lower than those of the CCl_4-DW and DW-CCl_4 groups. While the weight of the spleen, heart and testicles to the body weight of the CCl_4-DW and DW-CCl_4 groups were similar to that of the NC group, the CCl_4-HD and HD-CCl_4 groups showed a lower weight of organs than the NC and CCl_4-DW groups.

The kidney weights to body weight (%) in the CCl_4-HD and HD-CCl_4 groups were lower than those of the CCl_4-DW and DW-CCl_4 groups. Unlike the kidney weights, the weight of the spleen, heart and testicles to body weight (%) of the CCl_4-DW and DW-CCl_4 groups were similar to that of the NC group, and the weight (%) of the CCl_4-HD and HD-CCl_4 groups were lower than those of the NC and CCl_4-DW groups.

The liver weight to body weight (%) in the CCl_4-HD and HD-CCl_4 groups was lower than those of the CCl_4-DW and DW-CCl_4 groups, which is likely to suggest that the supplemented HD caused a better-damaged liver by CCl_4. Also, the finding that the liver weight and serum ALT activity in the HD-supplemented groups was lower compared to the distilled water-supplemented groups means that HD had a protective effect on liver damage.

Serum lipid content

The levels of triglyceride (TG), total cholesterol, HDL-cholesterol and LDL-cholesterol in serum are shown in Table 5. The level of serum triglyceride (TG) in all the experimental groups was lower compared with the NC group (Table 5). The HD-supplemented groups were similar in total cholesterol level to the NC group, but lower than the supplemented distilled water groups. On the other hand, HDL-cholesterol content in all the experimental groups was significantly decreased compared to that of the NC group. The decreasing degree was the lowest in the CCl_4-HD group and highest in

Table 4. Weight of organs and serum alt activity on rats supplemented with diluted herb distillate for 2 weeks before or after carbon tetrachloride administration

Internal organs	NC[1)]	CCl_4-DW[2)]	DW-CCl_4[3)]	CCl_4-HD[4)]	HD-CCl_4[5)]
Body wt (g)	266.5±8.50[a7)]	249.3±7.00[b]	250.6±4.90[ab]	254.5±5.00[ab]	258.0±7.20[ab]
Liver wt (%)	3.51±0.18[a]	3.77±0.17[a]	3.76±0.28[a]	3.66±0.20[a]	3.64±0.21[a]
Kidney wt (%)	1.21±0.10[a]	1.32±0.12[a]	1.35±0.10[a]	1.29±0.08[a]	1.26±0.08[a]
Spleen wt (%)	0.34±0.04[a]	0.35±0.06[a]	0.34±0.05[a]	0.29±0.08[a]	0.28±0.06[a]
Heart wt (%)	0.64±0.05[a]	0.62±0.02[a]	0.60±0.04[a]	0.59±0.04[a]	0.54±0.06[a]
Testicle wt (%)	1.31±0.14[a]	1.25±0.12[a]	1.28±0.08[a]	1.22±0.10[a]	1.24±0.12[a]
Serum ALT[6)]	20.2±2.6[d]	65.5±9.6[b]	86.7±10.2[a]	38.7±6.5[c]	69.8±10.5[ab]

[1~5)]See Fig. 1. [6)]Karmen unit/mL of serum.
[7)]Values are means±SE of 10 rats, different superscripts within a row (a~b) indicate significant difference ($p<0.05$).

Table 5. Lipid profiles and atherogenic index in serum on rats supplemented with diluted herb distillate for 2 weeks after or before carbone tetrachloride administration (mg/dL)

Groups[1)]	Triglyceride	Total cholesterol	HDL-cholesterol	LDL-cholesterol[2)]	Atherogenic index[3)]
NC	105.4±14.4[a4)]	104.9±9.5[b]	62.3±2.3[a]	21.5±8.2[c]	0.68±0.10[e]
CCl_4-DW	86.3±19.2[ab]	114.1±12.1[ab]	30.5±2.9[d]	66.3±9.2[ab]	2.74±0.08[a]
DW-CCl_4	79.6±14.0[ab]	127.6±11.1[a]	44.0±2.5[c]	67.7±10.4[a]	1.90±0.14[b]
CCl_4-HD	80.7±18.6[ab]	104.8±9.5[b]	49.8±1.5[b]	38.9±6.0[b]	1.10±0.10[d]
HD-CCl_4	71.8±13.8[b]	105.2±14.7[ab]	42.3±2.8[c]	48.5±10.2[ab]	1.49±0.12[c]

[1)]See Fig. 1.
[2)]LDL-cholesterol = Total cholesterol − HDL-cholesterol − (TG/5).
[3)]Atherogenic index = (Total cholesterol − HDL-cholesterol) / HDL-cholesterol.
[4)]Values are means±SE of 10 rats, different superscripts within a row (a~e) indicate significant difference ($p<0.05$).

the CCl_4-DW group. Also, LDL-cholesterol levels in all the experimental groups were significantly increased compared with that of the NC group. But the CCl_4-HD group was lower than the CCl_4-DW group. The atherogenic index, showing the same tendency to the LDL-cholesterol, was lowest in the CCl_4-HD group.

Serum TG is transported by very low density lipoprotein (VLDL), derived from the liver, to the extrahepatic tissues (16,17). It is well-known that the level of VLDL in serum is a parameter on the release of TG from the liver (18-20). In abnormal conditions such as liver damage, TG can not be released from the liver and accumulate in the liver due to the inhibition of VLDL apolipoprotein synthesis (18,20-22). Under these conditions, levels of serum TG were decreased and hepatic TG was increased (23,24). On the basis of the above results, in concert with the data in the present paper, we assume that the cause of the decreased serum TG level in the CCl_4-treated rats may be related to the inhibition of VLDL apolipoprotein synthesis in the hepatic tissue, and that HD may not restore the decreased serum TG level in the CCl_4-treated rats.

It is widely accepted that the liver mainly scavenged serum LDL derived from VLDL (25) and that the level of serum LDL is increased in hepatic-damaged tissue (20,24,26,27). In addition, activities of hepatic LCAT and hepatic HDL apolipoprotein synthesis are inhibited in the damaged liver (22,24,26,27). In general, herb distillate contains biologically active small molecules such as sesquiterpenoids, triterpennoids and acetylene derivatives (28). It has been shown that decursin in danggui modulates drug-metabolizing enzyme activities (29,30), and salvianic acid in dansam exhibits a protective effect on carbon tetrachloride-induced liver damage in rats (31). Therefore, those active components, which are presumably present in the herb distillate used in this study, may contribute to preventing CCL_4-induced liver damage. These results suggested that the herb distillate might be able to regulate levels of serum LDL- and HDL-cholesterol under conditions where liver damage occurs.

Contents of glutathione and lipid peroxide, and enzyme activities

The levels of hepatic glutathione and lipid peroxide, and enzyme activities are shown in Table 6. The content of glutathione was unchanged in all the experimental groups by CCl_4 administration, but the content of hepatic LPO was higher compared with the NC group and the content of LPO by supplementing HD decreased more greatly compared with the distilled water-supplemented groups. Total XOD activity was significantly changed in only the CCl_4-HD group. However, the activity of XOD type O was higher in all the experimental groups than the NC group. The HD-supplemented groups was lower than the distilled water-supplemented groups. On the other hand, the GST activity of CCl_4-DW, DW-CCl_4,

Table 6. Contents of hepatic glutathione (GSH) and lipid peroxide (LPO), and activities of xanthine oxidase (XOD) and glutathione s-transferase (GST) on rats supplemented with diluted herb distillate for 2 weeks after or before carbon tetrachloride administration

Groups[1)]	GSH (μmol/g)	LPO (MDA nmol/g)	XOD (uric acid nmol/mg protein/min)			GST (CDG[2)] nmol/mg protein/min)
			Total	O-type	O/T (%)	
NC	4.80±0.26[a3)]	14.01±0.34[c]	4.14±0.08[d]	1.03±0.02[d]	24.88±2.81[a]	370.8±32.3[a]
CCl_4-DW	5.09±0.24[a]	22.15±3.26[b]	4.31±0.04[c]	1.24±0.02[b]	28.77±1.60[a]	298.5±41.0[ab]
DW-CCl_4	5.28±0.23[a]	35.66±4.91[a]	5.15±0.07[a]	1.35±0.03[a]	26.21±2.89[a]	247.8±36.6[b]
CCl_4-HD	5.27±0.22[a]	17.41±2.26[bc]	3.86±0.05[c]	1.08±0.02[c]	27.98±2.29[a]	309.6±45.5[ab]
HD-CCl_4	5.10±0.23[a]	27.17±4.06[ab]	4.45±0.08[b]	1.20±0.02[b]	26.97±4.21[a]	278.2±29.0[b]

[1)]See Fig. 1. [2)]CDG: conjugated dinitrophenol glutathione.
[3)]Values are means±SE of 10 rats, different superscripts within a column (a~d) indicate significant difference ($p<0.05$).

CCl_4-HD, and HD-CCl_4 compared with the NC group were decreased by 19.5, 33.2, 16.5, and 25%, respectively.

XOD is classified into two forms: dehydrogenase (type D) using NAD^+ as an electron acceptor, and oxidase (type-O) using O_2 as an electron acceptor (11). The XOD in rat liver tissue is mainly an NAD^+-dependent dehydrogenase under normal conditions (11,32). Under pathological conditions, XOD type-D can be converted to XOD type-O either reversibly by sulfhydryl oxidation (33) or irreversibly by proteolytic cleavage (34). The XOD type-O uses molecular oxygen as an electron acceptor and consequently generates the superoxide anion (35), which participates in the generation of other reactive oxygen species (ROS), including hydrogen peroxide, hydroxyl radical and singlet oxygen. Excessive formation of ROS in the body results in diseases such as cancer, metabolic diseases, and inflammation. However, the ROS scavenging system, such as superoxide dismutase, catalase, glutathione peroxidase, GST and reduced glutathione (36-41), protects cells from the destructive effect of ROS. Nevertheless, an imbalance between ROS generating and scavenging systems induces an interaction between ROS and important cell components such as DNA, RNA, protein, and lipids, resulting in tissue damage (42-44).

In this study, the increase of lipid peroxide as a parameter indicating cell membrane damage (45) by CCl_4 in all the experimental groups resulted from excessively generated oxygen free radical by the increased XOD type-O. On the other hand, the decrease of lipid peroxide in the HD groups probably resulted from the inhibition of the D- to O-type conversion by antioxidative components of the herb distillate (28,31), as well as the prevention or inhibition of liver damage by scavenging oxygen free radicals. However, further detailed studies on the components in herb distillate are needed to confirm these findings.

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