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Effects of *Chrysanthemum boreale* M. Water Extract on Serum Liver Enzyme Activities and Kupffer Cells of Carbon Tetrachloride-Induced Rats

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Abstract Effects of water extract obtained from *Chrysanthemum boreale* M. (CE) on serum enzyme activities and Kupffer cells of carbon tetrachloride (CCl_4)-induced rats were investigated. Thirty-two healthy male Sprague-Dawley rats were divided into normal (N), CCl_4-induced (T), CE-supplemented (C), and CCl_4-induced and CE-supplemented (TC) groups. CCl_4 injection significantly increased aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase activities in serum. Significant increases in total cholesterol and triglyceride concentrations were also observed in CCl_4-induced rats. Oral administration of CE at 300 mg/kg body weight significantly decreased serum enzyme levels and suppressed CCl_4 hepatotoxicity-induced lipid profile changes. Histological findings showed fatty change, fibrosis and increased number of Kupffer cells in T group. Electron microscopic examination showed increased lysosome content and dilation of rough endoplasmic reticulum within Kupffer cells in T group, whereas CE supplement attenuated liver injury in CCl_4-induced liver. These results indicated CE could significantly alleviate CCl_4-induced hepatotoxicity injury.

Key words: *Chrysanthemum boreale*, carbon tetrachloride, Kupffer cell, hepatotoxicity, histological examination

Introduction

Living organisms are continuously exposed to oxidative damages caused by environmental factors such as ozone, nitrogen oxide, and xenobiotics, as well as by oxygen radicals produced endogenously via normal mechanisms (1). The underlying mechanisms in the toxicity of various xenobiotics such as carbon tetrachloride (CCl_4), acetaminophen, adriamycin, and alcohol involve free radical-mediated oxidation of biomolecules (2).

CCl_4 is a classical hepatotoxicant that causes rapid liver damage, progressing from steatosis to necrosis. Long-term administration of CCl_4, which causes chronic liver injury, is a widely accepted model for the production of hepatic fibrosis (3). The initial injury is mediated through the reductive metabolism by hepatic microsomal-mixed function oxidase to generate a hepatotoxic metabolite, trichloromethyl free radical (1). In the presence of oxygen, the radical rapidly interacts with hepatic lipids and cell proteins, and causes lipid peroxidation (2, 4). The resulting hepatic injury is characterized by the leakage of cellular enzymes into the bloodstream, triacylglycerol accumulation, liver fibrosis, and cell necrosis (2, 5). During liver fibrosis, the synthesis and deposition of extracellular matrix proteins are increased, and this accumulation of connective tissue inhibits normal functions of the liver (6). It is hypothesized that the fibrotic process involves several hepatocellular factors including Kupffer cells and various chemical mediators that are produced by these cells (7).

Kupffer cells act as a critical mediator of the inflammatory response during CCl_4-mediated liver damage, and provide new insight into the temporal molecular and biochemical changes (8). Kupffer cells, resident macrophages of liver, normally protect the hepatocyte against invading microorganisms and foreign molecules (9). It is possible that modest damage to parenchymal cells, due to the metabolism of CCl_4, participates in the activation of Kupffer cells (9, 10). The activated Kupffer cells release toxic secretory molecules such as prostaglandins, reactive oxygen species, and cytokines that may contribute to further damage of hepatocytes (9, 10). Consequently, the role of these cells has been confirmed by attenuating hepatotoxicity during steatosis, inflammation, and necrosis of liver selectively depleted of Kupffer cells (7).

A large section of the world's population relies on traditional remedies to treat a plethora of diseases, with medicinal herbs being indispensable due to low cost, easy access, and ancestral experience (11). Globally, a growing interest has emerged in rediscovering medicinal herbs as useful therapeutic agents for the prevention of hepatotoxicity (11, 12).

Chrysanthemum boreale Makino (CB) is a perennial plant widely distributed in wild fields and mountains of East Asia, including China, India, and Korea(13, 14). Along with other related species such as *C. indicum*, it is known as an important medicinal herb for the treatment of inflammation, and for analgestic purposes in Chinese traditional preparations (13, 14). The main chemical components of CB are camphor (15), flavonoid (16), and sesquiterpene (17).

Jang *et al.* (18) isolated analogues of cumambrin B from the flower of CB. Lee *et al.* (19) isolated a new guaianolide from CB and demonstrated its inhibition of etoposide-induced apoptosis in U937 cells. Kim *et al.* (15) reported the chemical composition of the essential oil of CB and its antibacterial activity. Han (13) identified two compounds, apigenin and linarin, from CB methanol extracts and reported that their free radical-scavenging

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activities were similar to that of α-tocopherol. Nam *et al.* (17) isolated sesquiterpene lactones from CB and reported their cytotoxic effect against Sarcoma 180 implanted in ICR mice. Recent studies demonstrated that CB could increase the anti-tumor and anti-cancer effects in experimental animals (17, 19).

In the present study, the oxidative damage induced by CCl_4 in rats was tested using the CB extract. The protective effect of the extract was also evaluated by histopathological analysis of liver and biochemical assays of hepatospecific enzymes of the rats.

Materials and Methods

The preparation of the water extract of CB CB was purchased from Yak Ryung Market, Daegu, Korea. The air-dried powdered flowers of CB were extracted with tenfold water under reflux and concentrated. The extract was kept at 4°C until use.

Animal and treatment Prior to use in experiments, 32 Sprague-Dawley male rats, each weighing 150±10 g, were subjected to a 1-week adaptation period. They were then divided into four groups of normal (N), CCl_4-treated (T), CB water extract-treated (C), CCl_4-injected CB water extract-treated (TC) groups. All animals were fed standard rat chow (Sam Yang Food Co., Wonju, Korea) and water ad libitum. They were kept in a temperature-controlled environment (20~22°C) with alternating cycle of 12 hr light and dark. The care of the animals was consistent with the National Institute of Health guidelines on the care and use of laboratory animals. Normal animals were given only the vehicle. Liver damage was induced experimentally by injecting CCl_4 intraperitoneally at 1 mg/kg in olive oil (1:1, v/v) once daily for 1 week. CE was fed at 300 mg/kg body weight. TC group was supplemented intragastrically with CE for 4 weeks after the administration of CCl_4, and the T group was given normal saline (10 ml/kg) during the same period.

Biological assays All rats were subjected to 1 day fasting before being sacrificed. They were lightly anesthetized with ethyl ether. Blood was collected from the abdominal aorta of each rat and was allowed to coagulate at room temperature for 1 hr. The blood was centrifuged at 4°C for 10 min to separate the serum and analyzed by Fully Automated Dry Chemistry System (Spoththerm: Daichi Kagaku Co., Japan).

Hepatocellular damage in the serum was estimated by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activities. The activities of AST and ALT were expressed in Karmen units, and those of LDH and ALP in Wrobleski units. Fresh hepatic tissues were carefully excised and homogenized in cold 0.05 M NaCl and adjusted into 10% minced tissue solution (w/v). The liver lipid was extracted by the Folch's method (20), and subjected to the same method used for the serum lipid analysis.

Histological examinations

Hematoxylin and eosin stain

After the blood was drained, the hepatic tissue was collected immediately from the same lobe of the liver and fixed in 10% neutral formalin solution for 24 hr. Subsequently, the hepatic tissue was dehydrated with a series of 75 to 100% ethanol solutions before being embedded in the paraffin wax. Cross sections (4 μm thickness) were cut and stained with hematoxylin and eosin, and examined by light microscope (Olympus Co., Tokyo, Japan).

Periodic acid-Schiff (PAS) stain

Sections of 4 μm-liver were immersed in a solution of 0.1 % periodic acid (Sigma, St. Louis, MO, USA) for 15 min at 56°C. The slides were washed in running tap-water and immersed in Schiff's reagent for 40 min. Subsequently, the sections were washed in running tap-water for 10 min, counter stained with hematoxylin, dehydrated in graded ethanol, cleared in xylene, and mounted in resinous medium. They were then observed by light microscope.

Immunohistochemical stain

Immunohistochemical stain was performed according to the method of Luckey and Peterson (6). Prior to immunohistochemical staining, each section was deparaffinized and rehydrated in a series of solvents (xylene, absolute ethanol, 95% ethanol, 70% ethanol, and water). Endogenous peroxidase activity was blocked by exposure to 0.1% phenylhydrazine HCl in phosphate buffered saline (PBS) for 20 min. Nonspecific antibody interactions were blocked with 7.5% bovine serum albumin (BSA) in PBS. Kupffer cells were detected with anti-CD68 antibodies (Dako Co., Glostrup, Denmark) incubated (1:400) overnight at 4°C in 7.5% BSA in PBS. Immunopositive interactions were indirectly performed with goat anti-rabbit IgG, conjugated with biotin (1:200 in PBS with 7.5% BSA, Sigma). Visualization of these interactions was performed with diaminobenzidine (Sigma). In control experiment secondary antisera were substituted for epitope-specific antisera.

Transmission electron microscopy (TEM)

A liver specimen (1 mm in diameter) was prefixed in paraformaldehyde and washed with 0.1M phosphate buffer. The specimen was postfixed in 1% osmium tetroxide in colloidine buffer (pH 7.4) for 1 hr, and dehydrated in a graded series of ethanol, followed by propylene oxide. Sections stained with uranyl acetate and lead citrate were examined using an electron microscope (H-7000, Hitachi Ltd, Tokyo, Japan).

Statistical analysis All results were expressed as mean ± standard deviation (SD). Duncan's multiple test of one-way ANOVA with SPSS program was used to compare the means among specific groups, with $p<0.05$ considered significant.

Results and Discussion

Body weight gain and the ratio of liver weight to body weight Body weight gain and the ratio of liver to body weight of rats fed for 5 weeks are shown in Table 1. Rats induced seven times with CCl_4 during 1 week period showed lower body weight gain (3.34 ± 0.44 g/day)

Table 1. Effect of *Chrysanthemum boreale* M. water extract on body weight gain and the ratio of liver weight to body weight of CCl_4-induced rats

Group[1)]	Body wt gain (g/day)	Liver wt/body wt ratio (%)
N	5.43 ± 0.12[a2)]	2.63 ± 0.19[a]
T	3.34 ± 0.44[b]	3.74 ± 0.35[c]
C	5.29 ± 0.26[a]	3.19 ± 0.17[a]
TC	4.67 ± 0.88[c]	2.99 ± 0.58[b]

[1)]N: Normal, T: CCl_4, C: CE, TC: CCl_4+CE
[2)]Means±S.D.(n=8).
Means followed by the same letter in the column are not significantly different ($p<0.05$).

compare to the normal group (5.43 ± 0.12 g/day). The treatment of CE resulted in a normal increase in the body weights, whereas that of the CCl_4-induced group increased 4.67 ± 0.88 g/day. As expected, exposure to CCl_4 resulted in a significant increase in the liver weight. CE treatment (300 mg/kg BW) significantly lowered the ratio of liver to body weight. Liver is a target organ for CCl_4 toxicity, and CCl_4 exposure increased liver weight in a weight-dose dependent manner (2, 3). Rajesh and Latha (12) investigated the anti-hepatotoxicity activity of polyherbal formulation in CCl_4-induced rats, and reported that the polyherbal formulation significantly lowered the liver enzyme activities, which had been increased by CCl_4 injection, and restored the body weight to normal level.

Serum enzyme activities The effects of CE supplementation on the CCl_4-induced elevation of serum AST, ALT, LDH, and ALP activities are shown in Table 2. CCl_4 (0.5 mL/kg, seven doses) caused hepatotoxicity in SD rats, as indicated by the increases in the serum AST, ALT, LDH, and ALP activities, as previously reported (Table 2) (21). Compared to the normal group, the Karmen units of AST and ALT in CCl_4-induced rats significantly increased to 325.63 ± 19.07 and 89.13 ± 10.55, respectively. However, the activities decreased with oral administration of CE at 300 mg/kg of body possibly due to the stabilization of the plasma membrane as well as the repairing of hepatic tissue damage caused by the administration of CCl_4, as supported by the finding that serum level of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (22).

Hepatic cells participate in a variety of metablic activities and contain a host of enzymes. AST and ALT are both produced by the liver and are required to metabolite amino acids. They are found at high concentrations in the cytoplasm, and AST, in particular, also exists in the mitochondria (23). Upon liver injury, transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane (24), which thereby increases the enzyme level in the serum. If injury involves organelles such as mitochondria, soluble enzymes such as AST, normally located in the organelle, will also be released. The elevated activities of AST and ALT in serum are indicatives of the cellular leakage and loss of the functional integrity of cell membranes in the liver (25).

Yadav and Dixit (26), upon induction of CCl_4 hepatotoxicity *in vivo* and *in vitro* to detect hepatoprotective activity of Kalanchoe *pinnata* Pers leaves, found that incubation of rat hepatocytes with 10 uL CCl_4 resulted in an induction of hapatotoxicity due to the increase in AST and ALT levels both *in vitro* and *in vivo*. They reported that the AST and ALT levels in culture hepatocyes and serum decreased significantly after treatment of the concentrate, an indication of the hepatoprotective activity of *K. pinnata* Pers. These results thus justify the use of *K. pinnata* Pers. in folk medicine for the treatment of liver dysfuction. The levels of LDH and ALP in serum showed the same tendency with those of AST and ALT. The elevation of the serum levels of LDH and ALP following the CCl_4 injection in SD rats was decreased by the administration of CE (Table 2). Ahmed *et al.* (27) investigated the hepatoprotective effect of 50% ethanol extract of *Lawsonia alba* Syn. Bark against CCl_4-induced oxidative stress. They demonstrated that the plant extract significantly ($p<0.001$) lowered serum LDH levels idose-dependently against the significant ($p<0.001$) rise of the enzyme evoked, when challenged with CCl_4.

Lipids changes in serum and liver tissue Tables 3 and 4 depict the concentrations of total cholesterol, LDL and HDL cholesterols, and triglyceride (TG) in serum and those of total cholesterol and TG in the liver. A remarkable increase in the concentrations of total and LDL cholesterols, and triglyceride was noticed in the serum of group T rats, which received CCl_4 alone. Significant increases were also observed in total cholesterol and triglyceride concentrations of CCl_4-induced livers. However, CE supplement after CCl_4 injection restored the contents of total cholesterol and TG to the levels of normal group. Previous reports indicate total cholesterol and TG contents increase in CCl_4-induced fatty livers (28). It is well known that CCl_4 administration induces the increased synthesis of fatty acids, while decreases the release of hepatic lipoproteins (29).

According to Recknagel and Lombardi (30), the accumulation of TG in livers of CCl_4-treated rats is not

Table 2. Effect of *Chrysanthemum boreale* M. water extract on the serum enzyme activities of CCl_4-induced rats

Group[1)]	AST	ALT	LDH	ALP
	(Karmen unit/L of serum)		(Wrobleski unit/L of serum)	
N	126.50 ± 10.68[a2)]	30.25 ± 4.40[a]	107.25 ± 36.78[b]	103.88 ± 10.49[a]
T	325.63 ± 19.07[c]	89.13 ± 10.55[c]	171.13 ± 33.22[b]	127.88 ± 7.26[c]
C	140.63 ± 9.05[a]	36.75 ± 3.15[ab]	115.75 ± 8.99[ac]	114.13 ± 3.98[b]
TC	203.13 ± 14.19[b]	42.87 ± 6.45[b]	137.38 ± 34.97[c]	117.88 ± 2.23[b]

[1)]N: Normal, T: CCl_4, C: CE, TC: CCl_4+CE
[2)]Means±S.D. (n=8)
Means followed by the same letter in the column are not significantly different ($p<0.05$).

Table 3. Effect of *Chrysanthemum boreale* M. water extracts on serum lipid profiles of CCl_4-induced rats (mg/dl of serum)

Group[1)]	Triglyceride	Total cholesterol(A)	HDL-cholesterol(B)	LDL-cholesterol	(B)/(A)×100(%)	A.I.[3)]
N	108.00 ± 8.50[b2)]	98.13 ± 9.17[ab]	15.28 ± 1.09[ab]	61.25 ± 8.76[a]	15.70[b]	5.46[b]
T	121.25 ± 6.88[c]	113.63 ± 6.93[c]	14.34 ± 0.88[a]	75.04 ± 6.36[b]	12.64[a]	6.94[c]
C	91.50 ± 4.34[a]	92.13 ± 4.55[a]	17.43 ± 1.06[c]	56.65 ± 3.78[a]	18.93[c]	4.31[a]
TC	112.00 ± 5.07[b]	101.00 ± 4.54[b]	16.25 ± 0.87[b]	62.35 ± 3.81[a]	16.10[b]	5.23[b]

[1)]N: Normal, T: CCl_4, C: CE, TC: CCl_4+CE
[2)]Means±S.D.(n=8).
Means followed by the same letter in the column are not significantly different($p<0.05$).
[3)]Atherosclerotic index = (Total cholesterol - HDL-cholesterol) / HDL-cholesterol
LDL-cholesterol = Total cholesterol - (HDL-cholesterol + Triglyceride/5)

Table 4. Effect of *Chrysanthemum boreale* M. water extract on total cholesterol and triglyceride of liver of CCl_4-induced rats (mg/g of tissue)

Group[1)]	Total cholesterol	Triglyceride
N	10.68 ± 0.74[b2)]	8.66 ± 0.53[a]
T	14.03 ± 0.57[c]	11.13 ± 0.52[b]
C	9.43 ± 0.53[a]	8.27 ± 0.57[a]
TC	11.02 ± 0.82[b]	10.66 ± 0.48[b]

[1)]N: Normal, T: CCl_4, C: CE, TC: CCl_4+CE
[2)]Means±S.D.(n=8).
Means followed by the same letter in the column are not significantly different($p<0.05$).

due to the interference with the formation of TG by the liver, but due to the inhibition or destruction of TG-secreting mechanisms. Ohta *et al.* (31) reported that the increased serum TG level in CCl_4-induced rats decreased with post administration of melatonin. In addition, lipid accumulation in the liver of rats as a result of hepatotoxicant administration was prevented by the supplementation of antioxidants such as vitamin E (32) or by herbal products (12, 33). The observed restoration of the CCl_4-evoked changes in the lipid profiles of serum and tissues show the protective nature of CE.

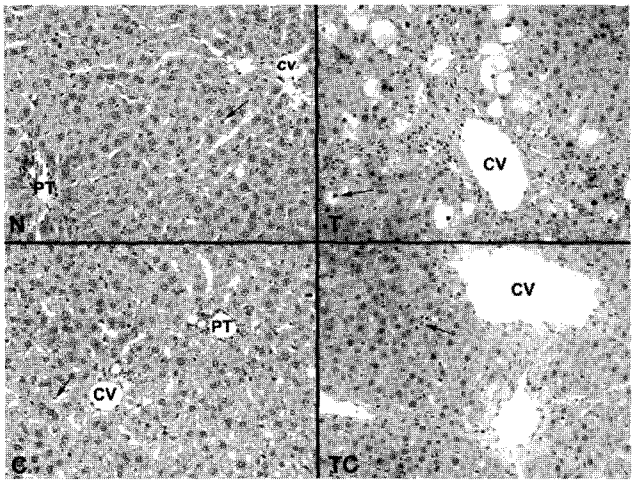

Fig. 1. Histological examination for observation of hepatocyte (hematoxylin and eosin stain, x100). N: Normal, T: N+CCl_4, C: N+CE, TC: CCl_4+CE, N: Normal hepatic lobular architecture is present. Portal tract (PV) and central vein (CV) are found. Kupffer cells (Kc, arrow) are noted in sinusoids. T: Moderate, macrovesicular fatty change is present. Inflammatory cells, mainly lymphocytes are present. Centrilobular fibrosis around the CV is present. Kc have increased and hypertrophied. TC: Fatty change is decreased. Mild fibrosis around CV is present. Kc have decreased more than in CCl_4 group. C: hepatic lobular architecture is normal.

Histopathologic examination Table 5 shows the results of histological examination on the hepatoprotective effect of CE after CCl_4 injection. Light microscopic examination by hematoxylin-eosin staining of hepatocytes revealed the normal group had a normal hepatic lobular architecture, portal tract (PT), central vein (CV), and Kupffer cells in sinusoid (Fig. 1). Histological findings showed CCl_4 injection induced hepatocyte necrosis with mild ballooning degeneration and inflammation around the central veins, and steatosis with the formulation of lipidic intracytoplasmic vacuoles (Fig. 1 and Table 5). Moderate fatty changes, fibrosis, and hyperthrophy of Kupffer cells were observed in the CCl_4 -induced group (Table 5). The hepatic cells of rats supplemented with CE after CCl_4 injection were normally arranged. Fibrosis, present around the central vein, was similar to that of the normal group except for the presence of mild ballooning degeneration and spotty necrosis (Fig. 1). The fatty changes were also reduced, and the number of Kupffer cells was decreased by the CE treatment. Figure 2 shows the glycogen contents as determined by PAS staining. In the normal group, PAS

Table 5. Effect of *Chrysanthemum boreale* M. water extract on histopathologic findings of liver tissue in CCl_4-induced rats

Group[1)]	Ballooning degeneration	Acidophilic degeneration	Inflammatory cell	Spotty necrosis	Zonal necrosis	Fatty change	Fibrosis	Cirrhosis
N	-	-	-	-	-	-	-	-
T	+	+	+	+	+	++	++	-
C	-	-	-	-	-	-	-	-
TC	+	-	-	+	-	-	-	-

[1)]N: Normal, T: N+CCl_4, C: N+CE, TC: CCl_4+CE
-: none, +: mild, ++: moderate

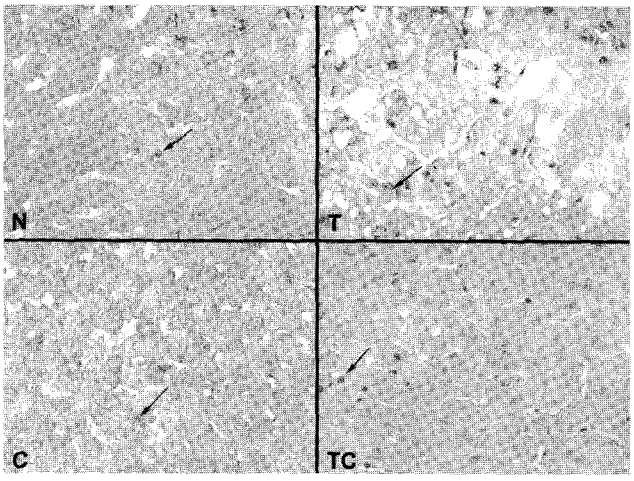

Fig. 2. Histological examination for observation of content of glycogen within the hepatocytes (Periodic acid Schiff stain, x200). N: Normal, T: N+CCl_4, C: N+CE, TC: CCl_4+CE, N: The content of glycogen within the hepatocytes is stained red by the PAS stain and Kc (arrow) are noted. T: The content of glycogen within the hepatocytes was decreased, and Kc are increased and hypertrophied. TC: The content of glycogen within the hepatocytes is increased more than that of the CCl_4 group. C: The glycogen within the hepatocytes is stained red.

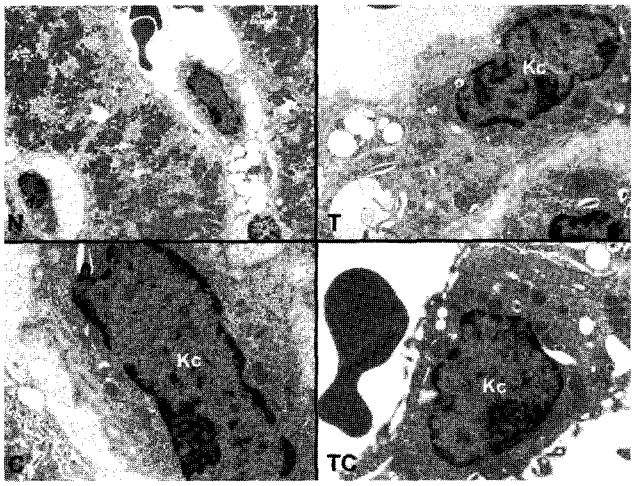

Fig. 4. Electron microscopic examination of Kupffer cells. N: Normal, T: N+CCl_4, C: N+CE, TC: CCl_4+CE, N: Kc are present in sinusoids. Cytoplasmic processes are present (TEM, x 1,500). T: Kc shows increased lysosomes and dilated rough endoplasmic reticulums (TEM, x 3,500). TC: Microorganells within Kc have decreased rather than those of the CCl_4 group (TEM, x 6,000). C: Mitochondria and rough endoplasic reticulums are present within the cytoplasm of Kc (TEM, x6,000).

stain stained the glycogen within the hepatocytes red, and the Kupffer cells were well noted in sinusoids. However, administration of CCl_4 only decreased the content of hepatic glycogen observed as pale red in Fig. 2 (T). In the CE-supplemented group, the content of glycogen within the hepatocytes was similar to that of the normal group. Immunohistochemical staining for CD68 revealed number of Kupffer cells increased as shown in T group (Fig. 3), whereas a decrease in the number of Kupffer cells within sinusoids was observed in the CE treated group.

Furthermore, electron microscopic examination for observation of Kupffer cells (Fig. 4, T) revealed CCl_4 injection increased the numbers and sizes of primary and secondary lysosomes in Kupffer cells. CCl_4 injections also increased the amount of euchromatin of Kupffer cells and dilation of the rough endoplasmic reticulum. However, CE supplement reduced the number of microorganelles within the Kupffer cells and the morphology of Kupffer cells became similar to that of the normal group (Fig. 4, TC). Mitochondria and rough endoplasic reticulums were present in the cytoplasm of Kupffer cells supplemented with CE.

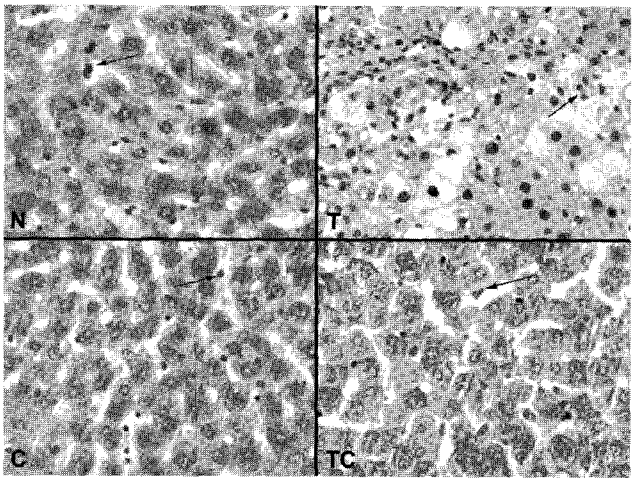

Fig. 3. Immunohistochemical stain for CD68 (x200). N: Normal, T: N+CCl_4, C: N+CE, TC: CCl_4+CE, N: Kc (arrow) shows that immunoreactivity for CD68 and Kc within sinusoids are present. T: Kc are increased and hypertrophied. TC: Kc within sinusoids have decreased rather than those of the CCl_4 group. C: Kc within sinusoids are present.

CCl_4 is one of the most widely used hepatic toxins for the experimental induction of hepatic fibrosis and cirrhosis in laboratory animals (2, 3). The CCl_4-induced fibrosis in experimental animals resembled the human fibrosis in some aspects of morphology and pathophysiology (2, 3, 6). It is now accepted that administration of CCl_4 results in liver injury characterized by increases in serum liver enzymes (21) and histological manifestations of inflammation and fibrosis (5, 8).

In the present study, elevation of the plasma levels of AST, ALT, LDH, and ALP (Table 2) following CCl_4 administration accurately reflected the hepatic injury(Table 5, Fig. 1). The histological findings showed moderate fatty changes and fibrosis, decrease of glycogen content, and an increase of Kupffer cells in CCl_4-induced groups. In this study, CCl_4 injections caused the activation of Kupffer cells, which was demonstrated by the increased number of Kupffer cells and size of lysosomes (Fig. 4, T). These changes were thought to be related to oxygen free radical mediated during the intoxification of a CCl_4-induced liver. The highly reactive free radicals rapidly interacted with hepatic lipids and induced liver damage.

When the CE extract was administered for 4 weeks after CCl_4-induced damage, a considerable reduction was observed in the elevated liver enzyme levels caused by CCl_4 toxicity (Table 2) and in the accumulation of fat globules (Table 5, Fig. 1). Restoration of glycogen content, and selective depletion of Kupffer cells by CE treatment

were also observed. The ameliorating effect may have been due to the inhibition of CCl_4-induced bioactivation or possibly the initial stages of CCl_4-induced cell damage leading to fatty accumulation was prevented, or both (34). Taken together, CE showed a potent protective effect on CCl_4-induced acute toxic liver injury.

Luckey and Petersen (6) induced the activation of Kupffer cells with CCl_4 in rats to elucidate molecular changes occurring in Kupffer cells upon liver injury. They demonstrated that CCl_4 administration increased both the number of resident macrophages and the gene expression of cytokines and tumor necrosis factor (TNF)-α in Kupffer cells. On the other hand, Tsukamoto *et al.* (35) reported that co-administration of α-tocopherol acetate and succinate suppressed the production of TNF-α in the CCl_4-mediated animal model. Luckey and Petersen (6) suggested that Kupffer cells were critical mediators in the inflammatory responses during CCl_4-induced liver injury damage, and that cytokine dysregulation reflected in Kupffer cell activation was associated with specific biochemical and molecular changes. In general, the primary functions of Kupffer cells are phagocytosis, processing of ingested material, antigen presentation, and secretion of biologically active products (8). Dhuley and Naik (36) investigated the protective effect of Rhinax, a herbal formulation, against CCl_4-induced liver injury and survival in rats, and reported that light microscopy of CCl_4-intoxicated livers showed typical changes including numerous pericentral necrosis, single cell necrosis, ballooned hepatocytes, and liver cells with signs of fatty degeneration. Additional administration of 80 mg/kg, p.o., of Rhinax histologically revealed the disappearance of cohesive focal necrosis, few scattered single cell necrosis, but obviously ballooned cells and cells with fatty degeneration. Venkateswaran *et al.* (33) investigated the effect of herbal formulation extracts on erythromycin estolate-induced hepatotoxicity in Wistar rats, and reported that histological examination showed congestion of portal vessels, dilation of blood vessels, infiltration of mixed inflammatory cell, and sinusoidal dilation in erythromycin estolate-induced livers. However, these changes were noticeably reduced in rats treated with the herbal formulation extract (33).

In addition to its antibacterial and cytotoxic effects, CB exerts free radical-scavenging activity. Histological finding of the present study indicated that CE itself was free of side effects on liver. On the basis of serum biochemical assays and histopathological examination, it can be concluded that CE has anti-hepatotoxic activity. Further investigations are necessary to isolate the hepatoprotective components of CE and establish their protective role through both biological and molecular approaches of the CCl_4-mediated injury model.

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