大韓獸醫學會誌(2011) 第51卷 第4號
Korean J Vet Res(2011) 51(4) : 259~265

<원 저>

Protective effect of ethyl acetate extract of *Ishige okamurae* against carbon tetrachloride-induced acute liver injury in rats

Sohi Kang[1,†], Wonjun Yang[1,†], Hanseul Oh[1,†], Yeonji Bae[1,†], Meejung Ahn[2], Min Chul Kang[3], Ryeo Kyeong Ko[3], Gi Ok Kim[3], Jun Hwa Lee[4], Jin Won Hyun[5], Changjong Moon[6], Taekyun Shin[1,*]

[1]*Laboratory of Veterinary Anatomy, College of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 690-756, Korea*

Departments of [2]*Anatomy, and* [5]*Biochemistry, College of Medicine, Jeju National University, Jeju 690-756, Korea*

[3]*Jeju Technopark, Jeju 690-121, Korea*

[4]*Department of Pediatrics, Samsung Changwon Hospital, School of Medicine, Sungkyunkwan University, Changwon 630-723, Korea*

[6]*Department of Veterinary Anatomy, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea*

(Received: August 17, 2011; Revised: September 26, 2011; Accepted: September 27, 2011)

Abstract : Several compounds and extracts isolated from a brown alga, *Ishige* (*I.*) *okamurae*, exhibit anti-oxidant and anti-inflammatory effects. The present study investigated whether the ethyl acetate (EtOAc) fraction of *I. okamurae* (EFIO) could ameliorate carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Sprague-Dawley rats were intraperitoneally (i.p.) administered with EFIO at 10 or 50 mg/kg per day for 2 consecutive days before CCl_4 injection (3.3 mL/kg, i.p.). Twenty four hours later, the rats were anesthesized with diethyl ether and dissected. Pretreatment with EFIO significantly reduced the increased serum levels of alanine aminotransferase and aspartate aminotransferase in CCl_4-treated rats. Pretreatment with EFIO also significantly inhibited the reduced activities of superoxide dismutase and catalase in the CCl_4-injured liver. Histopathological evaluations showed that hemorrhage, hepatocyte necrosis, inflammatory cell infiltration, and fatty degeneration induced by CCl_4 treatment were ameliorated by the administration of EFIO. Additionally, liver immunohistochemical analyses revealed the marked reduction in ED1-positive monocyte-like macrophages in EFIO-pretreated rats given CCl_4. These results suggest that EFIO ameliorates CCl_4-induced liver injury, possibly through the inhibition of oxidative stress.

Keywords : antioxidant, carbon tetrachloride, hepatoprotection, *Ishige okamurae*, serum chemistry

Introduction

Carbon tetrachloride (CCl_4) is a well-known hepatotoxicant that induces liver injury in experimental animals. This model has been widely used for the evaluation of the therapeutic potential of drugs as well as study of the mechanisms of liver injury, since it is similar to human liver disease from the standpoint of morphology to biochemical features of the cellular lesions [15].

Biochemically, when the metabolism of CCl_4 is initiated by NADPH-dependent cytochrome P-450 enzyme, trichloromethyl radicals ($\bullet CCl_3$) that are produced in liver microsomes react with O_2 to form trichloromethyl peroxyl radicals ($Cl_3COO\bullet$), which peroxidate membrane lipids [13]. The cleavage products of the lipid peroxides: malondialdehyde, 4-hydroxy-2-pentenal, 2,4-hexadienal, 4-hydroxy-2-nonenal, are toxic and cause the breakdown of the rough endoplasmic reticulum (rER) structure, decreased activity of rER enzymes and inhibit protein synthesis, which leads to an alteration in the fatty content of the liver that is linked to hepatocellular necrosis [5].

This CCl_4-induced hepatic injury is characterized by two sequential phases: a direct oxidative stress leading to hepatocyte death in the first phase [13], and secondary

*Corresponding author
Tel: +82-64-754-3363, Fax: +82-64-756-3354
E-mail: shint@jejunu.ac.kr
†These authors contributed equally to this work.

damage from activated hepatic macrophages (Kupffer cells) through the release of inflammatory mediators (*i.e.*, tumor necrosis factor alpha) [3, 4]. These are morphologically visualized in the form of the central lobular necrosis of the liver. Because of the direct involvement of oxidative stress in CCl_4-induced hepatic injury, it is conceivable that antioxidants may ameliorate CCl_4-induced liver damage.

Ishige (*I.*) *okamurae* (Phylum Phaeophyta, Class Phaeophyceae, Order Chordariales, Family Ishigeaceae) is an edible brown alga that has been collected from the coast of Jeju island of Korea [12].

Several studies have reported that the extracts and compounds originated from *I. okamurae* possess free radical scavenging activity [7, 8, 16] and anti-inflammatory activity [10, 11]. Thus, we considered that ethyl acetate (EtOAc) fraction *I. okamurae* (EFIO) may be useful in the prevention of various hepatic damages induced by oxidative stress and inflammation. However, little is known about the protective effect of compounds derived from *I. okamurae* against CCl_4-induced hepatic injury.

The aim of this study was to examine whether EFIO protects against CCl_4-induced hepatotoxicity in rats.

Materials and Methods

Fractionation of *I. okamurae*

The brown alga *I. okamurae* was collected from the coast of Sungsanri, Jeju Island, in July 2009. The samples were washed three times with water to remove salt, epiphytes, and sand attached to the surface, and then carefully rinsed with fresh water. The samples were dried at 60°C for 24 h in an oven and then ground in a grinder prior to extraction. The shade-dried whole plant of *I. okamurae* (2,900 g) was extracted with 80% aqueous methanol with stirring for 2 days at room temperature. The filtrate was concentrated under reduced pressure and freeze-dried to create a powder. The powdered extract (311.5 g) was suspended in water (2.0 L) and successively partitioned with n-hexane (*n*-Hex), methylene chloride (CH_2Cl_2), EtOAc, and n-butanol (*n*-BuOH). The EFIO was used for this experiment because EFIO contains anti-oxidant diphlorethohydroxy-carmalol [1, 8] as well as fucoxanthin [10].

Experimental animals

Female Sprague Dawley rats (150~200 g), 6~10-weeks-old, were purchased from Orient Bio (Korea). The rats were housed in plastic cages and maintained at 23 ± 2°C under a 12 h : 12 h light-dark cycle. Feed was 5L79 rat formula (PMI Nutrition, USA). Feed and water were given *ad libitum*. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at Jeju National University, Korea.

Optimal dose for *in vivo* preliminary study

Twenty five healthy female Sprague Dawley rats were randomly assigned to five experimental groups (five rats/each group). EFIO was dissolved in phosphate-buffered saline (PBS) and used for injection. To determine the optimal dose of EFIO, rats were intraperitoneally injected with 0, 10, 50, 100 and 200 mg/kg of EFIO once daily for 2 consecutive days. Body weights of the rats were checked daily for 1 week. Rats treated with over 100 mg/kg of EFIO showed a decreased body weight. In all subsequent experiments, the EFIO dose was 10 and 50 mg/kg.

Treatment with EFIO prior to CCl_4 injection

EFIO dissolved in PBS was used for intraperitoneal (i.p.) injection (10 or 50 mg/kg body weight) for 2 consecutive days before CCl_4 injection. As a control, only PBS vehicle was injected into rats that received CCl_4. The rats were divided (n = 5 per group) into a normal control, 10 mg/kg EFIO-treated group, 50 mg/kg EFIO-treated group, vehicle-treated CCl_4 group, 10 mg/kg EFIO-treated CCl_4 group, and 50 mg/kg EFIO-treated CCl_4 group. To induce acute liver injury, a 1 : 1 (v/v) mixture of the CCl_4 and sterile olive oil was injected i.p. (3.3 mL/kg). Rats were fasted and sacrificed 24 h after CCl_4 injection.

Preparation of serum

After 24 h after CCl_4 injection, the rats were anesthetized using diethyl ether for the sampling of the blood and liver. Blood samples were collected from heart or infraorbital venous plexus. These were allowed to coagulate at room temperature, and were centrifuged at 3,000 g for 15 min at room temperature to collect the serum fraction. The serum was separated from the blood and stored at 20°C before assay.

Alanine aminotransferase and aspartate aminotransferase assays

The serum levels of alanine aminotransferase (ALT)

and aspartate aminotransferase (AST) were measured as assays of liver function. ALT and AST levels were measured using commercially available kits (Fujifilm, Japan) according to the manufacturer's instructions and were evaluated using a Fuji Dri-Chem3500 Clinical Chemistry Analyzer (Fujifilm, Japan). Serum was diluted if the value of the measurement was out of range.

Detection of liver superoxide dismutase and catalase activities

The excised livers of experimental rats were immediately frozen and the tissue was homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4) to obtain a 1 : 9 (w/v) whole homogenate. The homogenates were then centrifuged at 11,000 g for 10 min at 4°C to remove cellular debris. The protein content of the supernatant was determined using the Bradford method. For detection of superoxide dismutase (SOD) activity, 50 µg liver protein was added to 500 mM phosphate buffer (pH 10.2) and 1 mM epinephrine. Epinephrine rapidly undergoes auto-oxidation at pH 10 to produce the pink-colored product adrenochrome, which was assayed at 480 nm using an ultraviolet/visible spectrophotometer operating in the kinetic mode. SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored at 480 nm, and the amount of enzyme required to produce 50% inhibition was defined as one unit of enzyme activity. The total SOD activity was expressed as units/mg protein [14]. For detection of catalase (CAT) activity, 50 µg of liver protein was added to 50 mM phosphate buffer (pH 7.0) and 100 mM hydrogen peroxide (H_2O_2), and the mixture was incubated for 2 min at 37°C. Following incubation, the absorbance of the mixture at 240 nm was monitored for 5 min. The change in absorbance was proportional to the breakdown of H_2O_2 and the CAT activity present in the sample was expressed as units/mg protein [2].

Histopathology

Liver tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax and cut into 5 µm-thick sections, which were stained with hematoxylin and eosin (H&E) for routine examination. To reveal the fatty changes, fixed frozen sections were stained with Oil red O, which detects neutral triglycerides and lipids.

Immunohistochemical analysis

Paraffin-embedded liver sections (5 µm-thick) were deparaffinized, treated with citrate buffer (0.01 M, pH 6.0) in a microwave for 3 min, and then treated with 0.3% H_2O_2 in methyl alcohol for 20 min to block endogenous peroxidase activity. After three washes with PBS, the sections were incubated with 10% appropriate sera including normal horse serum, and then with anti-rat monocyte-like hepatic macrophage primary antibody (ED1; Serotec, UK). Immunoreactivity was visualized with an avidin-biotin peroxidase reaction (Vector Elite; Vector Labs, USA). The peroxidase reaction was developed using a diaminobenzidine substrate kit (Vector Labs, USA). The sections were counterstained with hematoxylin before being mounted.

Statistical analyses

The data are reported as the mean ± SE. The data was analyzed using one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls post hoc test for multiple comparisons. In all cases, a p value < 0.05 was considered significant.

Results

Effect of EFIO on serum enzyme activity in CCl_4-induced acute liver injury

Biochemical analysis of serum enzymes was performed to verify the protective role of EFIO in CCl_4-induced hepatic injury. The activities of serum ALT and AST in normal controls were 71.7 ± 1.3 IU/L and 140.7 ± 1.9 IU/L, respectively (Table 1). There were no significant differences in the levels of serum ALT and AST between either 10 or 50 mg/kg EFIO-treated groups and normal controls. In vehicle-treated CCl_4-intoxicated rats, the activities of serum ALT (2,986.7 ± 1,070.8 IU/L) and AST (7,440.0 ± 2,404.7 IU/L) were significantly increased compared with those of the normal controls ($p < 0$ .05). Treatment with 50 mg/kg EFIO significantly reduced the activities of serum ALT and AST (206.7 ± 127.3 IU/L and 1,056.0 ± 470.5 IU/L, respectively; $p < 0.05$). These results were consistent with the view that EFIO ameliorates the increase of serum enzymes (ALT and AST) in CCl_4-induced liver injury.

Effect of EFIO on SOD and CAT activity in CCl_4-induced acute liver injury

To investigate anti-oxidant effect of EFIO on CCl_4-induced liver injury, the activity of the anti-oxidant enzymes SOD and CAT was evaluated in hepatic tissues.

Table 1. Effects of pretreatment with ethyl acetate fraction *Ishige okamurae* (EFIO) on carbon tetrachloride (CCl_4)-induced hepatotoxicity

Group	Serum ALT (U/L)	Serum AST (U/L)
Control	71.7 ± 1.3	140.7 ± 1.9
EFIO (10 mg/kg)	55.0 ± 14.0	162.3 ± 11.3
EFIO (50 mg/kg)	46.3 ± 13.4	207.3 ± 85.7
CCl_4 (3.3 mL/kg)	2,987.7 ± 1,071.9[a]	7,440.0 ± 2,404.8[a]
EFIO (10 mg/kg) + CCl_4	1,693.3 ± 618.1	6,073.3 ± 384.4
EFIO (50 mg/kg) + CCl_4	2,076.7 ± 127.3[b]	1,056.0 ± 470.5[b]

Rats were pretreated with EFIO (10 or 50 mg/kg, i.p.) once daily for 2 consecutive days. 24 h after the final treatment, the rats were treated with CCl_4 (3.3 mL/kg, i.p.). Hepatotoxicity was determined 24 h later by quantifying the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Each value represents the mean ± SE for three rats. [a]Significantly different from normal control at $p < 0.05$. [b]Significantly different from CCl_4-treated control at $p < 0.05$.

Table 2. Effects of pretreatment with ethyl acetate fraction *Ishige okamurae* (EFIO) on SOD and CAT in the liver of rats with carbon tetrachloride (CCl_4)

Group	SOD (U/mg)	CAT (U/mg)
Control	774.6 ± 55.1	1,519.4 ± 23.3
EFIO (50 mg/kg)	948.3 ± 31.8	2,236.2 ± 150.2[a]
CCl_4 (3.3 mL/kg)	255.5 ± 24.5[a]	433.2 ± 152.8[a]
EFIO (50 mg/kg) + CCl_4	445.6 ± 51.2[b]	1,202.2 ± 26.8[c]

Rats were pretreated with EFIO (50 mg/kg, i.p.) once daily for 2 consecutive days. 24 h after the final treatment, the rats were treated with CCl_4 (3.3 mL/kg, i.p.). Oxidative stress parameters were determined 24 h later by quantifying the superoxide dismutase (SOD) and catalase (CAT). Each value represents the mean ± SE for four rats. [a]Significantly different from normal control at $p < 0.001$. [b]Significantly different from CCl_4-treated control at $p < 0.05$. [c]Significantly different from CCl_4-treated control at $p < 0.001$.

We have chosen one group treated with EFIO (50 mg/kg) because this dose is effective in serum chemistry (Table 1) and compared EFIO (50 mg/kg) group with other control groups as shown in Table 2. The hepatic SOD activity in the vehicle + CCl_4 group was significantly reduced by approximately 33% as compared to the normal controls ($p < 0.001$). However, the SOD activity was significantly increased by 50 mg/kg EFIO treatment prior to CCl_4 treatment (EFIO + CCl_4) as compared to the vehicle + CCl_4 group ($p < 0.05$) (Table 2). Moreover, the activity of CAT in EFIO + CCl_4 group was significantly decreased by approximately 29% as compared to the untreated control group ($p < 0.001$). However, pretreatment with EFIO (EFIO + CCl_4) increased significantly the CAT activity as compared to the vehicle + CCl_4 group ($p < 0.001$) (Table 2).

Histopathological examination

Histopathological studies also provided important evidence supporting the biochemical analysis and liver antioxidant status. In the normal controls, liver sections showed normal hepatic cells with a well-preserved cytoplasm, a prominent nucleus, and central vein (Fig. 1A). The pathological changes in the livers were not found in rats treated with 10 or 50 mg/kg EFIO (data not shown). The livers of CCl_4-intoxicated rats revealed hepatic necrosis and hemorrhage, inflammatory cell infiltrating, and cell swelling (Fig. 1B). Compared with the lesions observed in the vehicle-treated CCl_4 control group, the hepatic lesions of the CCl_4-intoxicated rats pretreated with 10 mg/kg EFIO were markedly milder (Fig. 1C). Amelioration of hepatic necrosis in CCl_4-intoxicated rats pretreated with 50 mg/kg EFIO was evident in the liver (Fig. 1D). Liver tissues were further analyzed to assess the fatty changes in CCl_4-intoxicated rats (Fig. 2). In normal controls (Fig. 2A) and rats treated solely with either 10 or 50 mg/kg EFIO (data not shown), Oil-red-O staining did not reveal typical lipid droplets in the examined livers. In the vehicle-treated and CCl_4-intoxicated group, Oil-red-O staining revealed diffuse fatty degeneration throughout the liver (Fig. 2B). In contrast, pretreatment with either 10 mg/kg EFIO

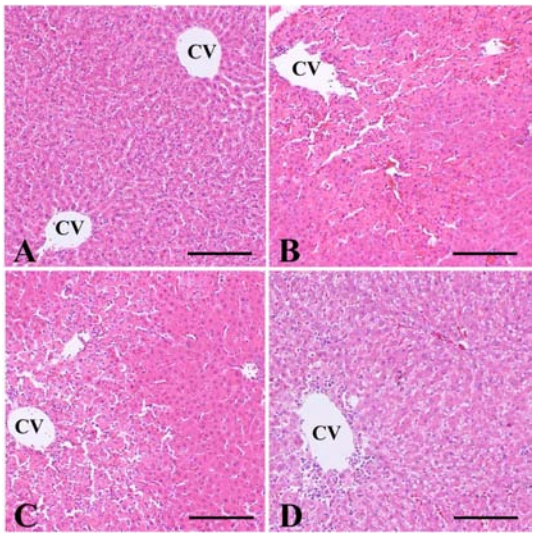

Fig. 1. Effect of EFIO on hepatic histology in CCl_4-intoxicated rats. Livers were sectioned and stained. (A) Normal control, (B) vehicle + CCl_4 (3.3 mL/kg) control, (C) EFIO (10 mg/kg) + CCl_4, (D) EFIO (50 mg/kg) + CCl_4. H&E stain, Scale bars = 100 μm.

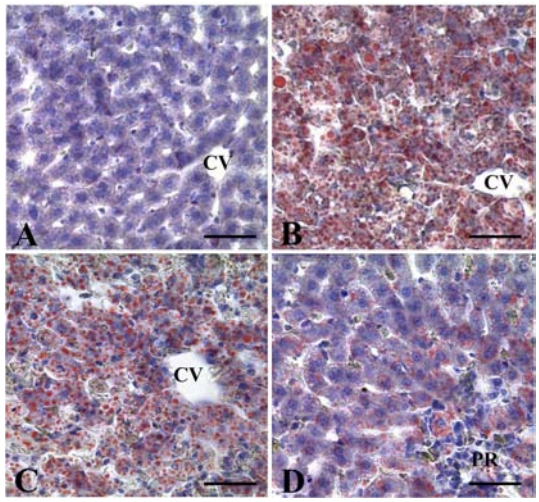

Fig. 2. Effect of EFIO on CCl_4-induced acute fatty change. Liver sections were stained. (A) Normal control, (B) vehicle + CCl_4 (3.3 mL/kg) control, (C) EFIO (10 mg/kg) + CCl_4, (D) EFIO (50 mg/kg) + CCl_4. Oil red O stain, Scale bars = 50 μm.

Table 3. Effects of EFIO on histopathology of CCl_4-induced liver injury in rats

Parameter	Group			
	Control	EFIO (50 mg/kg)	CCl_4 (3.3 mL/kg)	EFIO (50 mg/kg) + CCl_4
Hemorrhage[a]	–[c]	–	++	+
Hepatocyte necrosis[a]	–	–	+++	++
Infiltration of inflammatory cells[a]	–	–	+++	++
Fatty degeneration[b]	–	–	+++	+

Rats were pretreated with EFIO (10 or 50 mg/kg, i.p.) once daily for 2 consecutive days. 24 h after the final treatment, the rats were treated with CCl_4 (3.3 mL/kg, i.p.). Histopathological examination was performed 24 h after CCl_4 treatment. [a]For estimation of hemorrhage, hepatocyte necrosis, and infiltration of inflammatory cells, livers were sectioned and stained with hematoxylin and eosin by standard techniques. [b]For estimation of fatty degeneration, liver were sectioned and stained with Oil red O stain by standard techniques. [c]Grades: –, absent; +, trace (1~25%); ++, weak (26~50%); +++, moderate (50~75%); ++++, severe (75~100%).

(Fig. 2C) and 50 mg/kg EFIO (Fig. 2D) reduced the fatty change in the livers of rats treated with CCl_4. Especially, 50 mg/kg EFIO pretreatment markedly decreased the fatty changes of the periportal region (Fig. 2D). Histopathologic examinations (hemorrhage, hepatocyte necrosis, infiltration of inflammatory cells, and fatty degeneration) based on above histological findings were recorded and scored (Table 3).

Immunohistochemical localization of ED1-positive macrophages

To determine the effect of EFIO on the localization of monocyte-like hepatic macrophages (Kupffer cells) in CCl_4-induced acute liver injury, immunoreactivity of ED1-positive macrophages in the liver was assessed. ED1-positive reaction occurred only very rarely in normal controls (Fig. 3A). In vehicle-treated and CCl_4-intoxicanted rats, ED1-positive cells were uniformly distributed across the liver lobule following CCl_4 treatment (Fig. 3B). However, in samples from rats pretreated with 50 mg/kg EFIO, ED1-positive cells were markedly decreased and were localized almost exclusively in the centrilobular region of the liver (Fig. 3C).

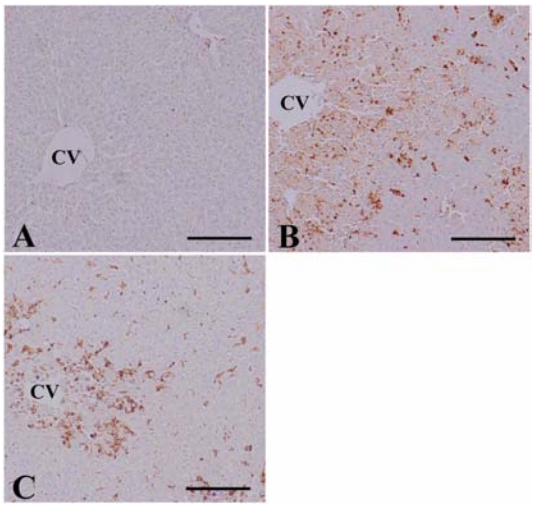

Fig. 3. Immunohistochemical staining of ED1-positive macrophages in the liver of rat with CCl_4 treatment. (A) Normal control, (B) vehicle + CCl_4 (3.3 mL/kg) control, (C) EFIO (50 mg/kg) + CCl_4. Scale bars = 200 μm.

Discussion

In the present study, the capability of EFIO to protect against CCl_4-induced hepatic injury was investigated in a rat model. It has been reported that the ethanolic extract of *I. okamurae* exert an anti-inflammatory effect by inhibiting nuclear factor-kappa B (NF-kB) [11]. Lots of anti-oxidant compounds including fucoxathin [10] and diphlorethohydroxycarmalol [1, 8] have been identified in the EFIO. Thus it is highly possible that EFIO modulates the liver injury induced by CCl_4. It is because the metabolites of CCl_4 formed by cytochrome P-450 enzyme, including trichloromethyl radicals ($\bullet CCl_3$) and trichloromethyl peroxyl radicals ($Cl_3COO\bullet$), have been demonstrated to initiate peroxidation and have effect on liver pathogenesis [13]. Many studies have revealed that a single compound from natural products reduced CCl_4-induced hepatic injury [6, 9]. The results favor the suggestion that a major hepatoprotective mechanism of such compounds is associated with their antioxidant abilities. *In vitro* and *in vivo* studies have shown that DPHC isolated from EFIO has anti-oxidative activity [1, 7, 8]. In the present study, EFIO treatment significantly prevented CCl_4-induced liver damage as evidenced by decreased serum activities of ALT and AST. Additionally, we also examined the liver activities of SOD and CAT, which are the major antioxidant enzymes responsible for elimination of reactive oxygen species. EFIO treatment restored SOD and CAT activities, most probably due to its strong free radical chelating potency. Therefore, we suggest that EFIO prevents CCl_4-induced injury in rat liver, possibly via the inhibition of oxidative stress.

In the present histological examinations, CCl_4-treated liver examined by H&E and Oil-red-O staining displayed characteristics of significant hepatotoxicity, including hemorrhage, hepatocyte necrosis, infiltration of inflammatory cells, and fatty degeneration, similar to previous histopathological findings [6, 9]. However, EFIO treatment significantly decreased these reactions in rat liver, suggesting that EFIO provides protection against CCl_4-induced hepatic injury.

In addition, CCl_4-induced injury is characterized by direct and indirect sequential phases in the liver [3, 4]. An indirect secondary damage originates from activated hepatic macrophages (Kupffer cells) through the release of inflammatory mediators [3]. An ethanol extract of *I. okamurae* and a fucoxanthin isolated from EFIO inhibit inflammation via inactivation of NF-kB transcription factor in macrophages [10, 11]. In the present study, in CCl_4-intoxicated rats, EFIO treatment altered the pattern of ED1 immunoreactivity from a widespread distribution to reduced total numbers and localization of the remaining cells around the central vein. This shift and the anti-inflammatory effect might contribute to the inhibitory effect of EFIO in CCl_4-induced hepatic injury.

In conclusion, EFIO is effective in alleviating CCl_4-induced liver injury in a rat model as evidenced by decreasing ALT and AST serum levels, increased SOD and CAT activities, and inhibition of histological damage and macrophage infiltration in the liver. The inhibitory effects of EFIO may be exploited in the use of the compound in the hepatoprotection against chemical-induced hepatotoxicity *in vivo*. However, further studies will be needed to establish the molecular mechanism of the protective effect of EFIO on hepatocytes in CCl_4-induced liver injury.

References

1. **Ahn M, Moon C, Yang W, Ko EJ, Hyun JW, Joo HG, Jee Y, Lee NH, Park JW, Ko RK, Kim GO, Shin T.** Diphlorethohydroxycarmalol, isolated from the brown algae *Ishige okamurae*, protects against radiation-induced cell damage in mice. Food Chem Toxicol 2011, **49**, 864-870.

2. **Carrillo MC, Kanai S, Nokubo M, Kitani K.** (−) Deprenyl induces activities of both superoxide dismutase and catalase but not of glutathione peroxidase in the striatum of young male rats. Life Sci 1991, **48**, 517-521.
3. **Edwards MJ, Keller BJ, Kauffman FC, Thurman RG.** The involvement of Kupffer cells in carbon tetrachloride toxicity. Toxicol Appl Pharmacol 1993, **119**, 275-279.
4. **elSisi AED, Earnest DL, Sipes IG.** Vitamin A potentiation of carbon tetrachloride hepatotoxicity: role of liver macrophages and active oxygen species. Toxicol Appl Pharmacol 1993, **119**, 295-301.
5. **Halliwell B, Gutteridge JMC.** The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med 1985, **8**, 89-193.
6. **Hayashi S, Itoh A, Isoda K, Kondoh M, Kawase M, Yagi K.** Fucoidan partly prevents CCl_4-induced liver fibrosis. Eur J Pharmacol 2008, **580**, 380-384.
7. **Heo SJ, Hwang JY, Choi JI, Han JS, Kim HJ, Jeon YJ.** Diphlorethohydroxycarmalol isolated from *Ishige okamurae*, a brown algae, a potent α-glucosidase and alpha-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. Eur J Pharmacol 2009, **615**, 252-256.
8. **Heo SJ, Kim JP, Jung WK, Lee NH, Kang HS, Jun EM, Park SH, Kang SM, Lee YJ, Park PJ, Jeon YJ.** Identification of chemical structure and free radical scavenging activity of diphlorethohydroxycarmalol isolated from a brown alga, *Ishige okamurae*. J Microbiol Biotechnol 2008, **18**, 676-681.
9. **Hsu YW, Tsai CF, Chuang WC, Chen WK, Ho YC, Lu FJ.** Protective effects of silica hydride against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem Toxicol 2010, **48**, 1644-1653.
10. **Kim KN, Heo SJ, Yoon WJ, Kang SM, Ahn G, Yi TH, Jeon YJ.** Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-κB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. Eur J Pharmacol 2010, **649**, 369-375.
11. **Kim MM, Rajapakse N, Kim SK.** Anti-inflammatory effect of *Ishige okamurae* ethanolic extract via inhibition of NF-κB transcription factor in RAW 264.7 cells. Phytother Res 2009, **23**, 628-634.
12. **Lee IK, Kang JW.** A check list of marine algae in Korea. Korean J Phycol 1986, **1**, 311-325.
13. **McCay PB, Lai EK, Poyer JL, DuBose CM, Janzen EG.** Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observation of lipid radicals *in vivo* and *in vitro*. J Biol Chem 1984, **259**, 2135-2143.
14. **Misra HP, Fridovich I.** The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972, **247**, 3170-3175.
15. **Pérez Tamayo R.** Is cirrhosis of the liver experimentally produced by CCl_4 and adequate model of human cirrhosis? Hepatology 1983, **3**, 112-120.
16. **Zou Y, Qian ZJ, Li Y, Kim MM, Lee SH, Kim SK.** Antioxidant effects of phlorotannins isolated from *Ishige okamurae* in free radical mediated oxidative systems. J Agric Food Chem 2008, **56**, 7001-7009.