BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 26(2), 218-223 (2018)

Hepaprotective Effect of Standardized *Ecklonia stolonifera* Formulation on CCl_4-Induced Liver Injury in Sprague-Dawley Rats

Jae-Hyuk Byun[1], Jun Kim[1] and Se-Young Choung[1,2,*]

[1]Department of Life and Nanopharmaceutical Sciences, Graduate School, [2]Department of Preventive Pharmacy and Toxicology, College of Pharmacy, Kyung Hee University, Seoul 02447, Republic of Korea

Abstract

The liver is an essential organ for the detoxification of exogenous xenobiotics, drugs and toxic substances. The incidence rate of non-alcoholic liver injury increases due to dietary habit change and drug use increase. Our previous study demonstrated that *Ecklonia stolonifera* (ES) formulation has hepatoprotective effect against alcohol-induced liver injury in rat and tacrine-induced hepatotoxicity in HepG2 cells. This present study was designated to elucidate hepatoprotective effects of ES formulation against carbon tetrachloride (CCl_4)-induced liver injury in Sprague Dawley rat. Sixty rats were randomly divided into six groups. The rats were treated orally with ES formulation and silymarin (served as positive control, only 100 mg/kg/day) at a dose of 50, 100, or 200 mg/kg/day for 21 days. Seven days after treatment, liver injury was induced by intraperitoneal injection of CCl_4 (1.5 ml/kg, twice a week for 14 days). The administration of CCl_4 exhibited significant elevation of hepatic enzymes (like AST and ALT), and decrease of antioxidant related enzymes (superoxide dismutase, glutathione peroxidase and catalase) and glutathione. Then, it leaded to DNA damages (8-oxo-2'-deoxyguanosine) and lipid peroxidation (malondialdehyde). Administration of ES formulation inhibited imbalance of above factors compared to CCl_4 induced rat in a dose dependent manner. Real time PCR analysis indicates that CYP2E1 was upregulated in CCl_4 induced rat. However, increased gene expression was compromised by ES formulation treatment. These findings suggests that ES formulation could protect hepatotoxicity caused by CCl_4 via two pathways: elevation of antioxidant enzymes and normalization of CYP2E1 enzyme.

Key Words: *Ecklonia stolonifera*, Hepatoprotective effect, Non-alcoholic liver injury, CYP2E1, Antioxidant enzymes, Carbon tetrachloride

INTRODUCTION

The liver, the largest and the essential organ, is responsible for digestive and excretory function, nutrition storage, and the synthesis of the new substances. The liver is also in charge of the detoxification of the exogenous xenobiotics, drugs, harmful chemical substances, and alcohol (Lee *et al*., 2007; Mihailović *et al*., 2013). The liver-related diseases are one of the most fatal diseases worldwide, and dietary habit changes (Guo and Choung, 2017) and increased drug use (Wang *et al*., 2009) result in the increased incidence rate of the liver diseases. The hepatic disease is divided into two types, the alcoholic and non-alcoholic. The alcoholic liver injury is characterized by the increased inflammatory cytokines due to the chronic alcohol intake, oxidative stress, lipid peroxidation, and acetaldehyde toxic and alcoholic liver induces the fibrosis of hepatocytes (Nanji *et al*., 1999). The non-alcoholic liver injury is caused by the obesity, diabetes, and drugs. The non-alcoholic liver injury induces fatty liver and damages the liver function (Kleiner *et al*., 2005). Recently, there has been increased number of researches on the drug-induced liver disease, and the interest in the metabolic enzyme and metabolic process within the liver has also increased. The animal liver injury model using CCl_4 is an established non-alcoholic fatty liver model, which has been used from the past (PARK *et al*., 2000; Achliya *et al*., 2004). This model is used as a valuable model in identifying the hepatotoxic protective effect of natural products.

The oxidative stress is the principal mechanism in the process of causing liver damage (Parola and Robino, 2001). The ROS generated from the alcoholic or non-alcoholic diseases attacks cell membranes, lipids, other metabolome and DNA (Roskams *et al*., 2003). Then, such damages cause inflam-

Open Access **https://doi.org/10.4062/biomolther.2017.199**

Received Oct 10, 2017 **Revised** Oct 11, 2017 **Accepted** Oct 12, 2017
Published Online Nov 13, 2017

***Corresponding Author**
E-mail: sychoung@khu.ac.kr
Tel: +82-2-961-9198, Fax: +82-2-961-0372

mation and apoptosis, and reduce the intercellular antioxidant ability (Kim and Choung, 2017). The consistent damages reduce the liver function and lower the cell regeneration ability, inducing gradual fibrosis of the liver (Weber *et al*., 2003). Several studies have reported the results showing the use of antioxidant substances in reducing the liver injury (Bruck *et al*., 2004; Reyes-Gordillo *et al*., 2007). Thus, the use of natural substances of antioxidant activities can be the one of the strategies for treatment of the liver diseases.

Ecklonia stolonifera (ES) is laminariaceous perennial brown algae and widely distributed throughout the places such as South Korea and Japan. ES is known for its antioxidant and anti-mutagenic activity. ES contains plentiful phlorotannins, which have anti-oxidative and anti-diabetic effects (Kang *et al*., 2004; Iwai, 2008). The previous *in vitro* study has figured out that ES has the most outstanding hepatotoxic protective effect among the various brown algae (Choi *et al*., 2015). After that, the liver protection effect was confirmed on the alcohol-induced rat model using the ES formulation (Bang *et al*., 2016). However, studies on non-alcoholic liver injury were insufficient. The purpose of this study, therefore, is to identify the protective effect of the ES formulation in regards to non-alcoholic liver injury and elucidate the related mechanism.

MATERIALS AND METHODS

Preparation of ethanolic formulation of ES

The ES formulation was obtained through our previous study and stored at -80°C until use (Choi *et al*., 2015; Bang *et al*., 2016). The amount phlorotannins of ES formulation was determined by HPLC as described previously (Bang *et al*., 2016).

Animals and treatment

All the animal experimental protocols were approved by the Institutional Animal Care and Use Committee guideline of Kyung Hee University (approval no. KHPASP(SE)-17-102). Five-week-old male Sprague-Dawley rats were purchased from CLS Bio (Bucheon, Republic of Korea). The rats were individually housed in standard cages at 21 ± 2.0°C in 50 ± 5% humidity with 12 h/12 h light/dark cycle. The rats were provided a laboratory diet and water *ad libitum*. After 1 week on the basal diet, the rats were randomly divided into six groups comprising nine rats (n=9) in each group as follows:

Normal control: rats were given 10 ml/kg saline per day
CCl_4: rats were given 1.5 ml/kg CCl_4 twice a week+10 ml/kg saline per day
Sil 100: rats were given 1.5 ml/kg CCl_4 twice a week+100 mg/kg (b.w.) of silymarin per day
ES 50: rats were given 1.5 ml/kg CCl_4 twice a week+50 mg/kg (b.w.) of ES formulation per day
ES 100: rats were given 1.5 ml/kg CCl_4 twice a week+100 mg/kg (b.w.) of ES formulation per day
ES 200: rats were given 1.5 ml/kg CCl_4 twice a week+200 mg/kg (b.w.) of ES formulation per day

Intraperitoneal administration with 1.5 ml/kg CCl_4 (1:1 mixed with peanut oil) was performed to induce liver injury (Dai *et al*., 2014). All rats except those in the normal group were pretreated 100 mg/kg silymarin or ES (50, 100, 200 mg/kg) every day for 21 days. Seven days after pretreatment, four times of 1.5 ml/kg CCl_4 (twice a week for 14 days) were injected (i.p.) to induced liver injury.

Measurement of liver index

Before dissecting the rats, we measured the rats' weights. The rats were anesthetized with isoflurane gas. We collected the blood samples from hepatic vein, and obtained livers to calculate liver-to-body weight ratio. The liver was washed with 0.9% phosphate-buffered saline (PBS, 1X; 135 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.4 mM KH2PO4, pH 7.4), and weighed.

Preparation of blood samples and tissue homogenates

Blood samples were centrifuged at 3,000 rpm for 15 min at 4°C. Hepatic tissues were homogenized in PBS buffer and centrifuged at 13,000 rpm for 15 min at 4°C. After obtaining the supernatant from the blood samples and homogenates, we performed subsequent analyses in blood samples and hepatic tissue homogenates.

Hepatotoxic injury assay

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were indicators of hepatotoxic injury. We measured ALT and AST in serum using commercial assay kit (Asan Pharmaceutical Co., Seoul, Korea). ALT and AST were measured at 505 nm using an ELISA reader (Bio-Tek Instruments, Inc., Winooski, VT, USA).

Total cholesterol and triglyceride estimation

We measured triglyceride (TG) and total cholesterol (TC) in serum and liver tissues. Folch method was used to formulation the TG and TC (Folch *et al*., 1957). TG and TC were determined using commercial assay kits (Asan Pharmaceutical Co.). TG and TC were measured at 550 nm using an ELISA reader.

Anti-oxidative enzyme activities and glutathione in liver tissues

To determine anti-oxidative enzyme levels and enzymatic activities, glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were measured using commercial assay kits according to the manufacturer's protocol. GSH and GPX assay kit were purchased from Elabscience Biotechnology (Bethesda, MD, USA). SOD assay kit was purchased from Cayman Chemicals (Ann Arbor, MI, USA). CAT assay kit was purchased from Biovision (Mountain View, CA, USA).

Measurement of MDA and 8-OHdG levels in liver tissues

Malondialdehyde (MDA) were measured using commercial assay kits according to the manufacturer's protocol. MDA assay kit was purchased from Elabscience Biotechnology.

8-OHdG is a biomarker of oxidative DNA damage from oxidative stress. To measure the 8-OHdG levels, we formulationed DNA from liver tissues using a DNeasy tissue kit. The 8-OHdG levels were measured using a commercial assay kit (R&D Systems, Minneapolis, MN, USA). The samples and anti 8-OHdG monoclonal solutions were added to each cell and incubated at 25°C for 1 hour. After incubation, the 96-well microplate was washed with 1X PBS containing 0.1% Tween 20 (PBST) and HRP-conjugate was added to each well. After incubation for 1 hour, we added substrate solution to each well and read the absorbance at 450 nm.

Table 1. Body weight, body weight gain and liver index in rats

	Control	CCl_4	Sil 100	ES 50	ES 100	ES 200
Initial body weight (g)	247.6 ± 10.6	247.8 ± 10.9	247.6 ± 10.5	247.8 ± 10.7	247.7 ± 10.6	247.8 ± 10.6
Final body weight (g)	348.3 ± 10.9	297.2 ± 10.8$^{\#\#}$	310.3 ± 12.4*	304.6 ± 14.4	307.4 ± 14.2	318.9 ± 11.6*
Body weight gain (g/21 days)	100.7 ± 7.6	49.4 ± 7.8$^{\#}$	62.7 ± 7.6	56.8 ± 8.4	59.6 ± 9.6	71.1 ± 8.1*
Liver weight (g/100 g of body weight)	12.6 ± 1.3	12.4 ± 1.5	11.8 ± 0.8	12.9 ± 2.1	12.8 ± 1.5	12.4 ± 1.1
Liver index (%)	3.6 ± 0.1	4.4 ± 0.2$^{\#\#\#}$	3.9 ± 0.1**	4.3 ± 0.4	4.1 ± 0.2*	4.1 ± 0.2*

The results are presented as the mean ± SD of three independent experiments (n=8). $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$. vs. normal control group, $*p<0.05$ and $**p<0.01$ vs. CCl_4 group.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Samples from the hepatic tissue were homogenized and total RNA formulationed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. cDNA was synthesized from RNA using a first-strand cDNA synthesis kit (TaKaRa ,Tokyo, Japan). Then cDNA was synthesized, quantitative real-time PCR (RT-qPCR) was performed with SYBR premix Ex Taq (TakaRa) using an ABI Step One Plus™ RT-q PCR machine (Applied Biosystems, Foster city, CA, USA). Reaction of the mixtures were incubated for initial denaturation at 95°C for 10 min, followed by 50 cycles at 95°C for 5 s, and primer Tm temperature for 20 s and 72°C for 30 s. gene expression level was normalized to glyceraldehyde 3-phosphated dehydrogenase (GAPDH).

Statistical analyses

All of the data were expressed as mean ± standard deviation (SD). Statistical analyses were determined by a one-way ANOVA using Tukey's post hoc test in Statistical Packages for the Social Science (SPSS) software (version 21.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Body weight and body weight gain in rats

Initial weight and final weight of the rats for 14 days was measured in the six groups. Variance analysis showed that body weight was reduced in the CCl_4 treated group compared to the normal group ($p<0.001$). In the final body weight, high dose of ES formulation group showed higher body weight compared to CCl_4 group ($p<0.01$) (Table 1). This was also explained as body weight gain. CCl_4 group showed significant low weigh gain compared to the normal group ($p<0.01$), however, the high dose of ES treatment inhibited reduction of weight gain induced by CCl_4 ($p<0.05$).

Effect of ES formulation on the rat liver index

The liver index was used to assess liver damage, and was significantly increased in the CCl_4 group compared with that of the normal control group ($p<0.01$; Table 1). Low dose of ES formulation did not show any statistical change in the liver index. However, elevation of the liver index was inhibited in both ES 100 and ES 200 group ($p<0.05$).

Effect of ES formulation on serum hepatic aminotransferases

First, we confirmed the hepatoprotective effect of ES formulation on CCl_4 induced liver injury. We estimated aminotransferases concentration in the serum. As presented in Fig. 1, administration of CCl_4 leaded a significant increase in AST and ALT levels compared to the normal control group ($p<0.001$). ES formulation treatment dose-dependently attenuated both aminotransferases levels. Both levels were significantly decreased in ESH group ($p<0.01$).

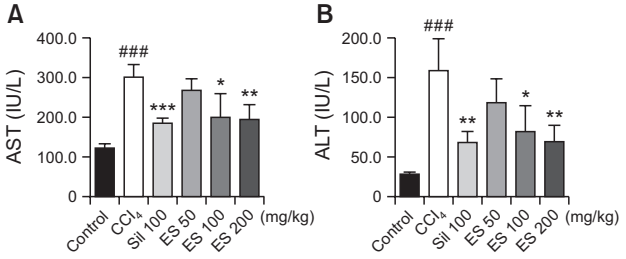

Fig. 1. Effect of ES formulation on serum hepatic aminotransferases. To evaluate the effects of ES formulation on hepatic functions, we measured the (A) serum AST and (B) ALT levels. The results are presented as the mean ± SD of three independent experiments (n=8). $^{\#\#\#}p<0.001$ vs. the normal control, $*p<0.05$, $**p<0.01$, $***p<0.001$ vs. the CCl_4 induced control. ALT, alanine aminotransaminase; AST, aspartate aminotransferase.

Effect of ES formulation on serum and hepatic tissue lipid profiles

The lipid profiles in all groups are shown in Fig. 2. The CCl_4 treated rats showed no significant change in serum TG level, while serum HDL cholesterol level was decreased significantly compared to the normal control group ($p<0.001$). However, ES formulation and silymarin treatment did not show any significant change in serum total cholesterol level. In all ES treated groups, serum TC level showed decreasing tendency in a dose dependent manner. But, the differences were not statistically significant. In the case of HDL-Cholesterol to total cholesterol ratio, CCl_4 administration exhibited a significant decrease compared to normal control group ($p<0.05$). However, the decrease was compromised by high dose of ES formulation treatment ($p<0.05$).

Hepatic TG and TC concentrations were increased in the CCl_4 group compared to normal control group ($p<0.001$ and $p<0.01$, respectively). Elevated hepatic TG level was significantly lowered from 100 mg/kg of ES formulation treatment. Increased hepatic TC level was inhibited in the ES formulation treated groups in a dose-dependent manner compared to CCl_4 group. In hepatic TG and TC levels, both ES 100 and Sil 100 groups showed similar effects compared to CCl_4 group

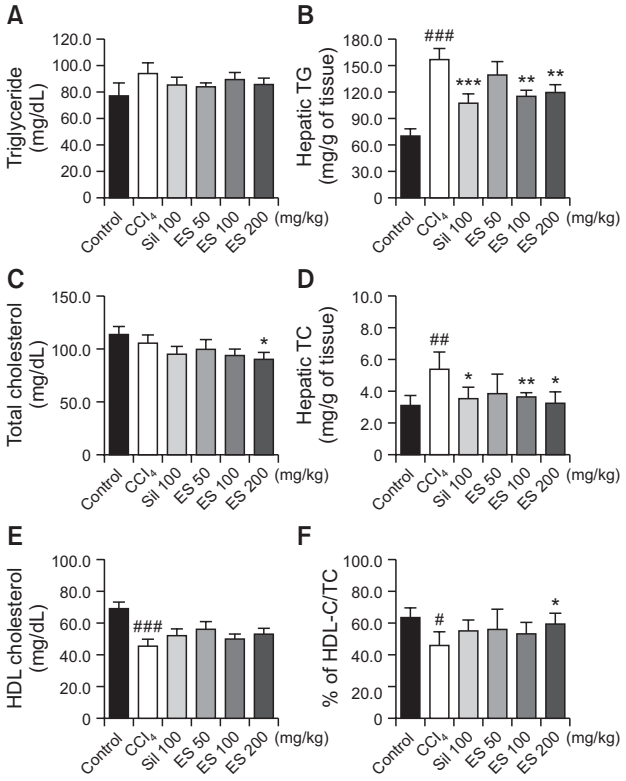

Fig. 2. Effects of ES formulation on triglyceride, total cholesterol, HDL cholesterol in serum and liver tissue. To evaluate (A) triglyceride, (B) hepatic TG, (C) total cholesterol, (D) hepatic TC, (E) HDL cholesterol, we investigated above factors in both serum and liver tissues. Then, we calculated (F) HDL cholesterol to total cholesterol. The results are presented as the mean ± SD of three independent experiments (n=8). $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. the normal control, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. the CCl_4 induced control. HDL, high density lipoprotein; TG, triglyceride; TC, total cholesterol.

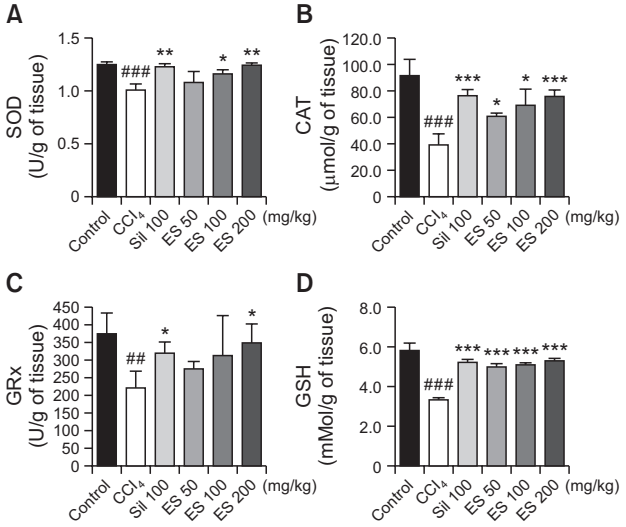

Fig. 3. Effect of ES formulation on the SOD, CAT, GPx and GSH levels in liver tissue. Activities of (A) SOD, (B) CAT and (C) GPx, and level of (D) GSH were estimated in the liver tissue. The results are presented as the mean ± SD of three independent experiments (n=8). $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. the normal control, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. the CCl_4 induced control. GSH, glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.

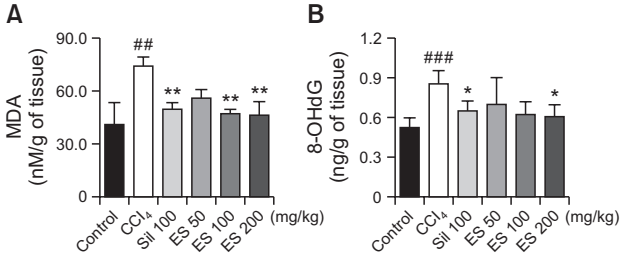

Fig. 4. Effect of ES formulation on MDA and 8-OHdG levels in liver tissue. Hepatic oxidative substances ((A) MDA and (B) 8-OHdG) were measured to verify DNA and lipid oxidation in liver tissue. The results are presented as the mean ± SD of three independent experiments (n=8). $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. the normal control, $^{*}p<0.05$, $^{**}p<0.01$ vs. the CCl_4 induced control. MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

($p<0.05$).

Effect of ES formulation on anti-oxidative enzymes and GSH in liver

To elucidate the protective mechanisms of hepatoprotection against CCl_4-induced liver injury, we measured the hepatic anti-oxidative enzymes activity and cellular antioxidant GSH (Fig. 3). The CCl_4 treated rats showed a significant decrease in SOD, CAT, GPx and GSH levels compared to the normal control group. Administration of ES formulation and silymarin inhibited the reduction of SOD, CAT, GPx and GSH activities. This results suggests that ES formulation boosts antioxidant conditions.

Effect of ES formulation on hepatic MDA and 8-OHdG in liver

In addition, we estimated oxidant substances (MDA and 8-OHdG) to identify DNA and lipid oxidation. As presented in Fig. 4, administration of CCl_4 caused a significant increase of MDA in the hepatic tissue compared to the normal control group ($p<0.01$). The ES formulation (100 and 200 mg/kg/day) and silymarin treatment reduced the elevated MDA levels in the hepatic tissue ($p<0.01$).

8-OHdG were measured to determine whether ES could inhibit oxidative stress-mediated DNA damage, administration of CCl_4 caused a substantial increase in 8-OHdG level compared to the normal control group ($p<0.001$). The elevated 8-OHdG level was significantly inhibited in the ES 200 and Sil 100 groups compared to the CCl_4 group ($p<0.05$).

Effect of ES formulation on the gene expression levels of CYP2E1 in liver

To verify the underlying mechanisms of ES formulation, we performed comparative real time qPCR to explain whether ES formulation regulates CYP2E1 gene in the hepatic tissue. Fig. 5 exhibited that CCl_4 treatment up-regulated the mRNA expression levels of CYP2E1 compared to the normal control group. However, administration of ES formulation (200 mg/kg/day) and silymarin significantly lowered the increased gene

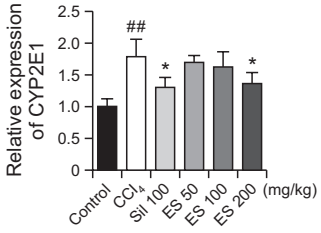

Fig. 5. Effect of ES formulation on the gene expression levels of CYP2E1 in liver tissue. CYP2E1 gene expression was evaluated in the liver tissue of rats, then, analyzed by comparative real-time PCR and normalized to GAPDH. The primer sequence was as follows: CYP2E1, 5'-TCCAACCTACCCCATGAAGC-3' (forward) and 5'-CCAACACAC AC ACGCTTTCC-3' (reverse); GAPDH, 5'-GCCAGCCTCGTCTCATAGACA-3' (forward) and 5'-AGAGA-AGGCAGCCCTGGTAAC-3' (reverse). The results are presented as the mean ± SD of three independent experiments (n=8). $^{\#\#}p<0.01$ vs. the normal control, $^{*}p<0.05$, vs. the CCl_4 induced control.

expression of CYP2E1 ($p<0.05$).

DISCUSSION

The previous studies proved the effectiveness of ES formulation in both alcoholic and non-alcoholic hepatotoxicity (Jung *et al*., 2014; Choi *et al*., 2015). The ES formulation showed inhibitory effect on fatty liver in the alcohol-induced rats (Bang *et al*., 2016). In addition, ES formulation exhibited hepatoprotective effect in tacrine-induced hepG2 cells (Choi *et al*., 2015). To identify the effect of ES formulation on non-alcoholic hepatotoxicity in animal models, we conducted this experiment using CCl_4 induced rat model. In the liver, CCl_4 is metabolized by CYP450 in microsome and generates the Cl_3CO_2 radical. Then, CCl_4 attacks the cell membrane directly to induce fatty degeneration. The CCl_4 also generates ROS consistently and reduces antioxidant enzymes, leading to lipid peroxidation (Recknagel *et al*., 1989; Jaeschke, 2000; Zhu and Fung, 2000; Weber *et al*., 2003). In recent studies, CCl_4-induced animal model showed a significant increase of AST and ALT levels in serum within a short period of time, and the model also showed coagulation necrosis and hepatocyte vacuolation in the hepatic lobules (Yang *et al*., 2008; Chen *et al*., 2013; Kemelo *et al*., 2017; Suzek *et al*., 2017). The purpose of this study is to identify the protective activity of ES formulation and to determine the underlying mechanism using the CCl_4-induced animal models.

In this study, the levels of AST and ALT in serum, where CCl_4 was administrated, were significantly increased. The CCl_4 administration also reduced GSH and antioxidant-related enzymes (SOD, CAT and GPx), and increased oxidative substances (MDA and 8-OHdG). The administration of ES formulation significantly prevented elevation of AST and ALT levels in a dose dependent manner. Furthermore, the ES formulation dose dependently inhibited the reduction of antioxidant-related enzymes caused by CCl_4. The ES formulation also dose dependently inhibited the generation of oxidative substances. In previous studies on alcoholic fatty liver, ES formulation showed dramatic hepatoprotective effect in rats (Bang *et al*., 2016). From this result, we have confirmed that ES formulation has protective effects on alcoholic and non-alcoholic liver injury.

The main consideration of non-alcoholic liver injury, especially drug-induced liver injury, is cytochrome p450. Certain drugs are mainly metabolized by cytochrome p450 in the liver to become active metabolome (biotransformation) and ROS is generated during the process. CYP2E1 is an enzyme involved in the metabolism of CCl_4, ethanol, number of anesthetics, and theophylline (Manyike *et al*., 2000). CYP2E1 is one of the enzymes mainly found in studies using CCl_4 induced animal model. Excessive substrate administration increases ROS production and leads to hepatotoxicity (Ha *et al*., 2005). In this study, gene expression of CYP2E1 in the CCl_4-induced liver significantly increased compared to the normal group. However, ES formulation administration dose dependently inhibited the increase in gene expression of CYP2E1. In previous studies using CCl_4 induced model, CYP2E1 protein levels were increased after treatment of CCl_4 in rat. Administration of *Chrysanthemum indicum L*. extract and proanthocyanidins inhibited elevation of protein expression level of CYP2E1 (Jeong *et al*., 2013; Dai *et al*., 2014). ES formulation showed similar hepatoprotective mechanism to both natural products. The result revealed that ES formulation regulates the enzyme changes of CYP2E1 and protects non-alcoholic liver injury.

To conclude, ES formulation showed hepatoprotective effect in two ways. 1) Inhibition of oxidative stress caused by CCl_4. 2) Inhibition of increase of CYP2E1 caused by CCl_4. On the basis of two pathways, ES formulation significantly restored antioxidant related enzyme levels and inhibited the generation of oxidants (MDA and 8-OHdG). In addition, ES formulation inhibited the increase of CYP2E1 caused by CCl_4 and restrained further oxidative stress. Therefore, ES formulation demonstrated effective liver protection effects on non-alcoholic liver injury, and thus holds the possibility of becoming the new treatment material for liver injury occurred in human.

REFERENCES

Achliya, G. S., Wadodkar, S. G. and Dorle, A. K. (2004) Evaluation of hepatoprotective effect of Amalkadi Ghrita against carbon tetrachloride-induced hepatic damage in rats. *J. Ethnopharmacol.* **90**, 229-232.

Bang, C.-Y., Byun, J.-H., Choi, H.-K., Choi, J.-S. and Choung, S.-Y. (2016) Protective effects of *Ecklonia stolonifera* extract on ethanol-induced fatty liver in rats. *Biomol. Ther. (Seoul)* **24**, 650-658.

Bruck, R., Aeed, H., Avni, Y., Shirin, H., Matas, Z., Shahmurov, M., Avinoach, I., Zozulya, G., Weizman, N. and Hochman, A. (2004) Melatonin inhibits nuclear factor κB activation and oxidative stress and protects against thioacetamide induced liver damage in rats. *J. Hepatol.* **40**, 86-93.

Chen, S., Zou, L., Li, L. and Wu, T. (2013) The protective effect of glycyrrhetinic acid on carbon tetrachloride-induced chronic liver fibrosis in mice via upregulation of Nrf2. *PLoS ONE* **8**, e53662.

Choi, J. S., Han, Y. R., Byeon, J. S., Choung, S. Y., Sohn, H. S. and Jung, H. A. (2015) Protective effect of fucosterol isolated from the edible brown algae, *Ecklonia stolonifera* and Eisenia bicyclis, on tert-butyl hydroperoxide- and tacrine-induced HepG2 cell injury. *J Pharm Pharmacol.* **67**, 1170-1178.

Dai, N., Zou, Y., Zhu, L., Wang, H.-F. and Dai, M.-G. (2014) Antioxidant properties of proanthocyanidins attenuate carbon tetrachloride (CCl_4)-induced steatosis and liver injury in rats via CYP2E1 regulation. *J. Med. Food* **17**, 663-669.

Folch, J., Lees, M. and Sloane-Stanley, G. (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Guo, Y.-R. and Choung, S.-Y. (2017) Germacrone attenuates hyperlipidemia and improves lipid metabolism in high-fat diet-induced

obese C57BL/6J mice. *J. Med. Food* **20**, 46-55.
Ha, K.-T., Yoon, S.-J., Choi, D.-Y., Kim, D.-W., Kim, J.-K. and Kim, C.-H. (2005) Protective effect of Lycium chinense fruit on carbon tetrachloride-induced hepatotoxicity. *J. Ethnopharmacol.* **96**, 529-535.
Iwai, K. (2008) Antidiabetic and antioxidant effects of polyphenols in brown alga *Ecklonia stolonifera* in genetically diabetic KK-A^{y} mice. *Plant Foods Hum. Nutr.* **63**, 163-169.
Jaeschke, H. (2000) Reactive oxygen and mechanisms of inflammatory liver injury. *J. Gastroenterol. Hepatol.* **15**, 718-724.
Jeong, S. C., Kim, S. M., Jeong, Y. T. and Song, C. H. (2013) Hepatoprotective effect of water extract from Chrysanthemum indicum L. flower. *Chin. Med.* **8**, 7.
Jung, H. A., Jung, H. J., Jeong, H. Y., Kwon, H. J., Kim, M.-S. and Choi, J. S. (2014) Anti-adipogenic activity of the edible brown alga *Ecklonia stolonifera* and its constituent fucosterol in 3T3-L1 adipocytes. *Arch. Pharm. Res.* **37**, 713-720.
Kang, H. S., Chung, H. Y., Kim, J. Y., Son, B. W., Jung, H. A. and Choi, J. S. (2004) Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. *Arch. Pharm. Res.* **27**, 194-198.
Kemelo, M. K., Pierzynová, A., Canová, N. K., Kučera, T. and Farghali, H. (2017) The involvement of sirtuin 1 and heme oxygenase 1 in the hepatoprotective effects of quercetin against carbon tetrachloride-induced sub-chronic liver toxicity in rats. *Chem. Biol. Interact.* **269**, 1-8.
Kim, J. and Choung, S.-Y. (2017) Pinus densiflora bark extract prevents selenite-induced cataract formation in the lens of Sprague Dawley rat pups. *Mol. Vis.* **23**, 638-648.
Kleiner, D. E., Brunt, E. M., Van Natta, M., Behling, C., Contos, M. J., Cummings, O. W., Ferrell, L. D., Liu, Y. C., Torbenson, M. S.,Unalp-Arida, A., Yeh, M., McCullough, A. J. and Sanyal, A. J. (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* **41**, 1313-1321.
Lee, C.-P., Shih, P.-H., Hsu, C.-L. and Yen, G.-C. (2007) Hepatoprotection of tea seed oil (Camellia oleifera Abel.) against CCl_4-induced oxidative damage in rats. *Food Chem. Toxicol.* **45**, 888-895.
Manyike, P. T., Kharasch, E. D., Kalhorn, T. F. and Slattery, J. T. (2000) Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. *Clin. Pharmacol. Ther.* **67**, 275-282.
Mihailović, V., Mihailović, M., Uskoković, A., Arambašić, J., Mišić, D., Stanković, V., Katanić, J., Mladenović, M., Solujić, S. and Matić, S. (2013) Hepatoprotective effects of Gentiana asclepiadea L. extracts against carbon tetrachloride induced liver injury in rats. *Food Chem. Toxicol.* **52**, 83-90.
Nanji, A. A., Jokelainen, K., Rahemtulla, A., Miao, L., Fogt, F., Matsumoto, H., Tahan, S. R. and Su, G. L. (1999) Activation of nuclear factor κB and cytokine imbalance in experimental alcoholic liver disease in the rat. *Hepatology* **30**, 934-943.
Park, E. J., Jeon, C. H., Ko, G., Kim, J. and Sohn, D. H. (2000) Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Pharm. Pharmacol.* **52**, 437-440.
Parola, M. and Robino, G. (2001) Oxidative stress-related molecules and liver fibrosis. *J. Hepatol.* **35**, 297-306.
Recknagel, R. O., Glende, E. A., Dolak, J. A. and Waller, R. L. (1989) Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther.* **43**, 139-154.
Reyes-Gordillo, K., Segovia, J., Shibayama, M., Vergara, P., Moreno, M. G. and Muriel, P. (2007) Curcumin protects against acute liver damage in the rat by inhibiting NF-κB, proinflammatory cytokines production and oxidative stress. *Biochim. Biophys. Acta* **1770**, 989-996.
Roskams, T., Yang, S. Q., Koteish, A., Durnez, A., DeVos, R., Huang, X., Achten, R., Verslype, C. and Diehl, A. M. (2003) Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. *Am. J. Pathol.* **163**, 1301-1311.
Suzek, H., Celik, I. and Dogan, A. (2017) Nephroprotective hepatoprotective potential and antioxidant role of carob pods (Cerotonia siliqua L.) against carbon tetrachloride-induced toxicity in rats. *Indian J. Pharm. Educ. Res.* **51**, 312-320.
Wang, K., Zhang, S., Marzolf, B., Troisch, P., Brightman, A., Hu, Z., Hood, L. E. and Galas, D. J. (2009) Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4402-4407.
Weber, L. W., Boll, M. and Stampfl, A. (2003) Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.* **33**, 105-136.
Yang, Y.-S., Ahn, T.-H., Lee, J.-C., Moon, C.-J., Kim, S.-H., Jun, W., Park, S.-C., Kim, H.-C. and Kim, J.-C. (2008) Protective effects of Pycnogenol® on carbon tetrachloride-induced hepatotoxicity in Sprague–Dawley rats. *Food Chem. Toxicol.* **46**, 380-387.
Zhu, W. and Fung, P. (2000) The roles played by crucial free radicals like lipid free radicals, nitric oxide, and enzymes NOS and NADPH in CCl_4-induced acute liver injury of mice. *Free Radic. Biol. Med.* **29**, 870-880.