J Vet Clin 29(6) : 441-446 (2012)

Journal of Veterinary Clinics
www.ksvc.or.kr

The Protective Effect of Ginger Aqueous Extracts on CCl_4-induced Hepatic Damage in Mice

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(Accepted: December 14, 2012)

Abstract : The purpose of present study is to observe the hepato-protective effect of ginger aqueous extracts on carbon tetrachloride(CCl_4)-induced mouse. Ginger groups received ginger aqueous extracts (500 mg/kg) orally for 3 days and given a single dose of CCl_4 (4 mL/kg). Silymarin group was treated with silymarin (50 mg/kg) orally for 3 days and then aministration of CCl_4 (4 mL/kg). Control group was only administered CCl_4 (4 mL/kg). In the ginger groups, the AST, ALT levels were significantly ($p < 0.05$) decreased compared to the control groups. Histopathological evaluation, hepatic parenchyma and kidney parenchyma of ginger groups were significantly ($p < 0.05$) decreased compared to control group. The results obtained in this study suggest that ginger aqueous extracts are able to protect the liver CCl_4-induced injury.

Key words **:** CCl_4, ginger, liver, mouse.

Introduction

Ginger (*Zingiber Officinale Roscoe*) is the root of a perennial herbaceous plant belonging to the family *Zingiberaceae*. It is widely cultivated in tropical regions such as India and Indonesia, and is extensively used as a culinary spice throughout the world given its unique scent and taste (20). Since ancient times, ginger has been used to treat indigestion, nausea, diarrhea, arthritis, rheumatoid arthritis, and hypertension. It has also been found that ginger exerts antioxidant, anti-inflammatory, and anti-carcinogenic effects, and helps prevent DNA damage (4,5). Patrick-Iwuanyanwu *et al* reported that gingerol has more a powerful antioxidant effect than vitamin E and that this compound removes −OH, superoxide radicals from the body, thereby reducing excessive fat oxide in hepatic cells (16). Ahmed *et al* reported that long-term dietary supplementation with ginger has hypoglycemic and hypolipidemic effects in rats and the antioxidant effect of ginger is as effective as that observed with ascorbic acid (1,2). The superoxide scavenging and tyrosinase inhibitory activity of ginger is well documented (4,13). Ginger is a potent antioxidant that may either mitigate or prevent the generation of free radicals. Moreover it is considered a safe herbal medicine with only a few insignificant side effects (9,19).

Carbon tetrachloride (CCl_4) is known to cause direct hepatic toxicity because it easily induces liver disease. Liver injuries induced by CCl_4 are mediated through the formation of reactive intermediates such as the trichloromethyl radical ($CCl_3\cdot$) and its derivative trichloromethyl peroxy radical ($CCl_3OO\cdot$), generated by cytochrome P450 of liver microsomes. These free radicals are thought to react with membrane lipids, leading to lipid peroxidation. Centrilobular necrosis and steatosis are two consequences of CCl_4-induced lipid peroxidation (5,8,17). Disintegration of hepatocytes membranes also results in subsequent release of marker enzymes of hepatotoxicity including aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP).

Silymarin, a hepatoprotective drug derived from plants, is currently used to treat a wide variety of liver diseases. This compound is extracted from the *Silybum marianum* fruit belonging to the plant family *Asteraceae*. It is commonly administered for the treatment of cirrhosis, chronic hepatitis, and other liver disorders associated with excessive alcohol consumption or environmental toxin exposure. As an antioxidant, silymarin may reduce free radical production and lipid peroxidation, thus ameliorating hepatotoxicity.

In this study, the protective effects of ginger aqueous extracts on CCl_4-induced liver damage in mice were evaluated and compared to those of silymarin.

Materials and Methods

Animals

One hundred adult male ICR mice were obtained from Orient Bio (Korea) and allowed to acclimatize 7 days prior to initiating this study. The mice (7 wks old, 37.5 ± 2.26 g) were housed in polypropylene cage (five mice per cage) and maintained in a temperature-controlled room (24 ± 1°C) with 12:

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12h light:dark cycles. Food and water were provided *ad libitum* throughout the experimental period.

Chemicals and Drugs

CCl_4 was purchased from Samchunlly Pharmaceutical Co., Ltd., olive oil was obtained from Oriental Chemical Industry Co., Ltd., and silymarin was acuquired from Asia Pharm Co., Ltd..

Extraction of ginger

Sample of ginger were peeled and air-dried for 2 days. The dried ginger was powdered by grinding, and 100 g of the powdered ginger was incubated with 1 L of distilled water at 37°C for 4 h in a waterbath. The extract was centrifuged at 3,000 rpm for 20 min and filtered using filter paper No. 2. Next, the filtrate was dried using a rotary evaporator (Laborota 4000, Heidolph, Germany) and then lyophilized using a freeze dryer. The extract was finally dissolved in distilled water at a concentration of 500 mg/ml.

Groups

One hundred adult mice were divided into 10 groups.

1. The normal group (Group 1) received oral doses of distilled water throughout the entire experiment ($n = 10$).
2. The control group (Group 2, 5, and 8) received distilled water orally for 3 days ($n = 10, 10, 10$).
3. The ginger group (Group 3, 6, and 9) received the ginger aqueous extract (500 mg/kg) orally for 3 days ($n = 10, 10, 10$).
4. The silymarin group (Group 4, 7, and 10) received orak doses of silymarin (50 mg/kg) for 3 days ($n = 10, 10, 10$).

Twenty-four hours after the last administration, the mice in groups 2 to 10 were treated with CCl_4 at a dose of 4 mL/kg (olive oil 1:1), p.o.

Biochemical analysis

On days 1 (Groups 2-4), 3 (Groups 5-7) and 7 (Groups 8-10) after CCl_4 administration, blood samples were obtained immediately after the animals were sacrificed. Plasma was separated by centrifugation and AST, ALT, BUN, and creatinine levels were measured using an Auto Dry Chemistry-analyzer (Spotchem^{TM} Sp-4000, Arkray Inc., Kyoto, Japan).

Histopathological evaluation

Tissue samples from the liver [left lateral lobes] and kidney were obtained, fixed in 10% neutral buffered formalin, and embedded in paraffin. Sections were then cut (3~4 μm) and stained with hematoxylin and eosin (H&E), and after that the histopathological profiles of each sample were observed under a light microscope (Nikkon, Japan). The percentages of degenerative regions in hepatic parenchyma (%/mm^2), the numbers of degenerative hepatocytes (numbers/1000 hepatocytes), percentages of degenerative regions in kidney parenchyma (%/mm^2), numbers of degenerative glomerulus (numbers/100 glomeruli) and numbers of abnormal kidney tubules (numbers/1000 tubules) were observed by histomorphometry, respectively.

Statistical analysis

All datas are presented as the mean ± SD. Statistical analysis was performed with Student's *t*-test and an ANOVA using SPSS for Windows (version 6.1.3; SPSS Inc., USA). A p-value of less than 0.05 was considered statistically significant.

Results

Biochemical analysis

Normal group showed no significant difference. (AST:52.1 ± 2.72, ALT:37.3 ± 5.44, BUN:16.2 ± 4.24, Creatinine: 0.82 ± 0.20)

Aspartate aminotransferase (AST)

After administration of CCl_4, AST levels of the control groups were significantly ($p < 0.05$) increased than normal group. However AST levels of the ginger groups (411.4 ± 43.10, 156.4 ± 24.02), silymarin groups (220.8 ± 57.09, 118 ± 32.77) were significantly ($p < 0.05$) decreased than the control groups (629.6 ± 108.98, 343.3 ± 38.32) on days 3 and 7. AST levels of the silymarin groups were significantly ($p < 0.05$) decreased than ginger groups on days 3 and 7.

Alanine aminotransferase (ALT)

After administration of CCl_4, ALT levels of the control groups were significantly ($p < 0.05$) increased than the normal group. In contrast ALT levels of the ginger groups (5225 ± 613.30, 188.4 ± 37.50, 51.4 ± 4.55) and silymarin groups (3124.3 ± 96.18, 90.2 ± 18.90, 22.6 ± 5.93) were significantly ($p < 0.05$) decreased than the control groups (11176 ±

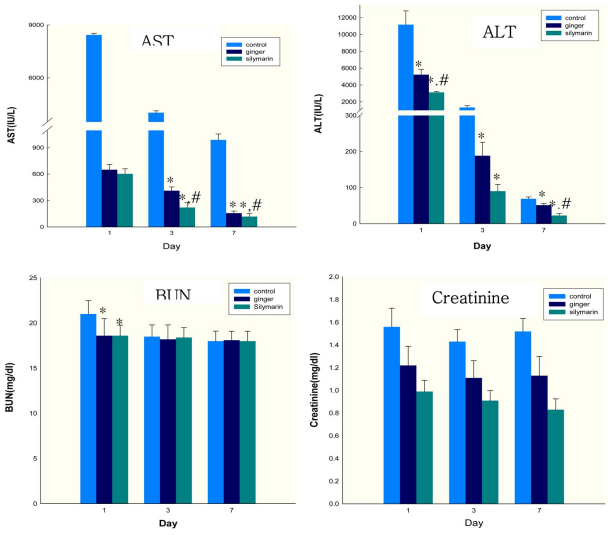

Fig 1. The changes of AST, ALT, BUN, Creatinine.
*means significant ($p < 0.05$) difference compared to Control group., #means significant ($p < 0.05$) difference compared to Ginger group.

1630.715, 1336.8 ± 214.25, 69.7 ± 5.21) during experiment. ALT levels of silymarin groups were significantly ($p < 0.05$) decreased than the ginger groups on days 1 and 7.

Blood urea nitrogen (BUN)

The BUN levels of control groups were increased than normal group. But BUN levels of ginger groups (18.6 ± 1.90), silymarin groups (18.6 ± 1.17) were significantly ($p < 0.05$) decreased than control groups (21 ± 1.49) on day 1.

Creatinine

Creatinine levels of the control groups were increased than the normal group. However creatinine levels of the ginger, silymarin groups showed no significant difference compared to the control group.

Histopathological evaluation

Vacuolation (deposition of lipid droplets) and ballooning of hepatocytes, appearance of acidophilic cells, inflammatory cell infiltration were detected in all CCl_4-treated groups (7,18). The most severe damages were detected on day 1 after administration, and then decreased over time. However, these CCl_4-induced hepatic damages were markedly inhibited by the treatment of ginger.

At histomorphometrical analysis, all two indices; degenerative regions and numbers of abnormal hepatocytes were significantly ($p < 0.01$) increased as compared with normal group in control group. However, these histomorphometrical indices related to the hepatic damages were significantly ($p < 0.01$ or $p < 0.05$) decreased by pre-treatment of ginger aqueous extracts from 3 days after administration as compared with control group, and from 1 day after administration in silymarin pre-treated mice, respectively (Table 1; Fig 2).

Table 1. Changes on the histomorphometrical analysis of hepatic parenchyma

Groups	CCl_4-induced hepatopathies	
	Percentage of hepatic degenerative regions (%/mm^2)	Numbers of abnormal hepatocytes (Numbers/1000 hepatocytes)
1 day after administration		
Normal	7.76 ± 3.29	45.40 ± 31.29
Control	85.92 ± 6.21[a]	662.20 ± 82.88[a]
Silymarin	68.69 ± 4.45[a,b]	504.40 ± 45.40[a,c]
Ginger	86.51 ± 9.01[a]	671.20 ± 109.79[a]
3 days after administration		
Normal	6.51 ± 1.93	31.60 ± 13.50
Control	50.65 ± 6.52[a]	384.00 ± 49.37[a]
Silymarin	27.19 ± 6.33[a,b]	187.40 ± 56.40[a,b]
Ginger	35.46 ± 6.43[a,c]	273.60 ± 46.46[a,b]
7 days after administration		
Normal	7.09 ± 2.48	29.80 ± 16.84
Control	39.87 ± 4.50[a]	253.40 ± 31.63[a]
Silymarin	17.27 ± 3.81[a,b]	115.20 ± 27.81[a,b]
Ginger	28.85 ± 3.12[a,b]	206.80 ± 9.68[a,b]

Values are sepressed as Mean ± S.D.
[a]$p < 0.01$ compared to normal; [b]$p < 0.01$ and [c]$p < 0.05$ compared to control.

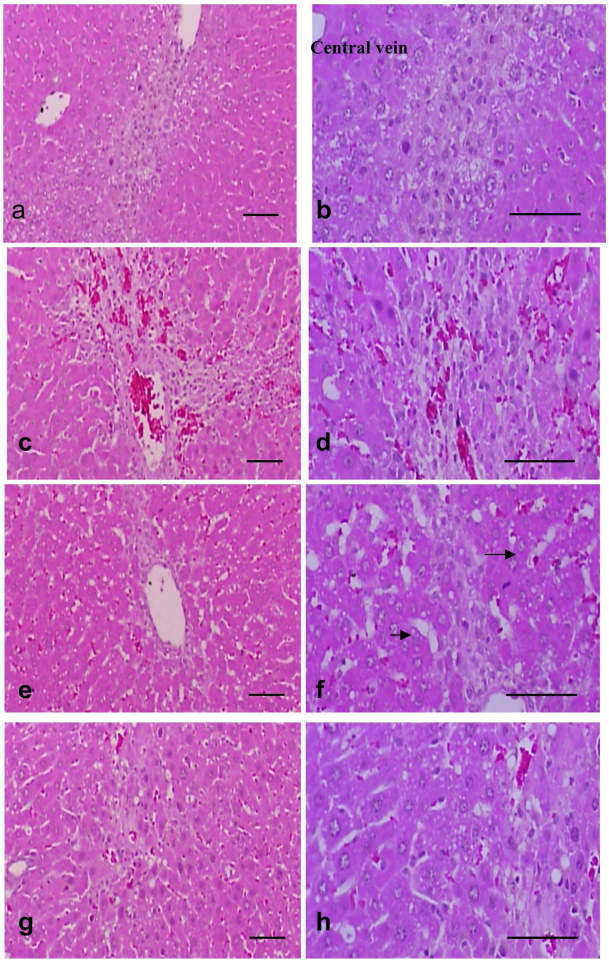

Fig 2. Changes on histological profiles of the liver in normal (a, b), control (c, d), Silymarin (e, f) and ginger (g, h) on day 7.

In the present study degeneration of kidney desquamation of tubular epithelium and atrophy, vasodilated atrophic changes of glomeruli were also observed after CCl_4 treatment. These CCl_4-related kidney damages were confirmed with histomorphometry as percentages of degenerative regions in kidney parenchyma, numbers of degenerateve glomerulus and numbers of abnormal kidney tubules were significantly ($p < 0.01$) increased as compared with normal group, respectively. The most severe damages were detected in 1 day after administration, and then decreased with time after administration in the present study. However, these CCl_4-treatment related kidney damages were dramatically and significantly ($p < 0.01$ or $p < 0.05$) inhibited by pre-treatment of ginger from 3 days after administration as compared with control

Table 2. Changes on the histomorphometrical analysis of kidney parenchyma

Groups	CCl_4-induced nephropathies		
	Percentage of kidney degenerative regions (%/mm^2)	Numbers of vasodilated glomeruli (numbers/100 glomeruli)	Number of abnormal tubules (numbers/1000 tubules)
1 d after administration CCl_4			
Normal	3.68 ± 1.83	2.60 ± 2.07	6.00 ± 4.36
Control	71.10 ± 6.33[a]	70.40 ± 6.15[a]	883.80 ± 32.61[a]
Silymarin	59.78 ± 2.57[a, b]	41.60 ± 6.48[a, b]	744.00 ± 105.06[a, c]
Ginger	68.10 ± 8.90[a]	72.00 ± 9.35[a]	832.40 ± 81.25[a]
3 d after administration CCl_4			
Normal	4.56 ± 1.40	4.40 ± 2.30	6.80 ± 5.31
Control	40.85 ± 5.24[a]	49.60 ± 6.43[a]	366.00 ± 49.39[a]
Silymarin	19.06 ± 2.74[a, b]	22.00 ± 6.89[a, b]	206.20 ± 11.73[a, b]
Ginger	31.53 ± 2.83[a, b]	28.80 ± 6.50[a, b]	245.00 ± 40.48[a, b]
7 d after administration CCl_4			
Normal	4.13 ± 1.87	3.20 ± 0.84	6.60 ± 2.30
Control	37.79 ± 5.79[a]	39.60 ± 4.72[a]	281.20 ± 55.93[a]
Silymarin	12.94 ± 1.95[a, b]	18.00 ± 3.16[a, b]	96.60 ± 10.26[a, b]
Ginger	27.81 ± 3.83[a, b]	26.80 ± 6.94[a, b]	184.20 ± 25.74[a, c]

Values are sepressed as Mean ± S.D.
[a]$p < 0.01$ compared to normal; [b]$p < 0.01$ and [c]$p < 0.05$ compared to control.

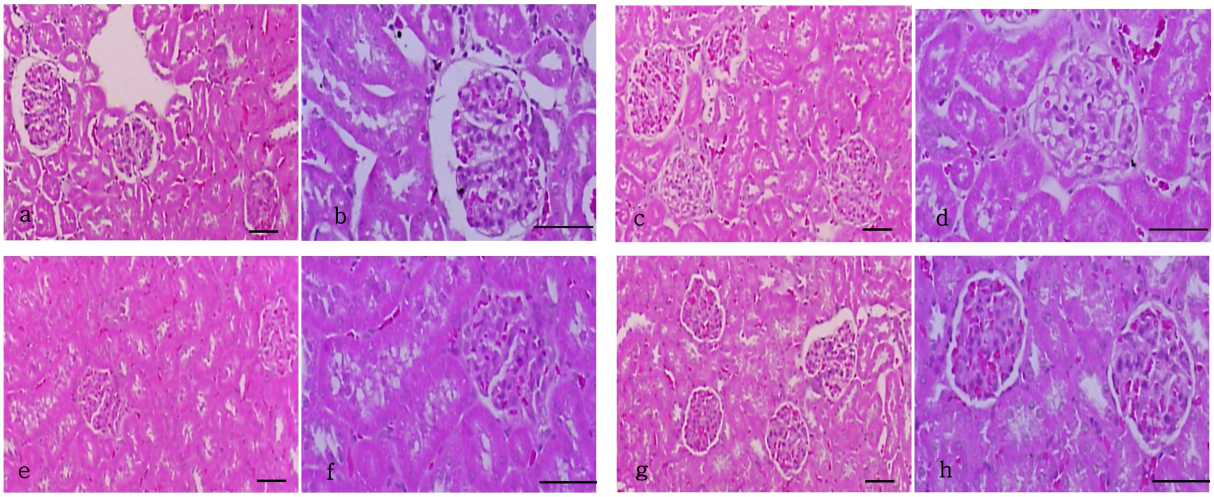

Fig 3. Changes on histological profiles of the kidney in normal group (a, b), control (c, d), silymarin (e, f) and ginger (g, h) group on day 7.

groups, and from 1 day after administration in silymarin pretreated mice, respectively (Table 2; Fig 3).

Discussion

Normal liver functions are characterized by balanced activities of AST, ALT, and ALP (used as serum marker enzymes), which are found at high concentrations in the cytoplasm of liver cells. Lysosomal instability due to CCl_4-induced hepatic injury leads to leakage of these marker enzymes into the blood stream. Dhananjay *et al* reported that AST, ALT, and ALP, concentrations are significantly increased while the levels of albumin and total protein are decreased in CCl_4-treated rats. Silymarin has also been found to significantly reduce elevated levels of liver enzymes while increasing the levels of albumin and total protein, indicating that this reagent possesses hepatoprotective properties. This might be due to the regeneration of hepatocytes and an absence of inflammatory infiltration (6).

Ajith *et al* reported that the mice were administered acetaminophen to induce liver damage, then prescribed ginger extracts. The results showed that AST, ALT, and ALP activ-

ity rates significantly decreased compared to those of the control group (3,13). In the present study, AST and ALT levels in the control groups were significantly ($p < 0.05$) increased compared to those of the normal groups, indicating liver damage. On the other hand, AST activity was significantly ($p < 0.05$) decreased on day 3 and 7 in groups pre-treated with silymarin or ginger. ALT activity was also significantly ($p < 0.05$) decreased in groups pre-treated with silymarin or ginger extracts during the experiment periods. Thus, pre-treatment with an aqueous extract of ginger appears to have a protective effect against CCl_4-induced liver damage.

Renal function is assessed by measuring creatinine and BUN levels. However, it is known that creatinine and BUN reflect the glomerular filtration rate poorly in mild or moderate renal impairment. When renal failure occurs, accumulation of these compounds is observed within the body, and concentrations in the blood, plasma, and serum can be used as indicators for measuring the degree of nitrogen waste retention and the state of renal failure (14). Yu *et al* reported that mice with acute renal failure have higher levels of serum creatinine and BUN compared to the controls (20). In the present study, BUN and creatinine levels of the control groups increased compared to those of the normal group. BUN levels were significantly ($p < 0.05$) decreased in ginger and silymarin groups on day 1. However creatinine levels did not change significantly. Ever since Montfort reported that liver damage is caused by CCl_4 in rats, CCl_4 has been the most widely used chemical to induce liver damage under experimental conditions. CCl_4 is oxidized to CCl_3 by the heterophilic oxidase system within the endoplasmic reticulum (ER) of hepatocytes. Next, it oxidizes polyenoic acid in the ER membrane and promotes lipid peroxidation, which results in deformation of the ER structure and degeneration of liver function (12). Na reported effects of jujube extract on liver cytotoxicity triggered by CCl_4 administration on rats (13). Furthermore, Patrick-Iwuanyanwu detected congestion in the central vein of the liver, centrolobular necrosis, lipid degeneration, and inflammatory cell infiltration in CCl_4-treated groups. However these symptoms were decreased in rats administered with ginger, garlic, and vitamin E (14). In this study, a certain degree of liver cell necrosis, hyperemia, hemorrhage, ballooning of the hepatocytes, inflammatory cell infiltration, and centrolobular necrosis were detected in a similar pattern. The most severe damage was observed on the first day after CCl_4 administration and decreased over time. Histomorphometry analysis showed that the hepatocyte degeneration rate and numbers of degenerated hepatocytes significantly decreased in groups administered the ginger aqueous extract compared to the control group. These results demonstrated that the ginger aqueous extract helped prevent CCl_4-induced liver damage.

Recently, natural materials have gaining interest as potential compounds for treating and protecting against diseases. Silymarin is currently used to protect the liver, but it is expensive and difficult for people to obtain in Korea. Ursodeoxycholic acid, another medicine specific for the liver, was first derived from natural materials. But it is now being chemically produced due to the high cost of the original raw materials. In contrast, ginger is produced throughout the korea, easily obtained, and easy to take in the form of food and drinks such as ginger tea. In summary, our findings indicate that ginger aqueous extracts can protect against acute CCl_4-induced liver and renal damage in mice.

Reference

1. Ahmed RS, Seth V, Pasha ST, Banerjee BD. Influence of dietary ginger (Zingiber officinales Rosc.) on oxidative stress induced by malathion in rats. Food Chem Toxicol 2000; 38: 443-450.
2. Ahmed RS, Suke SG, Seth V, Chakraborti A, Tripathi AK, Banerjee BD. Protective effect of dietary ginger (Zingiber officinales Rosc.) on lindane-induced oxidative stress in rats. Phytother Res 2008; 22: 902-906.
3. Ajith TA, Nivitha V, Usha S. Zingiber officinale Roscoe alone and in combination with α-tocopherol protect the kidney against cisplatin-induced acute renal failure. Food Chem Toxicol 2007; 45: 921-927.
4. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. Food Chem Toxicol 2008; 46: 409-420.
5. Aterman K. Toxic effect of carbon tetrachloride on the liver cell. Br J Pharmacol Chemother 1962; 19: 219-225.
6. Dhananjay W, Dilpesh J, Abhay KS, Rahup S. Hepatoprotective activity of symplocos racemosa bark on carbon tetrachloride-induced hepatic damage in rats. J Ayurveda Integr Med 2011; 7: 137-143.
7. Dogukan A, Akpolat N, Celiker H, Ilhan N, Halil Bahcecioglu I, Gunal AI. Protective effect of interferon-alpha on carbon tetrachloride-induced nephrotoxicity. J Nephrol 2003; 16: 81-84.
8. Fujii T, Fuchs BC, Yamada S, Lauwers GS, Kulu Y, Goodwin JM, Lanuti M, Tanabe KK. Mouse model of carbon tetrachloride induced liver fibrosis: Histopathological changes and expression of CD133 and epidermal growth factor. BMC Gastroenterol 2010; 10: 79-90.
9. Khaki AA and Khaki A. Antioxidant effect if ginger to prevent lead-induced liver tissue apoptosis in rat. J Med Plant Res 2010; 4: 1492-1495.
10. Khan SA, Priyamvada S, Farooq N, Khan S, Khan MW, Yusufi AN. Protective effect of green tea extract on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Pharmacol Res 2009; 59: 254-262.
11. Lim MK, Kim JW, Kim JE, Kim HT, Jung SJ, Kang MY, Lee KW. Therapeutic effect of Hovenia dulcis Thuunberg extracts and 6 types of herbal extracts on CCl_4-induced acute liver and kidney damages in rats. J Vet Clin 2007; 24: 400-405.
12. Masuda Y, Kikuzaki H, Hisamoti M, Nakatani N. Antioxidant properties of gingerol related compounds from ginger. Biofactors 2004; 21: 293-296.
13. Muriel P, Rivera-Espinoza Y. Beneficial drugs for liver diseases. J Appl Toxicol 2008; 28: 93-103.
14. Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of

butadiene in Sprague-Dawley rats. Urology 2003; 62: 353-356.
15. Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO. Prevention of CCl_4-induced liver by ginger, garlic and vitamin E. Pak J Biol Sci 2007; 10: 617-621.
16. Ruch RJ, Klauning JE, Schultz NE, Askari AB, Lacher DA, Pereira MA, Goldblatt PJ. Mechanisms of chloroform and carbon tetrachloride toxicity in primary cultured mouse hepatocytes. Environ Health Perspect 1986; 69: 301-305.
17. Shukla S, Bhadauria M, Jadon A. Effect of propolis extract on acute carbon tetrachloride induced hepatotoxicity. Indian J Exp Biol 2004; 42: 993-997.
18. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol 2002; 40: 1091-1097.
19. Valcheva-Kuzmanova SV, Popova PB, Krasnaliev IJ, Galunska BT, Belcheva A, Schunack W. Protective effect of BP 2-94, a histamine H3-receptor agonist prodrug, in a model of carbon tetrachloride-induced hepatotoxicity in rats. Folia Med (Plovdiv) 2004; 46: 36-41.
20. Yu SJ, Oh DJ, Yu SH. The investigation of macrophage infiltration in the early phase of ischemic acute renal failure in mice. Korean J Intern Med 2008; 23: 64-71.

사염화탄소 투여로 간독성을 유발한 쥐에서 생강열수추출물의 간보호 효과

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요 약 : 이 실험의 목적은 사염화탄소 투여로 간독성이 유발된 마우스에서 생강열수추출물의 간보호 작용을 알아보는 것이다. 생강투여군과 실리마린투여군은 사염화탄소(4 ml/kg)를 투여하기 전 3일동안 생강(500 mg/kg), 실리마린(50 mg/kg)을 경구 투여하였다. 혈청화학적 검사에서 생강투여군은 대조군과 비교하여 AST, ALT가 유의적 감소($p < 0.05$)가 인정되었으며, 조직병리학적 평가에서도 생강투여군은 대조군에 비해 간변성 및 신장변성이 유의적 감소($p < 0.05$)가 인정되었다. 이 결과 생강열수추출물의 경구투여가 사염화탄소로 유발된 간독성 마우스에서 간보호 효과가 있다고 생각된다.

주요어 : 생강, 사염화탄소, 간, 마우스