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Protective Effect of Korean Medicinal Plants on Ethanol-Induced Cytotoxicity in HepG2 Cells

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Abstract – The purpose of this study is to evaluate cytoprotective effect of Korean medicinal plants on alcohol-induced cytotoxicity in liver cells. Out of the 120 plant extracts tested in this study, 53 plant extracts enhanced alcohol-induced cytotoxicity in liver cells by 50~80%, while other 11 plant extracts including *Crataegus pinnatifida* reduced cytotoxicity by 1~68%. The results of DPPH free radical test and LDL lipid peroxidation test on the plant extracts that sharply reduced cytotoxicity in liver cells shows that *Crataegus pinnatifida* and *Cinnamomum cassia* had antioxidative effect. This study reports that the plant extracts that enhance or reduce ethanol-induced cytotoxicity in liver cells can be research objects as cytotoxic plants or cytotoxicity-protective plants.
Keywords – Ethanol, Cytotoxicity, Plant extract, Cytoprotective effect, DPPH, LDL

Introduction

Liver diseases including alcoholic steasis, viral hepatitis, cirrhosis and liver cancer are prevalent in about 10% of the world population (Bondy, 1992; Cederbaum, 2001; Mishra *et al*., 2011). This has triggered a lot of studies for the application of plant-originated natural substances, which has relatively low side effects, to liver diseases. At present, about 50% of medicines for liver diseases used for clinical purposes are natural substances or derivatives of natural substances (Paterson and Anderson, 2005; Teuten *et al*., 2005; Rollinger *et al*., 2006).

So far, silymarin contained in milk thistle (*Silybum marianum*) (Raškovic *et al*., 2011), glycyrrhizin contained in licorice (*Glycyrriza glabra*) (Ashfaq *et al*., 2011) and wogonin contained in *Scutellaria baicalensis (*Guo *et al*., 2007) has been reported as medicines for chronic viral hepatitis as well as curcumin contained in *Curcuma longa (*Rivera-Espinoza and Muriel, 2009) for carbon tetrachloride-induced cytotoxicity, reveratrol contained grapes (Rajasekaran *et al*., 2011) for prevention of liver cancer and rhein for hepatic fibrosis (Guo *et al*., 2002). Out of these substances, silymarin and naringenin are especially well-known for their inhibitory effect on alcohol-induced cytotoxicity in liver cells (Zhang *et al*., 2013). Since these cytotoxicity-protective compounds have antioxidative, antiviral and anticarcinogenic effects, they are expected to contribute to developing clinically significant medicines for liver diseases in the future (Ikeda *et al*., 2006; Ghosh *et al*., 2011).

There have been a lot of studies on plant extracts or compounds that have protective effect of substances such as CCl_4, galactosamine and paracetamol on cytotoxicity in liver cells (Girish *et al*., 2012). Nevertheless, there have been only a handful of reports on natural substances that have preventive effect on ethanol-induced hepatotoxicity (Kuma and Ali, 2000; Arteel *et al*., 2002; McKim *et al*., 2002). Therefore, this study has found plant extracts that have protective effect on ethanol-induced cytotoxicity in liver cells and provided the basic information on extracting alcoholic liver disease-preventive substances in the future based on the result of screening of bioactivity of 120 plant extracts. This study also reports the plant extracts that enhance ethanol-induced cytotoxicity in liver cells.

Experimental

General – 120 plant materials were obtained from the Korean herbal medicine market in Chuncheon, South Korea and each specimen was deposited in the herbarium of College of Pharmacy, Kangwon National University. Each dried plant material (100 g) was soaked in 300 mL of 80% methanol aquous solution at room temperature for 7 days. After filtration, the methanolic filtrate was

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evaporated to dryness under vacuum. These extracts without further purification were used to study their effect on cytoxicity, lipid peroxidation and scavenging activity of free radical. Materials such as methyl alcohol, ethyl alcohol, acetaminophen, vitamin C (vit-C), low-density lipoprotein (LDL) and dimetyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (MO, USA). Each specimen for free radical scavenging and antioxidation tests was dissolved in DMSO and final concentration of DMSO was adjusted to 1% (v/v) and the same amount of DMSO was used in the control experiment.

Cell culture method – The cell line used for this experiment was HepG2 cell (HB-8065^{TM}) purchased from ATCC and stored in a liquid nitrogen tank of College of Pharmacy, Kangwon National University. This cell was cultured according to the experimental purpose through frequent subcultures. For cell culture, MEM medium that contains 10% FBS, 1% L-glutamine, 1% penicillin-streptomycin. Most reagents used for cell culture were purchased from GIBCO (Life Technologies, CA, USA).

Ethanol-induced cytotoxicity and cytoprotective effect test – To evaluate cytotoxicity and cytoprotective effect, the cytotoxicity of ethanol in HepG2 cells was measured by using a microplate reader (Cole, 1986). 25,000 cells were put respectively in each well. After the culture in the medium 80~90 μl in a CO_2 incubator for 24 hours, ethanol 10 μl or the mixture of ethanol 10 μl and plant extract 10 μl was added to make 100 μl in total. Final concentration of plant extract was 0.4% in the culture with 1.3% ethanol. After the culture for 20 hours, MTT reagent 15 μl was added. After the culture for 4 hours, the medium was removed. After dissolution in DMSO 200 μl, the absorbance was measured at 570 nm. Vitamin C and acetaminophen were used under the same condition as the substances for comparison of activities. The cytoprotective effect (%) of plant extract was calculated by substracting the viability (%) of control from the viability (%) of treatment.

Free radical scavenging assay – DPPH method was used to test free radical scavenging ability of a plant extract, and vitamin C was used as the substance for comparison of activities. DPPH (1,1-Diphenyl-2-picryl hydrazil) contains radicals in molecules, which make a stable complex in combination with other free radicals. After adding the test solution 2 ml of the prescribed concentration, which was made by dissolving the extract in DMSO, in DPPH 2 ml of final concentration 60 μmol, mixing it for 5 minutes and leaving it for 20 minutes, the absorbance was measured at 520 nm (Fugita *et al*., 1988). Final concentration of plant extract was 150 μg/ml, and vitamin C was 7.5 μg/ml in the reaction mixture.

Antioxidative effect test by oxidized LDL – Antioxidative effect of oxidized LDL generated by lipid peroxidation by using Thiobarbituric acid (TBA) method (Ohtka *et al*., 1979). In the test group, LDL (2 mg/ml) 10 μl, 150 mM sodium chloride 470 μl and 100 μM copper sulfate 10 μl were added in the test solution 10 μl, which was made of the plant extract of the prescribed concentration. vitamin C was used as the substance for comparison of activities. Final concentration of plant extract was 150 μg/ml, and vitamin C was 7.5 μg/ml in the reacting solution. In the negative control group, DMSO 10 μl was added instead of the test solution. In the blank test group, distilled water 10 μl was added instead of the test solution. All the above solutions were made to be 500 μl. The reacting solutions were mixed immediately with a vortex mixer and cultured in a 37 ºC incubator for 3 hours. After putting each reacting solution 400 μl respectively in a 15 ml tube, stopping oxidation by dividing 4% BHT ethanol solution 25 μl, adding 10% phosphotungstic acid 500 μl and 0.7% thiobarbituric acid 250 μl dissolved in 0.5 M sulfuric acid, they were heated in a 95 ºC water bath for 50 minutes. After cooling in running water, vortex for 5 minutes by putting n-butanol 500 μl and centrifugation at 4,000 rpm for 15 minutes, the supernatant was measured at 535 nm.

Statistics process – Most experiments were conducted 3 times, and a significance test was conducted for acquired data by using Student's t-test.

Results

Effect of herbal medicine extracts on ethanol-induced cytotoxicity – As shown in Table 1 below, the activities of 120 herbal medicines in HepG2 cells were investigated to find natural substances that reduce or enhance alcohol-induced cytotoxicity in liver cells. The cell viability in 0.4% test solution added in 1.3% ethanol was different from the cell viability in 1.3% ethanol, which is the final concentration of the plant extracts. It has been confirmed that if (+) value gets higher, alcohol-induced cytotoxicity in liver cells is reduced more, while if (−) value gets higher, alcohol-induced cytotoxicity in liver cells is enhanced more. As shown in Table 1 below, out of the 120 investigated herbal medicines, 109 extracts, which compose of 91% of the total specimens, enhanced alcohol-induced cytotoxicity in liver cells by up to 79.7%. Among these, the plant extracts that enhanced cytotoxicity by over 75% are *Acanthopanax sessiliflorus*, *Polygala tenuifolia*, *Panax notoginseng*, *Chenastragalus membra-*

Table 1. Effects of Korean indigenous plant extracts on ethanol-induced cytotoxicity in HepG2 cells

Name of plant [#]	Part used	% Cell viability of treatment (A) [##]	% Cytoprotective effect(B) [###]	P
Crataegus pinnatifida	ramulus	152.50	68.2	**
Cinnamomum cassia	cortex	137.59	53.3	**
Pinus koraiensis	ramulus	101.05	16.8	**
Fagopyrum esculentum	semen	98.16	13.9	**
Asparagus cochinchinensis	rhizoma	96.64	12.4	**
Alpinia officinarum	radix	94.65	10.4	
Areca cathechu	semen	92.83	8.5	
Elsholtzia ciliata	herba	89.27	5.0	
Pueraria lobata	radix	88.16	3.9	
Plantago asiatica	semen	86.14	1.8	
Quisqualis indica	fructus	85.6	1.3	
Codonopsis pilosula	radix	83.23	-1.1	
Astragalus membranaceus	radix	82.78	-1.5	
pyrus pyrifolia var. culta (Makino)	flos	81.64	-2.7	
Rehmannia glutinosa	radix	81.15	-3.1	*
Polygonum multiflorum	radix	78.84	-5.4	**
Citrus unshiu	pericarpium	77.99	-6.3	**
Lithospermum erythrorhizon	radix	77.78	-6.5	*
Cnidium officinale	rhizoma	76.46	-7.8	
Helianthus annuus	semen	75.95	-8.3	
Aralia continentalis	radix	72.06	-12.2	**
Allium cepa	radix	72.01	-12.3	
Morus bombycis	folium	71.48	-12.8	**
Artemisia capillaris	herba	70.24	-14.0	**
Pinellia ternata	rhizoma	69.77	-14.5	*
Gastrodia elata	tuber	69.33	-15.0	**
Kalopanax pictus	ramulus	67.6	-16.7	**
Phaseolus radiatus	semen	65.64	-18.6	**
Prunella vulgaris	spica	65.59	-18.7	*
Trichosanthes kirilowii	radix	65.03	-19.3	**
Lycium chinense	fructus	63.73	-20.6	**
Nelumbo nucifera	folium	63.43	-20.9	**
Paeonia lactiflora	radix	63.19	-21.1	**
Citrus aurantium	fructus immaturus	59.87	-25.5	**
Pueraria lobata	radix	57.94	-26.3	**
Loranthus parasticus	ramulus	57.56	-26.7	**
Zizyphus jujuba	seed	57.43	-26.9	**
Cornus officinalis	fructus	55.59	-28.7	**
Dipsacus asperoides	radix	54.92	-29.4	**
Prunus mume	fructus	54.14	-30.1	**
Paeonia suffruticosa	cortex radicis	54.05	-30.2	**
Pinus thunbergii	folium	53.21	-31.1	**
Rhus javanica	Galla rhois	52.73	-31.6	**
Epimedium koreanum	herba	51.78	-32.5	**
Prunus japonica	semen	48.62	-35.7	**
Houttuynia cordata	herb	47.95	-36.3	**
Valeriana fauriei	radix et rhizoma	47.82	-36.5	**

Table 1. continued

Name of plant [#]	Part used	% Cell viability of treatment (A) [##]	% Cytoprotective effect(B) [###]	P
Morus alba	folium	46.51	-37.8	**
Acorus gramineus	radix et rhizoma	45.19	-39.1	**
Sophora japonica	flos	44.93	-39.4	**
Pinus koraiensis	folium	44.40	-39.9	**
Gastrodia elata	rhizoma	44.19	-40.1	**
Alpinia oxyphylla	fructus	42.64	-41.6	**
Poria cocos	poria	41.74	-42.6	**
Portulaca oleracea	herba	41.64	-42.6	**
Coptis japonica	rhizoma	40.19	-44.1	**
Nelumbo nucifera	semen	39.77	-44.5	**
Eucommia ulmoides	cortex	39.14	-45.2	**
Arctium lappa	fructus	38.63	-45.7	**
Codonopsis lanceolata	radix	38.21	-46.1	**
Saururus chinensis	herba	38.17	-46.1	**
Lycium chinense	cortex	37.03	-47.3	**
Brassica rapa	folium	36.08	-48.2	**
Lonicera japonica	flos	35.96	-48.3	**
Equisetum arvense	herba	35.20	-49.1	**
Platycodon grandiflorum	radix	35.10	-49.2	**
Hordeum vulgare	fructus germinatus	34.86	-49.4	**
Aloe barbadensis	juice	34.24	-50	**
Cephalonoplos segetum	herba	33.7	-50.6	**
Aralia continentalis	radix	32.91	-51.4	**
Polyporus umbellatus	hoelen	32.06	-52.2	**
Phyllostachys bambusoides .	silicea	27.87	-56.4	**
Atractylodes lancea	rhizoma	26.97	-57.3	**
Myristica fragrans	semen	26.70	-57.6	**
Kalopanax pictus	cortex	25.37	-58.9	**
Gardenia jasminoides	fructus	25.15	-59.1	**
Dendranthema indicum	herba	24.48	-59.8	**
Inula japonica	flos	22.81	-61.5	**
Bupleurum falcatum	radix	22.81	-61.5	**
Rubus coreanus	fructus	22.79	-61.5	**
Hepatica asiatica	herba	22.28	-62	**
Eucommia ulmoides	cortex	21.94	-62.4	**
Mentha arvensis	herba	21.53	-62.8	**
Cnidium officinale	rhizoma	21.39	-62.9	**
Taraxacum platycarpum	herba	19.73	-64.6	**
Saposhnikovia divaricata	radix	18.38	-65.9	**
Atractylodes japonica	rhizoma	18.22	-66.1	**
Leonurus japonicus	herba	18.1	-66.2	**
Peucedanum japonicum	radix	17.05	-67.2	**
Rhododendron mucronulatum	flos	16.96	-67.3	**
Asarum heterotropoides	rhizoma	16.82	-67.3	**
Carthamus tinctorius	flos	16.56	-67.7	**
Curcuma longa	radix	16.24	-68.0	**
Angelica dahurica	radix	15.35	-68.9	**

Table 1. continued

Name of plant #	Part used	% Cell viability of treatment (A) ##	% Cytoprotective effect(B) ###	P
Angelica tenuissima	radix	14.80	-69.5	**
Brassica deracea	folim	14.16	-70.1	**
Tribulus terrestris	fructus	13.36	-70.9	**
Acorus gramineus	rhizoma	12.46	-71.8	**
Citrus unshiu	pericaupium immaturus	12.35	-71.9	**
Pinus densiflora	pollen	12.31	-72.0	**
Euphorbia pekinensis	radix	12.24	-72	**
Poncirus trifoliata	fructus	12.19	-72.1	**
Thuia orientalis	immaturus semen	11.92	-72.4	**
Portulaca oleracea	herba	11.88	-72.4	**
Clematis mandshurica	rhizoma	11.41	-72.9	**
Trichosanthes kirilowii	radix	11.05	-73.2	**
Evodia rutaecarpa	fructus	10.36	-73.9	**
Polygonum multiflorum	radix	10.25	-74	**
Acanthopanax sessiliflorus	cortex	9.56	-74.7	**
Polygala tenuifolia	radix	9.26	-75	**
Panax notoginseng	radix et rhizoma	8.57	-75.7	**
astragalus membranaceus	radix	7.99	-76.3	**
Alisma orilutale	rhizoma	7.87	-76.4	**
Anemarrhena asphodeloides	rhizoma	7.48	-76.8	**
Gentiana triflora	radix et rhizoma	6.82	-77.5	**
Achyranthes japonica	radix	6.60	-77.7	**
Dimocarpus longan	arillus	5.04	-79.3	**
Schisandra chinensis	fructus	4.96	-79.3	**
Achyranthes japonica	radix	4.76	-79.5	**
Prunus mume	fructus	4.61	-79.7	**
Vitamin C		119.65	35.4	**
Trolox		99.73	15.4	**
Acetaminophen		62.68	-21.6	**

0.4% extract in 1.3% ethanol at final concentration,
Cell viability of 1.3% ethanol as positive control was 84.3 ± 2.3%,
Cytoprotective effect (B) = A – 84.3, *P < 0.05 **p < 0.01 : 1.3% ethanol vs sample treated (Student's t-test)

naceus, *Alisma orilutale*. *Anemarrhena asphodeloides*, *Gentiana triflora*l and *Achyranthes japonica*i. Especially, *Dimocarpus longan*, *Schisandra chinensis*, *Achyranthes japonica* and *Prunus mume* enhanced cytotoxicity by over 80%.

On the contrary, *Crataegus pinnatifida*, *Cinnamomum cassia*, *Pinus koraiensis*, *Fagopyrum esculentum*, *Asparagus cochinchinensis*, *Alpinia officinarum*, *Areca cathechu, Elsholtzia ciliata*, *Pueraria lobata*, *Plantago asiatica* and *Quisqualis indica* reduced ethanol-induced cytotoxicity. Among these, *Crataegus pinnatifida* and *Cinnamomum cassia* reduced cytotoxicity by 68.2% and 53.3% respectively. vitamin C and Trolox used for substances for comparison of activities reduced cytotoxicity in liver cells, while acetaminophen, which is a cytotoxic substance, enhanced cytotoxicity in liver cells. Among the investigated specimens, most plant extracts enhanced ethanol-induced cytotoxicity in liver cells, while a handful of plant extracts reduced ethanol-induced cytotoxicity in liver cells.

Antioxidative effect of *Crataegus pinnatifida* and *Cinnamomum cassia* – Table 2 and 3 show DPPH free radical scavenging effect and LDL antioxidative effect of *Crataegus pinnatifida* and *Cinnamomum cassia* extracts that have high protective effect on ethanol-induced cytotoxicity. The final concentration of *Crataegus pinnatifida* and *Cinnamomum cassia* extracts was 150 μg/ml. Table 2 shows that all of the two extracts have

Table 2. Free radical scavenging effect of *Crataegus pinnatifida* and *Cinnamomum cassia*

Treatment	ABS_{520nm}			Mean ± SD	p
Positive	0.283	0.273	0.270	0.275 ± 0.007	
Crataegus pinnatifida (150 μg/ml)	0.193	0.194	0.193	0.193 ± 0.001	< 0.01
Cinnamomum cassia (150 μg/ml)	0.132	0.136	0.143	0.137 ± 0.006	< 0.01
Vit-C (7.5 μg/ml)	0.177	0.174	0.178	0.176 ± 0.002	< 0.01

Table 3. LDL oxidation inhibition of *Crataegus pinnatifida* and *Cinnamomum cassia*

Treatment	ABS_{535nm}		Mean ± SD	p
Positive	0.133	0.136	0.135 ± 0.002	
Crataegus pinnatifida (150 μg/ml)	0.001	0.012	0.007 ± 0.008	< 0.01
Cinnamomum cassia (150 μg/ml)	0.044	0.038	0.041 ± 0.004	< 0.01
Vit-C (7.5 μg/ml)	0.020	0.025	0.023 ± 0.004	< 0.01

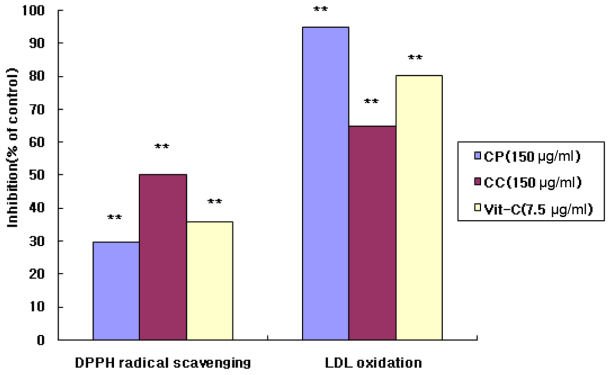

Fig. 1. Inhibition of DPPH radical scavenging and LDL oxidation of *Crataegus pinnatifida* (CP) and *Cinnamomum cassia* (CC). **P < 0.01, % inh = (positive – treated) / positive × 100

significant free radical scavenging effect ($p < 0.01$). vitamin C used as the substance for comparison of activities showed significant scavenging effect under similar level to the extracts in 7.5 μg/ml concentration. Table 3 shows that like DPPH free radical test, the final concentration of *Crataegus pinnatifida* and *Cinnamomum cassia* extracts was 150 μg/ml. *Crataegus pinnatifida* and *Cinnamomum cassia* had significant inhibitory effect ($p < 0.01$), showing as strong antioxidative effect as vitamin C (7.5 μg/ml) used as the substance for comparison of activities.

Fig. 1 shows the comparison between the antioxidative effect of *Crataegus pinnatifida* (150 μg/ml) and *Cinnamomum cassia* extracts (150 μg/ml) and that of Vitamin C (7.5 μg/ml). DPPH free radical scavenging effect was *Cinnamomum cassia* 50.2% > vitamin C 35.9% > *Crataegus pinnatifida* 29.7%. LDL antioxidative effect was *Crataegus pinnatifida* 94.8% > vitamin C 80.3% > *Cinnamomum cassia* 65.0%.

Discussion

Ethanol is a chemical substance that induces fatty liver, liver cirrhosis or liver cancer in human bodies. Therefore, it is required to find a proper pharmaceutically active material that reduces ethanol-induced cytotoxicity in liver cells. This study aimed at finding Korean plant extracts that have protective effect on ethanol-induced cytotoxicity in liver cells.

Table 1 shows that as a result of adding 0.4% plant extract in 1.3% ethanol, 109 out of the 120 plant extracts enhanced ethanol-induced cytotoxicity in liver cells, while only 11 reduced cytotoxicity. Therefore, it has been confirmed that most plant extracts enhance ethanol-induced cytotoxicity in liver cells. Acetaminophen, which is a cytotoxic substance used for the experiments of this study also enhanced ethanol-induced cytotoxicity in liver cells. Therefore, it is considered that when intaking ethanol-containing substances (including plant extracts, nutritive substances and medicines), alcohol-induced cytotoxicity is likely to be enhanced. Especially, it is required to study the synergism of ethanol-induced cytotoxicity incurred by all kinds of plant components intaken as liqueur. It is also worth to pay attention to some plant extracts that reduce ethanol-induced cytotoxicity as protective agents against cytotoxicity in live cells. Considering that antioxidants such as vitamin C reduce ethanol-induced cytotoxicity, it is expected that antioxidative components contained in these extracts have inhibitory effect on cytotoxicity. It is reported that polyphenol extract of cocoa or green tea has inhibitory effect on ethanol-induced liver damage (Arteel *et al*., 2002; McKim *et al*., 2002).

Cytotoxicity of ethanol is related to acetaldehyde or ROS generated by catalystic activities of alcohol

dehydrogenase and microsomal cytochrome P450 involved in the metabolism of ethanol [Arteel, 2003]. Alcohol, as a strong CYP2E1 inducing agent, generates superoxide radical and hydrogen peroxide in metabolic process, and is oxidized into acetaldehyde, generating free radicals (Arteel, 2008). These radicals causes lipid peroxidation and single strand breaks of DNAs along with various types of genetic toxicity including cytotoxicity and adduct formation (Navasumrit *et al*., 2001).

HepG2 cells used in this study are metabolizing cells subcultured in human liver cancer cells (Todaro, 1963; Westerink *et al*., 2007). Not quite sure but it is considered that the enhancement of ethanol-induced cytotoxicity in these cells was caused by the activities of plant extracts in liver cells, which caused the induction of CYP2E1 or the generation of active oxygen in metabolic process or otherwise by the inhibition of activation of antioxidant enzyme such as catalase and SOD of HepG2 cells.

Since long before, oriental societies where natural plants have been recoginized as relatively nontoxic components have used them as not only foods but also folk medicines and health functional foods. Indeed, there have been a lot of reports on the side effects of plants themselves or metabolites such as cytotoxicity in live cells (Bunchorntavakul *et al*., 2013). In the aspect of clinical medicine, it is reported that the intake of plant extracts such as *Polygonum multiflorum* induce hepatitis (Furukawa *et al*., 2010). In this study, *Polygonum multiflorum* was also a plant extract that strongly enhances ethanol-induced cytotoxicity by 74% (Table 1).

Meanwhile, only 11 plant extracts including *Crataegus pinnatifida* have been confirmed to reduce ethanol-induced cytotoxicity. Among these, *Crataegus pinnatifida* and *Cinnamomum cassia* had the best inhibitory effect. Vitamin C used as the substance for comparison of activities also reduced cytotoxicity. As a result of DPPH free radical test (Table 2) and LDL oxidation test (Table 3) on *Crataegus pinnatifida* and *Cinnamomum cassia* that reduced cytotoxicity most, their inhibitory effect has been confirmed. Vitamin C used as the substance for comparison of activities also had antioxidative effect in a low concentration level (7.5 μg/ml) (Fig. 1). Such a result shows that the inhibitory effect of *Crataegus pinnatifida* and *Cinnamomum cassia* extracts on the ethanol-induced cytotoxicity is highly associated with the antioxidative effect of these extracts.

Oxidative stress generated by the metabolism of ethanol and the reduction of antioxidant defence mechanism may cause liver damage (Nordmann, 1992; French, 2001). Among antioxidants such as vitamin C, Trolox or BHT, vitamin C had more protective effect on H_2O_2-induced cytotoxicity in NIH3T3 and HepG2 cells as well as inhibitory effect on oxidative DNA damage (Kim *et al*., unpublished). Intraperitoneal administration of vitamin C and vitamin E in rats before administering ethanol had more inhibitory effect on free radicals and DNA strand breaks than the ethanol-only group (Navasumrit *et al*., 2000). Therefore, it is considered that high antioxidative plant extracts reduce alcohol-induced oxidative cytotoxicity by scavenging ROS generated in alcohol oxidation process or otherwise by enhancing the activation of antioxidant enzyme such as superoxide dismutase, catalase and glutathione peroxidase.

Crataegus pinnatifida that contains polyphenols, procyanidines, chlorogenic acid and flavonoids (Chu *et al*., 2003; Cui *et al*., 2006) and *Cinnamomum cassia* that contains cinnamaldehyde and eugenol (Lin *et al*., 2003) have antioxidative effect. Therefore it is required to study CYP2E1 expression, ROS generation and activity change of antioxidant enzyme of the above two plant extracts in animal models as studies on the mechanism of ethanol-induced cytotoxicity (Isayama *et al*., 2003; Mikstacka *et al*., 2002; Ohashi *et al*., 2005).

It is reported that medicinal plants or herbal and dietary supplements induce a spectral diversity of cytotoxicity in liver cells. Therefore, it is required to understand their toxicity risk and pathological physiology and evaluate safety of natural products that enhance ethanol-induced cytotoxicity. Therefore, we need to be careful to use plant-originated extracts for cytological and medical purposes. This study has not resulted in detailed findings on plant extract's specific enhancing or reducing effect on ethanol-induced cytotoxicity.

We need to be careful with the use of plant extracts including those specified in this study such as *Dimocarpus longan*, *Schisandra chinensis*, *Achyranthes japonica* and *Prunus mume* for human bodies, especially, in case of oriental societies, with the intake of liqueur, which is made by maturing medicinal plants in ethanol, because of the toxicity of its congeners. In addition, it is also required to find natural substances such as *Crataegus pinnatifida* and *Cinnamomum cassia* that reduce ethanol-induced cytotoxicity in liver cells, investigate bioactive substances and derivatize them to develop cytoprotective substances.

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