Natural Product Sciences
9(2) : 87-90 (2003)

Screening of Hepatoprotective Activity of Medicinal Plant Extracts on Carbon Tetrachloride-induced Hepatotoxicity in Rats

Choon Sik Jeong*, In Ok Suh, Jin Ee Hyun[1], and Eun Bang Lee[2]

[1]*College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea*
[2]*Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea*

The purpose of this study was to find the effect of 36 traditional medicinal plant species on hepatoprotective activity screening. For this study, carbon tetrachloride (CCl_4) intoxificated rats were used. Test extracts were made with the traditional medicinal plants refluxed in 95% MeOH and orally administered to the rats. Sixteen species, such as *Mentha arvensis*, *Sophora japonica*, *Benincasa hispida*, *Lonicera japonica* (Lonicerae Flos), *Agaricus blazei*, *Epimedium koreanum*, *Aralia continentalis*, *Lithospermum erythrorhizon*, *Cimicifuga foetida*, *Gastrodia elata*, *Sanguisorba officinalis*, *Cephalonoplos segetum*, *Bupleurum falcatum*, *Alisma plantago-aquatica* var. *orientale*, *Lonicera japonica* (Lonicerae Folium) and *Sinomenium acutum* showed protective effect against increased serum alanine aminotransferase (ALT) and/or serum asparteate aminotransferase (AST) activities.

Key words – Medicinal plant, Hepatoprotective activity, carbon tetrachloride, serum aminotransferases

Introduction

In modern society, drug induced liver disease as well as impaired liver function of viral hepatitis are common (Park, 1991). The most frequently prescribed drugs for liver disease are made with natural resources: Sylimarin is obtained from *Silybum marianum* and dimethyl dimethoxy biphenylate (DDB) is synthesized from schizandrin, a component of Schizandrae Fructus. However, these drugs are not sufficient for the treatment of serious hepatic diseases.

Carbon tetrachloride (CCl_4) is a xenobiotic which produces hepatotoxicity in humans and animals. The hepatotoxicity induced by CCl_4 is assumed to the result of its reductive dehalogenation by the cytochrom P-450 enzyme system. The highly reactive free radical, trichloromethyl radical·CCl_3, combined to a molecular oxygen becomes trichloromethylperoxyl radical. Then trichloromethylperoxyl radical leads to impairment physiological functions of hepatocytes by attacking methyl carbon of polyenoic fatty acid and cell membrane phospholipid (McCay *et al.*, 1984; Butler, 1990; Chenery, 1981).

In order to test hepatoprotective activities of traditional medicinal plants, 36 species were selected based on Korean traditional prescription book 'Dongeuibogam' (Hur, 1989) and Encyclopedia of Chinese Herbs (Jiansu New College Medicine Eds., 1977). The purpose of this study is to examine hepatoprotective effect of the traditional medicinal plants and to obtain predominant candidates having potent hepatoprotective activity.

Materials and Methods

Herbal materials – The traditional medicinal plants used for the screening of hepatoprotective activity were purchased at the herbal market located in Jongro 5-ka in Seoul, Korea. The plants used for screening and yield of extracts are shown in Table 1. Test extracts were made with the plants refluxed in 95% MeOH three times for 4 hr at 70°C in a water bath.

Chemicals and instruments – The chemicals used in this study were carbon tetrachloride (Duksan Chem. Co., Ltd., Korea), olive oil (Shinyo Pure Chem. Co., Ltd., Japan), silymarin (Bukwang Pharmaceutical Co., Korea) and sodium carboxymethyl cellulose (CMC) (Junsei Chemical Co., Ltd., Japan). Alanine aminotransferase (ALT) Kit and aspartate aminotransferase (AST) Kit (Asan Pharm. Co. Ltd., Korea) were used to determine the enzyme quantity. Other reagents and solvents used for extraction were pharmaceutical or reagent grade.

The instruments used for manipulation and measurement of samples were centrifuge (Hanshin Medicals Co. Ltd., Korea), Dubnoff metabolic shaking incubator (Precision Scientific GCA Co., U.S.A.), UV/Visible spectrophotometer

*Author for correspondence
Fax. 82-2-901-8386, E-mail: choonsik@center.duksung.ac.kr

Table 1. The list of traditional medicine and yield of extract

Scientific name	Part used	Yield of extract (%)
Acanthopanax sessiliflorum (Araliaceae)	Bark	20.0
Aaricus blazei murill	Fungus	44.9
Alisma plantago-aquatica var. *orientale* (Alismataceae)	Root	31.0
Alnus japonica (Betulaceae)	Bark	13.9
Angelica gigas (Umbelliferae)	Root	47.1
Aralia continentalis (Araliaceae)	Root bark	25.0
Atractylodis japonica (Compositae)	Root	22.5
Benincasa hispida (Cucurbitaceae)	Seed	4.3
Bupleurum falcatum (Umbelliferae)	Root bark	23.3
Caesalpinia sappan (Leguminosae)	Wood	13.5
Cassia tora (Legbuminosae)	Seed	3.8
Cephalonoplos segetum (Compositae)	Herbs	13.3
Chrysanthemum indicum (Compositae)	Flower	31.2
Cibotium barometz (Dicksoniaceae)	Root	25.1
Cimicifuga foetida (Ranunculaceae)	Root	20.1
Curcuma zedoaria (Zingiberaceae)	Root	4.9
Epimedium koreanum (Berberidaceae)	Herbs	28.3
Eugenia caryophyllata (Mytraceae)	Flower	34.2
Forsythia viridissima (Oleaceae)	Fruit	15.3
Gastrodia elata (Orchidaceae)	Root	28.7
Gleditsia japonica (Leguminosae)	Fruit	39.2
Lithospermun erythrorhizon (Borraginaceae)	Root bark	51.7
Lonicera japonica (Caprifoliaceae)	Flower	23.8
Lonicera japonica (Caprifoliaceae)	Leaf	13.6
Mentha arvensis (Labiatae)	Herbs	22.6
Paeonia japonica (Ranunculaceae)	Root	25.2
Panax ginseng (Araliacae)	Root	25.3
Platycodon grandiflorum (Campanulaceae)	Root bark	28.6
Pueraria thunbergiana (Leguminosae)	Root	27.5
Rehmannia glutinosa (Scrophulariaceae)	Root	21.3
Sanguisorba officinalis (Rosaceae)	Root bark	48.2
Saposhnikovia divaricata (Umbelliferae)	Root bark	45.1
Scutellaria baicalensis (Labiatae)	Root bark	33.1
Sinomenium acutum (Menispermaceae)	Stem & Root	8.5
Sophora japonica (Leguminosae)	Root bark	26.0

(Jasco, Japan), and vacuum tray freeze dryer (Il Shin Engineering Co., Korea).

Animals – The animal used for this study were male Sprague-Dawley rats weighing 150-180g. The rats were purchased from Samyook Animal Laboratories, Kyunggi-do. Solid food (Samyang Yuji Co. Ltd., Korea) and tap water were supplied ad libitum. All rats were housed in a temperature-controlled (22-25°C) room and 12 h on and off lighting.

Treatments

Carbon tetrachloride-induced liver injury – The experiment was performed according to the partly modified method of Rao and Mehendale (1991). Carbon tetrachloride diluted in olive oil [CCl_4: Olive oil = 3:2 (v/v)] was intraperitoneally injected by the volume of 0.6 mg/kg for inducing hepatotoxicity except intact groups.

Administration of extracts – Thirty six extracts were suspended in distilled water with 0.5% CMC and orally administered to the rats in a volume of 0.5 ml/100 g (body weight). Control groups were received only 0.5% CMC solution. The extracts were administered 18 hr, 30 min before and 6 hr after carbon tetrachloride injection. After the final administration of extracts, all rats were fasted for 18 hr.

Measurement of serum aminotransferases and liver weight: The rats fasted for 18 hr were sacrificed with ether anesthesia. The blood sample obtained by heart puncture method was allowed to clot for 30 min at room temperature. Serum was obtained by centrifugation of the blood at 3000 rpm for 20 min and used for analyzation. Serum ALT and AST were measured with the enzyme kit by the method of Reitman and Frankel (1957). ALT and AST activity levels are presented by Karmen Unit that represents the amount of transaminase. Livers of the rats were weighed after washing with saline.

Statistical analysis: All data represent means±S.E. Statistical analyses of the data were performed using analysis of variance followed by Student's t-test. All data were evaluated at the $p<0.05$ level of significance.

Results and Discussion

The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie *et al.*, 1991). The ALT, AST activities and the ratio of liver weight to body weight are shown in Table 2. *Mentha arvensis* reduced ALT and AST by 108.7±10.8 (Karmen/ml) and 182.7±7.2, respectively. *Sanguisorba officinalis* and *Cephalonoplos segetum* significantly reduced ALT level by 101.0±9.8 and 112.7±4.3, respectively. *Bupleurum falcatum* reduced AST by 175.2±4.0 compared to the control group that showed ALT and AST level by 153.1±10.2 and 205.3±7.1, respectively.

Sophora japonica and *Benincasa hispida* reduced ALT and AST by 79.8±13.9, 157.1±3.7 and 67.0±9.2, 168.7±4.0, respectively, compared to the control group that showed ALT and AST level by 125.9±6.1 and 183.7±6.6, respectively.

Lonicera japonica (Lonicerae Flos), Agaricus blazei and *Epimedium koreanum* significantly reduced ALT by 71.3±9.4, 122.8±9.2 and 100.4±11.2 compared to control group's 149.5±6.8, and significantly reduced AST by 149.0±7.2, 215.8±2.0 and 181.3±11.7, respectively compared

Table 2. Hepatoprotective effect of MeOH extracts on CCl_4-induced hepatotoxicity

Treatment	Dose (mg/kg p.o.)	ALT activity Karmen/ml	AST activity Karmen/ml	Liver weight (% of B.W.[a)])
Intact	–	27.8± 2.4	82.6±13.3	2.0±0.2
Control	–	153.1±10.2[#]	205.3± 7.1[#]	3.7±0.1[#]
Caesalpinia sappan	1,000	142.0±16.1 (7.3)	189.6±12.3 (7.7)	3.6±0.1
Mentha arvensis	1,000	108.7±10.8* (29.0)	182.7± 7.2* (11.0)	3.5±0.2
Sanguisorba officinalis	1,000	101.0± 9.8** (34.1)	193.5± 8.6 (5.8)	3.4±0.1
Bupleurum falcatum	1,000	105.8±14.0 (21.4)	175.2± 4.0* (9.8)	4.1±0.2
Cephalonoplos segetum	1,000	112.7± 4.3* (16.2)	180.4± 9.5 (7.1)	3.7±0.1
Forsythia viridissima	1,000	144.5± 5.0 (–7.4)	187.0± 4.3 (3.7)	3.9±0.2
Silymarin	150	109.5±21.1* (28.5)	185.8±36.1* (9.5)	2.31±0.6
Intact	–	28.8± 2.3	72.6±12.2	2.1±0.2
Control	–	125.9± 6.1[#]	183.7± 6.6[#]	3.2±0.2[#]
Acanthopanax sessiliflorum	1,000	93.2±20.3 (25.9)	161.8± 8.6 (11.9)	3.2±0.2
Scutellaria baicalensis	1,000	101.5± 9.7 (19.4)	169.3± 7.5 (7.8)	2.8±0.2
Sophora japonica	1,000	79.8±13.9* (36.6)	157.1± 3.7** (14.5)	3.2±0.1
Benincasa hispida	1,000	67.0± 9.2* (32.1)	168.7± 4.0* (9.2)	3.5±0.1
Cibotium barometz	1.000	89.2±14.9 (9.6)	182.1± 3.8 (1.9)	3.2±0.2
Eugenia caryophyllata	1,000	78.1±12.5 (20.8)	176.4± 8.0 (5.0)	3.4±0.2
Silymarin	150	69.4±11.9* (29.6)	174.8± 6.0 (5.9)	3.5±0.2
Intact	–	21.4± 3.5	91.2±16.4	2.5±0.4
Control	–	149.5± 6.8[#]	225.2± 3.5[#]	4.0±0.6
Atractylodis japonica	1,000	106.2±12.4 (15.5)	172.0± 6.7 (1.7)	3.1±0.2
Gleditsia japonica	1,000	93.4±20.9 (25.7)	165.7± 9.6 (3.8)	3.8±0.1*
Lonicera japonica (Lonicerae Flos)	1,000	71.3± 9.4*** (43.3)	149.0± 7.2* (10.0)	3.4±0.2
Aaricus blazei murill	1,000	122.8± 9.2* (17.9)	215.8± 2.0* (4.2)	3.8±0.3
Alisma plantago-aquatica var. orientale	1,000	109.5±23.3 (26.8)	199.5± 5.1** (11.4)	4.3±0.2
Epimedium koreanum	1,000	100.4±11.2** (32.8)	181.3±11.7** (19.5)	3.8±0.2
Platycodon grandiflorum	1,000	146.7± 6.0 (1.9)	224.1± 3.9 (0.5)	3.9±0.2
Silymarin	150	75.2± 2.7*** (40.2)	151.6± 6.6* (9.2)	3.5±0.2
Intact	–	23.2± 3.5	89.3±15.2	2.6±0.4
Control	–	149.5± 6.8[#]	225.2± 3.5[#]	4.0±0.6
Aralia continentalis	1,000	65.8± 9.3* (56.0)	188.3± 5.6* (16.4)	3.6±0.1
Lithospermun erythrorhizon	1,000	99.6±15.6* (33.4)	201.6± 9.6* (10.0)	3.4±0.2
Lonicera japonica (Lonicerae Folium)	1,000	134.2± 4.5 (10.3)	204.0± 4.3** (9.4)	3.7±0.1
Sinomenium acutum	1,000	148.1±12.5 (1.0)	208.5± 3.6** (7.4)	4.0±0.6
Angelica gigas	1,000	116.5±28.2 (26.5)	179.6±10.2 (4.9)	4.2±0.1
Curcuma zedoaria	1,000	147.0± 5.4 (7.2)	197.4± 4.8 (–4.6)	5.4±0.1
Saposhnikovia divaricata	1,000	139.5± 8.2 (12.0)	182.1± 9.8 (3.5)	4.3±0.1
Silymarin	150	109.2±15.8* (27.0)	198.7±13.1* (11.8)	3.6±0.1
Intact	–	27.8± 2.3	90.6± 4.7	2.1±0.3
Control	–	141.4±15.7[#]	177.5±15.4[#]	3.9±0.4[#]
Germanium	200	145.0±15.1 (–2.8)	179.3± 8.9 (–1.1)	3.7±0.3
Chrysanthemum indicum	1000	130.8± 7.1 (7.8)	217.3±25.1 (–22.5)	3.8±0.1
Pueraria thunbergiana	1000	161.1± 8.4 (–14.2)	223.8±11.6 (–25.9)	4.1±0.2
Panax ginseng	1000	149.7±10.2 (–5.6)	213.4±14.7 (–20.3)	3.9±0.1
Alnus japonica	1000	159.5± 8.3 (–12.8)	219.7± 2.9 (–23.7)	3.8±0.1
Silymarin	150	104.1± 9.2* (22.6)	170.3± 6.8* (12.3)	3.3±0.2
Intact	–	28.6± 4.1	85.0± 4.8	2.5±0.5
Control	–	134.5± 8.8[#]	194.2± 6.5[#]	3.3±0.1[#]
Cassia tora	1000	122.8±14.1 (9.3)	183.8±16.8 (11.0)	3.4±0.2
Paeonia japonica	1000	111.3±25.1 (17.2)	174.2± 3.5 (10.3)	3.5±0.1
Rehmannia glutinosa	1000	129.3±20.4 (7.3)	186.6± 3.8 (4.1)	3.4±0.1
Cimicifuga foetida	1000	104.1± 7.7** (26.2)	131.5± 8.4** (32.5)	3.3±0.3
Gastrodia elata	1000	78.8±12.9* (41.8)	154.5± 4.5* (20.6)	3.2±0.1
Silymarin	150	95.0±12.2* (24.6)	157.5± 8.4* (14.2)	3.0±0.3

[#]Significantly different from the intact group (#$p<0.001$).
Significantly different from the control group ($p<0.05$. **$p<0.01$, ***$p<0.001$).
The results were expressed as mean±S.E. The figures in parentheses indicate inhibition percents.
[a)]B.W.: Body weight, n=6.

to the control group's 225.2±3.5. *Alisma plantago-aquatica var. orientale* reduced AST by 199.5±5.1.

Aralia continentalis and *Lithospermum erythrorhizon* reduced ALT and AST by 65.8±9.3, 99.6±15.6 and 188.3±5.6, 201.6±9.6, respectively. *Lonicera japonica* (Lonicerae Folium) and *Sinomenium acutum* reduced AST by 204.0±4.3 and 208.5±3.6, respectively compared to the control group that showed ALT and AST by 149.5±6.8 and 225.2±3.5, respectively.

Cimicifuga foetida and *Gastrodia elata* reduced ALT and AST by 104.1±7.7, 78.8±12.9 and 131.5±8.4, 154.5±4.5, respectively, compared to the control group that showed ALT and AST level by 134.5±8.8 and 194.2±6.5, respectively.

Briefly, *Mentha arvensis*, *Sophora japonica*, *Benincasa hispida*, *Lonicera japonica* (Lonicerae Flos), *Agaricus blazei*, *Epimedium koreanum*, *Aralia continentalis*, *Lithospermum erythrorhizon*, *Cimicifuga foetida* and *Gastrodia elata* significantly reduced ALT and AST compared to the control groups. *Sanguisorba officinalis* and *Cephalonoplos segetum* significantly reduced ALT compared to the control groups. *Bupleurum falcatum*, *Alisma plantago-aquatica* var. *orientale*, *Lonicera japonica* (Lonicerae Folium) and *Sinomenium acutum* significantly reduced AST.

The significant increase in liver weight observed when rats were treated with CCl_4 is consistent with the extablished fact that these compounds induced liver necrosis and an increase in hepatic weight, due to blockage in the synthesis of lipoprotein which carries triglyeride away from this organ, thus causing fat accumulation (Castro *et al.*, 1972; DeFerreyra *et al.*, 1974). Except *Benincasa hispida* and *Alisma plantago-aquatica* var. *orientale*, fourteen traditional medicinal plants which reduced ALT and AST activities are effective to decrease liver weight.

Herbal extracts that lowered both of ALT and AST activities can be candidates for further investigation. Also, other hepatotoxicity models induced by drugs which were not used in this study might be investigated with effective extracts in the future.

References

Butler, T. C., Reduction of carbon tetrachloride *in vivo* and reduction of carbon tetrachloride and chloroform *in vitro* by tissue and tissue constituent, *J. Pharmacol. Exp. Ther.* **134**, 311-319 (1990).

Castro, J. A., Cignoli, E. V., Castro, C. R. and Fenos, O. M. Prevention by cystamine of liver necrosis and early biochemical alterations induced by carbon tetrachloride. *Biochem. Pharmacol.* **21**, 49-57 (1972).

Chenery, R., George, M., and Krishna, G., The effect of ionophore A23187 and calcium on carbon tetrachloride-induced toxicity in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* **60**, 241-252 (1981).

Chenoweth, M. and Hake, C., The Smaller halogenated aliphatic hydrocarbons. *Ann. Rev. Pharmacol.* **2**, 363-398 (1962).

DeFerreyra, E. C., Castro, J., Diaz-Gomez, M. I., Acosta, N. D., Decastro, C. R. and DeFenos, O. M. Prevention and treatment of carbon tetrachloride hepatotoxicity by cystein: studies about its mechanism. *Toxicol. Appl. Pharmacol.* **27**, 555-568 (1974).

Hur, J., Dongeui Bogam, Namsan-dang, Seoul, 1989.

Jiansu New College Medicine Eds, Encyclopedia of Chinese Herbs, Shanghai Science & Technology Publication, Shanghai, 1977.

MacCay, P. B., Lai, E. K., Pyer, J. L., Dubose, C. M., and Janzen, E. G., Oxygen and carbon-centered free radical formation during carbon tetrachloride metabolism, *J. Biol. Chem.* **259**, 2135-2143 (1984).

Park, J. K., Human life science, Seoul National Univ. Press, 1991, pp. 449-461.

Rao C. V., and Mehendale H. M., Effect of colchicine on hepatobiliary function in CCl_4 treated rats. *Biochem. Pharmacol.* **27**, 2323-2332 (1991).

Reitman, S., and Frankel, S. Colchicine antimiosis for the determination of serum glutamic oxaloacetic and glutamic pyrubic transaminases. *Am. J. Clin. Pathol.*, **28**, 56-63 (1957).

Sallie, R., Tredger, J. and William, R., Drugs and the liver. *Biopharmaceutics and drug disposition* **12**, 251-259 (1991).

Wallach, J., Interpretation of diagnostic tests, 3th ed., Little & Brown and Co., Boston, 1978, pp. 57.

Widman, F. K., Clinical interpretation of laboratory test, 9th ed. F. A. Davis Co., Philadelphia, 1983, pp. 295.

(Accepted June 10, 2003)