Curative Effects of Extracts of *Hericium erinaceum* Hypha Cultivated with *Artemisia capillaris* (HEAC) and Their Primary Active Compounds on Rat Liver Disease

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Ethanol extract derived from *Hericium erinaceum* cultivated with *Artemisia capillaris* (HEAC) and its primary compound, scoparone, were utilized and incorporated in studying the protective effects on Carbon tetrachloride (CCl₄)-induced hepatic damage in male Sprague-Dawley rats. Male Sprague-Dawley rats were randomly divided into control, CCl₄, CCl₄+ursodeoxycholic acid (UDCA), CCl₄+silymarin, CCl₄+scoparone, CCl₄+HEAC, CCl₄+80% ethanol extract of *H. erinaceum* (HE), and CCl₄+80% ethanol extract of *Artemisia capillaris* (AC). Each group contained eight rats supplemented with UDCA, silymarin, scoparone, HEAC, HE, and AC with continuous normal diet after CCl₄ treatment. Physiological results shows control group gained weight 4.0 g day⁻¹ and that of CCl₄ group decreased 6.4 g day⁻¹. Supplementation of UDCA, silymarin, scoparone, HEAC, HE, and AC significantly decreased weight loss at 5.5, 4.1, 2.2, 5.5, and 4.2 g day⁻¹, respectively. Supplementation of UDCA, silymarin, scoparone, HEAC, HE, and AC significantly decreased serum alanine aminotransferase and aspartate aminotransferase activity as well as hepatic cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglycerides. Hepatic high-density lipoprotein (HDL)-cholesterol in CCl₄ group was reduced after CCl₄ treatment, and supplementation of UDCA, scoparone, HEAC, HE, and AC increased HDL-cholesterol level to that of control level. Atherogenic index and cardiac risks factor in CCl₄ group increased after CCl₄ treatment, and supplementation of tested compounds reduced both parameters. Taken together, HEAC and scoparone exerted protective effect against CCl₄-induced liver injury by attenuating hepatic lipid depots and reducing oxidative stress.

**Key words:** carbon tetrachloride, hepatoprotection, *Hericium erinaceum* cultivated with *Artemisia capillaris*, scoparone, silymarin, ursodeoxycholic acid

Carbon tetrachloride (CCl₄) has been classified as a possible human carcinogen, based on sufficient evidence obtained from experimental animals. Its primary metabolic pathway is cytochrome P450 (CYP) 2E1 in the liver, although it may also occur in other tissues [Wong et al., 1998]. CYP2E1 transforms CCl₄ to the bioactive metabolite form, producing the reactive trichloromethyl radical (CCl₃•) via the reductive dechlorination reaction [Zanger et al., 2000]. Under aerobic conditions, the trichloromethyl radical can act in response with oxygen to form the trichloromethylperoxyl radical (CCl₃OO•), and this highly reactive radical may attack membrane lipids in the active cells [Pohl et al., 1984]. The acute toxicity of CCl₄ has been intensively studied in animals, and its LD₅₀ value of 10,054 mg/kg body weight (bw) was reported [Dashiel and Kennedy, 1984]. Up to now, liver damage has been detected histopathologically in all treated animals as a loss of basophilic of the cytoplasm, fat, and hydropic degeneration, with occasional single-cell necrosis at high dose level [Korsrud et al., 1972]. Acute toxicity induced...
by CCl₄ in tested animals has been used for finding antioxidants which possess protective ability on liver damage, especially inhibiting lipid peroxidation by the reactive radicals on the membrane lipids.

Numerous nutritional compounds present in dietary vegetables including mushrooms prevent tumor formation by inhibiting CYP-dependent monooxygenase activity [Lee et al., 2003]. Our group previously reported some biological activities of *Hericium lucidum* extracts cultivated with *Artemisia iwayomogi* (HEAI) exerted inhibitory effects on the biotransformation of aflatoxin B₁ into aflatoxin B₁-8,9-epoxide, a potent human carcinogen, on proliferation of vascular smooth muscle cells, anti-complementary activity, and interferon-inducing activity [Lee et al., 2003; Choi et al., 2005]. HEAI also exhibited potent protective effect on the CCl₄-induced acute hepatitis in rats [Choi et al., 2005]. The protective effect was determined using biochemical parameters such as glutamic oxalacetic transaminase (GOT), glutamine pyruvic transaminase (GPT), and alkaline phosphatase (ALP). HEAI treatment significantly reduced GOT activity but not those of GPT and ALP in comparison with CCl₄ treatment alone [Choi et al., 2005]. HEAI-fed liver samples were histologically different from the liver samples without HEAI feeding before CCl₄ treatment [Choi et al., 2005].

In the present study, various physiological and biochemical parameters of rat liver after CCl₄ treatment with or without *Hericium erinaceum* cultivated with *Artemisia capillaries* (HEAC) supplementation and its primary component, scoparone, were investigated. The physiological parameters determined were body weight gain and feed efficiency ratio (FER), and biochemical parameters determined were aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, liver index, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride, atherogenic index, and cardiac risk factor. Normal group was classified as no chemical treatment only. Chemical treated groups were divided into aflatoxin B₁-extract-fed groups after CCl₄ exposure.

### Materials and Methods

**Chemicals.** CCl₄, olive oil, and scoparone (6,7-dimethoxycoumarin) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). UDCA was obtained from Daewoong Pharm (Seoul, Korea). Silymarin was obtained from Bukwang Pharm (Seoul, Korea). HEAC, *H. erinaceum*, and *A. capillaris* were supplied by Cosis Bio (Jincheon, Korea).

**Biological materials.** Male Sprague-Dawley rats (200–220 g) were housed under normal laboratory conditions (23±2°C, 55±15%, 12/12 h light-dark cycle) with free access to standard pellet (AIN-93G, Feedlab Korea, Guri, Korea) diet and water ad libitum during the experimental period. The room temperature was maintained at 25°C. HE, *A. capillaries* (AC), and HEAC were kindly supplied by H&M Bio Co., Ltd. (Chungju, Korea). Preparation of solid medium using AC for cultivating HE was performed following the method previously reported by Choi et al. [2008].

**Isolation and Identification.** HEAC (3.0 kg) was extracted two times with 80% ethanol (25 L) at room temperature for 2 days, and the extract was filtered. The resultant extract was combined and concentrated under reduced pressure at 40°C to yield about 11% (based on the weight of the dried HEAC). The 80% ethanol extract of HEAC (30 g) was sequentially partitioned into hexane (3.9 g), chloroform (8.2 g), ethyl acetate (2.9 g), butanol (2.3 g), and water-soluble (12.7 g) fractions for bioassay. The organic solvent fractions were concentrated to dryness by rotary evaporation at 40°C, and the water fraction was freeze-dried.

The dichloromethane fraction (10 g) was chromatographed on a silica gel column (70-230 mesh, 500 g, 5.5×70 cm; Merck, Darmstadt, Germany), and successively eluted with a stepwise gradient of dichloromethane/methanol (100/0, 90/10, 80/20, 70/30, 60/40, 50/50, and 0/100; v/v). Column fractions were analyzed by thin-layer chromatography (TLC; Silica gel 60, Merck, Darmstadt, Germany), and fractions with similar TLC patterns were pooled. The bioactive fraction (1.5 g) was successively rechromatographed on a silica gel column, using a stepwise gradient of n-hexane/ethyl acetate (90/10, 80/20, 70/30, 60/40, and 0/100; v/v). For further separation of the constituents, the active 60/40 fraction (500 mg) was fractionated by preparative high-performance liquid chromatography (HPLC; Spectra System P2000, Thermo Separation Products, San Jose, CA). The column was a 250×4.6 mm i.d. Cosmosil 5C₁₈-MS-II (Nacalai Tesque, Kyoto, Japan). The mobile phase was acetonitrile-water (25:75, v/v). The flow rate was 1.0 mL/min, the effluent was monitored at 254 nm, and the column temperature was set at 30°C. Finally, active principle I (1.68 mg) was isolated at the retention time of 15.2 min. Structural determination of the active isolates was made by spectroscopic analyses. 1H- and 13C-NMR spectra were recorded with a JNM-LA 400 F7 spectrometer (JEOL, Tokyo, Japan), and chemical shifts were given in δ (ppm). Correlation spectroscopy (COSY), heteronuclear multiple