J Korean Soc Appl Biol Chem (2012) 55, 159–163
DOI 10.1007/s13765-012-1023-y

ORIGINAL ARTICLE

Antiulcerogenic Activity of Scoparone on HCl/Ethanol-induced Gastritis in Rats

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Received: 9 November 2011 / Accepted: 13 January 2012 / Published Online: 30 April 2012

Abstract Protective effect of ethanol extract from *Hericium erinaceus* cultivated with *Artemisia capillaries* (HEAC) on gastric mucosal damage induced by 0.15 M HCl in ethanol in rats was evaluated. HEAC showed higher potent protective effect on gastritis with effective dose 50 (ED_{50}) value of 22.6 mg/kg compared those of selbex and stillen at 46.5 and 44.2 mg/kg, respectively, the presently used medicines for treating gastritis. ichloromethane fraction showed a dose-dependent protective effect on gastritis with ED_{50} value of 18.1 mg/kg. The biologically active component of dichloromethane fraction derived from HEAC ethanolic extract was characterized by spectroscopic analysis as scoparone with protective rate of 93% and ED_{50} value of 4.2 mg/kg on gastritis. Taken together, administration of HEAC and scoparone provided protective effect on the gastric lesion induced by ethanol-HCl and may therefore be a promising drug for treatment of gastritis and gastric ulcer.

Keywords *Artemisia capillaries* · *Hericium erinaceum* · gastritis · rat · scoparone

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Introduction

The generation of reactive oxygen species (ROS) caused by alcohol consumption has been referred to acute gastric lesions. Other factors such as stress, *Helicobacter pylori* infection, and administration of non-steroidal anti-inflammatory drugs also evoke gastritis and gastric ulcers, leading to gastric cancers. Gastric cancer has been recognized as the first leading cause of cancer death in Korea; thus attention on epidemiologic associations between risk factors and gastric cancer has increased. Risk factors including old age, male gender, *H. pylori* seropositivity, and smoking have been determined in Korean population for metaplastic gastritis, a precancerous lesion of gastric cancer (Choi et al., 2005). In relation to the risk factors in Korean gastritis, the prevalence of *H. pylori* infection dramatically increased, reaching up to 50% at 5 years of age, and 90% in asymptomatic adults over the age of 20 (Youn et al., 1996).

During the past decade in Korea, efforts have been concentrated on the discovery of new gastroprotective drugs from natural products (Huh et al., 2003; Lee et al., 2005) and some plants such as araloside A from *Aralia elata* and DA-9601 obtained from *Artemisia asiatica* are good natural sources to treat gastric ulcers (Huh et al., 2003; Lee et al., 2005).

HEAC is an ethanol extract of *Hericium erinaceus* cultivated with *Artemisia capillaries*, which has been used as a traditional oriental medicine with the anti-complementary activity (Lee et al., 2003). Recently, HEAC was found to have inhibitory effects on the biotransformation of aflatoxin B_1 to aflatoxin B_1-8,9-epoxide, which forms a single initial DNA adduct with the guanyl N_7 atom in the DNA sequence (Lee et al., 2003), and interferon-inducing activity (Lee et al., 2003). In addition, HEAC has inhibitory effect on the proliferation of human vascular smooth muscle cells (VSMCs) and protective effects on CCl_4-induced acute hepatic damage in rats (Choi et al., 2005; Choi et al., 2011).

Herein, HEAC was evaluated for gastroprotective effect in the

HCl ethanol-induced gastric lesions in rats. By bioassay-guided separation, a potential gastroprotective compound from HEAC was isolated and identified as scoparone.

Materials and Methods

Chemicals. EtOH and di-ethylether (ACS grade) were purchased from Duksan (Suwon, Korea). All chemical reagents used were from Sigma Chemical Co. (St. Louis, MO).

Biological materials. Male Sprague-Dawley rats (180–210 g) were housed in a temperature-controlled room with a 12 h light period and fed commercial solid food (Samyang Yuji Co. Ltd., Seoul, Korea) and tap water *ad libitum*. The test materials were suspended in 2% carboxymethylcellulose solution at 0.2 mL/100 g body weight. The doses of the test materials were chosen based on the yields obtained from the original extract or fractions. The room temperature was maintained at 25°C.

Isolation and Identification of scoparone. Scoparone was isolated and identificated as previously reported (Choi et al., 2011). HEAC was extracted two times with 80% ethanol at room temperature for 2 days and filtered. The resultant extract was combined and concentrated under reduced pressure at 40°C to yield about 11.0% (based on the weight of the dried HEAC). The 80% ethanol extract of HEAC was sequentially partitioned into hexane, dichloromethane, ethyl acetete, butanol, and water-soluble fractions for bioassay. The organic solvent fractions were concentrated to dryness by rotary evaporation at 40°C, whereas the water fraction was freeze-dried. The dichloromethane fraction was chromatographed on a silica gel open column (70–230 mesh, 500 g, 5.5 cm × 70 cm; Merck, Darmstadt, Germany), and successively eluted with a stepwise gradient of dichlormethane/methanol. Column fractions were analyzed by thin-layer chromatography (TLC; Silica gel G), and fractions with a similar TLC pattern were pooled. The bioactive fraction was fractionated by preparative high-performance liquid chromatography (HPLC; Spectra System P2000, Thermo Separation Products, San Jose, CA). The column was a Cosmosil $5C_{18}$-MS-II (250 mm × 4.6 mm (i.d.); Nacalai Tesque, Kyoto, Japan). The mobile phase was acetonitrile–water (25 : 75, v/v). The flow rate was 1.0 mL/min, and the effluent was monitored at 254 nm. Finally, scoparone was isolated at the retention time of 15.2 min. The spectral data of scoparone was: UV (CH_3OH) λ_{max} nm (logε) 345 (3.39), 295 (3.61), 235 (4.09); FT-IR: ν_{max} (Nujol) 3100 (aromatic C–H), 2900–2800 (CH_3), 1720 (ring C=O), 1600–1450 (aromatic C=C), 1200–1030 (C–O OCH_3), 1140 (cyclic ether C–O) cm^{-1}, EI-MS (rel. int. %): *m/z* 207 $[M + H]^+$ (9), 206 (100), 191 (46), 178 (26), 163 (37), 148 (5), 135 (23), 120 (10), 107 (21), 79 (20), 69 (20), 63 (7), 51 (17): ^1H NMR (500 MHz, $CDCl_3$): δ 3.81 (3H, s, 6-OCH_3), 3.87 (3H, s, 7-OCH_3), 6.30 (1H, d, *J*=9.6, H-2), 7.08 (1H, s, H-8), 7.26 (1H, s, H-5), 7.95 (1H, d, *J*=9.6, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.4 (6-O<u>C</u>H$_3$), 56.7 (7-O<u>C</u>H$_3$), 100.5 (C-8), 109.4 (C-5), 111.7 (C-10), 113.2 (C-3), 144.9 (C-4), 146.4 (C-6), 149.9 (C-7), 153.1 (C-9), 161.1 (C-2). The UV and NMR data of this compound were in agreement with those reported by Razdan et al. (1987) and Yamahara et al. (1989).

Ethanol/HCl-induced gastric lesion in rats. Gastroprotective activities of the samples were assessed using the ethanol–HCl induced lesion model as previously described (Schmeda-Hirschmann et al., 2002). Rats were randomly allotted into groups of eight animals each and fasted for 24 h with free access to water prior to the experiment. Fifty minutes after oral administration of samples and the currently used medicines for treating gastritis, selbex and stillen, all groups were orally treated with 0.2 mL of a solution containing 60% ethanol-0.15 M HCl (ethanol-HCl) for gastric lesion induction. Animals were sacrificed by an overdose of ether 1 h post-administration of ethanol-HCl, and the stomachs were excised and inflated by injection of saline (1 mL). The ulcerated stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. Gastric damage visible to the naked eye was observed in the gastric mucosa as elongated black-red lines, parallel to the long axis of the stomach similar to the ethanol-HCl-induced lesions in rats. The length (mm) of each lesion was measured and the lesion index was expressed as the sum of the length of all lesions. The gastritis pictures were obtained at this stage.

Histology of ethanol-HCl induced gastric lesions. The stomach of the rats subjected to gastric ulcers in the ethanol-HCl model with different treatments (normal, control, HEAC, scoparone, stillen) were pushed aside and opened through the large curvature for localization. The lesion was sectioned, and one sample was fixed in ALFAC solution containing alcohol, formaldehyde, and acetic acid for 24 h in 4°C. The samples were routinely processed for embedding in paraplast and cut into 7 μm-thick sections. These sections were stained with hematoxylin-eosin and periodic acid-Schiff (PAS) (Vocca, 1985). The samples wew analysed using a Leica microscope (Leica Qwin Software, Leica-England, Australia).

Statistical analysis. The experimental results were expressed as mean ± SD. A one-way analysis of variance (ANOVA) was used for multiple comparisons followed by application of Duncan's test. The data were considered significant if the probability was less than 0.05.

Results and Discussion

HEAC and its solvent fractions were assayed against gastric mucosal damage induced by ethanol-HCl solution. Significant differences were observed in the protective activity against the tested HEAC and solvent fractions when compared to the controls and the currently used medicines for treating gastritis, selbex and stillen. The protective effects of various fractions of HEAC at the concentration of 40 mg/kg of rats against gastritis are shown in Table 1. The hexane and dichloromethane fractions exhibited very strong protective activities against gastritis at the protection rate of

Table 1 Gastroprotective activity of various fractions obtained from the ethanol extract of *Hericium erinaceum* cultivated with *Artemisia capillaries* (HEAC) using ethanol/HCl-induced gastric lesion in rats

Treat	Dose (mg/Kg)	Gastric lesion rate (Mean ± SEM, %)	Inhibition rate (%)
Control	-	50.3±2.5	-
HEAC	40	14.9±7.2*	70.2
n-hexane		9.3±2.7*,**	81.6
dichloromethane		8.3±2.3*,**	83.6
ethyl acetate		18.9±2.4*	62.5
n-butanol		21.0±8.8*	58.3
water		41.1±2.5*	18.3

n =8 animals in each group: values are expressed as mean ± SD; in the same column, different Asterisk (*) are statistically different at $p < 0.05$; *$p < 0.05$, **$p < 0.01$, Significantly different from the control group.

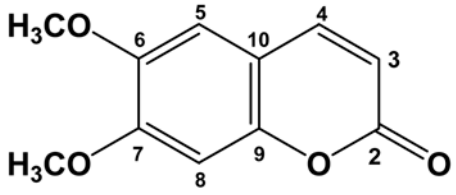

Fig. 1 Structure of scoparone.

81.6 and 83.6%, respectively. The ethyl acetate fraction also showed very strong activity against gastritis with the protection rate of 62.5%, whereas little protection activities were found in the butanol and water fractions. Due to its potent inhibitory activity against gastritis, the dichloromethane fraction was selected for further study. The dichloromethane fraction was separated into seven subfractions and very strong protective activity was observed with the active constituent, scoparone. The gastroprotective activity-guided fractionation with ethanol/HCl-induced gastric lesion in rats led to the isolation of one active principle identified as scoparone (Fig. 1) by means of spectroscopic analysis and direct comparison with the authentic compound. The compound showed protection activity with ED_{50} value of 4.2 mg/kg, whereas stillen and selbex showed 44.2 and 46.5 mg/kg against gastritis, respectively. Fig. 2 shows the images of severe gastritis after ethanol-HCl treatment without any drug as control. However, the currently used medicines, stillen and selbex, inhibited gastritis in rats. These findings show scoparone has a potent protective effect on the gastritis induced by ethanol-HCl treatment (Fig. 2).

H. erinaceus is a well-known traditional edible mushroom in Korea. The mushroom induceds maturation of dendritic cells (Kim et al., 2010), and its soluble components enhance NK cell activation via production of interleukin-12 in mice splenocytes (Yim et al., 2007). Recently, *H. erinaceus* polysaccharides have been found to induce activities of antioxidant enzymes and anti-skin aging activities (Xu et al., 2010). On the other hand, valuable constituents such as hericenone, erinacol, erinacine, and some terpenoids have been isolated from the mushroom, and their biological activities such as the stimulatory activities on nerve growth factor have been found (Lee et al., 2000; Kenmoku et al., 2002).

Similarly, *A. capillaries* possesses liver protective effects on CCl_4-induced liver fibrosis in rats (Park et al., 2000), and liver malondialdehyde levels were significantly lowered in rats treated with the ethanol-soluble portion of a hot-water extract of *A. capillaries* (Park et al., 2000). The inhibitory effects of *A. capillaries* on VSMC proliferation and intimal hyperplasia in the rat have been determined, and esculetin in the plant has been isolated as the primary compound responsible for the effects. The inhibitory activity of esculetin blocks cell proliferation *via* the inhibition of an upstream effector of Ras and downstream events

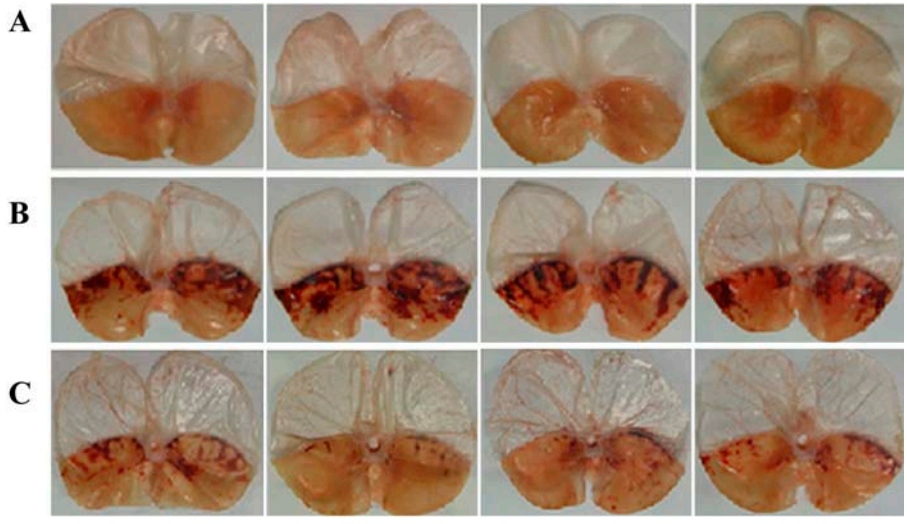

Fig. 2 Pictures of gastritis induced by ethanol-HCl solution in rat stomach. (A), normal stomachs; (B), control without any medicine on gastritis; (C), 40 mg/kg of scoparone.

Table 2 Gastroprotective activity of scoparone isolate dichloromethane fraction from the ethanol extract of HEAC using ethanol/HCl-induced gastric lesion in rats

Treat	Dose (mg/Kg)	Gastric lesion rate (Mean ± SEM, %)	Inhibition rate (%)
Control	-	50.3±2.5	-
Scoparone	40	3.4±1.4$^{*, **}$	93.2
Stillen	40	23.0±5.4*	54.2
Selbex	40	23.5±4.1*	53.2

Asterisk (*) are statistically different at $p < 0.05$; $*p < 0.05$, $**p < 0.01$, Significantly different from the control group. Eight animals were used in each experiment.

including p42/44 mitogen-activated protein kinase (MAPK) and phosphatidylinositol (PI) 3-kinase activations, immediate early gene expression, as well as nuclear factor (NF)-kappaB and activating protein (AP)-1 activations (Pan et al., 2003).

HEAC extracts showed similar liver protective activity against CCl_4-induced acute hepatic damage as compared to the extract of *A. capillaries*. Interestingly, this liver protective activity was not found in the methanol extract of *H. erinaceus* grown in media without *A. capillaries* (Choi et al., 2011). Therefore, co-cultivation of the mushroom with *A. capillaries* is important in providing the activity to the mushroom. In regard to the biological activity, HEAC shows a potent inhibitory activity on the proliferation of human VSMCs, whereas *H. erinaceus* grown in media in the absence of *A. capillaries* shows no inhibitory effects. The inhibitory effects exerted by HEAC may also be caused by new metabolites or constituents derived from *A. capillaries* (Choi et al., 2005).

In the present study, HEAC showed a strong inhibitory effect on ethanol/HCl-induced gastric mucosal damage in rats. The ED_{50} value of the inhibitory effect was stronger than those of stillen and selbex, the currently used medicine for treating gastritis. The extract was used for further isolation studies, and a coumarin was purified from the active fraction. The coumarin was characterized as scoparone by MS, ^{1}H-NMR and ^{13}C-NMR. The ED_{50} value of the coumarin on gastritis was only 4.2 mg/kg, ten times lower than those of stillen and selbex. Scoparone (Fig. 1) is one of the major coumarins in *Artemisia* sp., and several pharmacological reports of scoparone have shown the choleretic activity through increase of the bile secretion (Okuno et al., 1998), the marked inhibitory activity on the contractions induced by norepinephrine, 5-hydroxytryptamine, histamine and angiotensin II (Yamahara et al., 1989), anti-platelet activity (Okada et al., 1995), liver protective activity against CCl_4-induced acute hepatic damage (Choi et al., 2011), and the inhibitory activity on the expression of chemokines interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) in phorbol 12-myristate 13-acetate (PMA)-stimulated U937 cells to treat hepatitis and biliary tract infection in oriental countries (Jang et al., 2005). To the best of our knowledge this is the first report on the compound for treatment of gastritis. Additionally, a dramatic reduction in gastric lesions induced by HCl-ethanol treatment in rats has been found, showlng a dose-dependent effect

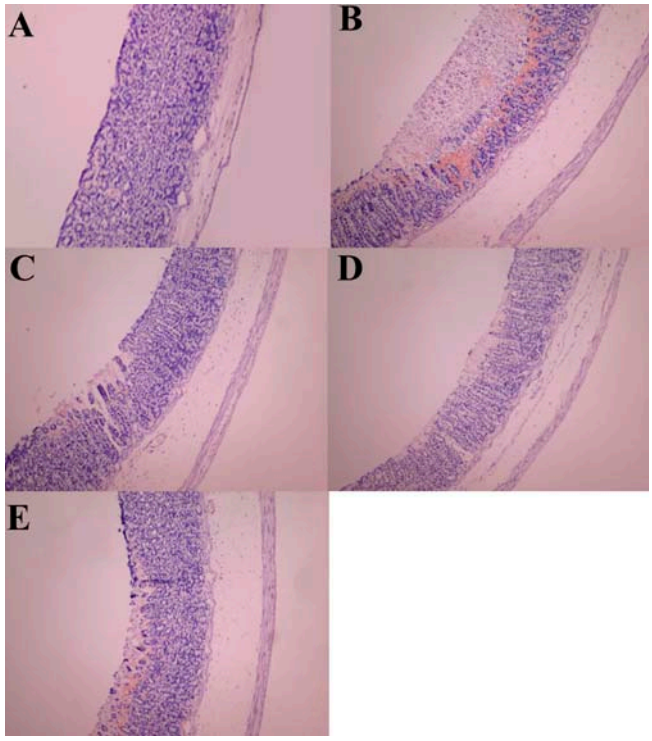

Fig. 3 Photomicrography of stomach gastric ulceration caused by ethanol-HCl solution. Gastric mucosal layer of rats stained with HE showing (A), normal epithelium from ethanol-HCl untreted rats; (B), damaged epitherlium from ethanol-HCl solution; (C), repaired epithelium after HEAC treatment; (D), repaired epithelium after scoparone treatment; (E), repaired epithelium after stillen treatment.

on the gastritis (Fig. 2 and Table 2). Furthermore, histological evidence suggests scoparone is able to repair the damaged mucosal layers induced by ethanol-HCl solution (Fig. 3).

Taken together, HEAC and scoparone showed strong inhibitory effects on gastritis induced by HCl-ethanol and are potent candidates for use as medicines to treat gastritis. Further studies are needed to assess the mode of activity of coumarins on the remedy of gastritis.

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