



Letter

Anti-inflammatory effects of *Houttuynia cordata* supercritical extract in carrageenan-air pouch inflammation model

Dajeong Kim^{1,#}, Dongsun Park^{1,#}, Jangbeen Kyung¹, Yun-Hui Yang¹, Ehn-Kyoung Choi¹,
Yoon-Bok Lee², Hyun-Kyu Kim³, Bang Yeon Hwang⁴, Yun-Bae Kim^{1*}

¹College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea

²Central Research Institute, Dr. Chung's Food Co., Ltd., Cheongju, Korea

³Institute of Life Science Research, Rexgene Biotech, Cheongweon, Korea

⁴College of Pharmacy, Chungbuk National University, Cheongju, Korea

Anti-inflammatory effects of *Houttuynia cordata* supercritical extract (HSE) were investigated in rat carrageenan-air pouch model. Oral administration of HSE (50-200 mg/kg) suppressed carrageenan-induced exudation and albumin leakage, as well as inflammatory cell infiltration at a high dose (200 mg/kg). Intraperitoneal injection of dexamethasone (2 mg/kg) only decreased exudation and cell infiltration, while indomethacin (2 mg/kg, i.p.) reduced exudate volume and albumin content without influence on the cell number. HSE lowered tumor-necrosis factor- α (TNF- α) and nitric oxide (NO), as well as prostaglandin E₂ (PGE₂). Dexamethasone only reduced TNF- α and NO, while indomethacin decreased PGE₂. The results indicate that HSE exhibits anti-inflammatory effects by inhibiting both TNF- α -NO and cyclooxygenase-2-PGE₂ pathways.

Key words: Carrageenan, inflammation, *Houttuynia cordata*, supercritical extract

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It has been reported that *Houttuynia (H.) cordata* extracts have diverse pharmacological effects including antiviral and antibacterial [1,2], antiallergic [3,4], antioxidant [5,6] and antimutagenic activities [5]. Thus, *H. cordata* Thunb has been used as an Oriental medicine for the therapy of inflammatory diseases such as ulcerative colitis [7].

Recent studies revealed that the constituents of *H. cordata* essential oil are methyl nonyl ketone, β -myrcene, β -pinene, α -pinene, α -terpineol and *n*-decanoic acid, and that the anti-inflammatory effects of this oil were also demonstrated [8]. Notably, the essential oil constituents and their concentrations varied with different extraction methods [2]. Recently, supercritical extraction technology has been adopted for the total extraction of constituents from natural plant sources.

In a previous study, we demonstrated the anti-inflammatory activity of *H. cordata* supercritical extract (HSE) in both macrophage cell line and a mouse model of carrageenan-induced air pouch inflammation [9], an *in vivo* model suitable for the analysis of diverse biochemical and pathological parameters [10,11]. In the present study, we evaluated the effectiveness of HSE in a rat model of air pouch inflammation, since the change in the inflammatory parameters was found to be different in mouse and rat models.

The aerial part of *H. cordata* was extracted for 2 hours under CO₂ supercritical conditions (temperature 60°C, pressure 400 bar). After separating the CO₂ solvent by reduced pressure, the extract (yield=1.5%) was collected [12]. The extract was dissolved in soybean oil, and orally administered at 4 mL/kg.

#These authors equally contributed to this work

*Corresponding author: Yun-Bae Kim, College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, 52 Naesudongro (Gaesin-dong), Cheongju, Chungbuk 361-763, Korea
Tel: +82-43-261-3358; Fax: +82-43-271-3246; E-mail: solar93@cbu.ac.kr

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Six-week-old male Sprague-Dawley rats (body weight 200-220 g; n=8/group) (Orient-Bio, Seongnam, Korea) were subcutaneously injected with 20 mL of sterile air into the back side to form a pouch [10,11]. After 2 and 5 days, the pouch was re-injected with 10 mL of air. Twenty four hours after the final air injection, HSE (20, 65 or 200 mg/kg) was orally administered, followed 30 min later by injection with 2 mL of lambda carrageenan (1% in saline; Sigma-Aldrich, St. Louis, USA) into the pouch. For comparisons, additional rats were given intraperitoneal injections of either dexamethasone (2 mg/kg; Sigma) or indomethacin (2 mg/kg; Sigma). The animal experiments were approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Research Center, Chungbuk National University, Korea.

The pouch was washed with 2 mL of cold saline after 6 hours, and the net volume of lavage fluid was recorded. Total numbers of inflammatory cells and albumin, a marker of vascular leakage, were determined using a coulter counter and a blood biochemistry analyzer, respectively. Tumor-necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Komabiotech, Seoul, Korea). The concentrations of NO and PGE₂ were determined by Griess reagent (Sigma) and enzyme immunoassay (EIA) using a Correlate-EIA kit (Assay Designs, Ann Arbor, Ann ArboUSA), respectively. The results were expressed as the mean \pm SD. Tests of significance were performed using Duncan's multiple-range test after one-way analysis of variance, with $P < 0.05$ as a criterion of difference.

Injection of carrageenan into rat air pouches significantly increased the exudate volume in the pouches and the albumin contents in the exudate (Table 1). However, oral treatment with HSE suppressed the carrageenan-induced

increases in both the exudate volume and albumin leakage in a dose-dependent manner, inhibiting by 28.4-43.2% and 71.4-128.6% at 20-200 mg/kg, respectively. For comparison, intraperitoneal administration of indomethacin (2 mg/kg) also reduced both the exudate volume (78.1%) and albumin content (200%). In contrast, dexamethasone (2 mg/kg) specifically suppressed exudates volume (83.8%), but not albumin leakage (14.3%).

Total white blood cells (WBC) in the exudate, including neutrophils, monocytes and lymphocytes, were greatly increased by carrageenan (Table 2). Interestingly, infiltrating inflammatory cells were suppressed to by a high dose of HSE (200 mg/kg) and dexamethasone, but not by indomethacin.

Carrageenan enormously enhanced major inflammatory cytokines TNF- α and IL-6 in the exudate (Table 3). Administration of HSE (65-200 mg/kg) and dexamethasone, significantly lowered the carrageenan-induced increases in TNF- α , while indomethacin was ineffective. In comparison, the increased IL-6 level was significantly attenuated only by dexamethasone. Interestingly, HSE (65-200 mg/kg) markedly blocked the carrageenan-induced increases in both NO and PGE₂. Dexamethasone specifically inhibited NO production, while indomethacin reversed PGE₂ concentration to the control level.

In comparison with the mouse air-pouch model [9], higher and lower responses to carrageenan exposure in exudate volume and albumin leakage, respectively, were observed in the rat model. That is, 1.80- and 3.88-fold increases in exudate volumes and 2.64- and 1.47-fold increases in albumin contents in mice and rats, respectively, were observed following carrageenan injection. An extract of *H. cordata* obtained under a supercritical condition markedly attenuated the secretion of both major inflammatory mediators, NO and PGE₂. The numbers of

Table 1. Effects of dexamethasone, indomethacin and *Houttuynia cordata* supercritical extract on carrageenan-induced exudation and inflammatory cell infiltration in rat air pouches (n=8)

Treatment	Exudate		Albumin	
	Volume (mL)	Inhibition (%)	Concentration (μ g/mL)	Inhibition (%)
Vehicle	0.33 \pm 0.11	-	0.15 \pm 0.12	-
Carrageenan alone	1.28 \pm 0.26*	-	0.22 \pm 0.06*	-
+Dexamethasone (2)	0.48 \pm 0.16#	83.8	0.21 \pm 0.14	14.3
+Indomethacin (2)	0.54 \pm 0.28#	78.1	0.08 \pm 0.04#	200.0
+HSE (20)	1.10 \pm 0.19	28.4	0.17 \pm 0.05#	71.4
+HSE (65)	0.94 \pm 0.21#	36.1	0.15 \pm 0.07#	100.0
+HSE (200)	0.87 \pm 1.14#	43.2	0.13 \pm 0.08#	128.6

HSE: *Houttuynia cordata* supercritical extract. *Significantly different from vehicle control, $P < 0.05$. #Significantly different from carrageenan alone, $P < 0.05$.

Table 2. Effects of dexamethasone, indomethacin and *Houttuynia cordata* supercritical extract on carrageenan-induced exudation and inflammatory cell infiltration in rat air pouches (n=8)

Treatment	Cells (1,000/ μ L)			
	WBC	Neutrophils	Monocytes	Lymphocytes
Vehicle	0.94 \pm 0.25	0.13 \pm 0.15	0.08 \pm 0.04	0.44 \pm 0.34
Carrageenan alone	23.81 \pm 4.11*	6.78 \pm 2.88*	1.94 \pm 0.94*	12.64 \pm 3.22*
+Dexamethasone (2)	13.94 \pm 5.44 [#]	3.41 \pm 2.21 [#]	1.11 \pm 0.76 [#]	7.21 \pm 1.25 [#]
+Indomethacin (2)	22.75 \pm 5.76	4.72 \pm 3.47	1.85 \pm 2.23	14.72 \pm 3.74
+HSE (20)	28.04 \pm 8.09	6.43 \pm 2.81	2.07 \pm 1.81	12.40 \pm 2.14
+HSE (65)	22.76 \pm 7.56	5.31 \pm 2.43	1.70 \pm 1.17	10.45 \pm 3.89
+HSE (200)	16.69 \pm 6.32	4.30 \pm 1.65 [#]	1.36 \pm 0.33 [#]	9.47 \pm 1.32 [#]

HSE: *Houttuynia cordata* supercritical extract, WBC: white blood cells. *Significantly different from vehicle control, $P < 0.05$. [#]Significantly different from carrageenan alone, $P < 0.05$.

Table 3. Effects of dexamethasone, indomethacin and *Houttuynia cordata* supercritical extract on the carrageenan-induced increases in cytokines and inflammatory mediators in rat air pouch exudates (n=8)

Treatment (mg/kg)	TNF- α		IL-6		NO		PGE ₂	
	Concentration (pg/mL)	Inhibition (%)	Concentration (pg/mL)	Inhibition (%)	Concentration (μ M)	Inhibition (%)	Concentration (pmole/mL)	Inhibition (%)
Vehicle	5.0 \pm 0.2	-	21.2 \pm 3.6	-	0.438 \pm 0.110	-	1,303.3 \pm 45.4	-
Carrageenan alone	1,018.2 \pm 150.7*	-	7,980.4 \pm 313.6*	-	0.865 \pm 0.185*	-	1,455.3 \pm 42.2*	-
+Dexamethasone (2)	279.8 \pm 58.3 [#]	72.9	3,632.5 \pm 84.0 [#]	54.6	0.224 \pm 0.143 [#]	150.1	1,381.3 \pm 88.1 [#]	48.7
+Indomethacin (2)	1,013.6 \pm 113.5	0.5	6,670.2 \pm 652.3	16.5	0.883 \pm 0.242	7.5	1,311.1 \pm 66.7 [#]	94.9
+HSE (20)	819.7 \pm 131.4	19.6	7,422.0 \pm 887.6	7.0	0.531 \pm 0.135 [#]	78.2	1,388.6 \pm 92.2	43.9
+HSE (65)	653.0 \pm 58.7 [#]	36.0	6,896.5 \pm 787.6	13.6	0.403 \pm 0.127 [#]	108.2	1,364.9 \pm 28.7 [#]	59.5
+HSE (200)	601.7 \pm 76.5 [#]	41.1	6,711.8 \pm 456.7	15.9	0.384 \pm 0.276 [#]	112.6	1,332.7 \pm 37.4 [#]	80.7

HSE: *Houttuynia cordata* supercritical extract, TNF- α : tumor-necrosis factor- α , IL-6: interleukin-6, NO: nitric oxide, PGE₂: prostaglandin E₂. *Significantly different from vehicle control, $P < 0.05$. [#]Significantly different from carrageenan alone, $P < 0.05$.

neutrophils, monocytes and lymphocytes increased by carrageenan were reduced following treatment with HSE (200 mg/kg). This was an effect obtained by dexamethasone, but not by indomethacin (1-2 mg/kg) [11,13]. Although migration of monocytes was suppressed only by a high dose (200 mg/kg) of HSE, the corticosteroid-like effect of HSE was confirmed by its inhibitory action on TNF- α and NO, which are the main inflammatory mediators from macrophages. Therefore, the effect of HSE on macrophages may come from mainly the suppression of signaling pathways, as supported by the highly-sensitive inhibition by HSE of NO secretion from RAW 264.7 cells [9].

In addition to the TNF- α -NO pathway, HSE exerted inhibitory activity on the *in vivo* release of PGE₂, which is produced from arachidonic acid via cyclooxygenase-2 (COX-2). It is assumed that HSE directly inhibits COX-2 or deactivates inflammatory cells expressing COX-2 [9]. Although there was a different sensitivity to NO and PGE₂, the production of PGE₂ was also markedly suppressed by HSE treatment in RAW 264.7 cells, mice and rats [9, the present study]. Such a difference between

in vitro and *in vivo* or between mice and rats studies may be due to the different animals and stimulators, i.e., lipopolysaccharide (LPS) and carrageenan.

There are few reports demonstrating the anti-inflammatory effects of *H. cordata*. Intravenous injection of *H. cordata* essential oils (including 22 constituents) reduced inflammatory responses in a carrageenan-induced pleurisy model, and attenuated xylene-induced ear edema [8]. Notably, our previous result shows that supercritical extract of *H. cordata* (IC₅₀ < 0.001%) is much superior to an aqueous extract (IC₅₀ = 0.1%) in the inhibition of NO production by RAW 264.7 cells [9,14].

Notably, supercritical extraction technology has been adopted for the total extraction of constituents from natural plant sources. We assessed the anti-inflammatory effects of HSE by analyzing mediators in the two major pathways of inflammation, TNF- α -NO and COX-2-PGE₂, in comparison with the synthetic steroid dexamethasone and a non-steroidal anti-inflammatory drug (NSAID) indomethacin. We demonstrated dual actions of HSE in the inflammatory process, dexamethasone- and indomethacin-like effects. Therefore, a supercritical

extract of *H. cordata* could be a good drug candidate for the relief of various types of inflammation responsive to corticosteroids or NSAIDs.

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