

## Short Communication

# Anti-inflammatory effects of a *Houttuynia cordata* supercritical extract

Sunhee Shin<sup>1,†</sup>, Seong Soo Joo<sup>2,†</sup>, Jeong Hee Jeon<sup>1</sup>, Dongsun Park<sup>1</sup>, Min-Jung Jang<sup>1</sup>, Tae-Ook Kim<sup>3</sup>, Hyun-Kyu Kim<sup>3</sup>, Bang Yeon Hwang<sup>4</sup>, Ki-Yon Kim<sup>5</sup>, Yun-Bae Kim<sup>1,5,\*</sup>

<sup>1</sup>College of Veterinary Medicine and Research Institute of Veterinary Medicine, and <sup>4</sup>College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea

<sup>2</sup>Division of Marine Molecular Biotechnology, Gangneung-Wonju National University, Gangneung 210-702, Korea

<sup>3</sup>Institute of Life Science Research, Rexgene Biotech, Cheongwon 368-885, Korea

<sup>5</sup>Department of Medicine, University of British Columbia, 2211 Wesbrook Mall, Vancouver, BC V6T 2B5, Canada

**Anti-inflammatory effects of *Houttuynia cordata* supercritical extract (HSE) were investigated in a carrageenan-air pouch model. HSE (200 mg/kg, oral) suppressed exudation and albumin leakage, as well as inflammatory cell infiltration. Dexamethasone (2 mg/kg, i.p.) only decreased exudation and cell infiltration, while indomethacin (2 mg/kg, i.p.) reduced exudate volume and albumin content. HSE lowered tumor-necrosis factor (TNF)- $\alpha$  and nitric oxide (NO), as well as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Dexamethasone only reduced TNF- $\alpha$  and NO, while indomethacin decreased TNF- $\alpha$  and PGE<sub>2</sub>. The suppressive activity of HSE on NO and PGE<sub>2</sub> production was confirmed in RAW 264.7. These results demonstrate that HSE exerts anti-inflammatory effects by inhibiting both TNF- $\alpha$ -NO and cyclooxygenase II-PGE<sub>2</sub> pathways.**

**Keywords:** carrageenan, dexamethasone, *Houttuynia cordata* extract, indomethacin, inflammation

Traditionally, *Houttuynia* (*H.*) *cordata* Thunb has been used as an Oriental medicine for the treatment of inflammatory diseases such as ulcerative colitis [3]. Previous studies showed that *H. cordata* extracts had antiviral and antibacterial [2,6], antiallergic [5], antioxidant and antimutagenic activities [1].

The major constituents of *H. cordata* essential oil included methyl nonyl ketone,  $\beta$ -myrcene,  $\beta$ -pinene,  $\alpha$ -pinene,  $\alpha$ -terpineol and *n*-decanoic acid. The anti-inflammatory effects of the oil were also demonstrated [7].

In the present study, we investigated the anti-inflammatory activity of *H. cordata* supercritical extract (HSE) in both

macrophages and a carrageenan-induced air pouch inflammation model [9,10].

The aerial part of *H. cordata* (10 kg) was extracted for 2 h under CO<sub>2</sub> supercritical conditions and the extract was collected as previously described [4]. The extract (yield = 150 g) was dissolved in soybean oil, and orally administered at 4 mL/kg.

For the effects of HSE on the secretion of inflammatory mediators, murine macrophages, RAW 264.7 cells (ATCC, USA;  $1 \times 10^6$  cells/mL), were incubated with HSE (final 0.001 ~ 1%) and lipopolysaccharide (LPS, final 2.5  $\mu$ g/mL) for 24 h. Levels of nitric oxide (NO) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) were measured by Griess reagent (Sigma, USA) and enzyme immunoassay (EIA) using a Correlate-EIA kit (Assay Designs, USA), respectively.

Male ICR mice (body weight 28 ~ 32 g; n = 8/group) were subcutaneously injected with 10 mL of sterile air into the back side to form a pouch [9,10]. After 2 and 5 days, the pouch was reinjected with 5 mL of air. Twenty four h after the final air injection, HSE (65 or 200 mg/kg) was orally administered, followed 1 h later by injection with 1 mL of lambda carrageenan (1% in saline; Sigma, USA) into the pouch. Dexamethasone (2 mg/kg; Sigma, USA) or indomethacin (2 mg/kg; Sigma, USA) were used as a positive control. 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 1 mL of cold saline after 6 h, 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 (TNF)- $\alpha$  and interleukin (IL)-6 were analyzed using ELISA kits (Komabiotech, Korea). Tests of significance were performed using Duncan's multiple-range test after one-way ANOVA with  $p < 0.05$  as a criterion of

\*Corresponding author

Tel: +82-43-261-3358; Fax: +82-43-271-3246

E-mail: solar93@cbu.ac.kr

<sup>†</sup>The first two authors contributed equally to this study.

difference.

LPS treatment increased the secretion of both NO and PGE<sub>2</sub> from macrophages, up to 25~30 fold of control levels, which were substantially suppressed by HSE in a concentration-dependent manner (Table 1).

Injection of carrageenan into mouse air pouches significantly increased the exudate volume and the albumin concentrations in the exudates (Table 2). Oral treatment with HSE suppressed the carrageenan-induced increases in the exudate volume and albumin leakage at 200 mg/kg (46.7% and 61.7%, respectively). Intraperitoneal administration of dexamethasone and indomethacin also reduced both the exudate volumes (67.1% and 60.0% respectively) and albumin contents (45.1% and 47.4% respectively). Total white blood cells (WBC) in the exudates were greatly increased by carrageenan. Interestingly, infiltrating inflammatory cells were suppressed to half level by HSE (200 mg/kg) and dexamethasone, but not by indomethacin.

Carrageenan enormously enhanced major inflammatory cytokines TNF- $\alpha$  and IL-6 in the exudates (Table 3).

Administration of HSE, dexamethasone and indomethacin significantly lowered the carrageenan-induced increases of TNF- $\alpha$ . In contrast, the increased IL-6 level was not significantly attenuated by any of the compounds. Interestingly, HSE nearly completely blocked the carrageenan-induced increases in both NO and PGE<sub>2</sub>.

HSE attenuated not only the secretion of both major inflammatory mediators, NO and PGE<sub>2</sub> but also carrageenan-induced inflammatory responses in animals. The numbers of neutrophils and lymphocytes increased by carrageenan were reduced following treatment with HSE. This was an effect obtained by dexamethasone, but not by indomethacin (1~2 mg/kg) [8,10]. The corticosteroid-like effect of HSE was confirmed by its inhibitory action on TNF- $\alpha$  and NO. Therefore, the effect of HSE might be due to the suppression of signaling activity, as supported by the highly-sensitive suppression by HSE of NO secretion from macrophages.

HSE exhibited an additional inhibitory activity on the *in vitro* and *in vivo* release of PGE<sub>2</sub>, suggesting that HSE directly inhibits COX II or deactivates inflammatory cells

**Table 1.** Effects of *Houttuynia cordata* supercritical extract (HSE) on the nitric oxide (NO) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production from RAW 264.7 cells stimulated with lipopolysaccharide (LPS)

Treatment	NO		PGE <sub>2</sub>	
	Conc. ( $\mu$ M)	Inhibition (%)	Conc. ( $\mu$ g/mL)	Inhibition (%)
Vehicle	1.20 $\pm$ 0.13	-	0.16 $\pm$ 0.03	-
LPS alone (2.5 $\mu$ g/mL)	42.10 $\pm$ 7.21*	-	3.98 $\pm$ 0.48*	-
+HSE (0.001%)	14.80 $\pm$ 2.13 <sup>†</sup>	66.7	3.74 $\pm$ 0.50	6.3
+HSE (0.01%)	14.5 $\pm$ 1.78 <sup>†</sup>	67.5	3.01 $\pm$ 0.42 <sup>†</sup>	25.4
+HSE (0.1%)	5.30 $\pm$ 0.90 <sup>†</sup>	90.0	2.42 $\pm$ 0.45 <sup>†</sup>	40.8
+HSE (1%)	2.00 $\pm$ 0.31 <sup>†</sup>	98.0	0.91 $\pm$ 0.14 <sup>†</sup>	80.4

\*Significantly different from vehicle control ( $p < 0.05$ ). <sup>†</sup>Significantly different from LPS alone ( $p < 0.05$ ). Conc.: concentration.

**Table 2.** Effects of dexamethasone, indomethacin and HSE on carrageenan-induced exudation and inflammatory cell infiltration in mouse air pouches (n = 8)

Treatment	Exudate		Albumin		Cells (1,000/ $\mu$ L)			
	Volume (mL)	Inhibition (%)	Conc. ( $\mu$ g/mL)	Inhibition (%)	WBCs	Neutrophils	Monocytes	Lymphocytes
Vehicle	0.65 $\pm$ 0.12	-	0.81 $\pm$ 0.12	-	0.86 $\pm$ 0.21	0.17 $\pm$ 0.06	0.07 $\pm$ 0.02	0.55 $\pm$ 0.15
Carrageenan alone	1.17 $\pm$ 0.12*	-	2.14 $\pm$ 0.22*	-	8.80 $\pm$ 1.32*	1.01 $\pm$ 0.19*	0.46 $\pm$ 0.17*	7.07 $\pm$ 1.58*
+Dexamethasone	0.82 $\pm$ 0.08 <sup>†</sup>	67.1	1.54 $\pm$ 0.43 <sup>†</sup>	45.1	4.54 $\pm$ 1.19 <sup>†</sup>	0.80 $\pm$ 0.23	0.20 $\pm$ 0.05 <sup>†</sup>	2.51 $\pm$ 0.75 <sup>†</sup>
+Indomethacin	0.86 $\pm$ 0.08 <sup>†</sup>	60.0	1.51 $\pm$ 0.12 <sup>†</sup>	47.4	8.54 $\pm$ 1.46	1.09 $\pm$ 0.29	0.38 $\pm$ 0.10	6.76 $\pm$ 2.08
+HSE (65 mg/kg)	1.07 $\pm$ 0.10	19.2	1.81 $\pm$ 0.43	24.8	8.13 $\pm$ 1.92	1.67 $\pm$ 0.28	0.74 $\pm$ 0.16	4.21 $\pm$ 1.39 <sup>†</sup>
+HSE (200 mg/kg)	0.93 $\pm$ 0.04 <sup>†</sup>	46.7	1.32 $\pm$ 0.21 <sup>†</sup>	61.7	4.94 $\pm$ 1.44 <sup>†</sup>	0.62 $\pm$ 0.21 <sup>†</sup>	0.43 $\pm$ 0.17	3.85 $\pm$ 0.85 <sup>†</sup>

\*Significantly different from vehicle ( $p < 0.05$ ). <sup>†</sup>Significantly different from carrageenan alone ( $p < 0.05$ ).

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

Treatment	TNF- $\alpha$		IL-6		NO		PGE <sub>2</sub>	
	Conc. (pg/mL)	Inhibition (%)	Conc. (pg/mL)	Inhibition (%)	Conc. ( $\mu$ M)	Inhibition (%)	Conc. (pmole/mL)	Inhibition (%)
Vehicle	5.0 $\pm$ 0.1	-	2.0 $\pm$ 0.4	-	0.391 $\pm$ 0.074	-	1,228.1 $\pm$ 43.8	-
Carrageenan alone	181.7 $\pm$ 50.0*	-	> 500.0*	-	0.774 $\pm$ 0.112*	-	1,439.5 $\pm$ 18.4*	-
+Dexamethasone	14.1 $\pm$ 5.8 <sup>†</sup>	94.9	442.8 $\pm$ 53.0	11.5	0.054 $\pm$ 0.023 <sup>†</sup>	188.0	1,411.3 $\pm$ 23.7	13.3
+Indomethacin	108.2 $\pm$ 22.4 <sup>†</sup>	41.6	473.8 $\pm$ 20.5	5.3	0.663 $\pm$ 0.203	29.0	1,222.3 $\pm$ 11.0 <sup>†</sup>	102.7
+HSE (65 mg/kg)	56.7 $\pm$ 15.2 <sup>†</sup>	70.7	> 500.0	0.0	0.412 $\pm$ 0.125 <sup>†</sup>	94.5	1,149.6 $\pm$ 51.6 <sup>†</sup>	137.1
+HSE (200 mg/kg)	79.1 $\pm$ 20.1 <sup>†</sup>	58.1	> 500.0	0.0	0.403 $\pm$ 0.187 <sup>†</sup>	96.9	1,218.5 $\pm$ 41.2 <sup>†</sup>	104.5

TNF- $\alpha$ : tumor-necrosis factor- $\alpha$ , IL-6: interleukin-6. \*Significantly different from vehicle control ( $p < 0.05$ ). <sup>†</sup>Significantly different from carrageenan alone ( $p < 0.05$ ).

expressing COX II. In spite of a less-sensitive inhibition compared to NO from macrophages, *in vivo* production of PGE<sub>2</sub> was fully suppressed by HSE treatment. Such a difference between *in vitro* and *in vivo* studies may be due to the different stimulators, LPS and carrageenan, respectively.

Notably, our result show that HSE (IC<sub>50</sub> < 0.001%) was much superior to an aqueous extract (IC<sub>50</sub> = 0.1%) in the inhibition of NO production by macrophages [10].

In summary, we first demonstrated the anti-inflammatory effects of HSE by analyzing mediators in the two major pathways of inflammation (TNF- $\alpha$ -NO and COX II-PGE<sub>2</sub>). Mechanistically, it was believed that HSE played a dual actions in the inflammatory process, dexamethasone- and indomethacin-like effects, implying that additional constituents inhibiting the COX II-PGE<sub>2</sub> pathway might be present in HSE, in addition to those acting on the TNF- $\alpha$ -NO pathway. Therefore, HSE could be a better drug candidate or adjunct than aqueous extracts for the relief of various types of inflammation that are responsive to corticosteroids or NSAID.

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## References

1. **Chen YY, Liu JF, Chen CM, Chao PY, Chang TJ.** A study of the antioxidative and antimutagenic effects of *Houttuynia cordata* Thunb. using an oxidized frying oil-fed model. *J Nutr Sci Vitaminol* (Tokyo) 2003, **49**, 327-333.
2. **Hayashi K, Kamiya M, Hayashi T.** Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus, and HIV. *Planta Med* 1995, **61**, 237-241.
3. **Jiang XL, Cui HF.** Different therapy for different types of ulcerative colitis in China. *World J Gastroenterol* 2004, **10**, 1513-1520.
4. **Lee HY, Kim YJ, Kim EJ, Song YK, Byun SY.** Red pigment from *Lithospermum erythrorhizon* by supercritical CO<sub>2</sub> extraction. *J Cosmet Sci* 2008, **59**, 431-440.
5. **Lee JS, Kim IS, Kim JH, Kim JS, Kim DH, Yun CY.** Suppressive effects of *Houttuynia cordata* Thunb (Saururaceae) extract on Th2 immune response. *J Ethnopharmacol* 2008, **117**, 34-40.
6. **Lu H, Wu X, Liang Y, Zhang J.** Variation in chemical composition and antibacterial activities of essential oils from two species of *Houttuynia* THUNB. *Chem Pharm Bull* (Tokyo) 2006, **54**, 936-940.
7. **Lu HM, Liang YZ, Yi LZ, Wu XJ.** Anti-inflammatory effect of *Houttuynia cordata* injection. *J Ethnopharmacol* 2006, **104**, 245-249.
8. **Oliveira de Melo J, da Conceição Torrado Truiti M, Muscará MN, Bolonheis SM, Dantas JA, Caparroz-Assef SM, Cuman RK, Bersani-Amado CA.** Anti-inflammatory activity of crude extract and fractions of *Nectandra falcifolia* leaves. *Biol Pharm Bull* 2006, **29**, 2241-2245.
9. **Romano M, Faggioni R, Sironi M, Sacco S, Echtenacher B, Di Santo E, Salmona M, Ghezzi P.** Carrageenan-induced acute inflammation in the mouse air pouch synovial model. Role of tumour necrosis factor. *Mediators Inflamm* 1997, **6**, 32-38.
10. **Wallace JL, Chapman K, McKnight W.** Limited anti-inflammatory efficacy of cyclo-oxygenase-2 inhibition in carrageenan-airpouch inflammation. *Br J Pharmacol* 1999, **126**, 1200-1204.