Antipruritic Effect of *Cnidii Monnieri* Fructus (Fruits of *Cnidium monnieri* CUSSON)

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Antipruritic effects of 70% ethanol extract (CM-ext) of *Cnidii Monnieri* Fructus (dried fruits of *Cnidium monnieri* CUSSON, Umbelliferae) were investigated. In mice, an oral administration of CM-ext (200 and 500 mg/kg) inhibited compound 48/80-induced scratching behavior without influence on spontaneous locomotion. Isopimpinellin (3) and osthol (1), coumarin derivatives isolated from CM-ext, showed an inhibitory effect on compound 48/80-induced scratching behavior.

Key words *Cnidium monnieri*; antipruritic effect; isopimpinellin; osthol

Pruritus is discomfort cutaneous sensation for patients having a skin disorder such as atopic dermatitis, and is often accompanied by scratching behavior. Antihistaminic, steroid and antiallergic drugs are used for the treatment of pruritus. During the course of our studies seeking antipruritic agents from natural resources, we developed an experimental mice model to treat a number of samples in a short time to evaluate the antipruritic effect.¹⁾ Using this model, namely compound 48/80-induced scratching behavior in ddY-mice, several herb medicines whose antipruritic effect was expected from various herbal literature were screened. Among them, *Kochiae* Fructus (dried fruits of *Kochia scoparia* L.) and *Cnidii Monnieri* Fructus (dried fruits of *Cnidium monnieri* CUSSON) were found to exhibit an antipruritic effect. Momordin Ic, an oleanane-type saponin, isolated from *Kochiae* Fructus was ascertained to be one of the active components.¹⁾ Recently, Basnet *et al.*²⁾ reported that a mixture of coumarin derivatives obtained from *Cnidii Monnieri* Fructus exhibited an antipruritic action on substance P-induced itching model ICR mice. However, inhibition of compound 48/80-induced scratching behavior in ddY mice by 70% ethanol extract (CM-ext) of *Cnidii Monnieri* Fructus has not been investigated in detail. The object of this report³⁾ is to examine the antipruritic components of CM-ext using compound 48/80-induced itching model mice.

MATERIALS AND METHODS

Plant Material *Cnidii Monnieri* Fructus (dried fruits of *Cnidium monnieri* CUSSON produced in China) was purchased from Yamada Yakken Co., Ltd. Pharmacy (Osaka) in 1999. A voucher specimen has been deposited at Kinki University, Osaka, (voucher number: CM-99001).

Preparation of CM-ext from *Cnidii Monnieri* Fructus The crushed fruits (500 g) of *Cnidium monnieri* were extracted twice with 70% EtOH (5 l each) under reflux for 1 h. The combined filtrates were concentrated under reduced pressure and lyophilized to give a brown powder (CM-ext) (73.7 g, yield; 14.7%).

Fractionation of CM-ext and Isolation of Osthol (1), Imperatorin (2), and Isopimpinellin (3) Melting points were determined on a Yanagimoto micro melting point appa-

ratus without correction. All chemical reagents were reagent grade and were purchased from Wako Pure Chemical Industries, Ltd. (Osaka), unless otherwise noted. The CM-ext (53.7 g) was suspended in water and then extracted successively with hexane, EtOAc, and 1-BuOH. After solvent extraction, the aqueous layer was concentrated under reduced pressure and lyophilized to afford an aqueous soluble fraction (26.7 g) (Chart 1). The hexane extract was dried over Na₂SO₄ and concentrated. The resulting crystalline precipitate (2.47 g) was collected by filtration, and its mother liquor was concentrated to give a hexane soluble fraction (3.9 g). The EtOAc extract was dried over Na₂SO₄ and concentrated to give an EtOAc soluble fraction (9.9 g). The 1-BuOH extract was concentrated to give a 1-BuOH soluble fraction (3.9 g). Antipruritic activity of each fraction was assayed by the compound 48/80-induced scratching behavior method described below. The data are shown in Chart 1. The most active crystalline precipitate (2.3 g) exhibiting three main spots on TLC [silica gel 60 F254 (Merck No. 1.05735), solvent system; hexane–EtOAc (3:1 v/v), detection; UV] was chromatographed on silica gel (No. 1.07734, Merck, Darmstadt, Germany) eluted with a gradient of hexane and EtOAc. The eluent was separated into 5 fractions (Fraction 1–5) by monitoring with TLC. Fraction 1 was recrystallized from diethyl ether–hexane to give osthol (1), 756 mg, colorless prisms, mp 82–83 °C. Fraction 3 was purified by rechromatography on silica gel (No. 1.05735, Merck, Darmstadt, Germany) eluted with benzene–EtOAc (10:1 v/v) and recrystallization from EtOAc–hexane to give imperatorin (2), 95 mg, colorless needles, mp 97–98 °C. Fraction 5 was recrystallized from EtOH–hexane to give isopimpinellin (3), 48 mg, pale yellow needles, mp 139–141 °C. The known compounds (1, 2, and 3) whose chemical structures are depicted in Fig. 1 were identified by comparison of their physical data (mp, IR, ¹H- and ¹³C-NMR) with reported values.^{4–7)}

Pharmacological Test Animals Male Wistar rats (180–200 g), male ddY mice (26–28 g), and male Hartley guinea pigs (200–250 g) were provided by SLC (Japan SLC, Hamamatsu). They were maintained in an air-conditioned room with lighting from 7 a.m. to 7 p.m. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. Laboratory pellet chows (Labo MR Stock and

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Fig. 2. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext) and Diphenhydramine (Diphen.) on Compound 48/80-Induced Scratching Behavior in Mice

Mice were dosed orally with test substances suspended in 0.2% CMC·Na 1 h before subcutaneous injection of compound 48/80 (100 mg/mouse). Immediately after the injection, the times of scratching were counted over a 10 min period. Each value represents the mean ± S.E. ($n=12-14$). Significantly different from control at **: $p<0.01$.

the mixture was incubated at 37 °C. After 10 min, 0.1 ml of compound 48/80 solution (0.2 mg/ml) was added, and the mixture was incubated at 37 °C for 10 min in a final volume of 2 ml. Cooling the mixture on ice terminated the reaction. The mixture was centrifuged at $150\times g$ and 5 °C for 5 min, then histamine in the supernatant fluid was assayed fluorometrically according to the method of Shore *et al.*¹⁰ The activity of the test substance on histamine release from mast cells induced by compound 48/80 was expressed as histamine release percentage. SCG was used as a reference drug.

Measurement of Histamine-Induced Contraction in Isolated Guinea Pig Ileum A guinea pig was sacrificed by a blow on the head and the ileum was isolated. A length of 1.5 to 2.0 cm was suspended in Tyrode's solution bubbled through with air in an organ bath maintained at 37 °C. Ileum contractions were isotonicly recorded by means of a lever loaded with 0.5 g on a smoke drum. The ileum was preincubated with CM-ext (10^{-4} g/ml) dissolved in 5% DMSO/Tyrode's solution or diphenhydramine (3×10^{-7} M) dissolved in Tyrode's solution for 3 min, and then histamine (10^{-10} — 10^{-5} M) was added and incubation was carried out for 3 min at 37 °C. The extent of contraction was estimated from the maximal contraction of the isolated ileum after the addition of histamine alone.

Statistical Analysis The experimental data were tested for statistically significant differences by Bonferroni/Dunn's method (Multiple Range Test).

RESULTS

Effect of CM-ext on Compound 48/80-Induced Scratching Behavior CM-ext (200 and 500 mg/kg, *p.o.*) inhibited the scratching behavior caused by compound 48/80 without influence on spontaneous locomotion. Diphenhydramine (50 mg/kg, *p.o.*) also showed such inhibitory effect without influence on spontaneous locomotion.

Effect of CM-ext on Spontaneous Locomotion CM-ext (200 and 500 mg/kg, *p.o.*) did not change the spontaneous locomotor activity of 10 mice per group. (Data not shown)

Effect of CM-ext on Compound 48/80-Induced Histamine Release Treatment with CM-ext (50, 200, 500 mg/ml) significantly suppressed the histamine release. A refer-

Fig. 3. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext) and Sodium Cromoglycate (SCG) on Compound 48/80-Induced Histamine Release from Peritoneal Mast Cells of Rats

Mast cells prepared from the peritoneal cavity fluid of rats were suspended in phosphate-buffered saline at 2.9×10^6 cells/ml. The mast cell suspensions treated with CM-ext or SCG were stimulated by compound 48/80 (10 mg/ml). Each value represents the mean ± S.E. ($n=3$). Significantly different from control at *: $p<0.05$, **: $p<0.01$.

Fig. 4. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext) and Diphenhydramine on the Concentration-Action Curves of Phasic Contraction by Histamine with the Isolated Ileum of Guinea Pig

○, control; □, CM-ext 10^{-4} g/ml; ●, diphenhydramine 3×10^{-7} M. Each value represents the mean of 3 experiments.

ence drug, SCG, at a concentration of 500 µg/ml also inhibited the release.

Effect of CM-ext on Histamine-Induced Contraction in Isolated Ileum In the experiment shown in Fig. 4, histamine by itself caused the contraction of isolated guinea pig ileum with an S-shaped log(dose)-response curve at concentration of 10^{-10} — 10^{-5} M. A reference compound, diphenhydramine (3×10^{-7} M) showed a competitive antagonistic effect. In the presence of CM-ext (10^{-4} g/ml), the dose-response curve was shifted to the right but altered slightly in shape.

Antipruritic Activity of Coumarin Derivatives Three known coumarin derivatives, osthol (1), imperatorin (2), and isopimpinellin (3), were isolated from CM-ext. On the antipruritic assay results shown in Table 1, isopimpinellin (3) was found to be an active constituent, whereas osthol (1) was less active than 3, and imperatorin (2) was ineffective.

DISCUSSION

Kuraishi *et al.*¹¹ reported that scratching behavior is caused dose-dependently by a dorsal subcutaneous injection of compound 48/80¹² in ddY male mice. It is well known that compound 48/80 releases many chemical mediators

Table 1. Effects of Coumarin Derivatives Isolated from *Cnidii Monnieri* Fructus and Diphenhydramine on Compound 48/80-Induced Scratching Behavior in Mice

Treatment	Dose (mg/kg)	Scratching times	Inhibition (%)
Control	—	41.1±6.0	—
Osthol	20	26.8±4.0*	34.7
	50	27.3±4.5*	33.6
	100	27.5±5.0*	33.1
Diphenhydramine	50	4.4±1.7**	89.3
Control	—	44.6±3.5	—
Imperatorin	20	48.2±7.1	-8.1
	50	39.7±5.0	11.0
	100	42.3±6.5	5.2
Isopimpinellin	20	26.5±7.8*	40.6
	50	26.8±5.4*	39.9
	100	16.2±3.4**	63.7
Diphenhydramine	50	3.0±1.1**	93.3

Mice were dosed orally with the test substances suspended in 0.2% CMC·Na 1 h before subcutaneous injection of compound 48/80 (100 µg/mouse). Immediately after the injection, the times of scratching were counted over a 10 min period. Each value represents the mean±S.E. (n=8–12). Significantly different from the control at *: $p<0.05$, **: $p<0.01$.

from mast cell.^{13–16} Although histamine is the major chemical mediator inducing itching, histamine did not induce scratching behavior in ddY mice as described in the previous paper.¹⁾

CM-ext significantly inhibited compound 48/80-induced scratching behaviors of ddY mice without influence on spontaneous locomotion. *Cnidii Monnieri* Fructus has been used to treat pain in female genitalia, impotence and suppurative dermatosis as an antipruritic agent in ancient China. *Cnidii Monnieri* Fructus has been reported to show antiallergic,¹⁷⁾ androgenic¹⁸⁾ and antibacterial⁶⁾ activities. Recently, Basnet *et al.*²⁾ reported that a so-called CS-3 fraction obtained from a methanol extract of *Cnidii Monnieri* Fructus showed an antipruritic action on the substance P-induced itching model of ICR mice, and that this effect may be due to additive or synergistic action of several coumarin derivatives, such as xanthotoxin, isopimpinellin, bergapten, imperatorin, osthol, and unidentified minor compounds.

The active components of CM-ext were isolated by activity guided fractionation and column chromatography monitoring with the inhibitory effect on compound 48/80-induced scratching behavior. An active principle was found to be a furocoumarin, isopimpinellin (**3**). Osthol (**1**) did not show any significant effect on substance P-induced itching model of ICR mice,²⁾ but showed a relatively strong inhibitory effect on compound 48/80-induced scratching behavior in ddY

mice. These results implied that there are some mechanistic differences between these two itching animal models due to the inducing agents and/or strains of mice. Coumarin derivatives, **1**, **2** and **3**, were isolated earlier from *Cnidium monnieri*,⁶⁾ but antipruritic activities of **1** and **3** on compound 48/80-induced itching model mice were found for the first time.

Some attempts to examine the biochemical inhibition mechanism of compound 48/80-induced scratching behavior by CM-ext revealed that CM-ext showed the inhibitory effects on release of histamine from mast cells induced by compound 48/80, and on the contraction of the excised guinea pig ileum induced by histamine. However, the question of whether the inhibitory effects of CM-ext are directly correlated with histamine during the course of compound 48/80-induced scratching behavior in ddY mice remains unanswered. Further detailed studies on the inhibition mechanism of CM-ext, **1** and **3** are in progress.

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