

Anti-allergic Effects of *Cnidii Monnieri* Fructus (Dried Fruits of *Cnidium monnieri*) and Its Major Component, Osthol

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Anti-allergic effects (types I and IV) of the 70% ethanol extract (CM-ext) obtained from *Cnidii Monnieri* Fructus (dried fruits of *Cnidium monnieri*) were investigated on 48 h homologous passive cutaneous anaphylaxis (PCA), 2, 4-dinitrofluorobenzene (DNFB)-induced contact dermatitis and picryl chloride (PC)-induced contact dermatitis in experimental animals. CM-ext showed inhibitory effects on these allergic models. Osthol isolated from CM-ext also had the inhibitory effects. These results suggested that *Cnidii Monnieri* Fructus might be useful as an agent for allergic diseases and that its anti-allergic effect was partially attributable to a coumarin derivative, osthol.

Key words *Cnidii Monnieri* Fructus; *Cnidium monnieri*; osthol; anti-allergic effect; antipruritic effect

Cnidii Monnieri Fructus, dried fruits of *Cnidium monnieri* CUSSON (Fam. Umbelliferae), has been used for treatment of pain in female genitalia, impotence and suppurative dermatitis as an antipruritic agent in ancient China. There have been reports on pharmacological studies of this dried fruit, such as its anti-allergic action,¹⁾ androgenic action²⁾ and antibacterial effect.³⁾ Recently, Basnet *et al.*⁴⁾ reported that a mixture of coumarin derivatives obtained from *Cnidii Monnieri* Fructus exhibited an antipruritic action on substance P-induced itching model in ICR mice. We have found that the 70% EtOH extract and two coumarin derivatives, osthol and isopimpinellin, isolated from the extract had inhibitory effects on cutaneous pruritus induced by compound 48/80 in mice without influence on spontaneous locomotion.⁵⁾ The target of the present study was to examine the effects of *Cnidii Monnieri* Fructus extract and its major component, osthol, on allergic responses related closely to pruritus using allergic model animals.

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 70% Ethanol Extract 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 brown powder (CM-ext) (73.7 g, yield; 14.7%).

Antigens and Chemicals The following materials were used in this study: egg albumin (OVA, Grade V, Sigma, St. Louis, U.S.A.), 2,4-dinitrofluorobenzene (DNFB), Evans blue, picryl chloride (PC), prednisolone, carboxymethylcellulose sodium (CMC·Na) (Nacalai Tesque, Kyoto), an inactive bacterial suspension of *Bordetella pertussis* (Wako Pure Chemical, Osaka), sodium cromoglycate (SCG, Funakoshi, Tokyo) and diphenhydramine hydrochloride (diphenhydramine, Tokyo Kasei, Tokyo). Osthol whose chemical struc-

ture depicted in Fig. 1 was isolated from CM-ext in our laboratories,⁵⁾ and identified by comparison of its physical data (mp, IR, ¹H-, and ¹³C-NMR) with reported values.⁶⁾

Animals Male Wistar strain rats (180–200 g) and female ICR strain mice (24–26 g or 30–32 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, Nihon Nosan Kogyo K.K., Yokohama) and water were freely available.

Forty-Eight-Hour Homologous Passive Cutaneous Anaphylaxis (PCA, Type I Allergic Model) Rat anti-OVA serum containing IgE was prepared by the method of Stotland and Share.⁷⁾ The rats were sensitized by an intradermal injection of 0.5 ml of saline suspension containing OVA (1.0 mg) and aluminum hydroxide gel (10 mg), followed by an intraperitoneal injection of 1.0 ml of an inactive bacterial suspension of *Bordetella pertussis* (2×10^{10} cells/ml). After 7 d, they were again sensitized by an intramuscular injection of 0.5 ml of the saline suspension, followed by an intraperitoneal injection of 1.0 ml of the inactive bacterial suspension. Fourteen days after the first sensitization, the rats were anesthetized with pentobarbital, blood was withdrawn from the carotid arteries, and rat anti-OVA serum was obtained in the usual manner. The anti-OVA IgE antibody titer was determined by 48 h homologous PCA in rats. Titers were expressed as the reciprocal ratio to the final dilution yielding a positive reaction measuring approximately 5 mm in diameter. The titer of the anti-OVA serum containing IgE was 1:32. The serum was stored at –80 °C until use.

The serum diluted 8-fold with physiologic saline was in-

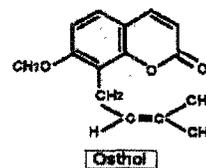


Fig. 1. Chemical Structure of Osthol

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jected intradermally on the shaved dorsal skin of rats in a 0.05 ml/site dose. Forty-eight hours after sensitization, the rats were challenged with 0.5 ml of saline solution containing OVA (2.0 mg) and Evans blue (5.0 mg) *via* the tail vein ($n=8$ to 10 per group). Thirty min later, the animals were sacrificed, and the dorsal skin was stripped off. A circular-cutting of dorsal skin (diameter, 10 mm) was used to measure the amount of blue dye. The amount of leaked dye ($\mu\text{g}/\text{site}$) was determined by the method of Katayama *et al.*⁸⁾ After alkaline fusion of the circular skin in 1.0 N KOH (1.0 ml) at 37 °C for 48 h, 0.6 N H_3PO_4 (2.5 ml) and acetone (6.5 ml) were added to the reaction mixture. Following vigorous shaking and centrifugation ($900\times g$, 10 min), spectrophotometric determination at 620 nm of the supernatant gave the amount of leaked dye ($\mu\text{g}/\text{site}$). Test substances suspended in 0.2% CMC·Na were administered orally 1 h before the antigen challenge. SCG dissolved in saline was administered intravenously 1 min before the challenge. The experimental results were expressed as the average amount of dye ($\mu\text{g}/\text{site}$) \pm S.E. ($n=8$ to 10 per group).

Dinitrofluorobenzene (DNFB)-Induced Contact Dermatitis (Type I Allergic Model) a) Ear Swelling Experiments: Dinitrophenyl-derivatized ovalbumin (DNP-OVA) was prepared according to the method described by Eisen *et al.*⁹⁾ and was used as an antigen. The biphasic cutaneous reaction was elicited by the method reported by Watanabe *et al.*¹⁰⁾ Mice weighing 24–26 g ($n=8$ to 12 per group) were sensitized by an intraperitoneal injection of a mixture of DNP-OVA (10 μg) and aluminum hydroxide gel (1 mg) in saline (0.2 ml). After one week, the mice were challenged by painting 10 μl of 0.1% DNFB solution in EtOH on the inside and outside of the right and left ears. Next day, the mice were again sensitized by the mixture of DNP-OVA (10 μg) and aluminum hydroxide gel (1 mg) in saline (0.2 ml). Then, after one week, they were challenged by painting of 0.1% DNFB on each ear. The thickness of the right ear was measured using a dial thickness gauge (Mitutoyo Co., Tokyo) immediately before, and 1 h [immediate phase response (IPR)] and 24 h [late phase response (LPR)] after the DNFB challenge. The ear swelling (increment of ear thickness) was expressed in percentage difference between the ear thickness (100%) immediately before the DNFB challenge and those 1 h (IPR) or 24 h (LPR) after the challenge. In a nonsensitized control group, sensitization procedures by DNP-OVA and administrations of test substances were omitted. Test substances, diphenhydramine, and prednisolone were suspended in 0.2% CMC·Na, and given orally 1 h before the DNFB challenge. The experimental results were expressed as the average percent change of ear swelling \pm S.E. ($n=8$ to 10 per group).

b) Scratching Behavior Experiments: In the above experiments, the number of scratchings on the DNFB challenged sites by hind paws of each mouse were counted for 1 h immediately after the DNFB challenge. The experimental results were expressed as the average number of scratchings \pm S.E. ($n=8$ to 12 per group).

Picryl Chloride (PC)-Induced Contact Dermatitis (Type IV Allergic Model) This procedure was in accordance with the method reported by Asherson and Ptak.¹¹⁾ Mice weighing 30–32 g were sensitized by topical application of 0.1 ml of 7% PC solution in EtOH to the shaved abdomen. After 6 d sensitization period, the mice were chal-

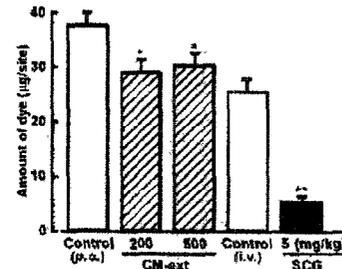


Fig. 2. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext) and Sodium Cromoglycate (SCG) on 48 h Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats

CM-ext suspended in 0.2% CMC·Na was orally administered to rats immunized with the rat anti-OVA serum 1 h before the challenge with the antigen. SCG dissolved in saline was intravenously administered 1 min before the challenge. Each value represents the mean \pm S.E. ($n=8$ to 10). Significantly different from the control at *, $p<0.05$, **: $p<0.01$.

lenged by painting of 0.02 ml of 1% PC solution in olive oil to the inside of the right ear. The ear thickness was measured immediately before and 24 h after the PC challenge, and calculated as percent change of ear swelling as described above. For the study of the effector phase of PC-induced contact dermatitis, mice showing an increment of percent change (over 25%) of ear swelling were chosen. Three days thereafter, the selected mice ($n=12$ to 14 per group) were sensitized again by application of 0.1 ml of 7% PC solution in EtOH to the shaved abdomen. Six days later, the mice received the last challenge with 0.02 ml of 1% PC solution in olive oil as described above. The ear thickness was measured again immediately before and 24 h after the last PC challenge, and calculated as percent change of ear swelling as described above. Test substances suspended in 0.2% CMC·Na were administered orally immediately before and 16 h after the last PC challenge. Prednisolone suspended in 0.2% CMC·Na was administered orally 16 h after the last PC challenge. The experimental results were expressed as the average percent change of ear swelling \pm S.E. ($n=12$ to 14 per group).

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 Forty-Eight-Hour Homologous PCA The dye leakage caused by PCA in rats was significantly decreased by CM-ext at doses of 200 and 500 mg/kg, *p.o.* as shown in Fig. 2. SCG at a dose of 5 mg/kg, *i.v.*, caused the inhibition.

Effects of CM-ext and Osthol on DNFB-Induced Contact Dermatitis As shown in Fig. 3, the challenge with DNFB in sensitized mice caused a biphasic skin reaction with two peaks (IPR and LPR) at 1 and 24 h. CM-ext (200 and 500 mg/kg, *p.o.*) as well as prednisolone (20 mg/kg, *p.o.*) significantly inhibited the ear swellings of IPR and LPR. Diphenhydramine (50 mg/kg, *p.o.*) showed the inhibitory effect only on IPR. Its major component, osthol at an oral dose of 20 mg/kg, also showed the inhibitory effect (Fig. 4).

The inhibitions of the scratching behaviors by CM-ext using the same animal model are shown in Fig. 5. Scratching

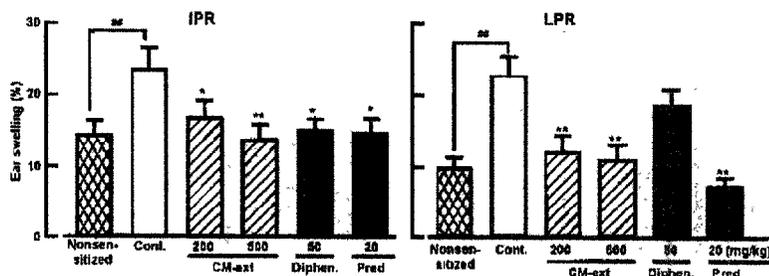


Fig. 3. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext), Diphenhydramine (Diphen.) and Prednisolone (Pred.) on Ear Swelling after DNFB Challenge in Actively Sensitized Mice

Test substances suspended in 0.2% CMC·Na were orally administered 1 h before DNFB challenge. Each value represents the mean±S.E. (n=8 to 12). Significantly different from the nonsensitized control at #: $p < 0.01$. Significantly different from the control at *: $p < 0.05$, **: $p < 0.01$.

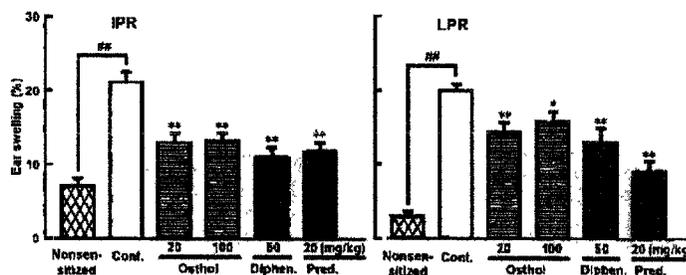


Fig. 4. Effects of Osthol, Diphenhydramine (Diphen.) and Prednisolone (Pred.) on Ear Swelling after DNFB Challenge in Actively Sensitized Mice

Test substances suspended in 0.2% CMC·Na were orally administered 1 h before DNFB challenge. Each value represents the mean±S.E. (n=8 to 10). Significantly different from the nonsensitized control at #: $p < 0.01$. Significantly different from the control at *: $p < 0.05$, **: $p < 0.01$.

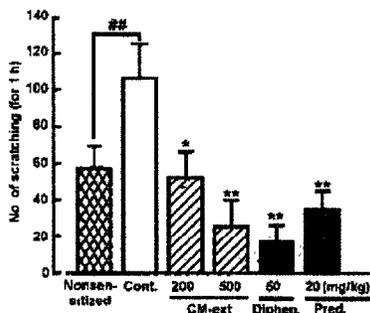


Fig. 5. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext), Diphenhydramine (Diphen.) and Prednisolone (Pred.) on Scratching Behavior after DNFB Challenge in Actively Sensitized Mice

Test substances suspended in 0.2% CMC·Na were orally administered 1 h before DNFB challenge. Each value represents the mean±S.E. (n=8 to 12). Significantly different from the nonsensitized control at #: $p < 0.01$. Significantly different from the control at *: $p < 0.05$, **: $p < 0.01$.

behavior in IPR was observed in sensitized mice after DNFB challenge, but not in LPR. CM-ext (200 and 500 mg/kg, *p.o.*) suppressed the scratching behavior as did diphenhydramine (50 mg/kg, *p.o.*) and prednisolone (20 mg/kg, *p.o.*).

Effects of CM-ext and Osthol on PC-Induced Contact Dermatitis As shown in Fig. 6, CM-ext at doses of 200 and 500 mg/kg had dose-dependent inhibitory effects on the effector phase in PC-induced contact dermatitis model mice. Osthol (100 mg/kg) and prednisolone (20 mg/kg) also showed the inhibitions (Figs. 6, 7).

DISCUSSION

In our studies seeking antipruritogenic agents from natural resources, we screened numerous herbal medicines in which

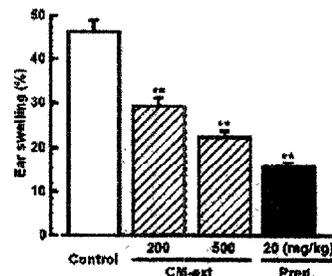


Fig. 6. Effects of 70% Ethanol Extract from *Cnidii Monnieri Fructus* (CM-ext) and Prednisolone (Pred.) on Effector Phase of Picryl Chloride-Induced Contact Dermatitis in Mice

CM-ext suspended in 0.2% CMC·Na was orally administered before and 16 h after the challenge. Prednisolone was orally administered 16 h after the challenge. Percentage of ear swelling was measured 24 h after the challenge. Each value represents the mean±S.E. (n=12 to 14). Significantly different from control at **: $p < 0.01$.

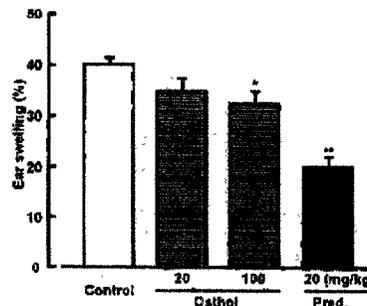


Fig. 7. Effects of Osthol, and Prednisolone (Pred.) on Effector Phase of Picryl Chloride-Induced Contact Dermatitis in Mice

Osthol suspended in 0.2% CMC·Na was orally administered before and 16 h after the challenge. Prednisolone was orally administered 16 h after the challenge. Percentage of ear swelling was measured 24 h after the challenge. Each value represents the mean±S.E. (n=12 to 14). Significantly different from control at *: $p < 0.05$, **: $p < 0.01$.

antipruritic effect will be expected from various herbal literatures.¹²⁾ Among them, *Kochiae Fructus* and *Cnidii Monnieri Fructus* were found to exhibit this effect.¹²⁾ In the previous paper, isopimpinellin and osthol, coumarin derivatives isolated from *Cnidii Monnieri Fructus*, were ascertained to be antipruritic components.⁵⁾ In this study, the effect of the dried fruits on various allergic responses related closely to itching and an exploration of active components were examined.

Some skin diseases such as atopic dermatitis and nettle rash are often accompanied by itching which is said to be induced by type I and IV allergic responses. Yamahara *et al.*¹⁾ found anti-type I and IV allergic effects of *Cnidii Monnieri Fructus* extract, and revealed that imperatorin is its active component. However, their report¹⁾ was not given on a pharmacological investigation for pruritus accompanied by allergic responses.

CM-ext inhibited 48 h homologous PCA in rats, a type I allergic model, and PC-induced contact dermatitis in mice, a type IV allergic model. These results are in reasonable agreement with those of Yamahara *et al.*¹⁾

Watanabe *et al.*¹³⁾ reported that repeated application of DNFB to an auricle of mice sensitized by DNP-OVA caused edemas of diphasic. It has been stated that the first phase edema similar to an occurrence of atopic dermatitis is IgE antibody dependent IPR appearing 1 h after application of DNFB, and the second phase edema is LPR appearing after 24 h.¹³⁾ In addition, it has been observed that mice made a scratching action at the site to which DNFB was applied during IPR.¹³⁾ IPR is an inflammatory reaction caused by release of chemical mediators from mast cells, whereas LPR is a cytokine-induced reaction.

CM-ext inhibited edemas in both IPR and LPR, and also, significantly inhibited scratching behavior accompanying IPR. As described earlier,⁵⁾ CM-ext did not change the spontaneous locomotor activity of mice. Diphenhydramine inhibited significantly both the edema and scratching behavior in IPR. Prednisolone pronouncedly inhibited both edemas in IPR and LPR, but the inhibitory effect on scratching behavior was very weak.

Osthol, a major component of CM-ext,⁵⁾ showed significant inhibitory effects on ear swelling after DNFB challenge. Thus part of the anti-allergic effect of CM-ext may be attributable to a coumarin, osthol. The anti-allergic activity of osthol was found for the first time.

In conclusion, it is clear that CM-ext has both anti-type I and IV allergic effects, and also has inhibitory effect on the scratching behavior accompanying the type I allergic model related closely to atopic dermatitis. In treating atopic dermatitis accompanied by itching, it is anticipated that the remedial value can be enhanced by combined use of a steroidal drug with *Cnidii Monnieri Fructus*, compared with use of steroid alone. Further studies on external applications of these dried fruits and their use in combination with diphenhydramine or prednisolone are in progress.

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