Studies on Kochiae Fructus IV. 1) Anti-Allergic Effects of 70% Ethanol Extract and Its Component, Momordin Ic from Dried Fruits of Kochia scoparia L.

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The 70% ethanol extract (KS-ext) from Kochiae Fructus (dried fruits of Kochia scoparia L.) has been screened for activity in experimental models of type I—IV allergy. In type I allergic models, KS-ext at doses of 200 and 500 mg/kg, p.o. exhibited an inhibitory effect on 48-h homologous passive cutaneous anaphylaxis (PCA) in rats, which is related to IgE, and 1.5-h heterologous PCA in mice, which is related to IgG. In a type III allergic model, KS-ext showed an inhibitory effect on direct passive arthus reaction (DPAR) in rats, while it had no inhibitory effect on reversed cutaneous anaphylaxis (RCA) in a type II allergic model. Furthermore, in a type IV allergic model, KS-ext had an inhibitory effect on the effector phase in picryl chloride-induced contact dermatitis (PC-CD). Also, its anti-pruritogenic component, momordin Ic (oleanane saponin) exhibited inhibitory effects on 48-h homologous PCA and PC-CD.

These results indicate that Kochiae Fructus not only inhibits humoral immunity but also influences cellular immunity, and should be recognized as a material for anti-allergic reactions. Also, the mode of its anti-pruritogenic activity may be mediated by anti-allergic action, and its active component may be partially attributed to momordin Ic.

Key words Kochia scoparia; type I—IV allergic reactions; momordin Ic; anti-allergic activity

Pruritogenic symptoms are subjective symptoms characteristic of skin diseases. A cause of pruritogenic symptoms would seem to involve an allergic reaction. It is known that typical diseases which cause pruritogenic symptoms involve skin trouble such as hives and atopy based on type I allergic reactions or a contagious dermatitis such as that produced by lacquer poisoning based on a type IV allergic reaction. Such pruritogenic symptoms accompanied by skin diseases can cause great mental stress, and the accompanying scratching behavior frequently worsens the skin disease. Kochiae Fructus, originated from the dried fruit of Kochia scoparia L., has been used for the treatment of skin diseases. In this series of studies on Kochiae Fructus, we reported that its 70% ethanol extract (KS-ext) exhibited anti-pruritogenic activity on a pruritogenic model induced by compound 48/80 in mice, and momordin Ic (3-O-[β-D-xylopyranosyl (1→3)-β-D-glucurono-pyranosyl]-oleanolic acid) was found to be its active compound, whereas momordin Ile (3-O-[β-D-xylopyranosyl (1→3)-β-D-glucuronopyranosyl]-28-β-D-glucuronopyranosyl-oleanolic acid) was ineffective.2) Therefore, Kochiae Fructus seems to be an effective anti-pruritogenic agent with anti-allergic activity.

The present paper presents a study of the inhibitory effects of 70% ethanol extract from Kochiae Fructus (KS-ext) against type I to IV allergic reactions and the extract’s active component.

MATERIALS AND METHODS

Materials Kochiae Fructus (fruit of Kochia scoparia L.), produced in Henan province, China, was obtained by Nippon Fum%matsu Yukuhin Co., Ltd. (Japan), and the origin was identified by Dr. Zhengtao Wang, China Pharmaceutical University.

Extraction and Separation The powdered fruits were extracted at about 80 °C for 1 h (two times) in 70% ethanol of decuple of the powder for the pharmacological tests. The extract (KS-ext) was evaporated and then frozen in dryness (yield; 13.4%). In order to clarify the active component (s), the crushed fruits were newly extracted with methanol (KM-ext) and separated by the method described in a previous paper3) (Chart 1).

Drugs The following drugs were used in this study: egg albumin (EWA, Grade V), cyclophosphamide (Sigma), sodium cromoglicate (DSCG, Funakoshi), dexamethasone, Evans blue, picryl chloride, prednisolone (Nacalai Tesque), Freund’s complete adjuvant (Difco), sheep red blood cell (SRBC, Research Foundation for Microbial Disease of Osaka University), and an inactive bacterial suspension of Bordetella pertussis (Wako).

Subjects Male Wistar strain rats (160—180, 180—200 g), male ddY strain mice (22—24 g), female BALB/c strain mice (18—20 g), male SD strain rats (150—180 g) and female ICR strain mice (18—20, 30—32 g) were provided by SLC (Japan SLC, Japan) and male JW strain rabbits (2.0—2.5, 2.5—3.0 kg) by KWL (Kiwai Laboratory Animals, Japan). 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 Labo R stock, Nihon Nosan Kogyo K.K., Japan) and water were freely available.

Pharmacological Tests Forty-eight-h Homologous Passive Cutaneous Anaphylaxis (PCA, Type I Allergic Model) Rat anti-EWA serum containing IgE was prepared by the method of Stottland and Sharet3) in male Wistar strain rats weighing 180 to 200 g. The rats were immunized with 1.0 mg of EWA and 10 mg of aluminum hydroxide gel suspended in 0.5 ml saline, and then 1.0 ml of an inactive bacterial suspension of Bordetella pertussis (2×10^10 cells/ml) was also injected intraperitoneally. Seven days after immunization, the rats were injected i.v. with a 1:1 dilution of rat serum and saline (A: 2 ml) and control rats were injected with saline alone (B: 2 ml). After a 6-h incubation at 37 °C, the back skin of each rat was injected with a 0.2 ml of a 1:1 dilution of rat serum and saline (A: 0.2 ml) and control rats were injected with saline alone (B: 0.2 ml). The PCA reaction was assessed after 30 min and 2 h incubation at 37 °C.

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later, the rats were immunized again by intramuscular injection of the same mixture and intraperitoneal injection of the bacterial suspension. Fourteen days later, the rats were anesthetized with pentobarbital, blood was withdrawn from the carotid arteries, and rat anti-EWA serum was obtained. The serum was stored at −80°C until use. The anti-EWA IgE antibody (1:32) was determined by PCA in Wistar rats. The PCA titer was expressed as the highest dilution causing a lesion more than 5 mm in diameter.

Antiserum diluted 8-fold with physiologic saline was injected intradermally into 2 sites on the shaved dorsal skin of male Wistar strain rats weighing 180 to 200 g in a 0.05 ml dose. Forty-eight h after sensitization, the rats were challenged with 0.5 ml of saline containing 2.0 mg of EWA and 5.0 mg of Evans blue via the tail vein. Thirty min later, the rats were sacrificed, the dorsal skin was removed for measurement of the blue area, and the amount of leaked dye was then determined colorimetrically after extraction with 1.0 N KOH and a mixture of acetone and phosphoric acid based on the method of Katayama et al.4) KS-ext suspended in 0.2% carboxymethylcellulose sodium salt (CMC·Na) was administered orally 1 h before the antigen challenge. DSCG dissolved in saline was administered intravenously 1 min before the challenge.

One and a Half-H Heterologous PCA in Mice (Type I Allergic Model) Anti-EWA serum containing the IgG antibody mentioned above was incubated at 56°C for 2 h to deactivate the IgE antibody. The antiserum diluted 4-fold with physiologic saline was injected intradermally in a 5 μl dose into the ear of ddY strain mice weighing 22 to 24 g. One and a half h after the sensitization, the mice were challenged with 0.25 ml of saline containing 0.25 mg of EWA and 2.5 mg of Evans blue via the tail vein. Thirty min later, the mice were sacrificed, and the ear was removed for measurement of the blue area. The amount of leaked dye was then determined colorimetrically after extraction with 1.0 N KOH and a mixture of acetone and phosphoric acid, based on the method of Katayama et al.4) KS-ext or prednisolone suspended in 0.2% CMC·Na solution was administered orally 1 h before the antigen challenge.

Formation of IgE Antibody On the basis of the method of Levine and Vaz,5) 1 mg EWA and 1.5 mg of aluminum hydroxide gel in 0.2 ml of saline was injected i.p. to BALB/c female mice weighing 18-20 g. Seven, 14 and 21 d thereafter, the mice were bled from eye grounds and serum was obtained to determine the serum IgE antibody. KS-ext was administered orally from the day of immunization. A standard drug, cyclophosphamide (dissolved in saline) was administered i.p. to the mice from the day of immunization. The anti-EWA IgE antibody was determined by PCA in Wistar rats. The PCA titer was expressed as the highest dilution causing a lesion more than 5 mm in diameter.

Reversed Cutaneous Anaphylaxis (RCA, Type II Allergic Model) Rabbit anti-rat serum was obtained by the method of Ungar et al.,6) briefly, 1 ml of normal rat serum was injected i.v. into rabbits weighing 2.0-2.5 kg, and this was repeated 10 times at 1-d intervals. One day after the final immunization, antiserum was obtained and stored at −80°C until use.

Based on the method of Ungar et al.,6) the RCA in rats was carried out as follows. Rabbit anti-rat serum (0.05 ml) containing 1% Evans blue or the same volume of physiologic saline containing 1% Evans blue was injected into the shaved dorsal skin of Wistar strain rats weighing 160-180 g. Two h later, the rats were sacrificed, and the dorsal skin was removed. The inflamed areas were cut out with a leather punch (12 mm in diameter). Each piece of skin was weighed immediately after removal. The swelling...
percentage was expressed by the following equation: Swelling % = \((W_f - W_i)/W_i \times 100\), where \(W_i\) is the weight of the inflamed site and \(W_f\) is the weight of the saline-injected site. KS-ext was administered orally 1 h before the injection of antiserum, and dexamethasone was administered orally 1 h before the injection.

**Direct Passive Arthus Reaction (DPAR, Type III Allergic Model)** Rabbit anti-EWA serum was prepared according to the method of Koda et al., briefly, 10 ml of Freund’s complete adjuvant was mixed with 10 ml of saline containing 20 mg of EWA. One ml of this mixture was intramuscularly injected into rabbits weighing 2.0—2.5 kg once weekly for 4 weeks. One week after the final injection, the rabbits were anesthetized with pentobarbital, blood was withdrawn from the carotid arteries, and rabbit anti-EWA serum was obtained. The serum was stored at -80°C until use.

Rabbit anti-EWA serum (0.5 ml/150 g body weight) was intravenously injected into male SD strain rats weighing 150—180 g via the tail vein. Thirty min later, 0.1 ml of saline containing 0.25 mg of EWA was intracutaneously injected into the right hind paw of the rats to induce an arthus reaction. The hind paw volume was measured from 1 to 5 h after the challenge of EWA at 1 h intervals, and the results were expressed as a percentage of the swelling compared with the initial hind paw volume. KS-ext and prednisolone were administered orally 1 h before the challenge.

**Sheep Red Blood Cell Delayed Type Hypersensitivity (SRBC-DTH, Type IV Allergic Model)** Female ICR strain mice weighing 18—20 g were sensitized by applying \(1 \times 10^8\) cells/mouse of SRBC to the back subcutaneously. After 4 d, the mice were challenged by injecting the right hind paw intradermally with SRBC to induce SRBC-DTH. The paw swelling was measured with a dial thickness gauge (Mitsutoyo, Japan) before and 24 h after the challenge, and the difference in swelling was calculated. KS-ext was administered orally before and 16 h after the challenge. Prednisolone was administered orally 16 h after the challenge.

**Picryl Chloride-Induced Contact Dermatitis (PC-CD, Type IV Allergic Model)** This procedure was in accordance with the method reported by Asherson and Ptak. Female ICR strain mice weighing 30—32 g were sensitized by applying 0.1 ml of 7% picryl chloride solution in ethanol to the shaved abdomen. After a 6-d sensitization period, the mice were challenged by painting the inside of the right ear with 0.02 ml of 1% picryl chloride solution in olive oil to induce PC-CD. To study the induction phase of PC-CD, the ear thickness was measured with a dial thickness gauge (Mitsutoyo, Japan) before and 24 h after the challenge, and the difference in thickness was calculated. In the study of the induction phase of PC-CD, KS-ext was administered orally from day -1 to day 6 after immunization. Prednisolone was also administered orally from day 0 to day 6 after immunization.

In the study of the effector phase of PC-CD, mice with a certain percentage (over 40%) of ear swelling after sensitization and challenge were chosen. Three days thereafter, they were subjected to sensitization and challenge again by the same procedure, and the percent-

age of ear swelling was again determined. KS-ext was administered orally before and 16 h after the challenge. Prednisolone was administered orally 16 h after the challenge. Furthermore, to determine the therapeutic effects of KS-ext and prednisolone on PC-CD, KS-ext and prednisolone were administered orally 24 h after the challenge. The ear swelling was measured from 2 to 10 h after administration at 2 h intervals.

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

**RESULTS**

**Forty-eight-h Homologous PCA** As shown in Fig. 1, the dye leakage caused by PCA in rats was significantly decreased by KS-ext at doses of 200 and 500 mg/kg, p.o. The control agent, DSCG, at a dose of 5 mg/kg, i.v., caused inhibition.

Furthermore, after a bioassay-guided fraction, momordin Ic as an anti-allergic component was isolated from KM-ext (Chart 1, Fig. 2). However, momordin Ic was ineffective.

**One and a half-h Heterologous PCA** As shown in Fig. 4, the dye leakage caused by PCA in mice was decreased by the oral administration of KS-ext. Momordin Ic also produced this inhibition (Fig. 5). The control agent, prednisolone, at a dose of 20 mg/kg, p.o., also caused the inhibition.

**Formation of IgE Antibody** KS-ext at doses of 50, 200 and 500 mg/kg, p.o., had no inhibitory effect on the formation of IgE antibody 1 or 2 weeks after the immunization. The control agent, cyclophosphamide (10 mg/kg, i.p.), showed strong inhibition (data not shown).

**RCA** KS-ext at doses of 50, 200 and 500 mg/kg, p.o., had no inhibitory effect on the skin swelling induced by RCA in rats. The control agent, dexamethasone, at a dose of 10 mg/kg inhibited this increase (data not shown).

![Fig. 1. Effects of 70% Ethanol Extract from Kochia Fruits (KS-ext) and Sodium Cromoglycate (DSCG) on 48 h Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats](image)
Fig. 2. Structures of Momordin Ic and Momordin IIc Isolated from Kochiae Fructus

Fig. 3. Effects of Momordin Ic and Momordin IIc Isolated from Kochiae Fructus and Sodium Cromoglicate (DSCG) on 48h Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats

Momordin Ic and momordin IIc suspended in 0.2% CMC-Na was orally administered to rats mediated by the rat anti-EWA serum 1h before the antigen challenge. DSCG dissolved with saline was intravenously administered 1 min before the challenge. Control was orally administered 0.2% CMC-Na or intravenously administered saline alone. Each value represents the mean ± S.E. (n = 7-8). Significantly different from control group at * p < 0.05; ** p < 0.01.

DPAR Figure 6 shows the effects of KS-ext and prednisolone on the DPAR reaction. KS-ext at a dose of 500 mg/kg was effective in reducing the paw swelling in rats induced by DPAR. Prednisolone at a dose of 25 mg/kg, p.o., also inhibited this reaction.

SRBC-DTH As shown in Fig. 7, KS-ext at a dose of 500 mg/kg inhibited the paw swelling induced by SRBC-DTH in mice.

PC-CD The effects of KS-ext and prednisolone on the induction phase of PC-CD were investigated (data not shown).

Fig. 4. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Prednisolone on 1.5h Heterologous Passive Cutaneous Anaphylaxis (PCA) in Mice

Test substances suspended 0.2% CMC-Na were orally administered to mice mediated by the rat anti-EWA serum 1h before the antigen challenge. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 10-13). Significantly different from control group at * p < 0.01.

Fig. 5. Effects of Momordin Ic Isolated from Kochiae Fructus and Prednisolone on 1.5h Heterologous Passive Cutaneous Anaphylaxis (PCA) in Mice

Test substances suspended in 0.2% CMC-Na were orally administered to mice mediated by the rat anti-EWA serum 1h before the antigen challenge. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 10-13). Significantly different from control group at * p < 0.01.
Fig. 6. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Prednisolone on Direct Passive Arthus Reaction (DPAR) in Rats

Test substances suspended in 0.2% CMC-Na were orally administered to rats 1h before the injection of antigen. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 8). Significantly different from control group at *p < 0.05; **p < 0.01. ...... Control. K.S-ext 50 mg/kg; ▲ KS-ext 200 mg/kg; ■ KS-ext 500 mg/kg; ◼ Prednisolone 25 mg/kg.

Fig. 7. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Prednisolone (Pred.) on Sheep Red Blood Cell-Induced Delayed Type Hypersensitivity (SRBC-DTH) in Mice

KS-ext suspended in 0.2% CMC-Na was orally administered immediately before and 16h after the application, and foot pad swelling percentage was measured 24h after the application. Positive control agent, prednisolone, was orally administered 16h after the application. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 15). Significantly different from control group at *p < 0.05; **p < 0.01.

Fig. 8. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Prednisolone (Pred.) on the Effector Phase of Picryl Chloride-Induced Contact Dermatitis (PC-CD) in Mice

KS-ext suspended in 0.2% CMC-Na was orally administered before and 16h after the challenge, and prednisolone was orally administered 16h after the challenge. Percentage of ear swelling was measured 24h after the application. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 15). Significantly different from control group at *p < 0.05; **p < 0.01. ...... Control. K.S-ext 50 mg/kg; ▲ KS-ext 200 mg/kg; ■ KS-ext 500 mg/kg; ◼ Prednisolone 20 mg/kg.

Fig. 9. Effects of Momordin Ic Isolated from Kochiae Fructus and Prednisolone (Pred.) on the Effector Phase of Picryl Chloride-Induced Contact Dermatitis (PC-CD) in Mice

Momordin Ic suspended in 0.2% CMC-Na were orally administered before and 16h after the challenge, and prednisolone was orally administered 16h after challenge. Percentage of ear swelling was measured 24h after the application. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 12). Significantly different from control group at *p < 0.05; **p < 0.01.

Fig. 10. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Prednisolone on the Therapeutic Effects of Picryl Chloride-Induced Contact Dermatitis (PC-CD) in Mice

Test substances suspended in 0.2% CMC-Na were orally administered 24h after the challenge. Percentage of ear swelling was measured 24h after challenge. Control was orally administered 0.2% CMC-Na. Each value represents the mean ± S.E. (n = 15). Significantly different from control group at *p < 0.05; **p < 0.01. ...... Control. K.S-ext 50 mg/kg; ▲ KS-ext 200 mg/kg; ■ KS-ext 500 mg/kg; ◼ Prednisolone 20 mg/kg.

shown).

Figure 8 and 9 show the effects of KS-ext, momordin Ic isolated from it and prednisolone on the effector phase of PC-CD. KS-ext (500 mg/kg, p.o.) and momordin Ic (50, 100 mg/kg, p.o.) had inhibitory effects on the ear swelling in mice induced by PC-CD. Prednisolone at a dose of 20 mg/kg also inhibited this ear swelling.

Finally, the effects of KS-ext and prednisolone on PC-CD were determined. Twenty-four h after the challenge, ear swelling reached a maximum. KS-ext and prednisolone were administered orally and the ear swelling at this point was 100%. The ear swellings at 2, 4, 6, 8 and 10 h after administration were measured. As shown in Fig. 11, KS-ext at a dose of 500 mg/kg significantly reduced
the swellings at 4h after the administration. A control drug, prednisolone, at a dose of 20 mg/kg, also exhibited an effect on the ear swellings from 2 to 10h after administration.

DISCUSSION

KS-ext (200, 500 mg/kg, p.o.) significantly inhibited the 48h homologous PCA, PCA reaction (known as type I allergic reaction), which is believed to be caused by chemical mediators such as histamine and leukotriene being released from mast cells, and by basophilis owing to an IgE antibody related mechanism. It also showed an inhibitory effect on the 1.5h heterologous PCA at a dose of 500 mg/kg. KS-ext did not inhibit IgE antibody production in EWA sensitized BALB/c mice. It can be considered that this inhibitory action of KS-ext against PCA observed in the present study is caused by an inhibition of release of chemical mediators or by antagonizing the physiological actions of chemical mediators.

KS-ext did not exhibit an inhibitory effect on RCA, which is classified as a type II allergic reaction. KS-ext (500 mg/kg, p.o.) also significantly reduced the rat paw swelling induced by DPAR, a model of type III allergic reaction in which an antigen–antibody complex activates the kinin system, forming anaphylatoxin with the participation of a complement, and further aggregating platelets to result in the injury of cells or tissue. The precise mechanism of action of KS-ext on type III allergic reaction is unclear, but it seems to be partly due to its anticomplement activity.

Type IV differs from types I through III in being a reaction of cellular mediated immunity closely related to the immune cell. This delayed type reaction is introduced by release of chemical mediators such as lymphokines derived from lymphocytes through contact with the corresponding antigen of T-cells sensitized with antibody and complement. KS-ext exhibited an inhibitory effect on the effector phase of SRBC-DTH and PC-CD, both type IV allergic models. Although KS-ext showed a weak curative effect on the swelling induced by PC-CD, it was ineffective on its induction phase.

The active component of Kochiae Fructus was followed by monitoring its inhibitory action against PCA reaction. Momordin Ic was isolated from the KM-ext of Kochiae Fructus as the active component. Momordin Ic also exhibited an inhibitory effect on the effector phase of PC-CD.

In conclusion, it was proved that KS-ext was effective on experimental models of type I, III and IV allergic reactions. One of its active components was estimated to be momordin Ic.

These results seem to indicate that a certain effectiveness of Kochiae Fructus (地蔵子 in Japanese) as described in the ancient herbal literature of China and Japan, is supported experimentally. Kochiae Fructus is especially expected to be effective against allergic diseases with pruritogenic symptoms since the anti-pruritogenic activity of KS-ext has been reported already.

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REFERENCES