Studies on Kochiae Fructus III. Antinociceptive and Antiinflammatory 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.) was screened for its activity on nociceptive and inflammatory responses in experimental animals. Although KS-ext at an oral administration of 500 mg/kg had an antinociceptive effect on writhing responses induced by acetic acid, it was ineffective on nociceptive response in the hot plate test. Oleanolic acid oligoglycoside, momordin Ic isolated from Kochiae Fructus significantly decreased the frequency of licking behavior within a unit of time at the late phase without affecting that of the early phase in the formalin test. Also, KS-ext inhibited the rise of vascular permeability induced by acetic acid, the increase of paw edema induced by carrageenan, histamine, serotonin or bradykinin and ear swelling induced by arachidonic acid. Momordin Ic also exhibited an inhibitory effect on carrageenin-induced edema. These results indicated that Kochiae Fructus has a peripheral antinociceptive effect mediated by antinflammatory action, and that its active component can be partially attributed to momordin Ic.

Key words: Kochia scoparia; oleanolic acid oligoglycoside; momordin Ic; antinociceptive activity; antiinflammatory activity

Kochiae Fructus (fruit of Kochia scoparia L.) has been used mainly for treatment of thermal skin diseases in the traditional Chinese system in medicine. In a previous paper, we reported that a 70% ethanol extract of Kochiae Fructus and momordin Ic, its main component was found to exhibit antipruritogenic activity. An inducing factor of pruritogenic symptoms is thought to be closely related to the pain and inflammation and the pruritogenic symptoms or to occupy a part of the pain accompanied by the inflammation. In case of nociceptive incidence for the body, substance P (SP) and carciton gene relating peptide (CGRP) released from a nerve end induce the release of histamine or serotonin from mast cells and the production of bradykinin in the vessels, followed by the resulting pruritogenic reaction with the pain and inflammation.

To determine the mode of action of the antipruritogenic activity found in our previous report, we report here a study on antinociceptive and antiinflammatory effects of 70% ethanol extract from Kochiae Fructus. The effects on those of momordin Ic isolated from the extract are also described.

MATERIALS AND METHODS

Materials Kochiae Fructus (fruit of Kochia scoparia L.) produced in Henan province, China was provided by Nippon Fumatsu Yakuhin Co., Ltd. (Japan) and its origin was identified by Dr. Zhengtao Wang of China Pharmaceutical University. The powdered fruits were extracted at about 80°C for 1 h (two times) in 70% ethanol of decouple of the powder for the pharmacological tests. The extract (KS-ext) was evaporated and then frozen in dryness (yield: 13.4%). Its major antipruritogenic constituent, momordin Ic, separated by the method described earlier was also used in this study.

Drugs The following drugs were used in this study:

arachidonic acid, bradykinin acetate (bradykinin), l-carrageenan, compound 48/80, 5-hydroxytryptamine (serotonin) (Sigma), diphenhydramine hydrochloride (diphenhydramine), 1-phenyl-3-pyrazolidinone (phenidone), pontamine sky blue (Tokyo Kasei), acetic acid, acetylsalicylic acid (aspirin), cyproheptadine hydrochloride (cyproheptadine), formalin, histamine dihydrochloride (histamine), indomethacin (Nacalai Tesque) and antipyrine (Hoei Yakko).

Subjects Male SLC:Wistar strain rats (150—180 g) and male SLC: ddY strain mice (18—20, 20—25 g) were used. 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 chews (Labo MR Stock and Nihon Nosan Kogyo K.K.) and water were given freely.

Acetic Acid-Induced Writhing Test Acetic acid-induced writhing test was performed by the method of Koster et al. Samples were orally given 1 h prior to an intraperitoneal injection of 1.0% acetic acid (0.1 ml/10 g mouse). The number of writhings and stretchings was counted for 10 min from 5 min after the injection.

Hot Plate Test Hot plate test was performed by the method described by Woolfe and Macdonald. Briefly, a mouse was placed on a hot plate maintained at 55 ± 1°C. The latency of nociceptive responses such as licking or jumping was measured. Only the mice that showed nociceptive responses within 15 s were used for the experiment. One h after the oral administration of samples, the latency of nociceptive responses was measured.

Formalin Test Formalin test was carried out according to the method of Shibata et al. One h after the oral administration of samples, 25 ml of 0.5% formalin in saline was subcutaneously injected to the right hind paw of mice. Each animal was then returned to the chamber and pain

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response was recorded for a period of 30 min. The summation of time (in seconds) spent in licking and biting responses of the injected paw during each 5 min block was measured as an indicator of pain response.

**Acetic Acid-Induced Vasculor Permeability** The method was based on that of Whittle. The ddY strain mice (20—25 g) were dosed orally with the test substances suspended in a 0.2% sodium carboxymethyl cellulose solution (CMC-Na) 1 h before the intravenous injection of 4% pontamine sky blue (10 ml/kg). Fifteen min after injection of the dye, 0.7% acetic acid (10 ml/kg) was injected intraperitoneally. Twenty min later, the mice were killed by dislocation of the neck, and the viscera were exposed after 1 min period to allow blood to drain away from the abdominal wall. Each animal was held by a flap of the abdominal wall, and the viscera were irrigated with 10 ml of saline over a petri dish. The washed matter was filtered through glass wool and transferred to a test tube. To each tube was added 0.1 ml of 1 N NaOH in order to clear any turbidity due to protein, and the absorbance was read at 590 nm with a Shimadzu model UV-160 spectrophotometer. Control animals were treated similarly, except that they received an oral dose of the vehicle alone. Vascular permeability was expressed in terms of absorbance value per mouse which leaked into the intraperitoneal cavity. Indomethacin was used as a standard drug.

**Carrageenin-Induced Edema** This method was based on that of Nakamura et al. Initial hind paw volume of Wistar strain rats (150—180 g) was volumetrically determined. A 1% solution of carrageenin in saline (0.1 ml/rat) was injected subcutaneously into the right hind paw 1 h after the samples had been administered orally. Paw volume was measured from 1 h to 5 h after the injection, and the edema was determined. The results were expressed as percentage of the swelling compared with the initial hind paw volume.

**Compound 48/80-Induced Edema** Following determination of initial hind paw thickness of the ddY strain mice (18—20 g), 5 μl of 0.1% compound 48/80 in saline was injected subcutaneously into the right hind paw 1 h after the test substances suspended in a 0.2% CMC-Na solution had been administered orally. Paw thickness was measured for up to 30 min at intervals of 10 min, and thickness of the edema was recorded. The results were expressed as the percentage of hind paw swelling as compared with the initial hind paw thickness. Diphenhydramine was used as a standard drug.

**Chemical Mediator-Induced Edema** Following determination of initial hind paw thickness of the ddY strain mice (18—20 g), 5 μl of 0.6% histamine (30 μg/mouse), 0.02% serotonin (1 μg/mouse) or 0.6% bradykinin (30 μg/mouse) in saline was injected subcutaneously into the right hind paw 1 h after the test substances suspended in a 0.2% CMC-Na solution had been administered orally. Paw thickness was measured for up to 30 min at intervals of 10 min, and thickness of the edema was recorded. The results were expressed as the percentage of hind paw swelling as compared with the initial hind paw thickness. Diphenhydramine or ciproheptadine was used as a standard drug.

**Measurement of Histamine-Induced Contraction in Isolated Guinea Pig Ileum** Each 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 isotonically recorded by means of a lever loaded with 0.5 g on a smoke drum. The ileum was preincubated with KS-Ext (10^-4 g/ml) dissolved in 5% dimethyl sulfoxide-Tyrode’s solution or diphenhydramine·HCl (10^-6 mmol/ml) dissolved in Tyrode’s solution for 3 min, and then histamine (10^-5—10^-4 mmol/ml) 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 (10^-4 mmol/ml) alone.

**Assay of Prekallikrein Enzyme Activity** Whole blood samples were collected from the heart of pentobarbital-anesthetized Wistar strain rats (180—220 g). Nine ml of the blood and 1 ml of sodium citrate (3.8%) were transferred into a plastic tube and centrifuged at 3000 rpm for 10 min to obtain plasma. Assay of prekallikrein enzyme activity was performed by the method of Ohishi and Katori. Citrated rat plasma (100 μl) was mixed with 800 μl of test solution [2% dimethyl sulfoxide/acetone—Tris—saline buffer (Buffer I; 0.02 M Tris·HCl; 0.15 M NaCl, pH 8.0)] and allowed to stand for 10 min at room temperature (about 25° C). Then, 100 μl of kaolin suspension (10 mg/ml Buffer I) was added and mixed vigorously for 5 s. Five min after the addition of kaolin, 50 μl aliquots of the reaction mixture were incubated for 10 min at 37° C with 1 ml of 50 mM Z-Phe—Arg—MCA in Buffer II (0.05 M Tris·HCl, 0.1 M NaCl, 0.01 M CaCl₂, pH 8.0) in the presence (tube A) or the absence (tube B) of 40 μg SBTI. The reaction was terminated by the addition of 2 ml of 17% acetic acid and the fluorescence was read at 460 (emission) and 380 (excitation) nm in a Hitachi F-4010 fluorescence spectrophotometer. The difference between the value in tube A and tube B was calculated as prekallikrein activity and the inhibitory percentage of the test substance was determined. FOY was used as a standard drug.

**Arachidonic Acid-Induced Ear Swelling** Arachidonic acid-induced ear swelling in mice was performed by the modified method of Young et al. The initial right ear thickness of ddY strain mice (18—20 g) was measured by a dial thickness gauge. One h after the oral administration of test substances suspended in 0.2% CMC-Na, each mouse was given 2 mg/ear of arachidonic acid solution (100 mg arachidonic acid/ml acetone) and thickness of the ear was measured 1 h thereafter. The control group received the vehicle. Phenidione was used as a standard drug and was dissolved in saline and administered intravenously.

**Statistical Analysis** The experimental data were tested for statistically significant differences by the Bonferroni/Dunn’s Multiple Range Test.

**RESULTS**

**Antinociceptive Effect by Acetic Acid-Induced Writhing Test** As shown in Fig. 1, KS-Ext at an oral dose of 500 mg/kg had an antinociceptive effect on the writhing responses induced by 1% acetic acid solution in mice. A positive control agent, antipyrine (100 mg/kg, p.o.), show-
ed an inhibitory effect.

**Antinociceptive Effect by Hot Plate Test**  KS-ext at oral doses of 50, 200, and 500 mg/kg did not have an antinociceptive effect in hot plate test (data not shown).

**Antinociceptive Effect by Formalin Test**  Antinociceptive activity of momordin Ic isolated from Kochiae Fructus was screened in a formalin test in mice. As shown in Fig. 2, momordin Ic (20, 50, and 100 mg/kg, p.o.) did not inhibit the duration of licking activity in the early phase, while it significantly inhibited the duration in the late phase. A reference antinociceptive agent, aspirin (200 mg/kg, p.o.) had similar effect.

**Antinflammatory Effect by Acetic Acid-Induced Vascula
permeability**  As shown in Fig. 3, KS-ext inhibited the increase of dye leakage induced by acetic acid in a dose response manner. A positive control agent, indomethacin 10 mg/kg, p.o., also reduced the leakage.

![Fig. 1. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Antipyrine on Acetic Acid-Induced Writhing in Mice](image1)

![Fig. 2. Effects of Momordin Ic Isolated from Kochiae Fructus and Aspirin on Hind Paw Licking in the Formalin Test in Mice](image2)

![Fig. 3. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Indomethacin (Indo.) on Acetic Acid-Induced Vascula
permeability in Mice](image3)

![Fig. 4. Effects of 70% Ethanol Extract from Kochiae Fructus (KS-ext) and Indomethacin on Carrageenin-Induced Edema in Mouse](image4)

**Antiinflammatory Effect by Carrageenin-Induced Paw Edema**  As shown in Fig. 4, KS-ext (200 and 500 mg/kg, p.o.) had a significant inhibitory effect on the edema. Momordin Ic also exhibited the inhibition on the edemas 1 and 3 h after the injection of carrageenin solution (Fig. 5). Strong inhibition was shown by the standard drug indomethacin (10 mg/kg, p.o.).

**Antiinflammatory Effect by Compound 48/80 (48/80)-
Induced Paw Edema**  As shown in Fig. 6, maximal edema was induced 10 min after injection of 48/80 solution in mice. KS-ext (500 mg/kg, p.o.) weakly inhibited the edema. The positive control agent, diphenhydramine (50 mg/kg, p.o.) exhibited strong inhibition.

**Antiinflammatory Effect on Chemical Mediators-Induced Paw Edema**  As shown in Fig. 7, maximal edemas were induced 10 min after injections of the chemical mediators, histamine, serotonin or bradykinin in mice. KS-ext strongly inhibited the edema induced by histamine, while the effects on serotonin- or bradykinin-induced edema were weak. The positive control agent, diphenhydramine (50 mg/kg, p.o.) significantly inhibited the edema.
Fig. 5. Effects of Momordin Ic Isolated from Kochiae Fructus (KS-ext) and Indomethacin on Carrageenan-Induced Edema in Mice

Wistar strain rats were dosed orally with test substances suspended in 0.2% CMC-Na solution 1 h before subcutaneous injection of 1% \( \varepsilon \)-carrageenan solution. Each value represents the mean \( \pm \) S.E. \((n=8)\). Significantly different from control group at *: \( p < 0.05 \), **: \( p < 0.01 \). --- Control, \( \bullet \) KS-ext 50 mg/kg, \( \Delta \) KS-ext 200 mg/kg, ■ KS-ext 500 mg/kg, • Indomethacin 10 mg/kg.

mg/kg, p.o.) or cyproheptadine (2 mg/kg, p.o.) exhibited inhibitory effects against the histamine, bradykinin-induced edema and serotonin-induced edema, respectively.

Antinflammatory Effect on Histamine-Induced Contraction in Isolated Guinea Pig Ileum Histamine at a concentration of \( 10^{-5} \) mmol/ml by itself caused the contraction of isolated guinea pig ileum. As shown in Fig. 8, KS-ext at concentrations of 10—300 \( \mu \)g/ml inhibited the contraction in a concentration-dependent manner and the 50% inhibitory concentration was 220 \( \mu \)g/ml.

Antinflammatory Effect by Prekallikrein Enzyme Activ-
momordin Ic from a Chinese crude drug. Kochiae Fructus originating from *Kochia scoparia* were investigated using experimental models on various nociceptive and inflammatory responses.

KS-ext exhibited an antinociceptive effect as shown by the inhibition of abdominal writhing responses in mice induced by acetic acid which activates a chemosensitive nociceptor in the abdominal cavity. Antinociceptive drugs are generally classified as central or peripheral at the sites of action. The results of this writhing test alone did not ascertain whether the antinociceptive effects are central or peripheral.

The effects of KS-ext or momordin Ic on a hot plate test or a formalin test were examined to determine the mode of the inhibitory effect of KS-ext on the nociceptive responses. In the hot plate test, KS-ext did not show the inhibitory effect. Woolfe and MacDonald describe that aminopyrine and antipyrine are effective only in very large doses, but phenobarbital sodium and aspirin had no analgesic action that was detected by this test.

It is known that formalin is useful to cause neurogenic pain and inflammatory pain and that its subcutaneous injection produces a biphasic pain response in rats. In the mice used the first phasic response (first phase) was observed from 0 to 10 min after its injection and the second phasic response (second phase) from 10 to 30 min. According to Dubussion and Dennis, centrally acting drugs inhibit the pain response in both phases equally, while the peripherally acting drugs inhibit the second phase only. The major antiprurritogenic component of Kochiae fructus, momordin Ic inhibited only the second phase. Also, KS-ext inhibited significantly the rise of vascular permeability of the dye caused by acetic acid administered intraperitoneally and the increase of paw edema induced by carrageenin.

These findings prove that the antinociceptive effect of KS-ext or momordin Ic is brought about through a peripheral site of action and is related to anti-inflammatory action.

The inhibitory effect of KS-ext or momordin Ic was relatively strong on the increased edema 1 h after the injection of carrageenin, but weak 3 h after the injection compared with the inhibition by indomethacin, a typical antiinflammatory agent. The biochemical mechanism for inflammatory reaction in the organism (the carrageenin edema) is not yet clear because various factors are involved in the induction of inflammation. However, chemical mediators such as histamine, serotonin, prostaglandin (PGs) and kinin are presumed to play an important part in the occurrence and development of inflammation. Edema induced by carrageenin is divided into a first phase with the participation of histamine or serotonin and a second phase in which PGs or bradykinin participate through the advance and retreat of swelling. It can therefore be presumed that KS-ext inhibits the production of chemical mediator or antagonizes the action of this mediator itself.

KS-ext strongly inhibited histamine- or serotonin-induced paw edema but was weak on compound 48/80 (histamine-releasing agent from mast cells)- and bradykinin-induced paw edemas. The inhibition of arachidonic acid-induced ear swelling was also weak. Accordingly, it is believed that KS-ext has especially inhibitory effects against chemical mediators, histamine and serotonin related to the first phase of carrageenin-induced paw edema.

These findings proved that the antinociceptive effect of KS-ext with antiinflammatory activity is brought about through a peripheral site of action and is antagonistic to chemical mediators such as histamine or serotonin which are related closely to the induction of pruritogenic symptoms. These results seem to indicate that a certain effectiveness of Kochiae Fructus (地 Mã££子 in Japanese) which has been described in the herbal literatures of China and Japan is supported experimentally.

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**REFERENCES**