Antiallergic and Anti-inflammatory Properties of the Ethanolic Extract from *Gleditsia sinensis*

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This study was carried out to determine the effects of the 70% ethanolic extract from the anomalous fruits of *Gleditsia sinensis* Lam. (AFGS) on experimental allergic reactions and inflammation. AFGS (200, 500, 1000 mg/kg, p.o.) dose-dependently inhibited the systemic anaphylactic shock induced by compound 48/80 in mice and cutaneous reactions induced by histamine or serotonin in rats. At doses of 500 and 1000 mg/kg, AFGS showed a clear inhibition on homologous passive cutaneous anaphylaxis in rats. *In vitro*, AFGS significantly reduced histamine release from rat peritoneal mast cells triggered by compound 48/80 at concentrations of 20 and 50 μg/ml. Moreover, AFGS (500, 1000 mg/kg, p.o.) showed a significant inhibition on the hind paw edema in rats and ear swelling in mice caused by carrageein and croton oil, respectively. It also clearly inhibited the vascular permeability induced by acetic acid in mice at a dose of 1000 mg/kg. These findings demonstrate that the ethanolic extract from the anomalous fruits of *Gleditsia sinensis* possesses antiallergic and anti-inflammatory activities, which may be mediated by reducing the release of mediators such as histamine from mast cells and weakening the inflammatory action of these mediators.

Key words  *Gleditsia sinensis*; passive cutaneous anaphylaxis; mast cell; inflammation; vascular permeability

In traditional Chinese medicine, various parts of *Gleditsia sinensis* Lam. (Leguminosae) are used for different remedies and express specific therapeutic values. The thorns of *G. sinensis* are used for the treatment of carbuncle, scabies and suppurative skin diseases, while the mature pods and anomalous fruits (fruits without seeds) are mainly used for treating apoplexy, headache, productive cough, asthma and scabies.1–5 The thorns of *G. sinensis* have been reported to show inhibitory effects on mast cell-dependent anaphylaxis and on the growth of nasopharyngeal carcinoma cells.6,7 However, little research has been done to identify the pharmacological properties of the pods and the anomalous fruits of *G. sinensis*.

Phytochemical investigations have shown that the anomalous fruits of *G. sinensis* contain over ten kinds of oleanane-type triterpenoidal saponins.4–6 Research has demonstrated that oleanolic acid, oleanane-type triterpenoidal saponins and herb medicines rich in this kind of saponin possess antiallergic and anti-inflammatory activities.8–12 Thus, the present study was undertaken to evaluate the effects of the 70% ethanolic extract from the anomalous fruits of *G. sinensis* on immediate allergic reactions and acute inflammation.

MATERIALS AND METHODS

Preparation of Extract The anomalous fruits of *Gleditsia sinensis* were purchased from an herbal market in Nanjing, China in October 2001. A voucher specimen (Dai 0103) is deposited in the Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University. Two hundreds grams of *Gleditsia sinensis* fruits were ground and refluxed with 70% ethanol (1.5 l) three times for 1 h. After filtration through filter paper, the clear supernatant was concentrated under reduced pressure at 45 °C with a vacuum rotary evaporator, and lyophilized to give a dry extract (74.1 g). The extract (AFGS) was freshly prepared in distilled water or physiological buffer saline (PBS) just before use.

Chemicals and Reagents Compound 48/80, ovalbumin (chicken egg, Grade V), croton oil, carrageein, 5-hydroxytryptamine hydrochloride (serotonin) and indomethacin were purchased from Sigma (St. Louis, MO, U.S.A.). Inactive bacterial suspension of *Bordetella pertussis*, o-phthalaldehyde (OPT), prednisolone and histamine dihydrochloride were from Wako (Osaka, Japan), and disodium cromoglycate (DSCG) was from Biomol (PA, U.S.A.). Other reagents used were of analytical grade. All reagents were dissolved in distilled water for oral administration or in physiological saline for subcutaneous and intraperitoneal administration.

Animals Male SD rats (weighing 180–220 g) and male ICR mice (weighing 18–20 g, 24–26 g) from the animal center of The Chinese University of Hong Kong were used. They were maintained on a standard pellet diet with free access to water, and housed in an air-contained room at 24±2 °C with lighting from 8:00 to 20:00.

Systemic Anaphylactic Shock Induced by Compound 48/80 in Mice The experiment was carried out according to the method of Shin et al.13) Mice were given an intraperitoneal injection of 10 mg/kg of compound 48/80. Mortality during the h after induction of the systemic anaphylactic reaction was monitored. AFGS was orally administered 1 h before the injection of compound 48/80.

Passive Cutaneous Anaphylaxis (PCA) in Rats Rat anti-ovalbumin serum containing IgE was prepared as earlier described.11) In brief, rats were immunized with 0.5 ml of suspension containing 1 mg of ovalbumin and 10 mg of aluminum hydroxide gel (s.c.) and 1 ml of inactive bacterial suspension of *Bordetella pertussis* (2×1010 cells/ml, i.p.), simultaneously. Seven days later, they were immunized again following the same procedure. Fourteen days later, rats were anesthetized with ketamine and xylazine, and blood was withdrawn from the carotid artery; then rat anti-ovalbumin serum was separated. The anti-OVA IgE antibody was determined by PCA in rats, and the titer (1 : 32) was expressed as...
the highest dilution causing a lesion more than 5 mm in diameter.

The antiserum diluted 4-fold with saline (50 μl) was intra-
dermally injected into 2 sites on the shaved dorsal skin of
rats. After 48 h, rats were challenged with 0.5 ml of saline
containing 2 mg of ovalbumin and 5 mg of Evans blue via the
tail vein. After 30 min, the animals were sacrificed, and the
skin surrounding spots were removed. After incubation with
1 K KOH, the dye that had leaked into the spots was extracted
with a mixture of acetone and phosphoric acid, and was de-
termined colorimetrically. AFGS was orally administered 1 h
before the challenge of ovalbumin. DSCG was intravenously
injected just before the challenge.

**Histamine Release from Rat Peritoneal Mast Cells** As
previously described, mixed rat peritoneal cells were col-
lected by peritoneal lavage and were purified by centrifuga-
tion through Ficoll density gradient. Purified mast cells were
washed and resuspended in PBS (NaCl 154 mM, KCl 2.7 mM,
CaCl2 0.9 mM, Na2HPO4 4 mM, KH2PO4 2.7 mM, glucose 5.6
mM and 0.1% bovine serum albumin). Mast cell preparations
were about 92% pure as assessed by toluidine blue staining.
Cell viability was confirmed to be around 90% before and
after experiments by the trypan blue exclusion test.

Purified mast cells (2×106 cells/ml) were preincubated at
37 °C for 10 min. Then, AFGS and DSCG dissolved in PBS
were added 5 min before activation by compound 48/80
(0.5 μg/ml). The reaction was stopped 10 min later by chill-
ing the test tubes in ice water. The supernatant and cell pel-
lets were then separated by centrifugation, and 0.05% Triton
100 was added to the cell pellets to liberate the residual his-
tamine. After addition of 0.036% OPT methanol solution, his-
tamine content in supernatant (Supernatant) and cell pellets
(Cell pellet) was determined spectrophuorometrically (Em
360 nm, Ex 450 nm). To estimate the spontaneous release of
histamine (Spontaneous), exactly the same procedure was
followed but without adding samples or compound 48/80.
The release percentage of histamine was calculated by the
following equation.

\[
\text{histamine release (\%)} = \frac{[\text{supernatant} - \text{spontaneous}]}{[\text{supernatant} + \text{cell pellet}]} \times 100\%
\]

**Skin Reactions Induced by Histamine and Serotonin in
Rats** Histamine- and serotonin-induced cutaneous reac-
tions were carried out as previously described. Briefly, 50
μl of histamine (2 μg) and serotonin (0.02 μg) were intra-
dermally injected into the spots on the shaved dorsal skin of
rats, respectively. Thereafter, 1 ml of physiological saline con-
taining 5 mg of Evans blue was immediately injected into the
tail vein. AFGS and prednisolone were orally administered 1 h
before challenge. Animals were sacrificed by bleeding 30
min after the induction of reactions, and the skin surrounding
each reaction spot was cut off, from which the extravasated
dye was extracted and assayed using the method described in
rat PCA.

**Hind Paw Edema Induced by Carrageeinin in Rats**
The acute hind paw edema was produced by subcutaneously
injecting 0.1 ml of 1% carrageeinin, suspended in sterile
physiological saline, into the right hind paw of the rats.
AFGS and indomethacin were orally administered 1 h prior
to the injection of carrageeinin. The rat pedal volume up to
the ankle joint was measured just before and 1 or 3 h after the
injection of carrageeinin. Data were expressed as a percent-
age increase of paw volume in relation to the initial values.

**Vascular Permeability Induced by Acetic Acid in Mice**
Mice received an intravenous injection of 0.5% Evans blue
saline solution and an intraperitoneal injection of 0.6% acetic
acid (10 ml/kg). After 20 min, the dye that leaked into the
peritoneal cavity was collected by lavaging with 10 ml of dis-
tilled water. After centrifugation, the amount of dye was
measured colorimetrically. AFGS and indomethacin were
orally administered 1 h prior to the injection of acetic acid.

**Ear Swelling Induced by Croton Oil in Mice** The cro-
ton oil ear test was performed as described. Each mouse
received 15 μl of an acetone solution of croton oil on the
inner surface of the right ear (75 μg croton oil/ear). After 6 h,
the animals were sacrificed by cervical dislocation, and
thickness of the ears was measured by a dial thickness gauge.
The swelling percentage was determined in relation to the
thickness of untreated left ears. AFGS and indomethacin
were orally administered 1 h prior to the application of croton
oil.

**Statistics** All values were presented as mean values ±S.D. The statistical significance between groups was cal-
culated by analysis of variance of the Student’s t-test. All p
values less than 0.05 (p<0.05) were considered significant.

**RESULTS**

**Effect on Systemic Anaphylactic Shock Induced by
Compound 48/80 in Mice** As shown in Table 1, an
intraperitoneal injection of compound 48/80 (10 mg/kg) re-
sulted in a fatal shock in 90% of mice, and AFGS pretreat-
ment (200, 500, 1000 mg/kg, p.o.) dose-dependently reduced
the mortality rate.

**Effect on Passive Cutaneous Anaphylaxis (PCA) in
Rats** AFGS (500, 1000 mg/kg, p.o.) and DSCG (2 mg/kg,
i.v.) clearly inhibited PCA in rats by 50%, 56.8% and 62.5%,
respectively. However, AFGS (200 mg/kg) did not affect
PCA (Fig. 1).

**Effect on Histamine Release from Rat Peritoneal Mast
Cells Induced by Compound 48/80** The spontaneous re-
lease of histamine from rat peritoneal mast cells was 9.4%.
Compound 48/80 (0.5 μg/ml) elicited about 80% histamine
release from mast cells. AFGS (20, 50 μg/ml) pretreatment
significantly reduced the release by 35.7% and 53.5%, re-
spectively. DSCG, a stabilizer of mast cell membrane, also
clearly reduced the histamine release at a concentration of
500 μg/ml. However, AFGS (5 μg/ml) did not affect the
release (Fig. 2).

<table>
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<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
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<th>Mortality (%)</th>
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<tr>
<td>Control</td>
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<td>10</td>
<td>90</td>
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<tr>
<td>AFGS</td>
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Mice were orally given AFGS 1 h prior to intraperitoneal injection of compound 48/80 (10 mg/kg). Mortality rate (%), within 1 h after the injection of this compound was represented as number of dead mice×100/total number of experimental mice.
Effect on Cutaneous Reactions Induced by Histamine and Serotonin in Rats

Cutaneous reactions were elicited by an intradermal injection of histamine (2 μg) and serotonin (0.02 μg), respectively. AFGS (200, 500, 1000 mg/kg, p.o.) dose-dependently reduced the skin reaction induced by histamine, and it also showed a clear inhibition on the skin reaction induced by serotonin at doses of 500 and 1000 mg/kg. Prednisolone, a steroidal anti-inflammatory agent, significantly inhibited the reactions at a dose of 20 mg/kg (Fig. 3).

Effect on Hind Paw Swelling Induced by Carrageenin in Rats

As shown in Fig. 4, a subcutaneous injection of carrageenin (1%, 0.1 ml) into the hind paw of rats produced 21.3% and 50.8% of swelling at 1 and 3 h after the injection, respectively. AFGS (200, 500, 1000 mg/kg, p.o.) reduced the hind paw swelling at 1 and 3 h in a dose-dependent manner. Indomethacin, a nonsteroidal anti-inflammatory agent, also significantly inhibited the hind paw swelling at a dose of 10 mg/kg.
**Fruits of Gleditsia sinensis** share a common mode of action on immediate allergic reactions with the thorns of *G. sinensis*.

Furthermore, the effect of AFGS on acute inflammation was observed. AFGS (500, 1000 mg/kg, p.o.) showed a significant inhibition on the hind paw edema in rats and ear swelling in mice caused by carrageenin and croton oil, respectively. It also showed a clear inhibition on the vascular permeability induced by acetic acid in mice at a dose of 1000 mg/kg. As mediators (e.g., histamine, serotonin and prostaglandins) that are secreted from mast cells play pivotal roles in the onset and development of inflammation, the inhibitory effects of AFGS on histamine and serotonin probably contribute to the anti-inflammatory action.

In conclusion, the 70% ethanolic extract from the anomalous fruits of *G. sinensis* exhibits an inhibitory effect on immediate allergic reactions and acute inflammation, which can be mediated by reducing the release of mediators from mast cells and weakening the inflammatory effects of these mediators. Further studies are needed to elucidate the precise mechanism of action and effective constituents of *G. sinensis*.

**Acknowledgement** Partial support was received with gratitude from the Innovation Technology Fund (AF/281/97) of the Government of the Hong Kong Special Administrative Region.

**REFERENCES**


