



Effects of a *Dictamnus dasycarpus* T. Extract on Allergic Models in Mice

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Received January 24, 2007; Accepted December 5, 2007; Online Publication, March 7, 2008
[doi:10.1271/bbb.70050]

The anti-allergic effect of a 70% ethanol extract from *Dictamnus dasycarpus* Turcz (DDT) was studied in mice. DDT at doses of 200 and 500 mg/kg inhibited the systemic anaphylactic shock induced by compound 48/80 in a dose-dependent manner. It also inhibited dose-dependently the scratching behavior induced by compound 48/80, histamine and serotonin. An increase in the vascular permeability induced by compound 48/80, histamine and serotonin was also inhibited by DDT. In an *in vitro* study, DDT inhibited the histamine released from rat peritoneal mast cells induced by compound 48/80. It seems likely from these findings that DDT was effective in antagonizing certain pharmacological effects induced by compound 48/80 that occurred *via* both histamine and serotonin released from mast cells. In conclusion, DDT may be effective in the relief of symptoms of allergic atopic dermatitis and other allergy-related diseases.

Key words: *Dictamnus dasycarpus* Turcz; anaphylactic shock; mast cell; scratching behavior; vascular permeability

Dictamnus dasycarpus Turcz (DDT) is a perennial herbal plant that mainly grows in the northern provinces of China, including Jilin, Heilongjiang, Liaoning and Hebei. The root bark of DDT is widely used as a medicinal herb in China, and it has been sold as an over-the-counter drug for the treatment of jaundice, cough and rheumatism.¹⁾ Du *et al.* have reported that DDT was used in the treatment of eczema, cutaneous pruritus and urticaria as an external and internal medicine.²⁾ In addition, it has been used as a component in Zemaphyte[®] (Phytopharm, Cambridge, UK), a standardized traditional Chinese herb formulation which has been reported to be effective in the treatment of atopic dermatitis in UK.³⁾

A phytochemical study of the root bark of DDT has demonstrated the presence of furoquinoline alkaloids,⁴⁾ limonoids,⁵⁾ flavonoids,^{2,6)} coumarins,⁷⁾ sesquiterpene and sesquiterpene glycosides.⁸⁾ Moreover, it has been

reported that the root bark of DDT showed a wide range of biological activities, such as the inhibition of human pathogenic fungi,¹⁾ enhancement of the cytotoxicity of microtubule inhibitors,⁹⁾ stimulation of the proliferation of T-cells¹⁰⁾ and cytotoxic activity against the A-549 (human lung adenocarcinoma) cell line.¹¹⁾ To our knowledge, however, there have been no reports on the anti-allergic activity of DDT.

We describe in this paper the effect of DDT on the anti-allergic properties characterized by the inhibition of histamine release from rat peritoneal mast cells, the inhibition of scratching behavior and the vascular permeability induced by compound 48/80 in ICR mice. We used rat peritoneal mast cells for studying histamine release. Sredni *et al.* have reported that mice peritoneal mast cells were almost the same as equivalent rat cells in both histological and functional properties.¹²⁾ In addition, rat mast cells are easily isolated and available compared to mice peritoneal mast cells.

Materials and Methods

Animals. Female ICR mice (6–10 weeks old) and male Wistar rats (7 weeks old) were obtained from Japan SLC, Shizuoka, Japan. The animals were housed in an air-conditioned room maintained at $24 \pm 2^\circ\text{C}$ with a relative humidity of $55 \pm 15\%$. They were given standard laboratory rodent feed (Oriental Yeast, Tokyo, Japan) and water *ad libitum*. All procedures involving the animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Chemicals and reagents. The following reagents were used in this study and their sources are shown in parentheses: histamine dihydrochloride (Sigma, St. Louis, MO, USA), compound 48/80 (Sigma), 5-hydroxytryptamine (serotonin; Sigma) and Evans blue (Wako, Tokyo, Japan). Compound 48/80, histamine and serotonin were dissolved in physiological saline and administered intradermally. The extract of DDT was

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freshly suspended in a 5% gum arabic solution and administered orally.

Preparation of the extract. The dried root bark of DDT was purchased from a local market in Changchun, China. Its identity was confirmed, as the herb of DDT, by anatomical observation, thin-layer chromatography (TLC) and direct comparison with authentic specimens which are stored in the Academy of Traditional Chinese Medicine and Materia Medica of Jilin Province. Two hundred grams of DDT root bark were ground and refluxed with 70% ethanol (600 ml) for 2 h. The extraction process was repeated 2 times. The extract was separated by passing through filter paper; the clear supernatant was concentrated under reduced pressure at 45 °C and lyophilized to give a dry extract (36.6 g). This dried extract of DDT was freshly dissolved in a 5% gum arabic solution before use.

Compound 48/80-induced systemic anaphylactic reaction. The experiment was carried out according to the method previously described by Shin *et al.*¹³⁾ Briefly, each mouse was given an intraperitoneal (i.p) injection of compound 48/80 (8 mg/kg) to evoke a systemic anaphylactic reaction. Either DDT or a vehicle was orally administered 1 h before the injection of compound 48/80. Mortality was monitored for 1 h after the induction of anaphylactic shock.

Compound 48/80-induced histamine release from isolated rat peritoneal mast cells. The peritoneal mast cells of male Wistar strain rats were harvested and purified by Percoll density centrifugation.¹⁴⁾ The collected mast cells (2.5×10^4 cells/tube) were then incubated with a physiological buffered solution (PBS; in mM: NaCl, 154; KCl, 2.7; CaCl₂, 0.9; N-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES), 5; glucose, 5.6; pH 7.4) for 10 min at 37 °C. The test drugs dissolved in PBS were added (0.1 ml) 10 min before compound 48/80 (to a final concentration of 0.5 µg/ml). The reaction was stopped 10 min later by cooling the tubes in iced water, before the tubes were centrifuged for 15 min at $200 \times g$. The histamine content was measured by means of a fluorometric assay.¹⁵⁾

Scratching behavior. Scratching behavior was observed by the same method as that used by Kuraishi *et al.*¹⁶⁾ DDT was dissolved in a 5% gum arabic solution and administered orally 1 h before the start of the behavioral observation. Compound 48/80 (10 µg/0.02 ml), histamine (100 nmol/0.02 ml) or serotonin (100 nmol/0.02 ml) was injected intradermally into the rostral part of the back of the mice. Immediately after the injection, the mice were placed in an observation chamber and their behavior was observed for 1 h. The scratching behavior was automatically detected and objectively evaluated with MicroAct apparatus (Neuroscience, Tokyo, Japan). A small magnet (1 mm diameter,

3 mm length) was implanted subcutaneously into both hind paws of a mouse under ether anesthesia at least 12 h before the measurement of scratching. The mice were placed in an observation chamber (11 cm in diameter, 18 cm high), which was surrounded by a round coil. The electric current induced in the coil by the movement of the magnets attached to the hind paws was amplified and recorded.¹⁷⁾

Vascular permeability of the skin. After the intradermal injection of 0.5 µg/0.02 ml of compound 48/80, 10 nmol/0.02 ml of histamine or 10 nmol/0.02 ml of serotonin into the rostral part of the back, a 2% Evans blue solution was intravenously injected into each animal. The animals were sacrificed 30 min later, and the diameter of the 'bluing' reaction at the injection site was measured.¹⁸⁾ DDT was orally administered 1 h before the experiment.

Statistical analysis. The data are presented as the mean $\pm$ S.E.M. Statistical significance was tested by a one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability value of less than 0.05 is considered to be significant.

Results

Effect of DDT on the systemic anaphylactic shock induced by compound 48/80

As shown in Table 1, an intraperitoneal injection of compound 48/80 (8 mg/kg) resulted in a fatal shock in all the mice, and the DDT pretreatment (200 and 500 mg/kg, p.o) dose-dependently reduced the mortality rate.

Effect of DDT on the histamine released from rat peritoneal mast cells induced by compound 48/80

Figure 1 shows the effect of DDT on the histamine released from rat peritoneal mast cells induced by compound 48/80. DDT dose-dependently inhibited the histamine released from mast cells, significant differences being observed at concentrations of 100 and 300 µg/ml.

Table 1. Effect of a *Dictamnus dasycarpus* Extract on Compound 48/80-Induced Systemic Anaphylaxis

Drug	Dose (mg/kg, p.o.)	Mortality (%)			
		10	20	60	(min)
Control		20	70	100	
DDT	100	0	40	100	
	200	0	30	80	
	500	0	20	80	

The *Dictamnus dasycarpus* extract was administered orally 1 h before injecting compound 48/80 (8 mg/kg). The mortality within 1 h after compound 48/80 injection is presented as the no. of dead mice $\times$ 100/total no. of experimental mice (n = 10).

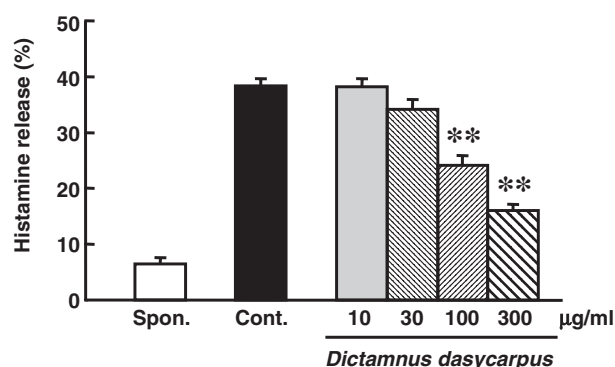


Fig. 1. Effect of a *Dictamnus dasycarpus* Extract on the Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80.

Each column and vertical bar shows the mean $\pm$ S.E.M. ($n = 10$). **, Significantly different from the control group at $P < 0.01$. Spon., spontaneous; Cont., control.

Effect of DDT on the scratching behavior induced by compound 48/80

Compound 48/80 was used at a dose of 10 μ g/site according to our previous report.¹⁸⁾ As shown in Fig. 2, DDT caused a dose-related inhibition of the scratching behavior induced by compound 48/80, a significant effect being observed at a dose of 500 mg/kg.

Effects of DDT on the scratching behavior induced by histamine and serotonin

As shown in Fig. 3A, DDT caused a dose-dependent inhibition of the histamine-induced scratching behavior, a significant effect being observed at a dose of 500 mg/kg. It also inhibited the scratching behavior induced

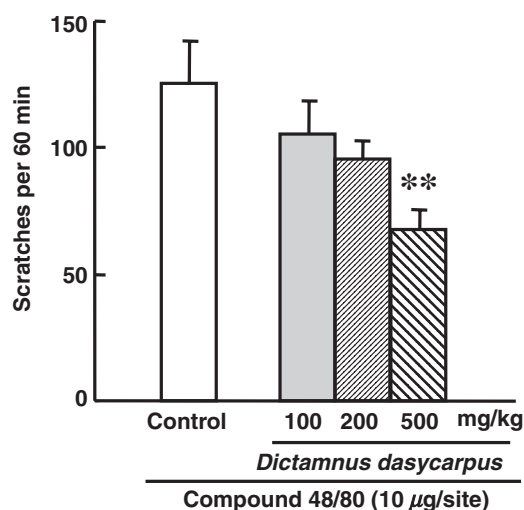


Fig. 2. Effect of a *Dictamnus dasycarpus* Extract on the Compound 48/80-Induced Scratching Behavior in ICR Mice.

The *Dictamnus dasycarpus* extract was administered orally 1 h before injecting compound 48/80 (10 μ g/site). Each column and vertical bar shows the mean $\pm$ S.E.M. ($n = 8-10$). **, Significantly different from the control group at $P < 0.01$.

by serotonin, a significant effect being observed at doses of both 200 and 500 mg/kg (Fig. 3B).

Effect of DDT on the vascular permeability induced by compound 48/80

Compound 48/80 was used at a dose of 0.5 μ g/site according to our previous report.¹⁸⁾ DDT at doses of 100 and 200 mg/kg caused no significant inhibition of the increased skin vascular permeability induced by

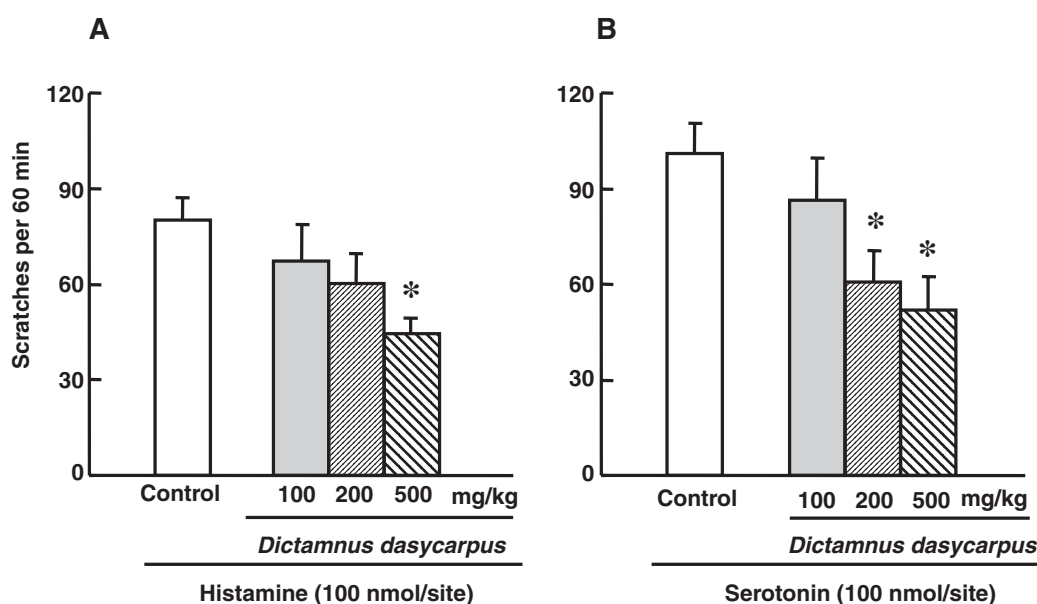


Fig. 3. Effect of a *Dictamnus dasycarpus* Extract on the Scratching Behavior Induced by Histamine (A) and Serotonin (B) in ICR mice.

The *Dictamnus dasycarpus* extract was administered orally 1 h before injecting histamine (100 nmol/site) or serotonin (100 nmol/site). Each value represents the mean $\pm$ S.E.M. ($n = 10$). *, Significantly different from the control group at $P < 0.05$.

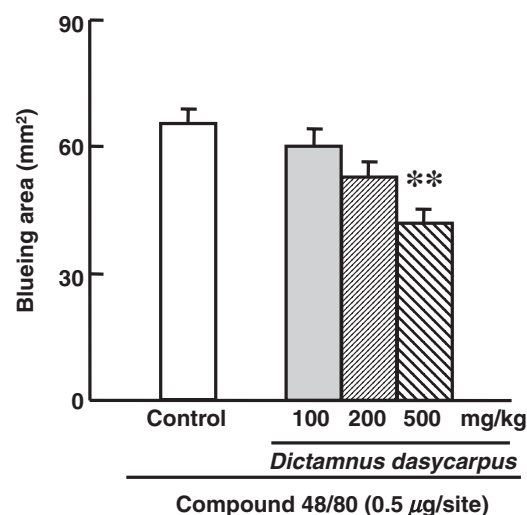


Fig. 4. Effect of a *Dictamnus dasycarpus* Extract on the Vascular Permeability Induced by Compound 48/80 in ICR mice.

The *Dictamnus dasycarpus* extract was administered orally 1 h before injecting compound 48/80 (0.5 µg/site). Each column and vertical bar shows the mean $\pm$ S.E.M. ($n = 8-10$). **, Significantly different from the control group at $P < 0.01$.

compound 48/80. However, at a dose of 500 mg/kg, it significantly inhibited the compound 48/80-induced vascular permeability (Fig. 4).

Effect of DDT on the vascular permeability induced by histamine and serotonin

DDT caused a dose-dependent inhibition of the histamine-induced vascular permeability, a significant effect being observed at a dose of 500 mg/kg (Fig. 5A).

It also reduced the vascular permeability induced by serotonin, a significant effect being observed at doses of both 200 and 500 mg/kg (Fig. 5B).

Discussion

Allergic diseases such as atopic dermatitis, food allergy and asthma are classified as a type I allergy. Atopic dermatitis is a chronic inflammatory skin disease that affects 10–20% of all children and 1–3% of adults.¹⁹⁾ The prevalence of atopic dermatitis has been increasing worldwide during the last few decades, especially in industrialized countries. In order to improve the quality of life for dermatitis patients, many efforts have been focused on screening herbs or finding new constituents from the herbs that exhibit an anti-pruritic effect.^{20,21)}

Itching, a sensation causing the urge to scratch, is the most significant outcome of atopic dermatitis. Mast cells play a key role in the immediate-type allergic reaction through the release of a number of mediators and cytokines. Among those, histamine is regarded as a principal mediator of the antigen-induced skin reaction.²²⁾ It is widely accepted that histamine derived from skin mast cells in response to various stimuli is an important mediator of human itching.²³⁾ Several studies *in vitro* as well as *in vivo* have confirmed that compound 48/80 potentially caused the release of histamine from mast cells, triggering such skin responses as scratching behavior and increased vascular permeability.¹⁶⁾ In addition, it is well recognized that serotonin is also released from rat mast cells by compound 48/80, and the representative serotonin antagonists, cyproheptadine

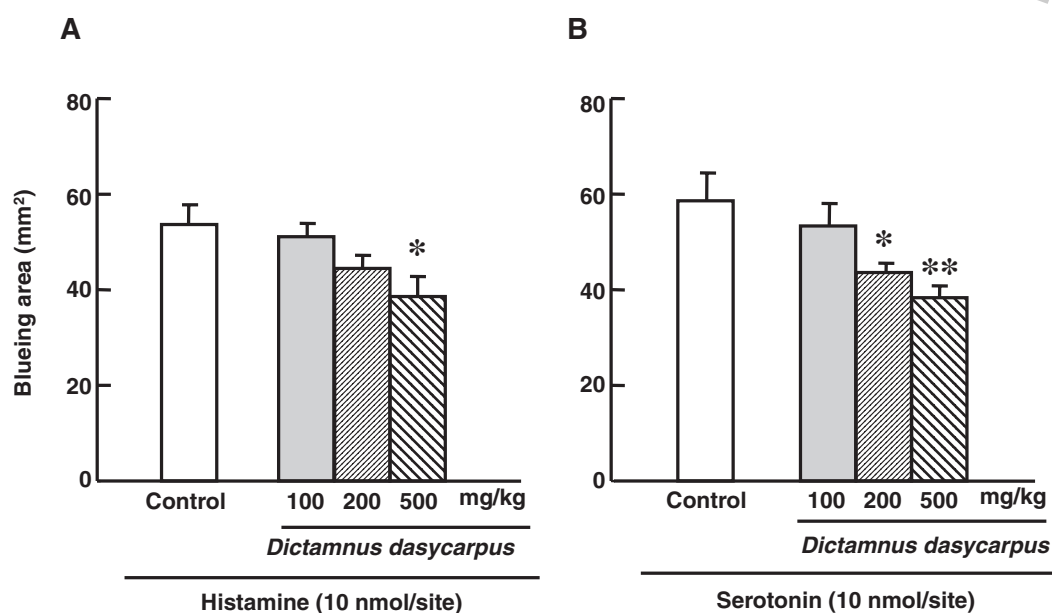


Fig. 5. Effect of a *Dictamnus dasycarpus* Extract on the Vascular Permeability Induced by Histamine (A) and Serotonin (B) in ICR Mice.

The *Dictamnus dasycarpus* extract was administered orally 1 h before injecting histamine (10 nmol/site) or serotonin (10 nmol/site). Each value represents the mean $\pm$ S.E.M. ($n = 8$). ***, Significantly different from the control group at $P < 0.05$ and $P < 0.01$, respectively.

and methysergide, caused a significant antagonistic effect of compound 48/80-induced scratching behavior.¹⁷⁾ We used from these findings histamine and serotonin as control drugs causing scratching behavior.

We first demonstrated that the 70% ethanol extract of the root bark of DDT inhibited the compound 48/80-induced systemic anaphylaxis reaction in ICR mice at doses of 200 and 500 mg/kg, and the histamine release from rat peritoneal mast cells at concentrations of 100 and 300 µg/ml. It is well known that compound 48/80, a classic secretagogue for mast cells, can induce a mast cell-dependent, non-specific anaphylactic reaction. The mechanism for the anaphylactic shock induced by compound 48/80 is considered to be due to the massive release of histamine from mast cells and basophils.²⁴⁾ In our present *in vitro* studies, DDT clearly inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80. Compound 48/80 is well known as a substance acting on the membrane of mast cells that causes degranulation.²⁵⁾ Therefore, it is reasonable to presume that the anti-anaphylactic action of DDT would be due to its membrane-stabilizing action on mast cells.

DDT also significantly inhibited the scratching behavior induced by compound 48/80 in ICR mice at a dose of 500 mg/kg. It is well known that histamine is present in a considerable amount in skin mast cells and has been thought to be an important mediator of itching.²⁶⁾ Serotonin is another important mediator responsible for pruritus in humans and has also been suggested to be involved in some pruritic diseases.²⁷⁾ It was found in the present study that DDT inhibited serotonin-induced scratching behavior more potently than the histamine-induced behavior. We next studied the effect of DDT on the vascular permeability induced by compound 48/80, histamine and serotonin. It was revealed that DDT inhibited not only the compound 48/80-induced vascular permeability, but also that induced by histamine and serotonin. These findings indicate that DDT had a direct inhibitory action on the inflammation induced by histamine and serotonin.

Several compounds isolated from DDT have also been reported to inhibit immediate allergic reactions. For instance, coumarins reduced the histamine release from rat peritoneal mast cells elicited by compound 48/80.^{28,29)} Wogonin, a kind of flavonoid, has been reported to inhibit the histamine release from peritoneal mast cells stimulated with A23187 and compound 48/80, and it also inhibited of the LTB₄ release from mast cells stimulated with A23187.³⁰⁾ These findings suggest that both coumarins and flavonoids might be active components responsible for the anti-allergic activities of DDT.

It seems likely from these findings that DDT would be effective in antagonizing some pharmacological effects induced by compound 48/80 that occurred *via* both the histamine and serotonin release from mast cells. This work provides experimental evidence for the folk use of DDT in the treatment of allergic diseases.

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