Antipruritic and Antiinflammatory Effects of Aqueous Extract from Si-Wu-Tang

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Si-Wu-Tang (SWT), a traditional Chinese formula, has been clinically used in the treatment of cutaneous pruritus, chronic inflammation, and other diseases. The present study was carried out to observe the antipruritic and antiinflammatory effects of SWT aqueous extract using compound 48/80 and picryl chloride (PC) models in mice. SWT (500, 1000 mg/kg *p.o.*) clearly reduced the scratching responses elicited by compound 48/80 in normal mice. At doses of 250 and 500 mg/kg, it inhibited the scratching responses induced by PC in mice actively sensitized with 2,4-dinitrophenol (DNP)-ovalbumin (OVA) plus alum. Furthermore, SWT (250, 500, 1000 mg/kg) significantly inhibited the footpad swelling caused by compound 48/80 in mice. In the biphasic ear skin reactions induced by PC in actively sensitized mice, SWT (250, 500 mg/kg) reduced the immediate—phase reaction, but did not affect the late—phase reaction. *In vitro*, SWT (50—500 μg/ml) showed a concentration-dependent inhibition of the histamine release induced by compound 48/80 from rat peritoneal mast cells. The crude drugs contained in SWT, Paeoniae Radix (25, 100 μg/ml), Rehmanniae Radix, and Chuanxiong Rhizoma (100 μg/ml), also showed a clear inhibition, but Angelica Radix did not at the concentrations examined. These findings indicate that SWT aqueous extract has antipruritic and antiinflammatory effects in mice. SWT inhibits histamine release from rat mast cells, and Paeoniae Radix probably plays a crucial role in the formula.

Key words Si-Wu-Tang; scratching behavior; biphasic skin reaction; swelling; histamine release

Pruritus, or itching, is a frequent and unpleasant symptom of cutaneous diseases (*e.g.*, atopic dermatitis, urticaria) and accompanies several systemic disorders (*e.g.*, chronic renal failure, cholestasis, diabetes mellitus). Itch-associated repetitive scratching often causes skin lesions and exacerbates the original disease such as atopic dermatitis. Inhibition of itch-evoked scratching is consistently beneficial for improving the quality of life of patients and treating the original disease. Unfortunately, there is no specific remedy available for this common symptom.

Si-Wu-Tang (SWT), consisting of Rehmanniae Radix, Angelica Radix, Chuanxiong Rhizoma, and Paeoniae Radix, is a well-known Chinese formula. It has been used for the treatment of gynecologic diseases (*e.g.*, dysmenorrhea, menoxenia, metrorrhagia, abortion), cutaneous diseases (*e.g.*, pruritus, urticaria, eczema, dermatitis), and chronic inflammation (*e.g.*, chronic nephritis, pelvic inflammation).[1)] Previous pharmacological research on SWT focused on the effects on experimental anemia, platelet aggregation, uterine contraction, and chronic inflammation.[1,2)] The present study was conducted to demonstrate the antipruritic and antiinflammatory effects of SWT in experimental animals.

MATERIALS AND METHODS

Animals Male ICR mice (body weight: 18—22 g) and male Sprague-Dawley (SD) rats (body weight: 200—250 g) were purchased from the Experimental Animal Center of China Pharmaceutical University and The Chinese University of Hong Kong, respectively. Animals were housed in an air-conditioned room maintained at 22—24 °C. Food and water were given *ad libidum*.

Preparation of Extract Rehmanniae Radix, Angelica Radix, Chuanxiong Rhizoma, and Paeoniae Radix were purchased from Tong-Ren-Tang (Nanjing, China). SWT was prepared by mixing the above four crude drugs in the same ratio. SWT (80 g) and each crude drug (20 g) were ground and soaked in 800 ml and 200 ml of water, respectively. After 30 min, they were refluxed twice for 1 h each. Water was evaporated and the extracts were dried to a powder. Extract yields are presented in Table 1. The extracts were freshly prepared with distilled water or phosphate buffered saline (PBS) at the desired concentrations just before use.

Chemicals Compound 48/80 and ovalbumin (grade III, OVA) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). 2,4-Dinitrophenol (DNP) and *o*-phthalaldehyde (OPT) were from Wako (Osaka, Japan). DNP-coupled OVA (DNP-OVA) was prepared according to the method described by Eisen *et al.*[3)] Picryl chloride (PC) and prednisolone were from Nakalai Tesque (Kyoto, Japan). Disodium cromoglycate (DSCG) was from BIOMOL (PA, U.S.A.). Terfenadine was from Yangzhou Pharmaceutical Factory (Yangzhou, China). Other reagents used were of analytical grade.

Scratching Behavior Induced by Compound 48/80 All experiments were performed between 12:00 and 18:00 in a quiet experimental room, and each treatment group was equally represented in each observation run. Successive runs were performed until the results of more than 5 animals/treatment were obtained. Before the experiments, the animals

Table 1. Extract Yields of Si-Wu-Tang (SWT) and Its Constituents

Extract	Botanical origin	Yield (%, w/w)
SWT		33.9
Rehmanniae Radix	*Rehmannia glutinosa* LIBOSCH	64.4
Angelica Radix	*Angelica sinensis* (OLIV.) DIELS	49.9
Chuanxiong Rhizoma	*Ligusticum chuanxiong* HORT.	32.6
Paeoniae Radix	*Paeonia lactiflora* PALL.	14.3

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were put into a plastic observation cage (30×45×20 cm) for about 10 min for acclimatization. Compound 48/80 (50 μg/mouse) in saline solution 0.1 ml was injected subcutaneously into the rostral part of the back. Immediately after injection, the animals were returned to the observation cages, with one mouse/cage. In accordance with the method described previously,[4,5] scratches on the injection site by the hind paws were counted for 10 min by observers who were unaware of the treatment of the animals. SWT extract and terfenadine were orally administered 1 h prior to the injection of compound 48/80. Control animals were orally administered the same volume of distilled water (20 ml/kg).

Scratching Behavior Induced by PC in Actively Sensitized Mice The experiment was conducted as reported previously,[6] with modification. In brief, mice were intraperitoneally injected with 3, 10, or 30 μg of DNP-OVA with 1 mg of aluminum hydroxide. Nonsensitized mice were injected with the same volume of physiological saline. After 14 d, scratching behavior was elicited by applying 30 μl of 1% PC in ethanol to both sides of the right ears of mice. Scratches on the right ears by the hind paws were counted for 1 h after PC challenge. SWT extract and terfenadine were orally administered 1 h before PC challenge. Control animals were orally administered the same volume of distilled water (20 ml/kg).

Hind Paw Swelling Induced by Compound 48/80 The procedure was similar to that described previously.[7] Briefly, after estimation of the initial thickness of the right hind paws, mice were orally administered SWT extract. Control animals were orally administered the same volume of distilled water (20 ml/kg). Positive control animals were orally administered terfenadine. After 1 h, 0.05% of compound 48/80 saline solution 10 μl was injected into the right hind paws using a microsyringe. The thickness of the right hind paws was measured using a dial thickness gauge 10 min after the injection. Hind paw swelling was expressed as the mean thickness difference between before and at 10 min after injection of compound 48/80.

Hind Paw Swelling Induced by PC in Actively Sensitized Mice Mice were actively sensitized with an intraperitoneal injection of 10 μg of DNP-OVA and 1 mg of aluminum hydroxide. Nonsensitized mice were injected with the same volume of physiological saline. After 14 d, skin reaction was elicited by applying 30 μl of 1% PC in olive oil to both sides of the right ears of mice. The reaction was evaluated by measuring the thickness of the right ears just before and 1, 4, 24, and 48 h after challenge. The skin swelling was expressed as the mean increase in ear thickness. SWT extract, terfenadine, and prednisolone were orally administered 1 h before PC challenge.

Histamine Release from Rat Peritoneal Mast Cells As previously described,[8] mixed rat peritoneal cells were collected by peritoneal lavage and purified by centrifugation through a Ficoll density gradient. Purified mast cells were washed and resuspended in PBS (NaCl 154 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM, Na_2HPO_4 4 mM, KH_2PO_4 2.7 mM, glucose 5.6 mM, 0.1% bovine serum albumin (BSA)). Cell viability was confirmed to be around 91% before and after experiments by the trypan blue dye-exclusion test.

Purified mast cells (2×10^6 cells/ml) were preincubated at 37 °C for 10 min. Then, SWT extract, included crude drugs 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 chilling the test tubes in ice water. The supernatant and cell pellets were then separated by centrifugation. Triton-100 0.05% was added to the cell pellets to liberate the residual histamine. After addition of OPT methanol solution 0.036%, histamine content in the supernatant and cell pellets were determined spectrofluorometrically (emission 360 nm, excitation 450 nm). To estimate the spontaneous release of histamine, exactly the same procedure without adding samples and compound 48/80 was followed. The release percentage of histamine was calculated by the following equation:

$$\text{histamine release (\%)} = (\text{supernatant} - \text{spontaneous}) / (\text{supernatant} + \text{cell pellet}) \times 100\ \%$$

Statistics All values are presented as mean±S.D. The statistical significance between groups was assessed by using one-way analysis of variance (ANOVA) followed by the *post hoc* Dunnett's test. *P* values less than 0.05 were considered to be significant.

RESULTS

Effect of SWT Aqueous Extract on Scratching Behavior Induced by Compound 48/80 Subcutaneous injection of compound 48/80 (50 μg/mouse) into the rostral part of the back elicited a significant scratching response in mice. The average scratching frequency in the 10 min after the injection of compound 48/80 was 109.2±24.6 times. SWT at doses of 500 and 1000 mg/kg significantly inhibited the scratching response, and the strongest effect was observed at the dose of 500 mg/kg. Terfenadine, a positive control drug, also significantly inhibited the scratching response at a dose of 5 mg/kg (Fig. 1).

Dose–Response Relationship of Scratching Behavior Induced by PC in Actively Sensitized Mice The scratching behavior after PC challenge was observed in actively sensitized and nonsensitized mice. The dose–response relationship and time course are summarized in Fig. 2. Mice actively sensitized with DNP-OVA 3, 10, and 30 μg with aluminum hydroxide scratched more than nonsensitized mice in the 1 h-

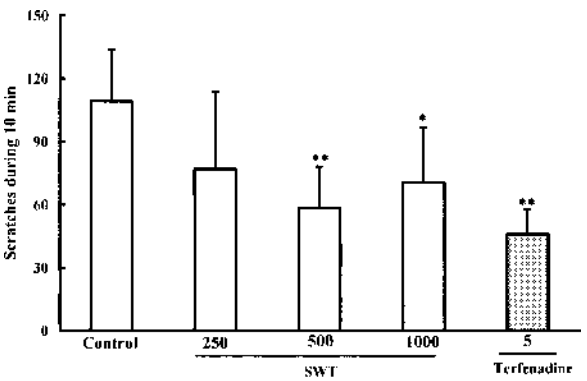

Fig. 1. Effects of Si-Wu-Tang (SWT) and Terfenadine on the Scratching Behavior Induced by Compound 48/80 in Mice

SWT aqueous extract and terfenadine were orally administered 1 h before the subcutaneous injection of compound 48/80 (50 μg/mouse). The number of scratches was counted for a period of 10 min after the injection of compound 48/80. Each value represents the mean±S.D. of 5—6 mice. $*p<0.05$, $**p<0.01$.

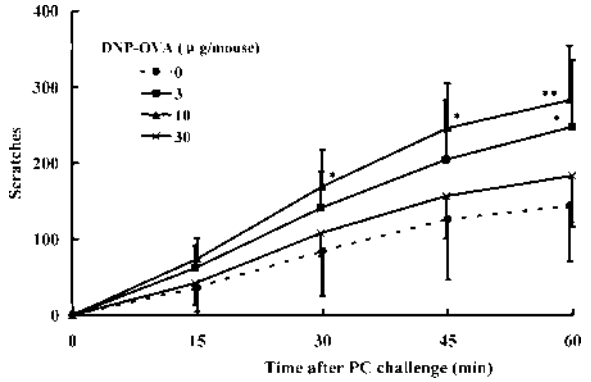

Fig. 2. Scratching Behavior Induced by PC in Mice Actively Sensitized with DNP-OVA and Aluminum Hydroxide

Mice were intraperitoneally injected DNP-OVA plus aluminum hydroxide 1 mg (sensitized) or saline (nonsensitized) 14 d before the challenge with 1% PC ethanol solution. Scratches of challenged ears with the hindpaws were counted in 15 min-intervals. Each value represents the mean±S.D. of 6 mice. $*p<0.05$, $**p<0.01$.

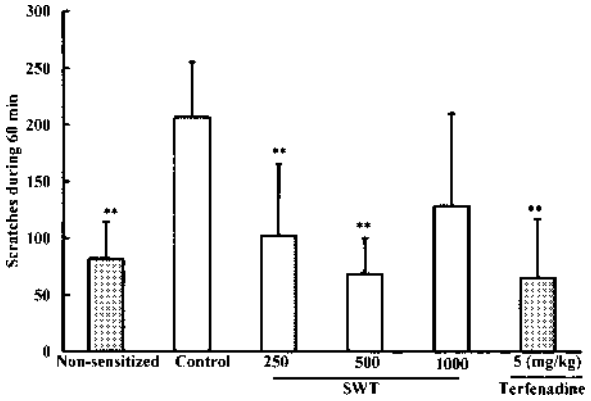

Fig. 3. Effects of Si-Wu-Tang (SWT) and Terfenadine on Scratching Behavior Induced by PC in Actively Sensitized Mice

Mice were intraperitoneally injected with DNP-OVA (10 μg/mouse) plus aluminum hydroxide (1 mg/mouse) or saline (nonsensitized) 14 d before challenge with PC ethanol solution. Scratches for 1 h after PC challenge were counted. SWT aqueous extract and terfenadine were orally administered 1 h before PC challenge. Each value represents the mean±S.D. of 6—8 mice. $**p<0.01$.

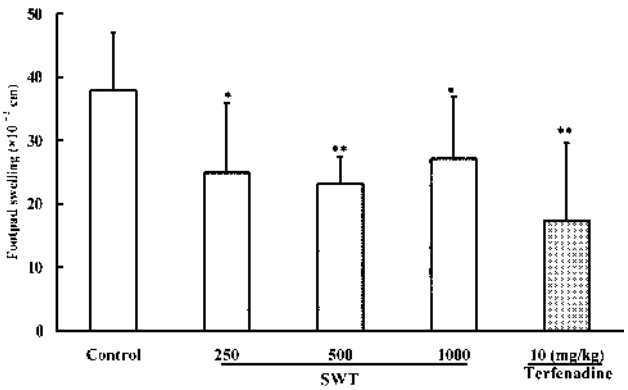

Fig. 4. Effects of Si-Wu-Tang (SWT) and Terfenadine on Footpad Swelling Induced by Compound 48/80 in Mice

SWT aqueous extract and terfenadine were orally administered 1 h before the subcutaneous injection of compound 48/80 (5 μg/site). The thickness of footpads was measured before and 10 min after challenge with compound 48/80. The increase in thickness represents footpad swelling. Each value represents the mean±S.D. of 8 mice. $*p<0.05$, $**p<0.01$.

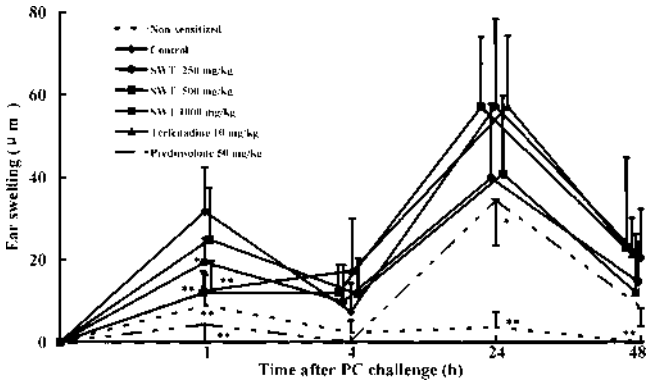

Fig. 5. Effects of Si-Wu-Tang (SWT), Terfenadine, and Prednisolone on Ear Swelling Induced by PC in Actively Sensitized Mice

Mice were intraperitoneally injected with DNP-OVA (10 μg/mouse) plus aluminum hydroxide (1 mg/mouse) or saline (nonsensitized). After 14 d, skin reaction was elicited by applying 30 μl of 1% PC olive oil solution on both sides of mouse ears. The thickness of ears was measured before and 1, 4, 24, and 48 h after challenge with PC. SWT aqueous extract, terfenadine, and prednisolone were orally administered 1 h before PC challenge. Each value represents the mean±S.D. of 6—8 mice. $*p<0.05$, $**p<0.01$.

period after PC challenge. Mice immunized with DNP-OVA 10 μg exhibited the most scratching, and that dose of DNP-OVA was therefore used in the subsequent experiments.

Effect of SWT Aqueous Extract on Scratching Behavior Induced by PC in Actively Sensitized Mice Mice actively sensitized with DNP-OVA 10 μg and aluminum hydroxide 1 mg showed more scratching than nonsensitized mice in the 1 h after PC challenge. SWT aqueous extract at doses of 250 and 500 mg/kg and terfenadine at a dose of 5 mg/kg significantly inhibited the scratching behavior in actively sensitized mice. The most effective dose for SWT was 500 mg/kg (Fig. 3).

Effect of SWT Aqueous Extract on Footpad Swelling Induced by Compound 48/80 Compound 48/80, injected subcutaneously into the footpads of mice, elicited significant swelling. SWT at doses of 250, 500, and 1000 mg/kg showed significant inhibition on the swelling, and the most effective dose was 500 mg/kg. Terfenadine (10 mg/kg) also markedly inhibited the swelling (Fig. 4).

Effect of SWT Aqueous Extract on Ear Swelling Induced by PC in Actively Sensitized Mice As shown in Fig. 5, a biphasic skin reaction with peaks at 1 h (immediate-phase reaction, IPR) and 24 h (late-phase reaction, LPR) after PC challenge was observed in actively sensitized mice, while only one peak at 1 h after PC challenge was observed in nonsensitized mice, which resembled the reaction caused by dinitrofluorobenzene (DNFB) in passively sensitized and nonsensitized mice.[6] Prednisolone, a steroid compound, showed a significant inhibition on the IPR and LPR at a dose of 50 mg/kg. SWT aqueous extract (250 and 500 mg/kg) clearly inhibited ear swelling in the IPR, while it did not at 1000 mg/kg. Furthermore, SWT did not affect the LPR at doses examined. Similarly, terfenadine (10 mg/kg) only obviously inhibited the IPR.

Effect of SWT Aqueous Extract on Histamine Release from Rat Peritoneal Mast Cells As shown in Table 2, SWT (50—500 μg/ml) inhibited histamine release induced by compound 48/80 in a concentration-dependent manner. To elucidate the effective ingredients of SWT, the effects of the four crude drugs in SWT on histamine release were observed. Paeoniae Radix was effective at 25 and 100 μg/ml, Chuanxiong Rhizoma and Rehmanniae Radix were effective

Table 2. Effects of Si-Wu-Tang (SWT), Its Constituents, and DSCG on Compound 48/80-Induced Histamine Release from Rat Peritoneal Mast Cells

Treatment	Concentration (μg/ml)	Histamine release (%)	Inhibition (%)
Control	—	64.0±1.9	
SWT	50	58.8±2.7*	8.1
	100	50.2±4.0**	21.6
	200	39.5±3.1**	38.3
	500	23.9±1.0**	62.6
DSCG	500	40.1±2.2**	37.3
Control	—	58.7±4.0	
Rehmanniae Radix	25	55.1±5.6	6.1
	100	47.1±2.2**	19.8
Angelica Radix	25	51.7±3.6	11.9
	100	52.0±4.2	11.4
Chuanxiong Rhizoma	25	56.0±6.0	4.6
	100	43.1±2.2**	26.6
Paeoniae Radix	25	43.8±5.3**	25.4
	100	26.3±3.8**	55.2
DSCG	500	33.4±5.5**	43.1

Purified mast cells (2×10^6 cells/ml) were preincubated at 37 °C for 10 min. Then test samples dissolved in PBS were added 5 min before activation by compound 48/80 (0.5 μg/ml). The reaction was stopped 10 min later. After centrifugation, histamine contents in the supernatant and cell pellets were determined. Each value represents the mean±S.D. of 3 experiments. $*p<0.05$, $**p<0.01$.

at only 100 μg/ml, and Angelica Radix was ineffective at the concentrations tested. The findings suggest that the inhibitory effect of SWT on histamine release from mast cells is mainly contributed by Paeoniae Radix.

DISCUSSION

Although the precise mechanism of cutaneous pruritus remains unclear, animal models of pruritus induced by compound 48/80, serotonin, substance P, and leukotriene B_4, have been reported successively.[4,5,9—11] Watanabe *et al*.[12] and Nagai *et al*.[13] reported that mice passively sensitized with anti-DNP IgE antibody or actively sensitized with DNP-OVA plus aluminum hydroxide exhibited IgE-mediated biphasic skin reaction with peaks at 1 h (IPR) and 24 h (LPR) after challenge with DNFB. Meanwhile, clear scratching behavior was observed in the IPR but not in the LPR in passively sensitized mice, and the frequency of scratching in sensitized mice was significantly higher than that in nonsensitized mice. In the present study, PC (trinitrochlorobenzene, an analogue of DNFB) was used as an antigen, and it also elicited a biphasic skin reaction, peaking at 1 and 24 h after challenge, and scratching behavior in the IPR but not in the LPR (data not shown) in actively sensitized mice. Prednisolone inhibited the ear swelling in the IPR and LPR, and the scratching behavior in the IPR. However, terfenadine, a selective antagonist of the histamine H_1 receptor, only inhibited the ear swelling and scratching behavior in the IPR but not the ear swelling in the LPR. These findings suggest that the skin reaction and scratching behavior in the IPR induced by PC in actively sensitized mice are mast cell- and histamine-dependent.

SWT aqueous extract significantly inhibited the scratching behavior and footpad swelling induced by compound 48/80 in mice. Similar to terfenadine, SWT only inhibited the skin reaction in IPR and accompanying scratching behavior in the PC-induced biphasic skin reaction in actively sensitized mice, but did not affect the ear swelling in the LPR. In addition, SWT (50—500 μg/ml) clearly reduced the histamine release induced by compound 48/80 from rat peritoneal mast cells. The individual crude drugs that make up SWT, Paeoniae Radix (25 or 100 μg/ml), Rehmanniae Radix, and Chuanxiong Rhizoma (100 μg/ml) also showed inhibition, but not Angelica Radix at the concentrations tested. These results indicate that SWT inhibits mast cell- and histamine-related scratching response and inflammation in mice. It also inhibits the histamine release from mast cells of rats, and Paeoniae Radix may play a crucial role in the formula.

In conclusion, SWT aqueous extract inhibits scratching behavior and inflammation induced by compound 48/80 and PC in normal and actively sensitized mice, respectively, and is useful for alleviating cutaneous pruritus in patients. Moreover, SWT significantly inhibits histamine release induced by compound 48/80 from rat peritoneal mast cells, and Paeoniae Radix probably plays a crucial role in the inhibition.

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REFERENCES

1) Xie M., "Modern Study of the Medical Formulae in Traditional Chinese Medicine," Xue Yue Press, Beijing, 1997, pp. 591—602.
2) Kojima S., Inaba K., Kobayashi S., Kimura M., *Biol. Pharm. Bull*., **19**, 47—52 (1996).
3) Eisen H. N., Elman B. S., Carsten M. E., *J. Am. Chem. Soc*., **75**, 4583—4585 (1953).
4) Kubo M., Matsuda H., Dai Y., Ido Y., Yoshikawa M., *Yakugaku Zasshi*, **117**, 193—201 (1997).
5) Kuraishi Y., Nagasawa T., Hayashi K., Satoh M., *Eur. J. Pharmacol*., **275**, 229—233 (1995).
6) Watanabe C., Satoh T., Tahara E., Murakami K., Hayashi K., Hase K., Andoh T., Kuraishi Y., Kodota S., Nagai H., Saiki I., *Biol. Pharm. Bull*., **22**, 26—30 (1999).
7) Matsuda H., Dai Y., Ido Y., Ko S., Yoshikawa M., Kubo M., *Biol. Pharm. Bull*., **20**, 1086—1091 (1997).
8) Kubo M., Matsuda H.,Tokuoka K., Ma S., Shiomoto H., *Biol. Pharm. Bull*., **17**, 262—265 (1994).
9) Dai Y., Ye W. C., Wang Z. T., Matsuda H., Kubo M., But P. P. H., *J. Ethnopharmacol*., **81**, 245—250 (2002).
10) Andoh T., Kuraishi Y., *Eur. J. Pharmacol*., **353**, 93—96 (1998).
11) Andoh T., Nagasawa T., Satoh M., Kuraishi Y., *J. Pharmacol. Exp. Ther*., **286**, 1140—1145 (1998).
12) Watanabe C., Hase K., Oku T., Koizumi F., Kadota S., Nagai H., Namba T., Saiki I., *Planta Med*., **64**, 12—17 (1998).
13) Nagai H., Sakurai T., Inagaki N., Mori H., *Biol. Pharm. Bull*., **18**, 239—245 (1995).