Natural Product Sciences
13(4) : 373-377 (2007)

Antipruritic Effect of Black Colored Rice

Sang-Jun Han[1], Hien Trung Trinh[1], Seong-Sig Hong[2], Su-Noh Ryu[3] and Dong-Hyun Kim*[1]

[1]*College of Pharmacy, Kyung Hee University, 1, Hoegi, Dongdaemun-ku, Seoul 130-701, Korea*
[2]*Rural Development Administration, Suwon-shi, Gyunggi-do 441-707, Korea*
[3]*Department of agricultural Science Korea National Open University, Seoul 110-791, Korea*

Abstract – Antiscratching behavioral effects of the water extract of two black colored rice (BCR) varieties [*Oryza sativa* L. cv. Heugjinjubyeo (HJ) and Josaengheugchalbyeo (JH)], major pigment of which is cyanindin 3-glucoside, were investigated. Orally administered BCRs' extracts exhibited potent inhibitory activity against scratching behaviors which were induced by compound 48/80 and histamine. The inhibitory effect of Josaengheugchalbyeo *in vivo* and *in vitro* were more potent than those of Heugjinjubyeo. These finding suggest that black colored rice, especially Josaengheugchalbyeo, may inhibit scratching behaviors and anaphylaxis reaction by stabilizing membrane.
Keyword – *Oryza sativa*, black colored rice, anti-allergic activity, antiscratching, RBL-2H3

Introduction

Pruritus (itch) is an unpleasant cutaneous sensation, which provokes the desire to scratch, can be local or widespread and associated with atopic dermatitis, uriticaria or systemic disorders (cholestasis, uraemia). Many endogenous chemical agents, like amines, proteases, growth factors, neuropeptides, opioids, ecosanoids and cytokines, can act as pruritogens (Hagermark, 1995; Lerner, 1994; Schmelz, *et al*., 1997). Scratching can cause skin lesions and contribute to severe psychological disturbances (Raid, 1955). Therefore, inhibition of this response is consistently beneficial for improving the quality of life. To evaluate the effect of itching-inhibitory agents, compound 48/80, substance P or histamine-induced scratching behavior mouse models were used (Inagaki, *et al*., 2002; Inagaki, *et al*., 2000a; Kuraishi, *et al*., 1995). However, there is no specific remedy available for this common symptom.

Rice (*Oryza sativa* L., Poaceae) is the staple food in many Asian countries (FAO, 2004). Varieties of rice include long-grain white, long-grain brown, glutinous brown, wild, basmati, brown basmati, jasmine, and rosotto. Although the most commonly consumed rice varieties have whitish pericarp, there are several colored varieties which have black (or red) caryopses mainly found in wild rice species. With growing concerns regarding national health and the expanding markets of health food, research in the industrial use of bioactive compounds from diverse crops have been extensively increased. Colored rice is broadly known as enriched rice with improved healthy properties (Oki, *et al*., 2002). Black colored rice (BCR) is rich in anthocyanins, especially cyanidin-3-glucoside (C3G) (Ryu, *et al*., 2003), having antioxidant activity, anti-inflammatory properties (Han, *et al*., 2004; Hu, *et al*., 2003) and antianaphylatic effect (Kim, *et al*., 1999a; Kim, *et al*., 1999b). However, its antiscratching behavioral effect was not thoroughly studied.

Therefore, in the present study, the antiscratching behavioral effect of BCR was investigated.

Experimental

Materials – Dulbecco's modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), ovalbumin (OVA), p-nitrophenyl-N-acetyl-β-D-glucosaminide, cremophor EL, azelastine, and compound 48/80 were purchased from Sigma Co. (St. Louis, MO, USA).

Extraction of BCRs – BCRs, Heugjinjubyeo (HJ) and Josaengheugchalbyeo (HJ), were grown at the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Gyeonggi-do, Korea, in 2006. Each rice cultivars were extracted three times with boiling water for 3 h, and filtered through Whatman #2 filter paper

*Author for correspondence
Fax: +82-2-957-5030; E-mail: dhkim@khu.ac.kr

followed by freeze drying. Each BCR samples were kept in –20 °C before using.

Animals – The male ICR and BALB/c mice (20 - 25 g) were supplied by the Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages, maintained at 20 - 22 °C and 50 ± 10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center) and allowed water *ad libitum*. All procedures relating to the animals and their care conformed to the international guidelines: Principles of Laboratory Animals Care (NIH publication no. 85-23, revised 1985).

Assay of scratching behavior frequency – The scratching behavioral experiment in male BALB/c mice (for compound 48/80) or ICR mice (for histamine) was performed according to the method of Sugimoto, *et al.* (1998). Male BALB/c mice were put into acrylic cages (22 × 22 × 24 cm) for about 10 min for acclimation. The rostral part of the skin on the back of mice was clipped, and 50 μg/50 μl of compound 48/80 or 300 μg/50 μl of histamine for each mouse was intradermally injected. The scratching agents were dissolved in saline and then used. Control mice received a saline injection in the place of the compound 48/80. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and, for the observation of scratching; their behaviors recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site by the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches for 1 s, and a series of these behaviors was counted as one incident of scratching for 60 min. The test agents (dissolved in 2% cremophor EL) were orally administered 1 h before the scratching agent.

Assay of analgesic activity – The analgesic activity was determined by using acetic acid-induced ICR mice (Suba, *et al*., 2005). The samples were orally administered prior to intraperitoneal administration of 0.7% acetic acid. The writhing produced in the mice was observed for 10 min (from 10 min to 20 min after administration of acetic acid).

Assay of β-hexosaminidase release from RBL-2H3 cells stimulated by IgE-antigen complex – The inhibitory activity of test agents against the release of β-hexosaminidase from RBL-2H3 cells and histamine of rat peritoneal mast cells was evaluated by a method reported previously (Choo, *et al*., 2003). Briefly, RBL-2H3 cells were dispensed into 24 well plates at a concentration of 2 × 10^5 cells/well using Dulbecco's modified Eagles medium (DMEM, Sigma Chemical Co., St. Louis, MO, USA) containing 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 0.5 μg/ml of mouse monoclonal IgE, and these were incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. Then the cells were washed twice with 500 μl of Siraganian buffer [pH 7.2, 119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM PIPES, and 40 mM NaOH], and incubated in 160 μl of Siraganian buffer [5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA were added] for an additional 10 min at 37 °C. Aliquots (40 μl) of test sample solution were added to each well and incubated for 10 min, followed by the addition of 20 μl of antigen (DNP-HSA, final concentration 1 μg/ml) at 37 °C for 10 min to stimulate the cells to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The reaction mixture was centrifuged at 3000 rpm for 2 min and 25 μl aliquots of the supernatant were transferred to 96-well plates and incubated with 25 μl of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured using a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration 0.1%). The percent inhibition of the release of β-hexosaminidase by the test material was calculated using the following equation, and IC_{50} values were determined graphically:

$$\text{inhibition (\%)} = \left(1 - \frac{T - B - N}{C - N}\right) \times 100$$

Control (C): DNP-HSA (+), test sample (–); Test (T): DNP-HSA (+), test sample (+); Blank (B): DNP-HSA (–), test sample (+); Normal (N): DNP-HSA (–), test sample (–). Under these conditions, it was calculated that 40 - 60% of β–hexosaminidase was released from the cells in the control groups by determination of the total β-hexosaminidase activity after sonication of the cell suspension.

Statistical analysis – All data are expressed as the mean ± standard deviation, and statistical significance was analyzed using a one way ANOVA, followed by a Student-Newman-Keuls test.

Results and Discussion

BCR have been reported to inhibit systemic anaphylaxis

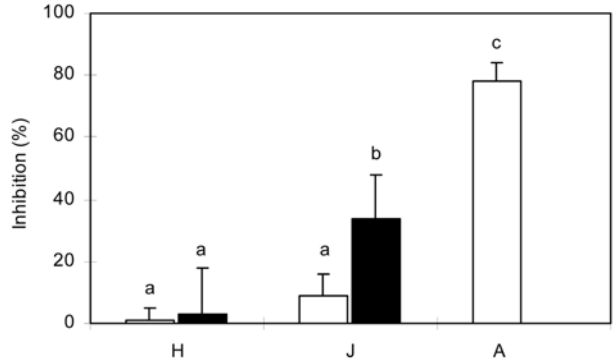

Fig. 1. Inhibitory effect of water extracts of Heugjinjubyeo (H) and Josaengheugchalbyeo (J) against compound 48/80-induced scratching behaviors. The BCR water extracts were orally administered 100 (white bar) and 200 mg/kg (black bar). Azelastine (A) was orally administered 100 mg/kg. In each case, the data shown were mean ± SD (means followed by the same letter are not significantly different at $p < 0.05$).

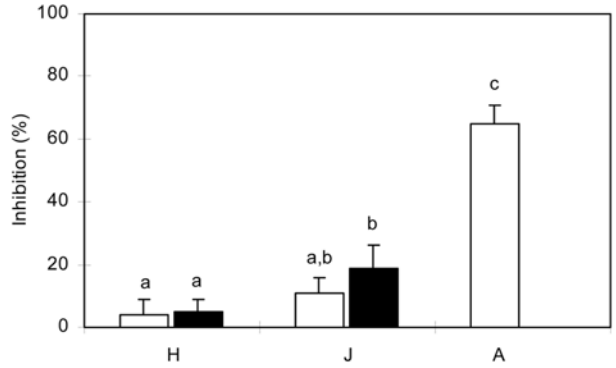

Fig. 2. Inhibitory effect of BCRs against histamine-induced scratching behaviors. The water extracts were orally administered 100 (white bar) and 200 mg/kg (black bar). H = Heugjinjubyeo; J = Josaengheugchalbyeo; A = azelastine. In each case, the data shown were mean ± SD (means followed by the same letter are not significantly different at $p < 0.05$).

reaction in mice by inhibiting the degranulation of mast cells or basophils (Kim, *et al*., 1999a; Kim, *et al*., 1999b). However, its anti-itching effect was not studied. Therefore, the antiscratching behavior effects of the representative BCRs, HJ and JH, were investigated (Fig. 1). When compound 48/80 was injected into the rostral part of the back skin of BALB/c mice, scratching behaviors were significantly induced. When BCRs were orally administered 1 h before the treatment of scratching agent compound 48/80, these BCR extracts inhibited the scratching behaviors. Of them, JH extract more potently inhibited it. However, its inhibitory potency was weak, compared with that of a commercially available azelastine. When histamine was used as an inducer of scratching behavior, histamine also induced the scratching behaviors (Fig. 2). BCR extracts also inhibited the

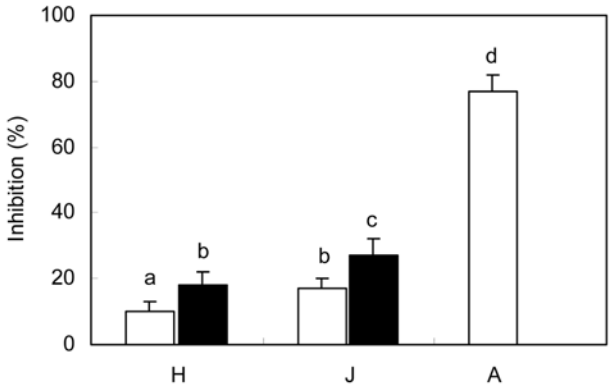

Fig. 3. Inhibitory effect of BCRs on acetic acid-induced writhing syndromes in mice. The BCR water extracts were orally administered 100 (white bar) and 200 mg/kg (black bar). A, orally administered 100 mg/kg of acetaminophen. In each case, the data shown were mean ± SD (means followed by the same letter are not significantly different at $p < 0.05$).

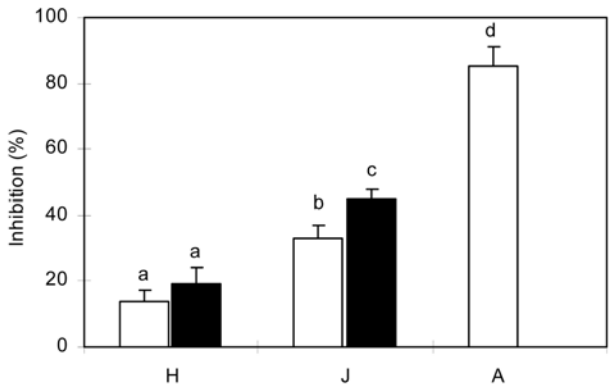

Fig. 4. Inhibitory effect of BCRs against the degranulation of RBL-2H3 cells induced by IgE-antigen complex. The concentrations of BCR water extracts (H: Heugjinjubyeo, J: Josaengheugchalbyeo) and azelastine (A) were 20 (white bar) and 100 μM (black bar). In each case, the data shown were mean ± SD (means followed by the same letter are not significantly different at $p < 0.05$).

histamine-induced scratching behavior. The water extract of JH more potently inhibited it than that of HJ.

To understand the inhibitory mechanism of BCR extract, their analgesic activities against acetic acid induced writhing syndromes in mice were measured (Fig. 3). BCRs inhibited writhing syndromes. HJ and JH at a dose of 100 mg/kg inhibited writhing syndromes by 10 and 17%, respectively. The analgesic activity of JH was more potently than that of HJ. And then we also measured their degranulation-inhibitory effects against RBL-2H3 cells induced by the IgE-antigen complex (Fig. 4). These BCRs potently inhibited the degranulation of RBL-2H3 cells. JH extract at a concentration of 200 mg/kg inhibited it by 48%. However, HJ showed the weak inhibition,

compared to that of JH.

Pruritus (itch) may be associated with atopic dermatitis, urticaria or systemic disorders (cholestasis, uraemia). The etiology of atopic dermatitis may be based on IgE-medicated pharmacological processes of a variety of cell populations, such as mast cells and basophils (Stevens and Austen, 1989). Degranulation of mast cells and basophils, with antigen-crosslinked IgE, releases histamine, prostaglandins, leukotrienes and cytokines (Benyon, *et al*., 1989; Ohmori, *et al*., 1990). These mediators activate chemotaxis and phagocytosis of neutrophils and macrophages, as well as induce itching (Andoh, *et al*., 2001; Rukwied, *et al*., 2000; Stevens and Austen, 1989). Antihistamines, NSAID, steroids and immunosuppressants are representative agents against these allergic diseases (Friedman, *et al*., 2002; Sakuma, *et al*., 2001; Schafer-Korting, *et al*., 1996; Simons, 1992). Among antihistamines, azelastine is an H1-receptor antagonist, but also decreases mediator release from mast cells and basophils. Disodium chromoglycate (DSCG) is a membrane stabilizer, whereas compound 48/80, a histamine releaser, is an activator of hyaluronidase (Cox, 1967). DSCG is mainly known to inhibit the release of chemical mediators from mast cells induced by the antigen-IgE antibody reaction. Steroids, betametasone and dexamethasone, are clinically used in the treatment of psoriasis and other skin disorders as a potent corticosteroid. Corticosteroids are well known to have potent anti-inflammatory effects, but topical use can cause intense skin atrophy, one of the serious side effects limiting their uses for chronic skin diseases. Repeated application of corticosteroids on dorsal skin of rats also causes dramatic skin atrophy (Schafer-Korting, *et al*., 1996). FK-506 and cyclosporine A are a potent immunosuppressant currently used for preventing allograft rejection (Sakuma, *et al*., 2001). FK-506 also suppressed the increase in ear thickness and epidermal thickness. However, it also exhibited side effects, such as severe nephrotoxicity and neurotoxicity. Therefore, foods and herbs have been advanced for allergic diseases, and its effectiveness has received increasing attention.

To evaluate effect of BCR extracts on scratching behavior mouse models, BCR extracts were orally administered to mice, and then these extracts inhibited scratching behaviors induced by compound 48/80 and histamine. Particularly JH more potently inhibited these scratching behaviors. JH also potently inhibited the degranulation of RBL-2H3 cells and showed analgesic effect against the writhing syndrome induced by acetic acid. In addition, Azuma, *et al*. (1976) reported that a membrane stabilizer tranilast, which inhibited the histamine release from rat peritoneal mast cells induced by antigens, as well as antigen-induced PCA reaction, possessed an inhibitory effect toward scratching behavior. Inagaki, *et al*. (2000b) reported that scratching behavior in mice was inhibited by μ-opioid antagonists. Therefore, we measured the analgesic activity of BCRs and azelastine against acetic acid- induced writing syndrome (pain behavior) in mice. Azelastine, which was shown to inhibit the histamine release by interfering with Ca^{++} influx in rat peritoneal mast cell (Chand, *et al*., 1983), and BCR showed the analgesic effect in mice induced by acetic acid. Its analgesic effect may be originated from inhibition of Ca^{++} influx.

These findings suggest that black colored rice, especially JH, may inhibit scratching behaviors and anaphylaxis reaction by stabilizing mem- brane, although the mechanism is sufficiently to be elucidated.

References

Andoh, T., Katsube, N., Maruyama, M., and Kuraishi, Y., Involvement of leukotriene B(4) in substance P-induced itch-associated response in mice. *J. Invest. Dermatol.* **117**, 1621-1626 (2001).

Azuma, H., Banno, K., and Yoshimura, T., Pharmacological properties of N-(3',4'-dimethoxycinnamoyl) anthranilic acid (N-5'), a new anti-atopic agent. *Br. J. Pharmacol.* **58**, 483-488 (1976).

Benyon, R.C., Robinson, C., and Church, M.K., Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli. *Br. J. Pharmacol.* **97**, 898-904 (1989)

Chand, N., Pillar, J., Diamantis, W., Perhach, J.L., Jr., and Sofia, R.D., Inhibition of calcium ionophore (A23187)-stimulated histamine release from rat peritoneal mast cells by azelastine: implications for its mode of action. *Eur. J. Pharmacol.* **96**, 227-233 (1983).

Choo, M.K., Park, E.K., Han, M.J., and Kim, D.H., Antiallergic activity of ginseng and its ginsenosides. *Planta Med.* **69**, 518-522 (2003).

Cox, J.S., Disodium cromoglycate (FPL 670) ('Intal'): a specific inhibitor of reaginic antibody-antigen mechanisms. *Nature* **216**, 1328-1329 (1967).

FAO, International year of rice 2004 - rice and human nutrition. vol. 2006. Food and Agriculture Organization of the United Nations, 2004.

Friedman, E.S., LaNatra, N., and Stiller, M.J., NSAIDs in dermatologic therapy: review and preview. *J. Cutan. Med. Surg.* **6**, 449-459 (2002).

Hagermark, O., Itch mediators. *Semin. Dermatol.* **14**, 271-276 (1995).

Han, S.J., Ryu, S.N., and Kang, S.S., A new 2-arylbenzofuran with antioxidant activity from the black colored rice (Oryza sativa L.) bran. *Chem. Pharm. Bull. (Tokyo)* **52**, 1365-1366 (2004).

Hu, C., Zawistowski, J., Ling, W., and Kitts, D.D., Black rice (Oryza sativa L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *J. Agric. Food Chem* .**51**, 5271-5277 (2003).

Inagaki, N., Igeta, K., Kim, J.F., Nagao, M., Shiraishi, N., Nakamura, N., and Nagai, H., Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80. *Eur. J. Pharmacol.* **448**, 175-183 (2002).

Inagaki, N., Nagao, M., Nakamura, N., Kawasaki, H., Igeta, K., Musoh, K., and Nagai, H., Evaluation of anti-scratch properties of oxatomide and epinastine in mice. *Eur. J. Pharmacol.* **400**, 73-79 (2000a).

Inagaki, N., Nakamura, N., Nagao, M., Kawasaki, H., and Nagai, H., Inhibition of passive cutaneous anaphylaxis-associated scratching behavior by mu-opioid receptor antagonists in ICR mice. *Int. Arch. Allergy Immunol.* **123**, 365-368 (2000b).

Kim, H.M., Kang, C.S., Lee, E.H., and Shin, T.Y., The evaluation of the antianaphylactic effect of Oryza sativa L. subsp. hsien Ting in rats. *Pharmacol. Res.* **40**, 31-36 (1999a).

Kim, H.M., Yi, D.K., and Shin, H.Y., The evaluation of antianaphylactic effect of Oryza sativa L. in rats. *Am, J, Chin, Med,* **27**, 63-71 (1999b).

Kuraishi, Y., Nagasawa, T., Hayashi, K., and Satoh, M., Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. *Eur. J. Pharmacol.* **275**, 229-233 (1995).

Lerner, E.A., Chemical mediator of itching. in: Berhard, J. (Ed.), Itch Mechanisms and Management of Prutitus. McGraw-Hill, New York, pp. 23-25, 1994.

Ohmori, H., Hikida, M., and Takai, T., Prostaglandin E2 as a selective stimulator of antigen-specific IgE response in murine lymphocytes. *Eur. J. Immunol.* **20**, 2499-2503 (1990).

Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I., and Sato, T., Polymeric procyanidins as radical-scavenging components in red-hulled rice. *J. Agric. Food Chem.* **50**, 7524-7529 (2002).

Raid, D.S., Pruritis of chronic cholestasis. *Q. J. Med.* **88**, 603-607 (1955).

Rukwied, R., Lischetzki, G., McGlone, F., Heyer, G., and Schmelz, M., Mast cell mediators other than histamine induce pruritus in atopic dermatitis patients: a dermal microdialysis study. *Br. J. Dermatol.* **142**, 1114-1120 (2000).

Ryu, S.N., Park, S.Z., Kang, S.S., and Han, S.J., Determination of C3G content in blackish purple rice using HPLC and UV-Vis spectrophotometer. *Korean J. Crop Sci.* **48**, 369-371 (2003).

Sakuma, S., Higashi, Y., Sato, N., Sasakawa, T., Sengoku, T., Ohkubo, Y., Amaya, T., and Goto, T., Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (Comparison with steroids). *Int Immunopharmacol* 1: 1219-1226 (2001).

Schafer-Korting, M., Schmid, M.H., and Korting, H.C., Topical glucocorticoids with improved risk-benefit ratio. Rationale of a new concept. *Drug Saf.* **14**, 375-385 (1996).

Schmelz, M., Schmidt, R., Bickel, A., Handwerker, H.O., and Torebjork, H.E., Specific C-receptors for itch in human skin. *J. Neurosci.* **17**, 8003-8008 (1997).

Simons, F.E., The antiallergic effects of antihistamines (H1-receptor antagonists). *J. Allergy Clin. Immunol.* **90**, 705-715 (1992).

Stevens, R.L. and Austen, K.F., Recent advances in the cellular and molecular biology of mast cells. *Immunol. Today* **10**, 381-386 (1989).

Suba, V., Murugesan, T., Kumaravelrajan, R., Mandal, S.C., and Saha, B.P., Antiinflammatory, analgesic and antiperoxidative efficacy of Barleria lupulina Lindl. extract. *Phytother. Res.* **19**, 695-699 (2005).

Sugimoto, Y., Umakoshi, K., Nojiri, N., and Kamei, C., Effects of histamine H1 receptor antagonists on compound 48/80-induced scratching behavior in mice. *Eur. J. Pharmacol.* **351**, 1-5 (1998).

(Accepted November 13, 2007)