Effects of Peppermint (*Mentha piperita* L.) Extracts on Experimental Allergic Rhinitis in Rats

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The present study was carried out to clarify the effects of extracts of the leaves of *Mentha piperita* L. on experimental allergic rhinitis. The 50% EtOH extract of peppermint inhibited histamine release from rat peritoneal mast cells induced by compound 48/80. The effect was dose-dependent and significant inhibition was observed at a concentration of 3 μg/ml. In addition, the 50% EtOH eluate separated from the 50% EtOH extract of peppermint by column chromatography (DIAION HP-20) was also effective in inhibiting histamine release at a concentration of 1 μg/ml. Nasal symptoms, sneezing and nasal rubbing induced by antigen challenge in actively sensitized rats were inhibited by oral administration of the 50% EtOH eluate. Significant inhibition of sneezing and nasal rubbing was observed at doses of 300 and 1000 mg/kg, *p.o.*, respectively. Furthermore, the 50% EtOH eluate inhibited dye leakage into the nasal cavity of rats induced by antigen in a dose-dependent manner. These results suggested that extracts of *Mentha piperita* L. may be clinically effective in alleviating the nasal symptoms of allergic rhinitis.

Key words *Mentha piperita* L.; histamine release; sneezing; nasal rubbing; experimental allergic model

It is widely accepted that allergic rhinitis is Type I allergy caused by an antigen–antibody reaction on the surface of mast cells located on the nasal mucosal membrane,[1—3] causing release of many kinds of chemical mediators such as histamine, leukotrienes, prostaglandins and serotonin from mast cells.[4] These mediators may act as a trigger to produce the symptoms of paroxysmal and repeated sneezing, rhinorrhoea and nasal obstruction. Antihistaminic, steroid and antiallergic drugs are used frequently as therapeutic drugs for treatment of allergic rhinitis.[5,6] On the other hand, systematic scientific studies have been also performed to examine the efficacy of traditional folk medicines and to isolate the biologically active compounds present in such medicines.[7] *Mentha piperita* L. has been reported to show anti-inflammatory,[8] antibacterial[9] and antifungal[10] activities. Recently, it was also demonstrated that peppermint oil is effective against Type I allergic reactions.[11] However, these biological activities are known to be caused by essential oil obtained by steam distillation from peppermint leaves.

Very little information is available as to whether residual ingredients not included in the essential oil from peppermint leaves and stems have antiallergic effects. Therefore, the present study was performed to clarify the effects of various peppermint extracts on experimental allergic rhinitis in rats.

MATERIALS AND METHODS

Animals Male Wistar rats (age; 7 weeks; body weight, 190—220 g) were obtained from Shimizu Laboratory Supplies (Kyoto, Japan). All of animals were maintained in an air-conditioned room with controlled temperature of 24±2 °C and humidity of 55±15%. The animals were allowed food and water *ad libitum.*

Extraction and Separation of *Mentha piperita* L. First, Essential oil was removed from peppermint leaves and stems by steam distillation, and the residual leaves and stems were dried. Then, dried powdered leaves and stems (1 kg) were extracted by reflux in 50% EtOH (10 l, 1 h). The filtrate was evaporated under reduced pressure to yield a dark brown extract (230 g). The extract was suspended in 50% EtOH and then defatted with *n*-hexane (1 l×3). After removal of the solvent, the residue that dissolved in H_2O was chromatographed on a highly porous polymer (DAIAION HP-20, Mitsubishi Kasei Co.,) column.

Chemicals The chemicals used and their sources were as follows: Aluminum hydroxide (LSL Co., Tokyo, Japan), gum arabic (Wako, Tokyo, Japan), compound 48/80 (Sigma, St. Louis, MO, U.S.A.), Evans blue (Wako), *o*-phthaldialdehyde (Sigma), ovalbumin (Sigma), tranilast (Schering Plough, Osaka, Japan).

Compound 48/80-Induced Histamine Release from Isolated Rat Peritoneal Mast Cells The procedure was similar to that described previously.[12] Briefly, rat peritoneal mast cells were harvested from the abdominal cavity of male rats and purified by Percoll density centrifugation. Briefly, physiological buffered saline (PBS; in mM: NaCl 154, KCl 2.7, $CaCl_2$ 5, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) 5; pH 7.4) was injected into the peritoneal cavity. The abdominal region was gently massaged for 90 s and peritoneal fluid was collected and centrifuged for 7 min at 100×***g***, 4 °C. After centrifugation, the cell pellets were pooled and washed twice in PBS(−) solution. The mast cell pellets purified by Percoll density centrifugation for 15 min at 200×***g***, 4 °C were washed twice in PBS(−) solution. Thereafter, equal numbers of mast cells (2.5×10^4 cells/tube) were preincubated in 0.8 ml of 0.1% glucose containing physiological salt solution [PBS(+)] for 10 min at 37 °C. The test drugs dissolved in PBS(+) were added (0.1 ml) 10 min before compound 48/80 (0.5 μg/ml). The reaction was stopped 10 min later by cooling the tubes in ice water. The tubes were centrifuged for 15 min at 200×***g*** and histamine content was measured in the supernatant and precipitate using an autoanalyzer (Technicon, Ireland).

Nasal Symptoms Induced by the Antigen Rats were actively sensitized by an injection of physiological saline containing 1 mg ovalbumin, 10^{10} cells of *B. pertussis* and

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2 mg of aluminum hydroxide into the four foot pads on the first day. Five days later, they were boosted by a subcutaneous injection of 1 ml of physiological saline containing 0.5 mg ovalbumin into the rostral parts of the back. Fourteen days later, local sensitization was performed every day by dripping ovalbumin in saline (1 mg/ml, 10 μl/nostril) into the bilateral nasal cavities using a micropipette. Effects of drugs on nasal symptoms after antigen challenge were evaluated with rats from 28 to 42 d old after general sensitization. Before the experiment, the animals were placed into an observation cage (32×24×18 cm) for 10 min for acclimatization. After nasal instillation of 10 μl of antigen dissolved in saline (1 mg/ml) into the bilateral nasal cavities, the animals were placed into the observation cage (1 animal/cage), and sneezing and nasal rubbing were counted for 30 min. Peppermint extracts were administered orally 1 h before antigen injection.

Nasal Symptoms Induced by Histamine Before the experiment, the animals were placed into an observation cage (32×24×18 cm) for 10 min for acclimatization. After nasal instillation of 10 μl (10 μmol/site) of histamine dissolved in saline into the bilateral nasal cavities, the animals were placed into the observation cage (1 animal/cage) and sneezing and nasal rubbing were counted for 30 min. Peppermint extracts were administered orally 1 h before histamine injection.

Antigen-Induced Nasal Hyperpermeability Experimental allergic rhinitis was induced according to the method of Kojima *et al.*[4] Rats immunized with the antigen were anesthetized with pentobarbital (35 mg/kg, i.p.) and the trachea was cannulated with a cannula to maintain an airway. Thereafter, polyethylene tubing (outside diameter, 1.09 mm; Intermedic, PE-20, Becton Dickinson and Co., Parsippany, NJ, U.S.A.) was inserted through the trachea to the posterior part of the nasal cavity. The other end of the polyethylene tubing was connected to a perfusion pump (Micro tube pump MP-3N, Tokyo Rikakikai Co., Ltd, Tokyo, Japan), and saline was perfused through the nasal cavity at a rate of 0.25 ml/min (37 °C). The oral cavity was filled with absorbent cotton. The effluent from the nostrils was collected for 10 min. Subsequently, 1% Evans blue (0.5 ml/100 g) was injected into the femoral vein, and 3 min after injection the effluent was collected for 10 min. The antigen was then perfused through the nasal cavity in the same manner, and the effluent was collected for 10 min. The effluent was centrifuged at 1200×***g*** for 10 min and Evans blue concentration in the supernatant was determined at 620 nm (Spectrophotometer, Type U-2000, Hitachi, Tokyo, Japan). Test drugs were administered orally (0.5 ml/100 g) 1 h before antigen challenge. The same volume of 5% gum arabic was administered to the control group.

Statistical Analysis The data are presented as means± S.E.M. Statistical significance was tested by one-way analysis of variance with Dunnett's test. A probability value less than 0.05 was considered as significant. IC_{50} values were calculated according to the probit method.

RESULTS

Effects of Peppermint Extract and Separated Fractions on Histamine Release from Peritoneal Mast Cells The average histamine release induced by compound 48/80 (0.5 μg/ml) was 51.0±1.4% (n=7) of the total content. The 50% EtOH extract was effective in inhibiting histamine release induced by compound 48/80, and significant differences were observed at concentrations of 10 μg/ml or more. The IC_{50} value for the 50% EtOH extract was 4.72 (2.54—7.65) μg/ml. H_2O, 50% EtOH and EtOH eluates caused dose-dependent inhibition of histamine release, and the 50% EtOH eluate was more effective than those of H_2O and EtOH eluate (Table 1).

Table 1. IC_{50} Values of Peppermint Extract and Separated Fractions for the Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80

Extracts	IC_{50} values (95% confidence limits) (μg/ml)
50% EtOH extract	4.72 (2.54—7.65)
H_2O eluate	14.2 (7.96—28.1)
50% EtOH eluate	2.55 (1.42—3.94)
EtOH eluate	6.71 (6.05—7.42)

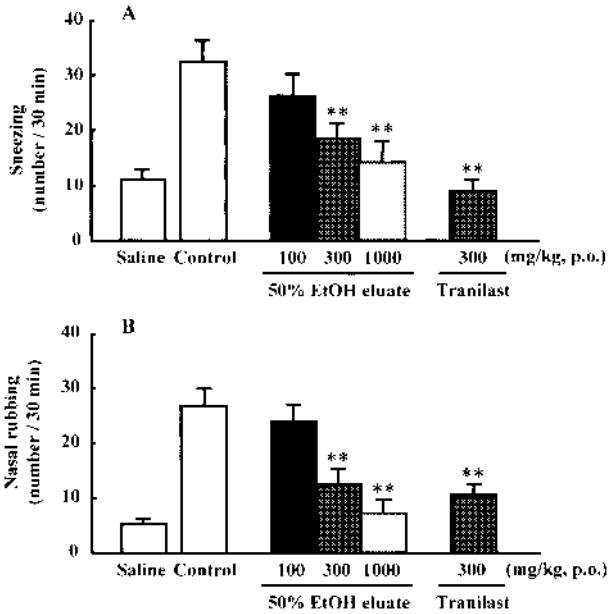

Fig. 1. Effects of 50% EtOH Eluate on Sneezing (A) and Nasal Rubbing (B) Induced by Antigen in Rats

Each column and vertical bar represents the mean±S.E.M. for 9 animals. **; $p<0.01$ compared with the control group (Dunnett's test).

Effects of the 50% EtOH Eluate on Sneezing and Nasal Rubbing Induced by Antigen The effects of the 50% EtOH eluate on the antigen-induced nasal symptoms are shown in Fig. 1. The average sneezing frequency induced by the antigen was 32.4±3.9 times/30 min. The 50% EtOH eluate caused a dose-related inhibition of this response and significant effects were observed at doses of 300 and 1000 mg/kg, *p.o.* Tranilast inhibited the antigen-induced sneezing at a dose of 300 mg/kg (Fig. 1A). Nasal rubbing was also inhibited significantly by the 50% EtOH eluate at doses of 300 and 1000 mg/kg. Tranilast significantly inhibited this response at a dose of 300 mg/kg (Fig. 1B).

Effects of the 50% EtOH Eluate on Sneezing and Nasal Rubbing Induced by Histamine Figure 2 shows the effects of the 50% EtOH eluate on nasal symptoms. The aver-

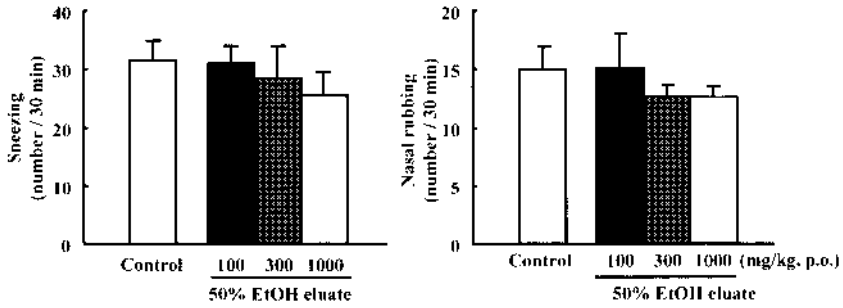

Fig. 2. Effects of 50% EtOH Eluate on Sneezing and Nasal Rubbing Induced by Histamine (10 μmol/site) in Rats
Each column and vertical bar represents the mean±S.E.M. for 10 animals.

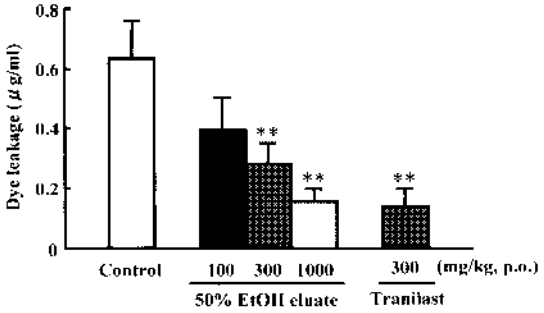

Fig. 3. Effects of 50% EtOH Eluate on the Antigen-Induced Increase in Dye Leakage into the Nasal Cavities of Actively Sensitized Rats
Each column and vertical bar represents the mean±S.E.M. for 9 animals. **; $p<0.01$ compared with the control group (Dunnett's test).

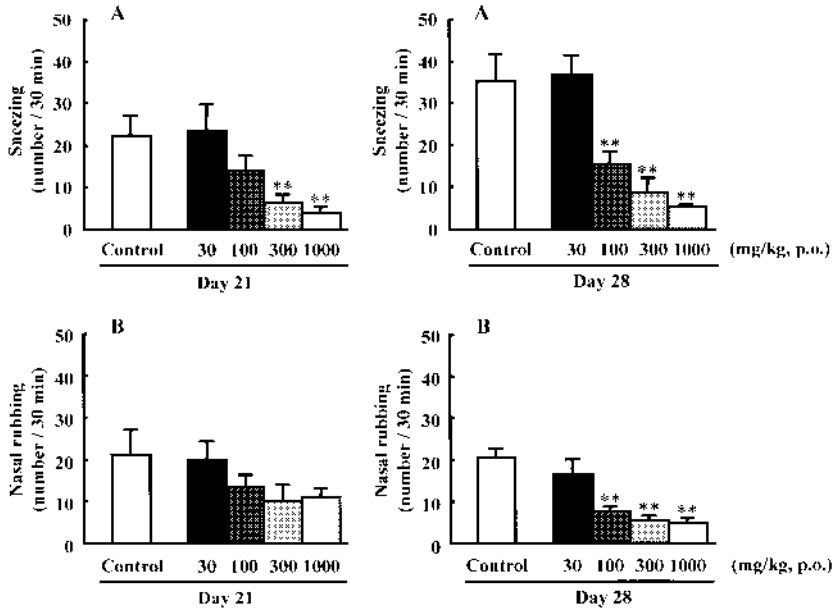

Fig. 4. Effects of Repeated Administration of 50% EtOH Eluate for 2 Weeks on Sneezing (A) and Nasal Rubbing (B) Induced by Antigen in Rats
Each column and vertical bar represents the mean±S.E.M. for 9 animals. **; $p<0.01$ compared with the control group (Dunnett's test).

age sneezing and nasal rubbing frequencies induced by histamine in rats were 29.7±6.8 and 13.5±2.5 times/30 min respectively. The 50% EtOH eluate showed no significant effect on this response.

Effects of the 50% EtOH Eluate on the Antigen-Induced Increase in Dye Leakage in the Nasal Cavities As shown in Fig. 3, the antigen-induced increase in dye leakage was inhibited by the 50% EtOH eluate in a dose-dependent fashion. Significant effects were observed at doses of 300 and 1000 mg/kg. Tranilast also significantly inhibited dye leakage at a dose of 300 mg/kg.

Effects of Repeated Administration of the 50% EtOH Eluate for 2 Weeks on Nasal Symptoms Induced by Antigen Figure 4 shows the effects of repeated administration of the 50% EtOH eluate on nasal symptoms induced by a local application of the antigen in rats. After general sensitization, local sensitization was performed once a day from 14 d to 28 d by the instillation of ovalbumin in saline (1 mg/ml, 10 μl/nostril) into the bilateral nasal cavities using a micropipette. The 50% EtOH eluate at doses of 30—1000 mg/kg (0.5 ml/100g) was administered orally every day from 14 d to 28 d at ten in the morning. Peppermint extracts

were given orally 1 h before the antigen challenge. On day 21 (7 d after the first oral administration), significant effects on sneezing were observed at doses of 300 and 1000 mg/kg. However, no significant effect on nasal rubbing was observed even at a dose of 1000 mg/kg. On day 28, both sneezing and nasal rubbing were significantly inhibited at doses of 100 mg/kg or more.

DISCUSSION

The results of the present study indicated that the 50% EtOH extract of peppermint leaves and stems significantly inhibited histamine release from rat peritoneal mast cells induced by compound 48/80. In addition, H_2O, 50% EtOH and EtOH eluates separated by column chromatography also showed similar effects, and the 50% EtOH eluate showed most potent effect (Table 1). These results indicated that the active component of the peppermint extract is contained in the 50% EtOH eluate.

Recently, we developed a new animal model of allergic rhinitis, and sneezing and nasal rubbing in this model are useful for evaluating the effects of antiallergic drugs on allergic rhinitis.[13] It was also found that the 50% EtOH eluate inhibited sneezing and nasal rubbing induced by antigen–antibody reaction. Saito *et al.*[14] proposed that chemical mediators including histamine released from mast cells cause sneezing and nasal rubbing. As shown in Table 1 and Fig. 2, the 50% EtOH eluate inhibited histamine release from rat mast cells, but it showed no significant effect on sneezing or nasal rubbing induced by histamine. Therefore, the effects of the 50% EtOH eluate on sneezing and nasal rubbing induced by antigen challenge may be largely due to inhibition of histamine release from mast cells in the nasal mucosa. Furthermore, the 50% EtOH eluate also decreased the amount of dye leakage induced by antigen exposure, suggesting that it suppressed the increase in nasal vascular permeability.

Repeated administration of the 50% EtOH eluate for 2 weeks inhibited sneezing and nasal rubbing. In addition, the effect of repeated administration was more potent than that of single administration. Ukai *et al.*[15] reported that repeated administration of DSCG showed more a marked prophylactic effect than single administration for treatment of rhinitis allergy to cedar pollen in humans. We anticipated similar findings when the 50% EtOH eluate of peppermint extract is used clinically.

In conclusion, the oral administration of peppermint extract inhibited both the nasal symptoms and the nasal vascular permeability induced by antigen challenge. Therefore, peppermint extract may be beneficial for the clinical treatment of allergic rhinitis.

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