

Effects of Hop Extracts on Nasal Rubbing and Sneezing in BALB/c Mice

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The effects of hop extracts (*Humulus lupulus* L.) on histamine release from rat peritoneal mast cells and human basophilic KU812 cells were studied. Hop water extract (HWE) and XAD-4 50% methanol fraction of HWE (MFH) inhibited histamine release from rat mast cells induced by compound 48/80 at concentrations of 100 and 10 μ g/ml, respectively. Almost the same findings were observed with A23187-induced histamine release from KU812 cells. Next, we studied the effects of hop extracts on antigen-induced nasal rubbing and sneezing in sensitized BALB/c mice. HWE caused a significant inhibition of nasal rubbing and sneezing at a dose of 500 mg/kg. MFH also inhibited nasal rubbing and sneezing dose-dependently. A significant difference was observed from 100 mg/kg in nasal rubbing and 200 mg/kg in sneezing. The effects of both extracts became clear after repeated administration. HWE and MFH significantly inhibited both nasal rubbing and sneezing, respectively, after consecutive treatment for 15 d at smaller doses compared with single administration. This finding indicates that the active component of hop is included in MFH, which was absorbed to Amberlite XAD-4 and eluted with 50% methanol. These results clearly demonstrated that hop extracts may be effective in the relief of symptoms of allergic rhinitis.

Key words hop extract; histamine release; compound 48/80; A23187; nasal rubbing; sneezing

The female flowers of hop (*Humulus lupulus* L.) have been used in the brewing industry to add bitterness and aroma to beer. Hop is also used in folk medicine as a tranquilizer, and in Japan, it is formulated in over the counter (OTC) drugs for depression of the central nervous system or the activation of gastric function. Moreover, it has been reported that the hop shows a wide range of the physiological roles, such as an inhibitory effect on the bone resorption,¹⁾ cancer chemopreventive activity,^{2,3)} inhibition of tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in mouse skin,⁴⁾ an estrogenic property of the phyto-estrogen, 8-prenylnaringenin,⁵⁾ and the inhibition of nitric acid production.⁶⁾

Allergic diseases such as hay fever, atopic dermatitis, food allergy and bronchial asthma are increasing in prevalence in most countries of the developed world. These allergic diseases are classified as type I allergies. Basophils and mast cells play a crucial role in the initiation of such types of allergies. On the other hand, it has been reported that tea,⁷⁾ peppermint⁸⁾ and tomato skin⁹⁾ showed antiallergic properties by inhibiting the degranulation of basophils and mast cells. Antiallergic properties of these plant extracts are attributable to their flavonoids and flavonoid glycosides. It is well known that hop contains polyphenols, including flavonoids and flavonoid glycosides.¹⁰⁾ However, relatively little is known about the antiallergic properties of hop extracts.

Therefore, we investigated the antiallergic properties of hop extracts by evaluating the inhibition of histamine release from rat peritoneal mast cells and human basophilic KU812 cells. The effects of hop extracts on the nasal rubbing and the sneezing in allergic rhinitis models were also studied in mice.

MATERIALS AND METHODS

Animals Female BALB/c mice (6–10 week-old) were obtained from Japan SLC, Inc., Shizuoka. 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 chow (Oriental Yeast, Tokyo) and water *ad libitum*. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Research Center.

Hop Sample Preparation Hop water extract (HWE): 500 g hop (*Humulus lupulus* L.) pellets were soaked in 9.5 l of distilled cold (4°C) water overnight. The hop pellets suspension was filtered through filter paper. The aqueous solution was concentrated under reduced pressure and then lyophilized. The yield was *ca.* 22%. XAD-4 50% methanol fraction of HWE (MFH): the HWE that dissolved in distilled water was applied to Amberlite XAD-4 (Organon; Tokyo, Japan). The column was washed with distilled water and then eluted with 50% methanol. The fraction eluted with 50% methanol was evaporated and lyophilized. The yield was *ca.* 4.5%.

Reagents The reagents used in the experiments were obtained from the sources shown in parentheses: compound 48/80 (Sigma, St. Louis, MO, U.S.A.), A23187 (Sigma), ketotifen fumarate (Sankyo, Tokyo, Japan), egg albumin (Sigma), alum (LSL Co., Tokyo, Japan) and *B. pertussis* (Sigma).

Histamine Release from Rat Peritoneal Mast Cells Peritoneal mast cells were harvested from peritoneal fluid of Wistar rat (Wistar male strain, obtained from Japan SLC) and purified to greater than 90% purity by percoll density gradient centrifugation. The collected mast cells (2.5×10^4 cells/tube) were then incubated with physiological buffer solution (in mM: NaCl 140, KCl 2.7, CaCl_2 0.9, glucose 5.6, HEPES 5, pH 7.4) for 10 min. Histamine release was evoked by adding 0.1 ml of compound 48/80 (final concentration; 0.5 μ g/ml) and incubating at 37°C for 10 min. The reaction was stopped 10 min later by cooling the tubes in ice water and the tubes were centrifuged at $200 \times g$ for 15 min. Histamine contents were measured by means of a fluorometric

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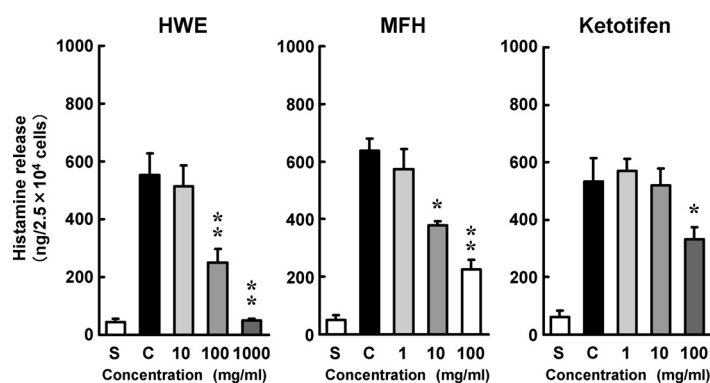


Fig. 1. Effects of Hop Water Extract, XAD-4 50% Methanol Fraction of Hop Water Extract and Ketotifen on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80

HWE: hop water extract, MFH: XAD-4 50% methanol fraction of hop water extract, S: spontaneous release, C: control. Each value represents the mean $\pm$ S.E.M. ($n=4$). *,** Significantly different from the control group with $p<0.05$ and $p<0.01$, respectively.

assay.¹¹⁾

Histamine Release from KU812 Cells KU812 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan). The cells were cultured in RPMI 1640 medium (Invitrogen Corp., Carlsbad, CA, U.S.A.) supplemented with 10% fetal bovine serum (Equitech-Bio, Kerville, TX, USA) at 37°C in a humidified atmosphere with 5% CO₂. The KU812 cells were washed twice with Tyrode buffer (in mM: NaCl 137, KCl 2.7, NaH₂PO₄·2H₂O 0.4, MgCl₂·6H₂O 1, NaHCO₃ 12, CaCl₂·2H₂O 1.8). Then, the cells were suspended in 0.5 ml Tyrode buffer to be 2×10^6 cells/ml. CaCl₂ (1 mM), calcium ionophore A23187 (final concentration: 0.5 mM) and test sample solution (10% v/v) were added into the cell suspension. The cell suspension was incubated at 37°C for 20 min and the reaction was stopped by cooling in an ice bath for 5 min. After centrifugation at 200 $\times g$ for 5 min at 4°C histamine concentration of the supernatant was determined using a fluorometric method.¹¹⁾

Sensitization The mice were sensitized by the injection of 0.2 ml of physiological saline containing egg albumin (0.1 mg), alum (1 mg) and *B. pertussis* (300 ng) into the peritoneal cavity on the first day. Five days later, they were boosted by the subcutaneous injection of 1 ml of physiological saline containing egg albumin (0.05 mg) in the back. Then, local sensitization was performed every day from day 18 by instilling egg albumin in physiological saline (50 mg/ml/nos-tril) into the bilateral noses using a micropipette.

Nasal Rubbing and Sneezing Behavior To evaluate nasal rubbing, each time the animal rubbed or touched the area near the nose with its forepaws, it was counted as one event. Touches around the eyes and the mouth were disregarded. Before the experiment, the animals were placed into an observation cage (32 $\times$ 22 $\times$ 20 cm) for about 10 min for acclimatization. The extracts of hop were administered orally. One hour later, 1 μ l of antigen (50 μ g) was instilled into the bilateral nasal cavities. The animals were placed into the observation cage (one animal/cage), and nasal rubbing and sneezing were counted for 30 min. Nasal rubbing and sneezing behavior were observed on day 39. HWE and FMH were administered orally every day from day 25 to day 39.

Statistical Analysis All values are expressed as the mean $\pm$ standard error of the mean (S.E.M.). Statistical evaluation of the results was performed by one-way analysis of

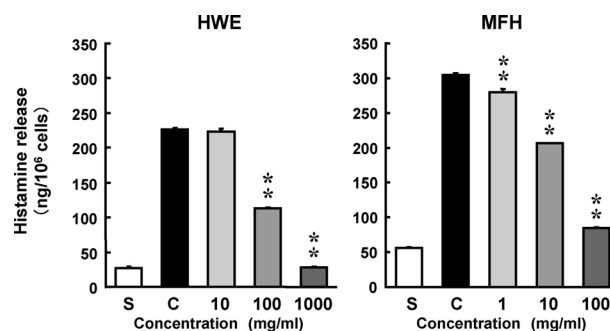


Fig. 2. Effects of Hop Water Extract and XAD-4 50% Methanol Fraction of Hop Water Extract on Histamine Release from Human Basophilic KU812 Cells Induced by A23187

HWE: hop water extract, MFH: XAD-4 50% methanol fraction of hop water extract, S: spontaneous release, C: control. Each value represents the mean $\pm$ S.E.M. ($n=3$). *,** Significantly different from the control group with $p<0.05$ and $p<0.01$, respectively.

variance (ANOVA) followed by the Bonferroni or Dunnett test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Histamine Release from Rat Peritoneal Mast Cells

Figure 1 shows the effects of the HWE, MFH and ketotifen on histamine release from rat peritoneal mast cells. In non treated cells with compound 48/80, the intracellular histamine concentration of rat peritoneal mast cells was 923.5 ± 10.3 ng/2.5 $\times 10^4$ cells. Both extracts caused a concentration-related inhibition of the histamine release induced by compound 48/80, and a significant effect was observed at 100 μ g/ml in HWE and 10 μ g/ml in MFH. Ketotifen also inhibited the histamine release, and the significant inhibition was observed at a concentration of 100 μ g/ml.

Histamine Release from KU812 Cells

Figure 2 shows the effects of the HWE and MFH on histamine release from KU812 cells. In non treated cells with A23187, the intracellular histamine concentration of KU812 cells was 440.0 ± 5.1 ng/10⁶ cells. Both extracts inhibited the histamine release induced by A23187, and a significant effect was observed at 100 μ g/ml in HWE and 1 μ g/ml in MFH.

Effect of HWE on Nasal Rubbing and Sneezing

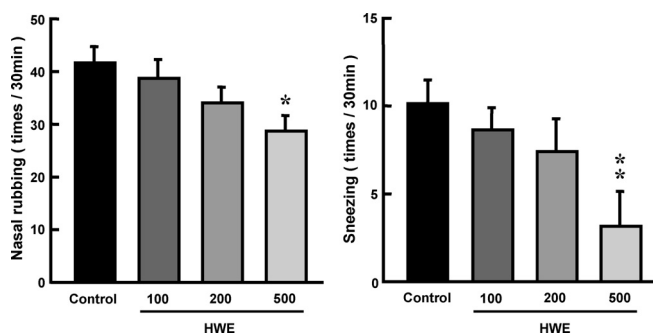


Fig. 3. Effect of Hop Water Extract on Nasal Rubbing and Sneezing Induced by Antigen

HWE: hop water extract. Each value represents the mean $\pm$ S.E.M. ($n=6-8$). **Significantly different from the control group with $p<0.05$ and $p<0.01$, respectively.

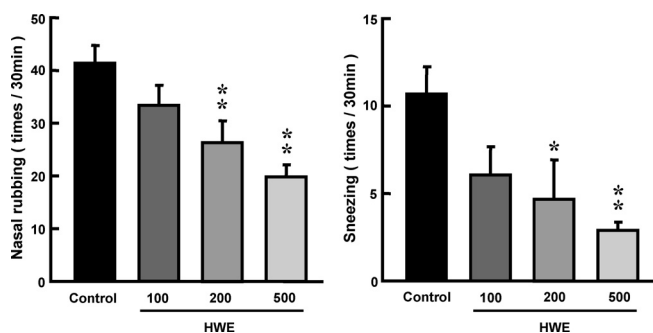


Fig. 4. Effect of Repeated Administration of Hop Water Extract for 15 d on Nasal Rubbing and Sneezing Induced by Antigen

HWE: hop water extract. Each value represents the mean $\pm$ S.E.M. ($n=6-8$). **Significantly different from the control group with $p<0.05$ and $p<0.01$, respectively.

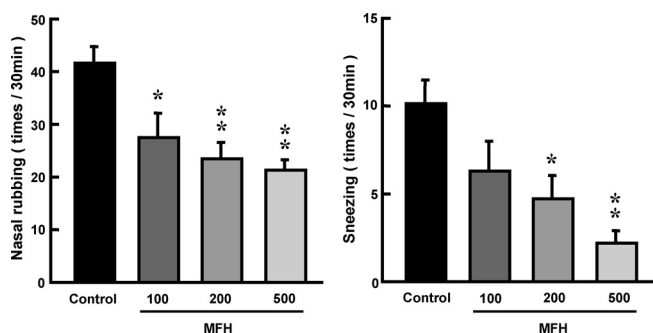


Fig. 5. Effect of XAD-4 50% Methanol Fraction of Hop Water Extract on nasal Rubbing and Sneezing Induced by Antigen

MFH: XAD-4 50% methanol fraction of hop water extract. Each value represents the mean $\pm$ S.E.M. ($n=6-8$). **Significantly different from the control group with $p<0.05$ and $p<0.01$, respectively.

Figure 3 shows the effect of HWE on nasal rubbing and sneezing induced by antigen. HWE caused no inhibitory effect on nasal rubbing at doses of 100 and 200 mg/kg; however, at a dose of 500 mg/kg, it caused a significant inhibition. Almost the same results were obtained with sneezing. When HWE was administered once a day for 15 d, the inhibitory effect became clear; and a significant effect on both behaviors was observed, even at a dose of 200 mg/kg (Fig. 4).

Effect of MFH on Nasal Rubbing and Sneezing

Figure 5 shows the effect of MFH on nasal rubbing and sneezing induced by antigen. MFH caused a significant inhibitory effect at a dose of 100 mg/kg on nasal rubbing and at

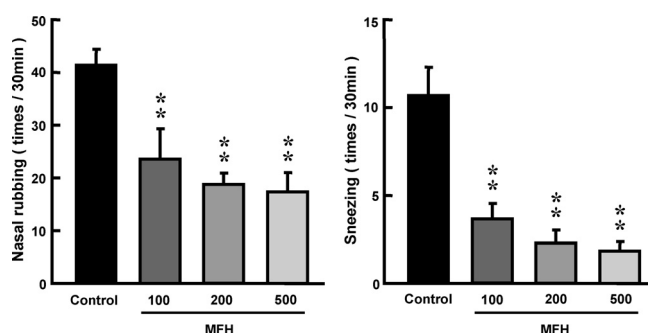


Fig. 6. Effect of Repeated Administration of XAD-4 50% Methanol Fraction of Hop Water Extract for 15 d on Nasal Rubbing and Sneezing Induced by Antigen

MFH: XAD-4 50% methanol fraction of hop water extract. Each value represents the mean $\pm$ S.E.M. ($n=6-8$). **Significantly different from the control group with $p<0.05$ and $p<0.01$, respectively.

200 mg/kg on sneezing. The effect of MFH became clear after repeated administration for 15 d, and a significant effect was observed at a dose of 100 mg/kg on both behaviors (Fig. 6).

DISCUSSION

In the present study, it was demonstrated that HWE and MFH significantly inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80 and from human basophilic KU812 cells induced by A23187. Although intracellular histamine concentration of rat peritoneal mast cells was relatively higher than that of KU812 cells, HWE and MFH inhibited the histamine release from both cells in the same manner. In addition to HWE and MFH, ketotifen which was able to prevent mast cell degranulation also inhibited the histamine release from rat peritoneal mast cells. The effect of MFH was more potent than HWE in histamine release from both mast cells and KU812 cells. This finding indicates that the active component of hop is included in MFH, which was absorbed to Amberlite XAD-4 and eluted with 50% methanol. It is well recognized that hop contains flavonoids, such as quercetin and kaempferol, and their glycosides.¹⁰ Moreover, it was reported that biologically active substances such as flavonoid and flavonoid glycosides were easily separated from other hydrophilic constituents by using highly porous polymer column.¹² We speculate therefore, that flavonoids and flavonoid glycosides in hop may contribute to the inhibition of histamine release from mast cells or basophilic KU812, although the detailed chemical structure of active components in MFH are under investigation. In association with these findings, it was reported that tea,⁷ peppermint⁸ and tomato skin⁹ have antiallergic properties by inhibiting the degranulation from basophilic and mast cells, and their flavonoids and/or flavonoid glycosides are its active components.

It goes without saying that the symptoms of allergic rhinitis are caused by the activation of mucosal mast cells of the nostril. This activation of mast cells is initiated by binding of the antigen to immunoglobulin E (IgE), and the cross-linkage of the IgE at the surface of mast cells triggers the release of many chemical mediators, such as histamine, leukotrienes and prostaglandins from these cells. Some investigations indicate that histamine caused sneezing and nasal rubbing by

its binding to the histamine H_1 -receptors on the sensory nerve endings.^{13–16} As a matter of course, there are some findings that histamine H_1 -receptor antagonists inhibited the symptoms of sneezing and nasal rubbing of the allergic rhinitis model in mice and rats.^{17,18}

The oral administration of HWE and MFH to the allergic rhinitis model mice, which are sensitized to egg albumin, significantly suppressed the sneezing and the nasal rubbing of these mice. Both extracts showed inhibition of the sneezing and nasal rubbing at a single dose of 100–500 mg/kg. We have reported that natural products, such as Brazilian propolis¹⁹ and Lo Han Kuo,¹¹ exert a stronger effect by consecutive administration; therefore, we studied the effect of hop extract by consecutive treatment for 15 d on both behaviors. As a result, it was found that repeated oral administration of both extracts resulted in more potent inhibitory effect than the single administration. As stated previously, both hop extracts inhibited histamine release from rat mast cells and basophilic KU812 cells. Therefore, it seems likely that the inhibitions of sneezing and nasal rubbing induced by the antigen occurred through histamine release inhibition from basophilic and/or mast cells.

From these results, it may be concluded that hop extract is effective in the relief of allergic symptoms when used in a clinical setting.

REFERENCES

- 1) Tobe H., Muraki Y., Kitamura K., Komiyama O., Sato Y., Sugioka T., Maruyama H. B., Matsuda E., Nagai M., *Biosci. Biotech. Biochem.*, **61**, 158–159 (1997).
- 2) Gerhauser C., Alt A., Heiss E., Gamal-Eldeen A., Klimo K., Knauff J., Neumann I., Scherf H. R., Frank N., Bartsch H., Becker H., *Mol. Cancer Ther.*, **1**, 959–969 (2002).
- 3) Chen W.-J., Lin J.-K., *J. Agric. Food Chem.*, **52**, 55–64 (2004).
- 4) Yasukawa K., Takeuchi M., Takido M., *Oncology*, **52**, 156–158 (1995).
- 5) Milligan S., Kalita J., Pocock V., Heyerick A., De Cooman L., Rong H., De Keukeleire D., *Reproduction*, **123**, 235–242 (2002).
- 6) Zhao F., Nozawa H., Daikonnya A., Kondo K., Kitanaka S., *Biol. Pharm. Bull.*, **26**, 61–65 (2003).
- 7) Tachibana H., Sunada Y., Miyase T., Sano M., Maeda-Yamamoto M., Yamada K., *Biosci. Biotech. Biochem.*, **64**, 452–454 (2000).
- 8) Inoue T., Sugimoto Y., Masuda H., Kamei C., *Biol. Pharm. Bull.*, **25**, 256–259 (2002).
- 9) Yamamoto T., Yoshimura M., Yamaguchi F., Kouchi T., Tsuji R., Saito M., Obata A., Kikuchi M., *Biosci. Biotech. Biochem.*, **68**, 1706–1711 (2004).
- 10) Stevens J. F., Miranda C. L., Buhler D. R., Deinzer M. L., *J. Am. Soc. Brew. Chem.*, **56**, 136–145 (1998).
- 11) Hossen M. A., Shinmei Y., Jiang S., Takubo M., Tsumuro T., Murata Y., Sugiura M., Kamei C., *Biol. Pharm. Bull.*, **28**, 238–241 (2005).
- 12) Inoue T., Sugimoto Y., Masuda H., Kamei C., *Biol. Pharm. Bull.*, **24**, 92–95 (2001).
- 13) Ohtsuka H., Okuda M., *Arch. Otorhinolaryngol.*, **233**, 227–235 (1981).
- 14) Raphael G. D., Meredith S. D., Baraniuk J. N., Druce H. M., Banks S. M., Kaliner M. A., *Am. Rev. Respir. Dis.*, **139**, 791–800 (1989).
- 15) Shelton D., Eiser N., *Clin. Otolaryngol. Allied Sci.*, **19**, 45–49 (1994).
- 16) Wang D., Smits J., Waterschoot S., Clement P., *Allergy*, **52**, 162–167 (1997).
- 17) Kayasuga R., Sugimoto Y., Watanabe T., Kamei C., *Int. Immunopharmacol.*, **2**, 745–750 (2002).
- 18) Sugimoto Y., Kawamoto E., Chen Z., Kamei C., *Immunopharmacol.*, **48**, 1–7 (2000).
- 19) Shinmei Y., Hossen M. A., Okihara K., Sugimoto H., Yamada H., Kamei C., *Int. Immunopharmacol.*, **4**, 1431–1436 (2004).