Clinical Effects of a Hop Water Extract on Japanese Cedar Pollinosis during the Pollen Season: A Double-Blind, Placebo-Controlled Trial

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Received March 19, 2007; Accepted April 20, 2007; Online Publication, August 7, 2007
[doi:10.1271/bbb.70157]

The clinical effects of oral administration of a hop water extract (HWE) on the improvement of Japanese cedar pollinosis (JCPsis) symptoms were investigated. In a double-blind, placebo-controlled trial, 39 subjects took a drink containing either 100 mg of HWE or a placebo for 12 weeks during the pollen season. Nasal symptoms (sneezing attacks, nasal discharge, and nasal obstruction) were assessed from the subjects’ diaries. A clinical examination and blood sampling were carried out before and 4, 8 and 12 weeks after the initiation of treatment. As a result, a significant difference was observed in the symptom score and in the symptom-medication score 10 weeks after the intervention in comparison with the placebo group. Improvements were observed in nasal swelling, nasal color, amount of nasal discharge, and characteristics of nasal discharge in the intervention group 12 weeks after the treatment. No significant eosinophil infiltration into the nasal discharge was apparent in the intervention group throughout the study period, although it was observed in the placebo group. These findings indicate that an oral administration of HWE may be effective in alleviating the allergic symptoms related to JCPsis.

Key words: allergy; Japanese cedar pollinosis; hop water extract; eosinophil; human clinical trial

Allergic diseases such as asthma, rhinitis, eczema and food allergies are reaching epidemic proportions in both the developed and developing world.1) The number of patients with Japanese cedar (Cryptomerica japonica) pollinosis (JCPsis), which is a type of seasonal allergic rhinitis, has recently been increasing in Japan. The prevalence of JCPsis is estimated to be approximately 16.2% of the Japanese population.2) Allergic rhinitis is associated with substantial morbidity, primarily in the context of reduced quality of life and productivity. The disease is usually treated with antihistamines, corticosteroids and so forth. However, there is a risk of side effects caused by the ingestion of these drugs.

Female hop flowers (Humulus lupulus L.) are used in the brewing industry to add bitterness and aroma to beer. Hops have a wide range of physiological functions such as an inhibitory effect on bone resorption,3) cancer chemopreventive activity,4,5) and the estrogenic property of the phytoestrogen, 8-prenylnaringenin.6) In our previous study, a hop water extract (HWE) significantly inhibited histamine release from human basophilic KU812 cells (in vitro). HWE contained quercetin glycosides such as isoquercitrin, rutin and isoquercitrin malonate, and kaempferol glycosides such as astragaline, kaempferol rutinoside and astragaline malonate. These flavonoid glycosides were responsible for the inhibition of histamine release.7) Furthermore, HWE significantly inhibited antigen-induced nasal rubbing and sneezing in egg albumin-sensitized BALB/c mice.8) Histamine release from antigen-sensitized BALB/c mice caused by the antigen challenge, and vascular permeability induced by compound 48/80 stimulation (in vivo).9)

There have been many reports on the antiallergic properties of the polyphenols in various foodstuffs such as epigallocatechin-3-O-methyl-gallate from green tea,10) luteolin-7-O-rutinidine from peppermint,11) naringenin chalcone from tomato skin,12) procyanidins from apple,13) astragalin from persimmon leaf,14) and rosmarinic acid from Perilla frutescens.15) These polyphenols show antiallergic properties by inhibiting degranulation from basophiles and mast cells. Of these, apple

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Abbreviations: JCP, Japanese cedar pollen; JCPsis, Japanese cedar pollinosis; HWE, hop water extract; SMS, symptom-medication score; CRP, C-reactive protein; OVA, ovalbumin; IL, interleukin; TNF, tumor necrosis factor
polyphenol and the extract of Perilla frutescens have been reported to alleviate the symptoms of allergic rhinitis in clinical trials.\textsuperscript{16,17)}

Little is known about the clinical effects of an oral administration of HWE to alleviate JCPsis symptoms. Therefore, in this study, a double-blind, placebo-controlled trial was carried out to verify the antiallergic effect of HWE in patients with JCPsis. We assessed its effects on the symptoms and nasal findings related to seasonal allergic rhinitis and eosinophil infiltration into the nasal discharge.

Materials and Methods

Subjects. Forty adults who had at least a 3-year clinical history of JCPsis-related symptoms such as sneezing, rhinorrhea, nasal blockage and eye irritation were selected. The patients were recruited on the basis of their clinical history of JCPsis and at least 2 of the following criteria: the presence of IgE specific for Japanese cedar pollen (JCP) in the serum, the presence of eosinophils in the nasal discharge, and a positive reaction to the nasal challenge of JCP. These subjects were evenly assigned to a placebo group or intervention group in terms of sex and age.

All subjects gave their informed consent to participate in this study. The study protocol was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Seikokai Clinic in Tokyo.

Administration of the hop water extract. This study was a double blind, placebo-controlled trial. Hop pellets, cultivated in the Saaz district of the Czech Republic, were used. HWE was prepared as previously described.\textsuperscript{7)}

The concentrations of quercetin and kaempherol aglycones in this HWE, as determined by the acid hydrolysis of flavonol glycosides, were 158 ± 21 μg/g and 186 ± 32 μg/g, respectively. The subjects in the intervention group were instructed to drink 1 bottle of a beverage which contained 100 mg of HWE in 350 ml bottle per day for 12 weeks, and the subjects in the placebo group were instructed to drink 1 bottle of a similar beverage without HWE per day for 12 weeks. This beverage was a sports drink whose ingredients were high-fructose corn syrup, citric acid, flavor, aspartame, and ascorbic acid. The two beverages were made indistinguishable in taste or appearance by adjusting the amount of the sour agent, sweeteners, and flavor. Only code numbers were printed on the bottled beverages. The nutritional contents of the test drinks per 100 ml were as follows: energy, 15 kcal; protein, 0 g; lipid, 0 g; carbohydrates, 3.7 g; and sodium, 43 mg. The test was performed according to the schedule shown in Fig. 1, the study being carried out between January 15, 2006 and April 22, 2006. Table 1 show the characteristics of the subjects in terms of sex, age and severity of pollinosis symptoms which were diagnosed by the physician in charge. One person in the placebo group dropped out of the study because of becoming constipated. Accordingly, the placebo group consisted of 19 people (7 men and 12 women; age, 27–58; mean age, 41.2 ± 2.1), the intervention group consisted of 20 people (7 men and 13 women; age, 27–53; mean age, 40.3 ± 1.6). Two subjects withdrew before the end of the clinical test for personal reasons. No significant difference was observed between the groups in the severity of pollinosis symptoms diagnosed by the physician in charge at the start of the study.
Clinical Effects of a Hop Water Extract on Japanese Cedar Pollinosis

Sneezing attacks

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>7/12</td>
<td>7/13</td>
</tr>
<tr>
<td>Age, years</td>
<td>41.2 ± 2.1</td>
<td>40.3 ± 1.6</td>
</tr>
</tbody>
</table>

**Table 1. Subject Characteristics**

**Table 2. Scoring System for Subject’s Nasal Symptoms**

<table>
<thead>
<tr>
<th>Symptom score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>Sneezing attacks</td>
<td>0</td>
<td>1–5</td>
<td>6–10</td>
<td>11–20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>Nasal discharge</td>
<td>0</td>
<td>1–5</td>
<td>6–10</td>
<td>11–20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>Nasal obstruction</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Violent</td>
</tr>
</tbody>
</table>

**Table 3. Scoring System for Subject’s Medication Score**

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral antihistamine or histamine-release suppressor</td>
<td>1</td>
</tr>
<tr>
<td>Nasal application of corticosteroids</td>
<td>2</td>
</tr>
<tr>
<td>Nasal application of vasoconstrictor or anticholinergic drug</td>
<td>1</td>
</tr>
<tr>
<td>Histamine-release inhibitive eyedrops</td>
<td>2</td>
</tr>
<tr>
<td>Corticosteroid eyedrops</td>
<td>2</td>
</tr>
<tr>
<td>Oral corticosteroid and oral antihistamine</td>
<td>3</td>
</tr>
</tbody>
</table>

**Assessment of symptoms.** Nasal symptoms (sneezing attacks, nasal discharge, and nasal obstruction) recorded in the subjects’ diaries were scored independently from 0 to 4 (Table 2). The highest score of sneezing attacks, nasal discharge, and nasal obstruction was used as the symptom score based on the method described by Okuda et al.18 with a slight modification. A medication score was used based on the guidelines of the Japanese Society of Allergology (Table 3). The sum of the symptom score and the medication score was used as the symptom-medication score (SMS).

**Clinical examination.** Local findings on the nasal cavity such as the swelling and color of the concha nasalis inferior mucosa, and the amounts and characteristics of nasal discharge were diagnosed and scored from 0 to 4 by the physician in charge before and 4, 8, and 12 weeks after the initiation of treatment. The onset of the pollen season for Japanese cedar, which is defined as the first count of at least two pollen grains/cm²/day on successive days at sampling sites, was recorded on the February 14, 2006 in the study region of Wakayama Prefecture. Figure 1 shows the daily average readings of Japanese cedar pollen and Japanese cypress pollen during the 2006 season from January 15 to April 23. There was no significant difference in days to develop the symptoms of JCPsis from the onset of the pollen season between the groups. It was 24.2 ± 2.4 days in the placebo group and 18.4 ± 2.4 days in the intervention group.

**Results**

**Clinical effect of HWE on the subjective symptoms**

The onset of the pollen season for Japanese cedar, which is defined as the first count of at least two pollen grains/cm²/day on successive days at sampling sites, was recorded on the February 14, 2006 in the study region of Wakayama Prefecture. Figure 1 shows the daily average readings of Japanese cedar pollen and Japanese cypress pollen during the 2006 season from January 15 to April 23. There was no significant difference in days to develop the symptoms of JCPsis from the onset of the pollen season between the groups. It was 24.2 ± 2.4 days in the placebo group and 18.4 ± 2.4 days in the intervention group.

**Clinical effect of HWE on subjective local findings of the nasal cavity**

The effect of the oral administration of HWE on the local findings on the nasal cavity such as the swelling and color of the concha nasalis inferior mucosa, and the amount and characteristics of the nasal discharge were investigated. Figures 3a–e show the nasal findings score...
and each local findings score (swelling and color of the concha nasalis inferior mucosa, and the amount and characteristics of the nasal discharge). No significant difference in these local findings on the nasal cavity between the groups was apparent throughout the test period. However, within the placebo group, all local findings scores in Weeks 8 and 12 was significantly worse than the scores before the treatment. On the other hand, within the intervention group, all local findings scores in Week 12 did not show any significant worsening in comparison with the score before the treatment, although that in Week 8 was significantly worse. These results indicate that a consecutive oral administration of HWE improved the exacerbation of the local findings in the nasal cavity caused by pollinosis after 12 weeks of administration.

Fig. 2. Weekly Summed Symptom Score (a), Medication Score (b), and SMS (c), from Week 0 (from January 22 to 28) to Week 12 (from April 16 to 22).

The t-test was used to analyze inter-group differences. Each value represents the mean ± standard error of the mean (SEM). ** Significantly different from the placebo group with \( P < 0.01 \).
Effect of HWE on the IgE antibody, CRP, and eosinophils in the nasal cavity

Blood total IgE and JCP-specific IgE in both groups were significantly higher after 12 weeks than the levels before treatment (Table 4). No significant change in either group blood CRP was observed (data not shown). As shown in Table 5, the number of eosinophils in the nasal cavity of the placebo group in Week 12 was significantly exacerbated in comparison with that before the treatment. However, there was no significant exacerbation in the number of eosinophils in the nasal cavity of the intervention group. These findings indicate that the consecutive oral administration of HWE prevented an increase in the number of eosinophils in the nasal cavity.

Fig. 3. Nasal Findings Score (a) and Each Local Finding Score for Swelling (b), Nasal Color (c), Amount of Nasal Discharge (d), and Characteristics of Nasal Discharge (e) before and 4, 8 and 12 Weeks after the Intervention.

The Wilcoxon test was used to analyze intra-group and inter-group differences. Each value represents the mean ± standard error of the mean (SEM). #, ## Significantly different from the value before the treatment in each group with \( P < 0.05 \) and \( P < 0.01 \), respectively. No significant difference in these local findings for the nasal cavity between the groups was apparent.
Discussion

In this clinical study, the oral administration of HWE significantly inhibited the increase in the weekly summed symptom score and SMS 10 weeks after the initiation of treatment, without affecting the amount of drugs during the study. The oral administration of HWE also improved such local nasal findings as the swelling and color of the concha nasalis inferior mucosa, and the amount and characteristics of nasal discharge 12 weeks after the treatment. Within the placebo group, the nasal local findings scores in Weeks 8 and 12 was significantly worse than the scores before the treatment. On the other hand, within the intervention group, these scores in Week 12 were not significantly worse than the scores before the treatment. On the other hand, within the intervention group, these scores in Week 12 were not significantly worse than the scores before the treatment. In the period from Weeks 10 to 12, both Japanese cedar pollen and Japanese cypress pollen was circulating in the air. In the 2006 pollen season, the total amount of cedar pollen in Wakayama city was about 20% less than that in an average year, and the total amount of cypress pollen was about 30% higher than that in an average year. For this reason, the reactivity against Japanese cedar pollen was about 30% higher than that in an average year, and the total amount of cypress pollen in Wakayama city was about 20% less than that in an average year. For this reason, the reactivity against Japanese cedar pollen was about 30% higher than that in an average year, and the total amount of cypress pollen in Wakayama city was about 20% less than that in an average year. For this reason, the reactivity against Japanese cedar pollen was about 30% higher than that in an average year, and the total amount of cypress pollen in Wakayama city was about 20% less than that in an average year. For this reason, the reactivity against Japanese cedar pollen was about 30% higher than that in an average year, and the total amount of cypress pollen in Wakayama city was about 20% less than that in an average year. For this reason, the reactivity against Japanese cypress pollen. There is therefore a possibility that HWE might have had a therapeutic effect against both cedar pollen and cypress pollen.

Furthermore, no significant increase of eosinophils in the nasal discharge was apparent in the intervention group during the clinical examination. On the other hand, eosinophils in the nasal discharge in Week 12 were significantly exacerbated in comparison with those before the treatment in the placebo group. The prominent increase of eosinophils represents the hallmark of allergic diseases. A correlation between the degree of eosinophilia and the degree of nasal symptoms during natural allergen exposure has been reported.\(^19,20\)

Activated mast cells play an important role in the pathogenesis of type I allergic diseases through an immediate reaction and late-phase inflammatory reaction. Chemical mediators such as histamine and leukotriens are released from mast cells immediately after the formation of cross-linked IgE mediated by the binding of multivalent antigens at the surface of the mast cells. These mediators collectively cause increased vascular permeability, vasodilation, bronchial and visceral smooth muscle contraction, and local inflammation.\(^21\) Histamine causes sneezing and nasal rubbing by binding to histamine H\(_1\)-receptors on the sensory nerve endings.\(^22\) Chemical mediators stimulate sensory nerve endings and then the induced afferent impulses are transmitted from the mucosa through the sensory fibers to the central nervous system, and then to the motor, autonomic, vasomotor and secretomotor nerve fibers. As a result, allergic symptoms such as nasal rubbing and sneezing occur.\(^22\) Histamine H\(_1\)-receptor antagonists have been reported to inhibit the symptoms of sneezing.

### Table 4. Total IgE and Japanese Cedar Pollen-Specific IgE in Serum

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Before 1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>0</th>
<th>12</th>
<th>18</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWE</td>
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<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>12</td>
<td>18</td>
<td>0.578</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
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<td>5</td>
<td>0</td>
<td>12</td>
<td>18</td>
<td>0.089</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
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<td>5</td>
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<td>5</td>
<td>18</td>
<td>0.014</td>
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<table>
<thead>
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<th>Time</th>
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<th>5</th>
<th>0</th>
<th>12</th>
<th>19</th>
<th>0.952</th>
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<tbody>
<tr>
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<td>11</td>
<td>19</td>
<td>0.368</td>
</tr>
<tr>
<td>Placebo</td>
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<td>2</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>11</td>
<td>19</td>
<td>0.379</td>
</tr>
<tr>
<td>Placebo</td>
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<td>3</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>19</td>
<td>0.110</td>
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</tbody>
</table>

\(^a\)Wilcoxon test was used to analyze intra-group and inter-group differences.

### Table 5. Eosinophils in Nasal Discharge

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>0+</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWE</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>11</td>
<td>19</td>
<td>0.368</td>
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<tr>
<td>Placebo</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>4</td>
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<td>11</td>
<td>19</td>
<td>0.379</td>
</tr>
<tr>
<td>Placebo</td>
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<td>3</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>19</td>
<td>0.110</td>
</tr>
</tbody>
</table>

\(^a\)Wilcoxon test was used to analyze intra-group and inter-group differences.

\(^*\)P < 0.05 vs placebo-group. \(^##\)P < 0.01 vs data before treatment in each group.

\(^b\)Data are shown as number or mean ± SEM.
and nasal rubbing in an allergic model in mice and rats.23)

In the late-phase inflammatory reaction, which is responsible for the induction of chronic allergic inflammation caused by the continued exposure to an allergen, activated mast cells produce such cytokines as interleukin (IL)-3, IL-4, IL-5, and tumor necrosis factor (TNF)-α. IL-3 promotes mast cell proliferation. IL-4 is the major stimulus for the production of IgE and for the development of Th2 cells from naïve helper T cells. IL-5 activates mature eosinophils and stimulates the growth and differentiation of eosinophils. TNF-α promotes the inflammation and infiltration of neutrophils.24) Cysteiny1 leukotrienes are chemoattractants for eosinophils by directly enhancing eosinophil infiltration into the inflammatory sites and indirectly increasing eotaxin, which is a chemoattractant for eosinophils, release from endothelial cells.25) Cationic granule proteins such as major basic protein and eosinophil cationic protein that are released from eosinophils injure normal tissue.26)

In our previous in vitro study, HWE significantly inhibited histamine release from human basophilic KU812 cells.7) Furthermore, in an in vivo study, an oral administration of HWE significantly inhibited the vascular permeability induced by compound 48/80 stimulation.9) Compound 48/80 is a potent activator of connective tissue-type mast cells and skin mast cells.20) Compound 48/80 stimulation of mast cells leads to the release of chemical mediators and causes an increase in vascular permeability at the stimulated site.27,28)

In this present clinical study, the oral administration of HWE did not affect the total and JCP-specific IgE concentrations in the serum. In our previous study using OVA-sensitized mice, 4 weeks continuous oral administration of HWE did not affect the total and OVA-specific IgE concentrations in the serum. Additionally, in many clinical trials evaluating the efficacy of food-stuffs for the improvement of seasonal and perennial allergic rhinitis, no significant total and allergen specific IgE decrease by their ingestion was apparent in comparison with the placebo group.17,29,30) In these clinical studies, patients having at least a several-year clinical history of JCPsis-related symptoms were recruited. They probably had sufficient allergen-specific IgE at the start of the clinical study. It therefore seems to be difficult to detect any significant difference in IgE concentration in the serum between the placebo group and intervention group. In our clinical study, total and JCP-specific IgE in the patients’ serum of the placebo group respectively increased by about 25% and 56% after the 12-week examination period. On the other hand, in animal experiments, total and allergen specific IgE in the serum dramatically increased several to dozens of times as a result of the allergen immunization, because the IgE concentration in non-sensitized animals is extremely low.9)

In this present study, total IgE in the subjects’ serum of the placebo group was significantly higher than that of the intervention group, while the difference in JCP-specific IgE between them was not significant. JCP-specific IgE was probably better correlated with JCPsis than total IgE, because no significant difference was apparent between them in the severity of pollinosis symptoms at the start of the study.

Based on these findings, the inhibitory effect of an oral administration of HWE on the exacerbation of allergic symptoms and local nasal findings, and on the eosinophil infiltration into the nasal discharge might have partially been due to the inhibition of mast cell activation by the orally ingested flavonoid glycosides contained in HWE.

In conclusion, the results of this study indicate that an oral administration of HWE may be an effective intervention for alleviating the allergic symptoms related to JCPsis.

References