Preventive and Curative Effects of *Gyokuheifu-san*, a Formula of Traditional Chinese Medicine, on Allergic Rhinitis Induced with Japanese Cedar Pollens in Guinea Pig

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***Gyokuheifu-san* (GHS; *Jade Windscreen Powder* in English, *Yupingfeng-san* in Chinese) is an herbal formula in traditional Chinese medicine that consolidates the superficial resistance to protect from invasion by external pathogenic influences. We evaluated the preventive and curative effects of GHS on allergic rhinitis induced by Japanese cedar pollens in guinea pigs, since the pollen can be considered one of external pathogens indicated by GHS. Guinea pigs were sensitized by intranasal instillation of cedar pollen extract with alum twice a day for 7 d, and the animals were then forced to inhale the pollens for challenge once a week for 5 weeks. We administered GHS once a day for 2 weeks in the period of sensitization to evaluate its preventive effect, or for 2 weeks from the 2nd to the 4th week of pollen inhalation, once pollinosis had begun, to evaluate its curative effect on allergic rhinitis. GHS significantly suppressed the frequency of sneezing induced by pollens and tended to reduce nose-scratching behavior after ceasing its administration in both designs of medicinal treatment. Tranilast, which is an anti-allergic drug we used as a positive control, could not suppress these rhinitic symptoms. GHS appears to have non-symptomatic and non-allopathic effects on allergic rhinitis. Our results suggest that traditional medicines have their own characteristics different from modern medicines, and the original pharmacological experiments are important to evaluate traditional medicines scientifically.**

Key words traditional Chinese medicine; allergic rhinitis; pollinosis; *Astragalus membranaceus*; *Atractylodes ovata*; *Saposhnikovia divaricata*

As a result of progress in modern medical treatment because of technological developments, acute diseases can be treated and mortality from disease has declined in industrialized countries. However, most of the modern medicines are allopathic and cannot cure chronic diseases such as hypertension, diabetes, allergic diseases or psychosomatic diseases. These drugs usually contain only one active compound that has sharp pharmacological activity, and the compounds sometimes cause unfavorable side effects. Due to the ineffectiveness of modern drugs against chronic diseases as well as the potential side effects, many patients choose to explore complementary/alternative medicines, and medicinal botanicals in particular.[1] Lately, traditional Chinese medicine (TCM) has received increasing attention as an alternative source of treatment for chronic diseases. TCM has the potential to treat patients holistically, without discriminating between body and spirit, by supporting the patient's own healing power.[2]

We chose to focus on holistic treatment of seasonal allergic rhinitis such as Japanese cedar pollinosis. Treating patients with pollinosis using modern medicine involves avoidance of allergen exposure and pharmacologic therapy using antihistamines, anticholinergic drugs or corticosteroids.[3] However, all these treatments are allopathic, and patients must continue taking these medicines in order to suppress the rhinitic symptoms such as sneezing, rhinorrhea and nasal congestion. We believe that TCM might cure the patients with pollinosis fundamentally, allowing them to discontinue pharmacologic therapy.

Gyokuheifu-san (GHS; 玉屏風散, *Yupingfeng-san* in Chinese, *Jade Windscreen Powder* in English) is a TCM formula that can be used to treat allergic rhinitis. GHS contains three herbal medicines: root of *Astragalus membranaceus* BUNGE (黄耆), rhizome of *Atractylodes ovata* DECANDILLE (白朮) and root of *Saposhnikovia divaricata* SCHISCHKIN (防風). In TCM theory, GHS invigorates *qi* and consolidates the superficial resistance, which increase patient's ability to protect from invasion by external pathogenic influences.[4] GHS is used to treat biomedically defined disorders such as upper respiratory tract infection, exacerbation of chronic bronchitis, mild bronchitis, hyperthyroid condition, autonomic dystonia and allergic rhinitis.[4] In this study, we examined the pharmacological effects of GHS on allergic rhinitis induced by Japanese cedar pollens, since the pollen can be considered one of external pathogens indicated by GHS. In order to evaluate its non-symptomatic effects, which are the greatest characteristics of TCM, we designed experimental protocols that can exhibit GHS's preventive and curative effects on this disease.

MATERIALS AND METHODS

GHS GHS in the form of a dried powder extract was supplied by Iskra Industry Co., Ltd. (Tokyo). The daily dose of GHS (1.5 g) contained extract of the mixture of following herbs: Astragalus Root (6 g), Atractylodes Rhizome (2 g), and Saposhnikovia Root (2 g), all of which are registered in Japanese Pharmacopoeia XIV.[5] These medicinal herbs were boiled in 6-times their weight of H_2O for 60 min, and then the decoction was lyophilized. Three-dimensional HPLC chromatogram of GHS is shown in Fig. 1. The quantitative analyses of marker compounds of each medicinal herb in GHS powder were conducted by HPLC as follows: calycosin (kindly provided by Iskra Industry) in Astragalus Root; column, Inertsil ODS-3, 4.6×250 mm; mobile phase, H_2O : CH_3CN : AcOH 70 : 30 : 0.1→60 : 40 : 0.1 (0→15 min, linear gradient); flow rate, 1.0 ml/min; column temperature, 40 °C; detection, UV 254 nm; retention time, 13.3 min: atractylenolide III (Matsuura Yakugyo Co., Ltd., Nagoya) in Atracty-

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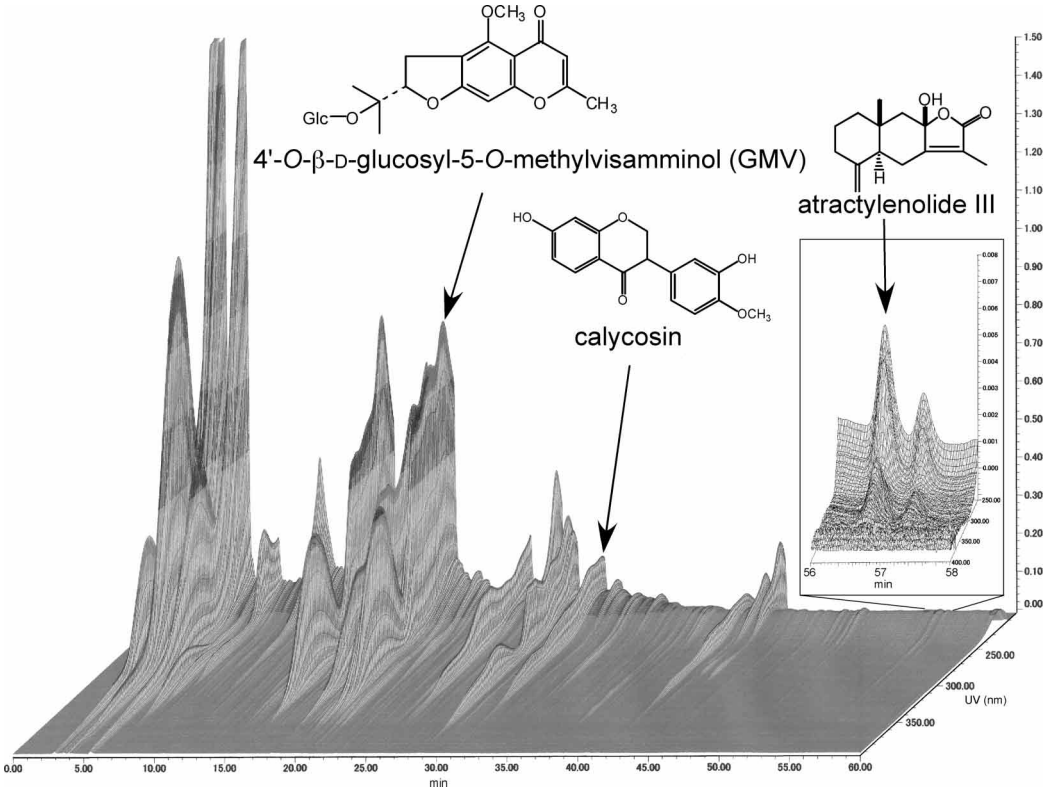

Fig. 1. 3D-HPLC Chromatogram of GHS

HPLC condition is following: sample, GHS (2 mg); column, Inertsil ODS-3 (GL Science, Tokyo), 4.6×250 mm; mobile phase, H_2O : CH_3CN : AcOH 90 : 10 : 0.1→40 : 60 : 0.1 (0→60 min), linear gradient; flow rate, 1.0 ml/min; column-temperature, 40 °C; detection, photodiode-array detector (Waters 2996, Waters, Tokyo), UV 200→400 nm.

lodes Rhizome; column, flow rate and column temperature were same as the method of calycosin; mobile phase, H_2O : CH_3CN : AcOH 42 : 58 : 0.1; detection, UV 220 nm; retention time, 10.5 min: 4′-*O*-*β*-D-glucosyl-5-*O*-methylvisamminol (GMV) (kindly provided by Iskra Industry) in Saposhnikovia Root; column, flow rate, column temperature and detection were same as the method of calycosin; mobile phase, H_2O : CH_3CN : AcOH 78 : 22 : 0.1; retention time, 12.3 min. GHS powder used in this study contained 0.011 (w/w)% of calycosin, 0.000385 (w/w)% of atractylenolide III, and 0.267 (w/w)% of GMV.

Experimental Animals Hartley guinea pigs (3 weeks old, male, 200—250 g) were purchased from Japan SLC (Hamamatsu). The animals were housed in a temperature-controlled room (23±1 °C) under a 12-h light–dark cycle (6:00 a.m.—6:00 p.m.), fed a standard laboratory diet (MF-2, Oriental Yeast Co. Ltd., Tokyo) and given water *ad libitum*.

Experimental Model for Allergic Rhinitis Induced with Cedar Pollens in the Guinea Pig The animal model for cedar pollinosis was produced by actively sensitizing a guinea pig model introduced by Nabe *et al.*[6] Cedar pollens (1×10^4 pollens/mg, kindly provided by Torii Pharmaceutical Co. Ltd., Tokyo) were suspended in phosphate-buffered saline (0.15 M, pH 7.6) at a concentration of 100 mg/ml and gently agitated at 4 °C for 48 h. After centrifugation (3000 rpm, 20 min, 4 °C), the supernatant was collected as cedar pollen extract. The protein concentration of the extract was measured using BCA Protein Assay Reagent (Pierce, Rockford, IL, U.S.A.). After the protein concentration was adjusted to 0.2 mg/ml, the extract was stored at −80 °C until use. Cedar pollen extract was adsorbed into $Al(OH)_3$ gel, which was prepared with 0.5 N NaOH and 0.5 N $Al_2(SO_4)_3$ as previously described,[7] at a concentration of 0.1 mg pollen protein and 0.1 g alum/ml in saline, and guinea pigs were sensitized by intranasal instillation of 5 μl of this solution into each nostril using a micro pipette 2 times a day for 7 d, as shown in Fig. 2. For the normal group, alum solution without pollen extract was instilled into each nostril. Prior to each sensitization, the upper airway mucosal surface was anesthetized by 4 min inhalation with 4% lidocaine hydrochloride solution (Xylocaine® for ophthalmology, AstraZeneka, Osaka) mists, generated by a nebulizer (NE-U22, Omron, Tokyo), to prevent the rapid elimination of the antigen. After that, the animals were intranasally challenged once a week for 5 weeks by inhalation of cedar pollens (3 mg/each nostril) using a hand-made inhalation apparatus.[8] Two of the authors who were blind to the details of the drug-treatment counted the frequency of sneezing and measured the length of guinea pig nose-scratching behavior following immediately after inhalation of the pollens for 1 h, and the average of the two authors' values was used as the data.

Animal Experiments Experiments no. 1 and 2 were conducted to evaluate preventive and curative effects of GHS on allergic rhinitis induced with cedar pollens in guinea pigs, respectively. In each experiment, guinea pigs were divided into 4 groups, the normal, control, GHS-treated and tranilast-treated groups (n=10—20). The dosages of GHS (0.3 g/kg, daily) and tranilast (0.05 g/kg, daily, kindly provided by Kissei Pharmaceutical Co. Ltd., Tokyo) were determined by using 10 times the amount of usual human dosages. In the experiment 1, the drugs were orally administered to the guinea pigs once a day from day −3 to 10. In the experiment

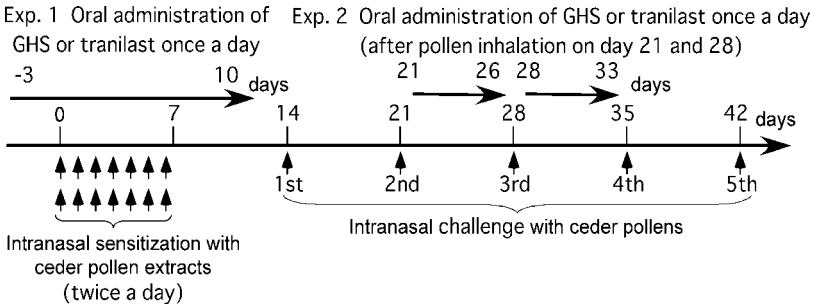

Fig. 2. Schedule for Sensitization with Cedar Pollen Extracts, Nasal Challenge by Inhalation of the Cedar Pollens and Administration of GHS and Tranilast in the Guinea Pig

2, the drugs were treated once a day from day 21 (2 h after pollen inhalation) to day 26, and from day 28 (2 h after pollen inhalation) to day 33 (Fig. 2).

Statistical Analysis Statistical analyses were performed by repeated one-way analysis of variance (ANOVA), combined with Dunnet's multiplex comparison analysis. A probability value of less than 0.05 was considered to be statistically significant.

RESULT

Experimental Animals In both experiments 1 and 2, there were no significant differences of body weight among the 4 experimental groups. The sensitization to pollen extract and the following inhalation of pollens in guinea pigs induced conspicuous symptoms of allergic rhinitis after the 2nd inhalation of pollens, and symptoms worsened until the 5th inhalation of pollens. These results were in accordance with previous results of Nabe *et al.*[7] In the normal group, minor symptoms of allergic rhinitis occurred following the inhalation of pollens without prior sensitization to the pollen extract.

Preventive Effects of GHS Experiment 1 was designed to evaluate the preventive effect of GHS on allergic rhinitis in guinea pigs. GHS was administered once a day from day −3 to 10, during which guinea pigs had been sensitized to the pollen antigen by the intranasal application of cedar pollen extract with alum. In this period, there were no symptoms of allergic rhinitis in any of the groups. After that, the inhalation of pollen induced the symptoms of allergic rhinitis such as sneezing and nose-scratching and sniveling. The 2nd to the 4th inhalation of pollens induced significantly less sneezing in the GHS-treated group than in the control group ($p<0.05$) (Fig. 3A). GHS also exhibited a tendency to decrease pollen-induced scratching in guinea pigs, but there were no statistically significant differences (Fig. 3B). On the other hand, treatment with tranilast showed no differences compared with the control group, but it tended to aggravate the symptoms of scratching behavior induced by the 2nd to the 4th inhalation, and there was significantly more sneezing when the 5th inhalation ($p<0.01$) was induced (Figs. 3A, B).

Preventive Effects of GHS on Pollinosis Experiment 2 was designed to evaluate the curative effect of GHS on allergic rhinitis in guinea pigs. GHS was administered once a day from day 21 (after the 2nd inhalation of pollens) to 26 and from day 28 (after the 3rd inhalation of pollens) to 33 to avoid detecting its symptomatic action. This scheme of the

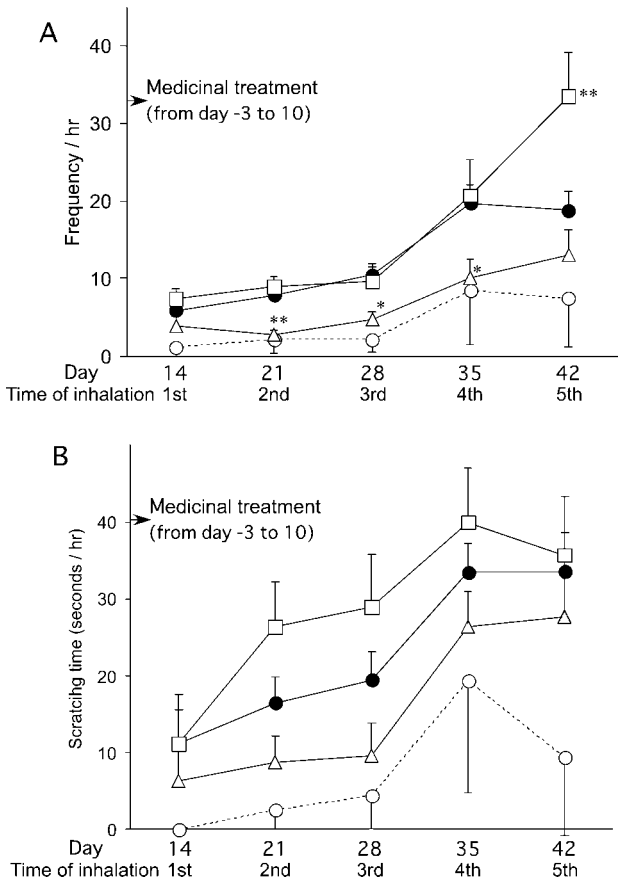

Fig. 3. Preventive Effect of GHS on Allergic Rhinitis Induced with Cedar Pollens (Experiment 1)

Frequency of sneezing (A) and period of nose-scratching (B) were counted for 1 h after the inhalation of pollens. Schedule of sensitization and challenge of cedar pollens and administration of GHS (0.3 g/kg, daily) or tranilast (0.05 g/kg, daily) was shown in Fig. 2. Open circle and dotted line, normal group; closed circle, control group; open triangle, GHS-treated group; open square, tranilast-treated group. Data were represented as mean±S.E. ($n=10$—20). $*p<0.05$ and $*p<0.01$ *vs.* control group.

dosage of GHS tended to suppress the symptoms of allergic rhinitis in guinea pigs, and there was a statistically significant difference ($p<0.05$) in the frequency of sneezing after the 5th inhalation compared with sneezing in the control group (Figs. 4A, B). Tranilast could not suppress the symptoms of allergic rhinitis in guinea pigs by this schedule of administration (Figs. 4A, B).

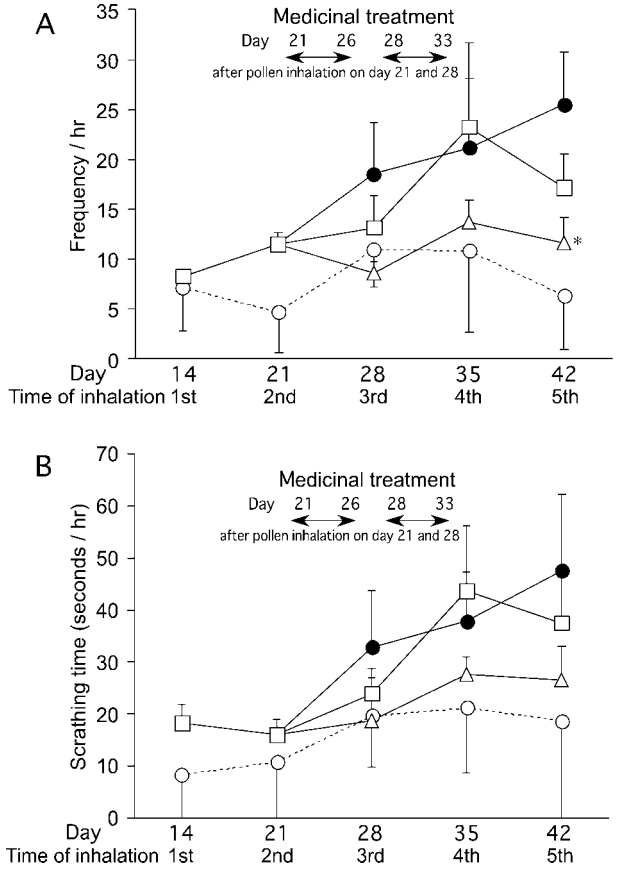

Fig. 4. Curative Effect of GHS on Allergic Rhinitis Induced with Cedar Pollens (Experiment 2)

Frequency of sneezing (A) and period of nose-scratching (B) were counted for 1 h after the inhalation of pollens. Schedule of sensitization and challenge of cedar pollens and administration of GHS (0.3 g/kg/daily) or tranilast (0.05 g/kg/daily) was shown in Fig. 2. Open circle and dotted line, normal group; closed circle, control group; open triangle, GHS-treated group; open square, tranilast-treated group. Data were represented as mean±S.E. (n=10—12). $*p<0.05$ *vs.* control group.

DISCUSSION

GHS is used clinically to treat allergic rhinitis including pollinosis. In this study, we used animal experiments to evaluate the effectiveness of this formula on Japanese cedar pollinosis according to the characteristics of TCM, which works by improving the patient's whole body and non-symptomatic action.

First, we evaluated the preventive effect of GHS on pollinosis. When GHS was administered to guinea pigs while they were being sensitized to cedar pollen antigen, the animals could escape from the aggravation of symptoms such as sneezing and nose-scratching induced by the inhalation of pollens. This result suggests that GHS might prevent patients from acquiring hyperreactivity to pollens by taking GHS before exposure to the pollens. Since IgE antibody is involved in the hyperreactivity, GHS might suppress IgE production. Wei *et al.* reported that cultured peripheral blood mononuclear cells collected from patients treated with Astragalus Root produced higher amounts of interferon-γ and interleukin-2, which are recognized as Th1 cytokines.[9] Since Th1 cytokines would cause a class-switching to B cells to produce immunoglobulins from IgA, IgG_1 and IgE to IgG_2,[10,11] GHS containing Astragalus Root might induce Th1 cytokines in guinea pigs and shift the production of the antibodies from B cells so that IgE would not be produced. Most of the chemical anti-allergic drugs do not work this way, that is, by the prevention from acquiring hypersensitivity and the inhibition of IgE production, so the results of this experiment should clarify the original pharmacological effect of TCM in improving the body's constitution.

In experiment 1, tranilast did not exhibit any therapeutic effects. This result is reasonable, because tranilast suppresses allergic reaction by inhibiting the release of chemical mediators such as histamine or leukotriens, and the period of medicinal treatment in this experiment would not be related to this action mechanism. On the contrary, the tranilast-treated group exhibited greater aggravation of the allergic symptoms than those of the control. In normal conditions, the regular immune system protects the body from external invaders and infectious disease. If subjects under the normal condition take anti-allergic or immunosuppressive medicine, the medications might reduce the immune functions and resistance in the body, which might let external antigens invade easily and induce increased production of antibodies in the body. Anti-allergic drugs are originally symptomatic and allopathic medicine, and they cannot be used for the prevention from allergy itself. Taking these medicines when one is not symptomatic may aggravate the symptoms thereby causing rather than solving problems.

The next experiment was conducted to evaluate the curative effect of GHS on allergic rhinitis. If the TCM can cure the disease fundamentally by improving the patient's body itself, its therapeutic effects might be continued after a break in taking the medicine. GHS was administered to pollen-sensitized guinea pigs with the onset of pollinosis, and GHS administration was stopped a day before pollen inhalation in order that its allopathic and temporal effects should not be detected. When guinea pigs were treated with GHS, their allergic rhinitic symptoms tended to be reduced. This tendency continued after the break in GHS treatment, and there was a statistically significant difference in the frequency of sneezing after the 5th inhalation of pollens between the control and GHS-treated groups. GHS appears to have fundamentally therapeutic effects on disease, which is one of the characteristics of traditional medicine, since the pharmacological effects of symptomatic drugs do not continue after a break in medicinal treatment. As regards these curative effects of GHS, it exhibited its preventive effect in this period, since the rhinitic symptoms were amplified by additional inhalation of pollens in this period, and GHS might suppress the next amplification of rhinitic symptoms by its preventive effect shown in experiment 1.

On the other hand, tranilast, which inhibits the release of chemical mediators from mast cells to exhibit anti-allergic effects, did not suppress the rhinitic symptoms induced by cedar pollens on this dosage scale. Takeuchi *et al.* reported that the time of maximum concentration of tranilast in blood (T_{max}) was 30 min, and the maximum suppressive effect on passive cutaneous anaphylaxis appeared 2—3 h after oral administration of tranilast in guinea pigs.[12] Therefore, the anti-allergic effect of tranilast does not persist, and may appear only when tranilast is administered just before the inhalation of pollens. In this experiment, it is reasonable that tranilast failed to suppress rhinitic symptoms induced with pollens in guinea pigs, since this drug is allopathic and symptomatic.

GHS contains 3 herbal medicines, Astragalus Root, Atractylodes Rhizome, and Saposhnikovia Root. In TCM theory, Astragalus Root is a powerful medicine for strengthening *qi* to activate body energy as well as to protect the body from external pathogens, and Atractylodes Rhizome strengthen the *spleen*, which is the source of *qi*.[13] This theory suggests that these herbs have immunostimulative activities, and some reports actually revealed the immunostimulative effects of Astragalus Root such as increase of antibody production,[14] stimulation for macrophage,[15] and anti-cancer.[16] However, if these herbs were a simple immunostimulator, it would worsen allergic diseases because allergy is a condition caused by excessively high immunoreactions. The major effect of Saposhnikovia Root in TCM theory is to expel and guard against the *wind* (the air containing pathogens),[13] and the combination of these 3 herbs finally strengthen the superficial *qi* running on the surface of the body to protect from external pathogens.[4] Since cedar pollens can be considered as external pathogens in pollinosis, GHS might strengthen the defense on nasal mucosa to protect from the invasion of the pollen antigens. Further studies are needed to evaluate the action mechanisms of GHS as well as the pharmacological effects of each medicinal herb in modern science.

In this study, we revealed that GHS, one of the herbal medicinal formulas in TCM, has non-symptomatic and holistic effects on allergic rhinitis, that is, preventive and fundamentally curative effects. There are few articles in pharmacology mentioning that medicinal treatments of this sort show some therapeutic effects, and we hope this study will contribute to the research evaluating experimental evidences of the result of using traditional medicine. This study supplies experimental evidences that GHS would be effective against allergic rhinitis and pollinosis. We are currently running an additional study to evaluate the characteristics of GHS and its componential herbal medicines about its suitable body condition based on TCM.

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