대한한의학회지 제27권 제2호(2006년 6월)
J Korean Oriental Med 2006;27(2):196-210

원 저

The Effect of *Gamitongkyutang* Distillate in Mice with Allergic Rhinitis

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Objectives : This study aimed to find the curative effect of *Gamitongkyutang* distillate in mice with allergic rhinitis.

Methods : Forty mice were divided into four groups : the normal group, the control group (allergic rhinitis elicited group), the sample Ⅰ group (*Gamitongkyutang* treated group after allergic rhinitis elicitation) and the sample Ⅱ group (distillate of *Gamitongkyutang* treated group after allergic rhinitis elicitation). Indexes of AR were investigated such as the histological changes of the nasal mucosa, the changes of eosinophil count, the changes of interleukin-4 (IL-4) secretion in the intranasal mucosa, the alteration of inducible nitric oxide synthase (iNOS) mRNA expression and the distribution of the nuclear factor kappa B (NF-κB). ANOVA test was used for statistical analysis ($p<0.05$).

Results : Loss of the cilium and the mucous secretion in the sample Ⅰ and Ⅱ groups was rare when compared to the control group. The segment of eosinophil was significantly decreased in the sample Ⅰ and Ⅱ groups when compared to the control group ($p<0.05$). A significant decrease of IL-4 mRNA expression was observed in the sample Ⅰ and Ⅱ groups when compared with the control group ($p<0.05$). Inhibition of iNOS induced by NF-κB p50 in the sample Ⅰ and Ⅱ groups was significantly superior to that in the control group ($p<0.05$). DGT and GT didn't affect AST and ALT.

Conclusions : GT was superior to DGT in the IL-4 secretion, eosinophil levels and iNOS production. However, considering the difficulty in taking herbal medicine, the DGT has a meaningful curative effect in mice with allergic rhinitis.

Key Words: allergic rhinitis, *Gamitongkyutang*, distillate, iNOS, STAT6, NF-κB.

Introduction

The traditional Oriental medicine, *Gamitongkyutang* (GT)[1], also called Jia mei tong qiao tang, contains sixteen species of medicinal plants and has been used as an herbal medicine for allergic disease, such as allergic rhinitis (AR), a common disease worldwide and increasing in prevalence. Recent reports suggest that GT is effective in anti-inflammatory, analgesic, anti-allergic, and antibacterial activity in vitro, the inhibition of the mast cell-dependent allergic reactions and the suppressing of inflammatory cytokine secretion[2-4].

Distillation of herbal medicine is a method of producing herbal decoctions, which results from the cooling of steam produced by decocting the medical herbs with cool water[5-7]. It recently has

· 접수 : 2006년 2월 21일 · 논문심사 : 2006년 2월 22일
· 채택 : 2006년 5월 25일
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been popular with children and sensitive adults because of convenience in taking the medicine and its almost tasteless, odorless and colorless properties.

In the food sector, the effect of distillate has been documented in a lot of studies[8-11]. However, in Oriental medicine, there were just a few cases[5-7]. In addition, the effect of using the distillate of herbal medicine internally has barely been evaluated.

In this study, the experiments were organized into three series. In the first series, the author evaluated the curative effect in the distillate of GT (DGT) as compared to GT for allergic rhinitis from the viewpoint of the state in the mucosa, the capillary and eosinophil levels; in the second series, the author evaluated the curative effect of DGT as compared to GT through examining the anti-allergic mechanism in the signal transducers and activators of transcription 6 (STAT6) activity and Interleukin-4 (IL-4) expression in vitro and in vivo; in the third series, the author evaluated the curative effect of DGT as compared to GT through examining the anti-inflammatory mechanism from the viewpoint of the nuclear factor kappa B (NF-κB) activity and inducible nitric oxide synthase (iNOS) expression in vitro and in vivo.

Materials and Methods

1. Animals

Four-week-old female BALB/c mice were purchased from the Dae-Han Experimental Animal Center (Seoul, Korea). Animals were adjusted to the environment for 2 weeks before the experiment, and mice weighing 20 g were used for experiments. Forty mice were classified into the normal group, the control group (allergic rhinitis elicited group), the sample Ⅰ group (*Gamitongkyutang* treated group after allergic rhinitis elicitation) and the sample Ⅱ group (distillate of *Gamitongkyutang* treated group after allergic rhinitis elicitation). GT and DGT were voluntary administered after first immunization.

2. Reagents

Ovalbumin (OVA: chicken egg albumin, grade V), phosphate buffered saline (PBS), aluminum hydroxide hydrate gel ($Al(OH)_3$), MTT solution (2mg/ml, [3-(4, 5-dimethylthiazol-2-y)-2, 5-diphenyl tetrazolium bromide]), dimethylsulfoxide (DMSO), 10% fetal bovine serum (FBS), phorbol-12-myristate-13-acetate (PMA), 4-tert-octylphenol (OP), lipopoly-saccharide (LPS), TRIzol reagent and 3.3'-diaminobenzidine (DAB) were purchased from Sigma (St. Louis, MO, USA).

Dulbecco's modified Eagle's medium (D-MEM), penicillin (1,000 unit/ml) and streptomycin (1,000 μg/ml) were used from Gibsco (Grand Island, NY, USA).

Rabbit anti-mouse macrophage inflammatory protein-2 (MIP-2, 1:100), goat anti-mouse IL-4 (1:250), rabbit goat anti-STAT6 (1:250), rabbit anti-mouse iNOS (1:250), nuclear factor (NF)-κB p65 (1:250), normal goat serum and biotinylated goat anti-mouse Ig G were from Santa Cruz Biotec (Santa Cruz, CA, USA).

Proteinase K and streptavidin peroxidase were obtained from DAKO (Glostrup, Denmark).

3. Preparation of GT and DGT

GT, which contains sixteen species of medicinal plants(table 1), was purchased from Dongguk University Gangnam Oriental Hospital (Seoul, Korea).

Table 1. Contents of *Gamitongkyutang* (GT)

Pharmacognostic nomenclature	Amount (g)
Sileris Radix	4.0
Angelicae Koreane Radix	4.0
Angelicae Tenuissimae Radix	4.0
Cimicifugae Rhizoma	4.0
Puerariae Radix	4.0
Cnidii Rhizoma	4.0
Atractylis Rhizoma	4.0
Angelicae Radix	4.0
Ephedrae Herba	4.0
Zanthoxyli Pericarpium	2.0
Asari Radix	3.0
Glycyrrhizae Radix	4.0
Platycodi Radix	6.0
Forsyyhiae Fructus	8.0
Lonicerae Flos	12.0
Zingiberis Rhizoma Recens	6.0
Total amount	77.0

The GT (77 g) was prepared by decocting the dried prescription of herbs with 4,500 ml boiling distilled water. The extraction, was decocted for approximately 3 hr, then filtered. The filtrate was concentrated in 900 ml by vacuum evaporation using the rotary evaporator, EYELA (Rika-kikai co., Tokyo, Japan) and kept at 4℃.

The DGT was distilled from GT by a vacuum extractor, cosmos-660 (Kyungseo machine co., Inchon, Korea) and also kept at 4℃.

4. Immunization

0.1% ovalbumin solution was made with OVA 1 ml, PBS 0.5 ml and aluminum hydroxide gel 0.5 ml.

Mice were immunized with the intraperitoneal injection of 0.1% ovalbumin solution by the method of Levine et al.[12] on the 1st, 7th, and 14th days in the control, sample I and sample II groups. From seven days after the last immunization, intranasal sensitization with 0.1% OVA solution was performed three times in one week on alternate days.

5. MTT assay

RAW 264.7 cells (5×10^3 cells/well) cultured in a 96-well plate, were incubated at 37℃ for 12 hr and were given a fresh change of serum-free medium without FBS. After 24 hr, cells were then treated with 10%, 50%, 100% (0.1mg/ml, 0.5mg/ml, 1mg/ml) concentrations of GT and incubated at 3 7℃ for additional 24 hr. The 2 mg/ml of MTT solution was added to the wells, and incubation was continued for another 4 hr. Solubilization solution, DMSO was added to each well. The absorbance was then measured with an Emax microplate reader (Molecular Devices co., Sunnyvale,

CA) at a test wavelength of 595 nm.

6. Cell culture

EL4 cell and RAW 264.7 cell were purchased from Korean Cell Line Bank(KCLB, Korea). These cells were maintained at subconfluence in a 95% air, 5% CO_2 humidified atmosphere at 37℃.

Cells were cultured in DMEM medium supplied with 10% FBS and antibiotics (100unit/ml penicillin, 100 μg/ml streptomycin).

7. Histological examination

Twenty-four hours after the challenge, mice were anesthetized with sodium pentobarbital solution. The animals were sacrificed, and their heads removed. They were fixed with 10% neutral buffered formalin (NBF) and vascular rinse for 24 hours and were decalcified with 10% EDTA solution for four weeks. Samples were embedded in paraffin, and frontal sections of the nose 5 μm thick were stained with hematoxylin and eosin to evaluate the number of eosinophils.

8. Investigation of curative effect in the damaged intranasal mucosa

1) Change in the goblet cells.

To investigate change of goblet cells, the sections were stained with periodic acid-Schiff (PAS) techniques for 20 minutes and applied with the hematoxylin and eosin (HE) contrast stain for one minute.

2) Change of capillary distribution in the lamina propria mucosa

Staining was done by Wright's stain solution for capillary distribution.

3) Change of angiogenic chemokine

MIP-2 were detected by immunohistochemical staining. The sections, which underwent proteolysis by proteinase K for five minutes, were applied with 10% normal goat serum as a blocking serum for one hour, treated with rabbit anti-mouse MIP-2 (1:100) at 4℃ in the incubation chamber for 72 hours and then linked with biotinylated goat anti-mouse IgG for 10 minutes. They were applied with streptavidin peroxidase for 10 minutes and were visualized with 0.05% DAB and 0.05M tris-HCl buffer solution (pH 7.4) containing 0.01% HCl. Hematoxylin was used for counterstaining. They were observed microscopically.

9. Effect of DGT on eosinophil count

Blood was collected from animals via cardiac puncture and counted using the Neubauer hemocytometer (Superior, Bad Mergentheim, Germany) after staining with Hinkelmann's solution.

Intranasal mucosa were applied with 91% Congo red reagent containing 0.01% NaOH for fifty minutes after nuclear stain using Mayer's hematoxylin solution.

10. Treatment mechanism through suppression of Th2 cell differentiation

1) Change of IL-4 mRNA expression

To compare the effect between sample Ⅰ and Ⅱ groups through suppressing the IL-4 mRNA expression, Th-2 skewed condition was provocated with PMA and OP in EL4 cell. EL4 cells (5×105 cells/well) were plated into 6 well plates. After 12hr, cells were treated with PMA (Final 1 ng/ml) and OP (Final 5 uM) for an additional 1 hr. At that point, 10% GT and 10% DGT was added to the wells respectively, and incubation was continued for another 24 hr.

Total cellular RNA was extracted using TRIzol

Table 2. The Primer of IL-4 and β-actin mRNA

Primer		Primer sequences	Product (bp)	No. of cycles
IL-4	sense	5'-TAGTTGTCATCCTGCTCTT-3'	404	35
	antisense	5'-CTACGAGTAATCCATTTGC-3'		
β-actin	sense	5'-GGAGAAGATCTGGCACCACACC-3'	840	35
	antisense	5'-CCTGCTTGCTGATCCACATCTGCTGG-3'		

Table 3. The Primer of iNOS as NF-κB Intricated Inflammatory Enzyme

Primer		Primer sequences	Product (bp)	No. of cycles
iNOS	sense	5'-TCTGCGCCTTTGCTCATGAC-3'	254	35
	antisense	5'-TAAAGGCTCCGGGCTCTG-3'		

reagent. cDNA synthesis was performed with RT-PCR kit (Promega, Madison, USA). PCR was performed to measure the relative differences in transcript levels of IL-4 along the temperature condition. PCR products were separated on 1-2% agarose gels with electrophoresis, and intensity of bands was quantified by image analysis. To estimate the accuracy in the RT-PCR kit, amplification of beta-actin was simultaneously carried out(table 2).

2) Change of IL-4 secretion in the mucosa

To evaluate the change of IL-4 secretion, immunohistochemical staining was performed with goat anti-mouse IL-4.

3) Change of STAT6 activity

Immunohistochemical staining was accomplished using the rabbit goat anti-STAT6 to investigate the activity of STAT6 in vivo.

11. Anti-inflammatory effect through suppression of NF-κ B activity

1) Change of iNOS mRNA expression

To compare anti-inflammatory effect between the sample Ⅰ and Ⅱ groups through the suppressing NF-κB activity, RAW 264.7 cells were treated with LPS.

RAW 264.7 cells, which were cultured in six-well plates for 12 hr, were pretreated with LPS (Final 1 ug/ml) for 2 hr to activate NF-κB and were incubated with 10% (0.1 mg/ml) concentrations of GT and DGT concentration for 24 hr.

Reverse transcriptase-polymerase chain reaction (RT-PCR) assay was used with the iNOS primer after extracting total RNA.

2) Change of iNOS expression in the intranasal mucosa

To investigate the distribution of iNOS, immunohistochemical staining was performed with rabbit anti-mouse iNOS.

3) Change of NF-κB distribution

Immunohistochemical staining was accomplished using NF-κB p65 (1:250) to investigate the distribution of NF-κB.

12. Activity of Transaminase (AST & ALT)

Discrete serum was measured using the aspartate aminotransferase(AST) & alanine transaminase (ALT) kit (Young-dong Pharm. co., Seoul, Korea).

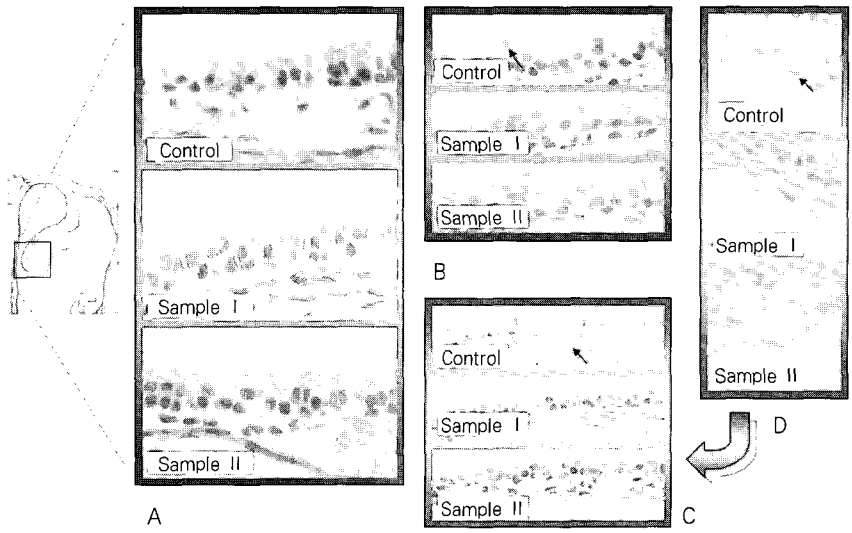

Fig. 1. The curative effect of GT and DGT for AR caused mucosa damages.
A. Mitigation of damage (H&E. ×1,000).
Damage to mucosa as disruption of cilia in respiratory cells was vacant mucose secreting cell were increased in the control group, but in the sample I and II group remained the same as in the normal configuration.
B. Configuration of goblet cells (PAS. ×600).
A decrease of PAS positive cells (arrow) was shown in the control group, but goblet cells occupied with neutral mucous were shown in the sample I and II groups.
C. Distribution of capillaries (Wright's stain, ×400).
The distribution of capillaries in mucosa (arrow) increased in the control group but decreased in the sample I and II groups.
D. Increase of CXC chemokine (MIP-2 immunohistochemistry, ×400).
The distribution of MIP-2 positive reacted cells (arrow) increased in the control group, but decreased in the sample I and II groups.

13. Statistical analysis & Image analysis

The results were expressed as mean ± standard deviation (S.D.) for the number of experiments. Statistical analysis was performed by analysis of variance(ANOVA) with SPSS 8.0. Probability values of less than 0.05 were considered significant.

Images were analyzed by Optima 5.2 (Optima Co., USA).

Results

1. Effect on the alleviation of intranasal mucosa damage

1) Histopathological changes

Damage of cilia, the secretion increment of goblet cell and infiltration of inflammatory cells in the mucosa of the concha near the nasal septum were observed.

The mucosa damage in the control group appeared to be more severe than in the sample Ⅰ and Ⅱ groups. The sample Ⅰ group was similar to the normal group in the nasal mucosa damage. The damaged nasal mucosa was observed partly in the sample Ⅱ group(fig. 1-A).

2) Change of goblet cells

After AR elicitation, the increase of the neutral mucous secretion and the collapse was detected in the goblet cells. PAS positive response in the goblet cells was highest in the sample Ⅰ group, followed by the sample Ⅱ and control groups. The goblet cells were almost empty in the control group(fig. 1-B).

3) Decrease of capillary distribution in the mucosa

Noticeable increase of capillaries in the intranasal mucosa was detected in the control group, whereas the number of capillaries was lower in the sample Ⅰ and Ⅱ groups than in the control group(fig. 1-C).

The number of MIP-2 positive cells decreased by 47.3% in the sample Ⅰ group and by 33.4% in the sample Ⅱ group as compared to that in the control group, whereas it increased by 352.2% in the control group as compared to that in the normal group.

The significant decrease in the number of MIP-2 positive cells was 21.0% larger in the sample Ⅰ group than that in the sample Ⅱ group. That of the sample Ⅰ group had a significant reduction of 21.0% compared with that of sample Ⅱ group(table 4, Fig. 1-D).

2. The change of eosinophil count

The eosinophil count in whole blood decreased significantly more in the sample Ⅰ and Ⅱ groups than in the control groups, whereas it increased significantly more in the control group than in the normal group.

The eosinophil count was the highest in the control group, followed by the sample Ⅱ group and the sample Ⅰ group. The eosinophil count decreased by 41.0% in the sample Ⅰ group and by 25.4% in the sample Ⅱ group as compared to that in the

Table 4. The Inhibition of MIP-2 in Sample Ⅰ and Ⅱ Group

Antibody	Group			
	Normal	Control	Sample Ⅰ	Sample Ⅱ
MIP-2 (pixel)	2084±128	12680±1032	6677±459*,#	8447±504*

(image analysis for 100,000 particles / range of intensity: 70-130)

*: P < 0.05 compared with control group
#: P < 0.05 compared with sample Ⅱ group
All values are mean ± standard deviation (S.D.).
Probability values of less than 0.05 were considered significant.

Table 5. The Inhibition of Th 2 cell Differentiation IL-4 and STAT6 in Sample Ⅰ and Ⅱ Groups

Antibody	Group			
	Normal	Control	Sample Ⅰ	Sample Ⅱ
IL-4 (pixel)	862±88	7444±147	2966±442*#	4663±361*
STAT6 (pixel)	1736±147	12478±1394	4159±138*#	7425±562*

(image analysis for 100,000 particles / range of intensity: 70-130)
*: P < 0.05 compared with the control group
#: P < 0.05 compared with the sample Ⅱ group
All values are mean ± standard deviation (S.D.).
Probability values of less than 0.05 were considered significant.

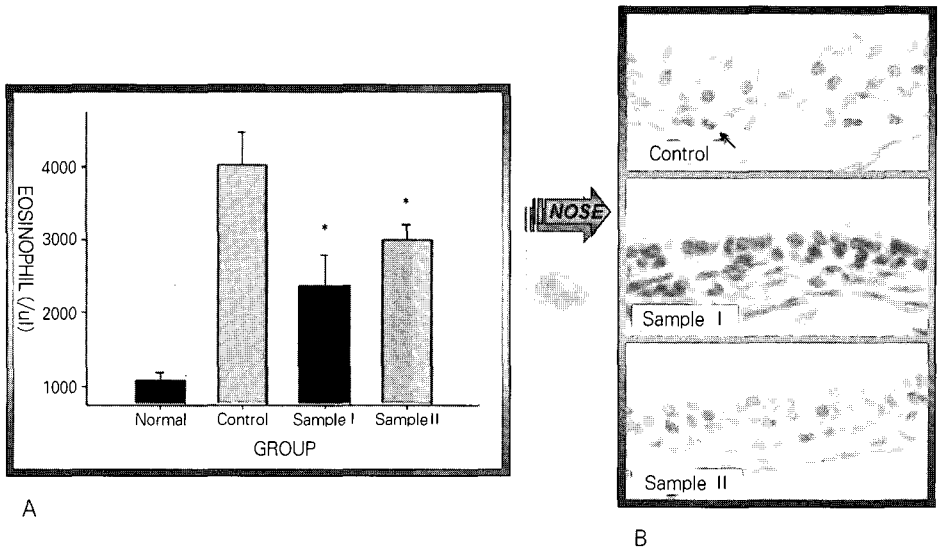

Fig. 2. The change of eosinophil count.
A. The decrease of eosinophils in the sample I and II groups after allergic rhinitis (AR) elicitation. The number of eosinophils in the sample I and II groups decreased more than in the control group, and this decrease had a probability in $P<0.05$ of ANOVA test.
B. The inhibition of eosinophils in mucosa of sample I and II groups. The infiltration of eosinophils in the sample I and II groups decreased more noticeably than that in the control group (Congo red, ×600).
*: $P < 0.05$ compared with the control group
#: $P < 0.05$ compared with the sample II group

control group, whereas it increased by 271.9% in the control group as compared to that in the normal group. The sample Ⅰ group had a noticeable reduction by 20.9% over the sample Ⅱ group in the eosinophil count(fig. 2).

A similar response was observed with eosinophil count in the intranasal mucosa.

The eosinophil count was the highest in the control group, followed by the sample Ⅱ group and the sample Ⅰ group(fig. 2).

3. The curative effect through suppression of Th 2 differentiation

1) Suppression of IL-4 mRNA expression

The expression of iNOS mRNA after PMA and OP decreased in EL-4 cells treated with GT and DGT, whereas it increased in EL-4 cells of the control group. It decreased by 30.0% in the sample Ⅰ group and by 21.2% in the sample Ⅱ group as compared to the control group(fig. 3-A).

2) Inhibition of IL-4 secretion

The number of IL-4 positive cells in the mucosa decreased in the sample Ⅰ and Ⅱ groups whereas it increased in the control group. It decreased by 60.2% more in the sample Ⅰ group and by 37.4% more in the sample Ⅱ group than in the control group, whereas it increased by 763.6% more in the control group than in the normal group.

The significant decrease was 21.0% larger in the sample Ⅰ group than that in the sample Ⅱ group(table 5, Fig. 3-B).

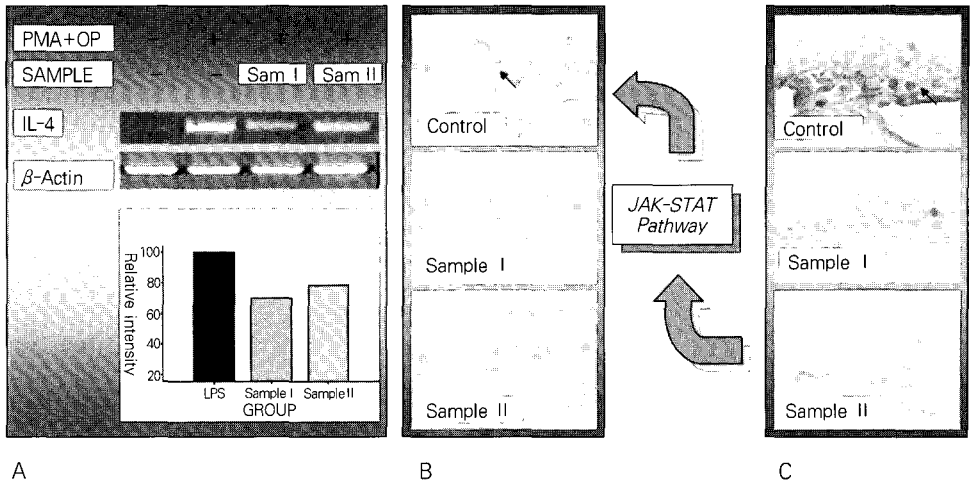

Fig. 3. The inhibition of Th 2 cell differentiation by GT and DGT.

A. In vitro (IL-4 mRNA expression)
The PMA (1 ng/ml) and OP (5 μM) induced IL-4 mRNA expression decreased in GT and DGT-treated EL4 cells.

B. In vivo (IL-4 immunohistochemistry, ×400)
The distribution of Interleukin-4 (IL-4) positive reacted cells (arrow) in mucosa increased in the control group, but decreased in the sample I and II groups. The IL-4 positive reaction appeared in nucleus around cytoplasm.

C. In vivo (STAT6 immunohistochemistry, ×400)
The distribution of signal transducers and activators of transcription 6 (STAT6) positive reacted cells (arrow) in mucosa increased in the control group, but decreased in the sample I and sample II groups. The STAT 6 positive reaction appeared in nucleus and cytoplasm around nucleus.

3) Inhibition of STAT6 activity

NF-κB p50 positive cells were shown strongly in the nucleus and in the cytoplasm surrounding the nuclear membrane.

The decrease of STAT6 positive cells was found in the sample I and II groups. It increased by 618.8% more in the control group than in the normal group but more decreased by 66.7% more in the sample I group and by 40.5% more in the sample II group than in the control group. The significant decrease was 42.6% larger in the sample I group than in the sample II group(table 5, Fig. 3-C).

4. Anti-inflammatory action through suppression of NF-κB activity

1) Suppression of iNOS mRNA expression

Decreased iNOS mRNA after LPS was found in RAW 264.7 cells treated with GT and DGT respectively. Expression of iNOS mRNA decreased by 32.9% in the sample I group and by 26% in the sample II group as compared to the control group(fig. 4-A).

2) Decrease in iNOS expression

The number of iNOS positive cells in the mucosa decreased in the sample I and II groups whereas it increased in the control group. It increased by 1330.58% more in the control group than in the normal group, whereas it decreased by 87.4% more in the sample I group and by

79.3% more in the sample II group than in the control group.

The significant decrease in the number of iNOS positive cells was 39.26% larger in the sample I group than in the sample II group (table 6, Fig. 4-B).

3) Inhibition of NF-κB activity

NF-κB p50 positive cells were strongly seen in the nucleus and in the cytoplasm surrounding the nuclear membrane.

The decrease of NF-κB p50 positive cells was shown in the sample I and II groups. The NF-κB p50 positive cells increased by 530.4% in the control group as compared to those in the normal group, but decreased by 68.2% in the sample I group and by 59.2% in the sample II group as compared to the control group. The significant decrease was 21.1% larger in the sample I group than in the DGT group(table 6, Fig. 4-C).

5. Activity of Transaminase (AST & ALT) in serum

There was no significant change in the activity of transaminase (AST & ALT) among the control, sample I and II groups.

Discussion & Conclusion

Allergic rhinitis is defined as any allergic reaction of the nasal mucosa which may occur perennially or seasonally. It is characterized by sneezing, swelling of the nasal mucosa with a profuse watery discharge, nasal obstruction, itching of the eyes, and lacrimination. It is a common disease worldwide and its prevalence is increasing with environmental pollution.[6,13]

Recently, the distillation of herb medicine has gradually increased in use by children and sensitive adults for convenience and its tasteless, odorless and colorless properties However, there are few

Table 6. The Inhibition of iNOS as Inflammation Cytokine by NF-κB p50 in the Sample I and II Group

Antibody	Group			
	Normal	Control	Sample I	Sample II
iNOS (pixel)	667±74	9,542±333	1,199±33*#	1,973±151*
NF-κB p50 (pixel)	1,061±40	6,689±324	2,129±90*#	2,732±145*

(image analysis for 100,000 particles / range of intensity: 70-130)
*: $P < 0.05$ compared with the control group
#: $P < 0.05$ compared with the sample II group
All values are mean ± standard deviation (S.D.).
Probability values of less than 0.05 were considered significant.

Table 7. The Changes of Transaminase (AST & ALT) in Serum

Transaminase	Group			
	Normal	Control	Sample I	Sample II
AST (kalmen unit/ml)	35.6±0.05	38.1±0.55	33.7±0.25	31.4±0.23
ALT (kalmen unit/ml)	51.3±0.81	59.3±0.10	47.0±2.19	58.8±0.40

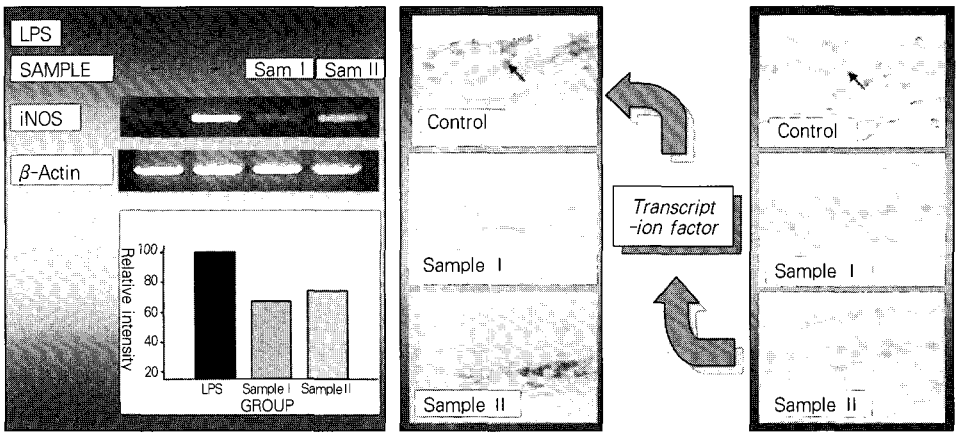

Fig. 4. The inhibition of iNOS expression by GT and DGT-induced suppression of NF-κB activation.
A. In vitro (iNOS mRNA expression)
The iNOS mRNA expression by LPS (200 ng/ml) was decreased in GT and DGT-treated RAW 264.7 cells.
B. In vivo (iNOS immunohistochemistry, ×400)
The distribution of iNOS positive reacted cells (arrow) in mucosa increased in the control group, but decreased in the sample I and II groups. The iNOS positive reaction appeared in cytoplasm and was shown as macrophage type.
C. In vivo (NF-κB p50 immunohistochemistry, ×400)
The decrease of NF-κB p50 positive reacted cells (arrow) was shown in the sample I and II groups.

reports on its effects when used by oral administration.

In the food sector, the effect of distillate has been documented in a lot of studies such as vaporized liquid of water-boiled pine needle, the distillation of *ginseng*, distilled pine-needle extracts, and distilled components from mustard seed[8-11]. In Oriental medicine, there were some cases, a study on apoptosis of cultivated wild ginseng distilled herbal acupuncture by controlled pH and electrolyte[5], a case of pompholyx treated with wet dressing by distilled solution of *Gamihwangryun-haedoktang*[6], a clinical study on the effect of *Tongkyutang* distillate[7] by spraying into the nasal mucosa on pediatric allergic rhinitis and so on. However, the effect of the internal distillate with the herbal medicine has barely been evaluated.

Thus, the author tried to find the effect of Oriental medicine distillate using GT, which was known to have the effect in vitro, in vivo and clinical leve[2-4].

In allergic rhinitis, the damage of the nasal mucosa are observed in the cilia, concha mucosa and the goblet cells such as the increase of mucous secretion and the immigration of inflammatory cells into the lamina propria mucosa[14-15]. In this experiment, these allergic states were also observed differently according to the condition of each group; damage to the nasal mucosa was rarely seen in the sample I and II groups but was intensely seen in the control group.

The marked increase in number of eosinophils is characterized in the nasal submucosa and

epithelium of AR[16]. In the late phase of the allergic reaction, eosinophils, which infiltrate into the tissues from post-capillary venules, release the granule cytotoxic proteins such as eosinophil cationic protein and major basic protein, which are presumed to produce the epithelial damage[17]. Eosinophils are well known to cause the increment of lavage fluid in nasal cavity with allergic rhinitis[18].

Ryoji Kayasuga et al.[14] Reported that eosinophils could be responsible for the organ damage in hypersensitivity reactions. As described in the results section, the number of eosinophils in the nasal mucosa of sample Ⅰ and Ⅱ groups significantly decreased as compared with that of control group. In addition, GT was more effective as compared to DGT in the decrement of eosinophils count. These findings suggest that GT and DGT are effective in the allergic rhinitis, and GT was more effective than DGT.

IL-4, which is secreted by several cell types including stimulated T-lymphocytes, mast cells, and basophils, is known to have the pleiotropic effects on the immune system[19-20]. IL-4 is essential for the induction of immunoglobulin E (Ig E) synthesis by activated B lymphocytes. It induces the proliferation of T lymphocytes and the differentiation of naive CD4+ T cells into Th2 cells, which produces a panel of cytokines including IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13[19-21]. The analysis of IL-4-deficient mice generated by gene targeting in embryonic stem cells has confirmed the importance of this cytokine in mediating many of immune responses[20,22-25].

Signal transducers and activators of transcription (STAT) proteins are a recently identified class of transcription factors responsible for mediating many cytokine-induced responses. Of the presently known STAT proteins, only STAT6 is activated in response to the cytokine IL-4[19,26]. Recent studies[27] have shown that STAT6 is associated with the Th2 differentiation and proliferation by IL-4, which affect the process of Ig E production from B cell. These reports demonstrate that, despite the existence of multiple signaling pathways activated by IL-4, STAT6 is essential for mediating responses to IL-4 in lymphocytes.

The results demonstrated that GT and DGT were effective in the suppressing of IL-4 mRNA, IL-4 generation and STAT6 activity in the cytosol with allergic rhinitis. These results suggest that the effects of DGT were two: one is inhibiting the secretion of IL-4 by suppressing the activity of STAT6 and furthermore regulating Th1/Th2 differentiation, the other is suppressing the allergic immune reaction by inhibiting the Ig E secretion of the activated B cell. In addition, the experiments suggest that GT is significantly more effective than DGT.

To find the effect of DGT compared to GT in the inflammatory action, the iNOS mRNA, iNOS and NF-κB activity were investigated. Nitric oxide (NO) is produced from L-arginine by constitutive and inducible nitric oxide synthase (iNOS) in various mammalian cells and tissues. It reacts with superoxide and yields peroxynitrite, which contributes to the etiology of cardiovascular disease and aging by promoting oxidative stress and inflammation processes. The iNOS, which is induced by either bacterial LPS of TNF-α and IFN-γ in macrophages, produces NO[28-30]. Expression of iNOS is closely related with the up-regulation of NF-κ B, whose sites have been identified in the promoter region of iNOS gene[30-32].

NF-κB is composed mainly of two proteins, p50

and p65. In unstimulated cells, NF-κB is present in the cytoplasm and is bound to the inhibitory protein IκB. Exposure of cells to various NF-κB activators such as LPS and TNF-α results in phosphorylation and degradation of the inhibitory protein IκB, leading to the release of NF-κB from IκB and its translocation into the nucleus[33-35].

The result demonstrated that iNOS mRNA, iNOS and NF-κB activity in the sample Ⅰ and Ⅱ groups decreased when compared with that in the control group. Similar conclusions have been reached about other types of plant such as those found in the Quercetin, platycodon saponin, methanol extract of Spiraea prunifolia var. simpliciflora root[32-34].

The studies demonstrate that GT and DGT were inhibitors of LPS-induced iNOS and iNOS mRNA expression in vitro and in vivo, and that this inhibition is apparently mediated by the blocking of NF-κB activation. These observations suggest that GT and DGT exert an anti-inflammatory action through suppressing of NF-κB activity. In addition, GT was more effective than DGT.

The analysis of the activity of transaminase (ALT & AST) showed that DGT did not affect the hepatic dysfunction.

In conclusion, these findings indicate that DGT is an inhibitor of IL-4 secretion, eosinophil levels and iNOS production, and that this inhibition is apparently mediated by the blocking of NF-κB activity and STAT6 activity. In addition, GT was superior to DGT in the IL-4 secretion, eosinophil levels and iNOS production. However, considering the difficulty in taking herbal medicine with children and sensitive adults, the DGT has a meaningful effect from the view of the anti-inflammatory action through regulation of the NF-κB activity and anti-allergic reaction through regulation of the STAT6 activity.

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