

전신과민성 식품 알레르기 마우스 모델에서의 刀豆 추출물의 항염 효과

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Anti-inflammatory Effects of *Canavalia gladiata* Extracts in a Systemic Anaphylaxis Food Allergy Mouse Model

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ABSTRACT

Objective : An allergy to peanuts is a major cause of fatal food-induced anaphylaxis, with food allergies becoming an increasingly important health research issue. Food allergy as clinical entity has been recongnized for many years, although there is yet no general concord as to the incidence of this symptom.

Methods : This study was undertaken to verify the effect of seeds of *Canavalia gladiata* (Jacq.) DC. extract (CGE) on the inhibition of allergic reactions using a cholera toxin and peanut extract-immunized food allergy mouse model. We determine whether the changes in rectal temperature were related to energy consumption owing to heat production in the body. Mast cell distribution and degranulation in the dermis and epidermis were observed with an optical microscope. Subsequently, Ara h1 levels in serum and interleukin (IL)-4, IL-10, and IFN- γ levels in cultured supernatants of splenocytes were measured.

Results : CGE treatment significantly attenuated the secretion of the Ara h1 antibody in serum and splenocytes. Ara h 1 was undetected in the cholera toxin and peanut extract-immunized food allergy mouse model. Improvement in ear tissue inflammation symptoms was the CGE experimental group. In the control group and peanut extract control group, the expression of mast cells was higher, whereas that in the CGE experimental group was significantly lower.

Conclusion : CGE causes suppression in a food allergy mouse model via the inhibition of Ara h1 secretion, and might be useful for developing functional health foods.

Key words : anti-allergy, *Canavalia Semen*, *Canavalia gladiata*, cholera toxin, food allergy, immunogenicity, Systemic Anaphylaxis

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Introduction

Adverse food reactions are abnormal reactions that occur after the ingestion of food. A food allergy is a hypersensitivity reaction mediated by an immunological mechanism¹⁾. Over the past two decades, the incidences of food allergies have steadily increased, with severity also increasing^{2, 3)}. In a previous meta-analysis study, the prevalence of self-reported symptoms compared to the prevalence of food allergies diagnosed by specific IgE or oral food challenge tests was significantly different. The prevalence rates of milk, egg, peanut, and seafood allergies diagnosed by oral food challenge tests were approximately 3.5%, which were much lower than the self-reported symptoms⁴⁾. The most common types of food allergies are to milk and eggs, with allergic reactions to peanuts increasing in recent years. An ISAAC study showed that eggs, milk, fish, shrimp, and crabs were the most common causes of allergic reactions^{5, 6)}. In a food-specific IgE study on patients with atopic dermatitis, which is a high food allergy risk group, allergies to eggs, milk, fish, crustaceans such as shrimp, soybean, peanuts, and wheat flour were common⁷⁾. A recent increase in allergic diseases, including food allergies, cannot be fully explained by genetic factors alone, and allergic diseases are multifactorial diseases that occur owing to a combination of genetic and environmental factors. A food allergy is also known as a food hypersensitivity, which is immunologically controlled by food and causes undesirable conditions in humans. Reduced ingestion of omega-3 fatty acids, oxidase, and vitamin D has been reported to increase food allergies^{8, 9)}. The characteristics of food allergens also play an important role in the development of allergies. The most common cause of food allergies is class 1 food allergen-soluble glycoproteins that are found in eggs, milk, nuts, crustaceans, wheat, and soybeans. These glycoproteins are 10 to 70 kD in size and are resistant to heat, acid, and proteolytic enzymes. These allergens are sensitized by ingestion via the gastrointestinal tract and cause systemic symptoms. Class 2 food allergens have a similar structure to pollens and are extracted from plants. These are mostly sensitized via the respiratory tract. They are highly sensitive to acid, heat, and proteases, and their structures are altered by ingestion or cooking, thus reducing antigenicity. Most class 2 allergens can be categorized into several specific protein groups^{10, 11)}. The time and route of exposure of food allergens also affect the occurrence of allergies. In animal experiments, allergen intake via the intestinal tract showed immune tolerance, yet sensitization via

the skin caused anaphylaxis¹²⁾. In addition, antigen sensitization has been reported to occur in the skin of patients with atopic dermatitis who have peanut allergies¹³⁾ and, in another study, food antigen-specific T cells were found in atopic dermatitis lesions¹⁴⁾. The gastrointestinal tract is protected from external materials by physical mechanisms such as intestinal mucous membrane cells, glycocalyx, gastric acid, intestinal enzymes, and peristalsis, and by immunological mechanisms such as normal bacterial flora, secretory IgA, and the reticuloendothelial system. Approximately 2% of ingested food allergens are absorbed into the blood via the barrier; however, these normally do not cause clinical symptoms in most people owing to oral tolerance to ingested allergens. Oral tolerance is defined as specific immunological nonresponsiveness to previously orally ingested allergens. Oral tolerance is acquired mainly by antigen presenting cells (APC) including intestinal epithelial cells (IEC), dendritic cells, and regulatory T cells. IEC are non-specific APC with no costimulatory signals or receptors, such as cytokines, that are required for T cell activation. IEC presents food allergens to T cells via the class II major histocompatibility complex, resulting in T cell anergy or T cell deletion and formation of oral tolerance. Dendritic cells are present in the Peyer's patch and induce tolerance by secreting interleukin (IL)-10 and IL-4. Regulatory T cells include T helper 3 cells, T helper 1 cells, and CD4(+)CD25(+) T cells. These regulate T cells and B cells via the transformation of growth factors (e.g., TGF- β) and IL-10 to induce oral tolerance¹⁵⁾. Recently, the effects of natural extracts on disease control have been actively studied; therefore, the effects of natural extracts such as traditional herbs have been attracting increasing attention. *Canavalia gladiata* (Jacq.) DC. belongs to the order Rosales and family Leguminosae, and is an annual vine plant, commonly known as the Jack bean or Sword bean. The origin of *Canavaliae Semen* (*C. gladiata*) is a tropical area in East Asia and it is cultivated in Korea; however, there have been no accurate statistics on the production and consumption of *Canavaliae Semen* (*C. gladiata*)^{16, 17, 18)}. In oriental medicine, *Canavaliae Semen* (*C. gladiata*) fruit has been used as a medicinal herb for the treatment of hiccups, vomiting, abdominal pain, kidney disease, and asthma. In folk remedies, it has been used to treat empyema, hemorrhoids, and purulent inflammation such as furuncle^{19, 20)}. In addition, *Canavaliae Semen* (*C. gladiata*) has been reported to have anticancer, antioxidant, and anti-diabetic effects, and to be effective against chronic nephritis^{21, 22, 23, 24)}. Recent studies have shown that *Canavaliae Semen* (*C.*

gladiata) enhances anti-inflammatory effects by improving the balance between CD4+ T and CD8+ T cells in atopic dermatitis^{25, 26}. Although many studies have revealed the efficacy of seeds of *C. gladiata*, there has been no study to date on its effect on food allergies. Therefore, the objective of the present study was to evaluate the effect of the seeds of *C. gladiata* extract (CGE) on food allergens.

Materials and methods

1. Experimental materials

The seeds of *C. gladiata* used in the present study was purchased from Soybean World Co., Ltd. (Jecheon in Chungcheongbuk-do, Korea) and validated by Professor Young-Chul Lee from the Department of Herbology at Sangji University. CGE was stored in a cryo-freezer (-84°C) (standard sample: CGS-70 EtOH and AL-70 EtOH) at the Institute of Traditional Medicine and Bioscience at Daejeon University and diluted to a certain concentration for use. Ethanol was purchased from Samchun Chemicals Ltd. (Pyeongtek, Korea); dimethyl sulfoxide was purchased from Sigma-Aldrich (St. Louis, MO, USA); and Dulbecco's modified Eagle's medium, fetal bovine serum (FBS), penicillin-streptomycin B (antibiotics), phosphate buffered saline

2. Animal experiment subjects

This study was approved by the Institutional Animal Care and Use Committee at Daejeon University (Approval No.: DJUAR2017-038) and was performed according to the regulations.

3. Cholera toxin induction and administration of study materials

Four-week-old female C57bl/6n mice were supplied from Orient Bio Co., Ltd. and acclimated to an experimental animal room for 1 week. The temperature of the room was adjusted to 20 ± 2°C and the relative humidity was adjusted to 50 ± 5%. Harlan feed (2018S, Harlan, USA) and drinking water were kept in the cage and were available for ad libitum feeding. The light: dark cycle was 12 h:12 h. Cholera toxin was administered orally to the C57bl/6n mice (n = 4/group) on day 0 and day 7 at a concentration of 10 µg/mouse to induce sensitization. CGE (5 mg/kg) was orally administered on day 0 and day 7, and CGE (10 mg/kg) was orally administered twice in a 30-min interval on day 21. On day 35, CGE (10 mg/kg) was orally administered and administered intraperitoneally at a concentration of 1 mg/mouse (Fig. 1). The mice were sacrificed after the last injection to measure the results from the experiment.

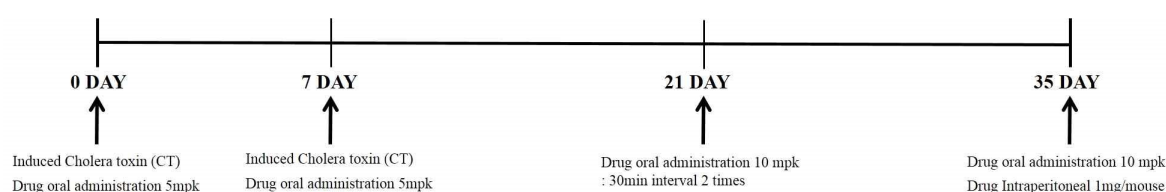


Fig. 1. Experimental plan of repeated cholera toxin induction

(PBS), and 0.25% trypsin-EDTA were purchased from Gibco (Carlsbad, CA, USA). The antibodies for fluorescence-activated cell sorting (FACS) analysis (R-Phycoerythrin (PE) anti-mouse fluorescein (FITC) anti-mouse CD4 (RM4-5, rat IgG2a), PE anti-mouse CD8 (53-6.7, rat IgG2a), PE anti-mouse CD69 (H1.2F3, rat IgG2a), FITC anti-mouse CD11b+F4/80+(aM, rat IgG2b), PE-cy5.5 anti-mouse B220 (RA3-6B2, rat IgG2b), and FITC anti-mouse NK1.1 (PK136, rat IgG2b)) were purchased from Biosciences (Franklin Lakes, USA). Mouse interferon gamma, IL-4 and IL-10 (R&D system, USA), and Ara h1-specific IgE enzyme-linked immunosorbent assay (ELISA) (INDOOR biotechnology, Virginia, USA) were also used.

4. Changes in rectal temperature in the cholera toxin-induced mouse model

To determine whether the changes in rectal temperature were related to energy consumption owing to heat production in the body, the white mice were transferred from the rearing room to the laboratory. Then, the body temperature was measured after 30 min of acclimation to the environment. A YTI -402 thermistor probe was inserted into the rectum of the experimental animals, held in place when approximately 8 cm was inserted, and remained in place for 20 s. Numerical values appeared on the digital YTI -43TG Telethermometer (Yellow Springs Instruments, Yellow Springs: OH), with rectal temperatures measured after 30 min, 60 min, and 90 min. Differences between the cholera toxin-induced

group, cholera toxin complex peanut extract administration group, and CGE administration group were confirmed.

5. Flow cytometry

After sacrificing the C57bl/6 mice, peripheral blood mononuclear cells (PBMC), separated splenocyte cells, and mesenteric lymph node (MLN) cells were centrifuged, and the pellets were used for analysis. Each separated cell was washed twice with FACS buffer and immunofluorescence staining was performed at 4°C. Each tissue was cultured for 30 min with anti-CD69-PE, anti-CD11b-FITC, anti-CD4-FITC, anti-CD8-FITC, anti-CD11b+F4/80+-FITC, and anti-B220-PE. After the reaction, the cells were washed three or more times with PBS and measured with a FACS Calibur® (BD Bioscience, San Jose, CA). The CD4+/CD8+, CD4+/CD25+, Gr-1+/CD11b+, and B220+/CD23+ cells were counted as a percentage (%) using the Cell Quest software program, and the wavelengths expressed in each cell were represented as dot plots.

6. Ara h1-specific IgE production inhibition assay

Total Ara h1-specific IgE concentration in the serum was measured using an ELISA. After completion of the experiment, the blood collected from the C57bl/6 mice was centrifuged, and the IgE concentration in the serum was measured using an Ara h1-specific IgE ELISA kit. Ara h1-specific IgE standard solution and samples were reacted in an antibody-coated well at room temperature for 2 h, and the cells were reacted with biotin-conjugated anti-IgE antibody solution for 2 h. The samples were then reacted with HRP-avidin solution for 1 h and with chromogenic substrate reagent for 20 min. A reaction stopper was added to stop the reaction and the absorbance was measured at 450 nm using ELISA (Molecular devices EMax).

7. Isolation and culture of mouse splenocytes

After completion of the experiment, the spleens of the C57bl/6 mice were aseptically removed, and the splenocytes were suspended. Then, cell debris was removed using mesh stainless steel sieve (Sigma Chemical Co., St. Louis, MO, U.S.A.). The cell suspension was centrifuged at 4°C and 1,500 RPM for 10 min. Then, the obtained cell pellets were centrifugally washed, placed in RPMI1640 medium containing 5% FBS, aliquoted to a 96-well plate coated with anti-CD3 antibodies, and cultured for 48 h. The cytokines present

were measured in the separated culture supernatant as outlined below.

8. Measurement of cytokine production in cultured mouse splenocytes

Cytokines (IFN- γ , IL-4, and IL-10) were measured in the culture supernatant of mouse splenocytes. The culture supernatant was collected from each cell culture well and centrifuged at 1,000 rpm for 10 min. Then, IFN- γ , IL-4, and IL-10 content were measured by ELISA. Biotinylated antibody reagent was added to each well in a 96-well plate and the supernatant was added. Then, the cells were cultured at room temperature. Streptavidin-HRP solution was added to each well, and the cells were cultured at room temperature for 30 min. A 100 μ L sample of TMB substrate solution was added to each well and the reaction was stopped after culturing in a dark room for 30 min. The absorbance was measured at 450 nm with an ELISA reader within 30 min of the reaction stopping. The concentration was calculated and analyzed for each well using a standard curve prepared using standard IFN- γ , IL-4, and IL-10.

9. Pathological observation of skin tissues

The ear tissue samples taken during the autopsy were fixed for 24 h. The tissues were rinsed with water, dehydrated, permeated, and infiltrated, and then paraffin embedding was performed. The tissues were cut into 4 μ m sections and stained with toluidine blue. Then, mast cell distribution and degranulation in the dermis and epidermis were observed with an optical microscope.

10. Statistical analysis

All measurements were expressed as the mean and standard error of the mean of representative values derived from independent experiments. Data were analyzed using the Statistical Package for the Social Sciences (SPSS; version 14.0, SPSS Inc, Chicago, IL, USA) and the significance of each group was $p < 0.05$, which was determined by Duncan's multiple range test and t -test.

Results

1. Rectal temperature changes in cholera toxin-induced animal model

There were no significant differences in rectal temperature among any of the experimental groups; however, the rectal temperature decreased after 30 min in the group sensitized with cholera toxin and administered peanut extract (Fig. 2).

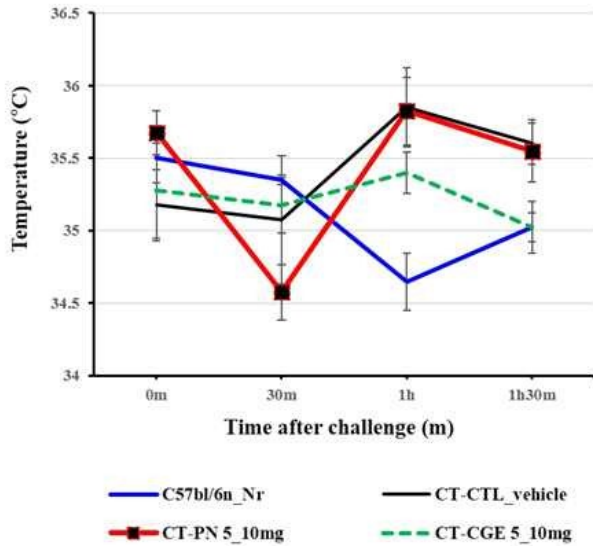


Fig. 2. Changes in rectal temperature (°C) in cholera toxin-induced mouse model. C57bl/6n_Nr: normal; CT_CTL: cholera toxin-induced control; CT-PN_5_10 mg: cholera toxin-induced peanut extract; CT-CGE_5_10 mg: cholera toxin-induced *Canavalia gladiata* extract. Data are expressed as mean ± SEM.

2. Determination of serum Ara h1 specific IgE secretion and IgE and cytokine secretion in splenocytes

In the cholera toxin-induced mouse model, Ara h1 specific IgE and inflammatory cytokines based on different CGE treatments were investigated. There are eight peanut protein antigens (Ara h1 to Ara h8), of which the major antigens are Ara h1, Ara h2, and Ara h3. The serum was separated to measure Ara h1-specific IgE, and production of inflammatory mediators (IFN- γ , IL-10, and IL-4) in the splenocytes was measured by ELISA. IFN- γ , IL-10, and IL-4 production in the splenocytes of the cholera toxin-induced control group was found to be higher than that of the normal group (Fig. 3). However, a significant increase was observed in the group administered the peanut extract, and the production of IL-10 and IL-4 showed a statistically significant increase. IFN- γ production decreased, whereas the production of IL-4 increased owing to a chronic disease reaction caused by an allergic mechanism. IgE production was increased by the imbalance of Th1/Th2 cells, which were shown by IFN- γ and IL-4. In addition, increased IgE is known to be a major problem in allergies.

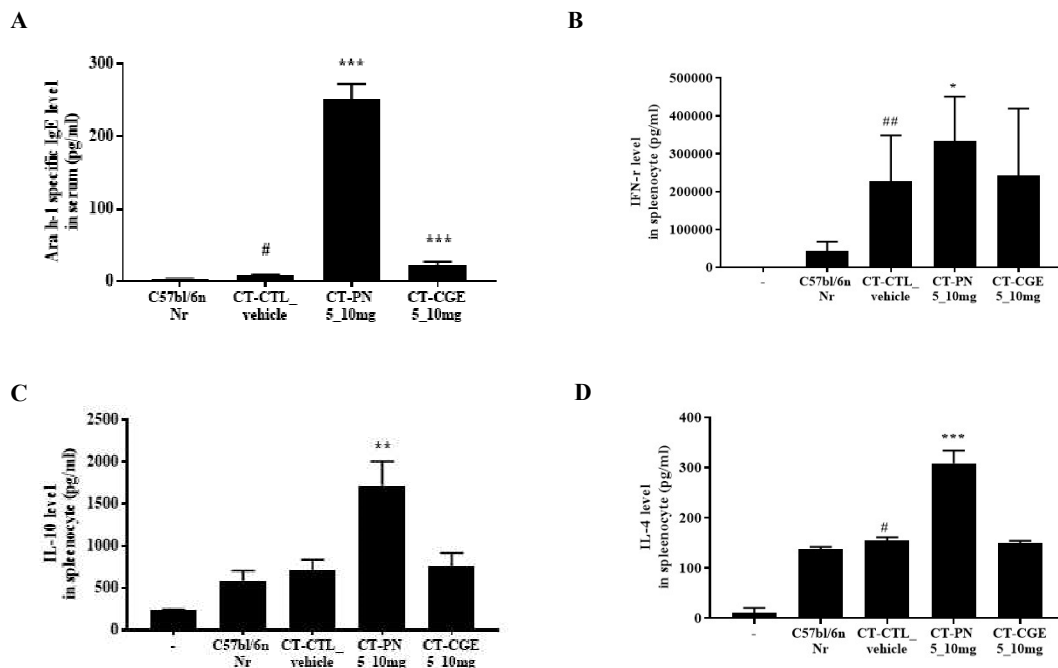


Fig. 3. Cholera toxin-induced peanut extract and *Canavalia gladiata* extract inhibiting IL-4, IL-10, and IFN- γ expression in splenocytes of cholera toxin-induced mouse model. The production of Ara h1-specific IgE in serum (A), IFN- γ (B), IL-10 (C), and IL-4 (D) in splenocytes were measured by ELISA. Data are shown as mean ± SEM. #p<0.05, ##p<0.005 vs Nor; *p<0.05, **p<0.01 and ***p<0.001 vs CTL.

3. Total PBMC, splenocytes, and MLN cells Number of the CGE of the cholera toxin– induced mouse model

Total PBMC, splenocytes, and MLN cells were counted using flow cytometry in the cholera toxin–induced mouse model and the total number of MLN and

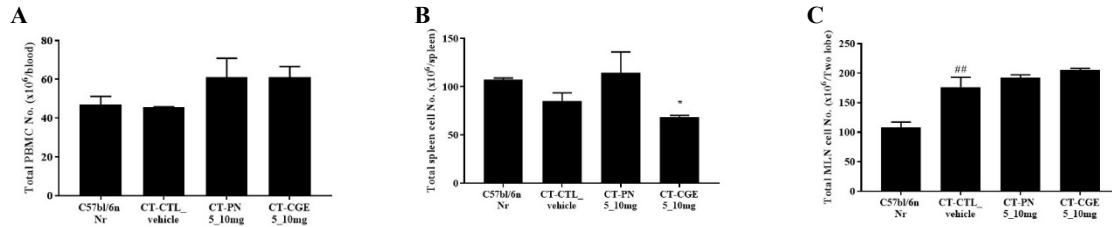


Fig. 4. Total PBMC, splenocytes, and MLN cells in each treatment group of the *Canavalia gladiata* extract of the cholera toxin–induced mouse model. Total PBMC (A), splenocytes (B), and MLN (C) cells were counted using flow cytometry. Data are shown as mean \pm SEM. ## p <0.005 vs Nor; * p <0.05 vs CTL

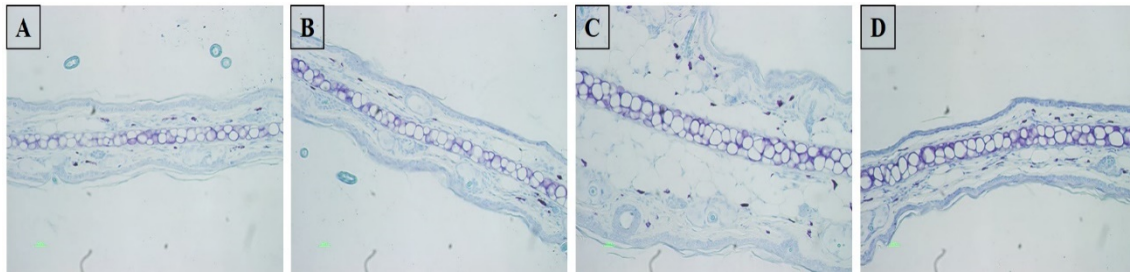


Fig. 5. Histopathology of ear tissue (toluidine blue staining) in mast cells of cholera toxin–induced systemic anaphylaxis. C57bl/6n_Nr: normal; CT_CTL: cholera toxin–induced control; CT-PN 5_10mg: cholera toxin–induced peanut extract; CT-CGE 5_10mg: cholera toxin–induced *Canavalia gladiata* extract.

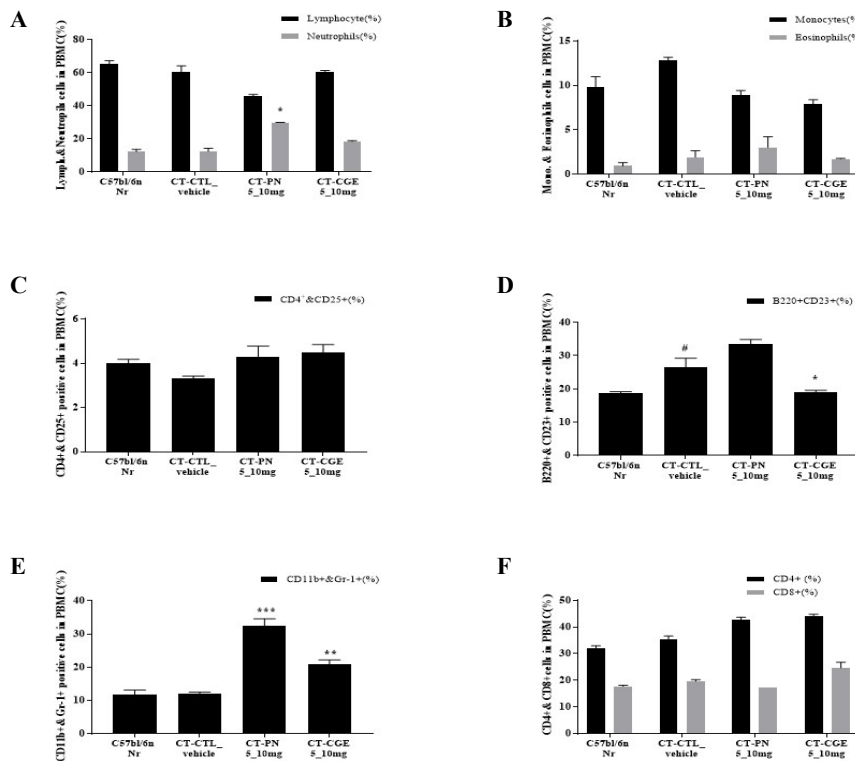


Fig. 6. Effects of CGE treatment on the changes in total absolute numbers of T cells in the PBMC of C57bl/6 mice in the cholera toxin–induced mouse model. The total absolute numbers of CD4+CD8+ (A), (B) and CD4+CD25+ (C), (D) and B220+CD23+ (E), (F) cells in the C57bl/6n mice. C57bl/6n_Nr: normal; CT_CTL: cholera toxin–induced control; CT-PN 5_10mg: cholera toxin–induced peanut extract; CT-CGE 5_10mg: cholera toxin–induced *Canavalia gladiata* extract. # p < 0.05 vs Nor; * p <0.05, ** p <0.01 and *** p <0.001 vs CTL.

splenocytes were higher in the cholera toxin-induced mouse group than in the other groups. However, the total number of MLN cells and splenocytes decreased based on the concentration of CGE orally administered to the mice (Fig. 4).

4. Inflammation symptom improvement by CGE administration in the cholera toxin-induced mouse model

Improvement in ear tissue inflammation symptoms were observed in the cholera toxin-induced group and the CGE experimental group. In the control group and peanut extract control group, the expression of mast cells was higher, whereas that in the CGE experimental group was significantly lower (Fig. 5).

5. Absolute total number of PBMC in cholera toxin-induced mouse model

In the cholera toxin-induced mouse model, PBMC were analyzed with flow cytometry and represented as lymph and neutrophil percentages (%), and the absolute

total number of cells in each tissue was calculated by applying the total number of cells. Neutrophils were three times higher in the PBMCs of the cholera toxin-induced control group administered peanut extract, whereas they were lower in the CGE experimental group, a level similar to that of the normal group (Fig. 6).

6. Absolute total number of MLN cells in the cholera toxin-induced mouse model

MLN cells in mice were analyzed as a percentage (%) by flow cytometry analysis, and the absolute total number of cells in each cell was calculated by applying the total number of cells. Eosinophils were higher in MLN cells from the cholera toxin-induced control group administered the peanut extract, whereas they were lower in the CGE experimental group. B220+CD23+ cells showed the same pattern.

7. Absolute total number of splenocytes in the cholera toxin-induced mouse model

Splenocytes in the mice were analyzed as a percentage

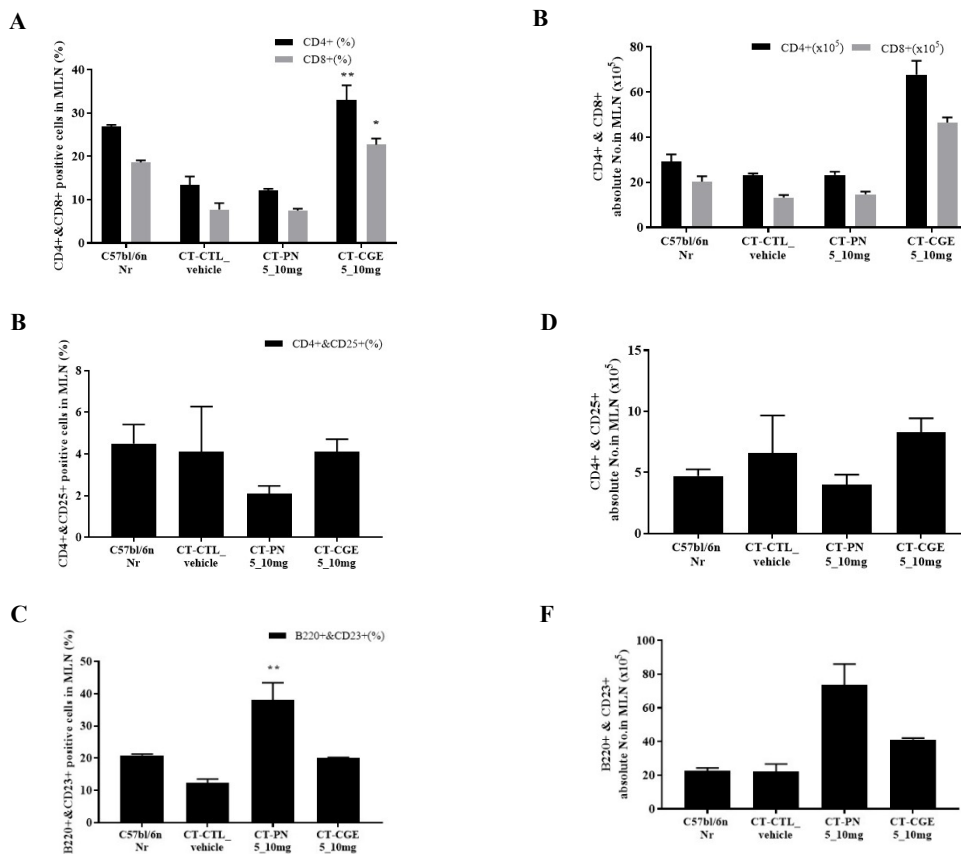


Fig. 7. Effects of CGE treatment on the changes in total absolute numbers of T cells in the MLN of C57bl/6 mice in the cholera toxin-induced mouse model. Total absolute numbers of CD4+CD8+ (A), (B) and CD4+CD25+ (C), (D) and B220+CD23+ (E), (F) cells in the C57bl/6n mice. C57bl/6n_Nr: normal; CT_CTL: cholera toxin-induced control; CT-PN 5_10mg: cholera toxin-induced peanut extract; CT-CGE 5_10mg: cholera toxin-induced *Canavalia gladiata* extract. *p<0.05 and **p<0.01 vs CTL.

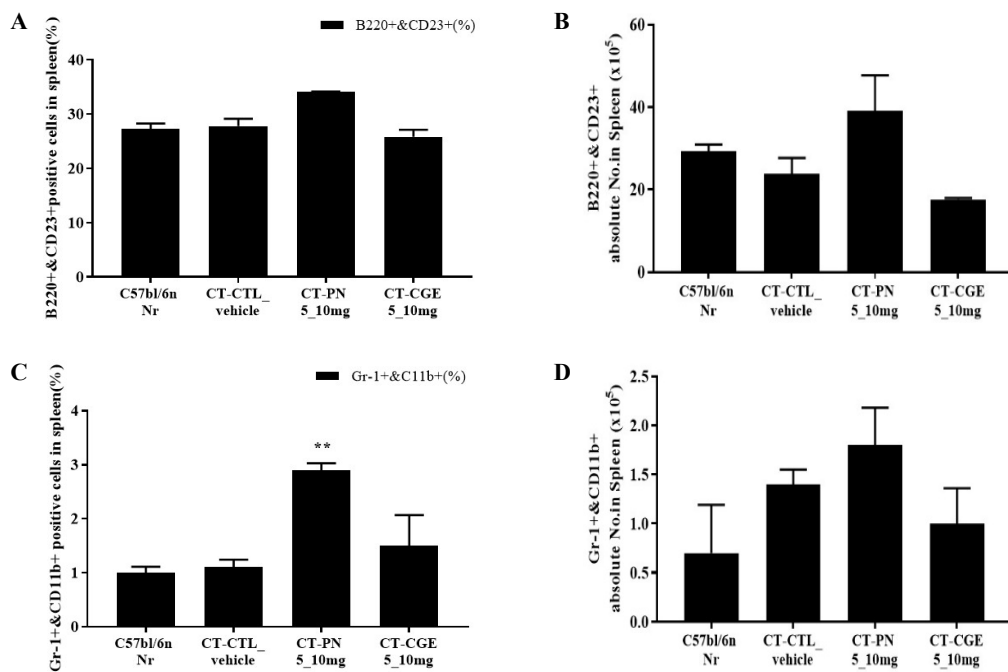


Fig.8. Effects of CGE treatment on the changes in total absolute numbers of T cells in the spleen of C57bl/6 mice in the cholera toxin-induced model. Total absolute numbers of B220+CD23+ (A), (B) and CD11b+Gr-1+ (C), (D) cells in the C57bl/6n mice. C57bl/6n_Nr: normal; CT_CTL: cholera toxin-induced control; CT-PN 5_10mg: cholera toxin-induced Peanut extract; CT-CGE 5_10mg: cholera toxin-induced *Canavalia gladiata* extract. **p<0.01 vs CTL.

(%) by flow cytometry analysis, and the absolute total number of the cells in each cell was calculated by applying the total number of cells. CD11b+/Gr-1+ cells were higher among splenocyte cells in the cholera toxin-induced control group administered the peanut extract, whereas they were lower in the CGE experimental group.

Discussion

C. gladiata is known to have various effects such as anti-inflammation and antioxidant activities^{26, 27, 28}. A previous study on immune regulation showed that fermented *C. gladiata* was effective in controlling the imbalance of Th1/Th2 and inhibiting inflammatory cytokines in an atopic dermatitis model^{29, 30}. Although an inflammatory response is a mechanism for regenerating injured tissues caused by infection, long-lasting inflammatory response persistently damages tissue, which in turn leads to disease such as cancer^{31, 32}. Although nitric oxide(NO) and reactive oxygen species are known to be involved in immune function activation, excessive production of NO, which is induced by lipopolysaccharide (LPS) or inflammatory cytokines during the inflammatory response, exacerbates the inflammatory response in terms of immune regulation³³, and ROS causes chronic inflammation and may result in autoimmune diseases such as inflammatory bowel

disease³⁴. In the present study, we investigated the anti-inflammatory activity of CGE in response to food allergens. CGE has been shown to have anti-inflammatory effects. Patients with peanut allergies may have associated allergies to food that has a similarity in terms of antigens or has no similarity. Peanuts are a common food that cause a serious food allergy. The most prominent symptom of a peanut allergy is an increase in the secretion of Ara h1 in the blood. There are eight known protein antigens in peanuts, Ara h1 to Ara h8, with the major antigens being Ara h1, Ara h2, and Ara h3. Thus, in the present study, we measured the secretion of Ara h1 in serum samples from the cholera toxin-induced experimental group administered CGE to determine the inhibitory effect of CGE on Ara h1 secretion. From this, Ara h1 secretion was found to be inhibited. In terms of peanut antigens, Ara h1 belongs to the 7S class, and Ara h3 belongs to the 11S class. The 2S albumin is one of the storage proteins with low molecular weight, which Ara h2 belongs to. Ara h1, which is resistant to heat and digestive enzymes, has a stable trimer structure. This structure is composed of interactions between monomers and the IgE binding epitope is present in the contact area. Ara h2 has a structure similar to that of a trypsin inhibitor. Although the sulfite bond is broken by heat treatment, the structure is not changed to a large extent. Thus, it

shows resistance to digestive enzymes and even protects Ara h1. Therefore, Ara h2 protects Ara h1 during heat treatment³⁵⁾. Peanut allergy has a higher Ara h1- and Ara h2-specific IgE detection rate than Ara h3-specific IgE detection rate. Thus, the antigenicity of Ara h1 and Ara h2 is higher. By measuring the production of serum cytokines (IL-4, IL-10, and IFN- γ), the secretion of these cytokines was found to be inhibited in the cholera toxin-induced experimental group administered CGE compared to that in the cholera toxin-induced control group administered the peanut extract. IL-10 is a cytokine that is produced in Th2 cells and controls the production of cytokines of Th1, thus regulating the balance of various inflammatory cytokine production. The viability of the host is significantly affected if pro-inflammatory cytokines such as IFN- γ are excessively secreted and not well-balanced with anti-inflammatory cytokines such as IL-10³⁶⁾. IFN- γ is a typical proinflammatory cytokine and is an early reactant that is secreted to prevent tissue damage. It is excessively released if it has not acted as a mediator for host defense, leading to an imbalance of anti-inflammatory cytokines such as IL-10 and adversely affecting immune function³⁷⁾. IFN- γ is a proinflammatory cytokine produced in Th1³⁸⁾ and inhibits the production of IL-10, an anti-inflammatory cytokine³⁷⁾. The mast cell expression was higher in the cholera toxin-induced control group administered the peanut extract, whereas mast cell expression was lower in the experimental group administered CGE. T cells are differentiated into helper T (CD4+) cells and cytotoxic T (CD8+) cells by recognizing antigen expressed by APC^{40, 41)}, and helper T cells stimulate B cells or cytotoxic T cells, activate macrophages, and produce various cytokines and antibodies. To determine the effect of CGE on food allergic activation, the number and activity of allergy immune cells were evaluated in the immune system (spleen and mesenteric lymphadenitis) after oral administration of CGE. B220+/CD69+, CD4+/CD8+T, and CD4+/CD25+T cell content in PBMC in mice and macrophage activity was lower in the CGE administered group than in the cholera toxin-induced control group administered the peanut extract. In addition, the distribution of CD4+/CD8+, CD4+/CD25+, and B220+/CD23+ cells were lower among MLN cells in the CGE experimental group than that in the control groups. Peanut allergy is a major cause of fatal food-induced anaphylaxis. The present study found that *C. gladiata* has an effect against the cholera toxin antigen in allergic reactions. The food allergy inhibitory effect present in the cholera toxin-induced group administered

the peanut extract indicates that CGE can prevent the acquired food allergic reaction.

Conclusion

In the present study, the effects of *C. gladiata* against cholera toxin antigen in food allergic reaction was studied. The cholera toxin-induced food allergy mouse model was used to confirm against the systemic anaphylaxis food allergic reactions effect of CGE. Splenocytes isolated from the cholera toxin-sensitized and peanut extract-induced experimental group administered CGE were cultured, and the secretion of IL-10, IL-4, and IFN- γ cytokines was measured. The secretion of IL-10, IL-4, and IFN- γ in splenocytes decreased after the administration of CGE. The secretion of Ara h1 in serum was also examined. The CGE administration group showed a significantly lower antibody secretion in serum than the control group. Therefore, CGE is a potential natural material that effectively inhibits food allergic reaction by reducing Ara h1 and inflammatory cytokines. CGE has an effect against the systemic anaphylaxis food allergic reactions.

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