

Preventive Effects of Saccharified Solution of Rice, *Oryza sativa* subsp. *japonica*, in Mouse Allergic Models

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We investigated the preventive effects of a saccharified solution of rice, *Oryza sativa* subsp. *japonica*, against type IV and type I allergy in mice. The oral and/or percutaneous administration of the saccharified rice solution prevented type IV allergy at doses of 0.3 and 0.5 ml/mouse and at a dose of 10 µl/ear, respectively. With oral administration, the solution also suppressed type I allergy at doses of 0.25 and 0.5 ml/mouse. In the case of type IV allergy, administration of the saccharified rice solution suppressed the ear levels of tumor necrosis factor- α and tended to suppress the ear levels of interleukin-12. The non-dialysate prepared from the saccharified solution showed similar effects to the original solution, suggesting that the molecular weights of the active components are higher than *ca.* 14000. The saccharified rice solution also enhanced the antioxidative activities in the serum of mice with type IV allergy. These results suggest that the saccharified rice solution may be a convenient beneficial food material for prevention of allergies based on suppression of the increases

in some proinflammatory cytokines and promotion of antioxidative activity.

Key words—rice, mouse, type IV allergy, type I allergy, cytokine

INTRODUCTION

The increasing incidence of allergic diseases around the world has become an important public health concern. Allergies can be divided into four types, type I – type IV, based on their mechanisms. The most frequent allergic diseases are those classified as type IV known as delayed type hypersensitivity via cell-mediated immune reaction and type I known as anaphylaxis via the humoral immune reaction. Typical symptoms of type IV allergy are contact dermatitis, while asthma and pollinosis are typical of type I allergy. We previously reported the suppressive effects of some natural products, such as methylated catechins in tea (*Camellia sinensis* L.)^{1–3)} and the ethanol extracts prepared from an edible mushroom (*Hypsizigus marmoreus*),^{4–6)} on mouse type IV and type I allergic models. These foods may be available to prevent allergies.

Rice (*Oryza sativa*) is a major food consumed around the world. In Japan, *Oryza sativa* subsp. *japonica* is also a principal food. Some proteins in boiled rice are known to cause allergic responses, such as rhinitis, asthma, and atopic dermatitis.^{7, 8)} In contrast, a rice-fluid extract prepared from Japanese rice showed antiinflammatory effects in *Helicobacter pylori*-associated gastritis.⁹⁾ We prepared a saccharified solution of Japanese rice for production of a food ingredient including glucose, minerals, amino acids, and some peptides. In this study, the preventive effects of the saccharified rice solution on mouse type IV and type I allergic models were examined.

MATERIALS AND METHODS

Preparation of Saccharified Rice Solution—A saccharified solution of rice (glucose 18.0% and maltose 3.7% in total sugars 23.2%, proteins 1.0%) was supplied by the Japan Agricultural Cooperatives Zennou Nagano (Nagano, Japan). In three solutions prepared, coefficients of variation (CV)

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of the sugar and protein contents were 4.6% and 12.1%, respectively. The preparation process was as follows. A constant amount of water was added to the raw rice (Koshihikari cultivar), followed by continuous treatment at high temperature and high pressure, resulting in collapse of the rice tissues and elution of the starch. The samples were saccharified by simultaneous processing with enzymes (amylase, *etc.*) and homogenization for several minutes.

Samples of 50 ml of the saccharified solution were dialyzed against 2000 ml of water for 48 hr with a cellulose tube (exclusion molecular weight *ca.* 14000; Wako Pure Chemical Ind., Osaka, Japan), which yielded 1.58 g of non-dialysate and 10.86 g of dialysate. Care should be taken in estimating the molecular weights of substances by dialysis as the values are only approximate.

Mouse Model of Type IV Allergy — Oxazolone-induced edema of the ear in mice was used as a model of type IV allergy.^{10, 11)} Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one), a sensitizer, was purchased from Wako Pure Chem. Ind. Male ICR mice, 4 weeks old, were purchased from Japan SLC, Inc. (Shizuoka, Japan). The hair of the abdominal region was carefully shaved off, and 0.1 ml of a 0.5% oxazolone solution in ethanol was applied to the skin (sensitization). Five days after sensitization, 20 μ l of a 0.5% oxazolone solution in acetone was applied to both sides of each animal's right ear (challenge). Twenty-four hours after the challenge, the mice were sacrificed under ether anesthesia, and circular parts (5.0 mm in diameter) were removed from both ears using a punching apparatus. The weights of the right (WR) and left (WL) ears were measured and the ear swelling ratio was calculated. In the case of oral administration, a saccharified rice solution was administered to mice 1 hr before the challenge at doses of 0.1, 0.3, and 0.5 ml/mouse. The non-dialysate (108.6 mg/mouse) and dialysate (15.8 mg/mouse) were also administered orally to mice. These doses were equivalent to those determined in 0.5 ml/mouse of the saccharified solution. Distilled water was added to adjust the whole volume of the administered solution to 0.5 ml. In the case of percutaneous administration, the samples were added to the challenge solution at doses of 2.5, 5, and 10 μ l/ear and applied to the ear. Distilled water was added to adjust the whole volume of the administered solution to 10 μ l. Hydrocortisone, a well-known steroid-type antiinflammatory agent, was assayed in the same

manner at a dose of 0.05 mg/ear. Control mice were administered only distilled water for the dissolution of the samples. The number of mice in each group was 4 or 6. The antiallergic activity was evaluated by comparing ear swelling ratios between the treated and control groups. The ear swelling ratio was calculated as follows: Ear swelling ratio (%) = $\{(WR_{\text{sample}} - WL_{\text{sample}}) / WL_{\text{sample}}\} / \{(WR_{\text{control}} - WL_{\text{control}}) / WL_{\text{control}}\} \times 100$.

Throughout the experiment, the animals were handled in accordance with The Guide for the Animal Experiments in Numazu National College of Technology, which is based on Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

Determination of Cytokines in Mouse Ears —

The levels of tumor necrosis factor- α (TNF- α) and interleukin-12 (IL-12) in the supernatant of 0.5% mouse ear homogenate in 0.04 M phosphate buffer (pH 7.4) were determined by enzyme-linked immunosorbent assay (ELISA) with a Mouse TNF- α ELISA Kit (Cosmo Bio Co. Ltd., Tokyo, Japan) and Mouse IL-12+p40 ELISA Kit (BioSource, San Jose, CA, U.S.A.), respectively.

Determination of Antioxidative Activities in Mouse Serum —

Mouse blood was taken and centrifuged at 1500 $\times g$ for 10 min, and the supernatant was used as the serum. Peroxyl radical scavenging activities in mouse serum were determined according to the method reported in our previous paper.¹²⁾ In this method, the combination of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator and a potentiometric titrator for determination of peroxide value (POV) was used. The antioxidative activity was expressed as μ g of ascorbic acid (AsA)-equivalent per mg of protein in the serum.

Determination of Sugars and Proteins —

The contents of sugars in the non-dialysate and dialysate of the saccharified rice solution were determined by the anthrone-sulfuric acid method.¹³⁾ Glucose was used as a standard. The contents of proteins in the non-dialysate and dialysate of the saccharified rice solution were determined by the method of Lowry.¹⁴⁾ Bovine serum albumin was used as a standard.

Mouse Model of Type I Allergy — The allergen ovalbumin (OVA) was purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.) and Freund's incomplete adjuvant (FIA) was purchased from

Wako Pure Chem. Ind. The antiallergic activity of the saccharified rice solution in mouse type I allergy was determined using the mouse abdominal wall method reported previously,¹⁵⁾ with slight modifications. Briefly, male ddY mice, 5 weeks old, were sensitized intraperitoneally with a 1 : 1 mixture of OVA (2 mg/ml N-saline) and FIA. The saccharified rice solution or distilled water as a control was administered orally to mice 9 days after initial exposure to OVA at doses of 0.25 and 0.5 ml/mouse. Distilled water was added to adjust the whole volume of the administered solution to 0.5 ml. Sixty minutes after administration of the sample, 0.1 ml of Evans blue dye solution (10 mg/ml N-saline) was administered intravenously. Within 5 min after injection of the dye, the abdominal skin of the mice was detached under ether anesthesia, without injury to the abdominal wall. Five minutes after injection of the dye, 50 μ l of OVA solution (5 μ g/site) was injected in the exposed abdominal wall. The animals were killed by cervical dislocation 7 min after challenge, and the abdominal wall was removed. The area of the abdominal wall permeated by blue dye was measured using a personal computer with a scanning apparatus. Throughout the experiment, the animals were handled in accordance with the Guide for the Animal Experiments in Numazu National College of Technology.

After all the above-mentioned experiments were performed three times and the reproducibility was confirmed, typical data were shown in this paper.

Statistical Analysis—Statistical analyses were performed with the nonparametric Mann-Whitney U test to determine the significance of differences between the appropriate experimental groups. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Prevention of Mouse Type IV Allergy by Administration of the Saccharified Rice Solution

We examined the antiallergic effects of the saccharified rice solution on type IV allergy in mice. The antiallergic activities of the saccharified solution administered orally at doses of 0.1, 0.3, and 0.5 ml/mouse and percutaneously at doses of 2.5, 5, and 10 μ l/ear are shown in Fig. 1. With oral administration, the saccharified rice solution showed significant antiallergic effects at doses of 0.3 and 0.5 ml/mouse. Percutaneous administration of the saccharified rice solution showed significant antial-

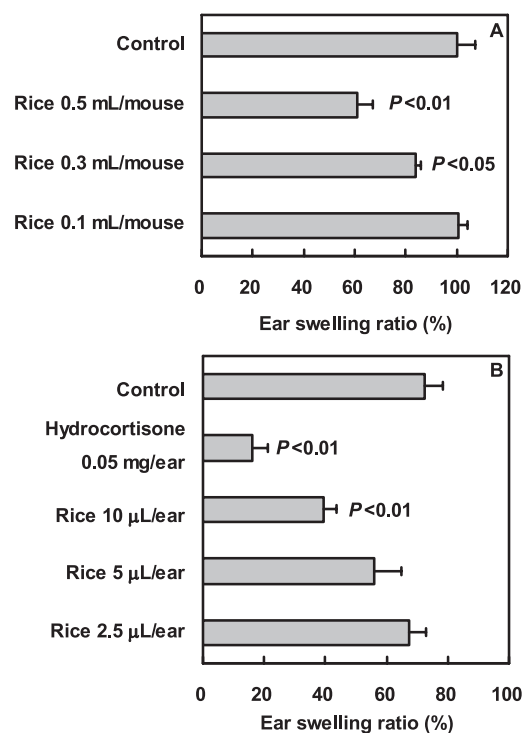


Fig. 1. Preventive Effects of Oral and Percutaneous Administration of Saccharified Rice Solution on Type IV Allergy in 5-week-old Male ICR Mice

A; oral administration, B; percutaneous administration. Means \pm Standard error of the mean (SEM). $n = 6$. Significant differences from control values are shown.

lergic effects only at a dose of 10 μ l/ear. The percutaneous administration of hydrocortisone as a positive control exhibited much strong antiallergic effects in comparison with the saccharified rice solution. These results suggest that oral and percutaneous administration of the saccharified rice solution was effective against type IV allergy in mice.

Suppressive Effects of the Saccharified Rice Solution on Ear Cytokine Levels in Mice with Type IV Allergy

The effects of the oral and percutaneous administration of the saccharified rice solution on the ear levels of TNF- α in mice with oxazolone-induced type IV allergy are shown in Fig. 2. Severe inflammation is a key process in the development of type IV allergy and TNF- α is one of the proinflammatory cytokines. Ear TNF- α levels were higher in mice with intense allergic symptoms than in normal controls. Oral administration of the saccharified rice solution significantly prevented this increase in mouse ears at doses of 0.3 and 0.5 ml/mouse, and percutaneous administration showed the same effects at a dose of 10 μ l/ear. These doses corresponded to

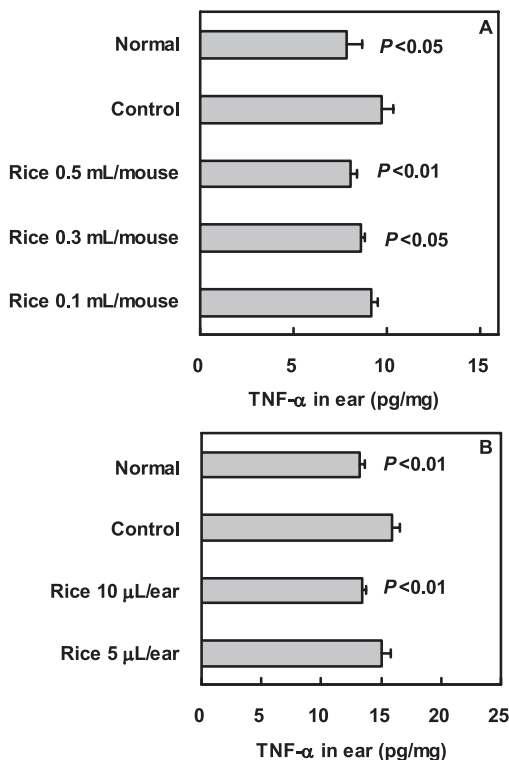


Fig. 2. Suppressive Effects of Oral and Percutaneous Administration of Saccharified Rice Solution on TNF- α Levels in the Ears of 5-week-old Male ICR Mice with Oxazolone-Induced Type IV Allergy

A; oral administration, B; percutaneous administration. Means \pm SEM. $n = 6$. Significant differences from control values are shown.

those at which significant antiallergic effects were observed. TNF- α secreted by type 1 T helper (Th1) cells and macrophages, has multiple actions such as the stimulation of macrophages, Th1 cells, and cytotoxic T lymphocytes (CTL).

The effects of oral and percutaneous administration of the saccharified rice solution on the ear levels of IL-12 in mice with oxazolone-induced type IV allergy are shown in Fig. 3. Th1 cells secreting TNF- α are differentiated from naive T cells by stimulation with IL-12, which is secreted by macrophage-like antigen presenting cells (APC). Ear IL-12 levels were higher in mice with intense allergic symptoms than in normal controls. This increase tended to be lowered by oral and percutaneous administration of the saccharified rice solution, especially at higher doses. However, no significant effects were observed. Therefore, the production and/or release of TNF- α from Th1 cells or macrophages were efficiently inhibited by the saccharified rice solution, and this resulted in preventive effects on type IV allergy.

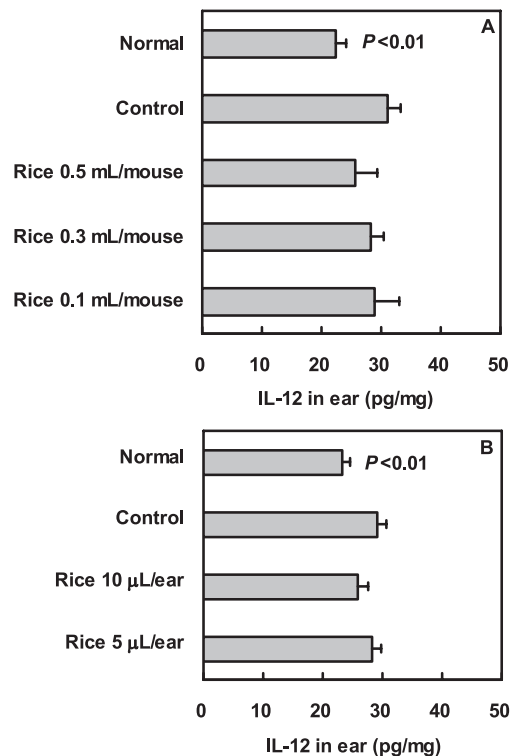


Fig. 3. Suppressive Effects of Oral and Percutaneous Administration of Saccharified Rice Solution on IL-12 Levels in the Ears of 5-week-old Male ICR Mice with Oxazolone-Induced Type IV Allergy

A; oral administration, B; percutaneous administration. Means \pm SEM. $n = 6$. Significant differences from control values are shown.

Increasing Effects of the Saccharified Rice Solution on Serum Antioxidative Activities in Mice with Type IV Allergy

The antioxidative activities in the serum of mice with oxazolone-induced type IV allergy are shown in Fig. 4. The activities in allergic mice (control mice) were equivalent to and/or slightly higher than those in normal mice. Serum antioxidative activities were significantly increased by oral administration of the saccharified rice solution at doses of 0.3 and 0.5 ml/mouse, and by percutaneous administration at a dose of 10 μ L/ear. The excess production of superoxide anion radicals and nitric oxide by macrophages and neutrophils is thought to provoke inflammation in the neighboring tissues.^{16, 17)} Previously, we reported that administration of the ethanol extract from *Hypsizigus marmoreus* enhanced antioxidative activities in mouse plasma and prevented mouse type IV allergy.^{5, 6)} The enhancement of the serum antioxidative activities in mice administered the saccharified rice solution may contribute to the antiallergic effects. Further investigations are nec-

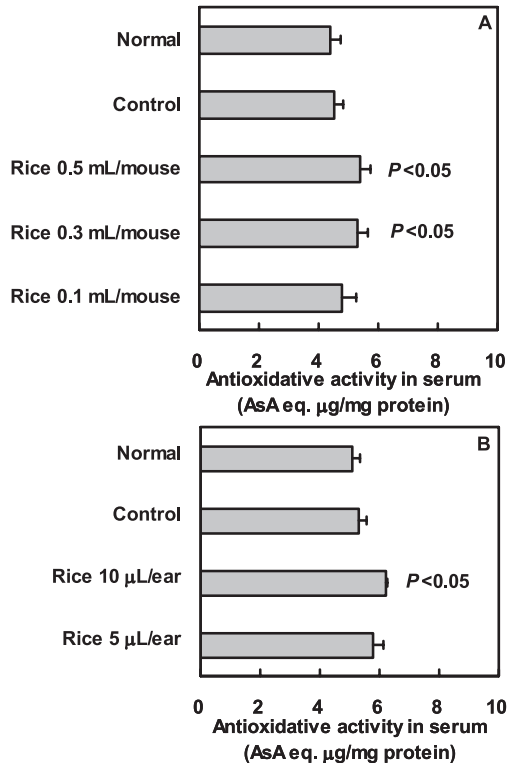


Fig. 4. Increasing Effects of Oral and Percutaneous Administration of Saccharified Rice Solution on Antioxidative Activities in the Sera of 5-week-old Male ICR Mice with Oxazolone-Induced Type IV Allergy

A; oral administration, B; percutaneous administration. Means \pm SEM. $n = 6$. Significant differences from control values are shown.

essary to clarify the mechanisms responsible for the serum antioxidative activities induced by administration of the saccharified rice solution.

Partial Characterization of Preventive Components in the Saccharified Rice Solution on Type IV Allergy in Mice

As shown in Fig. 1, oral administration of the saccharified rice solution at a dose of 0.5 ml/mouse significantly prevented type IV allergy in mice. This dose of the saccharified solution (0.5 ml) contained 15.8 mg of non-dialysate and 108.6 mg of dialysate. The anti-allergic activities of the non-dialysate and dialysate at doses of 15.8 and 108.6 mg/mouse, respectively, and the fluctuations of TNF- α and IL-12 levels in mouse ears are shown in Fig. 5. Only the non-dialysate administered orally exhibited a significant preventive effect on mouse type IV allergy to the same level as the original saccharified solution. Similar to the saccharified solution, oral administration of the non-dialysate significantly suppressed the increase in ear TNF- α levels in allergic mice. The increase in IL-12 levels also tended

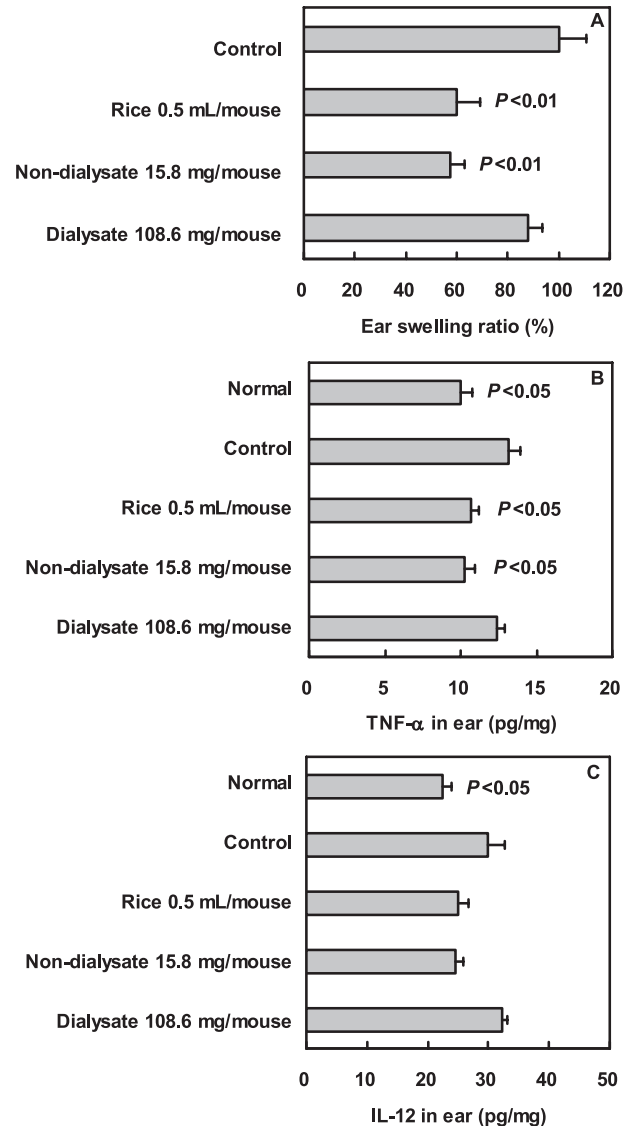


Fig. 5. Effects of Oral and Percutaneous Administration of Saccharified Rice Solution and the Non-dialysate and Dialysate on Ear Swelling Ratios and TNF- α and IL-12 Levels in the Ears of 5-week-old Male ICR Mice with Oxazolone-Induced Type IV Allergy

A; ear swelling ratios, B; TNF- α levels in ear, C; IL-12 levels in ear. Means \pm SEM. $n = 6$. Significant differences from control values are shown.

to be suppressed by the non-dialysate, although no significant effect was observed. Oral administration of the dialysate did not show suppressive effects on both type IV allergy and cytokine levels in mouse ears. Thus, the main active components appear to be contained in the high molecular weight fraction above *ca.* 14000. In solutions of the non-dialysate (15.8 mg/0.5 ml) and dialysate (108.6 mg/0.5 ml), the contents of sugars were 2.72 and 15.9% and those of proteins were 0.40 and 0.31%, respectively. The CV of the contents of sug-

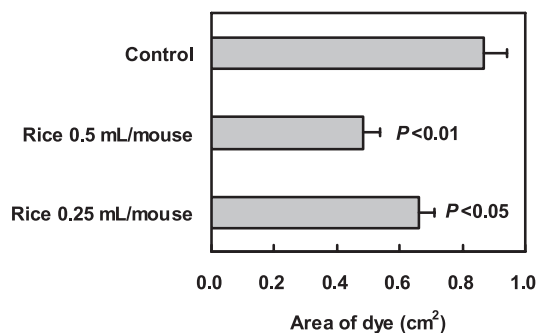


Fig. 6. Preventive Effects of Oral and Percutaneous Administration of Saccharified Rice Solution on Type I Allergy in 4-week-old Male ddY Mice

Means \pm SEM. $n = 6$. Significant differences from control values are shown.

ars in three non-dialysates and dialysates were 8.3 and 9.9%, respectively. The CV of the contents of proteins in them were 2.0 and 14.4%, respectively. Sugars such as starch in the saccharified rice solution would be mostly converted to low molecular weight saccharides such as glucose and maltose. Therefore, one of the active components in the non-dialysate of the saccharified rice solution would be residual polysaccharides, proteins, or peptides. Further investigations are required to determine the chemical structures of the active components.

As to the reproducibility of the ear swelling ratio, TNF- α levels, and IL-12 levels in mice orally administered the saccharified solution at a dose of 0.5 ml/mouse, each CV between the data in Figs. 1–3 and those in Fig. 5 was 1.7, 19.4, and 1.1%, respectively.

Prevention of Mouse Type I Allergy by Administration of the Saccharified Rice Solution

We examined the antiallergic effects of the saccharified rice solution administered orally at doses of 0.25 and 0.5 ml/mouse on type I allergy in mice, and the results are shown in Fig. 6. The saccharified rice solution showed significant antiallergic effects at both of doses. Further investigations are necessary to clarify the mechanisms and the active components involved in the preventive effects of the saccharified rice solution on mouse type I allergy.

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