



Inhibitory effect of chaga mushroom extract on compound 48/80-induced anaphylactic shock and IgE production in mice

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ABSTRACT

Chaga mushrooms (*Inonotus obliquus*) are hypothesised to exhibit general immune-potentiating, anti-inflammatory, and antitumor properties, but their anti-allergic activities are not fully understood. Therefore, this study investigated whether a chaga mushroom extract (C-HE) might have anti-allergic activity. This activity was assessed through the levels of the IgE Ab produced in response to an allergen (OVA). The administration of C-HE prophylactically inhibited the systemic anaphylactic shock induced by compound 48/80 in mice. The oral administration of C-HE significantly reduced the total IgE levels in mice and slightly affected the production of IgG1. Furthermore, spleen cell cultures harvested from OVA-sensitised mice that had received C-HE orally showed a significant increase in Th1-derived responses (IFN- γ production). Therefore, our results suggest that the chaga mushroom extract may be used as an anti-allergic functional food.

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1. Introduction

Although an immune reaction generally refers to the mechanism by which the body protects itself from infection, an abnormal immune response can occasionally be triggered by non-infectious environmental agents, inducing tissue damage or disease [1–3]. Cases of this nature that involve an abnormally severe immune response to non-infectious elements are known as hypersensitivity diseases. Hypersensitivity is divided into four categories (type 1 through type 4) depending upon the antigens, cells and symptoms that trigger the hypersensitivity reaction [1]. The medical condition that is commonly referred to as allergy is a type 1 immune hypersensitivity reaction [1,4]. This reaction is generally an antibody-mediated immune response caused by allergens that produce the IgE antibody, and its symptoms include anaphylactic shock and atopy [1,5]. An allergic reaction involves the symptoms that are induced by the release of inflammatory mediators in response to the cross reaction of the IgE antibody and the allergen [1,3,5]. This cross reaction arises when the IgE antibody, which is produced in response to an allergen and is initially combined with the mast cells inside the tissue, is re-exposed to the same antigen [1,5,6]. This cross reaction also leads to an abrupt decrease in blood pressure as a result of the expansion of blood vessels, the release of the inflammatory cytokine TNF- α , the contraction of muscles, and the shrinking of the bronchial

tubes, which can prove fatal [1,2]. In addition, it leads to the expansion of blood vessels through the production of prostaglandins, leukotrienes and other kinds of lipids; the contraction of the bronchial tubes; and the chemotaxis of blood vessels, such that within 5–10 min of degranulation oedema (commonly called a rash) appears [4,7]. In most cases, these symptoms are alleviated within an hour.

The production of IgE antibodies is attributed to a tendency to cause a Th2-type immune response to antigens, while the reasons that a Th2-type immune response is triggered in a given specimen include genetic factors, the nature of the antigen and exposure to antigens [2,6,8]. One of the characteristics of people who experience type 1 hypersensitivity is the production of an abnormal amount of the IgE antibody compared with ordinary people, who generally respond to the same antigens by producing IgG1 antibodies and a small amount of the IgE antibody. The precise reason why the IgE antibody is produced abnormally in response to the same antigen, aside from genetic factors, has yet to be fully explained [2,5,9].

Approximately 40% of people living in developed countries are categorised as having an atopic constitution that increases the production of IgE in response to allergens in the environment and not to germs that are the direct cause of infection [2,10]. Korea is currently facing a similar social crisis as the incidence of atopy increases [11]. Additionally, even if a given person does not suffer from hypersensitivity to a specific allergen, it is very likely that s/he may suddenly develop allergy or atopy [3]. As a rule, allergic symptoms are occurring with greater frequency in economically developed countries. One explanation

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for this trend is that the hypersensitivity response increases as a result of the changes in the balance of the adaptive immune mechanisms that combat the exposure to foreign antigens [2,10]. This increased response is caused by environmental factors, such as changes in the exposure to infectious diseases during childhood, pollution in the environment, and new eating habits. As a result, greater emphasis is being placed on the importance of dietary habits as one of the methods that can be adopted to overcome atopy, allergies, and other types of hypersensitivity reactions. This increased emphasis on dietary habits suggests the potential use of diet in the regulation or transformation of the immune reaction of helper T cells to antigens [3,10,11].

The chaga mushroom (*Inonotus obliquus*), which grows and lives on the sap of birch trees, is native to the birch forests in Siberia, Mongolia, Canada, the US, and Scandinavia at latitudes above 45° N [12]. The people of Siberia have used the extract of the chaga mushroom to treat cancer and digestive diseases for many years, and it has been reported that this chaga mushroom extract has no toxic effect on living organisms [12]. In addition, the chaga mushroom has a number of biological properties, including antiviral and antioxidant effects, the ability to stimulate the immune system, and anti-cancer activity [13,14].

This paper studied the effect exerted by the chaga mushroom extract on acquired immunity to ovalbumin (OVA) to determine its effect on type 1 hypersensitivity reactions.

2. Materials and methods

2.1. Preparation of chaga mushroom water extract

Chaga mushrooms originating from Russia were kindly donated by a functional polysaccharide research centre in Korea University. The crushed dry mushrooms (100 g) were placed in distilled water (1000 ml), and the mixture was stirred at 100 °C for 4 h [14]. After centrifugation at 15,000 g for 20 min, the extract was freeze-dried to obtain a powder (30 g), which was denoted C-HE. The C-HE stock solution was then prepared by dissolving the C-HE powder in PBS (50 mg/ml) and filtering the supernatant through 0.2-µm filters. The C-HE stock solution was then stored at 4 °C until use.

2.2. Mice

Specific pathogen-free 6-week-old female BALB/c mice were purchased from Jung Ang Lab Animal Inc. (Seoul, Korea). The mice were housed in a specific pathogen-free environment. The animal experiments were conducted in accordance with the guidelines established by the Animal Care and Use Committee of Yuhang University (P-080302). The mice were supplied with water and a pellet diet *ad libitum*.

2.3. Anaphylactic shock

Anaphylactic shock in mice was induced by the intraperitoneal administration of 8 mg/kg of compound 48/80. Prior to the induction of anaphylaxis, the animals were treated with C-HE, which was administered intraperitoneally 30 min before the administration of compound 48/80. The rate of mortality was observed in each group of animals [4].

2.4. Immunisation of mice

On days 0 and 21, three BALB/c mice per group were injected intraperitoneally (*i.p.*) with ovalbumin (OVA, 5 µg; Sigma-Aldrich, St. Louis, MO, USA) adsorbed in 1 mg of alum (Al(OH)₃) adjuvant (Sigma Aldrich) [15]. The mice immunised with OVA and with alum *i.p.* were injected with either 20 or 200 µg/mouse of C-HE. In the second experiment, the C-HE (0.1, 1 and 10 mg/mouse) was dissolved in water and administered orally once a day for 21 consecutive days.

The immunised mice were bled from the retro-orbital sinus 3 days after the second immunisation, and serum specimens were collected for the antibody test.

2.5. Measurement of OVA-specific IgG1 and total IgE by ELISA

Immediately after the sera samples were collected, the IgG1 OVA-specific levels were measured by ELISA [16]. Briefly, a 96-well microtitre plate (Nunc, Roskilde, Denmark) was coated with 20 µg/ml OVA in 50 µl coating buffer and maintained overnight at 4 °C. The plates were washed three times with PBST (PBS containing 0.05% Tween 20) and blocked with 2% skim milk dissolved in coating buffer at 37 °C for 2 h. After the plates were washed three times with PBST, 50 µl of the serum dilutions were added to the wells. The plates were then incubated at 37 °C for 2 h. After the plates were washed, peroxidase-conjugated goat anti-mouse IgG1 antibody (Invitrogen, Grand Island, NY, USA) was added to each well, and the plates were incubated for 1 h. The plates were then washed 3 times before the addition of the TMB (Sigma-Aldrich) substrate solution. The plates were then incubated for 30 min at room temperature in the dark. After the addition of 2 N H₂SO₄ as the stop solution, the optical densities of the wells were measured at 450 nm using an automated microplate reader (Bio-Tek Instruments, Winooski, VT, USA). The levels of total IgE in the serum were quantified using ELISA kits (BD Pharmingen, CA, USA) in accordance with the manufacturer's instructions.

2.6. Cytokine assay

Splenocytes were harvested from the immunised mice, and the cells (2×10^6) were cultured in the presence of OVA (20, 100 and 500 µg/ml) in 24-well plates at 37 °C in 5% CO₂ and humidified air. Three days later, the culture supernatants were harvested by centrifugation at 1000 rpm for 5 min, and the levels of the cytokines IFN-γ, IL-6, GM-CSF and IL-4 in the supernatants were quantified using ELISA kits (BD Pharmingen, CA, USA) in accordance with the manufacturer's instructions.

2.7. Statistical analysis

The statistical significance of a difference between the groups was calculated with Student's two-tailed *t* test.

3. Results

3.1. Stimulation of the suppression of anaphylactic shock

An experiment was conducted to determine whether C-HE can suppress the immediate hypersensitivity reaction caused by the administration of compound 48/80 to a mouse model to induce anaphylaxis. As shown in Table 1, 100% of the mice in the compound 48/80-treated group died. However, all of the mice in the experimental group that was administered with 2.5 mg of C-HE survived. In contrast, 80 and 100% mortality rates were observed 1 h after the administration of compound 48/80 to the mice in the groups that received 0.5 and 0.1 mg of C-HE, respectively.

Table 1
Effect of the chaga mushroom extract on the systemic anaphylactic reactions induced by compound 48/80.

Dose (mg/mouse)	Anaphylactic shock (mortality, %)			
	15 min	30 min	45 min	60 min
Control	0	60	100	100
C-HE	0.1	0	60	100
	0.5	0	20	40
	2.5	0	0	0

3.2. Effect of the intraperitoneal injection of chaga mushrooms on IgE production

Because the intraperitoneal injection of C-HE significantly suppressed the anaphylactic shock induced by compound 48/80, the effect of the immunisation of the test subjects with an intraperitoneal injection of both the allergen (OVA) and C-HE on the production of the IgE antibody was tested (Table 1). OVA is a well-known antigen that causes an immune reaction that is strongly characterised by a T-helper type 2 (Th2) response in many animal models [5,8,17]. To stimulate the Th2 immune reaction, alum was used as an adjuvant for OVA [17].

As shown in Fig. 1, mice immunised with OVA/alum and 500 µg of C-HE exhibited significantly lower total IgE and OVA-specific IgG1 levels compared with mice immunised with OVA/alum (control group); however, no significant difference was detected with the lower concentration (50 µg) of C-HE. Previous studies that have analysed the production of IgG1 and IgE, which are produced through the influence of the Th2-type cytokine IL-4, have shown that elements that exhibit anti-allergic activity simultaneously suppress the production of IgG1 and IgE in response to most antigens. Thus, the IgE and IgG1 results obtained in this study are in agreement with the findings reported by previous studies [6,18].

3.3. Effect of the oral administration of the chaga mushroom extract on the production of IgE

The oral administration of 10 or 1 mg of C-HE, which was performed simultaneous with the immunisation with the OVA antigen, significantly suppressed the production of IgE (Fig. 3). Although the effect of the oral administration of C-HE on the production of OVA-specific IgG1 antibodies was also C-HE-dose dependent, the corresponding reduction in the production of IgG1 antibodies induced by the oral administration of C-HE was weaker than that observed with the intraperitoneal injection of C-HE (Fig. 2). The IgG1 antibody plays the most important role in the host defence system against infection, which is marked by opsonisation [11,18]. Therefore, these findings strongly suggest the possibility that C-HE can be developed as a functional food with the abilities to suppress allergies and defend the body against infection [12,18].

3.4. Measurement of Th1- and Th2-type cytokines

The function of an effector B cell that participates in humoral immunity is regulated by cytokines, such as IL-2 and IFN-γ, which are produced by Th1 cells, or IL-4, IL-5, and IL-13, which are produced by Th2-type helper T cells [8,16,18]. Thus, to determine whether C-HE

can alter the Th-2 dominant immune response, we examined the OVA-specific Th1-type (IFN-γ) and Th2-type (IL-4) cytokine production by splenocytes of mice that had received an oral administration of C-HE.

As shown in Fig. 3, the production of the Th1-type cytokine IFN-γ was significantly accelerated in mice that had been immunised with OVA and received an oral administration of C-HE compared with mice that had only been immunised with the allergen. This effect was found to be dependent on the concentration of OVA used for the re-stimulation. However, no significant difference was detected in the production of IL-4, which is the representative Th2-type cytokine, between the two groups. Moreover, no significant differences were found in the production of GM-CSF and IL-6.

4. Discussion

Anaphylactic shock is caused by the release of inflammatory mediators resulting from the degranulation of mast cells [19]. The fact that C-HE is able to suppress the anaphylactic shock induced by the injection of compound 48/80 strongly suggests that it can counteract type 1 hypersensitivity (Table 1). It is well documented that compound 48/80 generally induces instability in the cell membrane and increases the amount of calcium admitted into mast cells. These effects reduce the level of cAMP inside the cell and induce a state of degranulation that results in the release of inflammatory mediators, including histamine, leukotrienes, serotonin, and platelet activation factor [5,7]. The degranulation of mast cells is the main cause of the development of immediate hypersensitivity. As shown in Table 1, the *i.p.* injection of C-HE suppressed the anaphylactic shock that was induced in mice by compound 48/80. This result suggests that C-HE can suppress the degranulation of mast cells and the release of inflammatory mediators by inducing membrane stabilisation and decreasing the membrane permeability in mast cells [5,7,19].

Because type 1 hypersensitivity reactions, including anaphylactic shock and atopy, are the major causes of the extrinsic allergic symptoms that are connected with the IgE antibody, excessive production of IgE is an important indicator of type 1 hypersensitivity [7]. In general, IgG1 antibodies responding to antigens are found in the blood, circulate throughout the body, and enter infected areas to control the defence of the organism against an antigen [11]. However, the IgE that is produced in an abnormal immune response is not circulated in the blood but exists on the surface of mast cell receptors (e.g., FcγRIII and FcεRI) [1,3,5]. When these IgE antibodies are re-exposed to the same allergen, the allergen induces an antigen–antibody reaction with the IgE attached to the mast cell [1,3]. This antigen–antibody reaction induces degranulation in mast cells, and the histamine and nitric oxide that are released as a result of this degranulation act on vascular endothelial cells to relax the smooth muscle cells in the blood vessels

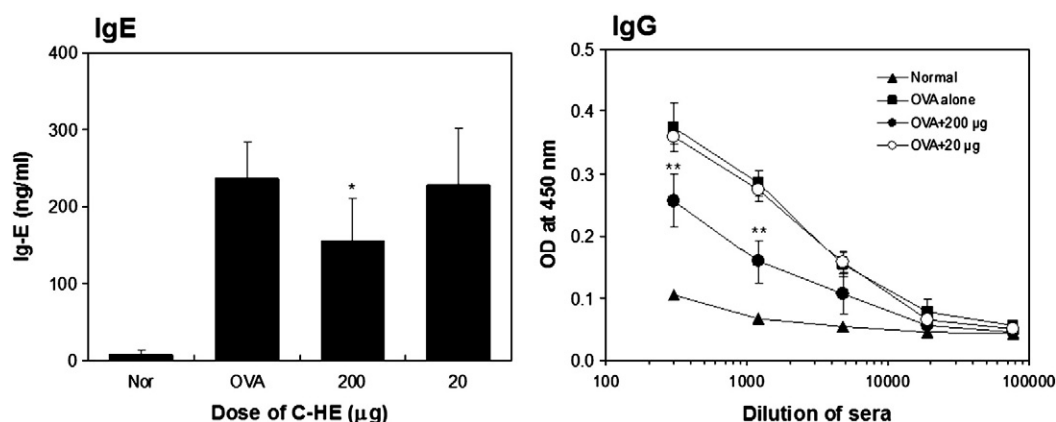


Fig. 1. Antibody production in the serum of mice immunised s.c. with the chaga mushroom extract and OVA. The data represent the means $\pm$ S.D. of 3 mice. * p < 0.5 and ** p < 0.01 compared with OVA-immunised mice.

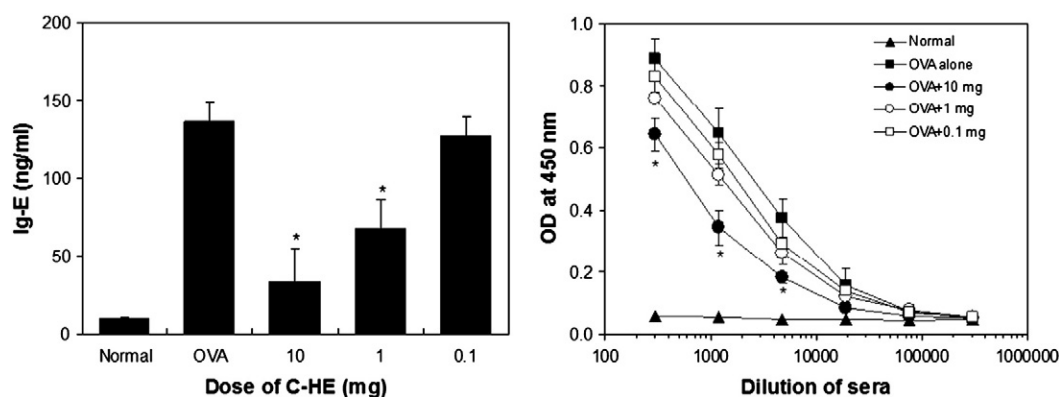


Fig. 2. Antibody production in the serum of mice immunised with the chaga mushroom extract p.o. before or after sensitisation against OVA s.c. The data represent the means $\pm$ S.D. of 3 mice. * $p < 0.01$ compared with OVA-immunised mice.

and thus promote vascular permeability [16]. Subsequently, the inflammatory cells exposed to various types of inflammatory cytokines induce the late allergy stage [5,17,20]. As a result, the IgE antibody that responds to antigens plays the most important role in the induction of immediate hypersensitivity reactions [16].

In an animal model, the Th2-dominant immune response induced by immunisation with OVA/alum is well documented [6]. The Th2 immune response is characterised by increased levels of IL-4, IL-5, and IL-13 and generally elevated serum levels of total and antigen-specific IgE and IgG1 [16]. Therefore, we attempted to determine the effects of C-HE on the IgE and IgG1 levels in OVA-immunised mice. As shown in Figs. 1 and 2, the IgE and IgG1 levels tended to decrease after the systemic and oral administration of C-HE, and these results are consistent with the findings reported by previous studies [6,16,18]. It is intriguing that, although the oral administration of C-HE potentially suppressed the production of the IgE antibody compared with the intraperitoneal

injection of C-HE, the oral administration of C-HE had less effect on the levels of IgG1 [16].

T cells play a central role in immunoregulation. T cells can be categorised as Th1, Th2, Th17 or Treg cells [21,22]. With regard to the regulatory mechanisms of humoral immune responses by helper T cells, the cytokines produced by the Th1 and Th2 subsets regulate the effector functions of B cells. A skewing of the Th1/Th2 balance in favour of Th1-mediated immunity is associated with an antiallergic effect [22]. During a humoral immune response, the Th1-type cytokines are involved in the production of IgG2-type antibodies by B cells and promote the cellular immune response [8,18]. Because the Th1 response is antagonistic to the Th2 response, the body will continue to trigger either a Th1-type or a Th2-type immune response once one of these responses becomes dominant [17]. Consequently, the fact that C-HE is able to suppress the production of the IgE antibody despite the fact that there was no change in the production of IL-4 indicates

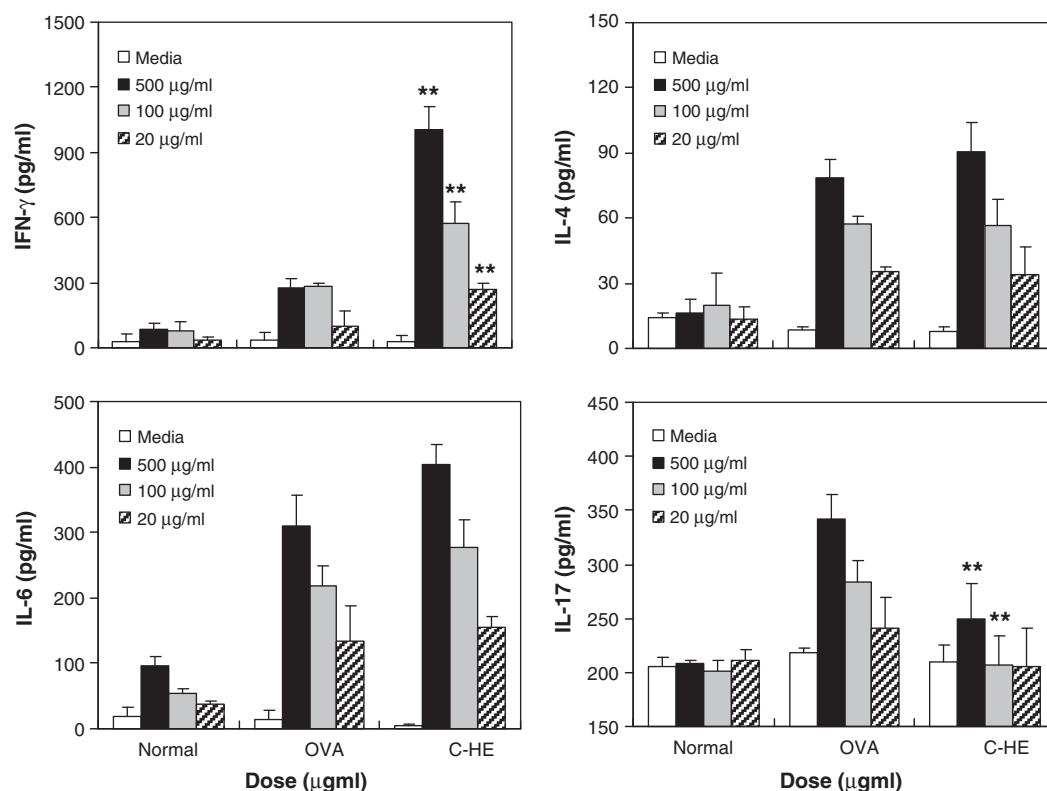


Fig. 3. Cytokines in the supernatants of cultured splenic cells from mice treated with the chaga mushroom extract p.o. before or after sensitisation against OVA s.c. The data represent the means $\pm$ S.D. ($n = 3$). * $p < 0.01$ compared with the OVA-immunised group. ** $p < 0.01$ compared with OVA-immunised mice.

that this effect is due to its ability to boost the production of IFN- γ , which is the classic Th1 cytokine [8,16,17]. In addition, IL-17A and TGF- β regulate the adaptive immune response against allergens [23]. A recent study suggests that IL-6 plays an important role in determining the type of adaptive immune response, primarily in the differentiation of effector CD4⁺ T cells. IL-6 can modulate the intensity of the immune response by promoting Th17 cell differentiation and inhibiting the development of Treg cells [22,23]. The critical role of IL-17 in allergy disease has recently been reported [22,23]. Collectively, IL-17 induces eosinophil- and neutrophil-rich conditions in an allergen-induced inflammation site. The reduced production of IL-17 observed in chaga-treated mice might decrease the inflammatory responses in this allergy model and may enhance the Th1-mediated response by shifting the T cell differentiation axis toward Th1 instead of Th17 [22,23]. Our results suggest that the administration of chaga might modulate the production of IgE in OVA-sensitized mice through antibody shifts in the immune homeostasis via mechanisms that are not yet elucidated.

Takano et al. [16] reported that the oral administration of aqueous peanut seed extracts (PSE) reduces the IgE and IgG1 levels, whereas apple polyphenols (AP) reduce the IgE level but not the IgG1 level. These results exhibit the same trends as our data. These researchers assumed that the anti-allergic activities of AP depend on the polyphenol content. To interpret these results, more detailed research is needed to refine the active ingredients, the dosage, and the period of administration [16].

In addition, the question of whether the orally ingested ingredients enter the immune organs in the small intestines known as Peyer's patches is the most important factor in the regulation of the mucosal immune response [8,19]. In order for orally administered elements to enter Peyer's patches, elements that appear during the digestive process or metabolic ingredients resulting from the action of microorganisms play a decisive role in allowing them to pass the M-cell zone [6,8]. Recent studies showed that the oral administration of components that activate the mucosal immune response, including probiotics, can buffer the allergic symptoms in OVA-sensitized mice [24,25]. In addition, Mirada et al. [26] reported that the serum IgE levels are higher in allergic children exposed to an allergen compared with non-allergic children and that the serum IgA levels are higher in non-allergic children compared with allergic children. The oral administration of the T cell epitope of ovomucoid induces a high level of IgA and a lower level of IgE [23], which suggests that the Th1 dominant immune response to the allergens can diminish the allergic symptoms. Consequently, to explain the changes in the production of antibody isotypes as a result of the oral administration of chaga mushrooms, it is necessary to investigate the components of chaga mushrooms that are capable of being absorbed by the intestinal immune system [6,8].

In conclusion, the experiments show that the oral administration of C-HE can suppress the OVA-induced production of the IgE antibody, which strongly suggests that C-HE is able to regulate the immune response to the allergen (OVA) in mice. Therefore, to develop chaga mushroom extracts into functional foods, detailed studies in the isolation of the active ingredients, the activation of the mucosal immune system, and the activation of the systemic immune system through the mucosal immune response must be conducted.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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