

Ganoderma modulates allergic asthma pathologic features via anti-inflammatory effects

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ABSTRACT

Ganoderma, a fungal genus, is a traditional medicine with immuno-modulating effects. Asthma is an inflammatory disease of airways, and the main trigger of asthma is allergic inflammation. In this study, the effects of Ganoderma (an anti-inflammatory agent) given via oral administration (G/O) or intraperitoneal injection (G/IP) on asthma was evaluated.

Forty BALB/c mice were divided into four groups, including the control, OVA-challenge, OVA-challenge + G/O, and OVA-challenge + G/IP. To determine AHR, the MCh challenge test was done. The levels of IL-1 β , -4, -5, -6, -8, -10, -12, -13, -17, -25, -33, -38, Cys-LT, LTB₄, and hydroxyproline were measured. Finally, lung histopathology was evaluated to determine eosinophilic inflammation, goblet cell hyperplasia, and mucus hypersecretion.

Treatment with G/O and G/IP could significantly reduce the levels of IL-1 β , -5, -6, -8, -17, -25, -33, and -38; the levels of IL-4 and IL-13 had no significant changes, but the levels of IL-10 and IL-12 were enhanced. The mice treated with G/O and G/IP showed decreased levels of Cys-LT, LTB₄, peribronchial and perivascular inflammation, but no significant changes were observed in AHR, hydroxyproline level, goblet cell hyperplasia, and mucus hypersecretion.

Ganoderma can be applied as an immunomodulatory and anti-inflammatory agent for managing asthma.

1. Introduction

Ganoderma, a fungal genus, is consumed as a traditional medicine in some countries of Asia for its beneficial health effects on a number of diseases. It is known as Lingzhi in China and as Ling-chi mushroom in other countries, used as a complementary and traditional medicine. During the last decade, Ganoderma has been used globally because of its biological effects. Considering the need for developing new treatments for complicated diseases and the growing interest of populations in

traditional medicine, it is essential to investigate the useful and possible deleterious effects of Ganoderma (Chen et al., 2004; Sui et al., 2016; Ren et al., 2021). Ganoderma has immunomodulatory effects mainly due to its polysaccharides. Both the intracellular and extracellular polysaccharides of Ganoderma have shown antioxidant activity (Chen et al., 2004; Sui et al., 2016). These polysaccharides have been shown to exert immunomodulatory effects on B and T cells. In fact, β -glucan of Ganoderma, which is not expressed in mammalian cells, is recognized as a PAMP by PRRs (Ren et al., 2021). Therefore, it can direct T cell immune

Abbreviation: AHR, Airway hyperresponsiveness; Akt, Protein kinase B (PKB); B, Lymphocyte B; BALf, BronchoAlveolar lavage fluid; Bcl, B-cell lymphoma/leukemia; Btk, Bruton's tyrosine kinase; Ca²⁺, Calcium; Card, Caspase recruitment domain family member; CD, Cluster of differentiation; CR, Complement receptor; Cys-LT, Cysteinyl leukotriene; DC, Dendritic cells; EOS, Eosinophil; ELISA, Enzyme-linked immunosorbent assay; ERK, Extracellular signal-regulated kinases; H&E, Hematoxylin and Eosin; G/IP, Ganoderma extract via intraperitoneal injection; G/O, Ganoderma extract via oral administration; GPCR, G protein-coupled receptor; IFN, Interferon; Ig, Immunoglobulin; IL, Interleukin; IP, Intraperitoneal; ITAM, Intracellular immunoreceptor tyrosine-based activation motif; JNK, c-Jun N-terminal kinases; Kg, Kilograms; LT, Leukotriene; Malt, Mucosa-associated lymphoid tissue; MAPK, Mitogen-activated protein kinase; MCh, Methacholine; Mg, Milligrams; ml, Milliliter; MR, Mannose receptor; NF- κ B, Nuclear factor kappa-light-chain-enhancer of activated B cells; Nk, Natural killer cells; OVA, Ovalbumin; PAMP, Pathogen-associated molecular patterns; PAS, Periodic Acid Schiff; PBS, Phosphate-buffered saline; Pg, Pictogram; PKC, Protein Kinase; ROS, Reactive oxygen species; PI3K, Phosphoinositide 3-kinase; PRR, Pattern recognition receptors; Syk, Spleen tyrosine kinase; Th, Lymphocyte T helper; TLR, Toll-like receptor; TNF, Tumor necrosis factor; Treg, Lymphocyte T regulatory.

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responses towards the Th1 branch.

Asthma is a common inflammatory disease of airways, especially in children. It is a complex lung disease presented by bronchial inflammation, bronchoconstriction, and dyspnea. When asthma attacks begin, airways' smooth muscle cells develop spasm accompanied with the stimulation of airways' sensory nerves and their mediator receptors on the cell surface. For the relaxation of smooth muscle cells, signaling from these receptors and nerves should be inhibited. The main trigger of asthma is allergic inflammation. Furthermore, Th2 and Th17 have important roles in asthma pathogenesis. While Th2 lymphocytes produce type 2 cytokines and manage allergic responses in airways, activated Th17 cells regulate pulmonary inflammation. The number of Th17 cells has been reported to positively correlate with pulmonary injury (Athari et al., 2017; Lankarani et al., 2017; Athari and Athari, 2014). Therefore, in the present study, the therapeutic effects of Ganoderma, as an anti-asthma and anti-inflammatory agent, were evaluated in mice models of asthma.

2. Material and methods

2.1. Animals and treatments

Forty 8-week-old male mice (BALB/c) were bred in an animal laboratory under standard conditions. The mice were divided into four groups. Mice in the control group were sensitized, challenged, and treated with PBS. In the OVA-challenge group, the animals were sensitized and challenged with OVA without Ganoderma treatment. The third group included OVA-challenged mice treated with Ganoderma extract (300 mg/kg via IP injection on days 25th and 29th). Finally, in the fourth group, OVA-challenged mice were orally fed with Ganoderma extract (300 mg/kg on days 25th and 29th). The preparation and dosage of the treatments were done according to previous reports (Wang et al., 2002; Andoh et al., 2010) that has been used for oral gavage only (Andoh et al., 2010) and the same dose was used as IP administration in this study. Also, a dose-response curve was not drawn in this study. Ganoderma extract was obtained according to previously described method (Wang et al., 2002). Mice models of airway inflammation and allergic asthma were produced according to a previously described method (Masoume Athari et al., 2018). Briefly, the mice were sensitized with OVA plus aluminum hydroxide via IP injections on day 1st and again on day 14th, followed by OVA challenge (aerosolized nebulizing) on days 24th, 26th, 28th, and 30th. The AHR test was done 30 min after the last OVA challenge on day 30th, and then the samples of blood, BALF, and the lung tissue were taken on day 31th.

2.2. MCh challenge

To assess AHR, the MCh challenge test was performed 30 min after the last challenge on day 30th by determining the Penh value. The mice were anesthetized (with ketamine and xylazine) and intubated, and then the tube was connected to a ventilator (Inspira ventilator, Harvard Apparatus, Holliston, MA, USA). All the groups were exposed to PBS and MCh aerosols with a series of doubling concentrations (6.25, 12.5, 25, and 50 mg/mL). Finally, the results were analyzed.

2.3. Cytokines

The levels of IL-1 β , 4, 5, 6, 8, 10, 12, 13, 17, 25, 33, and 38 were measured in BALF supernatant by ELISA kits according to the manufacturer's instructions (R&D, USA).

2.4. Eicosanoids

Cys-LT and LTB4 were assayed by ELISA kits (Cayman Chemical, Ann Arbor, USA) in BALF supernatant.

2.5. Hydroxyproline assay

The total collagen content of the lung was estimated by measuring hydroxyproline level. Saline solution (1.5 mL) was added to the peripheral part of the left lower lobe of the lung tissue (30 mg). After homogenizing the sample on ice, hydroxyproline content of the homogenate was measured by spectrophotometry (Ji et al., 2016).

2.6. BALF cells

BALF cells were separated to prepare slides by the cytospin method. The slides were stained with Giemsa, and eosinophil percentage was determined.

2.7. Histopathology

After separation, lung tissues were fixed to prepare histopathological slides, which were then stained with H&E and PAS. The slides were examined for inflammation, goblet cell hyperplasia, and mucus hypersecretion by light microscopy. Histopathological evaluation was performed by two pathologists who were blinded to the experimental group of the tissues. Mucus production was determined by assigning the score 0 to the absence of any observable stained mucus, as well as the score 0.05 for low-level mucus production; score 1 when approximately 25 % of the bronchial space was filled with mucus; score 2 for 50 %; score 3 for 75 %, and score 4 when 100 % of the bronchial space was filled with mucus. Moreover, goblet cells were quantified per 100 epithelial cells in several randomly selected microscopy fields (GCI), which was classified into four groups as follows: score 0 for GCI < 5%, score 1 for 5% $\leq$ GCI < 25 %, score 2 for 25 % $\leq$ GCI < 50 %, score 3 for 50 % $\leq$ GCI < 75 %, and score 4 for 75 % $\leq$ GCI $\leq$ 100 %. For peribronchial and perivascular inflammation, score 0 was given to the absence of eosinophils, score <1 for one incomplete layer, score 2 for 1–2 complete layers, score 3 for three complete layers, and score 4 for more than three complete layers (Athari et al., 2016; Athari et al., 2012).

2.8. Statistical analysis

The data were analyzed and presented as mean $\pm$ SD using SPSS version 21. One-way ANOVA was used as an inferential test, and the data of AHR were analyzed by two-way ANOVA. GraphPad prism was used to draw graphs, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. AHR

The Penh values for AHR significantly increased in the non-treated OVA-challenged group compared with the control group for all MCh concentrations ($p < 0.05$). G/O- and G/IP-treated OVA-challenged groups displayed no significant changes in the Penh value ($P > 0.05$) compared with the OVA-challenged group without G/O and G/IP treatments (Fig. 1).

3.2. Cytokines

The levels of IL-1 β , 4, 5, 6, 8, 13, 17, 25, 33, and 38 significantly increased in the OVA-challenged group in comparison with the control group ($P < 0.05$); however, a reverse trend was found for IL-12 ($P < 0.05$) and IL-10. Treatment with G/O and G/IP significantly reduced the levels of IL-1 β , 5, 6, 8, 17, and 38 compared with the non-treated OVA-challenged group ($P < 0.05$). There was no significant difference between the treated and non-treated OVA-challenged groups in terms of IL-4, 10, 12, 13, 25, and 33 levels ($P > 0.05$). Treatment with G/O and G/IP enhanced the levels of IL-10 and IL-12 compared with the non-

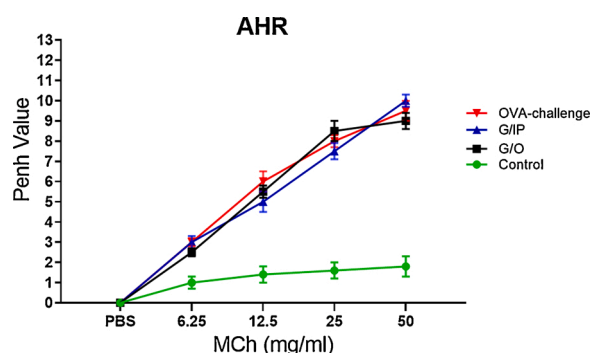


Fig. 1. The Penh value in the MCh challenge test. After being anesthetized, seven mice from each group were exposed to a series of doubling concentrations of MCh (PBS, 6.25, 12.5, 25, and 50 mg/mL) to study AHR changes on day 30. There was no significant difference ($p > 0.05$) between the treated and non-treated OVA-challenged groups.

treated OVA-challenged group, but the increase was not significant ($P > 0.05$).

3.3. Eicosanoids

The levels of Cys-LT and LTB₄ significantly decreased in the OVA-challenged groups treated with G/O (598.3 ± 38.4 and 78.4 ± 6.8 pg/mL, respectively) and G/IP (601.9 ± 44.7 and 69.7 ± 10.3 pg/mL, respectively) compared to the non-treated OVA-challenged group (952.4 ± 29.2 and 127.3 ± 11.9 pg/mL, respectively) ($P < 0.05$, Fig. 2).

3.4. Hydroxyproline

Treatment with G/O and G/IP had no significant effect on the hydroxyproline level in the lung compared with the non-treated OVA-challenged group ($P > 0.05$, Fig. 3).

3.5. BALf cells

Eosinophil percentage significantly ($P < 0.05$) increased in the OVA-challenged group ($71 \pm 11\%$) compared with the control group ($6 \pm 2\%$). Treatment with G/O and G/IP significantly ($P < 0.05$) diminished eosinophil percentage in BAL fluid (G/O: $27 \pm 8\%$ and G/IP: $25 \pm 9\%$).

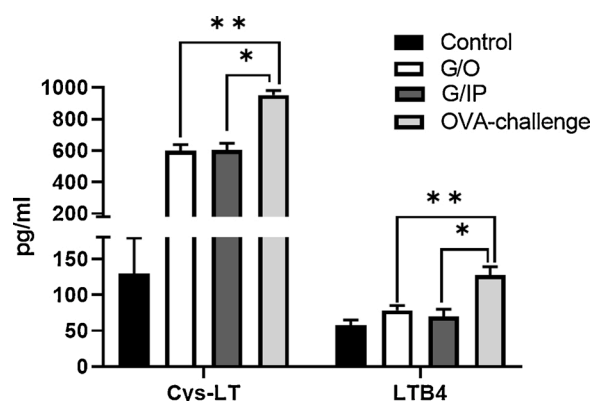


Fig. 2. Eicosanoid level. The levels of Cys-LT and LTB₄ were measured in the control, treated, and non-treated allergic OVA-challenged groups on day 31. There were seven mice in each group. Treatment with G/O and G/IP could significantly ($p < 0.05$) decrease Cys-LT and LTB₄ levels (* and ** symbols show significant differences between the groups, $p < 0.05$).

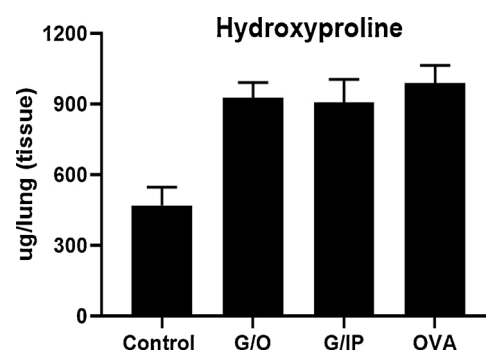


Fig. 3. Hydroxyproline assay. For estimating lung collagen content, hydroxyproline level was measured by spectrophotometry in all the experimental groups ($n = 7$ per group). There was no significant difference ($p > 0.05$) between the treated and non-treated OVA-challenged groups.

3.6. Histopathology

The number of goblet cells and mucus hyper-production significantly increased in the airways of OVA-challenged mice compared with the control group ($P < 0.05$). Treatment with G/O and G/IP had no significant attenuating effects on goblet cell hyperplasia and mucus hyper-production ($P > 0.05$). Nevertheless, G/O and G/IP treatment significantly decreased peribronchial and perivascular inflammation compared with the non-treated OVA-challenged group ($P < 0.05$, Figs. 4 and 5).

4. Discussion

Ganoderma, as a basidiomycete fungus, has been used in traditional Asian medicine for centuries. It was found that the polysaccharides purified from Ganoderma primed Th1-polarized adaptive immune response and could suppress allergic asthma development by controlling AHR, OVA-specific IgE and IgG1 production, airway inflammation, and Th2 cytokines' production (Pi et al., 2014; GuanMin-chang et al., 2014). The immunostimulatory activities of Ganoderma's polysaccharides are related to their structural characteristics, such as molecular weight and the composition, conformation, and branching degree of glycosidic bonds (Gao et al., 2020; Liu et al., 2016; Kuo et al., 2006). PS-F2, is a Ganoderma polysaccharide can act as an adjuvant, prime Th1 immune response and attenuate Th2-mediated immunopathology. PS-F2 reduces IL-4 level in BALF, stimulates DCs to produce IL-12 and IL-10 and also, effectively suppresses the development of allergic asthma in mice. Therefore, PS-F2 administration can suppress Th2-mediated airway inflammation and the development of AHR in asthma (Pi et al., 2014). In another study, organic selenium that was extracted from Ganoderma could control chronic airway inflammation and improve oxidant/antioxidant equilibrium in asthma and is able to counteract the asthma in an experimental asthma model by using a variety of testing systems (GuanMin-chang et al., 2014). In this study increased AHR was not controlled by G/O and G/IP treatment and similar result was observed in the IL-4 level between the treated and non-treated OVA-challenged groups. In our study, Ganoderma could not reduce IL-4 and in result, could not control early allergic reactions that are responsible for the immediate allergen-immune response and AHR. We could not find related reasons but it may be explained that in this study, we did not use pure PS-F2 or high dose of organic selenium in treatment protocol. Specific polysaccharides or organic trace elements may have effect of AHR and it needs more researches and deeper evaluations. On the other hand, CD4⁺ Th2 cells play a pivotal role in orchestrating ongoing immune-mediated allergic diseases, and in contrast, Th1 CD4⁺ cells can change the immune response balance from Th2 towards Th1 dominance. Therefore, predominant Th1 responses can modulate allergic reactions. Ganoderma supplementation has been reported to direct the Th1/Th2

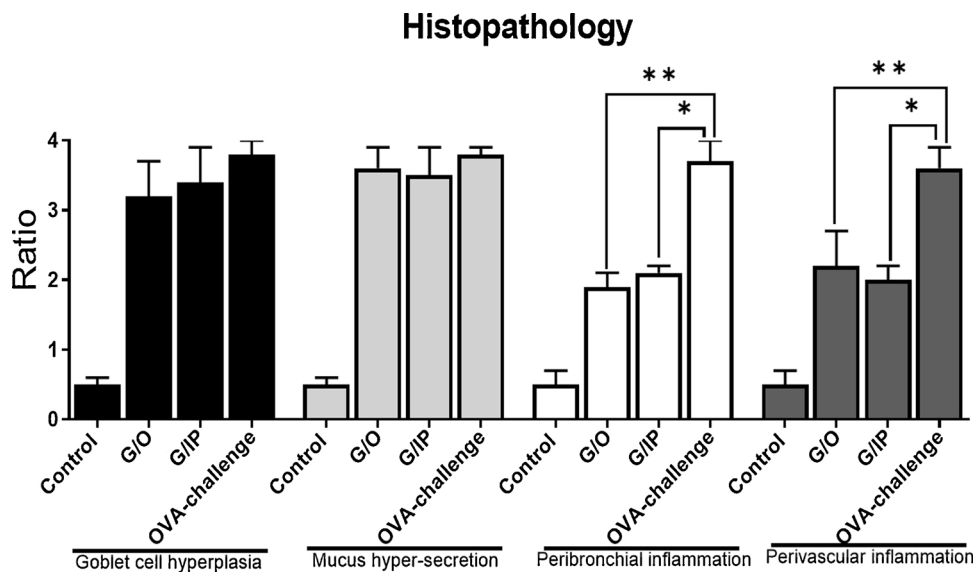


Fig. 4. Histopathological examination. Eosinophilic inflammation in the peribronchiolar and perivascular areas, hyperplasia of goblet cells, and mucus production were evaluated in all the experimental groups, each including seven mice. Treatment with G/O and G/IP could significantly ($p < 0.05$) decrease peribronchiolar and perivascular inflammation but had no significant ($p > 0.05$) effects on goblet cell hyperplasia and mucus production compared with the non-treated OVA-challenged group (* and ** symbols show significant differences between the groups, $p < 0.05$).

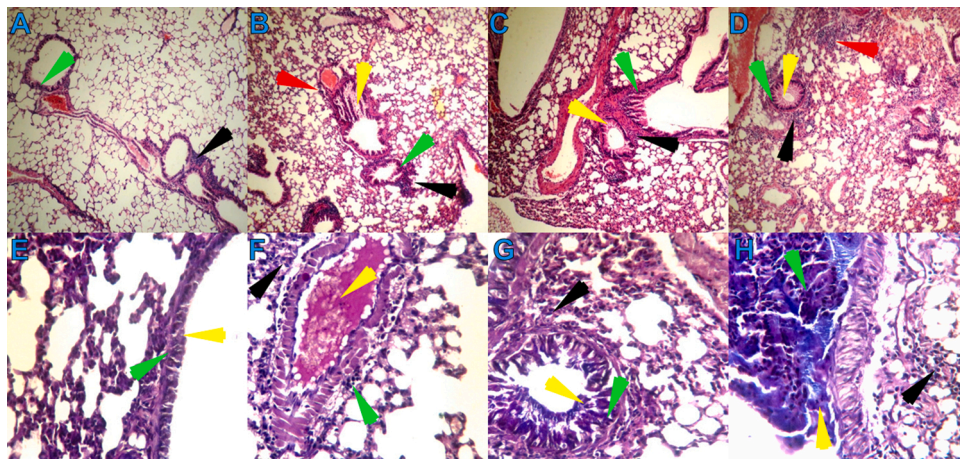


Fig. 5. Histopathology. Lung tissue sections were stained with H&E (A, B, C, and D in the first row, 100X) and PAS (E, F, G, and H in the second row, 400X). Eosinophilic inflammation is indicated around bronchi (the black arrow) and vascular areas (the red arrow). Goblet cell hyperplasia is shown by the green arrow, and the yellow arrow shows mucus secretion. A and E: control, B and F: G/O, C and G: G/IP, D and H: OVA-challenged group.

balance towards Th1-skewed responses (Hsiao et al., 2008). Ganoderma extract could activate cellular and humoral immunity, and its polysaccharides were reported to potentiate the production of cytokines, including IL-1 β , -6, -12, and TNF- α , by monocytes and macrophages and IFN- γ and TNF- α by T cells (Ren et al., 2021). In the present study, treatment with G/O and G/IP could reduce the levels of IL-1 β , 5, 6, 8, 17, and 38, reflecting the immunomodulatory effects of Ganoderma. However, G/O and G/IP treatments had no significant effects on the levels of IL-4, 13, 25, and 33, suggesting that the immunomodulatory effects of Ganoderma may be more pronounced on the Th1 than Th2 branch. It was noted that Ganoderma induced IFN- γ , TNF α , IL-12, 23, 10, and 17 but had no effect on IL-4 production (Ren et al., 2021), supporting our observations in terms of IL-4 and IL-17 levels. Therefore, treatment with Ganoderma seems to be effective in reducing the inflammation and wall thickness of airways.

Polysaccharides of Ganoderma (especially mannose and glucose) are recognized by cell surface receptors, such as TLR 2 and 4, dectin-1, CR3, MR, and scavenger receptors. Dectin-1 activation by Ganoderma's polysaccharides promotes glucan-independent binding to T cell subsets and is involved in triggering their proliferation and modulating cytokine and chemokine production (Ren et al., 2021). The activation of some

TLRs (including TLR-4, 7, and 9) results in IL-12 production, skewing the cytokine balance from Th2 to Th1 responses (Pi et al., 2014; Berovic et al., 2003). Ganoderma's polysaccharides exert immunomodulatory functions not only through binding to dectin-1, TLRs, and/or MR on mononuclear phagocytes, but also by interacting with CR3 on granulocytes and NK cells, indicating anti-inflammatory and immune-effector-cell enhancing effects. Also, Ganoderma's polysaccharides activate dendritic cells (DCs) and increase the production of IL-2 and IFN- γ (Ren et al., 2021; Huang and Ning, 2010; Pan et al., 2013). The immune stimulating effects of Ganoderma are amplified through TLR4/ROS/PI3K/Akt/MAPKs/NF- κ B-dependent pathways, proposing Ganoderma as an immunomodulatory agent. The TLR4/ROS/PI3K/Akt/MAPKs/NF- κ B signaling pathway is responsible for activating macrophage and inducing TNF- α production (Ren et al., 2021; Yin et al., 2009; Athari, 2019).

ERK1/2, JNKs, and p38 are the most common MAPK subunits, and the MAPK pathway can regulate immune responses. Moreover, the PI3K/Akt pathway, which can be activated by Ganoderma, shares a main role in controlling the differentiation and survival of DCs (Wanga et al., 2019). Ganoderma was reported to promote B cells' proliferation and increase antibody production. The bioactive polysaccharides of

Ganoderma possess B cell-stimulating activity, up-regulating CD71, CD25, PKC α , and PKC γ expression and antibody secretion (Ren et al., 2021; Athari, 2019). The binding of Ganoderma's polysaccharides to dectin-1 induces the receptor's ITAM and Syk phosphorylation, activating the PI3K/Akt pathway. Then Card9/Bcl10/Malt1 signalosomes activate the NF- κ B signaling route, which is linked with the innate and adaptive immune responses and GPCR signaling. Furthermore, dectin-1 interacts with Vav and Btk, which are related to phagocytosis initiation and autophagy (Ren et al., 2021; Athari, 2019; Esmailzadeh et al., 2020). In this study, we did not investigate allergy- and asthma-related signaling pathways; however, a number of inflammatory mediators such as Cys-LT and LTB4 were found to be decreased in the mice models of asthma treated with Ganoderma.

Recently, IL-33 treatment has been reported to have no impact on the expression of Th2-associated IL-4 (Luo et al., 2020; Lin, 2005). In this study, IL-33 and IL-4 decreased after Ganoderma treatment, but there was no significant difference in the levels of these cytokines between the treated and non-treated groups. Further, IL-5, as the main cytokine involved in eosinophilic inflammation, decreased significantly in both G/O- and G/IP-treated groups compared with the non-treated OVA-challenged group. Treatment with G/O and G/IP; however, could decrease neither goblet cell hyperplasia nor mucus production, but there were significant reductions in peribronchial and perivascular eosinophilic inflammation. These observations can be attributed to the control of IL-5 secretion from Th2 cells. On the other hand, failure to control goblet cell hyperplasia and mucus production may be related to persistently high levels of IL-13.

It was shown that the methanolic extract of Ganoderma inhibited scratching, an itch-related response, in mice models of allergy. The oral administration of Ganoderma extract could control allergic itch; however, mast cells and H1 histamine receptors were not the action sites of the antipruritic effects of Ganoderma, but it inhibited histamine release from mast cells (Andoh et al., 2010). In our study, treatment with Ganoderma had no effect on AHR, which may be attributable to its lack of effective role in controlling the impacts of allergic mediators on smooth muscle cells, leading to failure in attenuating airways' spasm and obstruction. However, Ganoderma spores, as airborne allergens, are associated with asthma attacks and increase the severity of allergic reactions, especially in environments with high humidity (Jedryczka et al., 2015). Generally, it should be noted that fungi and their modified orally consumable products may have allergenicity and inflammatory effects (Athari, 2013; Yavari et al., 2016).

In this study, Ganoderma could control LTs levels and also harness inflammation (around airways and vessels) in the asthmatic lung. G/O and G/IP had significantly anti-inflammatory effects and both of G/O and G/IP had similar effects on controlling and decreasing of inflammation. Ganoderma administration to the asthmatic mice could control inflammation (as main asthma pathophysiology) and can be used as anti-asthma agent to cure of airway inflammation and control of asthma pathology. It may have anti-remodeling effect and may prevent lung fibrosis that was not evaluated in this study. It was observed that route of Ganoderma administration (G/O or G/IP) in same dosage had no significant difference on control of allergen-inflammation in lung and can be used with both route according to conditions of the asthmatic patient. In conclusion, Ganoderma has anti-inflammatory effects, and these effects are not limited to asthmatic inflammation. Therefore, Ganoderma can be used in other inflammatory diseases; however, the effects of this fungus should be studied and confirmed in these conditions. In asthma, Ganoderma and its polysaccharides can be used to modulate the host's innate and cellular and humoral adaptive immunity responses. In our study, Ganoderma had no effects on the Penh value (an indicator of AHR) and hydroxyproline level (a lung remodeling factor), so more studies are needed in this area to confirm these observations. Moreover, as a complementary remedy, the molecular mechanisms of the anti-asthmatic and anti-allergic effects of Ganoderma need to be deeply investigated. The interactions of Ganoderma with multiple receptors on immune cells

should be further divulged. Also, the allergenicity of Ganoderma and its spores should be noted.

In this study, we had some limitations. In general, the Penh value is not a reliable parameter to determine AHR, and it is better to use specific airway resistance tests. However, we could not use specific airway resistance tests. Thus, our results regarding the effects of Ganoderma on bronchoconstriction responses (MCh and AHR) and airway sensory nerves may not be conclusive. In this study, we only determined eosinophil percentage in BALF, and other differential cell counts were not performed. Finally, we enrolled two additional OVA-challenged groups that were treated with Tris buffer, as the Ganoderma extract solution, via the oral and IP routes; nevertheless, the results of these groups were not presented because they showed no significant difference compared with the OVA-challenged group.

Ethics approval and consent to participate

All animal studies were approved by the ethical committee of the animal house of ix.med.vet.dep, 2021 (No. IX.MED.VET.DEP.REC.2021.300274.2).

Consent for publication

Not Applicable.

Availability of data and materials

Not Applicable.

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Authors' contributions

YL, ML, RW, BW, SSA, and JW participated in the design, laboratory testing, data analysis, and drafting the manuscript. JW and SSA supervised the study.

Declaration of Competing Interest

There is no conflict of interest.

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References

- Andoh, Tsugunobu, Zhang, Qun, Yamamoto, Takumi, Tayama, Manabu, Hattori, Masao, Tanaka, Ken, Kuraishi, Yasushi, 2010. Inhibitory effects of the methanol extract of Ganoderma lucidum on mosquito allergy-induced itch-associated responses in mice. *J. Pharmacol. Sci.* 114, 292–297.
- Athari, Seyyed Shamsadin, 2013. Immune response shifting of asthma in aging. *Middle-East J. Sci. Res.* 13 (4), 489–498.
- Athari, S.S., 2019. Targeting cell signaling in allergic asthma. *Signal Transduct. Target. Ther.* 4 (1), 1–19.
- Athari, Seyyed Shamsadin, Athari, Seyyed Moehyadin, 2014. The importance of eosinophil, platelet and dendritic cell in asthma. *Asian Pac. J. Trop. Dis.* 4 (S1), S41–S47.
- Athari, Seyyed Shamsadin, Babaloo, Zohre, Tehrani, Ali Asghar, Naderi, Mohammad Mahdi, Aghamohammadi, Negar, Khoshbakht, Rahem, Azimi, Ebrahim, Abdolipour, Mohammad, Badihi, Khorshid, 2012. Anti-inflammatory effects of silymarin against damages caused by U.V. irradiation. *Glob. Vet.* 9 (2), 149–153.
- Athari, Seyyed Shamsadin, Pourpak, Zahra, Folkerts, Gert, Garssen, Johan, Moin, Mostafa, Adcock, Ian M., Movassaghi, Masoud, Ardestani, Mehdi Shafiee, Moazzeni, Seyed Mohammad, Mortaz, Esmail, 2016. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. *Eur. J. Pharmacol.* 791, 811–820.

- Athari, Seyyed Shamsadin, Athari, Seyyede Masoume, Beyzay, Fateme, Movassaghi, Masoud, Mortaz, Esmaeil, Taghavi, Mehdi, 2017. Critical role of Toll-like receptors in pathophysiology of allergic asthma. *Eur. J. Pharmacol.* 808, 21–27.
- Berovic, Marin, Habijan, Jozica, Zore, Irena, Wraber, Branka, Hodzar, Damjan, Boh, Bojana, Pohleven, Franc, 2003. Submerged cultivation of *Ganoderma lucidum* biomass and immunostimulatory effects of fungal polysaccharides. *J. Biotechnol.* 103, 77–86.
- Chen, Hung-Sen, Tsai, Yow-Fu, Lin, Steven, Lin, Chia-Ching, Khoo, Kay-Hooi, Lin, Chun-Hung, Wong, Chi-Huey, 2004. Studies on the immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. *Bioorg. Med. Chem.* 12, 5595–5601.
- Esmaeilzadeh, Abdolreza, Tahmasebi, Safa, Athari, Seyyed Shamsadin, 2020. Chimeric antigen receptor -T cell therapy: applications and challenges in treatment of allergy and asthma. *Biomed. Pharmacother.* 123 (109685).
- Gao, Xiong, Qi, Jiayi, Ho, Chi-Tang, Li, Bin, Mu, Jingjing, Zhang, Yuting, Hu, Huiping, Mo, Weipeng, Chen, Zhongzheng, Xie, Yizhen, 2020. Structural characterization and immunomodulatory activity of a water-soluble polysaccharide from *Ganoderma leucocontextum* fruiting bodies. *Carbohydr. Polym.* 249 (116874).
- GuanMin-chang, Tang Wei-hong, Zhen, Xu, Jie, Sun, 2014. Effects of selenium-enriched protein from *Ganoderma lucidum* on the levels of IL-1 β and TNF- α , oxidative Stress, and NF- κ B activation in ovalbumin-induced asthmatic mice. *Evid. Based Complement. Altern. Med.*, 182817.
- Hsiao, Yi-Min, Huang, Yu-Lu, Tang, Sheau-Chung, Shieh, Gow-Jen, Lai, Jing-Ying, Wang, Po-Hui, Ying, Tsung-Ho, Ko, Jiunn-Liang, 2008. Effect of a fungal immunomodulatory protein from *Ganoderma tsugae* on cell cycle and interferon-gamma production through phosphatidylinositol 3-kinase signal pathway. *Process. Biochem.* 43, 423–430.
- Huang, Sheng-quan, Ning, Zheng-xiang, 2010. Extraction of polysaccharide from *Ganoderma lucidum* and its immune enhancement activity. *Int. J. Biol. Macromol.* 47, 336–341.
- Jedryczka, Malgorzata, Strzelczak, Agnieszka, Grinn-Gofron, Agnieszka, Nowak, Malgorzata, Wolskie, Tomasz, Siwulskif, Marek, Sobieralskif, Krzysztof, Kaczmarek, Joanna, 2015. Advanced statistical models commonly applied in aerobiology cannot accurately predict the exposure of people to *Ganoderma* spore-related allergies. *Agric. For. Meteorol.* 201, 209–217.
- Ji, Young Kang, Mi, Ran Jo, Kang, Hyeon Hui, Kim, Sung Kyoung, Kim, Myoung Sook, Kim, Yong Hyun, Kim, Seok Chan, Kwon, Soon Seog, Lee, Sook Young, Kim, Jin Woo, 2016. Long-term azithromycin ameliorates not only airway inflammation but also remodeling in a murine model of chronic asthma. *Pulm. Pharmacol. Ther.* 36, 37–45.
- Kuo, Mei-Chun, Weng, Ching-Yi, Ha, Choi-Lan, Wu, Ming-Juan, 2006. *Ganoderma lucidum* mycelia enhance innate immunity by activating NF- κ B. *J. Ethnopharmacol.* 103, 217–222.
- Lankarani, Kamran Bagheri, Honarvar, Behnam, Athari, Seyyed Shamsadin, 2017. The mechanisms underlying *Helicobacter Pylori*-mediated protection against allergic asthma. *Tanaffos* 16 (4), 251–259.
- Lin, Zhi-Bin, 2005. Cellular and molecular mechanisms of immuno-modulation by *ganoderma lucidum*. *J. Pharmacol. Sci.* 99, 144–153.
- Liu, Zhengguang, Xing, Jie, Zheng, Sisi, Bo, Ruonan, Luo, Li, Huang, Yee, Niu, Yale, Li, Zhihua, Wang, Deyun, Hu, Yuanliang, Liu, Jiaguo, Wu, Yi, 2016. *Ganoderma lucidum* polysaccharides encapsulated in liposome as adjuvant to promote Th1-bias immune response. *Carbohydr. Polym.* 142, 141–148.
- Luo, Ping, Deng, Shaorong, Ye, Hao, Yu, Xiaolan, Deng, Qing, Zhang, Yinjie, Jiang, Liya, Li, Jingjing, Yu, Yan, Han, Wei, 2020. The IL-33/ST2 pathway suppresses murine colon cancer growth and metastasis by upregulating CD40 L signaling. *Biomed. Pharmacother.* 127, 110232.
- Masoume Athari, S., Mehrabi Nasab, E., Shamsadin Athari, S., 2018. Study effect of *Ocimum basilicum* seeds on mucus production and cytokine gene expression in allergic asthma mice model. *Rev. Fr. Allergol.* 58 (7), 489–493.
- Pan, Ke, Jiang, Qunguang, Liu, Guoqing, Miao, Xiongying, Zhong, Dewu, 2013. Optimization extraction of *ganoderma lucidum* polysaccharides and its immunity and antioxidant activities. *Int. J. Biol. Macromol.* 55, 301–306.
- Pi, Chia-Chen, Wang, Hui-Yi, Lu, Chiu-Ying, Lu, Frank Leigh, Chen, Chun-Jen, 2014. *Ganoderma formosanum* polysaccharides attenuate Th2 inflammation and airway hyperresponsiveness in a murine model of allergic asthma. *SpringerPlus* 3 (297).
- Ren, Li, Zhang, Jie, Zhang, Tiehua, 2021. Immunomodulatory activities of polysaccharides from *Ganoderma* on immune effector cells. *Food Chem.* 340 (127933).
- Sui, Xiao-chen, Guo, Qing-bin, Xia, Yong-mei, Cui, Steve W., Shen, Jie, Zhang, Jue, Ding, Zhong-yang, 2016. Structure features of the intracellular polysaccharide from *Ganoderma lucidum* and the irrelative immune-anticancer activities of GLPs. *Bioact. Carbohydr. Diet. Fibre* 8, 43–50.
- Wang, Yuan-Yuan, Khoo, Kay-Hooi, Chen, Shui-Tein, Lin, Chun-Cheng, Wong, Chi-Huey, Lin, Chun-Hung, 2002. Studies on the immuno-modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. *Bioorg. Med. Chem.* 10, 1057–1062.
- Wanga, Hui, Yua, Qiang, Dinga, Xiaomeng, Hua, Xiaoyi, Houa, Kunyou, Liub, Xiaozhen, Niea, Shaoping, Xiea, Mingyong, 2019. RNA-seq based elucidation of mechanism underlying *Ganoderma atrum* polysaccharide induced immune activation of murine myeloid-derived dendritic cells. *J. Funct. Foods* 55, 104–116.
- Yavari, B., Sarami, S., Shahgaldi, S., Athari, S.S., Sharma, A., 2016. If there is really a Notable Concern about allergenicity of genetically modified foods? *J. Food Qual. Hazard. Control Rep.* 3 (1), 3–9.
- Yin, Guojun, Ardó, L., Thompson, K.D., Adams, A., Jeney, Z., G, 2009. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. *Jeney Fish Shellfish Immunol.* 26, 140–145.