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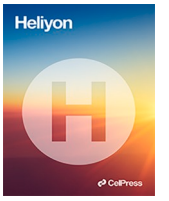

Influence of *Cordyceps militaris*-fermented grain substrate extracts on alleviating food allergy in mice

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ABSTRACT

Background: Cordyceps militaris is recognized as a tonic in traditional Chinese medicine, and there have been documented findings on the anti-allergic properties of its extract derived from the fruiting body. Due to the limited availability of wild *C. militaris*, a specialized grain substrate has been devised for the solid-state fermentation of its fruiting bodies. However, the fermented grain substrate is considered waste and usually used as feeds for animals. To achieve the sustainable development goals, *C. militaris*-fermented grain substrate (CFGS) was collected to prepare CFGS extracts. Further, the anti-allergic properties of these extracts were assessed with the aim of exploring novel applications.

Methods: The water extract and ethanol extract of CFGS were prepared, and their potential in alleviating allergic enteritis was assessed in mice with food allergy. Assessment of immunomodulatory effects included the measurement of serum antibodies and splenic cytokines. Additionally, influence of extracts on gut microbiota composition was examined through sequencing analysis of 16S rRNA gene from freshly collected feces of the mice.

Results: Daily administration of the water and ethanol extracts, at doses of 50 or 250 mg/kg body weight, demonstrated a notable alleviation of allergic diarrhea and enteritis. This was accompanied by a decrease in mast cell infiltration in the duodenum and a reduction in allergen-specific IgE production in the serum. Both extracts led to a significant decrease in IL-4 secretion. Conversely, there was an increase in IFN-γ, IL-10, and TGF-β secretion from splenocytes. Remarkably, allergic mice exhibited a distinct fecal microbiota profile compared to that of normal mice. Intriguingly, the administration of these extracts had varying effects on the fecal microbiota.

Conclusion: Taken together, these findings collectively indicate the potential of CFGS extracts as promising candidates for functional foods. These extracts show promise in managing allergic enteritis and modulating gut microbiota.

1. Introduction

Allergic diseases represent a substantial health concern in developed countries. In the last four decades, there has been a dramatic

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increase in the prevalence of allergic diseases [1]. Globally, 2 %–10 % of the population suffers from food allergy. Families of food allergy sufferers bear a considerable economic burden, including increased healthcare costs (an average of nearly $4184 per food-allergic child per year), costs associated with emergency medical care, hospitalization, and productivity losses [2]. Typical food allergy belongs to Type I hypersensitivity reactions, where allergens activate T helper (Th)2 cells, leading to immunoglobulin (Ig) E-mediated allergic reactions [3]. After ingestion, the allergens are internalized and degraded by mucosal dendritic cells, and the resulting peptide fragments move to the lymphatic T cell area. Upon interaction with naïve $CD4^+$ T cells, these cells undergo differentiation into Th2 cells. Th2 cells, along with associated cytokines like IL-4, play a pivotal role in fostering B cell proliferation and facilitating Ig class switching. This process ultimately leads to the production of IgE. Upon re-exposure to the same allergen, the allergen binds to IgE on mast cell receptors, triggering mast cell degranulation and the subsequent release of various mediators, such as histamine and prostaglandins, causing allergic reactions [4,5]. Ovalbumin (OVA) is the major allergenic protein in eggs, and the murine model of OVA-induced food allergy has been well established [5]. Currently, the recognized treatment methods for food allergy are avoiding allergenic foods and administering emergency medications in case of accidental exposure [6]. However, the available medications only alleviate symptoms and cannot provide a complete cure.

In a preceding investigation, we delved into the impacts of gut microbiota derived from mice with allergy on enterocytes, as well as the immune responses of T cells specific to allergen. Fecal strains isolated primarily belong to the genera *Clostridium*, *Streptococcus*, and *Bacteroides*, with a notable impact on enterocyte viability, leading to a significant reduction. Additionally, there is an increase in the proliferation of mesenteric lymph node cells and the production of IFN-γ and IL-4, whereas the secretion of inhibitory cytokines, such as IL-10 and TGF-β, was reduced. The findings reveal clear distinctions in the traits of gut microbiota when comparing regular mice and those prone to allergy. Importantly, fecal microbiota linked to food allergy could potentially trigger cytokine/chemokine signaling in the intestine and elicit T cell immune responses [7].

For millennia, *Cordyceps* species have served as medicinal fungi, employed in the treatment of a wide range of ailments. They are rich in natural bioactive compounds and possess multiple beneficial biological activities and nutritional value [8]. *C. militaris* operates as a tonic element within the realm of traditional Chinese medicine, exhibiting a variety of pharmacological properties that span a wide spectrum of effects [9–15]. The main bioactive components, *Cordyceps* polysaccharides and cordycepin, have been shown to have

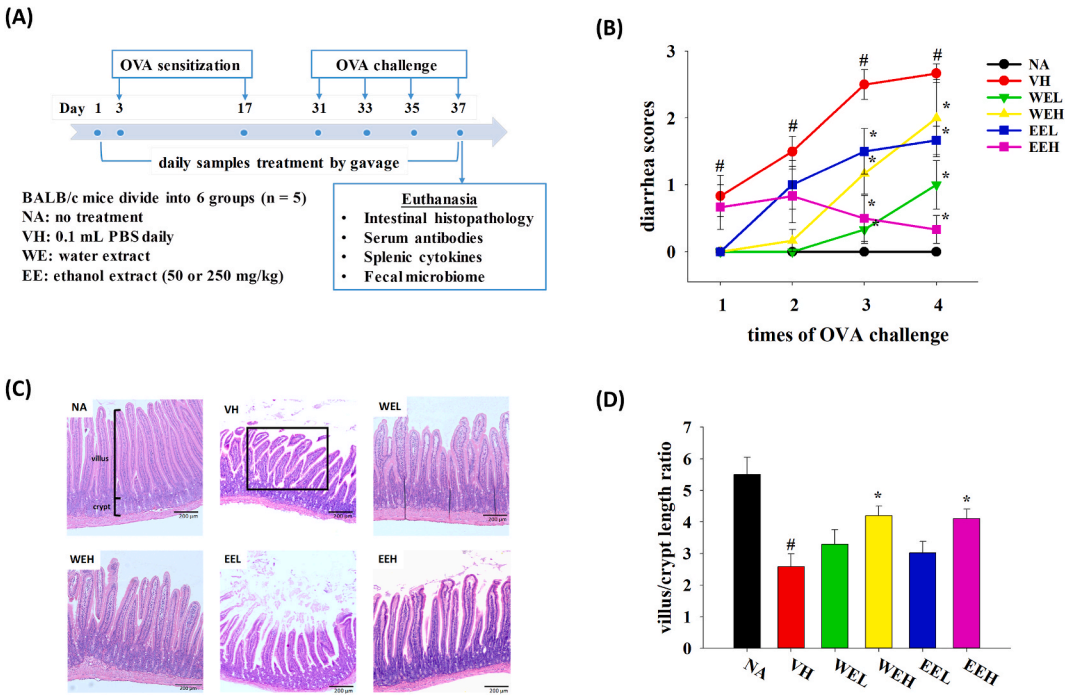

Fig. 1. Animal experiment protocol and diarrhea scores of mice. **(A)** Mice were randomly allocated into distinct groups, each comprising 5 mice. The groups included naive group (NA), PBS-treated (0.1 mL, daily via gavage) group (VH), as well as WEL, WEH, EEL and EEH groups (daily gavage with 0.1 mL, 50 or 250 mg/kg of water or ethanol extract of CFGS). With the exception of the NA group, all mice underwent OVA sensitization twice and subsequently received repeated OVA challenges via gavage every other day. Following the final OVA challenge, recently expelled feces were gathered for microbiota analysis. After euthanasia, the serum, spleen, and duodenal samples were harvested for subsequent experiments. **(B)** Severity of diarrhea were monitored for a duration of 30 min after each challenge, and diarrhea scores were assigned based on the following criteria, where 0 indicated regular feces, 1 denoted slightly soft feces, 2 represented loose feces, and 3 was assigned for diarrhea. **(C)** Displayed are illustrative images of duodenal sections stained with H&E, emphasizing noticeable villus edema and injury, as indicated within the designated region (outlined in black). (D) The calculation of the villus/crypt ratio was performed utilizing ImageJ software. The results are depicted as the mean ± SEM with a sample size of n = 5. Statistical significance ($^{\#}p < 0.05$) was found in comparison to the NA group, and a noteworthy difference ($^{*}p < 0.05$) was observed compared to the VH group.

numerous pharmacological activities, although there is currently no research and literature on their application in treating food allergy [9,14,16,17]. Certainly, the scarcity of wild *C. militaris* has prompted numerous studies to concentrate on extracting polysaccharides and cordycepin from artificially cultivated fruiting bodies or mycelial fermentation broth of *C. militaris* [17]. Although a specialized grain substrate has been devised for the solid-state fermentation of *C. militaris* fruiting bodies, the fermented grain substrate is considered waste and usually used as feeds for animals. In pursuit of sustainable development, the water extract and ethanol extract of CFGS were prepared, and their effects on alleviating food allergy were assessed in mice with food allergy. Moreover, the profile of immune response and gut microbiota was elucidated to uncover potential underlying mechanisms.

2. Materials and methods

2.1. Materials and preparation of CFGS extracts

Chemicals, including OVA, cell culture reagents, ELISA kits for cytokine and Ig measurements, and the powder of CFGS utilized in this study were the same as that employed in the previous studies [7,18]. The methodology for preparing the water extract mirrored that of our recent study, wherein we successfully extracted polysaccharides from CFGS [18]. On the other hand, the procedure for producing the ethanol extract remained consistent with the methodology outlined in the earlier study [19]. Composition analysis of CFGS was conducted by SGS TAIWAN (Taipei, Taiwan). The quantification of peptides, total sugar, and phenolics in both extracts was conducted following methodologies outlined in previous studies [20–22].

2.2. Animal experiment

We followed the guidelines outlined in the National Research Council's manual for the ethical treatment and utilization of laboratory animals throughout all animal trials. The procedures received approval from the Institutional Animal Care and Use Committee of the National Taiwan Ocean University (NTOU IACUC-109057). The approach and techniques employed to initiate food allergy were consistent with those detailed in the preceding investigation [7]. As depicted in Fig. 1A, female mice of the BALB/c strain, at the age of 5 weeks, were categorized into 6 groups, each consisting of 5 mice: the NA group (without any treatment), the VH group (treatment of 0.1 mL phosphate-buffered saline (PBS) by gavage every day), WEL and WEH groups (daily treatment of CFGS water extract at the dosage of 50 and 250 mg/kg body weight dissolved in 0.1 mL PBS by gavage), EEL and EEH groups (daily treatment of CFGS ethanol extract at the dosage of 50 and 250 mg/kg body weight suspended in 0.1 mL PBS by gavage). With the exception of the NA group, mice in the other groups underwent sensitization through intraperitoneal injection of OVA and aluminum hydroxide on day 3 and day 17. Subsequently, these mice were challenged by gavage with 50 mg of OVA on alternate days, starting from day 31 and continuing through day 37. Before each challenge, a fasting period of 3 h was observed, and the severity of diarrhea was evaluated within 1 h following the challenge. The assessment of allergic diarrhea intensity involved assigning scores ranging from 0 to 3 based on fecal consistency, where 0 indicated regular feces, 1 denoted slightly soft feces, 2 represented loose feces, and 3 was assigned for diarrhea. All mice were euthanized on day 37, and the duodenal tissues were gathered specifically for staining with hematoxylin and eosin (H & E) as well as toluidine blue. ImageJ software was employed to quantify the crypt/villus ratio and the number of mast cell infiltration in the stained sections [23–25]. Spleen specimens were separately isolated to generate suspensions of splenocytes. Furthermore, fresh feces were gathered following the ultimate challenge, earmarked for the utilization of next-generation sequencing (NGS) technology. This sequencing approach focused on the complete length of the 16S rRNA gene, in accordance with methodologies expounded in the prior research [7]. The methodology for management of splenocytes and quantification of cytokine levels within the splenocytes and Igs levels within the serum was the same as that reported in the previous studies [23–25].

2.3. Statistical analysis

A comparison between two groups was performed using Student's t-test (SigmaPlot V14, Systat Software Inc.). Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Effect of CFGS extracts on the mitigation of allergic diarrhea and enteritis

Throughout the experiment, no abnormal behavior or activity was observed in any of the mice, indicating that the administered dosage did not induce any noticeable adverse effects. The diarrhea scores of VH group escalated with each challenge, reaching a score of 3 at the 4th challenge. Nevertheless, the scores of mice treated with both extracts at 50 and 250 mg/kg showed statistically significant reductions when compared to the VH group at the 3rd and 4th challenges. This suggests that both water and ethanol extracts could effectively mitigate the severity of allergic diarrhea (Fig. 1B). Moreover, the evaluation of effects of both extracts on ameliorating allergic enteritis involved a thorough examination of tissue pathology. In the VH group, obvious villus edema and harm were apparent (Fig. 1C). Nonetheless, the groups subjected to treatment exhibited less severe histopathological alterations in comparison to the VH group (Fig. 1C). The ratio of villus height to crypt depth, a widely employed metric for gauging intestinal well-being [26], experienced a significant increase following the administration of water extract and ethanol extract at 250 mg/kg in contrast to the VH control group (Fig. 1D). This suggests the protective properties of CFGS extracts against allergic enteritis.

3.2. Impact of CFGS extracts on intestinal mast cell infiltration and serum OVA-specific IgE production

Since the initiation of mast cell degranulation and the following allergic enteritis is contingent upon the cross-linking of allergen-specific IgE on mast cells, the number of mast cells infiltrating the duodenal tissues, which is the first intestinal section exposure to food allergen, was determined. The outcomes from toluidine blue staining exhibited a noticeable rise in the abundance of mast cells within the duodenum of mice belonging to the VH group, in stark contrast to the mast cell count in the NA group (Fig. 2A and B). However, infrequent occurrences of mast cells were noted in mice subjected to the administered extracts (Fig. 2A and B). In addition, mice from the WEH, EEL, and EEH groups exhibited statistically reduced levels of OVA-specific IgE compared to those in the VH group (Fig. 2C). Accordingly, both water extract and ethanol extracts at 250 mg/kg could effectively suppressed allergen-specific IgE production and mast cell infiltration, revealing prevention against cross-linking of allergen-specific IgE on mast cells is one of potential mechanisms of CFGS extracts to alleviate allergic enteritis. Because OVA-specific IgE production was lowered by the treatment of CFGS extracts, the concentration of total IgG, the major isotype of antibody in serum to neutralize pathogen, was measured to understand whether the extracts influenced humoral immunity [27]. The concentration of total IgG showed no significant differences between each group (Fig. 2D), suggesting the extracts had limited impact on humoral immunity for protecting against pathogens.

3.3. Effects of CFGS extracts on regulating T cell-associated cytokine production

The balance between Th1/Th2 immune responses plays a critical role in the development of IgE-mediated hypersensitivity. Regulatory T (Treg) cells, producing IL-10 and TGF-β, play a pivotal role in preserving immune tolerance to allergens by inhibiting immune cell activation [28,29]. Accordingly, the impact of CFGS extracts on Th1, Th2 and Treg cell activation was addressed by assessing IFN-γ, IL-4, IL-10, and TGF-β production. Compared to that of the NA group, mice in the VH group exhibited elevated levels of IFN-γ, IL-4, and IL-10 secretion from OVA-stimulated splenocytes (Fig. 3A–C). Conversely, the generation of TGF-β was reduced in mice belonging to the VH group (Fig. 3D), revealing that the induction of food allergy may impair immune tolerance. Notably, treatment of water extract and ethanol extract, especially at the dosage of 250 mg/kg, significantly down-regulated IL-4 production and up-regulated IL-10, and TGF-β production (Fig. 3A, C and D), revealing the effects of both extracts on suppressing Th2 immune responses and restoring immune tolerance. Interesting, treatment of water extract at the dosage of 250 mg/kg obviously enhanced IFN-γ production (Fig. 3B), revealing potentially distinct action mechanisms of the water extract and ethanol extract. Although IL-10 could be secreted by Th2 cells, it is crucial to emphasize that IL-10 is not confined to a specific cell type and can also be produced by Treg cells, functioning as an anti-inflammatory cytokine to mitigate allergic enteritis [24,25]. Given the observed increase in TGF-β, an inhibitory cytokine associated with Treg cells, in the WEH, EEL, and EEH groups, it is suggested that the elevated IL-10 levels in these groups probably result from activated Treg cells. To further elucidate the source of IL-10, additional investigation using double staining, such as Foxp3/IL-10 and GATA3/IL-10, may be beneficial.

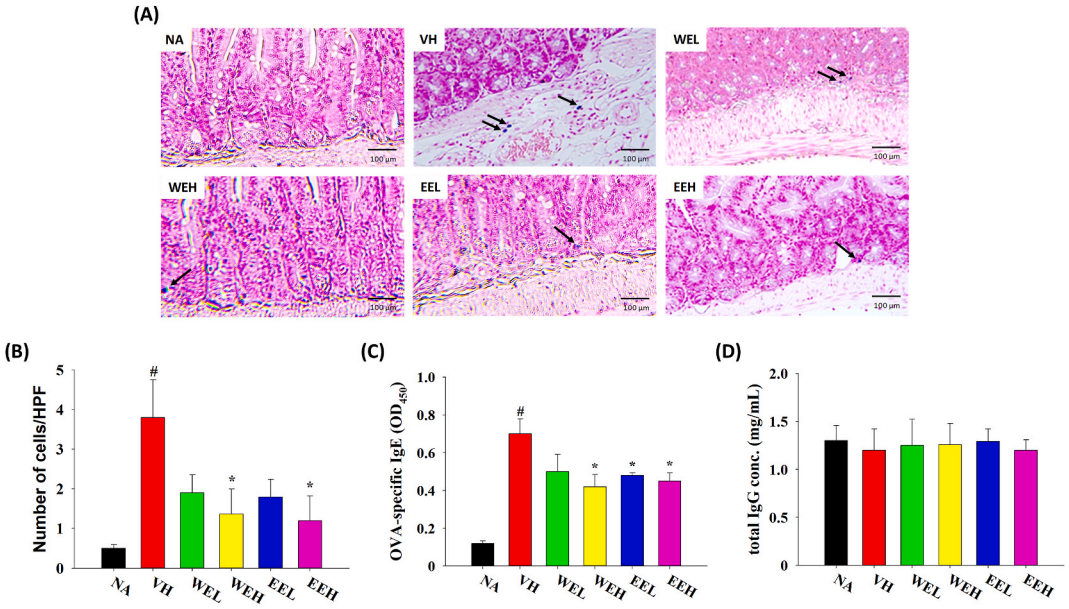

Fig. 2. Intestinal mast cell infiltration and serum antibody production. **(A)** Illustrative images of sections stained with toluidine blue are presented, with mast cells indicated by black arrows. **(B)** The quantification of mast cells per high-power field (HPF) was conducted using ImageJ software. Serum samples were gathered from each individual mouse, and the levels of immunoglobulins, encompassing **(C)** OVA-specific IgE, and **(D)** total IgG, were assessed. The results are depicted as the mean ± SEM with a sample size of n = 5. Statistical significance ($^{\#}p < 0.05$) was found in comparison to the NA group, and a noteworthy difference ($^{*}p < 0.05$) was observed compared to the VH group.

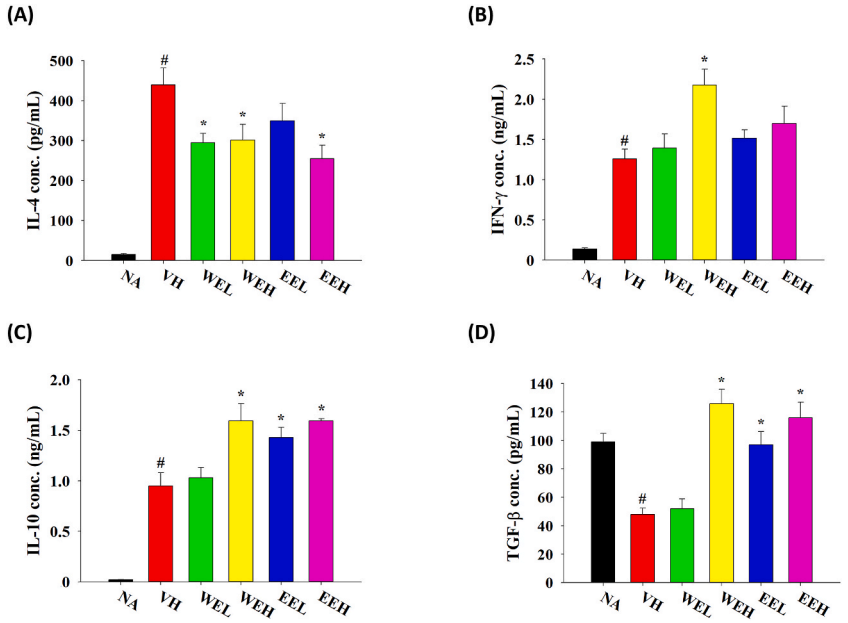

Fig. 3. Production of cytokines from splenocytes stimulated with OVA. Splenocyte suspensions from mice in each experimental group were cultured with OVA stimulation. The supernatants from these cultures were then analyzed to quantify the amounts of **(A)** IL-4, **(B)** IFN-γ, **(C)** IL-10, and **(D)** TGF-β. The results are depicted as the mean ± SEM with a sample size of n = 5. Statistical significance ($^{\#}p < 0.05$) was found in comparison to the NA group, and a noteworthy difference ($^{*}p < 0.05$) was observed compared to the VH group.

3.4. Impact of CFGS extracts on gut microbiota

As numerous studies pointed out the pivotal role of gut microbiota in the development of food allergy, fresh fecal samples from mice were collected to understand the profile of gut microbiota of each group. Compared to NA and VH groups, treatment of water extract and ethanol extract reduced the value of Faith's phylogenetic diversity (Faith's PD), a widely employed metric for assessing phylogenetic alpha diversity (Fig. 4A). [30]. Furthermore, the treatment groups exhibited higher values of unweighted UniFrac, a beta

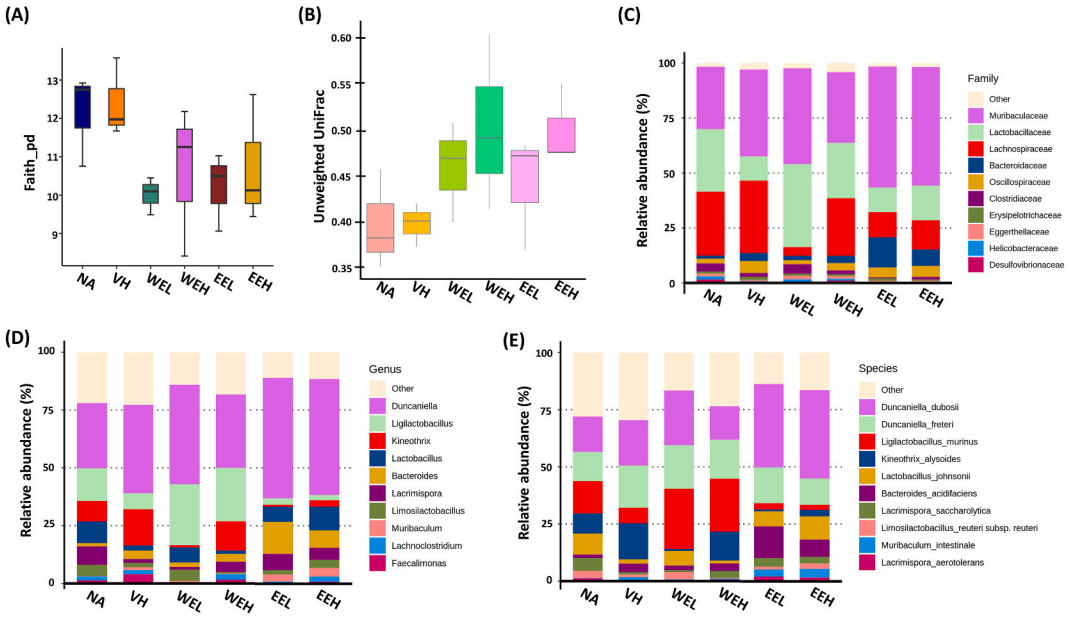

Fig. 4. Analysis of fecal microbiota. Following the last challenge, fresh feces were harvested from each mouse, and DNA extraction from the feces was performed for full-length 16S rRNA gene sequencing using NGS. **(A)** Faith's PD of alpha diversity, **(B)** Unweighted UniFrac of beta diversity, **(C)** family-level composition, **(D)** genus-level composition and **(E)** species-level composition are presented.

diversity measure utilizing phylogenetic information for sample comparison, in contrast to the NA and VH groups (Fig. 4B) [31]. The predominant bacterial families within the enteric microbiota were Muribaculaceae, Lactobaculaceae, and Lachnospiraceae (Fig. 4C). The VH group displayed a reduced relative abundance of Lactobaculaceae (Fig. 4C). In contrast to mice in the VH group, those treated with the water extract exhibited an increased relative abundance of Lactobaculaceae, while mice treated with the ethanol extract showed elevated levels of Muribaculaceae and Bacteroidaceae (Fig. 4C). In parallel, the relative abundance of *Ligilactobacillus*, *Lactobacillus, L. johnsonii* and *L. murinus* were increased in mice administrated with water extract, and that of *Duncaniella, Lactobacillus, Bacteroides, Lacrimispora, D. dobosii, L. johnsonii, B. acidifaciens* and *L. saccharolytica* was increased in mice treated with ethanol extract (Fig. 4D and E).

4. Discussion

CFGS employed in this study comprised 3.5 % moisture, 2.1 % ash, 16.6 % crude protein, 11.5 % crude fat, and 66.3 % carbohydrate. Moreover, CFGS comprised 33.5 % β-glucan, 1.1 % cordycepin and more than 0.02 % adenosine, which are known major bioactive components contained in *C. militaris* [32,33]. Moreover, the potential bioactive ingredients contained in both extracts were examined. The water extract comprised 88 % total sugar, 8.6 % peptides, and 4.1 % total phenols; the ethanol extract comprised 18.9 % total sugar, 17 % peptides, and 0.4 % total phenols. This indicates a distinct composition of bioactive ingredients between the two extracts, with polysaccharide emerging as the predominant component in the water extract but not in the ethanol extract (Table 1). In a prior study that also employed ethanol for *C. militaris* extraction, cordycepin and adenosine were identified as the principal components exerting biological activities [34]. Given that the extraction yield (mass of extract/mass of dry matter) of the ethanol extract in the current study approached 1 %, cordycepin and adenosine are recognized as the principal bioactive compounds in the ethanol extract. On the other hand, in a pilot study, we investigated different doses of the extracts, with a range consistent with those used in previous studies demonstrating the biological activities of cordycepin, polysaccharides, or extracts from *C. militaris* as described below. Notably, 50 mg/kg represented the lowest dose at which both extracts exhibited significant effects in mitigating allergic diarrhea. Adhering to the principles of the 3R framework for animal research, we selected doses of 50 and 250 mg/kg to explore and compare the potency of CFGS extracts.

Certain investigations have presented evidence regarding the potential anti-allergic properties found in extracts of the fruiting bodies of *C. militaris*. For instance, daily treatment with aqueous extract (4 g/kg) to OVA-induced asthma mice suppressed airway inflammation and serum IgE production [35]. In another study, the ingestion of *C. militaris* fruiting body ethanol extract (300 mg/kg) demonstrated noteworthy mitigation of allergic rhinitis symptoms induced by OVA in mice, including reduced instances of sneezing and scratching, as well as decreased eosinophil infiltration and inhibition of mast cell activation. These effects were achieved through the suppression of Ca^{2+} ion mobilization [36]. A specific compound known as β-sitostenone, present within the *C. militaris* fruiting body extract, exhibited the ability to hinder the release of β–hexosaminidase from activated mast cells [19]. Moreover, administering lactic acid bacteria-fermented aqueous extract of *C. militaris* (100 mg/kg) ameliorated allergic responses induced by IgE. This was evident through the reduction of ear swelling, attenuation of vascular permeability, and a decline in inflammatory cell infiltration observed in mice experiencing passive cutaneous anaphylaxis [37]. So far, only one study investigating the anti-allergic activity of *C. militaris* mycelium is available. Ingestion of *C. militaris* ethyl acetate extract (at dosages of 100, 300, 1000, and 3000 mg/kg) effectively suppressed allergic responses mediated by IgE in mast cells. Furthermore, this treatment demonstrated inhibition of passive cutaneous anaphylaxis reactions in mice [38]. On the other hand, Zhang et al., have demonstrated the anti-allergic effect of cordycepin (20–40 mg/kg) via up-regulation of Treg response and down-regulation of Th17 responses in OVA-induced asthma mice [39]. In the current study, CFGS, which contained *C. militaris* mycelium, was used for extraction. The effective dosage of CFGS extracts was within the range of that reported in the above studies. Notably, the present findings represent the initial evidence showcasing the effects of both the aqueous extract and ethanol extract of CFGS in mitigating allergic enteritis, modulating allergen-specific immune responses and reshaping gut microbiota.

Previous researches have revealed the influence of *C. militaris* on gut microbiota. For instance, intervention of *C. militaris* polysaccharides (400 mg/kg) over a span of 4 weeks yielded substantial outcomes. These included the reduction of obesity resulting from a high-fat diet, alleviation of associated hyperlipidemia and insulin resistance, and the mitigation of systemic inflammation. Concurrently, *C. militaris* polysaccharides exhibited the capacity to improve obesity-induced disruptions in gut microbial composition. Achieving this impact involved the restoration of diversity in gut microbiota and the augmentation of bacteria responsible for the production of short-chain fatty acids, concurrently decreasing the levels of bacteria linked to the progression of obesity [40]. Lee et al. have reported that the fruiting body, polysaccharides, and cordycepin extracted from *C. militaris* showcased distinct effects in modulating metabolic disorders and gut microbiota in mice fed with high-fat and high-sucrose diet [41]. In another study, administering cordycepin (50 mg/kg) via daily oral gavage for a duration of 4 weeks did not bring about changes in the diversity of gut

Table 1
Total sugar, peptide and total phenols contents of CFGS extracts.

Compositions	Water extract	Ethanol extract
Total sugar (%)	88.0 ± 3.2	18.9 ± 1.2
Peptide (%)	8.6 ± 0.2	17.0 ± 0.2
Total phenols (%)	4.1 ± 0.2	0.4 ± 0.0

Every value is presented as mean ± standard error (n = 3).

microbiota. However, it did induce a significant alteration in the proportional distribution of Bacteroidetes and Firmicutes in rats that had developed obesity due to a high-fat diet [42]. Cordycepin (25 or 50 mg/kg) and aqueous extract of fruiting body (1 or 1.5 g/kg) down-regulated parameters encompassing glucose and lipid metabolism, oxidative stress biomarkers, and inflammatory cytokines and resulted in a higher abundance of Firmicutes/Bacteroidetes in diabetic mice [43]. In an *in vitro* investigation, the hot water extract derived from *C. militaris* cultivated on soybean exhibited marked inhibitory effects on the growth of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *L. gasseri*. In addition, it demonstrated the capacity to promote the proliferation of *Bacteroides ovatus* and *Bifidobacterium longum*, both of which are recognized as beneficial bacteria within the human gut [44]. It has also been revealed recently that specific *Lactobacillus* species have been identified as capable of utilizing *C. militaris* as a carbon source and are able to generate postbiotics in the media [45]. While numerous studies have examined how *C. militaris* affects the gut microbiota in metabolic and inflammatory disorders, there is currently a lack of information regarding its role in allergic diseases. Nonetheless, it's worth noting that the development of allergic and immune responses is intricately linked to the composition of the gut microbiota [7]. Hence, an investigation into the fecal microbiota profile of treated mice was conducted. The administration of the water extract raised the relative abundance of *L. johnsonii* and *L. murinus*. These strains have been recognized as probiotics that combat allergies by influencing the functioning of dendritic cells and the differentiation of T cells [46,47]. On the other hand, treatment of ethanol extract resulted in a higher proportion of *D. dobosii*, *L. johnsonii*, *B. acidifaciens*, and *L. saccharolytica* within the microbiota. Notably, *B. acidifaciens* is recognized for its ability to alleviate allergic inflammation by impeding the cell surface expression of FcεRI on mast cells [48]. Nonetheless, the precise involvement of *Duncaniella* and *Lacrimispora* in allergy and inflammation remains uncertain. Given the constraints of this study, a more comprehensive investigation of the structural activity relationship will be necessary. This is crucial for comprehending the direct interactions between the CFGS extracts and immune cells, or its effects on the gut microbiota. Such insights are vital for unraveling the mechanisms behind the immunomodulatory and anti-allergic impacts.

5. Conclusion

This investigation yields significant insights into the anti-allergic attributes of extracts acquired from CFGS. The notable differences in the impact of the water extract versus the ethanol extract on allergic enteritis reduction, adjustment of allergen-specific antibodies and cytokines production, as well as alterations in the gut microbiota, are probably attributed to their distinct chemical compositions. The extracts from CFGS show promise as potential therapeutic agents for managing food allergy. Nonetheless, additional investigations are essential to acquire a comprehensive understanding of the underlying mechanisms and the long-term effects of these extracts in the context of allergic responses and immune regulation.

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Data availability

Data will be made available on request.

Ethics approval

The animal experiments strictly adhered to the guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals. Furthermore, they obtained approval from the IACUC of NTOU (NTOU IACUC-109057).

Consent for publication

Not applicable.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Jia-Shan Liu: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rong-Yi Huang:** Methodology, Investigation, Data curation, Conceptualization. **Yu-Jyun Wei:** Validation, Formal analysis. **Guo-Jane Tsai:** Supervision. **Chung-Hsiung Huang:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing

interests:Chung-Hsiung Huang reports financial support was provided by National Science and Technology Council, Taiwan.

Abbreviations

CFGS	*C. militaris*-fermented grain substrate
ELISA	enzyme-linked immunosorbent assay
Ig	immunoglobulin
IFN	interferon
IL	interleukin
NGS	next-generation sequencing
OVA	ovalbumin
Faith's PD	Faith's phylogenetic diversity
SEM	standard error of mean
TGF	tumor growth factor
T helper cell	Th cell
Treg cell	regulatory T cell

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