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Caraway extract alleviates atopic dermatitis by regulating oxidative stress, suppressing Th2 cells, and upregulating Th1 cells in mice

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ABSTRACT

Objective: To explore the anti-inflammatory and antioxidant effects of caraway on atopic dermatitis (AD) in mice.

Methods: AD was induced in two stages, including sensitization and challenge with the application of 2,4 dinitrochlorobenzene 2% and 0.2%, respectively. Clinical symptoms and histological analysis of the skin were assessed. The effects of caraway on oxidant/antioxidant parameters as well as Th1- and Th2-related cytokines were also evaluated.

Results: Caraway reduced the severity of dermatitis in AD-induced mice, as evidenced by significant inhibition of Th2-related cytokines (IL-4 and IL-13) and increased Th1-related cytokine (IFN-γ). Additionally, treatment with caraway significantly increased superoxide dismutase and catalase activity and decreased the malondialdehyde level in the serum of AD mice. Furthermore, caraway inhibited the differentiation of Th2 cells while favoring Th1 cell differentiation in the spleen *via* regulating their master transcription factors GATA3 and T-bet.

Conclusions: Caraway could improve AD autoimmune responses and could be considered a potential candidate to treat AD disease.

KEYWORDS: *Carum carvi* L.; Caraway; Atopic dermatitis; 2,4 dinitrochlorobenzene; Th2; Th1; Oxidative stress

1. Introduction

Atopic dermatitis (AD), also referred to as atopic eczema, is a chronic inflammatory disease of the skin that disrupts the barrier function of the skin[1]. AD is characterized by skin dryness, rash, pruritus, a pattern of eczema, and a relapsing course, as well as a family medical history[2]. Although the disease involves people of any age, it affects 20% of children and develops in 80% of children in early childhood/before the age of 6[3]. The prevalence of AD is increasing in the world and it is estimated that AD affects about one-fifth of all individuals in developed countries[4].

Significance

Carum carvi L. or caraway, a medicinal plant, is widely used as an antioxidant and anti-inflammatory remedy in traditional medicine. However, the effect of caraway on autoimmune diseases such as atopic dermatitis has not been reported. In our research, caraway reduced the severity of dermatitis in atopic dermatitis-induced mice by downregulating Th2 and upregulating Th1 responses. These effects were associated with antioxidant activity of caraway.

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Although the pathogenesis of AD is multifactorial, the research has revealed that aberrant autoreactive Th2 responses and their cytokines, IL-4 and IL-13, have a pivotal role in inflammation in AD[5,6]. Differentiation of naïve $CD4^+$ T cells into Th2 depends on the expression of GATA3, a transcription factor for Th2 cell differentiation, which promotes Th2 cells for the secretion of IL-4 and IL-13[7]. It is well known that IL-4 and IL-13 decrease the expression of filaggrin, a component of the skin barrier, by keratinocytes, as well as, the expression of antimicrobial peptides in the skin[8]. It has been documented that, in addition to the increased number of autoimmune Th2 cells in the AD skin lesions, barrier dysfunction is also involved in the pathogenesis of skin allergic diseases such as AD[9].

However, Th2 cell responses are not able to fully clarify the pathogenesis of AD. Recently, a number of studies have demonstrated that Th1 cells play an essential role in inflammatory and immune-mediated reactions in AD disease[10]. These cells are characterized by the transcription factor T-bet and the secretion of cytokines, including IFN-γ. Indeed, Th1 and IFN-γ have a pathologic role in the chronic phase of the disease, while Th2 and its related cytokines are involved in the early phase of the disease[11].

In spite of intensive investigations, the treatment of AD is still unknown. Nowadays, immune suppressant drugs are approved for AD treatment; however, these medications are partially effective and limited due to side effects and also are ineffective in some patients. Therefore, there is increasing interest in developing non-toxic and harmless herbal medicine for AD treatment.

Carum carvi L. (caraway) is a member of the Apiaceae family[12]. It has been used in traditional medicine to cure gastrointestinal disorders and also as a spice in foods. Caraway has also been known as an antioxidant and anti-inflammatory drug[13]. Anti-inflammatory effects of caraway have been documented in an animal model of colitis. In this colitis model, caraway extract decreased infiltration of leukocytes in the mucus of rats[14]. Additionally, the antioxidant effects of caraway have been documented in nephrotoxicity induced by gentamicin. The study showed caraway reduced DPPH radicals and malondialdehyde (MDA), and conversely increased catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase in animals[15]. Caraway has also been used in Indonesia for the treatment of inflamed eczema[16]. A topical ointment of caraway and caraway oil is also used for scabies and mycosis treatment[17]. According to the abovementioned properties of caraway, this study aimed to assess the effect of caraway on clinical and pathological symptoms of AD *in vivo*.

2. Materials and methods

2.1. Reagents

2,4 dinitrochlorobenzene (DNCB) was supplied by Sigma Aldrich; Merck KGaA (cat. no. 337339). TRIzol® was purchased from Roche Diagnostics GmbH, Mannheim, Germany. The cDNA synthesis kit (Takara Biotechnology, Otsu-Shiga, Japan) and the Real-Time SYBR Green kit (Prime mix EXTaq™, Takara Biotechnology, Otsu-Shiga, Japan) were employed for Real-Time qPCR analysis.

2.2. Experimental animals

BALB/c mice (female, 5-6-weeks-old), free of specific pathogens, were purchased from the Pasteur Institute of Iran (Tehran). Animals were kept at 23-25 ℃ with a (50±5)% relative humidity, and a 12 h light/dark cycle. A standard chow pellet diet and filtered tap water were given *ad libitum*.

2.3. Plant preparation and hydro-alcoholic extraction

Caraway seeds were collected at a high altitude from Kerman (Iran). The samples were identified and authenticated by a botanist at the Botany Research Division of Bahonar University of Kerman, Iran (*Carum carvi* number: 2767 MIR herbarium). The plant materials were dried and finely powdered. For hydro-alcoholic extraction, 100 g of powder was dissolved in ethanol-water (70/30, *v/v*). The solution was then placed on a shaker in the dark at room temperature for 72 h. The extract was filtered and evaporated under reduced pressure at 40 ℃ using a rotary evaporator until only a semisolid extract remained.

2.4. Study design

After one week of acclimation, to randomize the treatment or control groups, we labeled each mouse at the beginning of the study, then used a table of random numbers to select among the labeled mice. Then the mice were divided into five groups (n=8) as follows: The first group (control) was treated with the vehicle without induction of AD. The second group (AD group) was AD induced and orally treated with phosphate-buffered saline (PBS, pH 7.4). For the treatment groups, caraway extract (CE) was administered at 50, 100, or 200 mg CE/kg (low, intermediate, and high doses) every other day by gavage to mice in groups 3, 4, and 5, respectively. The doses were selected based on the findings of Keshavarz *et al*[14]. All animals in the study were treated from day 2 post-challenge until day 28. At the end of the experimental period (28 d), the mice were sacrificed according to animal ethics.

2.5. Induction of AD

AD was induced in two stages: in the first stage (sensitization), the dorsal skin of each mouse was shaved and sensitized with the application of DNCB. On days 1 and 4, the skin was painted with 100 μL of 2.0% DNCB in 4:1 (*v/v*) acetone/olive oil. In the second stage (challenge), the mice were challenged on days 7 and 11, by

application of 50 μL of 0.2% DNCB to the same areas[18] (Figure 1).

In the control and AD groups, the dorsal skin was painted with acetone/olive oil mixture [4:1 (*v/v*)] without or with DNCB in the same manner, respectively. In the treatment groups, AD was induced, and the mice were orally administered 50, 100, and 200 mg/kg of caraway in groups 3-5 for two weeks (day 14-28, Figure 1).

2.6. Evaluation of dermatitis severity score

The severity of dorsal skin was measured macroscopically in a blinded manner. The severity of dermatitis was evaluated based on the following symptoms: 1) erythema/hemorrhage, 2) scaling/dryness, 3) edema, and 4) excoriation/erosion. The clinical score of the AD-induced mice was graded as 0 (no symptoms), 1 (mild), 2 (moderate), and 3 (severe)[19]. The clinical score was recorded once a week by three trained researchers with no knowledge of the grouping of the mice.

2.7. Histopathological analysis

In order to analyze histopathological changes, on the 28th day after AD induction, the mice were anesthetized, and a tissue sample was taken from the skin of mice and fixed in 10% formalin. Then, the samples were embedded in paraffin, and tissue sections (5 microns thick) were prepared for histological analysis. The prepared slides were stained with hematoxylin and eosin (H&E) to assess leukocyte infiltration and the slides were assessed by evaluating three fields in a blinded manner.

2.8. Enzyme-linked immunosorbent assay (ELISA) for quantification of cytokines and IgE

To determine the effect of caraway on cytokines and IgE production, blood samples were collected at the end of the study by cardiac puncture. Sera were obtained from blood samples after centrifugation, and the levels of IgE, IL-4, IL-13, and IFN-γ were determined using mouse ELISA kits (eBioscience, San Diego, CA, USA), according to the manufacturer's instructions.

2.9. Measurement of oxidant and anti-oxidant activity of caraway

The effect of caraway on the oxidant and antioxidant parameters was determined by evaluating the levels of MDA, SOD, and CAT in the serum of AD mice.

To do this, blood samples were collected at the end of the study by cardiac puncture. Sera were obtained from blood samples after centrifugation and the levels of MDA, SOD, and CAT were determined using mouse ELISA commercial kits (eBioscience, San Diego, CA, USA), according to the manufacturer's instructions.

2.10. Quantitative real-time PCR (qPCR) for mRNA expression of transcription factors and cytokines

The effects of caraway on mRNA expression of Th transcription factors (*T-bet* and *GATA3*) and related cytokines (*IL-4*, *IL-13*, and *IFN-γ*) were determined by real-time PCR. The spleen cells were isolated from AD mice at the end of the study and total RNA was isolated using Trizol reagent (Roche Diagnostics GmbH, Mannheim, Germany), as per the manufacturer's guideline. The RNA was reverse-transcribed to cDNA using the Prime-ScriptTMRT reagent kit (Takara Biotechnology, Otsu-Shiga, Japan) according to the manufacturer's instructions.

Real-time PCR was performed on the Rotor-Gene Q (Qiagen Hilden, Germany) using Master Mix (Prime mix EXTaq™, Takara Biotechnology, Otsu-Shiga, Japan) with appropriate primer sequences that are depicted in Table 1.

The following reaction conditions were used for real-time PCR assay: initial denaturation and enzyme activation at 95℃ for 5 min, 35 cycle amplification at 95℃ for 45 s (denaturation), 60℃ for 45 s (annealing), and 72℃ for 45 s (extension). The expression of each gene was normalized to the expression of mouse *β-actin*, and then delta-delta Ct was used to calculate the relative quantification of genes.

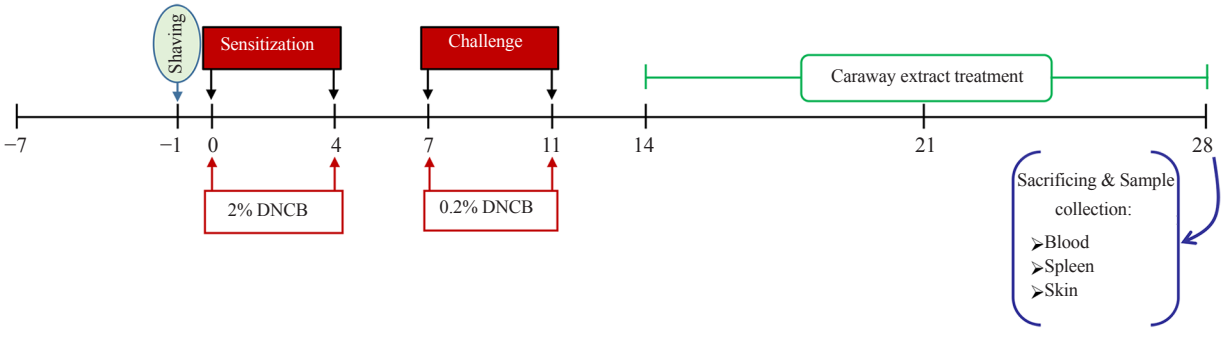

Figure 1. Schematic time-course of atopic dermatitis (AD) induction and caraway treatment in mice. DNCB: 2,4 dinitrochlorobenzene.

Table 1. The sequences of primers in the study.

Genes	Sequences
IL-4	F 5′- TCACAGCAACGAAGAACACCAC-3′
	R 5′- TCTGCAGCTCCATGAGAACACTA-3′
GATA3	F 5′- ACCACGTCCCGTCCTACTAC-3′
	R 5′- TCATACCTGGCTCCCGTGG-3′
T-bet	F 5′- CTTCCAACAATGTGACCCAGATGA-3′
	R 5′- AAGACGTGTGTGTTAGAAGCACT -3′
IL-13	F 5′- ACATCACACAAGACCAGACTCC-3′
	R 5′- ATTGGAGATGTTGGTCAGGGAA-3′
IFN-γ	F 5′- ATTGCCAAGTTTGAGGTCAACAA-3′
	R 5′- ATCTCTTCCCCACCCCGAAT-3′
β-actin	F 5′- CACTGTCGAGTCGCGTCC-3′
	R 5′- TCATCCATGGCGAACTGGTG-3′

2.11. Statistical analysis

The results of the analysis were expressed as mean±standard error of mean (SEM) by Graphpad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). A comparison of each group was carried out by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. $P<0.05$ was considered to be statistically significant.

2.12. Ethical statement

The study was approved by the Animal Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1399.287).

3. Results

3.1. Effects of caraway extract on skin symptoms in AD mice

To investigate the therapeutic potential of caraway against AD skin lesions, the severity of dermatitis was evaluated based on a scoring index. As shown in Figure 2, severe AD skin symptoms developed in the AD group with a total dermatitis score of 8.10±0.92 compared to the control group (0.50±0.24, $P<0.001$). However, treatment with caraway improved the skin lesions. As shown in Figure 2, caraway treatment at a high dose significantly reduced the severity of dermatitis compared to the AD group ($P<0.05$). Treatment with low and intermediate doses of caraway slightly improved AD symptoms without significant differences compared with the AD group (Figure 2).

3.2. Effects of caraway on the infiltration of inflammatory cells into the skin

Figure 3 shows that the skin of AD mice showed a massive leukocyte infiltration with several foci of inflammation compared with the control. In contrast, in the skin of caraway-treated mice, the number of inflammatory cells was lower than that in AD mice. The findings revealed caraway leads to a reduction in infiltration of inflammatory cells into the skin and the caraway-treated mice only showed slight signs of inflammation, confirming that these mice are largely protected against AD (Figure 3). The results showed that caraway could reduce the clinical severity of AD as a result of reduced cell infiltration into the skin.

3.3. Effects of caraway on the serum levels of cytokines

To evaluate the effect of caraway extract on Th responses, the serum levels of IL-4, IL-13, and IFN-γ were measured. As shown in Figures 4A and B, in the AD group, the secretion of IL-4, and IL-13 cytokines was significantly increased compared with the control group ($P<0.01$ for IL-4 and $P<0.05$ for IL-13), while the level of IFN-γ was decreased ($P<0.01$, Figure 4C). Caraway significantly reduced the level of IL-4 in a dose-dependent manner in comparison with AD mice ($P<0.05$, Figure 4A). There was also a statistically significant difference in the level of IL-4 between the treatment groups and the control ($P<0.05$, Figure 4A). In addition, in caraway-treated groups, the level of IL-13 was significantly decreased ($P<0.05$, Figure 4B) when compared with the AD group. In contrast, the level of IFN-γ was highly enhanced in the serum of mice treated with a high dose of caraway ($P<0.01$, Figure 4C). No significant differences were noted in IFN-γ production following caraway treatment at the doses of 50 and 100 mg/kg when compared with the AD group. These results indicate that caraway induces the suppression of Th2 response through the inhibition of IL-4 and IL-13 production and promotion of Th1 responses.

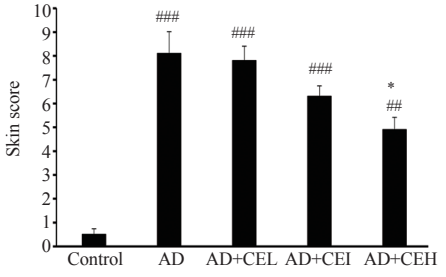

Figure 2. Effect of caraway on clinical scores of dorsal skin in AD-induced mice. Dermatitis score was evaluated based on erythema/hemorrhage, scaling/dryness, edema, and excoriation/erosion. Data are presented as mean±SEM of three independent experiments and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. $^{*}P<0.05$ versus the AD group and $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus the control group. AD: atopic dermatitis; AD+CEL: AD mice treated with 50 mg/kg caraway; AD+CEI: AD mice treated with 100 mg/kg caraway; AD+CEH: AD mice treated with 200 mg/kg caraway.

3.4. Effects of caraway on the serum level of total IgE

The AD group showed an elevated level of IgE, compared to the control group [(40.06±2.63) pg/mL versus (13.37±2.81) pg/mL, P<0.001, Figure 5]. Treatment with 50, 100, and 200 mg/kg of caraway led to a dose-dependent decrease in the level of IgE (Figure 5). The decrease was most significant in the mice treated with 200 mg/kg caraway [(14.66±1.95) pg/mL, P<0.01].

3.5. Effects of caraway on the level of oxidant and antioxidant parameters

As shown in Table 2, in the AD group, the level of MDA was significantly increased compared with the control group (P<0.05), while the activity of SOD and CAT was significantly decreased (P<0.01 for SOD and P<0.05 for CAT). Caraway reduced the level of MDA at a dose of 100 mg/kg, but not in the doses of 50 and 200 mg/kg. Moreover, increased SOD activity was observed in mice treated with 100 and 200 mg/kg of caraway (P<0.05 and P<0.01,

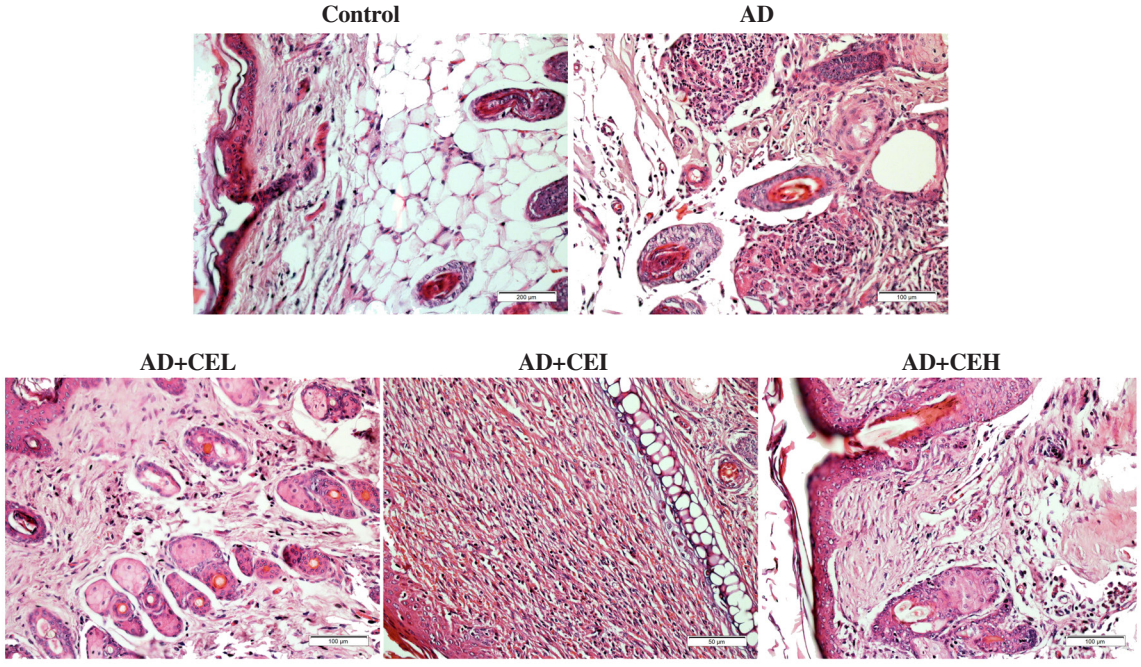

Figure 3. Effect of caraway on skin histology in AD mice. The skin from each mouse (collected on day 28) was fixed and embedded in paraffin. Sections (5-mm) were prepared and the tissues were then stained with H&E. The AD group shows severe inflammation and infiltration of immune cells into the skin compared to the control group. The caraway-treated groups dose-dependently reduce infiltration of inflammatory cells and signs of inflammation in the skin of mice. Magnification: ×20.

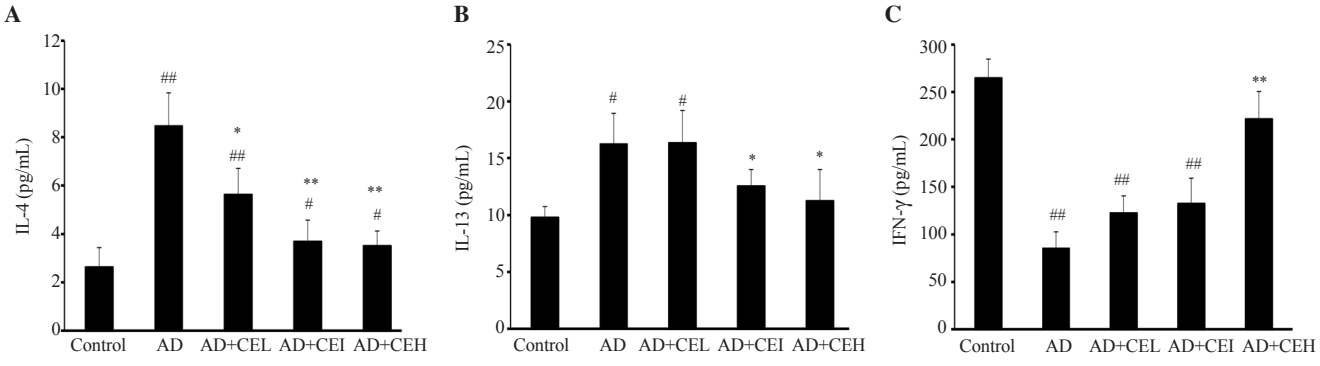

Figure 4. Effects of caraway treatment on key cytokines of Th1 and Th2. (A) IL-4; (B) IL-13; (C) IFN-γ. Data are presented as mean±SEM of three independent experiments and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. $^{*}P$<0.05, $^{**}P$<0.01 versus the AD group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus the control group.

respectively). Caraway at a high dose also markedly elevated the CAT activity (P<0.05, Table 2) with no significant differences found in the doses of 50 and 100 mg/kg.

Table 2. Effect of caraway extract on MDA level and SOD and catalase activities in the control and treatment groups.

Group	MDA (nmol/L)	SOD (U/mL)	Catalase (KU/L)
Control	1.25 ± 0.15	12.35 ± 1.49	51.28 ± 6.80
AD	1.83 ± 0.25$^{\#}$	3.30 ± 1.05$^{\#\#}$	31.76 ± 4.55$^{\#}$
AD+CEL	1.39 ± 0.19	4.81 ± 0.75$^{\#}$	32.80 ± 5.00$^{\#}$
AD+CEI	0.88 ± 0.18*	7.20 ± 1.20$^{*\#}$	30.05 ± 5.50$^{\#}$
AD+CEH	1.04 ± 0.20	9.70 ± 1.60**	48.30 ± 6.20*

Values are given as mean±SEM and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. MDA: malondialdehyde; SOD: superoxide dismutase. $^{*}P$<0.05, $^{**}P$<0.01 versus the AD group. $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus the control group.

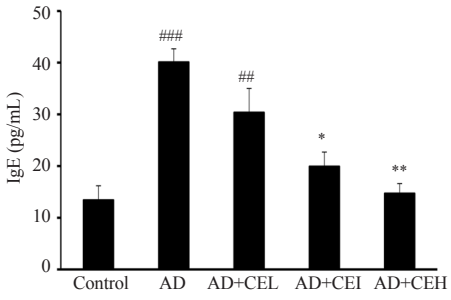

Figure 5. Effects of caraway treatment on the serum level of total IgE. Data are presented as mean±SEM and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. $^{*}P$<0.05, $^{**}P$<0.01 versus the AD group; $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 versus the control group.

3.6. Effects of caraway extract on the polarization of Th1 and Th2 responses in AD mice

To evaluate the therapeutic potential of caraway on the polarization of Th1 and Th2 responses, real-time PCR was performed. The AD group showed elevated expression of *GATA3* and reduced expression of *T–bet* in the spleen compared with the control mice (P<0.05, Figures 6A and B). However, caraway reversed the AD-induced changes in the expression of *GATA3* and *T–bet* (P<0.05).

Furthermore, treatment with caraway resulted in reduced expression of both *IL–4* and *IL–13* (Th2 signature cytokines) in a dose-dependent manner (Figures 6C and D), as well as increased the expression of *IFN–γ* (P<0.01, Figure 6E). As a result, caraway has a protective effect in the treatment of AD disease by suppressing Th2 cells and upregulating Th1 cells.

4. Discussion

The most important feature of AD is chronic skin inflammation, which is associated with barrier dysfunction of the skin[20]. Th2 and Th1 cells, as well as their signature cytokines (IL-4 and IL-13 for Th2, and IFN-γ for Th1), have a pivotal role in AD disease[21]. IL-4 and IL-13 can induce B cells to produce IgE, which in turn stimulates mast cells and eosinophils and induces a pro-inflammatory response, while Th1 cells, directly or indirectly, decrease inflammatory activities by inhibiting the Th2 responses[22]. Studies have shown

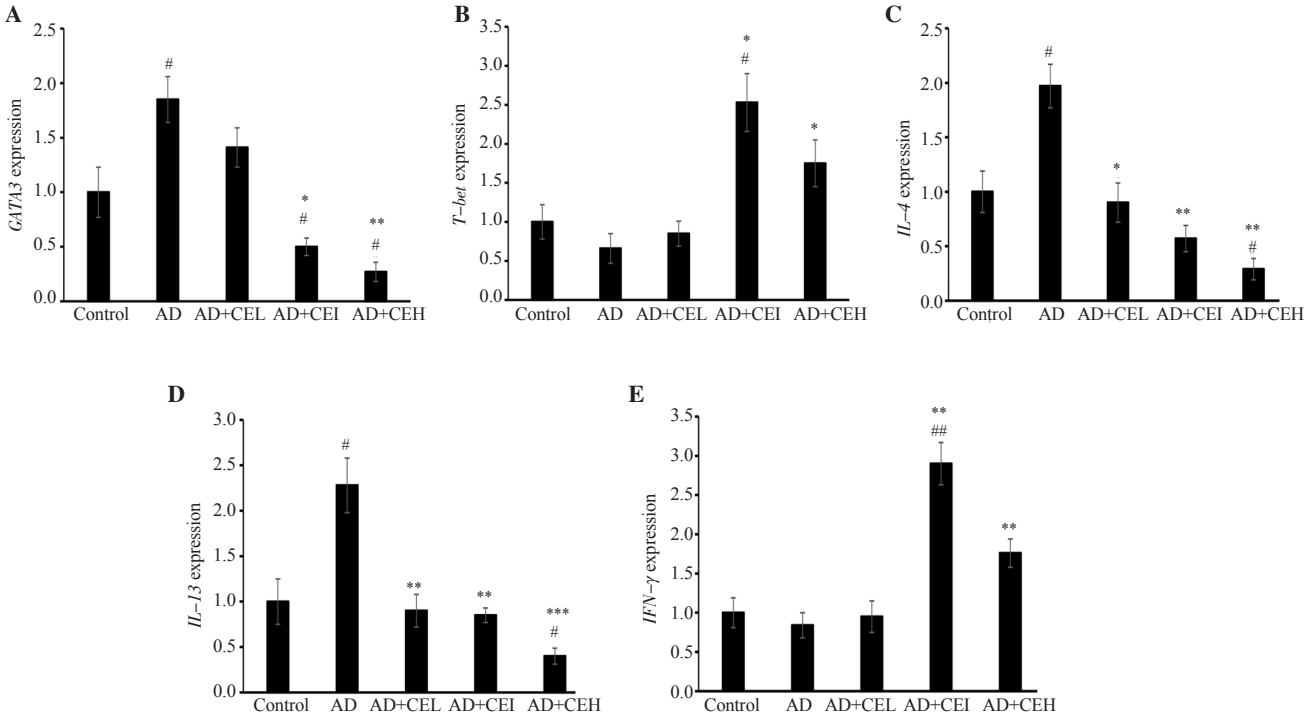

Figure 6. Effects of caraway on the expression of Th1 and Th2 transcription factors and related cytokines. The expressions of Th1 and Th2 transcription factors (*T–bet* and *GATA3*) and related cytokines (*IFN–γ*, *IL–4*, and *IL–13*) were determined by real-time quantitative PCR. *β–actin* was used as the housekeeping gene. Data are presented as mean±SEM of three independent experiments and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. $^{*}P$<0.05, $^{**}P$<0.01 and $^{***}P$<0.001 versus the AD group; $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus the control group.

that herbal medicine using plants with immunomodulatory and antioxidant potentials can modulate inflammatory diseases such as AD[23].

Caraway is a herbal medicine used for gastrointestinal disorders and pneumonia treatment in traditional medicine, and also as an appetizer and carminative in foods and beverages[24]. The results of this study demonstrated that caraway reduced the severity of skin lesions in AD mice. These changes were confirmed with reductions in the infiltration of inflammatory cells into the skin of mice treated with caraway. Such an outcome was consistent with the histological results of the study by Keshavarz *et al*. which reported that caraway decreased inflammation indicators, including inflammation extent and intensity, and damage to crypts in the colons of rats[14].

Moreover, in an animal model of paw inflammation, Seddighfar *et al*. reported caraway significantly alleviated paw edema in acute inflammation[25]. Similarly, the current results are in line with earlier studies which reported that caraway decreased inflammatory mediators, including prostaglandin E_2, cyclooxygenase-2, caspase-3 and IL-1β in an experimental model of chronic inflammatory bowel disease[26].

In addition, it is well established that caraway has antioxidant activity[27]. Studies have shown that there is a relationship between oxidative stress and reactive oxygen species (ROS) production in the pathogenesis of AD. These researches have revealed that oxidative stress is associated with increased lipid peroxidation products (*e.g.*, MDA) and reduction in antioxidant production in AD patients[28,29].

In the present research, the enhanced levels of MDA in AD mice demonstrated the presence of oxidative stress and enhanced ROS generation. In contrast, caraway reduced the levels of MDA in AD mice. This result is in agreement with the study of Dadkhah *et al*. which demonstrated that treatment of septic-induced rats with caraway oil caused a significant decrease in MDA level[30]. In animal models of ulcerative colitis and peptic ulcer, caraway administration resulted in a marked reduction of MDA, compared to the control group[31]. Additionally, Koppula *et al*. also indicated anti-stress activity of caraway in the stress-induced rat[32]. Therefore, modulation of MDA production by caraway might be essential to inhibit the pathogenesis of AD disease.

To assess the antioxidant activity of caraway, the research also evaluated SOD and CAT activity in mice treated with caraway. There is a well-known fact that ROS, such as superoxide anion (O_2^-), play essential roles in the pathogenesis of many diseases, including atherosclerosis[33], multiple sclerosis[34], Alzheimer's disease[35] as well as AD[36]. SOD, as an antioxidant enzyme, can convert superoxide anion to oxygen and hydrogen peroxide. Hydrogen peroxide is also known as a cell-damaging agent, which, in turn, is broken down into water by CAT[37]. Thus, SOD and CAT prevent oxidative stress in our bodies.

In the current study, caraway elevated SOD and CAT activity, especially at a high dose. In parallel with our results, previous studies indicated that caraway could promote SOD and CAT activity in *in vivo* models. Kamaleeswari *et al*. found that caraway significantly increased the level of SOD and CAT in the animal model of colon carcinogenesis, which was associated with an improvement in pathological lesions in the colon of rats[38]. Erjaee *et al*. have also reported that treatment with caraway led to an increase in antioxidant enzymes such as SOD and CAT in gentamicin-induced nephrotoxicity in an animal model[15].

Moreover, the antioxidant effect of *Carum copticum*, which has compounds similar to caraway, was studied by Alavinezhad *et al*. in collagen-induced arthritis. They showed that treatment of rats with *Carum copticum* significantly reduced the level of nitric oxide and elevated SOD activity[39]. As caraway (*Carum carvi*) and *Carum copticum* belong to the Apiaceae family and have similar components, it can be assumed that the antioxidant activity of caraway may be due to its main components, including monoterpene alcohols, linalool, carvacrol, carvone, and flavonoids. But it needs to be further verified in future studies.

On the other hand, the pathogenesis of AD is due to defects in immune cells and the polarization of the cells towards the Th2 response. The researchers have revealed that, in addition to the deviation of immune system towards Th2 cells, the increased numbers and malfunctioning of Th2 cells are involved in the immunopathogenesis of the disease[40]. Moreover, IL-4 and IL-13, as the main Th2 cytokines, play a crucial role in disrupting skin barrier function and promoting the autoimmune AD disease[41].

The results demonstrated that caraway decreases IL-4 and IL-13 secretion by Th2 cells while increasing IFN-γ production by Th1 cells. Our study also showed decreased *GATA3* expression in caraway-treated mice; hence, caraway can inhibit the production of Th2 cytokines through downregulation of *GATA3* and subsequently reduce Th differentiation to a Th2 subset.

Previous investigations demonstrated that polarization of naïve Th cells towards Th2 leads to aggravation of dermatitis[42]. Therefore, any drugs that leads to a reduction in the number and function of Th2 cells might be considered as a potential candidate in the treatment of AD.

In addition, the possible mechanism involved in AD disease is the expression of T-bet, a transcription factor for Th1 differentiation. Our results indicated that caraway improved AD disease by significantly enhancing the expression of *T–bet* and *IFN–γ*. These results are in agreement with the studies of other researchers which found that Th2 responses aggravate AD disease, while Th1 responses result in improvement in pathological symptoms of the disease[43].

In addition to T cells, B cells also play a role in the pathogenesis of AD through IgE synthesis, and in turn, the production of IgE is indirectly dependent on Th2 responses[44]. In the present study, the AD group markedly increased the level of IgE compared to the control group, while treatment with caraway ameliorated the effect of DNCB by significantly reducing the level of IgE in AD-induced mice.

So far, very few studies have been performed on the effect of

caraway on the level of IgE in inflammatory diseases, but the study by Moubarz *et al*., demonstrated that caraway attenuated diabetic complications in streptozotocin-induced diabetic rats and this protection was associated with reduced IgE production[45]. Several studies have shown that an increased level of IgE in patients with atopic dermatitis, and psoriasis, was associated with increased number of Th2 cells[46,47].

It is well documented that IL-4 is the most important cytokine to induce isotype switching to IgE by activated B cells[48]. According to the results, it can be concluded that the effect of caraway on the level of IgE is indirect *via* IL-4 and IL-13 production by Th2 cells.

In summary, the current research showed that caraway treatment effectively ameliorated the clinical and pathological symptoms of AD mice. Our findings also indicated caraway increased the expression of *T–bet* and decreased the expression of *GATA3*, demonstrating the immunomodulatory mechanisms of the drug. Although the present research showed that caraway could alleviate AD mainly through anti-oxidative and anti-inflammatory activity, further study should be carried out to investigate the active ingredient of caraway.

Conflict of interest statement

The authors declare no competing interests.

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Authors' contributions

FK, MRZ, and AK performed experiments, and VA collected data. AA and RN analyzed the data. SD performed pathological experiments, FK prepared manuscript and RN designed the study and revised the manuscript.

References

[1] Maliyar K, Sibbald C, Pope E, Sibbald RG. Diagnosis and management of atopic dermatitis: A review. *Adv Skin Wound Care* 2018; **31**(12): 538-550.

[2] Thijs J, Krastev T, Weidinger S, Buckens CF, de Bruin-Weller M, Bruijnzeel-Koomen C, et al. Biomarkers for atopic dermatitis: A systematic review and meta-analysis. *Curr Opin Allergy Clin Immunol* 2015; **15**(5): 453-460.

[3] DaVeiga SP. Epidemiology of atopic dermatitis: A review. *Allergy Asthma Proc* 2012; **8**(1): 126-128.

[4] Kowalska-Olędzka E, Czarnecka M, Baran A. Epidemiology of atopic dermatitis in Europe. *J Drug Assess* 2019; **8**(1): 126-128.

[5] Chiricozzi A, Maurelli M, Peris K, Girolomoni G. Targeting IL-4 for the treatment of atopic dermatitis. *Immunotargets Ther* 2020; **9**: 151-156.

[6] Bieber T. Interleukin-13: Targeting an underestimated cytokine in atopic dermatitis. *Allergy* 2020; **75**(1): 54-62.

[7] Stark JM, Tibbitt CA, Coquet JM. The metabolic requirements of Th2 cell differentiation. *Front Immunol* 2019; **10**: 02318.

[8] Strid J, McLean WI, Irvine AD. Too much, too little or just enough: A goldilocks effect for IL-13 and skin barrier regulation? *J Invest Dermatol* 2016; **136**(3): 561-564.

[9] Egawa G, Kabashima K. Barrier dysfunction in the skin allergy. *Allergol Int* 2018; **67**(1): 3-11.

[10]Su C, Yang T, Wu Z, Zhong J, Huang Y, Huang T, et al. Differentiation of T-helper cells in distinct phases of atopic dermatitis involves Th1/Th2 and Th17/Treg. *Eur J Inflamm* 2017; **15**(1): 46-52.

[11]Tokura Y, Phadungsaksawasdi P, Ito T. Atopic dermatitis as Th2 disease revisited. *J Cutan Immunol Aller* 2018; **1**(5): 158-164.

[12]Morcia C, Tumino G, Ghizzoni R, Terzi V. Carvone (*Mentha spicata* L.) oils. In: Preedy VR (ed.) *Essential oils in food preservation, flavor and safety*. Amsterdam: Academic Press; 2016, p. 309-316.

[13]Bourgou S, Bettaieb Rebey I, Dakhlaoui S, Msaada K, Saidani Tounsi M, Ksouri R, et al. Green extraction of oil from *Carum carvi* seeds using bio-based solvent and supercritical fluid: Evaluation of its antioxidant and anti-inflammatory activities. *Phytochem Anal* 2020; **31**(1): 37-45.

[14]Keshavarz A, Minaiyan M, Ghannadi A, Mahzouni P. Effects of *Carum carvi* L. (Caraway) extract and essential oil on TNBS-induced colitis in rats. *Res Pharm Sci* 2013; **8**(1): 1-8.

[15]Erjaee H, Azma F, Nazifi S. Effect of caraway on gentamicin-induced oxidative stress, inflammation and nephrotoxicity in rats. *Vet Sci Dev* 2015; **5**(2): 5896.

[16]Johri R. *Cuminum cyminum* and *Carum carvi*: An update. *Pharmacogn Rev* 2011; **5**(9): 63-72.

[17]Mahboubi M. Caraway as important medicinal plants in management of diseases. *Nat Prod Bioprospect* 2019; **9**(1): 1-11.

[18]Jang S, Ohn J, Kim JW, Kang SM, Jeon D, Heo CY, et al. Caffeoyl-Pro-His amide relieve DNCB-induced atopic dermatitis-like phenotypes in BALB/c mice. *Sci Rep* 2020; **10**(1): 1-9.

[19]Tang L, Li X, Wan L, Wang H, Mai Q, Deng Z, et al. Ameliorative effect of orally administered different linoleic acid/α-linolenic acid ratios in a mouse model of DNFB-induced atopic dermatitis. *J Func Foods* 2020; **65**: 103754.

[20]Yang G, Seok JK, Kang HC, Cho YY, Lee HS, Lee JY. Skin barrier abnormalities and immune dysfunction in atopic dermatitis. *Int J Mol Sci* 2020; **21**(8): 2867.

[21]Fania L, Moretta G, Antonelli F, Scala E, Abeni D, Albanesi C, et al.

Multiple roles for cytokines in atopic dermatitis: From pathogenic mediators to endotype-specific biomarkers to therapeutic targets. *Int J Mol Sci* 2022; **23**(5): 2684.

[22]Bretscher P. On analyzing how the Th1/Th2 phenotype of an immune response is determined: Classical observations must not be ignored. *Front Immunol* 2019; **10**: 1234.

[23]Alhazmi HA, Najmi A, Javed SA, Sultana S, Al Bratty M, Makeen HA, et al. Medicinal plants and isolated molecules demonstrating immunomodulation activity as potential alternative therapies for viral diseases including COVID-19. *Front Immunol* 2021; **12**: 637553.

[24]Deepak. Importance of *Cuminum cyminum* L. and *Carum carvi* L. in traditional medicaments-a review. *Indian J Traditional Knowledge* 2013; **12**(2): 300-307.

[25]Seddighfar M, Mirghazanfari SM, Dadpay M. Analgesic and anti-inflammatory properties of hydroalcoholic extracts of *Malva sylvestris*, *Carum carvi* or *Medicago sativa*, and their combination in a rat model. *J Integr Med* 2020; **18**(2): 181-188.

[26]Elsayed M, Hussein R. A comparative study of anti inflammatory and antioxidant activities of fenugreek (*Trigonella foenum–graecum*) and caraway (*Carum carvi*) aqueous and oil seed extracts in experimental model of ulcerative colitis. *World J Pharm Res* 2019; **8**: 251-266.

[27]Hajlaoui H, Arraouadi S, Noumi E, Aouadi K, Adnan M, Khan MA, et al. Antimicrobial, antioxidant, anti-acetylcholinesterase, antidiabetic, and pharmacokinetic properties of *Carum carvi* L. and *Coriandrum sativum* L. essential oils alone and in combination. *Molecules* 2021; **26**(12): 3625.

[28]Sivaranjani N, Rao SV, Rajeev G. Role of reactive oxygen species and antioxidants in atopic dermatitis. *J Clin Diagn Res* 2013; **7**(12): 2683-2685.

[29]Simonetti O, Bacchetti T, Ferretti G, Molinelli E, Rizzetto G, Bellachioma L, et al. Oxidative stress and alterations of paraoxonases in atopic dermatitis. *Antioxidants* 2021; **10**(5): 697.

[30]Dadkhah A, Fatemi F. Heart and kidney oxidative stress status in septic rats treated with caraway extracts. *Pharm Biol* 2011; **49**(7): 679-686.

[31]Shosha NNH, Fahmy NM, Singab ANB, Mohamed RW. Anti-ulcer effects of cumin (*Cuminum cyminum* L.), thyme (*Thymus vulgaris* L.), and caraway (*Carum carvi* L.) essential oils on peptic ulcer and ulcerative colitis models in rats. *J Herbmed Pharmacol* 2022; **11**: 389-400.

[32]Koppula S, Kopalli SR, Sreemantula S. Adaptogenic and nootropic activities of aqueous extracts of *Carum carvi* Linn (caraway) fruit: An experimental study in Wistar rats. *Aust J Med Herb* 2009; **21**(3): 72-78.

[33]Poznyak AV, Grechko AV, Orekhova VA, Chegodaev YS, Wu WK, Orekhov AN. Oxidative stress and antioxidants in atherosclerosis development and treatment. *Biology* 2020; **9**(3): 60.

[34]Vodjgani M, Salehi Z, Izad M. The influence of reactive oxygen species in the immune system and pathogenesis of multiple sclerosis. *Autoimmune Dis* 2020; **2020**: 5793817.

[35]Polak-Szabela A, Dziembowska I, Bracha M, Pedrycz-Wieczorska A, Kedziora-Kornatowska K, Kozakiewicz M. The analysis of oxidative stress markers may increase the accuracy of the differential diagnosis of Alzheimer's disease with and without depression. *Clin Interv Aging* 2021; **16**(16): 1105-1117.

[36]Bertino L, Guarneri F, Cannavò SP, Casciaro M, Pioggia G, Gangemi S. Oxidative stress and atopic dermatitis. *Antioxidants* 2020; **9**(3): 196.

[37]Ighodaro O, Akinloye O. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alex J Med* 2018; **54**(4): 287-293.

[38]Kamaleeswari M, Nalini N. Dose-response efficacy of caraway (*Carum carvi* L.) on tissue lipid peroxidation and antioxidant profile in rat colon carcinogenesis. *J Pharm Pharmacol* 2006; **58**(8): 1121-1130.

[39]Alavinezhad A, Boskabady MH. Antiinflammatory, antioxidant, and immunological effects of *Carum copticum* L. and some of its constituents. *Phytother Res* 2014; **28**(12): 1739-1748.

[40]Kim K, Kim H, Sung GY. An interleukin-4 and interleukin-13 induced atopic dermatitis human skin equivalent model by a skin-on-a-chip. *Int J Mol Sci* 2022; **23**(4): 2116.

[41]Humeau M, Boniface K, Bodet C. Cytokine-mediated crosstalk between keratinocytes and T cells in atopic dermatitis. *Front Immunol* 2022; **13**: 801579.

[42]Meng J, Li Y, Fischer MJ, Steinhoff M, Chen W, Wang J. Th2 modulation of transient receptor potential channels: An unmet therapeutic intervention for atopic dermatitis. *Front Immunol* 2021; **12**: 696784.

[43]Rerknimitr P, Otsuka A, Nakashima C, Kabashima K. The etiopathogenesis of atopic dermatitis: Barrier disruption, immunological derangement, and pruritus. *Inflamm Regen* 2017; **37**(1): 1-15.

[44]Lin YJ, Goretzki A, Schülke S. Immune metabolism of IL-4-activated B cells and Th2 cells in the context of allergic diseases. *Front Immunol* 2021; **12**: 790658.

[45]Moubarz G, Taha MM, Mahdy-Abdallah H. Antioxidant effect of *Carum carvi* on the immune status of streptozotocin-induced diabetic rats infected with *Staphylococcus aureus*. *World Appl Sci J* 2014; **30**(1): 63-69.

[46]Heeringa JJ, Rijvers L, Arends N, Driessen G, Pasmans S, Van Dongen J, et al. IgE-expressing memory B cells and plasmablasts are increased in blood of children with asthma, food allergy, and atopic dermatitis. *Allergy* 2018; **73**(6): 1331-1336.

[47]Kasumagic-Halilovic E. Total serum immunoglobulin E levels in patients with psoriasis. *Mater Sociomed* 2020; **32**(2): 105-107.

[48]Haase P, Voehringer D. Regulation of the humoral type 2 immune response against allergens and helminths. *Eur J Immunol* 2021; **51**(2): 273-279.

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