

ORIGINAL ARTICLE

Basic Mechanisms in Allergic Disease

Cinnamon extract inhibits allergen-specific immune responses in human and murine allergy models

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Abstract

Background: Ceylon cinnamon has been shown to possess anti-inflammatory properties in many diseases including allergic inflammation.

Objective: The aim of this study was to analyse in more detail the effects of cinnamon extract (CE) and its major compounds *p*-cymene and *trans*-cinnamaldehyde (CA) on allergen-specific immune responses in vitro and in vivo.

Methods: Therefore, monocyte-derived mature dendritic cells (DC) from grass or birch pollen allergic donors were pulsed with the respective allergen in the presence or absence of CE, *p*-cymene, CA or the solvent ethanol and co-cultured with autologous CD4⁺ T cells. Furthermore, basophil activation test was performed with or without CE or ethanol treatment. For the in vivo experiments, BALB/c mice were immunized with ovalbumin (OVA) and orally treated with CE or ethanol.

Results: Addition of CE, *p*-cymene or CA, but not ethanol significantly inhibited DC maturation and subsequent allergen-specific T cell proliferation as well as Th1 and Th2 cytokine production. Sulphidoleukotriene release and CD63 expression by basophils were also significantly diminished after addition of CE. In vivo, treatment of OVA-sensitized mice with CE led to a significant shift from OVA-specific IgE towards IgG2a production and to a strong inhibition of OVA-specific proliferation. Moreover, airway inflammation as well as anaphylaxis after intranasal or systemic allergen challenge was significantly reduced in CE-treated mice. Furthermore, topical application of CE prevented calcipotriol-induced atopic dermatitis-like inflammation in these mice.

Conclusions and Clinical Relevance: Taken together, our data indicate that the anti-inflammatory effect of cinnamon might be exploited for treatment of allergic inflammation, which needs to be further investigated.

KEYWORDS

allergy, cinnamon extract, dendritic cells, ovalbumin, Th1/Th2

1 | INTRODUCTION

The prevalence of IgE-mediated (atopic) allergic diseases like allergic rhinitis, allergic asthma, atopic eczema or food allergy has

strongly increased in the last decades reaching up to 25%-30% in industrialized countries. Atopic diseases are characterized by an imbalance of the T cell response, that is a dominance of Th2 versus regulatory T cells (Treg), against otherwise harmless antigens such as grass or tree pollen resulting in the production of allergen-specific IgE, which binds to FcεRI on basophils and mast

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cells. Crosslinking of IgE antibodies after allergen re-exposure leads to mast cell degranulation and release of histamine, tryptase and prostaglandins followed by the typical allergic symptoms. Pharmacological treatment with antihistamines and corticosteroids has been shown to reduce the symptoms but does not modify the course of disease. Allergen-specific immunotherapy (AIT) is the only curative treatment so far, but it is very long-lasting and not recommended for multiple sensitizations or food allergy (at present) due to the risk of severe systemic side-effects.¹⁻³ Many patients turn to complementary and alternative therapies like acupuncture, traditional Chinese medicine, homeopathy and herbal therapy for disease management with more or less evidence concerning their effectiveness.⁴⁻⁶

Cinnamon extract (CE) derived from the dried bark of the tropical evergreen tree *Cinnamomum verum* and its major compound *trans*-cinnamaldehyde (CA) have been reported to possess anti-oxidant, anti-microbial, anti-inflammatory, anti-diabetic, anti-cancer, cardioprotective and beneficial effects in arthritis, arteriosclerosis and Alzheimer's disease.⁷⁻¹³ Moreover, it has been demonstrated that CE and CA also exert anti-allergic properties by decreasing release and expression of mast cell-specific mediators.¹⁴⁻¹⁶ In a randomized double-blind placebo-controlled clinical trial, patients with seasonal allergic rhinitis were treated with ClearGuard™ tablets, containing a combination of cinnamon, acerola and Spanish needles, resulting in a reduced nasal symptom score after nasal allergen challenge and inhibited prostaglandin D2 release into nasal lavage fluid.¹⁷ Additionally, reduced serum IgE and histamine levels have been shown in a mouse model of allergic rhinitis after intranasal application of a hydroalcoholic CE.¹⁸ However, the effects of CE on parameters like anaphylaxis, airway or skin inflammation have not been investigated so far. It is also unclear whether CE also affects other cells of the immune system apart from mast cells and basophils. Using a human DC-T cell co-culture in vitro setting, we could show for the first time that CE and its major compounds *p*-cymene and CA strongly inhibited DC activation and subsequent T cell proliferation as well as Th1 and Th2 cytokine production. Furthermore, application of CE resulted in a significant reduction of anaphylactic reactions along with a decreased airway and skin inflammation in ovalbumin (OVA)-sensitized and calcipotriol-treated mice, a mouse model where asthma and atopic dermatitis-like inflammation can be studied simultaneously.¹⁹

2 | MATERIALS AND METHODS

2.1 | Patients

Leucocyte-enriched Buffy-Coats (transfusion centre, Mainz, Germany) were obtained from allergic donors suffering from allergic rhino-conjunctivitis and/or asthma to grass or birch pollen. Specific sensitization was documented by positive skin prick test and detection of allergen-specific IgE in the sera (t3 or g × 1 CAP class ≥3 measured with the ImmunoCAP® specific IgE blood test; Thermo Fisher Scientific, Phadia AB). The study was approved

by the local ethics committee (Landesärztekammer Rheinland-Pfalz). Informed consent was obtained from all subjects before the study.

2.2 | Preparation of cinnamon extract

Cinnamon extract (CE, endotoxin content <1 ng/mL, Maros Arznei) was prepared according to the following procedure: cinnamon powder (Cortex Cinnamomi (ceylanici) plv., product number 03809618, Caelo) was diluted 1:5 with 70% aqueous ethanol at room temperature in the dark, inverted once per day and filtered after 10 days. Its properties and identification control are published in the European Pharmacopoeia, 6th Edition (6.0/1819), 2008.

2.3 | Generation of monocyte-derived mature DC

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll Paque 1077 g/mL (Biochrom GmbH) density centrifugation from heparinized blood. CD14⁺ monocytes were enriched by incubation of 5×10^6 PBMC in a 12-well tissue-culture plate (Greiner) with 1 mL/well Iscove's modified Dulbecco's medium containing L-glutamine and 25 mM Hepes (IMDM, Gibco/Life Technologies Corp.) and 3% autologous heat-inactivated plasma at 37°C for 45 minutes. Non-adherent cells were washed up twice with pre-warmed PBS. The remaining monocytes were incubated with 1.5 mL/well IMDM, 2% autologous plasma, 10 ng/mL IL-4 (Miltenyi Biotec) and 200 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF, Leukine®; Immunex Corp.). On day 6, immature DC were pulsed with birch pollen or grass pollen allergen extract (10 µg/mL, Allergopharma) or tetanus toxoid (1% Tetanol® pur, Novartis Vaccines and Diagnostics GmbH) as control antigen and were stimulated with 10 ng/mL TNF-α, 10 ng/mL IL-1β (Miltenyi Biotec) and 1 µg/mL prostaglandin E2 (Cayman Chemical) to induce their maturation. DC were further treated with CE or its major compounds *p*-cymene and CA (both from Sigma-Aldrich) at the indicated concentrations, or the same amount of the solvent, 70% aqueous ethanol or 20 ng/mL IL-10 (DNAX). After 48 hours, supernatants of mature DC were collected for determination of cytokine production. Then, the cells were harvested, washed twice with PBS and used for T cell stimulation assays. Remaining DC were stained for CD83, CD86 and MHC-class II expression.

2.4 | Determination of cell viability

The effect of CE, *p*-cymene and CA on cell viability was analysed by WST-8 Cell Proliferation Assay Kit (Cayman Chemical). WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of metabolic active cells. Briefly, 2×10^4 immature DC were incubated in 96-well flat-bottom tissue-culture plates in duplicates in 100 µL IMDM in the presence or absence of titrated concentrations of CE, its major compounds, or the same amount of the solvent, 70% aqueous ethanol.

10% DMSO served as positive control for induction of cell death. After 12 hours, 10 μ L of WST-8 mixture was added for 2–4 hour and absorbance was measured at 450 nm.

2.5 | Isolation of CD4⁺ T cells and co-culture of T cells and autologous allergen-pulsed DC

Autologous CD4⁺ T cells were obtained from PBMC using antibody-coated paramagnetic MicroBeads (MACS, Miltenyi Biotec) according to the protocol of the manufacturer. Separation was controlled by flow cytometry (purity >98% CD4⁺ T cells). For the proliferation assay, 1×10^5 CD4⁺ T cells were co-cultured in 96-well flat-bottom tissue-culture plates in triplicates with 1×10^4 allergen- or tetanus toxoid-pulsed DC, treated with CE, *p*-cymene, CA, or 70% aqueous ethanol, in 200 μ L IMDM 5% autologous plasma. After 5 days, the cells were pulsed with 37 kBq/well of [³H]-thymidine (ICN) for 8 hours. [³H]-thymidine incorporation was measured in a beta counter (1205 Betaplate, LKB Wallac, Turku, Finland). For the cytokine assay, 5×10^5 CD4⁺ T cells and 5×10^4 allergen- or tetanus toxoid-pulsed DC, treated with CE, *p*-cymene, CA or 70% aqueous ethanol, were co-cultured in a 48-well tissue-culture plate in 1 mL IMDM, 5% autologous plasma. After one week, T cells were restimulated with freshly generated allergen- or tetanus toxoid-pulsed DC, and supernatants were harvested after 24 hours and stored at -80°C until analysis.

In some experiments, CD4⁺ T cells were stimulated with 0.5 μ g/mL anti-human CD3 mAb (UCHT1, R&D Systems) and 1 μ g/mL anti-human CD28 mAb (CD28.2, BD Biosciences) in the presence or absence of CE, its major compounds or 70% aqueous ethanol.

2.6 | Type I allergy and atopic dermatitis-like mouse model

All experiments described in this article were approved by the Animal Care Committee of Rhineland-Palatinate. 6- to 8-week-old female BALB/cJRj mice (Janvier Labs), which were kept under specific pathogen-free conditions in the Translational Animal Research Centre of the University Medical Centre of the Johannes Gutenberg-University in Mainz, were immunized i.p. with 20 μ g ovalbumin (OVA) grade VI (Sigma-Aldrich) diluted in 300 μ L 0.9% NaCl on days 0 and 10. From day 0 onwards, mice received an optimized concentration of 1 mL CE or 70% aqueous ethanol per kg body weight ad 100 μ L 0.9% NaCl every other day per gavage. On day 24, mice were challenged i.p. with 0.5 mg OVA and body temperature was measured after 20, 40 and 60 minutes. After 120 minutes, sera were collected and stored at -20°C until determination of MCPT-1 and OVA-specific IgE, IgG1 and IgG2a titres.

In some experiments, OVA-sensitized mice were topically treated on both ears with 1.25 nmol in 25 μ L ethanol per ear of the vitamin D3 analogue calcipotriol (MC903; Tocris Bioscience) or ethanol alone three times per week for a total duration of 12 treatments. In the CE-treated group, mice received 25 μ L CE per ear 10 minutes

prior to MC903 application. 24 hours after the last application, ear thickness was measured with an engineer's micrometer (Oditest; Kroeplin GmbH).

2.7 | Measurement of airway hyperreactivity by invasive body plethysmography

Mice were further challenged i.n. with 100 μ g OVA in 20 μ L 0.9% NaCl on days 24–26. 24 hours later, airway hyperreactivity was measured by administering increasing doses (3.125 to 50 mg/mL) of methacholine (MCh) into pentobarbital anesthetized and intubated mice using an invasive whole-body plethysmograph (Buxco Electronics, Inc). Data are reported as airway resistance (RI) above the baseline value taken before receiving MCh. Thereafter, lungs were lavaged via the tracheal tube with 1 mL PBS, and supernatants were stored at -80°C until determination of cytokines. Numbers of bronchoalveolar lavage (BAL) cells were counted after trypan blue exclusion. Differential cell counts were made from cytospin preparations stained with DiffQuick (Dade Behring AG).

2.8 | Cell preparation for in vitro restimulation and histology

After measurement of airway hyperreactivity, spleens from each group were dispersed through a 70- μ m nylon cell strainer (BD Biosciences). Then, erythrocytes were lysed using ACK lysing buffer (Lonza), and cells were washed twice with PBS. For proliferation assays, 5×10^5 splenocytes were cultured in 96-well flat-bottom tissue-culture plates in 200 μ L IMDM supplemented with 5% FCS in triplicates with or without the addition of 50 μ g/mL OVA. After 5 days, proliferating cells were evaluated as described above for human cell cultures. For cytokine production assays, 2.5×10^6 cells were incubated in a 48-well tissue-culture plate in 1 mL IMDM, 5% FCS, and supernatants were harvested 5 days later.

For histology, ears and lungs were removed and placed in formalin for paraffin sections. Staining was performed with Giemsa (Sigma-Aldrich) or periodic acid-Schiff (PAS, Merck) according to the manufacturer's instructions. Examination of slides and quantification of mucus-containing cells per mm basement membrane in the primary bronchus and medium-sized bronchi was performed using a microscope equipped with AxioCam ICc5 camera and ZEN 2012 (blue edition) software (Zeiss) in a blinded fashion.

2.9 | Quantification of cytokine production

Human IL-4, IL-10, IL-12p40, IL-13 (BD Biosciences), human IFN- γ (Mabtech AB), human IL-1 β , TNF- α (R&D Systems), mouse IL-4, IL-5, IL-10, IFN- γ (BioLegend) and mouse MCPT-1 (eBioscience) were measured by ELISA according to the instructions of the manufacturers of the employed pairs of antibodies.

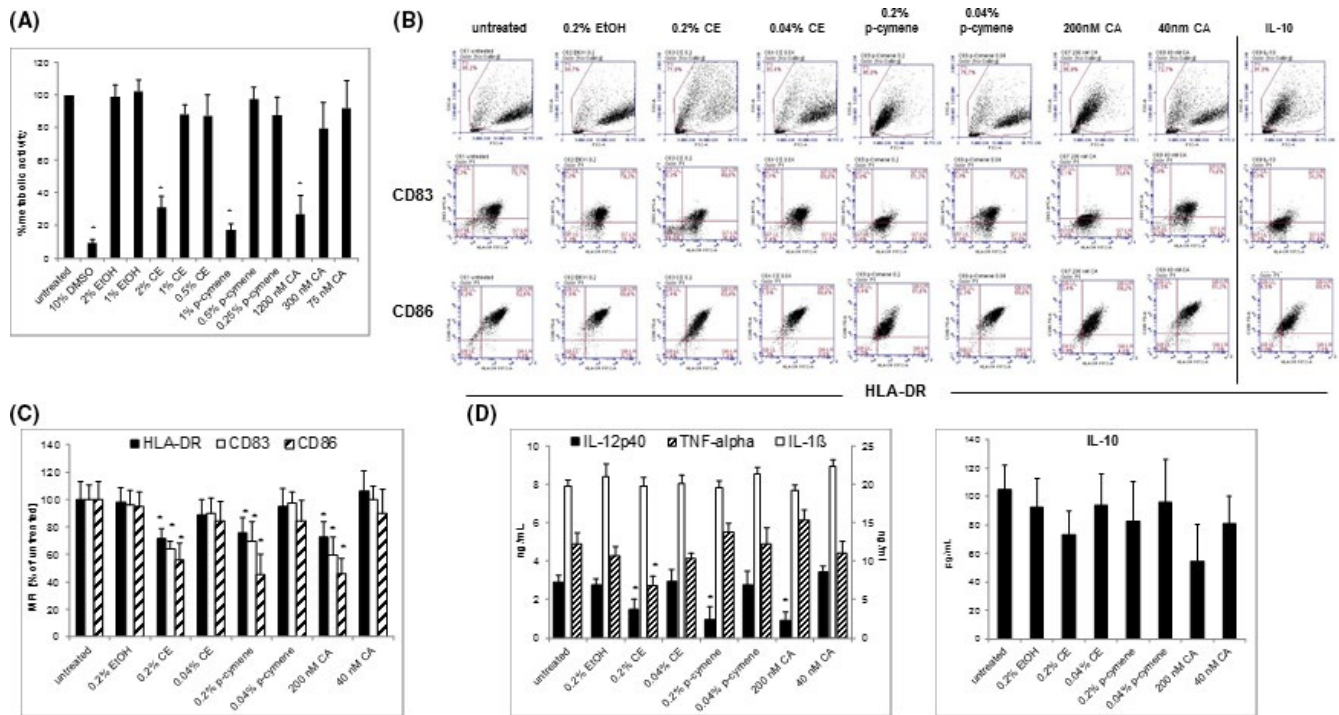


FIGURE 1 Effect of cinnamon extract (CE) and its major compounds on cell viability and DC activation. A, immature DC from birch or grass pollen allergic donors were treated with CE, *p*-cymene, CA or the solvent 70% aqueous ethanol, and cell viability was analysed by WST-8 Cell Proliferation Assay Kit 12 h later. B–D, immature DC were pulsed with birch or grass pollen or tetanus toxoid, matured and treated with CE, *p*-cymene, CA, IL-10 or 70% aqueous ethanol for 48 h. DC were stained with HLA-DR, CD83 and CD86 (B, C). Production of IL-1β, IL-10, IL-12p40 and TNF-α was determined in supernatants by ELISA (D). Shown are representative FACS plots (B) and the means ± SEM from 6 (10 for C) different donors, **P* ≤ .05 compared with untreated DC

2.10 | Determination of OVA-specific antibody titres in the serum

Anti-OVA IgE and IgG2a antibody concentrations in the serum from each mouse were measured by ELISA. Briefly, MaxiSorp plates (Nunc GmbH) were coated with OVA (25 µg/mL) and incubated with diluted sera (for IgG2a, 1:1000; for IgE, 1:20). Afterwards, detection was performed with biotinylated rat anti-mouse IgE (BD Biosciences) or goat anti-mouse IgG2a antibodies (SouthernBiotech) followed by horseradish peroxidase-conjugated streptavidin (Invitrogen Corp.).

2.11 | Determination of sulphidoleukotriene release and CD63 expression from activated basophils

Allergenicity was tested by sulphidoleukotriene release (LCT₄ and its metabolites LTD₄ and LTE₄) of activated basophils using the cellular antigen stimulation test (CAST®-2000 ELISA, Bühlmann Laboratories AG) according to the instructions of the manufacturer. Briefly, dextran (250 µL) was added to 1 mL EDTA-blood from birch or grass pollen allergic donors and incubated for 90 minutes at room temperature to allow sedimentation of erythrocytes. Then, peripheral blood leucocytes were centrifuged at 130 × *g* for 15 minutes and resolved in 1 mL stimulation buffer containing IL-3. 200 µL of this cell solution were stimulated with 50 µL allergen extract at different

concentrations or with an anti-IgE receptor antibody as positive control in the presence or absence of 0.1%–1% CE or 70% aqueous ethanol for 40 minutes at 37°C. Finally, the cells were centrifuged, the supernatants were stored at –20°C until quantitative analysis of sulphidoleukotriene concentration by ELISA, and CCR3 positive cells were analysed for CD63 expression by flow cytometry.

2.12 | Surface phenotyping by flow cytometric analysis

For analysis of human surface marker expression, 5 × 10⁵ T cells, 5 × 10⁴ DC or activated basophils were labelled with specific mouse anti-human mAbs for 20 minutes at 4°C. The following antibodies were used: AlexaFluor® 647-conjugated CD4 (MT310, Santa Cruz Biotechnology, Inc), phycoerythrin-conjugated CCR3 (5E8, BioLegend), CD83 (HB15e, BD Biosciences), CD86 [2331 (FUN-1, BD Biosciences)], fluorescein isothiocyanate-conjugated HLA-DR (L243, BD Biosciences), CD63 (MEM-259, ImmunoTools) and mouse IgG isotype controls (BD Biosciences). After incubation, the cells were washed and analysed by BD FACSria™ Flow Cytometer (BD Biosciences).

2.13 | Statistics

Statistical analysis was performed by GraphPad Prism 5.0 software (GraphPad Software). ANOVA was used to determine the levels of

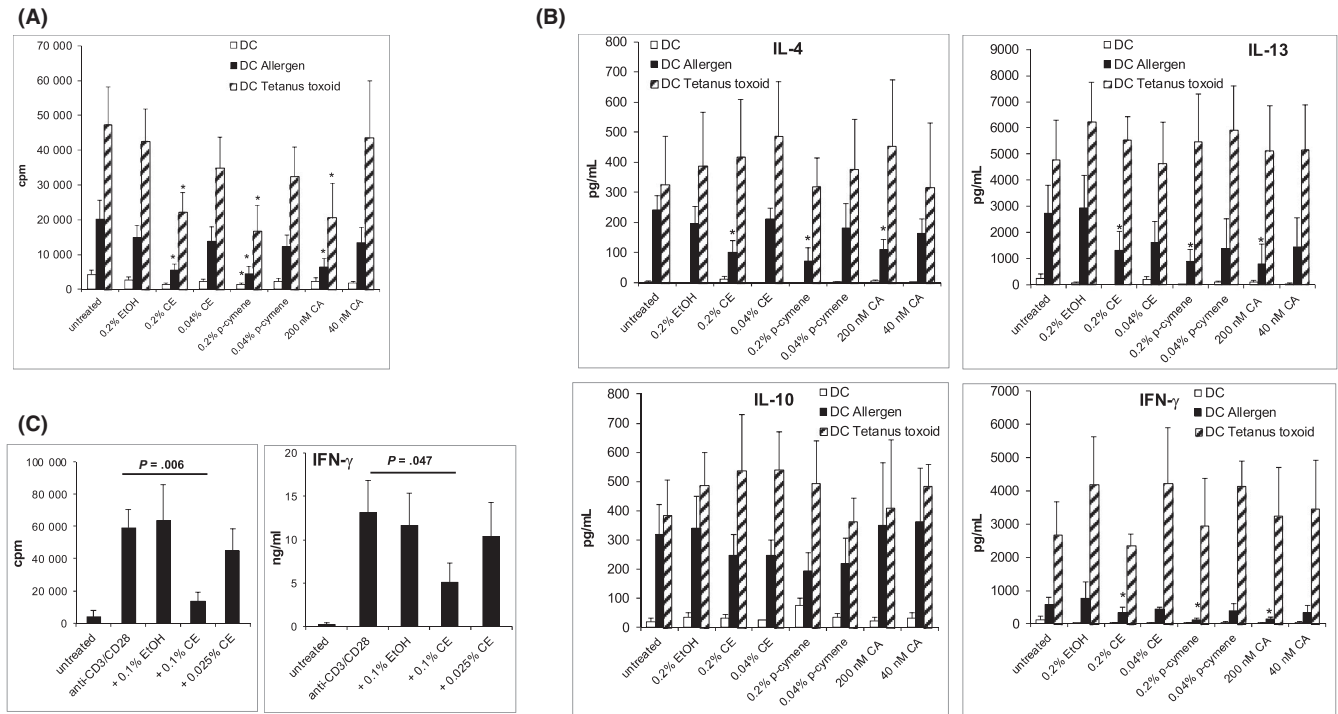


FIGURE 2 cinnamon extract (CE)-, *p*-cymene- or CA-treated treated DC exhibit reduced T cell stimulatory capacity compared with untreated or ethanol-treated DC. A–B, DC were treated as described in Figure 1B–D and co-cultured with autologous CD4⁺ T cells. After 5 days, proliferation was analysed by [³H]-thymidine incorporation (A). After 1 week, co-cultures were restimulated with freshly isolated mature DC, and supernatants were collected 24 h later for determination of cytokine production by ELISA (B). C, CD4⁺ T cells were stimulated with anti-CD3/CD28 mAbs in the presence or absence of CE, *p*-cymene, CA or 70% aqueous ethanol and proliferation and IFN- γ production was determined after 3 days. Results are expressed as means $\pm$ SEM from 10 (5 for C) different donors, * $P \leq .05$ compared with untreated DC

difference between experimental groups. Student 2-tailed paired *t* test was used to test the statistical significance of the results; $P \leq .05$ was considered to be significant. The number of experiments is indicated at the end of each figure legend.

3 | RESULTS

3.1 | CE and its major compounds *p*-cymene and CA inhibit human DC maturation and subsequent allergen-specific T cell stimulatory capacity in vitro

First, we determined the influence of CE, *p*-cymene, CA and the solvent 70% aqueous ethanol on cell viability of immature human DC by WST-8 assay, which revealed that concentrations up to 2% ethanol, 1% CE, 0.5% *p*-cymene and 300 nmol/L CA did not affect their metabolic activity (Figure 1A). To analyse whether CE and its major compounds can modify allergen-specific immune responses in vitro, CD4⁺ T cells from grass or birch pollen allergic donors were co-cultured with autologous allergen-pulsed mature DC, which were treated with the above tested non-toxic concentrations of CE, *p*-cymene, CA, the solvent 70% aqueous ethanol, or the anti-inflammatory cytokine IL-10 as positive control. In Figure 1B–D, it is shown that treatment of DC with 0.2% CE, 0.2% *p*-cymene or 200 nmol/L CA significantly inhibited DC maturation which was comparable to our previous finding for IL-10²⁰

and characterized by a reduced expression of the MHC-class II molecule HLA-DR, the DC maturation marker CD83, the co-stimulatory molecule CD86 and the production of IL-12p40 while the same concentration of ethanol had no inhibitory effect. The pro-inflammatory cytokine being present in the maturation cocktail, TNF- α , was also significantly inhibited by CE, but not by *p*-cymene, CA or ethanol treatment, while the other cytokine of the maturation cocktail, IL-1 β and IL-10 production was not affected at all (Figure 1C). Furthermore, allergen-specific proliferation of CD4⁺ T cells was significantly reduced after stimulation with CE-, *p*-cymene- or CA-treated allergen-pulsed DC compared with untreated or ethanol-treated DC (Figure 2A). The production of the Th2 cytokines IL-4 and IL-13 as well as the Th1 cytokine IFN- γ was also significantly inhibited (Figure 2B). Again, IL-10 production was not altered (Figure 2B). Of note, the anti-inflammatory effect of CE and its major compounds was also observed in DC-T cell co-cultures using the control antigen tetanus toxoid as stimulus (Figure 2A,B), and CE further inhibited anti-CD3/CD28 induced proliferation and IFN- γ production of CD4⁺ T cells (Figure 2C).

3.2 | CE decreases sulphidoleukotriene release and CD63 expression by human basophils in vitro

Next, we wondered whether CE also influences activation of basophils. Therefore, peripheral blood leucocytes from grass or birch

pollen allergic donors were stimulated with different concentrations of the respective allergen or anti-IgE receptor antibody as positive control in the presence or absence of CE or the solvent 70% aqueous ethanol at different concentrations, and basophil activation was analysed by ELISA and flow cytometry. Compared with untreated cells, the sulphidoleukotriene release by basophils after allergen and anti-IgE receptor stimulation was significantly reduced in the presence of 0.4% and 1% CE. Treatment of peripheral blood leucocytes with 1% ethanol also led to a significant but less pronounced reduction of this mediator, while the addition of 0.4% ethanol did not result in any inhibition (Figure 3A). Further analysis of the activation marker CD63 expressed on CCR3-positive basophils confirmed the strong inhibitory effect of CE- compared with ethanol-treatment (Figure 3B).

3.3 | Oral application of CE inhibits allergic lung inflammation and anaphylaxis in OVA-sensitized mice associated with increased allergen-specific IgG2a and reduced IgE production

To investigate the influence of CE in vivo, BALB/c mice were immunized with OVA and treated with an optimized concentration of 1 mL CE per kg body weight or the corresponding amount of 70% ethanol by gavage every other day. Possible negative effects of ethanol on OVA-sensitization of mice were excluded in preliminary experiments. In CE-treated mice, OVA-specific IgE production in mouse sera was significantly decreased compared with mice being gavaged with the solvent ethanol, while OVA-specific IgG2a production was significantly increased (Figure 4A). Additionally, systemic anaphylaxis measured by an increase of MCPT-1 levels in the sera and drop in body temperature after allergen challenge was significantly prevented by CE treatment (Figure 4B,C). Analysis of lung inflammation after intranasal allergen challenge further revealed a significant reduction of airway hyperreactivity and eosinophil numbers in the BAL

fluid together with a strong decrease in IL-4, IL-5 and IL-10 cytokine production (Figure 4D-F). In addition, histological examination of the lungs demonstrated a significantly decreased mucus production and goblet cell hyperplasia (Figure 4G). Furthermore, treatment of mice with CE significantly inhibited OVA-specific T cell proliferation after in vitro restimulation of splenocytes (Figure 5A). Production of Th1 and Th2 cytokines was also strongly reduced (Figure 5B).

3.4 | Cutaneous application of CE inhibits calcipotriol-induced atopic dermatitis-like skin inflammation

Finally, we analysed the impact of CE in a murine model of atopic dermatitis-like inflammation induced by topical application of the vitamin D3 analogue calcipotriol (MC903) on mice ears²¹ with or without prior treatment of the ears with CE. In Figure 6A,B, it is shown that ear swelling as well as epidermal hyperplasia and inflammatory cell infiltration was almost completely abolished in CE-treated mice.

4 | DISCUSSION

In the present study, we have analysed the anti-allergic potential of CE and its active compounds *p*-cymene and CA, both in vitro and in vivo. We demonstrate for the first time that CE, *p*-cymene and CA significantly inhibited DC maturation and subsequent allergen-specific immune responses in a human DC-T cell co-culture in vitro setting, but also proliferation of anti-CD3/CD28 stimulated T cells. This implies a general immunosuppressive function of CE comparable to previous results published by us and several other groups for IL-10.^{20,22} Additionally, we could show that CE not only significantly decreased sulphidoleukotriene release and CD63 expression of human basophils, but also anaphylactic reactions as well as airway hyperreactivity in OVA-sensitized and orally CE-treated mice. Numbers of eosinophils,

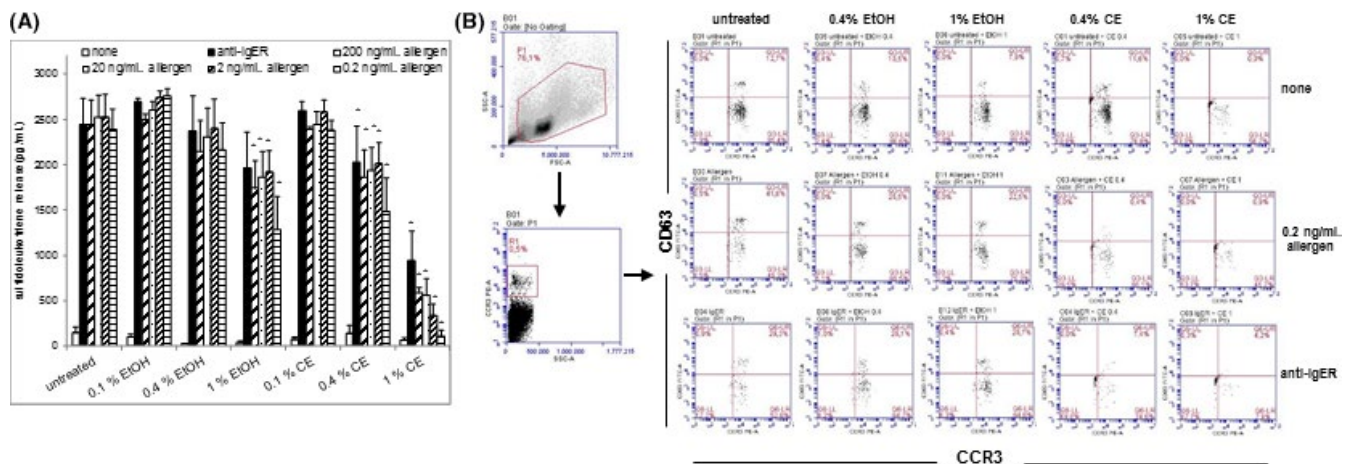


FIGURE 3 Decreased sulphidoleukotriene release and CD63 expression by human basophils after treatment with CE. Basophil-containing peripheral blood leucocytes from grass or birch pollen allergic donors were stimulated with different concentrations of the respective allergen or anti-IgER in the presence or absence of CE or 70% aqueous ethanol. A, sulphidoleukotriene release was quantified by ELISA. Shown are the means $\pm$ SEM from 5 different donors, * $P \leq .05$ compared with untreated cells. B, gating strategy and representative FACS blots of CD63 expression by CCR3-positive basophils

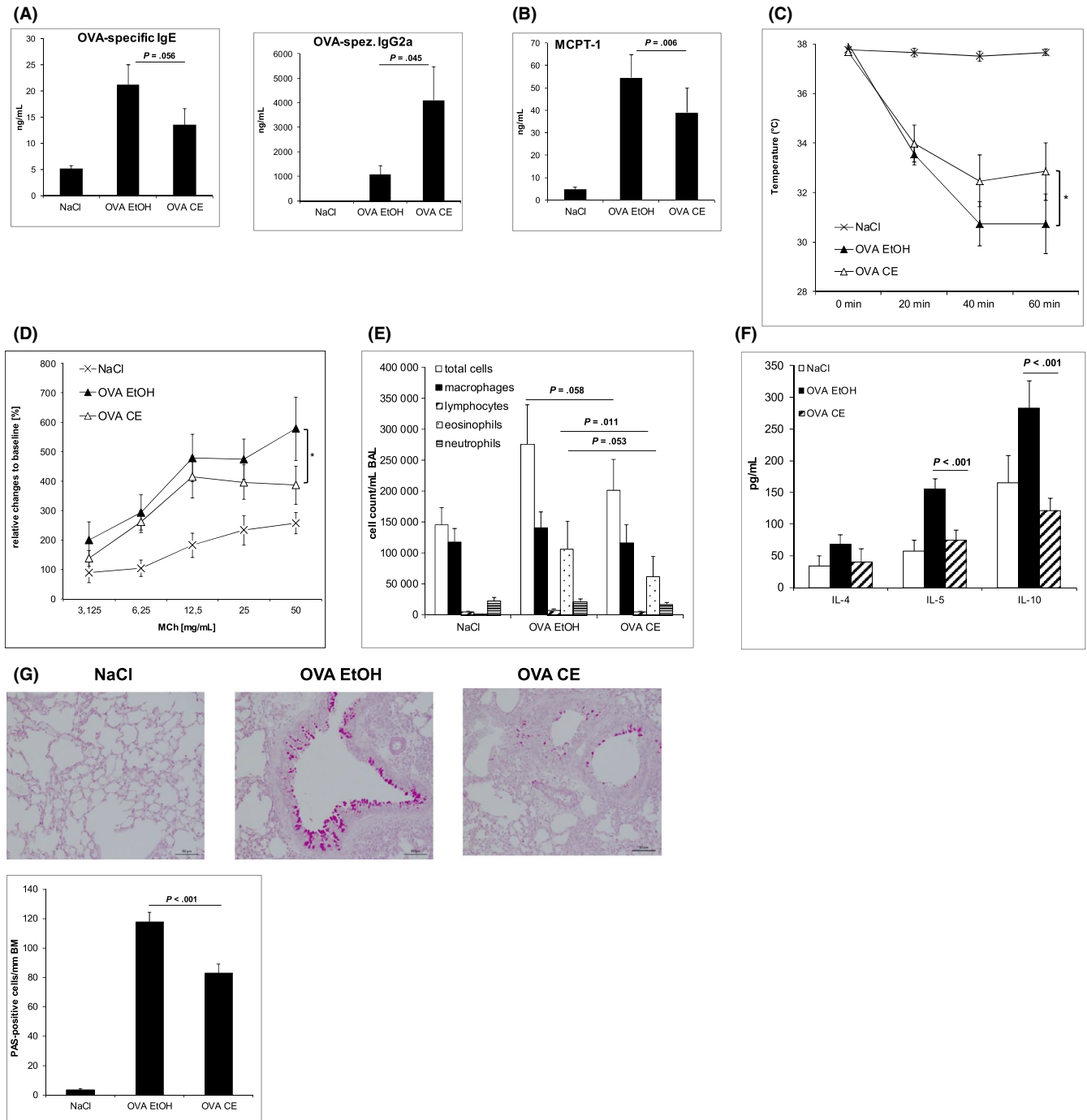


FIGURE 4 Reduced anaphylaxis and airway inflammation in OVA-sensitized mice by CE treatment. BALB/c mice were immunized i.p. with 20 µg OVA on days 0 and 10 in the presence or absence of CE or 70% aqueous ethanol given by gavage every other day from day 0 onwards. On day 24, anaphylaxis was induced by i.p. allergen challenge and OVA-specific IgE and IgG2a production (A), and MCPT-1 levels (B) were determined in the sera 2 h later by ELISA. Drop in body temperature was measured 20, 40 and 60 min after challenge (C). Mice were additionally challenged with the allergen i.n. for three subsequent days and airway resistance was measured by invasive body plethysmography 24 h later (D). Then, numbers of macrophages, lymphocytes, eosinophils and neutrophils (E) and Th2 cytokine production (F) were analysed in the BAL fluid and mucus-producing goblet cells per millimetre basement membrane were determined in PAS-stained paraffin-embedded lungs (G, lower panel). Shown are the means ± SEM of 5 (4 for C + D) independent experiments with 2-3 mice per group and micrographs of PAS staining (magnification × 200) of a representative result (G, upper panel), * $P \leq .01$

mucus-producing goblet cells and secretion of Th2 cytokines in the BAL fluid were also strongly reduced. Interestingly, IL-10 production itself was neither induced in vivo nor in vitro suggesting that other

regulatory factors may play a role. A further novel finding of our study was the almost complete inhibition of calcipotriol-induced atopic dermatitis-like skin inflammation by topical administration of CE, while

oral administration of CE had no effect in this disease model (data not shown). Thus, high local concentrations of CE are needed to prevent skin inflammation in this model, which could not be reached by the oral compared with the cutaneous application method even if pure

CE would also have been given due to the dilution throughout the bloodstream. Overall, all tested *in vivo* parameters were not further inhibited by feeding the mice with a 4.5 times higher, but still well-tolerated, CE concentration than our optimized one.

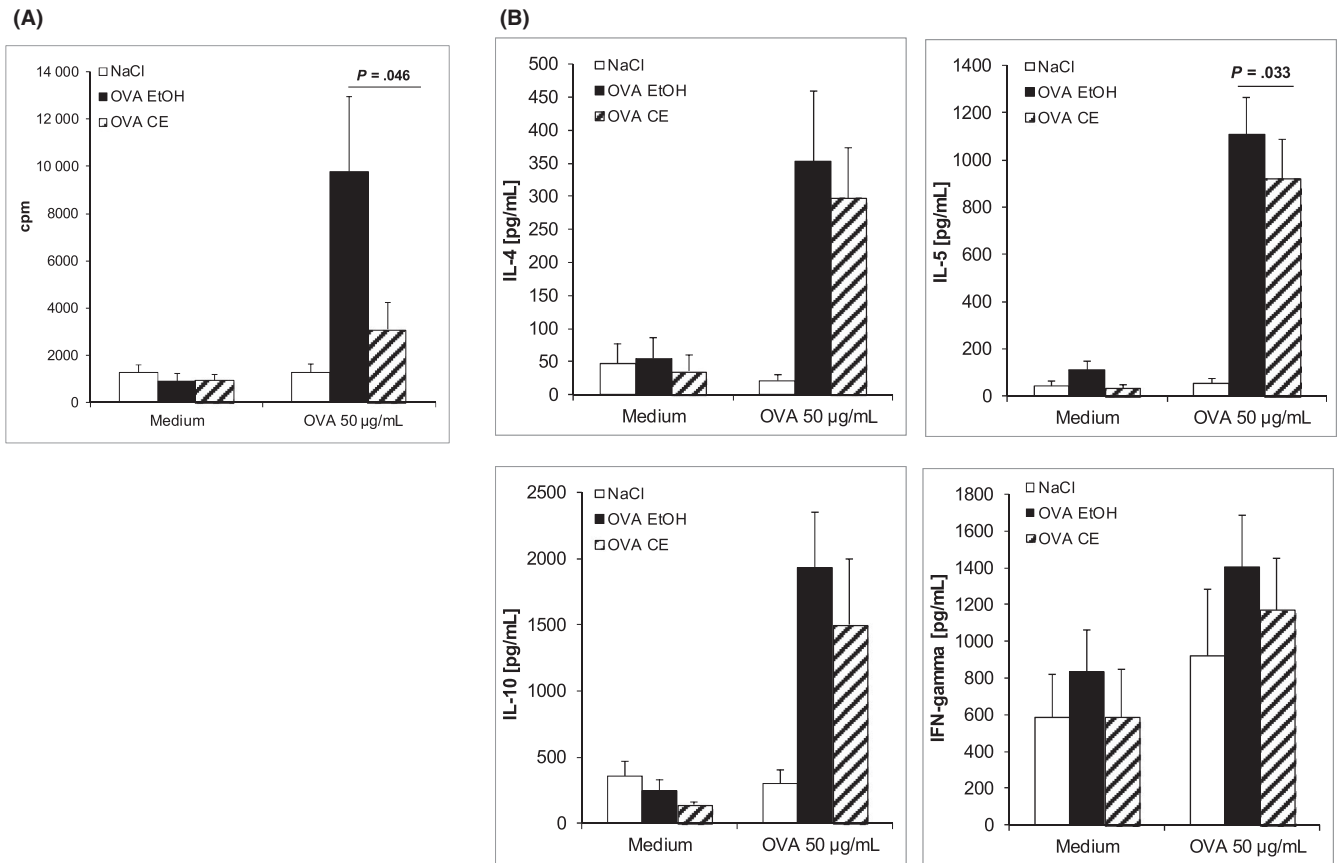


FIGURE 5 cinnamon extract (CE) treatment reduces OVA-specific proliferation of restimulated splenocytes. Mice were treated as described in Figure 4. At the end of the experiment, splenocytes were restimulated with OVA for 5 days. Proliferation was measured by [³H]-thymidine incorporation (A), and cytokine production was measured in the supernatants by ELISA (B). Shown are the means $\pm$ SEM of 7 independent experiments with 2-3 mice per group

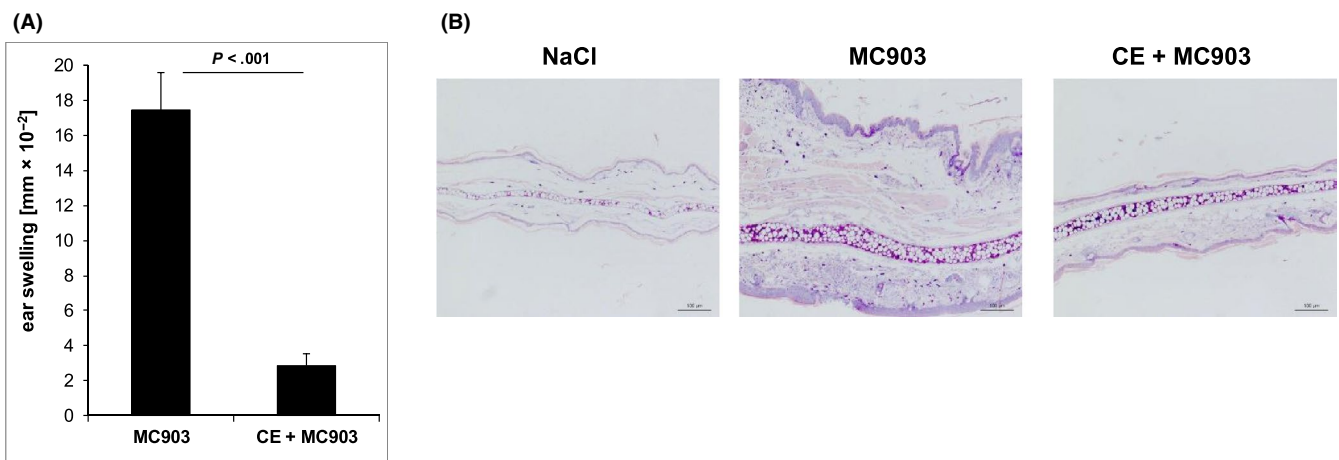


FIGURE 6 Inhibition of calcipotriol-induced atopic dermatitis by CE. Mice were treated as described in Figure 4. From day 0 onwards, they were further topically treated with the vitamin D3 analogue calcipotriol (MC903) on both ears three times per week. 10 min before MC903 application, mice ears were treated with CE or ethanol. 24 h after the 12th application ear swelling was measured (A) and paraffin-embedded ears were stained with Giemsa (B). Shown are the means $\pm$ SEM of 4 independent experiments with 2-3 mice per group (A) and micrographs of H&E staining (magnification $\times 100$) of a representative result (B)

Our data are in accordance with recent publications showing that CE and its major mediator CA prevented degranulation as well as de novo synthesis of inflammatory mediators in human mast cells isolated from intestinal tissue and in rat basophilic leukaemia cells.¹⁴⁻¹⁶ Additionally, following oral or intranasal treatment of mice with CE, a significantly decreased expression of mast cell-specific proteases and a reduced production of total IgE and histamine levels have been reported.^{14,18} In the study from Aswar et al¹⁸, OVA-sensitized and CE-treated mice further showed decreased nasal rubbing and sneezing after histamine challenge, but other IgE-mediated parameters were not measured. Notably, in our study, we addressed for the first time the effects of CE on anaphylaxis as well as lung and skin inflammation and could clearly demonstrate that all these inflammatory disorders were strongly inhibited by oral or topical treatment of mice with CE. The finding that CE completely prevented calcipotriol-induced atopic dermatitis-like skin inflammation supports a recent report demonstrating that cinnamon bark essential oil significantly inhibited the production of several inflammatory protein biomarkers in human dermal fibroblasts in vitro.²³ Inhibition of the aryl hydrocarbon receptor activation induced by dioxins or other environmental pollutants together with an anti-oxidant activity of CE and CA has further been shown in a human keratinocyte cell line.²⁴

Besides its anti-allergic property, cinnamon has also been described to possess anti-inflammatory effects in a number of mouse models such as inflammatory bowel disease, arthritis, diabetes, alcohol-induced fatty liver and experimental autoimmune encephalitis.^{7,25-28} Anti-inflammatory properties of CE are mediated by suppression of nitric oxide (NO), TNF- α , IL-1 β and IL-6 production as well as by blocking mitogen-activated protein kinase (MAPK) and nuclear factor- κ B (NF- κ B) activation.^{12,28-30} Direct suppression of LPS-induced toll-like receptor 4 (TLR4) oligomerization has been suggested as one mechanism for these inhibitory effects,^{31,32} but TLR4-independent upregulation of peroxisome proliferator-activated receptor gamma (PPAR-), a key regulator of glucose and lipid metabolism and anti-inflammatory genes, has also been described.³³ Importantly, our group recently identified several active compounds, among them *p*-cymene and CA, contributing to the strong anti-inflammatory effects of CE by interfering with downstream signalling pathways not only common for TLR4, but also for TLR2, such as the central NF- κ B/AP-1 signalling, which was shown by a similar reduction of the reporter gene in HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells.^{13,34} Further studies will be needed to better understand the exact underlying mechanisms and to test all these compounds in our allergy models.

In humans, safety and efficacy of CE has been shown in several phase I clinical trials, mainly in patients with type II diabetes mellitus, where a significant decrease in fasting plasma glucose, total cholesterol, triglycerides, LDL cholesterol, glycosylated haemoglobin and blood pressure could be observed after receiving 1-6 g of cinnamon capsules or powder daily for 40 days up to 4 months.^{8,9,35-37} Furthermore, cinnamon possessed therapeutic benefits on liver enzymes and high-sensitivity C-reactive protein in patients with non-alcoholic fatty liver disease.³⁸ Recently, improvement of clinical symptoms and inflammatory markers has been reported in patients

with rheumatoid arthritis.^{39,40} So far, there is only one double-blind placebo-controlled clinical study with a total of twenty allergic rhinitis patients where a combination phytotherapeutic product containing cinnamon was as effective as antihistamines regarding the reduction of the nasal symptom score after allergen challenge and even more effective in the prevention of prostaglandin D2 release into the nasal lavage fluid. However, whether this effect is mediated by cinnamon or the other two compounds remained unresolved.¹⁷ Furthermore, it has to be kept in mind that some adverse effects such as gastrointestinal disorders and allergic reactions after short or long term cinnamon consumption have been described. They were self-limiting in the majority of cases.⁴¹

In conclusion, our data suggest that cinnamon might be a promising anti-allergic therapeutic agent especially for atopic dermatitis patients but also in the context of anaphylaxis and airway inflammation, which merits further clinical investigation with larger cohorts.

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CONFLICT OF INTERESTS

The authors declare no financial or commercial conflict of interest.

AUTHOR CONTRIBUTION

KL, JS and IB conceived and designed the experiments; RO, JT, JM, PS and IB performed the experiments and analysed the data; AS and KL contributed reagents; IB wrote the manuscript; and all authors reviewed and approved the manuscript.

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