



## GINGER WITH HONEY PREVENTS TH2-MEDIATED IMMUNE RESPONSES IN A MOUSE MODEL OF AIRWAY INFLAMMATION

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### ABSTRACT

In traditional medicine, people use the association of ginger and honey to treat some respiratory disorder. The aim of the present study was to examine the effects of the association of ginger and honey on respiratory disorder in mice. Here we showed that ginger-honey can exert such functions *in vivo*, namely in a mouse model of Th2-mediated pulmonary inflammation. Intraperitoneal injections of Ginger-Honey aqueous extract (GHae) before airway challenge of ovalbumin (OVA)-sensitized mice resulted in a marked decrease in the recruitment of eosinophils to the lungs as attested by cell counts in bronchoalveolar lavage (BAL) fluids. Resolution of airway inflammation induced by GHae was accompanied by a suppression of the Th2 cell-driven response to allergen *in vivo*. Thus, IL-4 and eotaxin levels in the lungs were clearly diminished in ginger-honey-treated mice relative to their controls after allergen sensitization and challenge. Ginger-Honey can suppress Th2-mediated immune responses and might thus provide a possible therapeutic application in inflammation.

**Keywords:** Ginger, Honey, Airway Inflammation, cytokine

### INTRODUCTION

Many reviews have been devoted to specific aspects of ginger's actions.<sup>1</sup> For example, the review of Grzanna et al (2005) was on the use of ginger as an anti-inflammatory agent, while that of Shukla & Singh (2007) dealt with the cancer prevention properties of the crude drug.<sup>2,3</sup> The actions of ginger as a post-operative anti-emetic substance were the subject of a review by Chaiyakunapruk et al. (2006).<sup>4</sup> As ginger, honey is used to treat numerous diseases. Honey is used to treat peptic ulcers and gastritis, tooth extraction and oral surgery.<sup>5,6</sup> The treatment of infections of the urinary tract with honey has been reported at 4.6%.<sup>7</sup> Some bacteria that cause urinary tract infections, such as *Escherichia coli*, *Proteus* species and *Streptococcus faecalis*, proved susceptible to the antibacterial activity of honey.<sup>8</sup> The use of honey to relieve pain and disorder postnatal women were reported by 45.6% of people in Burkina Faso.<sup>7</sup> Researchers have demonstrated the antimicrobial activity of honey.<sup>9-11</sup> It is also used to treat skin disorders, abscesses, burns, and conservation of the grafted skin.<sup>12,13</sup> Ophthalmic applications of honey to treat blepharitis and keratitis, were reported by traditional healers.<sup>5,7,8</sup> The use of honey in treatment of oral diseases, such as pharyngitis, has been well described and is probably due to its antibacterial and anti-inflammatory activity.<sup>5,6,8,12,14</sup> Ginger is used in combination with the honey in some areas to relieve asthma. To justify the use of this complex, we propose to study the anti-inflammatory effects of ginger combined with honey in a model of murine allergic asthma.

### MATERIALS AND METHODS

#### Sample preparations of extracts

Rhizoma of *Zingiber officinale* Roscoe were collected in the market of Abidjan (Côte d'Ivoire) and dried at  $70 \pm 5$  °C. The plant was authenticated by an expert in Botany, Professor AKE-Assi Laurent of the "Centre National de Floristique de Côte d'Ivoire" at Félix Houphouët-Boigny University in Abidjan.

EZo (*Z. officinale* Rhizoma aqueous extract) preparation was previously described.<sup>15,16</sup> Plant material was ground into a

fine powder using a pestle and mortar and the powder was soaked in distilled water (1L) for 24 h at room temperature ( $27 \pm 3$  °C). The resulting solution was filtered and freeze dried. From a 50 g sample of ginger dried rhizome, 5 g of solid material (EZo, *Z. officinale* aqueous extract) was obtained (yield: 10 %).

To obtain Ginger-Honey aqueous extract (GHae), 100 g of powder of *Z. officinale* were collected with 100 g of honey and put to macerate in 500 ml of water for 24 hours under magnetic stirring. The aqueous suspension was dried in an oven at  $60 \pm 5$  °C for three days. The final product (GHae) was in the form of a paste of brown mass of 20 g.

Extracts (EZo and GHae) were stored at 4°C until use. The concentrations to be tested were prepared extemporaneously by dilution in distilled water.

#### Animals

NOD mice were bred and maintained in our animal facility under specific pathogen-free conditions whereas C57BL/6 mice were obtained from the Laboratory Animal Center Janvier (CERJ; le Genest-Isle, France). For experiments, 6-8 week old males weighing 20-25 g were used. Housing conditions and all *in vivo* experiments were performed according to the Institutional Ethical Committee of France and the guidelines established by the European Union on Animal Care (CEE Council 86/609).

#### Sensitization, airway challenge, and EZo treatment

Mice were sensitized by intraperitoneal injection (i.p.) of 100 µg of ovalbumin (OVA, INC Biomedicals, Inc, OH) emulsified in 1.6 mg of alum hydroxide (Merck, Darmstadt, Germany) in a total volume of 400 µl on day 1. Sensitized and naïve (NaCl) mice received aerosolized allergen challenge (50 mg OVA for 20 min) on 3 consecutive days (days 7, 8, and 9) using an ultrasonic nebulizer (Ultra-Neb99, Devilbiss). An hour before the first and/or the second challenge (days 7 and 8), OVA sensitized mice were injected i.p. with EZo at dose 360 mg/kg b.w.

#### Bronchoalveolar lavage (BAL) and lung homogenates

Animals were anesthetized by the intraperitoneal injection of urethane (ethylcarbamate, 2 g/kg b.w., Sigma Aldrich, Stenheim, Germany). After intubation, lungs were lavaged,

and bronchoalveolar lavage fluid (BALF) was recovered (0.5 ml of PBS; 5 times) through the trachea. Total leukocyte numbers were measured with a hemocytometer using Turk dye exclusion method. Differential cell counts were performed under light microscopy in a blinded manner, counting at least 200 cells according to the standard morphologic criteria on cytocentrifuged preparations (Shandon Cytospin 4, ThermoElectron Corporation) with a fast staining procedure (HAEME-Schnellfärbung, Labor+Technik Eberhard Lehmann, Berlin, Germany). Homogenates were prepared after cannulating the trachea and perfusing the airway. The lungs were then removed and vigorously vortexed. The

resulting cell suspension was homogenized and centrifuged for 10 min at 1600 g at 4 °C. Supernatants were removed and stored at -20 °C until cytokine measurement.

**Statistical analysis**

Statistical analysis was performed by using the Graph Pad Prism Software (version 3.03) to test whether treatment with ginger extracts leads to a significant reduction in several parameters associated with allergic disease. Results are expressed as means ± standard error of the mean (SEM). Student's t-test was used for analysis of significance among the groups. Values of  $p < 0.05$  were considered statistically significant.

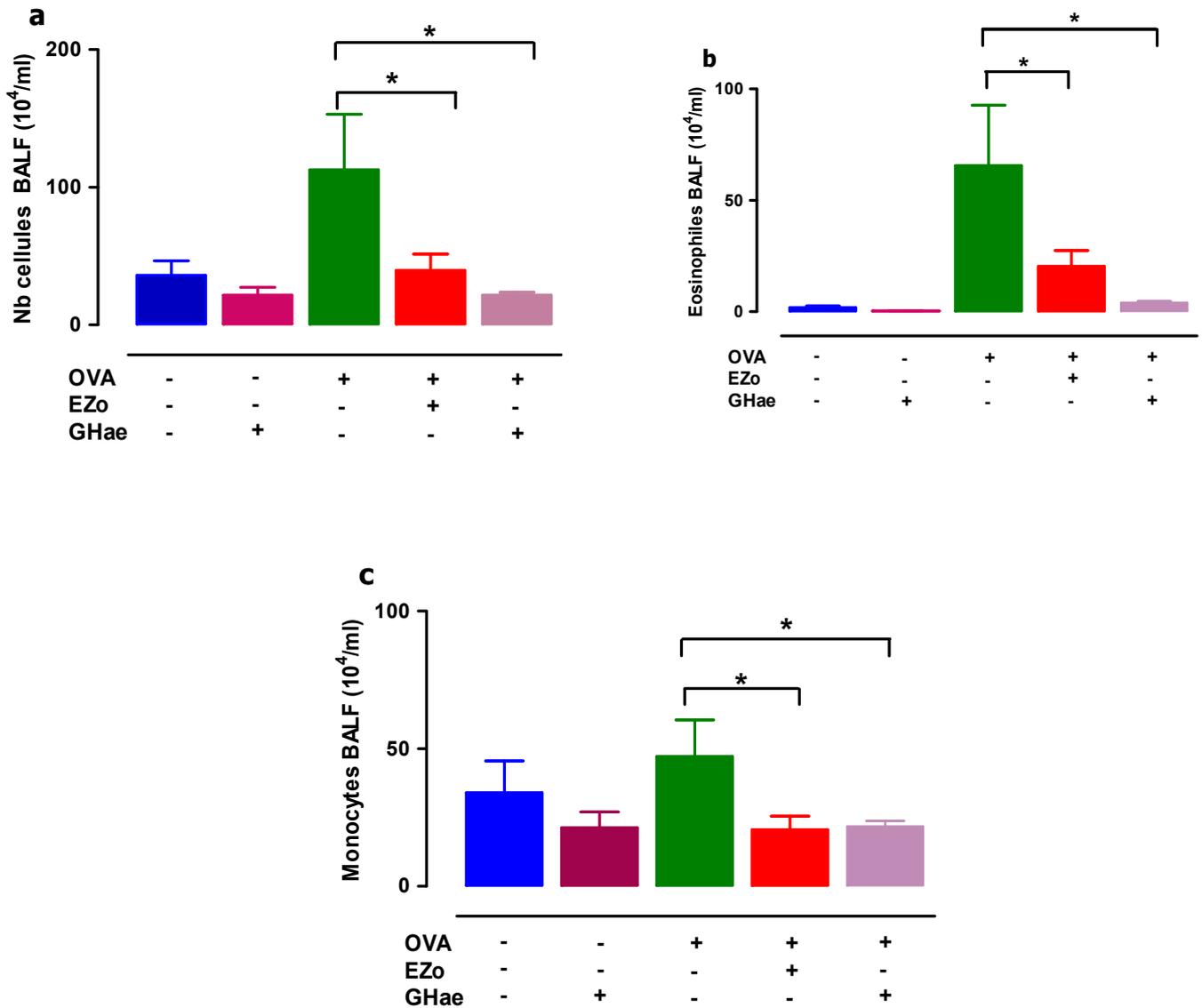


Figure 1: Inhibition of OVA-induced airway inflammation by GHae applied to NOD mice. NOD mice were sensitized with OVA and challenged with OVA or saline as described and received or not GHae (360 mg/kg b.w.) 1 h before the first and the second challenges. One day after the last challenge, the absolute number of total cells, eosinophils, monocytes and in the BALF was measured. (+): presence of drug; (-): absence of drug. Data shown are mean ± S.E.M. of 3 to 6 mice per group. \* $p < 0.05$ .

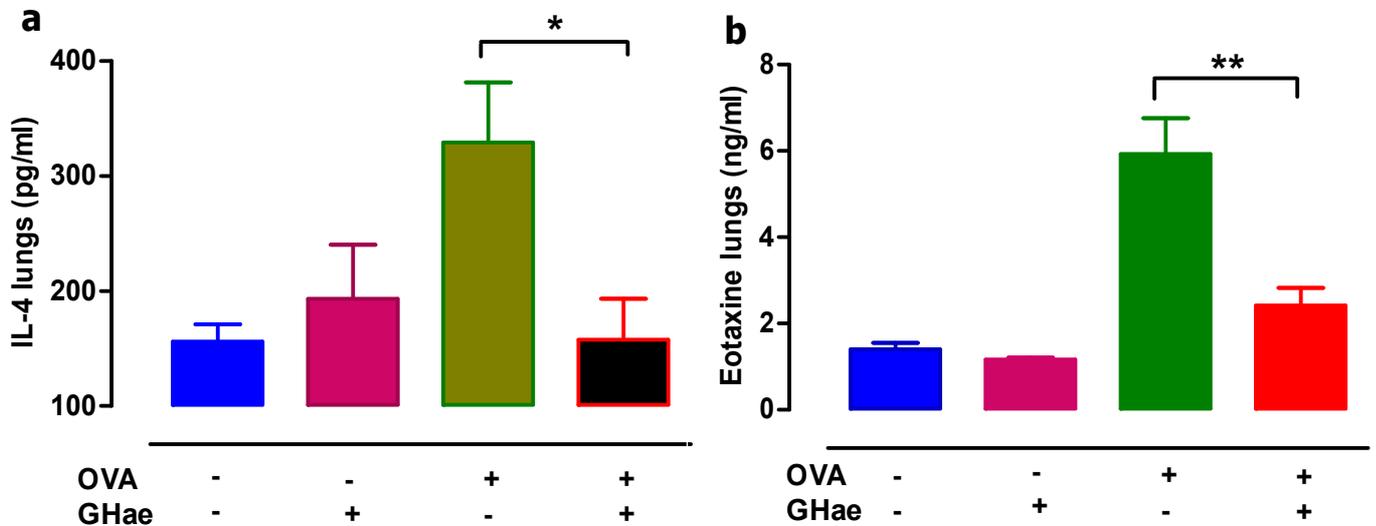


Figure 2: GHae inhibits OVA-induced cytokine production in NOD mice. NOD mice were sensitized with OVA and challenged with OVA or saline, and received or not GHae (360 mg/kg b.w.) 1 h before the first and second challenges. One day after the last challenge, BALF and lungs were collected. IL-4 and eotaxin protein levels were measured in BALF and lung tissue homogenate supernatants by ELISA. (+): presence of drug; (-): absence of drug. Data shown are mean ± S.E.M. of 3 to 12 mice per group. \*p < 0.05; \*\*p < 0.01.

**RESULTS**

**Inhibition of airway inflammation in OVA-immunized and-challenged mice treated with aqueous ginger extract**

Experimental OVA-dependent allergic asthma is characterized by airway eosinophilia and mucus production.<sup>17</sup> We have previously reported that the NOD mouse is a relevant model of experimental allergic asthma.<sup>18</sup> Thus, after sensitization and aerosol challenges with OVA, NOD mice develop eosinophilic airway inflammation. This typical feature of Th2-mediated pulmonary inflammation was clearly reduced in NOD mice treated with GHae used at the dose of 360 mg/kg b.w. The effect of GHae on the recruitment of inflammatory cells into BALF in OVA-challenged mice is shown in (Fig. 1).

In OVA-immunized and -challenged mice used as positive controls, total cell numbers 1 day after the last challenge were significantly increased. The frequency of inflammatory cells in decreasing order was: eosinophils and monocytes. The administration of GHae dramatically reduced the number of cells recruited, including eosinophils and monocytes. Altogether, these findings clearly show that treatment with GHae inhibits both eosinophilia and airway inflammation in OVA-immunized and -challenged mice.

**Production Th2 cytokine and eotaxin after OVA challenge is affected in GHae-treated NOD mice**

Allergic airway inflammation is accompanied by the generation of IL-4 as well as chemokines, such as eotaxin.<sup>19</sup> As shown in (Fig. 2), levels of IL-4 and eotaxin in BALF and lungs, respectively, were significantly reduced in OVA-immunized and -challenged NOD mice treated with GHae (360 mg/kg b.w.). GHae inhibits OVA-induced cytokine production in NOD mice.

**DISCUSSION**

Ginger and honey is used in traditional medicine in Côte d’Ivoire as a treatment for several diseases, including chronic inflammatory diseases.<sup>2,7</sup> Although the anti-inflammatory properties of ginger are well documented, our previous studies have evaluated the effects of this natural product on

the immune responses *in vivo*.<sup>15</sup> The objective of this study was to evaluate the effects of ginger associated with honey on airway inflammation *in vivo* in a mouse model of allergen-induced allergic asthma. Following sensitization, inhalation of OVA induces allergic inflammatory changes characterized by infiltration of inflammatory cells, mainly eosinophils, into regions around the airways and pulmonary blood vessels. The main finding in the present study was that these OVA-induced inflammatory changes in lung tissues were suppressed by ginger-honey extracts. In the present study, ginger associated with honey treatment led to a decreased production of IL-4 and eotaxin in lung tissues of OVA-sensitized and -challenged mice.

Thus, the inhibitory effects of ginger-honey extract on airway inflammation may be due in part to the decrease in IL-4 levels. The other pathway involves recruitment of eosinophils via release of eotaxin from Th2 cells or mast cells. Our results showed that the decrease in the number of eosinophils in BALF and lung tissue, and the number of mucus-producing goblet cells in the airway mucosa were concomitant with the decrease in eotaxin. Therefore, from all these data, it can be assumed that the inhibitory effects of ginger-honey extracts on airway inflammation are due to the decrease in Th2 cytokines and eotaxin. An important point is to know whether the inhibitory effects of ginger-honey apply to other cell types and molecules than those implicated in Th2-mediated inflammation. Although the predominant inflammatory cells recruited into asthmatic lung tissues are eosinophils, neutrophils and macrophages have been also found to be elevated in the BALF and lung tissues of OVA-sensitized and -challenged mice.<sup>20</sup> Here, we showed that ginger associated with honey inhibits the recruitment of eosinophils and macrophages too.

**CONCLUSION**

In our study, ginger-honey was administered before the induction of airway inflammation with allergen challenge and was effective in preventing its development. To summarize, our study provides the first evidence that ginger-honey

inhibits allergen-induced lung inflammation by reducing airway eosinophilia, Th2 cytokines and chemokines, thus supporting its anti-inflammatory role during the allergic response in the lung. Ginger-honey is therefore a promising new therapeutic approach for the preventive treatment of allergic asthma.

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#### ABBREVIATIONS

GHae: Ginger-Honey Aqueous Extract; EZo: *Zingiber officinale* Aqueous Extract; OVA: Ovalbumin; BAL: Bronchoalveolar Lavage; BALF: Bronchoalveolar Lavage Fluid.

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