Immunomodulatory properties of *Musa paradisiaca* L. inflorescence in Combined Allergic Rhinitis and Asthma Syndrome (CARAS) model towards NFκB pathway inhibition

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ARTICLE INFO

ABSTRACT

Musa paradisiaca L. (Musaceae), a tropical plant named banana is used as food and as medicine in Brazil. Banana inflorescence, popularly known as mangará, presents several biological activities including anti-inflammatory effects. Here, we demonstrated the immunomodulatory activity of banana inflorescence extract (HEM) on a mice model of combined allergic rhinitis and asthma syndrome (CARAS) and in human macrophages. The HEM inhibited the eosinophil migration, production of cytokines as IL-4, IL-5, IL-13, and IL-17A dependent on IFN-ɣ production in the airway. The mechanism of the extract was, in part, by the NF-κB signaling pathway inhibition. Besides, the HEM decreased expression of the CD86 and HLA-DR receptors on human M1 macrophages independently of M2 modulation. Therefore, we infer that the inflorescence, a disposable material from the banana crops, has anti-allergic property in the CARAS model and modulates the human macrophages, characterizing it as biologically important material for the production of phytomedicine.

1. Introduction

Musa paradisiaca L. popularly known as banana belongs to the *Musaceae* family and the genus *Musa*. The banana is among the main crops in the world and is the base of food for the tropical region population. Banana is a term that includes a large number of hybrids of species belonging to the *Musaceae* family spread around the world (Pereira & Maraschin, 2015), and in Brazil, *Musa paradisiaca* L. is a more frequent species (Correa et al., 2016).

Several studies of the *Musa* species have demonstrated the biological properties of its compounds as antioxidant, antidiabetic, cytotoxic and apoptosis, anti-microbial, anti-inflammatory, and cardiovascular protective effects (Arun et al., 2017; Bhaskar et al., 2012; Nisha & Mini, 2014). In addition, extracts of parts of *M. paradisiaca* L. have been used in Indian medicine to treat inflammation, rheumatism, colic, diabetes,

hypertension, and sepsis (Acharya et al., 2016).

The banana inflorescence is widely used as food in southern India, Malaysia, Taiwan, Srilanka, Indonesia, some of the African and South American countries (Lau et al., 2020). The banana inflorescence reduced blood glucose levels in diabetic rats, as well as HbA1C levels demonstrating its beneficial property in diabetes (Nisha & Mini, 2013). The Xokleng Indians, in the Southeastern of Brazil, use the banana inflorescence, popularly known as *mangará, coração da banana*, to ameliorate respiratory tract problems like asthma, but also in diarrhea, ulcers, menorrhagia and to maintain gastrointestinal health (Correa et al., 2016).

The banana inflorescence is rich in polyphenols (Lau et al., 2020) as phenolic acids as gallic acid, hydroxyl benzoic acid, protocatechuic acid, gentisic acid, vanillic acid, vanillin, caffeic acid, syringic acid, ferulic acid, p-coumaric acid, chlorogenic acid, sinapic acid and catechol

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(Ramu et al., 2014). Besides, flavonoids as catechin, epicatechin, quercetin, and rutin are found in banana inflorescence (Silva et al., 2017).

Brazil is the world's fourth-largest banana producer, after India, China, and Indonesia. The annual production reached 18.2 million tons of the fruit in an area of approximately 520.000 ha, of which more than 95% of the production is destined for the domestic market (Silva et al., 2017). Regarding Brazilian production, the disposal of the inflorescence is estimated at 1.12×10^5 tons, however, if treated properly would be able to surpass the revenue produced by the fruit, increasing the profitable capacity of the plant (Vilhena et al., 2019).

In this context, the banana inflorescence extract was analyzed in an experimental model of combined allergic rhinitis and asthma syndrome (CARAS) due to the world clinical importance of allergic respiratory diseases (Cavalcanti et al., 2020; Paiva Ferreira et al., 2019). Indeed, about 10 to 30% of the world population has allergic rhinitis and more than 300 million people have asthma. Also, about 40% of individuals with rhinitis may develop asthma at some point in their lives and about 80% of asthmatic patients have concomitant allergic rhinitis compromising their quality of life (Li et al., 2018; Tohidinik et al., 2019).

Up to now, there is no specific therapy to treat CARAS, and the usual treatment is the association of therapeutically protocols for asthma and rhinitis. Therefore, to contribute to the discovery of alternative treatment, the anti-inflammatory and immunomodulatory potential of the banana inflorescence extract was investigated in an experimental model of combined allergic rhinitis and asthma syndrome (CARAS), and human macrophages.

2. Material and methods

2.1. Banana inflorescence hydroalcoholic extract and toxicity assays

The hydroalcoholic extract of *Musa paradisiaca* L. (HEM) was obtained at the Phytochemical Laboratory of the Program for Natural and Bioactive Synthetic Products /Federal University of Paraíba / João Pessoa, Paraíba, Brazil. The inflorescence of *Musa paradisiaca* L. was collected from domestic crops after the formation of the fruits (geographical coordinates 7°08'14.6"S34° 52'26.1" W) in the Jaguaribe neighborhood, João Pessoa, Paraíba, Brazil. The fresh material (1.315 kg) was cut into small pieces to a percolator-type macerator (Amtech India Conical Percolator stainless steel 10,000 mL). The extraction was made with alcohol 80% and the percolator was kept at room temperature. After three days, the alcoholic solution was removed and a new alcohol solution was added for another three days. The extracted solution was subjected to rotary evaporation to remove the solvent and at the end of the process was obtained 147 g of the crude extract. The dry weight of the extract was obtained at 60 °C for a period of 24 h, and corresponded to 23.54% per 1 g of the crude extract (HEM). The HEM showed easy dispersion in saline solution, and two polar fractions (ethyl acetate and n-butanolic) of the extract were analyzed by HPLC-MS/MS (gradient mode). The mass spectrometer had an electrospray ionization source set in negative and positive ionization mode. The compounds disaccharide (HCl adduct), gallic acid, protocatechuic acid, 4-O-caffeoylshikimic acid I, ethyl gallate, quercetin-hexose, myricetin rhamno-hexoside, rutin, Hydroxycinnamic acid esters I, II, III, and kaempferol-O-rutinosídeo were identified by the interpretation of their fragmentation patterns (MS2 and MS3) and correlations of their corresponding data with scientific literature (Supplementary material). The toxicity assays for the HEM were carried out following the guidelines of the OECD, and the HEM presented LD50 > 5.000 mg/kg without genotoxicity or psychosomatic alteration.

2.2. Experimental model of combined allergic rhinitis and asthma syndrome (CARAS)

Female BALB/c mice (6–8 weeks old and weighing 20 to 25 g) and Wistar rats (200 to 250 g) were used for the experimental protocols. The animals were kept in polypropylene cages at a temperature of 21 ± 1 °C and were subjected to 12-hour light / dark cycles with free access to water and food throughout the experimentation period. The combined allergic rhinitis and asthma syndrome (CARAS) model was adapted from the Li and collaborators (Li et al., 2018). Mice (n = 5/per group) were ovalbumin-sensitized (50 μg/mL of OVA, grade V, SIGMA Chemical, St. Louis, MO, USA, 10 mg/mL Al(OH)$_3$, VETEC, Rio de Janeiro, RJ, Brazil) on days 0 and 12. The OVA-challenge (5% of OVA, grade II, SIGMA Chemical, St. Louis, MO, USA) was realized in an ultrasonic nebulizer and a sealed box from the days 19 to 23. On days 31 to 35, one hour before OVA-challenge, the animals were treated with the extract (HEM, 25, 50 or 100 mg/kg, orally), budesonide (BUD, 1 mg/kg, intranasal) or saline (basal group). The animals were supplied from the Research Institute for Drugs and Medicines (IPeFarM) production unit at UFPB/ João Pessoa-PB, Brazil. The Ethics Committee for Animal Use (CEUA)/ UFPB approved the experimental protocols (N°. 7948040618, ID 000261).

2.3. Rhinitis signs

The sneezing and nasal rubbing were quantified according to Cavalcanti and collaborators (Cavalcanti et al., 2020). Thereby, sneezing was identified as an explosive shortly exhalation and nasal rubbing was identified by itchy movements in the nasal region with the animal's forelimbs. The animals were observed for the rhinitis signs right after the last treatment and OVA challenge at day 35 of the CARAS experimental model. For the counting of the rhinitis signs each animal was placed in a transparent container and filmed for a period of 10 min.

2.4. Nasal hyper-reactivity induced by exogenous histamine administration

Nasal hyper-reactivity to exogenous histamine administration was determined by quantifying the clinical signs, sneezing and nasal rubs for 10 min, after administration of increasing doses of histamine (0.1, 1, 10, 100 and 1000 nmol/animal) by intranasal route. On day 35th, one hour after the OVA-challenge, 4 μL of the increasing doses of histamine were instilled in the nasal cavity in an interval of 60 min. among them and all exposure times were filmed for further evaluation (Cavalcanti et al., 2020).

2.5. Nasal (NALF) and bronchoalveolar (BALF) lavage fluids

On the 36th day of the CARAS protocol, the NALF and BALF were harvested as following: we added 1 mL or 1.5 mL of the cooled HBSS buffered solution into the nasal and lung respectively. The NALF and BALF were removed and put them into micro tubes, centrifuged (Centrifuge MP4R, IEC) at 1500 RPM, 4 °C for 10 min. The supernatants were harvested, and frozen at −20 °C for the cytokine quantification and the cells on the pellet were resuspended and counted the total and differential leukocytes. For the peripheral blood harvested, an incision in the tail vein was made and blood smearing was performed. The leukocytes was identified by the rapid panotic kit (Renylab Chemical and Pharmaceutic - São Pedro, Barbacena - MG, 36205-6660).

2.6. Cytokines and IgE-ovalbumin specific quantification

IL-4, IL-13, IL-5, IL-33, IL-10, IL-17A, and IFN-γ were quantified into the BALF by ELISA assay according to the manufacturer specification (BIOSCIENCE, Inc. Science Center Drive, San Diego, CA-USA) and the OVA-specific IgE titer was measure according to the methodology described by Cavalcanti and collaborators (Cavalcanti et al., 2020).

2.7. Histological analysis

The histological characteristics of the lung and nasal tissues were

assessed by collecting the organs right after the euthanasia. The heads were subjected to the EDTA descaling process for 14 days. The organs were embedded in the buffered formalin fixing solution for 24 h. Then, they were identified and submitted to histological processing in alcohol, xylol, and paraffin baths. Tissue sample cuts (5 μm) on slides were rehydrated for staining with hematoxylin-eosin (HE), Periodic Acid Shiff (PAS), Toluidine Blue (TB) or Gomori Trichrome (GT). The representative photomicrographs of each organ were collected at a software Motic Images plus 2.0. To perform the histological score, five slides from each animal group were analyzed for each color according to the evaluated parameter. Scores were assigned according to the level of changes following the classification determined by Cavalcanti and collaborators (Cavalcanti et al., 2020).

2.8. Human cell culture and treatment

Human primary monocytes (500.000 cells/wells) were isolated from healthy blood donors using a RosetteSep monocyte enrichment cocktail (Stem-Cell Technologies), as previously reported, with over 70–80% of CD14 + cells (Oliveira et al., 2012). Therefore, monocytes were plated with complete culture media (RPMI-1640 + Glutamax, Invitrogen, 10% heat-inactivated Fetal Bovine Serum-FBS, Lonza) and 1% penicillin G/streptomycin (P/S, Invitrogen) and maintained in a humidified incubator, at 37 °C and 5% CO_2. On the tenth day of the experiment, the cells were incubated with the media culture alone or with HEM (0.1, 1, 10, 100 or 1000 ug/mL), and on the thirteenth day they were submitted to different protocols. In addition, the cells were exposed to lipopolysaccharide (LPS) or IL-4 with or without the HEM filtered (0.45 μm millipore filter) (1, 10 or 100 μg/mL) and the expression of macrophage superficial molecules was evaluated by flow cytometry.

2.9. Cell viability and toxicity

The macrophages were incubated with HEM for 72 h and after this period it was added resazurin (1/10 of the volume of medium) in each well of the black plate with transparent background. After four hours, it was added 100 μL of the culture medium in each well of the plate and analyzed in a fluorescence spectrophotometer (Mx-Sinergy) with excitation at 530 nm and emission at 590 nm using GEN5 software. Then, the cells were fixed in paraformaldehyde (PFA 4%), stained with phalloidin (Cat #P3457 Termofisher), propidium iodide (Cat #P1304MP TermoFisher), or DAPI (Cat#62248 TermoFisher), and photographed using an immunofluorescence microscope and AxionVision software.

2.10. Flow cytometry analysis

Cells (2.5×10^5 cells) from the BALF of sick (OVA group), healthy (Basal group) or treated animals (HEM or BUD groups) were fixed, permeabilized, and stained according to the manufacturer's instructions. The anti-p-p65-NF-κB (Cat# 558,423 PE BD Phosflow) antibody was used for the intracellular labeling. Ten thousand events were analyzed by a flow cytometry (BD FACSCANTO II), Cell Quest software, Data was analysed with FlowJo software. The percentage of cells showing p-p65 for each sample was represented in dot-plot. For the human macrophages, the specific antibodies (anti-human-CD14-APC (Cat #21270146 Immunotools), anti-human-CD86-FITC (Cat #21480863 Immunotools) and anti-human-CD206-PE (Cat#12-2061-82 EBioscience), anti-human-HLA-DR-eFluor450 (Cat#564244 BD Horizon™) and anti-human-CD163-PE (Cat#556018 BD Pharmigen) were added in each well of the plate and incubated for 2 h. After this period, it was added PBS/2%FBS/0.01% sodium azide in each well and incubated for 30 min on ice and in the dark. The cells were washed three times with buffer solution, analyzed by flow cytometry (BD FACSCANTO II) and Cell Quest software and the data were analyzed with FlowJo. As negative controls, isotype-matched antibodies were used, to define background staining. Mean fluorescence intensity (MFI) values were calculated by subtracting the respective isotype control.

2.11. Ethics statement

The human biological samples used in this work and all the procedures followed to obtain them were according to the principles of the Declaration of Helsinki. Surplus buffy coats (BC) from healthy blood donors used for monocyte isolation were kindly donated by Serviço de Imunohemoterapia, Centro Hospitalar Universitário de São João (CHUSJ), Porto, Portugal. All experimental protocols were conducted following the approval and recommendations of the CHUSJ Ethics Committee for Health (reference 90/19). Buffy coats were provided anonymised and donor identification was not provided to researchers.

2.12. Statistical analysis

The statistical tests were chosen according to the type of sample. To analyze the normality of the results we used the D'Agostino & Pearson, Shapiro-Wilk and KS tests. To analyze the results of the *in vivo* experiments the non-parametric one-way analysis of variance (ANOVA) method followed by Tukey's post-test for multiple comparisons. To analyze the results of histological scores, we used the Kruskal-Wallis test followed by Tukey's post-test for multiple comparisons. To analyze the results of the in vitro experiments, we used the Friedman test followed by Dunns post-test to compare different groups. The results were analyzed using the GraphPad Prism software version 5.0 (GraphPad Software Inc., San Diego, U.S.A.), the values for $p < 0.05$ were considered significant and expressed as mean ± standard error of the mean (S. E.M.).

3. Results

3.1. Effect of HEM on inflammatory cell migration to the nasal, bronchoalveolar cavities and peripheral blood

Sick animals (OVA group) showed an increase ($p < 0.001$) in the total inflammatory cell infiltration in the NALF and the BALF as compared to healthy animals (basal group) (Fig. 1A, 1B, respectively). Treated animals (50 or 100 mg/kg of HEM) showed a significant ($p < 0.01$) decrease of inflammatory cells in NALF (Fig. 1A); however, the dose of 25 mg/kg of HEM did not affect cell migration. On the other hand, only the dose of 100 mg/kg of HEM was able to reduce significantly ($p < 0.01$) the total inflammatory cell migration in the NALF and the BALF (Fig. 1A and 1B). Therefore, the dose of 100 mg/kg of the extract was chosen to pursue with cell phenotype analysis. The eosinophil migration increased in the NALF (33.2 times), in the BALF (18.9 times), and in the peripheral blood of sick animals, and the HEM treatment was able to reduce this cell population in both cavities by 73,74% and 72,35%, respectively, as well as in the peripheral blood (Fig. 1C, 1D, 1E, respectively). Similar results were observed with the pattern drug budesonide (BUD).

3.2. Effect of HEM on rhinitis signs and on nasal hyper-reactivity induced by exogenous histamine

The rhinitis signs as nasal rubbing and sneezing were significantly ($p < 0.001$) developed in sick animals right after the OVA challenge as compared to healthy animals. On the other hand, the HEM or budesonide (BUD) treatment decreased ($p < 0.001$) the number of these two allergic rhinitis signals (Fig. 1F and 1G). These preliminary data indicated the anti-allergic potential of the extract in CARAS. Thereby, the nasal hyper-reactivity to exogenous histamine was analyzed by administrating increases doses of histamine in sick animals and counting both allergic rhinitis signals (Fig. 1H and 1I). The HEM or BUD treatment inhibited ($p < 0.001$) the development of the allergic rhinitis signals, therefore, decreasing the nasal hyperactivity to histamine (Fig. 1H and
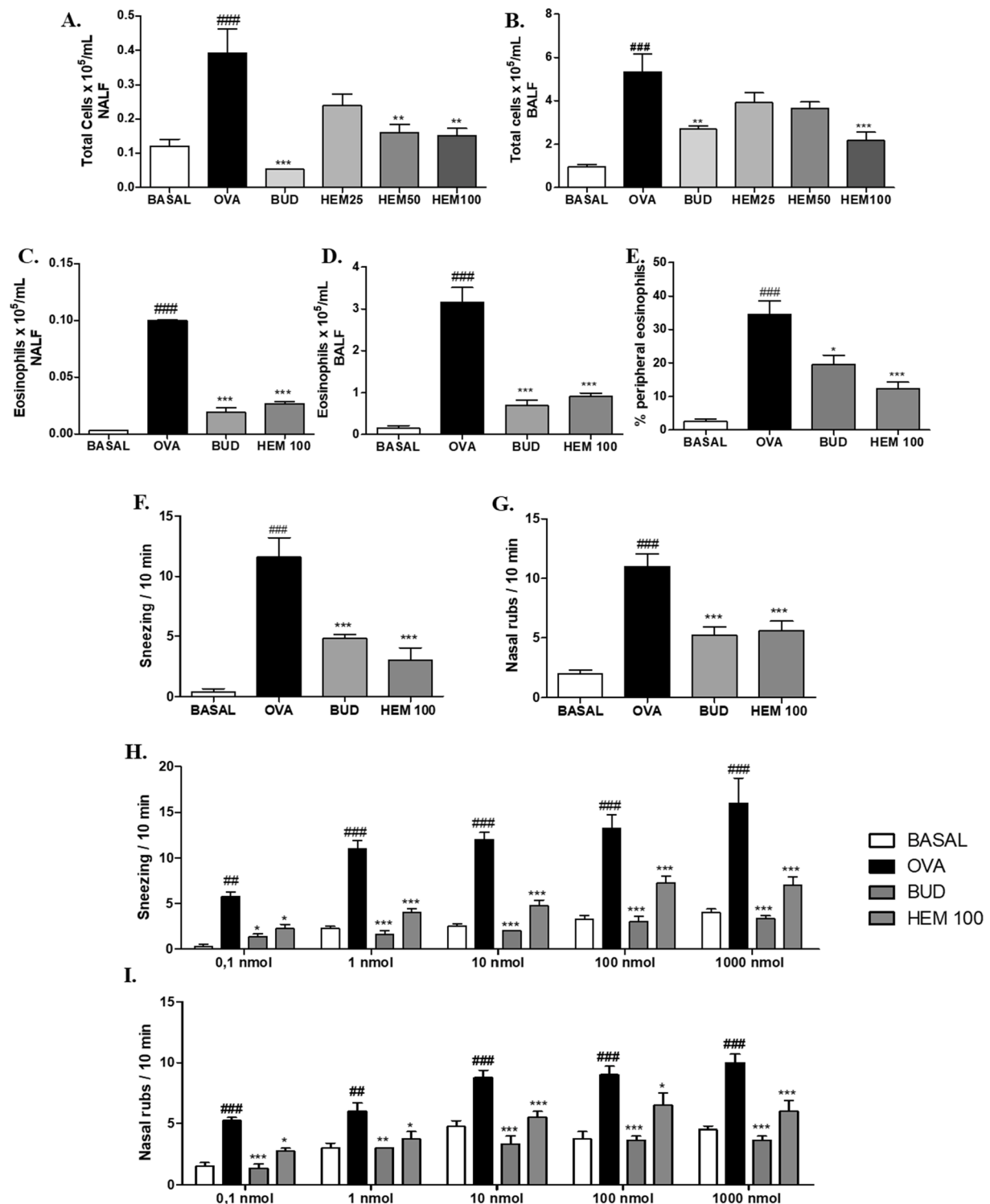
Fig. 1. Effect of *Musa paradisiaca* L. inflorescence hydroalcoholic extract (HEM) on inflammatory cells on airway tissues and blood, on rhinitis signs and nasal hyperactivity induced by exogenous histamine of in a combined allergic rhinitis and asthma syndrome (CARAS) in mice. (A, B) Number of total inflammatory cells in the nasal (NALF) and bronchoalveolar (BALF) fluids; (C, D) number of eosinophils in the NALF and the BALF, respectively; (E) relative number of eosinophils in peripheral blood; (F,G) Number of sneezing and nasal rubs on CARAS model; (H, I) number of sneezing and nasal rubs on exogenous histamine (0.1, 1, 10, 100 and 1.000 nmol)- induced nasal hyperactivity. The animal groups (n = 5) were distributed in healthy animals (BASAL), sick animals (OVA) and treated animals (HEM – 25, 50 or 100 mg/kg; budesonide – BUD – 1 mg/kg). The data were represented as mean ± SEM where the significant value for $^{###}p < 0.001$ was the comparison of the OVA group with the BASAL group and the significant value for $***p < 0.001$, $**p < 0.01$ or $*p < 0.05$ was the comparison of the treated groups (HEM or BUD) with the OVA group. Data were analyzed using one-way ANOVA followed by Tukey's post-test for multiple comparisons.

1I).

3.3. Effect of HEM on inflammatory signs of airway tissues

The nasal histological analysis of sick animals demonstrated cellular infiltration (blue triangle) in the nasal fossae region adjacent to the cartilaginous tissue, inflammatory vasodilation (green triangle) with increased vascular permeability, and loss of lining epithelium continuity (Fig. 2A, HE dye) as compared to nasal tissue of healthy animals. Also,

we observed, exacerbation of mucus production as a result of goblet cell hyperplasia (yellow star), increasing of mast cells in the nasal tissue (open red circle), and intense nasal tissue remodeling with collagen fiber deposition (red dot) (Fig. 2A, PAS, TB, and GT dyes, respectively). The quantification of the inflammatory parameters observed in the nasal tissues is in Fig. 2B. The HEM or BUD treatment of sick animals improved all the inflammatory parameters describe above (Fig. 2A and 2B). In the lung tissue of sick animals, we observed disruption of the lung architecture with perivascular (green triangle) and peribronchiolar
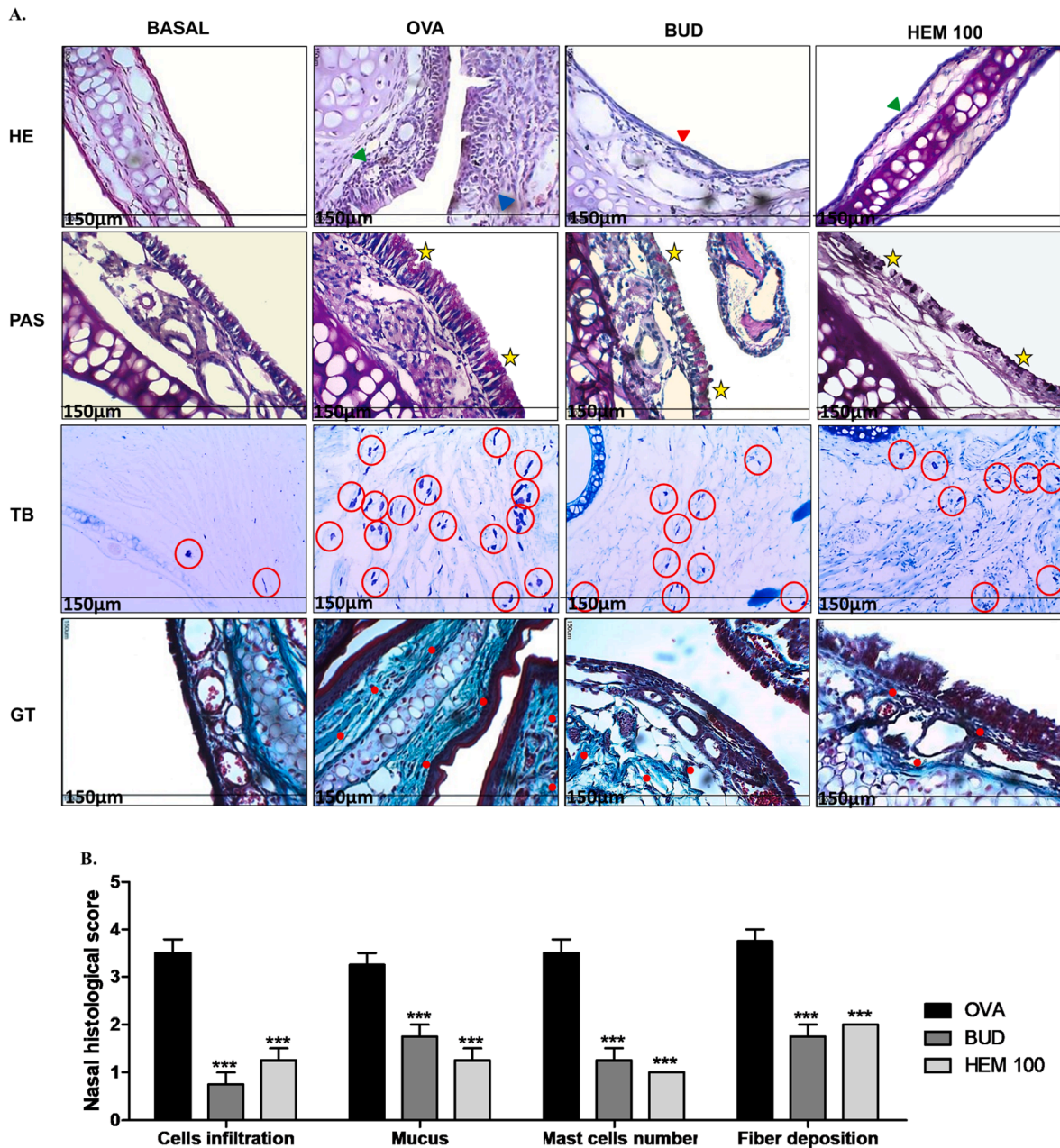

Fig. 2. Effect of *Musa paradisiaca* L. inflorescence hydroalcoholic extract (HEM) on inflammation parameters on airway tissues. Histological analysis (A, B) nasal tissues and inflammatory scores; (C, D) lung tissues and inflammatory scores (see material methods, section 2.7). The animal groups (n = 5) were distributed in healthy animals (BASAL), sick animals (OVA) and treated animals (HEM-100 mg/kg; budesonide – BUD 1 mg/kg). Photomicrographs are representative of five animal tissues per group. The hematoxylin and eosin (HE), periodic acid Shiff (PAS), toluidine blue (TB) and gomori trichrome (GT) stains were used to visualize any specific tissue alterations. The color signs on the photomicrographs indicate: the blue triangles, inflammatory cell infiltration; the green triangle, vasodilation; the red triangles, epithelium; yellow stars, mucus; the red circles, the mast cells; and the red points, deposition of collagen fibers. Histological analysis was performed using optical microscopy (objective 40x). The photomicrographs were obtained using the software Motic Images Plus 2.0 in an optical microscope (objective 10x and 40x). The histological scores were represented as mean ± SEM where the significant value for ***p < 0.001 or **p < 0.01 was the comparison of the treated (HEM or BUD) groups with the OVA group. Data were analyzed using one-way ANOVA followed by Tukey's post-test for multiple comparisons. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
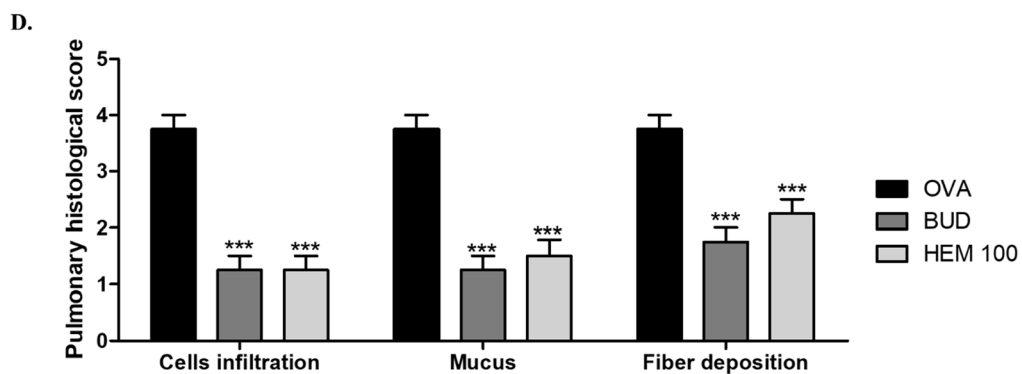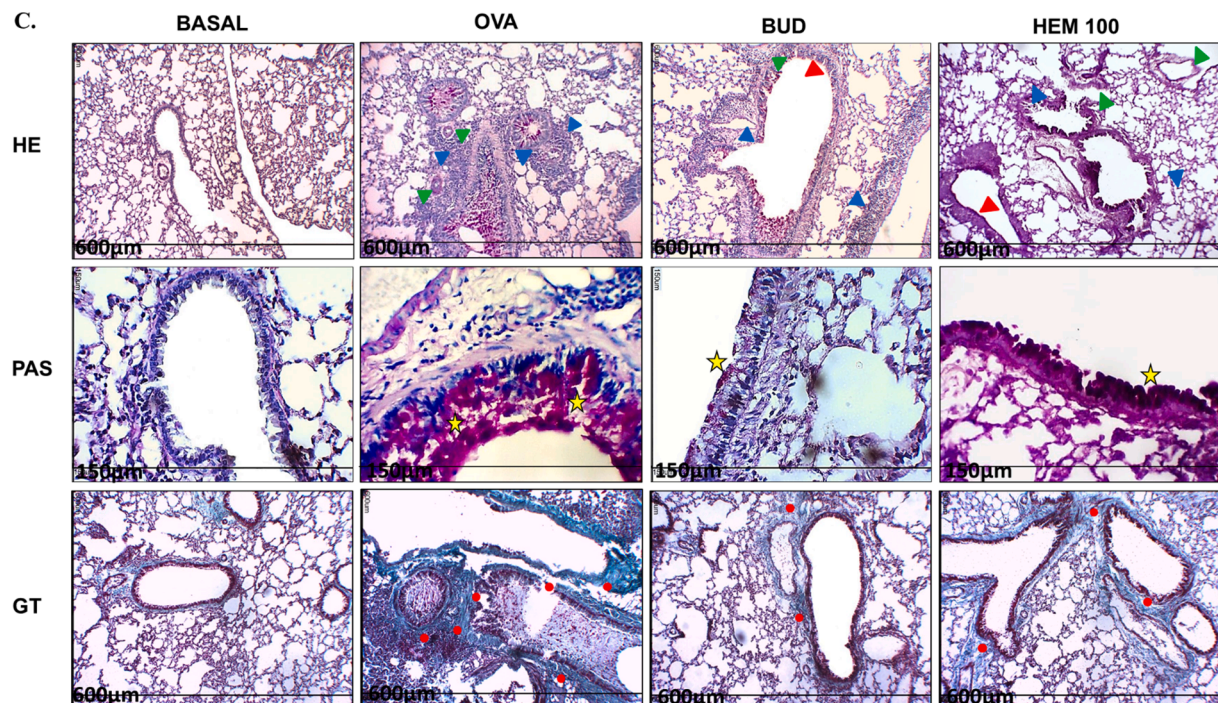
Fig. 2. (*continued*).

(blue triangle) cell infiltration (Fig. 2C, HE dye), decreasing of the bronchiole lumen with overproduction of mucus, and collagen fiber deposition mainly in the regions with sizeable inflammatory cells agglomerates. HEM or BUD treatment led to a significant (p < 0.001) decrease of the pulmonary tissue damage with the reestablishment of the lung architecture (Fig. 2C and 2D, PAS, and GT dyes, respectively).

3.4. Effect of HEM in cytokine production and serum OVA-specific IgE titer

The type 2 (IL-4, IL-13, IL-5), 3 (IL-17A) cytokines as well as IFN-γ were significant (p < 0.001) increases in the BALF of sick animals as compared to healthy animals (Fig. 3A, 3B, 3C, 3D, 3E, respectively). However, when these animals were treated with HEM or BUD presented a significant (p < 0.001) decrease of these cytokines independently of IFN-γ. The amount of IL-10 and IL-33 did not change among the groups (basal, sick, HEM or BUD) (Fig. 3F, 3G). Besides, the amount of OVA-specific IgE into the plasma of treated animals (HEM) was diminished (p < 0.001) as compared to sick animals (Fig. 3H).

3.5. Effect of HEM treatment on activated (p65) NF-κB in the BALF cells

The phosphorylated or activated form of (p p65)NFκB was evaluated in the cells of the BALF of all animal groups (Fig. A). Animals from the

OVA group showed an increase in the frequency of p-p65, in their cells, in about 51.6% as compared to the healthy animals (p < 0.001) (Fig. 4B). On the other hand, the HEM treated animals showed a reduction of 24.4% in the frequency of the activated molecule as compared to the OVA group (p < 0.001) (Fig. 4B).

3.6. Effect of HEM in human peripheral monocytes

Human peripheral blood monocyte-derived macrophages were exposed, in vitro, to nonfiltered HEM (0.1, 1.0, 10, 100 or 1.000 μg/mL) or filtered HEM (0.1, 1.0, 10 or 100 μg/mL) to analyze their immunomodulatory effect. The unfiltered or filtered HEM did not induce macrophage morphological alterations except the nonfiltered HEM at 1.000 μg/mL that induces disruption on the cell membrane and binucleation or cell death as illustrated in phalloidin-PI staining (Fig. 5A). Besides, resazurin reduction assay indicated that both (nonfiltered or filtered) HEM at 0.1, 1.0, 10, or 100 μg/mL led to metabolic activity values above 90%, while unfiltered HEM at 1.000 μg/mL decreased cell viability by 50% (Fig. 5B, 5C).

3.7. Immunomodulatory effect of HEM on human macrophages

The M1 / M2 macrophage phenotypes were analyzed by looking at the characteristic receptors (Fig. 6 A). The HEM treatment did not
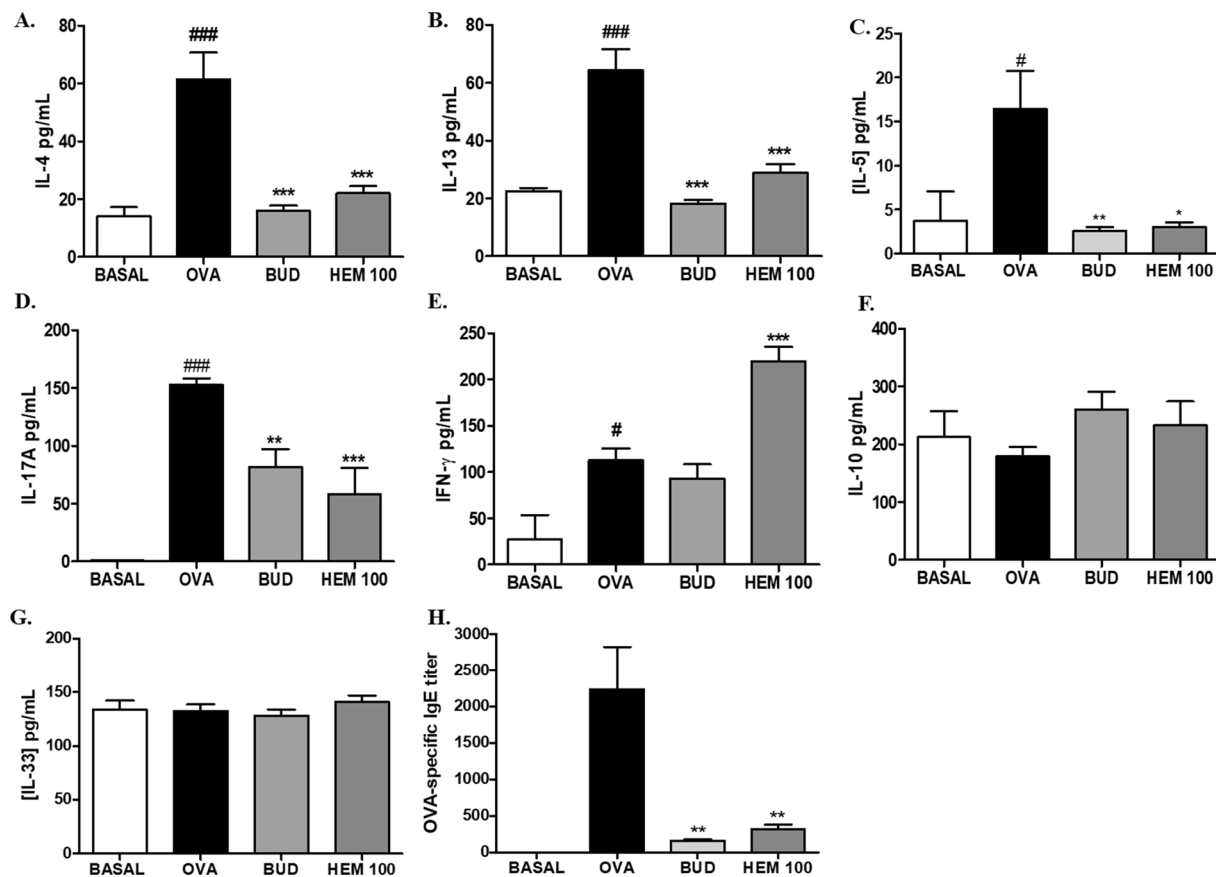
Fig. 3. Effect of *Musa paradisiaca* L. inflorescence hydroalcoholic extract (HEM) on cytokines and serum OVA-specific IgE titer. The cytokines IL-4 (A), IL-13 (B), IL-5 (C), IL-17A (D), IFN-ɣ (E), IL-10 (F) and IL-33 (G) were measured on the bronchoalveolar lavage fluid (BALF) and the OVA-specific IgE titer was measured on the serum of all animal groups. The animal groups (n = 5) were distributed in healthy (BASAL), sick (OVA) and treated animals (HEM-100 mg/kg; budesonide – BUD- 1 mg/kg). The data were represented as mean $\pm$ SEM where the significant value for ###p < 0.001, ##p < 0.01 or #p < 0.05 was the comparison of the OVA group with the BASAL group and the significant value for ***p < 0.001, **p < 0.01 or *p < 0.05 was the comparison of the treated groups (HEM or BUD) with the OVA group. Data were analyzed using one-way ANOVA followed by Tukey's post-test for multiple comparisons.

activate M1 or M2 phenotypes, as there were no increases of CD86 and HLA-DR receptors (M1 phenotype) or CD163 receptor (M2 phenotype) (Fig. 6B). Lipopolysaccharide (LPS)-stimulated macrophages presented an increase of CD86 (p < 0.05) and HLA-DR receptors (p < 0.001), and HEM at 100 µg/mL significantly (p < 0.05) decreased both markers (Fig. 6B). However, IL-4-stimulated macrophages in presence of HEM did not alter the amount of CD206 receptor, a characteristic IL4-stimulated macrophage marker (M2 phenotype) (Fig. 6C).

4. Discussion

In this study, we demonstrate the immunomodulatory and anti-inflammatory effects of the hydroalcoholic extract of *Musa paradisiaca* (HEM) inflorescence in an experimental model of combined allergic rhinitis and asthma syndrome (CARAS).

The HEM, oral treatment, reduced the allergic rhinitis signs as sneezing and nasal rubbing, nasal histamine-induced hyperreactivity, mucus production dependent on decreasing of goblet cell hypertrophy/hyperplasia and mast cell modulation. We also observed a decrease of airway inflammatory cell infiltration, mainly eosinophils, collagen fibers deposition in airway tissues, and decreasing of Th2 and Th17 cytokine profiles dependent on NF-κB downregulation, besides an increase of IFN-ɣ, a type 1 cytokine profile (Th1). At a systemic level, the HEM decreased the OVA-specific IgE serum titer and eosinophilia. However, the CARAS attenuation signs by HEM were independent of the regulatory cytokine profile (IL-10 or IL-33).

Besides, the in vitro experiments, using human activated

macrophages, showed that the HEM down-regulated the LPS-activated macrophages (M1 phenotype) by decreasing the CD86 and HLA-DR cell surface markers independently of the IL-4-stimulated macrophages (M2 phenotype) regulation. The HEM had low toxicity in human peripheral blood monocytes and did not activate the cells.

The clinical symptoms as sneezing, nasal friction, fluid leakage, edema formation, as well as wheezing, and shortness of breath of allergic rhinitis and asthma in CARAS are essential for allergic patient diagnosis. In experimental models of allergic rhinitis, a decrease of clinical signs, as sneezing and nasal friction are parameters to define the effectiveness of a drug, molecule, or extract treatment (Alrifai et al., 2014). Indeed, some studies using an OVA-induced allergic rhinitis experimental model and compounds from the banana inflorescence as kaempferol, gallic acid, and quercetin reduced the rhinitis signs by diminishing of allergen IgE production, mast cell histamine release, and by inducing the Th1-skewed cytokine production (Oh et al. 2013; Ding et al.2019; Fan et al. 2019). The reduction of these parameters by the HEM in animals with CARAS, demonstrates that the compound of the extract are acting on upper airway resident cells, on histamine receptors at nearby nerves, or through other receptors leading to rhinitis signs inhibition.

The nasal hyperreactivity induced by exogenous administration of histamine in allergic animals induces strong rhinitis signs. The HEM treatment reduced these rhinitis signs inhibiting the histamine effect. Thereby, banana inflorescence used by the Brazilian population to ameliorate upper respiratory allergies has scientific support. Scientific evidence that demonstrated the histamine inhibitory effect by
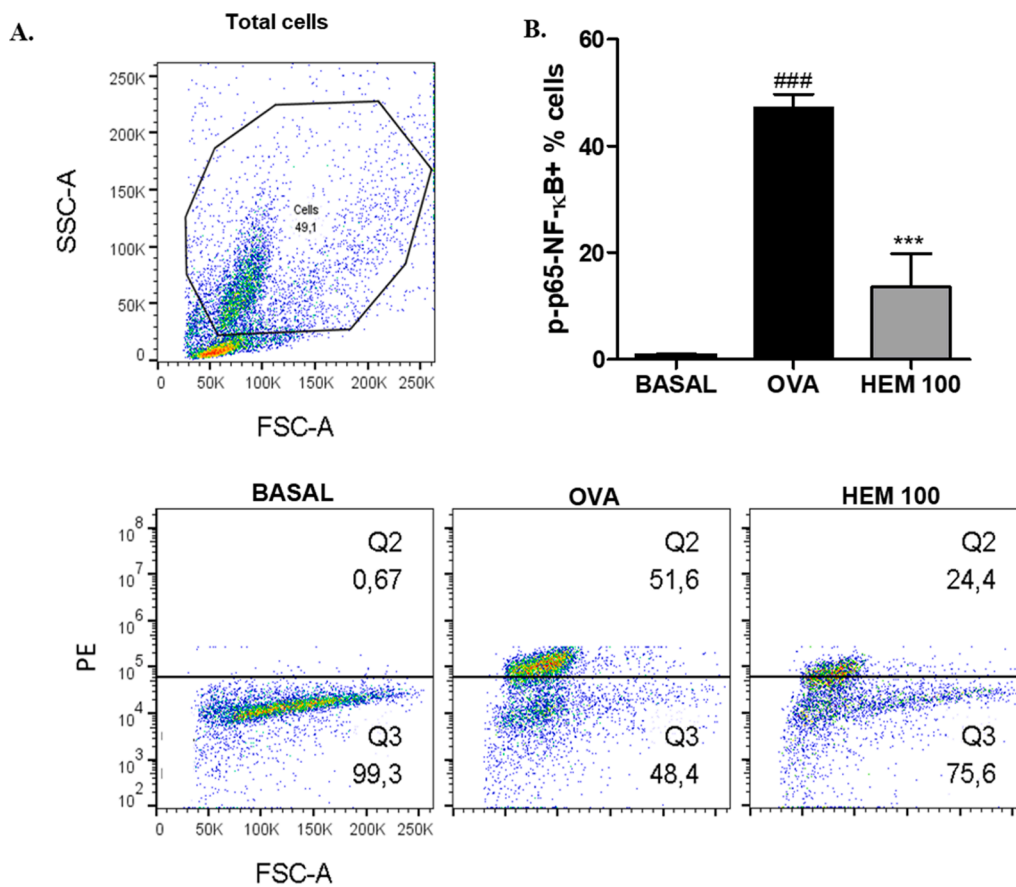
Fig. 4. Effect of *Musa paradisiaca* L. inflorescence hydroalcoholic extract (HEM) on the activation of the transcription factor NF-κB (p65) in all BALF cells (A). The horizontal axis represents the FSC-A of the cells and the vertical axis represents the fluorescence intensity. The animal groups (n = 5) were distributed in healthy (BASAL), sick (OVA) and treated animals (HEM-100 mg/kg). A gate was performed on the all populations of cells, and the percentage of p-p65-NF-κB + cells was analyzed. Values in each quadrant represent the percentage of cells in that quadrant. Bar graphs represent the percentage of expression (B) for the cell marker p-p65 in total 15 experimental animals. The data were represented as mean ± SEM where the significant value for ###$p < 0.001$ was the comparison of the OVA group with the BASAL group and the significant value for ***$p < 0.001$ was the comparison of the treated groups (HEM or BUD) with the OVA group. Data were analyzed using one-way ANOVA followed by Tukey's post-test for multiple comparisons.

compounds of banana came from a study of Kahraman and colleagues (Kahraman et al., 2003) that demonstrated the binding of the quercetin decrease mast cells and histamine levels leading to inhibition of its effect in a gastric ulcer experimental model. Recently, study has demonstrated that quercetin decreased histamine 4 receptor-induced calcium influx through the TRPV1 channel showing a molecular mechanism of the molecule in itching and inflammatory processes as well as in an unpleasant sensations (Yang et al., 2021). Corroborating this result, myricetin attenuated the histamine-induced vascular permeability and NF-κB activation (Vo et al., 2020).

The inflammatory cell migration, mainly eosinophils, to the airway tissues during allergic processes is a potential target for the control of the allergy (Paiva Ferreira et al., 2019). The cellular products released in the submucosa induce epithelial damage in the nasal tissue with the loss of its continuity as well as in lung tissues mainly in the perivascular, peribronchial, and peribronchiolar regions. Indeed, changes such as vasodilation and increases in vascular permeability promote micro edema in vascular regions. In this study, the HEM treatment of sick animals led to a decrease of eosinophil infiltration in the upper and lower airway tissues and peripheral blood, and ameliorate the morphological aspect of the airway tissues. These results indicate that the HEM is acting at least in vessels inhibiting the vasodilation or acting in endothelial cells diminishing the adhesion molecule expression. These studies will be carry out to demonstrate these hypotheses.

Some studies with chemical compounds from the banana as protocatechuic acid and rutin demonstrated that they decreased the inflammatory cell migration to the lung tissue of OVA-sensitized mice in the asthma experimental model. These effects were dependent on the inhibition of the phosphorylation of (p65) NF-κB and down-regulation of the Th17 cytokine profile, respectively (Liu et al., 2018; Wei et al., 2013). Even thought, we did not study isolated compounds from the extract, the HPLC analyses demonstrated that the HEM presents these compounds,

which may partially promote these effects.

In addition, activated eosinophils release mediators as eosinophilic cationic protein, main basic protein, eosinophil chemotactic factor (ECF), and various cytokines including the type 2 as IL-4, IL-5, and IL-13 (allergy hallmark) that lead to the exacerbation of allergy and chronicity of the inflammatory process in CARAS (Kaur & Chupp, 2019; Shin et al., 2019). The HEM was effective in reducing the type 2 cytokine production in sick animals towards a Th1 immune response with IFN-γ production without interfering with the regulatory cytokine profile (IL-33 or IL-10). The balance between Th1 and Th2 immune responses is the key for the immune system homeostasis however, in the allergic process the Th2 immune response is exacerbated, therefore its regulation by the Th1 immune response in the allergic patient improves the clinical symptoms (Wisniewski et al., 2018).

The HEM also decreased the production of IL-17A a type 3 cytokine profile. This cytokine is produced by several resident cells in allergic rhinitis and asthma and is implicated in asthma resistant to glucocorticoids (Palumbo et al., 2020). The presence of IL-17A into the bronchoalveolar lavage of allergic individuals demonstrates a synergism effect between type 2 and type 3 immune responses in severe allergic syndrome (Kamali et al., 2019). There are some studies demonstrating that the banana isolated compound, gallic acid decreased the IL-17A production dependent on the IFN-γ production. This inhibitory effect in allergic rhinitis is related to the blockade of the IL-17AR receptor diminishing the bronchial hyper responsiveness in an experimental model of severe asthma (Fan et al., 2019). Here we demonstrated that the extract of the disposable banana inflorescence is effective in inhibiting a type 3 cytokine putting it in a position of a potent material for the production of a phytomedicine to ameliorate the allergic ongoing symptoms.

Another hallmark of allergies is the production of a high amount of allergen-specific IgE that strongly binds to FcεRI of mast cells, basophils,
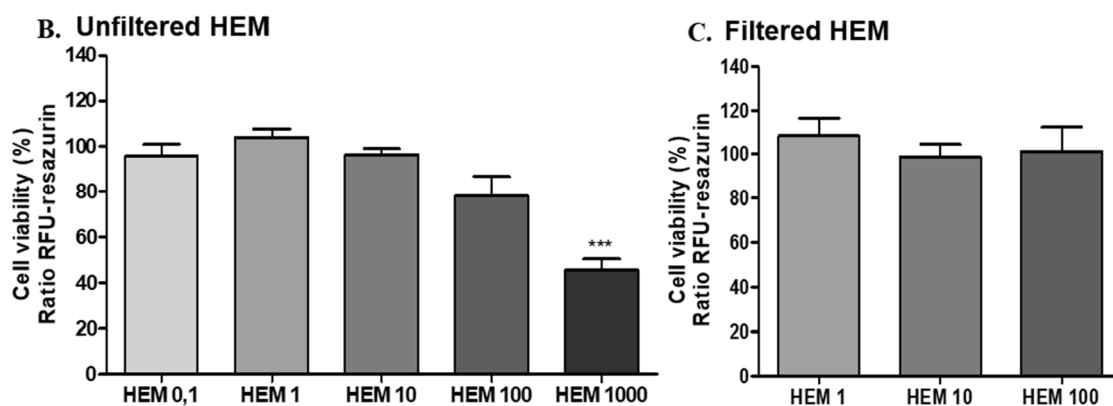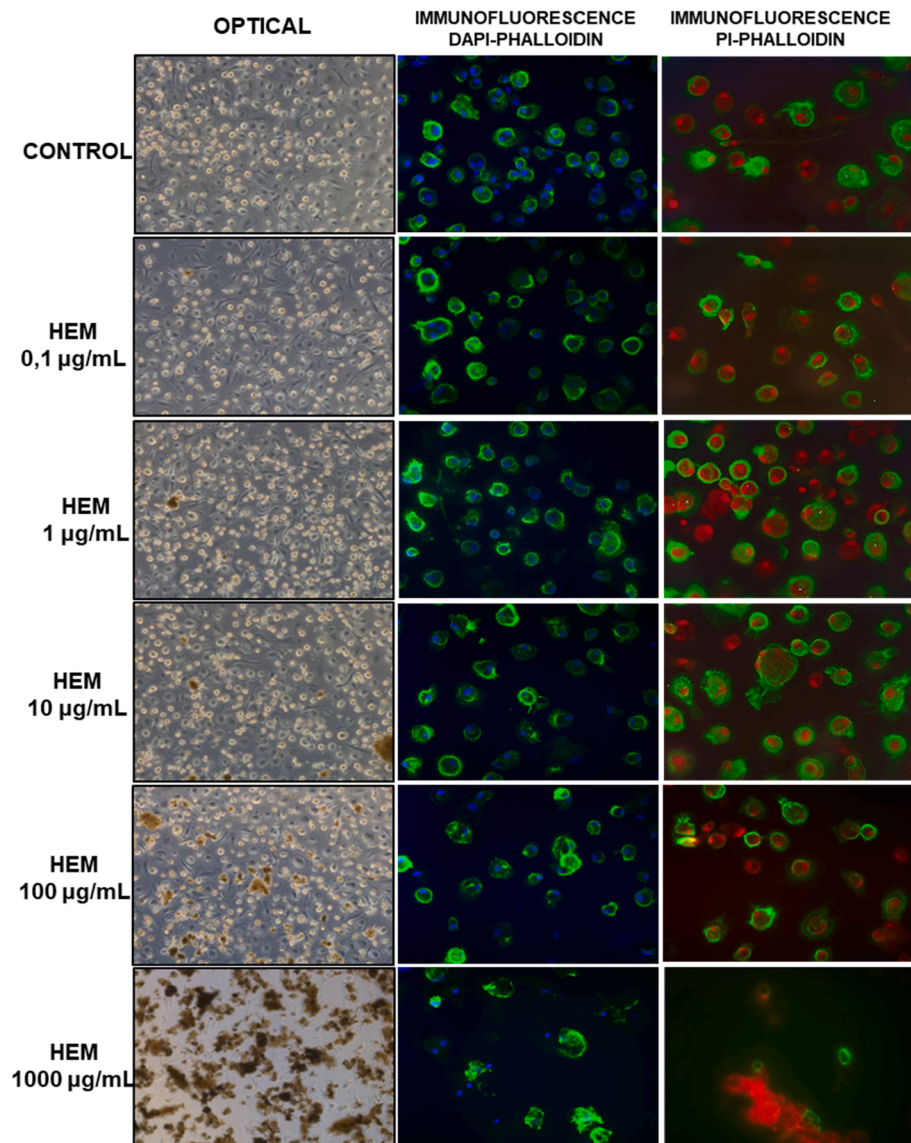
Fig. 5. Effect of *Musa paradisiaca* L. inflorescence hydroalcoholic extract (HEM) on human macrophages. The macrophages were incubated into wells of cell culture plates for three days and exposure to HEM (0.1, 1, 10, 100 or 1.000 µg/mL). A. photomicrographs of the cells and cells fluorescence stained with phalloidin and DAPI or PI and obtained in an optical microscope (objective 10X) and fluorescence microscope (objective 20X). Cell viability (B, C) was calculated using the ratio of RFU of resazurin (Relative Fluorescence Unit) of the cells in presence of unfiltered HEM or filtered HEM in relation to the RFU of resazurin of cells exposed only to the medium. The data were represented as mean ± SEM where the significant value for ***p < 0.001 was the comparison of the values of the lowest HEM concentration since it had 100% viability. The data were analyzed using the Friedman test followed by the Dunn's post-test for multiple comparisons.
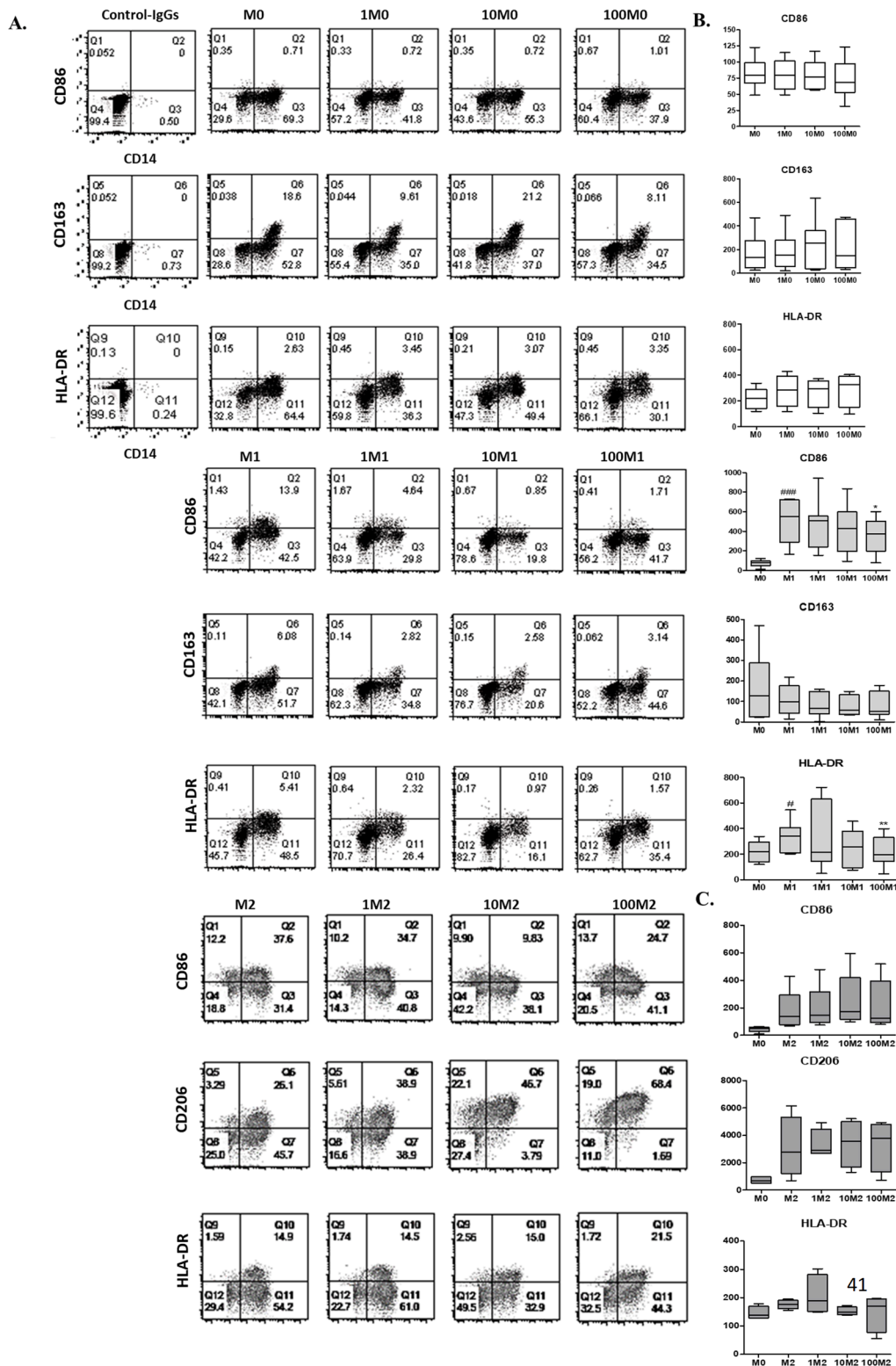
Fig. 6. Effect of *Musa paradisiaca* L. inflorescence hydroalcoholic extract (HEM) on the M1 / M2 macrophage phenotype profiles. Evaluation of the expression of surface molecules in monocytes and macrophages. Monocytes were exposed only medium (M0) or HEM at concentrations: 1, 10 or 100 mg/mL (1 M0, 10 M0, 100 M0). Monocytes were stimulated with LPS (macrophages M1) and exposed to HEM at the same concentrations: 1, 10 and 100 mg/mL (1 M1, 10 M1 and 100 M1). Monocytes were stimulated with IL-4 (macrophages M2) and exposed to HEM at the same concentrations: 1, 10 and 100 mg/mL (1 M2, 10 M2 and 100 M2). **A.** Dot-plots of representative donor. The cells were marked with anti-CD14, anti-CD86, anti-HLA-DR and anti-CD163 or anti-CD206. The mean fluorescence intensity (MFI) of the cells was obtained using a flow cytometry (BD FACSCANTO II) (B and C). The data obtained were analyzed using the software FlowJo and GraphPad Prim 5.0. The data were represented as mean ± SEM where the significant value for ###$p < 0.001$, ##$p < 0.01$ or #$p < 0.05$ was the comparison of the M1 or M2 cells with the M0 cells and the significant value for ***$p < 0.001$, **$p < 0.01$ or *$p < 0.05$ was the comparison of the HEM treated cells with the M1 or M2 cells. The data were analyzed using the Friedman test followed by the Dunns post-test for comparison between cells group.

and eosinophils, and once the allergen binds to the IgE promotes the degranulation process with releases of several vasoactive mediators. This study showed that HEM treatment of sick animals decreased the OVA-specific IgE serum titer and reduced the number of mast cells in the airway tissues. Currently, allergic patients that do not control the progression of the disease to classical therapies are treated with an immunobiological medicine that contain anti-IgE monoclonal antibodies (Bayar Muluk et al., 2019). This therapeutically strategy is expensive, thus a low cost-effective alternative therapy is in high demand, particularly in developing countries putting up the HEM as a potential medicine to be tested in the clinical trial.

In allergic processes, a variety of activated cells induce the translocation of the phosphorylate (p-p65) subunit of NF-κB to the nucleus and induces the gene transcription including inflammatory cytokines, enzymes as COX-2, LOX, iNOS gens that exacerbate the allergic reaction (Helal et al., 2019; Li et al., 2018). In this study, we showed that cells from the bronchoalveolar lavage (BALF) of HEM-treated sick animals decreased the cytoplasmic amount of the p-p65 unit of the NF-κB indicating a down modulatory effect of the extract on the NF-κB signaling pathway. Therefore, taken all these data together, we postulate that the HEM has an immunomodulatory effect by, at least in part, downregulating the NF-κB pathway in CARAS experimental model.

Finally, the HEM was tested in human macrophages, a cell population that is responsible in part for the production of inflammatory mediators that initiate the allergic inflammatory process (Tang et al., 2019). The unfiltered and filtered HEM showed low toxicity with the maintenance of the cell structure and nuclear membrane integrity. However, the unfiltered HEM highest concentration promoted macrophage death and filtered HEM at 1, 10, or 100 μg/mL showed low toxicity with cell metabolic activity above of 90%.

LPS-stimulated macrophages in presence of filtered HEM presented a decrease of two receptors (CD86 and HLA-DR) associated with the M1 macrophage profile independently of CD163 or CD206 regulation on IL-4-stimulated macrophages (M2 phenotype).

A previous report demonstrated that quercetin inhibited CD80, CD86, and HLA-DR receptors of dendritic cells (derived from monocytes) which are essential for the CD4 + T cell activation during the antigen presentation (Lin et al., 2017). Also, kaempferol down modulated the LPS-stimulated macrophages through the NF-κB pathway with inhibition of inflammatory cytokine production (Nam et al., 2017). Besides, the quercetin and rutin, regulated the balance M1/M2 macrophages in an diabetic experimental model and in vitro murne macrophage (RAW 264.7 cells) via the TRPM2 dependent calcium influx and iNOS, respectively (Balaji & Kavasseri Ganesan, 2020; Fu et al., 2020). Despite the HEM did not modulate receptors associated with the IL-4-stimulated macrophages (M2 phenotype), was able to down-regulate M1 macrophages attenuating, therefore, the inflammatory process.

Taken all data together, we postulate that the Brazilian disposable inflorescence of Musa paradisiaca L. (HEM) ameliorated the allergic inflammatory process in the CARAS experimental model acting as an immunomodulatory and anti-inflammatory product. The HEM inhibited the allergic parameters as eosinophil migration to the airway tissues, the Th2/Th17 profiles towards a Th1 profile dependent of NFκB down modulation, and decreased the allergen-specific IgE serum titer as well as allergic rhinitis signs. HEM also modulated the M1 macrophage by downregulating the CD86/HLA-DR cell surface expression independently of M2 macrophage profile modulation. Therefore, these data scientifically demonstrated that a disposable material of the banana crop could be a potential subtract for phytomedicine and be safety tested in clinical trials in the allergic process.

5. Conclusion

The hydroalcoholic extract (HEM) of Musa paradisiaca L. inflorescence from the Brazilian crop improved the allergic process on combined allergic rhinitis and asthma syndrome in mice by suppressing the

Th2 immune response towards the IFN-γ production and by down-regulating the M1 macrophage phenotype independently of M2 macrophage profile. The HEM mechanism of action is, in part, due to the decreasing of the activation of p65 subunit of the NF-κB at the nucleus of the inflammatory cells of the bronchoalveolar lavage fluid.

Ethical statement

CRediT authorship contribution statement

Francisco Allysson Assis Ferreira Gadelha: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Raquel Fragoso Pereira Cavalcanti:** Conceptualization, Methodology, Formal analysis. **Giciane Carvalho Vieira:** Conceptualization, Formal analysis. **Laércia Karla Diega Paiva Ferreira:** Methodology. **Gabriela Ribeiro de Sousa:** Methodology, Formal analysis. **José Maria Barbosa Filho:** Funding acquisition. **Mario A. Barbosa:** Resources, Funding acquisition. **Susana Gomes dos Santos:** Software, Formal analysis, Investigation, Resources, Writing - original draft. **Marcia Regina Piuvezam:** Conceptualization, Investigation, Resources, Writing - original draft, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104540.

References

Acharya, J., Karak, S., & De, B. (2016). Metabolite Profile and Bioactivity of Musa X Paradisiaca L. Flower Extracts. *Journal of Food Biochemistry, 40*(6), 724–730. https://doi.org/10.1111/jfbc.12263.

Alrifai, M., Marsh, L. M., Dicke, T., Kiliç, A., Conrad, M. L., Renz, H., & Garn, H. (2014). Compartmental and temporal dynamics of chronic inflammation and airway remodelling in a chronic asthma mouse model. *PLoS ONE, 9*(1), 1–9. https://doi.org/10.1371/journal.pone.0085839.

Arun, K. B., Thomas, S., Reshmitha, T. R., Akhil, G. C., & Nisha, P. (2017). Dietary fibre and phenolic-rich extracts from Musa paradisiaca inflorescence ameliorates type 2 diabetes and associated cardiovascular risks. *Journal of Functional Foods, 31*, 198–207. https://doi.org/10.1016/j.jff.2017.02.001.

Balaji, S., & Kavasseri Ganesan, K. (2020). Inhibition of inducible nitric oxide production by Caryota urens and its active constituents umbelliferone and rutin. *Journal of*

Ayurveda and Integrative Medicine, 10–13. https://doi.org/10.1016/j.jaim.2020.09.002.

Bayar Muluk, N., Bafaqeeh, S. A., & Cingi, C. (2019). Anti-IgE treatment in allergic rhinitis. *International Journal of Pediatric Otorhinolaryngology, 127*(May), Article 109674. https://doi.org/10.1016/j.ijporl.2019.109674.

Bhaskar, J. J., Mahadevamma, S., Chilkunda, N. D., & Salimath, P. V. (2012). Banana (Musa sp. var. elakki bale) flower and pseudostem: Dietary fiber and associated antioxidant capacity. *Journal of Agricultural and Food Chemistry, 60*(1), 427–432. https://doi.org/10.1021/jf204539v.

Cavalcanti, R. F. P., Gadelha, F. A. A. F., de Jesus, T. G., Cavalcante-Silva, L. H. A., Paiva Ferreira, L. K. D., Paiva Ferreira, L. A. M., Vieira, G. C., & Piuvezam, M. R. (2020). Warifteine and methylwarifteine inhibited the type 2 immune response on combined allergic rhinitis and asthma syndrome (CARAS) experimental model through NF-κB pathway. *International Immunopharmacology, 85*(May), Article 106616. https://doi.org/10.1016/j.intimp.2020.106616.

Correa, M., Mesomo, M. C., Pianoski, K. E., Torres, Y. R., & Corazza, M. L. (2016). Extraction of inflorescences of Musa paradisiaca L. using supercritical CO2 and compressed propane. *Journal of Supercritical Fluids, 113*, 128–135. https://doi.org/10.1016/j.supflu.2016.03.016.

Ding, Y., Che, D., Li, C., Cao, J., Wang, J., Ma, P., Zhao, T., An, H., & Zhang, T. (2019). Quercetin inhibits Mrgprx2-induced pseudo-allergic reaction via PLCγ-IP3R related Ca 2+ fluctuations. International Immunopharmacology, 66(July 2018), 185–197. https://doi.org/10.1016/j.intimp.2018.11.025.

Fan, Y., Piao, C. H., Hyeon, E., Jung, S. Y., Eom, J. E., Shin, H. S., Song, C. H., & Chai, O. H. (2019). Gallic acid alleviates nasal inflammation via activation of Th1 and inhibition of Th2 and Th17 in a mouse model of allergic rhinitis. International Immunopharmacology, 70(October 2018), 512–519. https://doi.org/10.1016/j.intimp.2019.02.025.

Fu, J., Huang, J., Lin, M., Xie, T., & You, T. (2020). Quercetin Promotes Diabetic Wound Healing via Switching Macrophages From M1 to M2 Polarization. *Journal of Surgical Research, 246*, 213–223. https://doi.org/10.1016/j.jss.2019.09.011.

Helal, M. G., Megahed, N. A., & Abd Elhameed, A. G. (2019). Saxagliptin mitigates airway inflammation in a mouse model of acute asthma via modulation of NF-kB and TLR4. *Life Sciences, 239*, Article 117017. https://doi.org/10.1016/j.lfs.2019.117017.

Kahraman, A., Erkasap, N., Köken, T., Serteser, M., Aktepe, F., & Erkasap, S. (2003). The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. *Toxicology, 183*(1–3), 133–142. https://doi.org/10.1016/S0300-483X(02)00514-0.

Kamali, A. N., Noorbakhsh, S. M., Hamedifar, H., Jadidi-Niaragh, F., Yazdani, R., Bautista, J. M., & Azizi, G. (2019). A role for Th1-like Th17 cells in the pathogenesis of inflammatory and autoimmune disorders. Molecular Immunology, 105(October 2018), 107–115. https://doi.org/10.1016/j.molimm.2018.11.015.

Kaur, R., & Chupp, G. (2019). Phenotypes and endotypes of adult asthma: Moving toward precision medicine. *Journal of Allergy and Clinical Immunology, 144*(1), 1–12. https://doi.org/10.1016/j.jaci.2019.05.031.

Lau, B. F., Kong, K. W., Leong, K. H., Sun, J., He, X., Wang, Z., Mustafa, M. R., Ling, T. C., & Ismail, A. (2020). Banana inflorescence: Its bio-prospects as an ingredient for functional foods. *Trends in Food Science and Technology, 97*, 14–28. https://doi.org/10.1016/j.tifs.2019.12.023.

Li, H. Tao, Chen, Z. Gui, Lin, Y. Sen, Liu, H., Ye, J., Zou, X. Ling, Wang, Y. Hong, Yang, H. Ling, & Zhang, T. Tuo. (2018). CpG-ODNs and Budesonide Act Synergistically to Improve Allergic Responses in Combined Allergic Rhinitis and Asthma Syndrome Induced by Chronic Exposure to Ovalbumin by Modulating the TSLP-DC-OX40L Axis. Inflammation, 41(4), 1304–1320. https://doi.org/10.1007/s10753-018-0779-6.

Lin, W., Wang, W., Wang, D., & Ling, W. (2017). Quercetin protects against atherosclerosis by inhibiting dendritic cell activation. *Molecular Nutrition and Food Research, 61*(9), 1–37. https://doi.org/10.1002/mnfr.201700031.

Liu, L. L., Zhang, Y., Zhang, X. F., & Li, F. H. (2018). Influence of rutin on the effects of neonatal cigarette smoke exposure-induced exacerbated MMP-9 expression, Th17 cytokines and NF-kB/iNOS-mediated inflammatory responses in asthmatic mice model. *Korean Journal of Physiology and Pharmacology, 22*(5), 481–491. https://doi.org/10.4196/kjpp.2018.22.5.481.

Nam, S. Y., Jeong, H. J., & Kim, H. M. (2017). Kaempferol impedes IL-32-induced monocyte-macrophage differentiation. *Chemico-Biological Interactions, 274*, 107–115. https://doi.org/10.1016/j.cbi.2017.07.010.

Nisha, P., & Mini, S. (2013). Flavonoid rich ethyl acetate fraction of Musa paradisiaca inflorescence down-regulates the streptozotocin induced oxidative stress,

hyperglycaemia and mRNA levels of selected inflammatory genes in rats. *Journal of Functional Foods, 5*(4), 1838–1847. https://doi.org/10.1016/j.jff.2013.09.003.

Nisha, P., & Mini, S. (2014). In vitro antioxidant and antiglycation properties of methanol extract and its different solvent fractions of Musa paradisiaca L. (Cv. Nendran) inflorescence. *International Journal of Food Properties, 17*(2), 399–409. https://doi.org/10.1080/10942912.2011.642050.

Oh, H. A., Han, N. R., Kim, M. J., Kim, H. M., & Jeong, H. J. (2013). Evaluation of the effect of kaempferol in a murine allergic rhinitis model. *European Journal of Pharmacology, 718*(1–3), 48–56. https://doi.org/10.1016/j.ejphar.2013.08.045.

Oliveira, M. I., Santos, S. G., Oliveira, M. J., Torres, A. L., & Barbosa, M. A. (2012). Chitosan drives anti-inflammatory macrophage polarisation and pro-inflammatory dendritic cell stimulation. *European Cells and Materials, 24*, 136–153. https://doi.org/10.22203/eCM.v024a10.

Paiva Ferreira, L. K. D., Paiva Ferreira, L. A. M., Monteiro, T. M., Bezerra, G. C., Bernardo, L. R., & Piuvezam, M. R. (2019). Combined allergic rhinitis and asthma syndrome (CARAS). *International Immunopharmacology, 74*(June), Article 105718. https://doi.org/10.1016/j.intimp.2019.105718.

Palumbo, M. L., Prochnik, A., Wald, M. R., & Genaro, A. M. (2020). Chronic Stress and Glucocorticoid Receptor Resistance in Asthma. *Clinical Therapeutics, 42*(6), 993–1006. https://doi.org/10.1016/j.clinthera.2020.03.002.

Pereira, A., & Maraschin, M. (2015). Banana (Musa spp) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health. *Journal of Ethnopharmacology, 160*, 149–163. https://doi.org/10.1016/j.jep.2014.11.008.

Ramu, R., Shirahatti, P. S., Zameer, F., Ranganatha, L. V., & Nagendra Prasad, M. N. (2014). Inhibitory effect of banana (Musa sp. var. Nanjangud rasa bale) flower extract and its constituents Umbelliferone and Lupeol on α-glucosidase, aldose reductase and glycation at multiple stages. *South African Journal of Botany, 95*, 54–63. https://doi.org/10.1016/j.sajb.2014.08.001.

Shin, N. R., Kwon, H. J., Ko, J. W., Kim, J. S., Lee, I. C., Kim, J. C., Kim, S. H., & Shin, I. S. (2019). S-Allyl cysteine reduces eosinophilic airway inflammation and mucus overproduction on ovalbumin-induced allergic asthma model. International Immunopharmacology, 68(October 2018), 124–130. https://doi.org/10.1016/j.intimp.2019.01.001.

Silva, A. M., Teixeira, J. H., Almeida, M. I., Gonçalves, R. M., Barbosa, M. A., & Santos, S. G. (2017). Extracellular Vesicles: Immunomodulatory messengers in the context of tissue repair/regeneration. *European Journal of Pharmaceutical Sciences, 98*, 86–95. https://doi.org/10.1016/j.ejps.2016.09.017.

Tang, H., Li, T., Han, X., & Sun, J. (2019). TLR4 antagonist ameliorates combined allergic rhinitis and asthma syndrome (CARAS) by reducing inflammatory monocytes infiltration in mice model. *International Immunopharmacology, 73*. https://doi.org/10.1016/j.intimp.2019.05.021.

Tohidinik, H. R., Mallah, N., & Takkouche, B. (2019). History of allergic rhinitis and risk of asthma; a systematic review and meta-analysis. *World Allergy Organization Journal, 12*(10), Article 100069. https://doi.org/10.1016/j.waojou.2019.100069.

Vilhena, R. de O., Marson, B. M., Budel, J. M., Amano, E., Messias-Reason, I. J. de T., & Pontarolo, R. (2019). Morpho-anatomy of the inflorescence of Musa × paradisiaca. Revista Brasileira de Farmacognosia, 29(2), 147–151. https://doi.org/10.1016/j.bjp.2019.01.003.

Vo, T. S., Kim, Y. S., Ngo, D. N., & Ngo, D. H. (2020). Myricetin from Rhodomyrtus tomentosa (Aiton) Hassk fruits attenuates inflammatory responses in histamine-exposed endothelial cells. *Process Biochemistry, 92*(February), 457–463. https://doi.org/10.1016/j.procbio.2020.02.004.

Wei, M., Chu, X., Guan, M., Yang, X., Xie, X., Liu, F., Chen, C., & Deng, X. (2013). Protocatechuic acid suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model. *International Immunopharmacology, 15*(4), 780–788. https://doi.org/10.1016/j.intimp.2013.01.010.

Wisniewski, J. A., Muehling, L. M., Eccles, J. D., Capaldo, B. J., Agrawal, R., Shirley, D. A., Patrie, J. T., Workman, L. J., Schuyler, A. J., Lawrence, M. G., Teague, W. G., & Woodfolk, J. A. (2018). TH1 signatures are present in the lower airways of children with severe asthma, regardless of allergic status. *Journal of Allergy and Clinical Immunology, 141*(6), 2048–2060.e13. https://doi.org/10.1016/j.jaci.2017.08.020.

Yang, C.-C., Hung, Y.-L., Li, H.-J., Lin, Y.-F., Wang, S.-J., Chang, D.-C., Pu, C.-M., & Hung, C.-F. (2021). Quercetin inhibits histamine-induced calcium influx in human keratinocyte via histamine H4 receptors. *International Immunopharmacology, 96*, Article 107620. https://doi.org/10.1016/j.intimp.2021.107620.