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Dietary intervention with purple passion fruit peel in asthma: insights into Th2-mediated responses in an ovalbumin-induced murine model

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ABSTRACT

Asthma is a chronic inflammatory airway disease in which Th2-driven immune responses and oxidative stress play key roles in its pathophysiology and are increasingly recognized as being modulated by diet. Purple passion fruit peel (PPFP), an agro-industrial by-product rich in dietary fiber and containing a diverse profile of bioactive compounds, including anthocyanins, represents a promising food-derived ingredient. However, most evidence on its effects in asthma relies on isolated extracts, and the mechanistic interplay of whole-food matrices remains poorly understood. In this study, female BALB/c mice were randomized into five groups: healthy control (CT), ovalbumin-induced asthma control (CA), positive control (dexamethasone), and asthma groups supplemented with 0.3% (PPFP-L) or 3.0% (PPFP-H) PPFP. Following intraperitoneal sensitization and intranasal OVA challenge, the effects of dietary supplementation on inflammatory mediators, oxidative stress markers, hepatic safety, and lung morphology were evaluated. Dietary inclusion of PPFP at 0.3% and 3.0% (non-cytotoxic in *Aliivibrio fischeri*) did not affect feeding behaviour, body weight, or hepatic toxicity markers. Supplementation at 3.0% significantly increased total reduced glutathione (41.7%) and the activities of catalase (19.8%) and superoxide dismutase (45.7%). Concurrently, systemic immunoglobulin E (15.9%) and pulmonary interleukin-4 (29.8%) and interleukin-5 (29.1%) levels were reduced, which was corroborated by decreased inflammatory cell infiltration in lung tissue. Conversely, lipid peroxidation and interleukin-13 production remained unaffected. These results indicate that PPFP mitigates oxidative stress primarily through the enhancement of antioxidant enzymatic homeostasis and modulation of Th2-driven systemic inflammation. Overall, PPFP emerges as a safe, minimally processed nutritional coadjuvant, supporting diet-based strategies to modulate chronic inflammation while promoting sustainable valorisation of food industry by-products.

1. Introduction

Asthma is a heterogeneous disease characterized by chronic airway inflammation and variable respiratory symptoms, as defined by the Global Initiative for Asthma (Global Initiative for asthma, 2025). It affects approximately 300 million people worldwide and represents a growing public health concern, particularly in low- and middle-income countries with limited access to healthcare (Asher et al., 2021; Mortimer et al., 2022).

The pathophysiology of asthma involves complex interactions between genetic, environmental, and immunological factors (Miller et al., 2021). T-helper cells type-2 (Th2)-mediated inflammation predominates in approximately 50% of mild to severe cases, and is characterized by the secretion of elevated levels of interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13), recruitment of eosinophils and other inflammatory cells, and immunoglobulin E (IgE) production (Al-Muhsen

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et al., 2011; Brusselle & Koppelman, 2022; Fahy, 2015; Habib et al., 2022). This immune response promotes airway remodelling, mucus hypersecretion, and intermittent airway obstruction (Barnes, 2018; Lambrecht & Hammad, 2015). In addition, the inflammatory response induces excessive production of reactive oxygen species (ROS), contributing to oxidative stress, epithelial damage, and exacerbation of bronchial hyperresponsiveness (Michaeloudes et al., 2022; Silveira et al., 2019). Current pharmacotherapy, primarily based on corticosteroids and β_2-agonists, is effective for symptom control but limited by dose-dependent adverse effects. Long-term or high-dose usage is associated with complications ranging from oropharyngeal candidiasis to systemic risks, including osteoporosis, susceptibility to infections, metabolic disorders, and neuropsychiatric events (Bleecker et al., 2020; de Benedictis et al., 2021; Heffler et al., 2018). These safety concerns, combined with steroid resistance in severe phenotypes, underscore the critical need for complementary therapeutic strategies. Dietary antioxidants, including vitamins C and E, carotenoids, and polyphenols, have been investigated as strategies to restore redox homeostasis and improve asthma control (Kirkham & Rahman, 2006; Rice-Evans et al., 1997). Epidemiological evidence links higher intake of antioxidant-rich foods to reduced asthma incidence and severity (Knekt et al., 2002; Shaheen et al., 2001), although clinical trials with isolated compounds have produced inconsistent outcomes (Michaeloudes et al., 2022; Patel et al., 2006; Pearson et al., 2004; Romieu et al., 1998). In contrast, some whole food matrices have shown promising results. A notable example is ginger (*Zingiber officinale*), whose bioactive constituents, such as 6-gingerol, 8-gingerol, and 6-shogaol, have been widely reported to ameliorate asthma symptoms by suppressing Th2-mediated inflammation and inducing airway relaxation (Kim et al., 2022; Townsend et al., 2013). Similarly, oral administration of an aqueous extract of purple passion fruit peel (PPFP) has been reported to alleviate respiratory symptoms in asthmatic individuals (Watson et al., 2008), and antioxidant effects of passion fruit peel flour have been observed in animal models, attributed to its fiber and phenolic content (Vuolo et al., 2020).

Recent evidence further highlights the contribution of dietary fiber to asthma modulation through the gut–lung axis, where microbially derived SCFAs support immune regulation and epithelial integrity, and reduced SCFA production is associated with worsened disease (Cait et al., 2018; Frati et al., 2018; Liu et al., 2025; Sdona et al., 2021; Song et al., 2024; Verstegen et al., 2021).

Passion fruit has traditionally been used in folk medicine to treat various conditions such as anxiety, insomnia, bronchitis, and asthma (Zibadi & Watson, 2008). *Passiflora edulis* Sims f. *edulis* (purple passion fruit) is the more cold-tolerant forma of *P. edulis*, cultivated in temperate, frost-free climates without intense summer heat (Fonseca et al., 2022). Its peel, which is rich in fiber (57.7 g/100 g DW), phenolic compounds (2.3–2.6 g gallic acid equivalents (GAE)/100 g DW), carotenoids (1.4 mg/100 g DW), and vitamin C (458 mg/100 g DW) (dos Reis et al., 2018; Fonseca et al., 2024), is commonly discarded, contributing to biomass waste and environmental impact (Fonseca et al., 2022).

Although PPFP has previously been investigated in several conditions, including asthma where its effects were mainly attributed to antioxidant and anti-inflammatory properties (Fonseca et al., 2022; Watson et al., 2008), the mechanisms underlying its action, and its histopathological impact on lung tissue, remain poorly understood. Moreover, previous studies have primarily focused on PPFP extracts, thereby excluding the dietary fiber fraction, which could play an additional beneficial role in asthma through modulation of the gut–lung axis. From both a processing and sustainability standpoint, the use of minimally processed PPFP may represent a more advantageous approach than extract-based formulations. Therefore, the aim of this work is to investigate the potential of dietary supplementation with minimally processed PPFP on inflammation and oxidative stress markers in an ovalbumin (OVA)-induced murine model that replicates the Th2-dominated immune response seen in human allergic asthma. Although OVA is not a common human allergen, this model is well-standardized and widely recognized for its high reproducibility and robustness in replicating key pathological features of human allergic asthma, particularly the Th2-driven inflammatory profile, eosinophilic infiltration, and IgE production (Aun et al., 2017). More specifically, the effect of two realistic PPFP dietary dosages (0.3 and 3.0%) on inflammatory markers (IgE, IL-4, IL-5, IL-13); oxidative stress parameters (lipid peroxidation, total reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) activity), and hepatic toxicity markers (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes) was evaluated to assess efficacy, route of action and safety.

2. Materials and methods

2.1. Chemicals

The main chemicals utilized in the experiment and analyses are described next. Ovalbumin (Grade V, Cat. #A5503, ≥98%), aluminium hydroxide ($Al(OH)_3$, Cat. #239186, 50.0–57.5%), dexamethasone (Cat. #D1756, ≥98%), bovine serum albumin (BSA, Cat. #A6003), Bouin's solution (Cat. #HT10132), Paraplast Plus (Cat. #P3683), reduced L-glutathione (Cat. #G4251), hypoxanthine (Cat. #4010CBC), xanthine oxidase (Cat. #X2252), nitrotetrazolium blue chloride (NBT, Cat. #93862), 5′5′-dithio-bis-2-nitrobenzoic acid (DTNB, Cat. #D8130) and thiobarbituric acid (TBA, Cat. # T5500) were obtained from Sigma Aldrich (St. Louis, MO, USA). Protein Assay Dye Reagent Concentrate (Cat. #5000006), sodium dodecyl sulfate (SDS, Cat. # 1610301) were purchased from BioRad Laboratories (Hercules, CA, USA). Malondialdehyde standard (MDA, Cat. #10009202) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

2.2. Preparation of purple passion fruit peel powder

PPFP powder was prepared according to Fonseca et al. (2024). Briefly, fruits were harvested from a purple passion fruit (*P. edulis* f. *edulis*) organic orchard situated in Paredes de Viadores, Portugal (41.118628, −8.106283). They were collected once they reached full and homogenous pigmentation and before they fell from the plant. After being collected, fruits were transported immediately to the laboratory, washed with tap water, their peduncles were removed, and they were left to dry at room temperature. Once dried, fruits were randomly selected at the point of optimum consumption (once they began to shrivel). Fruits were subsequently halved, and pulp separated from the peel with a spoon. Peel was freeze-dried (VirTis BenchTop K SP Industries, Warminster, PA, USA) and subsequently ground into powder with a PULVERISETTE 11 knife mill (Fritsch, Idar-Oberstein, Germany) at 10000 rpm for 60 s to obtain the PPFP powder. The mean particle size of this powder was determined (Laser Scattering Particle Size Distribution Analyzer LA-960, HORIBA) as 251.78 μm.

2.3. Characterization of phenolic compounds and carotenoids in purple passion fruit peel powder

PPFP was subsequently analysed for its specific content in flavonoids, phenolic acids, and carotenoids according to procedures already described (Nascimento et al., 2017; Pacheco, 2009; Santiago et al., 2010).

Flavonoids and phenolic acids analysis was performed according to Nascimento et al. (2017). For anthocyanins, PPFP powder (0.02 g) was extracted with 10 mL methanol:formic acid (90:10, *v*/v) in an ultrasonic bath, followed by centrifugation until decolorization. Extracts were dried and reconstituted in 100 μL of 5% formic acid in water:methanol (90:10, v/v). In the case of remaining flavonoids and phenolic acids, the sample was extracted with methanol:water (50:50, pH 2) for 1 h and centrifugation (5000 *g*, 10 min), followed by a second extraction with acetone:water (70,30, *v*/v). Supernatants were combined for HPLC

analysis. Hydrolyzed phenolic acids were obtained from the PPFP extracted residues via alkaline hydrolysis with 2 M NaOH containing 1% ascorbic acid and 10 mM EDTA at 61–63 °C for 60 min, followed by acid hydrolysis with 6 M HCl. The released phenolic acids were extracted with ethyl acetate, dried under N_2, and redissolved in methanol. All fractions were analysed using a WatersTM Alliance 2695 HPLC system, using a Waters™ 2996 photodiode array detector, with a ThermoTM Scientific C18 BDS (100 mm × 4.6 mm, 2.4 μm) column, using a gradient elution with phosphoric acid and acetonitrile. Quantification was performed by external calibration with laboratory-isolated standards, and identification by comparing their retention times and the absorption spectra UV–Vis_{max} with known extracts and literature.

Carotenoids were determined according to Pacheco (2009). Briefly, samples were extracted through maceration with celite and acetone, and the mixture was vacuum-filtered; the extraction was repeated until the sample lost its characteristic carotenoid colour. The combined acetone extract was transferred quantitatively into petroleum ether and washed multiple times with ultrapure water. The ether fraction was then saponified with 10% potassium hydroxide in methanol (10:90, v/v) for 16 h at room temperature in the dark. After saponification, the mixture was washed with water, dried over anhydrous sodium sulphate, and transferred to a volumetric flask. Total carotenoid content was determined spectrophotometrically at 450 nm using a UV–Vis spectrophotometer (UV-1800 model 92, Shimadzu Corporation, Kyoto, Japan), with petroleum ether as blank, and quantified according to the Beer-Lambert law. Chromatographic analysis of individual carotenoids was performed using a Waters™ Alliance 2695 HPLC system, using a Waters™ 2996 photodiode array detector and Empower™ software. Separation was achieved using a YMC™ C_{30} Carotenoid column (250 mm × 4.6 mm, 3 μm) at 33 °C, with a gradient elution of methanol and methyl tert-butyl ether. Carotenoids were identified by comparing retention times and UV–Vis spectra with laboratory-isolated standards (>99% purity) using calibration curves.

2.4. Evaluation of passion fruit peel powder toxicity by Aliivibrio fischeri bioluminescence assay

A. fischeri is a naturally bioluminescent marine bacterium whose light emission results from its metabolic processes. The disruption of cellular metabolism by toxic substances can be assessed by measuring changes in the bacterium's light emission. This makes it a fast, sensitive, and cost-effective method for evaluating the toxicity of a certain compound. To determine safe doses of PPFP in mice, toxicity assays were conducted using the bioluminescence assay with *A. fischeri*, following an established methodology (Kuznetsova et al., 2017; Matos et al., 2015; Salvador et al., 2017).

2.4.1. Bacterial growth conditions

Stock cultures of the bioluminescent *A. fischeri* ATCC 49387 (American Type Culture Collection, VA, USA) were maintained in 10% glycerol at −80 °C. Before each assay, a bacterial stock culture was transferred to 30 mL of Luria-Bertani salt (LBS) media (1% Tryptone, 0.5% yeast extract, 3% NaCl; pH 7.4) and grown overnight at 25 °C under stirring (120 rpm). Then, an aliquot (300 μL) was sub-cultured in LBS (30 mL) and incubated at the previously mentioned conditions until a stationary phase of 10^9 colonies forming units per mL (CFU/mL), corresponding to bioluminescent signal (in RLUs) of 10^9, was reached. This correlation between the CFU and the RLU of *A. fischeri* was already established and reported before (Salvador et al., 2017).

2.4.2. Toxicity assessment procedure

For all assays, an overnight culture of *A. fischeri* was initially diluted in phosphate buffered saline (PBS: 30 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 per litre; pH 7.4) to reach a final bioluminescent signal of approximately 10^7 RLU. Subsequently, 50 mL of this bacterial suspension was transferred into 100 mL sterilized Erlenmeyer flasks, with the appropriate amount of each compound added to attain final concentrations ranging from 0 (control - Ct) to 2000 mg/L of PPFP. The suspensions were incubated in the dark at 25 °C for 24 h with constant stirring at 120 rpm. To assess the toxicity of the PPFP on *A. fischeri*, 1 mL aliquots of treated and control samples were collected after 15, 30, 45, 60, and 120 min of incubation and the bioluminescence signal was measured using a luminometer (Promega Glomax 20/20 luminometer, Turner Designs, Inc., Madison, WI, USA) with peak wavelength detection at 420 nm, within a standard range of 300–650 nm. For each tested condition, three independent experiments with three replicate measures were conducted. Non-toxic concentrations of PPFP for mice were calculated using an interspecies correlation between *A. fischeri* and oral administration in mouse (Devillers et al., 2009) (**Eq. 1**).

$$\log(\text{PPFP concentration } A.fischeri) = 0.55 \times \log(\text{oral dose mouse}) - 0.13 \quad (1)$$

2.5. Animal experimentation

2.5.1. Ethics, animals, and conditions

The study was performed in accordance with the ARRIVE guidelines and followed the guide for the care and use of laboratory animals of the National Institutes of Health (NIH Publications No. 8023, revised 1978). The animal experiment was approved by the Ethics Committee on the Use of Animals of the University of Campinas (UNICAMP), protocol number 6347–1/2023.

Seven-week-old female BALB/c mice ($n = 50$) were obtained from CEMIB/UNICAMP and subsequently transported to LEB/FEA/UNICAMP, where they were acclimatized for one week in an experimental room with controlled temperature (22 ± 2 °C) and humidity (40%–60%), and 12 h/12 h dark/light cycles, starting the experimental protocol at eight weeks of age. Animals were grouped in five per cage and, during acclimatization, water was supplied ad libitum along with a commercial mouse feed (Nuvilab CR-1, Nuvital).

2.5.2. Establishment of the OVA-induced asthma model and treatment

Animals were randomly divided into five groups ($n = 10$/group): (1) normal control (CT), (2) asthma control (CA), (3) 0.5 mg/kg dexamethasone (DEX), (4) 0.3% diet supplementation with PPFP (PPFP-L) and (5) 3.0% diet supplementation with PPFP (PPFP-H). At the beginning of the experiment, all groups except CT were sensitized through the intraperitoneal injection of 50 μg OVA (emulsified with 1 mg of aluminium hydroxide in 200 μL of phosphate-buffered saline (PBS, pH 7.4)) on days 0, 7, and 14. CT group was subjected to the same procedure but only PBS was administered. Subsequently (from day 28 to 42), the animals were challenged with intranasal administration of OVA (50 μg/20 μL PBS) or PBS alone (CT), every two days after intraperitoneal anaesthesia with ketamine (80 mg/kg) and xylazine (10 mg/kg). Dexamethasone at a dose of 0.5 mg/kg, selected based on its proven efficacy in suppressing allergic airway inflammation (Liao et al., 2022) was administered daily via oral gavage to the DEX group, while sterile distilled water was used in the remaining groups. The schematic representation of the experimental protocol for the OVA-induced allergic asthma model establishment is shown in Fig. 1.

2.5.3. Diet composition

During sensitization, all groups received a standard AIN-93 M diet (Reeves et al., 1993). In the challenge phase (day 28 to 42), CT, CA and DEX groups continued with the same diet, while PPFP-L and PPFP-H groups diets were supplemented with 0.3 and 3.0% PPFP, respectively, while maintaining the remaining ingredients proportions (**Supplementary Material 1**). These concentrations were selected based on preliminary safety assessments (demonstrating non-cytotoxicity in *A. fischeri*) and translational relevance (Reagan-Shaw et al., 2008). Using the human equivalent dose (HED) (**Eq.2**) translation, based on body surface area (Reagan-Shaw et al., 2008), the selected dosages represent

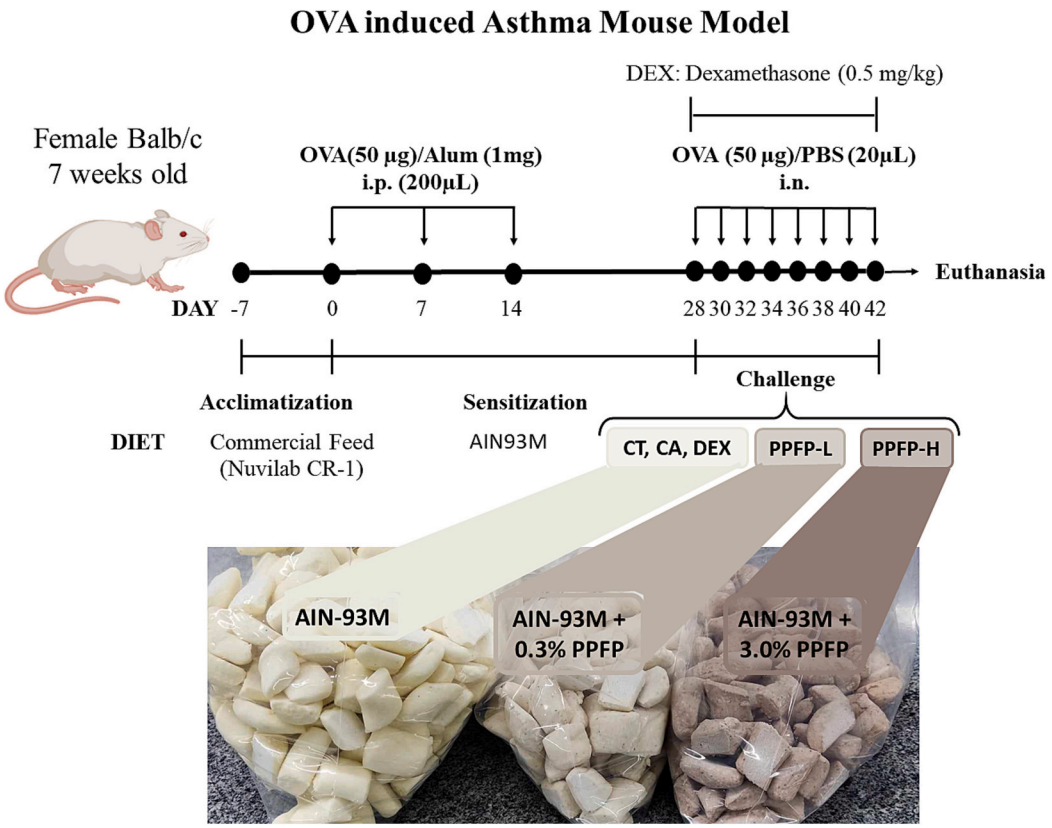

Fig. 1. Schematic representation of the protocol for ovalbumin-induced allergic asthma in mice. Experimental groups: CT – Control; CA – Control Asthma; DEX - Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-L – 3.0% Purple Passion Fruit Peel. i.p. – intraperitoneal; i.n. – intranasal. OVA - Ovalbumin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

two realistic human consumption scenarios: a low-dose dietary intake (~1.82 g/day) and a high-dose functional supplementation (~18.2 g/day) (**Supplementary Material 2**).

$$\text{HED}\ (mg/kg) = \text{animal dose} \times \frac{\text{Animal Km}}{\text{Human Km}} \quad (2)$$

Furthermore, the highest dose (3.0%) is aligned with the standard dietary inclusion range (1–5% *w*/w) widely adopted in functional food research using rodent models (R. D. P. D. Nascimento et al., 2023; Seymour et al., 2009). This established range ensures a sufficient delivery of bioactive compounds while preventing macro- and micronutrient imbalances, excessive fiber load, or palatability issues that could confound feeding behaviour. To verify this, diet consumption and animal body weight were monitored throughout the experiment.

2.5.4. Euthanasia and serum/tissue collection

Euthanasia of all animals was performed after 49 days of housing (7 days of acclimatization and 42 days of experiment), through the combined administration of ketamine (300 mg/kg of body weight) and xylazine (30 mg/kg of body weight) via intraperitoneal route, followed by cardiac puncture for blood collection. Serum was collected after centrifugation (10 min, 5000 rpm) and stored at −80 °C until analysis. The right lung was also collected, washed with saline solution, immediately immersed in liquid nitrogen, and stored at −80 °C until use. The left lung was collected, washed with saline solution and subsequently used for histological analysis.

2.5.5. Bronchoalveolar lavage fluid (BALF) collection

The bronchoalveolar lavage fluid (BALF) was collected according to (Kalidhindi et al., 2021). Briefly, the trachea was surgically exposed and cannulated with an 18-gauge cannula. The cannula was secured in place by tying a surgical thread around the trachea. The lungs were lavaged three times with 0.5 mL of ice-cold PBS. In each wash, the fluid was gently instilled and aspirated to maximize cell recovery. The recovered fractions were pooled in an Eppendorf tube (1.5 mL) and subsequently centrifuged at 1000 ×*g* for 10 min at 4 °C. The supernatant was collected and stored at −80 °C for cytokine analysis. BALF samples were subjected to protein quantification (mg of BSA/mL) according to Bradford assay (Bradford, 1976).

2.6. Inflammation markers and oxidative stress analyses

Lung tissue was weighed, homogenised in PBS buffer (0.5 g of tissue/mL) and centrifuged at 10000 rpm for 30 min. The supernatant was collected and subjected to protein quantification (mg of BSA/g of lung tissue) according to Bradford assay (Bradford, 1976). Supernatants were subsequently used for oxidative stress analyses.

The evaluated indicators of oxidative stress were CAT (Katals/mg of protein) and SOD (U/mg of protein) activity as well as GSH (nmol/mg of protein) and MDA (nmol/mg of protein) concentration. All the assays were performed in triplicate and samples absorbance readings were performed in a microplate (Synergy HT, Biotek, Winooski, VT USA) spectrophotometer coupled with Gen5™ 2.0 data analysis software.

Lipid peroxidation in lungs homogenate was estimated through the concentration of MDA determined by the thiobarbituric acid reactive substances (TBARS) method described by (Ohkawa et al., 1979), with adaptations. Briefly, samples or a standard were mixed with 8.1% SDS and working reagent (TBA, 20% acetic acid and 5% sodium hydroxide). This mixture was heated at 95 °C for 60 min and then cooled in an ice bath for 10 min. Afterwards, samples were centrifuged at 10000 rpm for 10 min at low temperature (4 °C). The absorbance of the supernatant was read at 532 nm using a clear 96-well microplate and their concentration was calculated through a standard curve (5–200 nmol/mL) of

MDA standard.

GSH was determined by first mixing a Tris (1 mM)/EDTA (2 mM) buffer with diluted homogenates (1:5) or a standard and read at 421 nm. This step was followed by the addition of DTNB, a 15 min incubation period at room temperature and a second reading at the same wavelength. L-glutathione reduced was used as standard (2.5–500 nmol/mL) and the GSH quantification was performed by subtracting the first reading data from the second one, followed by linear regression (Yoshikawa et al., 1993).

SOD activity was quantified according to (Winterbourn et al., 1975). Briefly, samples (1:2 dilution) and working solution (0.1 mmol hypoxanthine, 0.07 U xanthine oxidase, and 0.6 mmol NTB in phosphate buffer at 1:1:1 proportions) were added to a microplate, and the kinetic reaction was monitored at 560 nm for 10 min.

The CAT assay was performed according to (Aebi, 1984). In short, lung homogenate was diluted (1:10) in phosphate buffer (pH = 7.4) and H_2O_2 was used as a substrate. CAT activity was measured in each sample through absorbance at 240 nm after 3 min.

IgE (serum) and pro-inflammatory cytokines IL-4, IL-5, IL-13 (BALF) were quantified by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocol. ELISA kits for IL-4 (Cat. #900-K49K), IL-5 (Cat. #900-K406K) and IL-13 (Cat. #900-K207K) quantification were purchased from Peprotech (Rocky Hill, NJ, USA) and IgE ELISA Kit (Cat. #NBP318786) was purchased from Novus Biologicals (Centennial, CO, USA).

2.7. Hepatotoxicity markers analysis: ALT and AST enzymes

Serum was analysed using clinical kits LABORLAB (Guarulhos, São Paulo, Brazil) for determination of AST (Cat. #1770140) and ALT (Cat. #1770150) according to manufacturer's protocol.

2.8. Histological analysis

Left lung tissues from the mice of each experimental group were collected and fixed in Bouin's solution for 24 h. After fixation, the tissues were washed in 70% ethyl alcohol and dehydrated in successive increasing concentrations of alcohol, diaphanized in xylol, impregnated in liquid paraffin baths at 60 °C, and included in paraffin blocks. Subsequently, the samples were sectioned on a Hyrax M60 microtome (Zeiss, Oberkochen, Germany) with a 5 μm thickness on microscopy slides. To evaluate the lung lesions, the tissue slices were prepared and stained with hematoxylin and eosin (H&E); to analyse inflammatory cell infiltration in the bronchi and alveoli, according to the manufacturer's instructions using standard protocols. The slides were photographed using a Nikon Eclipse E-400 photomicroscope (Nikon, Tokyo, Japan) and each sample was analysed at 100, 200, and 400× magnification. Peribronchial and perivascular inflammation was assessed using a semi-quantitative scoring system on digitized micrographs, performed in a blinded manner. The severity of inflammation was graded on a scale of 0 to 3: 0 = no abnormality detected, 1 = minimal, 2 = mild, and 3 = severe.

2.9. Statistical analysis

Data of *A. fischeri* bioluminescence, diet consumption, oxidative stress and inflammatory parameters, AST and ALT were checked for normal distribution using the Kolmogorov–Smirnov test and for homogeneity of variance using the Brown–Forsythe test. Afterwards, the differences between treated and control samples were assessed by ANOVA and Tukey's multiple comparison tests (GraphPad Prism 9). A value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Polyphenol and carotenoid composition of purple passion fruit peel powder

PPFP proximate and micronutrient composition, as well as the total phenolic compounds (TPC) and antioxidant activity have been previously determined by our research group (Fonseca et al., 2024). The proximate composition of PPFP shows high carbohydrate content (831 g/kg DW), of which 69.4% is dietary fiber. Most of this fiber consists of cellulose (306 g/kg DW) and hemicelluloses (119 g/kg DW), with the remainder mainly pectin (73–329 g/kg DW) (Delvar et al., 2019; dos Reis et al., 2018; Thu Dao et al., 2020). PPFP was also found to be rich in micronutrients, notably vitamin C (4.58 g/kg DW) and vitamin E (0.049 g/kg DW). Spectrophotometric analyses also showed that PPFP contains an interesting amount of TPC (23–26 g GAE/kg DW) and a relevant antioxidant activity (13–15 g TE/kg DW and 13–16 g AAE/kg DW as determined by DPPH and ABTS assays, respectively). Due to this interesting TPC content and antioxidant activity, in this work, we have quantified the flavonoid, phenolic acids and carotenoids composition (Table 1) that may be associated with such bioactivity.

Among the quantified flavonoids (5510 μg/g), anthocyanins (4180 μg/g) represent the majority of their content with a 75.8% share, mainly due to cyanidin-3-glucoside (C3G) (4030 ± 70 μg/g). This is the

Table 1
Flavonoids, phenolic acids and carotenoids composition (μg/g DW) of purple passion fruit (*P. edulis* f. *edulis*) peel powder (PPFP) and literature (μg/g DW).

Compound	PPFP	Literature
	Flavonoids	
Flavonols		
Quercetin-3-glucoside*	593 ± 30	1670[1]; ✓[2]
Flavanols		
Catechin	720 ± 20	✓[2,3]
Flavones		
Luteolin*	22.43 ± 0.44	20.0[4]
Anthocyanins		
Cyanidin-3-glucoside	4030 ± 70	97.0%[a,5]; 290–5780[1,2,5]; ✓[2,8]
Unidentified acylated anthocyanin	42.9 ± 0.7	–
Pelargonidin-3-glucoside	40.3 ± 0.2	15.5[6]; 1.00%[a,5]; tr[1]
Peonidin-3-*O*-glucoside	68.0 ± 0.2	–
	Phenolic acids	
Gallic acid†	2.18 ± 0.05	–
Protocatechuic acid*	28.72 ± 1.27	✓[2]
4-Hydroxybenzoic acid†	75.95 ± 0.25	–
Vanillic Acid#	3.57 ± 0.12	–
Ferulic acid#	1.93 ± 0.03	–
p-Coumaric acid#	2.90 ± 0.10	–
	Carotenoids	
Cis-β-Carotene	0.61 ± 0.01 (13-*cis*); 0.82 ± 0.02 (9-*cis*)	–
β-Cryptoxanthin	1.53 ± 0.06	0.75[6]
Lutein	3.31 ± 0.15	3.67[6]
α-Carotene	1.35 ± 0.05	0.37[6]
β-Carotene	11.51 ± 0.28	7.16[6]
Lycopene	0.16 ± 0.01	–
Total Carotenoids	26.37 ± 0.86	12.4[6]

✓: Detected but not determined; tr: Trace amount; –: Data not available; [a] Relative amount of the total anthocyanin content; *Sum of free and hydrolysed fractions. #Hydrolyzed fraction. †Free fraction. [1] (Medina et al., 2017); [2] (Zibadi et al., 2007); [3] (Domínguez-Rodríguez et al., 2019); [4] (Ichimura et al., 2006); [5] (KidØy et al., 1997); [6] (dos Reis et al., 2018); [7] (Herrera-Ramirez et al., 2020); [8] (Jiménez et al., 2011).

compound responsible for the purple coloration of the peel and the one consistently found in the literature as the most abundant phenolic compound in PPFP (Fonseca et al., 2022). Furthermore, cyanidin-3-glucoside has shown antioxidant and anti-inflammatory properties mainly through its phase I and II metabolites, such as protocatechuic acid, phloroglucinaldehyde, vanillic acid, and ferulic acid (Tan et al., 2019). It has been demonstrated that these metabolites are able to regulate antioxidant enzymes (CAT, SOD, glutathione peroxidase (GPx), GSH) and modulate inflammatory signalling pathways, including nuclear factor-κB (NF-κB), extracellular signal-regulated kinase (ERK), and mitogen-activated protein kinase (MAPK) (Tan et al., 2019). Along with C3G, quercetin-3-glucoside (593 ± 30 μg/g) and catechin (720 ± 20 μg/g) constitute the most representative identified flavonoids in accordance with what has been previously reported (KidØy et al., 1997; Medina et al., 2017; Zibadi et al., 2007) while most representative phenolic acids identified include 4-hydroxybenzoic acid (75.95 ± 0.25 μg/g), that has not been reported before in PPFP, and protocatechuic acid (28.72 ± 1.27 μg/g).

The total carotenoids content found in this study (26.37 ± 0.86 μg/g) is approximately the double of that found by dos Reis et al. (2018) (12.4 μg/g DW) probably due to distinct pre and post-harvest conditions. However, the main carotenoids found were the same, namely β-carotene (11.51 ± 0.28 μg/g) followed by lutein (3.31 ± 0.15 μg/g).

In general, the analytical results show that PPFP is a good source of anthocyanins, mostly C3G, that can increase TPC content and antioxidant capacity when included in a standard diet.

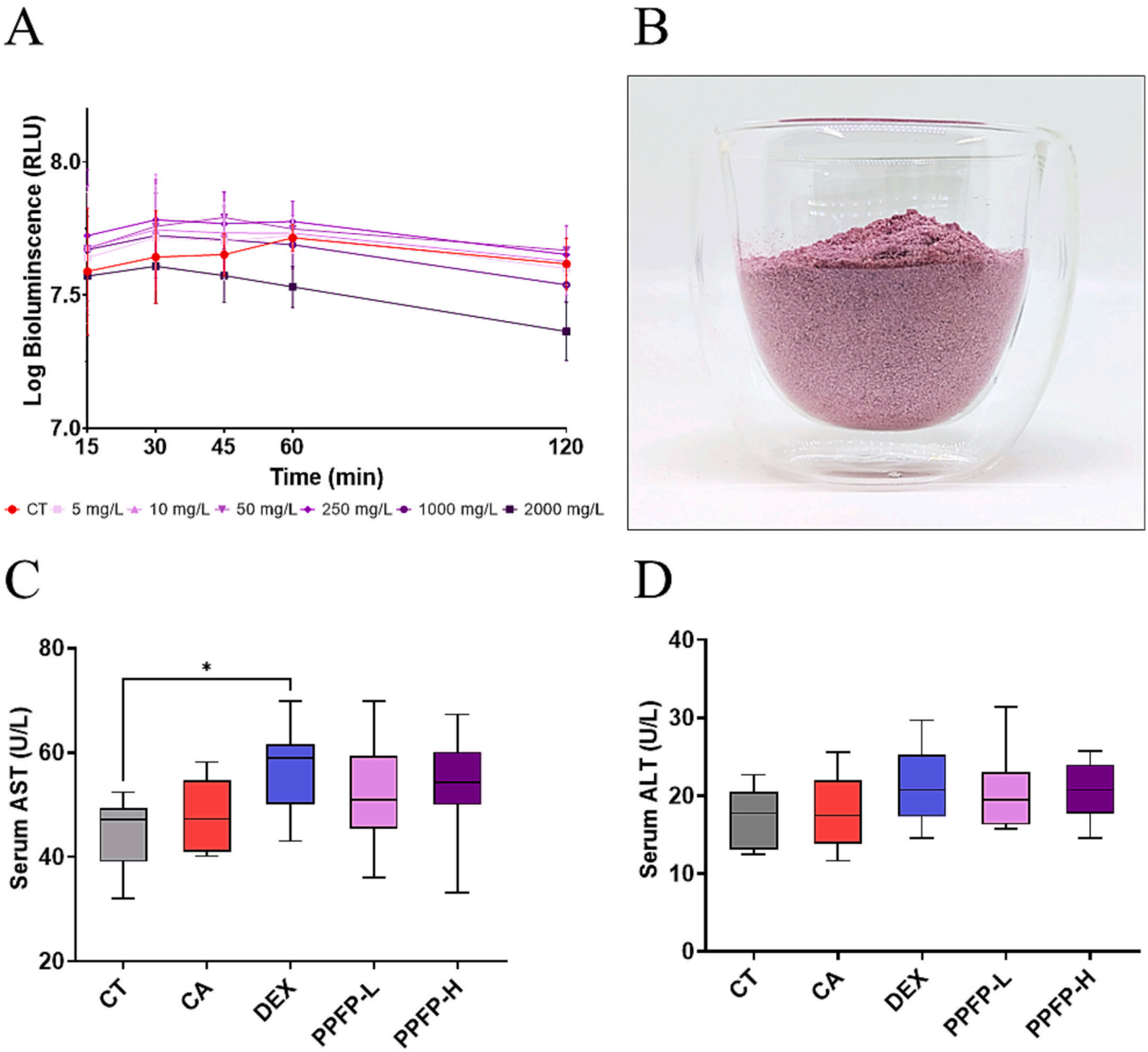

Fig. 2. PPFP toxicity evaluation with *A. fischeri* bioluminescence assay (A). Purple passion fruit peel powder (PPFP) used in diet supplementation of the animal experimentation (B). Toxicity evaluation of PPFP supplemented diet in the animal experimentation through the serum aspartate aminotransferase (AST) (C) and alanine aminotransferase (ALT) determination (D). CT – Control; CA – Control Asthma; DEX - Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-L – 3.0% Purple Passion Fruit Peel. * represents statistical significant differences between experimental groups ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Purple passion fruit peel powder toxicity evaluation

To evaluate PPFP toxicity and establish a maximum safe dosage to supplement the diet in the *in vivo* assay, an *A. fischeri* bioluminescence assay was performed. After confirming the correlation between the CFU and the bioluminescence signal of *A. fischeri*, the bacterium was exposed to PPFP (Fig. 2A) up to 120 min in concentrations ranging between 5 and 2000 mg/L (Fig. 2B). A control (0 mg/L) condition was also considered.

Fig. 2A shows that, until 120 min of incubation, concentrations of PPFP up to 1000 mg/L, were innocuous for *A. fischeri* since no statistically significant decrease in the bioluminescence signal was observed when compared to control. Only at a concentration of 2000 mg/L and after 120 min of incubation, a statistically significant viability reduction (3.16%, $p < 0.05$ vs CT) was observed. Thus, 1000 mg/L was selected as maximum safe dosage, and the corresponding maximum dosage was calculated by applying the **Eq. 1** of interspecies correlation between *A. fischeri* and mice, which translates into 360.7 g/kg of body weight (7.21 g for an average body weight of 0.02 kg). Therefore, the diets were prepared ensuring that PPFP doses did not exceed this value but also taking into consideration a realistic amount of PPFP that a person may incorporate in a balanced diet. For that reason, a 3.0% AIN-93 M supplementation in mice, which corresponds to 18.2 g of PPFP per day for a human (**Supplementary material 2**) was considered as a reasonable amount that a person may consume. This supplementation amount in mice (corresponding to 81.1 mg/L in *A. fischeri*) falls well within the established limit of toxicity (1000 mg/L).

Considering the substantial difference between the administered PPFP dosages and the determined safe limits, it was not expected to observe any toxicity effects of PPFP on mice during the in vivo experiment. In fact, AST and ALT quantification in mice sera (**Fig. 2C** and **D**) showed that no significant differences were observed in either enzymes activity when compared to the CT. The absence of significant changes in serum AST and ALT levels indicates that the compound did not cause detectable hepatocellular damage in mice after 14 days of dietary supplementation with PPFP up to 3.0%. While these findings suggest a favourable hepatic safety profile, more comprehensive and long-term safety studies would be necessary to entirely exclude chronic toxicity. According to OECD guidelines, repeated oral exposure of rodents for up to 28 days is considered subacute (OECD, 2025). Therefore, the 14-day supplementation protocol used in this study falls within the subacute range, allowing preliminary assessment of hepatotoxicity prior to subchronic or chronic evaluations.

3.3. Body weight, diet consumption and nutrients/compounds intake

Table 2 shows the average final body weight and the average diet consumption of each group in the challenge phase of the experiment. Based on these parameters and diet (**Supplementary Material 1**) and PPFP composition, energy, carbohydrate, fiber, TPC and vitamin C intake were estimated (Table 2).

Table 2
Comparison of final body weight, diet consumption, energy, carbohydrates, fiber, total phenolic compounds (TPC) and vitamin C intake of each group during the challenge phase of the experiment. Experimental groups: CT – Control; CA – Control Asthma; DEX - Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-L – 3.0% Purple Passion Fruit Peel.

Parameter	Experimental group				
	CT	CA	DEX	PPFP-L	PPFP-H
Final body weight (g)	18.50 ± 1.85[a]	19.25 ± 1.32[a]	18.43 ± 1.14[a]	18.28 ± 0.90[a]	18.69 ± 1.18[a]
Diet consumption (g/day)	2.60 ± 0.29[a]	2.60 ± 0.35[a]	2.42 ± 0.48[a]	2.39 ± 0.44[a]	2.32 ± 0.52[a]
Energy (kcal/day)†	10.1	10.1	9.40	9.28	8.99
Carbohydrates (g/kg/day)*	107.0	102.8	100.0	99.6	94.8
Fiber (g/kg/day)*	7.03	6.75	6.57	6.80	8.78
TPC (mg GAE/kg/day)*	–	–	–	9.61	91.2
Vitamin C (mg/kg/day)*	–	–	–	1.80	17.1

Results are expressed in mean ± standard deviation (SD). Statistical analysis performed by One-way ANOVA followed by Tukey; same letters indicate no statistical difference ($p < 0.05$). Experimental groups: CT – Control; CA – Control Asthma; DEX – Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-H – 3.0% Purple Passion Fruit Peel. Abbreviations: GAE: gallic acid equivalent, TPC: total phenolic compounds. † Calculated using the energy value of each diet: 3.88 kcal/g (AIN-93 M); 3.88 kcal/g (AIN-93 M + 0.3% PPFP); 3.87 kcal/g (AIN-93 M + 3.0% PPFP). *values estimated based on diet composition (Supplementary Material 1), on PPFP carbohydrate (831 g/kg DW), average TPC (24.5 mg GAE/g DW), vitamin C (4.58 mg/g DW) and fiber (694 g/kg DW) content, and group average diet consumption and body weight.

Average body weight at the end of the experiment ranged between 18.28 ± 0.90 g and 19.25 ± 1.32 g while the average diet intake of the experimental groups ranged between 2.32 and 2.60 g/day per animal. In terms of diet consumption, DEX (AIN-93 M), PPFP-L (AIN-93 M + 0.3% PPFP) and PPFP-H (AIN-93 M + 3.0% PPFP) groups showed a slight reduction compared to CT (AIN-93 M) and CA (AIN-93 M). However, this variation does not directly translate into the final group body weight observed. This is probably due to some slight variability in mice body weight prior to experiment. However, in both parameters, variability observed between groups is not statistically significant, suggesting that the PPFP supplementation did not markedly alter their feeding behaviour and body weight, thereby preserving the study validity and interpretability. These findings are consistent with other studies in which supplementation with powders of whole jabuticaba (5%), blueberries (10%) or strawberries (10%) to standard diets also did not change daily food intake or energy consumption (R. D. P. D. Nascimento et al., 2023; Prior et al., 2008).

Regarding estimated caloric intake, a slight variation that ranged between 8.99 (PPFP-H) and 10.1 kcal/day (CT and CA) is linked to diet intake variation since energy content is practically the same between the different diets (3.87–3.88 kcal/g). The same is observed for carbohydrate intake that ranged between 94.8 (PPFP-H) and 107.0 g/kg/day (CT). As seen before, these variations are not high enough to translate into significant body weight variability. In the current experiment, most of the fiber intake came from AIN-93 M formulation and thus remained similar for all groups (6.57–7.03 g/kg/day) with the exception of PPFP-H (8.78 g/kg/day), in which 3.0% PPFP supplementation contributed to approximately a 30% increase in fiber intake. Although this increase is not particularly substantial, it has been demonstrated that higher fiber intake enhances antioxidant defences and reduces inflammation in several animal models (Fang et al., 2017; Li et al., 2021; Yang et al., 2020).

PPFP-L and PPFP-H groups ingested an estimate of 9.61 and 91.2 mg GAE/kg/day of phenolic compounds and 1.80 and 17.1 mg/kg/day of vitamin C, respectively. Interestingly, a previous study supplemented Wistar rats diet with *P. edulis* peel flour with a similar TPC dose (11.72 mg GAE/kg/day), and reported a reduction in kidney lipid peroxidation, along with increased glutathione reductase (GR) activity and decreased GPx and SOD activities in the liver (da Silva et al., 2014). In line with higher phenolic intake of PPFP-H group in our work, another study administered an extract of jaboticaba to obese C57BL/6 J mice at doses of 50 and 100 mg GAE/kg/day. Over 14 weeks, this intervention conferred therapeutic benefits against obesity and related metabolic disturbances (Moura et al., 2021).

Despite some mixed results, several studies have shown an association between asthma and lower vitamin C concentration in blood and especially in lung fluids, likely due to increased oxidative stress and thus vitamin C supplementation has been tested for the treatment and/or management of asthma (Allen et al., 2009; Kelly et al., 1999). Jeong

et al. (2010) have shown that high dose of vitamin C (intraperitoneal injection of 3 or 5 mg twice, totalling a 6 or 10 mg administration throughout the experiment) exerted moderate anti-inflammatory effects in OVA-induced asthma BALB/c mouse model. In the current experiment, a comparable dose of total vitamin C (4.47 mg) was administered to the PPFP-H group.

3.4. Effects of purple passion fruit peel powder on inflammatory and antioxidant markers and histology

Inflammatory and antioxidant parameters were evaluated in mouse tissues after sacrifice at the end of the assay. Inflammatory markers (Fig. 3) include IgE concentration in serum and IL-4, IL-5 and IL-13 concentrations in BALF, while antioxidant markers evaluated (Fig. 4) include GSH concentration, CAT activity, SOD activity and MDA concentration in lungs.

Systemic (IgE in serum) and local (IL-4, IL-5 and IL-13 in BALF) inflammatory markers were determined to evaluate the impact of PPFP supplementation on inflammatory status in asthma. Serum IgE concentrations (Fig. 3A) were markedly elevated in CA group compared to CT (5.32 ± 0.70 vs. 1.13 ± 0.20 μg/mL, $p < 0.0001$), confirming successful allergic sensitization mediated by a Th2 response. Treatment with dexamethasone significantly reduced IgE levels (2.64 ± 0.40 μg/mL, $p < 0.0001$ vs. CA), while PPFP-H produced a more modest reduction (4.47 ± 0.48 μg/mL, $p < 0.05$ vs. CA). Although IgE levels in both groups remained above control values, this partial normalization indicates that PPFP, like dexamethasone, can suppress IgE synthesis. In the BALF, levels of IL-4, IL-5, and IL-13 were significantly increased in the asthma group compared with controls, reflecting robust activation of the Th2 axis, in line with the IgE data. Specifically, IL-4 concentrations (Fig. 3B) rose to 3.55 ± 0.63 ng/mg in the CA group compared to 1.70 ± 0.70 ng/mg in CT ($p < 0.0001$), while dexamethasone and PPFP-H treatment reduced IL-4 to 1.63 ± 0.70 ng/mg ($p < 0.001$ vs. CA) and 2.49 ± 0.71 ng/mg ($p < 0.05$ vs. CA), respectively. The same pattern was observed in IL-5 (Fig. 3C), which is also strongly upregulated in CA (14.4 ± 2.8 ng/mg vs. 6.5 ± 2.4 ng/mg, $p < 0.0001$) and decreased following treatment with dexamethasone (6.4 ± 2.0 ng/mg, $p < 0.0001$ vs. CA) and when supplemented with 3.0% PPFP (10.2 ± 3.5 ng/mg, $p < 0.05$ vs. CA). By contrast, IL-13 levels in BALF (Fig. 3D) that were increased by asthma induction (0.59 ± 0.1 in CA vs. 0.35 ± 0.07 ng/mg, $p < 0.0001$) and reduced by dexamethasone (0.41 ± 0.09 ng/mg, $p < 0.001$ vs. CA) were not significantly reduced by either PPFP dosages (0.49 ± 0.08 ng/mg in PPFP-H, ns vs. CA).

The observed results suggest that PPFP exerts selective effects on the Th2 cytokine network, effectively suppressing IgE, IL-4 and IL-5 but not IL-13. The reduction in IgE levels indicates inhibition of B-cell class-switch recombination, a process primarily driven by IL-4 signalling through the STAT6 pathway (Fahy, 2015; Ji & Li, 2023; Maspero et al., 2022). Similarly, the decrease in IL-5 levels suggests a suppression of Th2-mediated eosinophilic responses. This cytokine is essential for eosinophil recruitment, maturation, and survival, and its reduction indicates that PPFP may limit eosinophilic infiltration and associated tissue injury, a hallmark of late-phase asthmatic responses (Fahy, 2015; Ji & Li, 2023; Maspero et al., 2022). Since IL-13 is a critical driver of goblet cell metaplasia, mucus hypersecretion, and airway remodelling, the persistence of high IL-13 levels may indicate that PPFP alone is insufficient to significantly control the IL-13–MUC5AC axis, responsible for these pathological features (Fahy, 2015; Ji & Li, 2023; Maspero et al.,

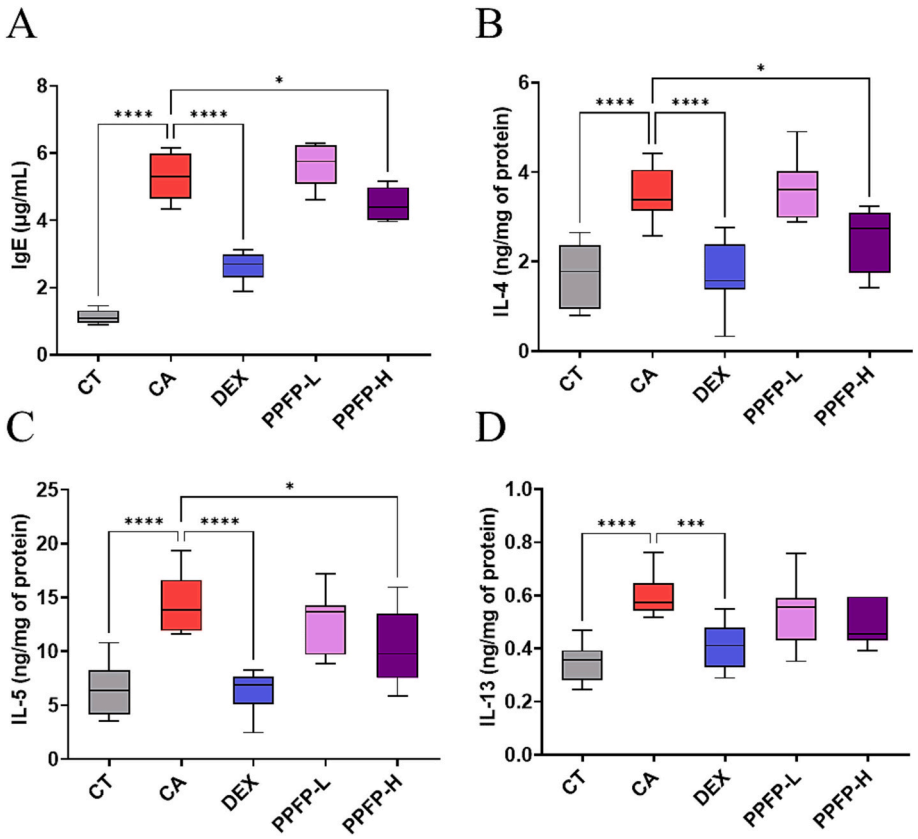

Fig. 3. Inflammation-related markers on serum and bronchoalveolar lavage fluid (BALF). Markers include immunoglobulin E (IgE) concentration on serum **(A)**; and interleukin 4 (IL-4) **(B)**, interleukin 5 (IL-5) **(C)**, and interleukin 13 (IL-13) **(D)** concentrations in BALF. Experimental groups: CT – Control; CA – Control Asthma; DEX - Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-L – 3.0% Purple Passion Fruit Peel. One-way ANOVA followed by Tukey ($p < 0.05$) was used to compare CA with remaining groups; only statistical differences between these groups are shown in the graphs (*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

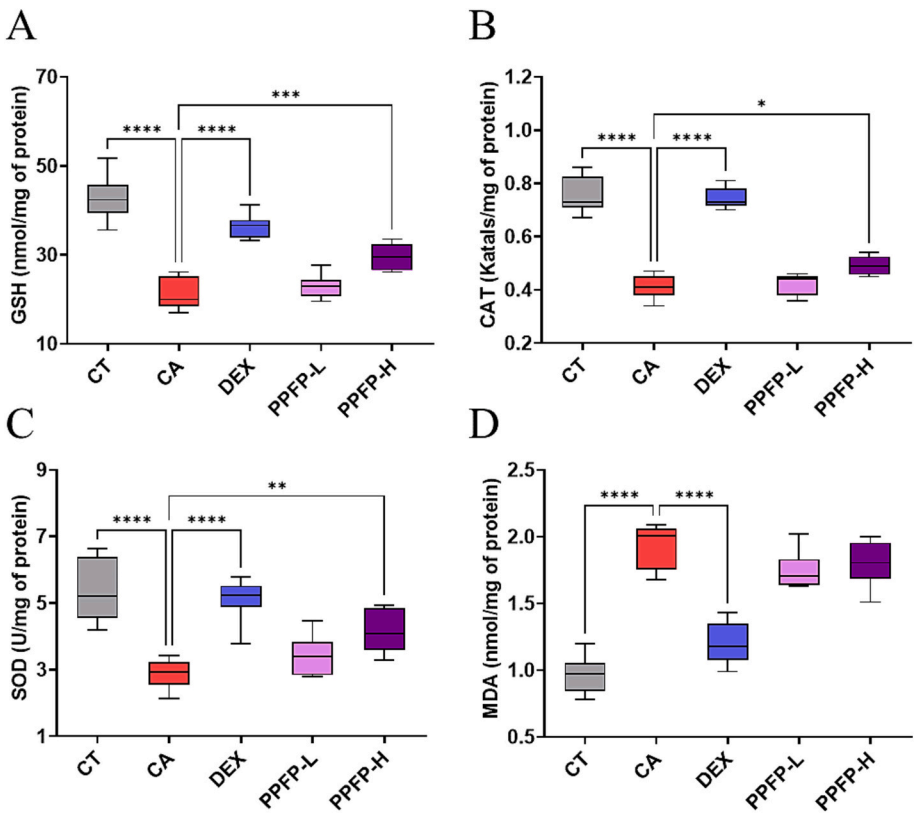

Fig. 4. Oxidative stress-related markers on lung tissue. Markers include total reduced glutathione (GSH) concentration **(A)**; catalase (CAT) activity **(B)**; superoxide dismutase (SOD) activity **(C)**; and malondialdehyde (MDA) concentration **(D)**. Experimental groups: CT – Control; CA – Control Asthma; DEX - Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-L – 3.0% Purple Passion Fruit Peel. One-way ANOVA followed by Tukey was used to compare CA with remaining groups; only statistical differences between these groups are shown in the graphs (*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2022). This persistence of IL-13 activity may contribute to persistent structural changes and partial therapeutic refractoriness, a phenomenon also described in asthma patients treated with anti-IL-4Rα therapies (e.g., dupilumab) (Castro et al., 2018; Svenningsen et al., 2023). These findings suggest a degree of pathway selectivity and point to the need for combination strategies or alternative interventions to achieve broader control of Th2-mediated airway pathology.

Besides the inflammatory profile, oxidative stress markers were also evaluated to assess whether the observed immunomodulation was accompanied by redox homeostasis restoration. Fig. 4A shows GSH levels were significantly reduced in CA compared to CT (20.88 ± 3.47 vs. 42.79 ± 4.68 nmol/mg protein, $p < 0.0001$), confirming that oxidative stress had been triggered by OVA-induced asthma. The same behaviour was observed in CAT (Fig. 4B) and SOD (Fig. 4C) activities that suffered a decrease of 46.1 and 46.9% respectively, in CA when compared to CT. Lipid peroxidation, measured by MDA levels (Fig. 4D), was markedly elevated in CA animals relative to CT (1.94 ± 0.17 vs. 0.97 ± 0.13 nmol/mg of protein, $p < 0.0001$) also corroborating an elevated oxidative membrane damage in CA group. As expected, treatment with dexamethasone (DEX group) markedly restored GSH concentrations (36.49 ± 2.57 nmol/mg protein), CAT (0.75 ± 0.04 Katals/mg protein) and SOD (5.13 ± 0.58 U/mg protein) activities, and MDA levels (1.21 ± 0.15 nmol/mg protein) towards CT values. Similar behaviour has been described in other studies, which is attributed to dexamethasone capacity to suppress inflammatory cell recruitment and ROS-generating enzymes, while also upregulating antioxidant defence systems (CAT, SOD, GSH), thereby restoring redox balance in the lung (Guan et al., 2020; Liang et al., 2022; Lu et al., 2025; Nounou et al., 2010).

While PPFP-L group didn't show a significant beneficial impact of PPFP on any oxidative stress marker, PPFP-H registered a partial improvement of GSH, CAT and SOD compared to CA. Although to a more modest degree than DEX, PPFP-H significantly increased GSH concentration (29.58 ± 2.97 nmol/mg protein, $p < 0.001$ vs. CA), CAT activity (0.49 ± 0.03 Katals/mg protein, $p < 0.05$ vs. CA) and SOD activity (4.19 ± 0.64 U/mg protein, $p < 0.01$ vs. CA). In the case of lipid peroxidation, not even the higher supplementation of PPFP was able to significantly reduce MDA concentrations in lung tissue. These findings are consistent with previous reports showing that pharmacological or nutraceutical interventions can restore endogenous antioxidant enzymes in allergic airway inflammation, although typically to a lesser extent than glucocorticoids such as dexamethasone (Chen et al., 2021; Lu et al., 2025; Yu et al., 2022). Interestingly, the elevation of enzymatic antioxidants did not translate into a significant reduction in lung MDA, a widely used marker of lipid peroxidation. While the limited specificity of conventional TBARS-based assays may contribute to these results, it is known that not all natural-sourced antioxidants equally protect lipid substrates from peroxidation (Ayala et al., 2014; Félix et al., 2020; Jardine et al., 2002). The ability of a compound to inhibit lipid peroxidation depends on factors such as redox potential, stability, and lipophilicity, which determine whether it can directly neutralize lipid radicals in membranes (Lü et al., 2010).

Thus, while PPFP was able to strengthen enzymatic defences against ROS, as reflected by the improvement of GSH, CAT, and SOD, its limited impact on MDA levels suggests that these effects do not derive from a potent direct antioxidant activity. Rather, the data allow us to hypothesize that the improvement in enzymatic antioxidant status might involve transcriptional regulation mediated by the major phenolic constituents. Specifically, the chemical characterization enabled the identification of C3G as the predominant flavonoid (4.03 mg/g).

Although the present study evaluated the whole PPFP matrix rather than testing the bioactivity of isolated chemical groups, C3G and its metabolites (protocatechuic and vanillic acids) are known to activate the Nrf2 pathway (Klaassen & Reisman, 2010; Tan et al., 2019), which governs the expression of the antioxidant enzymes we found to be upregulated (CAT, SOD). Therefore, it is plausible to infer that the observed enzymatic enhancement could be mechanistically linked to the presence of these compounds. The lack of significant reduction in MDA further reinforces the notion that the PPFP matrix may primarily enhance endogenous defences, rather than effectively neutralize ongoing lipid peroxidation directly.

Complementarily, the observed anti-inflammatory efficacy might be partially associated with the high dietary fiber content (69.4%) of the PPFP matrix, which is primarily composed of pectin and cellulose. As previously mentioned, literature indicates that the fermentation of these fibers by the gut microbiota yields SCFAs, which are increasingly recognized to modulate airway inflammation via the gut–lung axis (Cait et al., 2018). Taking this into consideration, we hypothesize that the overall beneficial effect of PPFP relies on a synergistic interplay of its multiple constituents. This potentially involves fiber-mediated systemic immunomodulation (reducing the inflammatory trigger) combined with polyphenol-mediated reinforcement of enzymatic antioxidant defences.

However, it must be emphasized that these mechanisms remain literature-supported hypotheses generated from the chemical composition of PPFP and the resultant systemic responses. Since no direct molecular evidence (such as Nrf2 protein expression analysis or SCFA quantification) was obtained in the present study, validating the precise role of the Nrf2 pathway and the gut-lung axis constitutes a critical priority for future mechanistic investigations. Ultimately, the current findings establish an association between the PPFP intervention and the observed immunomodulatory and antioxidant benefits, rather than proving direct molecular causation.

Histological analysis with H&E staining of lung sections revealed normal pulmonary architecture in the CT group, characterized by preserved alveolar spaces and absence of inflammatory infiltrates (Fig. 5). In contrast, OVA-challenged mice (CA group) exhibited pronounced peribronchial and perivascular inflammatory cell infiltration, airway smooth muscle thickening, goblet cell hyperplasia, and mucus hypersecretion. Treatment with dexamethasone markedly reduced these histopathological alterations, restoring lung morphology to a state comparable to the CT group. Supplementation with PPFP-H attenuated inflammatory infiltration and airway structural alterations compared to the CA group. However, some inflammatory cell infiltration, goblet cell hyperplasia, and mucus accumulation remained evident.

Histological analysis confirmed that OVA challenge induced hallmark features of allergic airway inflammation, consistent with previous reports of murine asthma models (Huang et al., 2021, 2024; Liao et al., 2022; Zhang et al., 2017). The attenuation of peribronchial and perivascular infiltration in PPFP-H–supplemented animals is aligned with the observed reduction in IL-5 and IgE, indicating suppression of eosinophilic inflammation. A limitation of our experimental design was the absence of direct differential cell counting in the BALF. However, it is well established that IL-5 is the key driver of eosinophil differentiation and recruitment. Therefore, we consider that the significant reduction in

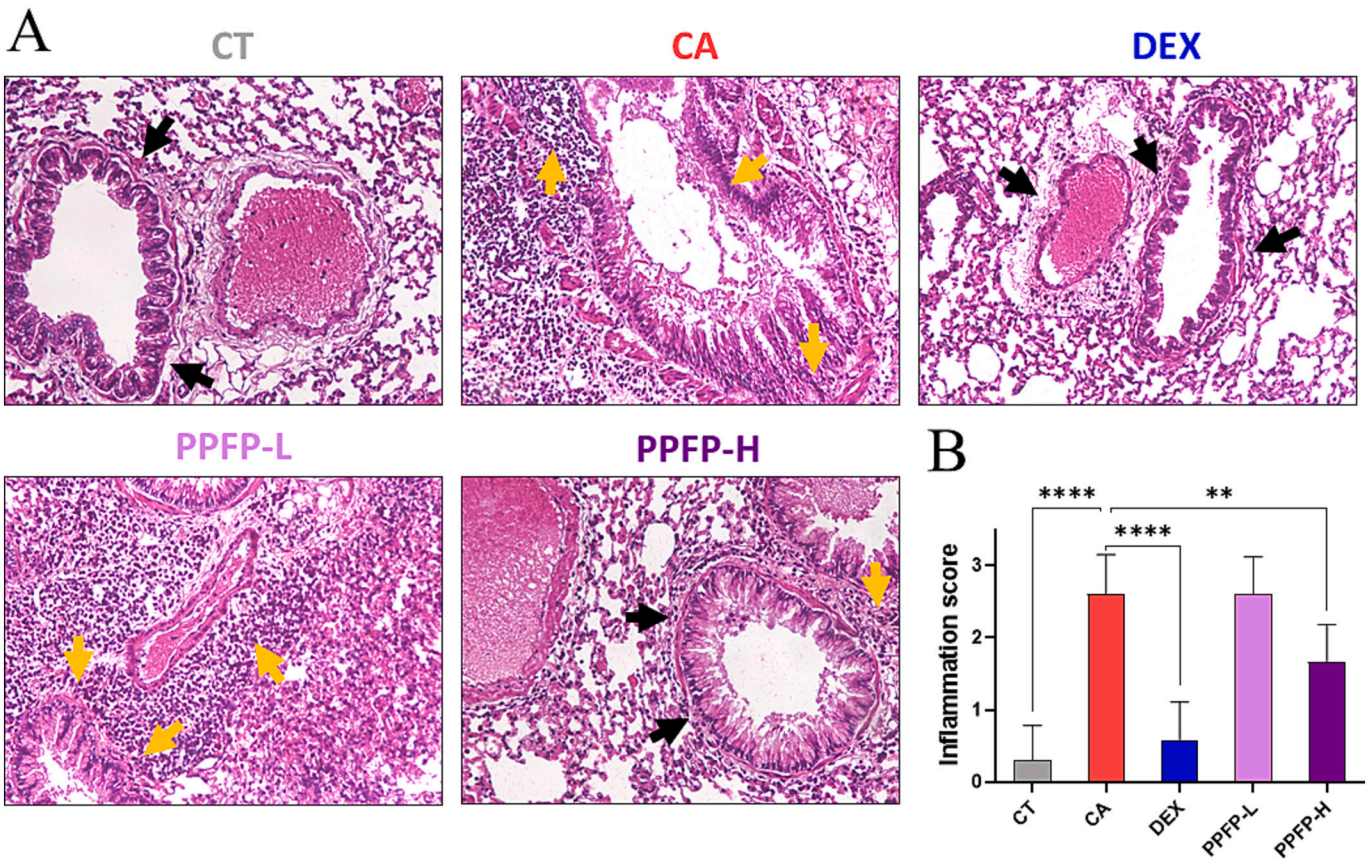

Fig. 5. Representative of histopathological changes in hematoxylin and eosin (H&E) stained lung tissue at 200× (**A**) obtained from mice of different groups: CT – Control; CA – Control Asthma; DEX - Dexamethasone (0.5 mg/kg); PPFP-L - 0.3% Purple Passion Fruit Peel; and PPFP-L – 3.0% Purple Passion Fruit Peel. Yellow arrows indicate peribronchial inflammatory cell infiltration and black arrows indicate areas of reduced inflammatory infiltrate and preserved bronchial structure. Semi-quantitative analysis of peribronchial and perivascular inflammatory cell infiltration (**B**) was performed using a grading scale of 0 (no inflammation) to 3 (severe inflammation). Data are presented as mean ± standard deviation (SD). ** $p < 0.01$; **** $p < 0.0001$vs CA group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

BALF IL-5 levels observed in the PPFP-H, combined with the reduction of inflammatory cell infiltration seen in the H&*E*-stained lung sections, provides robust evidence of the anti-inflammatory activity of the intervention. Regarding airway remodelling, although Periodic Acid-Schiff (PAS) staining was not performed to quantify mucus hypersecretion, we consider that cytokine data offers a clear mechanistic insight. The persistence of unaltered IL-13 levels in the treated groups strongly suggests that goblet cell hyperplasia was not reversed, since IL-13 is the primary driver of goblet cell metaplasia and MUC5AC upregulation (Kuperman et al., 2002; Wills-Karp et al., 1998). This "uncoupling" between the reduction of eosinophilic inflammation (IL-5/IgE) and the persistence of remodelling markers (IL-13) is a noteworthy observation. We speculate that this divergence could relate to the distinct cellular sources and signalling pathways governing these cytokines. Systemic markers such as IgE and IL-5 are heavily driven by circulating Th2 cells and B-cells, which are highly responsive to systemic immunomodulation, such as the SCFA-mediated effects arising from dietary fiber fermentation (Cait et al., 2018; Trompette et al., 2014). In contrast, airway IL-13 can also be abundantly produced by tissue-resident innate lymphoid cells (ILC2s) in the lung mucosa in response to local epithelial alarmins (Halim et al., 2012; Scanlon & McKenzie, 2012). It is plausible that the complex PPFP matrix, while effective at dampening systemic sensitization and reinforcing antioxidant defences, may not sufficiently reach or inhibit these specific localized mucosal pathways.

Given the anti-inflammatory and antioxidant effects observed in this study, a notable limitation is the absence of airway hyperresponsiveness (AHR) measurements. Because AHR is a hallmark functional parameter of asthma, the lack of these data limits our ability to claim full therapeutic efficacy. Consequently, the beneficial effects of PPFP reported herein must be strictly interpreted as an attenuation of the immunological and histopathological features of the disease, rather than a complete functional resolution. Evaluating lung function through plethysmography will be a crucial next step to confirm whether the reduction in eosinophilic inflammation and oxidative stress translates into functional improvements in lung mechanics and reduced bronchoconstriction.

Finally, it is important to emphasise the specific nature of the route of administration of dietary supplements in the context of standard asthma therapy. While first-line pharmacological treatments (e.g., corticosteroids, β_2-agonists) are typically administered via inhalation to maximize local bioavailability and minimize systemic effects, PPFP was administered orally. Therefore, this dietary intervention should be viewed as a preventive or adjuvant strategy rather than an acute treatment. However, the oral route is advantageous and necessary for dietary fiber-rich interventions, as it allows for interaction with the gut microbiota and the subsequent production of SCFAs, which are increasingly recognized to modulate airway inflammation via the gut–lung axis, representing a likely mechanism contributing to the efficacy of PPFP (Cait et al., 2018; Frati et al., 2018).

4. Conclusions

This study evaluated the in vivo potential of PPFP powder —a matrix rich in dietary fiber and containing a diverse profile of bioactive compounds — as a safe, minimally processed and sustainable dietary coadjuvant for mitigating inflammation and oxidative stress in asthma. Dietary supplementation with 0.3% and 3.0% PPFP (safety-validated and physiologically relevant dosages), did not modify mouse feeding behaviour or body weight and did not induce hepatotoxicity. While the 0.3% supplementation did not show any significant effect, 3.0% of PPFP enhanced enzymatic defences (GSH, CAT, SOD), reduced systemic IgE, and attenuated IL-4/IL-5–mediated Th2 responses, which was corroborated by the marked reduction in peribronchial and perivascular inflammatory cell infiltration observed in lung histopathology. Rather than a single isolated effect, the beneficial effect of PPFP likely stems from the combined interplay of its multiple constituents. Based on existing literature, the intervention may have enhanced enzymatic defences (GSH, CAT, SOD) through transcriptional activation (e.g., Nrf2 pathway) driven by polyphenols (particularly C3G), rather than simple direct radical scavenging, as evidenced by the stable MDA levels. At the same time, the attenuation of systemic IgE and IL-4/IL-5–mediated eosinophilic inflammation highlights an immunomodulatory effect, which we hypothesize could be reinforced by fiber-derived metabolites acting via the gut–lung axis. Despite its effects on IL-4, IL-5 and IgE, PPFP did not significantly modulate IL-13, thus indicating incomplete control of the IL-13–MUC5AC axis and persistence of mucus hypersecretion and goblet cell hyperplasia.

In summary, PPFP shows promise as a supportive nutritional strategy and a dietary adjunct to complement conventional therapies in asthma management, helping to mitigate Th2-driven airway inflammation and oxidative stress. It must be emphasized that this intervention is positioned as a nutritional coadjuvant, not as an alternative to acute pharmacotherapy, such as inhaled corticosteroids. Furthermore, while standard asthma medications rely heavily on inhalation to maximize rapid, localized efficacy, the oral administration route of PPFP is fundamentally essential to its proposed mechanism of action. This oral delivery allows the whole-food matrix, particularly its high dietary fiber content, to interact with the gut microbiota, a necessary step for the generation of metabolites that may be responsible for the systemic gut-lung axis immunomodulation. Nevertheless, future studies should focus on assessing AHR to confirm whether these immunological and antioxidant benefits translate into improved lung function. Additionally, long-term studies and combination-treatment evaluations with standard pharmacological drugs are also warranted.

Author contribution

A.M.A.F. performed the experimental work, validation, and formal analysis; wrote the original version, edited the final version, and prepared illustrations; R.P·N performed ELISA experimental work and review of the final version; L.B. contributed in animal experimentation; C. V. contributed to *A. fisheri* assay experimental work; A.A. review of the final version; R.G.B performed HPLC analysis; C.C.L review of the final version; M.R.M.J. was responsible for supervision, performed interpretation of the global results and review of the final version; A.J.D.S. was responsible for conceptualization, supervision, performed interpretation of the global results, writing, and review and editing of the final version; S.M.R. was responsible for conceptualization, supervision, writing, review and editing of the final version, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

CRediT authorship contribution statement

Alexandre M.A. Fonseca: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Roberto de Paula do Nascimento:** Writing – review & editing, Investigation. **Levi Bellinazzi:** Investigation. **Cátia Vieira:** Investigation. **Adelaide Almeida:** Writing – review & editing. **Renata Galhardo Borguini:** Investigation. **Cláudia C. Loureiro:** Writing – review & editing. **Mário R. Maróstica Junior:** Writing – review & editing, Validation, Supervision. **Armando J.D. Silvestre:** Writing – review & editing, Validation, Supervision, Conceptualization. **Sílvia M. Rocha:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.119503.

Data availability

Data will be made available on request.

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