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Lung injury caused by occupational exposure to particles from the industrial combustion of cashew nut shells: a mice model

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ABSTRACT

Cashew nut shells (CNS) is already used in the energy matrix of some industries. However, it is necessary to know the harmful health effects generated by exposure to pollutants of its combustion, especially in the workers exposed to industrial pollutants. In addition, it is known that the incidence of asthma grows among workers in industries, and due to its previously reported biological effects of anethole, these will also be objects of the present study. We used 64 Balb/C mice, randomly divided into eight groups. Groups were sensitized and challenged with saline or ovalbumin, then subjected to intranasal instillation of 30 µg PM_{4.0} (occupational exposure) from the combustion of CNS or saline, and then were subsequently treated with oral anethole 300 mg/kg or 0.1% Tween 80. Our results serve as a starting point for the development of public policies for the prevention of diseases in workers that are exposed to the pollutants coming from industries.

KEYWORDS

Anethole; cashew nut shells; occupational exposure; OVA-induced asthma; respiratory system

Introduction

There is increasing concern among the governments of leading nations about the possible adverse effects caused by air pollution, particularly in large urban centers. The use of biofuels as a source of energy seems to be the alternative to guarantee economic development, while decreasing pollution and its harmful effects on human health and the environment.

Data from the International Renewable Energy Agency show that, in relation to the estimated total capacity for energy production from renewable resources in 2017, Brazil ranked third in the world (128,293 MW), behind only China (618,803 MW) and the USA (229,913 MW), corresponding to 63.47% of the total capacity in South America. As for energy production capacity from renewable solid biofuels and waste, Brazil ranked first in the world (14,330 MW), corresponding to 86.05% of the total capacity in South America and 15.92% worldwide.¹

Brazil is one of the largest agricultural producers in the world and has great potential for the production of residual biomass, such as cashew nut shells (CNS), a cashew nut processing residuum. The cashew tree (*Anacardium occidentale* L.) is a tropical plant, native to Brazil and available in almost all of its territory.

The utilization of residual products from the processing of cashew nuts (such as CNS) as an energy source is already a reality in some industries. However, this requires the use of filters to contain toxic emissions and pollutants from exhaust gases arising from their combustion.²

Due to the increased use of biomass in the energy matrix of some industries, studies are relevant that aim to evaluate the effects that the industrial pollutants from the combustion process of biomass can cause in the health of its workers. Exposure to PM_{4.0} simulates an occupational hazard experienced by industry workers.³ In addition, it is known that the incidence of asthma grows among workers in industries.⁴

Asthma is a chronic inflammatory pathology of the airways, in which changes occur in various cells, such as eosinophils, mast cells, neutrophils, dendritic cells and T lymphocyte.⁵ Exposure to environmental pollutants and the induction or exacerbation of asthma are closely intertwined due to mechanisms such as direct effect on airways, toxic effect on respiratory epithelium, development of bronchial hyperresponsiveness, and decreased immune response.⁶

Asthma is rapidly diagnosed in most cases and usually responds to inhaled corticosteroids, adjusted for

symptoms and lung function. However, up to 40% of adult asthmatic patients remain symptomatic, and up to 5% have poorly-controlled asthma, despite multiple therapies,⁷ proving the need for alternative therapies to supplement the use of corticosteroids, such as Anethole.

Anethole (1-methoxy-4-propenyl-benzene, isoeustragole) is an alkoxypropenylbenzene derivative and an important flavoring component of the essential oils of more than 20 plant species.⁸ Anethole is commonly used as a flavoring agent in the food, beverage, cosmetic and medical liquids industries. Worldwide, traditional uses of plants that contain anethole include mainly antioxidant,^{9–11} anti-inflammatory,¹² antithrombotic,¹³ anesthetic,¹⁴ healing,¹⁵ and anti-carcinogenic⁹ substances, as well as and relaxing agents with effect on aortic rings.^{16,17}

Thus, in an attempt to determine the probable health effects of exposure to pollutants from burning CNS, we analyzed the effects of PM_{4.0} from CNS combustion exhaust gases on the respiratory systems of healthy mice, as well as in mice submitted to an OVA-induced asthma model. We also evaluated the benefits of treatment of these lung lesions using anethole. Due to its many favorable biological properties, especially its antioxidant and anti-inflammatory actions, anethole is a candidate for alternative therapy of asthma and its exacerbations caused by exposure to pollutants.

Materials and methods

Combustion process and aqueous suspensions for intranasal instillation

The process of combustion and collection of PM_{4.0} from the exhaust gases of the CNS used in this work, as well preparation of the aqueous suspensions for intranasal instillation of PM_{4.0} has been previously described.¹⁸

Animals

Balb/C mice (7–8 weeks of age), with a body mass of 25 ± 5 g and water and food *ad libitum*, were used in this study. Mice were housed in plastic cages under controlled environmental conditions. All use and care procedures had been previously approved by the animal ethics committee of the State University of Ceará (Protocol N° 3113976). Invasive procedures were performed under anesthesia, and every effort was made to minimize suffering.

Induction of murine model of asthma and experimental design

Mice were sensitized and challenged by Ovalbumin (OVA) (Sigma-Aldrich, St. Louis, MO, USA) according to a modified version of a previously described method.¹⁹ In short: BALB/c mice were sensitized with 100 µg of subcutaneous OVA, emulsified with 1 mg of aluminum hydroxide adjuvant (Sigma, MO, America), in 200 µL of phosphate buffered saline (PBS) on days 0, 7, 14. An intranasal challenge was performed, always under anesthesia with sevoflurane, to provoke pulmonary aspiration of the OVA solution. This challenge was performed on days 25, 26 and 27 with OVA (100 µg, diluted in 50 µL of DPBS). The negative control group received an equal volume of saline solution (NaCl 0.9%) subcutaneously (days 0, 7 and 14) and intranasally (days 25, 26 and 27).

The experimental design is shown in Figure 1. We used 64 Balb/C mice, randomly divided into eight groups (n = 8). In the SST, SSA, SMT and SMA groups, the mice were submitted to the negative asthma control protocol mentioned previously (27 days). These mice received either intranasal instillation of 30 µL of solution obtained from sonication of a clean glass-fiber filter (SST and SSA groups) or 30 µg PM_{4.0} from the CNS combustion exhaust gases diluted in 30 µL of saline solution (SMT and SMA groups) (day 28), and subsequent gavage with 200 µL of 0.1% Tween 80 in water (SST and SMT groups) or gavage with 200 µL of anethole (300 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) (SSA and SMA groups) (day 29). In the OST, OSA, OMT and OMA groups, mice were sensitized and challenged with OVA as previously mentioned (27 days) and then given intranasal instillation of 30 µL of solution from sonication of a clean glass-fiber filter (OST and OSA groups) or 30 µg PM_{4.0} from the CNS combustion exhaust gases diluted in 30 µL of saline solution (OMT and OMA groups) (day 28); animals were then submitted to gavage with 200 µL of 0.1% Tween 80 in water (OST and OMT groups) or gavage with 200 µL of anethole (300 mg/kg) (OSA and OMA groups) (day 29). All analyzes were performed 24 h after gavage with the vehicle or anethole (day 30).

The oral dose of 300 mg/kg anethole was chosen due to the use of an equivalent dose in previous studies evaluating its effects on the acute inflammatory response,^{20,21} in gastroprotective action²² and the lipopolysaccharide-induced acute lung injury in mice.²³

Respiratory system mechanics

24 hours after gavage with Tween or anethole, the animals were anesthetized with sodium pentobarbital

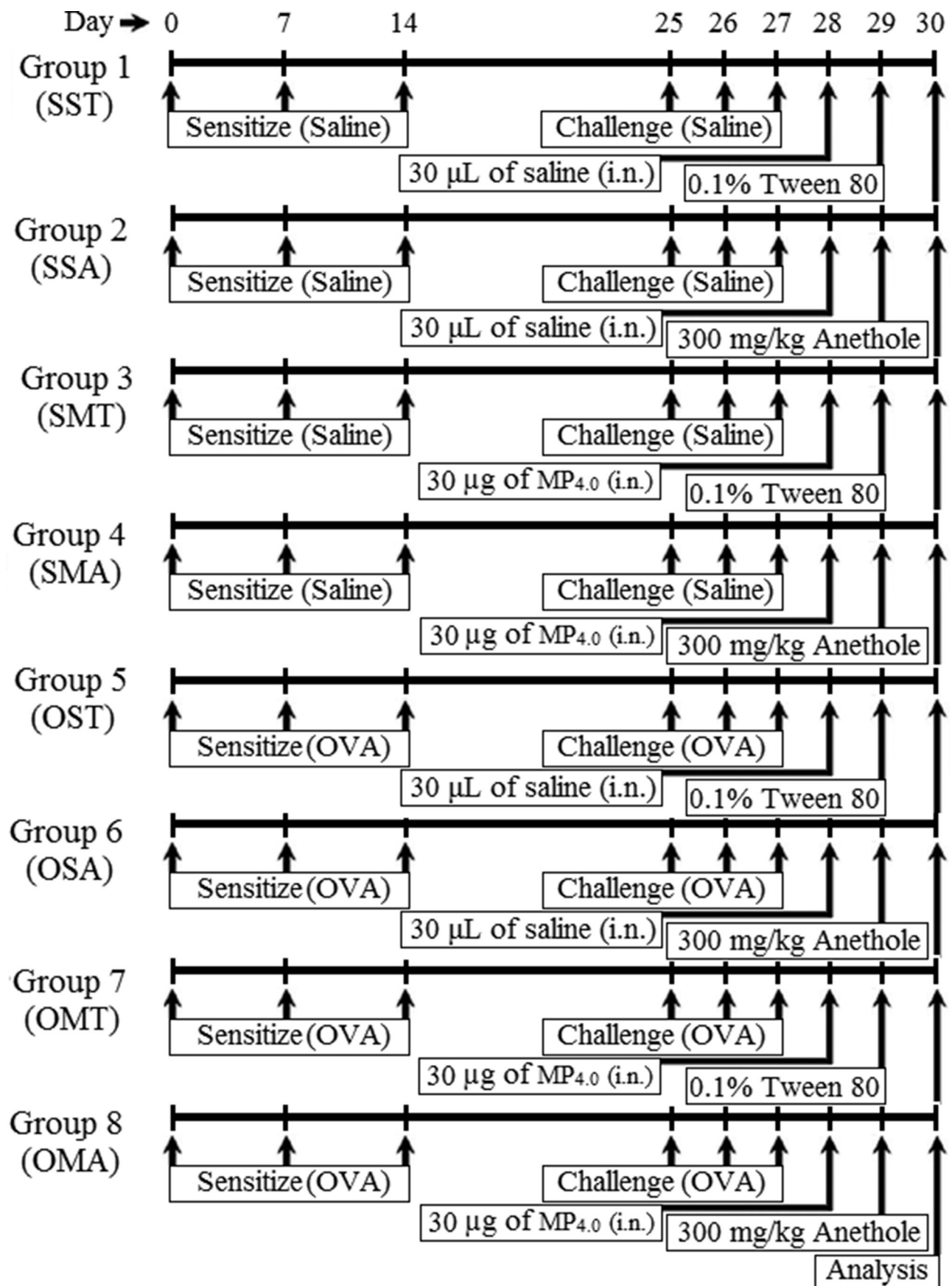


Figure 1. Schematic diagram of the experimental protocol.

(50 mg/kg, i.p., Hypnol® 3%, Syntect, Brazil) and tracheostomized. The animals were intubated with a 14-gauge cannula (Eastern Medikit, Delhi, India) that was then connected to a computer-controlled ventilator for small animals (Scireq© FlexiVent®, Montreal, QC, Canada). The animals were ventilated at the following baseline settings: respiratory frequency of 120 breaths/min, tidal volume of 10 mL/kg, pressure limit of 30 cmH₂O, and positive end-expiratory pressure (PEEP) of 3 cmH₂O. The mice were then paralyzed with pancuronium bromide (0.5 mL/kg, i.p., Cristália, Brazil).

Initially we standardized the mechanical history of the respiratory system with two deep inflations (DI, 6-s long, peak pressure: 30 cmH₂O) followed by ventilation during 5 minutes with the baseline settings. Soon after, the impedance of the respiratory system (Z_{rs}) was measured with the forced oscillation technique,²⁴ 12 sequential 30-s sampling intervals, for a total of 6 minutes.²⁵

The experimental Z_{rs} was fitted to the constant phase model as previously described:²⁶

$$Z_{rs} = R_N + I(2\pi f)i + \frac{G - Hi}{(2\pi f)^\alpha} \quad (1)$$

$$\alpha = \frac{2}{\pi} \tan^{-1} \left(\frac{H}{G} \right) \quad (2)$$

where R_N is the Newtonian resistance, which represents the central airways resistance, $i = \sqrt{-1}$, f is the frequency (Hz), I represents airway inertance, and G and H are respectively the dissipative and elastic properties of lung tissue.²⁴

Thereafter, starting at the functional residual capacity (FRC) defined by the PEEP, the FlexiVent delivered 7 inspiratory pressure steps for a total pressure of 30 cmH₂O, followed by 7 expiratory steps, pausing at each step for 1 s. At each step, plateau pressure (P) was recorded and related to the total volume (V) delivered, to produce a quasi-static PV (pressure-volume) curve. Static compliance (C_{ST}) was calculated as the slope of the curve (21). Five quasi-static PV curves were obtained to measure C_{ST} , an estimate of inspiratory capacity (IC), and PV loop area. Another forced oscillation technique ensued to determine respiratory system mechanics.

Methacholine challenge

Immediately after measurements of respiratory system mechanics, two DIs were performed, followed by 5 minutes of ventilation with baseline settings. Airway smooth muscle hyperresponsiveness was

evaluated by inhalation of methacholine (MCh) (Sigma-Aldrich, St. Louis, MO, USA), delivered by aerosol produced by an ultrasonic nebulizer (Inalasonic, NS, São Paulo, Brazil) coupled to the inspiratory line of the ventilator. For this purpose, 4 mL of MCh solution (30 mg/mL) were added to the nebulizer container.

Nebulization was carried out during 30 s under mechanical ventilation,²⁷ and the average amount delivered to the animal was 0.12 mg/kg of MCh solution. After nebulization, the aforementioned analysis was repeated (forced oscillation, 10-s sequential intervals for 4 min), followed by two DIs and another forced oscillation data gathering. Data regarding airway hyperresponsiveness (AHR) collected after nebulization of MCh are presented as ΔR_N , where Δ means the parameter after nebulization minus its value before MCh challenge. All Δ values were normalized using the pre-nebulization values.²⁸

Histological study

Slides containing left lung sections were stained with hematoxylin and eosin (HE) and examined by optical microscopy, according to their qualitative and quantitative aspects. An investigator blinded to the origin of the coded material examined the samples microscopically. Quantitative analysis was performed using an integrated eyepiece, with a coherent system consisting of a 100-point and 50-line grid coupled to a conventional light microscope. The fraction area of collapsed alveoli (alveolar morphometry), and the amount of polymorphonuclear (PMN) cells, as well as pulmonary tissue area, were determined by the point-counting technique.²⁹ Cellularity at 1000× magnification across 10-15 random non-coincident microscopic fields in each animal was assessed.

The air-space enlargement was quantified by the mean linear intercept length of the distal air spaces (L_m) in 30 randomly chosen fields of tissue sections per group.³⁰ The bronchoconstriction index (BCI) was determined in 10 non-coincident microscopic fields per animal, by counting the number of points in the airway lumen (NP) and intercepts through the airway wall (NI), using a reticulum and applying the equation $BCI = NI/\sqrt{NP}$. Only airways in which the long diameter did not exceed the short diameter by more than 20% were accepted for measurement.³¹ Alveolar morphometric analysis and determination of the bronchoconstriction index were done at 400× magnification.

Statistical analysis

Results are presented as mean $\pm$ SD, where n represents the number of samples. For comparison between groups, we used a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test or Bonferroni's Multiple Comparison Test. A difference was considered significant if $p < 0.05$.

Results

Figure 2 shows respiratory system mechanical data (constant phase model and quasi-static PV curve) of all groups. We observed changes between the OST, OMT and OMA groups compared to the SST group in all analyzed variables (one-way ANOVA followed by Student-Newman-Keuls test).

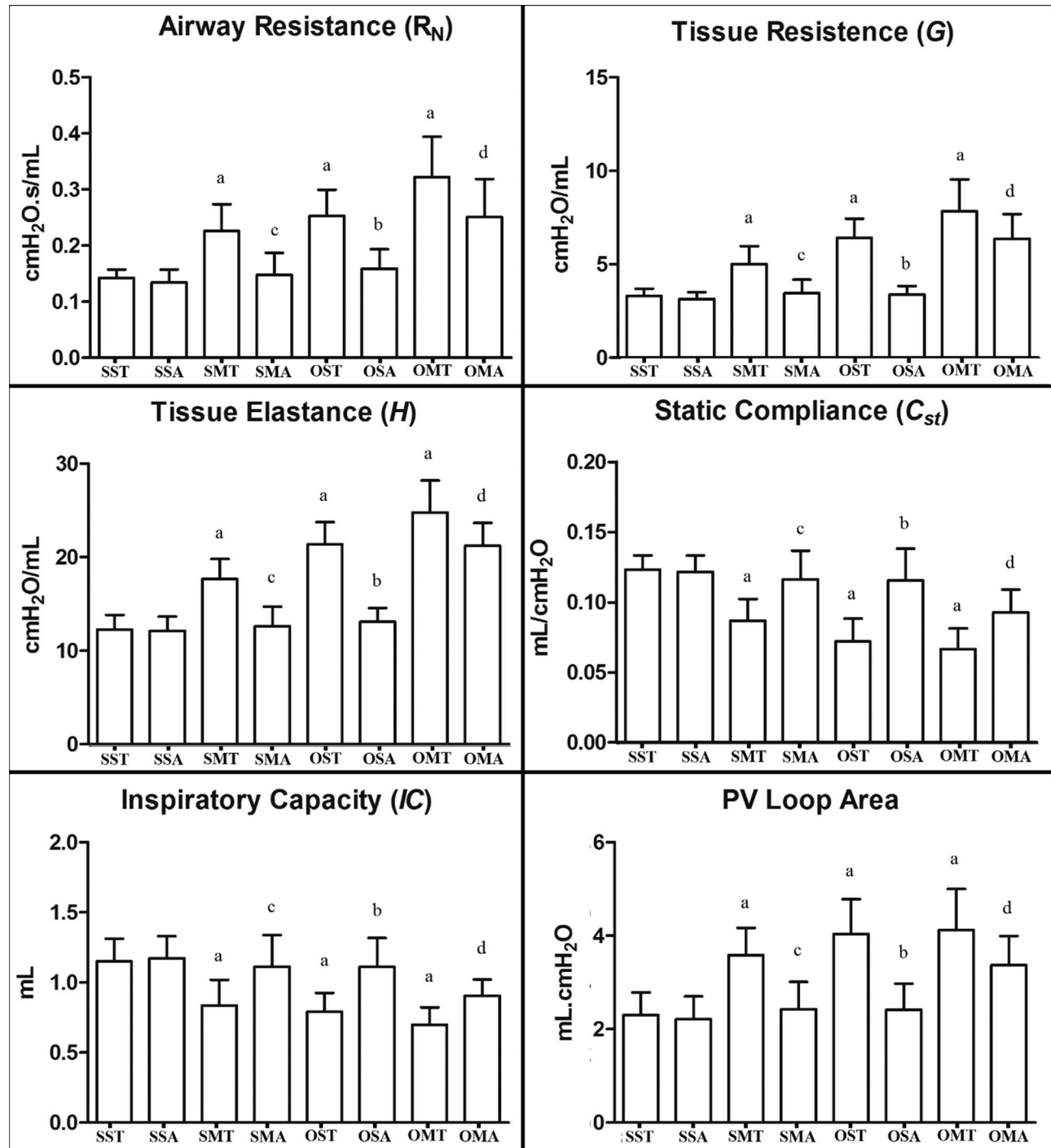


Figure 2. Differences between lung function parameters. Values are mean $\pm$ SD of all groups. ^a Different from SST group ($p < 0.05$). ^b Different from OST group ($p < 0.05$). ^c Different from SMT group ($p < 0.05$). ^d There was no difference from OMT group ($p > 0.05$). By one-way ANOVA followed by multiple comparisons corrected with Bonferroni's test.

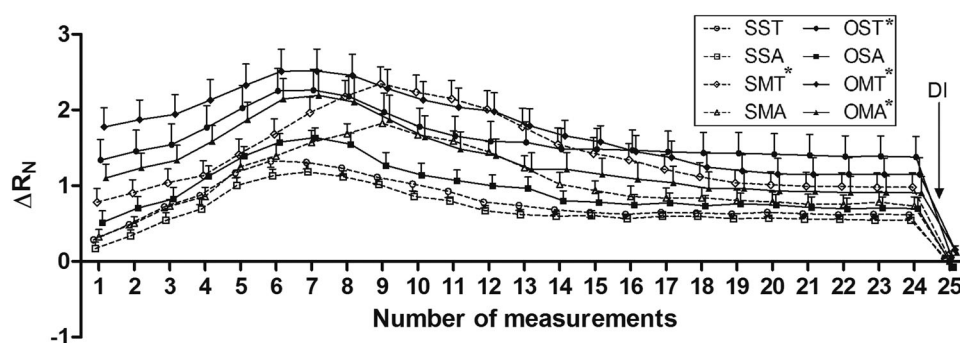


Figure 3. Change in mechanical parameters in SST (open circles), SSA (open squares), SMT (open lozenges), SMA (open triangles), OST (closed circles), OSA (closed squares), OMT (closed lozenges) and OMA (closed triangles) groups as a function of number of measurements after MCh nebulization (30 mg/mL for 30 s). Data are mean $\pm$ SEM. ΔR_N , lung tissue resistance after nebulization minus its corresponding value before MCh challenge; DI, deep inflation. *Represents statistically significant differences ($p < 0.05$) by one-way ANOVA followed by Bonferroni's multiple comparison test.

Figure 3 shows the time evolution of variations in ΔR_N after administration of MCh (30 mg/mL) in all groups. Changes were seen in the set of temporal measurements for the SMT, OST, OMT and OMA groups, in comparison to the SST group (one-way ANOVA followed Bonferroni's multiple comparison test), evidencing airway hyperresponsiveness (AHR).

Figure 4 depicts representative lung and airway (inserts) histological images from all groups. SMT, OST, OMT and OMA groups displayed thickened septae, cellular infiltrates, alveolar collapse and constricted airways (inserts). Alveolar collapse, thickened septa and cellular infiltration were observed in the photomicrographs of the pulmonary parenchyma from the SMT, OST, OMT and OMA groups.

Table 1 displays alveolar collapse, quantity of polymorphonuclear cells, mean alveolar diameter and bronchoconstriction index. We observed an increase in alveolar collapse, number of polymorphonuclear cells, mean alveolar diameter and bronchoconstriction index within the SMT, OST, OMT and OMA groups, when compared to the SST group. Altogether, these findings may suggest pulmonary inflammation and bronchoconstriction.

Discussion

Due to the harmful effects on the environment and on human health caused by the release of gases from the combustion of petroleum by-products, several countries have increased efforts to promote the use of renewable sources in their energy matrices. The use of biomass thus figures as an attractive alternative.

Brazil is one of the largest agricultural producers in the world, with great potential for the generation of residual biomass. Obtaining CNS begins through the process of extracting the cashew nut from the shell

(decortication), which occurs in cardol-filled tanks also containing 10% of the liquid extracted from the cashew nuts themselves. This is heated in boilers at a temperature of $\pm 800^\circ\text{C}$. The by-product of this stage is the nut, of great commercial value, and the CNS drenched in cardol, which holds great fuel potential.³²

Use of residual products from the processing of cashew nuts, such as CNS, as an energy source is already a reality in industries and craft centers. However, combustion of CNS promotes the release of pollutants such as CO_2 , CO , O_3 , NO_2 , SO_2 , PAHs, and particulate matter (PM); the mere presence of these substances in the air can transform them into pollutants, depending on the type of biomass burned (chemical composition) and the combustion method.³³

Particulate matter with an aerodynamic diameter equal to or less than $4\ \mu\text{m}$ ($\text{PM}_{4.0}$) can easily penetrate the respiratory system and reach the pulmonary alveoli, causing serious respiratory disease.³⁴ Due to the increasing use of biomass as an energy source and the need to know the possible health risks caused by exposure to its exhaust, this work used the $\text{PM}_{4.0}$ derived from the combustion of CNS as the object of study.

Exposure to $\text{PM}_{4.0}$ simulates an occupational hazard experienced by industry workers. The sampling method for determining $\text{PM}_{4.0}$ inhalation exposure in humans is well-established and is the most commonly-used method used in human studies. This method is also recommended by the Council of Labor Affairs (CLA) as the standard for measurement of breathable dust in the workplace.³ In our experimental model, acute exposure to particles from CNS combustion exhaust gases was used, by means of a single intranasal instillation of $30\ \mu\text{g}$ of $\text{PM}_{4.0}$ (SMT, SMA, OMT and OMA groups) diluted in $30\ \mu\text{l}$ of saline solution. Assuming that the mean particle

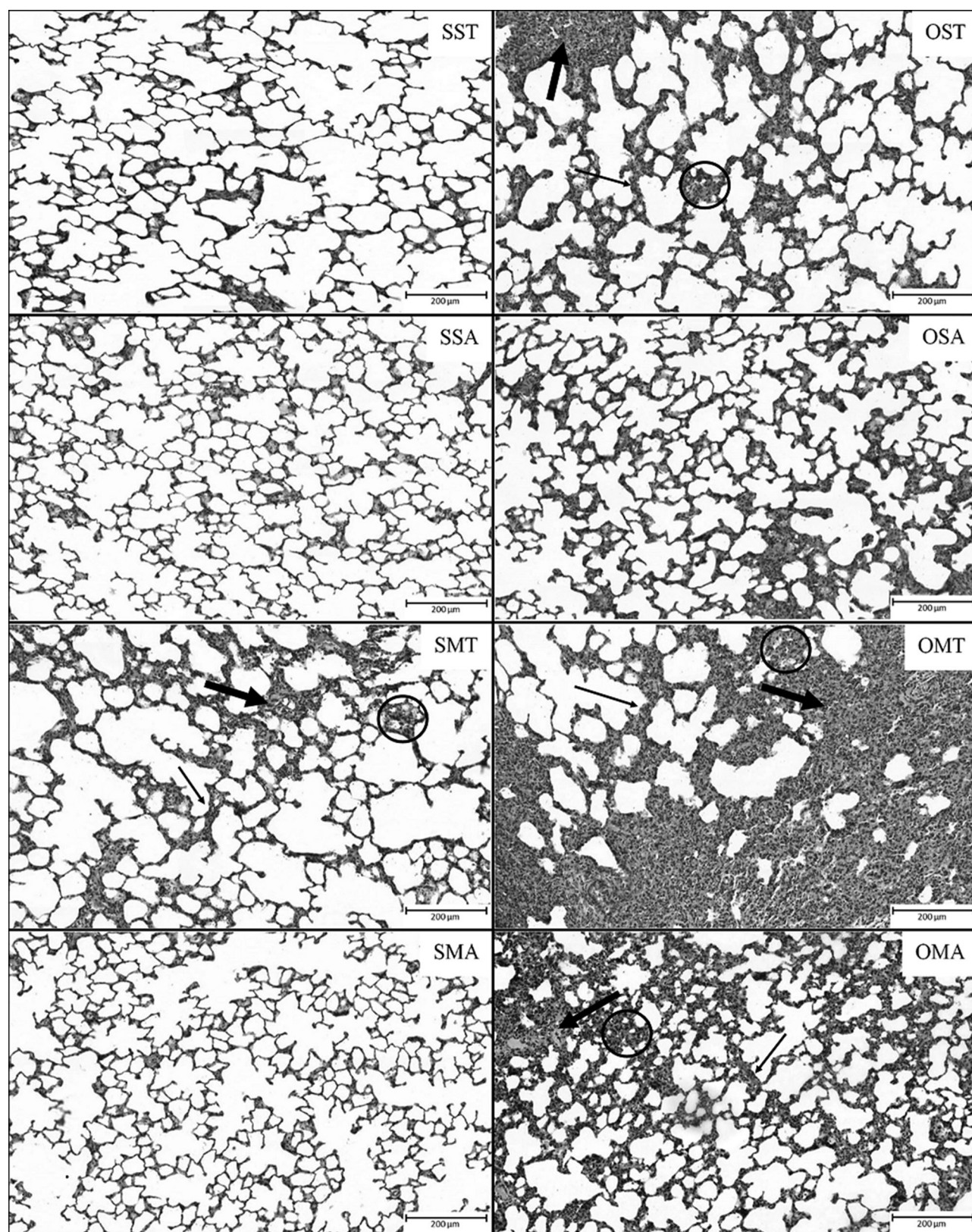


Figure 4. Photomicrographs of lung parenchyma and airway (inserts) stained with hematoxylin-eosin of ST, OT, SO100, OO100, SO300 and OO300 groups. Thin arrows: thickened septa; thick arrows: cellular infiltrate; circles: alveolar collapse.

Table 1. Morphometric parameters.

Groups	Alveolar Collapse (%)	PMN Cells ($\times 10^{-3}/\mu\text{m}^2$)	Mean alveolar diameter (μm)	BCI
SST	3.07 $\pm$ 0.72	13.39 $\pm$ 3.46	48.41 $\pm$ 3.59	1.97 $\pm$ 0.15
SSA	3.22 $\pm$ 0.52	12.35 $\pm$ 2.48	51.40 $\pm$ 7.29	1.92 $\pm$ 0.16
SMT	24.25 $\pm$ 3.83 ^a	25.95 $\pm$ 4.67 ^a	39.22 $\pm$ 4.40 ^a	2.30 $\pm$ 0.21 ^a
SMA	6.11 $\pm$ 2.91 ^c	16.61 $\pm$ 2.17 ^c	49.68 $\pm$ 4.60 ^c	2.05 $\pm$ 0.25 ^c
OST	24.21 $\pm$ 3.51 ^a	32.14 $\pm$ 6.50 ^a	34.21 $\pm$ 3.20 ^a	2.64 $\pm$ 0.23 ^a
OSA	9.02 $\pm$ 2.97 ^b	17.12 $\pm$ 4.67 ^b	45.40 $\pm$ 6.28 ^b	2.02 $\pm$ 0.17 ^b
OMT	31.81 $\pm$ 3.64 ^a	37.25 $\pm$ 8.66 ^a	30.72 $\pm$ 3.66 ^a	2.77 $\pm$ 0.37 ^a
OMA	28.85 $\pm$ 4.17 ^d	25.30 $\pm$ 4.45	35.95 $\pm$ 3.97 ^d	2.51 $\pm$ 0.29 ^d

Values are mean $\pm$ SD of SST, SSA, SMT, SMA, OST, OSA, OMT and OMA groups.

Data were collected in ten matched fields per mice.

By one-way ANOVA followed by the multiple comparisons corrected with the Bonferroni's test.

PMN, polymorphonuclear; BCI, bronchoconstriction index.

^aDifferent from SST group ($p < 0.05$).

^bDifferent from OST group ($p < 0.05$).

^cDifferent from SMT group ($p < 0.05$).

^dThere was no difference from OMT group ($p > 0.05$).

concentration in a cashew nut shell processing environment is $500 \mu\text{g}/\text{m}^3$ ^{3,35} and that the total volume of air inhaled by a mouse in 24 h is about 0.06 m^3 , exposure to a $30 \mu\text{g}$ dose is representative of 24 h of direct exposure in such an environment.

Asthma is a chronic inflammatory disorder of the airways.³⁶ Airway inflammation causes various symptoms often associated with widespread airflow obstruction, as well as an associated increase in airway responsiveness to a variety of stimuli.³⁷ Worldwide increases in the number of asthma cases over the past several decades have motivated intensive investigation of the role of environmental factors, including air pollution, in their development or exacerbation. Many of the stimuli that trigger or exacerbate asthma, including pollutants, can activate the production of oxidizing agents, inducing inflammation that produces asthma-like symptoms.³⁸

The inflammatory nature of asthma suggests that control of airway inflammation is the key to its treatment, regardless of its severity. Standard asthma anti-inflammatory therapy uses inhaled corticosteroids,^{39,40} and several studies have shown that this type of treatment modifies the characteristics of the inflammation.⁴¹ However, resistance to glucocorticoids may occur,^{42,43} making it necessary to search for alternative methods of treatment that may replace or supplement this therapy.

Demonstration of the anti-inflammatory action¹² and antioxidant properties^{9–11} of anethole was the object of this study, in order to investigate its therapeutic properties for the treatment of asthma in association with exposure to the $\text{PM}_{4.0}$ of CNS, a pollutant derived from the combustion of biomass.

Our results demonstrate significant changes in all respiratory mechanics variables analyzed for the SMT,

OST and OMT groups, as compared to the SST group (Figure 2). Specifically, comparison between SMT and SST confirms the existence of lung injury caused by exposure to $\text{PM}_{4.0}$ from the combustion of CNS. Comparison of OST *versus* SST groups confirms the OVA-induced lung injury, since comparison between OMT and SST confirms lung injury caused by OVA in association with the $\text{PM}_{4.0}$ from the combustion of CNS (Figure 2). Analysis of OSA *versus* OST and SMA *versus* SMT demonstrates that treatment with oral anethole at a dose of $300 \text{ mg}/\text{kg}$ was effective in the reversion of the OVA-induced injury, and injury caused by exposure to $\text{PM}_{4.0}$ from the combustion of CNS, respectively (Figure 2). However, comparison between OMA and OMT demonstrates that treatment with oral anethole at the same dose was ineffective for the reversion of lesions caused by OVA in association with $\text{PM}_{4.0}$ from the combustion of CNS (Figure 2).

Regarding respiratory system mechanics, R_N has been used as a good estimate of total airway resistance;⁴⁴ this variable showed a significant increase in the SMT group. This may occur because inhaled particles induce the expression of pro-inflammatory mediators and Ca^{2+} -dependent intracellular signaling pathways.⁴⁵ The presence of Ca^{2+} ions in the lungs plays a role in the regulation of various functions, such as mucus and surfactant secretion and control of the ciliary agitation frequency.⁴⁶ After cell stimulation, calcium is released from the endoplasmic reticulum, which in turn leads to an influx of calcium ions through the plasma membrane by means of calcium channels. This accumulation of calcium in the cytosol may lead to further narrowing or increased smooth muscle of the airways,⁴⁷ increasing the value of R_N . Significant changes in R_N in the OST and OMT groups were already expected. In the OST group, the use of the ovalbumin-induced asthma model was the probable cause of the airway inflammation and remodeling,^{48,49} which may result in an increased R_N . It is likely that the hypotheses raised to justify the increased R_N in the SMT and OST groups could be extrapolated to the OMT group.

Tissue Resistance (G) and tissue elastance (H) are related to the intrinsic properties of the lung tissue.⁴⁴ Significant changes in these variables can be explained by the influence that narrowing of the airways has on these parameters, resulting in distortion of the pulmonary parenchyma and closure of the small airways. This results in an effectively smaller lung with proportionally larger H and, consequently, G .⁵⁰ Where alveolar septum thickening and cell infiltrate occurs (Figure 4), alveolar collapse (Figure 4 and Table 1) and a

smaller mean alveolar diameter (L_m) (Table 1) may provide an increase in G and H in the animals of the SMT, OST, OMT and OMA groups. Similarly, increased values of G and H , were also found after instillations of other particles.^{51,52}

In the quasi-static PV curve, static compliance (C_{ST}) indirectly measures the degree of lung tissue distensibility; estimated inspiratory capacity (IC) quantifies the volume of air received by the lungs, up to a pressure of 30 cmH₂O; and the PV loop area provides an estimate of the amount of atelectasis (air-space closure) that existed before the PV loop manoeuvre.⁵³ To estimate IC and C_{ST} the pressure and volume data were measured in the upper flatter portion of the expiratory limb of the PV curve.

The reduction of C_{ST} suggests an increase in the static elastic recoil within the lung, probably due to remodeling.⁵³ This is most likely due to an accumulation of inflammatory material within the lung parenchyma (Table 1, PMN cells), causing an increase in friction. Decreased inspiratory capacity (IC) is consistent with the effective stiffening of lung tissue, as indicated by increased H . The higher PV loop area could be attributed to the elastic component of impedance, tissue changes, surfactant-associated mechanisms, and may also be associated with areas of atelectasis (Table 1, Alveolar Collapse (%), and Figure 4).

Airway hyperresponsiveness (AHR), was measured by the pulmonary response to MCh challenge, as assessed by ΔR_N (Figure 3). After challenge with MCh, we observed significant alterations in the SST, OST, OMT and OMA groups, when compared to the SST group, indicating AHR of the animals within these groups. AHR is associated with airway inflammation and is marked by eosinophilia, increased smooth muscle area, and goblet cell hyperplasia.⁵⁴ Hyperresponsiveness can also be attributed to the presence of inflammatory processes in the airways of these animals, which similar studies have reported as airway hyperresponsiveness following exposure to pollutants in models of acute and chronic allergic inflammation.^{28,55}

Several studies demonstrate the benefits of using anethole: oral administration in doses of 3, 10, and 30 mg/kg, inhibits paw edema elicited by carrageenan and specific pro-inflammatory mediators in swiss mice.¹² Anethole prevents LPS-induced lung inflammation in mice,²³ and some compounds also have antioxidant action.¹⁰ Oral treatment with anethole even for a prolonged time-seems to be devoid of specific side effects associated with non-steroidal anti-inflammatory drugs, such as hepatotoxicity, nephrotoxicity, and ulcerogenicity.⁵⁶

Our results demonstrate the benefits of using 300 mg/kg oral anethole in the treatment of mice submitted to an ovalbumin-induced asthma model (OSA group) as well as in lung injury induced by PM_{4.0} exposure from the combustion of CNS (SMA group). However, it was unable to reverse the lesions caused by association of pathogens (OMA group), according to the results referring to the mechanics of the respiratory system (Figure 2).

Our study has limitations: (i) the specific mechanism involved in oral anethole activity in the lung injury of mice submitted to an ovalbumin-induced asthma model (OSA group) or PM_{4.0} exposure from the combustion of CNS (SMA group) were not determined; (ii) an analysis of the exposure-response of MP_{4.0} from the combustion of CNS should have been performed; (iii) an analysis of the dose-response of oral anethole in the treatment of mice submitted to an ovalbumin-induced asthma model should have been performed. These limitations will be the aim of future studies of this research group.

Conclusion

In conclusion, oral anethole at a concentration of 300 mg/kg was able to attenuate lung injury in mice submitted to an ovalbumin-induced asthma model and subsequent exposure to PM_{4.0} from gas arising from the combustion of CNS. However, anethole was not able to attenuate the lesions caused by exposure to PM_{4.0} from the combustion of CNS in mice previously submitted to the ovalbumin-induced asthma model. Our results serve as a starting point for the development of public policies for the prevention of diseases in workers that are exposed to the pollutants coming from industries, as well as evaluating an alternative treatment for asthma or for pulmonary lesions caused by exposure to pollutants originating from biomass combustion.

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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