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FIXED OIL DERIVED FROM Caryocar coriaceum (PEQUI FRUIT) PREVENTS TISSUE AND FUNCTIONAL ALTERATIONS IN THE RESPIRATORY SYSTEM INDUCED HOUSEHOLD AIR POLLUTION ORIGINATING FROM BIOMASS

Allison Matias de Sousa[1]; *Fladimir de Lima Gondim*[2]*; *Gilvan Ribeiro dos Santos*[3]; *Marcelle Ferreira Moura*[1]; *Ruth Mesquita Ferreira*[4]; *Daniel Silveira Serra*[4]; *Antônia Torres Ávila Pimenta*[2]; *Mona Lisa Moura de Oliveira*[4]; *Francisco Sales Ávila Cavalcante*[4]

Abstract

The reuse of biomass residue can be in different ways, such as pellets obtained through the compaction of green coconut shell. Despite the advantages, studies relate the burning of biomass as the main source of household air pollution. Indoor pollution is also correlated with serious consequences for groups considered at risk, such as asthmatics. As a result, the population is looking for alternative and low-cost treatments through natural products with anti-inflammatory characteristics, such as the fixed oil of *Caryocar coriaceum* (CC). In the present study, we evaluated the effects of oral CC ingestion on the respiratory system of mice submitted to a model of chronic exposure to smoke from the combustion of coconut shell pellets (CSP) and submitted to the OVA-induced asthma model. We performed analysis of the gaseous composition in the exposure chamber and analyzes of different aspects of the respiratory system. CC could prevent inflammatory cell infiltration and alveolar collapse. Furthermore, it was able to avoid changes in the airway resistance, tissue resistance, elastance, compliance and inspiratory capacity. Our findings demonstrate the effects of long-term exposure to indoor pollution and suggest that dietary intake of CC may be a strategy to prevent respiratory diseases.

Keywords: *Caryocar coriaceum*. Pequi. Biomass. Household air. Indoor pollution. Asthma. Respiratory mechanics.

[1]Instituto Superior de Ciências Biomédicas, State University of Ceará, CEP: 60714-110, Fortaleza, Ceará, Brazil

[2]Departamento de Química Orgânica e Inorgânica, Federal University of Ceará, CEP: 60.440-900, Fortaleza, Ceará, Brazil

[3]Departamento de Física, Federal University of Ceará, CEP: 60440-900, Fortaleza, Ceará, Brazil

[4]Centro de Ciência e Tecnologia, State University of Ceará, CEP: 60741-000, Fortaleza, Ceará, Brazil

*Corresponding author: fladimir@dqoi.ufc.br

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1 Introduction

Environmental pollution, which includes household air pollution, accounted for 6.7 million deaths worldwide in 2019 (FULLER et al., 2022). Globally, 52% of the population relies on biomass as the primary fuel source for daily cooking (REHFUESS; MEHTA; PRÜSS-ÜSTÜN, 2006), particularly in rural households in developing nations. Roughly 3 billion individuals depend on solid fuel, such as coal or biomass, for domestic activities (WHO, 2018).

Biomass residue can be reused in various forms, such as pellets produced by compacting sawdust, rice husks, corn straw, cobs, sugarcane bagasse, cotton husks, coffee grounds, and green coconut shells under high pressure or temperature conditions (TAVARES; TAVARES, 2015).

The utilization of biomass brings forth numerous advantages, but studies have linked the combustion of biomass as a primary source of indoor air pollution. Indoor pollution, also known as indoor air pollution, encompasses pollutants similar to those found in tobacco smoke and outdoor air pollution. These pollutants have been associated with significant health implications, especially among vulnerable populations such as individuals with asthma (DI et al., 2017; SERRA et al., 2018; HASAN et al., 2019).

Respiratory diseases, in general, present characteristic symptoms including cough, excessive secretion, dyspnea, and airway obstruction. Consequently, the population, particularly those with low-income, seeks alternative and cost-effective treatments utilizing natural products to alleviate these symptoms. One such natural product is pequi oil, derived from the fruit of the pequi tree (*Caryocar coriaceum* Wittm., 1886), which is abundant in vitamins E and B, unsaturated fatty acids, and phenols.

Pequi oil is commonly used for wound healing (NASCIMENTO et al., 2015), alleviating rheumatic pain, treating gastric ulcers, sore throat, and cough (MATOS, 2007), as well as a therapeutic agent for bronchitis and asthma (SILVA; MEDEIROS-FILHO, 2006).

Given the anti-inflammatory and antioxidant properties of the fixed oil derived from *Caryocar coriaceum* (CC), this study aims to investigate the advantages of orally administering CC to mice exposed to smoke generated by the combustion of coconut shell pellets (CSP), simulating exposure to indoor pollutants. Furthermore, the effects of CC will be evaluated in mice with preexisting pulmonary pathology who are exposed to CSP.

2 Material and Methods

Animals

All animals received ethical and humane care, and the experiments were conducted in accordance with the following guidelines: ARRIVE; the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8523, revised 1985); and the National Council for Controlling Animal Experimentation of the Ministry of Science, Technology, and Innovation (CONCEA/MCTI), Brazil.

This study obtained approval from the Ethics Committee on the Use of Animals of the State University of Ceará (*Protocol No. 3529286-2018*). Invasive procedures were performed under anesthesia (see below), and every effort was made to minimize animal suffering.

Male BALB/c mice (7–8 weeks of age), 25 ± 5 g BW, had water and feed ad libitum, were used in this study. Mice were housed in plastic cages under controlled environmental conditions, in which a temperature of 20–22 ◦C, housed in a room with a 12 h light/12 h dark cycle. We used 64 animals randomly divided into eigth groups (n = 8): two groups asthma-induced, untreated (AVA) and CC-treated (ACCA); two groups exposed to smoke from pellet combustion, untreated (SVS) and treated with CC (SCCS); an asthma-induced group exposed to untreated pellet combustion smoke (AVS) and an asthma-induced group exposed to CC-treated pellet combustion smoke (ACCS); and two control groups, untreated (SVA) and treated with CC (SCCA) (Figure 1).

OVA-induced asthma model and treatment with the fixed oil of *Caryocar coriaceum* (CC)

Based on a modified approach (PARREIRA et al., 2012), ovalbumin (OVA; Sigma, MO, USA) was used to sensitize and challenge mice. In summary, the BALB/c mice in the positive control groups (AVA, AVS, ACCA, and ACCS) were subcutaneously sensitized with 100 µg of OVA, mixed with 1 mg of aluminum hydroxide (Sigma, MO, America) as an adjuvant in 200 µL of phosphate-buffered saline (PBS) on days 0 and 14.

After one week (day 21) following the final sensitization, intranasal OVA challenge was initiated, administering 40 µg of OVA diluted in 50 µl of PBS, three times a week for eight weeks. The intranasal challenge was always performed under anesthesia using sevoflurane to ensure pulmonary aspiration of the OVA solution.

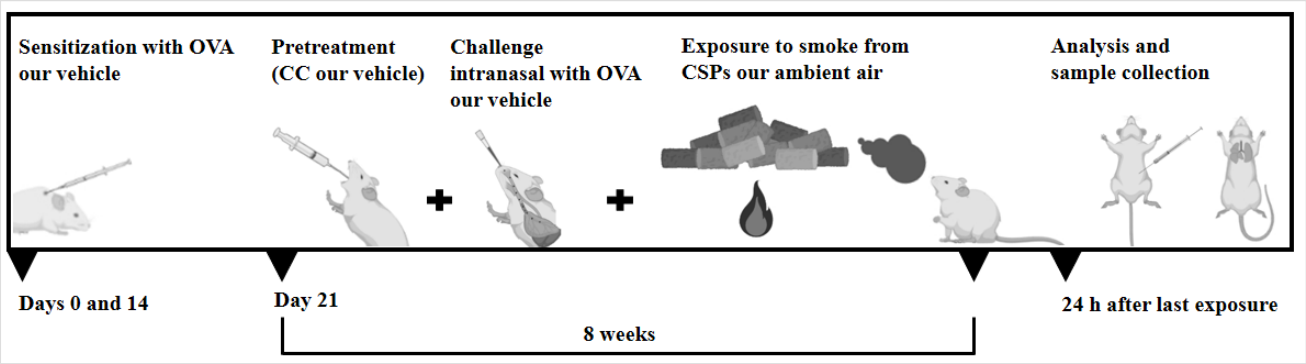

Figure 1. Experimental design.

The negative control groups (SVA, SCCA, SVS, and SCCS) received the same volume of PBS solution via subcutaneous injection (on days 0 and 14) and intranasally three times a week for eight weeks, without OVA and aluminum hydroxide.

For the treatment involving the administration of CC fixed oil, the groups (SCCA, ACCA, SCCS, and ACCS) received a daily oral dose of 50 µL of CC. The other groups (SVA, AVA, SVS, and AVS) were given the same volume of the vehicle (Tween-80 [1%] saline) orally. The CC doses used in this study were determined based on a previously published study (SERRA et al., 2020). Following the treatment, the animals were exposed to smoke generated from pellet combustion or exposed to ambient air.

Plant material and manufacture of coconut shell pellets (CSPs)

The coconut shell was provided by the ACP Nutrition Company located at the State University of Ceará - Av. Doctor Silas Munguba, 1700, Itaperi. After the in natura biomass collection, considering a moisture content around 85%, coconut shells were cut into pieces and subjected to a drying process through sunlight exposure for 72 h. The biomass was subsequently crushed using an electric grinder (TRF400F 2HP, Trapp) to obtain coconut shell. The powder from biomasse were then subjected to heat treatment at approximately 80°C for 20 min in a thermal oven, in order to protect the pellets from phytopathogenic microorganisms (SILVEIRA, 2008) (Figure 2).

Biomass powder was then distributed in cylinders (20 cm × 8 mm) and kept under 570 bar pressure (using a hydraulic jack) for 15 min in a manual pelletizer. The pellets were then removed from the cylinders and divided into approximately 2-cm-long pieces.

CSPs combustion model

A mouse model for exposure to the combustion gases of coconut shell pellets was created using an adaptation of the protocol proposed by SANTOS et al. (2021). To perform combustion tests on CSPs, a metal furnace (CMF) was adapted (Figure 2), with an iron base and a chimney where the smoke from combustion is dissipated. Tests were carried with 400 g of biomass (CSPs). All flows and temperatures of the combustion process were monitored using a datalogger (Fieldlogger®; Figure 2C) and a notebook (Figure 2D).

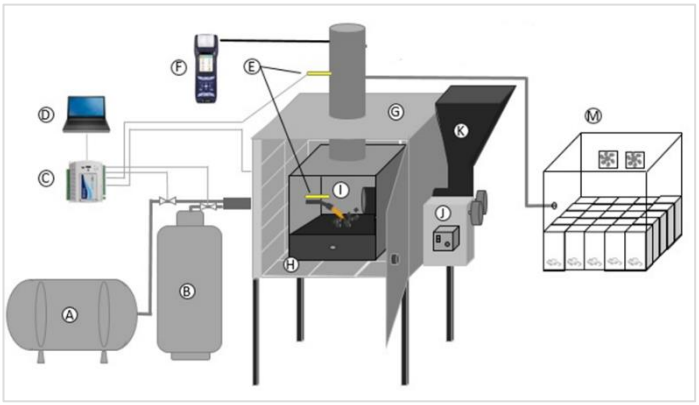

Figure 2. Coconut shell pellet combustion scheme. (A) Air compressor; (B) LPG; (C) Datalog; (D) Notebook; (E) Thermocouples; (F) Gas Analyzer; (G) Furnace; (H) Furnace thermal insulation; (I) Internal combustion chamber; (J) Pellet increase control; (K) Feeder Silo; (M) Animal exposure chamber.

For the initial ignition in the combustion tests, air compressor (Schulz - Pratic Air®; Figure 2A) and an auxiliary fuel represented by a gas cylinder (Figure 2B) containing liquefied petroleum gas (LPG) were used in order to quantify the air and LPG delivered during combustion. Gaseous product was collected by lead-line (stainless steel) inserted in the chimney (Figure 2F). These gases were directed to the animal exposure chamber.

The exposure chamber (387 mm high, 390 mm wide, 420 mm long) has a volume of approximately 63.5 L (Figure 2M), contains a rear fan and two side fans, used to homogenize gases.

Exposure to smoke from combustion of CSPs

The chronic protocol adopted (TESFAIGZI et al. 2002) for exposure to smoke from the combustion of green coconut pellets (CSPs) was carried out daily, lasting two hours (five times a week) for 8 weeks.

Exposure began on the 21st day of the asthma induction protocol (asthma positive and negative control groups). The animals in the groups (SVS, AVS, SCCS and ACCS) were placed in a box (Figure 2M) and exposed to smoke from the combustion of green coconut shell pellets, as previously described. The groups SVA, SCCA, AVA and ACCA were also placed in a box for the same period described above, but without exposure to smoke.

Respiratory system mechanics

Twenty-four hours after the last exposure to smoke generated by coconut shell pellets or to the surrounding air, the animals underwent anesthesia using 10% Ketamine Hydrochloride (100 mg/kg) and Xylazine Hydrochloride (10 mg/kg) and underwent a tracheotomy procedure. The animals were then intubated with a 14-gauge cannula (Eastern Medikit, Delhi, India), which was connected to a computer-controlled ventilator designed for small animals (Scirec©-flexVent®, Montreal, QC, Canada).

The animals were ventilated with initial settings, including a respiratory frequency of 120 breaths per minute, a tidal volume of 10 mL/kg, a maximum pressure limit of 30 cmH2O, and a positive end-expiratory pressure (PEEP) of 3 cmH2O. Subsequently, mice were rendered paralyzed using pancuronium bromide (0.5 mL/kg, i.p., Cristália, Brazil).

To begin with, we established a consistent mechanical pattern for the respiratory system by performing two deep inflations (DI) lasting 6 seconds each, with a peak pressure of 30 cmH2O. Subsequently, the animals were ventilated for a period of 5 minutes using baseline settings. Following this, we assessed the impedance of the respiratory system (Zrs) using the forced oscillation technique, employing a 3-second duration (HANTOS et al. 1992). This measurement was conducted over 12 consecutive 30-second sampling intervals, amounting to a total duration of 6 minutes.

Where R_N is the Newtonian resistance, which represents the central airway resistance, *I* represents airway inertance, tissue resistance (*G*) and tissue elastance (*H*) are respectively the dissipative and elastic properties of lung tissue (HANTOS et al. 1992).

Thereafter, starting at the functional residual capacity, the flexiVent delivered 7 inspiratory pressure steps for a total pressure of 30 cmH_2O, 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 (SALAZAR and KNOWLES, 1964). Two quasi-static PV curves were obtained to measure C_{ST}, an estimate of inspiratory capacity (*IC*), and PV loop area.

Histological study

The histological examination was conducted following previously established protocols (GONDIM et al., 2023). In summary, immediately after assessing the mechanics of the respiratory system, the left lung was maintained at functional residual capacity and fixed using Millonig's formaldehyde solution (100 mL HCHO, 900 mL H2O, 18.6 g NaH2PO4, 4.2 g NaOH).

Thin sections (4-µm-thick) of the left lung were prepared on slides and stained with hematoxylin and eosin (HE) for subsequent analysis under an optical microscope. A researcher, blinded to the origin of the samples, examined the slides, considering both qualitative and quantitative characteristics.

The quantification was carried out by employing an integrated eyepiece containing a coherent system comprised of a grid consisting of 100 points and 50 lines, which was attached to a traditional optical microscope.

The percentage of collapsed alveolar area (represented as a fraction of all points hitting alveoli) and the quantity of polymorphonuclear neutrophils (PMN) cells were evaluated using the point-counting method.

The enlargement of air spaces was measured by determining the average length of linear intercepts in distal air spaces (Lm) in 30 randomly selected fields of tissue sections per group (KNUDSEN et al., 2010). Cellularity (PMN cells) was observed at a magnification of 1000× in 10-15 randomly chosen non-overlapping microscopic fields in each animal.

Statistical Analysis

The statistical analysis was conducted using GraphPad Prism version 5.00 (GraphPad, San Diego, CA, USA). The data are expressed as mean ± standard deviation (SD), with 'n' representing the sample size.

To compare the groups, a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test was utilized. Statistical significance was determined at a p-value of less than 0.05.

3 Results

Table 1 shows the temperatures and concentrations of some of the gases in the interior of the test chamber during exposure to smoke from combustion of CSPs. Animals exposed to ambient air were subjected to an average temperature of 24.22 ± 0.23 °C, an average percentage of 21.00 ± 0.01 O_2, and to environment free of CO, NO_x, SO_2 and CH_4 gases.

Table 1. Gases concentration within the smoke exposure chamber from combustion of CSPs.

Gas	Gas Concentration (mean±SD)
O_2 (%)	20,784 ± 0,01074
CO (ppm)	20,25 ± 3,2976
NO_x (ppm)	2,31667 ± 0,99149
SO_2 (ppm)	0,51667 ± 0,34116

Values of exhaust gases from coconut shell pellet (CSP) combustion. Values are mean ± SD of O_2, CO, NO_X and SO_2.

Fixed oil of *C. coriaceum* was able to prevent changes in lung function parameters (Figures 3 and 4), as reflected by the absence in all cases of any significant difference between the ACCA, SCCS, ACCS and SVA groups.

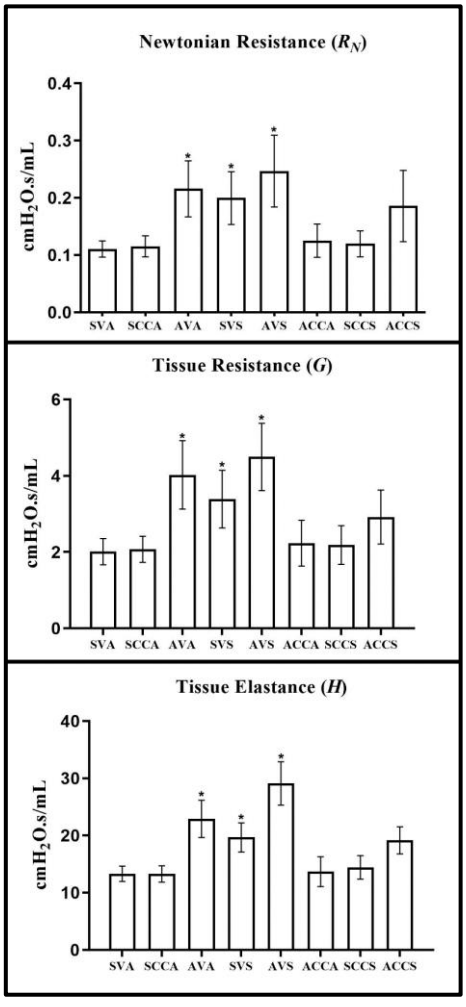

Figure 3. Lung function parameters based on the forced-oscillation technique. Values are mean ± SD of SVA, SCCA, AVA, SVS, AVS, ACCA, SCCS and ACCS (8 animals per group). The data were collected in ten matched fields per mice. * Different from SS group ($p<0.05$).

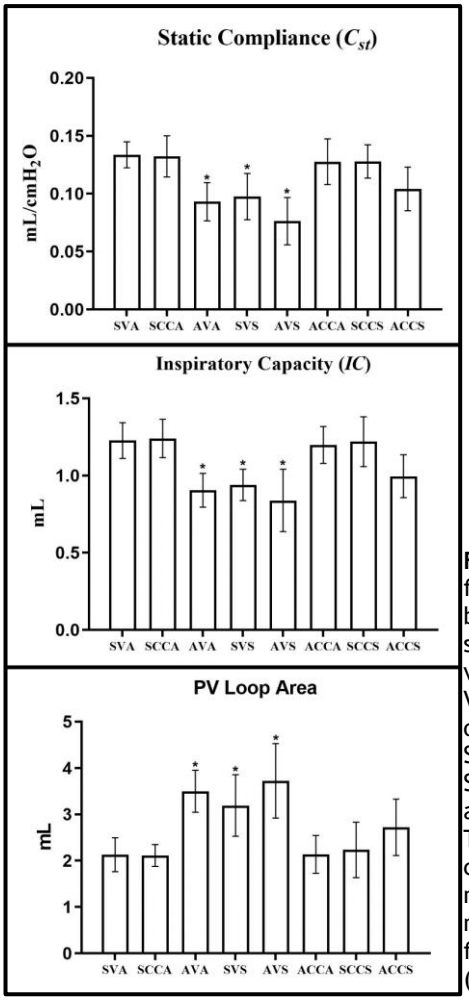

Figure 4. Lung function parameters based on the quasi-static pressure-volume (PV) curve. Values are mean ± SD of SVA, SCCA, AVA, SVS, AVS, ACCA, SCCS and ACCS (8 animals per group). The data were collected in ten matched fields per mice. * Different from SS group ($p<0.05$).

From the histological study, it was also possible to verify that the CC avoided the establishment of alterations at the alveolar level and attenuated the infiltrate of PMN, as seen in Table 2 and Figure 5.

Table 2. Alveolar collapse, amount of PMN and MN cells, mean alveolar diameter and collapsed alveoli.

Groups	Collapsed Alveoli (%)	PMN Cells (10^{-3}/μm^2)	Mean Alveolar Diameter (μm)
SVA	4,27 ± 2,17	14,60 ± 5,45	46,18 ± 4,81
SCCA	3,51 ± 0,70	14,03 ± 4,23	48,59 ± 9,45
AVA	27,25 ± 4,83*	29,95 ± 7,15*	34,79 ± 2,78*
SVS	26,11 ± 4,88*	22,20 ± 6,47*	39,17 ± 4,08*
AVS	32,90 ± 4,03	33,81 ± 13,16*	31,06 ± 3,57*
ACCA	10,56 ± 4,68	17,66 ± 5,36	43,84 ± 7,61
SCCS	9,53 ± 4,86	16,28 ± 2,63	45,59 ± 8,08
ACCS	22,69 ± 6,07	24,93 ± 4,67	41,87 ± 7,15

Morphometric parameters. Values are mean ± SD of SVA, SCCA, AVA, SVS, AVS, ACCA, SCCS and ACCS (8 animals per group). Data were collected in ten fields per mice. *Different from SVA group ($p<0.05$). PMN, polymorphonuclear.

Figure 5 depicts representative lung histological images. Alveolar collapse, thickened septa and cellular infiltration can be observed in lung parenchyma of groups SVA, SCCA, AVA, SVS, AVS, ACCA, SCCS and ACCS.

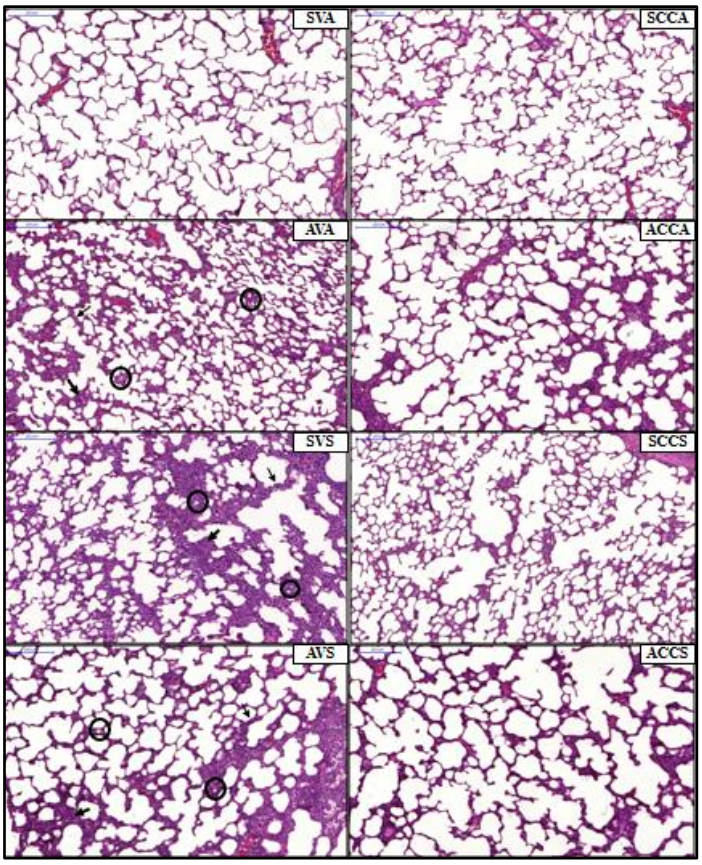

Figure 5. Photomicrographs of lung parenchyma stained with hematoxylin–eosin of SVA, SCCA, AVA, ACCA, SVS, SCCS, AVS and ACCS groups. Thin arrows: thickened septa; thick arrows: cellular infiltrate; and circles: alveolar collapse.

Our data showed that oral administration of CC in animals exposed to smoke from CSPs combustion, in asthmatic animals and asthmatic animals exposed to smoke from CSPs combustion was able to avoid the onset of lung injury, as reflected by the absence in all instances of any significant difference between ACCA, SCCS, ACCS and SVA groups.

4 Discussion

Fatalities resulting from pollutants, including those found in household air pollution, have witnessed a significant rise on a global scale in the last two decades (FISHER et al., 2021; FULLER et al., 2022). These pollutants primarily originate from biomass combustion, giving rise to particulate matter, carbon monoxide (CO), nitrogen dioxide (NO2), sulfur dioxide (SO2), and ozone (O3), which have direct implications for human well-being (WHO, 2018; MARAIS; WIEDINMYER, 2016).

Multiple research investigations have demonstrated a strong correlation between biomass combustion and various respiratory ailments (BALCAN et al., 2016; GONDIM et al., 2017; SERRA et al., 2017; SERRA et al., 2018).

In the present study, we developed a smoke exposure chamber, with the aim of mimicking an indoor environment where individuals are chronically exposed (8 weeks) to smoke from the combustion of coconut shell pellets, in addition to identifying the concentration of gases inside it.

From the comparison between the group exposed to smoke from combustion of CSPs (SVS) and the control group (SVA) our findings confirmed that exposure to smoke was able to promote tissue changes, with histological evidence of pulmonary inflammatory process and mechanical dysfunction of the respiratory system.

Respiratory mechanics were evaluated using the forced oscillation technique with a constant phase model (Figure 3) and a quasi-static pressure-volume (PV) curve (Figure 4). The constant phase model allowed us to assess Newtonian resistance (R_N), tissue resistance (*G*), and elastance (*H*) (BATES et al., 2009), while the quasi-static PV curve provided measurements of static compliance (C_{ST}), estimated inspiratory capacity (*IC*), and the area under the PV loop.

The analysis of the constant phase model parameters revealed a significantly elevated airway resistance (R_N) in the SVS group compared to the SVA group, indicating a greater overall central airway resistance in the former group. This finding suggests the presence of airway inflammation, leading to smooth muscle hyperreactivity and consequent narrowing of the airway diameter (MARTIN; DUGUET; EIDELMAN, 2000). Similar findings have been reported in previous studies investigating the inflammatory response in the bronchial wall following prolonged exposure to biomass smoke (CAPISTRANO et al., 2017; RAJ, 2014).

The presence of SO_2 in the exposure chamber (Table 2) may also have influenced the increase in R_N in the AVA, SVS and AVS groups (Figure 3), as this gas acts as an irritant product that affects the respiratory tract mucosa, causing increased bronchial reactivity and bronchoconstriction (ARBEX et al. 2012).

G and *H* values were found to be significantly higher in the AVA, SVS, and AVS groups compared to the SVA and SCCA animals. These results can be attributed to tissue alterations, including thickening and collapse of alveolar septa, as well as the presence of cellular infiltrates in the pulmonary parenchyma of animals from the AVA, SVS, and AVS groups (Figure 5).

The reduced mean alveolar diameter, increased number of collapsed alveoli, and elevated levels of PMN cells (Table 2) contribute to tissue remodeling and potentially account for the observed increase in *G* and *H* values. Similar findings have been reported in previous studies investigating the combustion of different biomass types (SERRA et al., 2018, 2021).

Simultaneously, the aforementioned constriction of the airways also impacts these parameters, leading to a distortion of the lung tissue, characterized by a relatively smaller lung volume and proportionally increased *H* value (WAGERS et al., 2002).

Another factor that may have caused the increase in *G* and *H* in the lungs was the presence of nitrogen oxides (NO_x) in the exposure chamber (Table 2). These oxides act as oxidizing molecules, reaching the most peripheral portions of the lungs, such as the bronchioles and alveoli, which can increase lung stiffness and susceptibility to infections and allergens (CANÇADO et al., 2006; ARBEX et al., 2012).

In addition to the gaseous compounds identified in the present study, it is noteworthy that the use of residential solid fuel is one of the main sources responsible for the emission of particulate matter (GORDON et al., 2023), able of causing important changes in lung function, such as a decrease in ventilatory flow and lung vital capacity (MULENGA; SIZIYA, 2019; STAPLETON et al., 2020).

These findings can be related to the results presented in the present study, where the analysis of the parameters obtained for the pressure-volume curves (Figure 4) showed a statistically significant decrease in C_{ST} and *IC*, as well as an increase in the PV curve area, when comparing the SVS group to the SVA group.

The reduction in inspiratory capacity (*IC*) provides further evidence of the pulmonary tissue becoming stiffer, as indicated by the elevated elastance (*H*) values (WAGERS et al., 2002).

The area under the pressure-volume (PV) curve reflects alterations in the surfactant distribution on the alveolar surface, and an increase in this area may suggest an expansion of atelectasis regions, possibly caused by the inflammatory process. This hypothesis can be supported by the inclusion of photomicrographic images (Figure 5, circles) and the notable increase in the count of pro-inflammatory polymorphonuclear cells (Table 2).

Furthermore, the larger area of the PV loop can be attributed to the elastic component of the respiratory system impedance, which is supported by the smaller C_{ST} found in the SVS group (Figure 4).

Studies report that exposure to environmental pollutants, such as particulate matter and NO_x, are related to the exacerbation of symptoms of pre-established respiratory diseases, such as asthma (ROVIRA et al., 2014; SERRA et al. 2021; IDAVAIN et al., 2019).

In this study, significant statistical differences were observed between the control group (SVA) and the group subjected to the OVA-induced asthma model (AVA), indicating the development of asthma. Although no statistical difference was found between the AVS (asthmatic group exposed to smoke) and AVA (asthmatic group not exposed to smoke), there was a noticeable trend towards exacerbation of tissue changes (Figure 5) and functional alterations in the respiratory system (Figure 3 and 4).

Previous studies have shown that fruits of the genus *Caryocar* have relevant antioxidant activity. For this reason, the habit of consuming fruits with high antioxidant potential becomes a beneficial routine practice for reducing the incidence of chronic diseases, such as lung cancer (COLOMBO et al., 2015).

Our research group showed that the fixed oil of *C. coriaceum* prevented acute lung injury in rats subjected to short-term exposure to passive cigarette smoke (SERRA et al. 2020).

In the current study, we investigated the effects of fixed oil of *C. coriaceum* on chronic exposure to CSP smoke and the chronic asthma induction protocol, both in a murine model.

Data referring to pulmonary histological analyzes demonstrated that pre-treatment with CC for 8 weeks was able to avoid tissue alterations (influx of polymorphonuclear pro-inflammatory cells, alveolar collapse and mean alveolar diameter) caused by CSP smoke, evidenced by the no significant difference between the SCCS and SVA groups (Table 2, Figure 3 and 4). In parallel, it was also possible to observe that the pre-treatment with CC for 8 weeks was able to avoid the same tissue changes in mice submitted to the chronic asthma induction protocol, evidenced by the absence of significant difference between the ACCA and SVA groups (Table 2; Figures 3 and 4). Similar anti-inflammatory effects were observed when animals induced to acute lung injury by exposure to LPS were pretreated with CC (COUTINHO et al., 2020).

In this perspective with the fact that there were no significant histological changes, there was also no significant difference in any of the respiratory system mechanics variables (R_N, *G*, *H*, C_{ST}, IC and PV loop area) among SCCS, ACCA and SVA groups (Figures 3 and 4).

Such findings of prevention of changes in lung function were also observed in a study that investigated the benefits of CC against the effects of exposure to cigarette smoke (SERRA et al. 2020).

It is hypothesized that the therapeutic properties of *C. coriaceum* are related to its high content of phenols and fatty acids, which contributes as a nutritional treatment for inflammatory and respiratory diseases (SENA et al., 2010; NI et al. 2015; DIPASQUALE et al., 2018). In addition, Tomiotto-Pellissier et al. (2018) demonstrated that the CC extract is able to promote the upregulation of Nrf2 expression, which massively stimulates the transcription of heme oxygenase-1 (HO-1), an important enzyme in the activation of antioxidant mechanisms (GOZZELINO; JENEY; SOARES, 2010).

5 Conclusions

In conclusion, we experimentally showed that exposure to smoke from the combustion of green coconut pellets causes changes in lung tissue and dysfunctions in the mechanics of the respiratory system. Our hypothesis is that the protective effect of *Caryocar coriaceum* fixed oil in preventing tissue changes and alterations in respiratory system mechanics in mice exposed to combustion smoke of green coconut pellets and mice subjected to the OVA-induced asthma model is primarily attributed to its ability to inhibit the infiltration of pro-inflammatory polymorphonuclear cells into lung tissue.

Collectively, our results indicate that incorporating *Caryocar coriaceu*m fixed oil into the diet could potentially serve as a preventive strategy against respiratory diseases of various origins.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

A.M.S., M.L.M.O. and D.S.S. conceived and designed research; A.M.S., G.R.S. and M.F.M. performed experiments; F.LG., R.M.F. and M.F.M. analyzed data; F.S.A.C., D.S.S. and F.L.G. interpreted results of experiments; D.S.S. and F.L.G. prepared figures and tables; A.M.S. and F.L.G drafted manuscript; A.T.A.P. and F.L.G. edited and revised manuscript; M.L.M.O., A.T.A.P. and F.S.A.C. approved final version of manuscript.

DECLARATION OF INTEREST

The authors disclose that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this manuscript.

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