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Effects of Exposure to Total Particulate Matter of Cashew Nuts Shell in the Respiratory System of Mice with Chronic Obstructive Pulmonary Disease

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Abstract

About 3 billion people are exposed to biomass fuel smoke compared to 1.1 billion people who smoke tobacco, suggesting that exposure to biomass smoke may be the most important global risk factor for Chronic obstructive pulmonary diseases COPD. Due to the increasing use of residual biomass as an energy source, there is a need to investigate the effects of exposure to total particulate matter from combustion exhaust gases from cashew nut shells (CNS) in more susceptible population groups. Thus, in the present study, we sought to investigate the effects of exposure to TPM of SNC in the Respiratory System of mice with COPD. To perform this investigation, C57black/6 mice were exposed to cigarette smoke or ambient air (CTRL group) for 60 days, and after this period the animals were submitted to 20 days of nasal instillation containing TPM (CS+TPM) or saline solution (CS). 24 h after last instillation, the animals were connected to a ventilator for small animals to perform the analyzes referring to the variables of the respiratory system mechanics. Our results demonstrated changes in all variables analyzed between the CS+TPM, CS and CTRL groups, and there was greater hyperresponsiveness in smooth muscle of the airways, demonstrating that the lung injury of animals caused by exposure to cigarette smoke is greater when associated with exposure to TMP from CNS combustion. In this way, increasing use of this biofuel in industries, craft centers and residences, can be can be harmful, especially to those with pre-existing respiratory disease.

Key words: Biomass, cashew nuts shell, chronic obstructive pulmonary disease, particulate matter, respiratory system.

I. INTRODUCTION

Global warming is one of the most debated environmental issues today, either because of the need for better knowledge on the subject, or for the analysis of its possible influences on life on earth. The increase in the temperature of the Earth's atmosphere coupled with the increase in the atmospheric levels of carbon dioxide (CO_2) are issues that need attention from the government and the scientific community, in an attempt to reverse the problems already present, and prevent them from increasing or become irreparable in the near future. One of the causes of global warming is the use of fossil fuels, a non-renewable resource.

Faced with this problem, the search for renewable energies has grown, highlighting the use of residual biomass. Most households in China, India and sub-Saharan Africa use biomass fuel for cooking, and in rural areas of Latin America the proportion ranges from 30% to 75% (EZZATI; KAMMEN, 2002). Thus, approximately 3 billion people worldwide are exposed to biomass combustion gases compared to 1.1 billion people who smoke tobacco, suggesting that exposure to biomass smoke may be the most important overall risk factor for chronic obstructive pulmonary disease (COPD) (LOPEZ *et al*., 2006). As one of the largest agricultural producers in the world, Brazil has great capacity to generate energy from the residual biomass of sugarcane, rice, coffee straw and cashew nut shells (CNS). Despite the use of CNS in boiler feed for thermal energy generation in some industries, the adverse health effects of the exhaust gases from the combustion process of CNS are poorly studied, mainly in analyzes of exposure to people with pre-existing respiratory diseases such as emphysema, characterized as a COPD.

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Although avoidable and treatable, COPD is still the fourth leading cause of death in the world, and it is estimated that there will be an increase in its incidence in the coming decades because of an aging population and an increase in its risk factors, such as irregular physical activity, poor eating habits, smoking and exposure to environmental pollutants (GOLD, 2018; ELWOOD *et al*., 2013).

It is estimated that about 5.2 million people die each year due to complications caused by smoking (WHO, 2005). In parallel, exposure to particulate matter (PM) is one of the factors most associated with adverse health effects (DOCKERY; HUECKEL-WENG; BIRBAUMER., 2009). Several studies have demonstrated the harmful effects of pollution and cigarette smoke on health (KENNEDY-FEITOSA *et al*., 2016; PETRUSCA *et al*., 2014; MAINALI *et al*., 2015; LEE *et al*., 2015; NAKAMURA *et al*., 2015), however, few investigated the association of these two factors, and we are not aware of studies that show the association between exposure to cigarette smoke and exposure to total particulate matter (TPM) from combustion exhaust gases from CNS. This fact is important because, in general, the acute effects secondary to PM exposure are mainly found in susceptible groups (with previous respiratory disease) and not in healthy populations (DONALDSON; MACNEE, 2001).

In view of the above, and due to the increasing use of residual biomass as an energy source, there is a need to investigate the effects of exposure to TPM from combustion exhaust gases from CNS in more susceptible population groups. Our hypothesis is that acute exposure to atmospheric pollutants, specifically TPM from the combustion of CNS, in individuals from susceptible groups (with pre-existing respiratory disease) may exacerbate the harmful effects on the respiratory system. Thus, in the present study, we sought to investigate the effects of exposure to TPM of SNC in the Respiratory System of mice with COPD. To perform this investigation, we used analyzes of respiratory mechanics *in vivo*, pulmonary parenchyma histopathology and morphometry *in vitro.*

II. MATERIALS AND METHODS

CNS Combustion System and TPM Collection

A CNS combustion system was developed to collect TPM from its exhaust gases (Fig. 1). For this collection, the CNS (400 g) were first placed in a cylindrical stainless-steel burner (Fig. 1A). Then, the initial combustion ignition of CNS was performed by supplying liquefied petroleum gas (LPG-Figure 1B) and ambient air from an air compressor (Fig. 1C). The combustion process of the CNS was accompanied by thermocouples (Fig. 1D) and flow transducers (Fig. 1E) connected to a data acquisition system (Field Logger-Figure 1F) for the analysis and control of temperature and LPG and air flows (Unpublished data), transferring the information to a notebook (Fig. 1G). The exhaust gases generated by CNS combustion were directed by a chimney (Fig. 1H) to a system containing 2 glass fiber filters (Fig. 1I), where the flow of 5 L/m was maintained by suction pump (AirChek® XR5000-Fig. 1J).

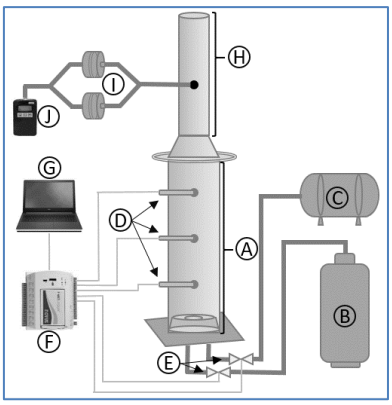

Fig. 1 CNS`s combustion system for collection of TPM. A - Biomass combustion reactor; B - LPG; C - Air compressor; D - Thermocouples; E - Flow transducers; F - Data acquisition system (Fieldlogger); G – Notebook; H - Chimney; I - glass fiber filters; J - Suction pump.

Preparation of the aqueous suspensions for intranasal instillation of TPM.

After collecting the TPM filters from the combustion exhaust gases of the CNS, the cleaned filters were placed in an oven at 50°C for 24 hours and weighed on analytical balance (FA-2104N). Then, the CNS combustion process was carried out to collect TPM. Subsequently, the TPM containing filters were put back into the oven at 50°C for 24 hours, and again weighed.

The filters were then packed in a Becker containing saline solution and sonicated for 8 hours in an ultrasonic sonicator (Q3350-QUIMIS®). After sonication, the filters were again placed in the oven at 50ºC for 24 hours and weighed. The efficiency of the extraction of the particles is calculated by the difference between the masses of the filters before and after the collection process (MAATZ *et al*., 2009). The final particle volume ratio was 1:1 (1 μg:1 μL), where we used 30 μg of TPM mass in 30 μL of the final solution.

Animals

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All animal use and care procedures were previously approved by the animal ethics committee of the State University of Ceará (Protocol No. 0681559/2018). Invasive procedures were performed under anesthesia and every effort was made to minimize suffering.

C57black/6 mice with body mass of 25±5 g and access to water and food *ad libitum*, were used in this study. To study COPD induced by cigarette smoke (CS), mice were exposed to 12 commercial cigarettes per day for 60 days using an inhalation chamber (40 cm long, 30 cm wide, and 25 cm high). The animals were placed in the inhalation chamber, housed inside an exhaust hood. The cigarettes were coupled to a 60 mL plastic syringe, and the CS was sucked into the syringe and then immediately expelled into the inhalation chamber. The animals were kept in this condition, with presence of CS in this environment, for 6 min. Then the inhalation chamber cap was removed, and the exhaust fan connected to evacuate the smoke for 1 min. Exposure to CS was repeated four times (4 x 6 min) with a 1 min escape interval after each exposure. This procedure was repeated three times a day (8h am, 12h am and 4h pm) (VALENÇA *et al*., 2008).

We used 24 animals randomly divided into three groups (Fig. 2). In the first group (n=8), the animals were exposed for 60 days to ambient air, and subsequently received intranasal instillation of 30 µL of solution from filter sonication in clean glass fiber in saline solution (0.9% NaCl) for 20 days (CTRL group). In the second group (n=8), the animals were submitted to the protocol of exposure to CS for 60 days and received intranasal instillation of 30 µL of solution from sonication of the clean glass fiber filter in saline solution for 20 days (CS group). In the third group (n=8), the animals were submitted to the protocol of exposure to CS for 60 days, and received intranasal instillation of 30 µg of TPM from the CNS combustion exhaust gases diluted in 30 µL of saline solution for 20 days (CS+TPM group).

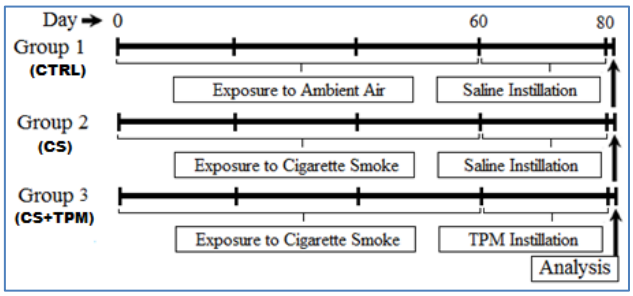

Fig. 2 Schematic diagram of the experimental protocol. In group 1 (CTRL), were exposed for 60 days to ambient air, and subsequently received intranasal instillation of 30 µL of solution from filter sonication in clean glass fiber in saline solution (0.9% NaCl) for 20 days. In group 2 (CS), were submitted to the protocol of exposure to cigarette smoke for 60 days and received intranasal instillation of 30 µL of solution from sonication of the clean glass fiber filter in saline solution for 20 days. In group 3 (CS+TPM), were submitted to the protocol of exposure to cigarette smoke for 60 days, and received intranasal instillation of 30 µg of TPM from the CNS combustion exhaust gases diluted in 30 µL of saline solution for 20 days. All analyses were performed on day 81.

Intranasal instillation

Exposure of the animals to the solution containing TPM (CS+TPM group) or saline solution (CTRL and CS groups) was performed via intranasal instillation during 20 days after protocol of exposure to cigarette smoke or ambiente air (CTRL group). Prior to intranasal instillation, the animals were sedated with sevofluorane (1 alveolar minimum concentration-AMC). The instillation causes a reflex of apnea followed by deep inspiration that leads the fluid into the lung. The animals received instillations containing 30 µg of TPM from the CNS combustion exhaust gases, diluted in 30 µL of saline solution (CS+TPM group), or 30 µL of clean filter fiber filter sonication solution (CTRL and CS groups). The technique used was effective to avoid wastage of the material. All analyzes were performed 24 hours after the last instillation (day 81).

Experimental Protocol

24 h after the last intranasal instillation of saline solution (CTRL and CS groups) or total particulate matter (CS+TPM), the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p., Hypnol® 3%, Syntect, Brazil) and tracheotomized. The animals were intubated with a 18-gauge cannula (Eastern Medikit, Delhi, India) that was then connected to a computer-controlled ventilator for small animals (Scirec©-*flexVent*®, Montreal, QC, Canada). The animals were ventilated at baseline settings: respiratory frequency of 120 breaths/min, tidal volume of 10 mL/kg, limiting pressure of 30 cmH_2O, and positive end-expiratory pressure (PEEP) of 3 cmH_2O. Animals were then paralyzed with pancuronium bromide (0.5 mL/kg, i.p., Cristália, Lindoia, MG, Brazil).

Initially we standardized the mechanical history of the respiratory system with two deep inflations (DI, 6-s long, peak pressure: 30 cmH_2O). Followed by 5 minutes of ventilation at baseline. Soon after, the impedance of the respiratory system (Z_{rs}) was measured with the forced oscillation technique (HANTOS *et al*., 1992), 12 sequential 30 s sampling intervals, for a total of 6 minutes (BATES, 2009).

The experimental Z_{rs} was fitted to the constant phase model as previously described (HIRAI *et al*., 1999):

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

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

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 (HANTOS *et al*., 1992).

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 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; KNOWLES, 1964). Two 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 DI were done, followed by 5 min of ventilation with baseline settings. Airway smooth muscle hyperresponsiveness was evaluated by inhalation of methacholine (MCh) (Sigma-Aldrich, St.Louis, MI, USA) delivered by aerosol produced by an ultrasonic nebulizer (Inalasonic, NS, São Paulo, Brazil) coupled to the inspiratory line of the ventilator. For such purpose, 4 mL of MCh solution (30 mg/mL) were added to the nebulizer container. The nebulization was carried out during 30 s under mechanical ventilation (XUE *et al*., 2019) and the average amount delivered to the animal was 1.2 mg/kg of MCh solution. After nebulization, the same previous analysis was repeated (forced oscillation, 30-s sequential intervals for 6 min), followed by two DI and another forced oscillation data gathering.

Histological study

Immediately after the determination of respiratory system mechanics, the rib cage was opened and Heparin (1000 IU) was injected in right ventricle of the heart. The trachea was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly euthanized the animals. The lungs were perfused with saline and, then, removed en bloc. The right lung was isolated, frozen in liquid nitrogen, and stored for biochemistry analysis; the left lung was kept at functional residual capacity and fixed in Millonig's formaldehyde (100 mL HCHO, 900 mL H2O, 18.6 g NaH2PO4, 4.2 g NaOH). 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, who was unaware of 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 or normal pulmonary areas, and the amount of polymorphonuclear (PMN) cells, as well as pulmonary tissue were determined by the pointcounting technique (WEIBEL, 1990). 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 (KNUDSEN *et al*., 2010).

Cellularity was assessed at 1000× magnification across 10-15 random non-coincident microscopic fields in each animal. Morphometric analysis and determination of bronchoconstriction index were done at 400× magnification. 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:

$$\text{IBC} = \frac{\text{NI}}{\sqrt{\text{NP}}} \quad \text{Eq. (3)}$$

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5.00 (GraphPad, San Diego, CA, USA). The pulmonary function data are presented as mean ± SD, where *n* represents the number of samples. Data normal distribution and homogeneities of variances were tested with Kolmogorov-Smirnov (with Lilliefors's correction) and Levene median tests, respectively. If both conditions were satisfied, Student's t-test was used. If any condition was refused, Mann-Whitney non-parametric test was used instead. A difference was considered significant if $p < 0.05$.

III. RESULTS

Our results concerning the analysis of respiratory system impedance (Z_{rs}), calculated from the forced oscillation technique, are presented in Figure 3. Some comparisons between the variables related to airway resistance (R_N), tissue resistance (G) and tissue elastance (H) of CTRL, CS and CS+TPM groups presented statistically significant differences ($p<0.05$).

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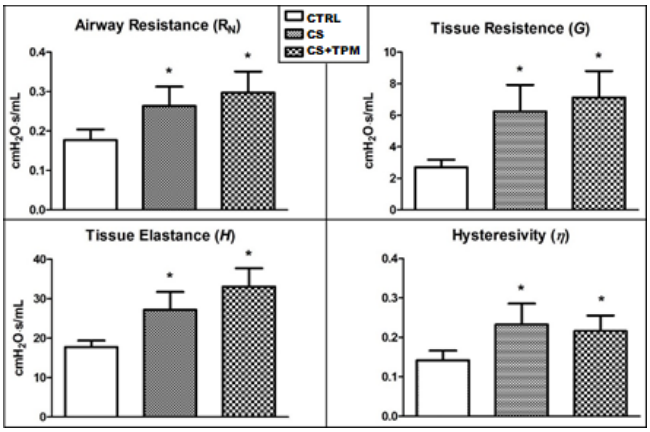

Fig. 3 Values of the variables of the constant phase model. Values for airway resistance (R_N), tissue resistance (G), tissue elastance (H) and hysteresivity (η) of the animals exposed to ambient air, CTRL group, to the cigarette smoke, CS group and to cigarette smoke and TPM from the combustion of cashew nuts shells, CS+TPM group. Values are represented by mean±standard deviation of the mean. * Represents statistically significant values in comparison to the control group ($p < 0,05$).

The results concerning the analysis of the volume pressure curve are presented in Figure 4. Some comparisons between the static complacency (C_{ST}), estimation of inspiratory capacity (IC) and PV loop area of the CTRL, CS and CS+TPM groups, presented statistically significant differences (p<0.05).

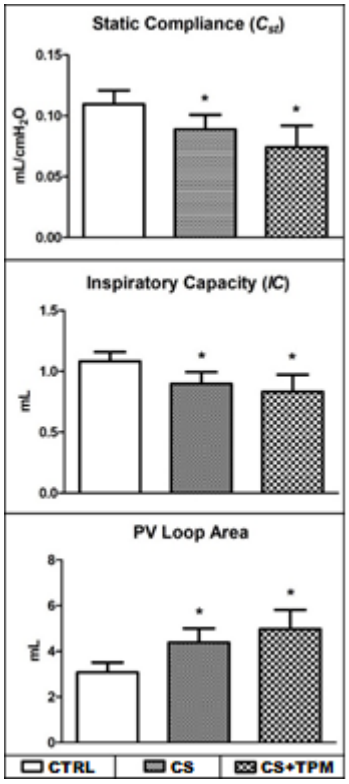

Fig. 4 Values referring to the variables collected from the PV curve. The static complacency (C_{ST}), estimation of inspiratory capacity (IC) and PV loop area, of the animals exposed to ambient air, CTRL group, to the cigarette smoke, CS group, and to cigarette smoke and TPM from the combustion of cashew nuts shells, CS+TPM group. Values are represented by mean±standard deviation of the mean. * Represents statistically significant values in comparison to the control group ($p < 0,05$).

Figure 5 shows the variations in ΔR_N after administration of MCh (30 mg/mL) in the groups. We observed an increase in the groups CS and CS+TPM in comparison to the CTRL group and an increase (ΔR_N) at several points of the groups CS and CS+TPM in comparison to the CTRL group, evidencing an airway hyperresponsiveness (AHR).

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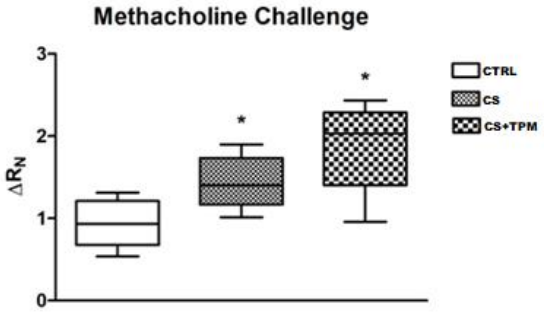

Fig. 5 Methacoline challenge as a function of number of measurements after MCh nebulization (30 mg/mL for 30 s) in animals exposed to ambient air, CTRL group, to the cigarette smoke, CS group, and to cigarette smoke and TPM from the combustion of cashew nuts shells, CS+TPM group. Values are represented by mean±standard deviation of the mean. * Represents statistically significant values in comparison to the control group ($p < 0,05$).

Figure 6 depicts representative lung and airway (inserts) histological images of the CTRL, CS and CS+TPM groups. There was the presence of alveolar collapse, thickened septa, cellular infiltration and constricted airways (inserts) in the photomicrographs of the pulmonary parenchyma of the CS and CS+TPM groups.

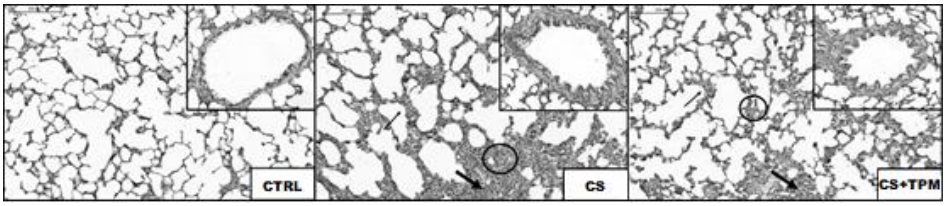

Fig. 6 Photomicrographs of lung parenchyma and airway (inserts) stained with hematoxylin–eosin of CTRL, CS and CS+TPM groups. Hin arrows: thickened septa; thick arrows: cellular infiltrate and circles: alveolar collapse.

Table 1 displays the alveolar collapse, amount of polymorphonuclear cells, mean alveolar diameter and bronchoconstriction index. We observed an increase in alveolar collapse, amount of polymorphonuclear cells, mean alveolar diameter and bronchoconstriction index of the CS and CS+TPM groups when compared to the CTRL group.

Table 1 Morphometric parameters.

Groups	Alveolar Collapse (%)	PMN Cells ($x10^{-3}/\mu m^2$)	Mean alveolar diameter (µm)	BCI
CTRL	4,77±3,06	8,45±1,45	37,25±3,55	1,90±0,10
CS	29,85±7,30 [a]	23,66±2,69 [a]	58,08±5,14 [a]	2,57±0,36 [a]
CS+TPM	39,91±4,74 [a]	25,79±3,79 [a]	51,43±5,84 [a]	2,84±0,44 [a]

Values are mean ± SD of groups CTRL, CS and CS+TPM. The data were collected in ten matched fields per mice. The CTRL group was submitted to the same procedures as the other groups, but without exposure to pollutants. In CS and CS+TPM groups, mice were were submitted to the protocol of induction to COPD by cigarette smoke. After the protocol of induction to COPD the CS+TPM group received intranasal instillation of TPM during the period of 20 days, while the CS group received intranasal instillation of saline. [a] Different from CTRL group ($p<0.05$).

IV. DISCUSSION

It is known that the environment in which the individual is inserted is relevant for the aggravation of diseases of the respiratory system (HANSEL *et al*., 2013). In this sense, in the attempt to simulate the exposure of people living in areas close to industrial centers or using biomass combustion for food cooking, with previous lung disease, the present study evaluated the effects of exposure to TPM by exhaust gases from CNS combustion, after the development of COPD induced by CS.

The energetic utilization of residual products from cashew processing, such as CNS, is already a reality in some industries. In particular, combustion exhaust gases from CNS promote the release of various pollutants, such as aldehydes, ketones and polycyclic aromatic hydrocarbons (PAHs), which are capable of promoting significant pulmonary changes (JOSINO *et al*., 2017). As well as TPM, which can easily penetrate the respiratory system and reach the pulmonary alveoli, resulting in diseases of respiratory diseases (WHO, 2005; LEWNÉ; NILS, 2007; XIAO *et al*., 2011), such as COPD.

The characteristics of human COPD can be induced in mice by the administration of proteases, particles and exposure to cigarette smoke. Although animal models do not perfectly replicate human COPD, prolonged exposure to cigarette smoke produces lesions consistent with the mild form of centrilobular emphysema observed in humans (WRIGHT; COSIO; CHURG, 2008).

In relation to our results, we assessed respiratory mechanics by the forced oscillation technique using a constant phase model (Figure 3), and quasi-static PV curve (Figure 4). In the constant phase model, the Newtonian resistance (R_N) represents a good estimate of the total central airway resistance,[14] and tissue resistance (G) and tissue elastance (H) are related to the intrinsic

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properties of the tissue, while hysteresivity (η) correlates with the degree of heterogeneity in lung ventilation (BATES, 2009; FREDBERG; STAMENOVIC, 1989).

In the quasi-static PV curve, the static compliance (C_{ST}) indirectly measures the degree of lung tissue distensibility, and estimate of inspiratory capacity (IC) quantifies the volume of air received by the lungs up to a pressure of 30 cmH_2O, while PV loop area provides an estimate of the amount of atelectasis (airspace closure) that existed before the PV loop manoeuvre (WEST, 2012). 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 increase in the value of R_N in groups CS and CS+TPM may represent greater narrowing of airway lumen or increased stiffness of smooth muscle of the airways in this groups compared to the CTRL group (SERRA *et al*., 2017). Our findings of histological analysis (Figure 6, insert) and lung parenchymal morphometry (Table 1, BCI) confirming narrower airways. In addition, the destruction of the alveolar wall, present in pulmonary emphysema (CS and CS+TPM groups), causes a distortion in the pulmonary parenchyma, reducing the tension that generates in the airway wall (BARNES; SHAPIRO; PAUWELS, 2003), causing the decrease of its lumen and consequent increase in the R_N.

Tissue resistance (G) and tissue elastance (H) are related to the intrinsic properties of the tissue. The increase of these parameters in the CS and CS+TPM groups may be related might be the change in tissue rheological properties (BATES, 2009). That could result from thickening of the alveolar septum and presence of atelectatic areas, as observed in our histological analysis (Figure 6), increased amount of collapsed alveoli, reduced alveolar diameter, and the increased number of polymorphonuclear cells (Table 1). The ratio of the dissipative (G) to the elastic (H) component of overall energy is named hysteresivity (η), and correlates with the degree of heterogeneity in lung ventilation (BATES, 2009; FREDBERG; STAMENOVIC, 1989). Therefore, the increase in η in the CS and CS+TPM groups may be related to the increase in ventilatory heterogeneities, probably caused by the changes in the R_N (BATES, 2009).

In relation to the analysis of the quasi-static PV curve (Figure 4), the chronic imbalance, influenced by smoking, provides high activity of proteases, low amounts of antiproteases, destruction of the extracellular matrix (ECM) and consequently increase of the mean alveolar diameter (SHAPIRO; INGENITO, 2005). With this, we would expect to see an increase in C_{ST} and CI in CS and CS+TPM groups, as a consequence of the loss of elastic recoil determined by the fibers of the pulmonary parenchyma.
However, our findings did not identify this behavior. Similar results were found by Kennedy-Feitosa and collaborators (2016). This fact can be explained due to parameters such as C_{ST} and CI are not commonly used in animal model studies to characterize COPD. For this, the mean alveolar diameter is used, being increased in both groups (Table 1). The increase in PV loop area in groups CS and CS+TPM, might be attributed to tissue changes (alveolar collapse, presence of PMN cells – Table 1) and possibly to a surfactant-associated mechanism (MÜLLER; SEIFART; BARTH, 1998).

Regarding pulmonary response to challenge with MCh (Figure 5), there was a statistically significant increase in CS and CS+TPM airway resistance compared to the CTRL group (Figure 3), evidencing a hyperresponsiveness of the smooth muscle of the airways. Hyperresponsiveness can also be attributed to the presence of inflammatory processes in the airways of these animals, where similar studies have reported airway hyperresponsiveness following exposure to cigarette smoke (LO *et al*., 2018) or pollutants (SERRA *et al*., 2017).

In order to analyze our hypothesis that the acute exposure to atmospheric pollutants, specifically TPM from the CNS combustion, in individuals of susceptible groups (COPD), can exacerbate the harmful effects of this pollutant in the respiratory system, we compared the mechanics data of the CS and CS+TPM groups with the CTRL group.
Regarding the parameters of the constant phase model, we observed in the CS group an increase of 33.2%, 56.7% and 34.7% in the values of R_N, G and H, respectively. In the CS+TPM group, this increase was 40.7%, 62.2% and 46.3%, in the R_N, G e H, respectively. Regarding the parameters of the quasi-static PV curve, we observed in the CS group an decrease of 23.2% and 29.8% in the values of C_{ST} and IC, and a 20.7% increase in the value of $PV\ Loop\ area$. In the CS+TPM group, this decrease was 47.3% and 38.1% in the C_{ST} e IC, and a 30.1% increase in the value of $PV\ Loop\ area$.
These results demonstrate the lung injury of the animals caused by exposure to cigarette smoke (CS group), but that this injury is greater when associated with exposure to TPM from the combustion of CNS (CS+TPM group). Evidenciating the harmful effects caused by the exposure that this pollutant, from the combustion of a residual biomass, causes in the respiratory system of animals with respiratory pathology.

V. CONCLUSION

Despite the encouragement to use renewable resources to the detriment of fossil fuel derivatives, care must be taken with the combustion of residual biomass. The increasing use of this biofuel in industries, craft centers and homes, warns of exposure to exhaust gases. The health of the surrounding population may be harmful, especially to those with pre-existing respiratory disease. These, because they have low resilience, are more susceptible to damages caused by exposure to pollutants. The present paper serves as an alert for government agencies to review some parameters of atmospheric emissions.

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