



CHANGES IN THE RESPIRATORY SYSTEM OF RATS EXPOSED TO GASES FROM THE COMBUSTION OF CASHEW NUTS SHELL

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ABSTRACT

The term biomass describes all organic matter that, when burned, decomposed or recycled, can directly and/or indirectly generate some form of mechanical, thermal or electrical energy. Brazil is one of the largest producers of agricultural products in the world, making it also one of the largest producers of residual biomass. Due to its importance in the cashew crop, there is a concern to find alternatives for an environmentally safe disposal for the residual biomass of the cashew nut processing, cashew nut shell (CNS). In view of the above, this work had the objective of evaluating the effects caused by acute exposure to CNS combustion emissions. For this, a CNS combustion reactor was produced to expose animals to exhaust gases. BALB / c mice, body mass of 25 ± 5 g, randomly divided into two groups, Control, exposed for 5 hours to ambient air, and CNS, exposed for 5 hours to CNS combustion exhaust gases were used. Our results show statistically significant changes in all variables analyzed (R_N, G, H, C_{ST}, CI , e PV loop area) of the CNS group in relation to the control group. The use of CNS as a biofuel can be feasible, but our results reinforce the urgent need to seek control methods for the exhaustion of these gases in the atmosphere. Further investigation is necessary in order to know safe parameters for individuals who are continuously exposed to CNS combustion exhaust gases.

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INTRODUCTION

The term biomass describes all organic matter that, when burned, decomposed or recycled, can directly and/or indirectly generate some form of mechanical, thermal or electrical energy. Thus, agricultural residues, animal waste, human waste and organic municipal waste can be used in processes to obtain energy from alternative sources (ANEEL, 2008). In Brazil, some biomass residues contribute to the growth of alternative energy production in the industrial sector, such as coffee grounds (Silva *et al.*, 1998), rice hull (Maffioletti and

Mota, 2013), sugarcane bagasse (Yiannis, 2011), eucalyptus foliage (Possel and Bell, 2012), and the cashew nuts shell (CNS) (Ndalila and Kaunde, 2014), the latter being our object of study. The cashew tree (*Anacardium occidentale L.*) is a tropical plant, native to Brazil and dispersed throughout most of its territory. Cashew is among the most cultivated fruit trees, standing out in the socioeconomic context of some underdeveloped tropical regions, due to the high nutritional and commercial value of its products, and the labor intensive character, whose production and industrialization guarantee a significant flow of income, making this culture an important source of job opportunities for the population (Ramos *et al.*, 2011). According to the International Nut & Dried Fruit (2016), Brazil is the fifth largest producer of cashew nuts in the world.

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The Brazilian cashew production reached 104,475 tons in 2015, corresponding to a harvested area of 585,966 hectares, the northeast region being responsible for 99.4% of the production (IBGE, 2015). In 2014, Brazil exported approximately 17,023 tons of cashew nuts (MDIFT, 2015). Obtaining the CNS begins through the withdrawal process almond cashew stalk (decorating), held in tanks with cardol, composed of 10% of the liquid extracted from cashew nut itself (CNL). This is heated in boilers at a temperature of ± 800 °C. The by-product of this stage is the almond, of great commercial value, and the CNS, drenched in cardol, which hold great potential fuel (Lima, 2008). The high calorific power of CNS (22,48 MJ/kg), is superior to other biomasses such as: rice straw (16,35 MJ/kg) and sugarcane bagasse (18,61 MJ/kg), making the CNS an attractive alternative compared to the use of other fuel sources used by industries, mini-factories and in handicraft productions (Figueiredo *et al.*, 2007), providing an alternative for its use and avoiding its disposal in the environment. However, the combustion of CNS promotes the release of some gases, such as: carbon dioxide (CO₂), carbon monoxide (CO), methane (CH₄), ozone (O₃), nitrogen oxides (NO_x), nitrogen dioxide (NO₂), sulfur dioxide (SO₂), polycyclic aromatic hydrocarbons (PAHs), Volatile organic compounds (VOCs), Besides other products considered pollutants (Lewne *et al.*, 2004; WHO, 2005). In view of the above, there is an urgent need to evaluate the effects caused by acute exposure to CNS combustion emissions, providing a better knowledge about its probable harmful effects on health, producing parameters of control and safety in its use as biofuel. For this, we analyzed the deleterious effects in the respiratory system of rats exposed to CNS combustion exhaust gases for 5 hours, through the analysis of variables related to the mechanics of the respiratory system of these animals.

MATERIALS AND METHODS

CNS combustion reactor

Cashew nut shell was supplied by cascaju agroindustrial S/A, located 65 km from Fortaleza in the municipality of Cascavel in Ceará, Brazil. The combustion and gas exposure system of the CNS (Figure 1). For the animal exposure process, the initial combustion ignition of CNS was performed by supplying liquefied petroleum gas (LPG- Figure 1B) and ambient air from an air compressor (Figure 1C). The combustion process of CNS was accompanied by thermocouples (Figure 1D) and flow transducers (Figure 1E) connected to a data acquisition system (FieldLogger-Figure 1F) for the analysis and control of temperature and LPG and air flows (unpublished data), directing information to a notebook (Figure 1G). During the combustion, 500 g of CNS was used every 1 hour of combustion, using a total mass of 2.5 kg during the 5 hours of combustion. The concentration of CO in the exposure chamber for the CNS group was monitored and adjusted every 10 minutes throughout the period, keeping the CO concentration at 10 ppm. The CNS used was not crushed in order to more closely simulate the characteristics of the biomass used in industrial furnaces and boilers.

Animais

This study followed all the rules in force for the maintenance of animal welfare. All the protocols used were previously approved by the ethics committee for the use of animals of the State University of Ceará (protocol N°3113976). We were used sixteen BALB/c mice, female, 8 weeks of age and body weight of 25 ± 5 g were housed in polypropylene cages with temperature of 20 °C to 24 °C with 12 hours light cycle and 12 hours dark, receiving food and water *ad libitum*.

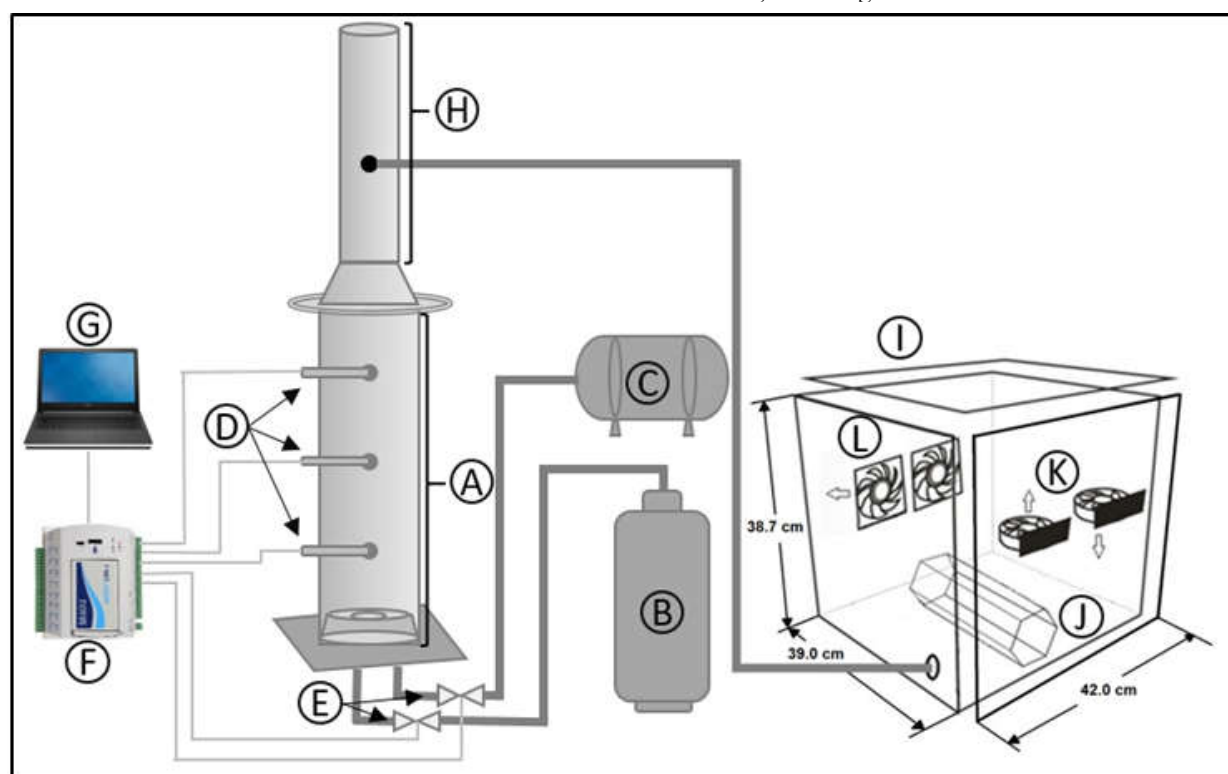


Figure 1. CNS combustion reactor for animal exposure (CNS group). A- Biomass combustion reactor; B-LPG; C- Air compressor; D- Thermocouples; E- Flow transducers; F- Data acquisition system (Fieldlogger®); G- Notebook; H-Chimney; I- Exposure chamber; J-Honeycomb unit to independently accommodate each animal; K- Mixing fans; L- Exhausts fans

The animals were randomly divided into two groups: one group exposed for 5 hours to ambient air (Control group, n=8), and another exposed for 5 hours to the CNS exhaust gas (CNS group, n=8). Exposure to exhaust gases from the CNS combustion was performed for 5 hours, an adaptation of the protocol proposed by Tesfaigzi *et al.*, (2002) used to investigate the sub chronic effects of exhaust gas exposure from wood smoke in rats.

Experimental protocol

24 hours after the end of the exposure period, 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 cmH₂O, and positive end-expiratory pressure (PEEP) of 3 cmH₂O. Animals were then paralyzed with pancuronium bromide (0.5 mL/kg, i.p., Cristália, Lidoia, MG, 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 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):

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

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

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 flexi Vent 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 (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. Another forced oscillation technique ensued to determine respiratory system mechanics.

Statistical analysis

Results are presented as mean \pm 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 $p < 0.05$.

RESULTS

During the exposure, the animals in the Control group were accommodated independently (Figure 1J) and exposed for 5 hours to the gases of the laboratory ambient conditions. These values were: 0 ppm for CO, 20.8 % for O₂, 0 ppm for NO, 0 ppm for SO₂ and average temperature of 25 °C. The animals in the CNS group were accommodated independently (Figure 1J) and exposed for 5 hours to the exhaust gases from the CNS combustion.

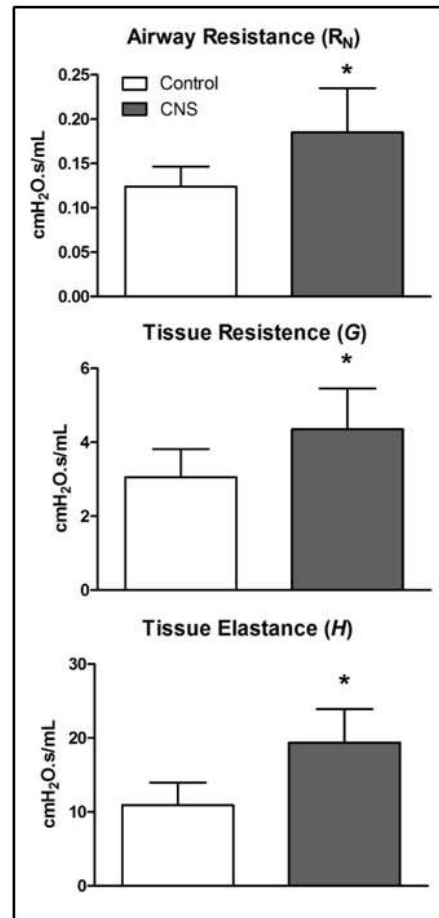


Figure 2. Values for airway resistance (R_N), tissue resistance (G) and tissue elastance (H), of the animals exposed to ambient air, Control group (white column), and to the combustion exhaust gases of CNS, CNS group (gray column). Values are represented by mean \pm standard deviation of the mean. * Represents statistically significant values in comparison to the control group ($p < 0.05$).

With average values of 8.9 ppm for CO, 20.2 % for O₂, 3.1 ppm for NO, 5.8 ppm for SO₂ and average temperature of 26.3 °C. In an attempt to avoid the intoxication of the animals by CO, the atmosphere of the CNS group was controlled so that this gas was kept below 10 ppm. Our results regarding the analysis of the impedance of the respiratory system (Z_{rs}), calculated from the forced oscillation technique, are presented in Figure 2. The values of the variables related to airway resistance (R_N), tissue resistance (G) and tissue elastance (H) of the CNS group in relation to the control group, presented statistically significant differences. The results concerning the analysis of the volume pressure curve are presented in Figure 3.

Table 1. Mechanical of respiratory system data of animals exposed to air and CNS. Values are mean \pm SD of animals exposed to ambient air (n = 8, Control group) and animals exposed to exhaust gases from the combustion of CNS (n = 8, CNS group). * $p < 0.05$, statistically significant difference.

Measure	Group	Value	pvalue (t student)
Newtonian Resistance (R_N) (cmH ₂ O·s/mL)	Control	0.123 \pm 0.022	
	CNS	0.185 \pm 0.049	$p = 0.0168^*$
Tissue Resistance (G) (cmH ₂ O/mL)	Control	3.05 \pm 0.76	
	CNS	4.35 \pm 1.09	$p = 0.0299^*$
Tissue Elastance (H) (cmH ₂ O/mL)	Control	10.93 \pm 3.05	
	CNS	19.39 \pm 4.51	$p = 0.0020^*$
Inspiratory Capacity (IC) (mL)	Control	1.45 \pm 0.15	
	CNS	1.06 \pm 0.20	$p = 0.0027^*$
Static Compliance (C_{ST}) (mL/cmH ₂ O)	Control	0.137 \pm 0.013	
	CNS	0.115 \pm 0.012	$p = 0.0102^*$
Loop Area (mL·mH ₂ O)	Control	4.17 \pm 2.07	
	CNS	7.16 \pm 2.64	$p = 0.0413^*$

The values of the variables related to the static complacency (C_{ST}), estimation of inspiratory capacity (IC) and PV loop area of the CNS group in relation to the control group, presented statistically significant differences. The absolute values, referring to the variables calculated from the analysis of the impedance of the respiratory system (Z_{rs}), and the PV curve of the groups exposed to the ambient air (control group) and to the combustion exhaust gases of the CNS (CNS group), are shown in Table 1.

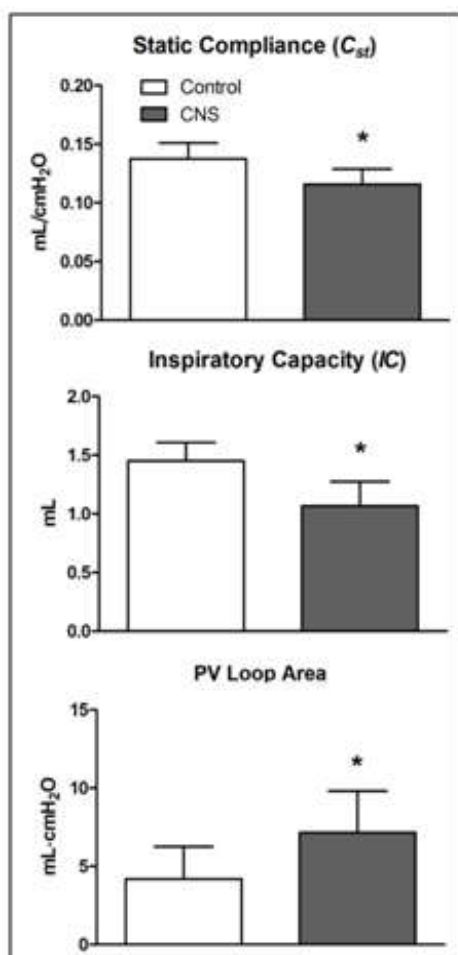


Figure 3. Values referring to the variables collected from the PV curve. The static complacency (C_{ST}), estimation of inspiratory capacity (IC) and PV loop area, of the animals exposed to ambient air, Control group (white column), and the combustion exhaust gases of CNS, CNS group (gray column). Values are represented by mean \pm standard deviation of the mean. * Represents statistically significant values in comparison to the control group ($p < 0,05$).

DISCUSSION

Although the literature presents works of exposition of animals to the most diverse pollutants, gases and particles generated by the exhaustion and combustion of some biomasses, we do not find works whose objective is to evaluate the probable harmful effects to the respiratory system, due to the direct exposure of animals to combustion exhaust gases from the CNS. However, there is evidence of its harmful effects on health, with regard to the combustion of CNS, the number of respiratory events, increased inhaled therapy, mutagenicity, genotoxicity and cytotoxicity (Cabral, 2010; Galvão *et al.*, 2014). The combustion of any biomass emits large amounts of carbon in the forms CO₂, CO, CH₄, particulate matter (PM) and their presence in the air, can transform them into pollutants, depending on the type of biomass burned (chemical composition) and the conditions of burning (Cieslinski, 2014). Exposure to these gases can result in acute and chronic repercussions in the respiratory system, these can occur in both previously healthy individuals and in patients with pre-existing disease (Galan *et al.*, 2003). Additionally, this exposure may potentiate immune system response to allergens by increasing epithelial permeability, recruitment and activation of inflammatory cells, and oxidative stress in the airways (Nagato, 2007).

The risk of lung cancer caused by the presence of PM and APHs (acid polycyclic hydrocarbons) present in the biomass combustion gases is higher than the corresponding risk from exposure to exhaust gases from motor vehicles (Sarigiannis *et al.*, 2015). The atmospheric pollution caused by biomass burning in the Brazilian Amazon region shows a positive association between the exposure of its pollutants and the occurrence of respiratory diseases, especially in the most vulnerable age groups (the elderly and children under 5 years of age) (Ignotti *et al.*, 2010). The MP present in the CNS combustion exhaust gases can undergo alveolar deposition and endocytosis, translocate the pulmonary epithelioendothelial barrier, expressing a high ratio between surface area and mass, reaching deeper into the respiratory tract, which can reach and damage the pulmonary parenchyma. Such as being absorbed into the bloodstream, which amplifies its biological toxicity (Shimada *et al.*, 2006; Furuyama *et al.*, 2009). Our results, in relation to the parameters obtained through the constant phase model, demonstrate a statistically significant increase in the values of the Newtonian resistance (R_N), tissue resistance (G) and tissue elastance (H) (Figure 2) in the CNS group compared to the Control group. The significant increase in R_N may be provided by the inhalation of the PM present in the exhaust fumes from the CNS combustion.

These particles, when inhaled induce the expression of pro-inflammatory mediators and Ca^{2+} dependent intracellular signaling pathways (Ermak and Davies, 2002). The biological functions of the Ca^{2+} ion in the lungs play a role in the regulation of various functions, such as mucus secretion, surfactant secretion and ciliary agitation frequency (Conway *et al.*, 2003). Changes in tissue resistance variables (G) and tissue elastance (H), may be related to the intrinsic properties of the tissue, causing alterations in the rheology of the pulmonary tissue, due to alterations of the extra cellular matrix, tissue remodeling and of other constituents (Bates, 2009). Alterations in G and H can also occur due to the increase in the R_N due to a narrowing of the airways, causing a distortion of the pulmonary parenchyma, resulting in an increase in the intrinsic stiffness of the tissue, causing elastance increase and the development of ventilatory heterogeneities throughout the lung, which causes an increase in tissue stiffness (Bates *et al.*, 1994). Another possibility for changes in the G and H variables is the presence of mucus in small caliber airways, which could lead to occlusions of these pathways, as the same effect of producing areas with atelectasis.

Regarding the parameters obtained through the realization of the volume pressure curve, we observed significant alterations in the parameters of static complacency (C_{ST}), estimation of inspiratory capacity (IC) and PV loop area (Figura 3) of the animals CNS group in relation to the Control group. The decrease in the C_{ST} parameter may be a reflection of the already discussed increase in tissue elastance (H). In experimental studies, inhalation of particles from combustion processes led to decreased lung compliance and an inflammatory response characterized by influx of polymorphonuclear cells and release of cytokines (Laks *et al.*, 2008; Mazzoli-Rocha *et al.*, 2008). The decrease in the inspiratory capacity (IC), indicating stiffening of the lung tissue, observed by the increase in H , assuming that the animals of the CNS group presented a greater effort in the inspiration. On the other hand, the increase of PV loop area can be explained by possible alterations in the distribution of surfactant on the alveolar surface, associated with the presence of alveolar edema in the lung of the CNS group. The PV loop area is determined by four processes: recruitment/de-recruitment, surface tension, stress relaxation, and gas absorption during PV assays.

Conclusion

Despite the environmental appeal for the energy utilization of a previously discarded biomass in the environment, our results demonstrate that an acute combustion exhaust exposure of the CNS was sufficient to cause changes in the respiratory system of mice. Our results reinforcing the urgent need to seek control methods for the exhaustion of these gases in the atmosphere. Further investigation is necessary in order to know safe parameters for individuals who are continually exposed to CNS combustion exhaust gases.

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