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Effect of particles of ashes produced from sugarcane burning on the respiratory system of rats

L.E.N. Ferreira [a], B.V. Muniz [a], T.O. Bittar [b], L.A. Berto [a], S.R. Figueroba [a], F.C. Groppo [a,*], A.C. Pereira [b]

[a] Department of Physiological Sciences, Piracicaba Dental School, University of Campinas – UNICAMP, Piracicaba, São Paulo, Brazil
[b] Department of Social Dentistry, Piracicaba Dental School, University of Campinas – UNICAMP, Piracicaba, São Paulo, Brazil

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ABSTRACT

The practice of burning sugarcane obtained by non-mechanized harvesting exposes workers and the people of neighboring towns to high concentrations of particulate matter (PM) that is harmful to health, and may trigger a series of cardiorespiratory diseases. The aim of this study was to analyze the chemical composition of the micro-particles coming from sugarcane burning residues and to verify the effects of this micro-particulate matter on lung and tracheal tissues. Micro-particulate matter (PM_{10}) was obtained by dissolving filter paper containing burnt residues in NaCl solution. This material was instilled into the Wistar rats' nostrils. Histological analyses (hematoxylin and eosin – HE) of cardiac, lung and tracheal tissues were performed. Inflammatory mediators were measured in lung tissues by using ELISA. The chemical composition of the particulate material revealed a large quantity of the phthalic acid ester, high concentrations of phenolic compounds, anthracene and polycyclic aromatic hydrocarbons (PAH). Histological analysis showed a reduction in subjacent conjunctive tissue in the trachea, lung inflammation with inflammatory infiltrate formation and reduction of alveolar spaces and a significant increase ($p < 0.05$) in the release of IL-1α, IL-1β, IL-6, and INF-γ in the group treated with PM_{10} when compared to the control group. We concluded that the burning sugarcane residues release many particles, which have toxic chemical compounds. The micro-particulate matter can induce alterations in the respiratory system.

1. Introduction

Environmental pollutants may trigger unfavorable responses, particularly in the human respiratory system, which is one of the main areas exposed to pollution (Gulisano et al., 1995; Foltinová et al., 2002). The burn of biomass is one of the major contributors to the production of toxic and greenhouse gases and accumulation of suspended particles all over the planet. It increases the levels of air pollution and affects hundreds of thousands of workers, especially in developing countries (Schwela et al., 1999).

Besides Brazil, Guatemala, Mexico, Costa Rica, United States, Philippines and India among others, use the burning practice as a pre-harvest measure in sugarcane fields. Brazil is the world's largest producer of sugarcane having 25% of the total worldwide cultivated sugarcane (França et al., 2012), being São Paulo state the largest Brazilian producer (da Silva et al., 2012).

Despite of the technological advances, the manual harvesting occurs in approximately 60% of the cultivated sugarcane (*Saccharum spp.*). This non-mechanized process of harvesting is usually preceded by burning procedures in order to simplify the process (Prado et al., 2012).

Sugarcane burning is one of the major producers of fine and ultrafine particles in the production areas during the cane-harvesting season (Lara et al., 2001). It is estimated that approximately 2.6 g of fine particulate matter are emitted per kg after burning dry-crop residues (França et al., 2012).

The concentration of particulate matter during the harvesting period registered in the atmosphere of urban areas, which are near to the sugarcane fields, is almost 2.5-times higher than the World Health Organization Air Quality Guidelines (WHO, 2005) recommendation for a maximum of 24-hours short-term exposure (Prado et al., 2012). This burning process produces ashes rich in particulate matter (PM). This pollutant is considered harmful and the cause of deleterious effects on human health (Arbex et al., 2007).

* Correspondence to: Av. Limeira, 901 – Bairro Areião CEP, 13414-903 Piracicaba, São Paulo, Brazil.
E-mail addresses: luiz.enferreira@gmail.com (L.E.N. Ferreira), bvm_bob@hotmail.com (B.V. Muniz), telmobittar@hotmail.com (T.O. Bittar), lucianaberto@hotmail.com (L.A. Berto), sfigueroba@uol.com.br (S.R. Figueroba), fcgroppo@fop.unicamp.br (F.C. Groppo), apereira@fop.unicamp.br (A.C. Pereira).

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Numerous epidemiological studies have reported consistent association between exposure to some air pollution particles of diameter lower than 10 μm (PM_{10}) and cardiorespiratory mortality/morbidity (Schwartz, 1997; Brunekreef and Holgate, 2002).

Fine and ultrafine particles play an important role in pathophysiological changes (Samet et al., 2000). Inhaled particles may lead to lung inflammation and subsequent release of soluble mediators that influence diverse physiological parameters (Donaldson et al., 2001). Inhalation of PM_{10} may stimulate the production of reactive oxygen species and inflammatory mediators by alveolar macrophages, epithelial cells and other pulmonary cells (van Eeden et al., 2001). *In vitro* studies using alveolar macrophages and epithelial cells from lungs exposed to PM_{10} particles, have demonstrated an amplification in the production of inflammatory mediators, resulting in alterations in many metabolic processes, also affecting phagocytic activity, cell proliferation and inducing apoptosis (Becker et al., 2003; Poma et al., 2006).

Therefore, we evaluated the chemical composition of the particulate material (PM_{10}) from residues of burning sugarcane, and its histopathological effects on the respiratory system of Wistar rats. In addition, the release of pro-inflammatory cytokines from the animal lungs was observed after seven days of exposure to PM_{10}.

2. Materials and methods

2.1. Animals

Young Wistar rats (*Rattus norvegicus albinus*) aging five to six weeks and weighing approximately 250–300 g were obtained from Multidisciplinary Center for Biological Investigation on Laboratory Animal Science (CEMIB–UNICAMP). The animals were kept under controlled environment at the Piracicaba Dental School vivarium with food and water *ad libitum*. The project was approved by the Ethics Committee on Animal Research of UNICAMP under Protocol number 104/2007 and the experiments were conducted in compliance with the principles established in the Guide for the Care and Use of Laboratory Animals (NRC, 2011).

2.2. Physicochemical analysis of the sugarcane particles

2.2.1. Particle sampling

In order to obtain the material, sugarcane (*Saccharum officinarum*) residues (dry leaves and barks) were burned in a specially designed oven. Paper filters (10 μm of pore size) adapted in the oven were used to collect the particulate matter during the burning process, as shown in Fig. 1. After completion of the burning process, the paper filters containing the retained particles were segmented in 10 g portions and diluted in 100 mL of 0.9% NaCl. As a control, a suspension was obtained diluting the same paper filter (10 g) without ashes residues in 100 mL of 0.9% NaCl. Both suspensions were sonicated for 20 min. Both solutions were re-filtered with 10 μm-pore size membranes (Millipore Corporation, MA, USA).

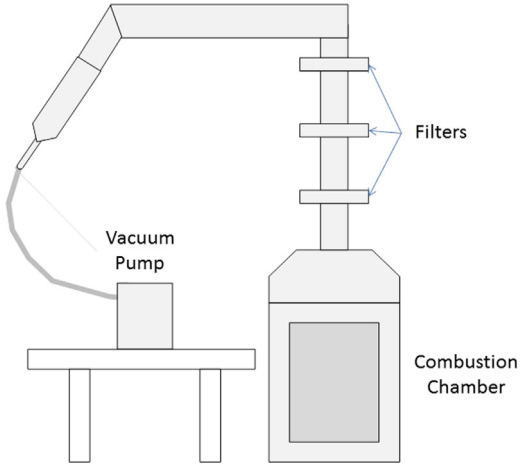

Fig. 1. Schematic drawing of the oven used to obtain particulate samples after burning sugarcane residues.

2.2.2. Gas chromatography

Chemical analysis was performed by gas chromatography coupled to mass spectrometry (GC–MS) after solid phase micro extraction (SPME). Briefly, 2 g of 0.9% NaCl was added in both suspensions in 15 mL flasks. Flasks were sealed and placed in a thermostatic bath at 70°C, and remained there under constant agitation for 30 min. After, SPME fibers (PDMS–DVB 65 μm) were introduced into each flask for 30 min, and introduced into the GC system.

GC–MS analysis was performed in an HP-6890 gas chromatograph coupled to HP-5975 mass selective detector with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The temperature of the injector and detector was 220 °C and 250 °C, respectively, while the column remained at 40 °C, 2 °C/min, and 200 °C. The gas (He) flow was 1.0 mL/min.

2.3. Animal exposure

Small amounts of PM_{10} suspensions were instilled daily in the nostrils (intranasal) of 14 rats for 7 days. The animals were divided into two groups of the following treatments:

1- Control group – instillation of 40 μL of the control suspension;
2- PM_{10} group – instillation of 40 μL of PM_{10} suspension.

For intranasal instillation of both control and PM_{10} suspensions, the animals were previously anesthetized by 2% xylazine 10 mg/kg and 10% ketamine 90 mg/kg, both administered intramuscularly.

2.4. Tissue histology and morphometry

After 7 days of treatment, all animals were euthanized by administration of sodium pentobarbital 50 to 100 mg/kg/i.p. After removal of the respiratory system, lungs were inflated to their maximum capacity, and one lung along with trachea and heart of each animal was kept in buffered phosphatase 4% paraformaldehyde solution (pH 7.2) for histopathological analysis. Those tissues were processed, and histological slides were obtained in longitudinal cuts of 6 μm. The slides were stained with hematoxylin and eosin (HE).

Slides were analyzed and photographed by using an optical photomicroscope (Leica DLMP) equipped with a digital camera (Leica DFC-280). Microscopic fields containing bronchiole, alveoli and blood vessels were selected to evaluate lung tissue. The inflammatory effect of the PM_{10} was observed by the percentage of the area relative to alveolar space, measured with the use of the Image J (National Institute of Mental Health, USA) software.

Measurements from the terminal portion of the smooth muscle up to the extremity of the calceiform cells and from the beginning of the hyaline cartilage up to the extremity of the calceiform cells were performed to evaluate the effects of PM_{10} on thickness of the tracheal tissue. In each field, measurements were performed in the central portion, and on the left and right margins. The total thickness was determined by the mean value of the three measurements. Leica Application Suite program (Leica Microsystems) was used to measure the morphometric parameters of the trachea.

2.5. Enzyme linked immunosorbent assay for cytokines

The other lung (not used to histopathological analysis) was stored at −80°C and used to measure the concentration of growth-related oncogene alpha (GRO-α), interleukin 1beta (IL-1β), 1alpha (IL-1α), six (IL-6), interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α).

Briefly, the 96-well plates were covered with 100 μL/well of capture antibodies for each cytokine, in accordance with the manufacturer's instructions (Peprotech Inc). The plates were incubated overnight at room temperature and washed with 0.05% Tween-20 in PBS thrice. Then, 300 μL of blocking buffer 1% BSA in PBS was added to each well. Plates were incubated for 1 h, and 100 μL of the samples (diluted 1:2) or of the standard solutions was added.

The plates were incubated at room temperature for 2 h. 100 μL of detection antibody and 100 μL of a conjugated avidin solution were added to each well. After 20 min, 100 μL of ABTS liquid substrate solution was added. The plates were maintained at ambient temperature and the colorimetric reaction was read in a spectrophotometer at a wavelength of 405 nm with correction of 650 nm.

2.6. Statistical analysis

Levene's and Shapiro–Wilk's tests were used to verify the homogeneity of variance and distribution of data, respectively. Tracheal tissue thickness were analyzed by one-way analysis of variance (ANOVA) and Tukey's test as the *post-hoc* test to assess the significance of differences between groups. Kruskal–Wallis' and