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FIXED OIL FROM PEQUI FRUIT (Caryocar coriaceum) PREVENTS LUNG CHANGES CAUSED BY VEHICLE POLLUTANTS

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Abstract

The exposure to diesel exhaust particles (DEP) in high-traffic environments is associated with significant alterations in the respiratory system. In parallel, it is assumed that the regular inclusion of compounds containing high levels of polyunsaturated fatty acids, such as pequi oil (*Caryocar coriaceum*), in the diet may help with disorders caused by these pollutants. The present work investigates the benefits of oral ingestion of fixed oil from *Caryocar coriaceum* (CC) on lung tissue and ventilatory mechanics in mice exposed to DEP, as well as its chemical composition. The CC, mainly composed of linoleic acid (49.13%), prevented the increase in the bronchoconstriction index and the infiltration of inflammatory cells in the pulmonary alveoli. Moreover, it was able to prevent changes in ventilatory parameters caused by DEP, such as airway resistance, tissue resistance, elastance, lung compliance, inspiratory capacity, and the area of the pressure-volume curve. Our findings demonstrated that the implementation of CC in the diet of mice exposed to DEP was responsible for preventing the establishment of histological and functional alterations in the respiratory system caused by these vehicular pollutants.

Keywords: Pollution. Diesel exhaust particles. *Caryocar coriaceum*. Fatty acids. Respiratory system.

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1 Introduction

Species of the genus *Caryocar* Linnaeus, 1771 are popularly known throughout all regions of Brazil by the name "pequi" (in English, souari nut). In the northernmost region of the Brazilian Northeast, the species *Caryocar coriaceum* Wittmack, 1886 is primarily identified, where its fruit is an indispensable product in the diet of the populations living around the areas where this species occurs (OLIVEIRA et al. 2008; MACIEL et al., 2018).

From the fruit pulp, an oil is extracted that, besides being used in industry and cooking, is empirically used as a medicinal product in the treatment of bronchitis, flu, and colds, among other ailments (RIBEIRO et al., 2014). Its antioxidant and anti-inflammatory properties can be explained by its high content of potentially bioactive substances, such as flavonoids and essential fatty acids (ALVES et al., 2017), which makes this compound attractive for scientific investigation regarding its potential use in the prevention and treatment of respiratory diseases.

Various factors can influence the incidence and exacerbation of these diseases, such as smoking and exposure to air pollution. According to the World Health Organization, one of the main pollutants that pose the greatest risk to human health is particulate matter (PM), which primarily comes from stationary sources, such as industries, or mobile sources, such as motor vehicles (WHO, 2016).

Recent studies link exposure to diesel exhaust particles (DEP) with the development of asthma (KHREIS; DE HOOGH; NIEUWENHUIJSEN, 2018), respiratory system cancer (RIBEIRO et al., 2019), chronic obstructive pulmonary disease (HENDRYX et al., 2019), oxidative stress, and acute pulmonary inflammation (CATTANI-CAVALIERI et al., 2019), along with significant changes in the ventilatory mechanics of the respiratory system (GONDIM; SERRA; CAVALCANTE, 2019).

Due to its aforementioned antioxidant and anti-inflammatory properties, it is believed that the regular inclusion of pequi oil in the diet may help reduce the inflammatory response caused by exposure to vehicle-derived particulate matter (AVES et al., 2017; SERRA et al., 2020; ALMEIDA-BEZERRA et al., 2022).

Given the presented context, the current study investigates the benefits of oral ingestion of fixed oil from *Caryocar coriaceum* (CC) as a preventive tool for respiratory diseases in mice exposed to particles emitted from diesel combustion. To this end, we investigated the effects of CC on in vivo respiratory mechanics and lung histopathology.

2 Material and Methods

Plant material

The CC was commercially acquired in July 2021 at the São Sebastião Market, a local market in Fortaleza, Ceará, Brazil. The species *Caryocar coriaceum* occurs in regions that include the states of Bahia, Goiás, Maranhão, Pernambuco, Piauí, Tocantins, and Ceará (BICALHO; AMORIM; 2021).

Analysis of *Caryocar coriaceum* fixed oil
Esterification reaction

For the esterification reaction, the IUPAC (International Union of Pure and Applied Chemistry) method was adopted (THOMPSON; ELLISON; WOOD, 2006)). In a 50 mL flask, 100 mg of the sample was added, solubilized in 3 mL of n-hexane and 0.2 mL of KOH 0.2N. The solution was vigorously shaken for 30 s. To finish, 4 mL of saturated NaCl solution was added, creating two phases. The solution was transferred to a separation funnel where the phases were separated. The organic phase containing the methyl esters was used for chromatographic analysis.

Analysis by gas chromatography coupled to mass spectrometry

Samples of CC were analyzed to identify their components. The fatty acid content was initially determined by adding a 0.1 mL aliquot of CC into a gas chromatograph coupled with a mass spectrometer (Shimadzu, model QP 2010), equipped with an Equity-tm5 column. Helium gas was used as the carrier gas with a flow rate of 1 mL/min, and the analysis was conducted in split mode at a ratio of 1:10 (ADAMS, 2017).

The injector temperature was set at 220°C. The temperature program started with an initial column temperature of 60°C, increasing at a rate of 3°C/min up to 240°C. The analysis time was 62 minutes. The mass spectrometer operated in electron ionization mode at 70 eV with a temperature of 240°C. Compound identification was performed using the mass spectra library database of NIST/EPA/NIH, comparing the results with those obtained from the sample, taking into account the fragmentation pattern of the sample and the similarity index between the analyzed spectra.

Preparation of solutions for intranasal instillation

The diesel particulate matter used in this study was collected from a vehicle belonging to the public transport fleet in the city of Fortaleza (state of Ceará, Northeast Brazil): the Mercedes-Benz™ OM-924 bus, operating under the E5 emissions standard, with electronic fuel injection control, operating with 10-ppm sulfur diesel fuel.

For DEP collection, glass fiber filters (20.3 cm wide and 24.4 cm long) were used in a high-volume air sampler (Hi-Vol 3000-Ecotec®) attached to the vehicles' exhaust pipes by means of a flexible aluminium tube. Data collection was performed with non-moving vehicles kept with the engine running under 2500 rpm during the sampling. Collections occured under the current national legislation for particulate matter collection, according to rules NBR 13412 (ABNT, 1995) and 9547 (ABNT, 1997).

Prior to DEP collection, clean glass fiber filters were heated in a thermal oven at 50°C during 24h for desiccation, and then subsequently weighed. The dried filters were then placed in the Hi-Vol sampler, and collection was carried out as previously mentioned. The filters were then again placed in the thermal oven at 50°C for another 24h, after which they were once again weighed. They were then cut into smaller pieces (10cm x 10cm) and placed in a beaker containing saline solution (0.9% NaCl) for sonication during 8h (QUIMIS® - Q3350).

After sonication, the filters were again placed in the thermal oven for 24h at 50° C and then weighed. Efficiency of DEP extraction was calculated considering the difference between filter masses before and after the collection process. The final particle to volume ratio (µg:µL) adopted in the study was 1:6 (1 µg:6 µL). The solution used for intranasal instillation in the test animals was 5 µg DEP dispersed in 30 µL saline. A second solution was obtained by sonication of small pieces of clean glass fiber filters in saline; this solution was then used for intranasal instillation in the groups not exposed to DEP (Figure 1).

Exposure and treatment protocols

All animals received humane care, and the experiments complied with the following guidelines: National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1985); and the National Council for Controlling Animal Experimentation of the Ministry of Science, Technology, and Innovation (CONCEA, 2013), Brazil. This study was approved by the Ethics Committee on the Use of Animals of the State University of Ceará (Protocol No. 0681311/2018). Invasive procedures were performed under anaesthesia (see below) and every effort was made to minimize suffering.

We used 32 animals randomly divided into four groups. In the first group (n=8), the animals received a daily oral treatment with 0.5 mL of vehicle (Tween-80 solution [1%]) followed by intranasal instillation of 30 µL of sonicated saline solution along with clean filters for 20 days (TS group). In the second group (n=8), the animals received a daily oral treatment with 0.5 mL of fixed oil from *Caryocar coriaceum* followed by intranasal instillation of 30 µL of sonicated saline solution along with clean filters for 20 days (CCS group).

In the third group (n=8), the animals received a daily oral treatment with 0.5 mL of vehicle followed by intranasal instillation of 5 µg of DEP in 30 µL of saline solution for 20 days (TD group). In the fourth group (n=8), the animals received a daily oral treatment with 0.5 mL of fixed oil from *Caryocar coriaceum* followed by intranasal instillation of 5 µg of DEP in 30 µL of saline solution for 20 days (OD group).

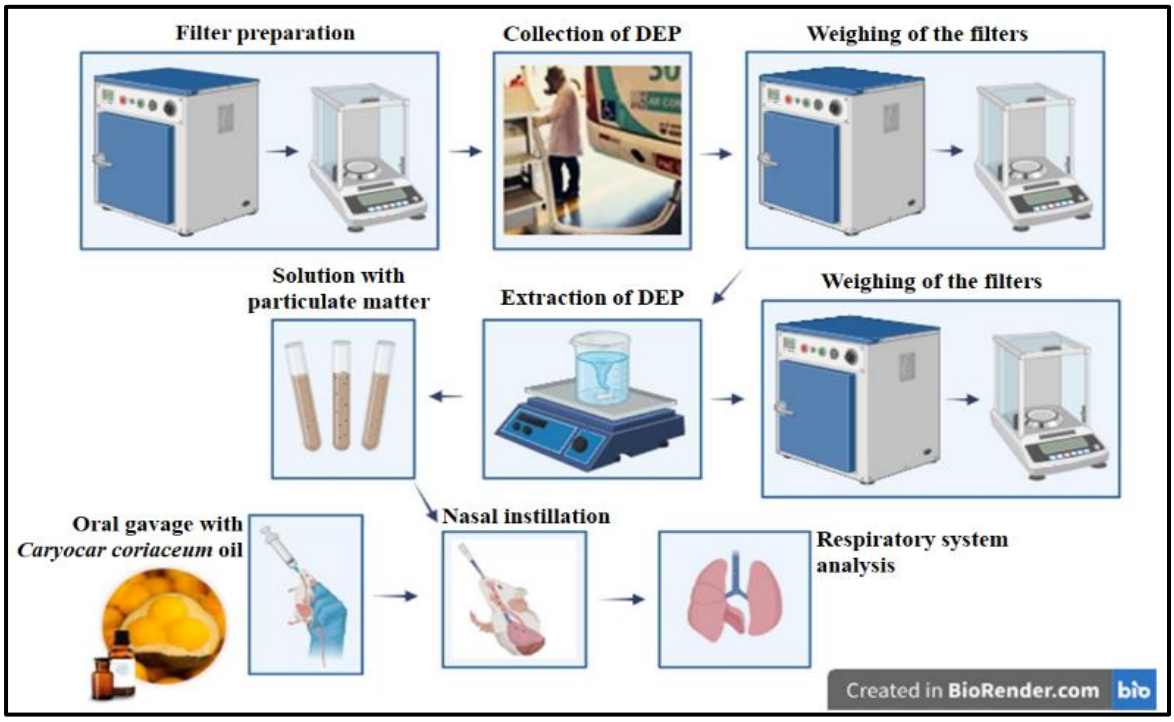

Figure 1. Methodologies for collection, preparation and exposure to DEP. Created with BioRender.com.

The intranasal instillation of 5 µg of DEP in 30 µL of saline solution simulates 24 hours of pollutant exposure in an urban area (Av. Treze de Maio - Benfica) with heavy vehicle traffic in the city of Fortaleza (LIMA, 2015). All analyses were performed 24 hours after the last instillation (day 21).

Respiratory System Mechanics

For the analysis of respiratory system mechanics after the treatment and exposure period, the animals were anesthetized (ketamine-100:10 mg/kg), tracheostomized, intubated (14-gauge cannula), and then connected to a computer-controlled mechanical ventilator (Scireq©-flexiVent®, Montreal, QC, Canada) for small animals. The animals were ventilated with initial settings and paralyzed with pancuronium bromide (0.5 mL/kg, i.p., Cristália, Brazil).

Immediately after a period of acclimation with the animals connected to the ventilator, respiratory system impedance (Zrs) was measured using the forced oscillation technique (HANTOS et al., 1992), with 12 sequential sampling intervals of 30 seconds each, totalling 6 minutes. Through the forced oscillation technique, we obtained data on Newtonian resistance (R_N), elastance (*H*) and tissue resistance (*G*). Next, two pressure-volume (PV) curves were obtained to measure static compliance C_{ST}, an estimate of inspiratory capacity (*IC*), and the area under the PV area.

Histological study

All procedures for histological analysis were previously reported (GONDIM; SERRA; CAVALCANTE, 2019). Immediately after determining respiratory system mechanics, the lungs were perfused with saline solution, then removed *en bloc* and kept at functional residual capacity, and fixed in Millonig's formaldehyde (100 mL HCHO, 900 mL H_2O, 18.6 g NaH_2PO_4, 4.2 g NaOH).

Slides containing lung sections were stained with hematoxylin and eosin (HE) and examined by optical microscopy. Quantitative analysis was performed using the number of polymorphonuclear cells (PMN), determined by the point-counting technique (WEIBEL et al., 1990). The bronchoconstriction index (BCI) was determined by counting the number of points in the airway lumen (NP) and the interceptions through the airway wall (NI), using a reticle and applying the equation BCI=NI/∫NP (SAKAE et al., 1994).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 7.00 (GraphPad, 2024).

Results are presented as mean ± SD, where *n* represents the number of samples. We conducted the Shapiro-Wilk normality test and the Levene's homogeneity of variance test to verify whether the data have a normal distribution and homogeneous variances, respectively. The results of the Shapiro-Wilk test indicated that the data distribution did not deviate significantly from normality ($p > 0.05$). Similarly, the Levene's test indicated that the group variances were homogeneous ($p > 0.05$). For comparison among groups, we used one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. A difference was considered significant if $p < 0.05$.

3 Results

The percentage composition of the CC obtained by gas chromatography/mass spectrometry is presented in Table 1, where the most representative value was found for linoleic acid (49.13%).

Table 1. Percentage composition of CC obtained by gas chromatography/mass spectrometry.

Compound	Molecular Mass	Yield %
Palmitic acid ($C_{17}H_{34}O_2$)	270	17.22
Linnoleic acid ($C_{19}H_{34}O_2$)	294	49.13
Elaidic acid ($C_{19}H_{36}O_2$)	296	29.13
Linoleic acid chloride ($C_{18}H_{31}ClO$)	298	0.71
Stearic acid ($C_{19}H_{38}O_2$)	298	3.81

Pequi oil was able to prevent tissue (Figure 2) and ventilatory (Figures 3 and 4) alterations in mice caused by exposure to DEP, as reflected by the absence of any significant difference between the TS and CCD groups in all cases.

Figure 2 presents data on the infiltrate of polymorphonuclear cells (PMN) and the bronchoconstriction index (BCI), showing an increase in the number of pro-inflammatory cells in the TD group (22.20±6.47) compared to the TS group (13.86±4.09), and an increase in BCI in the TD group (2.94±0.72) compared to the TS group (1.92±0.38). Similarly, it was identified that the group pre-treated with pequi oil and exposed to DEP was able to prevent the increase in BCI and PMN cell infiltrate, as no statistical difference was observed between the TS (PMN= 13.86±4.09; BCI= 1.92±0.38) and CCD groups (PMN = 17.83±3.42; BCI= 2.21±0.29).

Additionally, when comparing the TS group with the CCS group, no alterations were observed, confirming that the daily treatment with 0.5 mL of CC was not toxic to the respiratory system.

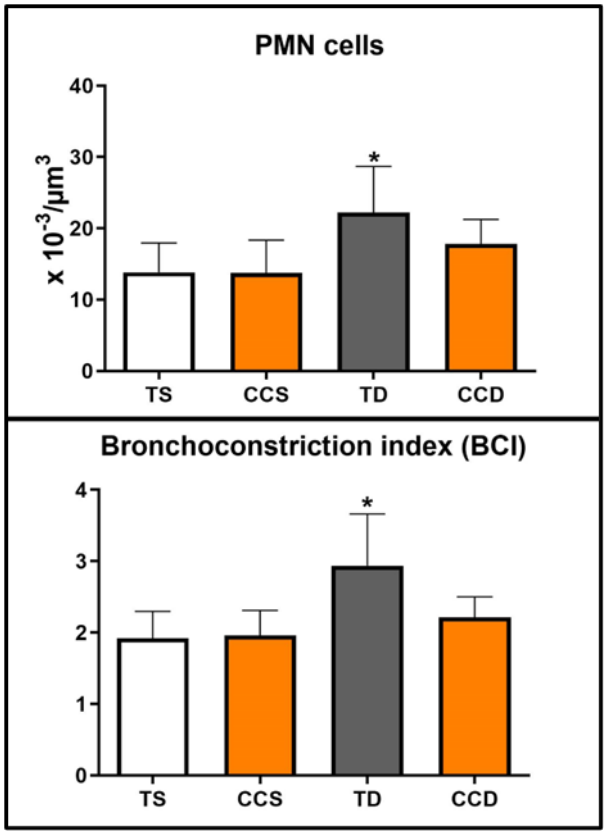

Figure 2. Histological analysis: polymorphonuclear cell count (PMN) and bronchoconstriction index (BCI). Values represented by the mean ± standard deviation of TD, CCS, TD and CCD (8 animals per group). Data were collected in ten corresponding fields per mouse. *Different from the TS group (p<0.05).

Figure 3. (respiratory system impedance) and figure 4 (Pressure-volume curve parameters) show data on the mechanics of the Respiratory System, where it is observed that pre-treatment with pequi oil was able to avoid changes in function ventilation caused by pollution, as identified in the following groups: TS (R_N = 0.137±0.021, G = 2.52±0.42, H = 16.11±1.73, C_{ST} = 0.124±0.010, CI = 1.22±0.14, PV area = 2.31±0.27), CCS (R_N = 0.132±0.020, G = 2.46±0.41, H = 16.98±1.69, C_{ST} = 0.122±0.012, CI = 1.23±0.17, PV area = 2.30±0.22), TD (R_N = 0.251±0.066, G = 4.50±0.82, H = 28.96±3.49, C_{ST} = 0.069±0.018, CI = 0.76±0.17, PV area = 3.82±0.64) e CCD (R_N = 0.186±0.057, G = 2.87±0.61, H = 19.19±2.06, C_{ST} = 0.101±0.017, CI = 1.15±0.25, PV area = 2.77±0.52).

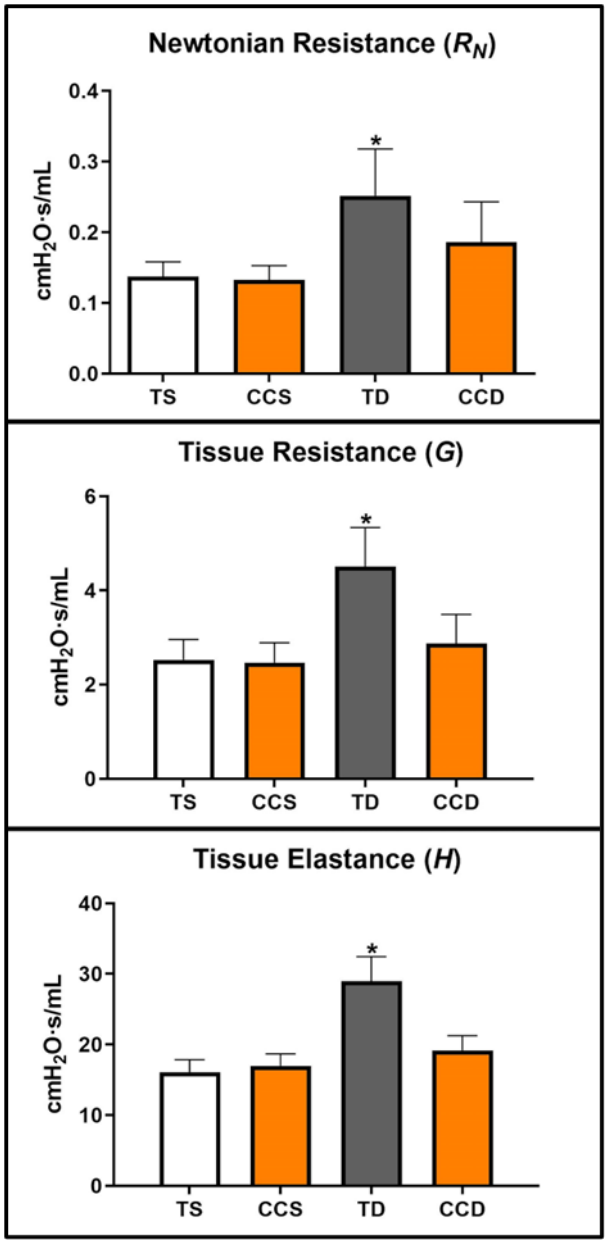

Figure 3. Respiratory system impedance parameters. Values represented by the mean ± standard deviation of TD, CCS, TD and CCD (8 animals per group). Data were collected in ten corresponding fields per mouse. *Different from the TS group (p<0.05).

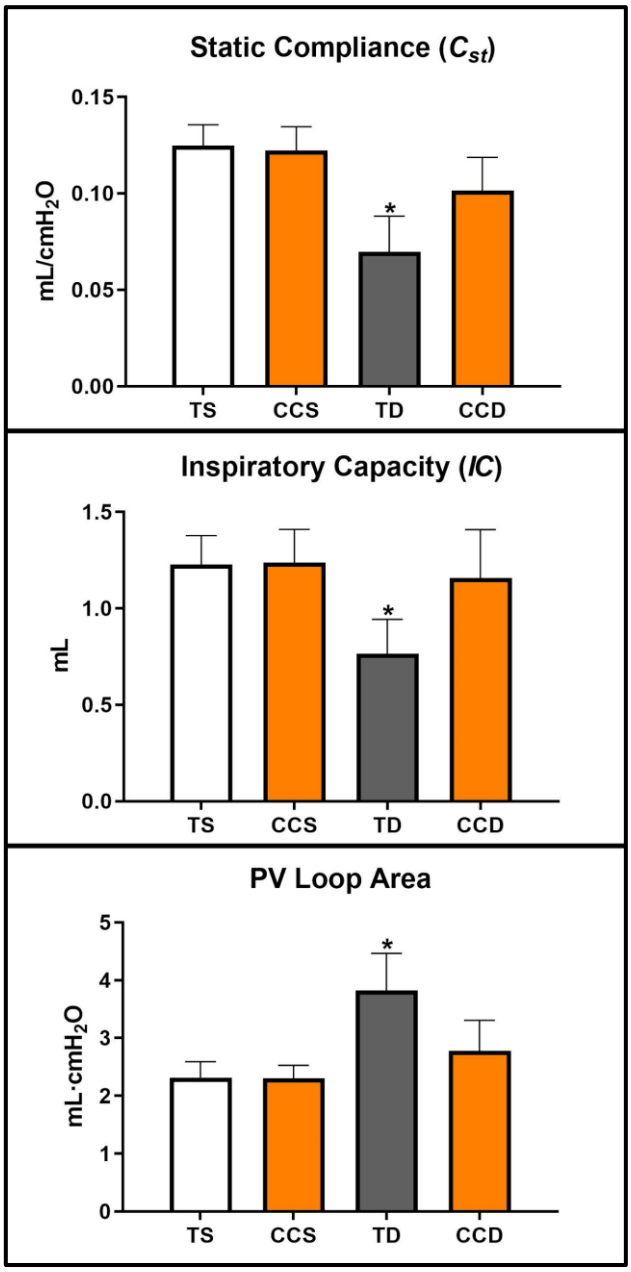

Figure 4. Pressure-volume curve parameters. Values are represented by the mean ± standard deviation of TD, CCS, TD and CCD (8 animals per group). Data were collected in ten corresponding fields per mouse. *Different from the TS group ($p<0.05$).

4 Discussion

In recent years, the study of the relationship between diet, health, and environmental factors has received increasing attention (VOGEL et al., 2020; SERRA et al., 2020; SOUSA et al., 2023).

Besides vehicle emission control policies and the search for cleaner energy alternatives, there is growing interest in complementary strategies to mitigate the negative effects of air pollution on human health, such as the development of particle filtration technologies, reduction of toxicological markers, engine operating conditions and fuel properties (GONDIM et al., 2021; RANA; SAXENA; MAURYA., 2022; ZHANG et al., 2023). In this context, nutrition and the use of plant-derived extracts have emerged as promising research areas.

In the present study, we aimed to verify the percentage composition of fatty acids in the fixed oil of *Caryocar coriaceum* and its potential attenuation of the effects of vehicle-derived DEP exposure on the respiratory system of mice.

In a previous study conducted by our research group ("Laboratório de Biofísica da Respiração", State University of Ceará, Ceará, Brazil), it was demonstrated that DEP, even at concentrations below those recommended by regulatory agencies, can cause morphological and functional alterations in lung tissue (GONDIM et al., 2021). In the present study, similar results are identified, with a notable infiltrate of pro-inflammatory polymorphonuclear cells (PMN) (Figure 2), airway narrowing (Figure 2), as well as changes in pulmonary ventilation parameters, such as compliance (Figure 4) and respiratory system elastance (Figure 3). Concurrently, these alterations were attenuated by the daily introduction of CC in the diet of mice exposed to vehicle-derived pollutants, as discussed further.

The chemical analyses of the fatty acids present in CC are presented in Table 1, where linoleic acid (49.13%), oleic acid (29.13%), and palmitic acid (17.22%) show the most representative values.

The consumption of seeds, nuts, and fixed oils of plant origin, and consequently the ingestion of fatty acids, becomes important as they are a source of essential polyunsaturated fatty acids (PUFAs) that are not synthesized in animals but are precursors of fatty acids important for organic homeostasis. PUFAs are classified as omega-3, omega-6, omega-7, and omega-9 based on the location of the first carbon double bond from the terminal methyl group of the molecule (KAR et al., 2023). Thus, linoleic acid (omega-6), the most prevalent PUFA in the human diet (BLASBALG et al., 2011), has antioxidant activities due to the presence of double bonds in its structure, allowing these molecules to act as potential reducing agents, neutralizing the oxidizing compounds present in DEP, which can cause oxidative damage and pulmonary inflammatory processes (HIURA et al., 1999; GONDIM et al., 2021; RANA; SAXENA; MAURYA., 2022).

In the present study, the results of pulmonary histological analyses revealed that the implementation of CC in the mice's diet for 8 weeks prevented tissue alterations, such as the influx of PMN and the bronchoconstriction index (BCI), induced by exposure to vehicular particles, as indicated by the absence of a significant difference between the TS and CCD groups (Figure 2).

PUFAs can regulate the gene expression of cytokines and pro-inflammatory mediators through the NF-κB pathway, which controls the expression of genes involved in the inflammatory response (CALDER, 2013; HERRERA-VIELMA et al., 2021; HARWOOD, 2023; KAR et al., 2023).

These interactions may explain the reduction in the influx of pro-inflammatory cells. Concurrently, regarding the beneficial effects observed in the BCI, PUFAs can also interact with plasma membrane proteins, influencing their fluidity and positively impacting the activity of receptors and ion channels (CALDER, 2015), whose functions are closely related to airway smooth muscle contractility.

Changes in tissue composition and architecture can disrupt biochemical and biomechanical processes, affecting the ability to perform specific functions. Thus, tissue deformations can reduce lung compliance and expandability during inspiration (LEDERER; MARTINEZ, 2018). Similarly, we identified a reduction in C_{ST} and IC and an increase in the area under the PV curve in the group exposed to DEP (TD), a phenomenon representing an increase in collapsed areas in the lungs.

In a study conducted by Zin et al. (2012), mice were subjected to a single exposure to particles from diesel vehicle combustion, which was sufficient to cause a significant increase in airway resistance (R_N), elastance (*H*) and tissue resistance (*G*) in the group exposed to DEP. The evaluation of respiratory mechanics through the forced oscillation technique in the present study demonstrated that the variables R_N, *H* and *G* also showed a significant increase when comparing the TD group with the TS group.

The increase in *G* and *H* (Figure 3), indicates stiffening of the lung tissue, presumably due to the increased infiltrate of PMN cells (Figure 2) and inflammatory process (ZIN et al., 2012; GONDIM; SERRA; CAVALCANTE, 2019), which justifies the decrease in IC (WAGERS et al., 2002).

Additionally, the increase in R_N values, as a response to airway narrowing, promotes the closure of small airways and subsequent distortion of the lung parenchyma (WAGERS et al., 2004), influencing tissue parameters (*G* and *H*), resulting in an effectively smaller lung.

In contrast, the group exposed to vehicular pollutants and pre-treated with CC did not show alterations in any of the above pulmonary mechanics parameters, as evidenced by the absence of a statistical difference between the CCD and TS groups (Figures 3 and 4).

This finding, which indicates the prevention of functional alterations, corroborates the results of the histological analyses, as we found that CC prevented a significant increase in the infiltrate of pro-inflammatory cells and an increase in the bronchoconstriction index.

5 Conclusions

In the present study, we demonstrated that exposure to particulate matter from diesel combustion, even at concentrations below the limits recommended by regulatory agencies, can cause pulmonary alterations at the tissue and functional levels.

However, by adding pequi oil to the diet of exposed mice, it was possible to prevent the establishment of histological and functional alterations in the respiratory system of the mice. These preclinical results suggest a future clinical investigation into the efficacy and safety of pequi oil as a dietary intervention to protect human respiratory health in polluted urban environments.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Fladimir de Lima Gondim: Investigation: Conducting the research and investigation process, specifically carrying out the experiments; Copywriting - Original Draft: Preparation, creation of the published work, writing specifically the initial draft; Writing - Proofreading and Editing: Critical review.
Marcelle Ferreira Moura; Allison Matias de Sousa; Ruth Mesquita Ferreira; Gilvan Ribeiro dos Santos; Ana Raquel Rodrigues de Oliveira: Investigation: Conducting the research and investigation process, specifically carrying out the experiments.
João Henrique Silva Luciano; Daniel Silveira Serra: Formal analysis: Application of statistical, mathematical, computational and other formal techniques to analyze or synthesize study data; Writing - Proofreading and Editing: Critical review.
Francisco Sales Ávila Cavalcante; Mona Lisa Moura de Oliveira; Antônia Torres Ávila Pimenta: Definition of terms: Conceptualization ideas; inclusion or evolution of comprehensive research objectives and goals; Methodology: Development or design of methodology; creating models.

DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this study.

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