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Effects of *Euterpe oleracea* Mart. (AÇAÍ) extract in acute lung inflammation induced by cigarette smoke in the mouse

Roberto Soares de Moura^{a,1}, Thiago Santos Ferreira^{c,1}, Alan Aguiar Lopes^b, Karla Maria Pereira Pires^b, Renata Tiscoski Nesi^c, Angela Castro Resende^a, Pergentino Jose Cunha Souza^d, Antônio Jorge Ribeiro da Silva^e, Ricardo Moreira Borges^e, Luis Cristóvão Porto^b, Samuel Santos Valenca^{c,*}

^a Departamento de Farmacologia e Psicobiologia – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

^b Programa de Pós-graduação em Biologia Humana e Experimental - Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

^c Programa de Pós-graduação em Farmacologia e Química Medicinal, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

^d Escola de Farmácia – Universidade Federal do Pará, Belém, Brazil

^e Núcleo de Pesquisa de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

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ABSTRACT

Short term inhalation of cigarette smoke (CS) induces significant lung inflammation due to an imbalance of oxidant/antioxidant mechanisms. Açai fruit (*Euterpe oleracea*) has significant antioxidant and anti-inflammatory actions. The present study aimed to determine whether oral administration of an açai stone extract (ASE) could reduce lung inflammation induced by CS. Thirty C57BL/6 mice were assigned to three groups ($n=10$ each): the Control + A group was exposed to ambient air and treated orally with ASE 300 mg/kg/day; the CS group was exposed to smoke from 6 cigarettes per day for 5 days; and the CS + A group was exposed to smoke from 6 cigarettes per day for 5 days and treated orally with ASE (300 mg/kg/day). On day 6, all mice were sacrificed. After bronchoalveolar lavage, the lungs were removed for histological and biochemical analyses. The CS group exhibited increases in alveolar macrophage (AMs) and neutrophil numbers (PMNs), myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase activities (GPx), TNF- α expression, and nitrites levels in lung tissue when compared with the control ones ($p < 0.001$ for all parameters). The AMs, PMNs, MPO, SOD, CAT, GPx and nitrite were significantly reduced by oral administration of ASE when compared with CS group ($p < 0.001$ for all parameters, with exception of AMs $p < 0.01$). The present results suggested that systemic administration of an ASE extract could reduce the inflammatory and oxidant actions of CS. Thus, the results of this study in mice should stimulate future studies on ASE as a potential agent to protect against CS-induced inflammation in humans.

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Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of worldwide morbidity and mortality. It is predicted that COPD will rank the third most common cause of death by 2020 (O'Donnell and Parker 2006; Sapey and Stockley 2006). COPD is characterized by a slow, progressive, and largely irreversible limitation in airflow. This condition arises due to chronic bronchitis and/or emphysema and is associated with an abnormal

inflammatory response of the lungs. Cigarette smoke (CS) is a complex admixture of more than 4700 chemical compounds and oxidants (Pryor and Stone 1993). CS is the major etiologic factor in the pathogenesis of COPD (Menezes et al. 2005). Although acute lung inflammation (ALI) caused by CS exposure does not model all aspects of COPD pathogenesis, the two processes do share certain hallmarks, including accumulation of inflammatory cells such as alveolar macrophages and neutrophils, and lung oxidative stress (Silva Bezerra et al. 2006).

The presence of CS in lung tissue is responsible for a increases the amount of oxidants in alveolar pockets from two sources; first, CS contains an impressive number of free radicals that are deposited in alveoli; second, CS increases the number of inflammatory cells in alveoli, and these spontaneously release oxidants (MacNee 2005). CS contains oxidants and free radicals both in the gas phase (alkyl, peroxyl, nitric oxide, and superoxide anion) and

* Corresponding author at: LIEC/ICB/CCS/UFRJ, Avenida Carlos Chagas Filho 373, Bloco J/sala25, Ilha do Fundão, CEP 21.941-902, Rio de Janeiro, R.J., Brazil.
Tel.: +55 21 2562 6733; fax: +55 21 2562 6734.

E-mail address: samuelv@ufrj.br (S.S. Valenca).

¹ These authors contributed equally to this paper.

the tar phase (semiquinone). These agents can stimulate alveolar macrophages and neutrophils to produce reactive oxygen species (ROS) and release cytokines that recruit inflammatory cells into the lung (MacNee 2000). The increase of ROS production due to CS can result in an imbalance between oxidants and antioxidants. This imbalance leads to an increase in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) (Valenca et al. 2008b), and myeloperoxidase (MPO) and a simultaneous decrease in the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) (Rahman and Adcock 2006). CS exposure is also associated with an increase in TNF- α (da Hora et al. 2005; Le Quemant et al. 2008; Valenca et al. 2008a; Valenca and Porto 2008), which is known induce the expression of inducible nitric oxide synthase (iNOS) (Moncada and Higgs 1993), which, in turn, increases the nitric oxide (NO) concentration.

The *Euterpe oleracea* Mart., popularly known as “açai”, is widely cultivated in the Amazon region of Brazil. Chemical studies have shown that the açai has a diverse composition of hydroxybenzoic acids, antioxidant polyphenolics, flavan-3-ols, and anthocyanins, predominantly cyanidin 3-*O*-rutinoside and cyanidin 3-*O*-glucuronide (Del Pozo-Insfran et al. 2004; Lichtenthaler et al. 2005; Rodrigues et al. 2006). Others studies have shown that consumption of the açai has various benefits attributed to its high superoxide anion scavenging properties, its anti-inflammatory potential, through the inhibition of cyclooxygenases 1 and 2 (Schauss et al. 2006), its vasodilator effects (Rocha et al. 2007), and its inhibition of NO production and iNOS activity and expression (Matheus et al. 2006). Therefore, the present study was undertaken to determine whether treatment with an açai stone extract could reduce the inflammatory and oxidative activities induced by CS in mice lungs.

Materials and methods

Reagents and animals

Bovine serum albumin (BSA), bromophenol blue, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), eosin, glycerol, hematoxylin, hexadecyltrimethylammonium bromide (HTAB), 2-mercaptoethanol, naphthylethylenediamide dihydrochloride, nicotinamide adenine dinucleotide phosphate (NADPH), oxidized glutathione, phosphoric acid, reduced glutathione, sodium acetate, sodium dodecyl sulfate (SDS), sodium nitrite, sulfanilamide, 3,3',5,5'-tetramethylbenzidine (TMB), triethanolamine, Tris-HCl, Tween 20, and 2-vinylpyridine were purchased from Sigma Chemical (St. Louis, MO, USA). Bradford reagent was purchased from Bio-Rad (Hercules, CA). Ethanol, formalin, hydrogen peroxide, potassium phosphate, and sodium chloride (NaCl) were purchased from Vetec (Duque de Caxias, Brazil). Eight-week-old, male, C57BL/6 mice (18–22 g) were purchased from the Instituto de Veterinária – Universidade Federal Fluminense (Niterói, Brazil). The mice were fed Purina chow and allowed unrestricted access to water in a controlled environment maintained at 18–22 °C, 50–70% relative humidity, and a 12-h light/dark cycle. Mice were allowed to acclimate for two weeks prior to experimental procedures.

Preparation of extract from *E. oleracea* (açai)

E. oleracea Mart. fruits were obtained from the Amazon Bay (Belém do Pará, Pará, Brazil) excicata number 29052 Museu Goeldi—Belem do Para. Hydroalcoholic extracts were obtained from a decoction of the stone. Approximately 200 g of açai stone were boiled in 400 ml of water for 5 min, mixed for 2 min, and then boiled again for 5 min. The decoction was cooled to room temperature and then extracted by addition of 400 ml of ethanol with shaking for 2 h. The extract was stored in dark bottles inside

a refrigerator (4 °C) for 10 days. After this maceration period, the hydroalcoholic extracts of açai were filtered through #1 Whatman filter paper, and the ethanol was evaporated using a rotary evaporator (Fisatom Equipamentos Científicos Ltda São Paulo, São Paulo, Brazil) under low pressure at 55 °C. The extract was then lyophilized (LIOTOP model 202, Fisatom Equipamentos Científicos Ltda São Paulo, São Paulo, Brazil) with temperature from –30 to –40 °C and vacuum of 200 mm Hg and frozen at –20 °C until use. Typically 100 g of stone yielded approximately 5 g of lyophilized extract. ASE was analyzed on a RP-18 column (250 mm \times 4 mm, 5 μ m particles) according to a procedure reported by Peng et al. (2001). Elution was made with solvents A [0.2% (v/v) phosphoric acid] and B [82% (v/v) acetonitrile, 0.04% (v/v) phosphoric acid]. Flow rate was 1 ml/min. DAD UV–vis absorption spectra were recorded on-line during HPLC analysis. The HPLC elution profile observed is strongly indicative of the presence of proanthocyanidins (Peng et al. 2001). The peak eluting at 37.2 min corresponds to catechin as confirmed by co injection of a standard and by comparison of the ultraviolet absorption spectrum. The late elution (at 54.7 min) and UV spectrum of the main peak were consistent with the presence of polymeric proanthocyanidins (Fig. 1).

CS exposure and procedures

To study CS effects, 20 C57BL/6 mice, 8-week-old, were exposed to 6 commercial full-flavored Marlboro cigarettes (10 mg tar, 0.9 mg nicotine, and 10 mg monoxide) per day for 5 days with the use of a smoking chamber, as described previously (Lanzetti et al. 2008; Valenca et al. 2008b, 2009). Briefly, animals were placed in the inhalation chamber (40 cm long, 30 cm wide, and 25 cm high), which had been placed inside an exhaust hood. In this condition, the exhaust fan was turned off. A cigarette was coupled to a plastic 60-ml syringe, and puffs of smoke were drawn into the syringe and then expelled into the inhalation chamber. One liter of smoke from each cigarette was aspirated with this syringe (20 puffs of 50 ml each), and each puff was immediately injected into the chamber. The animals were maintained in this smoke-filled air condition (\pm 3%) for 6 min. Then, the cover of the inhalation chamber was removed, and the exhaust fan of the hood was turned on to evacuate the smoke. Smoke was evacuated within 1 min. This exposure to CS was repeated twice (2 \times 6 min) with an exhaust interval of 1 min after each exposure. This procedure was repeated three times per day (morning, noon, and afternoon), which resulted in exposure to the smoke of six cigarettes over 36 min. Each cigarette produced 300 mg/m³ of total particulate matter in the chamber (measured by weighing material collected on Pallflex filters). Carboxyhemoglobin (COHb) levels were measured to confirm that the treatment was not toxic, as described previously (Valenca et al. 2008b).

Male C57BL/6 ($n = 30$) mice were assigned to three groups ($n = 10$ each): the CS-group was exposed to smoke from 6 cigarettes per day for 5 days; the CS + A group was exposed to smoke from 6 cigarettes per day for 5 days and received a daily treatment of 300 mg/kg ASE; and the Control + A group was exposed to ambient, smoke-free air and received a daily treatment of 300 mg/kg ASE. All the ASE treatments were performed by oral gavage once per day. All procedures were carried out in accordance with The Ethics Committee for Experimental Animals Use and Care (CEA) of Instituto de Biologia Roberto Alcântara Gomes/Universidade do Estado do Rio de Janeiro. The CEA follow guidelines from Intramural Animal Care and Use (ACU) program of the National Institutes of Health (NIH).

Bronchoalveolar lavage and lung homogenates

One day after the final ambient air or CS exposure, the mice were sacrificed by cervical displacement. Bronchoalveolar lavage (BAL) was performed in the left lungs of all animals. Briefly, the

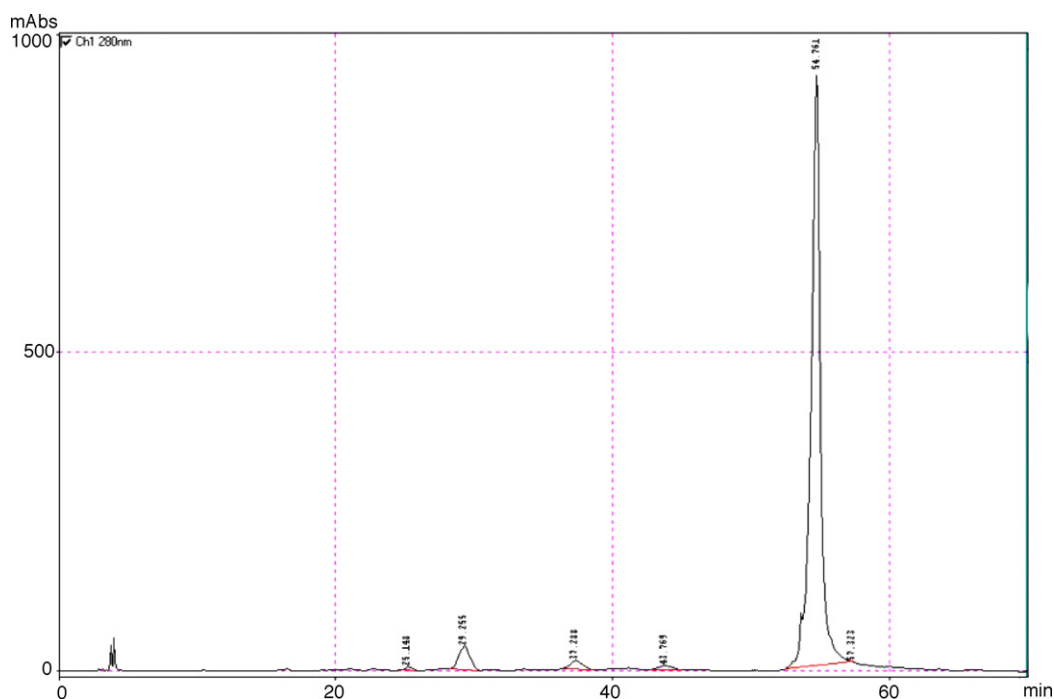


Fig. 1. The HPLC elution profile observed is strongly indicative of the presence of proanthocyanidins. The peak eluting at 37.2 min corresponds to catechin as confirmed by co injection of a standard and by comparison of the ultraviolet absorption spectrum. The late elution (at 54.7 min) and UV spectrum of the main peak are consistent with the presence of polymeric proanthocyanidins. Further evidences on the structure of the compounds present in ASE were provided by positive mode electrospray mass spectrometry. The sodiated ions detected at m/z : 601, 889, 1177, 1965, 1753 and 1893 are indicative of proanthocyanidins with 2, 3, 4, 5, 6 and 7 flavan units.

right lung was clamped, and a cannula was inserted into the trachea. The airspaces were washed with buffered saline solution (500 μ l) three times, and the flow-through (final volume 1.2–1.5 ml) was maintained on ice. Next, the BAL fluid was centrifuged, and the supernatant was collected and stored on ice for subsequent analyses of nitrite and MPO content. Then, the left lungs of each group were removed, and immediately homogenized (Homogenizer NovaTécnica mod NT 136, Piracicaba, Brazil) in 1.0 ml potassium phosphate buffer (pH 7.5). The homogenates were centrifuged at $7000 \times g$ (Centrifuge FANEM mod 243M, São Paulo, Brazil) for 10 min, and the supernatants were stored at -20°C for analyses of SOD, CAT, GPx, the GSH/GSSG ratio, and TNF- α expression. The total protein in the samples was determined by the Bradford method (Bradford, 1976).

Tissue processing and morphometry

The right ventricles of all mice were perfused with saline to remove the blood. Next, the right lungs of all animals were inflated with 4% phosphate buffered formalin (pH 7.2) at 25 cm H_2O pressure for 2 min and then ligated, removed, and weighed. Inflated lungs were fixed for 48 h before embedding in paraffin. Sagittal, 4- μ m serial sections of the right lungs were stained with hematoxylin and eosin (H&E) for histological analyses. Alveolar macrophage and neutrophil numbers were estimated by counting ten different random fields from lung sections present in three different slides. Two blinded investigators performed morphometry by counting coded sections.

Nitrite content

The nitrite levels in BAL fluid were determined by a method based on the Griess reaction (Green et al. 1982). A total of 100 μ l of sample was mixed with 100 μ l of Griess reagent (1% fanilamide in 5% phosphoric acid and 0.1% naphthylendiamide

dihydrochloride in water) and incubated at room temperature for 10 min. The absorbance was measured with a plate reader at 550 nm (Bio-Rad Microplate Reader model 680, Hercules, CA, USA). Nitrite concentrations in the samples were determined from a standard curve, generated by different concentrations of purified sodium nitrite.

Myeloperoxidase activity

The MPO activity in BAL fluid was measured with HTAB, TMB, and hydrogen peroxide (Suzuki et al. 1983). Initially, 100 μ l of sample was centrifuged with 900 μ l of HTAB at $14,000 \times g$ for 15 min. Then, 75 μ l of the supernatant was incubated with 5 μ l of TMB for 5 min at 37°C . Then, 50 μ l of hydrogen peroxide was added, and the mixture was incubated for 10 min at 37°C ; finally, 125 μ l of sodium acetate buffer was added. The reaction was measured with a microplate reader at 630 nm.

Superoxide dismutase, catalase, and glutathione peroxidase activities

SOD, CAT, and GPx activities were determined in lung homogenates. SOD activity was assayed by monitoring the inhibition of adrenaline auto-oxidation measured at 480 nm (Bannister and Calabrese 1987). CAT activity was measured by the rate of decrease in hydrogen peroxide concentrations measured at 240 nm (Aebi 1984). GPx activity was measured by monitoring the oxidation of NADPH at 340 nm in the presence of hydrogen peroxide (Flohe and Gunzler 1984).

Reduced glutathione/oxidized glutathione ratio

The GSH/GSSG ratio was estimated in lung homogenates by first reacting GSH and GSSG with DTNB, and measuring the 2-nitro-5-thiobenzoate (TNB) chromophore produced in the reaction

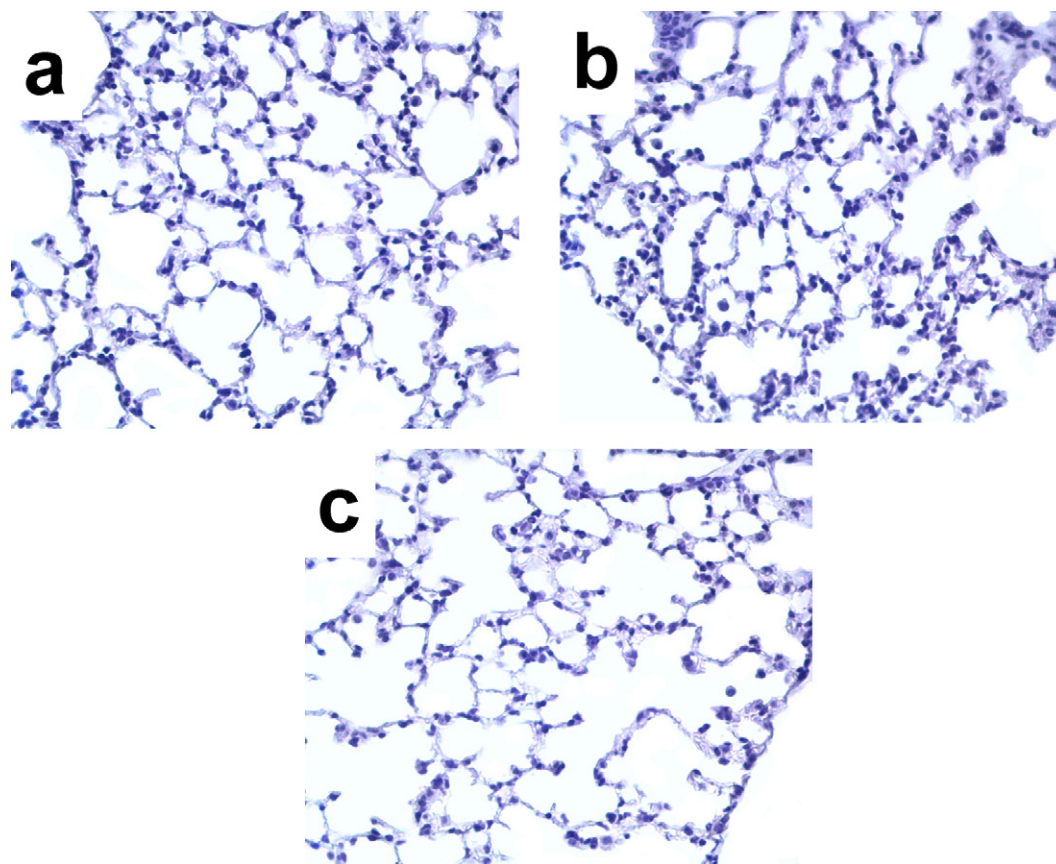


Fig. 2. Photomicrograph of lung sections stained with hematoxylin and eosin (400 \times). (a) Control + A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). (b) CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). (c) CS + A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE).

(Rahman et al. 2006). To determine GSSG, samples were treated with 2-vinylpyridine, which covalently reacts with GSH (but not GSSG). The excess 2-vinylpyridine was neutralized with triethanolamine. Then, the rate of formation of TNB, measured at 412 nm, was proportional to the concentration of GSSG in the sample. GSH or GSSG concentrations in the samples were determined from a standard curve, generated with different concentrations of purified GSH or GSSG.

Western blotting

Samples from lung homogenates were denatured in the sample buffer (50 mM Tris–HCl pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue) and heated in boiling water for 3 min. Samples (50 μ g of total protein) were resolved with 15% SDS polyacrylamide gel electrophoresis and the separated proteins were transferred to nitrocellulose membranes (Amersham Pharmacia Biotech GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). Rainbow markers (Amersham Pharmacia Biotech GE Healthcare Bio-Sciences Corp.) were run in parallel to estimate molecular weights. Membranes were blocked with Tween-TBS (20 mM Tris–HCl, pH 7.5, 500 mM NaCl, 0.5% Tween-20) containing 2% BSA, and incubated with goat anti-mouse primary TNF- α antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After extensive washing in Tween-TBS, the membrane was incubated with biotin-conjugated donkey anti-goat immunoglobulin G (SantaCruz Biotechnology) for 1 h. Then, the blot was developed with the Electrochemiluminescent Western Detection Reagent (Amersham Pharmacia Biotech GE Healthcare Bio-Sciences Corp.), according to the manufacturer's instructions.

Statistical analysis

Values for all measurements are expressed as means \pm SEM. Analyses of nitrite, MPO, SOD, CAT, and GPx were performed with a one way ANOVA, followed by a Tukey post hoc test ($p < 0.05$ was considered significant). Analyses of cell morphometry and GSH/GSSG ratios were performed with the Kruskal–Wallis test, followed by the Dunn's post hoc test ($p < 0.05$ was considered significant). GraphPad Prism software was used to perform the statistical analyses (GraphPad Prism version 5.0, San Diego, CA, USA).

Results

ASE reduced cell migration into the lung

Lung sections were examined by light microscopy to determine whether ASE was protective against the effects of CS. The Control + A group lungs exhibited normal-sized air spaces and thin alveolar septa (Fig. 2a). The CS-group lungs exhibited cells primarily in the air spaces, but no changes were observed in lung histoarchitecture (Fig. 2b). The CS + A group exhibited lung morphology similar to that of the control group (Fig. 2c).

Alveolar macrophages (AMs) and neutrophils (PMNs) were counted in lung sections stained with H&E (Fig. 3). The numbers of AMs and PMNs in the CS-group were significantly higher than those observed in the Control + A group ($p < 0.001$ for both). Remarkably, the numbers of AMs and PMNs in the CS + A group were significantly reduced compared to those observed in the CS-group ($p < 0.001$ for both). However, the number of PMNs in the CS + A group remained

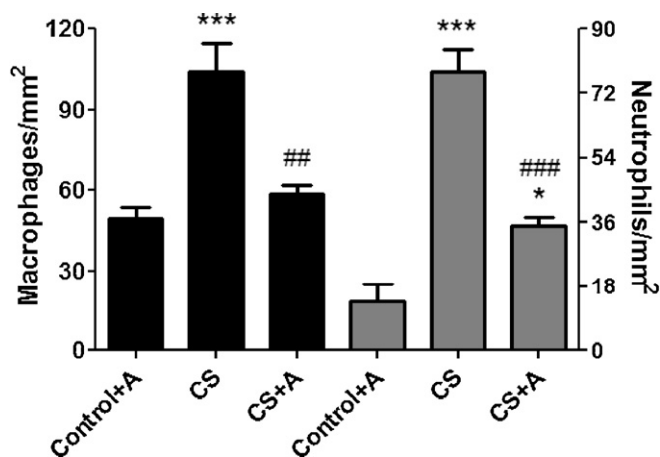


Fig. 3. Morphometry of alveolar macrophages and neutrophils in lung sections. Control+A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS+A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n=7$ for all groups) and were analyzed by Kruskal–Wallis followed with Dunn's post hoc test ($p < 0.05$). *Compared with Control+A group. #Compared with CS group. The symbols in the figure are * $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, *** $p < 0.001$.

significantly higher than that observed in the Control+A group ($p < 0.05$).

Effects of ASE on nitrite levels

The nitrite levels in BAL fluids were higher in the CS-group (Fig. 4) compared to the Control+A group ($p < 0.001$). However, nitrite levels in the CS+A group ($p < 0.001$) were lower than those observed in the CS-group.

ASE reduced myeloperoxidase activity

MPO activity in BAL fluids were higher in the CS-group (Fig. 5) compared to the Control+A group ($p < 0.001$). The MPO activity in the CS+A group was lower than that observed in the CS-group ($p < 0.001$).

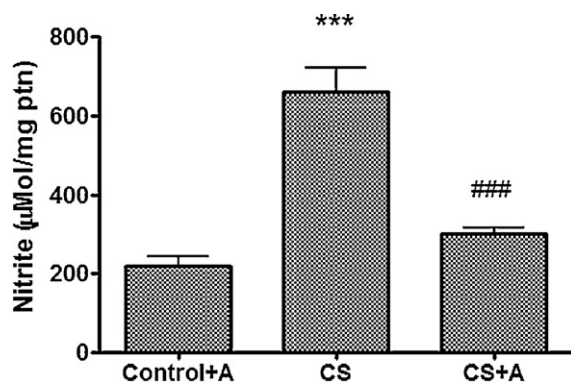


Fig. 4. Effects of ASE on the nitrite levels in bronchoalveolar lavage fluid. Control+A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS+A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n=10$ for all groups) and were analyzed by one-way ANOVA followed with Tukey post hoc test ($p < 0.05$). *Compared with Control+A group. #Compared with CS group. The symbols in the figure are ### $p < 0.001$, *** $p < 0.001$.

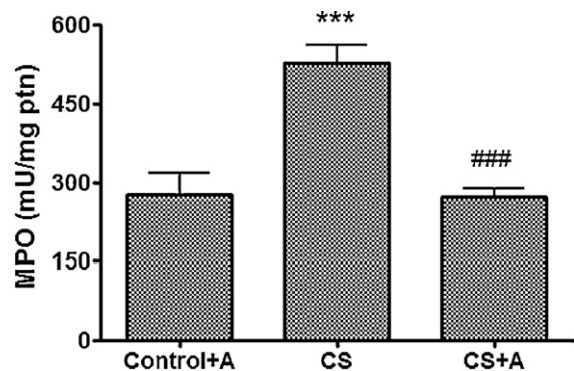


Fig. 5. Effects of ASE on the myeloperoxidase activity in bronchoalveolar lavage fluid. Control+A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS+A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n=8$ for all groups) and were analyzed by one-way ANOVA followed with Tukey post hoc test ($p < 0.05$). *Compared with Control+A group. #Compared with CS group. The symbols in the figure are ### $p < 0.001$, *** $p < 0.001$.

ASE reduced antioxidant enzyme activities

The SOD, CAT, and GPx activities (Figs. 6–8) in lung homogenates were higher in the CS-group compared to the Control+A group ($p < 0.001$). The SOD, CAT, and GPx activities in the CS+A group were lower than those observed in the CS-group ($p < 0.001$).

ASE increased the GSH/GSSG ratio

The GSH/GSSG ratio was slightly reduced in the CS-group compared to the Control+A group, but the difference was not significant (Fig. 9). However, the GSH/GSSG ratio in the CS+A group was significantly higher ($p < 0.05$) than that observed in the CS-group.

ASE reduced TNF- α expression

TNF- α expression was increased in the CS-group compared to the Control+A group (Fig. 10). The CS+A group exhibited reduced TNF- α expression compared to the CS-group.

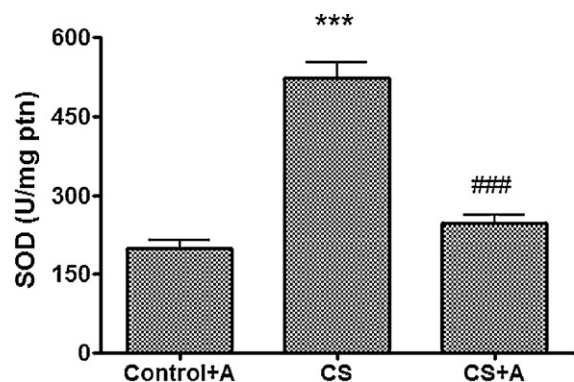


Fig. 6. Effects of ASE on superoxide dismutase activity in lung homogenates. Control+A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS+A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n=10$ for all groups) and were analyzed by one-way ANOVA followed with Tukey post hoc test ($p < 0.05$). *Compared with Control+A group. #Compared with CS group. The symbols in the figure are ### $p < 0.001$, *** $p < 0.001$.

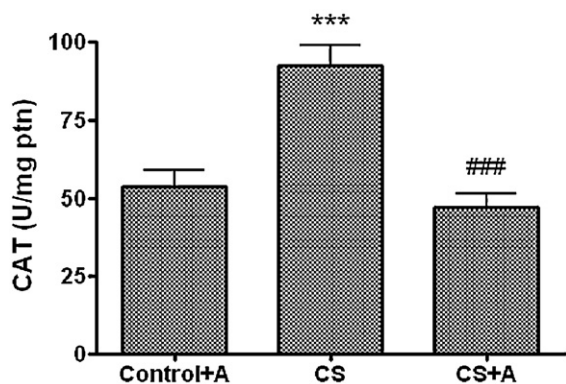


Fig. 7. Effects of ASE on catalase activity in lung homogenates. Control + A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS + A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n = 10$ for all groups) and were analyzed by one-way ANOVA followed with Tukey post hoc test ($p < 0.05$). *Compared with Control + A group. #Compared with CS group. The symbols in the figure are ### $p < 0.001$, *** $p < 0.001$.

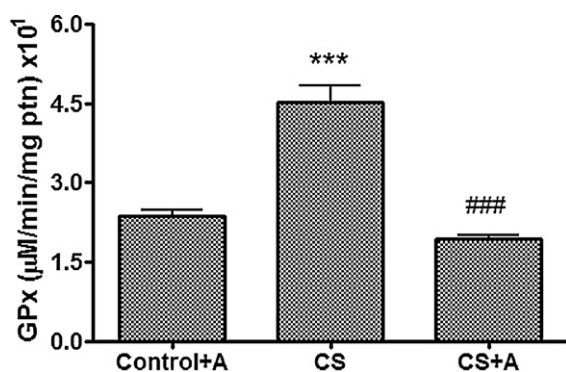


Fig. 8. Effects of ASE on glutathione peroxidase activity in lung homogenates. Control + A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS + A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n = 10$ for all groups) and were analyzed by one-way ANOVA followed with Tukey post hoc test ($p < 0.05$). *Compared with Control + A group. #Compared with CS group. The symbols in the figure are ### $p < 0.001$, *** $p < 0.001$.

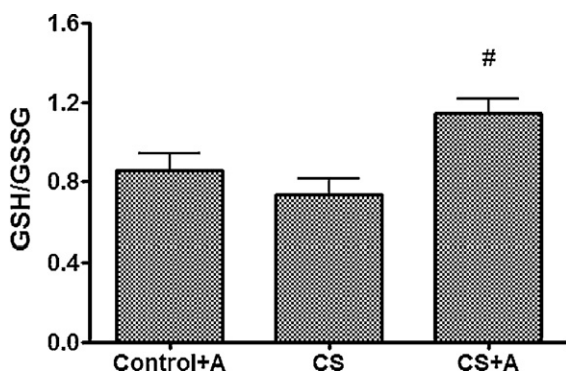


Fig. 9. Effects of ASE on GSH/GSSG ratio in lung homogenates. Control + A group (mice exposed to ambient air plus daily treatment with 300 mg/kg of ASE). CS group (mice exposed to six cigarettes per day for 5 days and treated with vehicle). CS + A group (mice exposed to six cigarettes per day for 5 days plus daily treatment with 300 mg/kg of ASE). Data are expressed as mean \pm SEM ($n = 7$ for all groups) and were analyzed by Kruskal–Wallis followed with Dunn's post hoc test ($p < 0.05$). #Compared with CS group. The symbol in the figure is # $p < 0.05$.

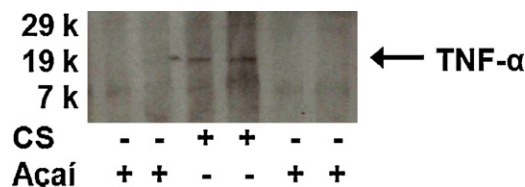


Fig. 10. Effects of ASE on TNF- α expression. Negative signal (–) indicates ambient air exposure and/or vehicle. Positive signal (+) indicates cigarette smoke exposure and/or ASE treatment.

Discussion

This study demonstrated for the first time that *E. oleracea* extract treatment significantly reduced pulmonary inflammation and oxidative stress markers. Previous studies have shown that antioxidants such as black tea, mate tea, vitamin C and vitamin E can mitigate the deleterious effects of CS in the lungs (Banerjee et al. 2007; Lanzetti et al. 2008; Panda et al. 2000; Silva Bezerra et al. 2006). Moreover, these studies used oral administration of antioxidants, as was done here.

The inflammatory response induced by CS includes the recruitment of inflammatory cells, primarily AMs and PMNs, which release proteolytic enzymes that lead to tissue destruction. TNF- α is also an important inflammatory mediator in diseases like COPD and acute respiratory distress syndrome (Le Quement et al. 2008). TNF- α is a pleiotropic cytokine shown to activate endothelial cells and neutrophils (Lauterbach et al. 2008). Previous analyses of leukocyte recruitment have most often been conducted several hours following intrascrotal injections of TNF and/or other inflammatory mediators. This leads to endothelial activation and adhesion receptor and chemokine expression that promote robust leukocyte–vessel wall interactions (McIntyre et al. 2003), which precludes the analyses of the role of TNF priming of leukocytes recruitment. Thus, a reduction of adhesion molecules may directly contribute to a reduction in leukocyte recruitment to the lungs of mice. In this study was found a reduction in both AMs and PMNs in the CS + A group compared to the CS-group. TNF- α expression was also reduced in the CS + A group compared to the CS-group. We suggested that ASE may reduce the expression of TNF- α and consequently, adhesion molecule expression. This, in turn, would reduce macrophage and PMN migration into the lung. Although the mechanism of action of ASE was not investigated in this study, could be speculate that the polyphenol compounds and proanthocyanidins could be responsible for reducing the expression of adhesion molecules, and consequently, lowering the inflammatory cell influx. The relationship between polyphenols and proanthocyanidins on the reduction of adhesion molecules was first demonstrated by Dell'Agli et al. (2004) and Garbacki et al. (2005), despite differences in experimental models of these studies compared to that presented here.

Eisenhut showed that NO contributed to toxicant-induced lung inflammation and injury (Eisenhut 2007). Moncada and Higgs showed that, under pathological conditions, NO production occurred after the induction and expression of iNOS in response to agents like TNF- α (Moncada and Higgs 1993). Those results were consistent with the reduced nitrite levels found in the CS + A group compared to the CS-group. Our results also corroborated other studies that showed that *Euterpe oleracea* Mart. extract was able to reduce the levels of NO and inhibit iNOS activity and expression (Matheus et al. 2006). Moreover, our finding that TNF- α expression was reduced concomitant with nitrite levels in the CS + A group was consistent with previous work (Moncada and Higgs 1993; Matheus et al. 2006).

MPO, the most abundant constituent within PMNs (Suzuki et al. 1983), had reduced activity in the CS+A group compared to the CS-group, consistent with the reduction in PMN numbers observed with morphometry. CS is also associated with increased MPO content in neutrophils and increased production of highly potent compounds, including hypochlorous acid and other ROS, which further increases the oxidative stress in lung.

SOD, CAT, and GPx are important antioxidant enzymes. SODs protect the lung against oxidative stress by reducing (O_2^-) to hydrogen peroxide, which is then converted to water by CAT or GPx (Pryor and Stone 1993). Surprisingly, was observed increases in SOD, CAT, and GPx in the CS-group. Because cigarette smoke contains many oxidants, and it still stimulates leukocytes to produce oxidants, we expected a reduction of enzymatic antioxidants (SOD, CAT and GPx). However, what we found was exactly the opposite—an increase of enzymatic antioxidants (SOD, CAT and GPx). Our hypothesis postulates that this reflected a disturbance in the balance between oxidants and antioxidants (Rahman and Adcock 2006), where this experimental model of ALI, the increase of oxidants (ROS) is proportional and parallel to an increase in its substrate, in this case SOD, CAT and GPx. In the CS+A group, the levels of SOD, CAT, and GPx were similar to controls, which probably reflected the maintenance of balance between oxidants and antioxidants. Previous studies have shown that açai extract had SOD-like activity and CAT-like activities (Lichtenthaler et al. 2005; Rodrigues et al. 2006; Schauss et al. 2006). On the other hand, did not was investigate whether ASE scavenged the excess ROS induced by CS or whether ASE normalized the production, release, and activity of SOD, CAT, and GPx.

Finally, the GSH/GSSG ratio is an important indicator of oxidative stress (Rahman et al. 2006). It was observed an increase in the GSH/GSSG ratio in the CS+A group compared to the CS-group. The reduction of the GSH/GSSG ratio found in the CS-group, though not statistically significant, reflected a tendency for increased oxidation of the thiol-containing molecule, GSH. On the other hand, the increased GSH/GSSG ratio in the CS+A group suggested that ASE prevented thiol radical oxidation. These data are corroborated with the reduced GPx activity found in the CS+A group compared to the CS-group. Therefore, ASE appeared to reduce the activity of GPx, which directly influenced the GSH/GSSG ratio. This finding indicated that ASE modulated the glutathione system.

Our results demonstrated that ASE treatment attenuated ALI induced by CS in mice. ALI is not emphysema, but displays the primary hallmarks of emphysema, including an inflammatory response and oxidative stress. ASE also reduced oxidative stress and TNF- α expression. More studies on the ASE mechanism of action are needed to clarify the antioxidant effects found in this study. In addition, it will be important to assess the potential of ASE treatment in a model of long-term CS exposure. The results of this study in mice should stimulate future studies on ASE as a potential agent to protect against CS-induced inflammation in humans.

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