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Anti-inflammatory actions of herbal medicines in a model of chronic obstructive pulmonary disease induced by cigarette smoke

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ABSTRACT

The effects of four medicinal herbs (*Arctium lappa, Plantago major, Mikania glomerata Spreng and Equisetum arvense*) with anti-inflammatory properties were evaluated in a chronic obstructive pulmonary disease (COPD) model. Wistar rats were exposed to cigarette smoke during 8 weeks and one of the groups was orally given a solution containing 4% of each alcoholic herbal extracts during the exposure period. Control group was not exposed to smoke or treated. Histopathological, immunohistochemical and biochemical analyzes were performed. Normal blood plasma levels of gamma glutamyl transferase indicated no toxicity of the administered herbal extracts. The treatment reduced leukocytes influx in bronchoalveolar lavage, mast cell and macrophages numbers in lungs, as well as prevented pulmonary congestion and tracheal metaplasia. Herbal mixture also decreased plasma inflammatory mediator levels and pulmonary expression of annexin A1 and nuclear factor-kβ. Our data indicate synergistic and protective effects of the used herbal medicines in animals exposed to cigarette smoke as a potential therapeutic strategy.

1. Introduction

The smoking habit is an important global health problem predisposing to the development of several cardiovascular and respiratory diseases [1]. Above all, smoking is strongly associated with chronic obstructive pulmonary disease (COPD), a serious condition characterized by progressive limitation of airflow, which is estimated to be the third leading cause of death by 2020 [2,3].

COPD is caused by an inflammatory process induced by the inhalation of harmful particles and gases, which leads to structural changes in the airways and alveoli. Different inflammatory cells, such as macrophages, mast cells, neutrophils and lymphocytes, participate in

Abbreviations: µL, microliter; µm, micrometer; ANOVA, analysisof variance; AnxAA1, annexinA1 Protein; BAL, pronchoalveolarlavage; C, control; CEUA, ethics committee for the use of animals; Cm, centimeter; COPD, chronicobstructive pulmonary disease; CS, cigarettesmoke-exposed and untreated; CS + phytotherapics, cigarettesmoke-exposed and treated with phytotherapics; DAB, diaminobenzine; ED-1, macrophageantibody; G, gram; GAMMAGT, gammaglutamyl transferase; HE, hematoxylin-eosin; IL-1β, interleukin-1beta; IL-6, interleukin-6; IL-10, interleukin-10; L, Liter; MCP-1, Monocytechemotactic protein-1; Mg, milligram; ML, mililiter; NF-kβ, nuclearfactor kappa beta; p, valueof p (significance of the statistical test); PBS, phosphatebuffered solution; Pg, picogram; RPM, rotationper minute; S.E.M, standarderror of mean = Standard error of mean; TNF-α, tumornecrosis factor-alpha; U/L, ultra/ liter; Vs, versus

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the disease development and are responsible for the release of several chemical mediators, which in the long term and imbalance of the immune response cause tissue damage and contribute to the lung function decline [2,4–6]

The influence of exposure to cigarette smoke in rats is a simple and widely used model to study smoking adverse effects and treatment possibilities of illnesses caused by this habit [7,8]. Although they differ in type and amount of cigarettes and time of exposure, several studies have been conducted to induce COPD in rats [7,9–11]

As the inflammatory mechanisms induced by smoking are related to development of different clinical conditions, one of the promising treatments is the anti-inflammatory therapy by medicinal herbs [12–16].

Among the plants related to the respiratory tract are *Mikania glomerata* Spreng and *Mikania laevigata*, which in addition to reducing inflammatory infiltration may be candidates for the prevention of oxidative lung damage caused by exposure to coal dust [17]. Studies also indicate the *Plantago major* efficacy in the treatment of the respiratory tract inflammation by controlling mast cells in asthmatic rats [18]. *Berberis vulgaris*, *Taraxacum officinale* and *Arctium lappa* were evaluated for their antimutagenic properties in various tumors, such as lung, kidney, brain and testicles, and showed important results when administered together. Control of proliferative activity in other tumors was also observed by the administration of a combination of *Chelidonium majus L.* and *Equisetum arvense* extracts [19].

In the face of COPD impacts and therapeutic potential of medicinal herbs, we evaluated the properties of *A. lappa, M. glomerata Spreng., P major and E. arvense* extracts administered in association in a COPD model.

2. MateriaL and methods

2.1. Animals

6-week-old Wistar rats were divided into 3 groups (n = 5/group), control (C), Cigarette smoke-exposed and untreated (CS), Cigarette smoke-exposed and treated with phytotherapics (CS + Phytotherapics) and kept in individual cages in a controlled environment (24–25 °C, 12 h light/dark cycle) with water and food ad libitum. All experimental procedures were conducted according to the guidelines for biomedical research stated by the Brazilian Societies of Experimental Biology and the European legislation on care and use of experimental animals (EU Directive 2010/63/EU for animal experiments; R.D. 53/2013) and approved by the Ethic Committee on Animal Use at Padre Albino Integrated College (Certificate nº 05/14). The experiments were designed to minimize the number of animals used and their suffering during the execution of the protocols. All animals were daily evaluated by the institution's veterinarian.

2.2. Preparation and standardization of herbal alcoholic extracts

Fresh leaves of *Arctium lappa, Plantago major, Mikania glomerata Spreng and Equisetum arvense* were collected in the Institution medicinal herbs garden and the vouchers specimens were deposited in the Institution herbarium. For the alcoholic extracts preparation, 40 g of each chopped dried herbs were placed, separately, in a Soxhlet (Prolab, São Paulo, Brazil) extractor with 160 mL of ethanol.

The standardization of the extracts was performed by the identification of chemical components as tannins, flavonoids, saponins and alkaloids by Ferric Chloride, Lead Acetate, Copper Acetate, Aluminum Chloride, Sodium Hydroxide reactions [20]. These analyzes indicated the presence of tannins and flavonoids.

Subsequently, each extract was evaluated for cytotoxicity in vitro [21] by the analysis of blood cells exposed to different concentrations (4%, 8%, 16%, 32% and 50%) and followed by the reading of absorbance at 540 nm in spectrophotometer. Cytotoxicity of 25% or greater was observed from the 8% concentration for all extracts, because of this, and knowing the possible synergistic action of medicinal herbs when administered together [16], the lowest extracts concentrations were selected.

2.3. Exposure to cigarette smoke and treatment protocol

The animals were exposed to the smoke of 10 commercial cigarettes (containing 0.8 mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide), one after another, twice a day (total of 20 cigarettes/day), for 8 weeks, considered the exposure time for initiation of morphological changes [22], in a specific apparatus. The apparatus consists of an animal containment system and a cigarette smoke release system with an external cigarette holder connected to a dynamic suction pump. The pump can be programmed so that periods of cigarette suction alternate with periods of suction of clean air to prevent suffocation [8]. The exposures were standardized and conducted at approximately the same time of the day. The control group was not exposed to the smoke.

The efficacy of herbal medicines in protecting against the inflammatory processes caused by smoking exposure was tested in one of the exposed to smoke groups. The animals were administered by gavage with 1 mL solution containing extracts of *E. arvense*. (4%), *P. major* (4%), *A. lappa* (4%) and *M. glomerata* Spreng (4%), twice a day, prior to the exposure to the cigarette smoke. The untreated-exposed to smoke rats received only the vehicle by gavage. After the exposure protocol, the animals were euthanized by overdose of anesthetic (ketamine, 40 mg/kg, i.p. and xylasin, 40 mg/kg, i.p.) [23]

2.4. Quantitative analyzes of bronchoalveolar lavage

In order to collect the bronchoalveolar lavage (BAL), the animals had the trachea cannulated and the right lung clamped. The left lung was washed 3 times with PBS and the obtained liquid was centrifuged for 10 min at 1500 rpm. The pellet was resuspended in 500 μL of PBS and 10 μL aliquots were stained in Turk (1:10) for counting the inflammatory cells in a Neubauer camera. Values were expressed as cell numbers $\times 10^3$/mL.

2.5. Biochemical analyzes of blood plasma

Blood was collected by cardiac puncture in heparinized syringes, placed in ependorffs, centrifuged for 15 min at 3000 rpm and the obtained plasma was used for glucose, cholesterol and gamma glutamyl transferase (gamma GT) dosages with commercially available kits (LAB Test, Minas Gerais, Brazil) and according to the manufacturer's instructions.

2.6. Histopathological analyzes and quantification of mast cells

The right lung and trachea were fixed in 4% formaldehyde, dehydrated in ascending order of alcohol and included in paraffin for histopathological analysis. Sections of 5 μm were stained with Hematoxylin-Eosin (HE). For the morphometric analyzes the measurements of the alveolar spaces were quantified by the average of 10 areas of each image obtained by the 40X objective in the Leica microscope (DM500). The areas were obtained using the Leica Image Analysis Software.

Lung sections were stained with 0.1% toluidine blue for mast cell evaluation according to their intact or degranulated morphological characteristics. Mast cell quantification was performed in 10 images per slide obtained by the 40X objective in the Leica microscope (DM500).

2.7. Immunohistochemical analyzes

Immunohistochemical studies were used to evaluate the expressions of the anti-inflammatory protein Annexin A1 (AnxA1) and nuclear

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