Biomedicine & Pharmacotherapy 99 (2018) 591–597

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

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

Lucas Possebon[a,b], Isabella de Souza Lima Lebron[a], Ligia Furlan da Silva[a], Julia Tagliaferri Paletta[a], Bruna Gabrieli Glad[c], Monielle Sant'Ana[d], Melina Mizusaki Iyomasa-Pilon[a], Helena Ribeiro Souza[a,b], Sara de Souza Costa[a,b], Giselda Pereira da Silva Rodriguesa[a], Maria de Lourdes Pereira[e], Andreia de Haro Moreno[a], Ana Paula Girol[a,b,*]

[a] Integrated College Padre Albino Foundation (FIPA), Catanduva, SP, Brazil
[b] São Paulo State University, (UNESP), Institute of Biosciences, Humanities and Exact Sciences (IBILCE), São José do Rio Preto Campus, Department of Biology, Laboratory of Immunomorphology, SP, Brazil
[c] Municipal Institute of Higher Education of Catanduva (IMES), Catanduva, SP, Brazil
[d] São Paulo Federal University (UNIFESP) São Paulo, SP, Brazil
[e] Aveiro Institute of Materials, University of Aveiro, Department of Biology and CICECO Aveiro Portugal

ARTICLE INFO

Keywords:
COPD
Histopathology
Inflammatory mediators
Phytotherapy
Smoking

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

* Corresponding author at: Department of Physical and Biological Sciences, Integrated College Padre Albino Foundation (FIPA), Rua dos Estudantes, 225, Catanduva, SP, Brazil.
E-mail addresses: lucas_possebon@hotmail.com, kelter.geromel@fipa.com.br (L. Possebon), isa0409lebron@gmail.com, marcia.gomes@fipa.com.br (I. de Souza Lima Lebron), ligia_lds@hotmail.com, cilene.hernandes@fipa.com.br (L. Furlan da Silva), jujupaletta@yahoo.com.br, renata.stuhi@fipa.com.br (J. Tagliaferri Paletta), brunaglad@hotmail.com, cep@fipa.com.br (B.G. Glad), monibiologia@yahoo.com.br, cidinha.marson@fipa.com.br (M. Sant'Ana), melmzk@gmail.com, melina.pilon@fipa.com.br (M.M. Iyomasa-Pilon), helena.riber@hotmail.com, maira.spina@fipa.com.br (H. Ribeiro Souza), sarah_sc_0705@hotmail.com, lurdinha.barbato@fipa.com.br (S. de Souza Costa), gi.psr@uol.com.br, marisa.stuchi@fipa.com.br (G. Pereira da Silva Rodriguesa), mlourdespereira@ua.pt (M.d.L. Pereira), ahmoreno@bol.com.br, natalia.biagi@fipa.com.br (A. de Haro Moreno), anapaulagirol@hotmail.com, anapaula.girol@fipa.com.br (A.P. Girol).

https://doi.org/10.1016/j.biopha.2018.01.106
Received 8 January 2018; Received in revised form 18 January 2018; Accepted 24 January 2018

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

factor (NF) k-β as well as to perform the macrophages quantification in lungs. Sections were processed for antigenic recovery with citrate buffer pH 6.0, blockade of the endogenous peroxidase activity and incubation with the rabbit polyclonal primary antibodies: anti-AnxA1 (1: 1000), anti- NFk-β (1: 200) and anti-ED-1 (1: 150) (Zymed Laboratories, Cambridge, UK) for 12 h. They were then incubated with the biotinylated secondary antibody (Histostain Kit, Invitrogen) and immersed in conjugated streptavidin peroxidase complex. The substrate diaminobenzidine (DAB Kit, Invitrogen) was used for the development and, thereafter, the sections were stained with Hematoxylin. Proteins were quantified by densitometry as arbitrary units from 0 to 255 using the Leica Image Analysis software image analyzer. Macrophages were quantified as previously described for mast cells.

2.8. Dosage of cytokines

The interleukins (IL)-1β, IL-6 and IL-10, tumor necrosis factor (TNF)-α and the monocyte chemoattractant protein (MCP)-1 were quantified in blood plasma using the rat cytokine MILLIPLEX MAP Kit (RECYTMAG-65K; Millipore Corporation, USA) according to manufacturer's instructions and analyzed on the LUMINEX xMAP MAGPIX equipment (Millipore Corporation, USA).

2.9. Statistical analyzes

The results were submitted to descriptive analysis and determination of normality. Afterwards, the Analysis of Variance (ANOVA) was used, followed by the Bonferroni test. All values were expressed as mean ± S.E.M. and P values less than 0.05 were considered statistically significant.

3. Results

3.1. Herbal extracts mixture controlled metabolic changes in blood plasma and inflammatory cells influx in bronchoalveolar lavage

Biochemical analyzes showed elevated blood plasma levels of glucose (Fig. 1A) and gamma GT (Fig. 1C) in untreated-exposed to smoke animals. But the administration of *A. lappa, M. glomerata Spreng., P major and E. arvense* extracts mixture decreased the levels of glucose ($p < .001$) (Fig. 1A), cholesterol ($p < .05$) (Fig. 1B) and gamma GT ($p < .001$) (Fig. 1C).

In BAL analysis a significant increase in macrophages (23.80 ± 1.655) ($p < .001$) (Fig. 2H) and lymphocytes (58.00 ± 5.822) ($p < .001$) (Fig. 2L) was observed in animals exposed to smoke without treatment compared to control (macrophage: 4.200 ± 1.594; lymphocyte: 10.80 ± 1.241). However, treated animals showed reduction in these cells (macrophages:13.00 ± 3.564, $p < 0.05$, lymphocytes: 30.20 ± 6.644, $p < 0.01$) (Fig. 2H and L).

3.2. Protective effects of herbal extracts mixture against lung and trachea tissue damages due to tobacco exposure

The histopathological analyzes showed enlargement of the intra-alveolar spaces, greater influx of inflammatory cells and pulmonary congestion in the lungs of the untreated-exposed to smoke group (Fig. 2E, F and D). These animals also showed tracheal metaplasia (Fig. 2G). All these alterations were reverted by herbal mixture treatment (Fig. 2I, J and K).

3.3. Decreased numbers of mast cells and macrophages in COPD model by herbal treatment

The mast cells were identified in lungs by their characteristic metachromatic cytoplasmic granules and observed according to their activation state as intact or degranulated cells. A large number of mast cells, mostly degranulated, were quantified in the untreated-exposed to smoke group (42.95 ± 0.6727; $p < .001$) compared to control (16.75 ± 3.052) and treated rats (22.60 ± 1.735; $p < .001$) (Fig. 3A, B, C and I).

Quantification of macrophages in lungs was performed by immunohistochemistry and the specificity of this analysis was confirmed by the reaction control. The cells were identified in the intra-alveolar spaces and tissue septa. Numerous macrophages were observed in the untreated animals (96.00 ± 9.567; $p < .001$) (Fig. 3E and H) compared to control (14.50 ± 2.424) (Fig. 3D and H). The extracts mixture administration decreased the lung macrophages (16.50 ± 5.037; $p < .001$) (Fig. 3F and H).

3.4. Phytotherapics administration reduces Annexin A1 and nuclear factor–kβ expressions

Untreated smoke-exposed animals showed increased expression of AnxA1 (177.6 ± 7.009; $p < .01$) (Fig. 4B) and NF-kβ (188.4 ± 6.283; $p < .001$) (Fig. 4E) in the lung compared to control (Fig. 4A and D). In contrast, the herbal extracts treatment promoted reduction of AnxA1 (130.7 ± 4.596; $p < .001$) (Fig. 4C) and NF-kβ (137.8 ± 3.852; $p < .001$) (Fig. 4F) expressions. The specificity of the immunolabeling was confirmed by the respective reaction controls (Fig. 4G).

3.5. Decreased levels of inflammatory mediators by herbal extracts mixture treatment

Increases in IL-1β ($p < .001$), IL-6 ($p < .001$), TNF-α ($p < .001$) and MCP-1 ($p < .001$) were found in the blood plasma of untreated-smoke-exposed animals (Fig. 5A, B, C and E).

Again, the herbal extract mixture administration promoted significant reduction of the pro-inflammatory mediators levels (Fig. 5A, B, C and E). Differently, the treatment lead to increased levels of the anti-inflammatory cytokine IL-10 ($p < .001$) (Fig. 5 D).

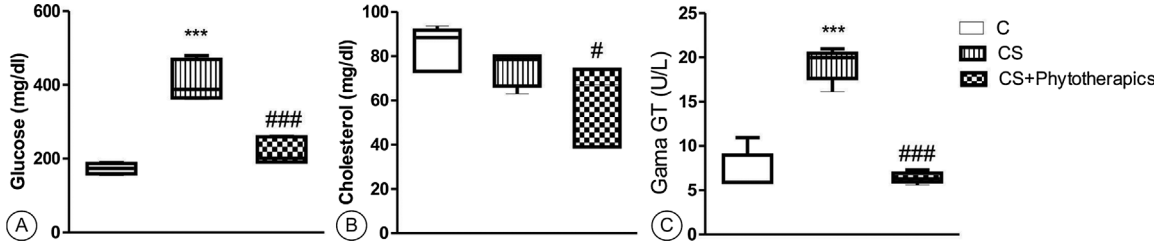

Fig. 1. Biochemical analyzes of blood plasma. Total Glucose (A), Cholesterol (B), Gamma GT (C). Data as median, minimum, maximum and quartiles. ***p < .001 vs C; # p < .05, ### p < .001 vs CS. C = control rats; CS = cigarette smoke-exposed untreated animals; CS + Phytotherapics = cigarette smoke-exposed and treated with phytotherapics.

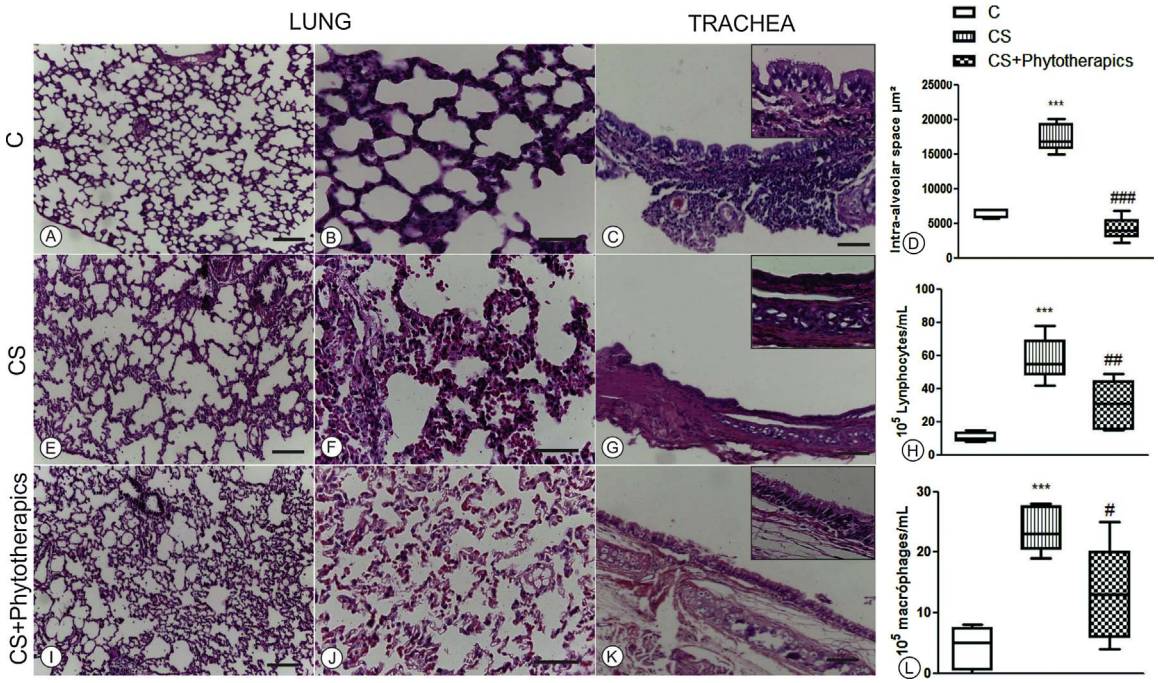

Fig. 2. Histopathological analyzes of the lungs. Control lung with normal appearance (A and B) lungs of untreated smoke-exposed rats (E and F). Reduction of lung inflammation and congestion in smoke-exposed animals treated with phytotherapics (I and J). Staining: HE. Bars: 10 µm. Measurement of intra-alveolar spaces. Increase in untreated group compared to control group and significant reduction by treatment (D). Data as Mean ± SEM. $^{***}p < 0.001$ vs C; ### $p < 0.001$ vs CS. Histopathological analyzes of trachea. Control with normal respiratory epithelium (C). Metaplasia in animals exposed to smoke and untreated (G) Preserved respiratory epithelium in treated animals (K). Details represent enlargements of the dashed areas. Color: HE. Bars: 10 µm. Quantitative analysis of bronchoalveolar lavage - Lymphocytes (H) and Macrophages (L). Results presented as median, minimum, maximum and quartiles. $^{***}p < .001$ vs C; ## $p < 0.01$ ### $p < 0.001$ vs CS. C = control rats; CS = cigarette smoke-exposed untreated animals; CS + Phytotherapics = cigarette smoke-exposed and treated with phytotherapics.

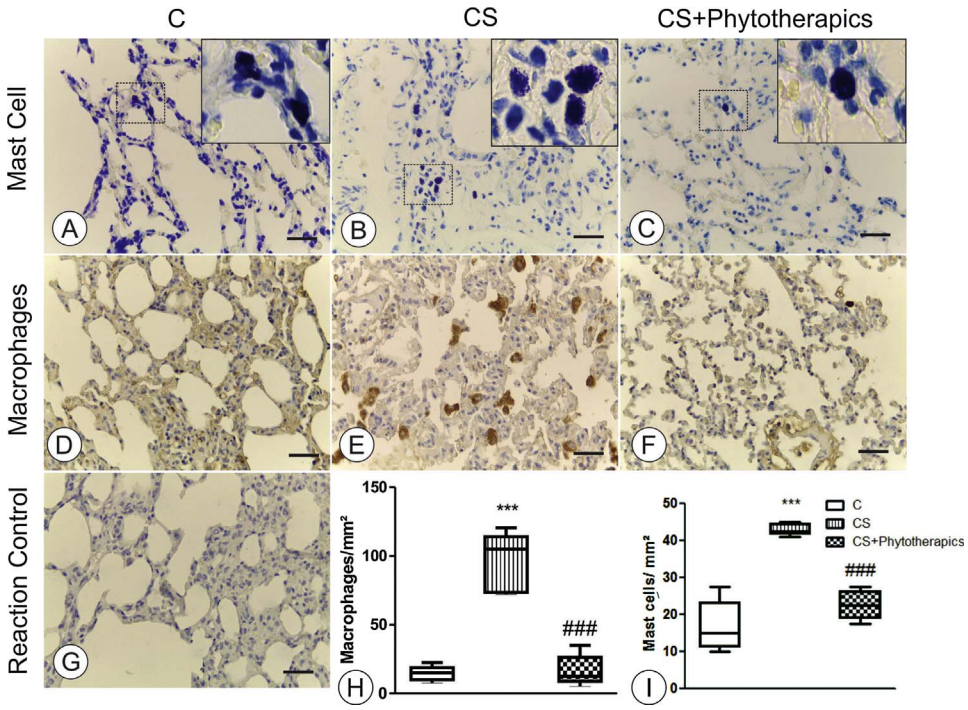

Fig. 3. Mast cells and macrophages in lung and trachea – Few mast cell in control (A) and treated animals (C), and increased of these cells in untreated smoke-exposed group (B), Staining: Toluidine blue. Few macrophages in the control (D) and treated animals (F). Numerous cells in the untreated group exposed to smoke (F). Counter-staining: Hematoxylin. 10 µm bars. Results presented as median, minimum, maximum and quartiles. $^{***}p < 0.001$ vs C; ### $p < .001$ vs CS. C = control rats; CS = untreated smoke-exposed animals; CS + Phytotherapics = smoke-exposed animals treated with herbal extracts.

4. Discussion

Smoking may result in chronic inflammation, such as COPD, with great morbidity and mortality [24]. The search for alternative anti-inflammatory treatments through medicinal plants is important and can be effective in the prevention and recovery of diseases caused by smoking [14].

Initially in this research we quantified the inflammatory cells in BAL. These analyzes showed influx of lymphocytes and macrophages in the untreated-exposed to smoke group, as also demonstrated in other studies using the COPD model induced by cigarette smoke [9,25]. However, the administration of *A. lappa, M. glomerata Spreng., P major and E. arvense* extracts mixture promoted decreased BAL leukocytes influx showing the anti-inflammatory effects of the herbal treatment.

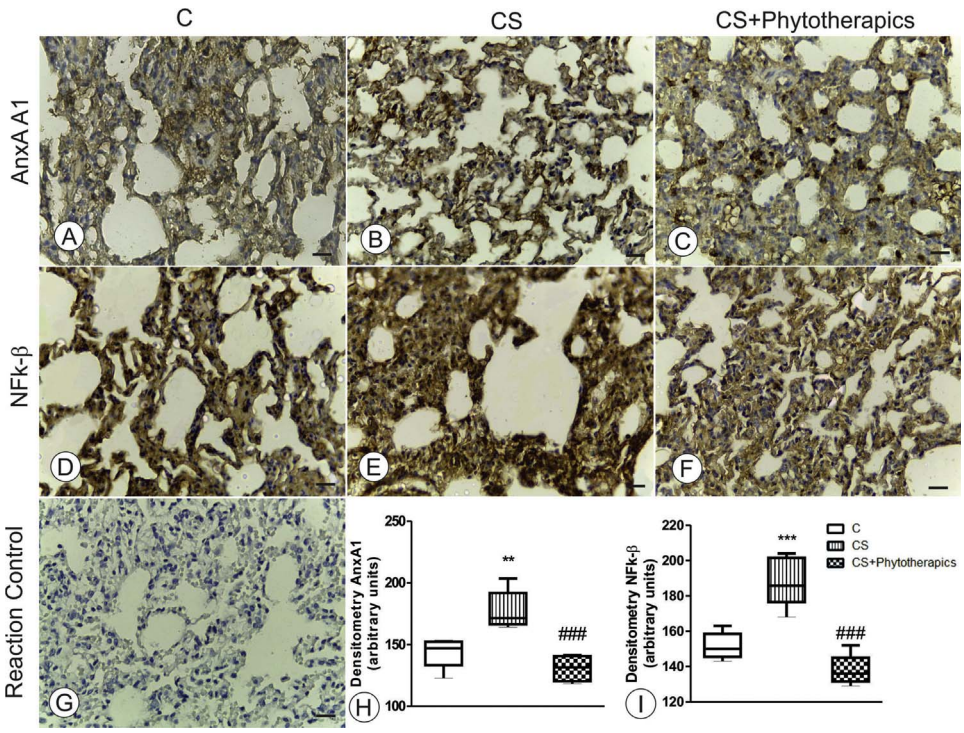

Fig. 4. Expression of the AnxA1 and NFk-β proteins in lung: Reduced expression of the proteins in the control group (A and D), increased immunolabeling in the untreated-exposed to smoke group (B and E) and reduction in the expression of AnxA1 and NFk-β after treatment with phytotherapics (C and F). Absence of immunolabeling on reaction control (G). Counter-staining: Hematoxylin. 10 μm bars. Data as median, minimum, maximum and quartiles. $^{**}p < .01$, $^{***}p < .001$ vs C; ### $p < .001$ vs CS. C = control rats; CS = cigarette smoke-exposed untreated animals; CS + Phytotherapics = cigarette smoke-exposed and treated with phytotherapics.

Our results are in line with researches that pointed the protective role of *M. glomerata* in the prevention of the oxidative lung damage in a coal dust exposure model [17] and reduction of inflammatory cells in lesions induced by *Bothrops jararaca* venom in rats [26].

Next, in the biochemical analyzes performed in blood plasma we found that the elevated levels of glucose, cholesterol and gamma GT observed in untreated animals were reduced by herbal mixture treatment. Cholesterol and glucose dosages alterations were also indicated in male and female offsprings exposed to tobacco smoke during lactation [12]. Once again, the used herbs prevented metabolic changes due to cigarette exposure and showed no toxicity, reinforcing their efficacy, safety and applicability. Another study also pointed that non toxic levels of P. major extracts were able to inhibit reactive oxygen species (ROS) production from activated human neutrophils suggesting the therapeutic potential of *P. major* [27].

Regarding the lung histopathological analyzes we observed important pulmonary alterations caused by the exposure to cigarette smoke as found by other investigators in the same model, indicating the tendency for worsening pulmonary function parameters in the smoker group [8]. Additionally, metaplasia was also observed in the untreated smoker group, a condition that compromises the morphophysiology of the organ and promotes the development of tumors [28]. Recruited neutrophils produce proteases which stimulate mucus secretion associated with chronic bronchitis, a major characteristic of COPD. Also, the recruited T lymphocytes cause cytolysis and apoptosis of alveolar epithelial cells, contributing to disease-related emphysema [5,6]. Again, our results indicated the protective effects of the treatments with *A. lappa, M. glomerata Spreng., P major and E. arvense* extracts mixture related to the influx reduction of inflammatory cells in lungs and preservation of the pulmonary architecture and tracheal epithelium. The anti-inflammatory role of phytotherapics used in different models of lung inflammation and fibrosis were also demonstrated in other studies [16,29].

Continuing the analysis of the inflammatory cells, we found a large

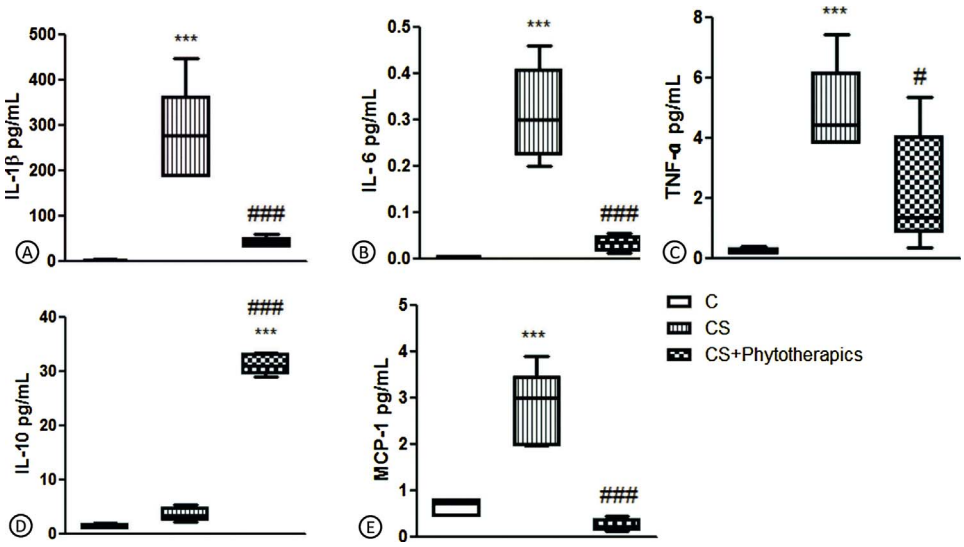

Fig. 5. Analysis of cytokines IL-1β (A), IL-6 (B), TNF-α (C) and IL-10 (D) and Chemokine MCP-1 (E) by MAGPIX in the control, untreated and treated smoke-exposed groups. Values obtained expressed as median, minimum, maximum and quartiles. $^{***}p < .001$ vs C; # $P < 0.05$; ### $p < .001$ vs CS. C = control rats; CS = cigarette smoke-exposed untreated animals; CS + Phytotherapics = cigarette smoke-exposed and treated with phytotherapics.

number of mast cells, mostly degranulated, in smoke-exposed animals without treatment compared to control and treated groups. Other researches indicated the involvement of mast cells in response to lesions and structural tissue remodeling in different inflammatory lung conditions [30]. However, mast cells related to cigarette smoke exposure may alter the cellular environment and assist in COPD progression [31]. Therefore, the reduction in the number of mast cells caused by the herbal treatment used in this study plays a protective role.

Similarly, numerous macrophages were observed in the untreated-smoke exposed group with significant reduction after the herbal treatment. Numerous mononuclear cells in the untreated group can be related to tissue destruction. Activated macrophages secrete various mediators of inflammation, which, if uncontrolled, can lead to tissue destruction and fibrosis characteristic of COPD [3,32].

To deepen histopathological studies we performed immunohistochemistry for AnxA1 and NF-kβ. The highest expression of the anti-inflammatory protein AnxA1 observed in the untreated-smoke-exposed group was reduced by treatment. Studies indicate that the receptor for formylated peptides-2 (FPR2) present in activated lung epithelial and inflammatory cells is particularly important in the resolution of COPD and can be modulated by AnxA1 and other agonists, such as serum amyloid A and lipoxin A4 [33,34]. Although AnxA1 plays an important anti-inflammatory role, the phytotherapics used in our model may have acted independently of this protein.

Our results also showed increased NF-kβ expression in the untreated group compared to the others, which shows the anti-inflammatory effect of the combined herbal medicines extracts in the studied model. The efficiency in reducing NF-kβ by the treatment with green tea (*Camellia sinensis*) has been observed in experimental uveitis and reinforces the therapeutic effects of phytotherapics in controlling inflammation [35].

Finally, we evaluated the blood plasma levels of inflammatory mediators. Elevated IL-1β, IL-6, TNF-α and MCP-1 levels were observed in the untreated smoke-exposed group, which may be associated with lung continuous inflammation promoted by inflammatory cells [36,37]. However, these inflammatory mediators were decreased in the treated animals. Reductions of TNF-α dosages have also been observed in former smokers receiving anticholinergic medicinal products [38]. Likewise, *A. lappa* extract was able to downregulate the IL-1β and TNF-α gene expression on H2O2 -induced macrophages, pointing this herbal extract anti-inflammatory activity by the modulation of key inflammation mediators [39].

Our analyzes also showed increased IL-10 levels in the herbal treated group, which indicates important modulation of this anti-inflammatory cytokine by the herbal treatment. The protective role of IL-10 on ventilation-induced lung injury in rats was associated to decreased number of neutrophils in BAL [40] reinforcing the organism mechanism of defense against the lung inflammatory process.

5. Conclusion

All together the results indicate the synergistic anti-inflammatory and protective effects of the associated *A. lappa, M. glomerata Spreng., P major and E. arvense* extracts in the management of the inflammatory response in a COPD model. The data open perspectives for further research on the use of these medicinal herbs as future alternative targets against the damages caused by the exposure to cigarette smoke.

Declarations of interest

None.

Funding

This work was supported by Faculdades Integradas Padre Albino (FIPA), Catanduva, SP, Brazil and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), SP, SP, Brazil (Process nº 2015/03359-5).

References

[1] F. Islami, L.A. Torre, A. Jemal, Global trends of lung cancer mortality and smoking prevalence, Transl. Lung Cancer Res. 4 (2015) 327–338.
[2] K.F. Rabel, S. Hurd, A. Anzueto, P.J. Barnes, S.A. Buist, P. Calverley, Y. Fukuchi, C. Jenkins, R. Rodriguez-Roisin, C. Van Weel, J. Zielinski, global initiative for chronic obstructive lung disease. global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary, Am. J. Respir. Crit. Care Med. 176 (2017) 532–555.
[3] R. Burkhardt, W. Pankow, Chronic obstructive pulmonary disease (COPD) - rational diagnostics and therapy, Pneumologie 70 (2016) 533–545.
[4] H. Sarir, P.A. Henricks, A.H. Van Houwelingen, F.P. Nijkamp, G. Folkerts, Cells, mediators and toll-like receptors in COPD, Eur J. Pharmacol. 585 (2008) (2008) 346–353.
[5] C. Compton, D. Mcbryan, E. Bucchioni, F. Patalano, The novartis view on emerging drugs and novel targets for the treatment of chronic obstructive pulmonary disease pulmonary, Pharmacol. Ther. 26 (2013) 562–573.
[6] G. Caramori, I.M. Adcock, A. Di Stefano, K.F. Chung, Cytokine inhibition in the treatment of COPD, Int. J. Chron. Obstruct. Pulm. Dis. 9 (2014) 397–412.
[7] W.Y. Geng, Z.B. Liu, N.N. Song, G.H. Zhang, W.Z. Jin, W. Zhou, L. Li, Y.X. Cao, D.N. Zhu, L.L. Shen, Effects of electroacupuncture at Zusanli (ST36) on inflammatory cytokines in a rat model of smoke-induced chronic obstructive pulmonary disease, J. Integr. Med. 11 (2013) 213–219.
[8] Kozma RDLH, E.M. Alves, V.A. Barbosa-De-Oliveira, F.D. Lopes, R.C. Guardia, H.V. Buzo, C.A. Faria, C. Yamashita, M. Cavazzana Júnior, F. Frei, M.J. Ribeiro-Paes, J.T. Ribeiro-Paes, A new experimental model of cigarette smoke-induced emphysema in Wistar rats, J. Bras Pneumol 40 (2014) 46–54.
[9] J.L. Wright, A. Churg, Animal models of COPD: barriers, successes, and challenges, Pulm. Pharmacol. Ther. 21 (2008) 696–698.
[10] H. Zheng, Y. Liu, T. Huang, Z. Fang, G. Li, S. He, Development and characterization of a rat model of chronic obstructive pulmonary disease (COPD) induced by side stream cigarette smoke, Toxicol. Lett. 189 (2009) 225–234.
[11] S. Zhou, J.L. Wright, J. Liu, D.D. Sin, A. Churg, Aging does not enhance experimental cigarette smoke-induced COPD in the mouse, PLoS one 8 (2013) e71410.
[12] P.C. Lisboa, P.N. Soares, T.C. Peixoto, J.C. Carvalho, C. Calvino, V.S.T. Rodrigues, D.N. Bernardino, V. Younes-Rapozo, A.C. Manhães, E. Oliveira, E.G. Moura, Effects of cigarette smoke exposure during suckling on food intake, fat mass, hormones, and biochemical profile of young and adult female rats, Endocrine 57 (2017) 60–71.
[13] F.B. Holetz, G.L. Pessini, N.R. Sanches, D.A. Cortez, C.V. Nakamura, B.P. Filho, Screening of some plants used in the brazilian folk medicine for the treatment of infectious diseases, Mem. Inst. Oswaldo Cruz. 97 (2002) 1027–1031.
[14] U. Härtel, E. Volger, Use and acceptance of classical natural and alternative medicine in germany–findings of a representative population-based survey, Forsch Komplement. Klass Naturheilkd 11 (2004) 327–334.
[15] R. Raschetti, F. Menniti-Ippolito, E. Forcella, C. Bianchi, Complementary and alternative medicine in the scientific literature, J. Altern. Complement. Med. 11 (2005) 209–212.
[16] S. Bahri, R.B. Alic, A. Abidia, S. Jameleddine, The efficacy of plant extract and bioactive compounds approaches in the treatment of pulmonary fibrosis: a systematic review, Biomed. Pharmacother. 93 (2017) 666–673.
[17] T.P. Freitas, P.C. Silveira, L.G. Rocha, G.T. Rezin, J. Rocha, V. Citadini-Zanette, P.T. Romão, F. Dal-Pizzol, R.A. Pinho, V.M. Andrade, E.L. Streck, Effects of Mikania glomerata Spreng. and Mikania laevigata Schultz Bip. ex Baker (Asteraceae) extracts on pulmonary inflammation and oxidative stress caused by acute coal dust exposure, J. Med. Food 11 (2008) 761–766.
[18] F. Farokhi, F. Khaneshi, Histophatologic changes of lung in asthmatic male rats treated with hydro-alcoholic extract of plantago major and theophylline, Avicenna J. Phytomed. 3 (2) (2013) 143–151.
[19] C. Di Giorgio, L. Boyer, M. De Meo, C. Laurant, R. Elias, E. Ollivier, In vitro and in vivo antimutagenic effects of DIG, a herbal preparation of Berberis vulgaris, Taraxacum officinale and Arctium lappa, against mitomycin C, J. Nat. Med. 69 (2015) 267–277.
[20] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoids contents in mulberry and their scavenging effects on superoxid radicals, Food Chem. 64 (1999) 555–559.
[21] J.F. Campos, D.T. De Castro, M.J. Damião, H.F. Vieira-Torquato, E.J. Paredes-gamero, C.A. Carollo, L.M. Estevinho, K. De Picoli-Souza, E.L. Dos Santos, The chemical profile of senna velutina leaves and their antioxidant and cytotoxic effects, Oxid. Med. Cell. Longev. (2016) 1–12.
[22] M.Y. Marcelino, N.L. Fuoco, C.A. De Faria, R.L.H. Kozma, L.F. Marques, J.T. Ribeiro-Paes, Animal models in chronic obstructive pulmonary disease—an overview, Exp. Lung Res. 40 (6) (2014) 259–271.
[23] J.B. Urban, R.C.T. Camargo, R.R. Costalonga, M.J.Q. Louzada, R.A. Fernandes, J.C.S. Camargo-Filho, The influence of passive tobacco exposure and physical exercise on bone tissue of young rats, Acta Ortop. Bras. 25 (2) (2017) 77–80.
[24] J.M. Leung, V. Chen, Z. Hollander, D. Dai, S.J. Tebbutt, S.D. Aaron, K.L. Vandemheen, S.I. Rennard, J.M. Fitzgerald, P.G. Woodruff, S.C. Lazarus, J.E. Connett, H.O. Coxson, B. Miller, C. Borchers, B.M. Mcmanus, R.T. Ng, D.D. Sin, COPD exacerbation biomarkers validated using multiple reaction monitoring mass spectrometry, PLoS One. 11 (8) (2016) e0161129.
[25] M. Bazett, A. Biala, R.D. Huff, M.R. Zeglinksi, P.M. Hansbro, M. Bosiljcic, H. Gunn,

S. Kalyan, J.A. Hirota, Attenuating immune pathology using a microbial-based intervention in a mouse model of cigarette smoke-induced lung inflammation, Respir. Res. 18 (2017) 92.
[26] V.B. Mourão, G.M. Giraldi, L.M. Neves, F.O. Gaspi, R.A. Rodrigues, A.A. Alves, M.A. Esquisatto, M.V. Mazzi, F.A. Mendonça, G.M. Santos, Anti-hemorrhagic effect of hydro-alcoholic extract of the leaves of Mikania glomerata in lesionsinduced by Bothrops jararaca venom in rats, Acta Cir Bras 29 (January) (2014) 30–37.
[27] E. Reina, N. Al-Shibani, E. Allam, K.S. Gregson, M. Kowolik, L.J. Windsor, The effects of plantago major on the activation of the neutrophil respiratory burst, J. Trad. Complement. Med. 3 (2013) 268-267.
[28] H.M. Rigden, A. Alias, T. Havelock, R. O'Donnell, R. Djukanovic, D.E. Davies, S.J. Wilson, Squamous metaplasia is increased in the bronchial epithelium of smokers with chronic obstructive pulmonary disease, PLoS one. 11 (5) (2016) e0156009.
[29] N.Y. Xu, C.J. Chu, L. Xia, J. Zhang, D.F. Chen, Protective effects of Rabdosia japonica var. glaucocalyx extract on lipopolysaccharide-induced acute lung injury in mice, Chin. J. Nat. Med. 13 (2015) 767–775.
[30] H. Virk, G. Arthur, P. Bradding, Mast cells and their activation in lung disease, Transl. Res. 174 (2016) 60–76.
[31] H. Li, T. Yang, Q. Ning, F. Li, T. Chen, Y. Yao, Z. Sun, Cigarette smoke extract-treated mast cells promote alveolar macrophage infiltration and polarization in experimental chronic obstructive pulmonary disease, Inhal. Toxicol. 27 (2015) 822–831.
[32] B.M. Sundblad, J. Jie, B. Levänen, K. Midander, A. Julander, K. Larsson, L. Palmberg, A. Lindén, Extracellular cadmium in the bronchoalveolar space of long-term tobacco smokers with and without COPD and its association with inflammation, Int. J. Chron. Obstruct. Pulmon. Dis. 11 (2016) 1005–1013.
[33] S. Bozinovski, D. Anthony, R. Vlahos, Targeting pro-resolution pathways to combat chronic inflammation in COPD, J. Thorac. Dis. 6 (2014) 1548–1556.
[34] O. Corminboeuf, X. Leroy, FPR2/ALXR agonists and the resolution of inflammation, J. Med. Chem. 22 (58) (2015) 537–559.
[35] Y.J. Qin, K.O. Chu, Y.W.Y. Yip, W.Y. Li, Y.P. Yang, K.P. Chan, J.L. Ren, S.O. Chan, C.P. Pang, Green tea extract treatment alleviates ocular inflammation in a rat model of endotoxin-induced uveitis, PLoS one. 9 (8) (2014) e103995.
[36] L.E. Crotty Alexander, S. Shin, J.H. Hwang, Inflammatory diseases of the lung induced by conventional cigarette smoke: A review, Chest 148 (2015) 1307–1322.
[37] T.E. Kastelein, R. Duffield, F.E. Marino, Acute immune-inflammatory responses to a single bout of aerobic exercise in smokers; the effect of smoking history and status, Front. Immunol. 6 (2015) 634.
[38] C.S. Berenson, R.L. Kruzel, S. Sethi, The impact of exogenous factors on respiratory pathogen-induced innate alveolar macrophage responses in COPD, Immunol. Invest. 45 (2016) 130–147.
[39] E. Pomari, B. Stefanon, M. Colitti, Effect of plant extracts on H2O2-induced inflammatory gene expression in macrophages, J. Inflamm. Res. 7 (2014) 103–112.
[40] J.Y. Wu, Z.H. Xiong, G.Z. Xiong, F.Q. Ding, J. Lei, S. Lu, Y. Li, G.M. He, L.L. Zhao, Z.J. Liu, Protective effect of interleukin-10 and recombinant human keratinocyte growth factor-2 on ventilation-induced lung injury in rats, Genet. Mol. Res. 14 (2015) 15642–15651.