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Article

Running head: *Pyrostegia venusta* and asthma

Aqueous *Pyrostegia venusta* (Ker Gawl.) Miers extract attenuates allergen-induced asthma in a mouse model via an antioxidant mechanism

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Abstract:

Objective: *Pyrostegia venusta* (Ker-Gawl.) Miers (Bignoniaceae) is a perennial invasive vine, distributed worldwide. In folk medicine, its parts are used for the treatment of inflammatory respiratory diseases. Extracts of *P. venusta* have antioxidant, antimicrobial, and antinociceptive properties. The aim of this study was to evaluate the effects of two extracts (aqueous and hydroethanolic) of *P. venusta* in the treatment of asthma in an animal model.

Methods: Balb/c mice were sensitized twice with ovalbumin (OVA) intraperitoneally (ip), one week apart, and after one week, challenged with OVA intranasally on four alternate days. Mice were treated ip with 300 mg/kg of aqueous or hydroethanolic extracts for seven consecutive days. Control groups received saline on the same days. Bronchial hyperresponsiveness, production of Th1 and Th2 cytokines, lung and airway inflammation, and antioxidant activity in lung tissue were assessed.

Results: Treatment with aqueous extract significantly decreased bronchial hyperresponsiveness, measured by total and tissue resistance and elastance. The administration of hydroethanolic extract did not reduce bronchial hyperresponsiveness. In addition, both extracts significantly reduced total cell and eosinophil counts in bronchoalveolar lavage. Both extracts did not change significantly IL-4, IL-5, IL-9, IL-13, IFN-gamma, and TGF-beta levels. Of note, only the aqueous extract significantly increased the total antioxidant activity and reduced lung inflammation.

Conclusion: Aqueous extract of *P. venusta* reduced bronchial hyperresponsiveness, lung and airway inflammation, probably via an antioxidant mechanism. These results demonstrate that *P. venusta* may have potential for asthma treatment.

Keywords: antioxidants, asthma, bronchial hyperreactivity, inflammation, phytotherapy.

Introduction

Asthma is one of the most common chronic diseases, affecting children and adults worldwide [1]. It is a heterogeneous inflammatory disease characterized by airway hyperresponsiveness, variable airflow limitation, and bronchial remodeling [1,2]. Numerous inflammatory markers are present in asthma and correlated with airway hyperresponsiveness and remodeling [3,4].

Activated Th2 cells and cytokines, including IL-4, IL-5, and IL-13, play a pivotal role in the pathogenesis of asthma. Additionally, some studies have found an imbalance between the reducing and oxidizing systems, with a predominance of an oxidative state [5-9]. Inflammatory cells can generate reactive oxygen species (ROS), and endogenous and exogenous reactive oxygen and nitrogen species are related to airway inflammation and asthma severity [5-9]. Moreover, the prevalence of asthma has increased in many countries, and exposure to oxidants could play a major role in its pathogenesis^[10,11]. Therefore, since antioxidants are required to counteract oxidative stress, therapies that increase antioxidant activity could be of great importance in asthma management^[8].

The treatment of asthma includes patient education and drug therapy, such as bronchodilators and corticosteroids^[2]. However, not all patients achieve disease control and some medications, especially corticosteroids in high doses, may have undesirable side effects^[2]. Thus, it is still necessary to search for new drugs, targeting specific pathways, such as oxidative stress.

Pyrostegia venusta (Ker-Gawl.) Miers (Bignoniaceae) is an invasive perennial vine with orange flowers, present worldwide. In folk medicine, its parts are used for the treatment of diarrhea, vitiligo, cough, and inflammatory and respiratory diseases, such as bronchitis and colds [12]. Previous studies have demonstrated the anti-inflammatory activity of a hydroethanolic extract from flowers of *P. venusta*, probably due to the presence of the flavonoid acacetin-7-O-glycopyranoside [13,14].

Additionally, extracts from *P. venusta* have antioxidant, antimicrobial, and antinociceptive properties [13,15,16]. The plant belongs to the family Bignoniaceae that contains numerous constituents such as naphthoquinones of the lapachol type, iridoid glucosides, alkaloids, flavones, triterpenes, polyphenols, tannins and seed oils [17,18]. Such compounds have antioxidant effects, serving as inhibitors or free radical scavengers [16].

Therefore, the aim of our study was to investigate the efficacy of two extracts of *P. venusta* in the treatment of asthma in an animal model.

Materials and methods

Plant material

P. venusta was collected in Jardinópolis, São Paulo, Brazil (21°4'33'' S, 47°44'48'' W), and identified by Dr. Inês Cordeiro (Botanical Institute, Secretariat for Infrastructure and Environment, São Paulo, Brazil). A voucher specimen (HPMU-1458) was deposited at the Herbarium of the University of Ribeirão Preto (UNAERP).

Preparation of plant material

Two extracts, aqueous and hydroethanolic, of *P. venusta* were prepared in the Biotechnology Laboratory of UNAERP, Ribeirão Preto, Brazil. The flowers were dried in an oven at 45°C for 24 hours and then grounded to 40 mesh particle size. The aqueous extract was prepared by adding one liter of boiling water to 100 g of the grounded flowers for 1 hour, filtered through filter paper, and then lyophilized. The hydroethanolic extract was prepared by adding one liter of 70% alcohol to 100 g of the grounded flowers and kept for 10 days. After, the solution was filtered through a filter paper and rota-evaporated.

Phytochemical evaluation of the extracts

The identities of verbascoside, isoverbascoside, quinic acid, and chlorogenic acid, present in the extracts, were confirmed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis using a Waters (Milford, MA, USA) Acquity UPLC *H-Class* system equipped with a PDA detector and a Waters Xevo TQ-S tandem quadrupole mass spectrometer with a Z-spray source operating in the positive mode. The aqueous and hydroethanolic extract injection volume was 5 μ L in a Sigma-Aldrich Ascentis Express C₁₈ column (100 x 4.6 mm i.d.; 2.7 μ m particle size) from SUPELCO Analytical. The mobile phase used for gradient elution consisted of 0.1% formic acid (solvent A) and methanol with 0.1% formic acid (solvent B) at a flow rate of 0.5 mL min⁻¹. The gradient elution program started with 3% B between 0 and 4 min, followed by 3-60% B between 4-15 min and 60-90% B between 15-19 min. Then, it returned to the initial condition (3% B) within the following 5 min. The source and operating parameters were optimized as follows: capillary voltage = 3.4 kV; Z-spray source temperature = 150°C, desolvation temperature (N₂) = 300°C, desolvation gas flow = 600 L h⁻¹, and mass range from *m/z* 100 to 700 in the full-scan mode.

Quantification of the aqueous and hydroethanolic extracts of *P. venusta* by HPLC-MS/MS

Quantification of quinic acid, chlorogenic acid, verbascoside, and isoverbascoside was carried out by HPLC-MS/MS in the multiple reaction monitoring (MRM) (Table 1) mode with the aqueous and hydroethanolic crude extract of *P. venusta*. Quinic acid (catalog n. 46944-U, Supelco) was purchased from Sigma-Aldrich (São Paulo, SP, Brazil), chlorogenic acid (catalog n. 00500590, HWI Analytik GMBH) was purchased from Pharma Solutions, while reference standards of verbascoside and isoverbascoside were furnished by Prof. Dr. Silvia H. Taleb-Contini. The working standard solutions were prepared by diluting the stock solutions (1.0 mg/mL) of the target compounds at followings concentrations: 5, 10, 50, 250, 1,250, 2,500, and 5,000 μ g/mL for 1 and 2, and 5, 25, 50, 125, 250, and 500 μ g/mL for 3 and 4.

Mice

Specific pathogen-free male Balb/C mice (6-8 weeks of age) were used and obtained from the local breeding facility of the Ribeirão Preto Medical School, University of São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil. Mice were housed in separate cages according to the treatment protocol. Water and food were provided *ad libitum*. Experiments were approved by the local Ethics Committee on Animal Use (n. 072/2013).

Allergen sensitization/challenge protocol

Mice were sensitized twice with 10 μ g of OVA (Sigma grade V, Sigma-Aldrich, Dorset, UK) and 1 mg of aluminum hydroxide (Al(OH)₃; Sigma-Aldrich, Dorset, UK), intraperitoneally (ip), seven days

apart. After one week, mice were challenged with nasal instillation of 50 μ L of OVA (10 μ g) under light anesthesia four times on alternate days.

Treatment with extracts of *Pyrostegia venusta*

The aqueous extract of *P. venusta* was diluted in saline 0.9%, and the hydroethanolic extract, in dimethylsulfoxide (DMSO) at 2.5%. Both extracts were administered for seven consecutive days, beginning with the first challenge, at a dose of 300 mg/kg/day. The decision of choosing this dose relied on results of previous studies [13-15]. Control groups received saline on the same days.

Measurement of *in vivo* respiratory mechanisms

Measurements of respiratory parameters were performed as previously described [19-21]. Briefly, 24 h after the last challenge, mice were anesthetized ip (10 mg/kg xylazine and 100 mg/kg ketamine), and a tracheal cannula was inserted. Mice were then connected to a small animal ventilator (FlexVent®, Scireq, Montreal, QC, Canada) and ventilated with a respiratory rate of 150 breaths/minute and a positive end-expiratory pressure (PEEP) of 3 cm H₂O. Then, pancuronium bromide (1.2 mg/kg) was administered and, after the animal was totally paralyzed, airway hyperresponsiveness was measured at baseline and after increasing concentrations (6.25, 12.5, 25, 50 mg/mL) of methacholine (Sigma-Aldrich, Dorset, UK) delivered via an ultrasonic nebulizer (Aeroneb® Lab, Aerogen Ltd, Galway, Ireland). Respiratory mechanics were measured with the forced oscillation technique, using the single compartment and the constant phase model, a mathematical model that allows to partition the changes into the large airway and peripheral compartments [21]. Airway hyperresponsiveness was expressed as total resistance (RRS), total elastance (ERS), tissue resistance (G), and tissue elastance (H).

Bronchoalveolar lavage and cell counting

After the measurement of respiratory parameters, the bronchoalveolar lavage (BAL) was processed as previously described [22,23]. Total cell counts were determined using a standard hemocytometer and trypan blue stain. After, cytospin preparation was stained with Panotico Rapido LB (Laborclin, Pinhais, PR, Brazil), and differential cell counts were determined by counting at least 300 cells.

Ovalbumin-specific IgE levels

After BAL collection, blood was collected and centrifuged. Then, the serum was collected and stored at -80°C for quantification of ovalbumin-specific IgE levels by ELISA (BD Bioscience Pharmingen, San Diego, CA, USA), as previously described [24].

Tissue preparation

The chest wall was opened, and saline was infused into the right ventricle to remove blood. Then, the lungs were removed, and the left lung was inflated with 10% formaldehyde at a transpulmonary pressure of 25 cm H₂O, and subsequently processed and embedded in paraffin blocks. The right lobe (50 mg) was homogenized in 1 mL of PBS containing protease inhibitor cocktail (Complete Inhibitor, Roche Diagnostics, Laval, PQ, Canada), and the supernatant collected and stored at -80°C for analysis of cytokine production.

Cytokine measurement

The levels of IL-4, IL-5, IL-9, IL-10, IL-13, interferon (IFN)-gamma, and tissue growth factor (TGF)-beta were measured by ELISA according to the manufacturer's protocols (BD Biosciences BD OptEIA™, San Diego, CA and eBioscience, San Diego, CA).

Antioxidant activity

The total antioxidant capacity (TAC) was assessed in the lung tissue supernatant by reduce radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) (Sigma-Aldrich, Dorset, UK), as previously described [25].

Histological analysis

Histological analysis was performed as previously described [22,23]. Sagittal tissue sections with a thickness of 5 µm were cut, and hematoxylin and eosin (H&E) staining was performed to evaluate peribronchial inflammation. Quantification was carried out on five airways with intact epithelial lining and a minimum to a maximum internal diameter greater or equal to 0.5. Measurements were performed using the software ImageJ (NIH, Bethesda, MA, USA). Peribronchial inflammation was quantified as the number of inflammatory cells, including granulocytes and mononuclear cells, present in an area extending from the basement membrane to the alveolar attachments, and expressed as the number of inflammatory cells per area.

Statistical analysis

The data were reported as means and standard errors and analyzed using GraphPad Prism software version 5.0 (GraphPad-Prism Software Inc., San Diego, CA, USA). Differences between groups were analyzed by one-way or two-way analysis of variance (ANOVA) followed by Bonferroni post-test, and Kruskal-Wallis test with Dunn's multiple comparison post-test when data were not normally distributed. Differences were considered statistically significant at $p < 0.05$.

Results

Identification and quantification of components isolated from aqueous and hydroethanolic extracts of *P. venusta*

Four substances, quinic acid, chlorogenic acid, verbascoside, and isoverbascoside (Figure 1, and Figures S1-S9) were identified and quantified in aqueous and hydroethanolic extracts of *P. venusta*. The amount of quinic acid was higher than the other substances in aqueous extract (69.5 ± 4.7 µg/mL) and hydroethanolic extract (64.2 ± 6.2 m µg/mL), while chlorogenic acid was present in smaller amounts (1.9 ± 0.1 µg/mL, in both extracts). The phenylpropanoid verbascoside was present in aqueous and hydroethanolic extracts at a concentration of 36.3 ± 2.5 µg/mL and 38.1 ± 2.1 µg/mL, respectively; while the isomer isoverbascoside was found at the concentration of 45.2 ± 5.9 µg/mL in aqueous extract, and 49.6 ± 3.7 µg/mL in hydroethanolic extract. The quantification of these components from extracts of *P. venusta* is shown in Table 2.

Only the aqueous extract of *P. venusta* reduces airway hyperresponsiveness

Lung mechanics is shown in Figure 2. OVA-challenged mice had a significant increase in airway hyperresponsiveness when compared to saline-challenged mice ($p < 0.05$). The administration of aqueous extract of *P. venusta* significantly decreased airway hyperresponsiveness, measured by RRS

($p < 0.01$), ERS ($p < 0.01$), G ($p < 0.01$), and H ($p < 0.001$). However, the hydroethanolic extract of *P. venusta* did not significantly decrease airway hyperresponsiveness when compared to OVA-challenged mice.

The administration of *P. venusta* aqueous and hydroethanolic extracts reduces total cell and eosinophil counts in the BAL

Total and differential BAL cell counts are shown in Figure 3. Total cell and eosinophil numbers in BAL were significantly increased in OVA-challenged mice compared to saline-challenged mice ($p < 0.001$). Mice treated with both extracts, aqueous and hydroethanolic, of *P. venusta* had a significant decrease in total cell and eosinophil counts when compared to OVA-challenged mice ($p < 0.001$).

***P. venusta* aqueous extract administration reduces lung inflammation**

OVA-challenged mice had a significant increase in the number of inflammatory cells in the peribronchial area as compared to saline-challenged group ($p < 0.001$). The administration of *P. venusta* aqueous extract significantly attenuated lung inflammation when compared to OVA-challenged mice ($p < 0.05$) (Figure 4).

Aqueous and hydroethanolic extracts of *P. venusta* do not significantly change inflammatory cytokines levels in lung homogenate

The levels of IL-4, IL-5, IL-13, and TGF-beta were significantly increased in OVA-challenged mice when compared to saline-challenged mice ($p < 0.05$). The administration of both extracts of *P. venusta* did not significantly decrease the levels of cytokines in lung homogenate. Levels of IFN-gamma, IL-9, and IL-10 did not reach statistical difference among groups (Figure 5).

OVA-specific IgE levels in serum

OVA-specific IgE levels were significantly increased in the OVA-challenged group when compared to the saline-challenged mice ($p < 0.001$). The treatment with aqueous and hydroethanolic extracts of *P. venusta* did not significantly change the levels of OVA-specific IgE (Figure 6).

***P. venusta* aqueous extract administration increases antioxidant activity in lung tissue**

The levels of TAC are shown in Figure 6. TAC was significantly increased in the group treated with aqueous extract of *P. venusta* when compared to OVA-challenged mice and its respective saline control ($p < 0.05$). Hydroethanolic extract of *P. venusta* did not significantly change TAC (Figure 6).

Discussion

Asthma is currently treated with synthetic drugs, such as bronchodilators, corticosteroids, leukotriene antagonists, monoclonal antibodies; however, they have a high production cost, and many asthmatics do not reach adequate control [26,27]. Additionally, approximately 5 to 10% of patients with severe asthma have a poorly controlled disease that could be refractory to the usual treatment [28]. Moreover, new biologic therapies have not been successful in all patients [29]. Therefore, an alternative or complementary approach to asthma treatment could be the use of medicinal plants

[30]. Blanc *et al.* [31] showed that 24% of asthmatic patients have already used herbs as additional therapy.

In the current study, for the first time, we examined the effects of *P. venusta* on asthma treatment in OVA-challenged mice. Furthermore, we evaluated two different standardized extracts of *P. venusta*, an aqueous and a hydroethanolic extract. The administration of aqueous extract attenuated bronchial hyperresponsiveness and the influx of inflammatory cells to the airway and lung, and increased the antioxidant capacity. In contrast, the hydroethanolic extract decreased airway inflammation but did not significantly change bronchial hyperresponsiveness and antioxidant capacity. The reduction in the number of total cells and eosinophils in BAL confirm the anti-inflammatory effect of *P. venusta* [13,14]. As asthma is an inflammatory disease, the reduction of inflammatory cells in the airway, especially eosinophils, is a significant outcome.

Previous studies have demonstrated, *in vitro* and *in vivo*, the antioxidant activity of *P. venusta* [16,32,33]. In our study, the aqueous extract of *P. venusta* significantly augmented TAC. Therefore, considering that both extracts did not change Th1/Th2 balance and allergen sensitization, we hypothesized that *P. venusta*, especially the aqueous extract, acts in the oxidative/antioxidant balance, mainly stimulating the antioxidant capacity.

The imbalance in ROS and antioxidants contribute to the pathogenesis of asthma [6,7,34]. Activated inflammatory cells, such as eosinophils, neutrophils, monocytes, and macrophages, can produce ROS, which is, at least partially, correlated with airway hyperresponsiveness [35,36]. On the other hand, low plasma levels of antioxidants are associated with more severe asthma [37]. Some studies have demonstrated that oral antioxidant supplementation to mild asthmatic patients improved lung function and decreased airway or systemic inflammation [8,38]. It has also been shown that the administration of vanillic acid, *Ziziphora clinopodioides*, or phytoconstituents (phyllanthin and hypophyllanthin) of *Phyllanthus amarus*, reduced immune-inflammatory response and oxidative stress in OVA-induced asthma [39-41]. However, given the poor pharmacokinetic properties that conventional antioxidants may have, some authors suggest the development of new nano-antioxidant therapies [42].

Several ROS and antioxidants can be assessed when studying oxidative stress. However, as some techniques are time-consuming, TAC evaluation has gained popularity among researchers [8]. Moreover, TAC is a useful index of overall non-enzymatic antioxidant potential and is positively correlated with lung function [8,43]. In our study, only aqueous extract of *P. venusta* significantly augmented TAC, suggesting that the antiasthmatic effects of *P. venusta* are, at least partially, mediated by its antioxidant activity.

The reduction of bronchial hyperresponsiveness observed with *P. venusta* aqueous extract may be related to the decrease of total cells and eosinophils in BAL, associated with an increase in the antioxidant capacity. The release of chemical mediators stimulates bronchial smooth muscle contraction and inflammation of the mucosa, causing edema and muscular spasm, simultaneously producing ROS [6,7,34]. Thus, the aqueous extract of *P. venusta* may have attenuated inflammation and hyperresponsiveness via increased antioxidant levels.

Several studies have shown that hydroethanolic extract of *P. venusta* has anti-inflammatory action [15,33], which was confirmed in our study. However, the reduction of inflammation was not sufficient to reduce bronchial hyperresponsiveness. As asthma is a complex heterogeneous disease with several mechanisms involved, in our model, inflammation was not directly linked to hyperresponsiveness [44,45]. Corroborating our findings, other studies have demonstrated that there is not always a direct correlation between hyperresponsiveness and inflammation [3,46].

Veloso *et al.* [13] analyzed the effects of a hydroethanolic extract of *P. venusta* on lipopolysaccharide-induced symptoms in mice at doses of 30, 100 and 300 mg/kg and demonstrated that the best response was obtained at the dose of 300 mg/kg. Although we did not perform a dose-response curve, we used the highest dose previously described. Further studies with larger doses and longer treatment are needed, especially to assess whether the hydroethanolic extract may also have a beneficial effect on the treatment of asthma.

One of the possible explanations for the action of *P. venusta* extracts is the chemical compounds present in this plant. *P. venusta* is rich in terpenoids, alkaloids, and tannins, among others, and all these compounds have antioxidant properties [17]. In addition, it contains a large quantity of flavonoids and phenylpropanoids that are natural antioxidants, helping to prevent the damage caused by oxidative stress [32].

Additionally, quinic acid and chlorogenic acid found in *P. venusta* extracts may have contributed to these effects. Quinic acid links to caffeic acid, resulting in chlorogenic acid, which has antioxidant, anti-inflammatory, and anti-asthmatic effects [47].

Previous studies on the structure-activity relationship of phenolic substances in different models have shown that the antioxidant activity of phenylethanoid glycosides is associated with the presence of the *ortho*-dihydroxyphenyl group [48]. Besides this, caffeic acid derivatives, such as chlorogenic acid, verbascoside, and isoverbascoside, have shown anti-inflammatory and anti-asthmatic effects [47].

Erdemli *et al.* demonstrated that caffeic acid phenethyl ester has potent anti-inflammatory and immunomodulatory effects [49]. In this way, we can suggest that both caffeoyl and phenethyl groups can be responsible for the anti-asthmatic effect of the substances found in the extracts of *P. venusta*.

One limitation of this study was that we did not evaluate the exact mechanism of action of *P. venusta*. Since a large number of compounds modulate nuclear factor kappa-B (NF- κ B) activity, including verbascoside, isoverbascoside, quinic acid, and chlorogenic acid [50-52], we cannot rule out the possibility that *P. venusta* extract could have acted via NF- κ B inhibition, besides increasing TAC. Another possible mechanism could be related to mitochondrial dysfunction, ROS formation, and lipid peroxidation [52-55]. Further studies with different methods are needed to better clarify its mechanism of action.

Conclusions/key findings

In conclusion, aqueous extract of *P. venusta* reduced bronchial hyperresponsiveness, lung and airway inflammation, probably via an antioxidant mechanism. These results demonstrate that *P. venusta* may have potential for asthma treatment and could be considered for future clinical trials.

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Declaration of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Martinez FD, Vercelli D. Asthma. *Lancet*. 2013 Oct;382(9901):1360-72.
2. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention, 2017. <http://www.ginasthma.org2017>.
3. Busse WW. The relationship of airway hyperresponsiveness and airway inflammation: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest*. 2010 Aug;138(2 Suppl):4S-10S.
4. Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. *J Allergy Clin Immunol*. 2011 May 31;128(3):451-462.
5. Mishra V, Banga J, Silveyra P. Oxidative stress and cellular pathways of asthma and inflammation: Therapeutic strategies and pharmacological targets. *Pharmacol Ther*. 2018 01;181:169-182.
6. Kirkham P, Rahman I. Oxidative stress in asthma and COPD: antioxidants as a therapeutic strategy. *Pharmacol Ther*. 2006 Aug;111(2):476-94.
7. Sahiner UM, Birben E, Erzurum S, et al. Oxidative stress in asthma. *World Allergy Organ J*. 2011 Oct;4(10):151-8.
8. Nadeem A, Chhabra SK, Masood A, et al. Increased oxidative stress and altered levels of antioxidants in asthma. *J Allergy Clin Immunol*. 2003 Jan;111(1):72-8.
9. Fatani SH. Biomarkers of oxidative stress in acute and chronic bronchial asthma [Article]. *Journal of Asthma*. 2014 Aug;51(6):578-584.
10. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. *Lancet*. 2014 May;383(9928):1581-92.
11. Flores KF, Bandoli G, Chambers CD, et al. Asthma prevalence among women aged 18 to 44 in the United States: National health and nutrition examination survey 2001-2016 [Article; Early Access]. *Journal of Asthma*. 2019:10.
12. Ferreira DT, Alvares PSM, Houghton PJ, et al. Chemical constituents from roots of *Pyrostegia venusta* and considerations about its medicinal importance. *Quimica Nova*. 1999;23(1).
13. Veloso CC, Bitencourt AD, Cabral LD, et al. *Pyrostegia venusta* attenuate the sickness behavior induced by lipopolysaccharide in mice. *J Ethnopharmacol*. 2010 Oct;132(1):355-8.
14. Veloso CC, Cabral LDM, Bitencourt AD, et al. Anti-inflammatory and antinociceptive effects of the hydroethanolic extract of the flowers of *Pyrostegia venusta* in mice. *Revista Brasileira de Farmacognosia* 2012;22(1).
15. Veloso CeC, de Oliveira MC, Oliveira CaC, et al. Hydroethanolic extract of *Pyrostegia venusta* (Ker Gawl.) Miers flowers improves inflammatory and metabolic dysfunction induced by high-refined carbohydrate diet. *J Ethnopharmacol*. 2014 Jan;151(1):722-8.
16. Roy P, Amdekar S, Kumar A, et al. In vivo antioxidative property, antimicrobial and wound healing activity of flower extracts of *Pyrostegia venusta* (Ker Gawl) Miers. *J Ethnopharmacol*. 2012 Mar;140(1):186-92.
17. Mostafa NM, El-Dahshan O, Singab ANB. *Pyrostegia venusta* (Ker Gawl.) Miers: a botanical, pharmacological and phytochemical review. *Medicinal & Aromatic Plants*. 2013;2(3).
18. Gachet MS, Schühly W. *Jacaranda*--an ethnopharmacological and phytochemical review. *J Ethnopharmacol*. 2009 Jan;121(1):14-27.
19. Borges MC, Marchica CL, Narayanan V, et al. Allergen challenge during halothane compared to isoflurane anesthesia induces a more potent peripheral lung response. *Respiratory Physiology & Neurobiology*. 2013 OCT 1 2013;189(1):144-152.
20. Marchica CL, Pinelli V, Borges M, et al. A role for decorin in a murine model of allergen-induced asthma. *Am J Physiol Lung Cell Mol Physiol*. 2011 Jun;300(6):L863-73.
21. Hantos Z, Daroczy B, Suki B, et al. Input impedance and peripheral inhomogeneity of dog lungs. *J Appl Physiol*. 1992 Jan;72(1):168-78.

22. Borges MC, Marchica CL, Narayanan V, et al. Allergen challenge during halothane compared to isoflurane anesthesia induces a more potent peripheral lung response. *Respir Physiol Neurobiol.* 2013 Oct;189(1):144-52.
23. Marchica CL, Pinelli V, Borges M, et al. A role for decorin in a murine model of allergen-induced asthma. *American Journal of Physiology-Lung Cellular and Molecular Physiology.* 2011 JUN 2011;300(6):L863-L873.
24. Fonseca VMB, Milani TMS, Prado R, et al. Oral administration of *Saccharomyces cerevisiae* UFMG A-905 prevents allergic asthma in mice. *Respirology.* 2017 Jul;22(5):905-912.
25. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem.* 2004 Apr;37(4):277-85.
26. Smith DH, Malone DC, Lawson KA, et al. A national estimate of the economic costs of asthma. *Am J Respir Crit Care Med.* 1997 Sep;156(3 Pt 1):787-93.
27. Olin JT, Wechsler ME. Asthma: pathogenesis and novel drugs for treatment. *BMJ.* 2014 Nov;349:g5517.
28. Kupczyk M, Wenzel S. U.S. and European severe asthma cohorts: what can they teach us about severe asthma? *J Intern Med.* 2012 Aug;272(2):121-32.
29. Gauthier M, Ray A, Wenzel SE. Evolving Concepts of Asthma. *Am J Respir Crit Care Med.* 2015 Sep;192(6):660-8.
30. Shergis JL, Wu L, Zhang AL, et al. Herbal medicine for adults with asthma: A systematic review [Review]. *Journal of Asthma.* 2016;53(6):650-659.
31. Blanc PD, Trupin L, Earnest G, et al. Alternative therapies among adults with a reported diagnosis of asthma or rhinosinusitis : data from a population-based survey. *Chest.* 2001 Nov;120(5):1461-7.
32. Pereira AM, Hernandez C, Pereira SI, et al. Evaluation of anticandidal and antioxidant activities of phenolic compounds from *Pyrostegia venusta* (Ker Gawl.) Miers. *Chem Biol Interact.* 2014 Dec;224:136-41.
33. Roy P, Amdekar S, Kumar A, et al. Preliminary study of the antioxidant properties of flowers and roots of *Pyrostegia venusta* (Ker Gawl) Miers. *BMC Complement Altern Med.* 2011;11:69.
34. Ahmad A, Shameem M, Husain Q. Relation of oxidant-antioxidant imbalance with disease progression in patients with asthma. *Ann Thorac Med.* 2012 Oct;7(4):226-32.
35. Dong CC, Yin XJ, Ma JY, et al. Effect of diesel exhaust particles on allergic reactions and airway responsiveness in ovalbumin-sensitized brown Norway rats. *Toxicol Sci.* 2005 Nov;88(1):202-12.
36. de Boer J, Meurs H, Flendrig L, et al. Role of nitric oxide and superoxide in allergen-induced airway hyperreactivity after the late asthmatic reaction in guinea-pigs. *Br J Pharmacol.* 2001 Aug;133(8):1235-42.
37. Misso NL, Brooks-Wildhaber J, Ray S, et al. Plasma concentrations of dietary and nondietary antioxidants are low in severe asthma. *Eur Respir J.* 2005 Aug;26(2):257-64.
38. Hoskins A, Roberts JL, Milne G, et al. Natural-source d- α -tocopheryl acetate inhibits oxidant stress and modulates atopic asthma in humans in vivo. *Allergy.* 2012 May;67(5):676-82.
39. Wu W, Li Y, Jiao Z, et al. Phyllanthin and hypophyllanthin from *Phyllanthus amarus* ameliorates immune-inflammatory response in ovalbumin-induced asthma: role of IgE, Nrf2, iNOs, TNF- α , and IL's. *Immunopharmacol Immunotoxicol.* 2019 Feb;41(1):55-67.
40. Bai F, Fang L, Hu H, et al. Vanillic acid mitigates the ovalbumin (OVA)-induced asthma in rat model through prevention of airway inflammation. *Biosci Biotechnol Biochem.* 2019 Mar;83(3):531-537.
41. Ahsan F, Shabbir A, Shahzad M, et al. Amelioration of allergic asthma by *Ziziphora clinopodioides* via upregulation of aquaporins and downregulation of IL4 and IL5. *Respir Physiol Neurobiol.* 2019 08;266:39-46.
42. Eftekhari A, Dizaj SM, Chodari L, et al. The promising future of nano-antioxidant therapy against environmental pollutants induced-toxicities. *Biomed Pharmacother.* 2018 Jul;103:1018-1027.

43. Yoon SY, Kim TB, Baek S, et al. The impact of total antioxidant capacity on pulmonary function in asthma patients. *Int J Tuberc Lung Dis.* 2012 Nov;16(11):1544-50.
44. Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *NEnglJMed.* 1990;323(15):1033-1039.
45. Bousquet J, Jeffery PK, Busse WW, et al. Asthma. From bronchoconstriction to airways inflammation and remodeling. *AmJRespirCrit Care Med.* 2000;161(5):1720-1745.
46. Janssen-Heininger YM, Irvin CG, Scheller EV, et al. Airway Hyperresponsiveness and Inflammation: Causation, Correlation, or No Relation? *J Allergy Ther.* 2012 Jan;2012(Suppl 1).
47. Kim HR, Lee DM, Lee SH, et al. Chlorogenic acid suppresses pulmonary eosinophilia, IgE production, and Th2-type cytokine production in an ovalbumin-induced allergic asthma: activation of STAT-6 and JNK is inhibited by chlorogenic acid. *Int Immunopharmacol.* 2010 Oct;10(10):1242-8.
48. Jiménez C, Riguera R. Phenylethanoid glycosides in plants: structure and biological activity. *Nat Prod Rep.* 1994 Dec;11(6):591-606.
49. Erdemli HK, Akyol S, Armutcu F, et al. Antiviral properties of caffeic acid phenethyl ester and its potential application. *J Intercult Ethnopharmacol.* 2015 2015 Oct-Dec;4(4):344-7.
50. Tian L, Su CP, Wang Q, et al. Chlorogenic acid: A potent molecule that protects cardiomyocytes from TNF- α -induced injury via inhibiting NF- κ B and JNK signals. *J Cell Mol Med.* 2019 Jul;23(7):4666-4678.
51. Jing W, Chunhua M, Shumin W. Effects of acteoside on lipopolysaccharide-induced inflammation in acute lung injury via regulation of NF- κ B pathway in vivo and in vitro. *Toxicol Appl Pharmacol.* 2015 Jun;285(2):128-35.
52. Lee JW, Seo KH, Ryu HW, et al. Anti-inflammatory effect of stem bark of *Paulownia tomentosa* Steud. in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and LPS-induced murine model of acute lung injury. *J Ethnopharmacol.* 2018 Jan;210:23-30.
53. Iyer D, Mishra N, Agrawal A. Mitochondrial Function in Allergic Disease. *Curr Allergy Asthma Rep.* 2017 May;17(5):29.
54. Tsai KL, Hung CH, Chan SH, et al. Chlorogenic Acid Protects Against oxLDL-Induced Oxidative Damage and Mitochondrial Dysfunction by Modulating SIRT1 in Endothelial Cells. *Mol Nutr Food Res.* 2018 06;62(11):e1700928.
55. de Moura Sperotto ND, Steffens L, Veríssimo RM, et al. Wound healing and anti-inflammatory activities induced by a *Plantago australis* hydroethanolic extract standardized in verbascoside. *J Ethnopharmacol.* 2018 Oct;225:178-188.

Table 1. Ion transitions, instrument settings and retention times for each quantified compound.

Compound	Precursor ion (m/z)	Product ion (m/z)	DP ^a (V)	CE ^b (eV)	RT ^c (min)
quinic acid	191.1	85.2	40	25	1.92
chlorogenic acid	353.2	191.3	40	25	2.11
verbascoside	623.1	161.1	40	35	4.08
isoverbascoside	623.2	133.1	40	53	4.45

^a DP = Declustering Potential

^b CE = Collision Energy (Ar was used as collision gas)

^c RT = Retention Time

Table 2 - Quantification of the isolated components from the aqueous and hydroethanolic extracts of *Pyrostegia venusta*.

	Aqueous extract	Hydroethanolic extract
Quinic Acid	69.5 ± 4.7 µg /mL	64.2 ± 6.2 µg /ml
Chlorogenic Acid	1.9 ± 0.1 µg /mL	1.9 ± 0.1 µg /mL
Verbascoside	36.3 ± 2.5 µg /mL	38.1 ± 2.1 µg /ml
Isoverbascoside	45.2 ± 5.9 µg /mL	49.6 ± 3.7 µg /ml

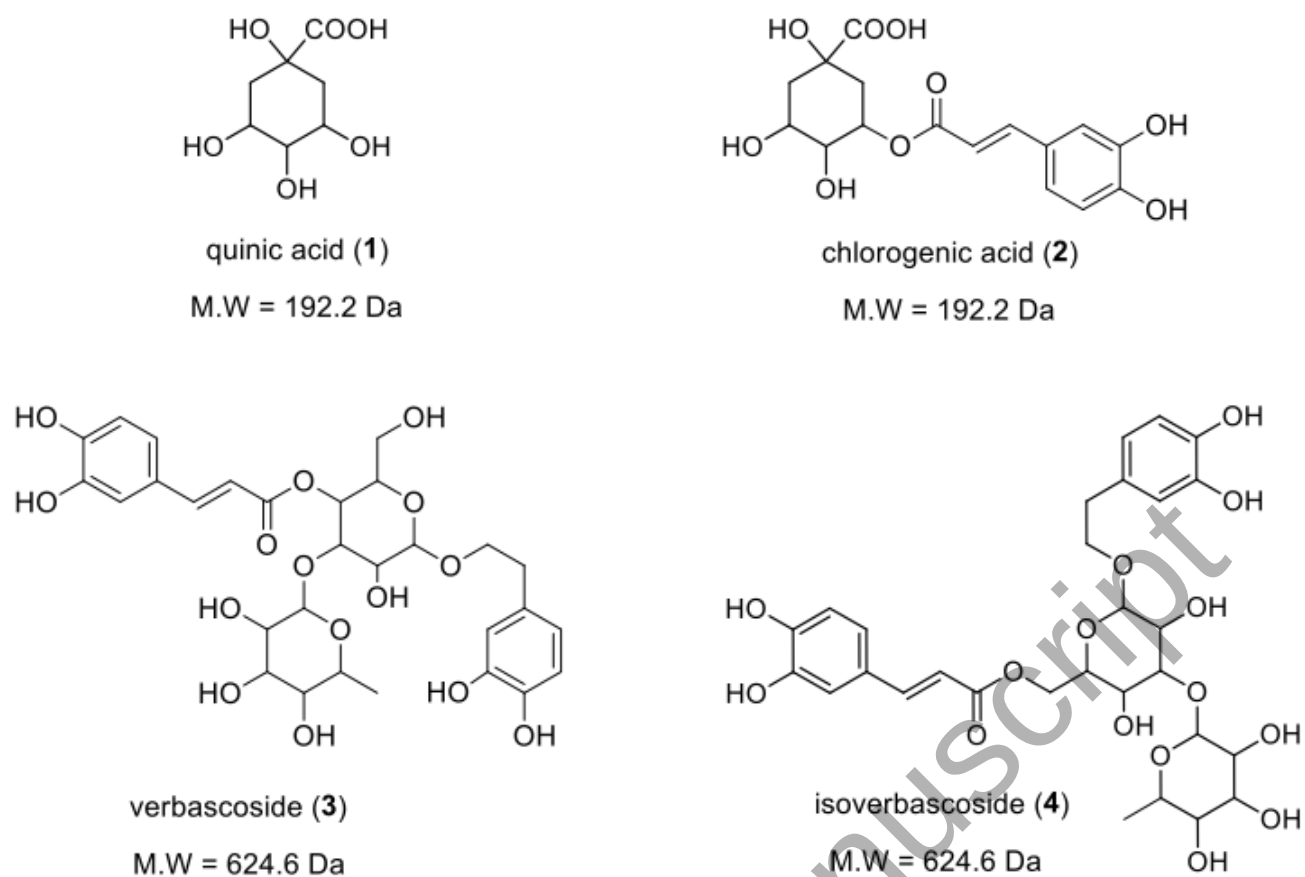


Figure 1: Quinic acid (1), chlorogenic acid (2), verbascoside (3), and isoverbascoside (4) detected in extracts of *Pyrostegia venusta*.

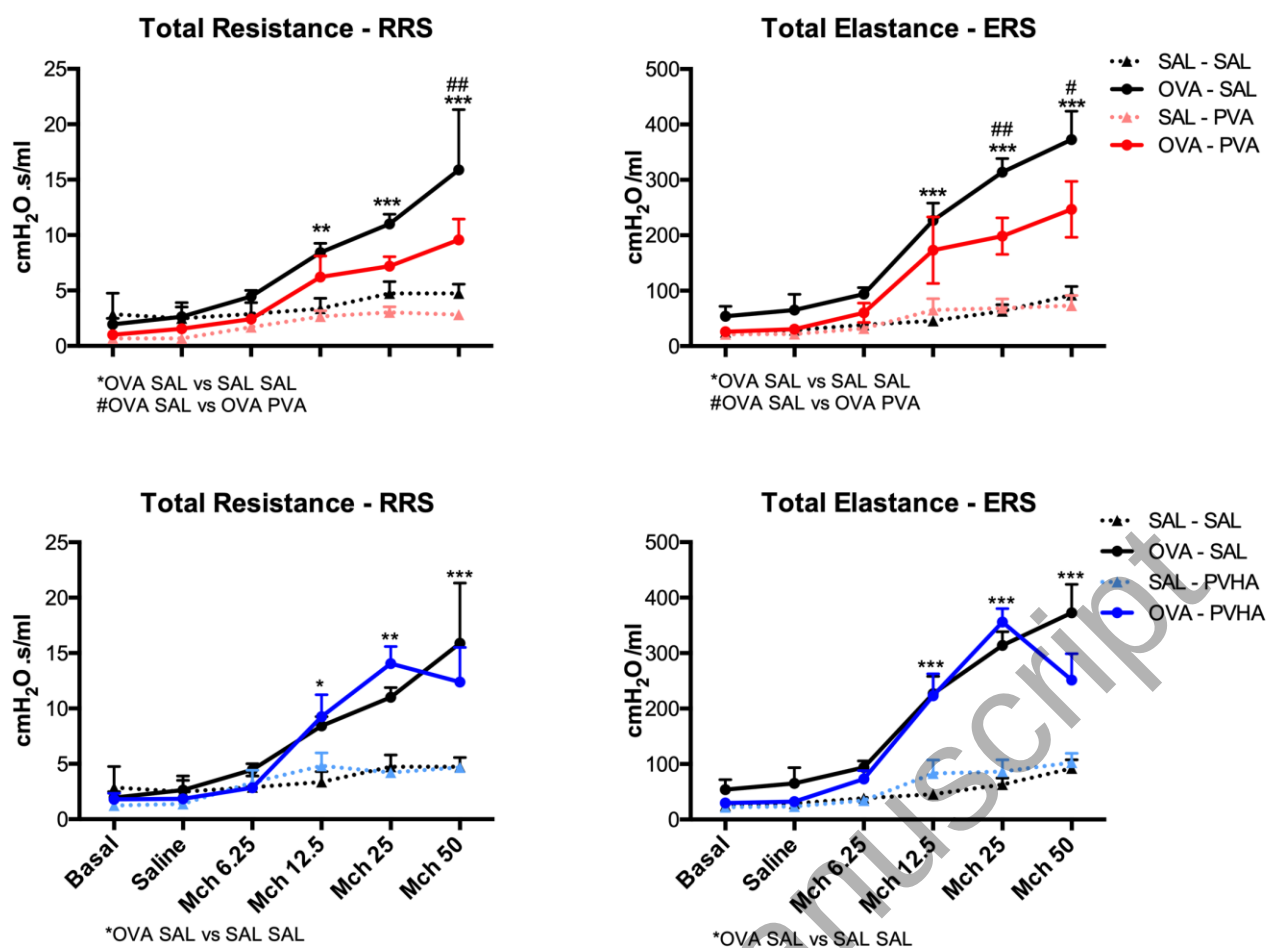


Figure 2. Effect of aqueous and hydroethanolic extracts of *Pyrostegia venusta* on airway hyperresponsiveness to methacholine in OVA-challenged mice. Airway responsiveness was measured in response to increasing concentration of methacholine (RRS: total resistance, ERS: total elastance). Values are shown as mean $\pm$ SEM (n = 8 to 10). *p < 0.05, **p < 0.01, ***p < 0.001.

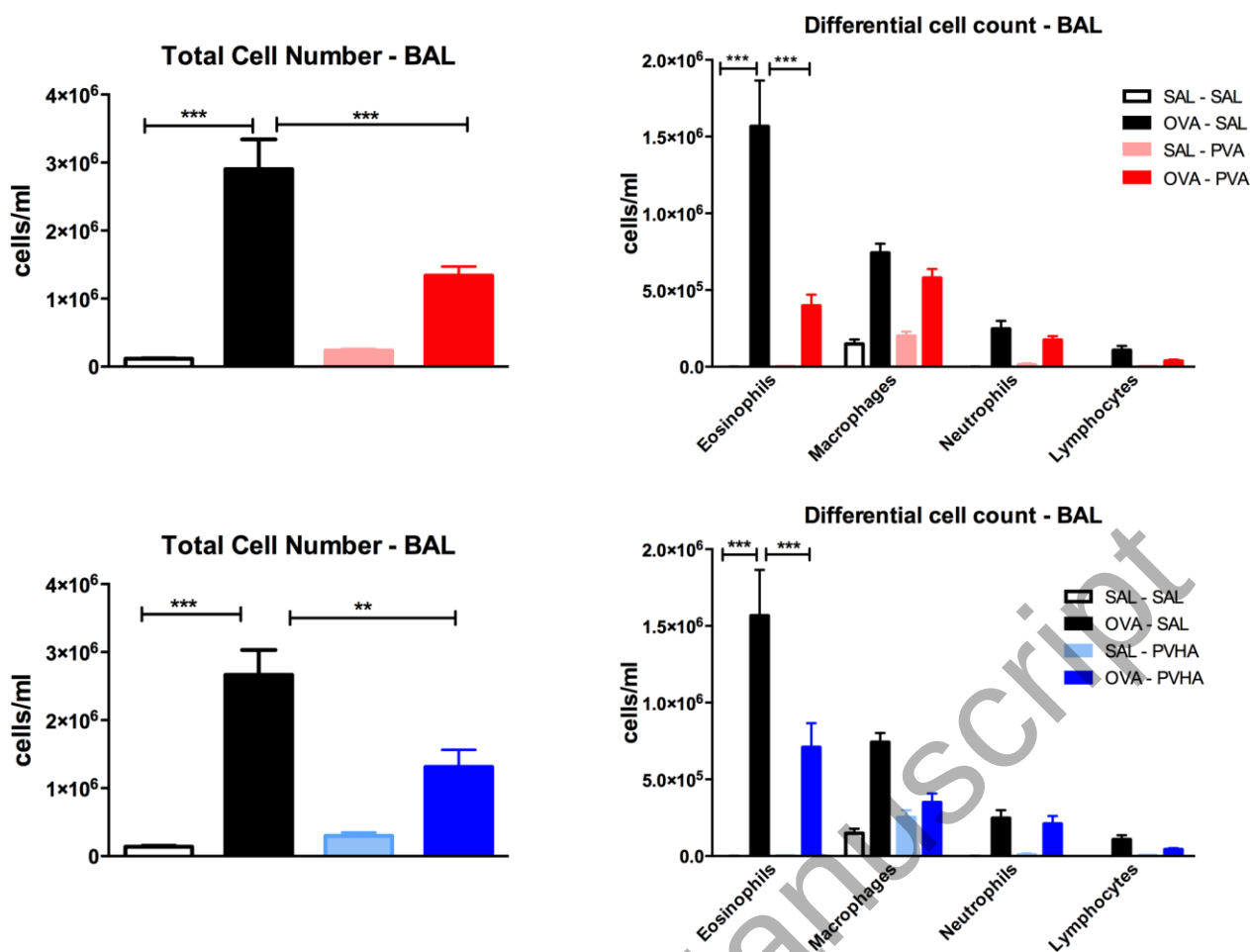


Figure 3. Effect of hydroethanolic and aqueous extracts of *Pyrostegia venusta* on total (A) and differential (B) cell counts in BAL fluid from OVA-challenged mice. Values are shown as mean $\pm$ SEM (n = 8 to 10). *p < 0.05, **p < 0.01, ***p < 0.001.

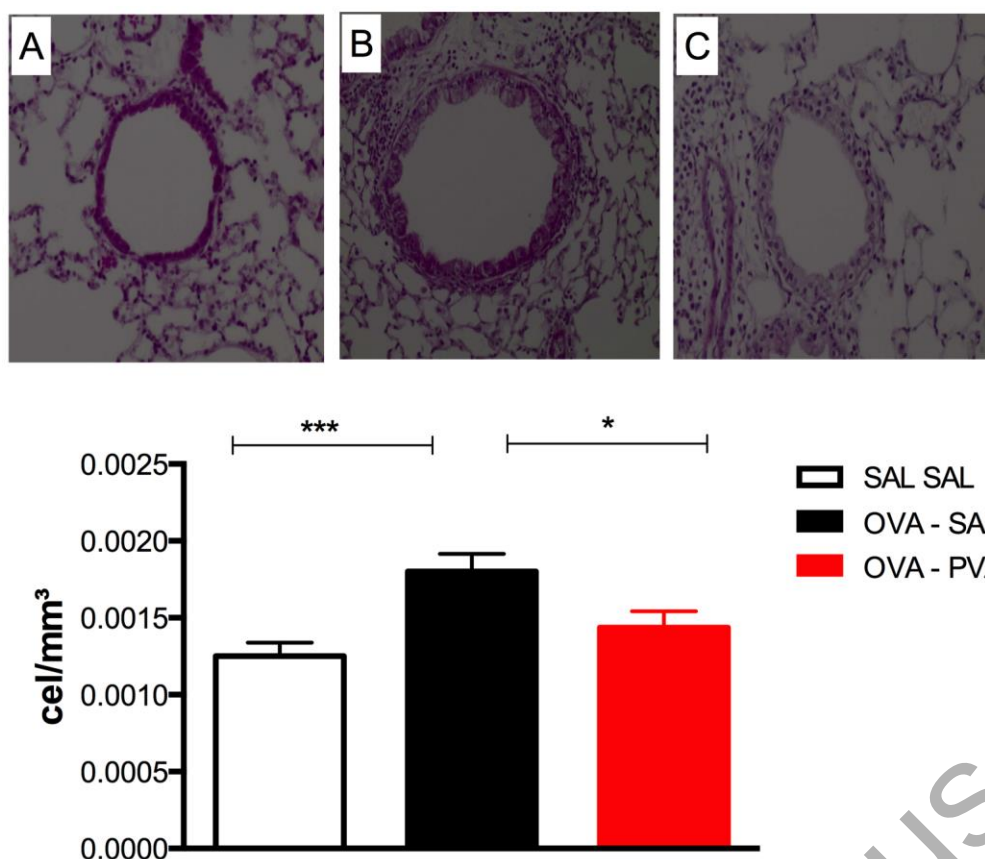


Figure 4. A-C: photomicrographs depicting tissue inflammation (hematoxylin and eosin). A: Saline-challenged mice. B: OVA-challenged, sham-treated mice. C: OVA-challenged, *Pyrostegia venusta* aqueous extract treated mice. Values are shown as mean $\pm$ SEM (n = 5-6). *p<0.05; ***p<0.001.

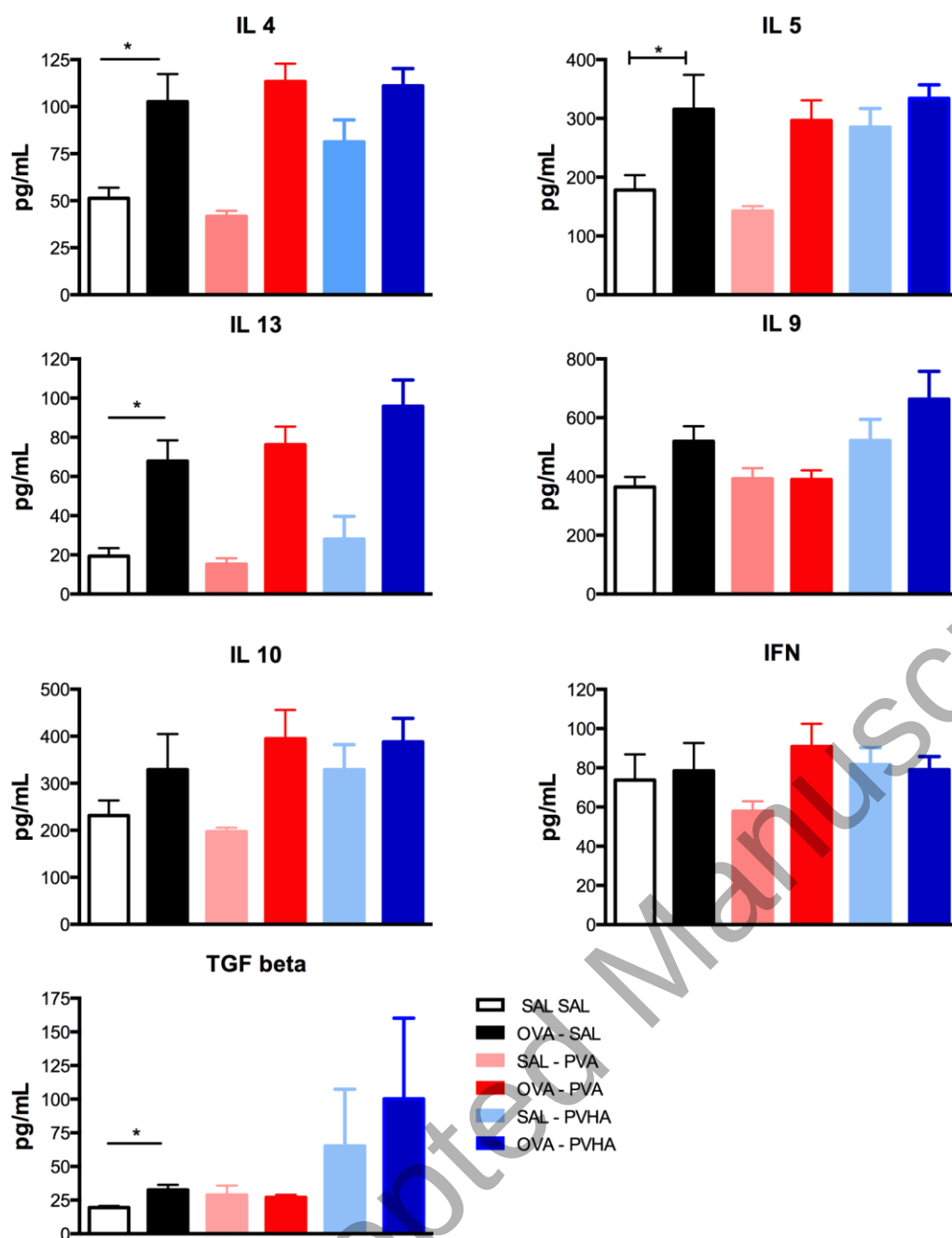


Figure 5. Effects of aqueous and hydroethanolic extracts *Pyrostegia venusta* on inflammatory cytokines levels in lung homogenate from OVA-challenged mice. Protein levels were measured by ELISA. Values are shown as mean $\pm$ SEM (n = 6 to 8). *p < 0.05.

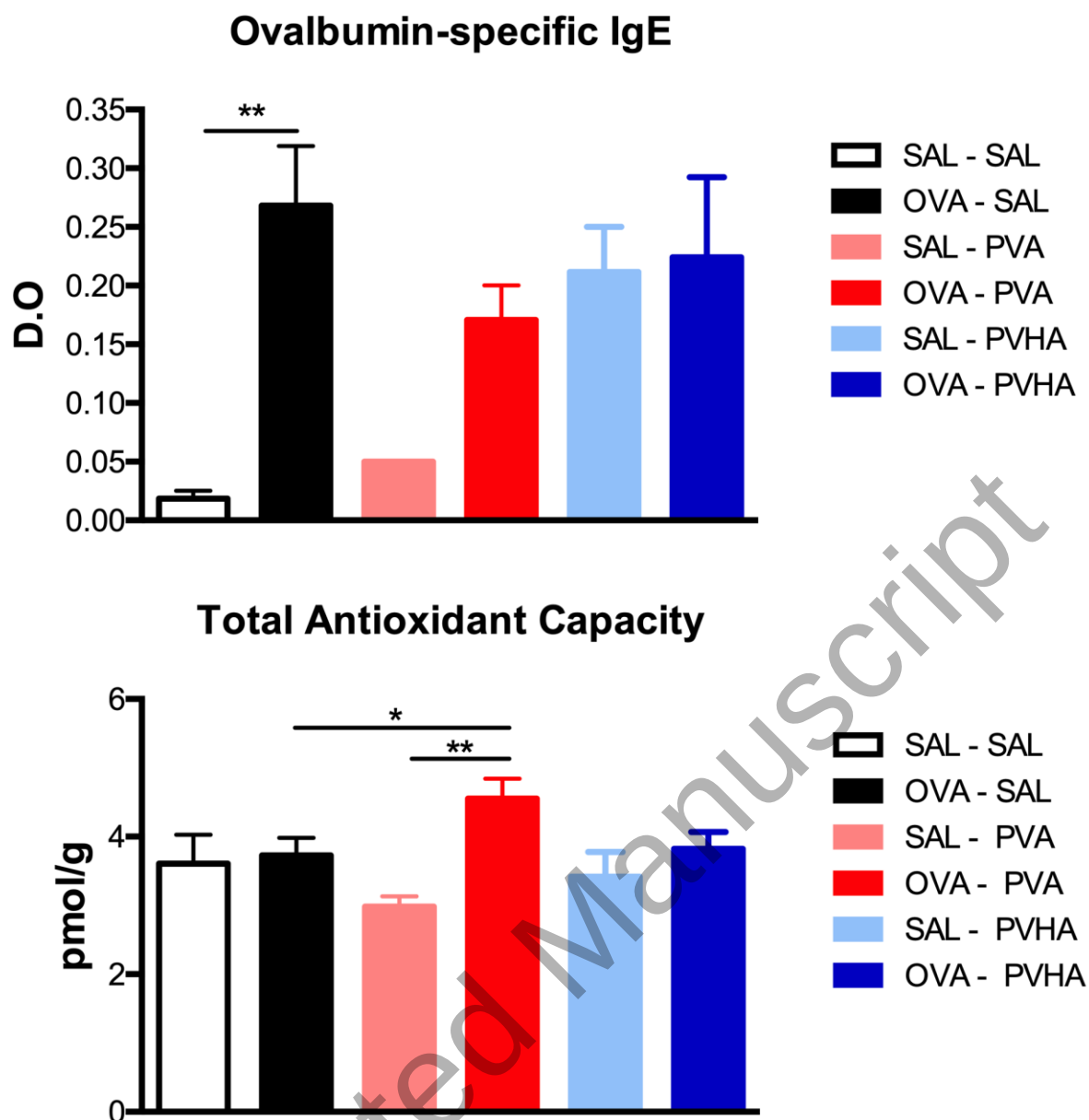


Figure 6. Effects of aqueous and hydroethanolic extracts of *Pyrostegia venusta* on OVA-specific IgE levels in serum and on total antioxidant activity in tissue lung from OVA-challenged mice. Values are shown as mean $\pm$ SEM (n = 6 to 8. *p < 0.05; ***p < 0.001).