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Full Length Research Paper

Dual effect of *Taraxacum officinale* leaves: Anticholinergic and inhibitory effect on inflammatory cells in ovalbumin-sensitized guinea-pigs

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Bronchospasm is an important characteristic of allergen-induced airway inflammation. *Taraxacum officinale* herb is believed to have several therapeutic effects including anti-inflammatory properties. The anticholinergic effect of the *T. officinale* leaves extract (TOLE) was determined in ovalbumin (OA)-sensitized guinea-pig trachea. The test drugs (TOLE or prednisolone) were administered orally 1 h prior to aerosolized 2% ovalbumin challenge. Isolated ovalbumin-sensitized guinea-pig tracheal was challenged with acetylcholine and OA in the absence and presence of TOLE. The blood samples were collected and neutrophils, lymphocytes and monocytes (eosinophils, basophils and macrophages) counts assayed. Prednisolone (2.5 mg/kg body weight (BW) orally) was used as reference standard. TOLE application showed significant antagonistic effect on contraction of trachea to both acetylcholine (35.10 ± 0.04) and OA (18.6 ± 1.5). Also, the extract reduced monocytes (0.62 ± 0.23), lymphocytes (3.4 ± 0.59) and neutrophils (3.65 ± 0.20) counts in OA-sensitized guinea-pigs. Thus, TOLE possesses anticholinergic and reduces neutrophil, eosinophil and basophil counts in ovalbumin-sensitized guinea-pigs.

Key words: *Taraxacum officinale*, inflammation, blood cell counts, anticholinergic.

INTRODUCTION

Increased responsiveness of trachea, bronchi and bronchioles to cognate stimuli is the hall mark of bronchial asthma. This is usually manifested as broncho-constriction and bronchial secretions which are outcome of an immediate hypersensitivity reaction (Ashenafi et al., 2008). Bronchial asthma affects about 7 to 10% of world population (Govindan et al., 1999). Bronchoconstriction is a key player in the pathophysiology of asthma. Increasing evidence suggests that eosinophils, basophils and other cellular elements are critical to the pathogenesis of asthma (Brightling et al., 2002; Busse and Rosenwasser, 2003). During allergen-induced airway inflammation, eosinophils release major binding proteins (MBP) from it cytoplasmic granules in the lungs which act as an allosteric antagonist to M_2 muscarinic receptors. M_2-receptors usually function as negative feedback by inhibiting the release of acetylcholine from parasympathetic nerves. However, dysfunctioning of M_2-receptors in asthmatic patients as a result of antagonistic activity of MBP causes an intense bronchoconstriction and mucus secretion in the airways (Roffel et al., 1990).

Anti-inflammatory agents, such as corticosteroids, β_2-adrenoceptor agonists, antimuscarinics and leukotriene receptor antagonists are used in the management of both bronchospasm and cellular activities in asthmatics.

Taraxacum officinale is an herbaceous perennial plant. A first reference to its application is reflected in its name, which is derived from the Greek words 'taraxis" for infla-

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mmation and ‘akeomai” for curative. In English speaking countries, *T. officinale* is commonly known as dandelion, from the French word “dent-de-lion”. This refers to the serrated leaves of the plant (Schütz et al., 2005). The first evidence for its therapeutic use by Arabian physicians’ dates back 10 and 11th centuries to treat liver and spleen ailments (Kroeber, 1950; Faber, 1958; Sweeney et al., 2005). The German physician and botanist Leonhard Fuchs (1543) described its use, among others, to medicate gout, diarrhoea, blister, spleen and liver complaints. In North American aboriginal medicine, infusions and decoctions of the root and herb were applied to remedy kidney disease, dyspepsia and heart-burn (Sweeney et al., 2005). Other folklore uses of the plant include laxative, arthritis, eczema, diabetes mellitus and bronchitis.

Pharmacological profiling of *T. officinale* has showed diuretic, cholerectic, anti-inflammatory, anti-oxidative, anti-carcinogenic, analgesic, anti-allergic, anti-hyper-glycemic and anti-thrombotic activities (Ahmad et al., 2000; Schütz et al., 2005). Various parts of the plant have been used in folk medicine to treat some diseases, such as hypertension, prostate, breast and uterine cancers. Studies have demonstrated that *T. officinale* has anti-inflammatory activity by eliciting its protective effect against cholecystokinin-induced acute pancreatitis in rats and suppression of both TNF-α and leukotriene B_4 formation in human neutrophils (Kashiwada et al., 2001; Seo et al., 2005). Furthermore, a recent study conducted by Yoon et al. (2010) using mouse macrophage cell line RAW 264.7, showed that, methanolic extract of *T. officinale* and its fraction inhibit lipopolysaccharide (LPS)-induced production of NO, pro-inflammatory cytokines and PGE_2 in a dose-dependent manner.

Phytochemical screening of the ethanolic extract of *T. officinale* showed evidence of phenolic compounds, and alkaloids are reported to have potential anti-asthmatic activities (Schütz et al., 2005).

The present study attempts to investigate the anti-cholinergic effect of *T. officinale* leaves and its effect on some inflammatory cells using ovalbumin sensitized guinea-pigs. The outcome of this study may provide the possible pharmacological effects of a natural plant product in allergen-induced asthma.

MATERIALS AND METHODS

Plant

The leaves of *T. officinale* were obtained from Botanical Garden of University of Ghana, Accra, in the month of October. The leaves were sent to the herbarium at Botany Department for identification and authentication.

Preparation of ethanolic extract

The leaves were washed thoroughly under tap water, air-dried, pulverized and macerated using 70% ethanol for 48 h. The supernatant was filtered and evaporated under reduced pressure at a temperature of 40 to 50°C in a rotary evaporator. The concentrated extract was defatted using petroleum ether, evaporated into mass syrup to remove left ethanol and freeze-dried. The weight of the freeze-dried *T. officinale* leaves extract (TOLE) was 9.605 g, a yield of 4.37%. The freeze-dried TOLE was reconstituted in distilled water and was stored at 4°C throughout the study.

Animals

Twenty male Noguchi strain guinea-pigs (350 to 500 g) were purchased from Noguchi Memorial Institute for Medical Research (NMIMR) Animal House, Accra, Ghana. The animals were quarantined in an air-conditioned room for 7 days at a temperature of 22 ± 1°C with relative humidity of 60 ± 1% and 12 h light/dark cycle at animal experimentation department of NMIMR. They were fed with autoclaved Sankofa goat and sheep pellet diet from Ghana Agro Food Company (GAFCO) and water *ad libitum* every morning throughout the study.

The study protocol was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School (UGMS), College of Health Sciences, University of Ghana. Throughout the entire study period, the animals were monitored for growth, health status and food intake capacity to be certain that they were healthy.

Sensitization and treatment of guinea-pigs

Noguchi strain male guinea-pigs used in the study were randomly put into four different groups of five animals each: group I (non-sensitized controls); group IIa (ovalbumin (OA)-sensitized controls); group IIb (OA-sensitized treated orally with 100 mg/kg body weight (BW) ethanolic extract of TOLE) and group IIc (OA-sensitized treated with prednisolone (2.5mg/kg BW orally) as reference standard). The dose of TOLE was determined based on previous study conducted by Tita et al. (1993). The dose of prednisolone, however, was extrapolated from the therapeutic dose.

All animals (except group I) were sensitized with two different doses of 10 mg OA and 30 mg aluminium hydroxide intraperitoneally and subcutaneously, each on day zero. Immune response boosting of antigen was done using 0.1 ml solution containing 1 mg OA dissolved in 0.9% saline intraperitoneally on day-14. The daily doses of drugs were started on day-22 and continued until day-56.

Ovalbumin challenge

On the 25th day through to 56th day, sensitized guinea-pigs were challenged with 2% aerosolized OA (0.2 g OA dissolved in 10 ml saline) for 10 min prior to 1 h of drug treatment. Group I animals were challenged with 0.1 ml of 0.9% saline for the same duration. The challenge was conducted in Perspex chamber (dimensions = 20 × 30 cm) connected to jet nebulizer. Figure 1 illustrates the schematic diagram of the experimental protocol indicating the events and durations.

Blood collection and cell counts

Guinea-pigs were anaesthetized using 50 mg/kg sodium pentobarbitone. Two milliliters of blood was drawn by cardiac puncture and transferred into an ethylenediaminetetraacetic acid (EDTA) test tube. An automated haematology analyzer (KX-2IN,

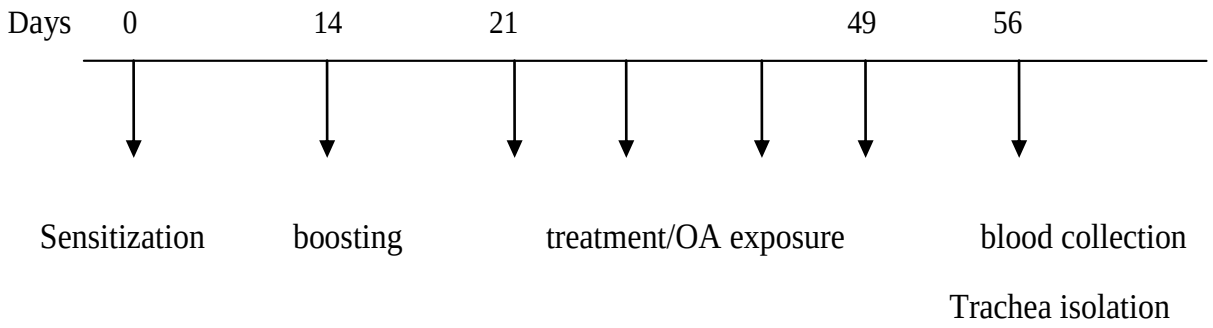

Figure 1. Schematic diagram of the experimental protocol indicating the events and durations

Sysmex Corporation, Japan) was used to estimate the counts of neutrophils, lymphocytes and monocytes (eosinophils, basophils and monocytes) in each blood sample.

Tracheal preparation

The trachea was surgically removed from guinea-pigs in each group. The isolated trachea was placed in Krebs' solution in a Petri dish with an aerator attached. Each trachea was cut into zig-zag chain (2 to 3 cm long) with a maximum of three cartilages in between. The zig-zag tracheal chain was suspended in a 50 ml organ bath filled with Krebs' solution as a physiological solution. the Krebs' solution was maintained at 37°C, ph 7.4, was and gassed with 95% o_2 and 5% co_2. the tracheal chain was suspended under isotonic condition of 1 g and was allowed to equilibrate for at least 1 h while washing with krebs' solution every 15 min.

Tracheal contractile response to acetylcholine

In each experiment, cumulative dose-responses for 0.1 to 6.4 mmol/L acetylcholine induced contraction of the tracheal chains were obtained by doubling the initial concentrations every 2 min. The contractile response of tracheal to each concentration of acetylcholine was calculated as a percentage of the maximum contraction. Tracheal contractile responses to acetylcholine were repeated 30 min after application of 100 and 200 µg/ml of TOLE. The contractile responses in percentages were plotted against negative log concentration of acetylcholine for both absence and presence of extract. The effective concentration of acetylcholine causing 50% maximum response (EC_{50}) in each experiment was determined. A graph of mean response versus the potency ($1/EC_{50}$) of acetylcholine was plotted to determine the magnitude of contractions.

Tracheal contractile response to ovalbumin

To assess tracheal contractile response to OA *in vitro*, 0.1 ml of 2% OA solution was added to the organ bath. The degree of tracheal chain contraction was recorded after 15 min. This was expressed as a percentage of the maximum contraction obtained from acetylcholine hydrochloride. The tracheal chain response to OA was repeated in the presence of 100 and 200 µg/ml of TOLE. The extract was applied on the tracheal tissues for 30 min prior to OA challenge.

Histological examination

Guinea-pigs were sacrificed by dislocating the neck bleeding. The lungs were swiftly excised from the thoracic cavity. They were immediately washed 4 times with 0.9% saline. The tissues were fixed with 10% neutral buffered formaldehyde (pH = 7.4), embedded in paraffin wax (56 to 60°C) and sectioned at 4 µm for histopathological examination. The sectioned tissues were stained with hematoxylin and eosin (H&E). Lung sections were evaluated microscopically using Olympus BX 51TF (Olympus Corporation, Tokyo, Japan) light microscope connected to a digital camera for morphology in the bronchioles. Images of selected sections were captured at 20× magnification.

Statistical analysis

The results were reported as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall F value was found to be statistically significant ($P < 0.05$), further comparisons among groups were made using the Benferroni post hoc test. All statistical analyses were performed using GraphPad prism demo 3 software.

RESULTS

Haematological studies

The counts of neutrophils ($P < 0.01$) and (monocytes) eosinophils, basophils and macrophages ($P < 0.05$) increased significantly, while that of lymphocytes ($P = 0.2586$) was not significant in OA-sensitized controls (group IIa) as compared to non-sensitized control. Treatment with TOLE or prednisolone reduced neutrophils and eosinophils, basophils and macrophages (monocytes) counts, but not the count of lymphocytes as illustrated in Figure 2.

OA-sensitized tracheal contractile response to acetylcholine

The $1/EC_{50}$ of acetylcholine in sensitized groups IIa, b and c were, 43.29 ± 0.09, 73.99 ± 0.04 and 87.15 ±

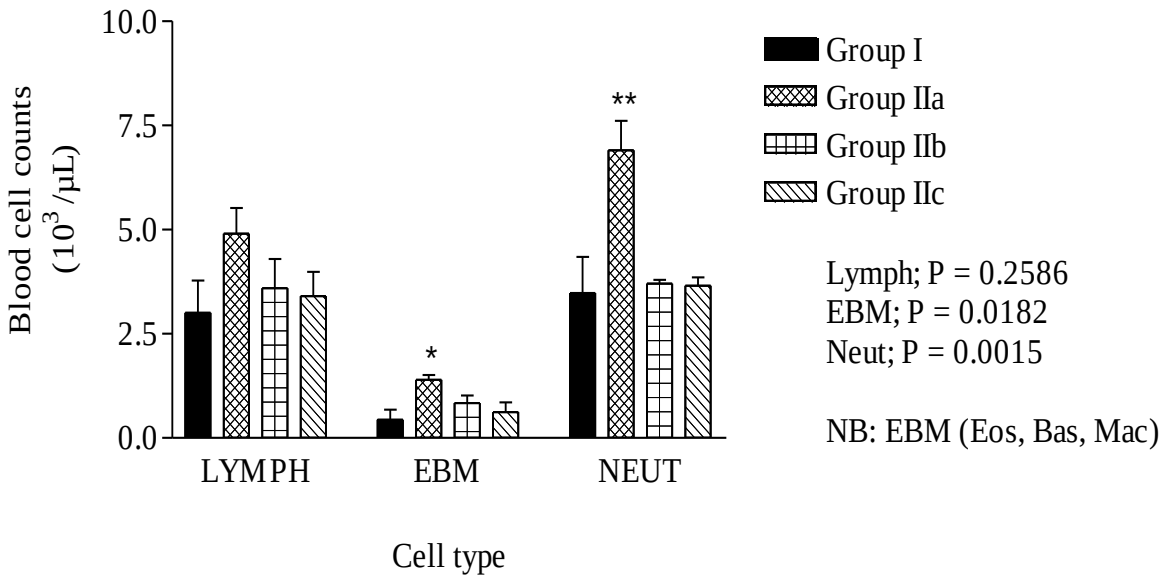

Figure 2. Blood cell counts: bar chart showing inhibitory effect of 30 days oral administration of TOLE on blood cell counts ± (SEM) 10^3/µl in OA-sensitized guinea-pigs (n = 5). NEUT= Neutrophils, LYMPH= Lymphocytes and EBM = Eosinophils, Basophils and Macrophages. **P < 0.01, *P < 0.05 versus non-sensitized control (Group I).

0.90%, respectively, and were significant (P < 0.0001) as compared to that of non-sensitized control 100 ± 0.40%. Application of 100 and 200 µg/ml reduced EC_{50} of acetylcholine in all groups as compared to those of plain acetylcholine (Figure 3).

Isolated OA-sensitized tracheal contractile response to OA

The tracheal contractile response to ovalbumin was expressed as a percentage of maximum contraction obtained with 1×10^3 mol/L of acetylcholine. The responses of OA-sensitized trachea to antigenic challenge were significantly higher (P = 0.0009) as compared to that of non-sensitized trachea. Application of 100 and 200 µg/ml TOLE to tracheal of OA-sensitized guinea-pigs reduced the magnitude of contraction to antigenic challenge with P = 0.0140 and 0.5717, respectively (Figure 4).

Histological examination

The photomicrographs of bronchiole of the treated groups showed reduced bronchoconstriction, infiltration of eosinophils and basophils, hypertrophy of trachealis muscle and peribronchial oedema as compared to the OA-sensitized control group. *T. officinale* leaves extract, however, reduced emphysema in OA-sensitized guinea-pigs better than prednisolone as shown in Figure 5.

DISCUSSION

In the current study, effects of TOLE on blood cell counts and isolated tracheal of OA-sensitized to acetylcholine and antigenic challenge were investigated.

In OA-sensitized guinea-pigs, exposure of the airways to aerosols of OA stimulates vagus nerve to release acetylcholine (Roffel et al., 1990), which binds to M_3-receptors in the bronchial walls causing broncho-constriction. Reactive oxygen species like hydrogen peroxide, increase airway responsiveness to constrictor agonists, such as acetylcholine and bradykinin (Asano et al., 2001). Also, infiltration of eosinophils into the walls of bronchiole produce major binding proteins (MBP) which causes dysfunction of M_2-receptor which ordinarily serves as a negative feedback to M_3-receptor (Jacoby et al., 1993). The dysfunctioning of M_2 is achieved by MBP acting as an allosteric antagonist to the receptors. The role played by cellular mediators; histamine, reactive oxygen species and major binding proteins in bronchioles of asthmatics increase the sensitivity of M_3-receptors to acetylcholine causing an intense bronchospasm.

Contractile responses of isolated trachea to acetylcholine and antigenic challenge were significantly inhibited by both direct application and 30 days oral administration of TOLE to OA-sensitized guinea-pigs. This may be due to anti-cholinergic activity of TOLE as reported by Schütz et al. (2005).

The anti-cholinergic activity of *T. officinale* reduced the potency of acetylcholine on the trachea of OA-sensitized guinea-pigs in a dose-dependent manner. Therefore,

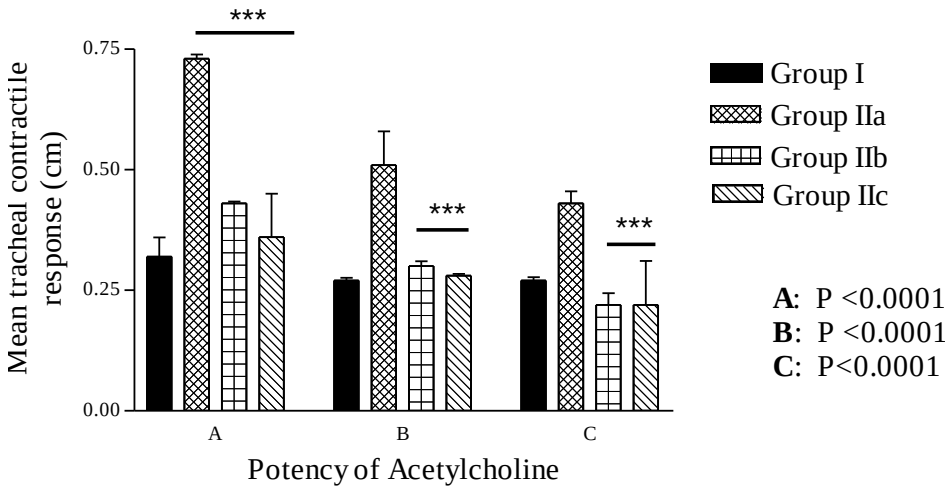

Figure 3. Inhibitory effect of TOLE on OA-sensitized tracheal contractile response to acetylcholine. Values are mean response ± SEM (cm) of OA-sensitized guinea-pigs (n = 5) tracheal to 10^3 molar acetylcholine. A = acetylcholine alone, B = acetylcholine plus 100 µg/ml TOLE and C = acetylcholine plus 200 µg/ml TOLE. ***P < 0.001 versus OA-sensitized control (group IIa).

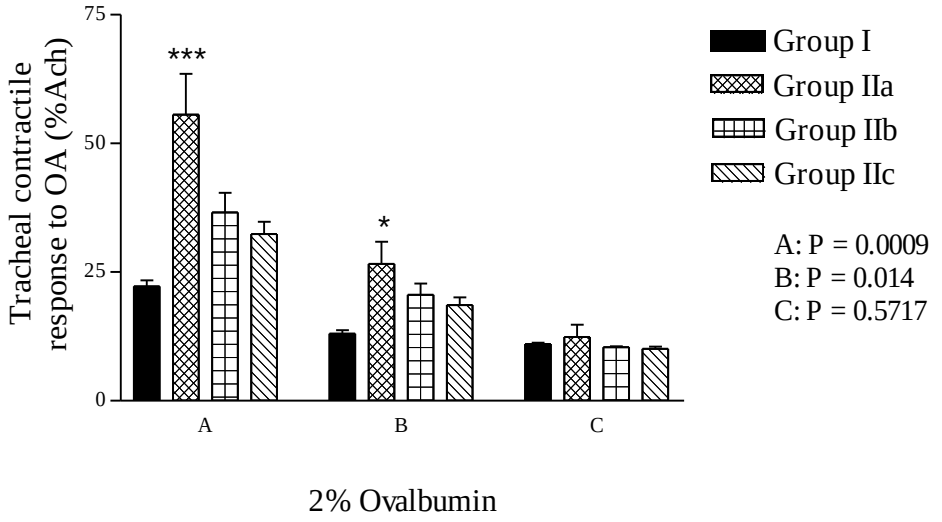

Figure 4. Inhibitory effect of TOLE on OA-sensitized contractile response to ovalbumin challenge. Values are mean response ± SEM to OA (percentage Ach) of OA-sensitized guinea-pigs (n = 5) relative to 10^3 molar acetylcholine. A = OA alone, B = OA plus 100 µg/ml TOLE and C = OA plus 200 µg/ml TOLE. ***P = 0.0009 and *P < 0.05 versus non-sensitized control (group I).

TOLE may be a potent bronchodilator in allergen-induced airway inflammation.

Also, the counts of neutrophils and eosinophils, basophils and macrophages (monocytes) were significantly higher in OA-sensitized control guinea-pigs. This was confirmed by the histological examination which showed infiltration of eosinophils and emphysema in the lungs. The current results agrees with other studies conducted in asthmatic animal models and human subject which showed elevated counts of neutrophils, eosinophils and basophils in the presence of specific antigens (Lewis et al., 2001; Fahy, 2009). TOLE inhibited infiltration of eosinophils and emphysema in the lungs. Furthermore, TOLE reduced the counts of monocytes and neutrophils in OA-sensitized guinea-pigs. However, the extract had no effect on lymphocytes in OA-sensitized guinea-pigs. This result is an indication that, TOLE could inhibit either the proliferation and/or secretions of eosinophils,

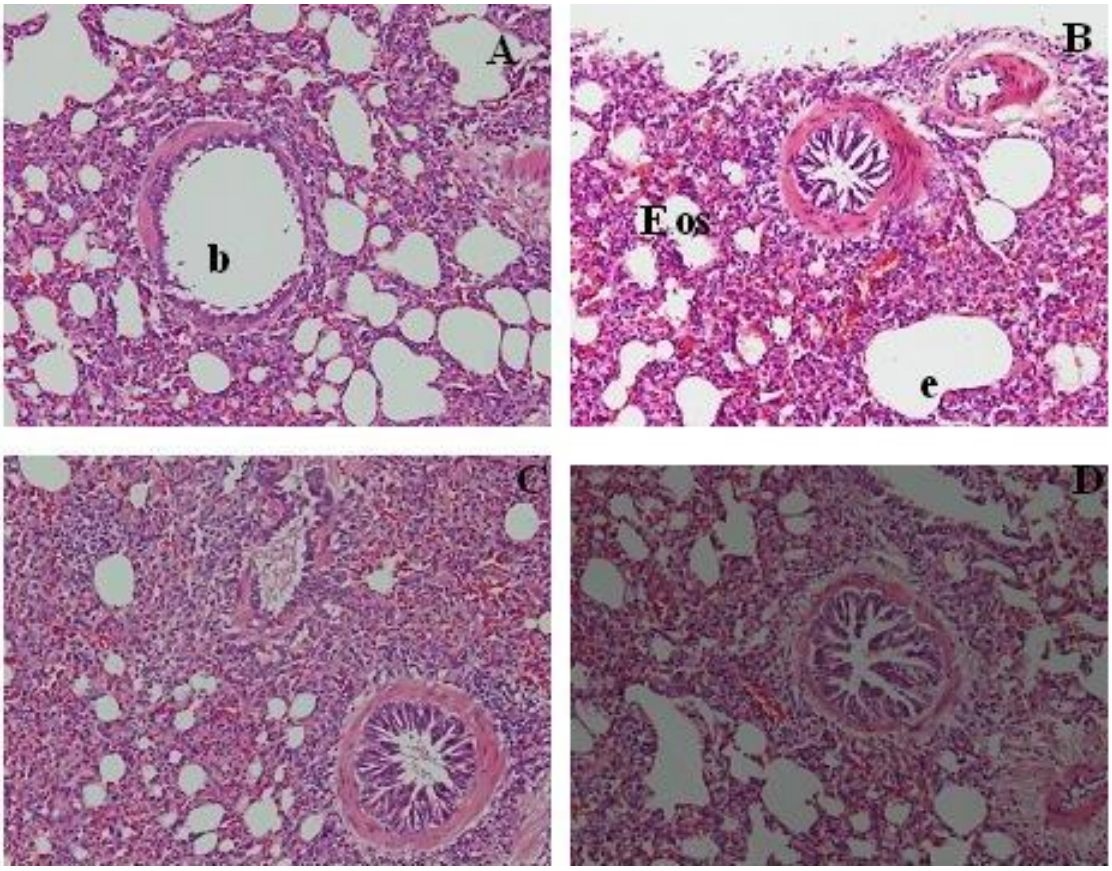

Figure 5. Histological effects of various treatments on bronchiole of guinea-pigs stained with haematoxylin and eosin. A: non-sensitized control (group I); B: OA-sensitized control (group IIa); C: OA-sensitized treated with TOLE (group IIb) and D: OA-sensitized treated with prednisolone (group IIc). The following abbreviations are used to describe salient pathological features on the photomicrograph: b (bronchiole), e (emphysema), Eos (eosinophil) and arrow (peribronchial oedema).

and neutrophils which are important mediators of airway immune diseases responsible for bronchospasm (Jacoby et al., 1993; Brightling et al., 2002; Busse and Rosenwasser, 2003).

The results of this study have demonstrated the anti-cholinergic activity of TOLE by significantly antagonizing acetylcholine on the trachea. Also, TOLE reduced eosinophil, basophil and neutrophil counts significantly in OA-sensitized guinea-pigs. The next aspect of this work would be focused on the histopathological changes on the lungs.

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