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Research Article

THE ANTI-INFLAMMATORY ACTIVITY OF *TARAXACUM OFFICINALE* LEAVES IN OVALBUMIN-SENSITIZED GUINEA-PIGS

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ABSTRACT

Objective: Inflammatory mediators, such as histamine and eicosanoids have been implicated in the pathophysiology of allergen-induced asthma including bronchospasm, vasodilation, increased vascular permeability, perivascular and peribronchial oedema, acute functional changes in the lungs and diarrhoea due to increase intestinal motility. This study aims to ascertain the anti-inflammatory effect of the *Taraxacum officinale* ethanolic leaf extract (TOLE) on pulmonary vascular permeability and H_1 -receptors in the ileum of ovalbumin(OA)-sensitized guinea-pigs.

Method: OA-sensitized guinea-pigs were challenged with 2% OA aerosols prior to 1 hr per os of drugs (TOLE or prednisolone). A piece of excised ileum was suspended in a tissue bath and challenged with histamine in the presence and absence of TOLE. Lungs were fixed in buffered formalin for histological studies using H & E stains. The results were reported as mean ± SEM. Statistical analysis was performed using one-way ANOVA and Benferroni post hoc test.

Results: The results showed a significant dose-dependent reduction in anti-histaminic activity ($p < 0.05$) on isolated guinea-pig ileum. Histopathological lesions such as perivascular oedema, hypertrophy of smooth muscles, infiltration of eosinophils and basophils were reduced in the lungs of TOLE treated group compared to OA-sensitized controls.

Conclusion: The study has shown that, TOLE has the potential to reduce pulmonary vascular permeability and intestinal motility in OA-sensitized guinea-pigs.

Keywords: Vascular permeability, Perivascular oedema, Anti-histaminic activity, Ovalbumin.

INTRODUCTION

Bronchial wall inflammation is reported as the principal pathophysiological abnormality which culminates in airway narrowing. Studies in allergic animal models have categorized the responsiveness into early and late phases depending on the type of predominant inflammatory mediator. In early phase allergic reaction, Immunoglobulin E (Ig E) bound to FcεRI on mast cells and basophil is cross-linked by repeated allergen exposure. The implicated cells, consequently, release pre-formed or rapidly synthesized chemical mediators. These mediators, such as histamine, elicit vasodilation, increased vascular permeability, perivascular and peribronchial oedema [1, 2] and acute functional changes in the airways. The Ig E mediated early phase reaction occurs within minutes of allergen exposure and could lead to a potential lethal anaphylactic shock. Late phase response, however, is triggered by cytokines and chemokines secreted from mast cells which causes recruitment of basophils and eosinophils into the epithelium and bronchiolar smooth muscle respectively in the lungs [1].

Corticosteroids, anti-histamines and mast cell stabilizers are the common therapeutic agents used to treat allergic asthma. These drugs block action of allergic mediators by preventing the activation of cells, degranulation processes or histamine-1 (H_1 -receptors). Bronchodilators such as β_2 – receptor agonists, antimuscarinics and leukotriene receptor agonists are also used to alleviate bronchospasm associated with allergic asthma. Although these therapeutics help to alleviate symptoms of allergic asthma, substantial undesired effects following prolonged use have been reported. For example, oral corticosteroids can cause general immune-suppression, skin fragility and Cushing's syndrome. Growth retardation in rapidly dividing cells such as embryonic cells and sedation are common side-effects of anti-histamines [3, 4]. There is therefore, the need to develop new anti-allergic therapies with satisfactory tolerability for long-term use. They could be beneficial for both early and late phases of allergic reactions.

Taraxacum officinale is a herbaceous perennial plant. A first reference to its application is reflected in its name, which is derived from the Greek words "taraxis" for inflammation 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 [1, 5]. The first evidence for its therapeutic use by Arabian physicians dates back 10th and 11th centuries to treat liver and spleen ailments [6, 7].

Pharmacological profiling of *T. officinale* has shown diuretic, cholerectic, anti-inflammatory, anti-oxidative, anti-carcinogenic, analgesic, anti-allergic, anti-hyperglycemic and anti-thrombotic activities [5, 8]. 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 B4 formation in human neutrophils [9, 10]. Furthermore, a recent study 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 [11].

Previous experiment conducted on *Taraxacum officinale* ethanolic leaf extract in our research laboratory showed dose-dependent anti-cholinergic activity on isolated trachea zig-zag chain, reduction in the blood counts of neutrophils, lymphocytes and monocytes (eosinophils, basophils) and bronchodilatory effect in ovalbumin-sensitized guinea-pigs [1]. The intestine of OA-sensitized guinea-pigs has been proven to respond extensively to histamine challenge leading to diarrhea in some allergic individuals. Additionally, exposure of sensitized animal models to cognate antigen increases the propensity of pulmonary vascular permeability due to retraction of vascular system especially the arterioles. The current study therefore, seeks to assess aspects of anti-inflammatory activities of TOLE on vascular permeability in the lungs and skin, and H_1 receptors in ileum of ovalbumin-sensitized guinea-pigs.

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 residual ethanol and freeze-dried. The weight of the freeze-dried *T. officinale* leaf 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), Korle-Bu, 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 (2.5 mg/kg BW orally) as reference standard). The dose of TOLE was determined based on previous study 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-21 and continued until day-56.

Ovalbumin challenge

On the 21st 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.

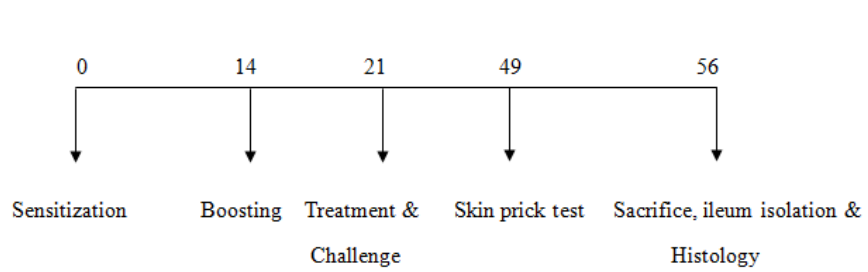

Fig. 1: Schematic diagram of the experimental protocol indicating the events and durations

Guinea-pig Skin Test

On day-49, back of each guinea-pig was shaven and injected intradermally with 1 % OA dissolved in 0.1 ml saline. The diameter of wheals formed in the skin of each animal was monitored and measured until complete disappearance of the oedema. The average skin oedema was calculated for each group and expressed as a percentage of the OA-sensitized control. Percentage of skin oedema relative to that of OA-sensitized control for each group was calculated (equation 1) and plotted on a bar chart (figure 2).

$$\text{Percentage skin oedema} = \frac{\sum \text{oedema in a particular group}}{\sum \text{oedema in group IIa}} \times 100\% \quad (1)$$

Guinea-pig ileum study

Guinea-pigs were sacrificed by dislocating the neck bleeding. The abdomen of each guinea-pig was opened and the ileum surgically removed. The isolated ileum was quickly placed in a petri dish containing Tyrodes' solution. The mesentery was trimmed away and 2-3 cm of the isolated ileum cut. Each end of the 2-3 cm piece of ileum was tied with a thread and mounted in an organ bath filled with 50 mL of Tyrodes' solution and gassed (95 % O_2 and 5 % CO_2). The guinea-pig ileum has relatively little spontaneous activity at physiological temperature. Therefore, it is possible to set a steady base-line by maintaining the temperature at 35 ± 0.5 ºC. The response of the ileum to graded doses of 10 µg/mL histamine was recorded using 3 min time cycle with a drug contact time of 30 sec. The experiment was repeated after application of 100 µg/mL and 200 µg/mL of TOLE for 30 minutes. A log-dose response curve was plotted for histamine and the EC_{50} values were determined. A graph of histamine potency ($1/EC_{50}$) on intestinal smooth muscle versus treatments was plotted to show the sensitivity of OA-sensitized guinea-pig ileum to histamine in the absence and presence of TOLE.

Histopathological study

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 bronchiolar microvasculature. Images of selected sections were captured at 400× 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 5 software.

RESULTS

Skin test

The skin test was used to assess the extent of inflammatory response in OA-sensitized guinea-pigs. A remarkable oedema was observed in all the sensitized groups except the non-sensitized controls (figure 2). The mean diameters of oedema observed after 30 minutes of intradermal injection of OA were 100 ± 0.12 %, 61.0 ± 0.04 % and 62.3 ± 0.10 % for groups IIa, IIb and IIc respectively. One-way ANOVA showed significant difference between the means of oedema for the three groups ($P < 0.0009$). Benferroni's multiple comparison tests confirmed the significant inhibition in oedema size for groups IIb and IIc compared to that of group IIa ($P < 0.01$). However, comparison of the oedema size between group IIb and IIc using Benferroni's post test showed no significant difference ($P > 0.05$). After 24 hrs of intradermal injection of OA, the mean diameters of oedema reduced to 98.5 ± 0.05 %, 53.3 ± 0.11% and 57.8 ± 0.07 % for groups IIa, IIb and IIc respectively. Again, one-way ANOVA showed significant difference between the means of oedema for the three groups ($P < 0.0005$) and confirmed by the Benferroni's multiple comparison tests (figure 2).

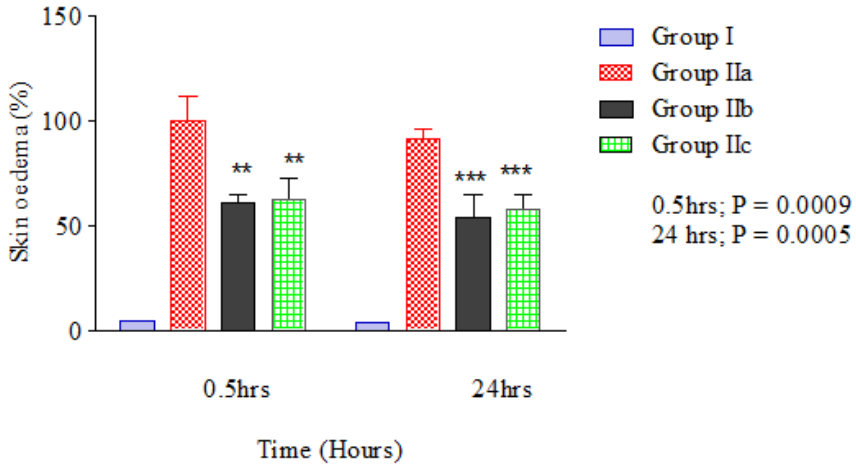

Fig. 2: Percentage skin oedema size with respect to time.

Keys: Group I = Non-sensitized control, Group IIa = OA-sensitized control, Group IIb = OA-sensitized + TOLE and Group IIc = OA-sensitized + prednisolone. ***P < 0.001 and **P < 0.01 versus OA-sensitized control (group IIa)

Guinea-pig Ileum Studies

The EC_{50} of OA-sensitized guinea-pig ileum to histamine were 4.54 ± 0.04 µmol/ml, 1.3 ± 0.04 µmol/ml, 1.82 ± 0.02 µmol/ml and 1.75 ± 0.03 µmol/ml for groups I, IIa, IIb and IIc, respectively (figure 3). One-way ANOVA showed significant differences in the magnitude of contractions to histamine ($P<0.0001$). Benferroni's multiple comparison test confirmed significant increase in sensitivity of ileum of OA-sensitized control (group IIa) to histamine compared to non-sensitized control (group I) with $P < 0.001$. However, 30 days oral administration of TOLE or prednisolone inhibited the magnitude of contractions of OA-sensitized ileum to histamine compared to that of OA-sensitized control significantly. Application of 100 µg/ml and 200 µg/ml of TOLE further remarkably inhibited the magnitude of contractions of OA-sensitized guinea-pig ileum to histamine in a dose-dependent fashion.

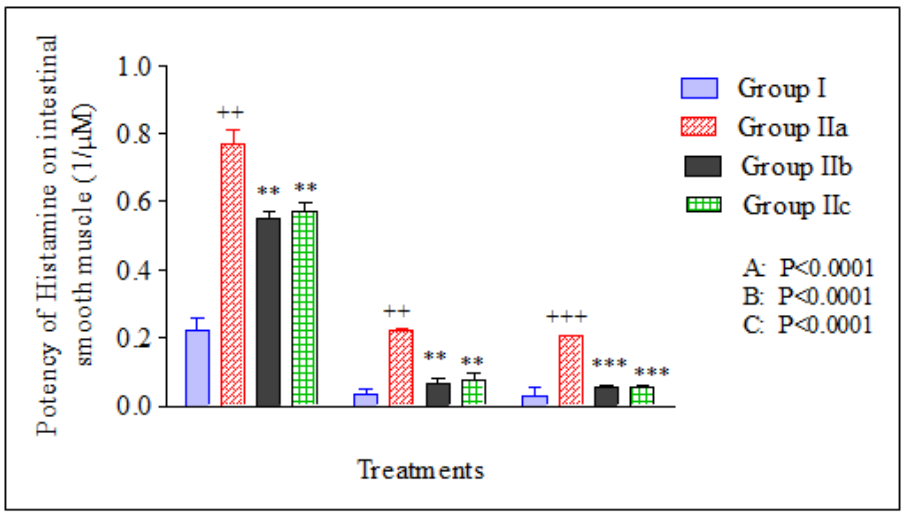

Fig. 3: OA - sensitized ileum contractile response to histamine

Keys: A = histamine alone, B = histamine plus 100 µg/ml TOLE and C = histamine plus 200 µg/ml TOLE. **P <0.001 and ***P < 0.001 versus group IIa; +++P < 0.001 and ++P < 0.01 versus group I.

Histopathological study

The photomicrograph of non - sensitized guinea-pig (figure 4A) showed no significant histological lesions in the artery except congestion of eosinophils. However, trapping of erythrocytes and infiltration of basophils into the endothelial lining of the blood vessels (v) were observed in OA - sensitized control (figure 4B). Furthermore, the smooth muscle of the artery was hypertrophied and infiltrated by eosinophils. There was also clear evidence of perivascular oedema (double arrow) which extended beyond the periphery of the artery. The photomicrograph of OA-sensitized guinea-pig treated with TOLE (figure 4C) indicated slight infiltrations of eosinophils and basophils into the smooth muscle and endothelium. The arrow at the top corner shows slight perivascular oedema but absence of smooth muscle response. Figure 4D showed extensive infiltrations of eosinophils and basophils into the smooth muscle and endothelium of the artery. There was also hypertrophy of the arteriolar muscle (blue double arrow) and a clear evidence of perivascular oedema (black double arrow).

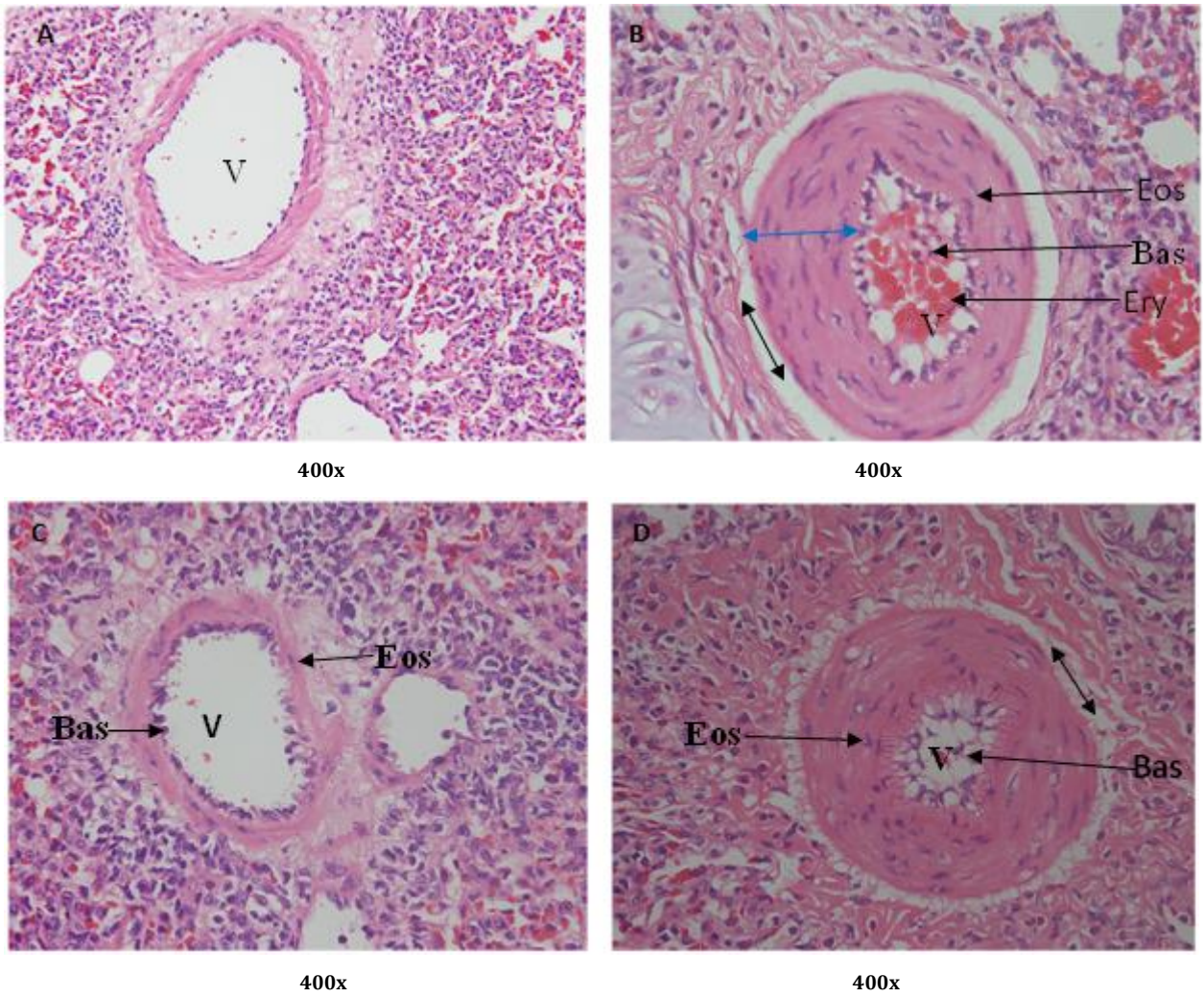

Fig. 4: Sections of H & E stained lungs showing arteriolar blood vessels.

Keys: Endothelium of artery (v), eosinophils (eos), basophils (bas), hypertrophied smooth muscle (blue double arrow) and perivascular oedema (black double arrow). Group I = non-sensitized guinea-pig challenged with saline (A), Group IIa = OA-sensitized guinea-pig challenged with aerosolized OA (B), Group IIb = OA – sensitized plus TOLE challenged with aerosolized OA (C) and Group IIc = OA-sensitized plus prednisolone challenged with aerosolized OA (D).

DISCUSSION

Airway inflammation is recognized as a key pathophysiology of bronchial asthma over the past decade [12, 13]. Bronchial asthma is now viewed primarily as an inflammatory disorder culminating into bronchial hyperreactivity and bronchospasm. Inflammation is an immunological defense mechanism characterized by rubor (redness), calor (warmth), tumor (oedema), dolor (pain) and functio laesa (loss of function). This can be elicited experimentally using stimuli such as infectious agents, ischemia, antigen-antibody interactions, chemicals, and thermal or mechanical injury [14]. The bronchial oedema is accompanied by increased vascular permeability during vascular remodeling in bronchial asthma. In this study, effect of TOLE on vascular permeability was evaluated on the skin, histamine receptors in the ileum and bronchial microvasculature of ovalbumin-sensitized guinea-pigs.

The guinea-pig skin prick test is a basic test to check the extent of inflammatory response to antigens in sensitized animals. In the current study, skin prick test was used to assess the effect of TOLE on mast cells, autonomic nerve endings and capillary blood vessels response to stimuli in ovalbumin-sensitized guinea-pigs. The response manifested as oedema in the skin of OA-sensitized guinea-pigs. The ability of TOLE to reduce oedema formation in the skin of OA-sensitized guinea-pigs after intradermal injection of ovalbumin could serve as potential anti-inflammatory activity of the extract. The development of oedema in the skin is biphasic process with first phase occurring within an hour and the second phase beyond an hour.

Preformed mediators such as cytoplasmic enzymes, histamine, and serotonin are released from mast cells during the first phase [15]. These preformed mediators are capable of enhancing vascular permeability, contraction of non-vascular smooth muscles, dilating precapillary sphincters and postcapillary venules [16]. The second phase is mediated by arachidonic acid metabolites including prostaglandins, leukotrienes and thromboxanes. The effects of these mediators are 10 folds higher than that of the preformed. Additionally, the test serves as an indicator for T-cell response in ovalbumin sensitized animal models [17]. Inhalation of aerosolized OA has been reported to induce inflammatory cell proliferation [18]. Proliferated T cells are differentiated into T helper 2 cells which secrete cytokines such as TNF α, IL-4, 5, 9 and 13 [17]. These mediators play important roles in the pathogenesis of allergic airway inflammation [19]. The inflammatory cytokines induce vascular permeability, tissue oedema, bronchoconstriction, massive leukocytes recruitment and inflammatory reaction in the mucosa of the lungs. Also, Kim *et al.* (2000) showed that both 100 and 1000 µg/ml of TOLE inhibit TNF α by hampering production of IL-1 in primary cultures of rat astrocytes stimulated with substance P and lipopolysaccharide [20]. Substance P together with other neuropeptides such as neurokinin A and calcitonin-gene related peptide are potent inducers of airway smooth muscle contraction, bronchial oedema, extravasation of plasma, mucus hypersecretion, and possibly infiltration of inflammatory cell and secretion by axon reflex mechanism [21]. This finding therefore shows that, TOLE has anti-inflammatory activity by inhibiting wheal formation in the skin of OA-sensitized guinea-pigs.

Histamine -1 (H_1) excitatory receptor is predominantly located in the skin, bronchioles and ileum of both man and animal models [12]. It is one of the key receptors responsible for vascular permeability in both skin and bronchioles of OA-sensitized guinea-pigs. In the present study, the anti-histaminic activity of TOLE was assessed using guinea-pig ileum. The H_1-receptors in ileum of OA-sensitized guinea-pigs contracted extensively to histamine challenge compared to that of non-sensitized guinea-pigs. This result concurs with study by Hicks and Sackeyfio (1972) which demonstrated that *in vivo* administration of histamine prior to sensitization using exogenous anaphylatoxin produced a significant increase in contraction of the guinea-pig ileum [22]. Increase motility of the OA-sensitized ileum to histamine could be responsible for the frequent defecation observed in the present study during OA challenge. This could be attributed to hyper-reactivity induced by OA on H_1-receptors in the ileum of sensitized guinea-pigs [12]. The magnitude of histamine-induced contraction in OA-sensitized guinea-pigs pre-treated with TOLE was reduced compared to OA-sensitized control and OA-sensitized pre-treated with prednisolone. The inhibitory effect of TOLE on ileal contractile response to histamine suggests that the plant extract has antihistaminic property. This finding agrees with a study conducted on hydroxymethanolic extract leaves of *adhatoda schimperiana* to assess contractile response of guinea-pig ileum to histamine due to presence of different phytochemical compounds [23]. The antagonistic effect of TOLE on OA-sensitized guinea-pig ileal contractile response to histamine was confirmed by the application of 100 µg/ml and 200 µg/ml of the extract. Thus, the antihistaminic property of TOLE could be responsible for reducing vascular permeability in the skin and bronchioles of OA-sensitized guinea-pigs. The anti-histaminic property of TOLE might also be exploited in the management of itching and diarrhoea which is observed in some allergic patients.

Inflammatory stimuli such as lipopolysaccharides and ovalbumin dilate arterioles and venules generating an increased vascular permeability in the lungs. As a result, fluid and plasma proteins are exudated to produce perivascular oedema. Mediators such as histamine, prostaglandins and leukotrienes are released in the course of vascular permeability [24]. The plasma protein leakage has been implicated to play essential role in the induction of thickness, engorgement and oedema of the airway wall, culminating into the narrowing of the endothelium which correlates bronchial hyperreactivity and airway inflammation [25]. Studies have shown that murine models of asthma exhibit increased vascular permeability associated with bronchial hyperreactivity and airway inflammation [26, 27, 28]. This was confirmed by our previous study, which demonstrated that, inhalation of OA aerosols increased bronchial responsiveness like, bronchoconstriction, hypertrophy of airway smooth muscle, infiltration of eosinophils and basophils, emphysema, peribronchial oedema in the lungs of OA-sensitized control guinea-pigs compared to non-sensitized controls [1]. In the current study, ovalbumin challenge showed histological lesions such as thickening of the arteriolar smooth muscle, and narrowing of the lumen in the microvasculature of the lung in sensitized guinea-pigs. Additionally, there were obvious evidence of perivascular oedema, infiltration of eosinophils into the tunica intima and tunica intermedia, and basophils into the lining of the lumen in the OA-sensitized controls. Surprisingly, pretreatment with TOLE prior to OA challenge inhibited vascular permeability to plasma fluid exudates, infiltration of eosinophils and basophils into the microvasculature of lungs. This therapeutic activity of TOLE could be attributed to the presence of several bioactive compounds in the extract with diverse pharmacological mechanism of actions [5, 8].

Infiltration of eosinophils to the lung is one of the hallmark characteristics of allergic asthma in both humans and animal models. In the lung, eosinophils can potentially perform a number of functions, including antigen presentation, and secretion of cytokines including IL-13 from mRNA that is preformed during development, IL-5 from preformed stores, TGF-β, and osteopontin, chemokines such as CCL-11, CCL22, matrix metalloproteinases (MMPs) granule mediators (e.g., erythropoietin and major basic protein), as well as leukotrienes (LTC_4, LTB_4) [29]. Eosinophils release major basic 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 [1, 30]. Previous study by Awortwe et al., 2011, confirmed that TOLE has anti-cholinergic activity in the trachea of OA-sensitized guinea-pigs [1]. The current result suggests the protective effect of TOLE which may be mediated by inhibition of eosinophil and basophil accumulation and release of products from these inflammatory cells.

CONCLUSION

The study has demonstrated the potential anti-inflammatory effects of TOLE through inhibition of H_1 receptors in the ileum and skin, and reduction of vascular permeability, hypertrophy of arteriolar smooth muscle, infiltration of eosinophils and basophils, and perivascular oedema in the lungs. Furthermore, the result of our preliminary phytochemical screening credited the inhibitory activity of TOLE on vascular permeability to the evidence of phenolics, flavonoids, alkaloids and tannins compounds. Although TOLE has exhibited some potential anti-inflammatory activities in OA-sensitized guinea-pigs at the dose used for this study, more investigations both *in vitro* and *in vivo* should be conducted to validate its folklore use in the management of asthma.

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