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Allergy and Ovalbumin-Induced Asthma in Rodents Is Attenuated by the Aqueous Stem Bark Extract of *Triplochiton scleroxylon* (K. Schum)

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ABSTRACT

The aqueous stem bark decoction of *Triplochiton scleroxylon* is used traditionally for the treatment of diabetes, lumbago, oedema and lactation failure. **Aim of the study:** To investigate the therapeutic value of the aqueous extract of the stem bark of *Triplochiton scleroxylon*(TS) in allergy and asthma. **Materials and methods:** The inhibitory effect of TS treatment on IgE-dependent mast cell degranulation was assessed in bovine serum albumin-induced cutaneous anaphylaxis. Its effect on non-immunologic reactions was investigated using compound 48/80-induced degranulation of pretreated rat mesentery mast cells. Ovalbumin-induced asthma model was employed to demonstrate TS effect on allergen- induced airway inflammation. **Results:** The study revealed a significant inhibition of dye extravasation in a local IgE-mediated allergic reaction. Pretreatment of ICR mice with TS (100-500 mg/kg) offered (34.3 - 70.4 %) inhibition of the reaction area observed 30 min after pinnae antigen challenge. Also, pretreatment of rat mesentery mast cells with TS (500-1000 µg/ml) showed (13.0 - 61.4 %) inhibition from compound 48/80-induced mast cell degranulation. TS treatment significantly suppressed key parameters of inflammatory damage induced by aerosolized ovalbumin challenge in Guinea pigs. Histopathological studies of lung sections demonstrated a suppression of inflammatory cell infiltration and goblet cell hyperplasia compared to the ovalbumin-sensitized and challenged control Guinea pigs. Differential blood cell count showed a marked reduction of key inflammatory cells such as eosinophils and lymphocytes at 100 -500 mg/kg TS and monocytes and basophils at the highest dose used. **Conclusion:** TS exhibits significant anti-allergic and anti-inflammatory effects with therapeutic potential for the treatment of asthma.

1. INTRODUCTION

Asthma, a known allergic disorder has shown a steady increase in incidence the world over with reported prevalence rates exceeding 300 million with annual deaths of about 250 people[1,2, 3]Asthma is associated with reversible airway obstruction, increased mucus production, airway hyper-responsiveness and airway inflammation[4]. There has been renewed scientific interest in the initial immune and cellular responses in the pathophysiology of allergic asthma in which cognate antigens also called allergens sensitize patients by eliciting the production of immunoglobulin E (IgE) type of antibodies. These antibodies remain in circulation in the blood or become attached to mast cells of the nasal or bronchial tissues and basophils. On re-exposure to the same antigen and cross-linking of bound immunoglobulin E (IgE) to surface receptors[5], the resulting antigen-antibody reaction in the early phase causes degranulation of the lung mast cells releasing powerful bronchoconstrictors like histamine, 5-hydroxytryptamine, prostaglandins example PGD, the cysteinyl leukotrienes (LTB_4, LTC_4 and LTD_4) and cytokines such as the interleukins, (IL-4, IL-5 and IL-13)[6]. These mediators of allergy sustain the late (delayed) phase of asthma and they activate additional inflammatory cells such as eosinophils, basophils, leukocytes and alveolar macrophages to release more of the LTs and ILs[7]. A progressive airway inflammation and remodeling is the consequence of these immune responses, the inflammatory cells and the mediators released. Airway inflammation, airway hyperreactivity (AHR), increased number of goblet cells, and mucus overproduction characterize asthma.

It is therefore not surprising that IgE-mediated and non-IgE-mediated mast cell activities have been identified as feasible therapeutic targets for the management of allergic and inflammatory diseases[8,9]. Mast cell stabilizers, anti-IgE medication and allergen-specific immunotherapy are all now considered important interventions in disease management[10]. The inhibition of T helper 2 (Th2) cell involvement has also shown promise in various studies[11].

Conventional long-term management of asthma is with the use of anti-inflammatory agents, particularly glucocorticoids. Though considered to be the most potent class of anti-inflammatory agents, a significant number of patients have shown poor or no response even at high doses[12]. Cases of various side-effects, receptor desensitization and patient non-compliance have also been widely reported[13]. These apparent limitations of current therapy, coupled with the renewed

interest in the allergic basis for asthma, have led to research into novel drugs with alternative mechanisms. For these reasons, plants with potential anti-asthmatic effects and possibly with reduced side effects have attracted the attention of researchers. One of such plants that is traditionally used in managing oedema and as an anodyne in lumbago and back-ache[14]is *Triplochiton scleroxylon* (family; Sterculiaceae). It is a large deciduous forest tree usually distributed in tropical West Africa. It is commonly referred to as African whitewood, African maple tree, *Wawa* in Ghana and *Obeche* in Nigeria.

This study investigates the potential effects of the aqueous extract of the stem bark of *Triplochiton scleroxylon*, on IgE-dependent and non-IgE-dependent mast cell degranulation. It also assesses the inhibitory effect of the extract on ovalbumin-induced airway inflammation in an experimental murine model of asthma.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Preparation of Plant Extract

The stem bark of *Triplochiton scleroxylon* was obtained from Kwahu-Asakraka, (6°38′15.0 N″;0°41′39.5″W), Eastern Region, Ghana and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. A voucher specimen (KNUST/HM1/2015/005) was prepared and deposited in the herbarium of the same department. The plant material was air-dried and pulverized to obtain 2 kg of powdered material. This was subsequently boiled in 4 L of distilled water for 30 min. The aqueous decoction was evaporated to dryness in an oven at a constant temperature of 45 °C. A dry paste-like extract (yield, 13% w/w) was obtained, reconstituted in normal saline (0.9%w/v, NaCl) when needed and referred to as the aqueous extract of *Triplochiton scleroxylon*, TS in this paper.

2.1.2 Animals

All animals were purchased from Noguchi Memorial Institute for Medical Research (NMIMR), Legon, Ghana and kept in the Animal House of the Department of Pharmacology, College of Health Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi. Animals

were housed under standard laboratory conditions (temperature 23 ± 2 °C with a 12 h light-dark cycle) while having access to standard commercial pellet diet from Ghana Agro Food Company Ltd (GAFCO) and water *ad libitum*. All animals were used once and humanely handled throughout the course of the study. All experimental procedures carried out were in accordance with the National Institute of Health Guidelines for Care and Use of laboratory animals and approved by the Department of Pharmacology (KNUST) Ethics Committee.

2.1.3 Chemicals and Reagents

Compound 48/80 (C2313); Evans Blue dye (PubChem CID: 6321418); Toluidine Blue O stain (PubChem CID: 7083); Aspirin (Acetylsalicylic acid) (PubChem CID: 2244); Dexamethasone (PubChem CID: 5473) and Ketotifen (PubChem CID: 3827) were purchased from Sigma-Aldrich (St Louis, USA). Bovine Serum Albumin, BSA, was purchased from PAA Laboratories (Germany).

2.2 Methods

2.2.1 *In vitro* mast cell stabilizing effect

A Wistar rat (250 g) was euthanized and pieces of its mesentery excised and placed in 6 petri dishes each containing 10 ml Ringer Locke solution. Into each petri dish, 0.1 ml of one of the following was added respectively; Ringer Locke solution, normal saline, ketotifen maleate (10 µg/ml) and TS (500, 750 and 1000 µg/ml) and incubated for 15 min at 37 ^{0}C. With the exception of the Ringer Locke-treated mesentery tissues, all other treated tissues were further incubated for 15 min with 0.1 ml Compound 48/80 (10 µg/ml). Tissues were transferred into a 4% buffered formalin solution containing 0.1 % Toluidine blue for 30 min. After fixation and staining of mast cells, the tissues were dehydrated by immersing in acetone and xylene through two cycles in each solvent and observed under high power resolution (×40) of a light microscope (Leica DM2500 M). Percentage mast cell degranulation for each treatment was calculated by counting the number of mast cells observed in different fields from a total of 100 mast cells counted.

2.2.2 Active Cutaneous Anaphylaxis

Male ICR mice (25-30 g) were immunized with 0.1 ml solution of an antigen (Bovine Serum Albumin, BSA, 0.05 mg/ml, s.c) at the start of the experiment. Immunization was repeated with

0.1 ml BSA (0.02 mg/ml s.c) on day 14. On day 21, each mouse received an intravenous injection of 0.2 ml of a 1 % solution of Evans blue dye *via* the tail vein. Mice were then randomized into 6 groups (n=5) and treated in one of the following respective regimen *p.o.* normal saline (5 ml/kg), dexamethasone (10 mg/kg), aspirin (100 mg/kg) and TS (100, 200 and 500 mg/kg). Both pinnae of each mouse were inoculated with BSA (0.1 mg/ml) using a 21 gauge hypodermic needle 1 h after drug treatment. The area of dye extravasation in the pinnae of each mouse was measured 30 min after the antigen challenge and expressed as the mean reduction in the area of extravasation of dye with reference to the saline-treated control.

2.2.3 Ovalbumin (OVA)-induced Asthma

Guinea pigs (300-350 g) were sensitized with intraperitoneal injection of 0.1 ml solution (2 mg ovalbumin emulsified in 10 mg aluminium hydroxide ($Al(OH)_3$) dissolved in 0.9% saline). A booster dose of 0.1 ml solution (1 mg ovalbumin dissolved in 0.9 % saline) was administered intraperitoneally on day 14. Sensitized Guinea pigs were challenged with 1% aerosolized ovalbumin daily for 10 min in a perspex chamber connected to a jet nebulizer, from day 21 to day 60. Guinea pigs were placed into 6 groups (n=5) as follows; Group 1 (naive), sham sensitization plus challenge with PBS, Group 2, sensitization plus challenge with ovalbumin and treated with saline, Group 3, sensitization plus challenge with ovalbumin and treated with dexamethasone (5 mg/kg), Groups 4-6, sensitization plus challenge with ovalbumin and treated with TS either 100, 200 or 500 mg/kg respectively 1 h before each challenge. Normal saline, dexamethasone or TS was administered daily, from day 21 to day 60. Guinea pigs were subjected to the following tests;

2.2.3.1 Skin Prick Test

On day 50, the lower dorsal regions of the Guinea pigs were shaved and injected intradermally with 0.1 ml 1% ovalbumin. Diameters of wheals formed were measured 1 h and 24 h, post-injection respectively. The average wheal diameters were calculated and expressed as a percentage inhibition of wheal formation with reference to the OVA sensitized and saline-treated group

2.2.3.2 Inflammatory cell count in blood

24 h after the last aerosolized ovalbumin exposure, Guinea pigs were sacrificed by cervical dislocation, the jugular vein was cut and blood allowed to flow freely into EDTA tubes for haematological assay.

2.2.3.3 Lung histopathology

24 h after the last exposure, animals were euthanized and lung tissues excised, fixed in 10% neutral buffered formalin for 24 h. Lung tissues were subsequently embedded in paraffin and cut into 5-µm sections. For the histopathological study, sections were deparaffinized, dehydrated and stained with either hematoxylin and eosin (H & E) stain or Periodic Acid Schiff (PAS) stain for identifying goblet cells. The tissue sections were observed under light microscope (Leica DM2500 M). A method previously described by Myou et al.[15] was used to assess the degree of airway inflammatory cell infiltration with a brief scoring system as follows; 0, no cell; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2-4 cell layers deep; 4, a ring of cells >4 cell layers deep.

Goblet cell hyperplasia in the airway epithelium was quantified based on a 5 point scale scoring system[16]as follows; 0, no goblet cells; 1, 25%of the epithelium; 2, 25-50% of epithelium; 3, 50-75 % of epithelium; 4, > 75% of epithelium. For each Guinea pig, 5 random sections from the left lower lung were analyzed and the average scores for each group calculated.

2.3 Statistical analysis

All results are presented as mean ± SEM. Data analysis was done using one-way analysis of variance (ANOVA). Multiple comparisons between treatment groups were performed using Dunnet's *post hoc* test. All statistical analyses were done using GraphPad for Windows version 6 (GraphPad Prism Software, San Diego, USA).

3. RESULTS

3.1 Effect of *Triplochiton scleroxylon* on *in vitro* Compound 48/80-induced mast cell degranulation

Microscopic observation of sections of mesentery tissues showed purple stained intact (1) and degranulated (2) mast cells (Plate 1A and B). Incubation of mesentery tissues with C48/80 for 15 min elicited profound mast cell degranulation in the saline-treated control compared to normal unchallenged tissue (Plate 1A and B). Quantification of the mast cells showed that pretreatment with TS (500, 750, 1000 µg/ml for 15 min) significantly inhibited Compound 48/80 elicited-degranulation as did ketotifen (10 µg/ml), a standard mast cell stabilizer compared to untreated naïve control (Fig 1). Percentage degranulation of mast cells was calculated by counting the number of degranulated mast cells observed in different fields from a total of 100 mast cells counted. TS (500-1000 µg/ml) exhibited 13.0% - 61.4% mast cell protection from C48/80-induced degranulation.

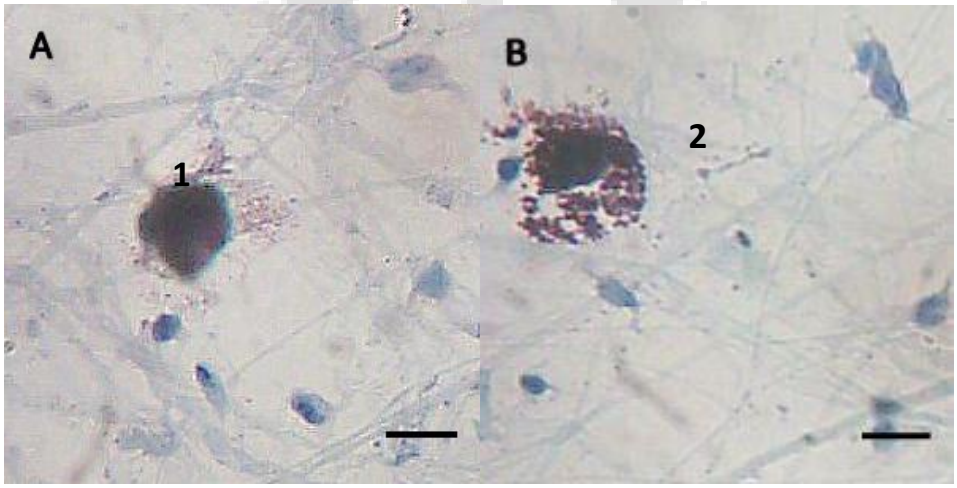

Plate 1. Effect of TS on C48/80- induced, rat mesentery mast cell degranulation.

A Wistar rat (250 g) was euthanized and pieces of its mesentery incubated for 15 min with 0.1 ml C48/80 (10 µg/ml). Tissues were transferred into a 4 % buffered formalin solution containing 0.1 % Toluidine blue for 30 min for mast cell staining (A and B). Intact mast cells (1) and degranulated mast cells (2) are denoted Tissues were either untreated or treated with ketotifen maleate (10 µg/ml) and TS (500, 750 and 1000 µg/ml). Scale bar represents 15 µm.

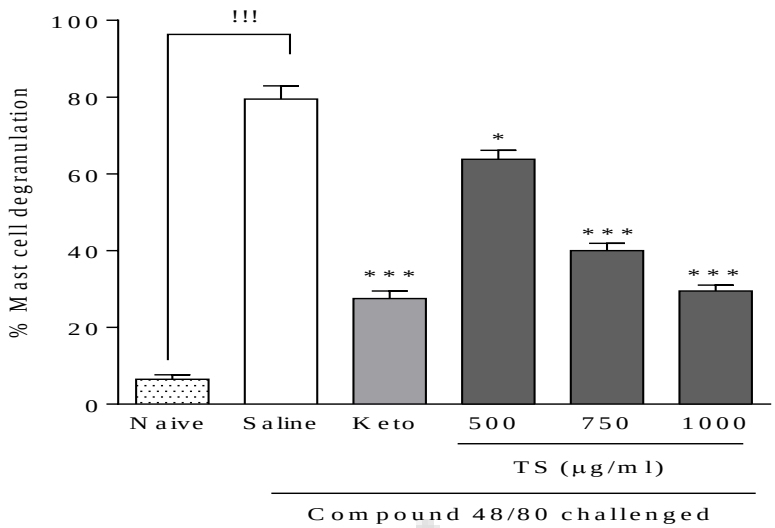

Fig.1. Effect of *Triplochiton scleroxylon* on C48/80-induced rat mesentery mast cell degranulation.

A Wistar rat (250 g) was euthanized and pieces of its mesentery incubated for 15 min with 0.1 ml C48/80 (10 µg/ml). Tissues were transferred into a 4 % buffered formalin solution containing 0.1 % Toluidine blue for 30 min for mast cell staining. Percentage degranulation of mast cells was quantified by counting the number of degranulated mast cells observed in different fields from a total of 100 mast cells counted. Values are expressed as mean ± SEM (n=5) ***P < 0.001, *P < 0.05 as compared to saline-treated control and $^{!!!}$P < 0.001 as compared to untreated/unchallenged naive control using One-way ANOVA followed by Dunnet's *post hoc* test. Tissues were either untreated or treated with ketotifen maleate (10 µg/ml) or TS (500, 750 and 1000 µg/ml).

3.2 Effect of *Triplochiton scleroxylon* on Active Cutaneous Anaphylaxis (ACA)

As expected, dexamethasone and aspirin-treated mice in which active cutaneous anaphylaxis had been induced presented significant reductions in the area of extravasation of the Evans blue dye relative to the saline-treated control animals. Dexamethasone (10 mg/kg) exhibited the highest inhibition of 78.0 ± 5.3% whereas aspirin (100 mg/kg) gave an inhibition of 66.5 ± 4.6 % (Fig. 2). Upon antigen challenge, pretreatment with TS at all doses(100, 200 and 500) mg/kg significantly (P < 0.5) inhibited the ACA reaction in previously sensitized mice by reducing the

dye extravasation by 34.3 ± 8.8 %, 63.9 ± 7.6 % and 70.4 ± 6.9 % respectively relative to the control response (Fig. 2).

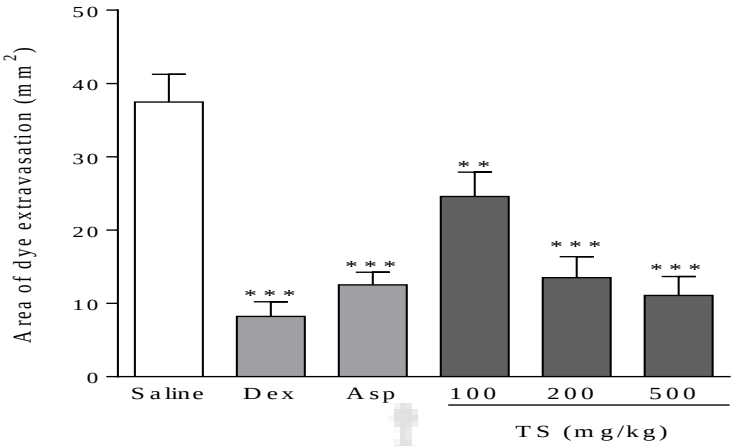

Fig.2. Effect of *Triplochiton scleroxylon* on Active Cutaneous Anaphylaxis in mice.

Male ICR mice (25-30 g) were immunized with an antigen, BSA twice as described in the method. On day 21, each mouse received an intravenous injection of 0.2 ml of a 1 % solution of Evans blue dye *via* the tail vein and challenged 30 min after with pinnae inoculation with BSA, 0.1 mg/ml. Average area of dye extravasation in both pinnae was measured. Saline 10 ml/kg, dexamethasone 10 mg/kg, aspirin 100 mg/kg and TS 100- 500 mg/kg were given prophylactically 1 h before challenge. Data is expressed as mean area ± SEM (n = 5) ***P < 0.001, **P < 0.01 as compared to saline-treated control using One-way ANOVA followed Dunnet's *post hoc* test)

3.3 Ovalbumin (OVA)-induced Asthma

3.3.1 Effect of Triplochiton scleroxylon on skin prick test

In the early phase inflammatory reaction, 1 h post ova challenge, the naïve (non-sensitized and unchallenged) control group had a mean wheal diameter of 7.5 ± 0.89 mm and this was significantly increased to 20.16 ± 0.60 mm in ovalbumin-challenged saline-treated control (Fig. 3). The reference drug, dexamethasone significantly suppressed the wheal diameter to 12 ± 0.82 mm. Likewise, TS treatment at 100, 200 and 500 mg/kg significantly inhibited the immediate inflammatory reaction recording wheal diameters of 14 ± 0.84 mm, 11.4 ± 0.68 mm and 12.0 ± 0.84 mm respectively (Fig. 3). In the late phase inflammatory reaction, 24 h post OVA challenge,

as expected, a significantly reduced mean wheal diameter of 1.3 ± 0.44 mm was recorded in the naïve animals that served as the normal non-sensitized and unchallenged control when compared to the mean wheal diameter in the early phase (Fig. 3). Statistically significant reductions in wheal diameter i.e 5.8 ± 0.37 mm were obtained for the dexamethasone-treated group. TS at 100, 200 and 500 mg/kg yielded wheal diameters of 8.8 ± 0.63 mm, 8.0 ± 0.41 mm and 7.0 ± 0.37 mm respectively when compared to the ova-sensitized and challenged saline-treated control group. Not surprisingly, there was no statistical difference in the ovalbumin-challenged saline-treated control at the two time points.

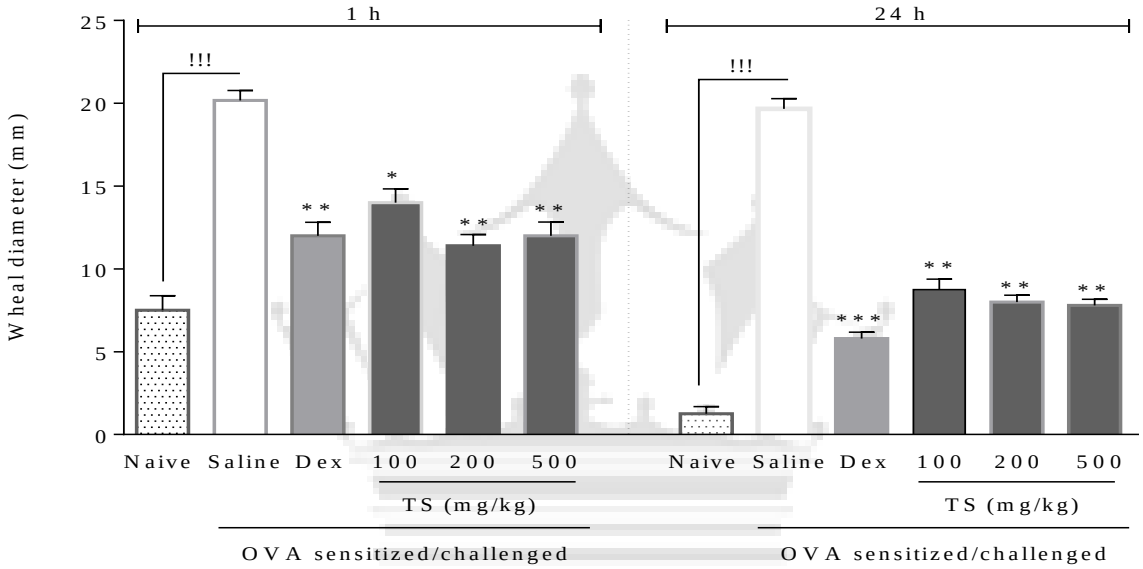

Fig. 3.Effect of *Triplochiton scleroxylon* on skin prick test.

Guinea pigs (300-350 g) were sensitized with an antigen, ovalbumin twice as described in the method and challenged with 1% aerosolized ovalbumin daily for 10 min from day 21.On day 50, the lower dorsal region of Guinea pigs were shaved and injected intradermally with 0.1 ml (1 % ovalbumin). Wheal diameters were measured 1 h and 24 h in naive non-sensitized/unchallenged Guinea pigs and in challenged groups treated with saline 10 ml/kg, dexamethasone 5 mg/kg or TS 100, 200 and 500 mg/kg. Data is expressed as mean wheal diameter ± SEM (n = 5) ***P < 0.001, **P < 0.01, *P < 0.5 as compared to saline-treated group. $^{!!!}$P < 0.001 as compared to non-sensitized unchallenged (naive) group using One-way ANOVA followed by Dunnet's *post hoc* test).

3.3.2. Effect of *Triplochiton scleroxylon* on inflammatory cell count in blood

Differential blood inflammatory cell count showed significantly increased numbers in peripheral blood of ovalbumin-challenged saline-treated control for eosinophils, lymphocytes, monocytes and basophils compared to the naïve non-sensitized and unchallenged normal control group (Fig. 4 A-D). The counts of these inflammatory cells in blood were suppressed significantly by dexamethasone (Fig. 4 A-D). At the administered dose of 100 – 500 mg/kg, the aqueous extract of *Triplochiton scleroxylon*, TS, significantly reduced the eosinophil and lymphocyte cell counts respectively with the lymphocyte count reduction showing dose-dependency (Fig. 4 A and B). Elevated monocyte and basophil counts respectively in the ova-sensitized and challenged saline-treated animals were significantly reduced at 500 mg/kg TS, while the 100 and 200 mg/kg TS doses showed reductions that were not significantly different from the saline-treated control (Fig. 4 C and D).

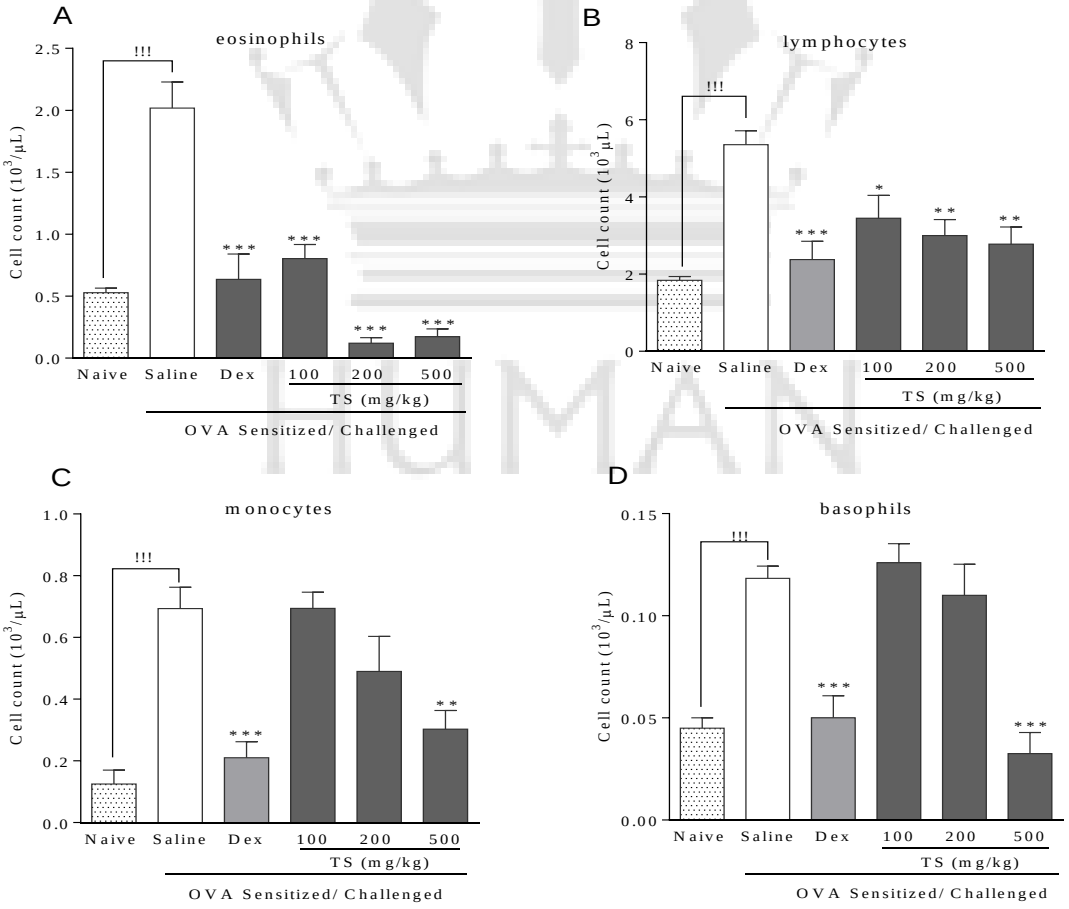

Fig. 4. Effect of *Triplochiton scleroxylon* on inflammatory cell count in blood.

Guinea pigs (300-350 g) were sensitized with an antigen, ovalbumin twice as described in the method and challenged with 1% aerosolized ovalbumin daily for 10 min from day 21. Blood was collected 24 h after the last exposure to ovalbumin and analyzed for differential blood cell count. Inflammatory cells; eosinophils (A), lymphocytes (B), monocytes (C), basophils (D) were counted. Animals were treated with saline 10 ml/kg, dexamethasone 5 mg/kg or TS 100, 200 and 500 mg/kg. Data is expressed as cell count (10^3/µL) ± SEM (n = 5) ***P < 0.001, **P < 0.01, *P < 0.5 as compared to saline-treated control; $^{!!!}$P < 0.001, $^{!!}$P < 0.01 as compared to non-sensitized/unchallenged (naive) control using One-way ANOVA followed by Dunnet's *post hoc* test.

*3.3.*3 Effect of *Triplochiton scleroxylon* on lung histopathology and inflammatory cell infiltration

The non-sensitized unchallenged control group presented with intact lung architecture (Plate 2A). Sensitization and challenge of the saline-treated group induced severe smooth muscle thickening, epithelial thickening and elongation, profuse inflammatory cell infiltration into alveolar air spaces and perivascular and peribronchiolar inflammatory cell accumulation (Plate 2B). Dexamethasone significantly reversed all features of inflammatory damage (Plate 2C) as did the administered doses of the extract (Plate 2D – F) with treatments showing a marked inhibition of smooth muscle hypertrophy and cell infiltration.

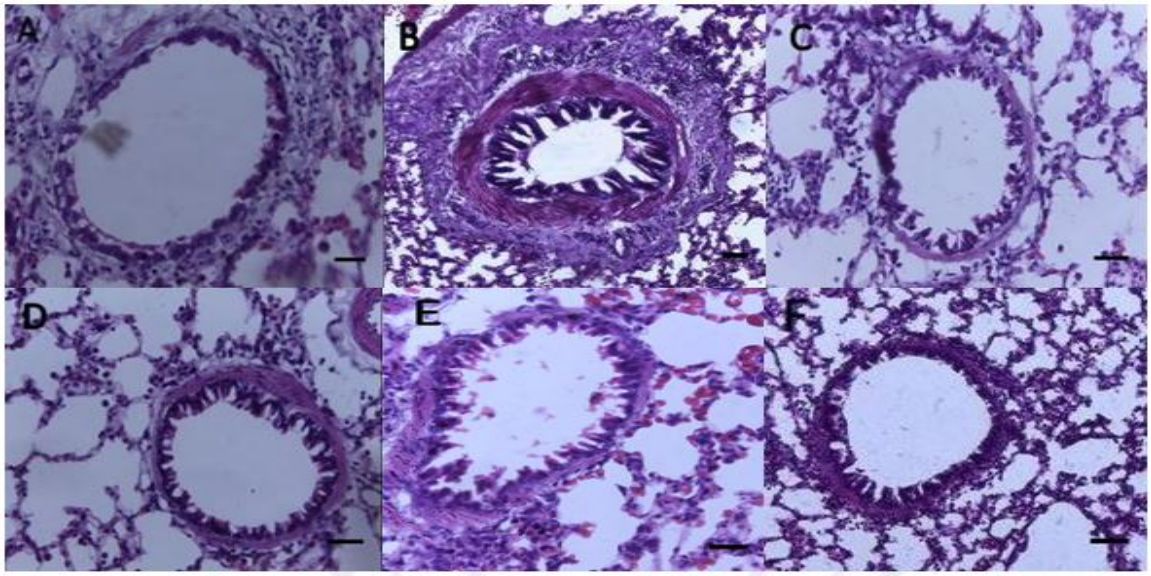

Plate 2. Effect of *Triplochiton scleroxylon* on lung histopathology.

Sensitized and non-sensitized Guinea pig lung tissues were excised 24 h after the last ovalbumin exposure, fixed, embedded and stained with H & E. Lung sections of naive Guinea pigs (A), OVA-challenged and saline-treated (B), OVA-challenged + 5 mg/kg Dex (C) and OVA-challenged + 100, 200 and 500 mg/kg TS respectively (D-F). Scale bar represents 50 µm.

H and E staining revealed significant infiltration of inflammatory cells, especially lymphocytes and eosinophils, in all sensitized/challenged treatment groups compared to naïve group. When quantified there was a severe accumulation of peribronchiolar inflammatory cell infiltration in saline treated control with an infiltration score of 3.67 ± 0.16. Dexamethasone reduced this score significantly to 1.46 ± 0.17. On treatment with the three doses of the extract a significant inhibition in the score to 1.80 ± 0.28, 1.80 ± 0.20 and 1.20 ± 0.14 were recorded for TS at 100, 200 and 500 mg/kg respectively (Fig 5).

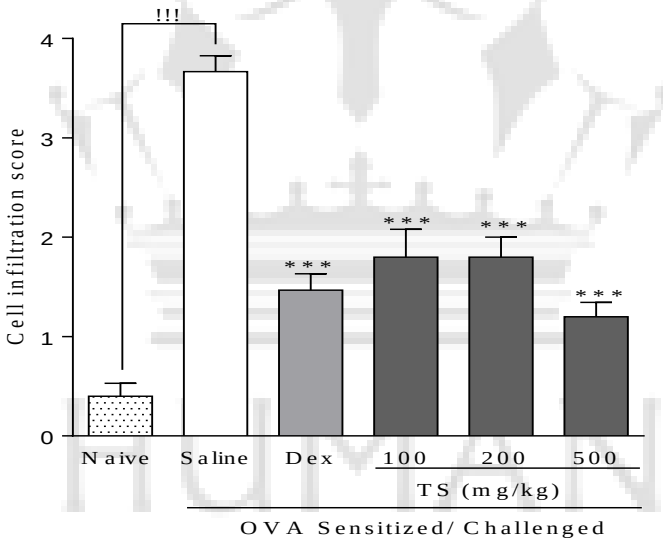

Fig.5. Effect of *Triplochiton scleroxylon* on inflammatory cell infiltration.

Sensitized and non-sensitized Guinea pig lung tissues were excised 24 h after the last ovalbumin exposure, fixed, embedded and stained with H & E to score inflammatory cells by a method described by Myou et al., (2003). Animals were treated with saline 10 ml/kg, dexamethasone 5 mg/kg or TS 100, 200 and 500 mg/kg. Data is expressed as mean infiltration score ± SEM (n = 5) ***P < 0.001, as compared to saline-treated group and $^{!!!}$P < 0.001 as compared to naive group using One-way ANOVA followed by Dunnet's *post hoc* test.

3.3.4 Effect of *Triplochiton scleroxylon* on goblet cell hyperplasia

The Periodic Acid Schiff (mucin specific) stain was employed to highlight the proliferation of goblet cells in the airway epithelium. The ovalbumin-sensitized and challenged saline-treated control group (Plate 3B) showed profound goblet cell hyperplasia (pink spots) associated with epithelial basement membrane thickening which was absent in lung sections of naive control Guinea pigs (Plate 3A). Lung sections from dexamethasone-treated group showed a marked inhibition of epithelial thickening (Plate 3C) as was observed with all three doses of TS (Plate 3D-F).

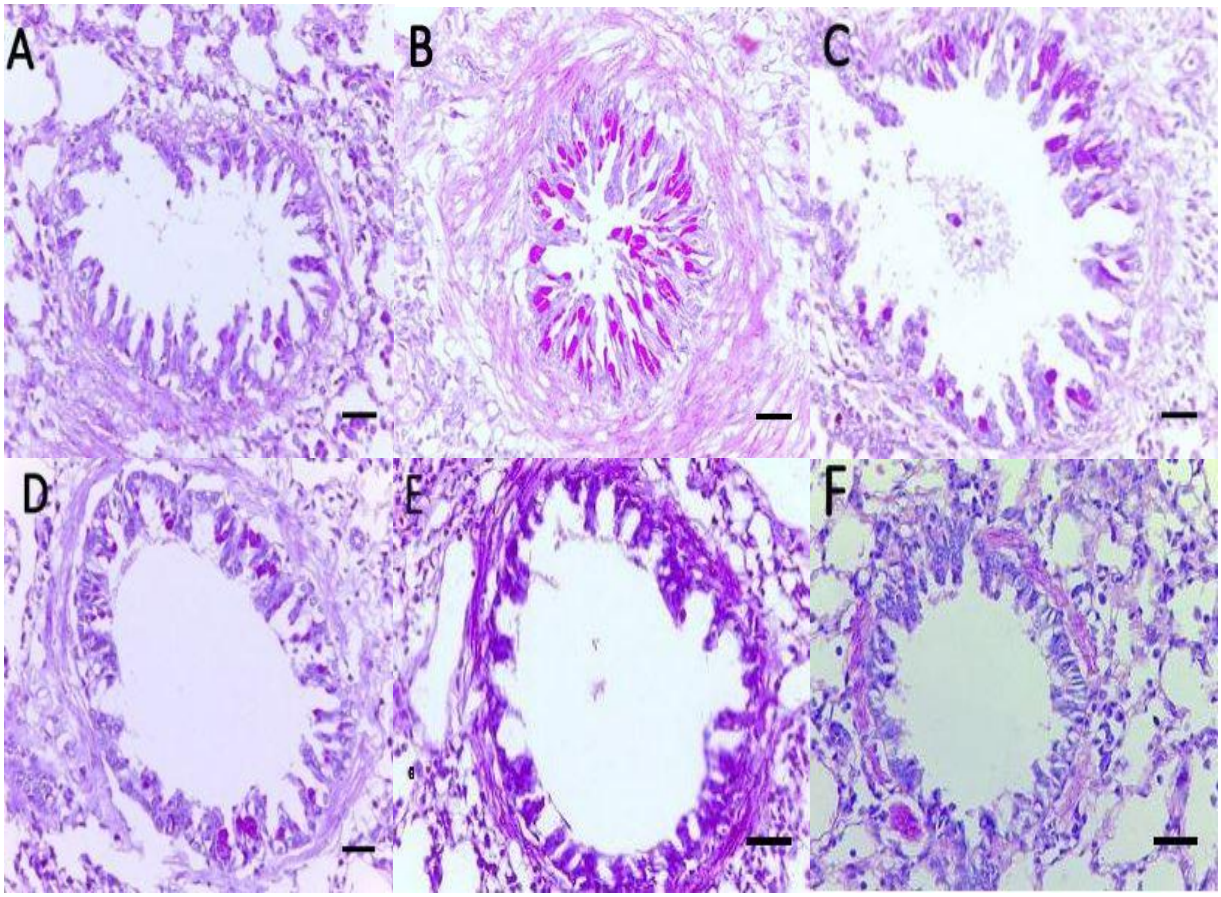

Plate 3. Effect of *Triplochiton scleroxylon* on goblet cell hyperplasia.

Sensitized and non-sensitized Guinea pig lung tissues were excised 24 h after the last ovalbumin exposure, fixed, embedded, and stained pink with Periodic acid Schiff (PAS) stain for goblet cell hyperplasia. Lung sections of non-asthmatic Guinea pig (A), OVA-challenged and saline-treated (B); OVA + 5 mg/kg Dex (C) and OVA +TS 100-500 mg/kg (D-F). Scale bar represents 50µm.

Pink stained goblet cells were quantified and presented in Fig 6. Ovalbumin-sensitized and challenged saline-treated group had a significantly increased staining score of 3.4 ± 0.16 relative to the naïve non-sensitized untreated control group with a staining score of 0.53 ± 0.16. Dexamethasone showed a marginal reduction of goblet cell proliferation of 2.8 ± 0.16 compared to the saline-treated group but this was not considered statistically significant. TS at 100, 200 and 500 mg/kg however significantly inhibited goblet cell proliferation with scores of 2.2 ± 0.17, 1.60 ± 0.27 and 1.27 ± 0.23 respectively.

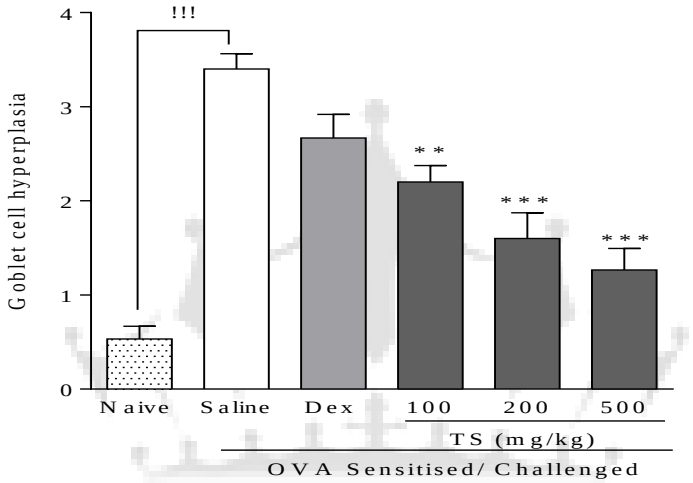

Fig. 6. Effect of *Triplochiton scleroxylon* on goblet cell hyperplasia.

Sensitized and non-sensitized Guinea pig lung tissues were excised 24 h after the last ovalbumin exposure, fixed, embedded and stained with Periodic acid Schiff (PAS) stain for goblet cell hyperplasia and quantified. Animals were treated with saline 10 ml/kg, dexamethasone 5 mg/kg or TS 100, 200 and 500 mg/kg. Data is expressed as mean goblet cell hyperplasia score ± SEM (n = 5) ***P < 0.001, **P < 0.01 as compared to saline-treated group and $^{!!!}$P < 0.001, as compared to naive group using One-way ANOVA followed by Dunnet's *post hoc* test.

4. DISCUSSION

This paper demonstrates that the aqueous extract of the stem bark of *Triplochiton scleroxylon* (TS) possesses significant anti-allergic properties and attenuates ovalbumin-induced airway inflammation in rodents. Airway inflammation, airway hyperreactivity (AHR), increased number of goblet cells, and mucus overproduction characterize asthma. Respiratory challenge with

ovalbumin (OVA) of sensitized mice has been shown by several laboratories to cause pulmonary pathology similar to that observed in human allergic asthma.

Microscopic observation and mast cell counts revealed that the pretreatment of mesenteric tissues with TS significantly inhibited C48/80-elicited degranulation. Compound 48/80 is a chemical known to induce non-immunologic degranulation of mast cells through disruption of the integrity of the lipid bilayer membrane and subsequent discharge of cytoplasmic secretory granules[17]. In the active cutaneous anaphylactic study, dye extravasation which is a direct consequence of changes in vascular permeability caused by inflammatory mediator release was markedly inhibited in the pinnae of mice treated with TS. The cutaneous challenge with an antigen in previously sensitized mice triggers an antigen-antibody reaction causing release of mast cell-derived proinflammatory mediators locally. TS suppressed the local allergic reaction arising from the antigen challenge possibly by inhibiting mast cell degranulation or by reducing effects of the released inflammatory mediators. Inhibition of this IgE-independent process, therefore, suggests that TS mast cell stabilizing mechanism may not depend entirely on the interference or modulation of IgE interactions with the mast cell.

We could show that chronic airway inflammation induced by ovalbumin challenge in previously sensitized Guinea pigs could be attenuated by TS administration. Ovalbumin is a reliable allergen for investigating IgE-mediated allergic reaction in animal models[18]. This method mimics a T helper 2 (Th2) cell-mediated allergic response that closely resembles asthma. It is characterized by inflammatory cell infiltration into lungs, airway hyperresponsiveness, mucus hypersecretion and airway inflammation[19]. The skin prick test showed reduced allergen sensitivity in TS-treated Guinea pigs. The resulting wheal formation, occurring as a result of intradermal ovalbumin injection was significantly inhibited. Lung sections of ovalbumin-challenged saline-treated Guinea pigs showed pathologies consistent with severe airway inflammation. Severe peribronchiolar and perivascular inflammatory cell accumulation, epithelial thickening and airway smooth muscle thickening were observed. Histopathological scores revealed marked improvement in TS-treated groups. Airway inflammation has been heavily linked directly and indirectly to worsening the asthma episode by reducing the airway caliber and increasing hyperresponsiveness and susceptibility to attacks[20]. Consequently, control of this process is highly relevant to asthma management. Airway inflammation is chiefly

dependent on activities of various inflammatory cells. For example eosinophils and other inflammatory cells in blood or bronchoalveolar fluid (BALF) have been found to be closely related to airway hyper-responsiveness (AHR) and airway inflammation[21]. In our study, we could demonstrate that the differential blood cell count showed reduced eosinophil, lymphocyte, basophil and monocyte levels in systemic circulation of TS-treated Guinea pigs. Though studies involving targeted blockade of eosinophils have failed to achieve overall control of asthma in the past[22,23], suppression of the immunologic events that control Th2 cell production, migration, infiltration and the chemotactic factors controlling these events are still viewed as having important therapeutic value[11,24]. The marked suppression of allergen sensitivity in the skin prick test and the inhibition of systemic proliferation of inflammatory Th2 cells, suggests TS exerts a possible modulatory effect on the underlying allergen-specific immune responses in ovalbumin-induced asthma. The specific mechanisms possibly involved are however beyond the scope of this study.

Also reported to be extensively implicated in the pathology of asthma are mucus plugs, thickened mucosal linings and goblet cell hyperplasia[25,26]. The mucin-specific PAS stain revealed severe proliferation of goblet cells and mucus occluded lumen in ovalbumin-challenged saline-treated Guinea pigs. TS treatment had significant inhibitory effect on this process, effectively attenuating ovalbumin-induced goblet cell hyperplasia. In this assay, we employed dexamethasone as a positive reference drug. Though dexamethasone has been reported in some studies to be effective in inhibiting goblet cell hyperplasia, it showed just a marginal effect in our study, although it significantly inhibited thickening of the basement membrane and epithelial layer. This is not surprising as it is consistent with earlier reports by Yilmaz et al.[27] where it was established that etanercept is more effective in lowering goblet cell number than dexamethasone in a mouse model of asthma. Studies by Kibe et al.[28] and Alimam et al.[29] in which dexamethasone failed to reduce goblet cell hyperplasia induced by IL-13, the major cytokine in allergen-induced goblet cell hyperplasia further points to the varied effect of dexamethasone on mechanisms of goblet cell proliferation.

Taken together, we could show that TS had inhibitory effects on both IgE-dependent and IgE-independent immediate allergic reactions and caused marked suppression of mast cell degranulation and key pathological features of asthma.

5. CONCLUSION

The aqueous stem bark extract of *Triplochiton scleroxylon* attenuates allergy and OVA-induced asthma in rodents.

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