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Anti-inflammatory and safety assessment of *Polyscias fruticosa* (L.) Harms (Araliaceae) leaf extract in ovalbumin-induced asthma

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Abstract

Background: *Polyscias fruticosa* is a plant used in the traditional management of asthma in Ghana. **Aim:** This study evaluated the anti-inflammatory property of an ethanolic leaf extract of *Polyscias fruticosa* and safety for use in ovalbumin-induced asthma. **Methodology:** The total and differential white blood cell counts, C-reactive protein level, and erythrocyte sedimentation rate were determined for blood samples obtained from Duncan Hartley guinea-pigs following sensitization (150 µg OVA + 100 mg aluminium hydroxide, I.P), OVA aerosol challenge, and treatment with 2 ml/kg normal saline, 10mg/kg prednisolone and 100, 250 or 500 mg/kg of the extract. An acute and delayed toxicity study was also conducted. **Results:** White blood cells and its differentials were significantly elevated ($P \leq 0.05$) after OVA-induced asthma. Treatments with the extracts and prednisolone significantly reduced ($P \leq 0.05$) elevated white blood cells and its differentials. Elevated serum C-reactive protein ($\geq 6.0 \pm 0.00$ mg/l) was reduced to within normal levels, and there was a significant decrease ($P \leq 0.01$) in erythrocyte sedimentation rates in PFE and prednisolone-treated animals following OVA-induced asthma. PFE showed no significant toxic effect at lower doses (NOAEL: < 1000 mg/kg) **Conclusion:** The ethanolic leaf extract of *Polyscias fruticosa* reduces white blood cell count and its differentials in blood, hence its anti-inflammatory effect; a useful property in the treatment of asthma.

Keywords: Anti-asthmatic, White blood cells, Ovalbumin, Erythrocyte sedimentation rate, C-reactive protein, Acute and delayed toxicity test.

Introduction

Asthma is a complex inflammatory disorder of the airways that involves the activation and release of inflammatory mediators resulting in the typical pathophysiological changes, i.e. bronchial inflammation and airflow limitation; leading to recurrent episodes of cough, wheeze, and shortness of breath.[1] In inflammation associated with asthma, the airway wall is infiltrated by T lymphocytes, eosinophils, macrophages/monocytes and mast cells, and sometimes neutrophils.[2, 3] In addition, there is the release of histamine and cysteinyl-leukotrienes from eosinophils and mast cells which induce bronchoconstriction, airway oedema, and mucus secretion.[4]

Although many drugs used in the treatment of asthma aim at reducing or blocking these inflammatory mediators, these drugs are very expensive and not readily available to all. In the face of these problems, efforts are being made worldwide to discover newer drugs that can nullify inflammation in asthma, and management of complications, with minimal side effects.

Many medicinal plants have shown interesting results in various target specific biological activities and as such have been suggested as potential in the treatment and management of asthma. One such plant is *Polyscias fruticosa* (L.) Harms (Araliaceae). Although there has been an increase of awareness and interest in medicinal plants in recent times,[5] a number of herbs are thought to possibly cause adverse effects.[6] To help curb the situation, the efficacy and safety of these plants must be ascertained before their recommendation for medical use. It is in this light that the anti-inflammatory property and the safety profile of an ethanolic leaf extract of *Polyscias fruticosa* in ovalbumin (OVA)- induced asthma in Duncan Hartley guinea

pigs was evaluated.

Materials and Methods

Polyscias fructicosa leaves were collected from Anwomaso, a suburb of the Kumasi Metropolis in the Ashanti Region of Ghana, (in December, 2013), and authenticated by Dr. George Henry Sam, of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana, where a voucher specimen (KNUST/HM/13/W010) has been deposited.

Figure 1: A picture of *Polyscias fruticosa* (L.) Harms showing the stem, leaves, flowers and friuts

Preparation of ethanolic leaf extract of *Polyscias fruticosa* (PFE)

The *Polyscias fruticosa* leaves were washed, shade-dried (26-30 ^{o}C) and pulverized into fine powder using a mechanical blender. A 3.0 kg quantity of the powder was soaked in 1 liter of absolute ethanol in a volumetric flask on a rotary mixer for 72 h. The filtrate obtained after extraction was concentrated in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland). The residue (58.2 g; Percentage yield: 1.94%) obtained was dried in a desiccator, labelled PFE and stored at 4^{o}C in a refrigerator for use in this study.

Phytochemical screening

Preliminary phytochemical screening was performed using standard phyto-analytical methods as described by Trease and Evans[7] and Sofowora[8].

Experimental animals

Healthy Duncan Hartley guinea pigs of either sex (300–500 g) were used for the experiment. They were maintained under ambient conditions of temperature (26 ± 4 ºC), relative humidity (60 ± 10%) and normal light/dark cycle. They were fed on commercially prepared pellet (Agricare Ltd, Kumasi, Ghana) and water *ad libitum.*

Induction of asthma

The induction of asthma was in two phases; a sensitization, and a challenge phase.[9] Guinea-pigs were sensitized by two bilateral intra-peritoneal injections (on days 1 and 7) of ovalbumin and aluminium hydroxide (150 µg: 100mg) in 1ml of normal saline. This was followed by an aerosol challenge on day 15. In the challenge phase the sensitized guinea pigs were put into five groups (n=4) and challenged by inhalation exposure to OVA aerosol (1% saline solution of OVA) for 30 min using Plexiglas (14×4 inch) chamber.

Experimental conduct and design

One day after the OVA-aerosol challenge, animals in groups 1-3 were given orally 100, 250, and 500 mg/kg PFE respectively, by gavage. Animals in groups 4 and 5 were treated orally with 10 mg/kg prednisolone (reference anti-inflammatory drug), and 2 ml/kg normal saline (non-drug treatment control) respectively. After an hour, the animals were sacrificed and blood samples collected were processed for total and differential white blood cell count, as well as erythrocyte sedimentation rate and C-reactive protein assay, as a measure of anti-inflammatory effect.

Total and differential white blood cell counts

Blood samples were obtained from all groups post-treatment and put into EDTA K3 tubes (Channel MED, China) for the hematological analysis using the Sysmex hematoanalyzer XS 1000i (Sysmex Instruments, Japan) at KNUST Hospital, Kumasi.

Determination of erythrocyte sedimentation rate (ESR)

Blood samples (2.5 ml) obtained from all groups were put into Trisodium Citrate ESR tubes (FL Medical, Italy), corked, and gently mixed for the ESR analysis. The sedimentation pipette was gently inserted into the citrate tube and by capillary action the anticoagulated blood rose to the 0 mm mark at the stopped top of the pipette. While steadily positioned in an upright vertical position, the rate at which the red blood cells fell in an hour (mm/h) was estimated.

C-reactive protein test

Blood from all groups were collected in tubes containing gel and clot activator (Channel MED, China) and were left to clot at room temperature. Clotted samples were centrifuged at 3000 rpm for 5 min, and collected sera were semi-quantitatively estimated for C-reactive proteins using the visual agglutination principle; protocol stated in the assay kit (Fortress Diagnostics Ltd, UK). The reagents in the kit were brought to room temperature and the CRP latex was re-suspended gently. A 50 µL quantity of the sample was placed on a circle in the test card. One drop of the latex reagent was added to the circle next to the sample to be tested. The reagent and sample were mixed in the circle using a sterile stirrer for two minutes, and readings were taken by macroscopic examination for the presence or absence of agglutination. Each run of test was validated with a positive and negative control. Specific values were obtained by performing serial dilutions from the positive samples by comparing with semi-quantitative values provided by the manufacturer of the diagnostic test kit. The presence of agglutination indicated a level of CRP greater or equal to 6 mg/l. An absence of agglutination indicated a level of CRP less than 6 mg/l.

Acute and Delayed Toxicity studies

Four-week-old male guinea pigs, weighing 300-350 g, were put in six groups of four and labelled A-F. The animals were fasted for 6 hours prior to dosing. Group A served as the control group and received no treatment. Groups B-F received

100, 250, 500, 1,000, or 2,000 mg/kg PFE by gavage, respectively. The animals were observed for clinical or behavioural signs of toxicity, changes in food and water consumption, and mortality, hourly for 8 h and daily thereafter for 14 days. The animal body weights were measured prior to dosing and on days 7 and 14 of the experimental period.

Ethical considerations

Institutional research clearance was obtained before the study was undertaken. Laboratory study was carried out in a level 2 biosafety laboratory. Protocols for the study were approved by the Departmental Ethics Committee. All activities during the studies conformed to accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). All the technical team observed all institutional biosafety guidelines for protection of personnel and laboratory

Data analysis

Graph Pad Prism Version 5.0 for Windows (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyses. Data was presented as mean ± SEM and also analyzed by one - way ANOVA followed by Bonferroni's multiple Comparison test (*post hoc* test). $P \leq 0.05$ was considered statistically significant in all analysis.

Results

Phytochemical screening

The ethanolic leaf extract of *Polyscias fruticosa* (L.) Harms was found to contain glycosides (saponins and cyanogenetic), alkaloids, and sterols (Table 1).

Table 1: Results of the phytochemical screening of PFE

Phyto-constituent	Presence/Absence
Alkaloids	+
Glycosides	+
Saponins	+
Cyanogenetic glycosides	+
Sterols	+
Tannins	–
Flavonoids	–
Coumarins	–

The + sign indicates the presence whereas the – sign indicates the absence.

Total and differential white blood cell count

Total and differential white cell counts were elevated ($P \leq 0.05$) with OVA-induced asthma. There were however resultant decrements ($P \leq 0.05$) with prednisolone and PFE treatments, with 250 mg/kg PFE dose giving better reduction of the elevated total and differential white cell count (Figure 2)

Erythrocyte Sedimentation Rate

Anticoagulated blood samples from sensitized animals treated with 100, 250 and 500 mg/kg of PFE, and 10 mg/kg of prednisolone recorded significantly low ($P \leq 0.01 – 0.001$) erythrocyte sedimentation rates whereas blood samples from OVA-sensitized, non-treated animals recorded the highest sedimentation rates (Figure 3).

C - reactive protein assay

Levels of C-reactive proteins in sera of animals treated with 100, 250, and 500 mg/kg PFE, and 10 mg/kg prednisolone, following OVA-sensitization was ≤ 6 mg/l; indicative of normal levels of the acute inflammation phase proteins. This result was comparable to that obtained from non-sensitized/non-treated animals. In the sensitized animals treated with normal saline, there was an elevated serum C-reactive protein level i.e. 12 mg/l (Table 2).

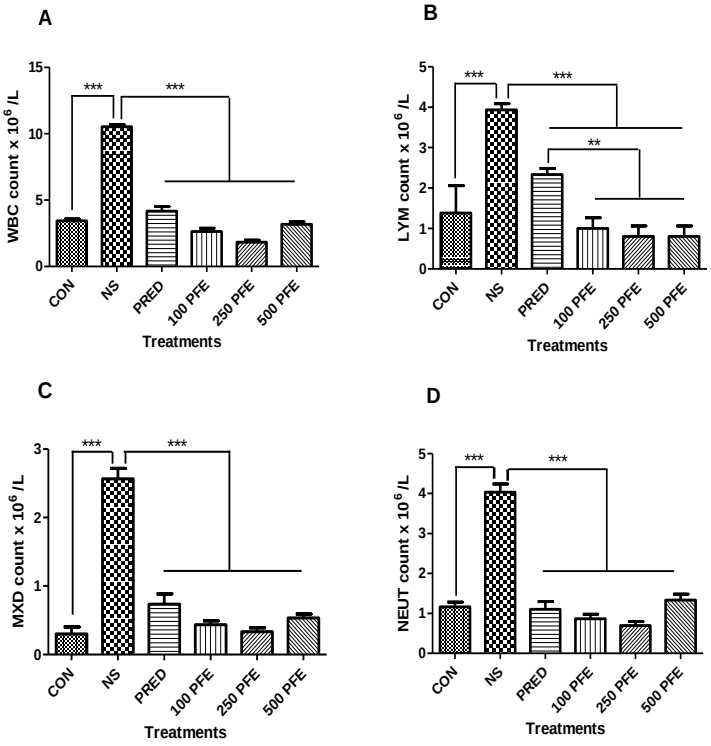

Figure 2: The effect of normal saline (NS), 10 mg/kg Prednisolone (PRED), and 100, 250, and 500 mg/kg PFE on (A) Total white blood cell count, and (B, C, D) differential white blood cell count in OVA-induced asthma in Duncan Hartley guinea pigs. ** $P \leq 0.01$, *** $P \leq 0.001$.

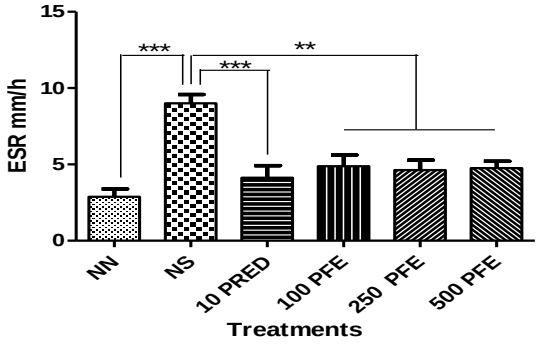

Figure 3: ESR of blood samples obtained from non-sensitized/non-treated guinea pigs (NN), as well as OVA-sensitized guinea pigs treated with 2 ml/kg Normal saline (NS), 10 mg/kg prednisolone, and 100, 250, and 500 mg/kg PFE. ** $P \leq 0.01$, *** $P \leq 0.001$.

Table 2: Serum concentrations of CRP in non-sensitized/non-treated animals, as well as OVA-sensitized guinea pigs treated with 2 ml/kg normal saline (NS), 10 mg/kg of prednisolone (PRED), and100, 250, and 500 mg/kg of PFE.

Treatment groups	Serum CRP LEVEL (mg/l)
Non- sensitized/non-treated	≤ 6 0 ± 0.00
2 ml/kg NS	> 6.0 but ≤ 12 0 ± 0.00
10 mg/kg PRED	≤ 6 0.± 0.00
100 mg/kg PFE	≤ 6 0 ± 0.00
250 mg/kg PFE	≤ 6 0.± 0.00
500 mg/kg PFE	≤ 6 0 ± 0.00

Values are means ± SEM (n=4) of semi-quantitative determination of CRP using the visual agglutination principle; protocol stated in the assay kit (Fortress Diagnostics Ltd, UK).

Acute and delayed toxicity studies

There were no toxic symptoms and mortality observed for groups treated with 100, 250, 500 and 1000 mg/kg PFE. However, cage-side observation recorded decreased activity, and reduction in food and water consumption in the group treated with 2000 mg/kg PFE. Here, a significant decrease ($P \leq 0.01 – 0.001$) in body weight was seen over the 14 day period for the study (Table 3).

Table 3: The effect of various concentrations of PFE on the body weights of guinea-pigs before, and on day 7 and 14 in an acute and delayed toxicity test

GROUPS	Before	Day 7	Day 14
Control	326.0 ± 21.04	343.0 ±21.97 ns	355.0 ± 22.64 ns
100 mg/kg	333.0 ± 22.25	339.0 ± 19.49 ns	350.4 ± 18.03 ns
250 mg/kg	335.0 ± 18.71	348.0 ±20.19 ns	350.0 ± 24.24 ns
500 mg/kg	333.0 ± 21.1	352.0 ± 20.19 ns	354.0 ± 21.61 ns
1000 mg/kg	339.0 ± 16.73	347.0 ± 18.23 ns	346.0 ± 10.84 ns
2000 mg/kg	331.0 ± 16.73	283.0 ± 28.85 **	262.0 ± 15.25 ***

Values are means ± SEM (n=4). ns implies $P > 0.05$ (for insignificant increments or decrements); ** implies $P \leq 0.01$, *** implies $P \leq 0.001$ (for significant increments). ANOVA followed by Bonferroni's multiple Comparison test (post-hoc test).

Discussion

In this study, OVA-sensitized guinea pig models were used to investigate the effect of oral administration of PFE in reducing inflammation in asthma by assessing total and differential WBC count, serum levels of C-reactive proteins, and erythrocyte sedimentation rate.

Asthma was induced in the guinea pigs by challenging OVA-sensitized animals intranasal with OVA. Late phase asthmatic response is characterized by mast cell degranulation leading to the release of inflammatory mediators such as cytokines, and an increase in leucopoiesis.[10, 11] Eosinophils, neutrophils, and lymphocytes release substances such as eicosanoids, metalloproteinase, elactase, lactoferins and reactive oxygen species which lead to contraction of airway smooth muscles and airway hyper-responsiveness.[12] OVA therefore induced an inflammatory response which was characterized by elevated total and differential WBCs.

OVA sensitization proceeds with the activation of mast cells and T-helper type 2 (Th2) cells in the airway when inhaled. These cells in turn act by inducing the production of mediators of inflammation such as histamine, leukotrienes and cytokines including interleukin-4 and interleukin-5. Interleukin-5 travels to the bone marrow and causes terminal differentiation of eosinophils. Circulating eosinophils enter the area of allergic inflammation and migrate to the lungs through interactions with selectins, and eventually adhere to endothelium through the binding of integrins to members of the immunoglobulin superfamily of adhesion proteins: vascular-cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). As the eosinophils enter the matrix of the airway through the influence of various chemokines and cytokines, their survival is prolonged by interleukin-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). The eosinophils on activation release inflammatory mediators, such as leukotrienes and granule proteins, to injure airway tissues. In addition, eosinophils can generate GM-CSF to prolong and potentiate their survival and contribution to persistent airway inflammation.[10-13] The persistent inflammation results in an increased ESR as well as an elevated serum CRP.

ESR in OVA-induced asthmatic animals treated with PFE and prednisolone reduced significantly to within normal levels. The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and their ability to resist the negative surface charges of the erythrocytes (zeta potential). During systemic inflammation and immune disorders such as chronic allergic asthma, there is an increase in proportion of fibrinogen and immunoglobulins in the blood. These bind, recruit and cause red blood cells to stick to each other (i.e. increase in red cell aggregation).[14] The red cells form stacks called 'rouleaux', which settle faster (i.e. higher sedimentation rate). Increase in ESR therefore indicates the presence of high plasma fibrinogen.[15]

Prednisolone is a synthetic corticosteroid used for their potent anti-inflammatory effects particularly in those conditions in which the immune system plays an important role e.g. arthritis, bronchitis and asthma. It acts by preventing the release of prostaglandins, kinins, histamine, liposomal enzymes, and complement system. Prednisolone increases the production of anti-inflammatory mediators such as Lipocortin 1, endopeptidases and endonucleases; and causes the inhibition of gene transcription for COX-2, cell adhesion molecules, and inducible NO synthase. It also reduces the synthesis and release of several pro-inflammatory cytokines such as IL-1, GM-CSF, IL-3, IL-4, IL-5, IL-6 and IL-8; reducing inflammatory cell activation, recruitment and infiltration, and decreasing vascular permeability.[16-18]

Therefore the significant decrease in ESR caused by PFE and Prednisolone indicates anti-inflammatory effect.

In establishing a better diagnosis of systemic inflammation, ESR is usually assessed together with CRP;[19] because the acute phase response produced by CRP develops in a wide range of acute and chronic inflammatory conditions like asthma.[20] It was therefore expected that levels of CRP would be approaching peak concentration after OVA-sensitization and challenge. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver. This explains why serum CRP level is a biomarker for systemic inflammation in addition to its use in monitoring systemic response to treatment of inflammation.[21] The fibrinogen produced in the liver is known to increase red cell aggregation thereby speeding up the ESR. An evaluation of CRP in serum from OVA-sensitized non-treated guinea pigs recorded the highest concentration; validating the efficacy of our sensitization protocol. The physiological role of CRP in the inflammatory process is to bind phosphocholine expressed on the surface of dead or dying cells in order to activate the complement system via the C1Q complex.[21]

In a further attempt to explain the mode of action of PFE in achieving its anti-inflammatory effect, reference was made to the phytoconstituents present in the extract. Saponins, sterols, glycosides including cyanogenetic glycosides, and alkaloids were found to be present. Saponins are well known for their anti-inflammatory and cell stabilizing activity.[22] Saponins act in one way by inhibiting the formation of cyclooxygenase, namely prostaglandins and thromboxanes.[23] Saponins also inhibit histamine, bradykinin and serotonin along with its antioxidant effects which in turn inhibits the formation of reactive oxygen species which are known to play an important role in inflammation.[24] Sterols possess a very strong anti-inflammatory activity by inhibiting pro-inflammatory cytokine IL-1 and 2 release and IL-6, consequently suppressing the release of histamine. Also, glycosides (e.g. luteolin) have been purported to be potent inhibitors of histamine release from mast cells, and inhibit the CD40 ligand expression by basophils and mast cells.[25]

The safety assessment revealed decreased locomotor activity, reduction in feed and water consumption, and hence a significant reduction in body weight at a dose of 2000 mg/kg. Decreasing locomotor activity could imply that PFE may have CNS depressant, anxiolytic and/or muscle relaxant effect. The extract could also have anorexiant effect. These would lead to a decrease in their feeding which could contribute significantly to weight loss. A possible PFE depressant activity on the limbic system, thalamus, and hypothalamus, could account for its anxiolytic effects. The extract is therefore safer to use at doses lower than 1000 mg/kg; as the No-Observable-Adverse-Effect-level (NOAEL) is within this range; to avoid CNS effects such as dizziness, drowsiness, and lethargy (among others) which would be associated with higher doses.

Conclusion

The ethanolic leaf extract of *Polyscias fruticosa* has potent anti-inflammatory effect; a useful property in the treatment of asthma. Its use however should be limited to lower doses as the NOAEL is less than 1000 mg/kg in Duncan Hartley Guinea-pigs.

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Conflict of Interest

Author declares no known conflict of interest whatsoever.

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