

Original Research Article

Effects of a Hydro-ethanolic Extract of *Scoparia dulcis* on Systemic Inflammation and Cachexia in an Allergic Asthma Model

ABSTRACT

Background: Chronic allergic lung diseases, such as allergic asthma and COPD, are often marked by increased systemic inflammatory responses and cachexia co-morbidities.

Aims: The study aimed at evaluating the systemic anti-inflammatory and anti-cachectic properties of a hydro-ethanolic extract of *Scoparia dulcis* (SDE) in an ovalbumin-induced allergic guinea-pig asthma model.

Place and Duration of Study: Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana; between October, 2014 and July, 2015.

Methodology: Allergic asthma was induced by challenging OVA-sensitized guinea-pigs intranasal with 1% Ovalbumin. Levels of C-reactive proteins in animal serum, erythrocyte sedimentation rate and erythrocyte levels, as well as the total and differential blood cell counts were determined (as measures of systemic inflammation) for blood samples obtained from OVA-induced allergic animals treated with 2 ml/kg normal saline, 10 mg/kg prednisolone and 50, 100 or 250 mg/kg of the plant extract. Changes in body weights for each treatment regime were also measured over the period.

Results: SDE showed a dose-dependent reduction ($P \leq 0.001$) in erythrocyte sedimentation rate, an increase ($P \leq 0.05$) in the body weights of OVA-induced allergic animals, and improved significantly ($P \leq 0.001$) levels of red blood cells after treatment. However, total and differential white blood cells were significantly elevated ($P \leq 0.05$) after the OVA-induced asthma. Treatments with the plant extract and prednisolone significantly reduced ($P \leq 0.05$) elevated white blood cells and its differentials, as well as elevated serum C-reactive proteins (> 12.0 but $\leq 24.0 \pm 1.0$ mg/l) to within normal levels in OVA-induced allergic guinea-pigs.

Conclusion: The hydro-ethanolic extract of *Scoparia dulcis* has significant systemic anti-inflammatory effects (via reducing erythrocyte sedimentation rate, C-reactive proteins, and white blood cell counts), as well as ameliorative effect on pulmonary cachexia. This makes it a possible remedy for allergic asthma and chronic obstructive pulmonary diseases.

Keywords: Allergic asthma, Anti-cachectic, Systemic anti-inflammation, C-reactive proteins, Erythrocyte sedimentation rate.

1. INTRODUCTION

Allergic asthma, one of the chronic inflammatory immune airway diseases in the human respiratory system, is characterized by several systemic factors such as elevated levels of inflammatory biomarkers like C-reactive proteins and fibrinogen, with an increase in blood leukocyte levels and reduction in erythrocyte numbers [1, 2].

Differential diagnostic values of erythrocyte sedimentation rate (ESR) and C-reactive proteins (CRP) in inflammatory and immune disorders are potential meaningful biomarkers for systemic disease differentiation [3]. Measuring and charting ESR and CRP values can therefore prove very useful in determining and monitoring disease progress of systemic inflammation or the effectiveness of treatment of inflammatory diseases, such as in allergic asthma [4]. Also, cachexia comorbidity is common in patients who have any of the range of illnesses classified as chronic allergic respiratory diseases such as COPD and allergic asthma, and is thought to be linked to an enhanced systemic inflammatory response [5].

Moreover, COPD and allergic asthma therapy is reported to lack satisfactory success due to adverse effects, drug unavailability and high cost in Ghana. It is therefore imperative that patients are seeking complementary and alternative medicines obtained from plant sources to treat asthmatic conditions [6]. Available data on *Scoparia dulcis* suggest and support its anecdotal usage in the traditional management of allergic asthma and chronic obstructive pulmonary diseases in Ghana [7, 8]. However, the effects of this herbal plant on systemic inflammation and pulmonary cachexia have not yet been studied. The present study, therefore, investigated the systemic anti-inflammatory and pulmonary anti-cachectic effects of the hydro-ethanolic extract of *Scoparia dulcis* (SDE) in OVA-induced allergic asthma guinea-pig model.

2. MATERIALS AND METHODS

2.1 Plant collection

The fresh aerial parts of *Scoparia dulcis* plant were obtained from Osene-Adikanfo, Faith Herbal Centre, Mampong in the Ashanti region of Ghana, in December, 2014. It was identified and authenticated by the Herbal Medicine Department of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Ghana; where a voucher specimen (KNUST/HM1/2013/S027) has been kept [7, 8].

2.2 Preparation of the hydro-ethanolic extract of *S. dulcis*

The fresh aerial parts of *S. dulcis* were shade-dried for two consecutive weeks (8:00am–6:00pm, 26–30°C), and later milled into powder. One kilogram (1 kg) of the powder was macerated in 9.0 L of water:ethanol (30:70) solvent for 72 h. The suspension was filtered and the ethanol evaporated off in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) and the concentrated extracts freeze-dried (Heto Power Dry LL3000, Jouan Nordic, Denmark) to obtain 27.65 g powdered material (percentage yield: 2.77%). The powdered material obtained, referred to in this study as the hydro-ethanolic extract of *S. dulcis* (SDE) was then stored at 4°C in a refrigerator. SDE was then reconstituted in a suitable vehicle for use [7, 8].

2.3 Chemicals and Reagents

Ovalbumin (OVA) and aluminium hydroxide (Alum) were purchased from Sigma Chemical Co. (St. Louis, MO, USA); whereas Prednisolone was obtained from Letap Pharmaceuticals Ltd. (Accra, Ghana).

2.4 Experimental animals

Healthy male Dunkin Hartley guinea pigs (300–450 g) were used for the experiment. Animals were housed in sanitized aluminium cages (70 x 42 x 28 cm) with a base dressing of wood chippings as bedding, fed on commercial pellet diet (Agricare Ltd, Tanoso-Kumasi, Ghana), and water *ad libitum*. They were kept under ambient conditions of temperature (26 ± 4 °C), relative humidity (60 ± 10 %) and normal light/dark cycle, for 10 days prior to experimentation.

2.5 Dosing of drugs to experimental animals

Dosing of the plant extract was done based on its known traditional usage. Dosing was done once daily by gavage, at a volume of 1 ml/kg in normal saline, for five consecutive days. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used as it is the intended human exposure route.

2.6 Phytochemical Analysis of SDE

Preliminary phytochemical analysis was carried out according to the methods described by Sofowora [9], to determine the various phytoconstituents of SDE.

2.7 Induction of Allergic Asthma

Allergic asthma was induced in healthy guinea pigs (6 experimental groups, n=4) by exposing them to OVA allergen. A suspension of OVA and Alum (1 mg: 100 mg) in 1 ml normal saline was used for active sensitization of guinea-pigs by intraperitoneal injection once a week for 3 weeks (Days 0, 7 and 14). This was followed by an OVA-aerosol challenge. The sensitized guinea pigs were challenged by inhalation

exposure to OVA aerosol (1% saline solution of OVA) on days 21 and 22 (5 min/day) using Plexiglas (14×4 inch) chamber and a Wright nebulizer. The whole exposure to the OVA allergen lasted 22 days, and the experiment then actualized. Each group of animals was thereafter treated with either 2 ml/kg normal saline (Vehicle), 10 mg/kg salbutamol, 10 mg/kg prednisolone, or 50, 100, or 250 mg/kg of SDE orally, single dose daily for 5 consecutive days (Days 24 – 28). Another group, without OVA exposure, was however kept as normal control (i.e. no OVA sensitization or challenge).

2.8 Determination of Systemic Anti-Inflammatory Effect

At the end of the experiment (i.e. Day 28), animals were sacrificed and blood samples collected and processed for haematological analysis, as well as erythrocyte sedimentation rate and C-reactive protein assaying (i.e. measures of systemic inflammation), as previously described by Koffuor et al. [10].

2.9 Determination of Anti-cachectic Effect

Body weights of the animals were taken at the start of the experiment (on Day 0), and later following sensitization and boosting (on Days 14 and 16), before OVA allergen challenge (on Day 20), while treatment (on Day 24), and after treatment (on Days 26 and 28). The percentage change in mean body weights for the duration was then calculated for each treatment regime.

2.10 Statistical analysis

Data obtained in all experiments were expressed as mean $\pm$ SEM. Statistical analyses were done by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test (*post hoc* test) using Graph-Pad Prism for Windows Version 5.0 (Graph-Pad Software, San Diego, CA, USA). Differences between means of treated groups and the control were regarded as statistically significant at $P \leq 0.05$.

3. RESULTS

3.1 Phytochemical analysis of SDE

The phytochemical screening of SDE showed the presence of tannins, alkaloids, phenols, glycosides, saponins and steroids.

3.2 Systemic Anti-Inflammatory Effects of SDE

3.2.1 Effect of SDE on Blood cell Levels

Total and differential white blood cell counts were elevated ($P \leq 0.01$), whilst erythrocyte levels reduced with OVA-induced allergic asthma (Figure 1). However, after prednisolone and SDE treatments, there were resultant decrements ($P \leq 0.01 - 0.001$) in white cell counts (i.e. Figures 1A and 2), and significant ($P \leq 0.001$) improvement in levels of red blood cells in OVA-induced allergic animals (i.e. Figure 1B).

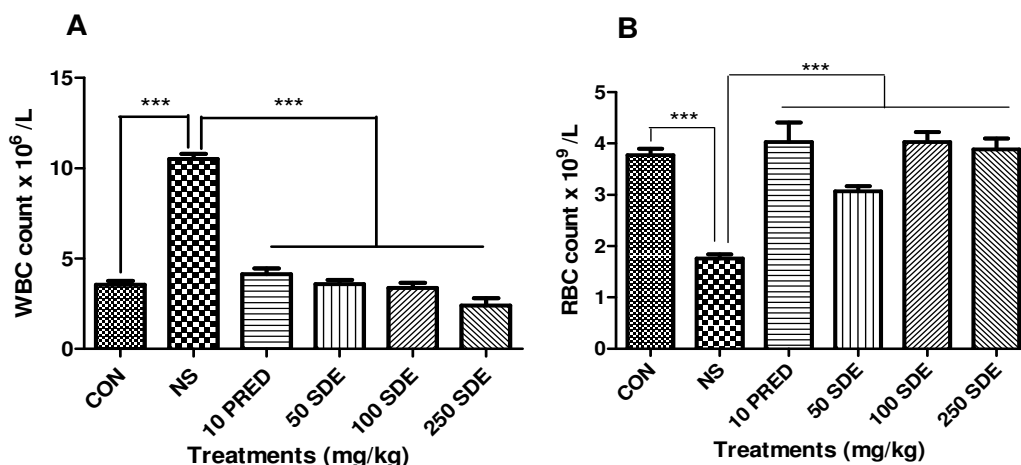


Figure 1:
The effect of SDE, Prednisolone (PRED), normal saline (NS), and Non-sensitized non-treated (CON), on total levels of (A) white blood cells and (B) red blood cells in an OVA-induced allergic asthma model.

*** implies $P \leq 0.001$. Values plotted are means $\pm$ SEM of $n = 4$.

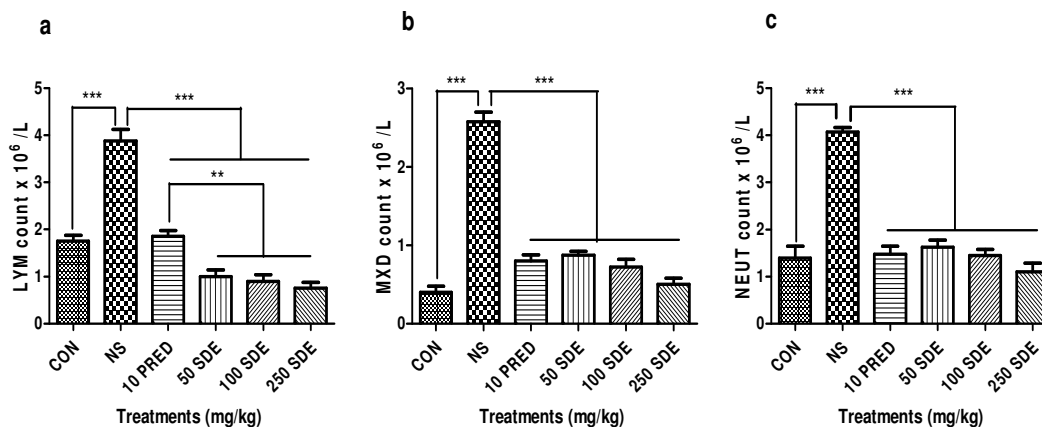


Figure 2: The effect of SDE, Prednisolone (PRED), normal saline (NS), and Non-sensitized non-treated (CON), on differential white blood cell counts (a – lymphocytes, b – eosinophils, c – neutrophils) in an OVA-induced allergic asthma model.

** implies $P \leq 0.01$; *** implies $P \leq 0.001$. Values plotted are means $\pm$ SEM ($n = 4$).

3.2.2 Effect of SDE on Erythrocyte Sedimentation Rate

Treatment of sensitized animals with 50, 100 and 250 mg/kg of SDE, and 10 mg/kg of prednisolone recorded significantly low ($P \leq 0.001$) erythrocyte sedimentation rates (as shown in Figure 3). Albeit, uncoagulated blood samples collected from OVA-sensitized, non-treated animals showed very high erythrocyte sedimentation rates.

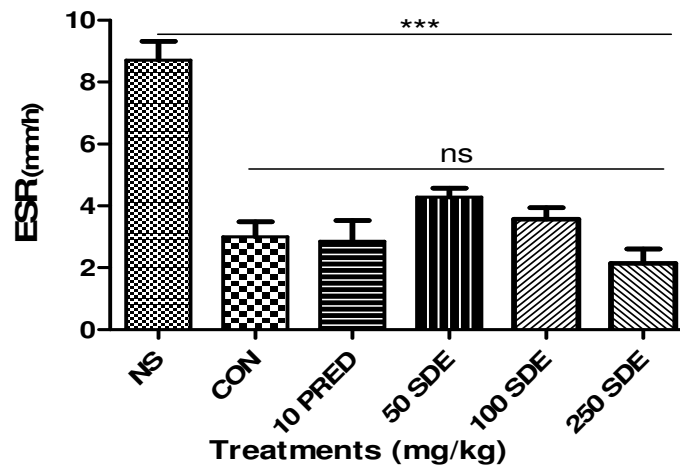


Figure 3: The Erythrocyte sedimentation rate of normal saline-treated (NS), non-sensitized non-treated control (CON), Prednisolone-treated (PRED), and SDE-treated guinea-pigs in an OVA-induced allergic asthma.

*** implies $P \leq 0.001$; ns implies $P > 0.05$. Values plotted are means $\pm$ SEM ($n = 4$).

3.2.3 Effect of SDE on C-Reactive Proteins

Serum C-reactive proteins in normal saline-treated control animals showed elevated levels after OVA sensitization and challenge, i.e. ≥ 12 mg/l. However, levels of C-reactive proteins in sera of animals treated with 50, 100, and 250 mg/kg SDE, and 10 mg/kg prednisolone, recorded ≤ 6 mg/l, comparable to that obtained from the non-sensitized, non-treated animals (Table 1); which is indicative of normal levels of the acute phase inflammation proteins.

Table 1: Sera concentrations of C-reactive proteins in non-sensitized non-treated (Control), normal saline-treated (NS), SDE-treated, and Prednisolone-treated (PRED), OVA-induced guinea-pigs of allergic asthma pattern.

Treatment groups	Serum CRP LEVEL (mg/l)
Control	$\leq 6.0 \pm 0.0$
2 ml/kg NS	> 12.0 but $\leq 24.0 \pm 1.0$
10 mg/kg PRED	$\leq 6.0 \pm 0.0$
50 mg/kg SDE	$\leq 8.0 \pm 2.0$
100 mg/kg SDE	$\leq 6.0 \pm 0.0$
250 mg/kg SDE	$\leq 6.0 \pm 0.0$

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

3.3 Anti-Cachectic Effect of SDE

There was a general decrease in body weights for all groups, after allergen sensitization and challenge, except for the non-sensitized non-treated control group. Prednisolone and SDE however stabilized and improved significantly ($P \leq 0.05$) the weights of animals after the treatment, comparable to the non-sensitized control, as shown in Figure 4.

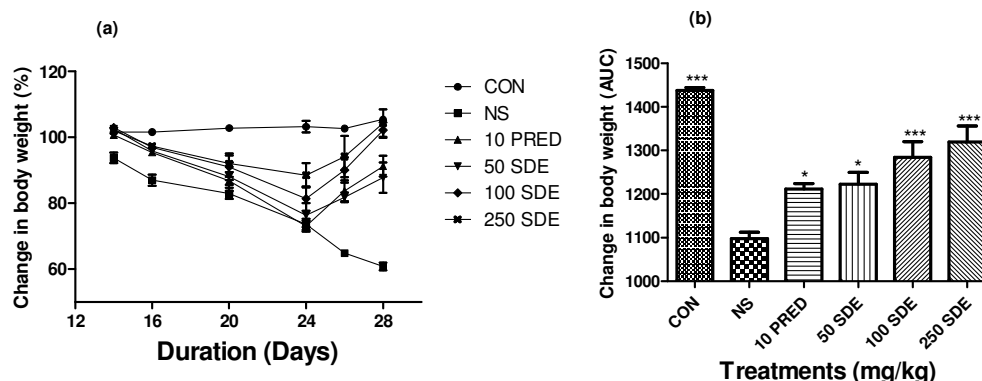


Figure 4: The percentage change in body weights with duration (a) and area under curve (b) of SDE-treated, Prednisolone-treated (PRED), normal saline-treated (NS), and non-sensitized non-treated control (CON) guinea-pigs in an OVA-induced allergic asthma pattern.

* implies $P \leq 0.05$; *** implies $P \leq 0.001$. Values plotted are means $\pm$ SEM of $n = 4$.

4. DISCUSSION

The present study investigated the effects of SDE on systemic inflammation, by assaying blood leukocyte and erythrocyte levels, erythrocyte sedimentation rate and C-reactive proteins, as well as its effect in improving body weight loss in OVA-induced allergic asthma disease in guinea pigs.

Ovalbumin (OVA) was used in the active sensitization and induction of allergic asthma in healthy guinea pigs, to cause systemic inflammation. Systemic inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules. Leukocytes (or white blood cells) are the cells of the immune system that are involved in defending the body against both infectious diseases and foreign materials. The total (increased/decreased) number of leukocytes in the blood is often an indicator of disease (such as occur in allergic asthma). OVA is known to initiate the activation of mast cells and T-helper type 2 (Th2) cells in the airway when inhaled. This leads to late phase allergic immuno-asthmatic responses characterized by mast cell degranulation (with the release of inflammatory mediators such as histamine), and an increase in leucopoiesis (i.e. increase formation of leucocytes in the body). In addition, the activated immune inflammatory cells induce the production of inflammatory cytokines such as IL-5, which regulates eosinophilic inflammation via the proliferation, differentiation and activation of eosinophils [11, 12]. OVA therefore induced an inflammatory response which was characterized by elevated total and differential WBCs. Again, WBCs (i.e. eosinophils, neutrophils and lymphocytes) on activation release inflammatory substances such as leukotrienes, granule proteins (e.g. metalloproteinase, elastase, lactoferrins) and reactive oxygen species. These mediators injure airway tissues causing contraction of airway smooth muscles, airway hyper-sensitivity and hyper-reactivity [10, 13]. Eosinophils can also generate granulocyte-macrophage colony-stimulating factor (GM-CSF) which prolongs and potentiates their survival, and contributes to persistent airway inflammation. This persistent inflammation results in an increased ESR and elevated serum CRP levels [10, 14]. Hence, levels of CRP and ESR were expected to be approaching higher concentrations after OVA-sensitization and challenge, as OVA inhalation and/or induction causes release of cytokines that trigger the synthesis of CRP and fibrinogen by the liver. An evaluation of sera from OVA-sensitized non-treated guinea pigs recorded the highest ESR levels and CRP concentrations; thus validating the efficacy of the sensitization/induction protocol.

Serum CRP and ESR levels are usually assessed together as biomarkers of systemic inflammation in establishing a better diagnosis for systemic inflammation, as the combination of the two measurements

helps improve diagnostic sensitivity and specificity [2]. Also, the acute phase response produced by CRP is known to develop in several acute and chronic inflammatory disorders including allergic asthma [10]. This helps explain why CRP and ESR are used in monitoring systemic response to treatment of inflammation. Rapid, marked increase in CRP is again known to occur in the plasma of chronic allergic pulmonary diseases, particularly during acute infective exacerbations [15]. This increment is due to a rise in the plasma concentration of IL-6 which is produced predominantly by leucocytes, particularly macrophages [3]. Also, elevated levels of inflammatory biomarkers such as C-reactive protein, fibrinogen and leukocyte count (WBCs), in individuals with chronic allergic lung diseases are reported to be associated with an increased risk of frequent exacerbations [16]. Increase in ESR levels has been shown to indicate the presence of high plasma fibrinogen and increased proportion of immunoglobulins in the blood, as fibrinogen is said to increase red cell aggregation and speed up the rate of erythrocyte sedimentation [10, 17]. An elevated plasma fibrinogen in a population is reported to be related with worse forced expiratory volume (FEV) and an increased risk of hospitalization for COPD and asthmatic patients [18, 19]. Hence, the ability of SDE to reduce the levels of elevated total and differential WBCs, ESR and C-reactive proteins in OVA-induced allergic guinea-pigs to within normal levels after treatment (i.e. Figures 1 – 3, Table 1) indicates its systemic anti-inflammatory properties, and possible curative effect on infective exacerbations in allergic asthma and other chronic allergic lung diseases such as COPD.

Moreover, numbers of erythrocytes in OVA-induced allergic animals reduced appreciably after OVA exposure and inhalation. However, there was significant increase ($P < 0.001$) in erythrocyte count after SDE and prednisolone treatments (i.e. Figure 1B); indicative also of the medicinal potential of SDE on systemic inflammation and/or anaemia. In some studies conducted, anaemia is reported to be an independent predictor of mortality in chronic immuno-inflammatory diseases, such as COPD and allergic asthma [20]. The anaemia is usually of the normochromic normocytic type, which is characteristic for most diseases of chronic inflammation; and this appears to be due to resistance to the effects of erythropoietin, the concentration of which is elevated in such situations [21, 22]. The level of haemoglobin is said to be strongly and independently associated with increased functional dyspnoea and decreased exercise capacity, and is therefore an important contributor to functional and vital capacity, quality of life, as well as proper pulmonary functioning and survival [23, 24].

Also, increased CRP and ESR are also related to health status and exercise capacity, and appear to be a significant predictor of body mass index. Body mass (particularly fat-free mass) is said to be related to the plasma levels of CRP, fibrinogen and TNF-R [17, 25]. Pulmonary cachexia is seen in patients who have any of the range of illnesses classified as chronic allergic respiratory diseases. Cachexia (or wasting syndrome) in any disease refers to a state of severely and pathologically low weight, due principally to the loss of mass of tissues other than fat, as a result of a chronic diseased state [5]. It is hypothesized that the increase in protein breakdown (in cachexia) coincides with an increase in protein “re-synthesis”, reflecting an inflammation-induced redirection of muscle protein in favour of synthesis of acute-phase proteins, such as CRP and fibrinogen [5, 25]. The systemic anti-inflammatory activity of SDE in reducing ESR and CRP levels implies that it might as well reduce inflammation-induced “re-synthesis” of acute-phase proteins, and hence would inhibit protein breakdown and subsequent cachexia; thus improving the body weights of OVA-induced diseased animals as observed (i.e. Figure 4).

Again, systemic inflammation and oxidative stress are known to be capable of inducing weight loss, and have become the primary focus of research into the genesis of wasting diseases in chronic obstructive lung diseases [26, 27]. The molecules said to be involved include TNF- α , IL-1 β , IL-6, reactive oxygen species (ROS), reactive nitrogen species (RNS), and C-reactive proteins [18, 28]. TNF- α has been shown to have direct catabolic effect on skeletal muscle and adipose tissue, and produces muscle atrophy through the ubiquitin–proteasome proteolytic pathway [29]. The mechanism involves the formation of reactive oxygen species leading to up-regulation of the transcription factor NF- κ B. NF- κ B is a known regulator of the genes that encode cytokines and the protein breakdown machinery in cells. The increased production of cytokines induces proteolysis and breakdown of myofibrillar proteins [30, 31]. Also, IL-1 β and IL-6 have been shown systemically to play a role in cachexia through suppression of food intake, by stimulating release of catecholamines and influencing macronutrient metabolism [28], whilst ROS and RNS are known to act as molecules capable of incurring tissue damage in pulmonary cachexia

[32]. Tsai et al. [33] showed that *S. dulcis* reduces the levels of tumour necrosis factor (TNF- α) and interleukin (IL-1 β) in inflamed tissues, as well as increasing the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd). The ability of the plant to increase the levels of SOD, GPx and GRd indicates a strong antioxidant activity, which may be partially responsible for some of the biological properties manifested by the plant extract. These strong antioxidant properties of *S. dulcis* implies it has the potential of scavenging free radicals and ROS/RNS produced in allergic asthma, and thereby modulating NF- κ B activity. Consequently, there would be reduced free reactive oxidative species and subsequent down-regulation of NF- κ B, which would lead invariably to a decrease production of cytokines and breakdown of myofibrillar proteins. These positive effects of SDE would indirectly also lead to increase in body weights of the OVA-induced allergic animals as observed. However, the effects of the plant extract on immuno-inflammatory mediators and Th2 cytokine levels are currently being investigated in our research laboratories.

5. CONCLUSION

The hydro-ethanolic extract of *Scoparia dulcis* has significant systemic anti-inflammatory and curative effects on chronic pulmonary disease wasting; making it a potential remedy in the management of allergic asthma and COPD.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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