Full Length Research Paper

Assessment of the anti-allergenic effects of *Scoparia dulcis* in asthma management

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Anti-allergics, including mast cell stabilizers, are used together with other anti-inflammatory drugs in the management of asthma, so as to prevent the release of pre-formed inflammatory mediators upon allergen exposure. This study was aimed at investigating the mast cell stabilizing and anti-anaphylactic effects of a 70% hydro-ethanolic extract of *Scoparia dulcis* (SDE) in murine models upon exposure to a known allergen. *In vitro* cytological and histological studies were conducted on guinea-pigs peritoneal cells and mesenteric tissues, respectively, to establish mast cell stabilization effect of the extract on compound 48/80-induced mast cell degranulation. The ability of SDE to protect mice against anaphylactic shock induced by compound 48/80 was also assessed. Preliminary phytochemical analysis was conducted on the extract using standard phyto-analytic procedures. Phytochemical screening showed the presence of tannins, alkaloids, glycosides, saponins, steroids and phenolic compounds. SDE showed significant inhibition ($P \leq 0.001$) against compound 48/80-induced degranulation in both the peritoneal and mesenteric mast cells of guinea-pigs (comparable to sodium cromoglycate and ketotifen fumarate). SDE also delayed the onset of symptoms of anaphylaxis in mice induced with compound 48/80, as well as reduced their mortality rate. The hydro-ethanolic extract of *S. dulcis* has significant mast cell stabilizing and anti-anaphylactic activities; making it a better adjunct in asthma management.

Key words: Anti-allergenic, Mast cell stabilization, Anti-anaphylactic, Compound 48/80, *Scoparia dulcis.*

INTRODUCTION

Asthma, as a complex chronic inflammatory disease of the airways, involves the activation of many inflammatory and structural cells (including mast cells) upon allergen exposure. This leads to degranulation of these immuno-

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inflammatory cells, causing release of inflammatory mediators such as histamine, prostaglandins, leuko-trienes, serotonin, tryptase and preformed cytokines. These chemical mediators cause immediate hypersensitivity reactions such as bronchial contraction, airway extravasations and anaphylaxis (Barnes, 2008; Kay, 1991).

Prevention of the release of these inflammatory mediators from mast cells is thereby a useful strategy in the management and treatment of allergic inflammatory diseases, such as asthma. Mast cell stabilizers act by blocking calcium channels and preventing the influx of Ca^{2+} ions in mast cells, stabilizing the membrane and subsequently inhibiting mast cell degranulation (Vogel, 2002), which is a prerequisite for the release of these inflammatory mediators, following allergen exposure in asthmatic attacks.

Besides, studies conducted on medicinal plants reveal that such herbal formulations possess significant target specific biological activities including mast cell stabilization, anti-inflammatory effect and bronchodilation; and as such might be useful in the treatment and management of asthmatic conditions. One of such herbal plant formulations is *Scoparia dulcis*, which is known to have muco-suppressant, anti-tussive, bronchodilatory and anti-inflammatory properties (Ofori-Amoah and Koffour, 2015; Koffuor et al., 2014). Though some studies on the plant in traditional asthma management are reported, a study on its anti-allergic effect is yet to be evaluated. This study, therefore, aimed at investigating the mast cell stabilizing and anti-anaphylactic properties of the hydro-ethanolic extract of *S. dulcis* using the compound 48/80-induced murine models, as a measure of its anti-allergenic properties.

MATERIALS AND METHODS

Plant collection

The fresh aerial parts of *S. dulcis* plant were obtained from Osene-Adikanfo, Faith Herbal Centre, Mamponteng 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.

Preparation of the hydro-ethanolic extract of *S. dulcis* (SDE)

The fresh aerial parts of *S. dulcis* were shade-dried for two consecutive weeks (8:00 am to 6:00 pm, 26 to 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 and reconstituted in a suitable vehicle for use.

Chemicals and reagents

Compound 48/80 and toluidine blue were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium cromoglycate (SCG) was obtained from Ashford Laboratory Ltd. (Macau), and Ketotifen fumarate from Novartis Pharma AG (Basel, Switzerland).

Experimental animals

Male Dunkin Hartley guinea pigs (300 to 450 g) were used for the mast cell stabilization studies, while C57 BL/6 mice (12 to 18 g) of either sex were used for the anti-anaphylactic experiment. All animals were housed in sanitized aluminium cages (70 × 42 × 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 ± 4ºC), relative humidity (60 ± 10%) and normal light/dark cycle, for 10 days prior to experimentation.

Ethical approval

Protocols for the study were approved (FPPS/PCOL/0011/2012) by the Committee on Animal Research, Publication and Ethics (CARPE). 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.

Dosing of drugs to experimental animals

Dosing of the plant extract was done once daily by gavage, at a volume of 1 ml/kg in normal saline, using the animals most recent recorded body weight. The oral route of administration was used, as it is the intended human exposure route.

Preliminary phytochemical analysis of *Scoparia dulcis*

Preliminary phytochemical analyses were carried out on the hydro-ethanolic plant extract (SDE) and the plant raw material (PRM) for possible phytoconstituents, using standard phyto-analytical methods as described by Trease and Evans (1989).

Mast cell stabilizing effect of SDE

The mast cell stabilization effect of SDE was studied using the extract's ability to protect against compound 48/80-induced degranulation of the peritoneal and mesenteric mast cells in guinea-pigs. These were carried out as previously described by Gohil and Mehta (2011), and Parmar et al. (2010), with some modifications.

Two guinea pigs were each injected with 15 ml of Tyrode's solution into the peritoneal cavity, and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was then made on each animal to expose the peritoneum, after they have been sacrificed by cervical dislocation. The pale peritoneal fluids were then aspirated into plastic centrifuge tubes using Pasteur pipette from the sacrificed animals. These were centrifuged at 1000×g for 15 min, and the supernatants discarded to reveal pale cell pellets. The cell pellets were re-suspended in fresh Tyrode solution and re-centrifuged, discarding the supernatant. Aliquots of cell pellets were then pre-incubated separately with 50, 100 or 500 µg/ml of SDE and 20 µg/ml sodium cromoglycate at 37°C for 15 min. In addition, intestinal mesenteries along with intestinal pieces were excised from sacrificed animals and kept in Tyrode's solution. The mesenteric pieces were cleared of adhering tissues, purified

and later pre-incubated separately with 50, 100 or 500 µg/ml of SDE, 20 µg/ml sodium cromoglycate and 10 µg/ml ketotifen furamate at 37°C for 15 min. Pre-treated cells and tissues were thereafter incubated with compound 48/80 (0.2 ml, 10 µg/ml) at 37°C for 10 min. The peritoneal cells were then carefully spread over glass slides, fixed in 95% ethanol and stained with 0.1% toluidine blue for 3 min, whilst the mesenteric tissues were placed in a 10% buffered formalin solution (to fix them), processed histologically to obtain thin sections, which were mounted on glass slides and stained with 1% toluidine blue for 3 min.

The slides were then washed under tap water to enhance the metachromatic staining of the mast cells by the toluidine blue. The stain was washed off and the slides dried in air. The slides were dipped in xylene, followed by the addition of DPX (Distrene, Plasticiser, Xylene) and finally cover-slipped. The mast cells were then counted using Leica DM 750 microscope (Leica Microsystems CM5 GmbH, Wetzlar – Germany) from randomly selected high power objective fields (40X magnification). The percent degranulation of the mast cells was calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted. The percentage inhibition of mast cell degranulation for each treatment was then determined in both the peritoneal cells and mesenteric tissues:

Percentage inhibition of Mast Cell Degranulation = $\{1 - (X / Y)\} \times 100$; (where, X = number of degranulated mast cells; Y = total number of mast cells counted).

Anti-anaphylactic effect of SDE

The anti-anaphylactic effect of SDE was also studied using compound 48/80-induced systemic anaphylaxis model in mice, as described by Patel et al. (2010).

Mice were allotted to five different treatment groups (n = 5). Group 1 was kept as normal control; Group 2 (that is, positive control) was pre-treated with 50 mg/kg sodium cromoglycate *per os*. Groups 3, 4 and 5 were pre-treated with 50, 100, and 250 mg/kg SDE orally, respectively. Compound 48/80 (0.5 ml, 8 mg/kg) was injected intraperitoneally into mice in all groups, 1 h after drugs administration, to induce anaphylactic shock. Animals were then observed for the onset of symptoms such as dyspnoea and twitches, collapse, duration of the persistence of symptoms (min), and mortality.

Statistical analysis

Data obtained in all experiments were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison tests (*post hoc* tests), using the software of Origin, Graph-Pad Prism for Windows Version 5.0 (Graph-Pad Software, San Diego, CA, USA). These were expressed as mean ± S.E.M. Differences between means of treated groups and the control were regarded as statistically significant at P ≤ 0.05.

RESULTS

Phytochemical analysis of SDE

The preliminary phyto-analytic screenings of the hydro-ethanolic plant extract (SDE) and the plant raw material (PRM) of *S. dulcis* showed the presence of tannins (condensed tannins), alkaloids, phenols, glycosides (free reducing sugars), saponins and steroids (as shown in Table 1).

Mast cell stabilizing effects of SDE

Compound 48/80 produced significant mast cell degranulation (93.08 ± 0.55%) in the guinea-pig mesenteric tissues, and (93.00 ± 0.94%) in the guinea-pig peritoneal cells. Pre-treatment of the mesenteric tissues and peritoneal cells with 100, 250 and 500 µg/ml of SDE, Sodium cromoglycate and Ketotifen fumarate inhibited significantly (P ≤ 0.001) the degranulation of mast cells, as shown in Figures 1 and 2.

Anti-anaphylactic effect of SDE

SDE protected mice significantly (P ≤ 0.001) against compound 48/80-induced anaphylactic shock in a dose-dependent manner. The onset of symptoms of anaphylaxis was delayed, and symptoms were less severe with reduced mortality rate, as compared to the control group (Figure 3).

DISCUSSION

The anti-allergenic effects of SDE were assessed using its ability to stabilize mast cells and prevent anaphylaxis upon exposure to a known allergen (that is, compound 48/80). The results showed that SDE has significant mast cell stabilizing and anti-anaphylactic effects.

Mast cell degranulation and systemic anaphylaxis were elicited with the synthetic Compound 48/80. This chemical, as well as other polybasic compounds, is reported to initiate the activation of signal transduction pathways which leads to mast cell degranulation and histamine release (Ennis et al., 1980). Compound 48/80 is also said to directly activate G-proteins, causing influx of Ca^{2+} ions into the mast cell, and increases the permeability of the lipid bilayer membrane by causing perturbation in the membrane (Mousil et al., 1990). This further causes activation of phospholipidase D, which leads to the generation of endogenous lysophosphatic acid receptor-activating phospholipids, and subsequently mast cell degranulation (Palomaki and Laitinen, 2006). Sodium cromoglycate, Ketotifen fumarate, and SDE all showed significant (P ≤ 0.001) mast cell stabilization effects (Figures 1 and 2). Sodium cromoglycate is a well-known mast cell stabilizer, whilst ketotifen fumarate is an anti-histaminic drug with known mast cell stabilizing properties. Sodium cromoglycate acts by blocking calcium influx and chloride ions, and thus helps prevent the release of inflammatory mediators (such as histamine) from bronchial mast cells in asthmatic airways. It also reverses the increased functional activation of leukocytes, inhibits leukocyte trafficking, as well as suppresses the activating effects of chemotactic peptides on neutrophils, eosinophils and monocytes (Heinke et al., 1995). On the other hand, Ketotifen fumarate is known to have calcium ion antagonistic property, inhibits the
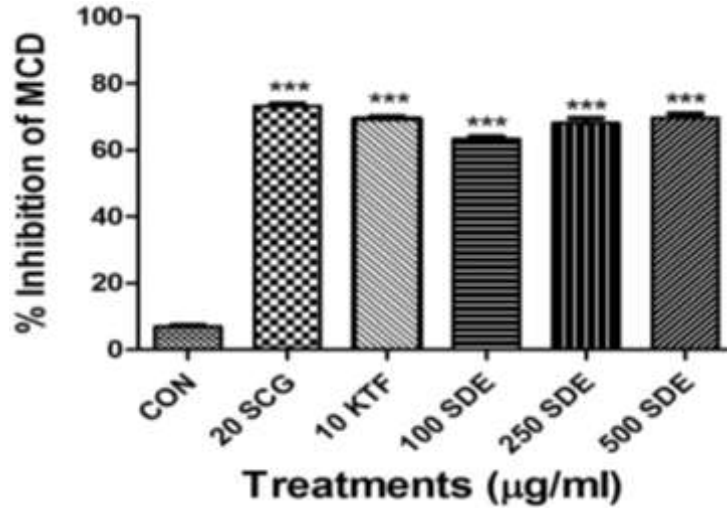
Figure 1. Inhibitory effect of SDE, Sodium cromoglycate (SCG), Ketotifen fumarate (KTF), and No treatment (CON), on compound 48/80-induced mast cell degranulation (MCD) in guinea-pig mesenteric mast cells. *** implies P ≤ 0.001. Values plotted are means ± SEM (n = 5).
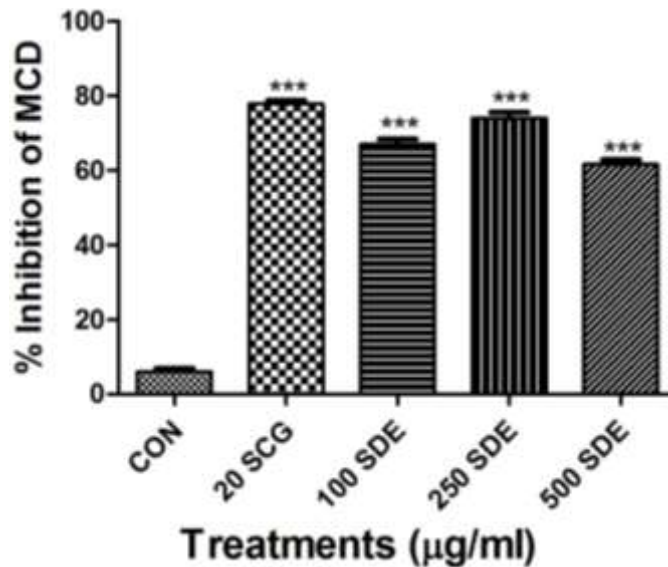

Figure 2. Percentage inhibition of compound 48/80-induced mast cell degranulation (MCD) by SDE, Sodium cromoglycate (SCG), and No treatment (CON) in guinea-pig peritoneal mast cells. *** implies P ≤ 0.001. Values plotted are means ± SEM (n=3).

release of mast cell mediators, and simultaneously blocks H1 receptors (Loeffler *et al.*, 1971), as well as inhibits the release of Platelet Activating Factor (PAF), prevents PAF-induced eosinophil accumulation and PAF-activated platelets development of airway hyper-reactivity (Mita and Shida, 1995). These properties are said to contribute to the anti-allergic activities and further ability of these reference drugs (sodium cromoglycate and ketotifen fumarate) to affect the underlying pathophysiology of asthma. Thus, the findings of this work have made inroads into possible modes of action of SDE, suggesting similarity between it and the known mast cell stabilizers, sodium cromoglycate and ketotifen fumarate.

The study again revealed significant protection of SDE against compound 48/80-induced anaphylactic shock (that is, Figure 3). This was characterized by decrease in
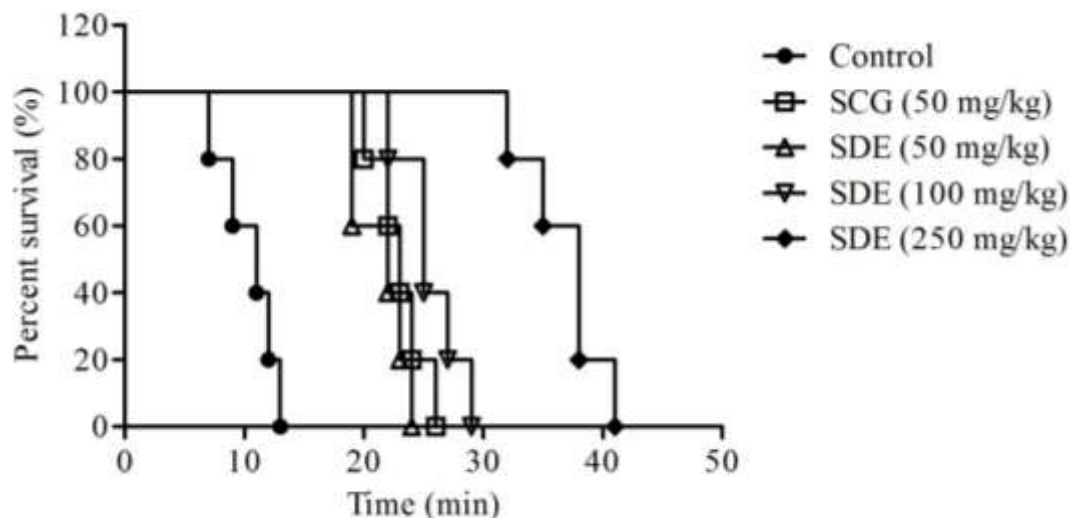
Figure 3. Effect of SDE, Sodium cromoglycate (SCG), and No treatment (Control) on compound 48/80-induced systemic anaphylaxis in mice. Survival rate was monitored for an hour after compound 48/80 administration. Data were analyzed using Log-rank (Mantel Cox) test; n=5. Survival curves were significant ($P \leq 0.001$).

Table 1. Phytochemical screenings of the hydro-ethanolic plant extract (SDE) and plant raw material (PRM) of *Scoparia dulcis*.

Phytoconstituents	SDE	PRM	Tests
Saponins	+	+	Frothing test
Alkaloids	++	+++	Dragendorff's test
Tannins	+	+++	Ferric chloride test
Steroids	++	++	Lieberman Burchard's
Triterpenes	_	++	Salkowski test
Flavonoids	_	+	Shinoda test
Reducing sugars (General glycosides)	++	++	Fehling's test
Anthraquinones	_	_	Ammonia test
Phenols	+++		Ferric chloride test
Polyuronoids	_		
Cyanogenic Glycosides	_		

Key: + = Detected; – = Not detected.

intensity, and delay in the development or onset of symptoms of dyspnoea, asphyxia, collapse and/or death. Anaphylaxis is a severe systemic allergic reaction with rapid onset inducing constriction of the trachea and preventing breathing (that is, causing anaphylactic shock); and hence may lead to asphyxia and/or death. It is caused by degranulation of mast cells and/or basophils, which is mediated by IgE (Schroeder et al., 1994).

Mast cells, basophils, and their various released mediators, play a pivotal role in the pathogenesis of allergic disorders. However, mast cells are the primary responders in allergic reactions, triggered by cross-linking of a high affinity IgE receptor on exposure to allergens or 'irritants'; releasing preformed and *de novo*-synthesized molecules such as histamine, serotonin, leukotrienes and various pro-inflammatory cytokines/chemokines (Gohil and Mehta, 2011; Bousquet et al., 2000). These are potent vasoactive and bronchoconstrictor agents, and they modulate local immune responses and inflammatory cell infiltration in the development of type I allergic reactions such as in allergic asthma (Okayama et al., 2007; Marone et al., 1997).

Systemic anaphylaxis is known to occur in three stages (Tripathi et al., 1979): viz (i) antigen-antibody interactions (that is, IgE bound to the surface of mast cells), (ii) IgE-

mediated mast cell disruption, and (iii) the liberation of various immune and inflammatory mediators. The anti-anaphylactic activity of SDE suggests that it might inhibit allergen-antigen-antibody interaction (that is, causes de-sensitization), or stabilizes mast cells, and prevents the synthesis and/or release of immuno-inflammatory mediators.

Results of the preliminary phytochemical analysis of the hydro-ethanolic extract of *S. dulcis* in this study is similar to earlier works done by Ofori-Amoah and Koffuor (2015); which confirms the presence of tannins, steroids, saponins, alkaloids, glycosides and phenols (that is, Table 1). Tannins have been identified to inhibit the release of histamine, bradykinin and serotonin from inflammatory cells (Jeffers, 2006), whilst steroids and saponins are known to possess anti-inflammatory and mast cell stabilizing activities, via inhibiting the synthesis of specific asthma markers such as prostaglandins, leukotrienes, histamine, bradykinin and serotonin (Desai et al., 2009; Cassileth and Lucarelli, 2003). Also, alkaloids and glycosides (for example, luteolin) have been reported to be potent inhibitors of histamine release from mast cells, and inhibit CD40 ligand expression by basophils and mast cells; which is required in the activation and differentiation of B cells into IgE-producing plasma cells (Inoue et al., 2002). Hence, the pharmacological properties of SDE observed in this study could be attributed to these phytochemical constituents present.

Besides, single mediator approach to asthma therapy is difficult as the disease process involved is complex. Subsequently, treatment of asthmatic symptoms is usually with inhaled beta-2 agonists, oral glucocorticosteroids, mast cell stabilizers, and leukotriene receptor antagonists. Asthma is thus well managed if the herbal formulation used has bronchodilating, mast cell stabilizing and anti-inflammatory effects, as well as potent inhibitory activity on mucus secretion and cough. Hence, the ability of SDE to elicit positive effects on mast cell stabilization and anaphylaxis, in addition to its bronchodilatory and anti-inflammatory properties (Ofori-Amoah and Koffuor, 2015; Tsai et al., 2011), and its suppressive effects on mucus secretion and cough (Koffuor et al., 2014), would make it a very potent remedy in the management of allergic asthma and COPD.

However, the effects of the plant extract on immuno-inflammatory mediators, released after mast cell degranulation, such as prostaglandins, leukotrienes and PAF need to be investigated in further studies.

Conclusion

The hydro-ethanolic extract of *S. dulcis* exhibits anti-allergic and membrane stabilizing properties by inhibiting mast cells degranulation and preventing anaphylaxis. These properties make it a probable remedy and an adjunct for asthma management.

Conflict of Interests

The authors have not declared any conflict of interest.

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