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Research Article

Inhibitory Effects of *Withania somnifera* on Mast Cell Degranulation and Mortality in Allergic Mouse Model

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Abstract

Background: Acute systemic anaphylaxis is a severe, acute allergic response to an allergen/antigen that is rapid in onset, characterized by life-threatening airway obstruction and hypotension - involving an inflammatory response of multiple organ systems. Medicinal plants have been used for centuries as a pharmacologic alternative/complementary mode of therapy in the prevention and treatment of many allergic disorders. The present study aims to evaluate the effects of WS extract on acute systemic anaphylaxis in mice.

Methods: Female BALB/c mice (20 - 25g) were divided into four groups (n = 8/group). On day 1, mice of all groups were sensitized with an intraperitoneal (i.p.) injection of 50 µg ovalbumin (OVA) an 1 mg of $AL(OH)_3$ in 0.5 ml saline, and on day 14, mice were challenged with 50 µg OVA in 0.5 ml saline. The mortality was checked on day 14 at 2 hr post-OVA challenge. 24 hr after the OVA challenge, all animals were anesthetized, the abdomen was cut-opened, and the mesentery was dissected from the small intestine. Fragments of mesentery were mounted on a glass slide, fixed and examined microscopically (x10) and the percentage of degranulated and intact mast cells was evaluated.

Results: After 2 hr of OVA challenge in mice, a higher mortality rate was observed in the disease control (OVA) group (75%). Pretreatment with a higher dose of WS extract (400 mg/kg) reduced mortality in mice to 37%, whereas the mortality in mice treated with a lower dose (200 mg/kg) was 50%. In addition, pre-treatment with WS extract (200 and 400 mg/kg) exerted significant percentage protection of mast cells' degranulation in a dose-dependent manner as compared to the disease control (OVA) group. These results were comparable with those seen after dexamethasone treatment (1 mg/kg).

Conclusion: Our study results indicated that WS extract showed significant protection of mast cell degranulation, and it reduced mortality rate in mice in a dose-dependent manner in an allergic mouse model. Thus, WS could have therapeutic potential in allergic conditions such as asthma.

Keywords: *Mast Cells; Anaphylaxis; Withania somnifera; Ovalbumin*

Introduction

Allergic disorders result from dysregulation of the immune system and complexly interacting cellular and humoral mechanisms are involved. Mast cells are of hematopoietic origin and play a pivotal role in the immune system and pathogenesis of allergic diseases. Mast cells are present throughout the body and particularly located at the junction point of the host and external environment at places of entry of antigen viz. gastrointestinal tract, skin, respiratory epithelium. In the lungs, mast cell progenitors are recruited upon antigen-induced

inflammation of the respiratory endothelium [1]. IgE-activated mast cells mediate acute hypersensitivity reactions via the release of a variety of cytokines, chemokines, and other mediators that regulate adaptive immune responses and tissue inflammation. Type 1 hypersensitivity is characterized by IgE antibody-mediated mast cell degranulation [2]. The high-affinity Fcε receptors (FcεRI) on mast cells bind to IgE making them highly responsive to inhaled antigens [3]. In bronchial asthma, mast cells are associated with chronic airway inflammation, cell infiltration, bronchoconstriction, and airway remodeling [4,5]. Medicinal plants have the source of many therapeutic agents in contemporary/modern medicine, and now rapidly emerging a viable alternative/complementary mode of therapy in the prevention and treatment of many allergic diseases, such as asthma [6]. The therapeutic efficacy of herbal combinations is attributed to multiple targeting of very complex and interdependent cellular and molecular pathways involved in the inflammatory process of asthma [7]. *Withania somnifera* (WS) is a well-documented Rasayana herb effectively used in Indian traditional systems of medicine with its constituent glycowithanolides contributing to the biological effects. The diverse pharmacological effects of the plant viz. anti-inflammatory, immunomodulatory and adaptogenic effects, make WS preparations a herbal agent with immense potential for treating a wide range of pathophysiological states [8]. One of the therapeutic strategies for bronchial asthma is prevention of mediator release from mast cells and the present study was designed to study the anti-anaphylactic activities of WS extract in OVA-induced mast cell degranulation in mice.

Materials and Methods

Drugs and chemicals

The standardized root extract of *Withania somnifera* (*WS)* was procured from Natural Remedies Pvt. Ltd., (Bengaluru, India). The aqueous extract was prepared from the dried mature root of *Withania somnifera* (L) Dunal (Family Solanaceae) by using water solvent. Physical and chemical parameters were tested and certified to have passed the prescribed standards. HPLC method was used to investigate the presence of the bioactive phytochemical markers in the root extract as well as to analyse their content. The total withanolide content was 2% in the portion of root dry extract which complied with the specification (≥ 1.5% total withanolides). The peaks at retention time of reference compounds; withanolide A and withanoside IV in the HPLC chromatogram were found at the similar retention time in sample extract. Ovalbumin (albumin from chicken egg white; OVA grade III) was purchased from Sigma Aldrich Chemical Co., USA. Dexamethasone (DEX) was purchased from Abbott Laboratories, India. All the buffers were freshly prepared, and all routine drugs/chemicals needed for the various assays were of high analytical grade and obtained from SRL Labs, New Delhi, India.

Experimental procedure

Female BALB/c mice (20 - 25g) were divided into four groups (n = 8/group) as follows: (i) disease control group (OVA-sensitized + challenged, and normal saline) (ii) positive control (OVA-sensitized + challenged and DEX 1 mg/kg i.p.), (iii) OVA-sensitized + challenged, and WS extract 200 mg/kg per oral; and, (iv) OVA-sensitized + challenged, WS extract 400 mg/kg per oral. The drug treatment groups (ii), (iii) and (iv) received WS extract doses or DEX from day 1 to day 14. Mice of all groups were sensitized with an i.p. injection of 50 µg OVA per mouse adsorbed to 1 mg of $AL(OH)_3$ in 0.5 ml of normal saline on day 1. On day 14, mice were challenged with an i.p injection of 50 µg OVA in 0.5 ml of NS, without $AL(OH)_3$, as described earlier (10) with some modification. The mortality was checked on day 14 at 2 hr post-challenge. After 24 hr (on day 15) of the antigen challenge, all animals were anesthetized, the abdomen was cut-opened, and the mesentery was dissected out from the small intestine. Fragments of mesentery were mounted on a glass slide and then cleaned with saline and stained and fixed for 15 minutes in a solution containing 5% acetic acid, 50% ethanol, 10% formaldehyde, and 0.2% toluidine blue. Degranulation of mast cell was evaluated by counting the percentage of mast cells with extruded granules, as the dye-stained granules were released from the cells and became extracellular (8 microscopic fields counted; 100 x magnification) [9]. The percent protection of mast cell degranulation was calculated by using the formula:

Percentage protection of mast cells' degranulation = (Y-X) / Y × 100

Where X = number of degranulated mast cells; Y = total number of mast cells counted.

Statistical analysis

The statistical analysis was carried out using the Graph Pad Prisms® software, version 8.3.0, San Diego, USA. The results were expressed as Mean ± standard error of mean (SEM). Data were analyzed using the one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test for inter-group comparisons. The per cent mortality data was analyzed by using the Chi-Square test with Yates modification. A value of $p < 0.05$ was considered the level of significance in all statistical tests.

Results

Microscopic examination of mice mesentery stained with 0.2% toluidine blue showed the appearance of degranulated mast cells (with extruded granules) as well as the intact mast cells. Counting was done at a magnification of ×100. OVA immunization (sensitization day 0 + challenge day 14) in mice (saline + OVA, disease controls, DC) induced appreciable degranulation of mast cells (count of 22.5 ± 3.09) as compared to the control (saline) group. In the WS (200 and 400 mg/kg) treatment groups (i.e. WS + OVA), there were dose related inhibitions of mast cell degranulation counts in the mesenteric strips - with the effects of higher dose (400 mg/kg) being more marked treatment groups (WS 200: 18.25 ± 1.31 and WS 400: 13.75 ± 1.75). Similarly, pretreatment of mice with the comparator drug, dexamethasone (DEX, 1 mg/kg) showed comparable reductions in mast cell degranulation as that seen after the treatment with doses of WS (Table 1). The mortality data in mice, 2hr post-challenge, showed that in the disease control group, there was a mortality of 75%, i.e. 6 of the 8 mice in the group could not survive the effect of OVA challenge and died because of acute systemic anaphylaxis. However, in the WS (200 and 400 mg/kg) treated group of mice, OVA challenge induced mortality rates were substantially lower viz. 50% (4 out of 8 mice survived) and 37.5% (6 out of 8 mice survived) respective ($p < 0.05$, in each case) - indicating protective effects of WS extract in these allergic mice. The WS data was comparable to the DEX (1 mg/kg) treated group, where the mortality rate was the lowest, i.e. 12.5% (Table 1). On calculating the percent protection, it was seen pretreatments with WS extract (200 or 400 mg/kg) exerted significant percentage protection of mast cells from degranulation, in a dose-dependent manner as compared to the disease control (OVA) group. The percent protections in mast cell degranulation were: 37.8% in DC group; 53.3% in the WS (200 mg/kg) and 61.7% in the WS (400 mg/kg) group ($P < 0.05$ and $P < 0.001$, respectively). A comparable % protection of 72.2% was also calculated in the DEX (1 mg/kg) group ($P < 0.05$). These results are summarized in figure 1.

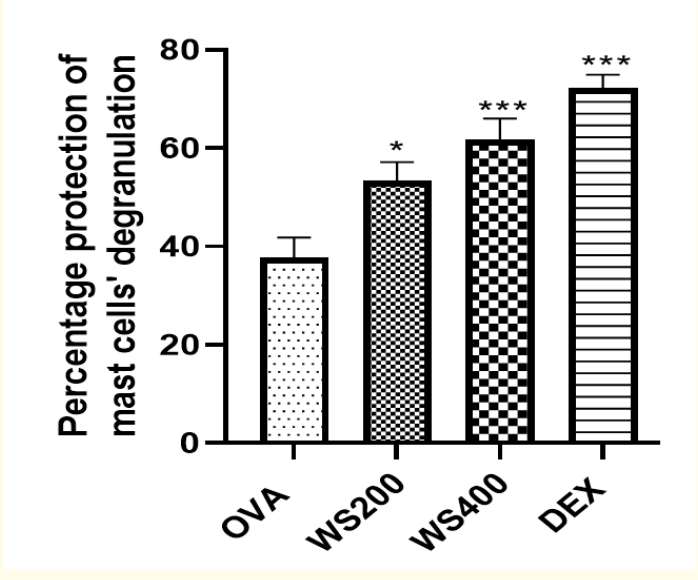

Figure 1: *Protective effects (%) of Withania somnifera extract (WS, 200 and 400 mg/kg) on OVA-induced mesenteric mast cell degranulation in mice. OVA- ovalbumin (disease controls); DEX-dexamethasone (1 mg/kg); All data are expressed as mean ± S.E.M. (n = 8/group). *p < 0.05, ***p < 0.001 compared with the disease control (OVA) group.*

Treatment group (mg/kg) (n = 8/group)	Degranulated mast cells (Mean ± SEM)	Mortality (%)
Saline + OVA (DC)	22.5 ± 3.0	75%
WS (200) + OVA	18.2 ± 1.3	50%
WS (400) + OVA	13.7 ± 1.7*	37.5%*
DEX (1) + OVA	12.0 ± 1.2*	12.5%*

Table 1: *Inhibitory effect of Withania somnifera extract on OVA-induced mesenteric mast cell degranulation and mortality in mice. DC - Disease Control; *p < 0.05, compared with DC group.*

Discussion

Anaphylaxis is a severe and potentially lethal allergic reaction to a variety of environmental stimuli including allergens/antigens. Murine models can elucidate the underlying mechanisms and pave the way for appropriate therapeutic options [10]. Mast cells are key initiators and modulators of allergic, anaphylactic, and other inflammatory reactions by induction of vasodilation, promoting of vascular permeability, recruitment of inflammatory cells, facilitation of adaptive immune responses, and modulation of angiogenesis, and fibrosis. Depending on the stimulus, these cells modulate the pattern of mediator release, modulate the amplification of allergic inflammation, and are involved in the resolution of the immune responses - all contributing to the regulation of health and disease [11].

Acute systemic anaphylaxis is a severe whole-body allergic response to an allergen that is rapid in onset, characterized by life threatening airway obstruction and hypotension and involving inflammatory reactions in multiple organ systems. In allergic reactions, allergens or irritants activate multiple inflammatory cells, including mast cells. These cells can produce different mediators, such as histamine, serotonin, and leukotrienes, which promote disease pathogenesis [12]. Mast cells in the airway smooth muscles secrete histamine, a potent vasoactive inflammatory mediator, that can act directly on airway smooth muscles and causes bronchospasm and is one of the proposed mechanisms for precipitation of acute asthmatic episodes [13]. Earlier studies have shown that the immunological response to OVA is mediated by the eosinophilic inflammation and mast cells degranulation [14]. In the current study, effects of WS extract (200 and 400 mg/kg) were assessed on allergic inflammation by using an allergic mouse model and its ability to protect mesenteric mast cell degranulation upon exposure to the allergen (OVA). Previous reports have established that OVA sensitization and challenge could induce mast cells activation and histamine release [15]. In an earlier study, the immunomodulatory activities of WS on OVA-induced active paw edema and sheep red blood cells (SRBC)-induced delayed type hypersensitivity were studied in mice. The results showed that WS extracts has effectively reduced OVA-induced paw edema, suggesting that WS may have a potential anti-inflammatory activity with respect to type-1 IgE-mediated anaphylactic reaction [16]. Another study also demonstrated that 500 mg of methanolic extract of WS inhibited antigen induced histamine release in mesenteric mast cells in rats [17]. Our results are consistent with these studies and in addition, showed that pretreatment with aqueous WS root extract exerted significant mast cell stabilizing and anti-anaphylactic effects against OVA-induced allergic mice model, as well as it reduced their mortality rate, in a dose dependent manner. The validity and relevance of these findings are further established by the fact that the comparator drug, dexamethasone, induced similar magnitude and character of effects on both parameters of acute systemic anaphylaxis seen in this allergic mouse model. Thus, WS could have significant anti-allergic activity through stabilization of mast cells and protection and protection against anaphylactic mortality.

Conclusion

In conclusion, in this study, we ascertained the anti-anaphylactic effects of WS extract in allergic mouse model. The ability of *Withania somnifera* extract (WS) to reduce mast cell degranulation and post-antigen challenge mortality in the allergic mouse model suggest anti-anaphylactic activity of the medicinal plant and this suggest that WS could have potential as a therapeutic strategy (stand alone or adjunct) in allergic disorders. These findings could have translational significance in respiratory allergies like bronchial asthma where mast cell membrane stabilization is considered as one of the treatment modalities in the prophylaxis of this disease.

Authorship Contribution Statement

N.H.A.: Methodology, investigation, data curation, and writing-original draft preparation.

S.R.: Performed a critical and substantial review of the manuscript.

A.R. and K.G.: Conceptualization, planning and critically reviewing of the manuscript.

All authors have read and approved the final manuscript.

Conflict of Interest

None.

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