Pharmacological Basis For Antianaphylactic, Antihistaminic And Mast Cell Stabilization Activity Of Ocimum Sanctum
G Sridevi, P Gopkumar, S Ashok, C Shastry

Citation

Abstract
Purpose: The present paper reports the antianaphylactic, antihistaminic and mast cell stabilization activity of Ocimum sanctum leaf extract on various experimental models. Methods: After optimizing the dose the extract was tested for its therapeutic activity using Wistar rats and Duncan Hartley guinea pigs. The antianaphylactic activity was investigated in rats using the active anaphylaxis model. The effect on mast cell stabilization was performed by ex vivo challenge of antigen in sensitized rat intestinal mesenteries. Antihistaminic activity was studied in guinea pigs using histamine-induced bronchospasm where preconvulsive dyspnea was used as an end point following exposure to histamine aerosol. Results: The findings from various studies reveal that the antihistaminic and antianaphylactic activity of extract which is mainly due to its mast cell stabilizing potential, suppression of IgE, and inhibition of release of inflammatory mediators Conclusion: Thus use of Ocimum sanctum leaves proved the strong rationale behind the mentioned therapeutic activities.

INTRODUCTION
Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Allergic diseases are responsible for significant morbidity and have severe economic impact. Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated reasons are increasing environmental pollution and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, changing lifestyles, and an increasing awareness of the disorders. Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulins, mast cells, and various autacoids in the etiopathogenesis of allergic conditions. Inspite of the voluminous literature on the subject, the treatment of allergic diseases continues to be far from satisfactory. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations owing to low efficacy, associated adverse events, and compliance issues.

Ocimum sanctum (Sanskrit:Tulasi; family: Labiaceae), is found throughout the semitropical and tropical parts of India. Ocimum sanctum commonly known as holy basil is a herbaceous sacred plant found throughout India. Different parts of the plant are traditionally used in Ayurveda and Siddha systems for the treatment of diverse ailments like infections, skin diseases, hepatic disorders and as an antidote for snake bite and scorpion sting. Indian material medica describes the use the plant in a verity of ailments. Different parts of plant like stem, flower, seed, leaves, root etc are known to possess therapeutic potential and have been used, by traditional medicinal practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive and antistress agent. Tulsi has also been used in treatment of fever, bronchitis, arthritis and convulsion.

A review of the literature mentions the use of plant in allergic conditions. However, there is no scientific data available to authenticate the folklore claim as well there is no scientific evaluation regarding its activity profile. Hence the present study was undertaken to evaluate the antianaphylactic, antihistaminic and mast cell stabilization activity of alcoholic extract of Ocimum sanctum leaves. The therapeutic activity of the test extract was studied on the active anaphylaxis, mast cell stabilization in rats, and histamine-induced bronchospasm in guinea pigs.
MATERIALS AND METHODS

PLANT MATERIAL

Plant leaves were collected locally. The leaves were authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (O.S 5/99) was deposited in the herbarium of our institute.

ANIMALS

Wistar rats (175-200 g) and guinea pigs (400-600 g) of either sex are procured from Indian Institute of Sciences. They are maintained under standard conditions (temperature 22 ± 2 °C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the “National Academy of Sciences” and published by the “National Institute of Health”.

CHEMICALS

Histamine and horse serum were procured from Sigma Chemicals and toluidine blue from Loba-Chemie, Mumbai. Elisa kit for IgE was supplied by Orion diagnostics, Espoc, Finland. All other chemicals and reagents were procured from Hi-Media Laboratories limited, Mumbai.

ACTIVE ANAPHYLAXIS

Twenty-eight rats were sensitized by injecting subcutaneously 0.5 ml of horse serum along with 0.5 ml of triple antigen containing 20,000 million Bordetella pertussis organisms (Serum Institute of India Ltd., Pune, India). The sensitized rats were divided into 4 groups of 7 each. Group I served as control and received water (vehicle). Groups II, III and IV were administered test extract at 100, 200, and 400 mg/kg respectively, orally, once a day for 14 days. On day 14, after 2 h of treatment, the rats were challenged with intravenous injection (tail vein) of 0.25 ml horse serum in normal saline. They were then observed for the onset of symptoms such as dyspnea and cyanosis, duration of the persistence of symptoms (min), and mortality. The severity of symptoms was scored. The optimal pharmacological effective dose, which is derived from this dose response study, was used for the remaining studies.

Serum total IgE was quantified with an ELISA protocol according to the manufacturer's instructions. Briefly, the plates were coated with affinity-purified rabbit anti IgE overnight at 4 °C and then blocked with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 1 h at 37°C. The serum samples and appropriate dilutions of a standard IgE preparation were placed in the wells, and the plates were incubated for 3 h at 4 °C. Sample blank wells were treated similarly but without serum. The bound IgE was detected with polyclonal goat anti IgE antibodies (incubation for 1 h at 37 °C), followed by HRP-conjugated rabbit anti-goat antibodies (incubation for 1 h at 37 °C). The plates were developed by the addition of O-phenylene diamine and read in an ELISA (Anthos HT-II, USA) plate reader at 490 nm.

MAST CELL STABILIZING ACTIVITY

Thirty-two rats were divided into four groups of eight animals in each group. Group I served as control and received vehicle (water). Group II (sensitized control group, received only water), Groups III (test extract) and IV (prednisolone) were sensitized by injecting 0.5 ml of horse serum subcutaneously along with 0.5 ml of triple antigen containing 20,000 million Bordetella pertussis organisms (Serum Institute of India Ltd., Pune). Group III were administered 400 mg/kg, p.o., once a day for 14 days. Group IV were administered prednisolone (reference drug) 10 mg/kg, p.o., for the same duration. On day 14, the rats were sacrificed 2 h after the treatment and the intestinal mesentry was taken out for the study on mast cells. Mesentaries along with intestinal pieces were excised and kept in Ringer Locke solution (NaCl 154, KCl 5.6, CaCl\(_2\) 2.2, NaHCO\(_3\) 6.0, glucose 5.55 mM/L of distilled water) at 37 °C. The mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained with 1.0% toluidine blue and examined microscopically for the number of intact and degranulated mast cells.

HISTAMINE-INDUCED BRONCHOSPASM IN GUINEA PIGS

Bronchospasm was induced in guinea pigs by exposing them to 1% histamine aerosol under constant pressure (1 kg/cm\(^2\)) in an aerosol chamber (24 × 14 × 24 cm) made of perplex glass. Of the two groups of six animals each, Group I served as control and Group II received test ethanolic extract 400 mg/kg, p.o., once a day for 5 days. The animals were exposed to 1% histamine aerosol under constant pressure (1 kg/cm\(^2\)) in an aerosol chamber on day 0 without any treatment. The
end point, preconvulsive dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsions. As soon as PCD commenced, the animals were removed from the chamber and exposed to fresh air. This PCD was taken as day 0 value. On days 1 and 5, 2 h after the administration of the drug, the time for the onset of PCD was recorded as on day 0.

STATISTICAL ANALYSIS
The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way ANOVA, followed by Bonferroni's multiple comparison post-hoc test or Chi-square test or unpaired Student's 't' test to find out the level of significance. P<0.05 was considered statistically significant. The analysis was performed using Graphpad Prism software package (Version 4.0).

RESULTS
EFFECT OF EXTRACT ON ANAPHYLACTIC SHOCK-INDUCED BRONCHOSPASM IN SENSITIZED RATS
Test extract protected the sensitized rats against anaphylactic shock in a dose-dependent manner. In control rats, intravenous antigen challenge (horse serum) caused shock in 100% of the animals, while in treated rats (400 mg/kg of etanolic extract), the onset of symptoms of shock was delayed (P <0.001), and symptoms were less severe (P <0.05) with reduced mortality (P <0.05). Results are represented in table 1. Test extract at the dose 300 mg/kg resulted in significant reduction of serum IgE levels (25.80 ± 4.85 ng/ml, P <0.001) as compared to sensitized controls (125.06 ± 9.66 ng/ml). Serum IgE levels in control group was 8.83 ± 0.84 ng/ml (P <0.001 as compared to sensitized control). Extract showed optimal pharmacological effect at 400 mg/kg dose. Hence, same dose of was used for further experimental models (Table-1).

Table 1: Effect of Ocimum ethanolic extract on anaphylactic shock- induced bronchospasm in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total score x 10</th>
<th>Onset of symptoms (min)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120</td>
<td>70</td>
<td>105</td>
</tr>
<tr>
<td>E.E. of O. Sanctum (100 mg/Kg)</td>
<td>116</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>E.E. of O. Sanctum (200 mg/Kg)</td>
<td>79</td>
<td>80</td>
<td>57</td>
</tr>
<tr>
<td>E.E. of O. Sanctum (400 mg/Kg)</td>
<td>68*</td>
<td>115*</td>
<td>41*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM except for mortality, which is expressed as percentage, n=7 in each group; Total score: F=50508, df =27, P=0.0050; Onset of symptoms: F=20.51, df = 27, P=0.0001. *P<0.05, # P<0.001 as compared to control. (ANOVA followed by Bonferroni’s multiple comparison post hoc tests for total score and onset of symptoms. Chi-square test for mortality).

MAST CELL STABILIZING POTENTIAL OF O. LEAF EXTRACT
Antigen challenge resulted in significant degranulation of the mesentric mast cells (approximately 88%, P <0.001). Pretreatment of sensitized animals with O.sanctum extract at 400 mg/kg, p.o., for 2 weeks resulted in a significant reduction in the number of disrupted mast cells (P <0.001) when challenged with horse serum. The effect of N. zeylanica extract was also comparable to the reference drug prednisolone (Table-2).
Pharmacological Basis For Antianaphylactic, Antihistaminic And Mast Cell Stabilization Activity Of Ocimum Sanctum

Table 2: Effect of O. on mast cell stabilization in sensitized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mast cells (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Disrupted</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>83.06 ± 3.70</td>
<td>16.94 ± 3.70</td>
<td></td>
</tr>
<tr>
<td>Sensitized control</td>
<td>12.31 ± 1.92</td>
<td>87.69 ± 1.92</td>
<td></td>
</tr>
<tr>
<td>E.E. of O. Sanctum</td>
<td>64.25 ± 9.51*</td>
<td>35.75 ± 9.51</td>
<td></td>
</tr>
<tr>
<td>Prednisolone (10mg/Kg)</td>
<td>69.19 ± 4.89*</td>
<td>30.81 ± 4.89*</td>
<td></td>
</tr>
<tr>
<td>One-way F</td>
<td>129.7</td>
<td>129.7</td>
<td></td>
</tr>
<tr>
<td>ANOVA df</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=8 in each group. * Significantly different from sensitized control (P<0.0001).

**EFFECT ON HISTAMINE-INDUCED BRONCHOSPASM**

O. sanctum ethanolic extract at 400 mg/kg, p.o., significantly prolonged the latent period of PCD (P <0.008) as compared to control, following exposure to histamine aerosols on day 5 (Table 3).

Table 3: Effect of O. ethanolic extract on histamine induced bronchospasm in guinea pigs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-convulsion dyspnea (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>188</td>
</tr>
<tr>
<td>E.E. of O. Sanctum (400mg/Kg)</td>
<td>186</td>
</tr>
<tr>
<td>P</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=6 in each group. * (P<0.008) as compared to control on day 5 (unpaired Students’ t’ test)

**DISCUSSION AND CONCLUSION**

Experimental animal model of asthma is characterized by allergen-induced immediate airway constriction and late airway reactivity to a pharmacological vasoconstrictor such as histamine and leukotrienes. Histamine is a central mediator in the pathogenesis of allergic and inflammatory disorders. In the present study, O. sanctum ethanolic extract prolonged the latent period of PCD in guinea pigs following histamine aerosol. This may be suggestive of an antihistaminic activity following treatment with O. sanctum extract. It also offered protection against anaphylactic shock-induced bronchospasm in rats.

Basophils, mast cells, and their preformed de novo synthesized mediators, play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration (17-18). Immunoglobulin E (IgE)-mediated mast-cell stimulation is an important initial event in the development of type I allergic reactions such as asthma and atopic disorders. Clinical studies have found a close association between asthma and serum IgE levels, as well as IgE-dependent skin test reactivity to allergens. Antigen challenge, in sensitized animals, results in the degranulation of mast cells, which is an important feature of anaphylaxis. O. sanctum ethanolic extract showed marked protection against the mast cell degranulation following antigen challenge in sensitized animals. Mast cell stabilizing activity of extract may be attributed to the presence of anti-inflammatory mediator release, which is known for their mast cell stabilizing potential against antigen-antibody reaction and/or due to the suppression of IgE antibody production, which is responsible for degranulation mast cells (9). This antianaphylactic and antihistaminic effect may be due to the stabilization of the mast cell membrane, suppression of IgE, and inhibition of pathological effects induced by the release of inflammatory mediators in test extract treated animals.

Experimental results indicated the potent benefits of O. sanctum in the treatment of asthma and related conditions. The findings from various studies reveal that the antihistaminic and antianaphylactic activity of extract which is mainly due to its mast cell stabilizing potential, suppression of IgE, and inhibition of release of inflammatory mediators. Thus use of O. sanctum leaves proved the strong rationale behind the mentioned therapeutic activities.

**ACKNOWLEDGEMENT**

Authors are greatful to Chemical Engg, department, National Institute of technology Surathkal, Karnataka for help in initial quantification of Phytoconstituents.

**References**

Author Information

G. Sridevi, Ph.D.
Department of Pharmaceutics, Srinivas College of Pharmacy

P. Gopkumar, Ph.D.
Department of Pharma chemistry, Srinivas College of Pharmacy

Shenoy Ashok, M.Pharm
Department of Pharmacology, Srinivas College of Pharmacy

C.S. Shastry, Ph.D.
Department of Pharmacology, Srinivas College of Pharmacy