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Combination treatment for allergic conjunctivitis – Plant derived histidine decarboxylase inhibitor and H1 antihistaminic drug

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ABSTRACT

Aim of present investigation was to study the effect of catechin and the combination of catechin and cetirizine in ovalbumin induced animal model of allergic conjunctivitis. Guinea pigs were divided into 5 groups: normal control, disease control, disease treated with catechin 100 mg/kg, disease treated with cetirizine 10 mg/kg, disease treated with combination of catechin and cetirizine, 50 mg/kg & 5 mg/kg respectively. Sensitization was carried out by intraperitoneal injection of ovalbumin for the period of 14 day. Simultaneously, catechin was administered orally for 14 days while, cetirizine was administered at the day of experiment. Determination of clinical scoring, mast cell and blood histamine content, histidine decarboxylase activity from stomach was carried out. Vascular permeability was measured by dye leakage after secondary challenge of allergen and conjunctival tissues were subjected for histopathological examinations. Treatment with catechin, cetirizine and combination showed significant ($P < 0.05$) decrease in clinical scoring and vascular permeability. While, catechin 100 mg/kg and catechin 50 mg/kg showed significant ($P < 0.05$) decrease in histamine content in mast and blood. The treatment also showed significant ($P < 0.05$) decrease in the histidine decarboxylase enzyme activity. However, cetirizine group did not show any difference in enzyme activity as well as histamine content. Histopathological examination also showed improvement in ulceration and decrease in edema and inflammation in all treatment groups. From the present study, we can conclude that catechin exhibits potent anti-allergic activity by histidine decarboxylase enzyme inhibition and combination shown significant anti-allergic activity at reduced dose by both enzyme inhibition as well as inhibition of histamine receptors.

1. Introduction

Allergic conjunctivitis is abnormal and altered immunological reaction induced by cross linking of allergen with IgE molecules on ocular surface which leads to release of histamine from mast cells and basophils by degranulation (Abelson et al., 1994). Current treatment options include; antihistamines, mast cell stabilizers, non-steroidal anti-inflammatory drugs, corticosteroids and immunosuppressants. These drugs however have a quick onset of action to relieve allergic reactions but lack a longer duration of action as well as associated with CNS (Central Nervous System) and CVS (Cardiovascular System) side effects (Small et al., 2007; Bourdin et al., 2009, Mihaibisca, 1997).

Histamine is a fundamental mediator released during the immediate allergic response from tissue mast cells and during the late phase response chiefly from recruited basophils. Histidine decarboxylase is an enzyme which catalyzes synthesis of histamine from histidine in mast cell and therefore H1 antagonist can be a choice in the treatment of allergic disease by preventing binding of histamine to the H1 receptors. The histidine decarboxylase inhibitors are agent which inhibits the conversion of histidine to histamine, and so, is thought to be beneficial through reduction of potentially damaging, histamine-related local immune response in allergic diseases. In the view of drawbacks of currently available synthetic antihistaminic drugs, it is reasoned that screening plant derived histidine decarboxylase inhibitor in combination with H1 antihistaminic drug which combines high selectivity with good oral efficacy and absence of CNS side effects could constitute a major therapeutic improvement in treatment of allergic diseases.

Catechin is a flavan-3-ol that is present in several plants such as *Acacia catechu, Camellia sinensis*. It has found to have beneficial effect in various diseases and disorders such as; aging, Parkinson's disease, stroke, obesity, diabetes, cancer and viral infection (Yilmaz,

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2006; Singh et al., 2011). Catechin reported to have enzyme inhibitory actions on histidine decarboxylase in *in vitro* study (Shimamura et al., 2007; Kawai et al., 2003). Therefore, the aim of the present investigation was to study effect of Catechin alone and in combination with cetirizine at reduced dose in animal model of allergic conjunctivitis.

2. Materials and methods

2.1. Materials

Catechin, ovalbumin, histamine hydrochloride, compound 48/80 was purchased from Sigma Aldrich Pvt. Ltd., India, cetirizine was purchased from Balaji drugs Ltd. India, o-phthalaldehyde, L-histidine, other chemicals were of analytical grade.

2.2. Experiment animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmedabad. 30 Hearty Guinea pigs (500–600 g) and 24 Balb/C albino mice were procured from Zydus Research Centre, Ahmedabad. Animals have been housed in the animal house of Nirma University, Ahmedabad under controlled conditions of temperature 23 ± 2 °C, relative humidity 55 ± 5%, and 12 h light and dark cycle.

2.3. Experimental protocol

Guinea pigs were divided into 5 groups normal control group, disease control group, disease treated with catechin (100 mg/kg, p.o.), disease treated with cetirizine (10 mg/kg, p.o.), disease treated with combination of catechin and cetirizine (50 mg/kg & 5 mg/kg respectively). Sensitization was carried out by intraperitoneal injection of 100mcg ovalbumin dissolved in 20 mg alum for fourteen days and ovalbumin challenge was given by instillation in conjunctiva on 14th day. Treatment of catechin was stared on day of sensitization till 14th day while, cetirizine was administered one hour before ovalbumin challenge. At the end of experiment, clinical scoring for physical characteristics was carried out (Yanni et al., 1999) (Table 1). Determination of vascular permeability was carried out by U V spectrometer of dye leakage from conjunctival tissues by tracing the jugular vein and injecting 0.4 ml Evans blue dye followed by subcutaneous injection of 0.1 ml 0.3% ovalbumin into the conjunctival tissue and measuring the extracted dye by excision of conjunctiva into extraction solution using UV spectrometer (Nakahara et al., 2000). Estimation of mast cell histamine content was done by isolation of mast cells from peritoneal cavity followed by degranulation with compound 48/80 and estimation of histamine content by spectrofluorimetry at 450 nm emission excited at 360 nm (Kim et al., 2006). Blood histamine content was measured by similar procedure using basophils instead of mast cells for histamine measurement (Barsoum and Gaddam, 1935). Eyes including eyelids and conjunctiva were exenterated and fixed in 4% paraformaldehyde for 24 h. Conjunctival tissues were cut into cross sections of 3-mm thick and stained with toluidine blue for detection of mast cells and eosinophils respectively.

Histidine decarboxylase assay was performed using the stomach homogenate. Homogenate was prepared in ice-cold 0.02 M phosphate buffer (pH 6.2) containing 1 μg/ml pyridoxal-5′-phosphate and 1 μg/ml dithiothreitol. Homogenate was subjected to centrifugation and supernatant obtained was used as the enzyme solution. 1 ml of the enzyme solution was incubated at 37 °C for 4 h with 0.1 μg/ml L-histidine. The enzyme reaction was terminated using $HClO_4$. The histamine formed is measured fluorimetrically (Ridzwan et al., 1990). Balb/c mice were used to determine the locomotor activity test of Catechin, cetirizine and the combination using determination of locomotor activity by photoactometer. Overnight fasted mice were administered drug and post an hour, animal was placed in the photoactometer and number of 'cut offs' were recorded for 30 min (Rakh and Chaudhari, 2010). Statistical analysis results are represented as mean ± S.E.M. Statistical analysis was performed using Graph pad prism 5 statistical software. Statistical differences between the means of various groups were evaluated using one way analysis of variance (ANOVA) followed by turkey's test. Data were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of treatment on clinical scoring for physical characteristics

Ovalbumin challenge showed significant increase in clinical score in disease control group as compared to normal control group, while treatment with catechin, cetirizine and combination of catechin and cetirizine showed significant ($P < 0.05$) reduction in clinical score compared to disease control group of animals. The combination has produced effect comparable to cetirizine 10 mg/kg (Fig. 1, Table 2).

3.2. Effect of treatment on vascular permeability

A significant increase in vascular permeability in disease control group was observed proceeding ovalbumin challenge as compared to normal control group, while the disease treated with catechin,

Table 1
Clinical scoring method for physical characteristics.

Feature	Characteristic	Clinical scoring
Conjunctiva redness	Normal	0
	Pink	1
	Red	2
	Dark red	3
Eyelid edema	No edema	0
	Lower lid edema	1
	Upper & lower lid edema	2
	Swollen, everted eye lids	3
	Swelling of both lids and side of face	4
Discharge	No discharge	0
	Glazed, glassy appearance	1
	Moist lids and surrounding hair	2
	Moist lids and surrounding hair, thicker mucous like	3