ORIGINAL ARTICLE

Preventive Effect of Nigella Sativa Essential Oil on Signs of Ocular Allergy Induced by Compound 48/80 in Rabbits

ZUJAJA ZAHEER*, SAMINA KAUSAR, NISAR AHMED, *NADEEM YAQOOB, SADIA CHIRAGH,*

ABSTRACT

Aim: To evaluate preventive anti allergic effect of *Nigella sativa* on signs of ocular allergy produced in rabbits by topical application of compound 48/80
Study design: Experimental study
Materials and methods: Twelve rabbits were divided in to two groups. Signs of ocular allergy were induced by compound 48/80 and the effect was seen without and with pretreatment with essential oil of Nigella sativa.
Results: the findings of present study show that oral pretreatment with Nigella sativa essential oil attenuates the ocular allergic response in rabbits that is produced by compound 48/80.
Conclusion: Our findings suggest that Nigella sativa essential oil has mast cells stabilizing property.
Key words: Nigella sativa, compound 48/80, ocular allergy

INTRODUCTION

An important undesirable side effect of immunity is the development, under some conditions, of allergy or other types of immune hypersensitivity.[1] Hypersensitivity can be classified as antibody-mediated or cell mediated (Type1-IV). Type I- III is antibody mediated while type IV is cell mediated. In general, immediate-type hypersensitivity reactions involve asthma, allergic rhinitis, conjunctivitis, urticaria, and anaphylaxis. Immediate or type I hypersensitivity is IgE mediated, with symptoms usually occurring within minutes following the patients encounter with antigens. A severe type I hypersensitivity reaction such as systemic anaphylaxis requires immediate medical intervention.[2]Signs and symptoms of ocular allergy are itching, injection, chemosis and mucous discharge[3].

Mast cells are the primary effector cells involved in an allergic or immediate hypersensitivity response[4] Activation of mast cells occurs in response to a challenge by a specific antigen against which the surface immunoglobulin E is directed, or by other IgE directed ligands. Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines[5]. Mast cell degranulation can also be elicited by the basic secretagogues. The most potent secretagogue include the compound 48/80, which is mixed polymer of phenethylamine cross linked by formaldehyde and a high dose of which induces almost a 90% release of histamine from mast cells[4].

Nigella sativa also known as black seed has long been used in traditional medicine, in conditions related to the respiratory and gastrointestinal system[6] as well as a natural remedy for various allergies[7]. The oil and seed constituents, in particular thymoquinone have shown promising medicinal properties in the treatment and prevention of a wide range of diseases[8]. Regarding treatment of allergies there is an increasing interest toward the potential health benefits of medicinal plants..

MATERIALS AND METHODS

Animals: Adult rabbits of either sex weighing 1–1.5 Kg were purchased from local market and kept in the animal house of PGMI for one week for acclimatization. They were fed on grass and grain; tap water was provided ad libitum.

Preparation of *Nigella sativa* essential oil: Fresh seed of *Nigella sativa* were purchased from local market. They were ground and subjected to hydro distillation using reverse Dean – Stark apparatus for 6 hours in PCSIR laboratories, Lahore. The extracted essential oil was dried over anhydrous sodium sulphate and stored at 4^{o}C for use. Yield of essential oil was 0.4% which is in accordance with international standard. Essential oil was dissolved in alcohol and diluted with distilled water before administration.

Induction and prevention of signs of ocular allergy: Twelve rabbits were divided into two groups, each group containing six rabbits. Signs of ocular allergy were induced by topical application of compound 48/80 (Sigma) which is mast cell degranulation agent. In rabbits of group I (n=6) right

**Deptt,. of Pharmacology K E Medical University, Lahore*
Deptt of Pharmacology, PGMI, Lahore
Correspondence to Dr. Samina Kausar, Assistant Professor Email: samina.zia@hotmail.com

eye served as control (C1) and 30 µl of distilled water was instilled in conjunctival sac. Left eye served as experimental eye (E1) and 30 µl (150µg) of compound 48/80 was instilled in the conjunctival sac.Eyes were examined before instillation and 10, 20 and 30 minutes after instillation of distilled water and compound 48/80.

Rabbits of group II (n=6) were administered essential oil of *Nigella sativa* 3 mg/Kg orally in the morning after over night fast. One hour after administration of essential oil, distilled water and compound 48/80 were instilled in right (CII) and left (EII) eye respectively and eyes were examined in the same manner as for group I.

Scoring of ocular allergy: Congestion, chemosis itching and lacrimation are signs of ocular allergy. We examined these sequelae as follows:

i. Congestion: Conjunctival redness was analyzed visually and scored as follows:

0. Absent
1. Barely visible vascular dilatation
2. 3-4 dilated vessels
3. 5-6 dilated vessels
4. Marked ciliary injection

ii. Chemosis: Swelling of conjunctiva was observed and scored as follows:

0. Absent
1. Swelling of palpebral conjunctiva
2. Swelling of palpebral and bulbar conjunctiva
3. Raised conjuctival appearance especially at limbal area
4. Ballooning of conjunctiva

iii Lacrimation: Tear formation was observed and scored as follows:

0. Normal appearance of eyes
1. Watery appearance of eyes
2. Tears on lid margin
3. Few tears rolling down
4. Running eyes

iv Itching: Irritation or itching was assessed by counting number of blinks/ minute

0. 0-2/min
1. 3-5/min
2. 6-10/min
3. 11-20/min
4. >20/ min or continued closure.

Scores of four parameters were added and cumulative score (0-16) was used for statistical analysis by Students't-test. p-value of ≤ 0.05 was considered significant. Miosis was also observed after topical application of compound 48/80 to the eye[9] probably due to direct activation of receptors on papillary sphincter muscle.[10] As this is not one of manifestations of ocular allergy, it was not analyzed statistically.

RESULTS

Signs of ocular allergy started appearing immediately after application of compound 48/80. Lacrimation and irritation were maximum at 10 minutes and then decreased. Congestion was maximum at 10 minutes and was sustained up to 30 minutes. Chemosis became maximum at 20 minutes and was sustained up to 30 minutes. Cumulative score was maximum at 10 minutes and this was used for statistical analysis.

Table 1 shows cumulative allergy score without and with *Nigella sativa* pretreatment. Without *Nigella sativa* mean score was highly significant as compared to control with p value <0.001. With pretreatment with essential oil of *Nigella sativa,* compound 48/80 produced signs of ocular allergy but of lesser magnitude. Mean score was significantly high with p value of < 0.005. When allergy score of experimental eyes without and with *Nigella sativa* were compared, results were again significant with p value <0.01. This shows that pretreatment with nigella sativa essential oil significantly reduced manifestation of allergic response to compound 48/80 although not able to abolish completely.

Table 1: Cumulative ocular allergy score without and with *nigella sativa* essential oil pretreatment

S. No	Without Nigella sativa		With Nigella sativa	
	C1	EI	CII	EII
1.	1	11	1	8
2.	2	12	1	5
3.	1	11	2	8
4.	2	14	2	7
5.	1	10	2	7
6.	1	10	1	6
Mean±SD	1.3±0.51	11.3±1.50	1.5±0.54	6.8±1.17
P-value	CI vs EI <0.001, CII vs EII <0.005, EI vs EII <0.01			

DISCUSSION

Ocular allergy is often encountered by allergists, ophthalmologists and primary care physicians. Search for new pharmacological agents will improve the efficacy and safety of ocular allergy treatment. In the current study preventive effect of *Nigella sativa* essential oil was observed on the signs of ocular allergy induced by compound 48/80.

Signs of ocular allergy started appearing immediately after topical application of compound 48/80 in the current study. These were lacrimation, irritation, congestion and chemosis. Conjuctival mast cells are important effector cells in seasonal allergic conjunctivitis via histamine and cytokine secretion[11]. It is well recognized that Compund 48/80 can induce a mast cell dependedent non specific anaphylactic reaction.Comound 48/80 is known to activate mast cell secretory processes by increasing the rate of

guanisine triphosphate gamma S binding to G proteins[12] which trigger activation of protein kinase C and Ca^{++} signaling which results in the release of histamine from these cells[13] by inducing the movement of granules to the plasma membrane followed by degranulation of mast cells and basophils[14]. Many studies have been conducted to find out the effect of various substances on histamine release from mast cells. *Nigella sativa* is one of such herb that is thought to have anti allergic effect.

The seeds of *Nigella sativa* commonly known as black seed or black cumin are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhea, dyslipidemia, inflammation and bronchitis. They are also used in food as a spice and a condiment. The seeds contain both fixed and essential oils, proteins, alkaloids and saponins. Much of the biological activity of the seeds has been shown to be due to thymoquinone,the major component of essential oil, which is also present in the fixed oil[15].

Many studies have been conducted on the effect of *Nigella sativa* seed extracts or oils on various body systems in vivo and in vitro. The aqueous extract of *Nigella sativa* has shown an anti inflammatory effect demonstrated by its inhibitory effects on carrageenan induced paw edema.[16] Studies have shown its inhibitory effect on histamine release from mast cells. In an experiment carried out on rat peritoneal mast cells, in vitro, it has been shown that nigellone, a carbonyl polymer of thymoquinone isolated from *Nigella sativa* seeds, was highly effective in inhibiting histamine release. The mechanism of action seems to be through decreasing intracellular calcium by inhibiting its uptake and stimulating its efflux, by an inhibition on protein kinase C. There is also indication for a mild inhibition of oxidative energy metabolism contributing to some inhibition of the release[17].

The present study has shown that oral pretreatment with *Nigella sativa* essential oil has reduced the severity of signs of ocular allergy. These results are in agreement with a study which showed that administration of thymoquinone suppressed the ocular symptoms and inflammatory cell infiltration in conjunctiva of mice[18]. These findings suggest that *Nigella sativa* essential oil has mast cells stabilizing property and prevents signs of ocular allergy induced by compound 48/80.

CONCLUSION

Considering these findings one can conclude that *Nigella sativa* could be of therapeutic potential in preventing diseases associated with hypersensitivity.

REFERENCES

1. Guyton AC, Hall JE, Text book of medical physiology , 12th ed. Mississippi: Saunders, 2011;34: 433-444
2. Lake DF, Briggs AD, Akporiaye ET. Immuno-pharmacology. In: Katzung BG, Masters SB and Trevor AJ , ed. Basic and Clinical Pharmacology. New York: Mc Graw Hill. 2009; 55: 963-986
3. Udell IJ, Abelson MB. Animal and human ocular surface response to a topical non immune mast cell degranulating agent (compound 48/80). Am J Opthalmol. 1981;91(2): 226-230.
4. Choi YH, Yan GH, Chai OH et al. Inhibitory effects of *Agaricus blazei* on mast cell mediated anaphylaxis like reactions. Biol. Pharm. Bull. 2006;29(7):1366-1371
5. Kalesnikoff J, Galli SJ. New developments in mast cell biology. Nat immunol. 2008; 9:1215-1223.
6. Majdalawieh AF, Hmaidan R, Carr RI. *Nigella sativa* modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. J Ethnopharmacol. 2010;131(2): 268-275.
7. Kalus U, Pruss A, Bystron J et al. Effect of *Nigella sativa*(black seed) on subjective feeling in patients with allergic dieases. Phytother Res. 2003; 17: 1209-1214.
8. Abd El Aziz AE, El Sayed NS, Mahran LG. Anti-asthmatic and anti-allergic effects of thymoquinone on airway induced hypersensitivity in experimental animals. JAPS 2011;01(08): 109-117.
9. StenbAck H, Krootilia K,. Mast cells in the anterior uvea of the rabbit.Intraocular effects of compound 48/80 in the rabbit. Exp Eye Res. 1992; 54 (2):247
10. Mandahl A, Brodin E, Bill A. Hypertonic KCl, NaCl and capsaicin intracamerally causes release of substance P like immunoreactive material into the aqueous humor in rabbits. Acta physiol. 1984; 120 (4): 579-84.
11. Galatowicz G, Ajayi Y, Stern M, Calder VL. Ocular antiallergic compounds selectively inhibit human mast cell cytokines in vitro and conjunctival cell infiltration in vivo. Clin Exp Allergy 2007; 37(11): 1648-1656.
12. Palomaki VA, Laitinen JT. The basic secretagogue compound 48/80 activates G proteins indirectly via stimulation of phospholipase D-lysophosphatidic acid receptor axis and 5-HT_{1A} receptors in rat brain sections. Br J Pharmacol.2006; 147:596-606.
13. Turner H, Kinet JP. Signaling through the high affinity IgE receptor Fc epsilon RI. Nature 1999; 402 (6760-supp): 24-30.
14. Nugroho AE, Ikawati Z, Sardjiman, Maeyama K. Effects of benzylidencyclopentanone analogues of curcumine on histamine release from mast cells. Biol Pharm Bull. 2009; 32:842-849.
15. Ali BH, Blunden G. Pharmacological and Toxicological properties of *Nigella sativa*. Phytother Res. 2003;17: 299-305.
16. Al-Ghamdi MS. The anti-inflammatory,analgesic and antipyretic activity of *Nigella sativa*. J Ethnopharmacol. 2001; 76(1): 45-48.
17. Chakravarty N. Inhibition of histamine release from mast cells by nigellone. Ann. Allergy 1993; 70(3):237.
18. Hayat K et al. Ameliorative effect of thymoquinone on ovalbumin induced allergic conjunctivitis in Balb/c mice.Curr Eye Res. 2011;36(7): 591-598.