



Comparative study of anti-allergic activity of two poly-herbal formulations in ova-challenged allergic rhinitis mice model

Richa Srivastava¹ · Sajal Srivastava¹ · Satya Prakash Singh²

Received: 17 November 2017 / Accepted: 27 September 2018

© Institute of Korean Medicine, Kyung Hee University and Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract

This study was to prepare a novel in situ gel system for nasal delivery of poly-herbal extracts and study its efficacy on the allergic rhinitis model. The main purpose is to compare the efficacy of poly-herbal nasal drops and in situ gel. A temperature depended in situ gel was developed and characterized with gellan gum as a carrier. The system was stable kept at 40 ± 2 °C for 6 months, and the micrographic results showed that in situ gel was safety without mucous irritation when given daily for 26 days to mice with allergic rhinitis that the effect of drops for the treatment. Extracts in gellan gum produced obviously effect on allergic rhinitis at the doses of 20 g/body following intranasal administration, and the efficacy was significantly superior to that of the drops ($p < 0.01$). This study uses Kruskal–Wallis test analysis of variance (ANOVA) followed by Dunn's test for the first time to develop, optimize the formulation and assess factors affecting the critical quality attributes. Histopathological study of the nasal mucosa suggested that the formulation was safe for nasal administration. The statistical difference in absolute bioavailability between drop and in situ gel by nasal route suggested that in situ gel had almost 21% increase in bioavailability for *Moringa oleifera* and for *Embelia Ribes* in combination there was a 16% increase over drop formulations. Thus the situ gel system is a promising approach for the intranasal delivery of polyherbal extracts for the therapeutic effect improvement.

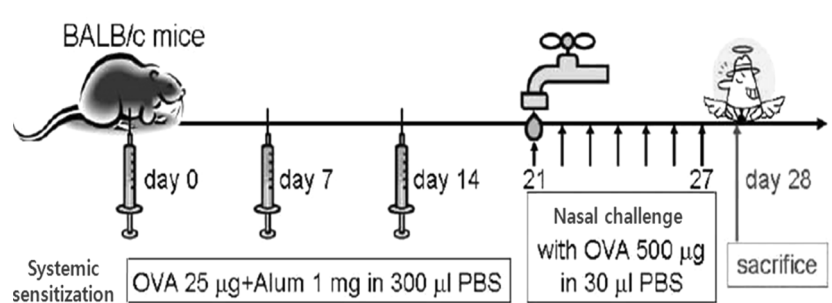
✉ Sajal Srivastava
ssrivastava2@lko.amity.edu

Richa Srivastava
richa_0306@rediffmail.com

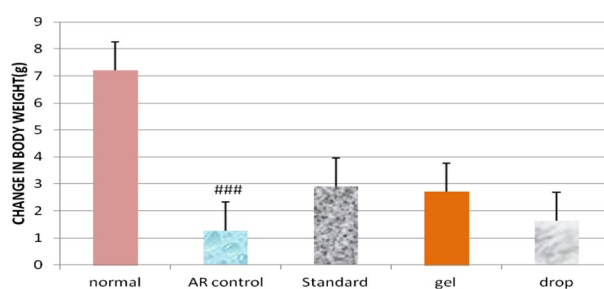
¹ Amity Institute of Pharmacy, Amity University Uttar Pradesh Lucknow campus, Gomti Nagar extension, Lucknow, UP 226028, India

² Pharmacy Department, Integral University, Lucknow, UP 226026, India

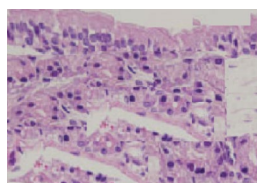
Graphical abstract



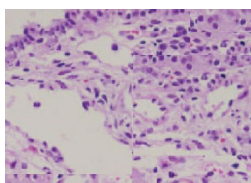
Source: KAPARD-KAAACI & West Pacific Allergy Symposium Joint International Congress



Effect of Nasal formulations on morphological alterations of change in body weight in OVA-induced sensitized mice



Allergic rhinitis induced



Allergic rhinitis treated

Keywords *Moringa olifera* · *Embelia ribes* · Gellan gum · Pluronic · In-situ · Allergic rhinitis · Polyherbal

Introduction

It is well known that allergic rhinitis is an inflammatory disorder of the higher airway, which is observed by means of sneezing, itching, congestion, rhinorrhea and lack of the feel of odor. AR is frequently involved in acute and chronic airway diseases such as bronchial asthma, usually caused by hypersensitivity to antigens, resulting in increase local inflammation as well as constriction in bronchitis, vasomotor change, and mucus hypersecretion. Among immune cells, mast cells play critical roles in the pathogenesis of allergic responses in AR. AR results from a complicated, allergen-pushed mucosal inflammatory technique, that is modulated via immuno-globulin E (IgE) and resulting from the

interaction between resident and infiltrating inflammatory cells variety of vasoactive and proinflammatory mediators, inclusive of cytokines (Settipane et al. 2013). The Pharmacotherapy of AR is multivariate, and plenty of interactive pathways are worried in the pathophysiology of AR. The one of a kind class of medicine which includes antihistamines, corticosteroids, mast cell stabilizers, decongestants, antileukotrienes and anticholinergics have been used (Leung and Hon 2008). The mast cells express the immunoglobulin FcεRI that trigger specific IgE response to antigen (Church and Levi-Schaffer 1997). Numerous herbs for asthma and AR have been carefully reviewed in comprehensive analyses recently, but their clinical value remains uncertain (Bielory and Lupoli 1999; Ziment et al. 2000).

One of the authors made an original clinical observation that herbal preparations were effective in treating AR and asthma (Hu et al. 1987; Shen et al. 2012; Hu et al. 1983). The recent invention is an aggregate of a polyherbal formula along with extracts of 2 herbs, extracted and delivered together in unique ratios at the side of the excipients to form an in situ nasal gel, and nasal drops organized from the extracts of a polyherbal, namely *Moringa olifera* and *Embelica ribes*. The gift invention is a type of herbal in situ mucoadhesive gel fabricated from formulating *Moringa olifera* and *Embelica ribes* with adhesives and polymers.

Compared with liquid nasal formulations, nasal in situ gels are instilled as low viscosity answers into the nasal cavity, and upon contact with the nasal mucosa, the polymer adjustments conformation generating a gel, so it can not simplest lengthen the contact time among the drug and the absorptive the mucosal sites in the nasal cavity, however also release the drug slowly and continuously, subsequently, hence, it is especially useful for those drugs used chronically. Although drops give faster onset of the movement, it cannot retain the recurrence and pain persistence. To conquer those constraints, nasal formulation is changed into gels at nasal mucosal temperature and consists of a bioadhesive polymer that lengthens the house time and accordingly the bioavailability of the gel. Several tries were made to supply triptans through formulating a gel using pluronic and poloxamers affecting important excellent attributes. The present examine uses Pluronic F127 and gellan gum preponderance of literature suggested the use of conventional optimization strategies in which confounding consequences of excipients over other prevents the proper assessment of the factors. The given study makes use of Kruskal–Wallis test analysis of variance (ANOVA) followed by Dunn's test to evaluate factors affecting the important high-quality attributes of thermoreversible in situ nasal gel and the nasal drops.

Materials and methods

Materials

Moringa olifera fresh leaves were collected and dried, *Embelia ribes* fruit (dreid) were brought from local market. The leaves and seeds were authenticated by State Ayurvedic College Lucknow with specimen no. RS 1 and RS 4 respectively. Pluronic F127 (PF127) was supplied from Sigma Aldrich Pvt. Ltd India, glycerine, gellan gum, Carbopol 934 were from SDFCL Pvt Ltd. India and potassium dihydrogen orthophosphate, acetonitrile, trimethylamine, ortho-phosphoric acid and sodium chloride were supplied by CDH, New Delhi (India).

Ovalbumin (Grade V), aluminium hydroxide and histamine dihydrochloride were purchased from Sigma-Aldrich

Co (St Louis, MO, USA). Sodium sulphate, acetone, alcohol, sulphanilic acid, sodium nitrite and trisodium phosphate were purchased from S.D. Fine Chemicals, Mumbai, India. Sulphanilamides, naphthalenediamine HCl and phosphoric acid were obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Xylometazoline (XLY) (Otrivine® nasal drops) was purchased from Vivimed Labs Ltd., India. Mouse IgE ELISA Kit (Cat. No. E99-115) was obtained from Bethyl Laboratories Inc., Montgomery, TX, USA. The culture of *Bordetella bronchiseptica* (Cat. No. NCIM2267, Batch NoATCC-14455) was obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. All the other chemicals and reagents used in this study were of analytical grade.

Animals

Adult swiss mice (18–22 g) were purchased from CDRI Lucknow. Mice were kept in quarantine for 1 week at standard laboratory conditions (Temperature: 24 ± 1 °C, relative humidity: 45–55% and 12:12 h light/dark cycle). The experiments were carried out between 10:00 AM to 5:00 PM. Animals had free access to standard chaw pelleted feed and water ad libitum. Experimental protocol (No: AIP/IAEC/07/2017) was approved by the Institutional Animal Ethics Committee. Animals were brought to testing laboratory 1 week before the experiments for adaptation purpose.

Preparation of extracts

Air dried and coarsely powdered (500 gm) of *Moringa olifera* leaves and *Embelia ribes* fruits, were weighed and their hydroalcoholic extracts were prepared separately, by using distilled water and ethanol. The extracts were then concentrated to dryness under reduced pressure and controlled temperature, respectively and they were preserved in a refrigerator.

Screening and optimization of pluronic concentration using 2² factorial design

Pluronic gel was prepared using cold technique described by (Schmolka 1972) varying amount of Pluronic F 127 (14, 16 and 18% w/v) was dissolved buffer (pH 5.5) with continuous stirring (RQ-122, Remi, India) and kept overnight at 4 °C. The final volume of the aqueous solution was made to 50 mL with the buffer containing pre-dissolved propylene glycol (0.5, 1.0 and 1.5% w/v). A recommended dose of *Moringa olifera* and *Embelia ribes* was added such that each 10 µL of nasal gel contains 2% w/v of each consecutively. The independent variables in 2² factorial design were pluronic and propylene glycol concentrations while, gelation temperature was dependent variable.

Preparation of thermo reversible in situ nasal gel

From preliminary screening, 18% w/v of pluronic containing 1.0% w/v propylene glycol gelling at 27.4 ± 0.2 °C was selected for further study. Thermoreversible gel (14% w/v of PF127) was prepared by method described earlier in the text and kept overnight at 4 °C. Final volume of aqueous solution was constituted to 50 mL with buffer containing recommended dose of pre-dissolved bioactive. Water soluble additives like mucoadhesive polymer (gellan gum (0.5, 1.0 and 1.5% w/v) and carbopol 934 ((0.5, 1.0 and 1.5% w/v)), PEG (4000 and 6000), osmotic agent (NaCl) and benzalkonium chloride (0.02% v/v) were dissolved prior to Pluronic F127 addition to optimize mixing.

Induction of allergic rhinitis

Induction of AR was performed with the help of sensitization solution containing OVA as per reported method [10]. Thirty swiss mice were divided into 5 groups of 6 each (G1–G5). G1 (normal group) was not sensitized and received saline (0.9% NaCl, 20 µL/nostril) twice daily during the experiment period. Remaining mice were sensitized with intraperitoneal injection of 500 µL of sensitization solution (containing 50 mg of OVA and 1000 mg of aluminium hydroxide and 0.5 mL of 1×10^{10} *Bordetella bronchiseptica* dissolved in 500 mL of saline) on days 1, 3, 5, 7, 9, 11 and 13 of the study. On day 14, mice from G2 to G5 were treated twice a day for next 7 days (day 14 to day 21) as follows: G2—AR control (negative control): OVA sensitized and received saline [20 µL/nostril, intranasal (i.n.)]; G3—XLY: positive control, received xylometazoline (0.5 mg/mL solution, 20 µL/nostril); G4—Thermoreversible nasal in situ gel: OVA sensitized (2 µg/kg, i.n.); G5—Nasal drop OVA sensitized (2 µg/kg, i.n.); On day 21, 1 h after the last dose of treatment, mice were challenged with i.n. administration of OVA (5%, 5 µL per nostril), and observations were recorded

Nasal signs (OVA challenge). After OVA or histamine challenge, nasal signs were evaluated within 10 min period after OVA challenge as described earlier (Wang et al. 2007). Number of sneezes and nasal itching motions (nasal rubbing) were recorded. Nasal discharge was scored as 0 = no discharge, 1 = the discharge reaches the anterior nasal aperture, 2 = the discharge overshoots the anterior nasal aperture and 3 = the discharge flows out. Redness was scored as 0 = no redness and 1 = redness. Nasal signs (histamine challenge). The mice were challenged with histamine dihydrochloride (10 µL per nostril of solution of 1 µmol/mL in physiological saline) on day 24 of study. The number of instances of nasal rubbing and sneezing during 10 min. period after challenge was counted and recorded.

Blood sample collection

On day 21, 2 h after OVA challenge, blood specimens were collected from the retro orbital plexus and serum was obtained by centrifugation 8350 rpm for 10 min at 4 °C. Samples were stored at 20 °C until biochemical and hematological measurements. Serum histamine level measurement. The histamine content of serum was measured by the o-phthalaldehyde spectrofluorimetric procedure. The fluorescent intensity was measured at 460 nm (excitation at 355 nm) using a spectrofluorometer, and histamine content was calculated.

Serum IgE level measurement

Total IgE titer in serum was evaluated using mouse IgE ELISA quantitation kit as per manufacturer's instructions. Serum samples were at 1:20 dilution, horseradish peroxidase (HRP) labeled rat anti-mouse IgE, and TMB substrate was used. Results were evaluated by the P/N value. Test was performed in duplicate to avoid false-negative and false-positive results, and average value was taken for final calculation.

Serum nitrite level measurement

The nitrite level was estimated as nitrite by reduction of nitrate by vanadium (III) combined with detection by the acidic Griess reaction as per reported procedure (Miranda et al. 2001). The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide and N-(1-naphthyl)ethylenediamine to produce a pink Azo-product with maximum absorbance at 543 nm. The concentrations were determined using a standard curve of sodium nitrate, and the results were expressed as microgram per millilitre.

Hematological measurements

Hematological parameters were measured on serum samples using an automated hematological analyser (BC2800, Golden Harvest Ltd.) with software specific to mice. The parameters such as haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width, mean platelet volume, white blood cells, polymorphonuclear leukocytes (PMN), lymphocytes, eosinophils, monocytes and platelet count were estimated.

Morphological measurement

Body weight (BW) of each mouse was recorded on day 0 and on alternate days thereafter during the experiment. On day 24, mice were sacrificed; organs (spleens and lungs)

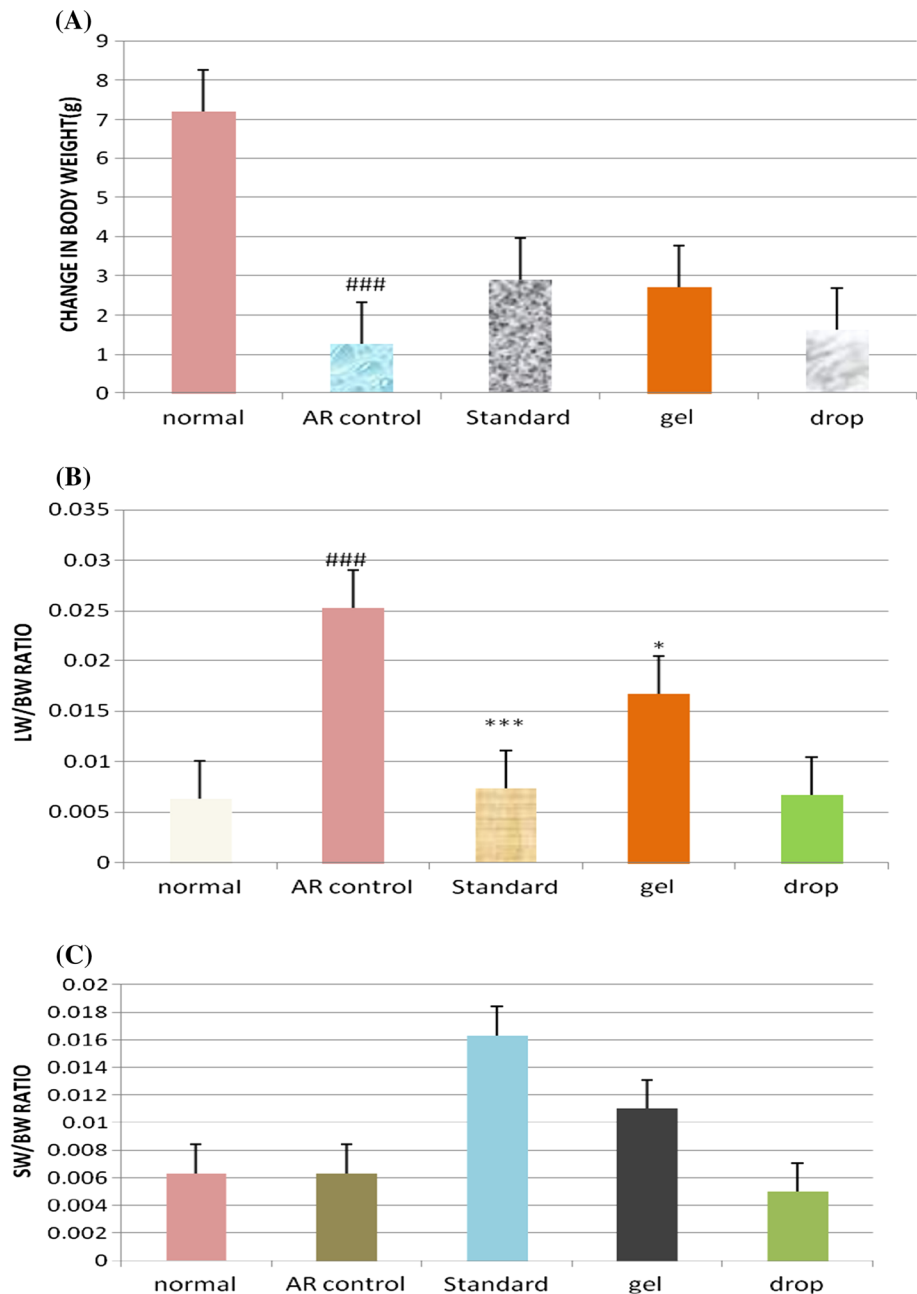
were removed and weighed as spleen weight (SW) and lung weight (LW), respectively. The relative weights of lungs and spleen were calculated as ratios of LW/BW and SW/BW, respectively, for each mice separately, refer Fig. 1 for the results.

Histological examination

On the last day, nasal mucosa and spleen tissues were dissected and stored for 24 h in 10% formalin for histological examination. The specimen was dehydrated and placed in xylene for 1 h (three times) and later in ethyl alcohol (70, 90

and 100%) for 2 h, respectively. The infiltration and impregnation were carried out by treating with paraffin wax twice, each time for 1 h. For nasal mucosa section preparations, sections (4 μ m) were cut at the level of incisive papillae, first and second palatal ridges, and stained with haematoxylin and eosin stains. Sections were examined under a light microscope to obtain a general impression of the histopathology features of specimen and infiltration of cells in epithelium and subepithelium. The intensity of inflammatory cell recruitment in the nasal mucosa was graded as grade 0 (not present or very slight), grade 1 (mild), grade 2 (moderate) and grade 3 (severe) as described in the literature (Yamasaki

Fig. 1 Effect of Nasal in situ gel on morphological alterations in OVA-induced sensitized mice. **a** Change in body weight (BW), **b** relative lung weight (LW) and **c** relative spleen weight. Data are expressed as mean $\pm$ SEM and analysed by separate one-way analysis of variance followed by Dunnett's test for each parameter. ### p < 0.001 as compared with normal group. * p < 0.05, ** p < 0.01 and *** p < 0.001 as compared with AR control group. Numbers in bracket indicate intranasal dose in microgram per kilogram



et al. 2001). For spleen section preparation, specimens were cut into sections of 3–5 µm thickness and were stained with haematoxylin and eosin. The specimen was mounted on slide by use of Distrene Phthalate Xylene as mounting medium.

Results

Statistical analysis

Data of each nasal symptom parameter were analysed by separate Kruskal–Wallis test analysis of variance (ANOVA) followed by Dunn's test. Data of each bio-chemical, hematological and morphological parameters were analysed by separate one-way ANOVA followed by Dunnett's test. The p value < 0.05 was considered significant when compared with AR control mice.

The data of effects of treatments on nasal signs (nasal rubbing and sneezing) in 10 min period after OVA challenge are presented in Table 1. OVA challenge caused statistically significant ($p < 0.001$) increase in the number of nasal rubbings and sneezing in AR control mice were compared with normal mice. XLY and In-situ nasal gel and nasal drop 2 µg/kg dose) pretreatment caused statistically significant ($p < 0.001$) prevention of OVA-induced elevated nasal rubbing and sneezing as compared with AR control mice. Intranasal challenge of OVA resulted in statistically significant increase ($p < 0.001$) in nasal discharge and redness scores in AR control mice as compared with normal mice. Pretreatment of XLY and in situ nasal gel shows resulted in statistically significant ($p < 0.01$) prevention of OVA-induced

elevated nasal discharge as compared with AR control mice. However, neither XLY nor nasal drop pretreatment showed prevention in OVA-induced nasal redness.

Effects on nasal signs (ova challenge)

The data on the effects of treatments on nasal signs (nasal rubbing and sneezing) in 10 min period after OVA challenge are presented in Table 1. OVA challenge caused statistically significant ($p < 0.001$) increase in the number of nasal rubbings and sneezing in AR control mice as compared with normal mice. XLY and in situ nasal gel (2 µg/kg, i.n.) pretreatment a used statistically significant ($p < 0.001$) prevention of OVA-induced elevated nasal rubbing and sneezing as compared with AR control mice. Intranasal challenge of OVA resulted in statistically significant increases ($p < 0.001$) in nasal discharge and redness scores in AR control mice as compared with normal mice. Pretreatment of XLY and in situ nasal gel (2 µg/kg, i.n.) but not a nasal drop in situ nasal gel (2 µg/kg, i.n.) resulted in statistically significant ($p < 0.01$) prevention of OVA-induced elevated nasal discharge as compared with AR control mice. However, neither XLY nor in situ nasal gel pretreatment showed prevention in OVA-induced nasal redness as compared with AR control mice.

Effects on serum histamine, IgE and nitrite levels

The effects of the pretreatments on levels of serum biochemical parameters after OVA challenge are presented in Table 2. OVA challenge caused statistically significant

Table 1 Effect of nasal formulations on nasal symptoms in OVA-induced sensitized mice

Nasal symptoms in 10 min period after	Normal	AR control	XLY	Nasal drop (2 µg/kg i.n)	In-situ nasal gel (2 µg/kg i.n)	Statistics (Kruskal–Wallis ANOVA)
OVA challenge						
Rubbing (number)	16.46 ± 2.57	62.33 ± 5.29####	30.16 ± 2.14***	41.68 ± 2.15*	28.11 ± 1.84***	H = 61.14, $p < 0.001$
Sneezing (number)	8.66 ± 2.31	43.33 ± 2.19####	18.59 ± 1.85**	25.11 ± 1.59**	14.33 ± 1.57***	H = 52.81, $p < 0.001$
Discharge (score)	0.13 ± 0.13	2.1 ± 0.17####	0.44 ± 0.17**	1.22 ± 0.232 ns	0.57 ± 0.19**	H = 46.90, $p < 0.001$
Redness (score)	0.11 ± 0.11	0.76 ± 0.14#	0.32 ± 0.16 ns	0.52 ± 0.19n	0.28 ± 0.14 ns	H = 45.90, $p < 0.001$
Histamine challenge						
Rubbing (number)	14.11 ± 1.27	68.50 ± 7.54####	23.83 ± 2.25**	57.83 ± 2.96 ns	34.50 ± 1.35*	H = 35.45, $p < 0.001$
Sneezing (number)	6.64 ± 1.64	54.83 ± 6.15####	19.67 ± 1.74*	32.83 ± 4.18 ns	23.17 ± 2.56*	H = 38.47, $p < 0.001$

Numbers in bracket indicate intranasal dose in microgram per kilogram. Data were represented as mean score ± SEM (n = 10) and analysed for each parameter separately by Kruskal–Wallis analysis of variance followed by Dunn's test

NS not significant, ANOVA analysis of variance

$p < 0.05$ v/s Normal

$p < 0.001$ v/s Normal

* $p < 0.05$ v/s AR control

** $p < 0.01$ v/s AR control

*** $p < 0.001$ v/s AR control

Table 2 Effect of nasal formulations on serum biochemical markers in OVA-induced sensitized mice

Parameter	Normal	AR control	XLY	Nasal drop (2 µg/kg i.n)	<i>In-situ</i> nasal gel (2 µg/kg i.n)	Statistics at d.f. (8, 36)
Serum histamine (µg/mL)	253.1 ± 35.18***	473.6 ± 38.7###	29.7 ± 45.79 ns	223.9 ± 19.21**	121.6 ± 10.57***	F = 12.79, $p < 0.001$
Serum IgE (ng/mL)	84.68 ± 15.42	5199.0 ± 32.85###	126.9 ± 23.42***	319.7 ± 21.02**	256.1 ± 31.28***	F = 33.49, $p < 0.001$
Serum nitrite (µg/mL)	13.12 ± 3.16	72.8 ± 6.65###	36.26 ± 6.54*	64.82 ± 7.13 ns	60.66 ± 7.01 ns	F = 6.12, ns

Numbers in bracket indicate intranasal dose in microgram per kilogram. Data are expressed as mean ± SEM (n = 10) and one-way analysis of variance followed by Dunnett's multiple comparison test

d.f. degrees of freedom

$p < 0.05$ v/s Normal

$p < 0.001$ v/s Normal

* $p < 0.05$ v/s AR control

** $p < 0.01$ v/s AR control

*** $p < 0.001$ v/s AR control

increase in serum levels of histamine, IgE and NO as compared with AR control mice. XLY pretreatment did not show statistically significant changes in levels of serum histamine as compared with AR control mice. However, the XLY treatment showed statistically significant ($p < 0.001$ and $p < 0.05$, respectively) prevention of elevated serum IgE and nitrite levels as compared with AR control mice.

In-situ nasal gel pretreatment at dose in situ nasal gel (2 µg/kg, i.n.) but nasal gel in situ nasal gel (2 µg/kg, i.n.) showed statistically significant ($p < 0.01$ and $p < 0.001$, respectively) prevention of elevated serum histamine and IgE levels as compared with AR control mice. None of the tested doses of in situ nasal gel (2 µg/kg, i.n.) show statistically significant effect on serum nitrite levels as compared with AR control mice.

Effects on hematological parameters

The effects of pretreatment on various hematological parameters after OVA challenge are presented in Table 3. Mice in AR control group showed statistically significant decrease in neutrophils, PMN and eosinophils count, whereas levels of lymphocytes were increased as compared with normal mice. Other hematological parameters did not show change in AR control mice as compared with the normal mice. XLY pretreatment did not show change in any hematological parameters except statistically significant ($p < 0.01$) prevention in MCHC elevation as compared with AR control mice. As compared with AR control mice, in situ nasal gel (2 µg/kg, i.n.) pretreated mice showed statistically significant increase in values of Hb, RBC count, PCV, MCV and eosinophils but not MCH, MCHC, red blood cell distribution width, TLC, PMN, lymphocytes, monocytes, platelet and mean platelet volume. However, nasal drop (2 µg/kg, i.n.) pretreatment

did not show significant change in any of the tested blood parameters.

Effects on morphological parameters. The data for treatment on changes in BW from baseline values are presented as Fig. 2a with $e(5, 54) = 11.15$, $p < 0.001$ (one-way ANOVA). The OVA-induced sensitized (AR control) mice showed statistically significant ($p < 0.001$) BW loss as compared with normal mice. Pre-treatment with the XLY and in situ nasal gel (2 µg/kg, i.n.) did not have any effect on BW as compared with AR control mice. The effect of treatments on relative weights of lungs (LW/BW ratio) is presented in Fig. 2b with $f(8, 45) = 4.11$, $p < 0.001$ (one-way ANOVA). The effect of treatments on relative spleen weight (SW/BW ratio) is presented in Fig. 2c with $f(8, 45) = 4.82$, $p < 0.001$ (one-way ANOVA). AR control mice showed statistically significant increase ($p < 0.001$) in relative weights of lungs and spleen as compared with normal mice indicating organ (lung and spleen) enlargements. XLY treatment caused statistically significant ($p < 0.001$) prevention of elevated relative weight of lung and spleen as compared with AR control mice. The elevation in relative weights of lungs was prevented by mice pretreated with in situ nasal gel (2 µg/kg, i.n.) in statistically significant ($p < 0.05$) manner, whereas nasal drop (2 µg/kg, i.n.) pretreatment showed statistically significant ($p < 0.01$) prevention of relative weight of spleen as compared with AR control mice.

Histological observations

Photomicrographs of representative sections of nasal mucosa from groups of mice are presented as Fig. 2. The section of nasal mucosa of AR control mice showed severe and aggravated infiltration of eosinophils (grade 3) into the nasal mucosa (Fig. 2b) as compared with section of normal mice (Fig. 2b) with no infiltration (grade 0). The plenty of

Table 3 Effect of nasal formulations on hematological alterations in OVA-induced sensitized mice

Parameter	Normal	AR control	XLY	Nasal drop (2 µg/kg i.n)	<i>In-situ</i> nasal gel (2 µg/kg i.n)	Statistics at d.f. (8, 36)
Hb (%)	10.00 ± 0.71	7.50 ± 1.16	6.90 ± 0.57	6.76 ± 1.20	9.60 ± 0.64***	F = 6.89, <i>p</i> < 0.001
RBC (X 106/mm ³)	7.97 ± 0.35	5.52 ± 0.34	5.02 ± 0.52	4.50 ± 0.81	6.24 ± 0.50*	F = 4.65, <i>p</i> < 0.01
PMN (%)	41.80 ± 5.10	36.40 ± 4.13#	25.20 ± 3.10	32.00 ± 4.43	47.20 ± 3.55	F = 6.50, <i>p</i> < 0.001
Lymphocytes (%)	41.44 ± 5.14	52.80 ± 4.26##	65.20 ± 3.13	69.00 ± 4.90	50.20 ± 3.33	F = 7.16, <i>p</i> < 0.001
Eosinophils (%)	3.96 ± 0.46	2.6 ± 0.97###	1.5 ± 0.30	1.92 ± 0.67	4.0 ± 0.81*	F = 4.09, <i>p</i> < 0.01
MCHC (%)	31.57 ± 1.48	32.34 ± 1.13	33.68 ± 1.18**	34.41 ± 1.27	31.13 ± 0.46	F = 3.35, <i>p</i> < 0.01
PCV (%)	28.06 ± 1.18	19.18 ± 2.14	25.62 ± 2.84	22.00 ± 3.70	33.06 ± 2.71**	F = 3.47, <i>p</i> < 0.01
MCV (fl/cell)	43.89 ± 1.37	41.46 ± 0.35	41.13 ± 0.91	41.40 ± 1.06	43.01 ± 0.41***	F = 2.04, <i>p</i> < 0.01
Monocytes (%)	2.00 ± 0.51	3.00 ± 0.64	2.80 ± 0.34	1.63 ± 0.88	1.60 ± 0.87	F = 0.76, ns
PLT (X 105/mm ³)	1.92 ± 0.29	4.91 ± 0.71	3.91 ± 0.61	3.89 ± 0.51	5.11 ± 0.16	F = 2.43, <i>p</i> < 0.05
MPV (FL)	5.18 ± 0.13	6.32 ± 0.53	6.42 ± 0.64	5.18 ± 0.74	6.45 ± 0.39	F = 8.77, <i>p</i> < 0.001

Numbers in bracket indicate intranasal dose in microgram per kilogram. Data are expressed as mean ± SEM (n = 10) and one-way analysis of variance followed by Dunnett's multiple comparison test

d.f. degrees of freedom, Hb haemoglobin, RBC red blood cells, PCV packed cell volume, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, RDW red blood cell distribution width, TLC, total leukocyte count, WBC white blood cells, PMN polymorphonuclear leukocytes, PLT platelet, MPV mean platelet volume

**p* < 0.05 v/s AR control

***p* < 0.01n v/s AR control

****p* < 0.001 v/s AR control

#*p* < 0.05 v/s Normal

##*p* < 0.01 v/s Normal

p < 0.001 v/s Normal

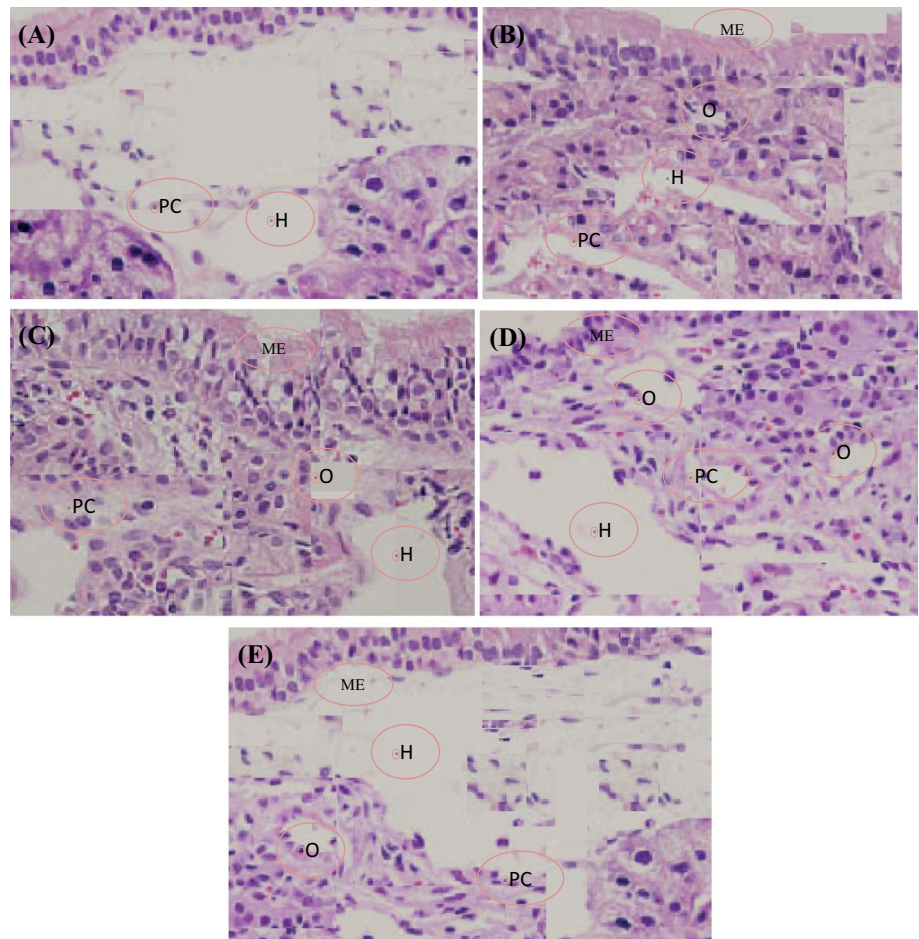
neutrophils exudates was seen in lumen, and the submucosa of AR control mice showed hyper-plasia of mucous secreting glands. Nasal mucosa of AR control mice also showed little ulceration. Section of nasal mucosa from the XLY-treated mice showed the normal basement membrane with mild infiltration of inflammatory (grade 1) and plasma cells (Fig. 2c). Section of nasal mucosa of nasal drop (c showed the presence of the inflammatory cells (grade 2) and ulceration in the mucosal membrane (Fig. 2d). In situ nasal gel nasal drop (2 µg/kg, i.n.) pretreated mice showed prevention of inflammatory cells as compared with AR control mice, which showed the presence of the hyperplasia of the mucous secreting glands (Fig. 2e). In situ nasal gel nasal drop (2 µg/kg, i.n.) showed normal basement membrane and reduction in amount of the lymphocytes, plasma cells. Photomicrographs of representative sections of spleen from groups of mice are presented as Fig. 3. The section of spleen from normal mice showed normal architecture of cells (Fig. 3a). Section from AR control mice (Fig. 3b) showed signs of spleen enlargement (hallmark of OVA-induced AR). These sections from spleen from AR control mice showed presence of hyperplasia of lymphoid tissue and follicles, thick fibrous capsule in periphery and large number of hemosiderin leyden macrophages (Fig. 3b). Spleen sections of mice treated with XLY and nasal drop nasal drop (2 µg/kg, i.n.) showed less number of mature lymphocytes (Fig. 3c, d,

respectively). Spleen section from mice treated in situ nasal gel showed far less enlargement of the spleen with far less lymphoid tissue hyperplasia, thinner fibrous capsule and fewer hemosiderin leyden macrophages (Fig. 3e).

Discussion

The array of laboratory animal models and species are used to judge potential treatment choices for AR. However, OVA-induced AR was found to be most reliable and reproducible laboratory animal model for allergic airway diseases. Inhalation is that the most typical route of substance entry inflicting several hypersensitivities like coryza (hay fever) and rubor. Therefore, in present study, we have a tendency to evoked AR in Swiss mice by sensitization followed by i.n. challenge with OVA. *In-situ* nasal gel nasal drop (2 µg/kg, i.n.) however, not nasal drop nasal drop at (2 µg/kg, i.n.) showed important interference of allergy-related changes in nasal signs, organic chemistry and microscopic anatomy as compared with AR management mice throughout gift study. The topical challenge with OVA is according to induce signs and symptoms almost like human AR (Shii et al. 2010). In our study, OVA challenge resulted in an increase in nasal signs (rubbing, sneezing, discharge and redness) in AR management cluster, that square measure in line according to the

Fig. 2 Photomicrograph of representative sections of nasal mucosa of OVA-induced sensitized mice. **a** Normal, **b** AR control, **c** nasal drop group (XLY), **d** nasal in situ gel, **e** Xylometazoline. Infiltration of eosinophils (E), hyperplastic mucous secreting glands (H), disturbance of mucosal epithelium (ME), edema (O) and plasma cells (PC). Sections stained with hematoxylin and eosin. Images (100× and respective inset 400× magnification)



characteristic signs of AR in Swiss mice (Wang et al. 2007). However, nasal pretreatment of in situ nasal gel showed important interference of OVA-evoked nasal signs except for redness. The inhibition of OVA-induced nasal signs showed by in situ nasal gel indicates inhibition of the discharge of mediators like amine or 5-HT by in situ nasal gel.

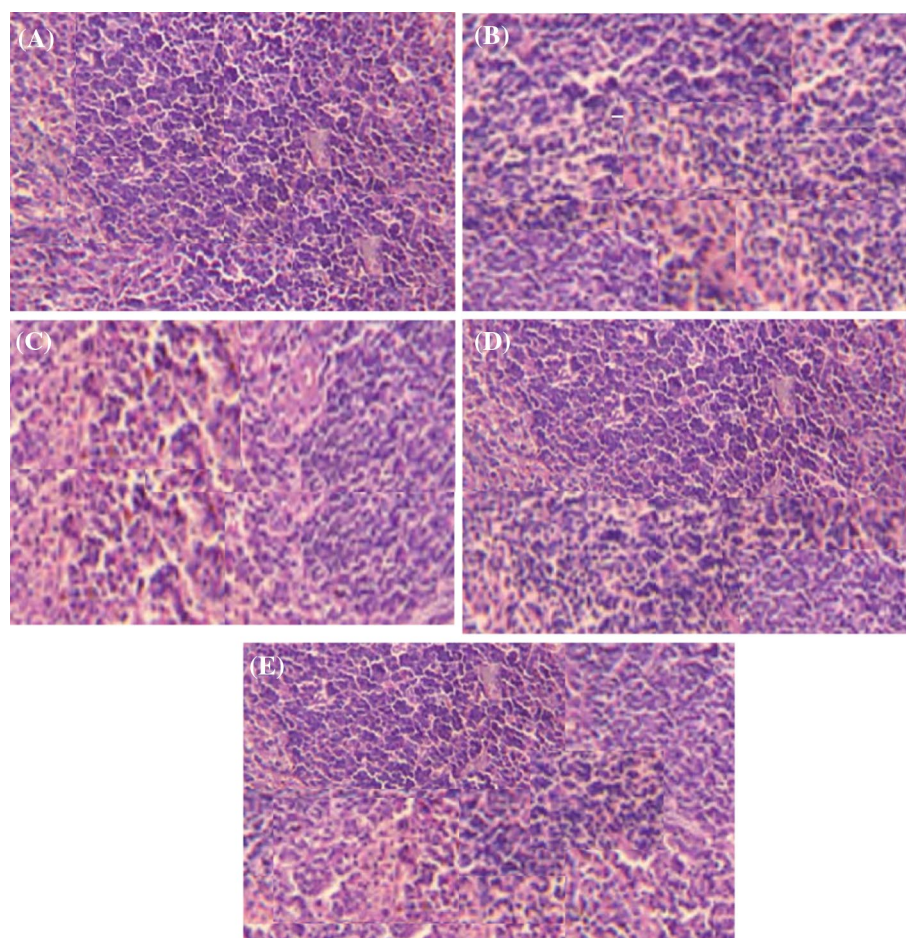
A hypersensitivity reaction is kind I hypersensitivity hypersensitive reaction mediated via activation of mast cells by Associate in Nursing substance and the subsequent unleash of amine. Elevated levels of amine caused an increase in vascular porosity, contraction of a sleek muscle, redoubled secretion and dropsy formation. Further, elevated amine levels once the exposure to the allergens within the AR patients square measure proverbial (Meltzer 2013). Therefore, we've investigated the effects of in situ nasal gel against amine challenge in OVA-induced supersensitized mice. In our study, amine challenge was found to induce innate reflex and rubbing reflex in AR management mice as compared with traditional mice. Treatment with in situ nasal gel considerably reduced the histamine-induced innate reflex and rubbing. Furthermore, within the gift study, in situ nasal gel conjointly showed dose-dependent interference in body fluid amine levels in substance challenged mice (Table 2).

Moreover, the thermoreversible nasal gel from different plant-based natural sources like apple and grapefruit extract was shown to possess somatic cell stabilization potential (Nakano et al. 2008). Therefore, the role of amine and/or somatic cell degranulation within the anti-allergic action of an in situ nasal gel is envisaged.

Intranasal sensitization and challenge with the macromolecule OVA involve IgE-mediated allergic airway unwellness within the higher and lower tract (Bahekar et al. 2008). In our study, AR management mice showed the sturdy increase in immune serum globulin levels. In-situ nasal gel showed dose-dependent interference of OVA-induced immune serum globulin increase as compared with AR management mice. These results indicated somatic cell stabilization and later interference of immune serum globulin and/or amine unleash as the primary mode of action for in situ nasal gel against OVA-induced AR.

The given study, AR management mice showed a steady increase in body fluid radical levels. NO is another vital intercessor in allergic inflammatory reactions like AR (Ciprandi et al. 2011). NO causes activation of G proteins couple receptor will increase nuclear factor- κ B (NF- κ B) concentration and stimulates protein production like

Fig. 3 Photomicrograph of representative sections of spleen of OVA-induced sensitized mice. **a** Normal, **b** AR control, **c** Xylometazoline (XLY), **d** Nasal in situ gel, **e** nasal drop group. Sections stained with hematoxylin and eosin. Images (100× and respective inset 400× magnification)



TNF- α in the nasal membrane (Alving and Malinowski 2010). Moreover, NO causes the white blood cell accumulation (Hebestreit et al. 1998) and enhances the chemotactic activity of eosinophils (Zanardo et al. 1997). However, nasal formulations pretreatment failed to forestall OVA-induced elevated body fluid radical, suggesting the absence of the role of NO or NF- κ B within the mechanism of action of TAPP-CZ. However, the recent study on *C. zeylanicum* bark extract according to the inhibition of unhealthy protein, TNF α , throughout in vitro and in vivo studies (Joshi et al. 2001) indicating the downstream effects of nasal formulations on TNF α inhibition while not moving NO. This has a look at is the first to document the efficacy of KOB03 in-mast mobile-mediated allergic responses. In well known, natural drug treatments possess many components with different biological houses (Jung et al. 2012). KOB03 is a decoction of five herbs with distinct pharmacological movements, and the principle compound, baicalin, has been identified by way of HPLC evaluation with a preferred compound. Baicalin, a flavonoid extracted from various herbal plants, is stated to have anti-allergic residences in atopic dermatitis (Yun et al. 2010) type IV hypersensitive reaction IgE-mediated

allergy (Kimata et al. 2000) and anaphylaxis. Eosinophils are known to manage nearby resistant and fiery reactions, and their aggregation in the blood and tissue is related to a few incendiary (Fulkerson and Rothenberg 2013). Eosinophils demonstrate cytotoxic activity in light of cationic proteins and eosinophil peroxidase (Tsunematsu et al. 2007). In the present examination, OVA challenge instigated soak increment in the number of eosinophils in the blood of AR control mice. Pre-treatment nasal formulations indicated gentle yet noteworthy counteractive action of eosinophils as contrasted and AR control mice. The histology of area of the nasal mucosa of AR control and nasal formulations treated rats additionally underpins these discoveries. Furthermore, the noteworthy increment was seen in blood parameters, for example, RBC includes, Hb, PCV and MCV nasal formulations regarded mice as contrasted and ordinary mice. These outcomes propose restraint of enlistment of eosinophils invasion at the nasal mucosa as a likely instrument of in situ nasal against OVA-incited AR in mice. Our outcomes are in accordance with prior reports of polymorphonuclear leukocyte (eosinophils and PMN) penetration restraint by numerous common polyphenols (Gonzalez-Segovia et al. 2008; Srivastava et al. 2010).

Conclusion

The in situ nasal gel formulation was demonstrated to be safe and effective in relieving the symptoms of allergic rhinitis as compared to nasal drop of the polyherbal extracts. The in situ nasal gel effects may be prolonged as some benefits could still be demonstrated at allergic rhinitis mice model. Its mode of action was noted to be associated with reduction in serum IgE. Further studies are required to confirm its efficacy and, to find the optimal dose, and to explain its more safety in a broader aspect.

Acknowledgements All the studies were performed at Amity Institute of Pharmacy, Amity University Lucknow campus Uttar Pradesh. We are highly thankful to the institute for providing us the platform to conduct our studies.

Compliance with ethical standards

Ethical statement This article does not contain any studies with human participants performed by any of the authors. Animal handling and experimental procedures were performed according to the guideline for Care and Use of Animals Laboratory (No: AIP/IAEC/06/2017) was approved by the Institutional Animal Ethics Committee.

Conflict of interest This manuscript described has not been published before; not under consideration for publication anywhere else; and has been approved by all co-authors.

References

- Alving K, Malinovski A (2010) Basic aspects of exhaled nitric oxide. *Exhaled Biomark* 49:1–31
- Bahekar PC, Shah JH, Ayer UB, Mandhane SN, Thennati R (2008) Validation of guinea pig model of allergic rhinitis by oral and topical drugs. *Int Immunopharmacol* 8:1540–1551
- Bielory L, Lupoli K (1999) Herbal interventions in asthma and allergy. *J Asthma* 36:1–65
- Church MK, Levi-Schaffer F (1997) The human mast cell. *J Allergy Clin Immunol* 99:155–160
- Ciprandi G, Signori A, Cirillo I (2011) Relationship between bronchial hyperreactivity and bronchodilation in patients with allergic rhinitis. *Ann Allergy Asthma Immunol* 106(6):460–466
- Fulkerson PC, Rothenberg ME (2013) Targeting eosinophils in allergy, inflammation and beyond. *Nat Rev Drug Discov* 12:117–129
- Gonzalez-Segovia R, Quintanar JL, Salinas E, Ceballos-Salazar R, Aviles-Jimenez F, Torres-Lopez J (2008) Effect of the flavonoid quercetin on inflammation and lipid peroxidation induced by *Helicobacter pylori* in gastric mucosa of guinea pig. *J Gastroenterol* 43:441–447
- Hebestreit H, Dibbert B, Balatti I et al (1998) Disruption of fas receptor signaling by nitric oxide in eosinophils. *J Exp Med* 187:415–425
- Hu GR, Shen ZY, Shi SZ et al (1983) Effect of the kidney reinforcing regimen on serum IgE and T cell subsets in the treatment of asthma. *J Tradit Chin Med* 5:33–36
- Hu GR, Shen ZY, Xu DS et al (1987) Lymphocyte adrenergic receptor function in asthmatic subjects with the treatment of Wen Yang Pill. *Chun Kuo Chung Hsi I Chieh Ho Tsa Chih* 7:71–74
- Joshi SS, Kuszynski CA, Bagchi D (2001) The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. *Curr Pharm Biotechnol* 2:187–200
- Jung HW, Jung JK, Cho CW, Kang JS, Park YK (2012) Antiallergic effect of KOB03, a polyherbal medicine, on mast cell-mediated allergic responses in ovalbumin-induced allergic rhinitis mouse and human mast cells. *J Ethnopharmacol* 142(3):684–693
- Kimata M, Inagaki N, Nagai H (2000) Effects of luteolin and other flavonoids on IgE-mediated allergic reactions. *Planta Med* 66:25–29
- Leung AK, Hon KL (2008) Seasonal allergic rhinitis. *Recent Pat Inflamm Allergy Drug Discov* 2:175–185
- Meltzer EO (2013) Pharmacotherapeutic strategies for allergic rhinitis: matching treatment to symptoms, disease progression, and associated conditions. *Allergy Asthma Proc* 34:301–311
- Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5:62–71
- Nakano N, Nishiyama C, Tokura T et al (2008) Procyanidin C1 from apple extracts inhibits Fc epsilon RI-mediated mast cell activation. *Int Arch Allergy Immunol* 147:213–221
- Schmolka IR (1972) Artificial skin: preparation and properties of Pluronic F-127 gels for treatment of burns. *J Biomed Mater Res* 6:571–582
- Settipane RA, Schwindt C (2013) Allergic rhinitis. *Am J Rhinol Allergy* 27(3_suppl):S52–S55
- Shen Y, Jia LN, Honma N, Hosono T, Ariga T, Seki T (2012) Beneficial effects of cinnamon on the metabolic syndrome, inflammation, and pain, and mechanisms underlying these effects a review. *J Tradit Complement Med* 2:27–32
- Shii D, Nakagawa S, Yoshimi M, Katsuta O, Oda T, Nakamura M (2010) Inhibitory effects of cyclosporine eye drops on symptoms in late phase and delayed-type reactions in allergic conjunctivitis models. *Biol Pharm Bull* 33:1314–1318
- Srivastava A, Greenspan P, Hartle DK, Hargrove JL, Amarowicz R, Pegg RB (2010) Antioxidant and antiinflammatory activities of polyphenolics from Southeastern U.S. range blackberry cultivars. *J Agric Food Chem* 58:6102–6109
- Tsunematsu M, Yamaji T, Kozutsumi D, Murakami R, Kimura S, Kino K (2007) Establishment of an allergic rhinitis model in mice for the evaluation of nasal symptoms. *Life Sci* 80:1388–1394
- Wang H, Zhang J, Gao C, Zhu Y, Wang C, Zheng W (2007) Topical levamisole hydrochloride therapy attenuates experimental murine allergic rhinitis. *Eur J Pharmacol* 577:162–169
- Yamasaki ML, Sasaki K, Mizutani N et al (2001) Pharmacological characterization of the leukocyte kinetics after intranasal antigen challenge in a guinea pig model of allergic rhinitis. *Inflamm Res* 50:474–482
- Yun MY, Yang JH, Kim DK, Cheong KJ, Song HH, Kim DH, Cheong KJ, Kim YI, Shin SC (2010) Therapeutic effects of Baicalein on atopic dermatitis-like skin lesions of NC/Nga mice induced by *dermatophagoides pteronyssinus*. *Int Immunopharmacol* 10:1142–1148
- Zanardo RC, Costa E, Ferreira HH et al (1997) Pharmacological and immunohistochemical evidence for a functional nitric oxide synthase system in rat peritoneal eosinophils. *Proc Natl Acad Sci USA* 94:14111–14114
- Ziment I, Tashkin DP (2000) Alternative medicine for allergy and asthma. *J Allergy Clin Immunol* 106(4):603–614