

## NOTES

### Suppressive effect of *Strychnos nux-vomica* on induction of ovalbumin-specific IgE antibody response in mice<sup>#</sup>

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*Strychnos nux-vomica* Linn. (SNV; Loganiaceae), a medicinal plant has been used as folk medicine for alleviating inflammation, joint pains and allergic symptoms. In the present study, we examined its possible immunomodulatory effect on induction of ovalbumin (OVA)-specific IgE antibody response in a murine model, as evaluated by passive cutaneous anaphylaxis (PCA). The OVA-specific IgE antibody response was significantly suppressed in BALB/c mice (H-2<sup>d</sup>), following intraperitoneal administration of aqueous stem extract of the plant along with OVA. Furthermore, the different doses of SNV extract were found to significantly suppress the induction of OVA-specific IgE antibody response. The anti-OVA IgE antibody response was suppressed in different haplotypes of mice viz., C57BL/6 (H-2<sup>b</sup>) and SWR/J (H-2<sup>k</sup>). However, preliminary findings revealed no significant change in the total IgG antibody response against OVA, as evaluated by ELISA. These results confirm the suppressive activity of *S. nux-vomica* on allergen-specific IgE antibody response and suggest its possible application in allergic conditions.

**Keywords:** *Strychnos nux-vomica*, Immunomodulation, Immunosuppression, IgE antibody response, Passive cutaneous anaphylaxis, ELISA

Allergic diseases are among the major diseases of the world and it is estimated that 22% of world population suffer from some form of allergic disease<sup>1</sup>, and their incidence being alarmingly increased in developing countries<sup>2,3</sup>. Four types of hypersensitivity reactions, types I-IV based on the underlying immunologic mechanism causing inflammatory reactions and tissue damage have been reported<sup>4</sup>. Type I constitute major class of allergic reactions

mediated by IgE antibodies that include asthma<sup>5</sup>, allergic rhinitis<sup>6</sup>, atopic dermatitis<sup>7</sup> and anaphylactic shock<sup>8</sup>. The pathological mechanism of IgE antibody-mediated allergy involves degranulation of mast cells, resulting in the release of chemical mediators such as histamine, leucotrienes and prostaglandins upon interaction of IgE antibody with Fcε receptor on mast cells<sup>9</sup>.

Several plants have been screened for anti-allergic properties. The extract of *Miscanthus sinensis* (Poaceae) inhibits IgE antibody production in mice against ovalbumin (OVA) as measured by passive cutaneous anaphylaxis (PCA)<sup>10</sup>. The aqueous extract of *Angelica polymorpha* (Umbelliferae) inhibits the DNP-OVA specific IgE antibody response in mice<sup>11</sup>. The alkaloid derivatives of *Chelidonium majus* Linn. (Papaveraceae) inhibit the anti-OVA IgE antibody response in mice<sup>12</sup>. Quillaja saponin from *Quillaja saponaria* Molina (Rosaceae) suppresses the anti-OVA IgE and IgG antibody responses<sup>13</sup>. The aqueous extract of *Withania somnifera* Dunal (Solanaceae) also downregulates the OVA-specific IgE antibody response, as evaluated by PCA<sup>14</sup>.

The aqueous stem extract of *Strychnos nux-vomica* (SNV, Loganiaceae) has been used by tribes of Visakhapatnam district for anti-rheumatic and antiallergic effects. Moreover, it has shown analgesic and anti-inflammatory activity in a rat model<sup>15</sup>. Thus, in the present study, the possible immunomodulatory activity on the murine humoral antibody response has been investigated by injecting stem extract of SNV intraperitoneally into mice along with OVA. The extract has been found to exhibit significant inhibitory activity on the induction of allergen-specific IgE antibody response, as evaluated by PCA.

### Materials and methods

#### Materials

Turkey egg albumin (OVA), Evans blue, trypan blue, tween-20, ortho-phenylene diamine (OPD), hydrogen peroxide, goat anti-mouse IgG conjugated to horseradish peroxidase were obtained from Sigma Chemical Co., St. Louis, USA. 96-well microtiter flat bottom ELISA plates were employed for the determination of humoral responses. All other general chemicals used were of analytical grade.

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### Animals

Eight-weeks old female inbred BALB/c (H-2<sup>d</sup>), C57BL/6 (H-2<sup>b</sup>) and SWR/J (H-2<sup>q</sup>) mice weighing 20-30 g and Wistar male rats weighing about 200-300 g were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India, for the induction of allergen-specific immune response and conducting the passive cutaneous anaphylaxis (PCA). The animals were housed under standard conditions and fed the pelleted experimental diet supplied by Rayans Biotech, Hyderabad, India and water *ad libitum*.

### Preparation of stem extract

Twigs of *S. nux-vomica* (SNV) tree were collected from Visakhapatnam district of Andhra Pradesh, India. The stem extract was prepared by slicing the washed young stems and the pieces were soaked in PBS (0.005 M phosphate, 0.075 M NaCl, pH 7.4) overnight. The soaked extract was centrifuged and the supernatant was separated and the protein concentration was estimated by Lowry's method<sup>16</sup>.

### Immunization and serum collection

Mice (n = 6 mice/group) were immunized intraperitoneally without adjuvant on days 0, 28, and 56 with 10 µg of OVA in PBS. Similarly, test groups of mice (n = 6 mice/group) were injected intraperitoneally with 10 µg of OVA along with 100 µg of SNV extract in PBS. Mice were bled from the tail vein on day 14 after primary immunization and day 7 after secondary and tertiary immunizations. The serum was analyzed for the determination of allergen-specific IgE antibody responses by PCA.

### Determination of OVA-specific IgE and IgG antibody response

The allergen-specific IgE antibody response elicited in mice was measured by the PCA on Wistar rats<sup>17</sup>. Briefly, antisera of mice were passively transferred intradermally on to the shaved dorsal skin of male rats in aliquots of 50 µl each in 2- or 4-fold serial dilutions. After 24 h, the animals were challenged intravenously through penal vein with 1.0 mg OVA in 1.0 ml PBS containing 0.5% Evans blue as a dye. The appearance of blue spots measuring greater than 5 mm was taken as a positive response and the reciprocal of highest dilution of antisera that gave blue spots was considered as an IgE antibody titer.

For determination of OVA-specific IgG antibody response, total IgG levels in antisera from both control and test groups of mice were assayed by ELISA<sup>17,18</sup>. Briefly, the 96-well microtiter plates were coated with 100 µl of OVA at a concentration of 100 ng/ml in 50 mM carbonate buffer (pH 9.6) and incubated overnight at 4°C. After incubation, the wells were washed thrice with PBS containing 0.05% tween-20 (PBS-T). The free binding sites in the wells were blocked with 300 µl of 3% skimmed milk powder in PBS for 10-12 h at room temperature. After washing the plates, the wells were further incubated with 100 µl of diluted sera (1:400 in PBS) in triplicates for 1 h at 37°C. The unbound serum constituents were washed off and the levels of total IgG were measured by incubating with 100 µl of goat anti-mouse IgG conjugated to horseradish peroxidase at a dilution of 1:1000 for 1 h at 37°C. Finally, the unbound conjugates were washed with PBS-T and 100 µl of freshly prepared substrate solution (10 ml of 100 mM citrate-phosphate buffer, pH 5.0 containing 4.0 mg of OPD and 10 µl H<sub>2</sub>O<sub>2</sub>) were added. The reaction was stopped after 5 min by adding 50 µl of 8 N H<sub>2</sub>SO<sub>4</sub>. The color developed was read at 492 nm using automatic ELISA reader.

### Results and Discussion

The adjuvant independent OVA-specific immune response was induced in BALB/c mice by injecting OVA alone and OVA along with the aqueous stem extract of SNV. As seen in Fig. 1, the intraperitoneal administration of aqueous extract

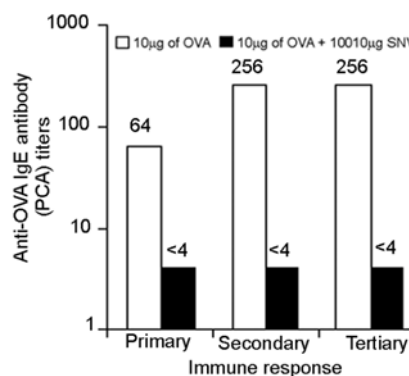


Fig. 1—Effect of SNV stem extract on induction of primary, secondary and tertiary allergen-specific IgE antibody response [Different groups of BALB/c mice were injected with 10 µg of OVA or 10 µg of OVA plus 100 µg of SNV on days 0, 28 and 56. Antisera collected on days 14, 35 and 63 were evaluated for OVA-specific IgE antibody response by PCA. Values represent mean ± SD of three experiments]

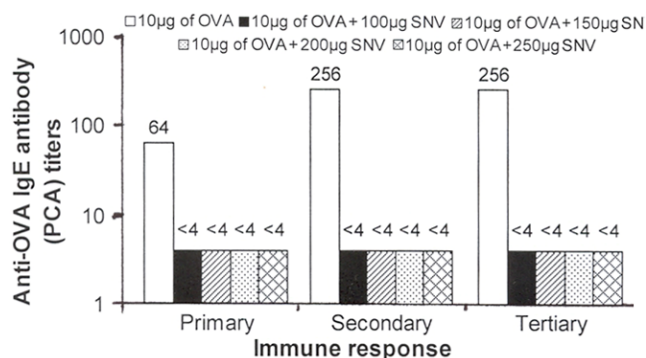


Fig. 2—Effect of SNV stem extract on OVA-specific IgE antibody response [Different groups of BALB/c mice were injected with 10 µg of OVA or 10 µg OVA plus different doses of SNV (100, 150, 200, 250 10 µg) on days 0, 28 and 56. Antisera were collected on days 14, 35, 63 and OVA-specific IgE antibody response was measured by PCA. Values represent mean ± SD of three experiments]

suppressed (PCA titers less than 4) the induction of OVA-specific primary, secondary and tertiary IgE antibody responses, as evaluated by PCA. The OVA-specific IgE antibody response was significantly inhibited with 100, 150, 200 and 250 µg of extract (in terms of protein concentration), confirming the suppressive activity of the SNV (Fig. 2). Furthermore, down-regulation of IgE antibody response against OVA was observed in different haplotypes of mice (H-2<sup>d</sup>, H-2<sup>b</sup>, H-2<sup>q</sup>), implying the suppressive activity of SNV was independent of genetic differences (Fig. 3).

Cyclosporine A, isolated from a fungus *Tolypocladium inflatum* Gams at higher doses is reported to decrease all the isotypes against OVA<sup>19</sup> and the extract of mycelium fungus *Polyporus squamosus* (Polyporaceae) inhibits both IgE and IgG antibody response to OVA<sup>20</sup>. Unlike these agents, SNV was found to suppress the OVA-specific IgE antibody response but not OVA-specific total IgG antibody response (Fig. 3). Oral administration of *Hochu-ekki-to*, a traditional Chinese herbal medicine also suppresses IgE antibody response without influencing IgG<sub>1</sub>, and IgG<sub>2a</sub><sup>21</sup>. Dexamethasone on oral administration also selectively inhibits the anti-OVA IgE and IgA, but not IgG (or) IgG1 levels<sup>19</sup>. The above results of suppressing the IgE antibody response by SNV suggest the presence of antiallergic principle in the plant.

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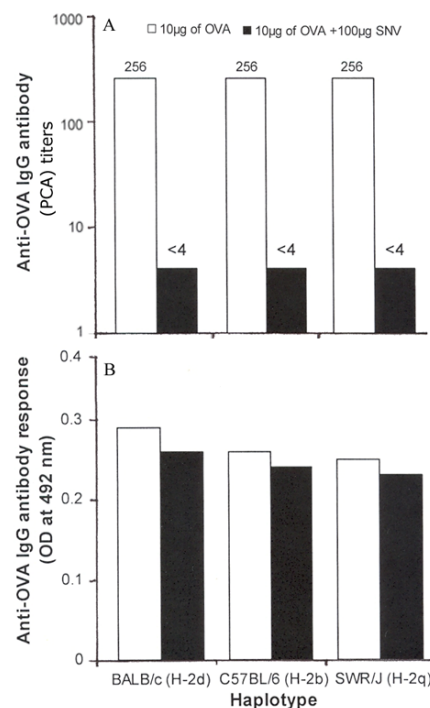


Fig. 3—Effect of SNV stem extract on induction of OVA-specific IgE antibody response in different haplotypes of mice [Different haplotypes of mice viz. H-2<sup>d</sup>, H-2<sup>b</sup>, H-2<sup>q</sup> were injected with 10 µg of OVA or 10 µg of OVA plus 100 µg of SNV on days 0 and 28. Antisera collected on day 35 were evaluated for OVA-specific secondary IgE antibody response by PCA (A) and IgG antibody response by ELISA (B). Values represent mean ± SD of three experiments]

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#### References

- Warner J O, Kaliner M A, Crisci C D, Giacco D S, Frew A J, Liu G H, Maspero J, Moon H B, Nakagawa T, Potter P C, Rosenwasser L J, Singh A B, Valovirta E & van Cauwenberge P (2006) *Int Arch Allergy Immunol* 139, 166-174
- Law M, Morris M K, Wald N, Luczynska C & Burney P (2005) *Bmj* 330, 1187-1188
- Von Hertzen L C & Haahtela T (2004) *Allergy* 59, 124-137
- Goldsbey R A, Kindt T K & Osborne B A (2003) *Hypersensitive reactions*. In: *Kuby Immunology*, 5<sup>th</sup> edn, pp 361-388, W H Freeman & Company, New York
- Goldstein R A, Paul W E, Metcalfe D D, Busse W W & Reece E R (1994) *Ann Inter Med* 121, 698-708
- Nathan R A, Meltzer E O, Selner J C & Storms W (1997) *J Allergy Clin Immunol* 99, S808-S814
- Hanifin J & Rajka G (1980) *Acta Dermatol Venereol* 92, 44-47
- Bochner B S & Lichtenstein L M (1991) *N Eng J Med* 324, 1785-1790
- Metcalfe D D, Baram D & Mekory Y A (1997) *Physiol Rev* 77, 1033-1079

- 10 Xu H X, Kadota S, Hattori M, Takahashi T, Kojima Y & Namba T (1993) *Planta Med* 59, 529-532
- 11 Sung C P, Baker A P, Holden D A, Smith W J & Chakrin L W (1982) *J Nat Prod* 45, 398-406
- 12 Wyczolkowska J, Michon T & Nowicky J W (1996) *Drugs Exp Clin Res* 22, 195-200
- 13 Katayama S & Mine Y (2006) *J Agric Food Chem* 54, 3271-3276
- 14 Srinivasulu A, Kumar S P & Rao A R (1999) *Pharm Biol* 37, 253-259
- 15 Yin W, Wang T S, Yin F Z & Cai B C (2003) *J Ethnopharmacol* 88, 205-214
- 16 Lowry O H, Rosenbrough N J, Farr A L & Randall R J (1951) *J Biol Chem* 193, 256-275
- 17 Duddukuri G R, Rao Y V, Rao D N & Rao A R (2001) *Indian J Clin Biochem* 16, 89-94
- 18 Vasudeva R Y, Duddukuri G R, Sunil G B & Rao A R (2002) *Pharm Biol* 40, 175-178
- 19 Puignero V, Salgado J & Queralt J (1995) *Int Arch Allergy Immunol* 108, 142-147
- 20 Babakhin A A, Majoul L A, Vedernikov A A, Leskov V P, Pisarev V M, Babakhin A A, Logina NYu, Gushchin I S, Nolte H & DuBuske L M (1997) *Allergy Asthma Proc* 18, 301-310
- 21 Suzuki T, Takano I, Nagai F, Fujitani T, Ushiyama K, Okubo T, Seto T, Ikeda S & Kano I (1999) *Biol Pharm Bull* 22, 1180-1184