Effect of sodium glycocholate on development of tolerance to
Parthenium hysterophorus extracts

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Sodium glycocholate, a penetration enhancer showed significant enhancement of IgG levels in Balb/c mice when administered intranasally with P. hysterophorus pollen extract.

Parthenium pollen containing parthenin, a major sesquiterpene lactone is primarily responsible for allergic contact dermatitis. It is the higher molecular weight proteins of the pollen, which are responsible for allergic rhinitis in Parthenium sensitive patients. The intranasal route has been extensively studied for immunotherapy in patients suffering from allergic rhinitis to Ambrosia artemisiifolia and Parietaria judaica. This route being a non-invasive route with high permeability and lower enzymatic barrier has attracted the delivery of many polypeptide drugs like oxytocin, desmopressin, and calcitonin etc. But it has not been as successful with larger peptides with molecular weights greater than 10 kDa (ref. 7). It was during these studies that the use of penetration enhancers to enhance the intranasal delivery of polypeptides was suggested.

The penetration enhancer effect of sodium glycocholate for peptide hormones given through the intranasal route is reported. Since the major allergens in the Parthenium pollen extracts are in the range of 14kDa-64kDa, it was thought of to study sodium glycocholate for any possible effect in enhancing the intranasal tolerance to Parthenium pollen.

Materials — Tween 20, hydrogen peroxide (Glaxo Laboratories India Ltd., Bombay, India); O-phenylenediamine (Aldrich Laboratories, WI, USA); mouse IgG, Rabbit anti-mouse IgG, bovine serum albumin, sodium dodecyl sulphate, sodium bicarbonate, sodium glycocholate (Sigma Chemical Co., St Louis, USA); Avidin-horse radish peroxidase conjugate (Vector Laboratories Inc, Burlingame, CA, USA); 0.22 µM Millipore filter (Millipore Corp, Bedford, MA, USA); polystyrene microtiter plates (96-well, flat bottom) with lid stand sterile, 96-well tissue culture plates (Coster, Cambridge, MA, USA); Coomassie-brilliant blue G-250 (Biorad Laboratories, Hercules, CA, USA) were used.

Animals — Inbred BALB/c mice of 6-8 week of age were purchased from National Institute of Nutrition Animal Facility, Hyderabad, India. Mice were maintained in pathogen-free conditions under 12/12 hr L: D cycle and were given food and water ad libitum.

Antigens — Parthenium pollen (1g) was defatted prior to extraction using 3 successive portions of 20 ml ether. It was then extracted overnight in 50 ml PBS (pH 7.2) by end to end mixing at 4°C. The extract was spun down at 10,000 rpm for 5 min and the supernatant was dialyzed against PBS. The dialyzed extract was filtered through 0.45 µm Millipore filter and used for immunization.

SDS-PAGE profile — Resolution of proteins was achieved on a 12.5% polyacrylamide gradient gel and silver staining was carried out to visualize the separated proteins.

Total protein — Total protein content was measured using the Bradford's dye-binding method using bovine serum albumin as standard.
skin prick test (SPT) — The test was carried out to ascertain the potency of the allergic extract compared to a histamine reaction in patients sensitive to *P. hysterophorus*. It was carried out on the volar surface of the forearm of patients suffering from allergic rhinitis as well as control subjects. A 50% glycerol solution in PBS (pH 7.4) and histamine hydrochloride (1mg/ml) dissolved in PBS-Glycerol (1:1 v/v) served as negative and positive controls respectively. The reaction was designated as positive if the net wheal diameter with the extract was greater than 3 mm than the negative control.

The following grading schedule was followed:

1+: wheal between 1-3 mm larger in diameter than PBS control;
2+: wheal between 3-5 mm larger in diameter than PBS control;
3+: wheal between 5-7 mm larger in diameter than PBS control; and
4+: wheal over 7 mm diameters larger than the control.

Immunization — The BALB/c mice were grouped into active group and control group containing 5 mice each. The active group was administered with fresh extract of *P. hysterophorus* containing 1% sodium glycocholate and the control group received only *P. hysterophorus* (1:50 w/v) extract. A total of 20μl of the extract was instilled into both the nostrils of the mice with a micropipette. The mice were immunized on every alternative day (9 times) over a period of 25 days. On the 25th day, the mice were bled and antigen specific serum IgG was measured using the Avidin-Biotin micro ELISA technique. The dilutions of serum used (1:1600, 1:3200, 1:6400, 1:12800) in the estimations were chosen from previous studies.

Statistical analysis — The results are expressed as mean ± SE. The significance of the data was evaluated by Student's *t*-test. *P* < 0.01 was considered to indicate statistical significance.

The SDS-PAGE profile of the extract showed proteins of molecular weight 14, 21, 31, 45 and 67 kDa (Fig. 1). The total protein content as estimated using the Bradford's dye-binding method was found to be 0.35 μg/μl of the (1:50 w/v) extract of *Parthenium hysterophorus*. The potency as ascertained by the SPT indicated a reaction of Grade +2 when compared to a positive control of histamine hydrochloride in *Parthenium* sensitive patients. The production of antigen specific IgG through intranasal immunization to *Parthenium* pollen antigens was significantly enhanced (*P* < 0.01) on addition of 1% sodium glycocholate (Fig. 2).

![Fig. 1 — SDS-PAGE analysis of extracts of *P. hysterophorus*](image)

![Fig. 2 — Serum IgG levels in control and active group at different dilutions.](image)
The intranasal route offers great promise for delivery of antigens because of its high permeability and low enzymatic barrier. Being a non-invasive route, it also increases patient compliance, which has been a consistent problem with immunotherapy (IT) given through parenteral route. Since *Parthenium* pollen is an inhalant allergen, its administration through the target organ i.e. nose reflects the route of sensitization and allergic immune responses in humans.

Although the mechanisms of action of allergen specific IT are not well defined, many studies suggest the development of IgG “blocking” antibodies and a suppression of IgE antibody production to be responsible for clinical efficacy of the therapy\(^4\)\(^5\). It is on this basis that we estimated IgG antibody levels to study the effect of sodium glycocholate on the development of intranasal tolerance to *Parthenium*.

Immunity to small pox, scarlet fever and diphtheria has been produced by merely a nasal application of the respective toxins. Hormones like progesterone, testosterone, estradiol etc also have achieved a rapid and complete absorption through this route\(^16\). But it has not been as successful for proteins with molecular weights greater than 10 kDa. However the intranasal bioavailability of such peptides can be enhanced using penetration enhancers like sodium glycocholate along with parenteral route. Since 

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cholate, which may be responsible for the enhance-
ment of IgG levels as seen in the present studies.
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References