In the present study active allergenic ingredients were separated from Prosopis juliflora pollen grains. It was found that Prosopis juliflora pollen allergens were rapidly diffused from pollen grains during extraction in physiological saline. Efforts were made to examine the different chemical constituents of Pj crude extract. Major chemical constituents of crude Pj extract was protein. The amount of Carbohydrate and Nitrogen was one fourth and one sixth of total protein content, respectively. Phosphate, sulfhydryl group and sialic acid were also assayed in crude extract to understand the chemical nature of the allergens.
Separation of the allergenic activity in Prosopis juliflora pollen into a highly active fraction and less active fraction was performed by gel filtration. In gel filtration, six molecular weight proteins were isolated whose molecular weights varied between daltons 81,000 to 13,000. Thus, it suggests that Pj pollen extract contains six molecular weight proteins.

Polyacrylamide gel electrophoresis of Pj pollen crude extract was performed and six bands were obtained. Further PAGE of pooled gel fractions gave single band. Thus, it confirms the gel filtration finding that Pj pollen extract contains six fractions and it also suggests the purity of each fraction.

SDS polyacrylamide disc gel electrophoresis of crude Pj extract gave six bands and molecular weight was similar to the molecular weight of different fractions of gel filtration. Thus, it further confirms the finding of gel filtration. Each pooled fraction was again used for SDS-PAGE and single band was obtained. Thus, it suggests that each molecular weight protein was monomeric and homogenous.
Nine peaks were obtained in DEAE cellulose ion exchange chromatography, by stepwise increase of NaCl molarity (0.05 to 0.6 M). Thus DEAE cellulose chromatography further purify the allergenic components of Pj pollen extract which is evident from the separation of extra three fractions. Extra fractions might be due to separation of allergenic components from non-allergenic components.

Both in vivo and in vitro tests were performed for quantitative and qualitative test of allergenicity of different allergens and crude extract. A quantitative skin test was performed in the present study to test the allergenicity of crude extract in sensitized guinea pigs. The wheal-flare (immediate response) and erythema-redness (late response) were observed from intradermal skin test by using different doses on different sensitizing days. It was observed that 50 μg/ml was the optimum sensitizing dose and maximum sensitizing days was 15 days when maximum response was observed after giving challenging dose. Whereas, in 7 days sensitized guinea pigs 25 μg/ml pollen extract gave maximum early and late response. Reason of this dose shifting in guinea pigs sensitized for period from 50 μg/ml to
25 µg/ml remains to be studied. However, it is suggested that this dose shifting could be due to elicitation of reaginic antibodies and suppression of reaginic antibodies through a feedback mechanism or suppressor T cells. This skin testing was proved very fruitful for measurement of allergenicity and to establish a ratio of allergenicity between allergens in order to arrive at a safe and effective dose regimen.

A dose dependent histamine release was assayed and found 5.0 x 10^{-1} µg/ml and 5.0 x 10^{-2} µg/ml antigen (allergen) released more than 50 per cent histamine. Even 10^{-4} µg/ml of allergen was sufficient to release histamine. Maximum histamine was released when 15 days sensitive guinea pig leukocytes were induced by Pj pollen allergen followed by 45 days and 7 days sensitized guinea pigs. This was also investigated that optimum histamine release was at 25 minutes.

Thus, it suggests that the release of histamine was an active process resulting from stimulation of living cells by specific allergens. Here shifting of histamine release in sensitized guinea pigs for different periods remains to be understood. However, the difference in histamine release at different periods of
sensitization of guinea pigs is thought to be due to different sensitivity of peripheral blood derived leukocytes mediated via homocytotropic antibodies and blocking antibodies or suppressor T cells.

Allergen activity of different fractions was tested by prick test, histamine release test, immuno-diffusion test, immunoelectrophoresis test, Radioallergosorbent test and passive cutaneous anaphylaxis test.

Fractions obtained from gel filtration and ion exchange chromatography were tested by prick test. It was observed that pigmented fraction having molecular weight 20,000 in gel filtration, i.e., fraction E and fraction III, and IV in DEAE cellulose chromatography gave maximum response (wheal formation). Thus, it appears from this result that major allergens is glycoprotein.

In vitro histamine test was also performed to assess the allergenicity of different gel fractions. Results indicated that fraction E released maximum (more than 50 per cent) of histamine when incubated with leukocytes.

In vivo and in vitro immunological tests were performed to test the nature of allergens.
diffusion analyses revealed that antigens reacting with polyspecific anti-Pj serum occurred in all protein fractions obtained by gel filtration. Maximum sharpness and thick band was observed in fraction E, which supported the earlier findings that allergenic antigens are of lower molecular weight, i.e., peak E.

Two dimensional immunoelectrophoresis was performed for the identification and quantitation of allergens. It was found that Pj extract contains at least 7 proteins in which two were major allergens.

Assessment of allergen activity of different fractions was shown by crossed immunoelectrophoresis and two dimensional cross electrophoresis. It was found that fraction E showed strong (sharp) band. Thus, it appears that fraction E may be the main allergens of Pj pollen.

The allergenic activity of crude Pj pollen extract and gel fractions were evaluated by radio-allergosorbent test (RAST).

It was observed that incorporation of radio-labelled $^{125}$I-IgE varied according to the concentrations (0.01 μg to 1 μg) of Pj pollen extract. RAST inhibition test was performed to verify the preferential
binding of the guinea pigs Pj antiserum neutralized allergens to the paper disc after the incorporation of $^{125}$I-IgE antibodies. The main allergenic fraction E gave maximum RAST inhibition (58%/1 μg). Therefore, present observation suggests that IgE or IgE like antibodies seem to be responsible for Pj antigen induced allergy in guinea pigs.

In vivo passive cutaneous anaphylaxis (PCA) elicitation and inhibition were performed to know the chemical nature and the mode of action of the allergens. It was observed that gel fraction allergens from Pj pollen extract inhibited the PCA reaction completely at a concentration of 75 μg/ml. Lowest dose (15 μg/ml) of all the fractions (C, D, B, A and F), except fraction E, did not inhibit PCA reaction whereas lowest dose (15 μg/ml) of fraction E inhibited PCA reaction significantly. Thus, it appears that fraction E is major allergen. Inhibition of PCA and RAST might be mediated by 'haptene' like compounds.