

ANALYSIS OF ALLERGENICITY AND HYPERSENSITIVITY OF *PROSOPIS JULIFLORA* POLLEN GRAINS IN GUINEA PIGS

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ABSTRACT

Allergenic activity of *Prosopis juliflora* pollen has been tested by *in vivo* intracutaneous skin test and by *in vitro* histamine release test. The intracutaneous skin test showed a biphasic effect i.e. early wheal-flare response and late erythema-redness in guinea pig sensitized with various concentrations (100, 50, 25, 5 and 1.5 $\mu\text{g/ml}$) of *P. juliflora* pollen extract for three different periods (7, 15 and 45 days). *In vitro* histamine release assay in *P. juliflora* sensitive guinea pig leukocytes showed dose-dependent histamine release, which was maximum in 15 days of sensitization.

INTRODUCTION

PLANT pollens are the most common causes of seasonal allergic diseases. Allergenicity of hundreds of Plant pollen grains has been verified¹⁻³. *P. juliflora* is one of the most common trees of arid region of tropical and subtropical regions. Its plantation is under active consideration by the Government of India for wood waste energy sources and reclamation of the desert. Pollen grains of *P. juliflora* shrubby trees are known to be allergenic in nature⁴⁻⁶ but the test of allergenicity is limited to skin prick test in sensitized patients⁶. The chemical nature and the active principle of *P. juliflora* pollen and its mechanism of allergenicity are still unknown. Therefore, in this study an attempt is made to study allergenicity of *P. juliflora* pollen grains by direct intracutaneous immediate skin response (wheal-flare) and late skin response (erythema-redness). To understand the mechanism of skin reaction, *in vitro* histamine release at different dose, time and duration has been estimated in sensitized guinea pigs.

MATERIALS AND METHODS

Preparation of crude pollen extract: Active allergenic ingredient from *P. juliflora* pollen was extracted in phosphate buffer-saline as described by Belin⁷. The extract was centrifuged at 15,000 rpm and the yellow coloured supernatant was sterilized by passing through a 0.45 μm filter. The protein content was measured by the method of Lowry *et al*⁸.

Sensitization and hypersensitivity test: Guinea pigs weighing 300–350 g were sensitized with different concentrations (100, 50, 25, 5 and 1.5 $\mu\text{g/ml}$) of

P. juliflora pollen allergen extract and 0.5 ml Freund's complete Adjuvant by the method of Freund and McDermott⁹. A challenging dose of 0.05 ml of 0.5 $\mu\text{g/ml}$ *P. juliflora* pollen extract was administered intracutaneously at three sites, on abdomen or back of guinea pigs after 7, 15 and 45 days of sensitization. The diameter of wheal-flare and erythema-redness was measured in comparison to that obtained with physiological saline control as described by Voorhorst¹⁰.

Histamine release test: *In vitro* histamine release was assayed in leukocytes (1.5×10^6 population) from 7, 15 and 45 days *P. juliflora* sensitized guinea pigs incubated with different concentrations (5.0×10^{-1} ; 5.0×10^{-2} ; 5.0×10^{-3} ; 1.0×10^{-3} ; 5.0×10^{-4} ; 1.0×10^{-4} $\mu\text{g/ml}$) of *P. juliflora* extract at 37°C for 1 hr by the method of May *et al*¹¹.

RESULTS

***In vivo* immediate hypersensitivity test by intracutaneous skin test:** Immediate response as wheal-flare was observed for 0–1 hr after administration of a challenging dose 0.05 ml of 0.5 $\mu\text{g/ml}$ *P. juliflora* pollen extract to 7, 15 and 45 days guinea pigs sensitized by various concentrations of *P. juliflora* pollen extract (figure 1). Maximum wheal-flare was observed at 20 min after giving challenging dose in all the three groups of guinea pigs. Maximum wheal-flare response was observed in 25 $\mu\text{g/ml}$ *P. juliflora* pollen extract sensitized guinea pigs for 7 days. At 50 $\mu\text{g/ml}$ sensitizing dose maximum response was obtained in 15 days and 45 days sensitized guinea pigs. Late response was observed for 3–12 hr as erythema-redness at the same site of early response (figure 2). Similar to the observations of early response, maximum late

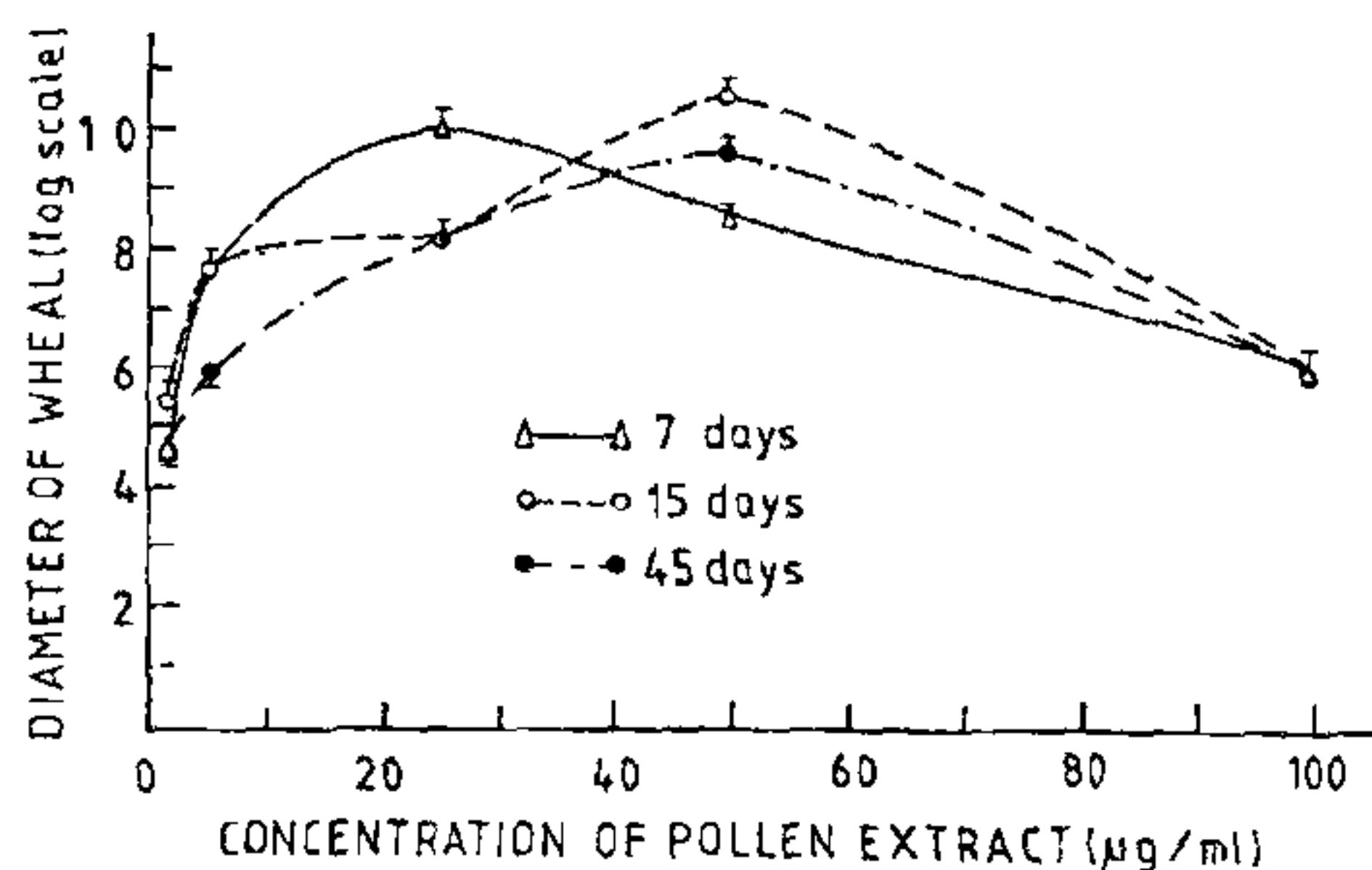


Figure 1. Changes in diameter (in mm) of wheal-flare induced *P. juliflora* pollen extract in guinea pigs sensitized with various concentrations (1.5 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$) *P. juliflora* pollen extract for different periods. Wheal-flare was expressed in log scale in comparison to 0.1 M saline as control. Each point represents the mean of not less than 10 values, the SEM is shown by vertical bars.

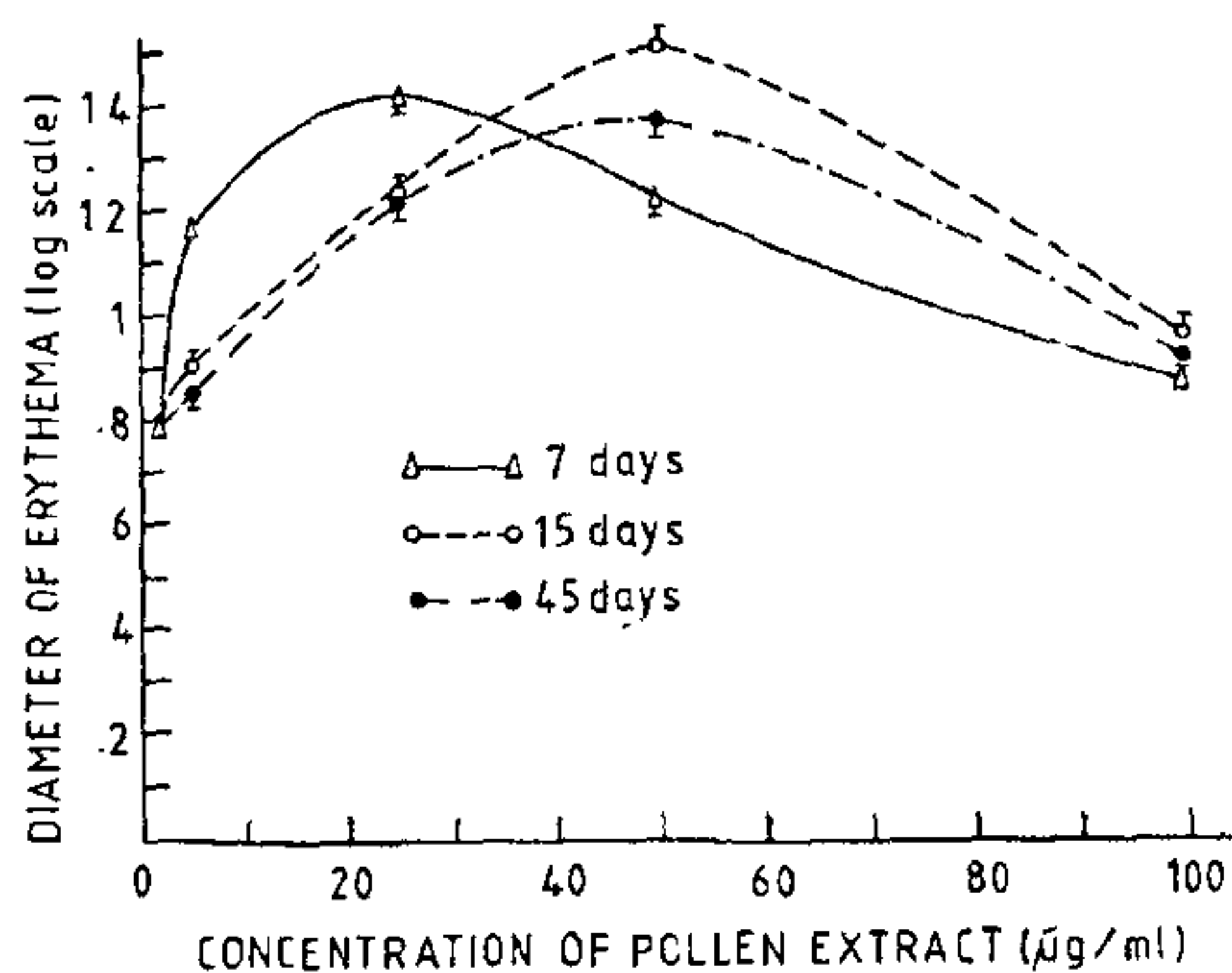


Figure 2. Changes in diameter (in mm) of erythema-redness induced *P. juliflora* pollen extract in guinea pigs sensitized with various concentrations (1.5 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$) *P. juliflora* pollen extract for different periods. Erythema-redness was expressed in log scale in comparison to 0.1 M saline as control. Each point represents the mean of not less than 10 values, the SEM is shown by vertical bars.

response (erythema-redness) was observed in 7 days guinea pigs sensitized by 25 $\mu\text{g/ml}$ of *P. juliflora* pollen extract, and in 15 days and 45 days by 50 $\mu\text{g/ml}$.

In vitro histamine release test: Histamine release

induced by various concentrations of *P. juliflora* pollen extract in leukocytes from guinea pig sensitized by 50 $\mu\text{g/ml}$ of *P. juliflora* pollen extract for 7, 15 and 45 days was determined (figure 3). A detectable quantity of histamine release was observed following the treatment with as little as 10^{-4} $\mu\text{g/ml}$ of *P. juliflora* pollen extract in all the three sensitized groups of guinea pig leukocytes; 50% or more of histamine was found to be released on incubation with the *P. juliflora* pollen extract concentrations of 5.0×10^{-1} $\mu\text{g/ml}$ and above.

It was further observed that the maximum release of 69% of histamine in leukocytes incubated with 5.0×10^{-1} $\mu\text{g/ml}$ *P. juliflora* pollen extract was found in 15 days of *P. juliflora* pollen sensitized guinea pigs (figure 3).

DISCUSSION

Guinea pig has been chosen as animal model to test the allergenicity caused by allergens from plant because antibody formation in it is similar to human beings. Its proneness to sensitization and for studying the regulatory mechanisms involved in reaginic and formation of blocking antibodies is well recognised¹²⁻¹⁴. So far, there are no reports of intracutaneous skin testing of pollen allergenicity in guinea pigs except for an indirect one on biphasic skin reactions at different sites of guinea pig evoked by intracutaneous administration of 2,4-dinitro-

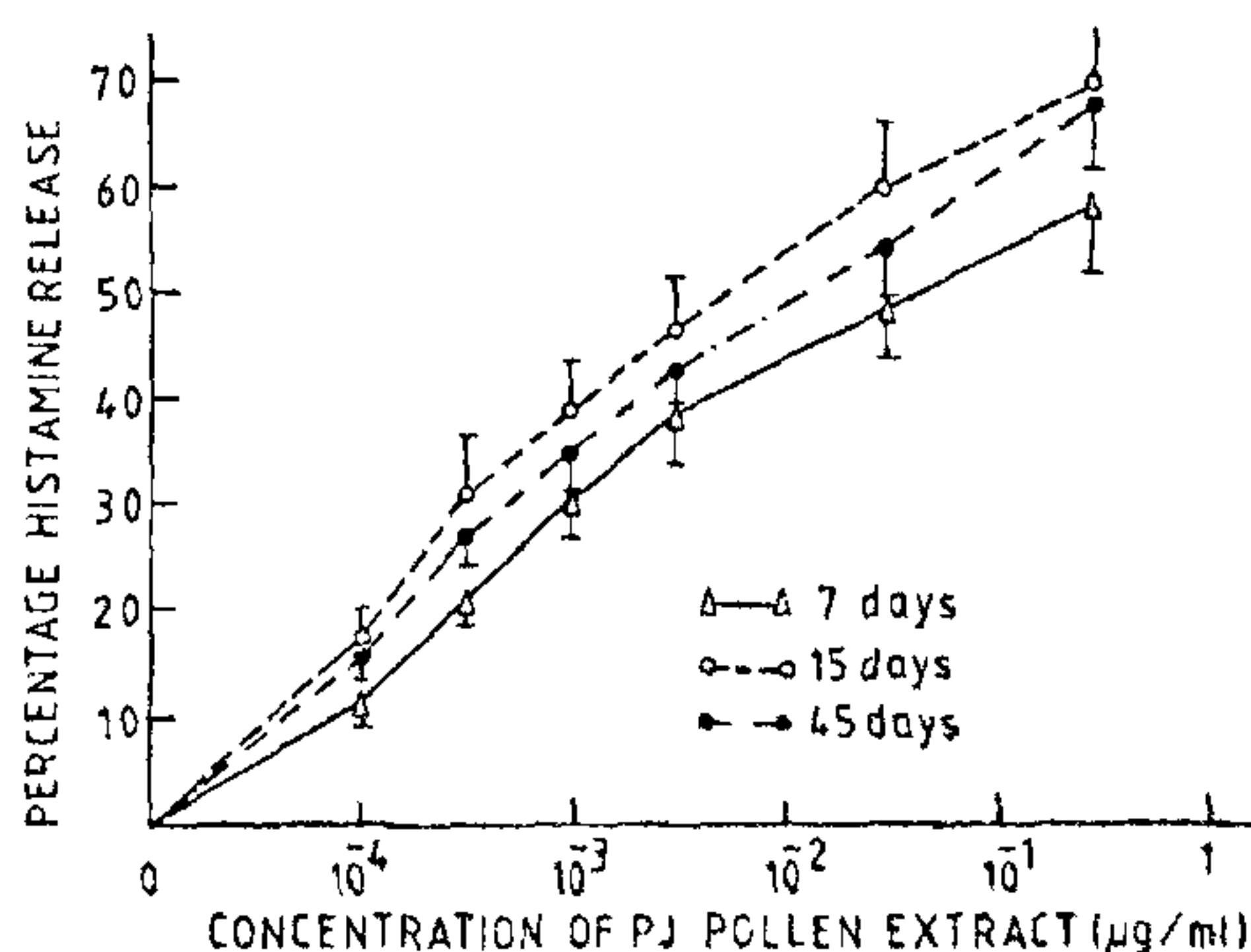


Figure 3. Percentage histamine release induced by different concentrations of *P. juliflora* pollen extract (5.0×10^{-1} to 1.0×10^{-4} $\mu\text{g/ml}$) in leukocytes from guinea pigs sensitized for different periods. Each value is an average of 5-6 experiments. SEM is shown by vertical bars.

chlorobenzene¹⁴ (DNCB). The reason for early and late responses is considered to be due to capillary leakages of histamine which was mediated by reaginic antibodies^{11,13-15}. The reason for dose shifting in different sensitized group remains unknown. The presence of allergen specific suppressor T-cells or feed-back regulatory mechanism of reaginic and blocking antibodies might be responsible for this^{16,17}.

One of the significant findings emerged that the release of histamine in *P. juliflora* pollen sensitized guinea pig leukocytes was directly proportional to the concentration of *P. juliflora* pollen extract. This is in agreement with the earlier reports of histamine release, assayed in man and guinea pig's leukocytes with ragweed pollen and *P. juliflora* pollen allergens^{11,18-20}. It is thought that the release of histamine by pollen allergen sensitive leukocytes could be due to the cell fixed reaginic antibodies (IgE or IgG_Y) and specific receptors on leukocytes which interact with *P. juliflora* allergen and induce the release of histamine^{20,21}. This is one of the first reports of time based histamine release from pollen sensitive guinea pig leukocytes with pollen allergens.

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