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INVESTIGATION OF IMMUNOLOGICAL RESPONSE OF EGYPTIAN BEE VENOM PREPARATION IN RABBITS

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Abstract

The repeated exposure of the immune system to bee venom as antigen leads to significant increase in immune response. In this study different immunological schedules were tried to reach maximum immunological effect with the least allergic side effect either by using and aqueous or emulsified preparation of bee venom. Three groups of male rabbits of one year old each were used for a period of four weeks. The blood samples were with drown before bee venom injection and weekly after its infection for determination of IgG and IgE. The results showed that injection of Egyptian bee venom in an aqueous form have the ability to boost the level of immune response without significant allergic reaction.

Key words: Egyptian bee venom, Rabbits, Immunology, Allergic side effects, Human safety.

Introduction

Apis mellifera (Hymenoptera: Apidae) is the commonest of the 7-12 species of honey bees worldwide (Engel, 1999). Stinging Hymenoptera included three main groups: ants, bees, and wasps, some people, mainly children the insect sting is a painful but transitory episode, and can have fatal consequences (Abdel-Rahman *et al,* 2015). Bee venom is a group of multiple allergens with wide range of molecular weight led to T cell and B cell response according to venom concentration rising to different immunological response (Akdis *et al,* 1997; Kettner *et al*, 1999). Tsicopoulos *et al.* (1989) studied the specific cell-mediated and humeral immune responses of 14 children allergic to honeybee venom. They found that an 8-day rush venom immunotherapy induced an increase in T proliferative and T suppressive cell-specific activities with antibody variations, an increase in specific IgG4, and a decrease in specific IgE were observed one year later. Elfiky *et al.* (2020) found that bee venom therapy is the specified form of apitherapy, and required the expertise of a well-trained physician. These data indicated that there are interrelationships between the cell-mediated immunity and the antibody responses in honeybee allergy. In this experiment rabbits were injected intradermally according to different immunization schedules with different diluents (aqueous preparation and emulsified preparation), with determination of IgG, and IgE before and during the immunization schedule which last for six weeks, to determine the most suitable immunization schedule for stimulation of immune system with a minimal risk or hypersenstivity arising from bee venom injection.

Material and Method

In this experiment 36 male New Zealand Rabbits, weighing 3-4kg (three groups, 12 rabbits each) were used for determination of immunological effect of bee venom in aqueous or emulsified preparation. All animals were kept under the same management with regard to nutrition, housing, and the necessary hygienic measures. During the period of the study, which lasted for 6 weeks, the rabbits were injected by bee venom according to experimental design for each group. They were keenly and regularly observed and any ill-health condition or ailment was registered and clinically interpreted. Rabbits were divi ded into three groups and kept under different immunization schedules (Bernstein *et al*, 1989) and sensitized (Przybilla and Rueff, 1999), which were adopted by VACSERA experiences as follows:

GI: Rabbits were injected intradermal with 100µg bee venom emulsified with complete

Freund's adjuvant for up to 6 weeks. Immunization course was continued by weekly injection with the same quantity of the emulsified venom, before every injection a blood sample withdrawn to estimate both the anti-IgG, and IgE antibodies titers. GII: Rabbits were injected intradermal with 50µg bee venom emulsified with complete Freund's adjuvant for up to 6 weeks. Immunization course was continued by weekly injection with the same quantity of the emulsified venom, before every injection a blood sa-mple withdrawn to estimate both the anti-IgG, and IgE antibodies titers. GIII: Rabbits were injected intradermal with 100µg bee venom in aqueous preparation for up to 6 weeks. Immunization course was continued by weekly injection with the same quantity of the emulsified venom, before every injection a blood sample withdrawn to estimate both the anti-IgG, and IgE antibodies titers.

Statistical analysis; All data were computerized and statistically analyzed.

Results

In GI which received 100µg venom emulsified with complete Freund's adjuvant, IgG mean value increased sharply to a maximum level of about 1250- mg%, while IgE mean value was vibrated and reaches 78 IU/ml after the 6th injection. In GII which received 50µg emulsified venom, IgG mean value increased to maximum level and reached 1250 mg%, but IgE was vibrated and reached 63IU/ml. In GIII which received 100µg venom as aqueous preparation both IgG & IgE titers were estimated and the mean value of IgG increased gradually from 664mg% to 830 mg% during the experimental period, but the mean value of IgE decreased to its minimum level of about 19 IU/ml.

Details were given in table (1) and figures (1, 2, & 3).

Table 1: IgG and IgE values in Rabbits immunized by bee venom preparations

Time	Group I (100 µg aquas prep.)		Group II (50 µg emulsified prep.)		Group III (100 µg emulsified prep.)	
	IgG (mg/dl)	IgE (IU/ml)	IgG (mg/dl)	IgE (IU/ml)	IgG (mg/dl)	IgE (IU/ml)
Before injection	664±28.75	13.28±0.60	718±10.4	22±0.72	684±16.8	24±0.81
After 1 week	752*±21.9	50.0**±1.81	1094*±14.7	27.0±0.78	1026**±22.3	25.0±0.57
After 2 week	700±24.36	58.0***±1.88	1050*±16.2	44.0*±2.08	1250***±36.6	82.0***±3.26
After 3 week	770*±20.31	26.0*±1.32	1050*±19.7	57.6**±1.84	1256***±24.4	60.8**±1.83
After 4 week	770*±22.19	22.0*±0.85	1050*±18.9	77.0***±3.62	1256***±22.9	58.0**±1.88
After 5 week	830**±29.13	20.5±0.51	1250**±14.9	70.0***±1.95	1250***±37.2	71.0***±1.86
After 6 week	830**±27.11	19.5±0.29	1250**±11.3	63.0***±1.46	1250***±35.0	78.0***±4.38

Discussion

The repeat exposure of the immune system to bee venom as antigen led to significant increase in immune status manifested by increase in IgG and decrease in allergic reaction as manifested by IgE decrease (Bellinghausen *et al,* 1997). In this study, it was better to give the maximum immunological effect with the least allergic manifestations, either by aqueous or emulsified preparation of bee venom.

In GIII received 100µg venom as an aqueous preparation both IgG and IgE titers mean values of IgG increased gradually from 664mg/dl to 830mg/dl at the study end while IgE mean value decreased to 19.5IU/ml. This agreed with Artemov (1958) who reported that bee venom in aqueous preparation was immunogenic substance

In GI & GII received venom emulsified with Complete Freund's adjuvant, IgG mean value increased sharply to a maximum level of 1250mg/dl and IgE mean value reached 63IU/ml after the 6th injection in the first group injected by 50µg emulsified venom, and 78IU/ml in GII ones injected by 100µg emulsified venom. Thus, the sharp increase in IgG was due to the effect of bacterial adjuvant, complete Freund's adjuvant (CFA), which acted mainly by stimulating the suitable cytokines formation (Fontes *et al,* 2017). The IgE antibodies are antigen-specific triggers of allergic reactions play important role in protective immunity against parasites exerting regulatory influences in expression of its receptor (Burton and Oettgen, 2011).

IgE was increased in both indicated allergic reaction by adding adjuvant (Cossu *et al,* 2017). Elfiky *et al.* (2023) by histopathology & immunology found that bee venom by intradermal injection in 0.1mg in aqueous solution was safe without side effects. Nainu *et al.* (2021) reported that bee products have long been used in traditional healing practices to treat many disorder types, including cancer and microbial-related diseases. El-Fiky *et al.* (2022) found that the bioactive components of bee venom can be used as an alternative to improve humans' health. Kwon *et al.* (2022) reported that BV has anti-breast cancer activity. El Naggar *et al.* (2023) reported that BV is a promising Apitherapy for chronic toxoplasmosis markedly enhanced by loaded metal organic frameworks.

Conclusion

The outcome data showed that injection of bee venom in aqueous preparation have the ability to boost the general level of immune activity without significant allergic reaction especially after bee venom immunotherapy.

Recommendation

For human safety, the bee venom must be administered in an aqueous preparation.

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Explanation of figures

Fig. 1: Determination of IgG and IgE injected by 100ug aquous bee venom preparation.
Fig. 2: Determination of IgG and IgE injected by 50ug emulsefied bee venom.
Fig. 3: Determination of IgG and IgE injected by 10 ug emulsefied bee venom.