Pharmaceutical Biology
1999, Vol. 37, No. 4, pp. 253–259

1388-0209/99/3704-0253$15.00
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SUPPRESSIVE EFFECT OF *WITHANIA SOMNIFERA* ROOT EXTRACT ON THE INDUCTION OF ANTI-OVALBUMIN IgE ANTIBODY RESPONSE IN MICE

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ABSTRACT

Withania somnifera *Dunal (Solanaceae) (WS), a well known Indian herbal drug, was examined for its effect on downregulation of antigen-specific IgE antibody response in mice. The extract prepared from the dried roots of WS (WSE) was administered intraperitoneally along with ovalbumin (OVA), a classical allergen, in the presence of aluminum hydroxide as an adjuvant. It exerted a significant suppression of OVA-specific IgE antibody production in BALB/c mice (H–2^d) as determined by passive cutaneous anaphylaxis (PCA). The extract also inhibited the production of OVA–specific IgE antibody, when administered 24 h prior to, and 6 h after, immunization. Further, WSE not only downregulated OVA-specific IgE antibody response in other haplotypes of mice such as C57Bl/6 (H–2^b) and SWR/J (H–2^q), but it also suppressed the antigen-specific IgE antibody response against other allergens tested in BALB/c mice. Thus, the basic concept for its application in the alleviation of different IgE mediated immunopathological conditions is supported.*

INTRODUCTION

According to Coombs and Gell, hypersensitivity reactions are classified into 5 types (i.e., Type – I, II, III, IV and stimulatory), based on the particular immunological mechanism that damages tissue(s) (Kuby 1994). The development of allergic symptoms varies from individual to individual and depends on the sensitivity to a variety of cross-reactive allergens with its involvement to different parts of the body. The most common forms of allergic diseases are allergic rhinitis (Blumenthal et al., 1992), urticaria (Reinmann et al., 1982), diarrhoea (Walker, 1987) and asthma (Burrows et al., 1989). About 15% of the population suffers from atopic allergy. IgE antibodies have been implicated in the immunopathological condition of Type-I hypersensitivity reactions. There is also clear evidence of a direct relationship between the IgE antibody titers on the one hand, and the density of high affinity FcE (receptors on the cell surface of mast cells, basophils, eosinophils and Langerhans cells on the other, in the development of allergic symptoms (Geert et al., 1995; Capron et al., 1984).

In the traditional Indian system of medicine (Ayurveda), the roots of *Withania somnifer*a Dunal (Solanaceae) have been reported to possess potent anti-inflammatory and antiasthmatic properties (Anbalgan & Sadique 1981, 1985; Kirtikar & Basu, 1944). However, the identification and isolation of pharmacological and immunologically active compounds from water-soluble extracts and the mechanism of action of these compounds are poorly explored. In the present study, the aqueous extract prepared from the roots of WS has been studied for identification of immunomodulatory effects using turkey egg ovalbumin (OVA) as an allergen. The extract was found to downregulate the anti-OVA IgE antibody response in a murine model.

Keywords: Ovalbumin, IgE antibody, PCA, *Withania somnifera*.

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MATERIALS AND METHODS

Preparation of Extract

The roots were procured from authorized local medicinal plant distributors and were authenticated with the help of taxonomists of the Botany Department, Andhra University, Visakhapatnam. A specimen sample was kept with the Department of Biochemistry. The dried and cleaned roots of WS were powdered and a 30% extract was prepared in double distilled

water. The extract was centrifuged and lyophilized. The lyophilized material was resuspended in phosphate buffered saline (PBS; 0.005 M phosphate, 0.075 M NaCl, pH 7.4) and the final concentration of the extract (WSE) was adjusted to 1.0 mg/ml and stored at –20°C until it was used to evaluate the inhibitory effect on the induction of OVA-specific IgE antibody response.

Animals

Eight to ten week-old female BALB/c (H–2^d), C57Bl/6 (H–2^b) and SWR/J (H–2^q) mice weighing 20–25 g and male Wistar albino rats weighing 300–350 g were procured from the breeding facilities of National Institute of Nutrition, Hyderabad, India and were housed under experimental conditions and allowed to feed with pelleted diet (supplied by Hindustan lever Ltd, Bangalore, India) and water *ad libitum*.

Immunization and Bleeding

Mice were immunized intraperitoneally (i.p.) with 1.0 µg of OVA (Sigma Chemical Company, USA), 100 µg extract of cowpea green seeds [*Vigna sinensis* Engl. (Leguminaceae)], finger millet [*Eleusina coracana* Geartn. (Poaceae)] seeds or edible parts of sea prawn (*Penaeus monodon*) along with 1.0 mg of aluminum hydroxide as an adjuvant, in a final volume of 0.5 ml of PBS. However, the test groups of mice received single or different concentrations of WSE along with the antigens mentioned above. Each group in all the experiments consisted of 4 mice. The mice were immunized on days 0, 28 and 56, and bled on days 14, 35 and 63, after primary immunization from the tail vein as per the protocol shown in Figure 1, unless otherwise stated. The sera collected were stored at –20°C until further use.

Measurement of IgE Antibody

Antigen-specific IgE antibodies were measured by passive cutaneous anaphylaxis (PCA), as described earlier (Duddukuri et al., 1997). Briefly, 50 µl of 4–fold serial dilutions of the antisera was injected intradermally onto the dorsal skin of the male rats and 24 h later they were challenged with 1.0 mg of OVA or 5 mg of other allergenic extracts containing 0.5% Evans blue dye in 1.0 ml of PBS through the penal vein. The skin was removed after 30 min and the highest serum dilution giving a 5 mm diameter blue spot was noted and considered as the positive PCA titer. The PCA titers were the arithmetic mean of the serum dilution in two determinations.

RESULTS

Induction of OVA-specific IgE Antibody Response

To check the immunomodulatory effect of WSE, an adjuvant dependent OVA-specific IgE antibody response was induced in mice by using different concentrations (0.1, 1.0, 10.0 and 100.0 µg) of OVA. As shown in Figure 1, it was found that a 1.0 µg dose was optimum to induce a significant immune response. Hence, in all the further studies, unless otherwise stated, a 1.0 µg OVA dose was used to induce the antibody response.

Effect of WSE on the Induction of OVA-specific IgE Antibody Response

The results in Figure 2 demonstrate a dose-dependent effect on the suppression of OVA-specific IgE antibody by the i.p. administration of WSE (0.01, 0.1, 0.5 and 1.0 mg) along with OVA. A complete suppression (PCA titers < 2) was observed with 0.5 and 1.0 mg of WSE, hence 0.5 mg was taken as a standard dose for under-

Table 1. Different groups of mice [BALB/c (H–2^d), C57Bl/6 (H–2^b) and SWR/J (H–2^q)] were immunized i.p. with 10.0 µg of OVA in the presence or absence of 0.5 mg of WSE and bled as per the protocol shown in Figure 1. OVA specific IgE antibody concentrations were determined by PCA and expressed as anti-OVA IgE antibody titers.

Haplotypes of mice	Treatment	Anti-OVA IgE antibody titers		
		Primary	Secondary	Tertiary
H-2^d	10.0 µg OVA + 0.5 mg WSE	< 2	< 2	< 2
	10.0 µg OVA	256	1024	4096
H-2^b	10.0 µg OVA + 0.5 mg WSE	ND	< 4	< 4
	10.0 µg OVA	ND	4096	8192
H-2^q	10.0 µg OVA + 0.5 mg WSE	ND	< 4	< 4
	100 µg OVA	ND	2048	6192

ND - Not Determined.

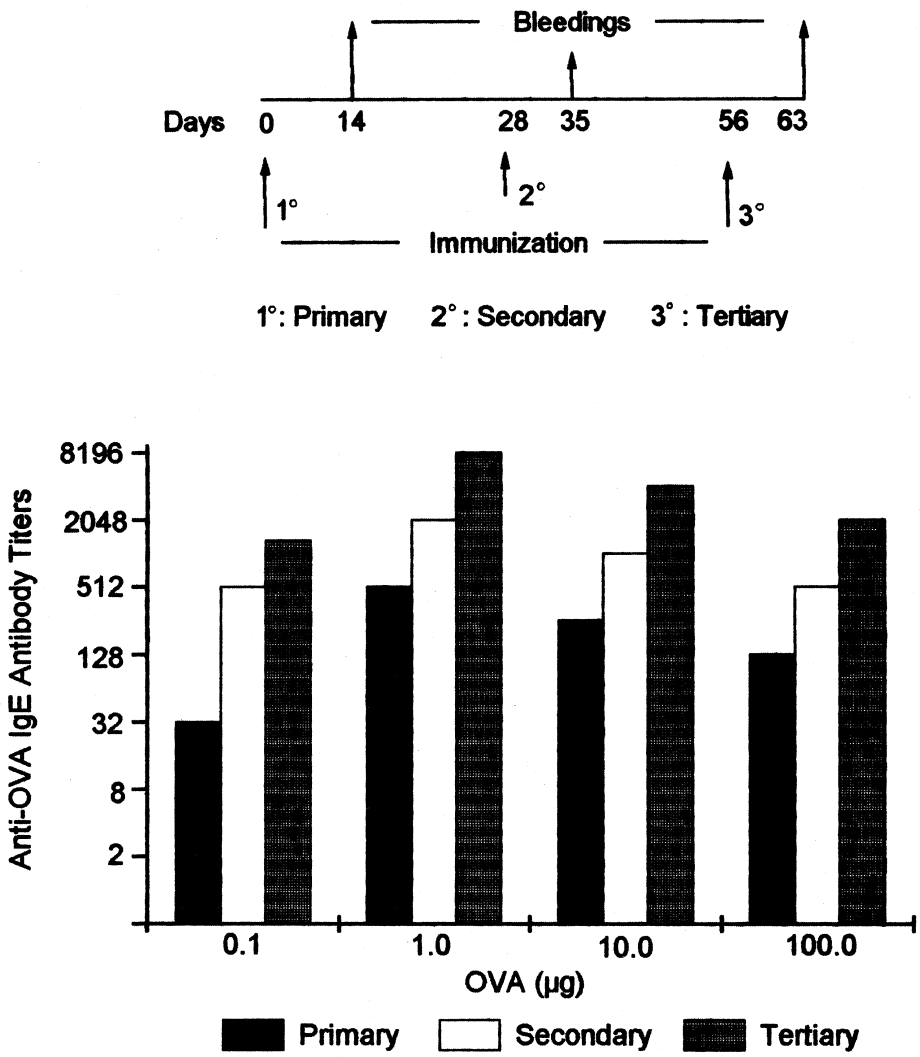

Fig. 1. BALB/c mice were immunized on days 0, 28 and 56 with 0.1, 1.0, 10.0 and 100.0 µg of OVA in presence of adjuvant (alum) and were bled 14 days after primary and 7 days after secondary and tertiary immunization. The sera were used for the estimation of anti-OVA IgE antibody titers by PCA.

taking other studies. The results in Figure 3 demonstrate that the administration of WSE (Group A) exerted an inhibition on the induction of secondary anti-OVA IgE antibody response by more than 60% against the control groups B and C which received only OVA or PBS, respectively, on day 28 as a booster dose. The results in Table 1 reveal that simultaneous administration of WSE along with OVA in different strains of mice [BALB/c ($H–2^d$), C57Bl/6 ($H–2^b$) & SWR/J ($H–2^q$)] induced a significant downregulation of OVA-specific IgE antibody responses. Furthermore, it also downregulated the induction of antigen specific IgE antibody response against 100 µg of cowpea, finger millets or prawn extracts, as shown in Figure 4. The WSE prepared in boiling water also inhibited the induction of anti-OVA IgE antibody response (data not shown).

Kinetic Effect of WSE on the Induction of Anti-OVA IgE Antibodies

To observe the kinetics of WSE effect on the elicitation of OVA-specific IgE antibody, different groups of mice were injected with 0.5 mg of WSE at time intervals of 48, 36, 24, 12 and 6 h before and 1, 6 and 12 h after immunization with OVA. These mice were bled 14 days after primary and 7 days after secondary immunization. The % suppression of IgE antibody in different test groups is shown in Figure 5 and the data demonstrate the groups that received WSE between 24 h prior to and 6 h after immunization exhibited a notable reduction of OVA-specific IgE antibody response.

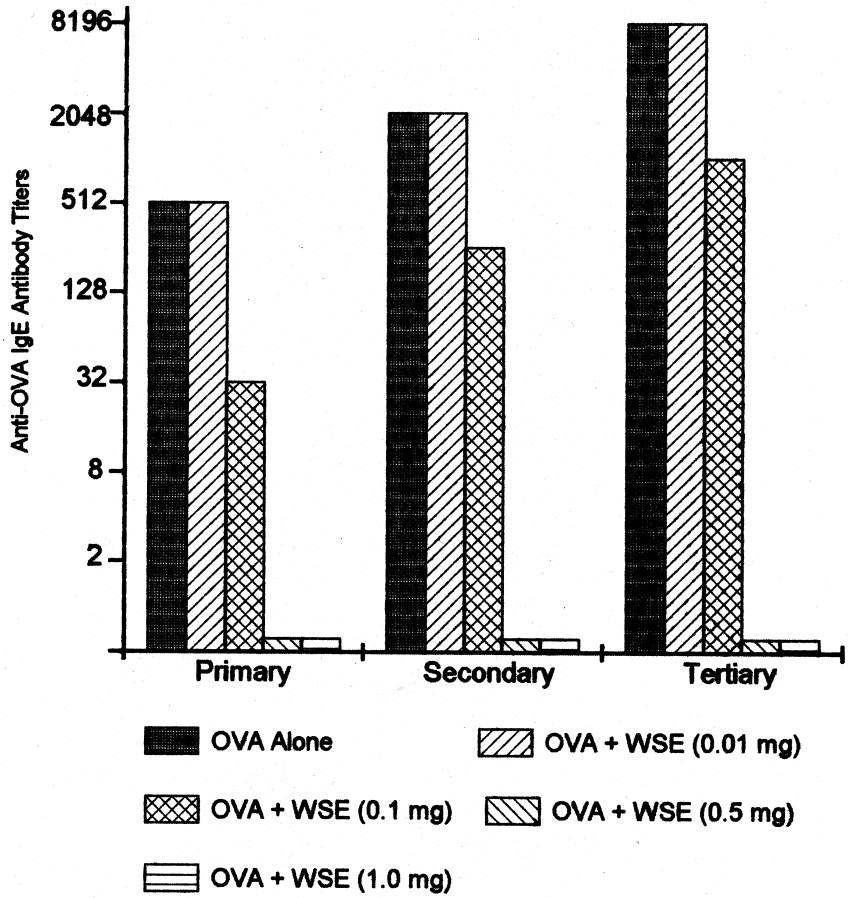

Fig. 2. Four groups of mice received 0.01, 0.1, 0.5 and 1.0 mg of WSE along with 1.0 µg of OVA in presence of alum and the other group received only OVA plus adjuvant, as per the protocol shown in Fig.1. The sera collected were analyzed for IgE antibodies specific to OVA by PCA.

DISCUSSION

In India, the roots of WS are traditionally used in the treatment of a variety of disorders such as bronchitis, asthma, chronic infections and inflammatory conditions without the knowledge of its mechanism of action and the principal compound(s) responsible. Thus far many compounds have been isolated from different parts of WS having different biological activities (Kirtikar & Basu, 1944; Atal et al., 1975). Previously it was reported that withanolides and solasodines isolated from the toluene extract of WS suppress mitogen-induced splenocyte activation in *in vitro* cultures (Bahr & Hansel, 1982). However, in the present study, the experimental data reveals that the administration of an aqueous extract of WS exerted a dose-dependent down-regulation of OVA-specific IgE antibody production in mice, as shown in Figure 2. It is known that the suppression of an ongoing immune response is more difficult than the suppression of a primary immune response, either by using modified antigens or plant extracts (Athota, 1989; Hang-XiXu et al., 1993). However, the experimental data in Figure 3 clearly demonstrate that WSE suppressed the ongoing secondary response specific to OVA by more than 60%. Furthermore, the suppressive effect of WSE is not restricted either to a single antigen or a single strain of mouse as evident from the results in Figure 4 and Table 1. Finally, as inferred from the results in Figure 5, to achieve significant suppression of immune response, one should administer WSE any time between 24 h prior to 6 h after antigen immunizations. The results indicated that, in addition to previously reported properties, WS aqueous extract has a potent effect on down-regulation of the antigen-specific IgE antibody response. The immunosuppressive compound(s) of WSE seem to be heat stable as an extract prepared in a boiling water bath also exerted downregulation of the anti-OVA IgE antibody response. At present, the isolation, immunological and biochemical characterization of immunosuppressive compound(s) from WSE is underway.

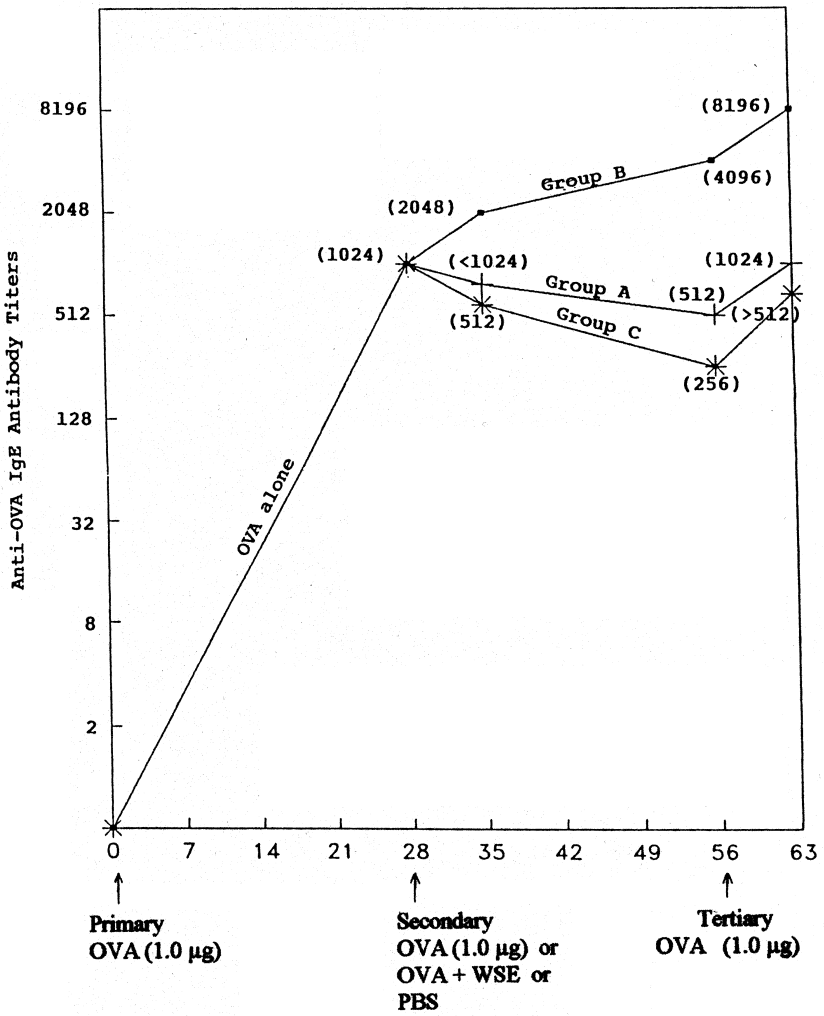

Fig. 3. Three groups of mice (A, B, C) received 1.0 µg of OVA on day 0. On day 28 group A was treated with 0.5 mg WSE along with OVA, group B received only OVA, while group C received only PBS. Again on day 56 all groups of mice received only 1.0 µg OVA and were bled on days 28, 35, 56 and 63 for determination of PCA titers.

ACKNOWLEDGMENTS

The authors would like to thank the DST, New Delhi for financial support.

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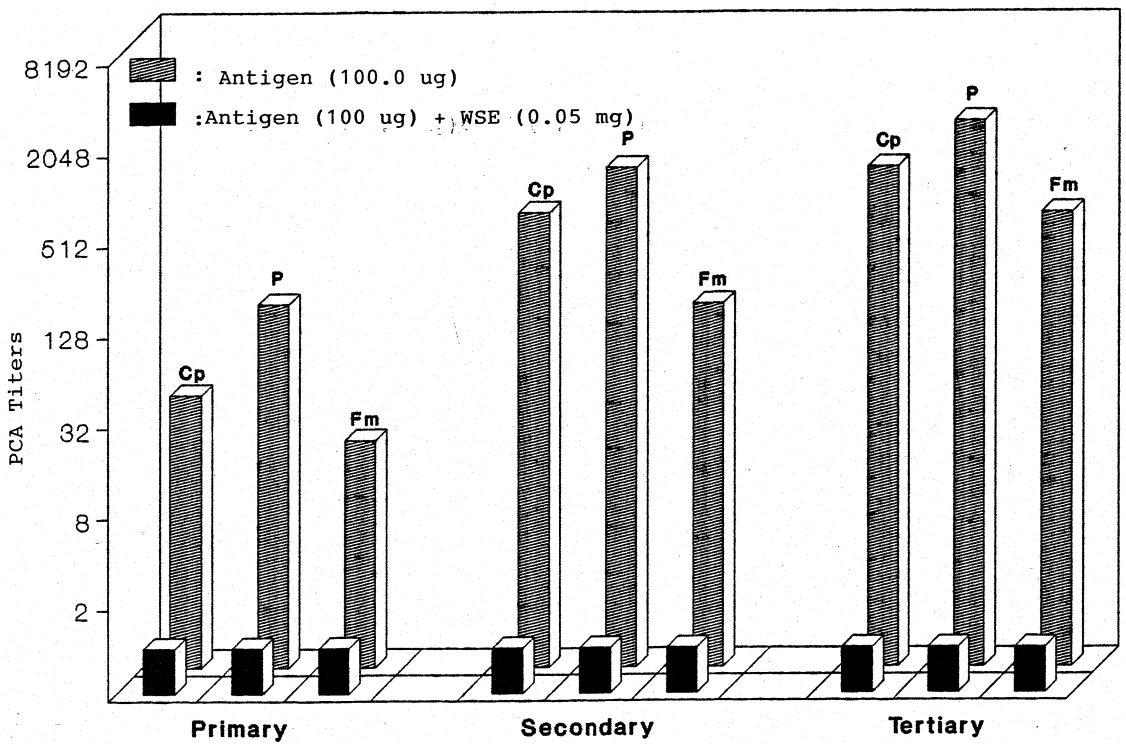

Fig. 4. Different groups of mice were immunized with 100 µg of cowpea (CP), finger millets (FM) or prawn (P) extracts mixed with alum in presence or absence of 0.5 mg WSE and bled from the tail vein as per the protocol shown in Fig.1. Anti-OVA IgE antibody levels in the sera were estimated by PCA.

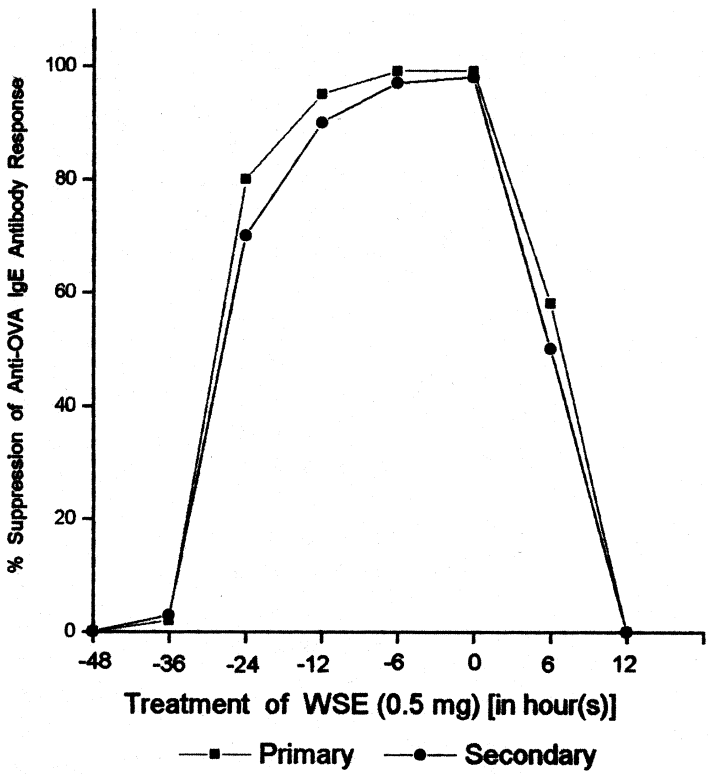

Fig. 5. Six groups of mice at 48, 36, 24, 12, 6 and 1 h before and three groups of mice at 1, 6 and 12 h after immunization with 1.0 µg OVA were treated with 0.5 mg WSE and bled on day 14 after primary and day 7 after secondary immunization. The positive control group of mice were immunized only with OVA on day 0 and 28 and were bled similarly to the other groups. The results are expressed as % suppression of anti-OVA IgE antibody titers.

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Accepted March 17, 1999