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Original Research Article

Effect of some indigenous Plant sources on the elicitation of Antigen-specific Antibody Response

Y. Vasudeva Rao
Biochemistry, Institute of Agriculture, Visva-Bharati, Sriniketan-731236, India.

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Abstract: Several indigenous plants sources, namely, *Achyranthes aspera*, *Bryophyllum pinnatum*, *Croton bonplandianum*, *Cissus quadrangularis*, *Piper longum*, *Phyllanthus asperulatus*, *Vanda roxburghii*, *Cuscuta europaea*, *Argemone mexicana*, *Momordica charantia*, were screened for the immunomodulating activity. Ovalbumin along with the aqueous extract of each plant source was injected intraperitoneally into different group's mouse, and to the control group only ovalbumin was given. Antigen-specific antibody *response* was determined by enzyme linked immunosorbant assay (ELISA). Among these plant sources, aqueous extracts of *Achyranthes aspera Piper longum* and *Coscuta europaea* have significantly enhanced the induction of OVA-specific antibody response, and the aqueous extract of *Mimordica karantia* has significantly suppressed the induction of OVA-specific antibody responses in murine system.

Keywords: Plant sources; Ovalbumin; Antigen; Antibody response; ELISA

Introduction

The modulation of immune response has been studied using varieties of natural and synthetic agents. Natural sources, such as saponins isolated from the cortex of *Quillajasaponaria molina* were demonstrated to boost the elicitation of antigen-specific IgG subclasses of antibody levels in mice but not stimulated the production of IgE antibodies (Kensil *et al*., 1991). The aqueous fraction of the 50% ethanolic extract of *Nyctanthes arbor-tristis* showed strong stimulation of antigen-specific and non-specific immunity in mice as evidenced by enhancement of humoral and delayed type hypersensitivity response to sheep red blood cells (Puri *et al*., 1994). The formulation comprising *Boerhavia diffusa, Tinospora cordifolia, Berberis aristata, Terminalia chebula* and *Zingiber officinale* enhanced the humoral immunity in golden hamsters as evidenced by the hemagglutination titre (Sohni and Bhatt, 1996). Ethanolic extract and purified diterpene andrographolides of *Andrographis paniculata* were found to induced significant stimulation of antibody response to sheep red blood cells in mice (Puri *et al*., 1993). The antibody response was augmented in BALB/c and C3H/HeJ mice by AS-VII from *Alsophila spinulosa* (Kao *et al*., 1994). *Oldenlandia diffusa*, a Chinese medicinal herb enhanced the production of immunoglobulins in mice (Yoshida *et al*., 1997). The n-Butanol fraction and epimedian C from aerial parts of *Epimedium hunanense* significantly enhanced the response of spleen antibody forming cells to near normal in mice treated with an immunosuppressant, hydrocortisone acetate (Liang *et al*., 1997. Synthetic sources, such as dimerized lysozyme (KLP-602) can stimulate the cellular and humoral defense mechanisms in fish (Siwicki *et al*., 1998). A series of 7, 8-disubstituted guanosine derivatives were found to augment the antibody response to sheep red blood cells (Reitz *et al*., 1994). A synthetic peptide, PimucV3 was found to stimulate a strong antibody response to the linked peptide or to a co-injected protein (Deprez *et al*., 1995). Peptidoglycan monomer linked with zinc has markedly enhanced the antibody production to sheep red blood cells in mice (Radosevic *et al*., 1995). Monophosphoryl lipid A has been found to exhibit the adjuvant activity in both the cellular and humoral immunity (Ulrich and Myers, 1995).

Honey has been found to suppress the induction of allergen-specific humoral antibody response in mice (Govinda *et al*., 1997). Root extract of *Withania somnifera* was found to suppress the induction of anti-ovalbumin IgE antibody response in mice (Srinivasulu *et al*., 1999).

In this study we have screened some indigenous plant sources for immuno-modulatory activity in murine model.

***Corresponding Author:**
Dr. Vasudeva Rao Y
Biochemistry, Institute of Agriculture,
Visva-Bharati, Sriniketan-731236, India.

Materials and Methods

Experimental Animals

Eight weeks old female mice, namely BALB/c (H-2^d) were used in this experiment. After acclimatization mice were divided into different groups, with four mice per each group. Pelleted rat food and water was provided daily to all groups. Temperature ranged from 37^0C to 30^0C during the experiment.

Preparation of aqueous plant extracts

Several indigenous plants sources, namely, *Achyranthes aspera*, *Argemone mexicana*, *Bryophyllum pinnatum*, *Cissus quadrangularis*, *Croton bonplandianum*, *Cuscuta europaea*, *Momordica charantia*, *Piper longum*, *Phyllanthus asperulatus*, *Vanda roxburghii*, were collected from Srikakulam and Visakhapatnam districts of Andhra Pradesh. Each plant extract was prepared separately and not mixed with other plant sources. For preparation of each extract all available parts of the plant were taken. A measured amount of the plant was ground to paste in a mortar with addition of measured amount of PBS (p^H 7.4). After fine grinding the content was centrifuged at 10000xg for 5 minutes, the supernatant was collected and kept in refrigerator for use and the sediment was discorded. The protein content of each plant extract was determined by Lowry method (Lowry *et al*., 1951).

Antigen & Dose of antigen

Ovalbumin (OVA) Sigma Chemical Co., USA, was used as an antigen. 10 µg of OVA/mouse in PBS was given.

Antigen administration

All groups of mice were injected intraperitoneally on days 0, 28 and 56 as primary, secondary and tertiary immunizations. The control group mice were injected with OVA alone. The test groups of mice were injected with OVA along with 100 µg (in terms of protein concentration) of each plant extract.

Sampling

Mice were bled from the tail vein, on day 14 after primary immunization and day 7 after secondary and tertiary immunizations. After collection blood was allowed to clot at room temperature and centrifuged. The serum was collected and kept in refrigerator for further analysis.

Enzyme Linked Immunosorbent Assay (ELISA)

The antigen-specific-antibody level present in serum samples was measured by ELISA. Briefly, the wells of the microtiter plates (F96-Maxisorp Nunc Immunoplates, Nalge Nunc International, Denmark) were coated with 100 µl of OVA (100 ng/well) in 50 mM carbonate-bicarbonate buffer p^H 9.6. After 12 h of incubation at 4^0C, the wells were washed three times with PBS containing 0.05% Tween-20. The free binding sites were blocked by adding 300 µl of PBS containing 3% skimmed milk powder (Hindustan Lever Ltd., Mumbai), per well, and incubating for 12 h at 37^0C. The wells were then washed and 100 µl of serum, diluted 1:400 in PBS, was added to each well and incubated for 1 h at 37^0C. For determination of antigen-specific IgG antibodies in serum, after washing the wells 100µl of horseradish peroxidase conjugated goat antimouse IgG (Sigma), diluted 1:1000 in PBS, was added to each well and incubated for 1 h at 37^0C. The wells were washed, 100 µl of substrate containing 4 mg of o-phenylenediamine dihydrochloride (Sigma) and 10 µl of hydrogen peroxide in 10 ml of 100 mM citrate-phosphate buffer p^H 5.0, was added to each well. After developing the color, the reaction was terminated by adding 50 µl of 4 N sulfuric acid to each well. The optical density was measured at 490 nm in an automatic microplate reader (Bio Rad model 550).

Results and Discussion

Several indigenous plants sources, namely, *Achyranthes aspera*, *Bryophyllum pinnatum*, *Croton bonplandianum*, *Cissus quadrangularis*, *Piper longum*, *Phyllanthus asperulatus*, *Vanda roxburghii*, *Cuscuta europaea*, *Argemone mexicana*, *Momordica charantia*, were screened for the immunomodulating activity, in two different sets of experiments. Antigen-specific antibody response, i.e., anti-OVA IgG antibody response was determined by enzyme linked immunosorbant assay (ELISA). Primary samples were not collected but secondary and tertiary samples were collected and analyzed. Among the plant sources tested, *Achyranthes aspera*, *Piper longum* and *Coscuta europaea* were found to possess immunostimulatory activity. Antigen-specific antibody level was found significantly ($P<0.05$) higher in these three plant treated group than the control group (Figs. 1 & 2). The secondary response of *Achyranthes aspera*, *Piper longum* are

much higher than even the tertiary response of the control group mice. The secondary and tertiary responses of *Achyranthes aspera* treated group are 78% and 150% higher compared with the control group secondary and tertiary responses, respectively (Fig. 1). The secondary and tertiary responses of *Piper longum* treated group are 64% and 108% higher compared with the control group secondary and tertiary responses, respectively (Fig. 1). The secondary and tertiary responses of *Coscuta europaea* treated group are found to be 38% and 97% higher compared with the control group secondary and tertiary responses, respectively (Fig. 2).

Though the secondary antibody response in mice treated with *Croton bonplandianum* and *Vanda roxburghii* extracts were found to be higher compared with the secondary response of control, but their tertiary response was nearly similar or their increment is negligible compared to that of the control tertiary response (Fig. 1). The secondary response of the mice treated with *Croton bonplandianum* and *Vanda roxburghii* are 47% and 51% higher compared with the control group secondary response, respectively. There was little or negligible difference between secondary and tertiary responses of mice treated with *Croton bonplandianum* and *Vanda roxburghii*. Only 5% and 10% increment was observed from secondary to tertiary response in *Croton bonplandianum* and *Vanda roxburghii* treated groups, but there was 41% increment from secondary to tertiary response in control group.

We could collect only the secondary samples from the groups of mice treated with *Bryophyllum pinnatum, Cissus quadrangularis*, *Phyllanthus asperulatus*. These mice did not survive beyond this period and all the mice in these groups were died, may be due to the treatment with the plant extract, or the dose of the injected plant extract may me much higher than the tolerance. The secondary response of the group of mice treated with *Bryophyllum pinnatum* shown to be 33% higher than the secondary control, and this increment is nearly equal to the tertiary response of the control group. The secondary response of the mice treated with *Cissus quadrangularis* is similar to that of the control group secondary response, but the secondary response of the mice treated with *Phyllanthus asperulatus* was found to be suppressed by 9% compared to the secondary response of control group (Fig. 1).

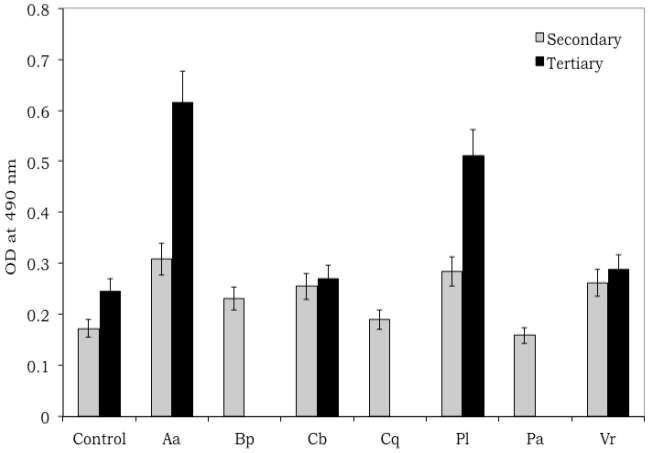

Fig. 1: Effect of different aqueous plant extracts on induction of antigen-specific murine humoral antibody response. Aa = *Achyranthes aspera*, Bp = *Bryophyllum pinnatum*, Cb = *Croton bonplandianum*, Cq = *Cissus quadrangularis*, Pl = *Piper longum*, Pa = *Phyllanthus asperulatus*, Vr = *Vanda roxburghii*.

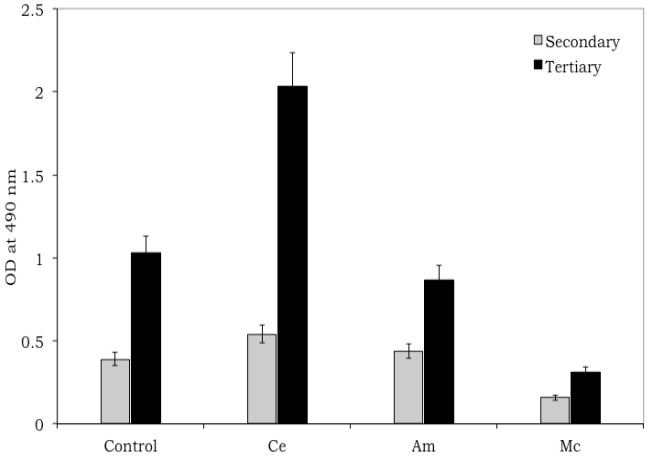

Fig. 2: Effect of different aqueous plant extracts on induction of antigen-specific murine humoral antibody response. Ce = *Cuscuta europaea*, Am = *Argemone mexicana*, MC = *Momordica charantia*.

It was observed that there was no significant change in the secondary and tertiary responses in the mice treated with *Argemone mexicana* compared with the control group. The secondary response of *Argemone mexicana* treated group was found to be 12% higher than the control secondary response, and the tertiary response was 16% lower than the control tertiary response (Fig. 2).

It was observed that there was a significant suppression ($P<0.01$) in secondary and tertiary antibody responses in the mice treated with *Momordica charantia* compared

with the control group. The secondary response of *Momordica charantia* treated group was found to be 60% lower than the control secondary response, and the tertiary response was 70% lower than the control tertiary response (Fig. 2).

In this study, it was found that intraperitoneal administration of aqueous extracts of *Achyranthes aspera Piper longum* and *Coscuta* has significantly enhanced the induction of OVA-specific antibody responses in mice as determined by ELISA. Also it was found that intraperitoneal administration of aqueous extract of *Momordica charantia* has significantly suppressed the induction of OVA-specific antibody responses in murine system.

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