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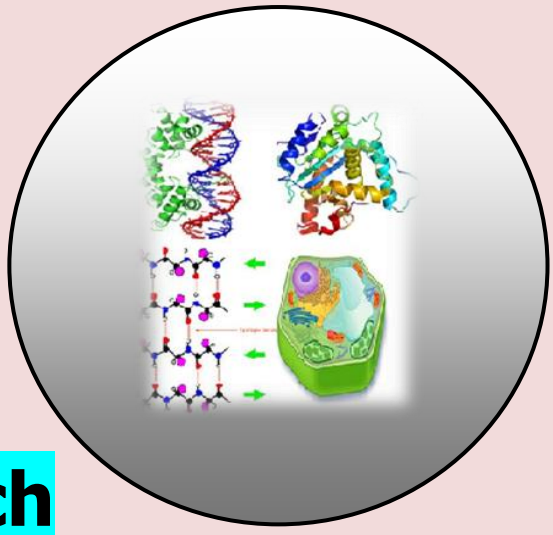

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RESEARCH PAPER

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Screening of different Plant Sources on the Modulation of Murine Antibody Response

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ABSTRACT

Different indigenous plants sources, namely, Achyranthes aspera, Bryophyllum, Croton banplandianum, Cissus quadrangularis, Piper longum, Phyllanthus asperlutus, Vanda roxburgi, Cuscuta, Argiman mexicana, Mimordi cakarantia, were screened for the immunomodulating activity in murine system. Ovalbumin along with the aqueous extract of each plant source was injected intraperitoneally into different groups mouse, and to the control group only ovalbumin was given. Antigen-specific antibody response was determined by enzyme linked immunosorbent assay (ELISA). Among these plant sources, aqueous extracts of Achyranthes aspera Piper longum and Coscuta has significantly enhanced the induction of OVA-specific antibody response, but aqueous extract of Mimordi cakarantia has significantly suppressed the induction of OVA-specific antibody responses in murine system.
Keywords: Indigenous plants, immunomodulation, antigen and antibody response.

INTRODUCTION

The immune system is the defense force of the individual and is very crucial in sustaining the life of the individual. The immune response can be modified by various natural and synthetic sources. Natural sources, such as Leaf extract of *Capparis zeylanica* has showed to stimulate the specific and non-specific immunities in mice (Agrawal *et al.,* 2010). Methanolic extract of *Aegle marmelos* fruit (FEAM) possesses potential for augmenting immune activity by cellular and humoral mediated mechanisms (Patel *et al.,* 2010). Ethanolic extract of the leaves of *Spilanthes acmella* has showed immunostimulatory activity by stimulating the macrophage activity (Savadi *et al.,* 2009). *Euphorbia hirta*, a pantropic herb has been reported to be immunologically active in mice (Ramesh and Padmavathi, 2010). Aqueous extract of *Malvastrumtri cuspidatum* showed immunostimulatory effect in rats (Bhadoriya *et al.,* 2012). Methonalic extract of *Murraya koenigii* has showed immunomodulatory activity by stimulating humoral immunity and phagocytic function (Shah *et al.,* 2008). Oral administration of the aqueous extract of *Ocimum sanctum* showed immunomodulatory effect by increasing antibody production in rat (Jeba *et al.,* 2011). *Oldenlandia diffusa* has enhanced the production of immunoglobulins in mice (Yoshida *et al*., 1997). The fractions of *Epimedium hunanense* significantly enhanced the response of spleen antibody forming cells to near normal in mice treated with an immunosuppressant (Liang *et al*., 1997.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). Ethanolic extract and purified diterpene andrographolides of *Andrographis paniculata* were stimulated the antibody response to

sheep red blood cells in mice (Puri *et al*., 1993). Saponins isolated from the cortex of *Quillaja saponaria molina* were demonstrated to stimulate the antigen-specific IgG subclasses of antibody levels in mice (Kensil *et al*., 1991). The antibody response was augmented in mice by AS-VII from *Alsophila spinulosa* (Kao *et al*., 1994). The formulation comprising different plant sources, viz., *Boerhavia diffusa, Tinospora cordifolia, Berberis aristata, Terminalia chebula* and *Zingiber officinale* enhanced the humoral immunity in golden hamsters as evidenced by the hemagglutinationtitre (Sohni and Bhatt, 1996). The crude extracts of amla (*Emblica officinalis*) and shankhpushpi (*Evolvulus alsinoides*) were caused immunosuppression in adjuvant induced arthritic rats (Ganju *et al.,* 2003).Honey has been found to suppress the antigen-specific humoral antibody response in mice (Duddukuri *et al*., 1997). Root extract of *Withania somnifera* was found to possess the immunomodulatory activity by suppressing the anti-ovalbumin IgE antibody response in mice (Srinivasulu *et al*., 1999). In the present study, I have screened some locally available plant sources for their effect on the immunity in murine system.

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*, *Argiman mexicana*, *Bryophyllum*, *Cissus quadrangularis*, *Croton banplandianum*, *Cuscuta*, *Mimordi cakarantia*, *Piper longum*, *Phyllanthus asperlutus*, *Vanda roxburgi*, were collected. 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 and Dose of antigen

Ovalbumin (OVA) 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

The antigen-specific-antibody level present in serum samples was measured by ELISA. Briefly, the wells of the microtiter plates 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 100 µl of horseradish peroxidase conjugated goat-anti-mouse 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 dihydro chloride (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.

RESULTS AND DISCUSSION

A. aspera, *Bryophyllum*, *C. banplandianum*, *C. quadrangularis*, *P. longum*, *P. asperlutus*, *V. roxburgi*, *Cuscuta*, *A. mexicana*, *M. karantia*, were screened for the immunomodulating activity. Antigen-specific antibody response, i.e., anti-OVA IgG antibody response was determined by enzyme linked immunosorbent assay (ELISA). secondary and tertiary samples were collected and analyzed for specific antibody response. Among the plant sources tested, *A. aspera*, *P. longum* and *Coscuta* 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 and 2). The secondary response of *A. aspera*, *P. longum*are much higher than even the tertiary response of the control group mice. The secondary and tertiary responses of *A. 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 *P. 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* treated group are found to be 38% and 97% higher compared with the control group secondary and tertiary responses, respectively (Fig. 2).

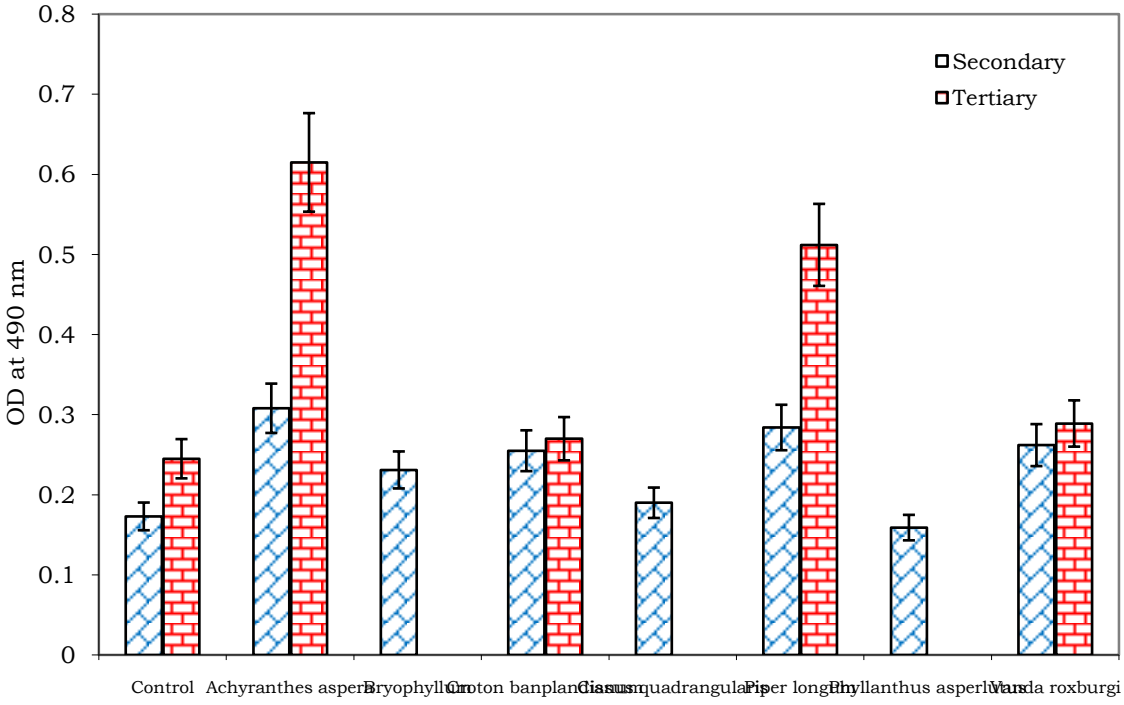

Figure 1. Effect of different aqueous plant extracts on induction of antigen-specific murine humoral antibody response.

Though the secondary antibody response in mice treated with *C. banplandianum* and *V. roxburgi* 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 *C. banplandianum* and *V. roxburgi* 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 *C. banplandianum* and *V. roxburgi*. Only 5% and 10% increment was observed from secondary to tertiary response in *C. banplandianum* and *V. roxburgi* treated groups, but there was 41% increment from secondary to tertiary response in control group.

We collected only the secondary samples from the groups of mice treated with *Bryophyllum, C. quadrangularis*, *P. asperlutus*. These mice did not survive beyond this period and all the mice in these groups were dead, 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* 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 *C. quadrangularis* is similar to that of the control group secondary response, but the secondary response of the mice treated with *P. asperlutus* was found to be suppressed by 9% compared to the secondary response of control group (Fig. 1). It was observed that there was no significant change in the secondary and tertiary responses in the mice treated with *A. mexicana* compared with the control group. The secondary response of *A. 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).

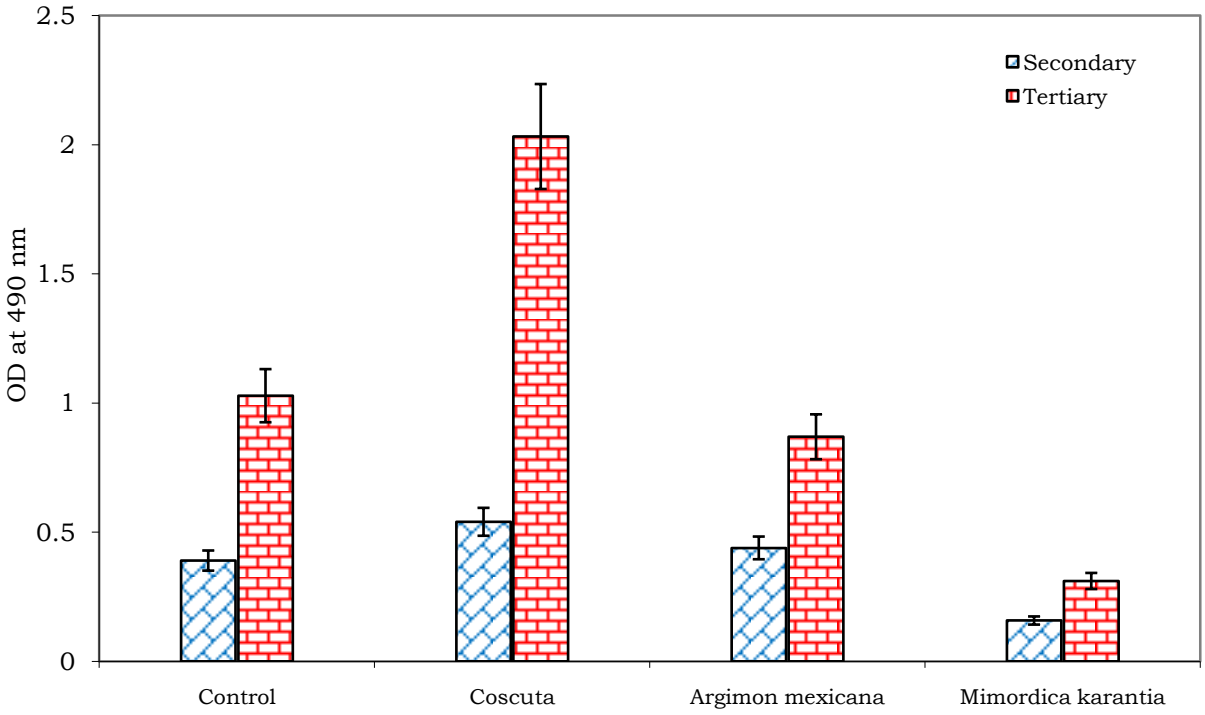

Figure 2. Effect of different aqueous plant extracts on induction of antigen-specific murine humoral antibody response.

It was observed that there was a significant suppression ($P<0.01$) in secondary and tertiary antibody responses in the mice treated with *M. karantia* compared with the control group. The secondary response of *M. karantia* 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).

A. aspera P. longum and *Coscuta M. karantia* were significantly shown to possess immunomodulatory activity. In this study, it was found that intraperitoneal administration of aqueous extracts of *A. aspera P. longum* and *Coscuta* has significantly stimulated immune response as evident by enhanced antigen-specific antibody production; but intraperitoneal administration of aqueous extract of *M. karantia* has significantly suppressed the immune response, which was evident by suppressed antigen-specific antibody production in murine system. Other plants tested did not show any significant immunomodulatory activity.

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