Immunostimulant activity of certain plants used in Indian traditional medicine

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Certain plants products widely used in the traditional medicine system of India were studied for immunostimulant activity. In the present study, strong stimulation of antigen specific and non specific immunity was evidenced by increase in haemagglutinating antibody (HA) titre, plaque forming cells (PFC) and in macrophage migration index (MMI), in mice fed with 50% ethanolic extract of these plants. The observation provides scientific basis for wide spread use of these plant products which are likely to play a role in Indian traditional medicine as tonic for rejuvenation therapy and chronic ailments.

Key words: Macrophage migration index, haemagglutinating antibody, immunostimulant.

INTRODUCTION

Immunostimulant activity has been demonstrated in several plants used in the traditional medicine of India, China and European countries for rejuvenation therapy and treatment of chronic ailments (Atal et al., 1986; Wagner, 1983, 1984a, b). Strong immunostimulant activity has been reported from this laboratory in Panax pseudoginseng (Araliaceae) (Dua et al., 1989), Picrorhiza kurroa (Scrophulariaceae) (Puri et al., 1992), Andrographis Paniculata (Acanthaceae) (Puri et al., 1993), Nyctanthes arbortristis L. (Nyctanthaceae) (Puri et al., 1994), Curculigo orchioides (Hypoxidaceae) (Lakshmi et al., 2004), and certain dry fruits and plant material, namely: Prunus amygdalus (Rosaceae), Euryale ferox (Nymphaceae), Buchanania lanzan (Anacardiaceae), Phoenix dactylifera (Arecaceae) and Zingiber officinale (Zingiberaceae) given to mothers after child birth and to invalids in both of whom the immune status is low (Puri et al., 2000).

In addition to the immunostimulant activity, P. kurroa was also shown to provide non specific protection against Leishmania donovani infection in hamsters (Puri et al., 1992). A. paniculata and N. arbortristis have also shown antileishmanial (Tandon et al., 1991) and antimalarial (Misra et al., 1992), respectively. “Pipalli Rasayana” an Ayurvedic preparation consisting of Piper longum (Piperaceae) and Butea monosperma (Fabaceae) was shown to be effective in the management of giardiasis in patients (Agarwal et al., 1997). In experimental giardiasis, a curative effect of the rasayana was accompanied by stimulation of specific and nonspecific immune responses (Agarwal et al., 1994). P. Longum, the main constituent of Pipalli rasayana was later shown to be effective against experimental giardiasis accompanied by stimulation of specific and nonspecific responses in the animals (Tripathi et al., 1999).

The present communication reports on the immunostimulant activity of certain other plants used in the Indian traditional medicine as tonics for invigorating
health and for a variety of ailments.

MATERIALS AND METHODS

Collection and extraction of plant material
The seeds, leaves, flowers or stem of various plants were collected from different localities and identified by the Botany Division of this Institute. The voucher specimens have been deposited in the herbarium of the Institute. The materials were shade dried and extracted separately with 95% ethanol. The ethanol extracts were concentrated under reduced pressure below 50°C in a rotary evaporator to yield viscous mass which were dried separately under high vacuum to remove last traces of the solvent.

Experimental animals and treatment
BALB/c albino mice of either sex, weighing 20 to 25 g were used in the present study. The animals were kept on a standard pellet diet (Lipton, Mumbai, India) and water ad libitum. The plant extracts were fed orally at a dose of 25 mg/kg/day for 7 consecutive days.

Immunostimulant activity
The immunostimulant activity was determined using: (1) macrophage migration index (MMI) which is a correlate of macrophage activation and cellular immunity, (2) haemagglutination antibody (HA) titre and (3) plaque forming cell (PFC) count, both of which are parameter of antigen specific humoral immunity.

Macrophage migration index (MMI)
The test was performed according to the method of Saxena et al. (1991). Briefly peritoneal exudate cells (PEC) collected from the extract treated and control mice, packed in a haematocrit capillary tubes of 7.5 mm length and uniform bore (1.0 to 1.2 mm) were allowed to migrate in migration chamber filled with complete Roswell Park Memorial Institute (RPMI-1640). The area of PEC migration was marked on Whatmann filter paper using Camera Lucida and weighed separately. The ratio of the migration area of PEC from the extract treated group (A1) to that from the untreated group (A2) has been expressed as macrophage migration index.

\[ MMI = \frac{A_1}{A_2} \]

Humoral immune response
Groups of the extract treated and untreated control mice were injected each with a suspension of sheep red blood cells (SRBC) using 1 x 10⁸ cells per mouse intraperitoneally: (a) Haemagglutinating antibody (HA) titre and (b) plaque forming cell (PFC) counts were determined 4 days after the immunization with SRBC.

Haemagglutination antibody (HA) titre: Two fold dilutions of sera from the immunized mice were prepared in 0.15 M phosphate buffer saline (PBS) pH 7.0 and 50 µl of each dilution was aliquotted into 96 well microtitre plates. Twenty-five microtitre of fresh 10% SRBC suspension in PBS was then dispensed into each wells, mixed thoroughly and the titre plates were incubated at 25 to 30°C for 2 h and then examined for agglutination. The reciprocal of the highest dilution of the test serum giving 50% agglutination has been expressed as the HA titre.

Plaque forming cell (PFC) count: The assay was carried out after the technique of Jerne and Nordin (1963). Spleen cells were separated from the extract treated and control animals 4 days after the immunization with SRBC, in RPMI-1640 medium, washed twice with the medium and suspended in complete RPMI-1640 medium containing fetal calf serum. Glass petri dishes (25 mm) were layered with 1.2% agarose in 1.5 M NaCl to form the bottom layer. A mixture of 2 ml 0.6% agarose in RPMI-1640 medium at 42°C, 0.1 ml suspension of 20% SRBC and 1 x 10⁸ spleen cells in 0.1 ml were layered over the bottom layer and the petri dishes incubated at 37°C for 45 min. Two milliliters of 1:10 diluted fresh guinea pig serum was then added and the incubation continued for 45 min, washed twice with the medium and resuspended in complete RPMI-1640 medium containing Fetal calf serum. Glass petri dishes (25 mm) were layered with 1.2% agarose in 1.5 M NaCl to form the bottom layer. In a mixture of 2 ml 0.6% agarose in RPMI-1640 medium at 42°C, the plaques were counted immediately and the value has been expressed as counts per 10⁵ spleen cells.

RESULTS AND DISCUSSION
The data on immunostimulant activity of 18 plants studied have been presented in Table 1 in which the plants have been arranged in order of their macrophage activating macrophage migration index (MMI). As evident an form, the data in which the animals were treated with the extracts of the first six plants showed appreciably high MMI values in the range of 2.3 to 3.2 times higher than the control untreated animals. The maximum value of 3.2 was recorded for Tinospora cordifolia (Menispermaceae), Achyranthes asper (Amaranthaceae) Occimum sanctum (Lamiaceae), Tephrosia purpurea (Fabaceae), Eclipta alba (Asteraceae) and Luffa echinata (Cucurbitaceae) also showed quite high values between 2.8 to 3.0. Cinnamon tamala (Lauraceae), Cichorium intybus (Asteraceae) and Zizyphus sativa (Rhamnaceae) showed MMI values between 1.5 and 2.0. The rest of the plants showed only marginal or no activation of macrophages.

The humoral immune response in terms of haemagglutinating antibody (HA) titre was highest for Cassia occidentalis (Caesalpiniaeae) with mean HA titre of 1638 followed by O. sanctum, E. alba., Evolvulus alsinoides (Convolvulaceae), Zingiber officinale and Pueria tuberosa, (Fabaceae) which gave mean HA titre of 1443 each. T. purpurea , L. echinata, Foeniculum vulgare (Umbelliferae) and Memordica diocea (Cucurbitaceae) also showed a high HA titre with mean value 1228. The titre for T. cordifolia and A. asper (Compositae) which elicited highest macrophage activity response gave rather low antibody response in terms of HA titre and C. tamala and C. intybus (Compositae) which showed significant macrophage activation failed to
Table 1. Cell mediated and humoral immune response in certain plants used in Indian system of medicine.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Plants</th>
<th>Cell (MMI) mediated immune response</th>
<th>Humoral immune response</th>
<th>Medicinal uses of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HA titre mean±SD</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Tinospora cordifolia</td>
<td>3.2</td>
<td>614.4±228.97</td>
<td>Bitter tonic, general debility, febrifuge anti-rheumatic</td>
</tr>
<tr>
<td>2</td>
<td>Achyranthus asper</td>
<td>3.0</td>
<td>716.8±280.4</td>
<td>Antisheumatic, cough &amp; chest disorders, febrifuge</td>
</tr>
<tr>
<td>3</td>
<td>Tephrosia purpurea</td>
<td>2.9</td>
<td>1228.8±457.94</td>
<td>Tonic, anti asthmatic, anti rheumatics, bronchitis, diseases</td>
</tr>
<tr>
<td>4</td>
<td>Ocimum sanctum</td>
<td>2.9</td>
<td>1433.6±560.8</td>
<td>Stimulant, bronchitis, anti asthmatic</td>
</tr>
<tr>
<td>5</td>
<td>Eclipta alba</td>
<td>2.8</td>
<td>1228.8±457.94</td>
<td>Stimulant, bronchitis, anti asthmatic, anti gonorrhoeic</td>
</tr>
<tr>
<td>6</td>
<td>Luffa echinata</td>
<td>2.3</td>
<td>1443.6±560.8</td>
<td>Stimulant, bronchitis, anti asthmatic, anti gonorrhoeic</td>
</tr>
<tr>
<td>7</td>
<td>Evolvulus alsinoides</td>
<td>2.3</td>
<td>1443.6±560.8</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
<tr>
<td>8</td>
<td>Amoora rohituka</td>
<td>2.3</td>
<td>614.4±228.97</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
<tr>
<td>9</td>
<td>Cinnamon tamala</td>
<td>2.0</td>
<td>307.2±114.48</td>
<td>Stimulant, anti rheumatic, cold, anti gonorrhoeic</td>
</tr>
<tr>
<td>10</td>
<td>Cichorium intybus</td>
<td>2.0</td>
<td>358.4±140.21</td>
<td>Tonic in fever, spleen and liver disorders, anti rheumatics,</td>
</tr>
<tr>
<td>11</td>
<td>Zizyphus sativa</td>
<td>1.5</td>
<td>460.8±114.48</td>
<td>Tonic, chronic bronchitis, soar throat, rheumatism.</td>
</tr>
<tr>
<td>12</td>
<td>Zingiber officinale</td>
<td>1.3</td>
<td>1443.6±560.8</td>
<td>Stimulant, anti rheumatic, affections of threat head and chest.</td>
</tr>
<tr>
<td>13</td>
<td>Foeniculum vulgare</td>
<td>1.2</td>
<td>1228.8±457.94</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
<tr>
<td>14</td>
<td>Cassia occidentalis</td>
<td>1.0</td>
<td>1638.4±560.86</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
<tr>
<td>15</td>
<td>Pueraria tuberosa</td>
<td>1.0</td>
<td>1443.6±560.86</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
<tr>
<td>16</td>
<td>Memordica diocea</td>
<td>1.0</td>
<td>1228.8±457.94</td>
<td>Gout -rheumatism, spleen and liver disorders, febrifuge,</td>
</tr>
<tr>
<td>17</td>
<td>Solanum nigrum</td>
<td>0.9</td>
<td>716.8±280.4</td>
<td>Febrifuge, bronchitis, liver disorders, anti asthmatic</td>
</tr>
<tr>
<td>18</td>
<td>Hedychium spicatum</td>
<td>0.8</td>
<td>912.6±228.97</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
<tr>
<td>19</td>
<td>Control</td>
<td>1.0</td>
<td>307.2±114.48</td>
<td>Anti gonorrhoeic, stimulant, cordiotonic cornivirulent,</td>
</tr>
</tbody>
</table>

*p< 0.01; *p < 0.05.

elicit HA response. On the other hand, C. occidentalis, Z. officinale, F. vulgare, P. tuberosa, M. dioca and H. spicatum (Zingiberaceae) which showed little or no macrophage activation, elicited significantly high HA titre.

The humoral immune response in terms of plaque forming cell (PFC) counts was highest with C. occidentalis followed by Z. officinale (160), P. tuberosa (150), O. santnum (130), E. alba (130), T. cordifolia (126), A. asper (126), M. dioca (125), S. nigrum (120) and E. alsinoides (120). The PFC count was significant with other plants except for C. tamala and C. intybus which like HA titre failed to elicit PFC response over the control value.

The last column of the table shows the medicinal uses of the plants taken up for the present investigation (Husain et al., 1992). Plants 1, 3, 4, 5, 9, 10, 11, 14, 15 and 18 have been used as tonics for invigorating health. Of these plants, plant 5 also prevents ageing and premature greying of hairs. Plants 2, 3, 4, 7, 11, 12 and 17 have been used against chronic bronchitis and diseases of chest. All these later plants with the exclusion of plants 11 and 12 have also been found useful against allergy-asthma. Plants 1, 3, 9 and 13 have been used in gonorrhoea and plants 1, 6, 14 and 16 against jaundice. Plants 1 and 14 have been found to be antileprotic, plant 3 antisyphilitic, plants 6 antitubercular and plant 8 antitumor. Plants 1, 2, 3, 8, 9, 10, 11, 12, 15 and
16 have been found to be effective against rheumatic disorders.

It may be recalled that plants 1 to 11 of this study stimulate both cellular and humoral immune response of the body, with the exception of plants 9 and 10 which stimulate only cellular immune response. All other plants, particularly plant 12 to 18 stimulates only humoral immune response. Although all the ailments for which these plants have been used may not require stimulation of the immune response of the body, certain ailments or condition do require immunostimulation or immuno-regulation for effective cure. As evident from the existing literature (Atal et al., 1986; Wagner, 1984a, b), the plants used as tonics or stimulants for invigorating health and for treatment of chronic bronchitis, sour throat, and jaundice of viral origin, tuberculosis, leprosy, gonorrhoea and tumor are very much likely to require immunostimulaton. The plants used against chronic cases of fever may also require stimulation of immune response. But in some cases the curative effect may also be due to the presence of antipyretic substances in some plants.

Allergy such as asthma (Holt and Sly, 2003; Crompton and Mc Hardy, 1991) and rheumatism (Nuki, 1991) are yet other disorders which develop due to alterations in the immunological system of the body. In allergic asthma, individuals start producing Ig E-type of antibodies instead of the normal IgG-type, which in turn are responsible for the allergic manifestations. Type-4 allergic manifestations are ascribed to derangement of cell-mediated immunity involving T-cells. In rheumatoid disorders, circulatory antiglobulins to antibodies (rheumatoid factor) formed as a result of immunological imbalance become attached to the bone joints. There is also evidence of T-Cell alterations in persons genetically predisposed to rheumatism. The immunostimulants, therefore, are most likely to be beneficial in these disorders.

Spleen is one of the secondary lymphoid organs where lymphocytes can interact with each other and with the antigens since, phagocyte, macrophages, antigen presenting cells, mature T and B lymphocytes were collected together in this organ (Ferguson, 1991a). Disorders of spleen may accompany impairment of immune system.

Similarly, kuffer cells which have microbicidal activity are located in liver and they may be affected in liver disorders (Ferguson, 1991b). In view of these facts, it is most likely that the curative effect of these plants in spleen and probably also in liver disorders may also be due to their immunostimulant activity.

REFERENCES


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