Evaluation of Immunomodulation Potential of Red Velvet Mite, *T. grandissimum* Using Swiss Albino Mice

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Abstract: The present study was carried out to find out immunomodulatory potential of red velvet mites, *T. grandissimum* against chosen antigen (SRBC) using animal model, swiss albino mice. Immunological parameters such as antibody titre, delayed type hypersensitivity, lymphocyte sub population are used to evaluate immunomodulatory potential of test sample. Oral administration of the methanol extracts of *T. grandissimum* (100mg/kg body weight, 150mg/kg body weight) for 7 days around immunization, caused a dose-related decrease in DTH (40% and 50% respectively) reactivity in mice. This indicated the effectiveness of red velvet mite extracts to inhibit DTH reactivity. The augmentation of the humoral response as evidenced by an enhancement of antibody responsiveness to SRBC in mice as a consequence of RVME administration indicates the enhanced responsiveness of lymphocytes subsets involved in antibody synthesis. The results proved that the extracts of the mites enhanced humoral immune response. The antibody titre value of control mice showed 5.6±0.4 log titre, but mite extracts 100 mg/kg/bw and 150 mg/kg/bw administered mice showed 7.8±0.6 and 8.8±0.5 log titre values respectively. From the present investigation confirmed the immunomodulatory potential of red velvet mites.

Key words: Antibody, immunomodulation, *T. grandissimum* and Swiss albino mice.

INTRODUCTION

Animals have been used as medicinal resources for the treatment and relieve of a myriad of illness and diseases in practically every human culture. The phenomenon of zootherapy represents a strong evidence of the medicinal use of animal resources (Costa-Neto, 2005). Science has already proven the existence of immunological, analgesic, antibacterial, diuretic, anesthetic and antirheumatic properties in the bodies of arthropodan group of animals (Conconi Julieta and Pihofose, 1988). Insects had been used in many parts of the world for various illness (Costa-Neto et al., 2000, Trowell 2003, Solavan et al., 2004, Wilsanand et al., 2007). It is well known that the annual global trade in animal-based medicinal products account for billions of dollars per year (Kunin and Lawton, 1996). About 150 prescription drugs currently in use in United States of America, 27 have animal origin (World Resources Institute, 2000). Over 500 species of insects, mites and spiders are used as medicines to cure both common and complicated ailments in Chhattisgarh, India (Oudhia, 1995). For example, the oil from the red velvet mite (*Trombidium grandissimum* Koch 1867) is useful for paralysis. As paralysis is one of the immunity related disease, it is believed that the extracts of the red velvet mites could influence immunity. As there is no scientific validation of this immunomodulation effect of red velvet mite, the present study was planned to find out the immunomodulating potential in the red velvet mites, *Trombidium grandissimum*.

MATERIALS AND METHODS

For the experimental study, Swiss albino mice (BALB/c) were selected and fed with the whole body extracts of red velvet mites (100 and 150 mg/kg/b.w/day) for the immunostimulatory experimental studies.

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Antibody Estimation:
The mice were immunised with SRBC (0.5×10⁴ cells per ml per 100g, ip) on day 0. The animals were then divided into three groups, each group comprising of six mice. Animals in one group were injected with normal saline while the second and the third group received red velvet mite extract daily at a dosage of 100mg/kg to 150mg/kg body weight per day 1 to 6. On day 7, the animals were lightly anaesthetized with ether and blood was collected from retro-orbital plexus. The serum was separated and the haemagglutination titre was estimated using microtitre plates. Two-fold dilutions (0.025ml) of sera were made in microtitre plates with saline. Two each well 0.025ml of 1% (v/v) SRBC was added. The plates were incubated for 1h at 37°C and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre, which was expressed in a graded manner, the minimum dilution (1/2) being ranked as 1. The different groups were compared for statistical analysis.

Cell Mediated Immune Response:
Cell mediated immune response in mice on treatment with the extracts of red velvet mites was traced using Delayed type hypersensitivity assay (DTH).

Delayed Type Hypersensitivity:
The DTH response to sheep red blood cells (SRBC) in mice was detected following the method of Dhasarathan et al., 2008. The mice were sensitized with 10% SRBC (1×10⁶ cells) at day 0 and day 7 subcutaneously (s.c) in the right foot pad region. They were divided into two groups: one group was fed with the extracts of red velvet mite (at the dose level of 100mg/kg b.w and 150mg/kg b.w) for 7 days. The other group was control group that were fed with standard pellets only. On day 9, both groups were challenged with 1×10⁶ SRBC cells intradermally into the right foot pad of each mouse, while PBS was injected into the left hind paw. The increase in foot pad thickness was measured 24h later using a vernier calliper. The degree of DTH reaction was expressed as the percentage increase in foot pad thickness over the control values.

B and T Cell E-Rosette Assay:
The mice were treated with the extracts of red velvet mites (RVME). Serum sample was taken from control and RVME treated mice from retroorbital plexus. About 5-10 ml of blood collected from control and RVME treated mice was introduced into sterile flask containing five sterile glass beads and defibrinated. The defibrinated blood over layer on lymphoprep solution and centrifuged. The interphase (containing lymphocytes) was removed using pipette. Lymphocytes were loaded into the activated nylon wool column. Then the column was held vertically above an eppendorf tube, now hot saline (about 60°C) was slowly dripped into the column. The hot saline passing out of the column was collected in the eppendorf tube, which contain T lymphocytes. 0.2 ml of the saline containing lymphocytes (from the eppendorf tube containing T cell) was taken in a separate eppendorf tube. To this 0.2 ml of 1% SRBC was added and then the mixture was centrifuged for 12 minutes at 1600 rpm. After centrifugation the sample were incubated in an ice box or refrigerator (at 4°C) for 5 minutes. After cold incubation, the pellet in the eppendorf tube was resuspended by gentle flushing with a Pasteur pipette. Then a drop of it was taken in a clean dry slide, observed and enumerated T cells under the microscope (20x/40x) for rosettes. Number of T cell rosettes formed were observed among hundred lymphocytes observed was tabulated, after hot saline elution, cold saline then dripped through the column. The column was gently squeezed to release the adhered B cells (repeat twice). The cold saline dripping out of the column was collected in another eppendorf tube. About 0.2 ml of the saline containing B lymphocyte (from the eppendorf tube containing B cell) was taken in a separate eppendorf tube. To this 0.2 ml of 1% SRBC was added and then the mixture was centrifuged for 12 minutes at 1600 rpm. After centrifugation, the sample were incubated in a refrigerator (at 4°C) for 5 minutes. After cold incubation, the pellet in the eppendorf tube was resuspended by gentle flushing with a Pasteur pipette. Then a drop of it was taken in a clean dry slide, observed and enumerated B cells (at the microscope (20x/40x) for rosettes. Number of B cell rosettes formed were observed among hundred lymphocytes observed was tabulated.

RESULTS AND DISCUSSION

Red velvet mite extract administered to mice produced a significant (P<0.05) increase in antibody titre as in Table 1. The antibody titre increased from 5.6±0.3 in control to 7.8±0.4 in velvet mite extract (100mg/kg feed) given animals and 8.7±0.4 in 150mg/kg administered group. The present study was carried out to findout the effect of red velvet mites extracts of the anti-SRBC antibody titre. The results proved that the extracts of the mites enhanced humoral immune response.
Table 1: Effect of red velvet mites extracts (RVME) on humoral immune response (anti-SRBC antibody) in Swiss albino mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Anti SRBC antibody titre (mean ± S.E.)</th>
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<tbody>
<tr>
<td>Control</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td>RVME (100mg/kg body weight)</td>
<td>7.8±0.6</td>
</tr>
<tr>
<td>RVME (150mg/kg body weight)</td>
<td>8.8±0.5</td>
</tr>
<tr>
<td>RVME - Extract of red velvet mite; P&lt;0.05</td>
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</tbody>
</table>

Cell Mediated Immune Response:

Oral administration of the methanol extracts of T. grandissimum (100mg/kg body weight, 150mg/kg body weight) for 7 days around immunization, caused a dose-related decrease in DTH reactivity in mice. The drug treatment caused a significant decrease in DTH (Table-2). This indicated the effectiveness of red velvet mite extracts to inhibit DTH reactivity. The interaction of sensitized T-cells with presented antigen is known to be associated with the release of mediators such as histamine, products of arachidonic acid metabolism (Griswold et al., 1982). Therefore, the inhibitory action could be due to the influence of fraction on the biological mediators. Mungantiwae et al., 1999. suggested that the decreased DTH reactivity may be due to the simultaneous presence of high titres of antibodies, promoting the elimination of antigen by non-presenting phagocytes. In the present study, antibody titre was high in RVME treated mice.

The augmentation of the humoral response as evidenced by an enhancement of antibody responsiveness to SRBC in mice as a consequence of RVME administration indicates the enhanced responsiveness of macrophages and B-lymphocytes subsets involved in antibody synthesis (Benacerraf, 1978). Thus the augmentation of humoral response to SRBC confirm the pivotal role played by the macrophages in coordinating the processing and presentation of antigen to B cells, the augmentation of humoral response to SRBC invivo reveals that the methanol fraction of T. grandissimum enhanced the effect by facilitating these processes.

Table 2: Effect of the extracts of red velvet mites on SRBC-induced DTH in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(mg/kg)</th>
<th>Foot pad thickness Mean% edema at 24h</th>
<th>%Change in DTH reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>32.00±0.24</td>
<td></td>
</tr>
<tr>
<td>RVME 100</td>
<td>18.54±0.36</td>
<td>42.06</td>
<td></td>
</tr>
<tr>
<td>RVME 150</td>
<td>14.37±0.13</td>
<td>55.09</td>
<td></td>
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</table>

n=6 per group: results expressed as mean ± SE
Significance test applied - student ‘t’-test, P<0.01

T Cell and B Cell Count:

Total number of T cells and B cells in control and in red velvet mite extract administered mice were counted, using e-rosette assay. The results are presented in table 3.

T and B cell count in the red velvet mites extract fed mice were increased. This indicates a promotion in lymphopoiesis in mice. The present study indicates that the red velvet mite is having some immune potentiating bio-active compounds in its body and further research is needed.

Table 3: T cell and B-cell counts in control and red velvet mite’s extract treated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage occurrence of T and B cells</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T-cells</td>
</tr>
<tr>
<td>Control</td>
<td>39.7± 3.2</td>
</tr>
<tr>
<td>RVME(100mg/Kg body weight)</td>
<td>40.2± 4.3</td>
</tr>
<tr>
<td>RVME(150mg/Kg body weight)</td>
<td>41.6± 3.4</td>
</tr>
</tbody>
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REFERENCES


