Immunomodulatory activity of ethanol extract of *Sonerila tinnevelliensis* Fischer (Melastomataceae) whole plant in mice

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**Abstract**

**Background:** Immunomodulation is a process, which alters the immune system of an organism by interfering with its functions. This interference results in either immunostimulation or immunosuppression. An immunomodulator is a substance that helps to regulate the immune system. Keeping in this view, efforts have to be directed to modulate the immune responses, to permit effective treatment of various ailments associated with immune system and thus the development of a safe and effective immunomodulator for clinical use.

**Methods:** *Sonerila tinnevelliensis* Fischer an ethnomedicinal plant was studied for its immunomodulatory activity. Immunomodulatory activity of different doses of ethanol extract of *S. tinnevelliensis* was evaluated in Swiss albino mice. Mice were treated with two doses (200 and 400mg/kg body weight) for 5 days. Body weight, relative organ weight, delayed type hypersensitivity (DTH) response and Haemagglutinin titre (HT) were studied in various groups of animals.

**Results:** The results obtained show a significant increase \( p < 0.05 \) in body weight and relative organ weight of spleen, liver and kidney at dose of 400mg/kg. The *S. tinnevelliensis* extract elicited a significant increase \( p < 0.05 \) in the DTH response at dose of 400mg/kg. In the HT test, the plant extract showed a stimulatory effect at all doses. The dose of 400mg/kg significantly \( p < 0.05 \) increases the WBC count, compared with the control group.

**Conclusions:** Overall, *S. tinnevelliensis* showed a stimulatory effect on both humoral and cellular immune functions in animal models.

**Keywords:** *Sonerila tinnevelliensis*, Delayed type hypersensitivity, Haemagglutinin Titre, Flavonoid.

**Introduction**

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of nonspecific system, that is, granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence both immune stimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world [15]. Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system [19]. To overcome these problems a number of drugs from natural source either herbal or mineral have been used as to alter the human immune system [14]. There are several medicinal plants employed in different system of medicine throughout the world to improve the immunological disorders.

*Sonerila* includes approximately 175 species. *Sonerila tinnevelliensis* is used to cure liver diseases and gastritis. Its leaf extract is orally administered to cure body swelling by Kanikaran. Decoction of fresh leaves is consumed on an empty stomach once in a day to get relief from rheumatic complaints. A handful of fresh leaves consumed on an empty stomach once in a day for 12 to 15 days to get relief from rheumatic problems. The biological activities such as *in vitro* antioxidant, antiinflammatory, hepatoprotective, anticancer and antidiabetic activities were...
reported [6-10]. However, the immunostimulatory potential of *S. tinnevelliensis* plant on immune system has not yet been explored. Therefore, the objective of the present study was to study the immunomodulatory activity of *S. tinnevelliensis* whole plant.

**Materials and Methods**

**Plant material**

The whole plant of *Sonerila tinnevelliensis* Fischer was collected from Agasthiarimalai Biosphere Reserve, Western Ghats, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin for further reference.

**Animals**

Study was conducted in Swiss albino female mice (20 - 25 g). The animals were bred and maintained under standard laboratory conditions (temperature 25 ± 2 °C and light period of 12 h). The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water ad libitum. Study was carried out as per IAFC approval No: 82/ PHARMA/SCRI, 2010.

**Treatment protocol**

The plant extract was administered i.p. for 5 days at doses of 200 and 400 mg/kg body weight. The dose volume was 0.2 ml. The control animal group received the same volume of normal saline and left untreated. The animals were divided into four groups (Groups I - IV). Each group comprised of a minimum of five animals. The control group (Group I) was given normal saline and the treatment groups were given the whole plant extract of *S. tinnevelliensis* at the doses of 200 mg/kg and 400 mg/kg body weight (Groups II and III) for five days, respectively. Group IV mice were given dexamethasone, at 20mg/kg body weight. The animals were humanized 24 h after the last dose. Body weight gain (percentage) and relative weight of kidney, liver and spleen (organ weight/100 g of body weight) were determined for each animal.

**Assessment of humoral immune functions**

Animals within the experimental groups were challenged with 0.2 mL of 10% sheep red blood cells (SRBC), i.p., on the 10th day of the initiation of experiment. The haemagglutinin titre assay was also studied in these animals.

**Assessment of haematological and liver marker enzymes**

Red blood cell (RBC) count, haemoglobin (Hb) content and White blood cell (WBC) count were measured from freshly following tail vein blood. Total bilirubin was determined as described by Balistrei and Shaw [1]. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalo transaminase (SGOT) and alkaline phosphatase were determined by the method of King and Armstrong [5].

**Statistical analysis**

All values were expressed as mean ± standard error of mean (S.E.M) and comparison between the groups were made by Analysis of Variance (ANOVA). The Data were analysed using the statistical analysis system SPSS (SPSS Software for windows release 10.0; SPSS Inc., Chicago IL, USA).

**Results**

After treatment with two different doses (200 and 400 mg/kg body weight) of whole plant ethanol extract of *S. tinnevelliensis* for 5 days, the Swiss albino female mice were evaluated for immunomodulatory activity. Body weight, relative organ weight, delayed type hypersensitivity (DTH) and haemagglutinin titre (HT) were studied in all the treated animal groups.

**Effect of plant extract on Body weight and relative organ weight**

In the present study treatment with the whole plant ethanol extract of *S. tinnevelliensis* was effective in increasing the body weight and also the weight of spleen, liver and kidney (Table 1).

**Table 1: Effect of whole plant ethanol extract of *Sonerila tinnevelliensis* (ST) on the body weight and relative organ weight**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Treatment type and Dosage</th>
<th>Body weight and relative weight of organs (mean± SE) in g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body weight</td>
</tr>
<tr>
<td>Group I</td>
<td>Saline (Normal control)</td>
<td>23.56±1.38</td>
</tr>
<tr>
<td>Group II</td>
<td>ST extract (200 mg/kg b. wt.)</td>
<td>26.93±1.14</td>
</tr>
<tr>
<td>Group III</td>
<td>ST extract (400 mg/kg b. wt.)</td>
<td>24.88±1.45</td>
</tr>
<tr>
<td>Group IV</td>
<td>Dexamethasone (20 mg/kg b. wt.)</td>
<td>24.88±1.45</td>
</tr>
</tbody>
</table>

Each Value is SEM of 6 individual observations: *Comparison between normal control and drug treated groups. Level of significance: * p <0.05; ** p <0.01.
Effect of plant extract on humoral immunity parameters
In the haemagglutinin titre (HT) (Table 2), doses 200 mg and 400 mg/kg showed titre value of 3.98 and 5.64 respectively, while the titre value of control was 2.73, thus showing a significant increase in the titre values with doses of 200 and 400 mg/kg in the treated groups ($p < 0.05$).

Effect of plant extract on cell mediated immunity parameters
The plant extract at dose of 400mg/kg elicited a significant ($p < 0.05$) increase in DTH response (Table 2), compared to the control animals. In this study, dexamethasone (Group IV) decreased DTH response, compared to the control group.

Table 2: Effect of whole plant ethanol extract of Sonerila tinnevelliensis (ST) on DTH response in comparison with dexamethasone and on the HT titre by using SRBC as the antigen in mice

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Treatment type and Dosage</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Foot Pad Edema (mm)</td>
</tr>
<tr>
<td>Group I</td>
<td>Saline (Normal control)</td>
<td>0.34±0.018</td>
</tr>
<tr>
<td>Group II</td>
<td>ST extract (200 mg/kg b. wt.)</td>
<td>0.41±0.024ns</td>
</tr>
<tr>
<td>Group III</td>
<td>ST extract (400 mg/kg b. wt.)</td>
<td>0.59±0.054*</td>
</tr>
<tr>
<td>Group IV</td>
<td>Dexamethasone (20 mg/kg b. wt.)</td>
<td>0.53±0.018*</td>
</tr>
</tbody>
</table>

Each Value is SEM of 6 individual observations: *Comparison between normal control and drug treated groups. Level of significance: * $p <0.05$; ns: not significant.

Effect of plant extract on blood parameters and liver enzymes
There was no significant elevation in the levels of SGOT, SGPT and ALP as a result of treatment with S. tinnevelliensis (Table 3). Total bilirubin content was slightly increased. No significant difference in blood parameters was recorded in various test groups. The doses of 200 and 400mg/kg increased the WBC count, compared with the control group.

Table 3: Effect of whole plant ethanol extract of Sonerila tinnevelliensis (ST) on the haematological parameters and serum liver marker enzymes in drug treated mice

<table>
<thead>
<tr>
<th>Treatment Groups Type and Dosage</th>
<th>Haematological parameters</th>
<th>Biochemical (serum) parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb (g/dl)</td>
<td>RBC (x106/mm2)</td>
</tr>
<tr>
<td>Group I Saline (Normal Control)</td>
<td>14.01±0.94</td>
<td>3.50±0.16</td>
</tr>
<tr>
<td>Group II ST extract (200 mg/kg b. wt.)</td>
<td>11.84±0.76*</td>
<td>3.57±0.45</td>
</tr>
<tr>
<td>Group III ST extract (400 mg/kg b. wt.)</td>
<td>13.60±0.17</td>
<td>4.86±0.51*</td>
</tr>
<tr>
<td>Group IV Dexamethasone (20 mg/kg b. wt.)</td>
<td>12.55±0.19</td>
<td>3.96±0.28</td>
</tr>
</tbody>
</table>

Each Value is SEM of 6 individual observations: *Comparison between normal control and drug treated groups. Level of significance: * $p <0.05$;

Discussion
Modulation of the immune response in the course of stimulation or suppression may help in maintaining a disease – free state. Drugs or agents which improve host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy. Immunomodulatory activity of ethanol extract of S. tinnevelliensis was explored by evaluating its effect on haemagglutinin titre (HT) and delayed type hypersensitivity (DTH) response. In the present study, ethanol extract of S. tinnevelliensis whole plant showed an overall stimulatory effect on the immune functions in mice. Stimulatory effects were observed on both humoral and cellular immunity. In HT test, the plant showed an increase response in all doses, but this increase was significant only in dose 400 mg/kg. This activity could be due to the presence of flavonoids or saponins which augment the humoral response, by stimulating the macrophages and B-lymphocytes subsets involved in antibody synthesis. It appears that 400 mg/kg is the optimum dose in mice in humoral immunity.

In DTH test, the DTH response, which directly correlates with cell mediated immunity (CMI), was found to be highest at the maximum dose (400 mg/kg) tested in the extract. The mechanism behind this elevated DTH during the CMI responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lymphokines, recruiting more scavengers cells to the site of reaction. An increase in DTH response indicates a stimulatory effect of the plant which has occurred on the lymphocytes and accessory cell types required for the expression of this reaction.

The phytochemical screening of S. tinnevelliensis whole plant revealed the presence of flavonoid, tannin, phenolic compound, terpenoid and saponin. Some phytoconstituent are able to regulate immune responses like polysaccharides, polyphenolls, flavonoids, alkaloids, saponins and omega fatty acids. The presence of flavonoids and saponins may be responsible for the observed immunostimulatory activity.
acid. These types of components are reported to exhibit immunomodulatory activity in various experimental models \[20\]. *S. tinnevelliensis* whole plant has stimulated both humoral as well as cellular arms of immune system. This plant also is rich source of flavonoids and phenols which may act as immunostimulatory.

**Conclusions**

The studies have demonstrated immunostimulatory properties of ethanol extract of *Sonerila tinnevelliensis* whole plant in various *in vivo* experimental methods. Further studies to elucidate the exact immunostimulatory mechanism of *Sonerila tinnevelliensis* need to be explored.

**References**