4.1 REVIEW OF *LEPTADAENIA RETICULATA*

*Leptadaenia reticulata* (Retz.) Wight. & Arn. (family Asclepiadaceae) is a climber. It is commonly known as Jivanti, Swarnjivanti or Dori. The botanical identity of the drug made from this plant is highly disputed. *L. reticulata* as the source of the drug and real Jivanti. Ayurvedic formulary of India also accepts this as the true drug plant (Chunekar, 1999).

*L. reticulata* is distributed in tropical and subtropical parts of Asia and Africa, Burma, Sri Lanka, Malayan peninsula, Philippines, Mauritius and Madagascar. In India, it is found in Gujarat, Punjab, Himalayan ranges, Khasia hills, Konkan, Nilgiris, South India, Sikkim, Deccan and Karnataka. The true centre of origin of this plant is not known, but its oldest description in *Atharva Veda* suggests Indian origin. *L. reticulata* is a climber/liana having stem with cork-like, deeply cracked bark with numerous branches, among which the younger ones are glabrous (Chemexcil, 1992). Seed germination takes place from June to September after one or two showers of rain under field conditions. Flowering takes place throughout the year. Fruit formation takes place only during December–February. The maturation of fruits/seeds continues up to mid-May and dispersal of seeds takes place during June–July.

**Classification:** USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Data from 07-Oct-06] Dassanayake, & Fosberg (1980).

Kingdom : Plantae  
Class : Angiospermae  
Order : Gentianales  
Family : Asclepiadaceae  
Subfamily : Asclepiadoideae  
Tribe : Ceropegieae  
Subtribe : Leptadeniinae  
Genus : *Leptadenia*  
Species : *Reticulata*

**Indian synonames:**
Chapter-IV

Immunomodulatory Studies of *Leptadaenia reticulata*

Bengoli - Bhadjivai
English - Leptadenia
Gujarati - Dodi, Radarudi
Hindi - Dodi
Kannada - Hiriyahalle
Marathi - Hiranvel
Sanskrit - Jivanti
Tamil - Palaikkodi
Telagu - Kalasa

**PHYTOCHEMICAL STUDIES**

*Leptadaenia reticulata* contains many important phytoconstituents of plants. Previously reported chemical constituents of *Leptadaenia reticulata* are α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentriacontanol (Krishna *et al.*, 1975), a triterpene alcohol simiarenol (Subramanian *et al.*, 1977) and apigenin (Sastry *et al.*, 1985). Pregnane glycosides reticulatin, deniculatin and leptaculatin have also been isolated from the aerial parts (Srivastav *et al.*, 1995). Pregnane glycosides reticulatin, deniculatin, leptaculatin isolated from aerial parts which on hydrolysis give calogenin tocopherols. Other are acetyl alcohol, lupanol 3-O diglucoside, lepididine 1, saponins, flavonoid, luteolin, diosmtn and tannin. Leaves contain two resins and also a bitter neutral principle, albuminous and colourinsing matter, Ca-oxalate, glucose, carbohydrate and tartaric acid (Sastry *et al.*, 1985). The preliminary phytoconstituent studies of aerial part of *L. reticulata* consist of 6-7% moisture, 17.5% total nitrogen, different flavonoids, moisture (6-7%); total ash (5.5 to 6.5%); insoluble ash (0.1%), calcium (0.6%), sodium and potassium calculated as chlorides (2.16 to 2.24%), reducing sugars aldohexos, ketohexoses and pentoses, other constituents like proteins, gums, a steam volatile unidentified ferric (Fe+++ ) greening substance and a substance which holds reducing sugars molecules in glycosidal linkage.
Pharmacological activities:

It is considered to be a *Rasayana* (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body (Daniel, 2006). Its medicinal use dates back to about 4500 to 1600 BC, as mentioned in *Atharva Veda*, Kanda eight, Sukta two. The *Atharva Veda* mentioned its uses as a life and strength giver, propagator of milk and useful in many other ailments. Charaka described it as an important rasayana drug, capable of maintaining youthful vigour and strength, and Vagbhata incorporated it among the ten drugs that constitute the Jivaniya gana or the vitalizing group. It promotes health and vigour, improves the voice, alleviates the three dosas—vata, pitta and kapha, and cures eye diseases, haematemesis, emaciation, cough, dyspnoea, fever, burning sensation, dysentery, night-blindness, poisonous affections and tuberculosis (Sivarajan and Balachandran, 1999). Anjaria *et al.*, 1997 mentioned it as a stimulant, galactagogue, eye tonic, astringent, prolapse of uterus, vagina, controlling habitual abortion and maintain pregnancy. Its restorative property makes it an important ingredient in the preparation of ‘Chyawanprash’ (an Ayurvedic tonic). The leaves, paste and roots are taken orally with water to cure gangrene by the Bhils of southern Rajasthan (Singh and Pandey, 1998) Kirtikar and Basu (Kirtikar and Basu, 1994) mentioned it as a stimulant and tonic. Alcoholic (50%) extract of roots and leaves shows antibacterial activity against Gram-positive and Gram-negative bacteria.
Aqueous extract of the stem of this plant demonstrated vasodilator, transient, inotropic, chronotropic and prolonged hypotensive effect in dogs (Anjaria et al., 1960). A number of studies have been carried out on its galactogogue property. On lactating rats, its ether extract was found to increase lactation (Anjaria et al., 1975). The lactogenic effect of the plant was also studied on Gir cows (Anjaria et al., 1974). It showed an increase in the secretions of the accessory sex organs in the mice (Sud et al., 1983). Another formulation Leptaden, has been shown to provide effective treatment in cases of deficient lactation and lack of lactation in humans (Habla and Sitaratna, 1972). The extract of the leaf shows antibacterial and antifungal activities (Patel and Dantwala, 1958).

The alcoholic extracts (50%) of the leaves and roots are reported to be active against Micrococcus pyrogenes, Bacillus megatherium, Escherichia coli, Salmonella typhi, Proteus vulgaris and Trychophyton rubrum. The entire plant has been clinically tested and found useful in treatment of habitual abortion in women. Aqueous extract of the aerial parts has been reported to produce prolonged and pronounced hypotensive effect in dogs (Rastogi and Mehrotra, 1999). The antioxidant, wound healing, galactagogue and lactogenic activity of the plant has been reported (Diallo et al., 2002 and Anjaria et al., 1975).

**Acute toxicity studies:**

Acute toxicity study was carried out on whole plant extract of Leptadenia reticulata. The extract was found to be safe up 3000mg/kg of body weight. The oral acute toxicity study was performed using the up and down procedure (OECD guidelines).
4.2 RESULT OF LEPTADAENIA RETICULATA

4.2.1 Qualitative chemical tests

The aqueous extracts of *Leptadaenia reticulata* showed the presence of Carbohydrate, Glycosides, Saponin, Phenolic compound, Flavonoids, Steroid, Terpenoids and Tanin and the ethanolic extract showed presence of Protein, Alkaloid, Tanin, Flavonoids, Steroid, Terpenoids, Saponin, and Glycosides.

4.2.2 TLC Studies

Thin layer chromatographic studies were performed for aqueous and ethanolic extracts of *Leptadaenia reticulata*. Aqueous extracts of *Leptadaenia reticulata* have shown best separation in n-Butanol: Glacial acetic acid: Distilled water (4:1:5) and give ten spots ($R_f$ value-0.10, 0.15, 0.27, 0.31, 0.50, 0.58, 0.64, 0.080, 0.91, 0.97). Spots were visualized by Ninhydrin reagent.

Ethanolic extract of the drug best separates in Chloroform: Acetone: Formic acid (74+15+5) and eight spots ($R_f$ value-0.08, 0.19, 0.30, 0.33, 0.79, 0.94, 0.97, 0.99). Spots were visualized by Anisaldehyde-sulphuric acid reagent.

4.2.3 Toxicity Studies

In toxicity test with *Leptadaenia reticulata*, no mortality was recorded in both the extracts.

4.2.4 Carbon Clearance Test

Carbon Clearance depends on time and it was calculated as phagocytic index of time interval between the treated groups of animals compared with the control group. The mean phagocytic index of control (Group I) was found to be 1.007 ± 0.020. The crude aqueous extract of *Leptadaenia reticulata* treated groups III and IV were elevated as 1.195 ± 0.038 (P<0.025) and 1.431 ± 0.054 (P<0.001), while group I was observed slightly on lower side as 0.989 ± 0.061 when animals treated with 100,
150 and 50mg/kg b.wt intraperitoneally for seven days (Table 4.2.1 and Fig 4.2.1).

The ethanolic extract had given significantly increased phagocytic index as 1.465 ± 0.067 (P<0.001), 1.625 ± 0.055 (P<0.001) and 1.700 ± 0.049 (P<0.001) respectively with 50, 100 and 150mg/kg b.wt intraperitoneally for seven days (Table 4.2.2 and Fig 4.2.2).

4.2.5 Delayed Type Hypersensitivity Test

Delayed Type Hypersensitivity response to SRBC was calculated as a measure of paw volume (in mm) for each animal and compared with control group I which was injected 2ml of 5% Normal saline intraperitoneally for seven days. Paw volume was calculated after 24, 48, 72 and 96 hrs. The decreased value for group II, III and IV after 24 hrs were found to be 1.58 ± 0.027 ml, 1.55 ± 0.016 ml and 1.51 ± 0.011 ml and after 48 hrs there were 0.92 ± 0.019 ml, 0.094 ± 0.028 ml and 0.89 ± 0.018 ml (P<0.05) after 72 hrs it was 0.64 ± 0.013 ml, 0.58 ± 0.012 ml and 0.52 ± 0.015 ml (P<0.05) and finally after 96 hrs paw volume reduced significantly 0.24 ± 0.015 ml (P<0.05), 0.23 ± 0.014 ml (P<0.05) and 0.20 ± 0.021 ml (P<0.025) respectively (Table 4.2.3 and Fig 4.2.3).

Animal treated with crude ethanolic extract showed reduced paw volume after 24, 48, 72 and 96 hrs. The decline in paw volume for group II, III and IV after 24 hrs were found to be 1.41 ± 0.028 ml (P<0.05), 1.35 ± 0.019 ml (P<0.05) and 1.21 ± 0.013 ml (P<0.025) and after 48 hrs there were 0.88 ± 0.024 ml, 0.83 ± 0.011 ml (P<0.05) and 0.82 ± 0.025 ml (P<0.05) after 72 hrs it was 0.58 ± 0.015 ml, 0.55 ± 0.017 ml and 0.54 ± 0.019 ml and finally after 96 hrs paw volume where paw volume does not showed any significant change and it was 0.29 ± 0.012 ml, 0.28 ± 0.023 ml and 0.28 ± 0.028 ml respectively (Table 4.2.4 and Fig 4.2.4).

4.2.6 SRBC Agglutination Test

Agglutination titer to sheep red blood erythrocyte was calculated and compared with Group I (control). Group II, III and IV were treated
with crude aqueous extract orally for ten days (50, 100, 150mg/kg b.wt) and on 10th day agglutination titer were observed in various serum dilution (X: 20, X: 40, X: 80, X: 160, X: 320). An increased was observed at the dose of 50, 100 and 150mg/kg b.wt. (Table 4.2.5 and Fig 4.2.5).

With crude ethanolic extract Group I was given 5% normal saline and agglutination titer was compared with treated one. Group II, III and IV that received the crude ethanolic extract at the dose of 50, 100 and 150mg/kg b.wt respectively, decrease was observed in the animals of group III and IV which received 100 and 150mg crude ethanolic extract/kg b.wt. while group II did not showed any change in the activity. (Table 4.2.6 and Fig 4.2.6)

4.2.7 Drug Induced Myelosuppression Using Cyclophosphamide

The Group I was control and received as usual 2 ml of 5% of normal saline and various hematological observations were taken. In them the mean haemoglobin was 13.11 ± 0.12 gms/dl, mean RBC count was 4.65 ± 0.154 million/mm³ and mean WBC count was 13.27 ± 0.425 thousand/mm³. Neutrophils count was 53.10 ± 1.51%, Lymphocytes count was 40.82 ± 1.08%, Monocytes count was 2.45 ± 0.38%, Eosinophil count was 2.35 ± 0.51% and platelets count was 3.23 ± 0.243 lacs/mm³. In Group II cyclophosphamide (3mg/kg b.wt) were administered and there was a significant decrease in all hematological parameters studied except Neutrophils and Monocytes count which were slightly increased. Mean haemoglobin was 8.25 ± 0.29 gms/dl (P<0.025), mean RBC count was 3.72 ± 0.121 million/mm³ (P<0.05) and WBC count was 11.16 ± 0.241 thousand/mm³ (P<0.05). Mean Neutrophils count was 59.84 ± 0.89%, Lymphocytes count was 33.59 ± 0.85% (P<0.05), Monocytes count was 3.01 ± 0.26%, Eosinophil count was 2.41 ± 0.24% and platelets count was 2.40 ± 0.456 lacs/mm³ (P<0.05).

Group III, IV and V were administered crude aqueous extract of (50, 100 and 150mg/kg b.wt) with cyclophosphamide intraperitoneally,
in them the mean was found to be 11.02 ± 0.22 (P<0.05), 11.78 ± 0.16 (P<0.05), 12.41 ± 0.19 gms/dl (P<0.05) respectively. Mean RBC count was 3.95 ± 0.094, 4.11 ± 0.173, 4.24 ± 0.117 million/mm³ (P<0.05), mean WBC count was 11.79 ± 0.151, 12.16 ± 0.114, 12.87 ± 0.512 thousand/mm³ (P<0.05), Neutrophils count was 57.57 ± 1.12%, 55.43 ± 1.20%, 55.13 ± 0.97%, Lymphocytes count was 35.13 ± 0.99%, 38.43 ± 1.02% (P<0.05), 38.98 ± 0.74% (P<0.05), Monocytes count was 2.14 ± 0.18% (P<0.05), 2.58 ± 0.43%, 2.87 ± 0.23%, Eosinophils count was 2.23 ± 0.56%, 2.32 ± 0.39%, 2.38 ± 0.42% and platelets count was 2.84 ± 0.215, 2.86 ± 0.483, 2.98 ± 0.381 lacs/mm³ (Table 4.2.7 and Fig 4.2.7 to 4.2.14).

In another sets of experiments Group III, IV and V were administered crude ethanolic extract of (50, 100 and 150mg/kg b.wt) with cyclophosphamid intraperitoneally, in them the mean haemoglobin was found to be 9.84 ± 0.15, 10.05 ± 0.50, 10.13 ± 0.38 gms/dl respectively. Mean RBC count was 3.79 ± 0.142, 3.75 ± 0.219, 3.68 ± 0.087 million/mm³, mean WBC count was 11.54 ± 0.326, 12.03 ± 0.257, 12.38 ± 0.249 thousand/mm³ (P<0.05), Neutrophils count was 55.52 ± 0.72%, 57.67 ± 1.17%, 57.91 ± 0.76%, Lymphocytes count was 33.81 ± 0.57%, 34.66 ± 0.41%, 35.27 ± 0.39%, Monocytes count was 2.37 ± 0.33% (P<0.05), 2.49 ± 0.48%, 2.64 ± 0.28%, Eosinophils count was 2.61 ± 0.37%, 2.65 ± 0.42%, 2.77 ± 0.36% and platelets count was 2.74 ± 0.511, 2.82 ± 0.349, 2.91 ± 0.410 lacs/mm³ (Table 4.2.8 and Fig 4.2.15 to 4.2.22).

4.2.8 Cytokines (IL-2 and IL-6) Assay

Cytokines (IL-2 and IL-6) level were observed in all Groups (I, II, III and IV) and treated Groups were compared with control (Group I). The IL-2 and IL-6 levels were observed as 24.21 ± 1.352 and 30.58 ± 2.846pg/ml respectively for control. Crude aqueous extract had given relatively higher IL-2 level as 35.47 ± 3.861, 51.34 ± 6.589 (P<0.05),
72.92 ± 7.195pg/ml (P<0.025) and IL-6 level was found stable as 31.61 ± 1.837, 34.89 ± 3.453, 33.48 ± 3.007pg/ml respectively with 50, 100, 150mg/kg b.wt. (Table 4.2.9 and Fig 4.2.23).

Crude ethanolic extract had also given higher IL-2 level as 32.15 ± 4.273, 45.24 ± 5.581 (P<0.05), 61.86 ± 5.162pg/ml (P<0.05) and IL-6 level were found stable as 29.91 ± 3.454, 31.07 ± 2.106, 34.83 ± 4.813pg/ml respectively with 50, 100, 150mg/kg b.wt. (Table 4.2.10 and Fig 4.2.24).

4.2.9 Electrophoresis Study of Serum Protein Profile

For analysis of potential protein complexes, serum from mice was used. Standard protein molecular marker was also run with mice serum to estimate molecular weight of separated protein bands. Results demonstrate that a total of 8 major gel bands could be clearly distinguished after Coomassie blue staining in control and treated groups. Bands 3 and 8 were observed thicker and darker in all treated groups for crude aqueous and ethanolic extract with comparison to control. Many other bands were present in the gel, but they were not distinguishable. So along with the original gel photograph, a sketch representation was also made of that gel (Fig 4.2.25 and 4.2.26).
Table 4.2.1: Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Phagocytic Activity in Carbon Clearance Test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean absorbance ± SD</th>
<th>Phagocytic Index(k) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>15 min</td>
</tr>
<tr>
<td>I. Control</td>
<td>0.3361 ± 0.008</td>
<td>0.2413 ± 0.019</td>
</tr>
<tr>
<td>II. Crude Aqueous extract (50mg/kg body wt.)</td>
<td>0.3289 ± 0.024</td>
<td>0.2366 ± 0.017</td>
</tr>
<tr>
<td>III. Crude Aqueous extract (100 mg/kg body wt.)</td>
<td>0.3232 ± 0.012</td>
<td>0.2172 ± 0.019</td>
</tr>
<tr>
<td>IV. Crude Aqueous extract (150 mg/kg body wt.)</td>
<td>0.3185 ± 0.022</td>
<td>0.1984 ± 0.018</td>
</tr>
</tbody>
</table>

Where, n=6 swiss balb-c mice per group, tabular value represents mean ± S.D. (* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 4.2.1 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Phagocytic Activity.
Table 4.2.2: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Phagocytic Activity in Carbon Clearance Test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean absorbance SD</th>
<th>Phagocytic Index(k) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>15 min</td>
</tr>
<tr>
<td>I. Control</td>
<td>0.3361 ± 0.008</td>
<td>0.2413 ± 0.019</td>
</tr>
<tr>
<td>II. Crude Ethanolic extract</td>
<td>0.2886 ± 0.013</td>
<td>0.1773 ± 0.011</td>
</tr>
<tr>
<td>(50mg/kg body wt.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Crude Ethanolic extract</td>
<td>0.2794 ± 0.027</td>
<td>0.1629 ± 0.028</td>
</tr>
<tr>
<td>(100 mg/kg body wt.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV. Crude Ethanolic extract</td>
<td>0.2648 ± 0.029</td>
<td>0.1512 ± 0.015</td>
</tr>
<tr>
<td>(150 mg/kg body wt.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D. (* P<0.05, ** P<0.025, and ***P<0.001)

Figure: 4.2.2 Effect of Ethanolic Extract of *Leptadaenia reticulata* on Phagocytic Activity.
Chapter-IV  
Immunomodulatory Studies of *Leptadaenia reticulata*

**Table 4.2.3: Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Delayed Type of Hypersensitivity.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw Volume (ml) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hrs</td>
</tr>
<tr>
<td>I. Control</td>
<td>1.59 ± 1.010</td>
</tr>
<tr>
<td>II. Crude Aqueous extract</td>
<td>1.58 ± 0.027</td>
</tr>
<tr>
<td>(50mg/kg body wt.)</td>
<td></td>
</tr>
<tr>
<td>III. Crude Aqueous extract</td>
<td>1.55 ± 0.016</td>
</tr>
<tr>
<td>(100 mg/kg body wt.)</td>
<td></td>
</tr>
<tr>
<td>IV. Crude Aqueous extract</td>
<td>1.51 ± 0.011</td>
</tr>
<tr>
<td>(150 mg/kg body wt.)</td>
<td></td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.  
(* P<0.05, ** P<0.025, and ***P<0.001)

**Figure: 4.2.3 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Delayed Type of Hypersensitivity.**

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Department of Zoology, Dr. H.S. Gour Central University, Sagar (M.P.)
### Table 4.2.4: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Delayed Type of Hypersensitivity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw Volume (ml) ± S.D.</th>
<th>24 Hrs</th>
<th>48 Hrs</th>
<th>72 Hrs</th>
<th>96 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>1.59 ± 0.010</td>
<td>1.01 ±</td>
<td>0.64 ±</td>
<td>0.31 ±</td>
<td>0.016</td>
</tr>
<tr>
<td>II. Crude Aqueous extract (50mg/kg body wt.)</td>
<td>1.41 ± 0.028*</td>
<td>0.88 ±</td>
<td>0.58 ±</td>
<td>0.29 ±</td>
<td>0.012</td>
</tr>
<tr>
<td>III. Crude Aqueous extract (100 mg/kg body wt.)</td>
<td>1.35 ± 0.019*</td>
<td>0.83 ±</td>
<td>0.55 ±</td>
<td>0.28 ±</td>
<td>0.023</td>
</tr>
<tr>
<td>IV. Crude Aqueous extract (150 mg/kg body wt.)</td>
<td>1.21 ± 0.013**</td>
<td>0.82 ±</td>
<td>0.54 ±</td>
<td>0.28 ±</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

### Figure: 4.2.4 Effect of Ethanolic Extract of *Leptadaenia reticulata* on Delayed Type of Hypersensitivity.
Table 4.2.5: Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Agglutination Titre to SRBC

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Dilution in Normal Saline± 50 µl antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X: 20</td>
</tr>
<tr>
<td>I. Control</td>
<td>+</td>
</tr>
<tr>
<td>II. Crude Aqueous extract (50mg/kg body wt.)</td>
<td>+</td>
</tr>
<tr>
<td>III. Crude Aqueous extract (100 mg/kg body wt.)</td>
<td>+</td>
</tr>
<tr>
<td>IV. Crude Aqueous extract (150 mg/kg body wt.)</td>
<td>+</td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

**Figure: 4.2.5** Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Agglutination Titre to SRBC.
Table 4.2.6: Effect of Crude Ethanol Extract of *Leptadaenia reticulata* on Agglutination Titre to SRBC

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Dilution in Normal Saline±50 µl antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X: 20</td>
</tr>
<tr>
<td>I.</td>
<td>Control</td>
</tr>
<tr>
<td>II.</td>
<td>Crude Ethanol extract (50mg/kg body wt.)</td>
</tr>
<tr>
<td>III.</td>
<td>Crude Ethanol extract (100 mg/kg body wt.)</td>
</tr>
<tr>
<td>IV.</td>
<td>Crude Ethanol extract (150 mg/kg body wt.)</td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D. (*P<0.05, **P<0.025, and ***P<0.001)

Figure: 4.2.6 Effect of Ethanol Extract of *Leptadaenia reticulata* on Agglutination Titre to SRBC.
Chapter-IV  Immunomodulatory Studies of *Leptadaenia reticulata*

**Figure: 4.2.7** Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Haemoglobin Concentration.

![Graph showing effect of Crude Aqueous Extract on Hb Gms/dl Mean±SEM](image)

**Figure: 4.2.8** Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on RBCs count.

![Graph showing effect of Crude Aqueous Extract on RBC Mean±SEM Million/mm3](image)
Figure: 4.2.9 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on WBCs Count.

![Bar chart showing WBC Mean±SEM in different groups](image)

Figure: 4.2.10 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Neutrophills Percentage.

![Bar chart showing Neutrophills % Mean±SEM in different groups](image)
Chapter IV

Immunomodulatory Studies of *Leptadaenia reticulata*

Figure: 4.2.11 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Lymphocytes Percentage.

![Lymphocytes % Mean±SEM](image)

Figure: 4.2.12 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Monocytes Percentage.

![Monocyte % Mean±SEM](image)
Figure: 4.2.13 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Eosinophills Percentage.

![Eosinophill Count % Mean±SEM](image)

Figure: 4.2.14 Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Platelets count.

![Platelets Lacs/mm3 Mean±SEM](image)
Chapter-IV Immunomodulatory Studies of *Leptadaenia reticulata*

Figure: 4.2.15 Effect of Ethanolic Extract of *Leptadaenia reticulata* on Haemoglobin concentration.

![Graph of Hb Gms/dl Mean±SEM](image)

Figure: 4.2.16 Effect of Ethanolic Extract of *Leptadaenia reticulata* on RBCs Count.

![Graph of RBC Mean±SEM Million/mm3](image)
Chapter IV

Immunomodulatory Studies of *Leptadaenia reticulata*

**Figure: 4.2.17** Effect of Ethanolic Extract of *Leptadaenia reticulata* on WBCs Count.

![Graph showing WBC Mean±SEM thousand/mm3](image)

**Figure: 4.2.18** Effect of Ethanolic Extract of *Leptadaenia reticulata* on Neutrophils Percentage.

![Graph showing Neutrophils % Mean±SEM](image)
Figure: 4.2.19 Effect of Ethanolic Extract of *Leptadaenia reticulata* on Lymphocytes Percentage.

![Graph showing Lymphocytes % Mean±SEM](image)

Figure: 4.2.20 Effect of Ethanolic Extract of *Leptadaenia reticulata* on Monocytes Percentage.

![Graph showing Monocyte % Mean±SEM](image)
Chapter-IV  
Immunomodulatory Studies of *Leptadaenia reticulata*

**Figure: 4.2.21** Effect of Ethanolic Extract of *Leptadaenia reticulata* on Eosinophills Percentage.

**Figure: 4.2.22** Effect of Ethanolic Extract of *Leptadaenia reticulata* on Platelets count.
Table 4.2.9: Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Cytokines (IL-2 and IL-6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-2 concentration in mice serum (pg/ml)</th>
<th>IL-6 concentration in mice serum (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>I. Control</td>
<td>24.21 ± 1.352</td>
<td>30.58 ± 2.846</td>
</tr>
<tr>
<td>II. Crude Aqueous extract (50mg/kg body wt.)</td>
<td>35.47 ± 3.861</td>
<td>31.61 ± 1.837</td>
</tr>
<tr>
<td>III. Crude Aqueous extract (100 mg/kg body wt.)</td>
<td>51.34 ± 6.589*</td>
<td>34.89 ± 3.453</td>
</tr>
<tr>
<td>IV. Crude Aqueous extract (150 mg/kg body wt.)</td>
<td>72.92 ± 7.195**</td>
<td>33.48 ± 3.007</td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D. (* P<0.05, ** P<0.025, and ***P<0.001)

**Figure: 4.2.23** Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Cytokines (IL-2 and IL-6)
Table 4.2.10: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Cytokines (IL-2 and IL-6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-2 concentration in mice serum (pg/ml)</th>
<th>IL-6 concentration in mice serum (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>I. Control</td>
<td>24.21 ± 1.352</td>
<td>30.58 ± 2.846</td>
</tr>
<tr>
<td>II. Crude Ethanolic extract (50mg/kg body wt.)</td>
<td>32.15 ± 4.273</td>
<td>29.91 ± 3.454</td>
</tr>
<tr>
<td>III. Crude Ethanolic extract (100 mg/kg body wt.)</td>
<td>45.24 ± 5.581*</td>
<td>31.07 ± 2.106</td>
</tr>
<tr>
<td>IV. Crude Ethanolic extract (150 mg/kg body wt.)</td>
<td>61.86 ± 5.162*</td>
<td>34.83 ± 4.813</td>
</tr>
</tbody>
</table>

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.
(* P<0.05, ** P<0.025, and ***P<0.001)

**Figure: 4.2.24** Effect of Ethanolic Extract of *Leptadaenia reticulata* on Cytokines (IL-2 and IL-6)
4.3 DISCUSSION OF *LEPTADAENIA RETICULATA*

*Leptadaenia reticulata* (family Asclepiadaceae) is a climber found in forests and gardens near thorny trees. *L. reticulata* as the source of the drug and is real Jivanti. Ayurvedic formulary of India also accepts this as the true drug plant. Jivanti is considered as stimulant and tonic in Ayurvedic literature. Its medicinal use dates back to about 4500 to 1600 BC, as mentioned in *Atharva Veda*, Kanda eight, Sukta two. The *Atharva Veda* mentioned its uses as a life and strength giver, propagator of milk and useful in many other ailments. Charaka described it as an important rasayana drug, capable of maintaining youthful vigour and strength, and Vagbhata incorporated it among the ten drugs that constitute the Jivaniya gana or the vitalizing group. It promotes health and vigour, improves the voice, alleviates the three dosas – vata, pitta and kapha, and cures.

*Leptadaenia reticulata* is well known for its tonic, restorative and stimulant property in the Indian system of medicine. This plant is distributed in the southern parts of India. The main constituents reported are stigma sterol, beta-sitosterol, flavonoids, pregnane glycosides and proteins (Singh *et al.*, 2003). Aerial parts of *Leptadaenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue, antimicrobial and anti-inflammatory activity. Seeds of *L. reticulata* are reported to contain hyperoside, a flavonoid glycoside. *L. reticulata* is claimed to have hypotensive effect in dogs. Antioxidant principles derived from plants are reported to have antitumor activity (Ruby *et al.*, 1995).

Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Wagner and Proksch, 1983). Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said...
to be true immunomodulation (Bamunnarachi and De Silva 1989). The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported. Such drugs are well tolerated by the patient, may be developed into alternative coadjuvants in the treatment of disorders caused by an exaggerated or unwanted immune response, such as in autoimmune diseases, allergies, glomerulonephritis, chronic hepatitis, etc. (Rossi-Bergmann et al., 1994).

The carbon clearance assay was used to evaluate the effect on reticuloendothelial cell mediated phagocytosis (Jayathirtha and Mishra 2004, Gokhale et al. 2003). When ink containing colloidal carbon is injected intravenously, the macrophages engulf the carbon particles of the ink. Rate of clearance of (carbon particles) ink from blood is known as phagocytic index. The extract produced an increased in phagocytic index suggesting its effect on reticuloendothelial system.

Crude aqueous extract of Leptadaenia reticulata showed significant immunostimulant activity in carbon clearance test by increasing phagocytic index in a dose dependent manner. Crude aqueous extract, increased the phagocytic index significantly as $1.195 \pm 0.038 \ (P<0.025)$ and $1.431 \pm 0.054 \ (P<0.001)$. The crude ethanolic extract also enhanced the phagocytic index in dose depended manner which was $1.465 \pm 0.067 \ (P<0.001)$, $1.625 \pm 0.055 \ (P<0.001)$ and $1.700 \pm 0.049 \ (P<0.001)$ respectively with 50, 100 and 150mg/kg b.wt. Increase in phagocytic index indicates that phagocytosis is increasing. Stimulation of phagocytosis is influenced by the activation of macrophages, the activated macrophages secrete a number of cytokines, which in turn stimulate other immune cells (Nose et al., 1998). In the same experiment a dose of 50mg/kg b.wt of crude aqueous extract did not show any significant increase or decrease in the phagocytic effect. This suggests that the active substance, which stimulates the immune system, either is absent or present in such a low concentration that no invocation to phagocytes is generated significantly.
The results depict that aqueous extract of *Leptadaenia reticulata* has immunomodulatory activity. Both, crude aqueous extract and crude ethanolic extract have the phytocontents for chemostimulation of phagocytosis. Significant clearance of carbon particles from the blood of treated animal in dose dependent manner is observed. The component(s) of the extract activate the receptors to remove antigen (here the carbon particles) through pinocytosis as the antigen is very small. In case of mouse CRI, CRI2, CR3, CR3b and CR3bi are the main receptors. The phenols, flavonoids, terpenes and saponins as reported by Veropptta, 2001 are responsible to incite them, which in turn eliminate carbon particles or phagosome phagocytosed made to assume the area of plasma membrane of neutrophil and monocytes increase but the microscopical examination of the blood of control and treated animals, show no change in size of monocytes either of cell. Neutrophils or monocytes, which are main phagocytic leucocytes, take up particles through minimum 40 receptors expressed on their surface. These receptors are for IgG complement, mannose and galactose terminated oligosaccharides. It is supposed that many of the receptors become active due to the exposure of the extracts.

The pre-existing and newly formed IgG may be playing their role in the identification of the antigen, activation of MoRC. IgG receptors, and the attachment of the receptors to facilitate phagocytosis. Many Flavones increase phagocytosis through complement C3 and C1. Flavonoids are present in the extract of the plants. Besides them some other compounds are also there which work in association of flavonoids to activate CR3b and CR3bi receptor of phagocytes and ligation of complements with the receptors (Kandaswami and Middleton, 1994, Estrada *et al.*, 2000).

Delayed type Hypersensitivity required the specific recognition of given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. DTH is a part of the process of graft rejection, tumor immunity and most important, immunity to many intracellular microorganisms. It can also be due to activation of complement, release
of reactive oxygen or nitrogen species by activated phagocytes and pro-inflammatory cytokines (Smith and Kroes, 2000). Delayed type hypersensitivity (DTH) is antigen specific and cause erythema induction at the site of antigen infection in immunized animals. The histology of DTH can be different for different species but the general characteristics are influx of immune cells at the site of injection, macrophages and basophills in rat’s induction become apparent within 24-72 hrs (Poulter et al., 1982).

Delayed Type Hypersensitivity Test was done to study the effect at crude aqueous and crude ethanolic extract on cell-mediated immune response to paw edema in 24, 48 hrs and then after 72 and 96 hrs paw volume significantly decreased when compared with control.

The reduction in paw volume may be because of a quick action of various enzymes, hormones etc on the invader, simultaneously phagocytosis increased because of activated macrophages and hence reduction in paw volume was observed. Reduction in paw volume after 24 hr. and onwards point to the fact that saponins and similar compounds increase the metabolic activity of the neighboring cells to release metabolites and activated macrophages eliminate the causative agents hence the edema gradually reduces. The increase in paw volume, in response to infiltration of CD4 line of T-lymphocytes and as usual diapedesis of mononuclear macrophages and liberation of edema causing substances for example serotonin, prostaglanddulin E, cytokines etc. The infiltration of lymphocytes is possibly because of the compounds, which perhaps observed the cell-mediated immune response. Extract of Leptadaenia having potent activity to involve cell- immune response. This indicates that aqueous extract and ethanol extract contain amines and multiple hormonal substances like lymphokines. These hypersensitive responses particularly by attracting and activating macrophages (Roitt, 1988, Kulkarmi and Desai, 2001, Ray et al., 1996). Aqueous extract of plant contains saponins and according to Liu et al., 1995, Shaibata, 1977 and Verotta, 2001 saponins are
immunostimulatory agents.

Antibodies, product of B-lymphocytes and plasma cells, are central to humoral immune responses. IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization and neutralization of toxins (Miller et al., 1991). Immunoglobulin like IgM can overcome electric barrier and get cross-linking with red blood cells, which lead to subsequent agglutination. The augmentation of the humoral immune response to SRBCs by plant extracts as evidenced by an increased in the antibody titer in rats indicated that enhanced responsiveness of T and B lymphocyte subsets, which is involved in the antibody synthesis (Benacerraf, 1978). The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation to antibody secreting plasma cells (Ose and Muenster, 1968).

An increase in humoral immune response was observed. Agglutination titer to SRBC fraction also showed agglutination titer up to the same level. Aqueous extract of the plant contained proteins, oligosaccharides and their conjugated compound besides β-sterols, saponins, flavonoids, flavones etc. the antigenicity to elicit antibodies of first two compounds is well known, but ethanol soluble fraction is devoid of some compounds, other compounds are equally potent for the synthesis of immunoglobulins. Red blood cell at neutral pH possesses negative ions that form cloud, which repel one another Immunoglobulins like IgM can overcome the electric barrier and get cross-link with red blood cells, this leads to subsequent agglutination. From the above results it is possible that there is an enhancement in the level of IgM and IgG because antibody tire against SRBC were raised. In many plants similar activities and increase titer of IgM etc. are observed (Rezaeipoor et al., 2000, Frier et al., 2003). The responsible chemicals were alkaloids flavonoids, polysaccharides and polypeptides as suggested by Gao et al., 1996, Liu et al., 1995 and Pinilla and Luu, 1999.

The crude ethanolic extract caused a decrease in the agglutination
titer. Crude ethanolic extract at the doses of 100, 150mg/kg b. wt. showed agglutination titer only up to X: 40 and the ethanolic extract suppresses humoral immune response and interfere with antibody formation so antibody is formed insignificantly, affects agglutination titer against SRBC titer. The study of the results depict that the ethanolic extract surprisingly show almost no change in agglutination titer, perhaps the amount of the compounds that can invoke antibody synthesis is not enough to incite T4 and B lymphocytes or such compounds are not in the extract. This cannot be ignored that immunosuppression may be caused by the other contents of the extract.

Cyclophosphamide suppresses humoral, cellular, non-specific and specific cellular immune response. When animal was treated with cyclophosphamide then haemoglobin (Hb), RBC counts, WBC count, Lymphocyte% and Platelet count all are reduced significantly (Doherty, 1981, Gill and Liew, 1978, Habibullah et al., 1979). The suppressive effect of cyclophosphamide was protected by the administration of aqueous extract and their ethanol soluble and ethanol insoluble fraction. Flavonoids in biological systems tend to adhere with the molecules of cyclophosphamide this causes to increase the size of the molecules and prevent its entry to the stem cells. As already stated that such compounds are detected in the plant extract besides this some more compounds are there which are not only negating the effect of cyclophosphamide, but also accelerating the total WBC and haemoglobin count. The crude ethanol extract did not make any significant elevation in the hematological parameters taken for study on the other hand crude aqueous extract of 100, 150 mg/kg b.wt. showed significant increase in the haemoglobin, RBC count, WBC count; Lymphocytes and platelet count in a dose dependent manner. This suggests that the constituent of the plant preventing the access of cyclophosphamide to the stem cells so that synthesis of haemoglobin, WBC and RBC is not inhibited. Another point is that the compound as are reutilizing this immunosuppressant before it could act upon haemopoetic and myeloid tissue and its effective
amount is present in 100/150 mg of extract. The crude aqueous extract also enhanced the number and activities of various immune cells and protected the animal from the adverse effect of cyclophosphamide.

On the other hand crude ethanolic extract of *Leptadaenia* showed a mixed effect, sometimes the values of blood parameters increase or decrease. The ethanolic extract showed a dual nature, stimulatory as well as suppressive effect. In some case extract protected the animal from the effect of cyclophosphamide but at certain doses of the extract only it showed a slight reduction in the given values. In addition to carbohydrates, glycosides and saponins, proteins also contribute to a larger extent to immunostimulation more activity was observed with aqueous extract as compare to ethanolic extract (Steven, 2000).

Cytokines are essential mediators of cell-to-cell signals in physiological and pathological immune responses and in the inflammatory response. Under normal conditions, these cytokines act as crucial signals in the development of appropriate defenses. However, exaggerated or prolonged release can lead to pathological conditions. Both crude aqueous and ethanolic extract enhanced IL-2 levels in a dose dependent manner while the IL-6 showed almost stable levels.

The qualitative analysis of IL-2 in both control and experimental animals was assessed and correlated with significant increase in WBCs count/lymphocyte count in experimental animals. So for IL-6 is concerned its increase can be correlated with increase paw volume. The cytokines whenever increase in very low concentration a definite effect is produced. IL-2 is formed to increase with many plants extracts as reported by Ganguli *et al.*, 2001 and Bone, 1996. These low molecular weight proteins activate the receptors on lymphocytes to cure these sensitivity a growth and activity. IL-2 also stimulates other cellular effectors like GCFs and GMCSF although these factors are not estimated but an indirect conclusion about their increase can be made which
inhibits WBCs count and paw edema test. Probably the glucosteroids and flavones which are present in good quantity in the extracts, they are directly or indirectly responsible to elevate the cytokines.

Bacterial products or the by-products of opsonins can activate macrophages or monocytes directly. Regardless of the initiating event, monocytes or macrophages are usually the cells that elicit the response cascade in the acute phase of inflammation (Baumann and Gauldie, 1994). Thus, these cells play a crucial role in the induction of both immune and inflammatory responses.

Activated monocytes release a broad spectrum of cytokines, which can induce the subsequent cytokine cascade. Interleukin 1 (IL-1), tumor necrosis factor (TNF), and IL-6 are biologically active peptides produced by monocytes, notably in response to endotoxin (Baumann and Gauldie, 1994, Durum and Oppenheim, 1993). These soluble factors initiate and maintain the acute phase of the inflammatory response, whereas the IL-1 receptor antagonist (IL-1ra) and IL-10 are anti-inflammatory cytokines, which are also produced by monocytes (Durum and Oppenheim, 1993, Malefyt et al., 1991).

IL-6 is a pleiotropic cytokine involved in the regulation of the immune response, the acute-phase reaction, and haematopoiesis. IL-6 inhibits the production of LPS-induced TNF-a and IL-1b by cultured human monocytes and in mice in vivo (Tilg et al. 1994). IL-6 is also known to increase the amounts of TNF receptors in hepatocytes. Thus, IL-6 belongs to the category of anti-inflammatory cytokines (Tilg et al., 1994). The enhancement of IL-6 production by crude aqueous extract may be involved in the suppression of TNF and IL-1 synthesis.

*Allium sativum* (garlic), like many of the plants demonstrated to show the effects on multiple cytokines. Garlic lowered IL-6 in an *in vitro* human model (Hodge, 2002).

Known as anthrapachaka in the Ayurvedic system, *Tylophora*
**Chapter-IV**

**Immunomodulatory Studies of Leptadaenia reticulata**

*asthmatica* is traditionally used in the treatment of asthma, allergies, and autoimmune disorders (Bone, 1996). Tylophora demonstrates a biphasic effect on IL-2 secretion. Ganguly *et al.* demonstrated this effect in an *in vitro* model. Using the same model throughout their investigations, a lower dose of *T. asthmatica* increased IL-2 levels, while more than a thousand-fold increase in dose reduced IL-2 levels, demonstrating a paradoxical response to the same exogenous stimulus (Ganguly *et al.*, 2001).

Song *et al.* (2000) had reported an increase in activated B cells, together with a suppression of IFN-γ levels, in response to *Astragali* extracts. However, IL-6 mRNA expression was found to be suppressed. More recently, Young *et al.* (2003) have shown that water-soluble extracts of *Astragalus* radix were able to stimulate the proliferation of splenic lymphocytes, as well as increase the mRNA expression of the cytokines (IL-1, IL-6 and TNF). The extracts also increased IL-1 and IL-2 as well as macrophage activity in the immunosuppressed mouse (Song *et al.*, 2000).

Many constituents of *Ginseng* stimulate the immune system: protopanaxadiol and protopanaxatriol induce proliferation of lymphocytes and cytokines (Jae *et al.*, 2002); rhamnogalacturonan II ginsenosides (Kwang *et al.*, 1998) stimulate IL-6 activity; and, ginsan (an acidic polysaccharide) induces activities of IL-1, IL-12, TNF-α and IFN-γ (Song *et al.*, 2002).

The electrophoretic pattern of drug treated animal is almost similar to that of serum proteins electrophoretic pattern of control. Both the extracts of *Leptadaenia reticulata* did not elicit any response for the formation of new serum protein. Possibly the technique may not be show much sensitive to detect the new protein which appeared in very little concentration in blood as the constituents of the extracts did not produced new protein. This cannot be ignored that the constituents of both the extracts exhibited a definite immunostimulatory effect as
evidence by various parameters for immunostimulation increased phagocytic index and SRBC test go in favour of the drugs for immunostimulation. The increased concentration of serum protein (The reading is not incorporated here) also points to the fact that the liver and probably the plasma cells also produced to increase the concentration of serum protein. Serum of the drug treated animal showed a more darkened spots in comparison to that of control. This shows that the constituents of the drug having effects either on liver are on B lymphocytes and plasma cells.

Moraes et al. (1994) suggested that the blockade of lymphocyte proliferation by a component isolated from A. tenella may contribute to the plant anti-inflammatory properties since stimulated lymphocytes secret cytokines which attract neutrophils during the inflammatory process. The results of the paw edema assay prompted the hypothesis that crude aqueous extract has components with immunosuppressive activity alongside others, which can be immunostimulatory. Several other plant extracts have also been shown to have simultaneous immunosuppressive and immunostimulatory effects (Yamaguchi, 1992, Mediratta et al., 2002). Overall results with the Leptadaenina reticulata showed its immunostimulant as well as immunosuppressant nature.