5.1 INTRODUCTION

Natural products of plant and animal origin offer vast resources of newer medicinal agents with potential in clinical use (Saudi et al., 1996). Some of these are believed to promote positive health and maintain organic resistance against infection by re-establishing the body’s equilibrium and conditioning the body tissue (Fulzele et al., 2003). The immune system is designed to protect the host from invading pathogens and to eliminate Disease (Sharma et al., 2011). The immune system is a remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and to eliminate disease. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders (Richard et al., 2003).

The natural resistance of the body against infection can be enhanced by the use of herbal drugs (Atal et al., 1986). Several herbal preparations that can enhance the body’s immune system status are extensively being used in the indigenous system of medicines. There is an upsurge in the clinical usage of indigenous drugs as they are free from serious side effects. Large number of plants having a known immunomodulatory activity (Dua et al., 1989). The interactions of large molecules in biological systems play an important part in many life processes.

immunomodulating activity of the seeds of the plant *Celastrus paniculatus*. The phytochemical constituents like diterpenoids, steroids, proteins and tannins are considered to exhibit micropotential.

The immune system itself is very much more complex, having at its centre the reaction of a host’s antibodies with invasive antigens (Reynolds and Dweck, 1999). However there is no study to expose the immunomodulating activity of *C. rotundus*.

*Cyperus rotundus* L.(Family : Cyperaceae, Koraikizangu in Tamil, Thunga in Telugu, Dutgrass in English) is a perennial sedge distributed throughout India. It is pestiferous perennial weed with dark green glabrous culms, arising from underground tubers. The genus *Cyperus rotundus* includes common weeds found in upland and paddy fields in temperate to tropical regions. In Asian countries, the rhizomes of *Cyperus rotundus*, which are used as traditional folk medicines for the treatment of stomach and bowel disorders and inflammatory diseases, have been widely, investigated (Gupta *et al.*, 1971; Singh *et al.*, 1971; Weenen *et al.*, 1990). It is also home remedy for indigestion, disorders of stomach. The tubers are used in Ayurvedic medicine and have been mentioned in ancient texts for various ailments (Agarwal *et al.*, 2005). In this present chapter an attempt has been made to evaluate the immunomodulatory potential of the medicinal plant *Cyperus rotundus* L.

5.2 PLANT MATERIAL

The rhizomes of the *Cyperus rotundus* Linn. were harvested from outfield near Nagercoil, Kanyakumari District, Tamil Nadu, South India in the month of October. Botanical identification was carried out by Department of Botany, Sri Paramakalyani College, Alwarkurichi(Ref SPKCZ/17), South India. Fresh plant roots were rinsed severally with clean tap water to make it dust and debris free. Then the roots were dried
in the shady condition at the room temperature for 15 days until they become crispy while still retaining the brownish coloration. Dried roots were ground in an electric chopper and made into coarse powder, stored in an air tight container in a refrigerator prior to subsequent analysis.

5.3 PREPARATION OF THE PLANT EXTRACT

Ethyl acetate and methanol extracts were obtained by using soxhlet apparatus. The two types of extract, with different polarities were concentrated by evaporating it to dryness under reduced pressure by rotary vacuum evaporator to obtain the respective extracts and each residue was stored at 4 °C. These two extracts were resuspended in Dimethyl sulfoxide. The extracts were then stored at -18 °C until further analysis.

Treatment protocol

Group I – Normal control received equivalent volume of 0.17 Sodium Carboxy Methyl Cellulose as a vehicle.

Group II – Rats were treated with pyrogallol (100mg/kg/7 days) (disease control)

Group III- Rats were treated with pyrogallol (100 mg/kg/7 days) and was followed with the oral treatment of methanol extract of *Cyperus rotundus* L.rhizomes (200 mg/kg) from the eighth day unto 14th day.

Group IV – Rats were treated with pyrogallol (200 mg/kg) for 7 days and was followed with the oral treatment of ethyl acetate extract of *Cyperus rotundus* L.rhizomes (200 m/kg) from the eighth day unto 14th day.

Group V – Rats were treated with Pyrogallol (200mg/kg w/v) for seven days and was followed with the treatment of Vitamin E suspension (150mg/kg) from the eighth day unto 14th day.
5.4 PREPARATION OF ANTIGEN

The antigen used for the study was different forms of Sheep Red Blood Cells (SRBC).

Sheep Red Blood corpuscles (SRBC)

Sheep blood was collected in Alsevier’s solution from a nearby slaughter house without contamination. To avoid allogenic difference, the same sheep blood was used throughout the study. After 15 days of storage at 4 °C in Alsevier’s medium, the Sheep Red Blood Cells (SRBC) were washed thrice with sheep blood isotonic saline (0.15N). A suspension of 25% SRBC was prepared using isotonic saline medium shortly before use. (By counting in a haemocytometer, 0.1ml of 25% SRBC must contain 5-6 x 10^8 cells ml^-1)

5.5 ALSEVIER’S SOLUTION

Alsevier’s solution was prepared by dissolving the chemicals in one litre of distilled water and autoclaving at 15 pounds for 7 minutes.

Composition of Alsevier’s solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>20.5g</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>8.0g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.2g</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.005g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00ml</td>
</tr>
</tbody>
</table>

5.6 ANIMALS AND TREATMENT

For the experimental study, Wistar rats were chosen. The rats were obtained from Madras Medical College, Chennai and reared in laboratory under standard conditions of light and darkness (12 -12 h) and temperature (24 °C ± 2 °C). The rats were fed Standard
laboratory mice pellet feed (Lipton Ltd., Mumbai) (Consisting protein 15-17%, fat 4-5%, carbohydrate 45 – 55%, fiber 15%, vitamin A 7000 IU/kg, vitamin E 40 mg/kg, vitamin K, 2mg/kg, vitamin B1 mg/kg Hawk-Oser salt 11 mg/kg) and water *ad libitum* to all the animals. The access to animal room was limited and kept to minimum.

For the experimental study rats weighing 150 to 210 gm (30 days old) were recruited from the acclimatized stock. The rats were grouped into several groups and each group with six individuals. These animals were housed in a specially designed (polyethylene cage) cage with provision for systematic supply of pellets and water. The animals were trained to take water from a feeding bottle kept in cage.

*C. rotundus* extract (MECR and EACR) extract and standard drug treatment was given to animals for 3 weeks. During treatment pellet feed and water were given in *ad libitum*. Food consumption, general condition and other symptoms were observed daily and body weights were recorded weekly. For treatment with MECR and EACR, the doses for the treatment were fixed for each group (200mg/kg).

**5.7 IMMUNIZATION**

The test animals were divided into equal groups for stimulation with sheep red blood cells (SRBC). For stimulation with antigen, the rats were immunized (i.p) with SRBC suspended in normal saline (0.15 M). Approximately 25x10^6 cells ml^-1 were administered for the primary and 50x10^6 cells ml^-1 for the secondary immunization two weeks after the primary dose. Unstimulated mice were treated similarly except immunization with SRBC antigen. The stimulated rats were treated with different types of *C. rotundus* extract. For each extract (MECR and EACR) and control groups, triplicate experiments were maintained.
5.8 EXPERIMENTAL SYSTEM

Blood samples of stimulated rats were collected on the 14th and 21st days following plant extract treated by cardiac puncture, after anesthetizing the rats with chloroform. The serum was separated for each group separately and kept at -20 °C, till analyzed. Sodium citrate (2.8g/100ml) was used in collecting whole blood and leukocyte rich plasma for lymphocyte subset numeration.

5.9 HAEMATOLOGICAL INVESTIGATION

To find out the effect of plant extract on the total and differential count of blood cells, Red Blood Cells, and Haemoglobin content of rats were studied.

5.10 IMMUNOLOGICAL ASSAYS

a) Humoral immune response
   1. Antibody titre

b) Cell – mediated immune response (CMI)
   I. Delayed type hypersensitivity (DTH)
   II. T cell E-rosette assay : to estimate of T lymphocytes

5.11 HAEMAGGLUTINATION ANTIBODY TITRE (HA) (SRBC-ANTIGEN)

The rats were divided into five groups consisting of six animals each. Rats in group I (control group) received vehicle only for 7 days. Groups II received immune suppressive drugs pyrogallol (100mg/kg/7days). Rats in treatment group III were given Vitamin E(150 mg/kg) (immunostimulatory drug) daily from the 8th day upto 21 days. Group IV and V were given plant extract MECR and EACR 200mg/kg day from the 8th day to 14th day. On 14th and 21st day of study, rats from all the groups (i.e group I to V were immunized and challenged respectively, with SRBCs in normal saline (0.1ml of 20% SRBCs) intraperitonially.
Blood was withdrawn on 14th and 21st day from heart puncture method, by using chloroform to give mild anesthesia to all rats group. The blood obtained was centrifuged to raise serum, normal saline was used as a diluent and SRBCs count was adjusted to (0.1ml of 20% SRBCs). Each well of a microtitre plate was filled initially with 25 µl of saline and 25 µl serum was mixed in the first well of microtitre plate. Subsequently the 25 µl diluted serum was removed from first well and added to the next well to get twofold dilutions of the antibodies present in the serum.

Further twofold dilutions of this diluted serum were similarly carried out till the last well of the first row (11th well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 20 µl SRBC (0.1% of SRBCs) were added to each of these dilutions and the plates were incubated at 37 °C for one hour and then observed for haemoagglutination (Agarwal et al., 2001). The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titers were expressed in the graded manner, the minimum dilution (1/2) being ranked as I, and mean ranks of different groups were compared for statistical significance.

5.12 DELAYED TYPE HYPERSENSITIVITY (DTH) RESPONSE IN RATS

On 14th day of the study, all the groups I to IV were immunized with SRBCs (0.1 ml of 20% SRBC i.p.) in normal saline. On day 21st all animals from all the groups were challenged with 0.03 ml of 20% SRBCs in subplantar region of right hind paw. Foot pad oedema in rat was used for detection of cellular immune response (Fulzele et al., 2003). On 21st day, injection of 0.1 ml of 20% SRBCs in the subplantar region of right hind paw in the volume of 0.03 ml and normal saline in left hind paw in same volume as well as the extract treated (MECR, EACR 100mg/kg).
Footpad reaction was assessed every 24 and 48 h on 22\textsuperscript{nd} and 23\textsuperscript{rd} day, in terms of increase in the thickness of footpad as a result of hypersensitivity reaction due to oedema, the thickness of the right hind footpad was measured using vernier caliper. The footpad reaction was expressed as the difference in the thickness (mm) between the right foot pad injected with SRBC and the left footpad injected with normal saline.

5.13 T CELL-E ROSETTE ASSAY

Blood was collected from plant extract treated, control and standard drug treated rat as mentioned in earlier section. T cell count in the blood is carried out by the following method.

Five to ten ml of blood was collected and it was introduced into sterile conical flask/ beaker containing (4-5) sterile glass beads. It was then continuously swirled until no sounds were heard from the beads. This indicated that all the fibrins have adhered to the beads. This blood was considered as defibrinated blood. This defibrinated blood was taken and diluted with equal volume of physiological saline. Three ml of the lymphoprep solution was taken in a centrifuge tube. The tube was kept in slanting position and 9 ml of diluted blood was slowly added along the sides of the centrifuge tube using Pasteur pipette. Care was taken so that the FICON layer of the lymphoprep solution present in the centrifuged tube was not disturbed.

The content of the centrifuge tube was then centrifuge at 1600 rpm for 20 min. The interphase (containing lymphocytes) was removed using pipette. The cells were washed with 1ml saline and excess FICON was removed. The sample was again washed with 1 ml of saline after centrifugation the supernatant was decanted by inverting the tube over a filter paper after all saline was drained; the pellet was then resuspended in 300 µl of RPMI 1640 medium.
Twelve to fourteen centimeter of drinking straw was cut. One end of the straw was slantly cut and sealed by slightly heating the tip in a flame. Nylon wool fibers were finely teased using a pair of forceps and the teased fibers were packed (loosely) into the straw. Adding 5 ml of physiological saline the straw with packed nylon wool column was washed. A small opening was made at the sealed end of the straw to drain the physiological saline. After washing with physiological saline, the nylon wool was then filled with 3 ml of RPMI 1640 medium in a horizontal position. The nylon wool column was kept in the incubator (at 37 °C for 30 minutes) in horizontal position. This process activates the nylon wool column.

Resuspended lymphocytes were loaded into the activated nylon wool column. Then the column was held vertically above an eppendorf tube, then hot saline (about 60°C) was slowly dripped into the column. The saline passing out of the column was collected in the eppendorf tube, which contain T lymphocytes. About 0.2 ml of the saline containing T lymphocyte (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 icebox 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 the T cells under the microscope (20x/40x) for rosette. Number of T cell rosettes formed were observed and tabulated.
Table 5.1
Haematalogical parameters of normal methanol and Ethyl acetate extracts of C. rotundus treated groups

<table>
<thead>
<tr>
<th>S.No</th>
<th>Hematological Parameters</th>
<th>Control Group (G1)treated with 0.1% CMC</th>
<th>G2 Treated with 100 mg/kg pyrogallol</th>
<th>G3 treated with Pyrogallol(100mg/kg) and Methanol extract of Cyperus rotundus (100mg/kg)</th>
<th>G4 treated with Pyrogallol(100mg/kg) and ethyl acetate extract of Cyperus rotundus(100mg/kg)</th>
<th>G5 treated with Pyrogallol(100mg/kg) and Vitamin E suspension(100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Differential count</td>
<td>13.80±0.00</td>
<td>8.70±0.00*</td>
<td>16.10±0.00*</td>
<td>13.20±0.00*</td>
<td>17.29±0.01*</td>
</tr>
<tr>
<td></td>
<td>(cells/cumm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Polymorph (%)</td>
<td>63.67±0.33</td>
<td>55.67±0.33*</td>
<td>58.67±0.33</td>
<td>61.33±0.33</td>
<td>68.33±0.33</td>
</tr>
<tr>
<td>3</td>
<td>Lymphocytes (%)</td>
<td>38.00±0.00</td>
<td>34.00±0.00</td>
<td>34.00±0.00</td>
<td>41.33±0.33</td>
<td>43.33±0.33</td>
</tr>
<tr>
<td>4</td>
<td>Hb(mgs)</td>
<td>13.67±0.33</td>
<td>9.00±0.00*</td>
<td>14.00±0.00*</td>
<td>12.33±0.33</td>
<td>16.00±0.00</td>
</tr>
<tr>
<td>5</td>
<td>Erythrocyte sedimentation rate(mm) ½ hrs</td>
<td>1.00±0.00</td>
<td>5.00±0.00</td>
<td>3.00±0.00</td>
<td>2.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td></td>
<td>1 hrs</td>
<td>2.00±0.00</td>
<td>7.00±0.00</td>
<td>2.00±0.00</td>
<td>3.00±0.00</td>
<td>2.00±0.00</td>
</tr>
</tbody>
</table>

*P<0.01
Table 5.2
Evaluation of SRBC induced Delayed Type Hypersensitivity in Wistar rats treated with the extract of *Cyperus rotundus*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Different groups of treated animals</th>
<th>DTH response (mm of mean paw edema thickness at different Time Intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>1</td>
<td>Normal Group (G1) treated with 0.1% CMC</td>
<td>4.20±0.13</td>
</tr>
<tr>
<td>2</td>
<td>Immunosuppression with pyrogallol</td>
<td>2.20±0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-47.62)</td>
</tr>
<tr>
<td>3</td>
<td>Immunosuppression with Pyrogallol (100 mg/kg) and treated with Methanol extract of <em>Cyperus rotundus</em> (200mg/kg)</td>
<td>4.30±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+95.46)</td>
</tr>
<tr>
<td>4</td>
<td>Immunosuppression with Pyrogallol (100mg/kg) and treated with ethyl acetate extract of <em>Cyperus rotundus</em> (200mg/kg)</td>
<td>4.57±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+107.73)</td>
</tr>
<tr>
<td>5</td>
<td>Immunosuppression with Pyrogallol (100mg/kg) and treated with Vitamin E suspension (200mg/kg)</td>
<td>4.83±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+119.55)</td>
</tr>
</tbody>
</table>

(Each data represents the mean ±SE of 6 Wistar rats Percentage change from Normal group and Immunosuppression was given in parenthesis)
Fig. 5.1 DTH response before and after C. rotundus treatment

- Normal Group (G1) treated with 0.1% CMC
- Immuno suppressed with pyrogallol
- Immuno suppressed with Pyrogallol (100 mg/kg) and treated with Methanol extract of Cyperus rotundus (100mg/kg)
- Immunosuppressed with Pyrogallol (100mg/kg) and treated with ethyl acetate extract of Cyperus rotundus (100mg/kg)
- Immunosuppressed with Pyrogallol (100mg/kg) and treated with Vitamin E suspension (100mg/kg)

DTH response (mm of mean paw edema thickness at different Time Intervals) 24 h
DTH response (mm of mean paw edema thickness at different Time Intervals) 48 h
5.14 RESULTS AND DISCUSSION

The effect of various extracts of *C. rotundus* [MECR, EACR] was tested in normal, Pyrogallol control, vitamin E control, Methanolic and ethyl acetate extract treated groups. Haematological indices such as, TC, differential count of WBC (DCW), haemoglobin (Hb) and, ESR were estimated in control and extracts treated groups. (Table- 5.1)

In the normal rats Total count of WBC was 13.80±0.00 cells /cumm. But the quantum of different species of WBC was polymorph 67.67±0.33, and Lymphocytes 38.0±0.00 percent. Haemoglobin level was 13.67±0.33 mgs. ESR rate \( \frac{1}{2} \) h was 1.00 and for one hour it was 2.00. In the pyrogallol treated groups a significant. (P<0.01) reduction was noticed in DC (8.70 cells/cumm). Similarly Haemoglobin level was also reduced (9.00±0.00 p< 0.01).

Co -administration of pyrogallol along with MECR and EACR showed an elevation in DC, and Hb levels (p<0.05) when compared with pyrogallol control. The elevation was however less than pyrogallol and Vit. E treated groups. The enhancement of DC and Hb in ethyl acetate group was less than MECR group-ESR rate was altered due to pyrogallol administration. But it was brought back to normal level by the treatment of MECR and Vitamin E(Plate 6 and 7).

5.14. a. DTH reaction

Delayed Type hypersensitivity reaction (DTH) was estimated in normal, Paracetamol treated, MECR, EACR and VIT.E treated groups. DTH is a type of cell mediated immune response. In the control group treated with vehicle CMC the paw edema was 4.20 ±0.13 and 4.80±0.06 mm at 24 and 48 h respectively. In the rats
administered with immunosuppressive drug pyrogallol, DTH response in terms of paw edema was reduced (i.e), 0-47.6 and 0-49.7% respectively after 24 and 48 h.

In the rats that were treated with immune suppressive drug and subsequently with *C. rotundus* extract (MECR and EACR) the development of paw edema due to antigenic challenge was increased when compared to immunosuppressive drug treated Imps. (Table-5.2). The increase was 95.46 and 41.28 percent after 24 and 48h in MECR treatment and 107.73 and 92.12% on EACR treated groups at 24 and 48h respectively.

DTH response is an expression of cell mediated immunity. As the rats were treated with immune suppressive drug pyrogallol, the DTH reaction was reduced. The percentage reduction was 47.62 and 49.79 after 24 and 48h. When the rats administered with immunosuppressive drugs were treated with *C. rotundus* extract, the suppressed immune response regained its lost vigor. The paw edema an index of DTH activity showed a increase of 107.73 and 92.12 percent after 24 and 48h respectively during MECR treatment, Also the recovery was 119.55 and 93.75 percent after 24 and 48h. During Delayed type hypersensitivity reaction (DTH) T- Cells got sensitized when challenged by the antigen SRBC. The sensitized T- Cells are transformed into lymphoblast cells. Lymphoblast cells secrete lymphokinnes attracting more phagocytes/macrophages to the site of reaction. Hence an increase in DTH response was of the stimulated effect of MECR and EACR on lymphocytes and other cells required for the expression of reaction.

When the elevation of immune response was compared with the standard immunity enhancing drug Vit. E, the extracts of *C. rotundus* is effective to stimulate cell mediated immunity. Phenolic compound and other phytochemicals in *C. rotundus* had induced the immunity elevation.
Table 5.3

Antibody titre value and the T-cell rosette formation in Wistar rats treated with the extracts of *Cyperus rotundus*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Different groups of treated animals</th>
<th>T Cell rosette formation</th>
<th>Antibody titre value (SRBC Challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14th day</td>
<td>21st day</td>
</tr>
<tr>
<td>1</td>
<td>Normal Group (G1) treated with 0.1% CMC</td>
<td>84.00±2.08</td>
<td>88 ±0.2</td>
</tr>
<tr>
<td>2</td>
<td>Immuno suppression with pyrogallol (100 mg/kg)</td>
<td>37.67±1.20** (-55.16)A</td>
<td>31.10±0.2** (-64.70)A</td>
</tr>
<tr>
<td>3</td>
<td>Treated with pyrogallol (200mg/kg) and Methanol extract of <em>Cyperus rotundus</em> (200mg/kg)</td>
<td>58.67±0.33** (55.75)B</td>
<td>56.15±0.6** (79.52)B</td>
</tr>
<tr>
<td>4</td>
<td>Treated with pyrogallol (100mg/kg) and ethyl acetate extract of <em>Cyperus rotundus</em> (200mg/kg)</td>
<td>71.00±0.58** (88.48)B</td>
<td>68.47±.001** (120.16)B</td>
</tr>
<tr>
<td>5</td>
<td>Treated with pyrogallol (100mg/kg) and Vitamin E suspensión (200mg/kg)</td>
<td>88.00±0.58** (112.37)B</td>
<td>71.56±0.4* (130.09)B</td>
</tr>
</tbody>
</table>

A- The percentage change over in normal rat is given in parenthesis

B- Percentage change over in immune suppressed rats

** P<0.001

* P <0.01
Chapter Title 5.14.b Antibody Production

Antibody production in response to antigenic challenge is a type of humoral immune response. Antibody production in normal, immune suppressive drug and *C. rotundus* extract treated rats were estimated. When the rats were given pyrogallol and given priming dose of antigen on 7th day antibody production was found to be 29.07 and 36.0 present in 7th and 14th day treated rats. The immunity response after second dose or booster dose was less.

In the *C. rotundus* extract treated rats antibody titre was elevated and both primary and secondary immune response was high and the rats recovered from the effect of pyrogallol (Table- 5.3).

In the rat treated with MECR after immunesuppression responded well to extract when compared with immunity suppressed group and the increase was 117.07, 114.03 and 122.44 percent in MECR, EACR and Vit E treated groups respectively after 24h. Also after 48 h the antibody production was elevated when compared with pyrogallol group.

The antibody production depends on B-cells. Hence the extract of *C. rotundus* has some compounds that had stimulated the clone proliferation and functional mechanism of B-Cells.
5.14.c T CELL – ROSETTE ASSAY

In the present study, the extracts *C. rotundus* was found to enhance immunity. Both cell mediated and humoral immune responses were modulated by the extracts of *C. rotundus* T-cell number was reduced by 55.16 percent when normal rates were given pyrogallol. But the administration of pyrogallol improved the suppressed T-cell count. The improvement in T-cell counts on exposure to *C. rotundus* extracts of 55.75% for methanol extract and 112.37 for vitamin E extract treatment.

Administration of the extracts of *C. rotundus* also influenced antibody titre formation. Antibody production after SRBC antigenic challenge got suppressed due to the treatment with pyrogallol. But from the suppressed antibody production an elevation was observed when methanol, ethyl acetate and Vitamin E was given.

T-Cells are involved in cell mediated immunity. T-helper cells, T-Cytotoxicity Cells, T-suppressor cells and other species of T-Cells are important constituents of defence system. T-Cell count in the rat was estimated using rosette formation assay. The number of T-Cell rosettes formed for normal rat was 84.00±2.08 and 88.10±0.2 on 14th and 21st day after antigenic challenge. In the immunosuppressed rat the number of rosettes formed by T-Cell was 37.67±1.20 and 31.1±0.02 on 14th and 21st day after antigenic challenge. But when the immune suppressed rats were treated with *C. rotundus* extract. T cell rosette formation was significant increased and it was 71.00±0.58 and 68.17±0.001 on 14th and 21st days respectively (Table-5.3). From the results it was concluded that *C. rotundus* is a immune booster and the root extracts have the compounds that stimulate the formation of both B and T cells(ie), It has modulated both cell mediated and antibody mediated immune responses in wistar rats.

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