Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypotonicity induced membrane lysis can be taken as an in vitro measure of anti inflammatory activity of the drugs or plant extracts.

Bacopa monniera (Coastal Waterhyssop, Brahmi, Thyme-leafed gratiola, Water hyssop) is a perennial, creeping herb whose habitat includes wetlands and muddy shores. It commonly grows...
in marshy areas throughout India, Nepal, Sri Lanka, China, Taiwan, and Vietnam, and is also found in Florida, Hawaii and other southern states of the USA where it can be grown in damp conditions by the pond or bog garden. This plant has a number of uses in Ayurveda. It is a traditional treatment for epilepsy and asthma. The other reported activities include sedative, antioxidant, vasoconstrictor and anti inflammatory. Phytochemical analysis of B. monniera plant extracts revealed the presence of various biochemical compounds such as alkaloids, betulic acid, stigmastarol, beta-sitosterol, bacopasaponins, tetracyclic triterpenoid saponins, hersaponin. Since triterpenoids and flavonoids have remarkable anti inflammatory activity, so our present work aims at evaluating the in vitro anti inflammatory activity of B. monniera by HRBC membrane stabilization.

**MATERIAL AND METHODS**

**Collection of Plant Material**

The fresh whole plant of Bacopa monniera was collected from Araku valley situated near Visakhapatnam, Andhra Pradesh, India.

**Chemicals**

All chemicals and reagents used were of analytical grade or purest quality.

**Extraction and Preparation of methanolic extracts**

10 gm of powder of the plant was packed in thimble flask and 250ml of methanol was added in 1 litre round bottom flask. Then the Soxhlet assembly was set up to complete 10 to 15 cycles. The solvent was distilled at lower temperature under reduced pressure, after that the extract was filtered and filtrate was concentrated using water bath to get the crude extract which is stored in freezer for future use. The percentage yield of methanolic extract of Bacopa monniera is 15.47 %.

**Preparation of Human Red Blood Cells (HRBC) Suspension**

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosalone (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosalone.

**Heat Induced Hemolysis**

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37°C for 30 min and centrifuged respectively. The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

The percentage of hemolysis of HRBC membrane can be calculated as follows:

\[ \% \text{ of Hemolysis} = \frac{(O.D \text{ of Test sample})}{(O.D \text{ of Control})} \times 100 \]

The percentage of HRBC membrane stabilisation can be calculated as follows:

\[ \% \text{ of Protection} = 100 - \frac{(O.D \text{ of Test sample})}{(O.D \text{ of Control})} \times 100 \]

**RESULTS AND DISCUSSION**

The inhibition of hypotonicity induced HRBC membrane lysis i.e., stabilization of HRBC membrane was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilisation for methanolic extracts of B. monniera and Diclofenac sodium were done at 50, 100, 250, 500, 1000, 2000 µg/ml. Methanolic extracts of B. monniera are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (50 2000µg/ml) as shown in Table 1. It showed the maximum inhibition 92.92±1.41% at 2000µg/ml respectively, when compared to diclofenac sodium that is 98.76±1.26% at 2000µg/ml respectively. With the increasing concentration the membrane hemolysis is decreased as shown in Figure 1 and membrane stabilisation / protection is increased as
Table 1: Effect of *B. monniera* and Diclofenac sodium (standard) on percentage of HRBC membrane hemolysis and membrane stabilization

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>% of Hemolysis of <em>B. monniera</em></th>
<th>% of Stabilisation of <em>B. monniera</em></th>
<th>% of Hemolysis of Diclofenac sodium</th>
<th>% of Stabilisation of Diclofenac sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>31.34±1.02</td>
<td>68.66±1.13</td>
<td>47.18±1.31</td>
<td>52.81±1.73</td>
</tr>
<tr>
<td>100</td>
<td>27.08±0.78</td>
<td>72.92±0.77</td>
<td>23.47±0.79</td>
<td>76.54±1.12</td>
</tr>
<tr>
<td>250</td>
<td>23.46±0.45</td>
<td>76.54±1.06</td>
<td>18.68±1.65</td>
<td>81.32±1.08</td>
</tr>
<tr>
<td>500</td>
<td>18.65±1.21</td>
<td>81.35±0.86</td>
<td>14.34±0.23</td>
<td>85.67±0.71</td>
</tr>
<tr>
<td>1000</td>
<td>11.76±0.95</td>
<td>88.24±0.92</td>
<td>7.43±1.11</td>
<td>92.58±1.09</td>
</tr>
<tr>
<td>2000</td>
<td>7.08±1.17</td>
<td>92.92±1.41</td>
<td>1.24±0.87</td>
<td>98.76±1.26</td>
</tr>
</tbody>
</table>

Each value represents means ± SD (n=3).

Fig. 1: Effect of *B. monniera* and Diclofenac sodium (standard) on percentage of HRBC membrane hemolysis

Fig. 2: Effect of *B. monniera* and Diclofenac sodium (standard) on percentage of HRBC membrane stabilisation
shown in Figure 2. Hence anti inflammatory activity of the extracts was concentration dependent.

**CONCLUSION**

Stabilization of the HRBCs membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti inflammatory action of *B. monniera* Therefore; our present in vitro studies on *B. monniera* extracts demonstrate the depression of inflammation. Due to the presence of active principles such as flavonoids, bacosaponins, triterpenoids and related polyphenols may be responsible for this activity. Hence, *B. monniera* can be used as a potent anti inflammatory agent.

**REFERENCES**