Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Pergularia Daemia

Sandeep Akkam*1, Dr. C. Madhusudhan Chetty2, D. Chinna Babu3, V. Vijay Kumar4, Y. Chaitanya Kumar5, N. Swaroop Reddy6

Department of Pharmacy Practice, Santhiram college of Pharmacy, Nandyal, Andhra Pradesh, India.

ABSTRACT
The plant Pergularia daemia has been traditionally used for treating various diseases. The present study was aimed to investigate the anti-inflammatory activity of aqueous extract of the whole plant of Pergularia daemia by human red blood cell (HRBC) membrane stabilization method. The prevention of hypo tonicity induced human red blood cells (HRBC) membrane lysis was taken as a measure of the anti-inflammatory activity. Phytochemical assay was done to find the main active constituents. Among all the concentrations 400 μg/ml showed significant anti-inflammatory activity and 78.97% protection and the standard drug diclofenac showed 86.68% protection. IC50 values were calculated and found to be 217 μg/ml and 200μg/ml for plant extract and standard diclofenac respectively. The results obtained in the present study suggest that Pergularia daemia can be a potential source of anti-inflammatory agents.

Key words: Pergularia daemia, anti-inflammatory activity, HRBC, IC50.
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[2,3]. 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 [4].Red blood cell membrane (HRBC) by hypo toxicity induced membrane lysis can be taken as an in vitro Measure of anti-inflammatory activity of the drugs or plant extract.

The plant Pergularia daemia (Family: Asclepiadaceae) is known as “Uttaravaruni” in Sanskrit and “Utranajutuka” in Hindi. In ethno medicinal practices the traditional healer use Pergularia daemia (Asclepiadaceae) as anthelmintic, emetic, thermogenic, expectorant, antipyrretic and laxative. Leaves juice is given in catarhal affections, asthma, and infantile diarrhoea and is applied to inflammatory swelling in combination lime [5]. Aerial parts of the plant used for snake bite [6]. Latex of this plant used for boils and sores [7]. Fresh roots of plant used as an abortifacient [8] and used to treat gonorrhoea [9]. The latex or a decoction of the roots were used in many countries as a medicine to treat several illnesses, such as venereal diseases, arthritis, muscular pains, asthma, rheumatism, snake-bites. The latex may also be used as a fish poison [10] and toothache [11]. Plant has been documented for presence of presence of triterpenes, saponins cardenolides and alkaloids [12]. The present study was aimed to investigate the anti-inflammatory activity of aqueous extract of the whole plant of Pergularia daemia by human red blood cell (HRBC) membrane stabilization method.

2. Materials and Methods

Collection of plant material:
The leaves of Pergularia daemia were collected from the local areas of Nellore district, identified and authenticated by Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University and Tirupathi.

Extract Preparation:
The leaves were dried under shade and powdered. Powdered plant (200g) was extracted in Soxhlet apparatus using water for 18hrs. The extract was filtered using a whatman filter paper No.10. The filtered extract was then evaporated under vacuum below 45°C in a vacuum drier to give a final yield of 14.98g (7.49% w/w).

Phytochemical screening:
In order to identify the chemical components of the plant, A variety of indicators including Dragendorff, and Wagner for detection of alkaloids, vanillin sulphuric acid and vanillin phosphoric acid for terpenoids, ferric chloride for phenol components, natural product polyethylene glycol (NP/PEG) for flavonoids and kef and blood agar tests for saponins was used in this screening.

3. Results and Discussion

Phytochemical screening:
The aqueous extract of Pergularia daemia was studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. The RBC membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is play an important role in limiting the inflammatory response by preventing 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. Various concentrations of the plant extracts (50,100,200,300,400 g/ml) were used for the study.

Among all the concentrations used 400 g/ml showed significant anti-inflammatory activity and showed 78.97% protection of HRBC in hypotonic solution compared with standard diclofenac which showed 86.68% protection. The results are tabulated (Table 1). IC50 values was found to be 217 g/ml and 200µg/ml for plant extract and standard diclofenac respectively. With the increasing concentration the membrane haemolysis is decreased as and membrane stabilisation / protection is increased as shown in graph1. Hence anti-inflammatory activity was concentration dependent.
Table 1: Phytochemical screening results of Aqueous extract of Pergularia daemia

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Categories of compounds</th>
<th>Tests, reagent used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Vanillin sulphuric acid reagent</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Terpenoids and phenyl propanoids</td>
<td>Vanillin phosphoric acid</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoids</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic compounds</td>
<td>Natural product reagent</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>blood agar tests</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Membrane Stabilization Test by (HRBC) Suspension Method

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Concentration of plant extract µg/ml</th>
<th>Absorbance at 237 nm</th>
<th>% Haemolysis</th>
<th>% Protection of sample</th>
<th>IC50 VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.084±0.023</td>
<td>74.39%</td>
<td>25.61%</td>
<td>217µg/ml</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.102±0.054</td>
<td>68.90%</td>
<td>31.1%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>0.152±0.067</td>
<td>53.65%</td>
<td>46.35%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>0.196±0.020</td>
<td>40.24%</td>
<td>59.76%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>0.259±0.034</td>
<td>21.03%</td>
<td>78.97%</td>
<td></td>
</tr>
</tbody>
</table>

5. References


4. Conclusion

Stabilization of the HRBCs membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti inflammatory action of Pergularia daemia. Therefore, our present in-vitro studies on Pergularia daemia extracts demonstrated the depression of inflammation. Active principles such as flavanoids, glycoside, tri terpenoids, phenol may be responsible for this activity. Hence, Pergularia daemia can be used as a potent anti-inflammatory agent.