IN VITRO ANTI INFLAMMATORY ACTIVITY OF PLECTRANTHUS AMBOINICUS (LOUR) SPRENG BY HRBC MEMBRANE STABILIZATION

K.Nirmala Devi*, K.Periyanayagam 1

Address for Correspondence
*1 Department of Pharmacognosy, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Srivilliputhur-626190.
2 Department of Pharmacognosy, Madurai Medical College, Madurai -625020.
E.mail devinirmala1980@yahoo.co.in

ABSTRACT:
Plectranthus amboinicus (Lour) Spreng is a dicotyledonous plant belonging to the family Lamiaceae. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, epilepsy. The work on the chemical composition of the leaves revealed the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin. Since many flavonoids have remarkable anti inflammatory activity the present work aims at evaluating the anti inflammatory activity of P.amboinicus by HRBC membrane stabilization. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity. The anti inflammatory activity of the ethanolic extract was comparable to that of the standard drug Hydrocortisone. The percentage protection for ethanolic, aqueous extracts and hydrocortisone were 68.20, 60.60 and 77.11 respectively at 500 mcg/ml. The ethanolic extract of P.amboinicus has significant anti inflammatory activity in comparison to aqueous extract of the same plant.

KEY WORDS: Anti-inflammatory, Plectranthus, Coleus amboinicus, Coleus aromaticus, HRBC membrane stabilization.

INTRODUCTION:
Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti inflammatory action. (Tripathi. K.D, 2004) Irrespective of the type of injury the inflammatory response is suppressed by glucocorticoids. This is the basis of most of their clinical uses. Glucocorticoids interfere on several steps in the inflammatory response. Corticoids are only palliative, do not remove the cause of inflammation: the underlying disease continues to progress while manifestations are dampened. They favor spread of infections as capacity of defensive cells to kill microorganisms is impaired. They also interfere with healing and scar formation. Peptic ulcer may perforate asymptptomatically. Indiscriminate use of corticoids is hazardous. Other than corticosteroids the NSAIDs (Non Steroidal Anti- Inflammatory Drugs) are also used to treat inflammation. The major mechanism of action of the NSAIDs is the inhibition of prostaglandin (PG) synthesis or preferential or selective cox-2 inhibition. Due to inhibition of PG synthesis it may produce toxicities like gastric mucosal damage, bleeding, inhibition of platelet function: delay/prolongation of labour, asthma and anaphylactoid reaction in some individuals. (Tripathi K.D 2004). The inhibition of cox-2 may enhance the cardiovascular risk. The plant Plectranthus amboinicus (Coleus amboinicus, Coleus aromaticus) commonly known as country borage is a dicotyledonous plant belonging to the family Lamiaceae (Wealth of India, 1950, Warrier P.S) It is a large succulent aromatic perennial herb. Much branched,
fleshy highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed throughout India, cultivated in the gardens. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, bronchitis, hiccough, and epilepsy. (Chopra R.N, 1956, Kirtikar K.R, 1975, Nadkarni, 1996) The phytochemical study reveals the presence of various flavonoids like quercetin, luteolin, apigenin, salvigenin and genkwanin. (Ram.P.Rastogi, 1970) Since many flavonoids have remarkable anti inflammatory activity (Bonta. I.L, 1969) the present work aims at evaluating the anti inflammatory activity of *P.amboinicus* by HRBC membrane stabilization.

**MATERIALS AND METHODS:**

**Plant material:**
The leaves of *P.amboinicus* were collected in August 2007 at the local areas of Erode district, Tamil Nadu, India. The plant material was identified, authenticated by the Botanical survey of India, Coimbatore and a voucher specimen (PCG PA 20) was deposited in the herbarium of the Department of Pharmacognosy, The Erode College of Pharmacy, Erode, Tamil Nadu.

**Preparation of extracts:**
The leaves were garbled and dried under shade and powdered. The powder was loaded in to soxhlet extractor and subjected to extraction with distilled water and ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. The % of yield values obtained were 26.79 and 5.43 for aqueous and ethanol respectively.

**ANTI-INFLAMMATORY ACTIVITY:**
The HRBC membrane stabilization has been used as a method to study the anti inflammatory activity. (Gandidasan.R, 1991) Blood was collected from healthy volunteers. The collected blood was 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 and packed cells were washed with isosaline (0.85%, pH 7.2) and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug (at various concentration as mentioned in table1), 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Hydrocortisone sodium was used as the reference drug. Instead of hyposaline2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula,

\[
\text{Optical density of drug treated sample} \times 100
\]

\%

Protection = \frac{100 - \text{Optical density of control}}{\text{Optical density of control}} \times 100
RESULTS AND DISCUSSION:
The lysosomal enzymes released during inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. (Rajendran Vadivu, 2008)
Since HRBC membrane are similar to lysosomal membrane components the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti inflammatory activity of drugs. The results were reported in table 1. It was observed from the table1 and figure 1 that the ethanolic extract shows significant anti inflammatory activity at the concentration of 500 mcg/ml which is comparable to the standard drug hydrocortisone sodium. The anti inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased. The ethanolic extract of P.amboinicus has significant anti inflammatory activity in comparison to the aqueous extract of the same plant.

Table 1: Anti inflammatory activity of P.amboinicus at various concentrations

<table>
<thead>
<tr>
<th>S.NO</th>
<th>CONCENTRATION (µg/ml)</th>
<th>Activity (% protection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ethanolic extract of P.amboinicus</td>
</tr>
<tr>
<td>1</td>
<td>Control - - -</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>48.3 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>51.2 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>57.4 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>400</td>
<td>62.0 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>68.2 ± 0.02</td>
</tr>
</tbody>
</table>

(Values are expressed as SEM of 3 readings)

IN VITRO ANTI INFLAMMATORY EFFECT OF P.amboinicus

![IN VITRO ANTI INFLAMMATORY EFFECT OF P.amboinicus](image-url)
CONCLUSION:
The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage. (Murugasan, 1981)

From the above study it was concluded that the ethanolic extract of *P. amboinicus* has significant membrane stabilization property compared to the aqueous extract of the same plant and it was comparable to the standard drug Hydrocortisone sodium.

REFERENCES:
1. Anonymous. The Wealth of India. 1950; Vol II; CSIR; New Delhi; 308.
8. Ram P.Rastogi, Mehrotra B.N. Compendium of Indian Medicinal Plants.