ANTI-INFLAMMATORY ACTIVITY OF ARTEMISIA PALLENS BY HUMAN RED BLOOD CELL MEMBRANE STABILIZATION METHOD

Arjun. K1*, Sangeetha.M1, R.Kamalraj2
1Sri Ramachandra University Porur, Chennai – 600 116.
2R&D, Hospira a Pfizer Company, IKKT, Tamilnadu-602117

ARTICLE INFO

ABSTRACT

Artemisia pallens was studied for anti-inflammatory action by HRBC Membrane Stabilization Method. Various Extracts of Aerial Parts of Artemisia Pallens with serious of dose concentration (10-1000 μg/ml) were studied for the anti-inflammatory action and compared against the standard Diclofenac sodium. The percentage protection was found to be petroleum ether (60.93%), ethyl acetate (63.89%), ethanol (69.96%), chloroform (64.55%), and hydro alcohol (66.17%). The results of the present study suggest that ethanol extract of Artemisia pallens at the concentration of 1000 (μg/ml) has %inhibition of 69.96% which is similar to Diclofenac sodium and it may uses as a potent anti-inflammatory agent.

 Corresponding author
 Arjun. K
 Department of Quality assurance,
 Sri Ramachandra university Porur,
 chennai – 600 116.
 +91-9940025966.
 arjunkonakalla10@gmail.com.

Copyright © 2016 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.iajpr.com
INTRODUCTION

Artemisia pallens, Dhavanam (Tamil: தவணம், Marathi: दवण), is an aromatic herb. In genus of small herbs or shrubs, xerophytic in nature. Dhavanam is imported worldwide for its sweet fragrance and illustrous medicinal values and is used to glorify the idol of Lord Shiva in India. With much regional importance, Davana essential oil gained global attention only in the mid of the 20th century after which it has earned a special spot in the hearts of perfumers’ and flavorists’, particularly in United States and Japan where it is a flavor ingredient for bakery, tobacco and beverage products.

The flowers, stem and leaves of this plant are used as an effective antiseptic and disinfectant for quick treatment of wounds, cuts and infectious ailments such as cough, cold, measles etc. Davana essential oil has also been a part of other Complementary and Alternative medicinal practices like Unani and the Traditional Chinese Medicine (TCM). It is found in the southern part of India especially to the states of Karnataka, Tamilnadu, Andra pradesh and Maharashtra.

In current study the anti-inflammatory activity was investigated by HRBC membrane stabilization method. Since the lysosomal enzymes released during inflammatory condition produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The anti-inflammatory agents act by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal enzymes. Since the human red blood cell membrane is similar to lysosomal membrane components, the prevention of hypo tonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of a drug. The present study was therefore undertaken to investigate some of the folkloric claims especially the use of the plant extract of Artemisia pallens as a treatment of anti-inflammatory by HRBC stabilization method.

MATERIALS AND METHODS

Drugs and Chemicals

The following drugs and chemicals were used. Alsever’solution(spectrum chemicals), Dextrose, 2 % (aquapure &perfect che, Sodium citrate, 0.8%, Citric acid, 0.05%, Sodium chloride, 0.42%, Isosaline, 0.85%, Ph 7.2, Phosphate buffer, 0.2M, Hyposaline, 0.36%, Dimethyl sulphoxide (DMSO) and all the other chemicals used were of the analytical and highest purity grade from standard companies.

Plant Materials

The aerial parts of Artemisia pallens were collected from the Sengottai, Tirunelveli, Tamil Nadu, India in the month of October 2013. The plant material was identified and authenticated by Mr. V.Chelladurai, Retired Research officer-botany, C.C.R.A.S, Govt. of India, Tirunelveli. The Collected plant material was free from diseases and also free from contamination of other plants. The air dried aerial parts of the plant were cleaned and reduced to powder form with the help of mechanical grinder, after which 150 gm of powdered sample was exhaustively extracted in an aspirated bottle with petroleum ether, chloroform, ethyl acetate, ethanol and hydro alcohol for about 72, 48 and 24 hours by maceration technique. The plant material was separated by filtration and the extract was concentrated (by Rota vapour, Büchi, Switzerland) and lyophilized to preserve it.

Anti-Inflammatory Activity

Preparation of HRBC Suspension

4ml of venous blood was collected from healthy volunteers and mixed with equal volumes of alsever’s solution and centrifuged at 3 r and the packed cells were washed with isosaline and a 10% (v/v) suspension was made with isosaline.

Preparation of Assay Mixture

The assay mixture contains 1ml of phosphate buffer [pH 7.4, 0.15M], 2ml hyposaline [0.36%], 0.5ml HRBC suspension [10% (v/v)] and 0.5ml of various extracts of different concentrations (10,50,100,200,400,800,1000mcg/ml) were prepared in DMSO and standard Diclofenac sodium (100 mg) was used to compare the data.

Procedure

Alsever solution was prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% of sodium chloride in distilled water. 10 ml of blood was collected from healthy volunteer (Human Ethics committee approval number: CSP/13/DEC/32/234). The collected blood was mixed equal volumes of Alsever’s solution. The blood was centrifuged at 3000 rpm and the packed cells were washed with isosaline and 10% (v/v) suspension was made. The different concentration of drug samples were prepared The assay mixture contained the drug, 1 ml phosphate buffer, 2 ml hyposaline, 0.5 ml HRBC suspension. Diclofenac sodium as the reference drug and 2 ml of distilled water as control. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. Percentage of protection was calculated using the following equation:

\[
\text{Percentage of protection} = \frac{100 - \text{OD of treated sample}}{\text{OD of control}} \times 100
\]
RESULTS AND DISCUSSION

The various extracts of the aerial parts of *Artemisia pallens* were subjected to *in vitro* anti-inflammatory activity in various concentrations i.e., 10, 50, 100, 200, 400, 800, 1000 mcg/ml. The percentage protection was found to be petroleum ether (60.93%), ethyl acetate (63.89%), ethanol (69.96%), chloroform (64.55%), hydro alcohol (66.17%). The petroleum ether extract and hydro alcohol extract shows the bi phase response. The ethanol extract, chloroform extract and ethyl acetate extract shows the dose dependent response. Hence from the performed study it was proved that ethanolic extract was more potent and the order of potency was Ethanol > hydro alcohol > chloroform > ethyl acetate > petroleum ether.

The IC50 values were found to be 324μg/ l for etroleum ether, 385 μg/ l for Chloroform, 356μg/ l for Ethyl acetate, 203μg/ l for ethanol and 285μg/ l for hydro alcohol extracts.

<table>
<thead>
<tr>
<th>% inhibition</th>
<th>Petroleum Ether</th>
<th>Chloroform</th>
<th>Ethyl Acetate</th>
<th>Ethanol</th>
<th>Hydro Alcohol</th>
<th>Diclofenac Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (μg/ml)</td>
<td>10</td>
<td>9.89</td>
<td>11.23</td>
<td>13.37</td>
<td>15.06</td>
<td>14.02</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>15.68</td>
<td>16.37</td>
<td>19.32</td>
<td>21.73</td>
<td>22.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>23.76</td>
<td>24.84</td>
<td>27.08</td>
<td>29.12</td>
<td>29.13</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>30.37</td>
<td>33.72</td>
<td>39.12</td>
<td>37.18</td>
<td>39.21</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>42.83</td>
<td>45.73</td>
<td>46.37</td>
<td>48.13</td>
<td>47.62</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>62.37</td>
<td>53.87</td>
<td>55.12</td>
<td>57.64</td>
<td>66.17</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>55.75</td>
<td>64.55</td>
<td>65.89</td>
<td>69.96</td>
<td>57.36</td>
</tr>
<tr>
<td>IC50</td>
<td>324</td>
<td>385</td>
<td>356</td>
<td>203</td>
<td>285</td>
<td>156</td>
</tr>
</tbody>
</table>

**Table.1: Anti Inflammatory Activity of Various Extracts of Aerial Parts of Artemisia Pallens.**

Fig -1: Comparison flow charts of % inhibition of Anti Inflammatory Activity of Various Extracts of Aerial Parts of *Artemisia Pallens*

CONCLUSION

The aerial parts of *Artemisia pallens* were subjected to *in vitro* anti-inflammatory activity by HRBC membrane stabilization method in various concentrations i.e., 10,50,100,200,400,800,1000 μg/ml. All the extracts showed the positive response as compared to the standard Diclofenac sodium. The petroleum ether extract and hydro alcohol extract shows the bi phase response. The ethanol extract, chloroform extract and ethyl acetate extract shows the dose dependent response. In specific the present study suggest that ethanol extract of *Artemisia pallens* at the concentration of 1000 (μg/ml) has %inhibition of 69.96% which is similar to Diclofenac sodium and it may uses as a potent anti-inflammatory agent.

ACKNOWLEDGEMENT

The authors are grateful and wish to acknowledge the management of *Sri Ramachandra University Porur, Chennai* for providing facilities.

List of abbreviation

- HRBC - human red blood cells
- DMSO - dimethyl sulfoxane
- IC - inhibitory concentration
- μg - Micro gram
- Ml - Milliliter
Conflict of Interest:
We have no conflict of interest to declare. This research has not been submitted for publication nor has it been published in whole or in part elsewhere.

REFERENCE