In-vitro anti inflammatory activity of Salix caprea Linn. (goat willow) by HRBC membrane stabilization method.

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ABSTRACT

Ethnopharmacological Relevance: Salix caprea L. is belonging to family Salicaceae and commonly known as goat willow. Salix caprea is a common species of willow native to Europe and western and central Asia. It is a folkloric medicinal plant used to treat rheumatoid arthritis, malaria, various hemorrhages, gout, neuralgia and intestinal diseases. It is also used as an antipyretic, analgesic, antibacterial, haemostatic, sedative and antiinflammatory agent.

AIM of study: The present work aims to evaluate the anti inflammatory activity of salix caprea flower by HRBC membrane stabilization method. MATERIALS and METHODS: Flowers of Salix caprea were powdered and successively extracted with petroleum ether, ethyl acetate, methanol, hydroalcohol and aqueous extracts. These extracts were subjected to identify the anti-inflammatory potential of Salix caprea by HRBC membrane stabilization method. RESULTS: The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure for the anti inflammatory activity. The percentage protection of petroleum ether, ethyl acetate, methanol, hydroalcohol and aqueous extracts were 45.57, 61.10, 66.79, 60.49, 68.39% at 400µg/ml respectively and Diclofenac sodium hold the percentage protection 77.39% at 200µg/ml. CONCLUSION: Salix caprea holds the promising anti-inflammatory activity. The hydroalcohol, methanolic extracts showed most and aqueous, ethyl acetate extracts showed intermediate while petroleum ether extract showed least percentage protection as compared to Diclofenac sodium.

Key words: Anti-inflammatory, Salix caprea Linn., HRBC membrane stabilization.

INTRODUCTION

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide. Evaluation of the effects of these plants on organs and systems has contributed to the development of the scientific basis for their therapeutic application and also has enriched considerably the therapeutic arsenal for the treatment of a number of diseases. Inflammation is associated with a large range of mediators that initiate the inflammatory response, recruit and activate other cells to the site of inflammation and subsequently resolve the inflammation. Salix caprea is a common species of willow native to Europe and central Asia. It is a folkloric medicinal plant used to treat rheumatoid arthritis, malaria, various hemorrhages, gout, neuralgia and intestinal diseases. It is also used as an antipyretic, analgesic, antibacterial, haemostatic, sedative and antiinflammatory agent. The work on the chemical composition of the plant revealed the presence of various flavonoids like kaempferol, luteolin, apigenin, naringenin quercetin isothamnolin, luteolin 7-0-glucopyranoside, baccoside, salicaprin, aastragalin, quercemeritin, quercetin 3,7-di-o-glucoside and isouqueretin were isolated from salix caprea flower. Since many flavonoids have remarkable anti inflammatory activity. The present work aims to evaluate the anti inflammatory activity of salix caprea by HRBC membrane stabilization.

MATERIALS AND METHODS:

Plant material: The Flowers of salix caprea were collected in April (2010) at the SMPU of RRIUM Srinagar, India. The plant material was identified, authenticated by SMPU of RRIUM Srinagar, India and a voucher specimen (1536) was deposited in the herbarium of the RRIUM Srinagar, India.

Preparation of extracts: The Flowers were garbled and dried under shade and powdered. The powdered material was successively extracted with petroleum ether, ethyl acetate, methanol, hydroalcohol in soxhlet apparatus and aqueous extraction was done by decoction method using distilled water. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in desiccators. The percentage yield were 2.10, 1.40, 8.92, 9.18 and 10.3 in petroleum ether, ethyl acetate, methanol, hydroalcohol and aqueous extracts respectively. The qualitative analysis of various extracts of salix caprea flowers has indicated the presence of terpenoids, flavonoids, phenolic, and steroids types of compounds. Since these compounds are of pharmacological interest, prompted us to evaluate Salix caprea for possible anti-inflammatory.

In-vitro Anti-inflammatory activity

Human Red Blood Cell (HRBC) membrane stabilization method: The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosolaine and a 10% suspension was made. Various concentrations of extracts were prepared (200 and 400µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was spectrophotometrically at 560 nm. Diclofenac (100 and 200µg/ml) was used as reference standard and a control was prepared by omitting the extract.

The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

\[
\text{Percentage Protection} = \frac{\text{Optical density of control} - \text{Optical density of drugs treated sample}}{\text{Optical density of control}} \times 100
\]

Statistical Analysis:

All observations were presented as Mean ± SEM. The data was analyzed by student’s t-test or one-way ANOVA followed by Dunnet’s test. *p<0.05, **p<0.01, ***p<0.001 was considered as significant.

RESULTS

Effect of extracts on HRBC membrane stabilization:

Extracts were evaluated for anti-inflammatory activity by HRBC membrane stabilization method and we found that the percentage protection for petroleum ether, ethyl acetate, methanol, hydroalcohol and aqueous extracts were 45.57, 61.10, 66.79, 60.49, 68.39% at 400µg/ml respectively and Diclofenac hold the percentage protection 77.39% at 200µg/ml. The hydroalcohol, methanolic extracts showed most and aqueous, ethyl acetate extracts showed intermediate while petroleum ether extract showed least percentage protection as compared to Diclofenac. All extracts were showed significant activity with reference to standard drug (Diclofenac).

Table: 1 Effect of extracts on HRBC membrane stabilization

<table>
<thead>
<tr>
<th>Samples (µg/ml)</th>
<th>Percentage protection</th>
</tr>
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<tbody>
<tr>
<td>Control (Distilled water)</td>
<td>-</td>
</tr>
<tr>
<td>SDS 100</td>
<td>71.29 ± 1.40**</td>
</tr>
<tr>
<td>SDE 200</td>
<td>77.62 ± 2.40**</td>
</tr>
<tr>
<td>PEE 200</td>
<td>43.00 ± 3.21**</td>
</tr>
<tr>
<td>PEE 400</td>
<td>45.57 ± 1.85**</td>
</tr>
<tr>
<td>EAE 200</td>
<td>35.08 ± 2.00**</td>
</tr>
<tr>
<td>EAE 400</td>
<td>61.10 ± 3.28**</td>
</tr>
<tr>
<td>ME 200</td>
<td>62.39 ± 3.00**</td>
</tr>
<tr>
<td>ME 400</td>
<td>66.99 ± 2.27**</td>
</tr>
<tr>
<td>HAE 200</td>
<td>62.83 ± 1.60**</td>
</tr>
<tr>
<td>HAE 400</td>
<td>68.19 ± 1.20**</td>
</tr>
<tr>
<td>AQE 200</td>
<td>59.41 ± 1.77**</td>
</tr>
<tr>
<td>AQE 400</td>
<td>60.49 ± 1.88**</td>
</tr>
</tbody>
</table>

Where, n=6, Values are expressed as Mean ± S.E.M. SDS 100 = Standard Diclofenac sodium of 100 µg/ml, SDE 200 = Standard Diclofenac sodium of 200 µg/ml, PEE 200 = Petroleum ether extract of flowers of Salix caprea Linn. of 200 µg/ml, PEE 400 = Petroleum ether extract of flowers of Salix caprea Linn. of 400 µg/ml, EAE 200 = Ethyl acetate extract of flowers of Salix caprea Linn. of 200 µg/ml, EAE 400 = Ethyl acetate extract of flowers of Salix caprea Linn. of 400 µg/ml, ME 200 = Methanol extract of flowers of Salix caprea Linn. of 200 µg/ml, ME 400 = Methanol extract of flowers of Salix caprea Linn. of 400 µg/ml, HAE 200 = Hydroalcohol extract of flowers of Salix caprea Linn. of 200 µg/ml, HAE 400 = Hydroalcohol extract of flowers of Salix caprea Linn. of 400 µg/ml, HAE 200 = Hydroalcohol extract of flowers of Salix caprea Linn. of 400 µg/ml, HAE 400 = Hydroalcohol extract of flowers of Salix caprea Linn. of 400 µg/ml. The results were expressed as Mean ± SEM and statistically analyzed by student’s t-test and one-way analysis of variance (ANOVA) followed by Dunnet’s test with level of significance set at* p<0.05, **p<0.01.
**DISCUSSION:**

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. 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. During inflammation lysosomal enzymes are released that 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. 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 extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extracts may stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bacterial enzymes and proteases, which cause further tissue inflammation and damage. From the above study it was concluded that extracts of *Salix caprea* has significant membrane stabilization property.

**REFERENCES**


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