**INTRODUCTION**

Inflammation is a self defense mechanism of the body to protect against reaction to infection, irritation or allergens or any other harmful irritation. It is a part of the host defense mechanisms. It is known to be involved in the inflammatory reactions such as release of histamine, bradykinin, prostaglandins, extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease condition. The critical role of inappropriate inflammation is becoming accepted in many diseases that affect man, including cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infection and cancer[1]. Stabilization of lysosomal membrane is important in inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil for example bactericidal enzymes and proteases, which cause tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane[2]. 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 by hypotonicity induced membrane lysis can be taken as an in-vitro measure of anti-inflammatory activity of the untapped drugs or plant extracts. *Skimmia anquetilia* is an aromatic gregarious shrub belonging to family Rutaceae. It is mostly found in Western part of Himalayas and Kashmir in India. Traditionally, the leaf infusion of *S. anquetilia* is taken for treatment of headache, freshness and general fever[1,3]. The leaves of *S. anquetilia* are aromatic and known to contain linalool, geraniol, pinene, scopoletin, skimmianine, umbelliferone[3]. The present investigations are scientifically validated in vitro anti-inflammatory effects using human red blood cell membrane stabilization and anti-arthritic activity by protein denaturation assay methods.

**ABSTRACT**

Current investigations were carried out for the validation of in-vitro anti-inflammatory and anti-arthritic property of leaves of *Skimmia anquetilia* using red blood cells membrane stabilization and protein denaturation methods respectively. Defatted ethylacetate extracts at different concentration levels (50, 100, 200 and 400 mg/ml) were used in these studies. Dose dependent inhibition of protein denaturation was found 92.41% at 400 mg/ml of extracts and 96.21 % at 100 mg/ml of acetyl salicylic acid as standard in antiarthritic study. Similarly, in membrane stabilization methods, maximum effect found 90.70 % at 400 mg/ml of extracts and 94.88 % at 100 mg/ml of diclofenac sodium as standard for anti-inflammatory evaluation. The results concluded that, ethyl acetate extract of *S. anquetilia* leaves has shown significant (*P<0.05) anti-inflammatory and anti-arthritic effects.

**KEYWORDS:** Skimmia anquetilia, In-vitro anti-inflammatory, Anti-arthritic activity, Membrane stabilization assay, Protein denaturation method
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 3000 rpm. The packed cells were washed with isosalone and a 10% suspension was made.

Ethyl acetate extracts of *S. anquetilia* leaves was prepared and evaluated at different levels 100, 200 and 400 mg/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 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated with UV spectrophotometer at 560 nm. Diclofenac (50 mg/mL) was used as reference standard and a control was prepared by omitting the extracts. The experiment was performed in triplicate. The percentage of HRBC membrane stabilization or protection was calculated using the formula mentioned below:

\[
\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} \times 100
\]

**In-vitro Anti-arthritic Activity**

**Inhibition of protein denaturation method**

Inhibition of protein denaturation method was performed as described by Kumari *et al.*[5]. The suspension test mixture (2.5 ml) consisted of 2.2 ml bovine serum albumin (5% aqueous solution) and 0.3 ml of *S. anquetilia* leaves extract at different concentration levels 100, 200 and 400 mg/mL. The samples were incubated at 37°C for 30 min. After cooling the samples, 7.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured UV spectrophotometer at 660 nm for control test. 0.5 ml distilled water was added instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

\[
\text{Percent inhibition} = \frac{\text{Abs. of Control} - \text{Abs. of treated}}{\text{Abs. of Control}} \times 100
\]

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (50 mg/ml) treated samples.

**RESULTS**

Human red blood cell membrane stabilization method reflects the effect of drugs on cellular membrane i.e. red blood cell. Since HRBC membrane are similar to lysosomal membrane components[5,6]. The prevention of hypotoxicity induces HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. The ethyl acetate extract of *S. anquetilia* showed significant anti-inflammatory activity at the concentration of 400 mg/ml which is comparable to the standard drug diclofenac sodium (100 mg/ml). *In-vitro* anti-inflammatory activity of the extracts showed concentration dependent activity.

<table>
<thead>
<tr>
<th>Extracts/ Drug</th>
<th>Concentration (mg/ml)</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>EESA*</td>
<td>50</td>
<td>22.18±0.12</td>
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<tr>
<td></td>
<td>100</td>
<td>36.11±1.10</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>74.31±2.35</td>
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<tr>
<td></td>
<td>400</td>
<td>90.70±3.12</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>100</td>
<td>94.88±2.52</td>
</tr>
</tbody>
</table>

*EESA* - Ethyl acetate extracts of *S. anquetilia* leaves. Data’s statistical significance found as: *P< 0.01, *P< 0.05

**Table 1: Effect of ethyl acetate extract of *S. anquetilia* leaves (EESA) on HRBC membrane stabilization anti-inflammatory activity**

Table 2. Effect of ethyl acetate extract of *S. anquetilia* leaves (EESA) on Protein denaturation method for anti-arthritic activity

**Table 2: Effect of ethyl acetate extract of *S. anquetilia* leaves (EESA) on Protein denaturation method for anti-arthritic activity**

Human red blood cell membrane stabilization results are tabulated in Table 1. The results of 50, 100, 200 and 400 mg/ml of EESA showed concentration dependent activity respectively i.e. 22.18 %, 36.11 %, 74.31 % and 90.70%. Among all the concentration, EESA 100 mg and 400 mg were found statistically significant (*P<0.05). All the results of test drugs were compared with control and standard drug diclofenac sodium which showed 94.88% protection (Table 1).

**CONCLUSION**

The present investigations are scientifically validated *in vitro* using human red blood cell membrane stabilization and protein denaturation assay methods, it supported to traditional claim as
anti-inflammatory and anti-arthritic activity respectively. It can be concluded that the ethyl acetate extract of leaves of *Skimmia anquetilia* can be further used for *in-vivo* activities related to anti-inflammatory and anti-arthritic activity.

**CONFLICTS OF INTEREST**

No potential conflicts of interest were disclosed.

**REFERENCES**