INVITRO ANTI-INFLAMMATORY ACTIVITY OF MYXOPYRUM SMILACIFOLIUM BLUME (OLEACEAE)

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ABSTRACT
Inflammation can cause various physical dysfunctions. The study was aimed to evaluate the anti inflammatory activity of both ethanol and aqueous extract of the leaves of Myxopyrum smilacifolium Blume by HRBC membrane stabilization method and protein denaturation, using different concentrations (25, 50,100, 200 µg/ml). In this study membrane lysis and protein inhibition was taken as the measure of anti-inflammatory activity. Both results are compared with standard diclofenac, Myxopyrum smilacifolium showed significant membrane stabilizing activity of 69.60% and protein inhibition activity of 91.54% at concentration of 200 µg/ml. The result obtained from this study suggests that plant is a good natural source for anti-inflammatory therapy.

KEYWORDS: Myxopyrum smilacifolium, Anti-inflammatory, HRBC, Protein denaturation.

INTRODUCTION
Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body’s nonspecific internal systems of defense,
the response of a tissue to an accidental cut is similar to the response that results from other
types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion [1].

When tissue cells become injured they release kinins, prostroglandins and histamine. These
work collectively to cause increased vasodilatation (widening of blood capillaries) and
permeability of the capillaries. This leads to increased blood flow to the injured site. These
substances also act as chemical messengers that attract some of the body's natural defense
cells a mechanism known as chemo taxis [2].

Drugs which are in use presently for the management of pain and inflammatory conditions
are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g.
hydrocortisone. All of these drugs possess well known side and toxic effects. Several
experimental protocols of inflammation are used for evaluating the potency of drugs. The
management of inflammation related diseases is a real issue in the rural community; the
population in these areas uses many alternative drugs such as substances produced from
medicinal plants [3]. On the contrary many medicines of plant origin had been used since long
time without any adverse effects. Exploring the healing power of plants is an ancient concept.
For centuries people have been trying to alleviate and treat disease with different plant
extracts [4].

Myxopyrum smilacifolium Blume (Family-Oleaceae) is an important medicinal plant widely
used in indigenous system of medicine in India. The leaves are astringent, acrid, sweet,
thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha,
vata, cough, asthma, rheumatism, nostalgia, consumption, fever, otopathy, neuropathy and
cuts and wounds [5, 6]. Earlier the plant has been studied for its antimicrobial [6], antioxidant,
wound healing activity [7].

MATERIALS AND METHODS

Collection of plant material
The leaves of Myxopyrum Smilacifolium Blume was collected from Agricultural
University, Odakkali, Perumbavoor Ernakulam district, Kerala (India) in the month of
September 2013 and authenticated by Dr. Radhika, Govt Ayurvedic College Pariyaram,
Kannur. The leaves were dried in shade at room temperature. The dried leaves were subjected
to size reduction by using mixcy to obtain coarse powder.
Preparation of extract

**Ethanol extract:** The shade dried powdered leaves (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The ethanol was concentrated in vacuum to a syrupy consistency. The percentage yield of extract was found to be 3.75%.

**Aqueous extract:** The aqueous extract was prepared using fresh powder by maceration process. 100gm of the powdered drug was taken in a 2000ml conical flask with 500ml of distilled water and 10ml chloroform is added as preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc is discarded and airtight container in its filtrate dried under hot air oven at 45°C to semisolid mass. These were stored in refrigerator below 10°C. The percentage yield of extract was found to be 5%.

**The human red blood cell (HRBC) membrane stabilization method**

The Blood was collected from healthy human volunteers who were not taken any NSAIDs for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water) and centrifuged at 3000rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline.

Various concentrations of extracts were prepared (25, 50, 100 and 200µg/ml) using distilled water and to each concentration 1ml of phosphate buffer (0.15M, pH 7.4), 2ml hyposaline (0.36%) and 0.5ml of HRBC suspension were added. It is incubated at 37°C for 30min and centrifuged at 3000 rpm for 20min. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac was used as reference standard and a control (distilled water) was prepared omitting the extracts [2,3].

The % haemolysis was calculated by assuming the haemolysis produced in presence of distilled water of as 100% [8].

\[
\text{Inhibition} (\%) = \left( \frac{V_t}{V_c} - 1 \right) \times 100
\]

\[V_t = \text{Absorbance of test sample}, \quad V_c = \text{Absorbance of control}\]
Inhibition of protein denaturation

The anti-inflammatory activity of Myxopyrum smilacifolium Blume was studied by using inhibition of protein denaturation technique \cite{9,10}. The reaction mixture (5ml) consist of 0.2 ml of egg albumin (from fresh hen’s egg), 2.8ml phosphate buffered saline (pH:6.4) and 2ml of varying concentration of test extract. So that final concentrations become 25, 50, 100, 200µg/ml. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2°C in an incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660nm by using vehicle as blank. Diclofenac at the final concentration of (50, 100µg/ml) was used as reference drug and treated similarly for determination of absorbance.

The Percentage inhibition of protein denaturation was calculated as follows:
Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

RESULTS AND DISCUSSION

The phytochemical screening subjected to detect the presence of some secondary plant metabolites following standard procedure shown in Table 1. Ethyl alcohol extract revealed the presence of carbohydrates, steroids, terpenoids, flavanoids, tannins and poly phenols, while aqueous extract showed presence of carbohydrates, saponins, alkaloids, flavanoids, tannins and poly phenols.

Table 1: Phytochemical screening of plant material Myxopyrum smilacifolium

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Ethanol Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) : Present  (-) : Absent

Membrane stabilization

The HRBC membrane stabilization has been used as a method to study the invitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane \cite{11, 12} and its stabilization implies that the extract may well stabilize lysosomal membranes. 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 causes further tissue inflammation and damage upon extra cellular release. The lysosomal released during inflammation produce a various disorders. The extra cellular activity of these enzymes are 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.

Both extracts showed significant anti inflammatory activity in a concentration depended manner. Aqueous extract at a concentration of 200µg/ml showed 69.60% protection of HRBC in hypotonic solution compared with standard diclofenac which showed 97.059% protection at 100µg/ml.

Table 2: Invitro anti-inflammatory activity of Myxopyrum smilacifolium Blume.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. (µg/ml)</th>
<th>% Inhibition</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hemolysis</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>25</td>
<td>9.80</td>
<td>68.927</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16.17</td>
<td>70.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>46.58</td>
<td>80.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>20.58</td>
<td>91.54</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>25</td>
<td>10.29</td>
<td>58.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>18.13</td>
<td>72.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>56.37</td>
<td>77.09</td>
<td></td>
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<tr>
<td></td>
<td>200</td>
<td>69.60</td>
<td>86.46</td>
<td></td>
</tr>
<tr>
<td>Standard. Diclofenac</td>
<td>50</td>
<td>69.60</td>
<td>92.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>97.059</td>
<td>96.71</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Invitro anti-inflammatory activity of Myxopyrum smilacifolium Blume by HRBC membrane stabilization method.
Protein denaturation

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 80.07% was observed at 100 µg/ml and 91.54% at 200µg/ml. Diclofenac showed the maximum inhibition 96.71% at the concentration of 100 µg/ml (Table2).

![Invitro anti-inflammatory activity of Myxopyrum smilacifolium Blume by Protein denaturation.](image)

**Figure 2. Invitro anti-inflammatory activity of Myxopyrum smilacifolium Blume by Protein denaturation.**

**CONCLUSION**

In the present study, results indicate that the ethanol and aqueous extract of Myxopyrum smilacifolium Blume possess anti-inflammatory properties. This activity may be due to the strong occurrence of polyphenolic compound such as alkaloids, flavanoids, tannins, steroids and phenols. Further investigations are required to find out active component of the extract.

**REFERENCES**


