EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF ALCOHOL AND AQUEOUS EXTRACT OF *EPIPHYLLUM OXYPETALUM* LEAVES

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ABSTRACT

**Objective:** The main objective of the study is to evaluate the anti inflammatory activity of alcohol and aqueous extract of *Epiphyllum oxypetalum* leaves. **Methods:** The study was carried out by using invitro method which included human red blood cell membrane stabilisation and inhibition of protein denaturation method. The animal study was also carried out in albino wistar rats. **Results:** The percentage inhibition was seen maximum in 300 µg/ml concentration of alcohol and aqueous extract by using invitro methods whereas the percentage inhibition in alcohol and aqueous extract in animal study were found be maximum at 600mg/kg and 200mg/kg respectively. **Conclusion:** The alcohol extract and aqueous extract of *Epiphyllum oxypetalum* leaves exhibit anti inflammatory activity.

**KEYWORDS:** anti inflammatory, *Epiphyllum oxypetalum*, membrane stabilisation, protein denaturation.

INTRODUCTION

The inflammatory responses involve a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue break down and repair which are aimed at host defense and usually activated in most disease conditions. [1] The mechanism of inflammation injury is attributed in part to release of reactive oxygen species from activated neutrophil and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. [2]
Inflammation is the condition associated with many of the disease states and this review elaborate the medicinal plants, their parts used in the effective management of inflammation and its associated conditions.

Medicinal plants constitute important components of flora and are widely distributed in different region of India. Medicinal plants consists of various phytochemicals like alkaloids, flavonoids, polyphenols, glycoside. These bioactive compounds from a medicinal plants have shown many pharmacological activities. Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles in against various diseases.

Nowadays much interest has arisen in the search of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agents without too many side effects.

*Epiphyllum oxypetalum* is a member of the cactaceae family and considered to be popular as an ornamental plant. It has various other names like Brahmakamal, Wijayakusuma, Queen of night, Dutchman’s pipe. *Epiphyllum oxypetalum* has been used as a traditional medicinal plant since a long time, especially in treatment of injury and inflammation. People generally use *Epiphyllum oxypetalum* fresh collision and affix it on the skin as a swelling and wound healing.

The chemical content of *Epiphyllum oxypetalum* in plants has a potent power to stifle the pain and is able to neutralize blood clotting. The phylloclades contain some active ingredients and show anti bacterial activity. The stem is also used medicinally to cure dropsy and cardiac affections. Flower also has a power that can speed up the cook wound abscesses. Also it is used in bloody phlegm and cough, uterine bleeding and shortness of breath. The previous research reports that *Epiphyllum oxypetalum* has some potential activities to reduce pain as well as to neutralize the blood clotting. So present study was undertaken to investigate the anti-inflammatory activity of alcohol extract and aqueous extract of *Epiphyllum oxypetalum* leaf by in vivo and in vitro methods.
MATERIALS AND METHODS

Collection of plant material
The fresh leaves of *Epiphyllum oxypetalum* were collected from the nursery and local garden in Ratnagiri, Maharashtra. The collected leaves were identified and authenticated by Dr. Harshad Pandit from Guru Nanak Khalsa College, Matunga.

Preparation of plant extract
The fresh leaves were washed under running tap water, shed dried and coarsely powdered in a mechanical grinder.

Preparation of alcohol extract
- The powder was extracted with absolute ethanol in soxhlet extractor at temperature 40-50°C. The extract was dried on water bath at 60°C.

Preparation of aqueous extract
- The powder was weighed approximately and was added in distilled water. The extraction process was carried out at 80°C for 4-5 hrs. Then the extract was filtered hot using a muslin cloth and dried on water bath at 60°C.

Experimental animals
Adult rats of Albino Wistar strain of both sex weighing 150-200g were selected. They were fed with commercial pellet diet and water *ad libitum*. Before the treatment the rats were acclimatized to the laboratory conditions for a week. Animals were housed in polypropylene cages with not more than four animals per cage under standard laboratory conditions (temperature 25°C ± 2°C) and relative humidity 50±5% with alternating 10 h dark/14 h light photoperiod. Approval from the institutional animal ethics committee for the use of animals in the experiments was obtained. The protocol of the study was approved by an animal ethics committee of the department and the experiments were carried out as per the guidelines of CPCSEA.

Experimental design
The studies will be carried out by using six groups of six animals
- **Group I** – Control group received normal saline (0.5% Sodium CMC solution)
- **Group II** – Rats were received 200 mg/kg of alcohol extract
- **Group III** – Rats were received 400 mg/kg of alcohol extract
- **Group IV** – Rats were received 600 mg/kg of alcohol extract
- **Group V** – Rats were received 200 mg/kg of aqueous extract
Group VI – Rats were received 400 mg/kg of aqueous extract
Group VII – Rats were received 600 mg/kg of aqueous extract
Group VIII – Rats were received Standard drug (Aspirin 10 mg/kg p.o.)

Inflammation was induced by a 0.1 ml injection of 1% w/v suspension of carrageen nan in saline to the plantar surface of right hind paw. Test and standard drugs were administered orally to the respective groups 60 minutes prior to carrageenan injection. The change in the inflammatory reaction was measured using plethysmometer on various time intervals (0, 1, 2, 3, 5 hr) and compared with control group. The right hind paw edema inhibition at different doses of test drug and standard drug was calculated by the following formula -

\[
\% \text{ inhibition of paw edema} = \frac{(V_t - V_o)_{control} - (V_t - V_o)_{treated}}{(V_t - V_o)_{control}} \times 100
\]

Where, \( V_t \) - rat paw volume at time ‘t’,
\( V_o \) - initial rat paw volume (before carrageenan injection),
\( (V_t - V_o)_{control} \) - edema produced in control group and
\( (V_t - V_o)_{treated} \) - edema produced in treatment.

Invitro evaluation of anti inflammatory activity

1) Membrane stabilisation method

Preparation of Human Red Blood Cells (HRBC) Suspension - Fresh whole human blood was collected and mixed with anticoagulants. The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.\(^9,10\)

Heat Induced Hemolytic Activity: The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula below.\(^9,11\)

\[
\% \text{ Inhibition} = \frac{(\text{Absorbance}_{control} - \text{Absorbance}_{test})}{\text{Absorbance}_{control}} \times 100
\]
2) Inhibiton of albumin denaturation

The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min. after cooling the samples the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows,

\[
\text{% Inhibition} = \left( \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right) \times 100
\]

Where, \(\text{Abs}_{\text{control}}\) - absorbance without sample
\(\text{Abs}_{\text{sample}}\) - absorbance of sample extract / standard.[9]

Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups (p<0.05). Means between treatment groups were compared for significance using Duncan’s new Multiple Range post test for in vitro tests and Dunnett’s test for animal study.

RESULTS AND DISCUSSION

Animal study

Carrageenan induced paw edema is most commonly used as an *in vivo* model of inflammation to assess the anti-edematous effect of the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin and bradykinin on vascular permeability. The late phase of the inflammatory response has been shown to be due to potentiating effect of bradykinin on mediator release and prostaglandins, producing edema after mobilization of the leukocytes. Nitrous oxide (NO) is a potent vasodilator and is also involved in carrageenan-induced edema, which may be related to its ability to increase vascular permeability and edema through changes in local blood flow.[11]
Table 1: Anti inflammatory activity of *Epiphyllum oxypetalum* leaves extract by carrageenan induced rat paw edema method

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose</th>
<th>Paw edema volume in ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>1hr</td>
<td>2hr</td>
<td>3hr</td>
<td>5hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol Extract</td>
<td>200 mg/kg</td>
<td>0.47±0.03</td>
<td>0.80±0.04</td>
<td>0.68±0.05*</td>
<td>0.50±0.04#</td>
<td>0.52±0.03*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.53±0.02</td>
<td>0.87±0.04</td>
<td>0.80±0.04</td>
<td>0.67±0.02</td>
<td>0.58±0.03*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>600 mg/kg</td>
<td>0.50±0.00</td>
<td>0.82±0.02</td>
<td>0.70±0.03*</td>
<td>0.65±0.02</td>
<td>0.57±0.02*</td>
<td></td>
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</tr>
<tr>
<td>Aqueous Extract</td>
<td>200 mg/kg</td>
<td>0.45±0.02</td>
<td>0.72±0.04*</td>
<td>0.62±0.04*</td>
<td>0.50±0.03#</td>
<td>0.47±0.02*</td>
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<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.52±0.02</td>
<td>0.83±0.02</td>
<td>0.70±0.03*</td>
<td>0.67±0.02*</td>
<td>0.55±0.02*#</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>600 mg/kg</td>
<td>0.57±0.02</td>
<td>0.90±0.03*</td>
<td>0.70±0.04</td>
<td>0.65±0.02</td>
<td>0.57±0.02*#</td>
<td></td>
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<tr>
<td>Standard drug</td>
<td>10 mg/kg</td>
<td>0.40±0.04</td>
<td>0.78±0.08*</td>
<td>0.67±0.08*</td>
<td>0.58±0.07#</td>
<td>0.50±0.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.34±0.02</td>
<td>0.73±0.02</td>
<td>0.77±0.04</td>
<td>0.82±0.03</td>
<td>0.45±0.02</td>
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</tbody>
</table>

*P > 0.05, **P < 0.05, # P < 0.01

Table 2: Percentage inhibition of rat paw edema by alcohol and aqueous extracts of *Epiphyllum oxypetalum* leaves

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose</th>
<th>Percentage Inhibition</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1hr</td>
<td>2hr</td>
<td>3hr</td>
<td>5hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol Extract</td>
<td>200 mg/kg</td>
<td>14.89</td>
<td>49.02</td>
<td>71.93</td>
<td>53.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>14.89</td>
<td>37.25</td>
<td>71.93</td>
<td>53.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>600 mg/kg</td>
<td>19.15</td>
<td>52.94</td>
<td>75.44</td>
<td>38.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>200 mg/kg</td>
<td>27.27</td>
<td>58.33</td>
<td>89.28</td>
<td>66.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>13.63</td>
<td>54.16</td>
<td>67.85</td>
<td>33.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>600 mg/kg</td>
<td>9.09</td>
<td>66.66</td>
<td>82.14</td>
<td>66.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard drug</td>
<td>10 mg/kg</td>
<td>2.13</td>
<td>37.25</td>
<td>61.40</td>
<td>7.69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Invitro evaluation of anti inflammatory test

1) Membrane stabilisation method

Stabilization of RBCs membrane was studied which further establishes the mechanism of anti-inflammatory action of ethanolic and aqueous extract of *Epiphyllum oxypetalum*. Both the extracts were effective in RBC membrane stabilisation. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The alcohol and aqueous extract stabilised the RBC membrane to varying degree (Table 2). The aspirin, standard drug showed the maximum inhibition 77.55% at 100µg/ml.

2) Inhibiton of protein denaturation

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 extract protein
denaturation was studied. Both the plant extracts were found to effective in inhibiting heat induced albumin denaturation. Maximum inhibition 39.45% and 55.70% was seen at concentration 300µg/ml in alcohol and aqueous extract respectively. Aspirin, standard anti inflammatory drug showed maximum inhibition 72.10% at concentration 100µg/ml.

Table 3: In vitro evaluation of anti inflammatory activity of alcohol and aqueous extract of *Epiphyllum oxypetalum* leaves

<table>
<thead>
<tr>
<th>Dose</th>
<th>Inhibition of Protein denaturation (% inhibition ± SEM)</th>
<th>Membrane stabilization (% inhibition ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alcohol extract</td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>23.93±1.12</td>
<td>36.38±0.99</td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>30.81±0.92</td>
<td>45.45±1.80</td>
</tr>
<tr>
<td>300 µg/ml</td>
<td>39.45±1.51</td>
<td>55.70±1.03</td>
</tr>
<tr>
<td>Standard drug</td>
<td>72.10±1.78</td>
<td>77.55±1.02</td>
</tr>
</tbody>
</table>

CONCLUSION

The alcohol extract and aqueous extract of *Epiphyllum oxypetalum* leaves exhibited anti inflammatory activity. It was concluded from the animal study and in vitro methods that the aqueous extract of *Epiphyllum oxypetalum* showed more anti inflammatory activity than alcohol extract.

REFERENCES

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