RESEARCH ARTICLE
Experimental evaluation of anti-inflammatory effect of ethanolic extract of *Vanilla planifolia* seeds in Wistar rats

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ABSTRACT

Background: Inflammation is a protective response of the body to the harmful stimuli. Inflammation can be either acute or chronic, always associated pain, redness and loss of function. *Vanilla planifolia* (VP) is the aromatic plant, as per literature, it has anti-inflammatory activity, which has not been tested as per modern medicinal parameters. Non-steroidal anti-inflammatory drugs are commonly used for the treatment but have many adverse effects such as gastritis, hepatitis etc. Therefore, there is always a search for new safe drug. Aim and Objectives: The aim of the study is to evaluate the anti-inflammatory activity of VP seeds in acute and chronic animal model of inflammation. Materials and Methods: Rats weighing 150–200 g of either sex were included in the study. Acute Anti-inflammatory activity tested with carrageenan-induced paw edema and chronic with cotton pellet-induced granuloma model. Animals were divided into five groups – Gr-I Control, Gr-II Vehicle control, Gr-III Diclofenac sodium, Gr-IV (VPLD), Gr-V (VPHD). Drug treatment was given 1 h before carrageenan injection. Paw volume measured at different time interval with plethysmometer. In chronic model, drug treatment was given for 7 days after pellet implantation. On 8th day, pellets removed and dried in oven. Weight of wet and dry pellets from all the groups compared with vehicle control. Data obtained was analyzed with Graph pad prism 6. Results: Reduction in paw volume started in all drug treated groups after 1 h of treatment. Paw volume was significantly reduced ($P < 0.001$) in group III, IV & V in 5 h. reached to near normal. In chronic model, VPLD showed decrease in wet pellet ($P < 0.01$) and dry pellet weight ($P < 0.05$) significantly. VPHD was more effective in reducing wet pellet ($P < 0.001$) and dry Pellet weight ($P < 0.01$). Similar results were seen on the left side. Conclusion: VP seed extract showed promising anti-inflammatory effect in both models of inflammation.

INTRODUCTION

Inflammation is a protective response of the body to the harmful stimuli such as infectious agents, thermal, chemical, physical agents, and ischemia.[1] The cardinal features of inflammation are redness, warmth, swelling, and pain.[2] Inflammation can be either acute or chronic inflammation.[3,4] Tissue damage causes the vascular and cellular changes such as dilation of venules, increase in vascular permeability, and infiltration of histamine, cytokines and other inflammatory components.[5] Pain is always associated with inflammation, an unpleasant sensation but essential for the diagnosis.

Pain and inflammation is usually treated with Non-steroidal anti-inflammatory drugs like Diclofenac, Aspirin, Ibuprofen, Naproxen, Celecoxib, Ketorolac etc. associated with the acute or chronic condition. However, there are many adverse effects such as epigastric pain, nausea, vomiting and gastric ulcers, bleeding, melena encountered with its long-term use.
which limits use. Therefore, there is always a need of more effective and safe drug.\footnote{[6]}

Many natural substances and herbal products are claimed to be effective in reducing pain and inflammation.\footnote{[7]} They are tapped for their potential use in these conditions using modern medicinal parameters.

*Vanilla planifolia* (VP) belongs to the family Orchidaceae, is available for commercial as well as medicinal purpose.\footnote{[8]} The active substance in VP is vanillin, a methylprotocatechuic aldehyde (4-hydroxy-3h (methoxy) benzaldehyde) Maximum part is present in the Vanilla beans and other constituents are vanillic acid, anisaldehyde, hydroxy benzoic acid, anisic acid, anisyl alcohol, caproic acid, phenol ether, lactone. This herbal agent is claimed to be effective as anti-inflammatory and have analgesic activity.\footnote{[9]}

The present study was undertaken with the aim to evaluate the anti-inflammatory activity of ethanolic extract of VP seeds.

**Objectives**

The study aims to evaluate the anti-inflammatory activity of VP in acute and Chronic animal model of inflammation.

To compare it with standard drug Diclofenac sodium.

**MATERIALS AND METHODS**

The study was started after getting approval from Institutional Animal Ethics Committee of Bharati Vidyapeeth Medical College (Approval Letter No. IAEC/BVDUMC/1607/2020/002/008) Pune.

**Chemicals**

Organic vanilla seeds powder was obtained from authentic Ayurvedic oushadhalaya., Diclofenac, Ketamine (with letter from the anesthetist), Betadine, Normal saline- Was obtained from the Chemist.

**Surgical Instruments**

Scissors, forceps, artery forceps scalpel, needles, catgut.

**Other instruments**

Soxhlet Apparatus, Water bath, Plethysmometer, Oven (Thermostat).

**Plant Material**

**Preparation of extract\footnote{[10]}**

The soxhlet apparatus filled with the 300 ml of ethanol into the round bottom flask.

The thimble containing vanilla sample (20 gms) kept into extracting tube and attached to the flask containing solvent. A condenser unit attached with the extraction tube and water passed through it and Soxhlet apparatus fixed on heating mantle. The heating mantle switched on and the temperature set as per requirement and the flask containing solvent heated.

The solvent start evaporating and falls in the extraction tube after condensing. Remove extraction from the heating mantle and cool the solution, transferred it into the porcelain dish.

The rectangular water bath filled with water and heated with the temperature as per requirement [generally set 100°C]. Placed in the porcelain dish on the rectangular water bath and after some time gradually increased the temperature of water, ethanol gets evaporated and a like gelly substance remains in the porcelain dish. After complete drying, it was used for the study.

**Standard Drug**

Market preparation of Diclofenac tablets was used as positive control.

**Animals Used**

Albino Wistar Rats of either sex weighing - 150–200 g were used for the study.

Animals were obtained from B.V.D.U. Medical College, Central animal house recognized by CPCSEA (Regd.No. 258/PO/ReBi/S/2000/CPCSEA), Pune 43. Housed at 25°C ± 2°C in clean polypropylene cages in batches of three animals per cage. 12 h day and night cycle maintained. Rodent food from Pranav agro industries and aquaguard water given *ad libitum*.

**Methods**

*For anti-inflammatory activity*

1. Acute inflammation- Carrageenan induced paw edema
2. Chronic inflammation- Cotton pellet granuloma.

**Carrageenan Induced Paw Edema\footnote{[11]}**

Thirty adult male Wistar albino rats (150–200 g) were used in the study.

Animals were divided into five groups (*n* = 6). Baseline paw volume recording was carried out using a digital plethysmometer.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>I</td>
<td>Control- No treatment</td>
</tr>
<tr>
<td>II</td>
<td>Disease control</td>
</tr>
<tr>
<td>III</td>
<td>Standard control- Diclofenac sodium (10 mg/kg body weight)</td>
</tr>
<tr>
<td>IV</td>
<td>Vanilla seed extract X (100 mg/kg)</td>
</tr>
<tr>
<td>V</td>
<td>Vanilla seed extract 2 X (200 mg/kg)</td>
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</tbody>
</table>
Drug treatment was given according to the groups to all the animals. Sixty minutes after administration the vehicle/drug treatment, left hind paw edema was induced by subcutaneous injection of 0.1 ml of 1% carrageenan (freshly prepared in normal saline) in the animals of Groups II, III, IV and V. Paw volume was again measured at 1 h, 2 h, 3 h, 4 h, 5 h and 24 h post-carrageenan administration. Paw edema was determined [Figure 1].

**Anti-inflammatory Activity - Chronic Inflammation**

*Cotton pellet granuloma* [11]

Wistar albino rats with 150–200 g. weight and of either sex were included in the study. Animals were divided into four groups (*n*=6).

<table>
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<tr>
<td>I</td>
<td>Disease control</td>
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<td>Vanilla seed extract 2 X (200 mg/kg)</td>
</tr>
</tbody>
</table>

**Inflammation induced by cotton pellet implantation.**

**Method of pellet implantation** [12]

- Small pellets of 50 ±1 mg were prepared and sterilized in sterilizer
- The skin below the nape of the neck on scapular region was shaved
- Animal was anesthetized with Ketamine in 100 mg/kg dose
- Skin was disinfected with 70% ethanol
- Incision was taken on left side
- By passing a blunt forceps subcutaneous tunnels was prepared
- A sterilized cotton pellet of 50 mg was placed through the incision in the tunnel in the scapular region
- Incision closed with sutures
- Same procedure was performed on the Rt side [Figure 2].

Drug treatment was given according to groups to all the animals for seven days. The rats were anesthetized with ketamine on 8th day. Incision on the skin was made and pellet was separated carefully and removed.

**RESULTS**

**Anti-inflammatory Activity-Acute Inflammation**

In normal control, there was no increase in the paw volume. Baseline 0 h reading of all the groups was almost similar. In Vehicle control group, paw volume kept on increasing up to five hours but gradually decreased over the period 24 h. One hour after the drug treatment the paw volume not increased significantly in VPHD group. Effect of VPHD was more marked than other drug treated groups in 1 h but the significant effect of Diclofenac was seen in 2 h and VPLD appeared 3 h. Significant difference in paw volume in Diclofenac (*P* < 0.05), VPLD (*P* < 0.05) and VPHD (*P* < 0.01) in 4th h and Diclofenac (*P* < 0.001), VPLD (*P* < 0.001) and VPHD (*P* < 0.001) in 5th h in comparison with vehicle control. In 24 h Paw volume was reached to normal values [Table 1].

**Anti-inflammatory Activity-Chronic Inflammation-Right Side Pellets**

Pellet weight of the Vehicle control rats was high in wet as well as in the dry form. In standard control (Diclofenac) rats both wet (*P* < 0.001) and dry (*P* < 0.001) weight was significantly less in comparison with vehicle control. Low dose VP (VPLD) showed decrease in wet pellet (*P* < 0.01) and dry pellet weight (*P* < 0.05). High dose of VP (VPHD) was more effective in reducing wet pellet weight (*P* < 0.001) and dry pellet weight (*P* < 0.01) [Figure 3].

Pellet weight of the vehicle control rats was high in wet as well as in the dry form. In standard control rats both wet (*P* < 0.001) and dry (*P* < 0.001) weight was significantly less in comparison with control. Low dose VP showed decrease in wet pellet (*P* < 0.01) and dry pellet weight (*P* < 0.01). High dose of VP was more effective in reducing wet pellet weight (*P* < 0.01) and dry pellet weight (*P* < 0.001) [Figure 4].

**DISCUSSION**

Inflammation is a complex set of interactions can arise in any tissue in response to traumatic, infectious, post-ischemic,
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Toxic or autoimmune injury. Mainly the cellular and molecular components are involved in the acute inflammatory response to infection and to a lesser extent to tissue injury. Typical presentation of the inflammatory response includes redness, pain, swelling and heat. Depending on the trigger, the inflammatory response has a different physiological purpose and pathological consequences. The process normally leads to recovery from infection and healing. However, if targeted destruction and assisted repair are not properly phased, inflammation can lead to persistent tissue damage by leukocytes, lymphocytes, or collagen. Systemic chronic inflammation, which occurs in a wide variety of diseases, including type 2 diabetes, cardiovascular diseases and even in cancer.

As per traditional literature VP has antispasmodic, anti-inflammatory and analgesic activity, it has been evaluated for the anti-carcinogenic, anti-oxidant activity but not for the anti-inflammatory activity of the seeds ethanolic extract.

The search of new therapeutic agent for anti-inflammatory action is fruitful when an appropriate model is used and the results obtained are reproducible and reliable and the obtained results furthered to the clinical trial.

Acute inflammation is characterized by the vascular changes such as changes in the blood flow, increased microvascular permeability, and formation of cellular exudate whereas cellular changes lead to formation of phagosome. In the present study, we used carrageenan-induced paw edema model to study the acute effect of the VP in inflammation. This is a well-established, reliable model having greater reproducibility and depicts the changes occurring in the human. Sulfated sugars in the carrageenan leads to the activation of complement system and release of various pro-inflammatory mediators.

In our study, the inflammatory changes started reducing from one our maximum effect remained upto 4–5 h and at 24 h it became near normal. VP showed powerful anti-inflammatory potential by inhibiting the early release of mediators of inflammation.

### Table 1: Effect of VP seed extract on paw volume

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>Dose (mg/kg)</th>
<th>Baseline</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NC)</td>
<td>No Treatment</td>
<td>1.08±0.09</td>
<td>1.06±0.09</td>
<td>1.07±0.08</td>
<td>1.07±0.08</td>
<td>1.07±0.08</td>
<td>1.07±0.08</td>
<td>1.07±0.08</td>
</tr>
<tr>
<td>(VC)</td>
<td>NS- 1 ml</td>
<td>1.02±0.11</td>
<td>1.28±0.08*</td>
<td>1.32±0.06*</td>
<td>1.36±0.05*</td>
<td>1.38±0.06*</td>
<td>1.41±0.05*</td>
<td>1.11±0.10*</td>
</tr>
<tr>
<td>(Diclo)</td>
<td>10 mg/kg</td>
<td>1.08±0.12</td>
<td>1.26±0.10*</td>
<td>1.21±0.11</td>
<td>1.20±0.11</td>
<td>1.20±0.02</td>
<td>1.18±0.04*</td>
<td>1.09±012</td>
</tr>
<tr>
<td>(VPLD)</td>
<td>100 mg/kg</td>
<td>1.03±0.06</td>
<td>1.25±0.06*</td>
<td>1.25±0.05*</td>
<td>1.24±0.07</td>
<td>1.21±0.02*</td>
<td>1.19±0.02*</td>
<td>1.10±0.07</td>
</tr>
<tr>
<td>(VPHD)</td>
<td>200 mg/kg</td>
<td>1.04±0.08</td>
<td>1.20±0.07</td>
<td>1.18±0.06</td>
<td>1.19±0.09</td>
<td>1.16±0.06*</td>
<td>1.12±0.06*</td>
<td>1.08±0.09</td>
</tr>
</tbody>
</table>

Values expressed as Mean±SD, n=6 Data analyzed by one-way ANOVA followed Tukey’s test. P<0.05, \*P<0.01, **P<0.001 Paw Volume in comparison with control. *P<0.05, **P<0.01, ***P<0.001 Paw Volume in comparison with vehicle control. VP: Vanilla planifolia.

Figure 2: Chronic inflammation – Cotton pellet granuloma

Figure 3: Effect of Vanilla planifolia on pellet weight right side

Figure 4: Effect of Vanilla planifolia on pellet weight left side
inflammation. With the high dose of VP, it was more evident after one hour of treatment. Yukio Murakami showed COX 2 inhibitor activity of Vanillin in invitro study. vanillin, a potent inhibitor of LPS (lipopolysaccharide)-induced COX-2 gene expression suggesting that the potent anti-inflammatory activity of vanillin attributed to its phenol function characterized by the higher bond dissociation Enthalpy value. Eun-Ju LI et al. studied anti-inflammatory activity of vanillin in acetic acid-induced permeability model in mice.

Cotton pellet-induced granuloma model represents the pathological events in chronic inflammation, transudative, and proliferative elements can be measured. Chronic inflammation is characterized by granulomatous changes. Wet pellets absorb the fluid and dry pellet represents the weight of granulomatous tissue.

VP showed reduction in wet and dry weight of the pellets, indicating that it was effective in reducing transudate and granulomatous tissue formation respectively. Anti-inflammatory activity attributed to decrease in the levels of inducible nitric oxide synthase (iNOS). Arya et al. also showed that vanillin reduced the induction of iNOS mRNA in a concentration-dependent manner. VP seeds contains phenolic compounds (4-hydroxy-3-methoxybenzaldehyde) are responsible for various biological properties like antioxidant, anti-inflammatory, anticancer and anti-atherosclerotic effects. In addition, VP has been studied for antioxidant property reducing the oxidative stress by ROS scavenging property and improving the condition.

CONCLUSION

The potent anti-inflammatory activity of the ethanolic extract of VP seeds in high dose was confirmed in the present study. Low dose showed the comparable anti-inflammatory activity with Diclofenac sodium. It seems VP, a promising anti-inflammatory agent which needs to be further evaluated to explore its clinical potentials.

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REFERENCES


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