Investigation of Anti-inflammatory Activity of *Passiflora nepalensis* Against Carrageenan Induced Inflammation in Rats

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**Abstract:** This study was intended to evaluate the anti-inflammatory activity of the whole plant of *Passiflora nepalensis*. The anti-inflammatory activity study was carried out by using carrageenan induced paw edema model. The methanolic extract of whole plant of *Passiflora nepalensis* was administered at different doses such as 150 and 225 mg/kg body weight and the study was compared with standard drug Aspirin (300mg/kg). The extract exhibited significant anti-inflammatory activity, which supports the traditional medicinal utilization of the plant.

**Key words:** *Passiflora nepalensis* · Anti-Inflammatory · Carrageenan · Edema

**INTRODUCTION**

There are 500 species in the Genus *Passiflora*. In 1529 Spanish discovered *Passiflora* which comes from a Latin word “Passio” which was a symbol for “Passion of Christ” [1]. They are common for warm and tropical areas [2]. The synonyms of *Passiflora nepalensis* are Passion fruit (English), Krishna Fal (Hindi), Pansara (Bengali) and Garindal (Nepali) [3]. In West India, Mexico, Netherlands, South America, Italia and Argentina, *Passiflora* is widely used as a traditional medicine. In Eastern India, *Passiflora nepalensis* is commonly found which belongs to the family Passifloraceae [1]. It is commonly found in Sikkim, Himalayan region, Assam and Bengal of India [3]. Various chemical constituents are present in *Passiflora* like phenols, glycosyl flavonoids and cyanogenic compounds. Vitexin has been isolated from methanolic extract of *Passiflora nepalensis* which is one of the crucial glycosyl flavonoid [1-3]. Isovitexin, orientin, isoorientin, apigenin, kaempferol and quercetin, carbohydrates, amino acids, benzoypyrones, cyanogenic glycosides such as gynocardin, pyrone derivatives such as maltol and ethyl maltol are also the chemical ingredients of Passion flower [4]. The medicinal value of Passion flower is present in Ayurveda, Siddha and Unani systems of medicine [2]. *P. nepalensis* is used as a cure for hypertension and inflammation. It has potent antioxidant activity [5]. *Passiflora edulis* is useful as a sedative and to cure central disorders such as anxiety and insomnia [3]. It has negative chronotropic effects. It has hypolipidemic activity which is stated by rural community of Sikkim State. It also acts as protective agent against renal ischemia/reperfusion [2]. *Passiflora nepalensis* treats anxiety, opiates withdrawal, insomnia, attention- deficit hyperactivity disorder and cancer [4]. The juice of *P. nepalensis* is used in the manufacturing of candy, squashes, cordials, syrups, carbonated beverages and jellies [3]. *Passiflora nepalensis* is grown as an ornamental plant in gardens. It is a climber with pale pink flower and its fruit is edible when ripe [2].

Inflammation is one of the major conditions associated with various diseases [6]. Rheumatoid arthritis is one of the inflammatory disorders [7]. Drugs which are used currently for inflammatory disorders and management of pain are salicylates, corticosteroids but they are associated with various side effects like intestinal tract ulcers, erosion of stomach lining [5, 8]. The present study focuses on evaluating the anti-inflammatory activity of *Passiflora nepalensis* in carrageenan induced rat paw edema [9].

**MATERIALS AND METHODS**

**Plant Material:** The whole plant of *Passiflora nepalensis* Walp. Passifloraceae, was collected in the month of October from Eastern India (Sikkim Himalayas) and...
identified by Dr. K. Gauthaman of Pharmacognosy Department, Himalayan Pharmacy Institute, Sikkim, India. A voucher specimen number HPI 168 was deposited in the departmental herbarium.

**Extraction:** The whole plant was dried in shade and powdered (no. 60 mesh) and 100 g of the dried powder was extracted successively with petroleum ether, chloroform and methanol in Soxhlet apparatus. The weight of methanolic extract after drying was calculated as 15.4 g.

**Animals:** Male Sprague-Dawley rats weighing 300-350 g were used for the experiment. The rats were kept in suitable environmental conditions and fed with standard diet and water ad libitum. The experiment was carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The institute animal ethical committee has given approval for conducting animal experiments (HPI/08/60/IAEC/0060).

**Acute Toxicity Study:** The acute toxicity study of *Passiflora nepalensis* was performed (Turner, 1965; Veerappan et al. 2007). The dead animals obtained from primary screening studies, LD50 value determination experiments, & the acute studies were subjected to post mortem studies. The external appearance of the dead animals, the appearance of the viscera, heart, lungs, stomach, intestine, liver, kidney, spleen and brain were carefully noted & any apparent & significant features or differences from the normal were recorded [1].

**In vivo Anti-inflammatory Activity:**

**Hind Paw Edema Method:** The anti-inflammatory activity is determined in Male Sprague-Dawley rats using 6 animals in each group. The animals were injected with carrageenan (1% w/v suspension in 0.9% saline) in right hand foot under plantar anesthesia.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control treated with saline</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Positive group treated with Aspirin</td>
<td>300 mg/kg, p.o.</td>
</tr>
<tr>
<td>3</td>
<td>Extract low dose</td>
<td>150 mg/kg, p.o.</td>
</tr>
<tr>
<td>4</td>
<td>Extract high dose</td>
<td>225 mg/kg, p.o.</td>
</tr>
</tbody>
</table>

- Group I: The control group was given same volume of normal saline as in the test group orally.
- Group II: The positive group was treated with acetylsalicylic acid (Aspirin) orally before one hour of the carrageenan injection.

Groups III and IV: The test groups of rats were given 150 mg/kg and 225 mg/kg of methanolic extract of *Passiflora nepalensis* orally one hour before the carrageenan injection.

The inflammation was quantitated in terms of ml i.e. the volume of water replaced by edema using a plethysmometer immediately before carrageenan injection and then 1, 3, 5 hours after carrageenan injection. The percentage inhibition of edema was calculated for each group with respects to the control group. The anti-inflammatory activity was calculated by following formula:

\[
\left(\frac{V_c - V_i}{V_c}\right)\times 100
\]

Where \(V_c\) and \(V_i\) are denote mean increase in paw volume of control group and treated groups respectively [5, 10, 11].

**Statistical Analysis:** All the results were expressed as mean ± standard error (SEM). Data were analyzed using one-way ANOVA followed by Dunnett’s test. \(p<0.001\) and \(p<0.05\) were considered as statistically significant.

**RESULTS**

**In vivo Anti-inflammatory Activity:** The anti-inflammatory activity was compared between both the doses of *Passiflora nepalensis* along with control group. From the result it can be noticed that methanolic extract of *Passiflora nepalensis* has shown significant anti-inflammatory activity by decreasing paw volume at 3 hrs. and 5 hrs. compared with control. The result obtained in anti-inflammatory activity is represented by the following graph:

![Graph showing anti-inflammatory activity of methanolic extract of *Passiflora nepalensis*](image-url)

**Fig. 1:** Effect of methanolic extract of *Passiflora nepalensis* on carrageenan-induced paw edema in rats.
**DISCUSSION AND CONCLUSION**

In the present study, the anti-inflammatory activity of the methanolic extract of *Passiflora nepalensis* has been evaluated by anti-inflammatory models. The inhibition of carrageenan induced inflammation in rats is a reported model for evaluating anti-inflammatory activity of the drugs. The development of carrageenan induced edema has 2 steps; the first step occurs within one hour of carrageenan inflammation and there is release of cytoplasmic enzymes, histamine and serotonin from the mast cells. The second step (> 1.0 h) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two steps is provided by kinins.

Since the extract significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract, because the carrageenan inflammatory model basically reflects the actions of prostaglandins. This effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme, which catalyses the synthesis of cyclic endoperoxides important in the formation of prostaglandins. Dextran-induced paw edema has been reported to be mediated mainly by histamine and serotonin released by the mast cells. The release of these inflammatory mediators results in marked vascular changes: including vasodilation, increased permeability and an increase of blood flow, eventually leading to an increase in paw size. Many flavonoids and alkaloids have been found to exhibit anti-inflammatory effects. Acute toxicity studies revealed no mortality at a dose of 225g/kg. Hence, the extract was classified as irrelatively harmless when administered orally.

In conclusion, the methanolic extract of *Passiflora nepalensis* has been shown to be effective against acute inflammation in a dose related manner. This present study supports the claim in the use of the methanolic extract of *Passiflora nepalensis* in traditional medicine for the treatment of inflammatory conditions.

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