INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of chronic illnesses. Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It characterized by Pain, Redness, Immobility (loss of function), Swelling and Heat. There are a number of synthetic non steroidal anti inflammatory drugs (NSAIDs) currently available for use in the management, control and treatment of inflammation. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also posses many side effects like gastric irritation, anorexia, diarrhea, rashes, stomach ulcers, GIT bleeding, kidney damage, liver damage, hypertension etc. Therefore, there is a great need for the development of alternative therapies particularly herbal therapies that are believed to be effective, safe and economical.

Raphanus sativus is an annual herb, consumed as vegetable. It belongs to the family Brassicaceae commonly known as Fiji or muli.

Anti-Inflammatory Activity of *Raphanus sativus* L in Acute and Chronic Experimental Models in Albino Rats

SHOBHA KAMBLE, Md. ZUBAIR AHMED¹, S. RAMABHIMAIAHA² and PRABHAKAR PATIL

Department of Pharmacology Al-Ameen Medical College, Bijapur, India.
¹Department of Pharmacology Prathima Institute of Medical Science, Karimnagar, India.
²Department of Pharmacology Navodaya Medical College, Raichur, Karnataka, India.
*Corresponding author E-mail: drmshobha22@gmail.com

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ABSTRACT

Inflammation is a localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury. Edema formation, leukocyte infiltration and Granuloma formation represent such components of inflammation. *Raphanus sativus* L. ‘Radish’ locally known as Fiji, is a Brassicaceous plant of cabbage family which has been reputed for its beneficial medicinal properties. The present study was undertaken to evaluate the anti-inflammatory effect of freshly squeezed Raphanus Sativus leaf and root juice (FRJ) in acute and chronic experimental models in albino rats. The freshly squeezed Raphanus Sativus leaf and root juice (FRJ) was studied in carrageenan induced hind paw edema in albino rats and paw volume was measured plethysmographically at 0 and 3 hr after injection of 1% carageenan in right hind paw and compared with control and standard drug, diclofenac. The drug was also investigated in formalin induced edema model in rats by injecting 2% formalin in right hind paw. Degree of inflammation was measured plethysmographically on day 1 and day 7 and compared with control and standard drug, diclofenac. All the drugs were administered orally, control group received 4% gum acacia, standard group received diclofenac 10mg/kg, and test groups received FRJ in doses of 3 ml. Both radish leaf juice and root juice significantly (p<0.01) reduced carageenan and formalin induced paw edema in rats but radish leaf juice compared to root juice produced more significant anti-inflammatory effect in both acute and chronic models of inflammation. However the anti-inflammatory effect of radish leaf juice is less than standard drug diclofenac sodium.

Key words: *Raphanus sativus* L, Inflammation, Diclofenac, Carageenan, Formalin.
Greek name of the genus Raphanus means “quickly appearing”. The different extracts contained alkaloids, glycosides, saponins, tannins carbohydrates, phenolic compounds, flavonoids, amino acids and volatile oil. The aqueous, methanolic anhydrophobic radish extracts or specific phytochemicals that are present in radishes including glucosinolates and isothiocyanates, phenolic acids and anthocyanins.

Almost all parts of the plant including leaves, seeds and roots are utilized in medicine. The fresh juice obtained from leaves are diuretic, laxative, roots are used for urinary complaints, haemorrhoids, gastroduodenal pains and various gastric ailments. The seeds are expectorant, digestive, diuretic, laxative and carminative. The extracts of Raphanus Sativus have been reported to have hepatoprotective, cardio protective, anticancer, antimicrobial and anti-urolithiatic activities. The extract also has an inhibitory effect on lipid peroxidation by increasing the activity of enzymatic antioxidants like catalase and also by increasing or maintaining the levels of glutathione. The radius juice also possesses an antigastric ulcer effect, being presumably attributed to its phenolic, terpenoidal and sulphurated constituents through preventing the accumulation of excessive free radicals and protecting the gastric tissue against noxious chemical challenges. Raphanus Sativus has been used in various abdominal and inflammatory disorders, although very limited information is available to support this. Hence the present study was undertaken to evaluate the anti-inflammatory activity of freshly squeezed Raphanus Sativus leaf and root juice (FRJ) in carrageenan and formalin induced hind paw edema in albino rats.

MATERIALS AND METHODS

The fresh radish was purchased from the local vegetable market and authenticated by an expert taxonomist.

Animals

Albino rats of either sex, weighing between 200 to 250gm were maintained in animal house and they were divided in to 4 groups of 6 animals each. Prior to the experimentation they were acclimatized to housing conditions for at least one week period of time to adjust to the new environment providing with food and water ad libitum. In order to avoid the influence of diurnal variation, all the experiments were carried out at same time of the day i.e. between 9 a.m. to 5 p.m.

Group I – Control & received 4% gum acacia suspension.
Group II – Standard & received Diclofenac sodium 10mg/kg suspension in 4% gum acacia.
Group III – Test & received 3 ml of freshly squeezed Raphanus sativus root juice.
Group IV – Test & received 3ml of freshly squeezed Raphanus sativus leaf juice.

Dose selection and route of administration:

The freshly squeezed juice of radish (FRJ) was administered at oral doses of 3 ml per 200 g b.w. This dose was selected based on preliminary experiments that showed pharmacological effects in animals. Moreover, the herbal medicine practitioners commonly used the radish in the form of juice, therefore, the present dosage form was adopted in this study. All the drugs were administered orally. All the experiments were conducted as per the norms approved by Institutional Animal Ethics Committee.

Carrageenan - induced Hind paw oedema in rats

The anti-inflammatory effect of FRJ was evaluated using carrageenan-induced paw edema in rats. Animals were divided into four groups of six animals in each group. In all groups acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rat and paw volume was measured plethysmometrically at 0 and 3hr after carageenan injection. Animals were premedicated with vehicle (4% gum acacia) or freshly squeezed radish leaf and root juice(3 ml) or diclofenac (10 mg/kg) orally one hour before injection of carageenan. Mean increase in paw volume was measured and percentage of inhibition was calculated.

Formalin-induced paw oedema in rats

This method is also based on the plethysmometric measurement of oedema produced by injection of 0.1ml of 2% formalin into the sub-plantar area of the hind paw of rat. Albino rats were divided into four groups of six animals in each and treated with either
of 4% of gum acacia suspension or freshly squeezed radish leaf and root juice (3 ml) or diclofenac (10 mg/kg). All the drugs were administered orally one hour prior to formalin injection and continued for 7 days. The second injection of formalin was given on third day. The paw volume was measured by plethysmometer on day 1 and day 7. The difference in paw volume on day 1 and day 7 was considered as inflammatory oedema. Volume changes in standard group and test groups were compared with that of control group and the percentage of inhibition of inflammation was calculated.

\[
\text{Percentage of inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

\(V_c\) = Volume of paw oedema in control animals.
\(V_t\) = Volume of paw oedema in FRJ treated animals.

### Statistical Analysis

The results were presented as mean ±S.E.M and subjected to one way ANOVA test & Post Hoc test. P values less than 0.05 were considered significant.

#### RESULTS

**Carageenan induced paw oedema**

The anti-inflammatory effect of FRJ in carageenan-induced paw oedema at the end of 3h is presented in table-1 and graph 1. FRJ significantly reduced paw volume (P<0.01) at 3h compared to control, the percentage of inhibition with root juice is 28.46% and leaf juice 41.90%. The reference drug, diclofenac inhibited the paw oedema by 51.12% (P<0.01). Thus the fresh juice of root and leaf of Radish has significant anti-inflammatory effect compared to control group, but leaf juice has more significant effect than root juice and both the juices showed less significant anti-inflammatory effect when compared to standard drug diclofenac sodium.

**Formalin-induced paw oedema**

The anti-inflammatory effect of FRJ in formalin-induced paw oedema at the end of 7 day is presented in table-2 and graph 2. The percentage of inhibition of oedema at the end of 7 day with freshly squeezed root juice is 30.43% and with leaf juice is 42.10% (P<0.01) as compared to control rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Increase in paw volume after 3h(ml) (Mean ± SEM)</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4% gum acacia</td>
<td>1.48 ± 0.03</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10mg/kg</td>
<td>1.30 ± 0.02*</td>
<td>51.12</td>
</tr>
<tr>
<td>Radish root juice</td>
<td>3ml</td>
<td>1.47 ± 0.05*</td>
<td>28.46</td>
</tr>
<tr>
<td>Radish leaf juice</td>
<td>3ml</td>
<td>1.23 ± 0.04*</td>
<td>41.90</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, number of animals used are six each group, *P <0.01.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Paw volume increase on day 7(ml)</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4% gum acacia</td>
<td>1.29 ± 0.01</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10mg/kg</td>
<td>1.12 ± 0.02*</td>
<td>60.86</td>
</tr>
<tr>
<td>Radish root juice</td>
<td>3ml</td>
<td>1.34 ± 0.01*</td>
<td>30.43</td>
</tr>
<tr>
<td>Radish leaf juice</td>
<td>3ml</td>
<td>1.3 ± 0.02*</td>
<td>42.10</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, number of animals used are six each group, *P <0.01.
Diclofenac sodium also exerted significant (P<0.01) inhibitory action (60.86%) on oedema formation. In this model also both the juices showed significant anti-inflammatory effect compared to control group, but leaf juice showed more significant effect than root juice and when compared to diclofenac both juices showed less significant anti-inflammatory effect.

**Fig. 1: Carrageenan induced paw edema in Rats**

**Fig. 2: Formalin induced paw edema in Rats**

Percentage inhibitions of formalin-induced rat paw oedema at the end of 7 days treatment with diclofenac sodium and Radish juice.
Thus in both the models Raphanus sativus leaf juice has more significant anti-inflammatory effect compared to root juice.

**DISCUSSION**

The results of present study show that the FRJ possesses significant anti-inflammatory effect on acute and chronic inflammatory models in albino rats.

The inhibition of Carrageenan-induced inflammation in rats is an established model to screen compounds for potential anti-inflammatory activity. It is well known that Carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after Carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3 – 4 h after Carrageenan injection), Kinin and prostaglandins are involved. Our results revealed that administration of freshly squeezed *Raphanus sativus* Linn juice inhibited the paw volume after third hour and during all phases of inflammation, which is probably due to inhibition of different aspects and chemical mediators of inflammation.

The hydro alcoholic extract of Raphanus sativus showed potent anti-inflammatory activity that may be due to the presence of flavonoids, phytosteroids and tannins and also due to inhibition of main inflammatory mediators like histamine, serotonin, prostaglandins, bradykinin, angiotensin, tachykinin, platelet activating factor and substance-p. Hence it is concluded that the *Raphanus sativus* possesses significant anti-inflammatory activity against carrageenan induced paw edema in rats.

It is well known that inhibition of formalin-induced paw oedema in rat is one of the most suitable test procedure to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis.\(^\text{13}\) Injection of formalin subcutaneously in to hind paw of rats produced localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by later tissue mediated response.\(^\text{15}\) Thus formalin induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. Formalin-induced paw edema in rats represents the proliferative phase of inflammation FRJ showed significant anti-inflammatory activity in this model. Therefore it appears to act by inhibiting proliferative phase and thus could be an effective anti-proliferative agent.

This study provides evidence that the freshly squeezed juice of radish possesses an anti-inflammatory effect due to its urosolic acid & oleunolic acid.\(^\text{13}\)

- Vitamin C in radishes is an antioxidant and anti-inflammatory, and has been shown to have a positive effect on symptoms of asthma because of its anti-inflammatory properties.
- Radish is a great source of vit C, riboflavin, folate, rich in minerals potassium, magnesium, sulphur, iron, and iodine.

In conclusion FRJ significantly inhibited inflammation in carrageenan and formalin induced hind paw edema in albino rats. The phytochemical screening showed the presence of flavonoids, anthocyanins and sulfurated constituents. The results showed Radish leaf juice compared to root juice produced more significant anti-inflammatory effect in both acute and chronic models of inflammation. However the anti-inflammatory effect of radish leaf juice is less than standard drug diclofenac sodium.

**REFERENCES**


