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Research Article

ANTI-INFLAMMATORY ACTIVITY OF SPINACIA OLERACEA

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Abstract

The present study deals with the investigation of phytochemically evaluated ethanolic and aqueous extracts of leaves of *Spinacia oleracea* for its anti-inflammatory activity. The anti-inflammatory activity was evaluated by carrageenan induced rat paw oedema method for acute inflammation and cotton pellet granuloma method for chronic inflammation. The standard drug used was Indomethacin (20 mg/kg) for both the models. In both methods, ethanolic as well as aqueous extract at a dose level of 1100 mg/kg has shown significant activity which is comparable to that of the standard.

Keywords: - Spinacia oleracea, Indomethacin, Anti-inflammatory, Carrageenan

Introduction

Inflammation (Latin, *inflammare*, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue [1].

Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [2].

Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of the inflammation.

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Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of Carrageenan-induced inflammation [3], whereas prostaglandins are detectable in the late phase of inflammation [4].

Spinacia oleracea is an edible flowering plant in the family of Amaranthaceae. In Hindi it is known as palaak and in english as Spinach. It is native to central and southwestern Asia. The leaves are alternate, simple, ovate to triangular-based, very variable in size from about 2-30 cm long and 1-15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. It is used as carminative and laxative. In experiments it has been shown to have hypoglycaemic properties [5]. It has been used traditionally in the treatment of difficult breathing, inflammation of the liver and jaundice [6].

Objective of Research

A large numbers of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. It is believed that current analgesia-inducing drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side-effects and potency [7].As a result, a search for other alternatives seems necessary and beneficial. Traditional and folklore medicines play an important role in health services around the globe. Ayurveda, the traditional medicinal system in India, describes certain plants which strengthen the host immune system.

Material and Method

Experimental animals: Albino Wistar rats weighing between 200-250g were used. Institutional Animal Ethics Committee approved the experimental protocol**.** Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Plant material: The leaves of Spinacia oleracea were collected from the local area of Meerut district and identified and authenticated by Dr. Anjula Pandey, Taxonomist, National Herbarium of Cultivated Plants, New Delhi. Voucher specimens have been kept in National Herbarium of Cultivated Plants, New Delhi and Department of Pharmaceutical Technology, MIET for future reference.

Extraction

The leaves were dried under shade, reduced to moderately coarse powder, loaded into soxhlet extractor and was subjected to successive extraction with Petroleum Ether, Benzene, Chloroform, Ethanol and Water to get different extracts.

Preliminary Phytochemical Studies

The ethanolic and aqueous extracts were then subjected to qualitative phytochemical screening for the identification of the phytoconstituents. Ethanolic and aqueous extracts showed positive tests for the presence of flavonoids. As traditionally, the aqueous paste or the aqueous extract of the plant is used to cure inflammation, the anti-inflammatory activity of the ethanolic and aqueous extract of the plant at 1100 mg/kg dose level [8] is being reported here.

Anti-Inflammatory Activity [9]

Acute inflammation

Carageenan induced rat paw oedema

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control, group 2 received indomethacin 20mg/kg suspended in 1% sodium carboxymethyl cellulose. Groups 3 and 4 were treated with 1100mg/kg of ethanolic and aqueous extracts of *Spinacia oleracea* suspended in Tween 80/ethanol/saline (1:1:10). Edema was induced by injecting 0.1 ml of a 1% solution of carrageenan in saline into the subplantar aponeurosis of the right hind paw of the rats. The vehicle, extracts, and the standard drugs were administered orally 60 min prior to the injection of the phlogestic agent. The volumes of edema of the injected and the contralateral paws were measured at 1, 2, 3, 4, 5 h after the induction of inflammation using a plethysmograph to calculate the percentage of anti-inflammatory activity.

In the above model, % inhibition of Oedema was calculated as follows:-

% Inhibition of Oedema = (1-Vt/Vc) x 100

where ,Vt is the inflammatory increase in paw volume of the rats of treated groups.

Vc is the inflammatory increase in paw volume of the rats of control groups.

Chronic inflammation

Cotton pellet granuloma

The animals were grouped as described above to study the anti-inflammatory activity. The groups were fasted and treated with drugs/doses similar to that of carrageenan-induced hind paw edema. Sterile cotton pellets each weighing 30 ± 5mg were prepared and sterilized in a hot air oven at 123°C for 3 h. Each animal was placed under light ether anesthesia and subcutaneously implanted with four cotton pellets, one each into both the axillae and the groin region under aseptic conditions. The drugs were administered orally for seven days starting from the day of implantation of the pellets. All the animals had free access to drinking water and food. On the 8 th day, all the animals were sacrificed and the implanted cotton pellets were recovered, cleaned of surrounding tissues, and blotted with filter paper. These cleaned pellets were weighed and dried in a hot air oven overnight at 70°C and the dry weights were noted [10].

Percentage inhibition of Granuloma Pouch in rats was calculated using the following formula:-

$$\% \text{ Inhibition} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

Satistical Analysis [11]

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p<0.05$ were considered significant.

The results are summarized in the tables given below

Table 1: Details of Qualitative Phytochemical Tests

Tests	Ethanol Extract	Aqueous Extract
1.Tests for sterols		
a. Test solution+Conc.H_2SO_4	**-ve**	**-ve**
b. Libermann Buchard's Test	**-ve**	**-ve**
c. Test solution + sulphur	**-ve**	**-ve**
d. Salkowski test	**-ve**	**-ve**

2.Tests for Glycosides		
a. Keller Killiani's Test	**-ve**	**-ve**
b. Balget's Test	**-ve**	**-ve**
c. Bromine water Test	**-ve**	**-ve**
d. Legal's Test	**-ve**	**-ve**
e. Raymonds Test	**-ve**	**-ve**
3. Test for Saponins		
a. Haemolytic Test	**-ve**	**-ve**
b. Foam Test	**-ve**	**-ve**
4. Test for Proteins		
a. Xanthoproteic Test	**-ve**	**-ve**
b. Millon's Test	**-ve**	**-ve**
c. Biuret Test	**-ve**	**-ve**
d. Ninhydrin Test	**-ve**	**-ve**
5. Test for Tannins		
a. Gelatin Test	**-ve**	**-ve**
b. Ferric Test	**-ve**	**-ve**
6. Test for Alkaloids		
a. Dragendroff's Test	**-ve**	**-ve**
b. Mayer's Test	**-ve**	**-ve**
c. Hanger's Test	**-ve**	**-ve**
d. Wagner's Test	**-ve**	**-ve**
7.Test for Carbohydrates		
a. Barfoed's Test	**-ve**	**-ve**
b. Benedict's Test	**-ve**	**-ve**
c. Molisch's Test	**-ve**	**-ve**
8. Test for Flavonoids		
a. Shinoda Test	**+ve**	**+ve**
b. Alkaline Reagent Test	**+ve**	**+ve**
c. Ferric Chloride Test	**+ve**	**+ve**
d. Lead Acetate Test	**+ve**	**+ve**
e. Zn-HCl reduction Test	**+ve**	**+ve**

+ve *indicates positive result* ***–ve*** *indicates negative result*

Table 2: Effect of ethanolic and aqueous extracts of *Spinacia oleracea* on carrageenan induced rat paw oedema

Groups	**Dose (mg/kg)**	**Paw Volume After Carrageenan Injection**									
		1 hr.		**2 hrs.**		**3 hrs.**		**4 hrs.**		**5hrs.**	
		EV	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**	**EV**	**EI (%)**
Control	-	0.17± 0.0084	-	0.22± 0.0071	-	0.28± 0.0125	-	0.33± 0.0096	-	0.39± 0.0123	-
Indomethacin	20	0.15± 0.0049	11.76 %	0.17± 0.0057[d]	22.72 %	0.13± 0.0080[d]	53.57 %	0.11± 0.0047[d]	66.66 %	0.10± 0.0030[d]	74.35 %
Ethanolic	1100	0.16± 0.0047	5.88 %	0.20± 0.0093	9.09 %	0.16± 0.0073[d]	42.85 %	0.15± 0.0060[d]	54.54 %	0.14± 0.0087[d]	64.10 %
Aqueous	1100	0.16± 0.0057	5.88 %	0.19± 0.0079[b]	13.63 %	0.15± 0.0057[d]	46.42 %	0.13± 0.0071[d]	60.60 %	0.12± 0.0060[d]	69.23 %

Values are expressed as mean ± SEM. (n=6)

[a]$p<0.05$, [b]$p<0.02$, [c]$p<0.01$,[d]$p<0.001$ as compared to control group.

Table 3: Effect of ethanolic and aqueous extracts of *Spinacia oleracea* on Cotton pellet granuloma

S. No.	Groups	Dose (mg/kg)	Wet Weight (mg)	Dry Weight (mg)
1	Control	-	397.7±1.2441	100.8±2.1882
2	Indomethacin	20	285.35±0.9917[d]	45.51±1.573[d]
3	Ethanolic	1100	360.61±1.0292[d]	57.9±1.7886[d]
4	Aqueous	1100	344.15±1.3492[d]	51.96±1.6052[d]

Values are expressed as mean ± SEM. (n=6)

[d]$p<0.001$ as compared to control group.

Discussion

The present study shows that both ethanolic and aqueous extracts of *Spinacia oleracea* possesses anti-inflammatory activity in carrageenan induced rat paw oedema method. The activity profile of extract closely resembled to that of sIndomethacin. Carrageenan induced paw oedema was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carrageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamaine and serotonin and the delayed phase is sustained by the leukotrienes and prostaglandins. To further verify the anti-inflammatory activity of the extracts and its effects on the proliferative phase of inflammation, cotton pellet granuloma formation was used. The ethanolic and aqueous extracts at a dose level of 1100 mg/kg showed a significant inhibitory effect on granuloma formation. This study revealed that both the extracts were active against the inflammation induced by a foreign body. This effect of extract was less pronounced than that of indomethacin.

Conclusion

The carageenan induced edema in rats was reduced to a lower level after supplemention of *Spinacia olearcea*. The findings with *Spinacia oleracea* are significant as the preparation is highly cost effective. As *Spinacia oleracea* is used as a dietary vegetable it is easily available all over the world therefore it is worthwhile to conduct detailed studies in order to explore the full potential of this plant in reducing inflammation in humans from the point of view of cost and availability for people at all socioeconomic levels.

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