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## Anti-Inflammatory Activity of Aqueous Extract of *Beta Vulgaris L.*

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**ABSTRACT:** The present study deals with the investigation of phytochemically evaluated aqueous extract of leaves of *Beta vulgaris* 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 (10 mg/kg) for both the models. In both methods, aqueous extract at a dose level of 1000 mg/kg has shown significant activity which is comparable to that of the standard.

**KEYWORDS:**

*Beta vulgaris*, Indomethacin, Anti-inflammatory, Carrageenan

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### INTRODUCTION

Inflammation is defined as local response of living mammalian tissue to injury due to any agent. Inflammation manifests usually in form of painful swelling associated with some changes in skin covering the site [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. 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].

*Beta vulgaris* (Linn.), belonging to chenopodiaceae, family is a native of South Europe, extensively cultivated as an article of food and especially for the production of sugar, and presents many varieties. It is a tall, succulent plant, about 2 feet high, with large, fleshy, glossy leaves, angular stems and numerous leafy spikes of green flowers [5].

There are some reports indicating the potential hepatoprotective, antioxidant and anti-inflammatory activities of *Beta vulgaris*, though without any scientific proof [6].

Since no detailed scientific literature is available on anti-inflammatory activity of *Beta vulgaris*, therefore the present work was undertaken.

### MATERIAL AND METHOD

#### Experimental animals

Albino Wistar rats weighing between 200-250g were used. Institutional Animal Ethics Committee

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approved the experimental protocol (Ethical clearance number: 711/02/a/CPCSEA). 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 *Beta vulgaris* L. var. *bengalensis* Roxb. 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 (No. NHCP/NBPGR/2010-26/920) 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 and macerated with hot water for 48 hours to get aqueous extract. The aqueous extract was concentrated to dryness using rotary evaporator, giving yield as 23.41% (w/v) and preserved in a refrigerator. Aliquot portions of the aqueous extract of *Beta vulgaris* were weighed and suspended in an appropriate volume of Tween 80 (2%, v/v) for use on each day.

### Preliminary Phytochemical Studies

The aqueous extract was then subjected to qualitative phytochemical screening for the identification of the phytoconstituents. The aqueous extract showed positive tests for the presence of flavonoids, carbohydrates, pentose, amino acids, saponins, tannins and steroids. As traditionally, the aqueous paste or the aqueous extract of the plant is used to cure inflammation, and the anti-inflammatory activity of the aqueous extract of the plant at 500 and 1000 mg/kg dose levels [7] is being reported here.

## ANTI-INFLAMMATORY ACTIVITY

### 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 10mg/kg suspended in 2% Tween 80 (v/v)[8, 9]. Groups 3 and 4 were treated with 500 mg/kg and 1000 mg/kg of aqueous extract of *Beta vulgaris* suspended in 2% Tween 80. Edema was induced by injecting 0.1 ml of a 1% (w/v)

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 [10].

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

$$\% \text{ Inhibition of Oedema} = (1 - V_t/V_c) \times 100$$

where,

$V_t$  is the inflammatory increase in paw volume of the rats of treated groups.

$V_c$  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  $30 \pm 5$  mg were prepared and sterilized in a hot air oven at  $123^\circ\text{C}$  for 3 h. Each animal was placed under light with ether anesthesia and subcutaneously implanted 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<sup>th</sup> 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  $70^\circ\text{C}$  and the dry weights were noted [11].

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

$$\% \text{ Inhibition} = (\text{Control} - \text{Test}) / \text{Control} \times 100$$

### Statistical Analysis [12]

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 statistical software used was Jindal Sigma stat (2.03v).

## RESULT

**Table 1:** Effect of aqueous extract of *Beta vulgaris* 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	-	0.16± 0.0036	-	0.21± 0.0036	-	0.26± 0.0049	-	0.29± 0.0097	-	0.31± 0.0125	-
Indomethacin	10	0.17± 0.0047	6.25	0.18± 0.0047 <sup>c</sup>	14.28	0.16± 0.0057 <sup>c</sup>	38.46	0.14± 0.0086 <sup>c</sup>	51.72	0.13± 0.0060 <sup>c</sup>	58.06
Aqueous	500	0.165± 0.0042	3.125	0.205± 0.006 <sup>1</sup>	2.38	0.24± 0.0072 <sup>a</sup>	7.69	0.26± 0.0036 <sup>b</sup>	10.34	0.27± 0.0057 <sup>b</sup>	12.9
Aqueous	1000	0.17± 0.0057	6.25	0.20± 0.0057	4.76	0.18± 0.0047 <sup>c</sup>	30.76	0.16± 0.0062 <sup>c</sup>	44.82	0.15± 0.0036 <sup>c</sup>	51.61

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

<sup>a</sup>p<0.05, <sup>b</sup>p<0.02, <sup>c</sup>p<0.001 as compared to control group.

EV- Oedema Volume and EI- Oedema Inhibition.

**Table 2:** Effect of aqueous extract of *Beta vulgaris* on Cotton pellet granuloma

S. No.	Groups	Dose (mg/kg)	Dry Weight (mg)
1	Control	-	106.95±0.91
2	Indomethacin	10	54.98±0.5115a
3	Aqueous	500	100.56±0.4153a
4	Aqueous	1000	62.55±0.4089a

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

<sup>a</sup>p<0.001 as compared to control group

## DISCUSSION

The present study shows that aqueous extract of *Beta vulgaris* possesses anti-inflammatory activity in carrageenan induced rat paw oedema. The activity profile of extract at 1000 mg/kg closely resembled to that of indomethacin. 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 histamine and serotonin and the delayed phase is sustained by the leukotrienes and prostaglandins [13]. 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 aqueous extract at a dose level of 500 and 1000 mg/kg showed a significant inhibitory effect on granuloma formation. This study revealed that the aqueous extract was 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 supplementation of *Beta vulgaris*. The findings with *Beta vulgaris* are significant as the preparation is highly cost effective. As *Beta vulgaris* 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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