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Comparative Study of Anti-Inflammatory activity of Petroleum Ether and Ethanolic extracts of Brassica Juncea

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Abstract: Inflammation is a primary physiological defence mechanism that help body to protect itself from infection, burns, toxic chemicals or other noxious stimuli. Anti inflammatory drugs may exert their effects by scavenging oxidants and decreasing formation of reactive oxygen species by activated phagocytes. *Brassica juncea* has been used since ancient times and it is popularly known as mustards. The aim of present study was to evaluate the anti inflammatory activity of petroleum ether and ethanolic extracts of *Brassica juncea* against carrageenan induced paw edema. Acute toxicity study was performed up to 2gm/kg p.o and animals did not show any mortality and behavioral changes. Hence we selected 250 & 500 mg/kg p.o. as low and high doses. Both the extracts inhibited carrageen induced paw edema in a dose dependent manner and among the two extracts ethanolic extract shows better anti-inflammatory activity when compared to petroleum ether extract.
Keywords: Anti-inflammatory, Petroleum ether extract, Ethanol extract, *Brassica juncea* , Carrageenan, Plethysmometer.

INTRODUCTION:

Inflammation is a part of biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants.[1] Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.[2] Drugs presently in use for the management of inflammation are associated with well known side and toxic effects.[3] Purified natural compounds from plants can serve as a template for the synthesis of new generation anti-inflammatory drugs with low toxicity and higher therapeutic value.[4] Traditionally *Brassica juncea* was used as anti-inflammatory, anti-oxidant[5], antifungal[6] and anti-microbial[7] . The plant *Brassica juncea* itself can grow from two to eight feet tall, with racemes of small yellow flowers. These flowers are usually up to 1/3" across, with four petals each. The leaves are covered in small hairs; they can wilt on hot days, but recover at night. crushed seeds have pungent odour[8]. Literature review also indicated that antiinflammatory property of this species in not been

clinically evaluated so far[9]. So the present study was aimed to evaluate the anti inflammatory potency of petroleum ether and ethanolic extracts of *Brassica juncea* against carrageenan induced paw edema in rats.

MATERIALS AND METHODS

Plant Material

The dried seeds of *Brassica juncea* belonging to the family *Brassicacea* were collected at the local areas of Ananthapur district, Andhra Pradesh, India. The plant material was identified and authenticated by Dr.B.Ravi Prasad Rao, M.sc., Ph.D., Department of Botany, Sri Krishnadevaraya University, Ananthapur and voucher specimen (riper -05/11) was preserved in Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education And Reseach.

Processing of sample

The dried seeds were pulverized into fine powder and used for extraction.

Preparation of extracts

Petroleum ether and ethanol extract: The powdered crude drug was loaded into the soxhlet extractor and subjected to extraction with petroleum ether and ethanol separately. After the extraction, the solvent was distilled off and the extracts were concentrated to dryness at room temperature. The yield values, texture and colour obtained were stated in table 1.

Phytochemical screening

Both petroleum ether and ethanolic extracts of *Brassica juncea* were subjected to prelimnary phytochemical screening.

Drugs and chemicals:

Ibuprofen (Abbott pvt.ltd.,Goa), Carrageenan (Hi Media labs pvt ltd.), Petroleum Ether (Thermo Fisher Scientific pvt ltd) and Ethanol.

Experimental Animals: Male Wistar rats weighing about 150-200 gms were used in the experiments for anti-inflammatory activity. The animals were kept in climatized environment (25 ± 3°C), with light/dark control each 12 hours (7 a.m. to 7 p.m.). The animals were placed in cages. They were kept without any food 12 hours before the experiments, and water was ad libitum.

Ethical Approval

The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/007/2011) has approved the experimental protocol at post graduate department of pharmacology, Raghavendra Institute of Pharmaceutical Education Research, Ananthapur, Andhra Pradesh, India.

Animal groups: The animals were grouped into 7 groups (n=6).

Group-I	Normal	Vehicle p.o.
Group-II	Negative Control	1% Carrageenan s.p.
Group-III	Standard	Ibuprofen 10mg/kg p.o.
Group-IV	Test-1	Petroleum ether extract 250 mg/kg p.o.
Group-V	Test-2	Petroleum ether extract 500 mg/kg p.o.
Group-VI	Test-3	Ethanolic extract 250 mg/kg p.o.
Group-VII	Test-4	Ethanolic extract 500 mg/kg p.o.

Table: 1 Colour, , texture and % Yield of petroleum ether and ethanolic extracts of *Brassica juncea.*

Plant part	Type of Extract	Colour	Texture	% Yield
Brassica juncea seeds	Petroleum ether extract	Yellowish brown	Semisolid	10 %w/w
	Ethanolic extract	Yellowish brown	Gummy	25 %w/w

Rats Paw Edema Induced By Carrageenan:
The phologistic agent carrageenan 0.1ml of 1% w/v was injected to right hind paw plantar surface of rats . Sterile saline solution (0.9%, 0.1 ml) was injected to left paw as the control reference for the tested paw. The foot volumes of the animals were determined by plethysmometer. This method was used to evaluate the effectiveness of the extract in the inhibition of the inflammatory process by comparison with the negative control and the standard drug, Ibuprofen 10mg/kg, p.o. The results were obtained by measuring the volume difference between the right and the left paws.

$$\% \text{ edema inhibition} = \frac{[\text{Paw volume of control} - \text{Paw volume of test}]}{\text{Paw volume of control}} \times 100$$

% edema inhibition is calculated to determine the efficacy of the extracts.

STATISTICAL ANALYSIS
The results were expressed as mean ± S.E.M. The differences were compared using One Way Analysis Of Variance (ANOVA) and subsequently followed by Bonferroni's test. [10]

Table: 2 Phytochemical screening of petroleum ether and ethanolic extracts of *Brassica juncea.*

Phytochemicals	ETHANOLIC EXTRACT	PETROLEUM ETHER EXTRACT
Carbohydrates	+	_
Proteins	+	_
Amino acids	+	_
Glycosides	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	_

+ = Present, - = Absent

Table: 3 Anti inflammatory activity of petroleum ether and ethanolic extracts of *Brassica juncea.*

TREATMENT	PAW VOLUME (% INHIBITION OF PAW EDEMA)				
	30 min	60 min	120 min	180 min	240 min
NORMAL	0.2±0.001	0.2±0.001	0.2±0.001	0.2±0.001	0.2±0.001
NEGATIVE CONTROL (1%CARRAGEENAN s.p.)	0.25±0.008	0.51±0.005####	0.59±0.005####	0.68±011####	0.48±0.008####
STANDARD IBUPROFEN 10mg/kg p.o.	0.25±0.005	0.45±0.020** (11.7)	0.37±0.008**** (37.2)	0.37±0.008**** (45.5)	0.20±0.005**** (45.8)
PETROLEUM ETHER EXTRACT					
250 mg/kg p.o.	0.26±0.005	0.44±0.008** (13.72)	0.50±0.005**** (15.25)	0.55±0.008**** (19.12)	0.45±0.005 (6.25)
500 mg/kg p.o.	0.26±0.003	0.39±0.003**** (23.53)	0.42±0.008**** (28.81)	0.47±0.005**** (30.88)	0.34±0.005**** (29.16)
ETHANOLIC EXTRACT					
250 mg/kg p.o.	0.26±0.005	0.40±0.003**** (21.57)	0.46±0.005**** (22.03)	0.52±0.008**** (23.53)	0.45±0.008 (6.25)
500 mg/kg p.o.	0.25±0.003	0.38±0.003**** (25.49)	0.40±0.003**** (32.20)	0.44±0.008**** (35.29)	0.26±0.005**** (45.83)

P< 0.001 When compared to normal

** P<0.05 When compared to negative control **** P<0.001 When compared to negative control

RESULTS AND DISCUSSION

Carrageenan induced inflammation is most commonly used as an experimental model for evaluating the anti-inflammatory potency of compounds or natural products because it produces reproducible results.[11] The petroleum ether and ethanolic extracts of *Brassica juncea* seeds were subjected to preliminary phytochemical investigations (Table.2) where ethanolic extract revealed the presence of aminoacids, carbohydrates, proteins, glycosides, alkaloids, tannins and flavonoids and petroleum ether extract revealed the presence of glycosides, alkaloids and flavonoids [12]. Then, the extracts were subjected to anti-inflammatory activity by means of carrageenan induced edema in rat's hind paw.[13] There was a significant increase in paw edema of rats in the negative control group. However, in test groups, ethanolic extract showed a more significant reduction in the paw edema followed by petroleum ether extract (Table.3). So, the present activity may be due to presence of alkaloids. [14]The possible mechanism of action of alkaloids might suppress the antigen and mitogen induced lymphocyte proliferation, natural killer cell cytotoxicity, histamine release by mast cells, interleukin-1 secretion by human monocytes.[15]

From the above results it was concluded that both extracts had shown dose dependent antiinflammatory activity and among the two extracts ethanolic extract possess more significant anti-inflammatory activity than petroleum ether extract. However, further investigations are needed to explore the exact active constituents and mechanisms responsible for the anti-inflammatory activity.

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REFERENCES:

1. Amritpal Singh, Samir Malhotra, Ravi Subban, Anti-inflammtory and analgesic agents from Indian Medicinal Plants, International Journal of Integrative Biology, Vol.3, 2008.
2. Denko CW, A role of neuropeptides in inflammation.In:Whicher, J.T and Evans, S.W. Biochemistry of inflammation, 1992, 177-181.
3. Mahesh S., Paschapur, M.B. Patil, Ravi kumar, Sachin, Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. Male flowers in experimental animals, Journal of Medicinal plants Research, Vol.3(2), 2009, 49-54.
4. M.Anil kumar, Ethanomedicinal plants as anti-inflammatory and analgesic agents, Ethanomedicine: A source of complementary therapeutics, 2010, 267-293.
5. Indian Medicinal plants by Krithikar, K.R and Basu B.D VOL-1.
6. A GRAS Notification submitted by the Canola Council of Canada, Winnipeg, Manitoba to the United States Food and Drug Administration, Washington, D.C. in accordance with proposed Section 170.36 of the Federal Food, Drug, and Cosmetic Act September, 1999.
7. A *Brassica juncea* chitinase with two-chitin binding domains show anti-microbial properties against phytopathogens and Gram-negative bacteria. Plant Signaling & Behavior 3:12, 1103-1105; December 2008.
8. Post,George Edward (1900). "Mustard".in james Hastings. A dictionary of bible11 Duke,J.A. 1979. Ecosystematic data on economic plants. Quart. J. Crude Drug Res. 17(3–4):91–110.
9. Indian medicinal plants by Orient Longmann part-I, coll no AVS 1055.
10. Thompson WR, Weil CS. On the construction of tables for moving average interpolations. Biometrics, 8, 1952, 51–54.
11. Winter CA, Risley EA and Nuss GW,Carrageenan induced oedema in hind paws of the rat as an assay for anti-inflammatory drugs, Proc soc Exp Biol. Med. 52, 1962, 544-552.
12. Antibacterial qualities and phytochemical screening of the oils of *Curcubita pepo* and *Brassica juncea.* Journal of Medicinal Plants Research Vol. 3(5), pp. 429-432, May, 2009.
13. Niemegeers CJE. The activity of suprofen on nystatin induced paw oedema in rats. Arzneimittel-Forschung.,23,1975, 1516–1519.

14. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain, 30, 1987, 103-114.
15. P.Rambabu, K.Venkata ramana, R.S.Ganapathy, 'Evaluation of anti-inflammatory activity of *Ziziphus glabrata* stem bark', International journal of pharmaceutical biological sciences, Vol.4 (2), 2010,35-38.

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