ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *IPOMOEA CARNEA* JACQ

Md. Saifuddin Khalid[*1], Rajnish Kumar Singh[1], I.V. Narasimha Reddy[1], Shah Jinesh Kumar[1]. B. Sunil Kumar[2], G.N. Santosh Kumar[3], K. Srinivas Rao[4].

1. Dept. of Pharmacology, Luqman College of Pharmacy, Gulbarga, Karnataka.
2. Dept. of Pharmaceutical Chemistry, NET College of Pharmacy, Raichur, Karnataka, India.
3. Dept. of Biochemistry, Gulbarga University, Gulbarga, Karnataka, India.
4. Dept. of Biochemistry, Novodaya Institute of Medical Sciences, Raichur.

Corresponding author address:
[*]Md. Saifuddin Khalid
Assistant Professor,
Department of Pharmacology,
Luqman college of Pharmacy,
P.O. Box No: 86,
Old Jewargi Road, Behind P.& T Colony,
Gulbarga- 585102, Karnataka, India.
Email Id: khalid2568@yahoo.com
Mobile: + 91-9845242820.

Summary

The aim of present study was to assess the anti-inflammatory activity of aqueous extracts of *Ipomoea carnea* leaves. Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Therefore, the search for a better tolerated anti-inflammatory agent appears to be a necessity. Ipomoea carnea is used for the treatment of skin disease in India. Anti-inflammatory activity was screened by carrageenan (0.1%) induced rat paw edema method. The mature green leaves of *Ipomoea carnea* were collected and authenticated. For screening of anti-inflammatory activity, the extracts were administered orally at a dose of 250 mg/kg and 500 mg/kg body weight. Animals were divided into six groups of 6 animals each. Group 1 served as control and group 2 as reference standard (Etoricoxib 6 mg/kg), group 3 and 4 animals were treated with 250 mg/kg and 500 mg/kg aqueous extract respectively. Paw volume was significantly ($p<0.01$) reduced in test treated groups (500 mg/kg body weight) as compared to control group. Present study revealed that the plant *Ipomoea carnea* leaves possesses a significant anti-inflammatory activity as evidences in Carrageenan induced paw edema method.

Key words: Inflammatory disease; Ipomoea carnea leaves; Aqueous extract; Etoricoxib; Paw edema.

Introduction

The plant *Ipomoea carnea* (Besharam, Behaya) is a large, diffuse or struggling shrub with milky juice, leaf ovate cordate, entire, acuminate, flower large campanulate, pale rose, pink or light violet in lax, dichotomously branched axillary and terminal, pedunculate cymes; Fruits glabrous capsule; Seed silky, belonging to family Convolvulaceae[1-3]. It is well distributed in India and found particularly in Chhattisgarh and Madhya Pradesh[4-6].The plant is commonly known as Besharam, Behaya and used for skin troubles successfully. The milky juice of Beshram is used for the treatment of Safed Dag (Leucoderma)[7].The juice is collected and applied externally on affected parts, anti-inflammatory. It is used to decrease the teratogenic effect resulting from cyclophosphamide[8]. Aqueous extract of *Ipomoea carnea* shows neuromuscular blocking activity[9]. It used as aphrodisiac, purgative and cathartic[10]. The leaves of *Ipomoea carnea* contain 1-3 flavonol glycosides and Ergine (D-Lysergic acid amide) [11]. Polyhydroxylated alkaloids were isolated from the leaves, flowers and seeds[12]. Chromatographic separation of the leaf extract resulted in the isolation of swainsonine, 2-epilentiginosine, calystegines B (1), B (2), B (3) and C (1) and N-methyl-trans-4-hydroxy-l-proline and beta sitosterol[13-15]. Carrageenan is a sulphated mucopolysaccharide extracted from the seaweeds Chondrus spp. and Gigartina spp, commonly known as Irish moss or carrageen moss. It has been used in the rat for inflammation models: foot pad inflammation or paw edema model [16]. After exhaustive literature survey it was aimed to screen the anti-inflammatory activity of the leaves of *Ipomoea carnea*.

Methods

Plant material:

The plant Ipomoea carnea is widely found throughout India. The plant herbarium specimen was identified and authenticated by Mr. P. G. Diwakar, Joint Director, Botanical Survey of India, Western circle-7, Koregaon Road, Pune -1 on dated 11/01/2011, Voucher No. RASICA4. The leaves were dried in shade at room temperature. The dried leaves were coarsely powdered, stored in airtight container until used and packed in soxhlet apparatus. Extraction of leaves of Ipomoea carnea was carried out by using soxhlet apparatus. Polar solvent petroleum ether, chloroform, ethanol and water were used according to the polarity.

Experimental animals:

Healthy albino Wistar rats of age between 10-15 weeks of either sex were used after approval of the institutional ethics committee. The animals were maintained at a temperature controlled, well ventilated animal room for a period of 7 days prior to the experimental period. They were kept on standard pellet diet and water ad libitum. Surgical procedures on animals were done under strict aseptic conditions, under light ether anesthesia.

Acute toxicity study:

Aqueous extracts of *Ipomoea carnea* leaves were studied for acute toxicity at doses of 5mg/kg, 50mg/kg, 300mg/kg, 500mg/kg and 2000mg/kg. As per OECD 420 guideline dose of 2000mg/kg showed the toxic symptoms, so according to OECD guideline 420, it is considered as a LD_{50} cutoff value. Doses selected for pharmacological studies by fixed dose methods are 250mg/kg and 500mg/kg[17].

Evaluation of anti-inflammatory activity:

Carrageenan-induced paw edema in rats:

Albino wistar rats of either sex weighing 100-150g were divided in to four groups. Each group consisted of 6 rats. The animals were starved overnight. Group I served as control and received 0.2 ml of 2% gum acacia. Group II served as standard and received Etoricoxib intraperitoneal (6mg/kg body weight) [18]. Group III received the aqueous extract at the dose of 250 mg/kg body weight orally. Group IV received the aqueous extract at the dose of 500 mg/kg body weight orally. After one hour of administration of the test formulation and the drug, a sub-plantar injection of 0.1 ml of 1% w/v suspension of Carrageenan into the plantar side of the left hind paws. The paw was marked with ink at the level of the lateral malleoulus and immersed in mercury up to this mark. The paw volume was measured plethysmographically before the injection and after the injection for 0h, 1h, 2h and 3h interval gap. Each observation was repeated thrice and average of these observations was considered.

The average foot swelling in test as well as standard groups was compared with that of the control group and the % edema was calculated by using the formula:

% Edema = $(C_t - C_o / C_o) \times 100$
Where, C_t = Average paw volume of treated group.
C_o = Average paw volume of control group.

Statistical analysis:

The data is expressed as mean±SEM and subjected to students't' test and the level of significance was set at $p<0.01$.

Results

The result of Anti-inflammatory activity of Ipomoea carnea on carrageenan-induced inflammation is shown in Table-1 and Figure-1. Paw volume was significantly ($p<0.01$) reduced in test treated groups (250 mg/kg body weight and 500 mg/kg body weight) as compared to control group. The inhibition of paw edema was found to be 14.7% at the dose of 250mg/kg and 39% at 500mg/kg. The aqueous extract of Ipomoea carnea exhibited significant anti- inflammatory activity against carrageenan-induced rat paw edema. The aqueous extracts of Ipomoea carnea leaves with a dose of 500mg/ kg showed the maximum anti-inflammatory activity. Etoricoxib as a reference standard inhibited the edema formation due to carrageenan to an extent of 39% at the dose of 6 mg/kg i.p. Etoricoxib and aqueous extract of Ipomoea carnea exhibited significant anti-inflammatory activity against carrageenan-induced rat paw edema. The edema formation

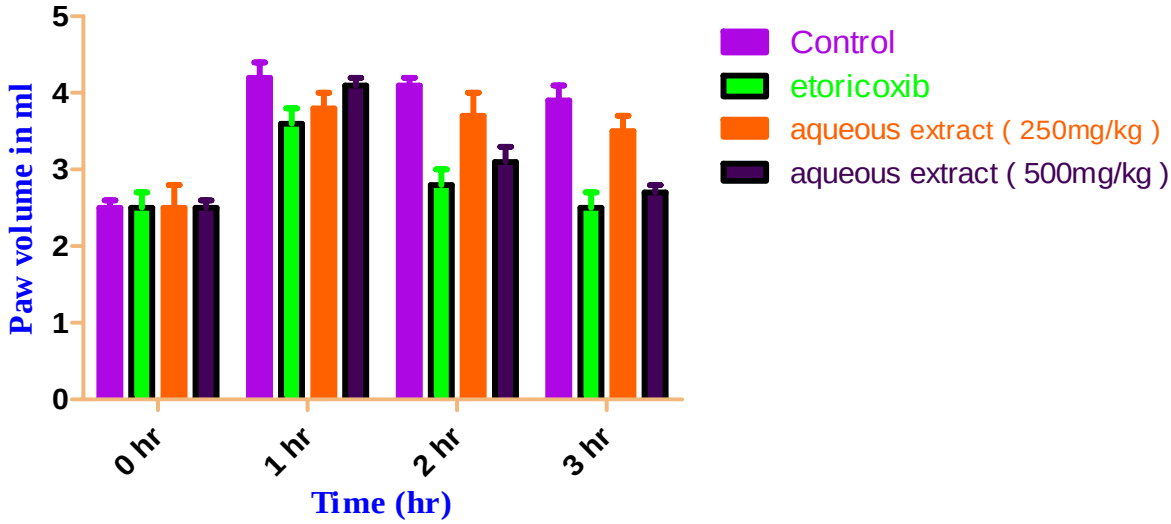
was greatly inhibited between 2-3 hours after sub-planter injection of carrageenan in all treated groups.

Table 1
Anti-inflammatory activity of aqueous extract of *Ipomoea carnea* leaves on carrageenan induced rat hind paw edema.

Treatment	Dose(mg/kg)	Mean paw volume(ml) at				%Inhibition of edema after 3hr
		0h	1h	2h	3h	
Control	0.2ml	2.5± 0.1	4.2 ± 0.3	4.1 ± 0.6	3.9 ± 0.4	-
Etoricoxib	6mg/kg	2.5± 0.2	3.6± 0.2	2.8± 0.2	2.5± 0.2**	39
Aqueous extract	250mg/kg	2.5± 0.2	3.8± 0.2	3.7± 0.3	3.5± 0.2*	14
Aqueous extract	500mg/kg	2.5± 0.1	4.1 ±0.1	3.1± 0.2	2.7± 0.1**	37

Values are mean ± SEM; n=6 *P<0.05, **P<0.01compared with normal control.

Figure 1
Anti-inflammatory activity of aqueous extract of *Ipomoea carnea* leaves on carrageenan induced rat hind paw edema.



Discussion

Edema represents the early phase of inflammation in carrageenan induced paw edema and is the simple stand most widely used acute inflammatory model for studying anti-inflammatory agents. The enzymes included cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), phospholipase A (2) (PLA (2), 5-lipoxygenase (5-LO) and 12-lipoxygenase (12-LO) of which leaves of *Ipomoea carnea* along with all of the other plant extracts showed inhibitory activities against at least one of the enzymes in various percentages depending upon the concentrations. The aqueous extract of *Ipomoea carnea* (500mg/kg) exhibited significant anti- inflammatory activity at 3rd hour against carrageenan-induced rat paw edema.

Conclusion

The aqueous extract of *Ipomoea carnea* leaves possesses anti-inflammatory property with the dose dependent effect carried out on experimental model. Between the two doses studied, aqueous extract of *Ipomoea carnea* at a dose of 500mg/kg was found to possess better anti-inflammatory activity as compared to Etoricoxib (6mg/kg). At this stage, it is difficult to say which component(s) of the extract are responsible for this anti-inflammatory activity. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

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References

1. Frey R. Ipomoea carnea sp. fistulosa (Martius ex Choisy) Austin: Taxonomy. Biology and ecology reviewed and inquired. 1995; 36(1): 21-48.
2. Compendium of Indian Medicinal Plant. Central Drug Research Institute. Publication and Information Directorate. New Delhi. 1979; 2: 390-391.
3. Indian Herbal Pharmacopoeia. Mumbai: Indian Drug Manufacture Association; 2002. P.498-99.
4. Ekka R Neeli, Dixit VK. Ethno-pharmacognostical studies of medicinal plants of Jashpur district Chattisgarh. Int Jour of Green Phar 2007; 1(1): 2-4.
5. Dwivedi SN. Traditional health care among the tribals of Rewa District of Madhya Pradesh with special reference to conservation of endangered and vulnerable specie. Econ Taxon Bot 1999; 23(2): 315-320.
6. Dwivedi SN. Ethonobotanical studies and conservation strategies of wild and natural resourses of Rewa district of Madhya Pradesh. J Econ Taxon Bot 2003; 27(1): 233-244.
7. Varghese E. SVD. Applied Ethnobotany- A case study among the Kharias of Central India. New Delhi: Deep Publications; 1996.

8. Phillips O, Gentry AH, Reynal L, Wilkin P, Gulvez DCB. Quantitative Ethnobotany & Amazonian Conservation. Conser. Bio 1994; 8: 225-248.
9. Shrivastava S, Dwivedi S, Dubey D and Kapoor S, Traditional herbal remedies from Madhya Pradesh used as oral contraceptives- A field survey, Int. Jour. of Green Pha. 2007; 1(1): 18-22.
10. Ved GA, Kinhal K, Ravikumar MK, Vijaya S, Indresha JH. Threat Assessment and Management prioritization for Medicinal Plants of Chattisgarh & Madhya Pradesh. FRLNI. Bangalore. India; 2004.
11. Pankaj O. Major Ipomoea Species (Morning glories) of Chhattisgarh. India: Natural Occurrence. Traditional Medicinal Knowledge and Trade; 2003.
12. Maria de FA, Patricia F de F, Jose MBF. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Brazilian Journal of Pharmacognosy 2007; 17(1): 114-140.
13. Umar S, Junior P, Wichtl M. Isolation and identification of agroclavin and alpha-dihydrolysergol from leaves of Ipomoea fistulosa. 1980; 40(4): 328-332.
14. Dubey P, Khare N, Gupta PC. A new flavonoid glycoside from the leaves of Ipomoea fistulosa. Curr Sci 1982; 51: 351-352.
15. Haraguchi M, Gorniak SL, Ikeda K, Minami Y, Kato A, Watson AA, et al. Alkaloidal components in the poisonous plant. Ipomoea carnea (Convolvulaceae). Journal of agricultural and food chemistry 2003; 51(17): 4995-4998.
16. Lundeberg, T., Alstergren, P., Appelgren, A., Appelgren, B., Carleson, J., Kopp, Sand Theodorsson, E.: A model for experimentally induced temperomandibular joint arthritis in rats: effects of carrageenan on neuropeptide-like immunoreactivity. Neuropeptides 1996; (30): 37– 41
17. Mrs. Prema Veeraraghavan. Expert consultant, CPCSEA, OECD guide line No. 420, 2000.
18. Behal N, Singh KS, Sharma P and Sanyal SN. Effect of non-steroidal anti-inflammatory drug etoricoxib on the hematological parameters and enzymes of colon and kidney, Nutr Hosp 2009; 24(3):326-332.