

**ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC STEM EXTRACTS OF  
*RUBIA CORDIFOLIA* LINN. IN RATS**

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**ABSTRACT**

In the present Study of Ethanolic extract of Stem of *Rubia cordifolia* Linn.(Rubiaceae) was screened for anti-inflammatory activity in carrageenan induced paw oedema rats. The effect was assessed by Difference in paw oedema volume, before & after the low & high dose administration of the extract in Rats. Ethanolic extract of *Rubia cordifolia* stem (20 & 40 mg./kg./ml.) were administered orally. Anti-inflammatory effects were compared with Standard drug- Indomethacin (10mg./kg/ml.). These observations helped us to conclude that Ethanolic Extract high dose is endowed with anti-inflammatory property.

**KEYWORDS:** Anti-inflammatory, Indomethacin, *Rubia cordifolia*, Plethysmograph.

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## INTRODUCTION

Inflammation is the tissue reaction to infection, irritation or foreign substance. It is a part of the host defense mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins. The development of non-steroids in overcoming human sufferings such as Rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property<sup>1</sup>.

*Rubia cordifolia* Linn. (Rubiaceae) is commonly known as Manjistha, Majith etc. It is distributed throughout the lower hills of Indian Himalayas in the North & Western Ghats in the south and Japan, Indonesia, Ceylon, Malay, Peninsula, Java & tropical Africa in moist temperate & tropical forests, upto an altitude of 3500 m. It is a large genus of hardy climbers with perennial root stocks distributed in the temperate & tropical zones. About 15 species occur in India. Some of these are Indian madder (*R. cordifolia*), Naga madder (*R. sikkimensis*) & European madder (*R. tinctorum*)<sup>2,3</sup>.

The major Phytoconstituents reported in this plant include free Alizarin & its glucosides, Purpurin, Xanthopurpurin, Munjistin, Ruberythric acid, Pseudo-purpurin, Physcion, Nordamnacanthal, Rubicoumaric acid, Mollugin, Furomollugin, various Anthraquinone glycosides such as- 1-hydroxy-2-methyl anthraquinone, 1,4-dihydroxy-6-methyl anthraquinone etc., cyclic hexapeptides as RA-V, RA-VII, cyclic heptapeptides as RA-III, RA-IV etc<sup>4,5,6</sup>.

The Literature review revealed that antimicrobial, anticancer, Hypoglycaemic, Haemostatic, antipyretic, analgesic, antihelminthic, anti-inflammatory, In purifying blood, Diuretic, In liver complaints, In pains of joints, Uterine pains, In Rheumatoid arthritis property of *Rubia cordifolia* have been studied scientifically<sup>7,8,9</sup>.

So far no systematic study has reported for anti-inflammatory property of stem extract of *Rubia cordifolia*. In the present study effort has made to establish the scientific validity to the anti-inflammatory property of *Rubia cordifolia* stem extract using Indomethacin & Carrageenan induced paw oedema model in Rats.

## MATERIALS & METHODS

### Plant Material & Extraction Procedure

The dried Stems of *Rubia cordifolia* collected from a well known herbal material suppliers of Chandigarh, Punjab, India during October, 2007 and authenticated by National Bureau of Plant Genetic Resources department of IARI, Pusa- New Delhi, India. A Voucher Specimen of the plant was deposited in NBPGR herbarium under the No.-NHCP/NBPGR/2008/9/3026. The air dried stems of *Rubia cordifolia* reduced to coarse powder and around 2 kg. of dried powder was extracted separately with 95% v/v alcohol by continuous hot percolation process using Soxhlet apparatus. Finally, dried stems of *Rubia cordifolia* were macerated in Chloroform water IP for aqueous extract. After the effective extraction, Solvent were concentrated at Room temperature in reduced pressure using a rotary evaporator and water was removed by freeze drying to become semisolid residue<sup>10,11</sup>.

### Animals

Albino rats (150-250 gm. Each) of either sex kept under standard environmental conditions (25±2°C under 12 h light & 12 h dark cycles) in polypropylene cages. Standard pelleted feed & drinking water were provided *ad libitum* throughout the experimental period. The animals were acclimated to laboratory conditions one week prior to the initiation of experimental work. The protocol was approved by the Ethics committee & the CPCSEA under the no.-IAEC/CPCSEA-385.

## ANTI-INFLAMMATORY STUDIES

### Carrageenan induced hind 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/ml. suspended in 1% sodium carboxymethyl cellulose. Group 3 and 4 were treated with 20 and 40 mg./kg/ml. of ethanolic extracts of *Rubia cordifolia* Stems (EERCs) suspended in Tween 80/ ethanol / saline (1:1:10). Oedema was induced by injecting 0.1 ml. of a 1% solution of carrageenan in saline into the subplantar region of the right hind paw of the rats. The vehicle, extracts and the standard drugs were administered 60 min. prior to the injection of the phlogestic agent. The volumes of oedema 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 paw oedema inhibition<sup>11</sup>.

## EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

### Determination of Inhibition of Paw oedema

The percentage inhibition of rat paw oedema was calculated and compared with that of standard indomethacin. Indomethacin produced a 76.79% inhibition of paw oedema when observed after 3 hours of carrageenan injection. The alcoholic extract (high dose) of *Rubia cordifolia* significantly inhibited the paw oedema 39.13% inhibition when compared to the saline group after three hours of carrageenan injection. In case of (Low dose) of alcoholic extract of *Rubia cordifolia*, which gave a 29.01% inhibition of paw oedema, however they didn't attain the statistical significant value compared to saline treated group.

### Statistical Analysis

The values are expressed as mean  $\pm$  S.E.M. Statistical Analysis was performed using ANOVA (one way) followed by Student's t-test  $p < 0.05$  was considered to be significant or we can say that the values are significantly different from the control or saline group at  $P < 0.05$ .

## RESULTS & DISCUSSION

The alcoholic extract of the stems of *Rubia cordifolia* has been tested for their possible anti-inflammatory activity in Albino rats of four groups, each group containing six animals of either sex weighing between 150-250 gm. The first group received saline which served as control. The second group was given standard NSAID indomethacin drug (10mg./kg./ml) orally, which served as standard anti-inflammatory agent.

The third & fourth groups received the low dose (20mg./kg./ml.) and high dose (40mg./kg./ml.) of alcoholic extract suspension of test drug *Rubia cordifolia* respectively, orally. The percentage inhibition of rat paw oedema was calculated and compared with that of standard indomethacin. Indomethacin produced a 76.79% inhibition of paw oedema when observed after 3 hours of carrageenan injection. The alcoholic extract (high dose) of *Rubia cordifolia* significantly inhibited the paw oedema 39.13% inhibition when compared to the saline group after 3 hours of carrageenan injection.

In case of (low dose) of alcoholic extract of *Rubia cordifolia*, which gave a 29.01% inhibition of paw oedema, however, they didn't attain the statistical significant value compared to saline treated group. According to statistical analysis of anti-inflammatory data, we can say that the values were significantly different from the control or saline group at  $P < 0.05$  (ANOVA, followed by Student's t-test).

## CONCLUSION

It is concluded that Ethanolic extract of stem of *Rubia cordifolia* possess significant anti-inflammatory activity against experimentally induced paw oedema in rats. This may be due to the presence of reported active Phytoconstituents & their influence on the prostaglandins pathway. Further research, to isolate anti-inflammatory principle & exact mechanism involved, is needed.

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**Table I: Effect of EERCS on Carrageenan-Induced hind Paw Oedema**

S.No.	Treatment	Dose (mg/kg/ml)	Mean difference in Paw volume(ml.) ± S.E.M.				
			1 h	2 h	3 h	4 h	5 h
1.	Vehicle ( Control )	-	0.51 ± 0.02	0.70 ± 0.01	0.81 ± 0.02	0.73 ± 0.04	0.69 ± 0.004
2.	Standard (Indomethacin)	10	0.14 ± 0.01 a*(73.03)	0.19 ± 0.01 a*(72.57)	0.19 ± 0.01 a*(76.79)	0.16 ± 0.02 a*(78.02)	0.17 ± 0.01 a*(75.22)
3.	EERCS ( Low dose)	20	0.41 ± 0.003 a*(19.29)	0.59 ± 0.01 a*(14.85)	0.58 ± 0.01 a*(29.01)	0.47 ± 0.02 a*(35.02)	0.47 ± 0.02 a*(33.52)
4.	EERCS ( High dose)	40	0.33 ± 0.01 a*(35.43)	0.52 ± 0.01 a*(26.00)	0.49 ± 0.01 a*(39.13)	0.32 ± 0.01 a*(56.73)	0.39 ± 0.01 a*(42.71)

EERCS: Ethanolic extract of *Rubia cordifolia* stem

Values are mean ± S.E.M., n= 6 animals in each group, a= represents a comparison between group 2, 3, 4 v/s group1. Values within parenthesis represent the percentage protection or paw oedema inhibition. The values were significantly different from control at P< 0.05 (ANOVA, followed by student's t-test).

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