Anar Patel et al /J. Pharm. Sci. & Res. Vol.2 (12), 2010,809-813

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of Anti inflammatory and Analgesic activity of roots of *Rubia cordifolia* in rats.

Anar Patel [1], Timir Patel [1], Carol Macwan[1], Mayuree Patel [1], Khushbu Chauhan [1], Jatin Patel[2]

[1]Faculty of Pharmacy, Dharmsinh Desai University, College road, Nadiad-387001, Gujarat.
[2] F&D, Alembic research centre, Baroda, Gujarat.

ABSTRACT

The present study was aimed to investigate the analgesic and anti-inflammatory effect of the methanolic extract of root of *Rubia cordifolia* in rats. *Rubia cordifolia* (100-300 mg/kg, p.o.) was evaluated for its anti-inflammatory activity by carrageenan induced rat paw edema and *Rubia cordifolia* (200-400 mg/kg) for its analgesic activity by tail flick method. *Rubia cordifolia* (100-300 mg/kg, p.o.) showed significant ($P<0.05$) reduction in the paw edema produced by the carrageenan and significant ($P<0.05$) increased reaction time in tail flick test.

Key words: *Analgesic activity, Anti inflammatory activity, Rubia cordifolia roots.*

INTRODUCTION

The herb *Rubia cordifolia* is usually categorized as GRAS (generally recognized as safe) (1). *Rubia cordifolia Linn* (Rubiaceae), an ayurvedic herb is popularly known as Indian Madder, majit, manjishtha (2,3,4). It is distributed throughout the lower hills of Himalayas in the North and Western Ghats in the South India, Japan, Indonesia, Ceylon, Peninsula, Java and in tropical Africa moist temperate and tropical forests (2,3). *Rubia cordifolia* is perennial, herbaceous, climber with very long, cylindric, roots with a thin red bark (2). Stems are long, rough, grooved and become slightly woody at the base. Generally root, leaves, fruits, stem etc. of the plant *Rubia cordifolia* are used for their therapeutic properties. Manjith has been used as a dye, in skin care and treatment and it has been used internally in disorders of the urinary tract. Roots are traditionally used as anti-inflammatory, astringent, tonic, antiseptic, diuretic, deobstruent, antidysenteric, blood purifier, anthelmintic, analgesic, hepatoprotective etc. (1,2)

MATERIAL AND METHODS

Chemicals

Carrageenan solution (1%w/v), Sodium carboxy methyl cellulose (vehicle for anti inflammatory activity), Diclofenac sodium (standard). All the chemicals required were purchased from Dutt Enterprise, Nadiad.

Procurement of plant material.

The *Rubia cordifolia* root was collected at Maharaja Enterprises, Ahmedabad and authentified at Bioscience Department, Gujarat University, Ahmedabad. After collection roots were washed thrice with water, dried under shade for a period of one month, powdered, passed through 60# sieve and stored in air tight container.

Preparation of the alcoholic extract.

The coarse powder 200 gm of dried *Rubia cordifolia* root was defatted with petroleum ether and then taken with 1000 ml methanol. It was subjected to reflux for 3 cycles. The extract was concentrated on water bath, stored in air tight container, in dry place. (5)

Animals.

Healthy male Wistar rats (200-250g) were housed in CPCSEA approved animal house in groups of five in polypropylene cages. They were maintained at 25 ± 2° C, relative humidity of 45 to 55% and under standard environmental conditions (12hrs light 12 hrs dark cycle). The animals had free access to food and water ad libitum. All the procedures were performed in accordance with the Institutional Animal Ethical Committee constituted as per the directions of the CPCSEA. The study was carried out between 9.00 am and 4.00 pm.

PHARMACOLOGICAL STUDIES:

1. Analgesic Activity:

1.1 Experimental design:

In this study, a total of 30 rats were used. The rats were divided in to five groups of six rats each.

Group I: Control group (Normal untreated rats)

Group II: Standard Drug Diclofenac Sodium (10mg/kg)

Group-III, IV, V: Administration of different doses of alcoholic extract of *Rubia cordifolia* root (200 mg/kg, 300 mg/kg & 400 mg/kg respectively)

1.2 Evaluation of Analgesic activity:

Wistar male rats (200-250 g) housed under standardized animal house conditions were used. Before administration of the test compound or the standard the normal reaction time was determined. The tail flick latency was assessed by the Analgesiometer (Inco, India). The strength of the current passing through the nacked nichrome wire was kept at 2-3 Amps of constant temperature of 55°C was maintained throughout. The distance between the heat source and the tail skin was kept at 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut- off reaction time was fixed at 25 sec to avoid tissue damage (6, 7). The escape reaction which was the end point of this test regarded as a complex phenomenon mediated by the brain (8). Results were compared with control group

2. Anti-inflammatory activity

2.1 Experimental Design

In anti-inflammatory study, a total of 30 rats were used. The rats were divided in to five groups of six rats each.

Group I: Control group (Normal untreated rats)

Group II: Standard Drug Diclofenac Sodium (10mg/kg)

Group III, IV, V: Administration of different doses of alcoholic extract of *Rubia cordifolia* root (100 mg/kg, 200 mg/kg & 300 mg/kg respectively)

2.2 Evaluation of Anti-inflammatory activity (9, 10):

The methanolic extract was evaluated for anti-inflammatory activity on a carrageenan-induced rat paw edema model. Inflammation was produced in rats (Male, Wistar, weighing 200-250 g) using 100 µL of 1% carrageenan (wt/ vol) in distilled water. Thirty minutes later of carrageenan injection, methanolic extract of *Rubia cordifolia* root was given orally. The increase in paw volume was measured plethysmometrically before time 0, 1, 3 and 6 hours after carrageenan administration. All groups were treated with 0.1 ml of 1% W/V carrageenan solution. Results were compared with control group. The difference between the two readings was taken as the volume of the edema and % inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 1 - (A-X / B-Y) * 100$$

Where,

A= Mean paw volume of treated rats after administration of carrageenan at time 't'

X= Mean paw volume of treated rats before administration of carrageenan

C= Mean paw volume of treated control rats after administration of carrageenan at time 't'

Y= Mean paw volume of treated control rats before administration of carrageenan

Statistically analysis:

Results were expressed as Mean ± SEM. Statistically differences was determined by Analysis of variance methods (ANOVA) by using statistical computer software Graph Pad Prisom. Only those value showing statistical differences $p<0.05$ considered as statistical significant.

RESULTS:

1. Result of Analgesic activity:

Rubia cordifolia (200 & 300 mg/kg) significantly increased the reaction time for the thermal induced pain in treated with as compared to normal rats. Hence, it is speculated that apart from inhibition of chemical mediators of inflammation, ***Rubia cordifolia*** may also modulate the pain response in the central analgesic activity. Results are mentioned in Table 1 and Figure 1.

Table 1: Effect of methanolic extract of *Rubia cordifolia* root & Diclofenac sodium on thermal stimulus-induced pain (tail flick test) in male wistar rats.

Treatment	*Mean Reaction Time [Sec]*						
	0 hr	*½ hr*	*1 hr*	*2 hr*	*3 hr*	*4 hr*	*6 hr*
Normal Rats	5.0 ± 0.577	5.55 ± 0.619	5.11 ± 0.61	5.50 ± 0.67	4.83 ± 0.48	6.00 ± 0.51	**4.30 ± 0.49**
Diclofenac Sodium treated rats [10mg/kg,*p.o*]	5.83 ± 0.40	9.33 ± 0.49*	9.66 ± 1.17*	9.88 ± 1.07*	9.83 ± 1.22*	9.60 ± 0.66*	**6.60 ± 0.80***
Methanolic Extract of Rubia cordifolia root.							
100 mg/kg	5.33± 0.49	7.50 ± 1.23	9.60 ± 1.17*	7.33 ± 0.61*	9.30 ± 0.49*	8.00 ± 0.68#	**5.10 ± 0.47**
200 mg/kg	5.60 ± 0.80	9.33 ± 1.22*	9.66 ± 1.20*	8.55 ± 1.43*	9.55 ± 0.56*	9.00 ± 0.85*	**5.60 ± 0.04***
300 mg/kg	5.29 ± 0.68	10.61 ±1.37*	11.03 ±1.68*	10.13± 1.36*	9.97± 1.37*	9.93 ± 0.73*	**5.13 ± 0.37***

Each values are represented as Mean ± S.E.M. n=6, # $p<0.05$, * $p<0.01$ as compared to control group.

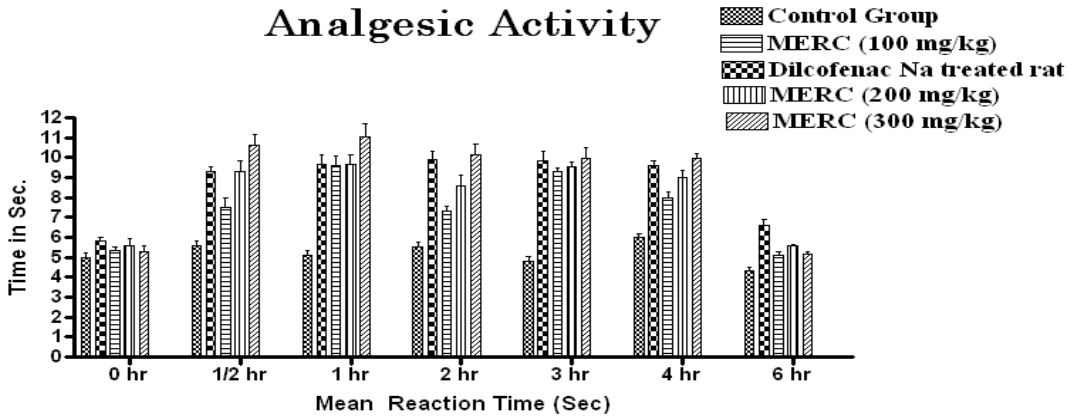

Figure 1: Effect of methanolic extract of *Rubia cordifolia root* & Diclofenac sodium on thermal stimulus-induced pain (tail flick test) in male wistar rats. Mean reaction time was measured. n=6, # $p<0.05$, * $p<0.01$ as compared to control group

Table 2: Effect of methanolic extract of *Rubia cordifolia* root on Carrageenan Induced Hind Paw Edema of Rat.

Group	*PAW VOLUME (ml)*							
	30 min		*1 hrs*		*3 hrs*		*6 hrs*	
	ml	*% I*	*ml*	*% I*	*ml*	*% I*	*ml*	*% I*
Control	1.4±0.08	-	1.56±0.02	-	1.61±0.03	-	1.06±0.04	-
STD(10 mg/kg)	1±0.07#	22.3	0.7±0.05*	49.71	0.53±0.04*	64.94	0.43±0.06*	**95.36**
100 mgkg	1.56±0.13	3.80	1.21±0.09*	29.92	0.98±0.09*	48.56	0.98±0.08*	**72.13**
200 mg/kg	1.16±0.11	15.57	0.9±0.06*	41.81	0.73±0.03*	58.85	0.63±0.04*	**87.23**
300 mg/kg	**1.5±0.02**	**12.44**	**1.08±0.07***	**35.57**	**0.86±0.08***	**52.75**	**0.86±0.06***	**80.78**

I = Inhibition, N=6 in each group. #P<0.05, *P<0.001 compared to control

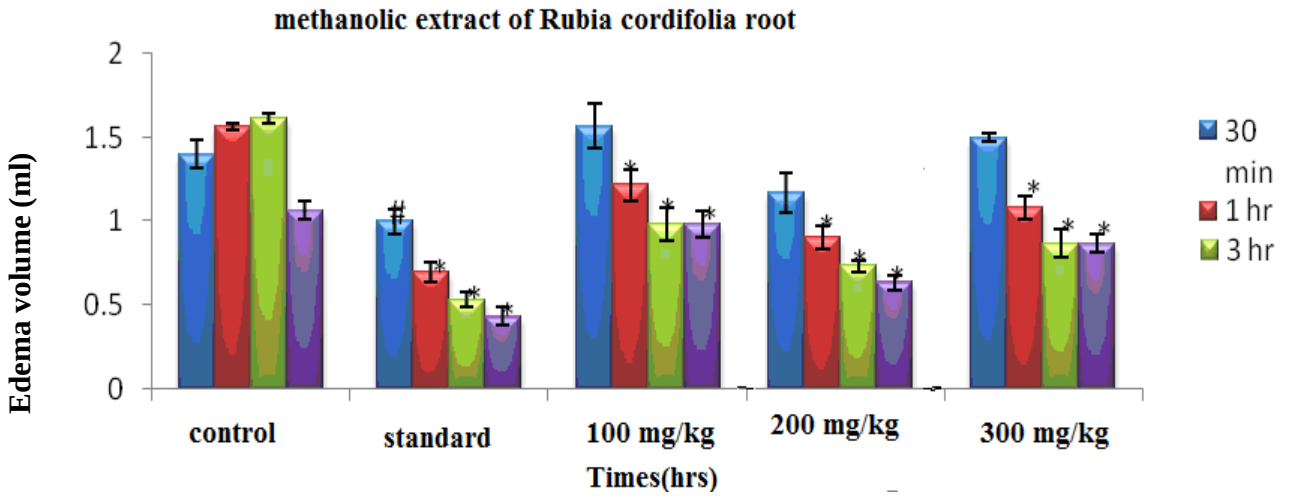

Figure 2: Effect of methanolic extract of *Rubia cordifolia* root on carageenan induced Inflammation in rat paw edma as edema volume (ml) v/s Time (hrs).

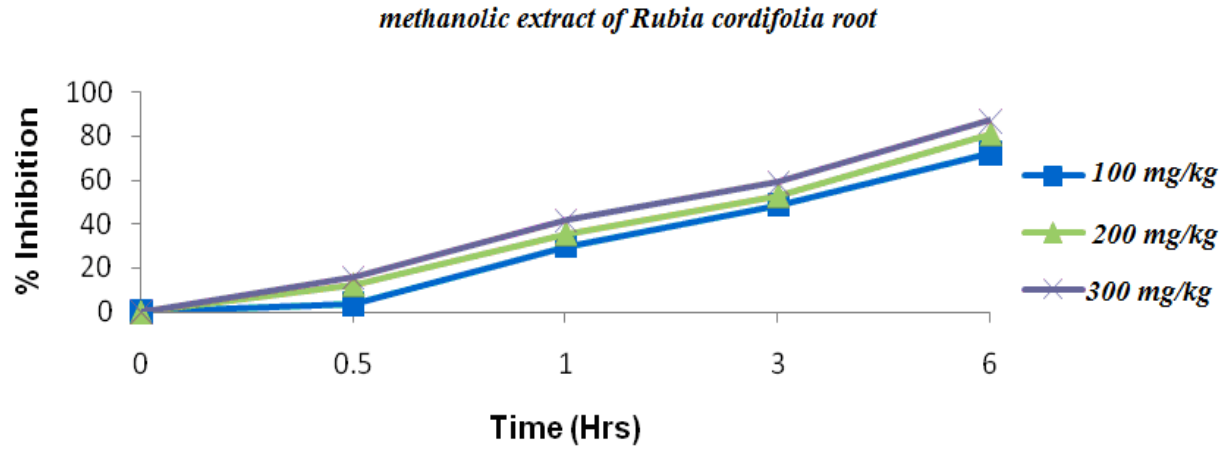

Figure 3: Effect of methanolic extract of *Rubia cordifolia* root on carageenan induced Inflammation in rat paw edma as % Inhibition v/s Time (hrs).

2. Result of Anti-inflammatory activity:

In the acute inflammation model, methanol extract of *Rubia cordifolia* roots in doses of 100, 200 and 300 mg/Kg., p.o. produced inhibition of paw edema. The test and the standard drug produced significant inhibition of paw edema as compared to the control. The results are also showed graphically in Fig. 2&3. The extract was tested at three different dose levels. The results showed that methanolic extract with a dose of 200 mg/Kg showed maximum 87.23% (Table 2) of inhibition on carrageenan induced rat paw edema at end of 6 hrs respectively (Table 3). The present results indicate that the efficacy of this extract with above doses had an efficient therapeutic activity in acute inflammatory conditions.

Table 3: % Inhibition of paw edema

Dose (mg/Kg, p.o.)	*Methanol*
100mg/Kg	72.13
200 mg/Kg	87.23
300 mg/Kg	80.78

DISCUSSION:

The analgesic activity may be attributed due to the presence of glycosides, alkaloids and other bioactive compounds. the present study demonstrated that Methanolic extract of *Rubia cordifolia* has intrinsic analgesic activity which needs to be investigated with more information on the bioactive principles responsible for the action. The results indicate that the MERC possesses significant analgesic activity. Furthermore, Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substance which peak at 3 hrs. The methanolic extract of *Rubia cordifolia* root was evaluated by carrageenan induced rat paw edema. They produced significant inhibition of rat paw edema induced by carrageenan. The inhibition was however, less than that of standard drug, Diclofenac sodium.

CONCLUSION:

Methanolic extract of *Rubia cordifolia* shows significant analgesic and antinflammatory activities. But, analgesic and anti-inflammatory mechanisms of its root as well as active principles involved are not known. Ongoing studies in this laboratory include mechanism of action of this plant roots as well as activity guided isolation of active principles from the root.

ACKNOWLEDGMENT:

Authors are thankful to Dr. A.K. Saluja and Mr. Girish Patel their guidance and support.

REFERENCES

[1]. Elizabeth M. Williamson, 2002, *Major herbs of Ayurveda*, Published by Churchill Livingstone, 13-15, 69-75, 257-260.

[2]. K.R.Kirtikar, B.D.Basu, 1980, *Indian Medicinal Plants* vol II, International Book, Distributors, Dehradun, Sec edition, p. 1305-1307.

[3]. C.P. Khare, 2004, *Encyclopedia of Indian Medicinal Plants, Rational Western Therapy & other Traditional Usage, Botany,* Springer Verlag Berlin Meidelberg, p. 406- 407.

[4]. Anonymous, 2005, *Quality Standards of Indian Medicinal Plants*, Indian council of Medical research, New Delhi, V. 3, 78-87, 307-315.

[5]. Mukharji Pulok K., 2002, *Quality Control of Herbal Drugs,* An approach to Evaluation of Botanicals, Business Horizons Pharmaceutical Publishers, New Delhi, first edition, 600-604.

[6]. Von Voigt lander PF, 1982, Pharmacological alteration of pain: The discovery and evaluation of analgesics in animals, In: Lednicer D (Ed) *Central Analgesics,* John Wiley & Sons, New York, 51–79,

[7]. Davies O.L., Raventos J, 1946, A method for evaluation of Analgesic activity using rats, *Br. J. Pharmacol.,* vol 1, 255, Carried through Subrata K. Mandal, Boominathan R., *Indian journal of experimental biology,* July 2005, vol-43,662-663

[8]. Vogel H. Wolfgang, Julgen Sandow,2002, *Drug Discovery and Evaluation, Pharmacoligical assays*, 9th edition, Springer, Germany,670-723

[9]. Sarıgüllü Özgüney I, Yeşim Karasulu H, Kantarcı G, Sözer S, Güneri T, and Ertan G., 2006, Transdermal Delivery of Diclofenac Sodium Through Rat Skin From Various Formulations, *AAPS Pharm SciTech*.; 7(4): Article 88.

[10]. Winter CA, Risley EA and Nuss GV, 1962, Carrageenan-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs, *Proc Soc Exp Biol Med*. ;111:544-547.