Research Article

Evaluation of Anti-Nociceptive Activity of Ethanolic Extract of Stem of *Rubia Cordifolia* in Wistar Albino Rats

Ahamed Shaheen Yasar S[1], Sridevi K[2], Nagapati Prabhakar Bhat[2], Roopa P. Nayak[2]

[1]Department of Pharmacology, SRM Medical College Hospital and Research Centre, Kattankulathur, Chennai – 603 203.
[2]Department of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore – 575 018, India

(Received: 06-01-2026 Revised: 23-02-2026 Accepted: 02-03-2026)

Corresponding Author: ***Ahamed Shaheen Yasar S*** Email: ***sshaheen94@yahoo.com***

ABSTRACT

Background: The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage". The primary goal of analgesia is to eliminate the cause of pain but this is not always possible so analgesics are used to treat pain symptomatically. This study aims to scientifically validate the anti-nociceptive activity of stem of *Rubia cordifolia* using rodent models.

Materials and methods: Ethanolic extract of *Rubia cordifolia* (EERC) stem was used for evaluating the anti-nociceptive activity in Wistar albino rats. Ethanolic extract of *Rubia cordifolia* stem was used at a dose of 100 and 200 mg/kg. Distilled water 10 ml/kg was used as vehicle control. Anti-nociceptive activity was evaluated by performing hot water tail immersion test and Eddy's hot plate method comparing with Diclofenac (10mg/kg) as the standard drug. The data was analyzed using one way ANOVA (analysis of variance) followed by Tukey Krammer test. P value <0.05 was considered as significant.

Results: Ethanolic extract of *Rubia cordifolia* stem at a dose of 200mg/kg showed a significant analgesic efficacy in hot water tail immersion test and Eddy's hot plate method. Additionally, the results were closely comparable to the standard drug diclofenac.

Conclusion: Ethanolic extract of *Rubia cordifolia* stem shows promising anti-nociceptive activity in Wistar albino rats, suggesting that it could be a valuable natural alternative for pain relief but further studies are necessary to confirm its safety and effectiveness in broader clinical settings.

Keywords: Pain, Anti-nociceptive, Ethanolic extract of *Rubia cordifolia* stem, Wistar albino rats.

1. INTRODUCTION

Pain, one of the most common health problems associated with sensory experience is multidirectional, inherently unpleasant, poorly defined and disabling in many medical conditions. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [1]. The effective management of pain is essential in clinical practice, as it significantly impacts patient quality of life and functional outcomes. Although several classes of analgesics such as NSAIDs, opioids, anticonvulsants, and muscle relaxants are available, their use is often limited by suboptimal efficacy and adverse effects, particularly with long-term use. This has led to increased interest in identifying safer and more effective alternatives. Therefore, there is a growing need to explore novel analgesic agents, especially from natural sources, that offer improved safety profiles and therapeutic efficacy [2].

The World Health Organisation (WHO) recently defined traditional medicine as the body of knowledge, skill and practices derived from theories, beliefs and experiences that are unique to various cultures. They can be rationalised and

used in the preservation of health as well as in the prevention, diagnosis, improvement and treatment of physical and mental illness [3]. The presence of natural chemical ingredients such berberine, morphine, psilocin, and vincristine4 as well as natural chemicals for drug manufacture are necessary for the number of ways that plants have been used as medicines. Nicotine, quinine, tubocurarine, colchicine, and other substances are used for medicinal purposes [4]. *Rubia cordifolia* is a medicinal plant known for its medicinal properties since ancient times. It is also known as Madder or Indian madder or Common madder. Manjistha is a known popular term for this plant. It is widely distributed throughout India mostly in the hilly regions. Different parts of this plant such as roots, leaves and stem are mainly used in traditional medicine practice. Previous studies have shown that root extract of *Rubia cordifolia* possess anti-inflammatory, neuroprotective, hepatoprotective, antidiabetic, antibacterial, radioprotective, antioxidant and antitumor aktivity [5]. Phytochemical analysis on root extract of *Rubia cordifolia* isolated major bioactive compound anthraquinones and their glycosides and showed significant analgesic activity. However the same constituents were also present in stem extract of this plant [6]. In the literature review there were no studies found on the analgesic effect of stem extract of this plant. Therefore, the present study helps to evaluate the analgesic activity of stem extract of this plant and to scientifically validate its efficacy. Hereby in this study our objective is to evaluate the central anti-nociceptive activity of the ethanolic extract of the stem of *Rubia cordifolia* in Wistar albino rats using Eddy's hot plate and Hot water tail immersion and to compare the analgesic activity of the extract with standard drug diclofenac.

2. MATERIALS AND METHODS

This study was carried out in the Ethnopharmacology Laboratory, Department of Pharmacology, Yenepoya Medical College, Mangalore. The approval from the Institutional Animal Ethics Committee (IAEC) (No: YU/IAEC/04/2023) was obtained prior to starting the experiment.

Equipment

The equipment used in this experiment includes soxhlet apparatus, Eddy's hot plate, weighing balance, beakers, spatula, muslin cloth, water bath, glass mortar and pestle, test tubes, glass rod, Whatman filter paper, disposable syringes, oral gavage, gloves, cotton, stopwatch, adhesive tape, stand and container.

Animals

In this study, male and female Wistar albino rats weighing 200–250 gm and aged 4-5 months were employed. The departmental animal house (Registration no: 347/PO/ReBi-S/Rc-L/01/CPCSEA) was used to keep the animals under standard conditions. The temperature was kept at 24+/-^{o}C and there was a 12:12 light: dark cycle. Water was available at all times and the animals were fed a regular pellet diet. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for seven days. Guidelines issued by the "Committee for Control and Supervision of Experiments on Animals" (CCSEA) were followed while handling the animals.[7]

Drugs and chemicals

Distilled water (Vehicle control), Diclofenac (Standard drug), Ethanolic extract of *Rubia cordifolia* (EERC) stem

Plant material

The plant known as *Rubia cordifolia* was obtained from a forest located in Agumbe, Karnataka, India and authenticated by a botanist at Pilikula Development Authority, Mangalore.

Preparation of the extract

After seven days of shade drying, the *Rubia cordifolia* stem was ground into a coarse powder. 200g of stem powder were taken in Soxhlet apparatus for extraction. The solvent used was 95% ethanol. The temperature was kept between 60 and 70^{o}C. The extraction process took four days in total. For three days, the concentrated extract was stored in a water bath. The extracted dark brown product had a weight of 8g and a yield of 4%. Before being utilized, the extracted material was kept at 0°C. Animals were given varying dosages of ethanolic extract of the stem

of *Rubia Cordifolia* (EERC) which had been diluted in distilled water.[8]

Sample size and Grouping

A total of 18 Wistar albino rats was randomly divided into 4 groups with 3 animals each in group 1 & 2 and 6 animals each in group 3 & 4 of either sex. (Table 1). Group 1 & 2 animals were shared for 6 animals each.

Experimental procedure

The study was carried out using two animal models which are widely followed for screening analgesia in rodents – Hot water tail immersion test and Eddy's hot plate method.

Hot Water Tail Immersion Test (HWTI)

Wistar albino rats (male and female – equal numbers i.e. 3 each) weighing between 200-250g were used. Animals were subjected to pre-testing and tail flick movement was recorded. After 30 min of dose administration, the lower 5 cm of rat tail were marked and immersed in a beaker of water maintained at around 55 + 1°C and reaction time for tail curling or flicking movement was recorded using a stopwatch for each group at 30, 60, 120 min. After each test the tail was carefully dried. Results were compared with control group and standard drug [9, 10].

Eddy's Hot Plate Method (EHPM)

Wistar albino rats (male and female – equal numbers i.e. 3 each) weighing 200-250g were used. Animals were subjected to pre-testing and nociceptive activity was recorded. After 30 min of dose administration, the animals were placed on Eddy's hot plate maintained at 55 + 1°C for a maximum time of 30s. Latency to exhibit the nociceptive response such as licking their fore and hind paws or jumping was determined for each group at 30, 60, 120 min. A cut-off time of 60s to avoid tissue damage was maintained. Results were compared with control group and standard drug [9, 10].

Statistical Analysis

Observations made in HWTI and EHPM were compiled and tabulated. Data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple comparison test using GraphPad Prism version 6.0. SPSS version 24.0 was used for data verification and additional statistical validation. A p-value < 0.05 was considered statistically significant. Values were considered statistically significant when, P-value < 0.05, P-value < 0.01 was considered as highly significant and P-value < 0.001 was considered as very highly significant [11, 12].

3. RESULTS

In the hot water tail immersion test for screening of central analgesic activity, standard drug Diclofenac (10mg/kg), EERC 1 (100mg/kg) and EERC 2 (200mg/kg) showed increase in tail withdrawal time which was extremely significant for standard and EERC 2 at the end of 30 minutes, 1 hour and 2 hours. It was very significant for EERC 1 (100mg/kg) at the end of 30 minutes and 2 hours, both the results were compared to standard & control group (Table 2).

In the Eddy's hot plate method for screening of central analgesic activity, standard drug Diclofenac (10mg/kg) and EERC 2 (200mg/kg) showed increase in the paw withdrawal time which was extremely significant statistically at the end of 30 minutes, 1 hour and 2 hours. It was very significant for EERC 1 (100mg/kg) at the end of 30 minutes and 2 hours, both the results were compared to standard and control group (Table 3).

4. DISCUSSION

The goal of this study was to assess the ethanolic extract of *Rubia cordifolia* stem's analgesic potential in experimental animal models. The result show that, when the extract was given to rats in pain models at intervals of 0 minutes, 30 minutes, 60 minutes and 120 minutes, there was a notable analgesic effect. EERC doses of 100 mg/kg and 200 mg/kg were pharmacologically evaluated using Eddy's hot plate method and Hot water tail immersion test.

Behaviors like the tail flick response observed in the hot water tail immersion test, use a response phenotype structured at the spinal level. The animal's tail is moving as a result of a poly-synaptic reflex, which is triggered by an abrupt, high-intensity thermal input. The reaction exhibited in Eddy's hot plate which is hind paw withdrawal is considered a supraspinal organized response since it necessitates intricate motor coordination [13]. Study done by Marasini D *et*

al., investigated the analgesic properties of *Ficus religiosa* stem bark and leaves, demonstrating a dose-dependent increase in latency times at doses of 200 and 400 mg/kg. The leaves exhibited greater analgesic activity, attributed to their higher phytochemical content. Ethanolic extracts of the root cortex (EERC) at doses of 200 mg/kg and 100 mg/kg also showed dose-dependent effects, with the 200 mg/kg dose displaying comparable efficacy to diclofenac. Both studies confirmed central antinociceptive action using Eddy's hot plate method [14]. Study done by Chhetri *et al.*, evaluated the analgesic potential of the aqueous extract of *Diploknema butyracea* (ADBB), which exhibited a dose-dependent increase in reaction times, with a 200 mg/kg dose demonstrating effects equivalent to diclofenac. The analgesic action of ADBB is centrally mediated. Similar findings were observed with EERC, where the 200 mg/kg dose produced significant, dose-dependent analgesic effects. Both studies emphasized the role of flavonoids, tannins, and glycosides as key contributors to the analgesic properties [15]. Study done by Anandhalakshmi *et al.*, demonstrated the analgesic activity of hydroalcoholic extract of *Costus pictus* leaves using tail clip and hot plate methods. Significant increase in pain threshold, especially at 400 mg/kg, comparable to the standard drug. Comparable findings with EERC, where the 200 mg/kg dose showed significant anti-nociceptive action using the hot plate method. Both emphasize a strong dose-dependent response [16]. Study done by Ghauri *et al.*, investigated the aqueous methanolic extract of *Euphorbia granulata*, which demonstrated central and peripheral analgesic activity. At a dose of 200 mg/kg, the latency time significantly increased, showing 61% analgesic potential compared to tramadol. Additionally, EERC at 200 mg/kg also exhibited significant analgesic activity with a dose-dependent effect. The findings from both studies confirm central analgesic mechanisms [17]. Study done by Liu *et al.*, (evaluated the analgesic activity of methanolic root bark extract of *Ottonia anisum* using Eddy's hot plate. Dose-dependent effect observed at 50–200 mg/kg, with the greatest effect at 200 mg/kg. Similar to EERC, which showed significant anti-nociceptive action at 200 mg/kg using Eddy's hot plate method. Both studies highlight the dose-dependent analgesic effects of plant extracts [18].

Although the present study demonstrates promising anti-nociceptive activity, no acute or chronic toxicity studies were conducted. Further investigations, including toxicity profiling, dose optimization, pharmacokinetic studies and clinical trials are necessary to establish the safety and efficacy of *Rubia cordifolia* stem extract for therapeutic use.

Table 1: Sample size and Grouping

S.no	Groups	No. of Wistar albino rats
1	Vehicle Control - Distilled water	3
2	Standard Drug - Diclofenac – 10mg/kg po *	3
3	Test Drug EERC stem 1 – 100mg/kg po **	6
4	Test Drug EERC stem 2 – 200mg/kg po **	6
	Total	18 Wistar albino rats

*Standard dose, **Test dose, po –per oral, Group 1 & 2 animals were shared for 6 animals each

Table 2: Showing the effect of Diclofenac, EERC 1 and EERC 2 on tail curl / flick (in sec) by Hot water tail immersion test.

Groups	Drugs	0 min	30 min	60 min	120 min
1	Distilled water	5.0 ± 0.566	4.60 ± 0.619	5.16 ± 0.61	5.08 ± 0.67
2	Diclofenac 10mg/kg po	5.83 ± 0.40	9.30 ± 0.48***	9.61 ± 1.16***	9.80 ± 1.12***
3	Test Dose EERC stem 1 – 100mg/kg po	5.33 ± 0.48	7.52 ± 1.22**	9.60 ± 1.17***	7.32 ± 0.62**
4	Test Dose EERC stem 2 – 200mg/kg po	5.60 ± 0.80	9.23 ± 1.22***	9.41 ± 1.20***	8.54 ± 1.42**

Values were considered statistically significant when, *P<0.05 – significant, **P<0.01 - very significant, ***P<0.001 - extremely significant, EERC – Ethanolic extract of *Rubia cordifolia*, po – per oral

Table 3: Showing the effect of Diclofenac, EERC 1 and EERC 2 on paw withdrawal time (in sec) by Eddy's Hot Plate method.

Groups	Drugs	0 min	30 min	60 min	120 min
1	Distilled water	6.13 ± 0.566	5.83 ± 0.618	6.0 ± 0.60	6.12 ± 0.66
2	Diclofenac 10mg/kg po	6.83 ± 0.40	10.23 ± 0.46***	10.41 ± 1.18***	10.75 ± 1.10***
3	Test Dose EERC stem 1 – 100mg/kg po	6.33 ± 0.48	8.41 ± 1.20**	8.64 ± 1.16**	9.10 ± 0.64**
4	Test Dose EERC stem 2 – 200mg/kg po	6.61 ± 0.80	9.83 ± 1.18**	10.25 ± 1.18***	10.33 ± 1.44***

Values were considered statistically significant when, *P<0.05 – significant, **P<0.01 - very significant, ***P<0.001 - extremely significant, EERC – Ethanolic extract of *Rubia cordifolia*, po – per oral

5. CONCLUSION

Traditional herbal remedies are integral to primary healthcare but require scientific validation. The study concluded that the ethanolic extract of *Rubia cordifolia* stem exhibits significant analgesic activity at doses of 100 mg/kg and 200 mg/kg, comparable to diclofenac, potentially providing a natural alternative to conventional pain relief medications. The findings highlight the therapeutic potential of EERC warranting further research into its mechanisms of action and possible clinical applications.

Limitations: However, this is a preliminary study, further clinical studies have to be carried out to assess the dose-related variation in the analgesic response, the active principle and the precise mechanism of action in charge of the analgesic activity. These models frequently focus on acute or nociceptive pain, ignoring the complexities of chronic or neuropathic pain observed in humans. Reflex-based evaluations fail to capture the emotional and psychological aspects of pain. Furthermore, high drug doses tolerated by rats can lead to inaccurate efficacy or toxicity predictions. Ethical concerns and variability in experimental conditions further adds to the limitations.

Acknowledgement

I thank all the staff members of the Department of Pharmacology, Animal House staff, Lab Assistants and workers for their constant support for successful completion of this study.

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Funding Information

No Funding was received for this research work.

Ethical Information

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Yenepoya Medical College (Approval No: YU/IAEC/04/2023). All experimental procedures were conducted in accordance with CCSEA guidelines for the care and use of laboratory animals.

References

1. Barrett KE, Barman SM, Brooks HL, Yuan J. Ganong's review of medical physiology. 26th ed. New York: Mcgraw-Hill Education; 2019. p292
2. Kurlekar PN, Bhatt JD. Study of the antinociceptive activity of fluoxetine and its interaction with morphine and naloxone in mice. *Indian journal of Pharmacology*. 2004; 36(6):369.
3. World health organisation. *Traditional, complementary and integrative medicine*. Available from: www.who.int/traditional-complementary-integrative-medicine/en/ [Accessed on: 12th June 2024]
4. Balandrin MF, Klocke JA, Wurtele ES, and Bollinger WH. Natural Plant Chemicals. *Sources of Industrial and Medicinal Materials. Science.* 1985;228:1154-1160.
5. Briskin, D. P. Medicinal Plants and Phytomedicines, Linking Plant Biochemistry and Physiology to Human Health. *American Society of Plant Physiology*. 2000;124:507-514.
6. Verma A, Kumar B, Alam P, Singh V, Gupta SK. Rubia cordifolia-a review on pharmacognosy and phytochemistry. *International Journal of Pharmaceutical Sciences and Research.* 2016;7(7):2720–31.
7. CPCSEA. Compendium of CPCSEA 2018. Committee for the Purpose of Control and Supervision of Experiments on Animal; p 157.
8. Shekhar TC , Bahuguna Y M , Singh Vijender: Anti-inflammatory activity of ethanolic stem extracts of *Rubia Cordifolia Linn.* in rats: *International Journal of*

Research in Ayurveda & Pharmacy. 2010;1(1):126-130.

9. Vogel HG. Analgesic, anti-inflammatory and anti-pyretic activity. In: drug discovery and evaluation: Pharmacological Assays. Vol 1.3rd ed. New York: Springer Verlag; 2008. p1013.
10. Gupta SK. Drug Screening Methods. 3rd edition. New Delhi: Jaypee brothers medical publishers Pvt Ltd; 2015. p476-94.
11. Mahajan BK. Methods in Biostatistics. New Delhi: Jaypee Brothers Medical Publishers Pvt Ltd; 2010. p138–47.
12. Pagano M, Gauvreau K. Principles of Biostatistics: Analysis of Variance. 2nd ed. Pacific Grove (CA): Duxbury; 2022. p279.
13. Yaksh TL, Woller SA, Ramachandran R, Sorkin LS. The search for novel analgesics: targets and mechanisms. *F1000prime reports.* 2015;7:56.
14. Marasini DR, Pandey JI, Sharma LP, Paudel LA, Gyawali RO, Rokaya RK, Giri PM, Khadka RB, Aryal PR, Bhandari RA. Analgesic activity of bark and leaves of *Ficus religiosa L.* from Nepal. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2020;12(7):32-5.
15. Bahadur S, Khatri D, Parajuli K. Antioxidant, Anti-Inflammatory, and Analgesic Activities of Aqueous Extract of *Diploknema butyracea* (Roxb.) H.J. Lam Bark. *The Scientific World Journal.* 2020:1–6.
16. Anandhalakshmi A, Madhav Rao C, Sarath Babu K. Preclinical evaluation of analgesic activity of hydroalcoholic extract of *Costus pictus* leaves in Wistar abino rats. *Natl J The National Journal of Physiology, Pharmacy and Pharmacology.* 2022;12(07):1079-1083.
17. Ghauri MA, Iqbal L, Raza A, Hayat U, Atif N, Javeed A. In vivo anti-inflammatory, antipyretic, analgesic activity and in vitro anti-proliferative activity of aqueous methanolic extract of Euphorbia granulata Forssk. *Future Journal of Pharmaceutical Sciences.* 2021;7(1).
18. Liu M, Wang H, Yue Q, Liu J. Effects of Ottonia anisum plant extract on local anesthetic, analgesic, anti-inflammatory and HCl-induced acute lung injury activities: a study in animal models. *Bioresources and Bioprocessing.* 2023;10(1).