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ANALYSIS OF THE CENTRAL AND PERIPHERAL MECHANISMS UNDERLYING THE ANALGESIC EFFECTS OF THE RUBIADIN

SOMADE PM[1*], CHOPADE AR[2] AND SOMADE PP[3]

1: Dept. of Physiology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India

2: Department of Pharmacology, Rajarambapu College of Pharmacy, Kasegaon, 415404, India

3: Intern, Krishna Institute of Medical Sciences, Karad, Maharashtra, India

***Corresponding Author: Dr. Prakash M. Somade: E Mail: saishaprakash@gmail.com**

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ABSTRACT

Aim and objective of the study:

The central and peripheral mechanisms underlying the analgesic effects of Rubiadin, a major isolated phyto constituent of *Rubia cordifolia* Linn, was evaluated in mice using the acetic acid-induced writhing and hot plate tests.

Materials and methods:

a) Writhing was induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg body wt.) and the number of muscular contractions were counted for 30 min following acetic acid injection with animals treated with Rubiadin.

b) The assay was performed according to the classical hot-plate technique of Eddy and Leimbach. The parameter evaluated was the latency time for paw licking and jumping responses after exposure to the hot plate surface.

Results:

Results showed that Rubiadin administered intraperitoneally can significantly attenuate acetic acid-induced writhing in mice in a dose-dependent manner. In the hot plate latency test, Rubiadin showed common activity in prolonging duration time and caused marked inhibition of acetic acid induced pain.

Conclusions: These findings of the current study imply the involvement of both peripheral and central antinociceptive mechanisms in observe analgesic potential of Rubiadin.

Keywords: Rubiadin, analgesic activity, writhing test & hot plate test

INTRODUCTION

Rubiadin, is a anthraquinone that has been isolated from the roots of *Rubia cordifolia* Linn (Family- Rubiaceae) **[1]**. *Rubia cordifolia* is an important medicinal plant which is used for treatment of wide ailments in Ayurvedic system of medicine **[2, 3]**. Rubiadin, isolated from the roots of *Rubia cordifolia* was found to have potent antioxidant property **[4]**. In addition, rubiadin also have been found to inhibit lipid peroxidation **[5]**. The plant *Rubia cordifolia* have been reported for anti-inflammatory **[6],** immunomodulatory **[7]**, anticonvulsant and anxiolytic **[8]** and anti-tumor activities **[9]**.

In a detailed study by Roa *et al*., indicates that rubiadin has a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats **[10]**. While the recent study by Shen *et al*., showed that a close correlation existed between chemical fingerprints with analgesic and anti-inflammatory activities, and alizarin, 6-hydroxyrubiadin, purpurin and rubiadin might be the active constituents of *Rubia cordifolia* L. extract **[11]**.

However, the mechanisms that underlying their analgesic effect still remains unclear. In the present study we therefore attempt to evaluate the central and peripheral mechanisms underlying the analgesic effects of Rubiadin in mice using the acetic acid-induced writhing and hot plate tests.

MATERIALS AND METHODS

Plant material

The Rubiadin [1,3-dihydroxy-2-methylanthracene-9,10-dione] purchased (Product code: R004, Lot. no. : T19D079; CAS No: 117-02-2) from Natural Remedies Pvt. Ltd., Bangalore. Purity of Rubiadin was determined by the manufacturer by HPLC area normalization and was certified above 94.80%.

Experimental Animals

Swiss albino mice (25-30 g) were used for the study. The groups of animals (n = 4) animals were maintained under standard environmental conditions and were fed with standard diet and water ad libitum. Twenty-four hours before the experiments, they had access only to water ad libitum. A prior approval [Approval number-RCP/18-19/P-20] was obtained from the Animal Ethics Committee of Rajarambapu College of Pharmacy, Kasegaon for the study. RCPK is registered under Committee for the Purpose of Control and Supervision

of Experiment on Animals (CPCSEA), Govt. of India.

Acetic acid-induced writhing test [12-13]

The assay was performed according to the classical technique of Koster *et al*. with slight modifications. Writhing was induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg body wt.) into a group of 4 mice. Animals were treated with Rubiadin at dose of 50, 100 & 200 mg/kg, i.p. 10 -15 min before the injection of 0.6% acetic acid. Control animals received a similar volume of vehicle (DMSO). Aceclofenac was used as positive control and administered intraperitoneally at a dose of 30 mg/kg. The number of muscular contractions was counted for 30 min following acetic acid injection. Data represent the average of the total number of writhes observed for 30 minutes are expressed as mean ± SEM (standard error of mean).

Hot plate test [14-15]

The assay was performed according to the classical hot-plate technique of Eddy and Leimbach the method described previously with little modification. The parameter evaluated was the latency time for paw licking and jumping responses after exposure to the hot plate surface. The hot plate temperature was maintained at 55 ± 1 ºC. The animal was kept on the hot plate until it lifted one of its hind paws. The response was determined over 150 min after the injection of the Rubiadin and the data represent the mean reaction time for the animals. Latency time was recorded and the results are expressed as the hot plate analgesic index.

The Rubiadin was administered intraperitoneally at doses of 50, 100 & 200 mg/kg body wt. The standard (Pentazocine lactate, 10 mg/kg body wt.) was administered to by intraperitoneal route. The latency time for paw licking was recorded as pain threshold when mice were exposed to the hot plate surface which is kept at 55 ± 1 °C. Basic pain threshold was measured, then all treatments were given 10-15 min before the thermal stimulus and the response was determined at 30, 60, 120 and 180 min. Pain threshold inhibition (%) = (Pt − P0) × 100/P0, P0 and Pt separately presents basic pain threshold and pain threshold at time interval.

Statistical analysis

All the Statistical calculation were performed using Graphpad Prism software version 6.01, © 1992-2012. Results are presented as means + SEM (standard error of mean). Data was analyzed statistically using Dunnett's Multiple Comparison test, with the level of significance set at $p < 0.05$.

RESULTS

Writhing test

In the writhing test, which is more sensitive for non-steroidal analgesics, the effect of Rubiadin was significant at studied doses as compared to the control and standard. The intraperitoneal injection of Rubiadin reduced, the number of stretching episodes induced by the intraperitoneal injection of acetic acid (0.6%). **Figure 1** shows the effect of the Rubiadin on acetic acid-induced writhing in mice. The administration of Rubiadin significantly inhibited writhing response in a dose dependent manner. Rubiadin produced 80.00 %, 86.857 % and 89.143 %, inhibition of writhing at dose of 50, 100 and 200 mg/kg respectively. Whereas the standard drug acelofenac produced 96.00% inhibition of writhing.

Hot plate test

The intraperitoneal injection of Rubiadin increased the reaction time of the mouse to the hot plate painful stimulus **(Figure 2)**. Rubiadin showed maximum analgesic activity at for 200 mg/kg dose. The reaction time (paw licking / jumping response) in rats pretreated with Rubiadin (50, 100 and 200 mg/kg) and Pentazocine (10 mg/kg) were found to be highly elevated, when compared to the control group rats. The duration of analgesic effect was significant as compared to the control and reference drug pentazocine which significantly increased the reaction time at each time point of testing. Effect of Rubiadin expressed as the % inhibition threshold of pain response to heat stimuli induced by hot plate is summarized in **Figure 3**.

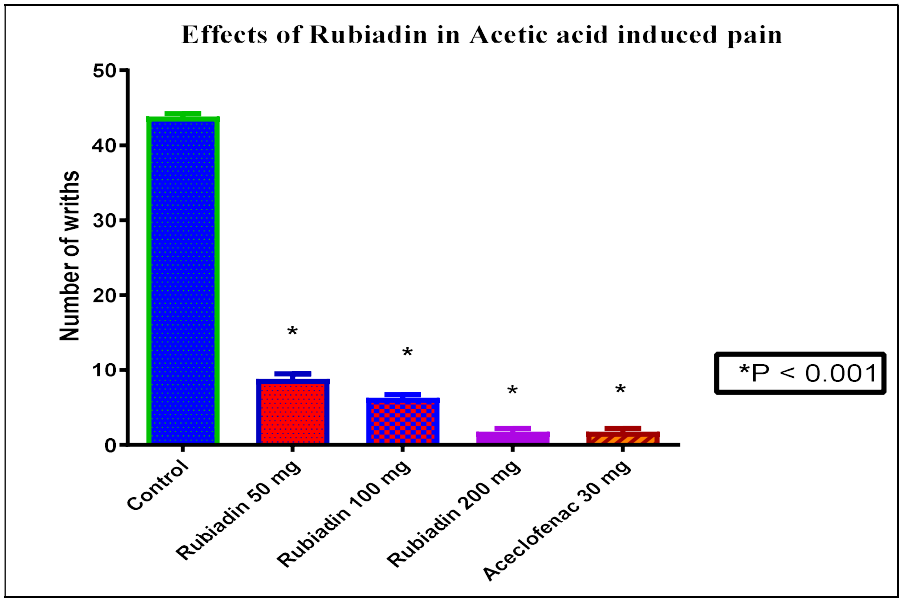

Figure 1: Peripheral analgesic effect of Rubiadin

Effect of Rubiadin expressed as number of wriths induced by intraperitoneal administration of a 0.6% acetic acid solution in mice (n = 5). Negative Control was DMSO (vehicle) and positive control was aceclofenac. The results are shown as mean ± SEM of writhing movements at 30 minutes and a significant difference from the control group is shown as *P < 0.001

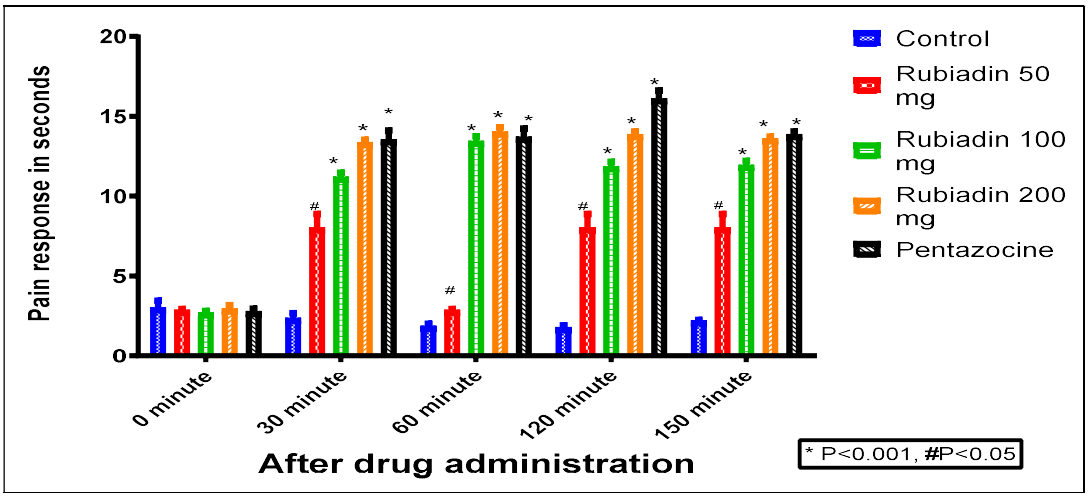

Figure 2: Central nervous system analgesic effect of Rubiadin, Effect of Rubiadin expressed as pain response in seconds induced by hot plate in mice (n = 5). The results are shown as mean ± SEM and a significant difference from the control group is shown as *P < 0.001; # P<0.05

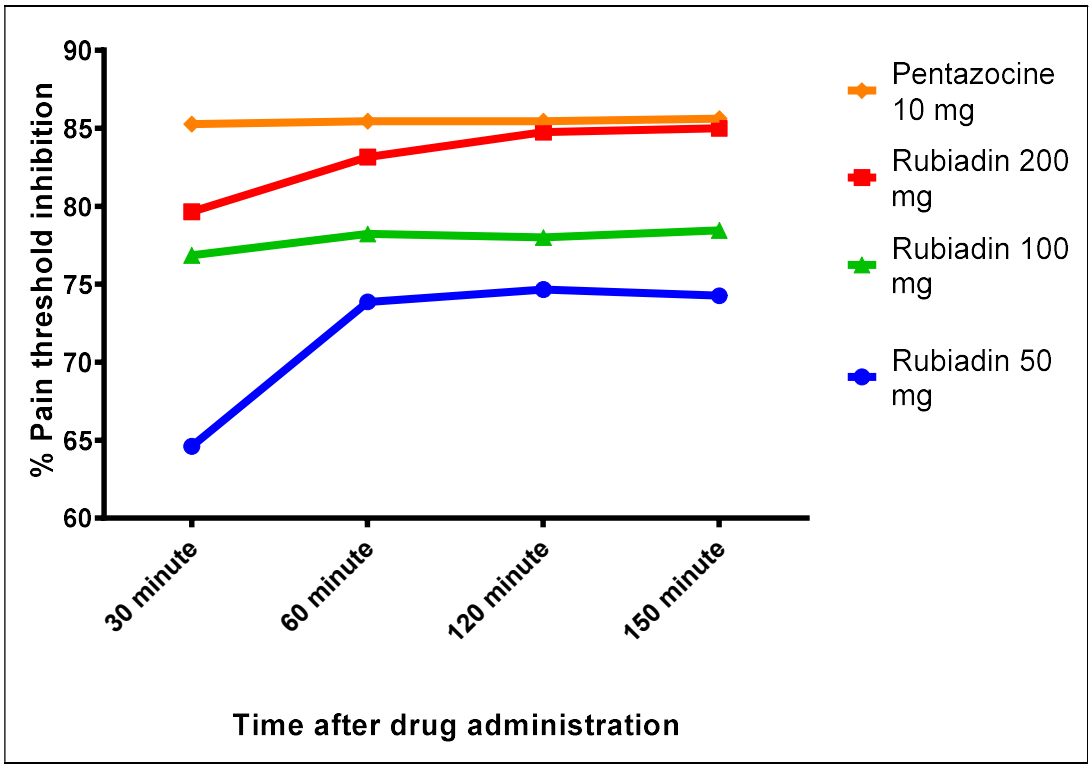

Figure 3: % Pain threshold inhibition of Rubiadin in hot plate test, Effect of Rubiadin expressed as the % inhibition threshold induced by hot plate in mice (n =5). The results are shown as average mean inhibition at that particular time point

DISCUSSION

The previously reported results show that the active principles present in *Rubia cordifolia* L. extract, exhibit potent and long-lasting systemic analgesic effect when analyzed in various models of inflammation and pain **[11]**. By both hot plate and writhing tests, we showed that the Rubiadin has marked analgesic properties.

Peripheral mechanisms of Rubiadin: Deraedt *et al*. have described the quantification of prostaglandins in the peritoneal exudates of rats by radioimmunoassay, obtained after intraperitoneal injection of acetic acid **[16]**. They found high levels of prostaglandins PGE2α e PGF2α during the first 30 min after acetic acid injection. Nevertheless, it was found that intraperitoneal administration of acetic acid induces not only the liberation of prostaglandins, but also the liberation of sympathetic nervous system mediators [**16-17]**. Therefore, we may state that anti-inflammatory substances may also be involved in peripheral analgesic activity. Rubiadin inhibited acetic acid induced writhing in mice. It can therefore be suggested that the analgesic effect of the extract is peripherally mediated. Rubiadin produced a reduction in the number of writhes at all the tested three doses in present study, but the most significant effect was obtained at the dose of 200 mg/kg body wt. **(Figure 1)**.

Central mechanisms of Rubiadin: The other algesimetric test used was hot plate test that reveals the central analgesic response. The hot plate model has been used to study centrally acting analgesics. In this model, sensory nerves sensitize the nociceptors and the involvement of endogenous substances such as prostaglandins are minimized **[18, 19]**. The Rubiadin showed a significant increase in the pain threshold, corresponding to a significant increase in the percentage of protection, as compared to the control group and the pentazocine standard **(Figure 2)**.

From the results, it is clear that analgesic effect of the Rubiadin was significant by both measures (in the acetic acid-induced model and hot plate model). Therefore, the analgesic effect of the Rubiadin may possess dual mechanisms, bearing both peripheral and central analgesic properties.

Overall, it can be concluded that the Rubiadin possesses central and peripheral analgesic activity, probably mediated by the inhibition of prostaglandin synthesis, as well as by central inhibitory mechanisms.

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Conflicts of interest- We all the authors declare no conflict of interest.

REFERENCES

[1] Nilambari Deshkar, Shrikant Tilloo, and Vipinchandra Pande, A Comprehensive Review of Rubia Cordifolia Linn Pharmacognosy Review, 2008,2,3,124-134.

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

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

[4] Tripathi, Y.B., Sharma, M.K., Manickam, M., Rubiadin, a new antioxidant from Rubia cordifolia. Indian Journal of Biochemistry and Biophysics 1997.34, 302–306.

[5] Tripathi, Y.B., Sharma, M., Comparison of the antioxidant action of the alcoholic extract of Rubia cordifolia with rubiadin. Indian Journal of Biochemistry and Biophysics 1998.35, 313–316.

[6] Antarkar, D.S., Chinwala, T., Bhatt, N., 1983. Anti-inflammatory activity of Rubia cordifolia Linn. in rats. Indian Journal of Pharmacology 15, 185–188.

[7] Joharapurkar, A.A., Deode, N.M., Zambad, S.P., Umathe, S.N., 2003.Immunomodulatory activity of alcoholic extract of Rubia cordifolia Linn. Indian Drugs 40, 179–181.

[8] Kasture, V.S., Desmukh, V.K., Chopde, C.T., 2000. Anticonvulsant and behavioral actions of triterpine isolated from Rubia cordifolia Linn. Indian Journal of Experimental Biology 38, 675–680.

[9] Adwankar, M.K., Chitnis, M.P., Khandalekar, D.D., Bhadsavale, C.G., 1980. Anti-cancer activity of the extracts of Rubia cordifolia Linn. Indian Journal of Experimental Biology 18, 102–106.

[10] Guntupalli M. Mohana Rao, Chandana V. Rao, Palpu Pushpangadan, Annie Shirwaikar, Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. Journal of Ethnopharmacology, 2006, 103, 484–490.

[11] Shen C, Liu C, Song X, Zeng W, Lu Z, Zheng Z, Zhan R, Yan P, Evaluation of analgesic and anti-inflammatory activities of Rubia cordifolia L. by spectrum-effect relationships, J Chromatogr B Analyt Technol Biomed Life Sci. 2018; 1090:73-80.

[12] Koster R., Anderson M., De Beer E.J., Acetic acid for analgesic screening, Fed. Proc. 1959, 18, 412.

[13] Chopade A R, Sayyad F J. 2013. Analysis of the central and peripheral mechanisms underlying the analgesic effects of the extracts of *Phyllanthus amarus* & *Phyllanthus fraternus*. Asian J. Pharm. Res., 3(1), 10-14.

[14] Eddy, N.B., Leimbach, D., Synthetic analgesics II. Dithienylbutenyl and dithienylbutylamines, J. Pharmacol. Exp. Ther. 1953, 45, 339.

[15] Ghosh MN. Fundamentals of Experimental Pharmacology. 2 nd rev. ed. Calcutta: Scientific Book Agency; 1984. p. 144-58.

[16] Deraedt R, Jouquey S, Delevallee F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol., 1980, 61: 17–24

[17] Duarte JDG, Nakamura M, Ferreira SH., Participation of the sympathetic system in acetic acid induced writhing in mice. Brazilian J Med Res., 1988, 21: 341–343.

[18] Ranadran K, Basinath L. A critical Analysis of the Experimental Evaluation of Nociceptive reactions in Animals. Pharm Research. 1986; 3: 253–270.

[19] Woolfe G, MacDonald AD. The evaluation of analgesic action pethidine hydrochloride. J Pharmacol Exp Ther. 1994; 80: 300.