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Effect of Riboflavin Supplementation on Anti-Nociceptive Effect of Diclofenac in Animal Models of Acute Pain

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ABSTRACT

The influence of Riboflavin supplementation (50mg/kg and 100mg/kg) on anti-nociceptive effect of Diclofenac is assessed in acute pain models of Albino Mice. Reaction latency on hot plate, Tail Flick Latency in immersion test and Licking time in early and late phase after subcutaneous Formalin injection were observed before and after the administration of diclofenac alone and in combination with two doses of Riboflavin. There is 64.85% increase in reaction time in diclofenac treated group. 68.66% and 75.78% increase in 50 mg/kg and 100 mg/kg Riboflavin supplemented Diclofenac administered groups respectively. The percentage inhibition in Tail Flick Latency is almost same in all the three groups treated. The addition of high dose (100mg/kg) of Riboflavin to Diclofenac significantly decreased the licking time in late phase of Formalin injection. The study revealed that there was no increase in the reaction latency on Hot Plate and Immersion Test but there is significant decrease in licking time in late phase of the Formalin tests Riboflavin supplementation with Diclofenac is not able to ameliorate central pathway mediated nociception but having role in inflammatory induced nociception.

INTRODUCTION

Nociception or pain perception is the response of sensory nervous system to noxious stimuli. This includes sensory detection, transduction, and neural transmission of noxious events to the central nervous system by the stimulation of sensory cells called nociceptors[1]. Nociception triggers a variety of physiological and behavioural responses and usually results in a subjective experience of pain in reactive organisms [2]. The basic science of pain research and the preclinical research on pain treatments essentially depends on a battery of tests available to study nociception employing naive animals. The concerned tests that use thermal stimulation include the tail flick test, the hot or cold plate tests and the radiant heat paw-withdrawal test. Nociceptive tests can also rely on the stimulus threshold necessary to elicit an avoidance behaviour.

Vitamins are well known for their supplementation in deficiency states but some of the vitamins themselves have other preventive and therapeutic potential [3-5]. Vitamin C [6], Vitamin D [7] and B complex vitamins [8,9] have a primary or supplementary role in attenuating pain in preclinical and clinical models. Riboflavin from this group is effective in Migraine prophylaxis [10] and has a direct and indirect anti-inflammatory activity through vitamin D metabolism. It exacerbates the anti-nociceptive effect of morphine [11,12]. But Riboflavin effect in acute pain models and its possible potentiation effect on anti-nociceptive action of NSAIDs have not been reported. So, it was thought prudent to carry out an experimental evaluation of possible potentiation of analgesic effect of Diclofenac by Riboflavin supplementation in acute pain models in albino mice.

MATERIALS AND METHODS

Chemicals and Drugs

Diclofenac sodium (Gift sample from NATCO), Riboflavin (Chemzone Pharma), Formalin (S.D. fine chemicals, India).

Animals

Swiss albino male mice weighing 20–24 g was used in this study. They were housed individually in polypropylene cages containing sterile paddy husk as bedding throughout the experiment. They were given standard pellet diet and water *ad libitum.* The mice were maintained under standard condition at temperature of 25 ± 10℃ , 60 ± 5% relative humidity

and 12 hours light dark cycle. A gap of 1 week was kept for acclimatization of animals. All experiments were carried out according to the guidelines for the care of laboratory animals. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of RBVRR Women's College of Pharmacy, Hyderabad.

Experimental Design

Animals were divided into four groups each having six animals. All the test drugs are dissolved in normal saline and administered through oral route. One hour after the administration of drugs, tests for nociception should be carried out.

Group I: Control animals (Normal saline)

Group II: Diclofenac (10 mg/kg in Saline)

Group III: Diclofenac + Riboflavin (50 mg/kg in saline)

Group IV: Diclofenac + Riboflavin (100 mg/kg in saline)

Eddy's Hot Plate Test

The temperature of the hot plate was set at 55 ± 0.5°C. The mice that showed fore paw licking, withdrawal of the paw(s), or a jumping response within 15s on the hot plate were selected for this study 24 hours prior to the experiment, and the pre-treatment latency was recorded. The animals were treated orally with respective drugs as per the experimental design. Sixty minutes after the oral administration, the reaction time was again recorded. A cut off time of 15s was used to avoid damage to the paw. The percentage increase or decrease in reaction time (as index of analgesia) at each time was calculated [13].

$$\text{Percentage increase in reaction time} = \frac{Rt - R0}{Rt} X100$$

Rt = Average reaction latency after treatment

R_0 = Average reaction latency before treatment

Tail Immersion Test

The lower 5 cm of the mouse tail was immersed in warm water kept constant at 55 ± 0.5°C. The latency between tail submersion and deflection of the tail was recorded. The pre-

treatment latency was recorded 24 hours prior to the experiment. The animals were treated orally with the test drugs as per the experimental design. Sixty minutes after oral administration, the reaction time was again recorded. A cut-off time of 15 seconds was used to avoid tail tissue damage in mice [14].

$$\text{Percent Inhibition} = \frac{\text{Post treatment latency} - \text{pretrearment latecy}}{\text{Cutoff time} - \text{pretreatment latency}}$$

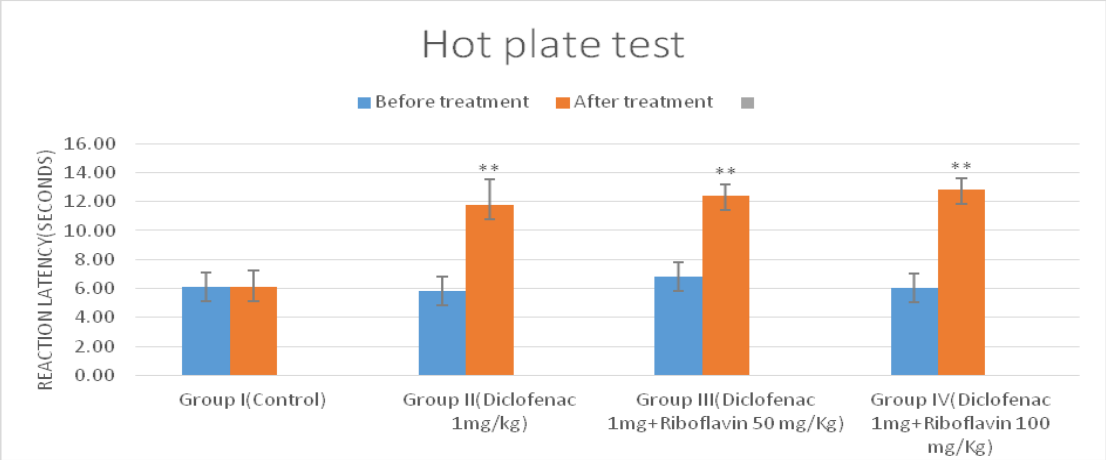
Formalin Test

Thirty minutes after treatment with standard and test drugs, 20 µl of 1% formalin was injected subcutaneously under dorsal surface of the hind paw and the time spent for licking the paw injected with formalin was counted for 30 min post formalin injection and considered as indicative of the pain stimuli. The formalin test has two distinctive phases possibly reflecting different types of pain. The first phase peaked at 5 min and the second phase at 20-30 min after formalin injection. This represented neurogenic and inflammatory responses, respectively [15].

RESULTS

Eddy's Hot Plate Test

The reaction time on Hot Plate is increased significantly in Diclofenac treated animals when compared to their baseline values. Both the doses of riboflavin supplementation along with Diclofenac significantly increased the reaction latency of animals on Eddy's Hot Plate in a dose dependent manner. But this increase is not significant compared to the exclusive diclofenac administration. There is 64.85% increase in reaction time in diclofenac treated group. 68.66% and 75.78% increase in 50 mg/kg and 100 mg/kg riboflavin supplemented diclofenac administered groups respectively (Figure No.1).

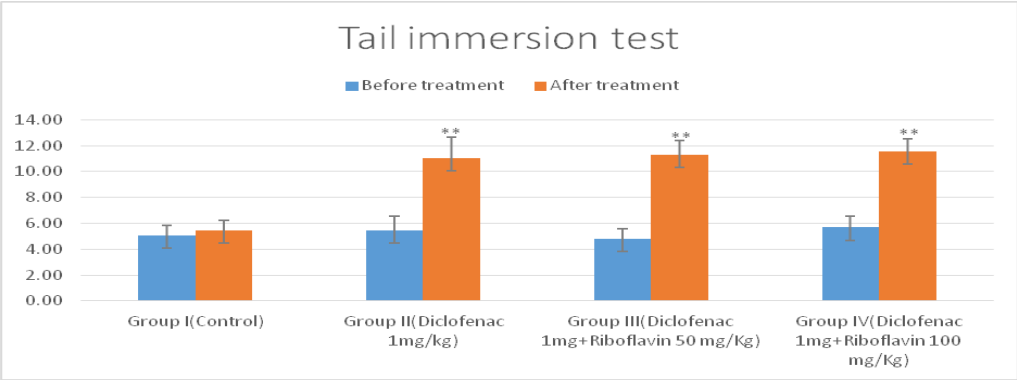


Given values are Mean ± SEM. * P < 0.05 ** P < 0.01 compared to Control group and baseline values (before treatment).

Figure No. 1: Effect of Riboflavin Supplementation on Reaction Latency of Diclofenac Treated Mice on Eddy's Hot plate

Tail Immersion Test

In tail flick latency test treatment with Diclofenac increased the tail flick latency compared to control group but the Riboflavin supplementation in low and high doses doesn't increase the tail flick latency beyond exclusive Diclofenac administration. The % inhibition in tail flick latency is almost same in all the three groups treated. It was 58.68%, 63.63% and 62.88% respectively in exclusively Diclofenac treated group, Diclofenac and 50mg of Riboflavin treated group and Diclofenac and 100 mg of Riboflavin treated group (Figure No.2).

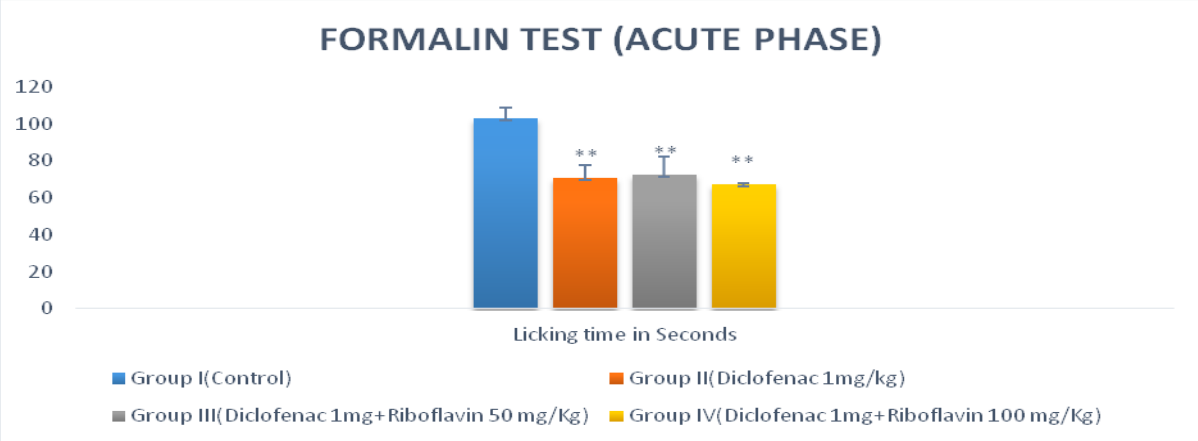


Given values are Mean ± SEM. * P < 0.05 ** P < 0.01 compared to control group and baseline values (before treatment).

Figure No. 2: Effect of riboflavin supplementation on Tail Flick Latency of Diclofenac Treated Mice in Hot Immersion Test

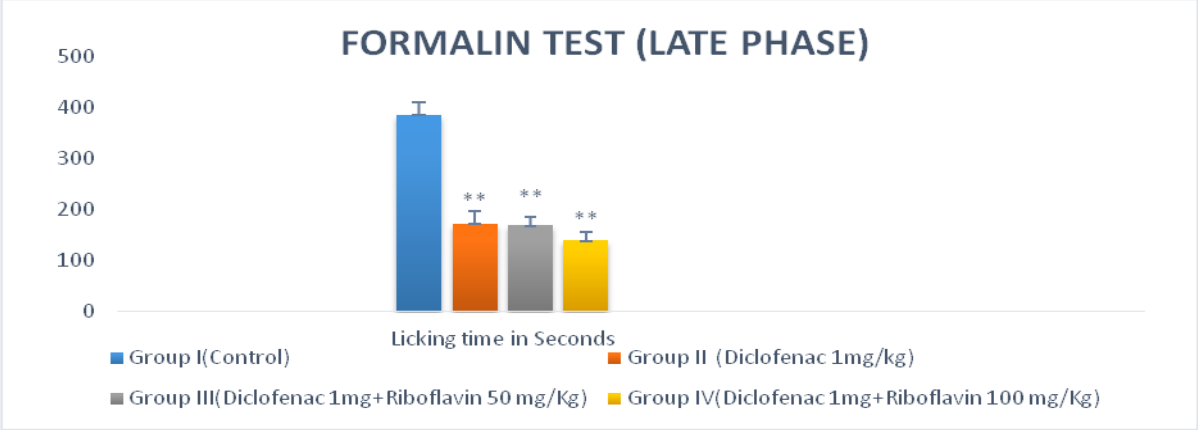
Formalin Test

As shown in Figure No.3 a, the evaluation of the licking response in the acute phase of the formalin test showed decreased licking time in comparison with the control groups. There was a significant difference between control and test groups. But the addition of riboflavin (50 mg/kg and 100 mg/kg) doesn't significantly alter the licking time. In the chronic phase also, there is a significant decrease in the licking response in all the test groups compared to control animals. The addition of high dose (100mg/kg) of Riboflavin to Diclofenac significantly decreased the licking time in late phase.



Given values are Mean ± SEM. * P < 0.05 ** P<0.01 compared to Control group.

Figure No. 3a: Effect of Riboflavin Supplementation on Licking Time in Acute Phase of Formalin Test in Diclofenac Treated Mice



Given values are Mean ± SEM. * P < 0.05 ** P < 0.01 compared to Control group.

Figure No. 3 b: Effect of Riboflavin Supplementation on Licking Time in Late Phase of Formalin Test in Diclofenac Treated Mice

DISCUSSION

Riboflavin or vitamin B2 makes up a part of the vitamin B group, which in recent studies shows a growing implication in the treatment of some pathology that imply pain management. Anti-nociceptive and anti-inflammatory activities of riboflavin in different experimental models have been studied [16]. Hot Plate test is supposed to have supraspinal integrated responses. Diclofenac increased the reaction latency on hot plate. The mechanism of anti-nociception induced by Diclofenac involves an activation of α2-adrenoceptors at spinal and supraspinal levels [17]. It is known that the tail immersion/tail-flick response appears to be a spinal reflex, and is regarded as a specific screening method for centrally acting analgesics. Increased reaction time in the tail flick test by Diclofenac is consistent with the interpretation that its analgesic property might have a central origin [18]. But the addition of Riboflavin in either of the doses hasn't significantly increased the reaction latency on Hot Plate and Tail Flick latency in Hot Immersion Test in Diclofenac treated animals. As Diclofenac has central analgesic action along with peripheral nociceptive action [19]. It increased the pain threshold of the animals on Hot Plate and Hot Water Immersion. But the addition of Riboflavin has not shown any significant improvement in pain threshold in both the tests indicating that it might not possessing significant role at the level of spinal and supraspinal nociceptive pathways. In late phase of Formalin test the addition of Riboflavin in high dose significantly decreased the licking time. So, supplementation of Riboflavin to Diclofenac can involve inhibition of the synthesis and/or action of inflammatory mediators since it was not observed in either Hot Plate model or Tail Immersion. Demonstrated ATP modulation by Riboflavin can also be attributed to the anti-nociceptive action. Further it can be confirmed by assessing its potential in the treatment of different painful inflammatory models.

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