

Original Article

Combined Antinociceptive Effects of Alpha-Tocopherol and Diclofenac in Acetic Acid-Induced Writhing Test

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Abstract

Background: The search for effective analgesic combinations with improved efficacy and fewer side effects is an ongoing challenge in pain management. **Objective:** The purpose of the present study was to determine the combined analgesic efficacy of alpha-tocopherol, a potent antioxidant, and diclofenac, a widely used nonsteroidal anti-inflammatory drug (NSAID), using the acetic acid-induced writhing test in rats. **Methodology:** This prospective experimental study was conducted in the Department of Physiology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from January to December 2013. 15 male Long Evans rats, weighing 180 to 250 grams, were collected from Bangladesh Institute of Research and Rehabilitation for Diabetic Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka, Bangladesh for this purpose. On the basis of vitamin and drug administrations, the rats were divided into three 3 groups (5 rats in each). Group A received normal saline, group B received diclofenac sodium, group C received combination of diclofenac sodium with α -tocopherol. All the groups received single dose and equal volume through intraperitoneal route 1 hour before the test. Just one hour after administrations, rats of each group were subjected to the acetic acid induced writhing test. After the completion of experiments, all the rats were sacrificed immediately to decrease their sufferings. **Results:** This study suggests that combining α T and DS in a single dose may be more beneficial than administering DS alone in reducing nociceptive and inflammatory pain. **Conclusion:** The results indicate a synergistic interaction between the two compounds, suggesting a potential role for alpha-tocopherol as an adjuvant in pain therapy.

Keywords: Pain; analgesic α -tocopherol; writhing test

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Introduction

Pain is a complex physiological response, often associated with inflammation and oxidative stress. It is an unpleasant emotional and sensory experience¹. The most frequent reason for visiting a doctor is pain and suffering, which is consequently widespread. In a variety of medical

conditions, pain is a common presenting sign and is linked to acute tissue damage, disease, or intervention^{2,3}. Vital physiological sensation nociceptive pain is brought on by stimulation of peripheral nerve fibers. Nociceptors have different thresholds or sensitivities. Which acts as a crucial tissue damage early warning system. Nociceptive pain can

be mechanical, thermal, chemical, or electrical³⁻⁴. Diclofenac, a cyclooxygenase (COX) inhibitor, is effective in managing inflammatory pain but is associated with gastrointestinal and cardiovascular side effects^{5,6}. α -tocopherol is a fat-soluble vitamin and widely known to be one of the reactive oxygen species (ROS) scavengers and a drug that has been shown to reduce the pain responses induced by various causes in animal pain models and also in human it has shown promise as an antioxidant with anti-inflammatory properties⁷⁻⁹.

To detect the severity of pain, its measurement is necessary. This study aims to evaluate the antinociceptive effects of alpha-tocopherol in combination with diclofenac sodium using the acetic acid-induced writhing test in rats, a well-established model for peripheral nociception. This test is sensitive to opiates as well as non-opiate analgesics¹⁰. However, there is not enough information currently available to make any definitive judgments on this topic. It has not been reported that the analgesic effects of DS and α T together are comparable to that of individual administration of DS on nociceptive pain.

Methodology

Study Settings and Population: This prospective experimental study was conducted at Bangabandhu Sheikh Mujib Medical University (BSMMU) in the Pain Laboratory of the Department of Physiology between January 2013 to December 2013. Fifteen (15) apparently healthy adult male Long Evans rats weighing 180-250g were collected from Bangladesh Institute of Research and Rehabilitation for Diabetic Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka, Bangladesh.

Study Procedure: It was 12/12 light/dark for the rats. The rats' thermo-neutral zone was regulated between 27 and 28°C. All rats had free access to a conventional laboratory diet and cooled boiled water. They were held there for seven (7) days before the trial to acclimate. To avoid circadian effects, all trials were conducted between 08:00 and 16:00 hours¹¹.

Allocation: On the basis of vitamin and drug administrations, all the rats were divided into three (3) groups (5 rats in each). The control group received normal saline at a dose of 5ml/kg body weight, one experimental group received diclofenac sodium at a dose of 10mg/kg body weight, and another experimental group received DS and α T at doses of 10mg/kg body weight and 500mg/kg body weight, respectively. 1 hour before the test, each group got a single dosage of the same volume (1ml)

administered intraperitoneally. They were treated to an acetic acid induced writhing test just one (1) hour after injection. All the groups received single dose and equal volume through intraperitoneal route. Pain assessment was done by Writhing test^{12,13}.

Follow Up and Outcome Measures: On the day of experiment, the rats were placed in a plexiglas observation chamber (30× 30× 20 cm³) for acclimatization. Then all the rats were administered by normal saline or DS or α T or combined dose of DS and α T intraperitoneally. One hour after administrations, 1ml of 2% acetic acid was injected intraperitoneally using a insulin syringe. Immediately after the injection, the latency time and the number of writhes were counted upto 60 minutes of observation period. Finally % analgesic activity was calculated by using following formula^{14,15}.

$$\% \text{ Analgesic activity} = \frac{\text{Mean writhing count (control group) - treated group}}{\text{Mean writhing count of control group}} \times 100$$



Figure 1: Writhing test showing writhing response.

Latency Time: Period between acetic acid administration and the onset of the first writhing.

Writhing: Abdominal muscle contraction followed by hind limb extension.

Statistical Analysis: The results are expressed as mean standard error of the mean, and the data were evaluated statistically using ANOVA and the Bonferroni's post hoc test. The level of significance for interpreting the results was determined to be $p \leq 0.05$.

Ethical Clearance: All experiments and animal care were conducted under the guidelines established in the 'Manual for the Care and Use of Laboratory Animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b 2002) and was approved by the BSMMU's institutional review board (IRB).

Results

Latency time in writhing test: The results were expressed as mean±SE and the data were statistically analyzed by Independent Student test. In the interpretation of results p 0.05 was accepted, as the level of significant. The mean±SE of duration of latency time in acetic acid induced writhing test after administration of vitamin and drug were 5.5 ± 0.63, 11.2 ± 0.34, and 14.6±0.43 minutes in group A, B, and C, respectively. The percent increments of this variable were 113.12 ± 21.03, 49.73± and 178.85 ± 30.46 in group B, and C, respectively in comparison to that of control. In this study, the differences of the mean values of among the groups were statistically significant (p≤0.05). Again, the mean values as well as percent increments were significantly higher (p≤0.05) in all the study groups than that of control. Moreover, the value of group C was significantly (p≤ 0.001) higher than that of group B.

Table 1: Mean Latency Time in Writhing Test in Different Groups of Rats (n=20)

Groups	n	Latency Time	
		Minutes	% Increment
A	5	5.5 ± 0.63 (4 to 7)	-
B	5	11.2 ± 0.34 (10 to 12)	113 ± 21.03 (64.29 to 175)
C	5	14.6 ± 0.43 (13 to 15.5)	178.85 ± 30.46 (114.29 to 275)

Data were expressed as mean±SE. Figures in parentheses indicate ranges. Percent (%) change was done in comparison to control (A); Statistical analysis was done by ANOVA followed by Bonferroni’s Post Hoc test; Group A: with Normal Saline (control); Group B: with Diclofenac Sodium (10 mg/kg); Group C: with Diclofenac Sodium (10 mg/kg) + α-tocopherol (500 mg/kg); ***=significant (p≤ 0.001); **=significant (p≤0.01); n=number of rats.

Table 2: Comparison of Different Groups

Groups	P value
A vs B vs C	0.000***
A vs B	0.000***
A vs C	0.000***
B vs C	0.000***

Number of Writhes in Writhing Test: In this study, the mean±SE number of writhes in the writhing test were 71.8 ± 2.92, 33.6 ± 0.5, and 24.6 ± 1.07 Frequency / 60 minutes in group A, B, and C, respectively. The percentages of analgesic activity were 0, 53.2, and 65.73 % in group A, B, and C respectively. In addition, the percent reductions of this variable were -52.97 ± 1.57 %, -18.69 and -65.43 ± 2.4 % in group B, and C, respectively, in comparison to that of control. The differences of this mean value among the groups were statistically significant (p≤0.001). Again, the mean values as well as the percent reductions were significantly (p≤0.01) lower in all the study groups than that of the control. Moreover, the value of group B was significantly (p≤0.001) lower than that of group D.

Table 3: Mean Writhing Value in Different Groups of Rats (n=20)

Groups	n	Number of Writhes Frequency /60 min	%Analgesic activity	% Reduction
A	5	71.8 ± 2.92 (67 to 83)	0	-
B	5	33.6 ± 0.5 (32 to 35)	53.2	- 52.97 ± 1.57 (-59.04 to -50.72)
C	5	24.6 ± 1.07 (22 to 28)	65.73	- 65.43 ± 2.4 (-71.08 to -58.21)

Data were expressed as mean±SE. Figures in parentheses indicate ranges. Percent (%) change was done in comparison to control (A); Statistical analysis was done by ANOVA followed by Bonferroni’s Post Hoc test; Group A: with Normal Saline (control); Group B: with Diclofenac Sodium (10 mg/kg); Group C: with Diclofenac Sodium (10 mg/kg) + α-tocopherol (500 mg/kg); ***=significant (p≤0.001); **=significant (p≤ 0.01); n=number of rats.

Table 4: Comparison of Different Groups

Groups	P value
A vs B vs C	0.000***
A vs B	0.006**
A vs C	0.000***
B vs C	0.000***

Discussion

Pain is a complex, multidimensional perception. The severity of pain does not correlate with the degree of tissue damage and if not managed properly it affects a person’s quality of life.16 Many studies have beenfound throughout the world to replace or at least to reduce the dose or duration

of drugs therapy, by intervening alternate pain medications. Good analgesic effect with this vitamin (500mg/kg) as a single dose in rats is found and it has been reported that toxic dietary level of this vitamin is more than 16g/kg¹⁷. Moreover, it has also been reported that, 10 mg/kg diclofenac might be the optimal dose to get effective response against nociceptive and inflammatory pain as well as inflammation in rat models¹⁷⁻¹⁸.

Now a day's administration of combination of analgesics with antioxidants in pain treatment are applied to decrease the doses of analgesics and to prevent the negative impact of reactive oxygen species¹⁹. But still the information's regarding this matter is not enough to reach any final conclusion. Many experimental studies have shown analgesic and anti-inflammatory effects of α T. Significant reduction of the nociceptive pain was found after supplementation of this vitamin in rats²⁰⁻²¹. Investigators from different countries were also reported these types of findings in rats and mice²²⁻²⁴.

In this study, combined administration of α T and DS have shown more effectiveness in lowering pain and inflammation than individual intervention of DS, as evidenced by more decrements of all the study variables. Though the mechanisms involve for lowering the pain and inflammation cannot be explained exactly. This may be due to activation of different pain lowering pathway by α T and DS as both the mechanisms are involved and added together and causes more effectiveness²⁵.

Conclusion

The combination of alpha-tocopherol and diclofenac demonstrates significant synergistic antinociceptive effects in the acetic acid-induced writhing test. Further studies are needed to explore the molecular mechanisms underlying this interaction and its potential clinical applications in pain management.

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