Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

Original Research ISSN: 3060-7663 Doi: 10.22103/VCBR.2025.24547.1032

Evaluation of the Combined Analgesic Effect of Diphenhydramine and Ketoprofen Compared to Morphine in Mice

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Article history:

Received: 04 December 2024
Revised: 26 January 2025
Accepted: 02 February 2025
Published: 08 February 2025

Keywords:

Analgesic effect
Diphenhydramine
Ketoprofen
Morphine
Mice

Abstract Morphine is a significant and effective pain relief medication that has been of interest for a long time. However, the rapid onset of tolerance and dependence on morphine and other opioids remains a critical limiting factor in their use. Numerous studies have investigated the mechanisms involved in opioid dependence, leading researchers to focus on alternative medications with fewer side effects. The aim of the present study was to investigate the combined analgesic effects of diphenhydramine and ketoprofen compared to morphine in mice. Thirty mice were randomly divided into five groups of six and analgesia was induced through intra-peritoneal injection as follow, control group (normal saline), standard group receiving 10 mg/kg morphine, treatment group 1 receiving 2.5 mg/kg diphenhydramine, treatment group 2 receiving 2.5 mg/kg ketoprofen, and treatment group 3 receiving 2.5 mg/kg diphenhydramine plus 2.5 mg/kg ketoprofen. Following injection, pain assessment was conducted using the hot plate test and formalin tests. The results indicated that the combined use of ketoprofen and diphenhydramine exhibited significant analgesic effects compared to the control group with fewer side effects, suggesting they could serve as alternatives to morphine. However, further studies are needed to evaluate the side effects of ketoprofen and diphenhydramine.

Introduction

Diphenhydramine is an ethanolamine derivative and an H_1 histamine receptor antagonist widely used to alleviate symptoms of allergic reactions such as itchy skin disorders, conjunctivitis, nasal mucosal inflammation, nausea and vomiting, motion sickness, dizziness, insomnia, and symptomatic treatment of coughs and colds; it also possesses local anesthetic properties [1,2].

Anti-inflammatory drug (NSAID) medications inhibit various levels of cyclooxygenase enzymes, thereby suppressing prostaglandin production and resulting in antipyretic, analgesic, and anti-inflammatory effects. To date, two cyclooxygenase enzymes have been identified that are produced by different genes [3]. The cyclooxygenase enzyme is continuously produced in most body tissues and is essential for many physiological functions including blood supply to the gastrointestinal tract and kidneys as well as the blood clotting process. cyclooxygenase 2 is an inducible enzyme

produced in response to inflammatory mediators [4].

Morphine is a potent opioid derived from opium and is the most important active compound in opium; its concentration in dried opium ranges from approximately 4% to 21%. Morphine was the first alkaloid extracted from opium in 1803. Its mechanism of action occurs through its effects on the central nervous system by reducing pain sensation [5].

The aim of the present study was to investigate the combined analgesic effects of diphenhydramine and ketoprofen compared to morphine in mice.

Materials and methods

Animals

This study was conducted on 30 mice weighing between 25-35 g and maintained under standard conditions with a 12-hour light/dark cycle at a temperature of 25 ± 2°C with adequate access to food and water. The research followed ethical guidelines for working with laboratory animals (Ethical approval number: BAU, 140011) and involved random assignment into five groups of six mice each. Analgesia was induced through intra-peritoneal injection as follows: Control group received normal saline; Standard group received 10 mg/kg morphine; Treatment group 1 received 2.5 mg/kg diphenhydramine; Treatment group 2 received 2.5 mg/kg ketoprofen; Treatment group 3 received 2.5 mg/kg diphenhydramine plus 2.5 mg/kg ketoprofen. Pain assessment was conducted after injection using the hot plate test and formalin tests [6,7].

Hot plate test

The hot plate was set at 55 ± 2°C. The animal was placed on this hot surface while reaction time was measured in seconds until it responded to the pain stimulus by licking its paws or jumping out of the plastic chamber. Mouse behavior was observed for up to 45 seconds; if no response was observed within this period, the animal was removed from the plate. This assessment was conducted at intervals of 5, 15, 30, 60, and 90 minutes after drug administration [6].

Formalin test

To conduct the formalin test and record pain behaviors, animals were acclimatized in a formalin testing chamber for thirty minutes before drug injection. After fifteen minutes post-drug administration, a 2.5% formalin solution (20 microliters) was subcutaneously injected into the right paw using an insulin syringe. The animal was then placed in a transparent 30×30×30 cm box with a mirror positioned at a 45-degree angle for observation. Pain responses were assessed every fifteen seconds using a scoring system from 0 to 3: Score 0: Balanced walking with even weight distribution on both paws. Score 1: No weight bearing on the injected paw but no walking difficulties. Score 2: Lifting the painful paw with no floor contact. Score 3: Licking or vigorously shaking the painful paw. Pain scores were recorded in twelve 5-minute blocks over 60 minutes, and the average pain score was calculated. This method provides a structured assessment of pain responses in animals under experimental conditions [7].

Statistical analysis

Statistical analyses were conducted using Prism statistical software version 9.1.0. This included descriptive tests, one-way ANOVA, and repeated measures ANOVA. The normality of the data was assessed using the Kolmogorov-Smirnov test, with most variables showing a normal distribution.

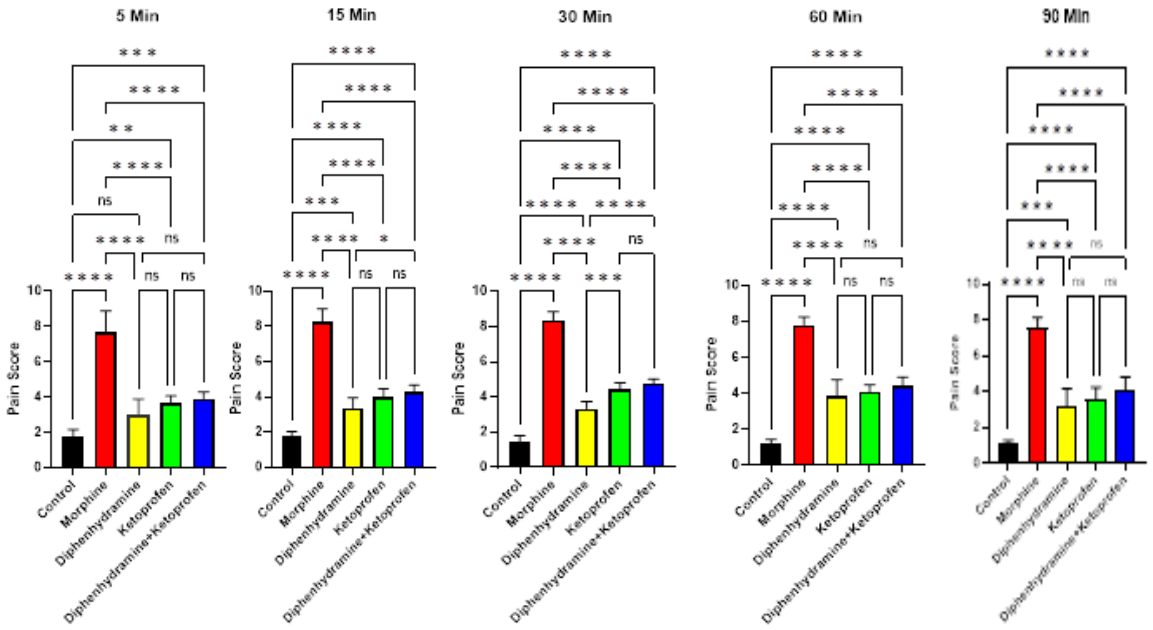

Fig 1: The results of the hot plate test at different time points (5, 15, 30, 60, and 90 minutes) after drug administration.

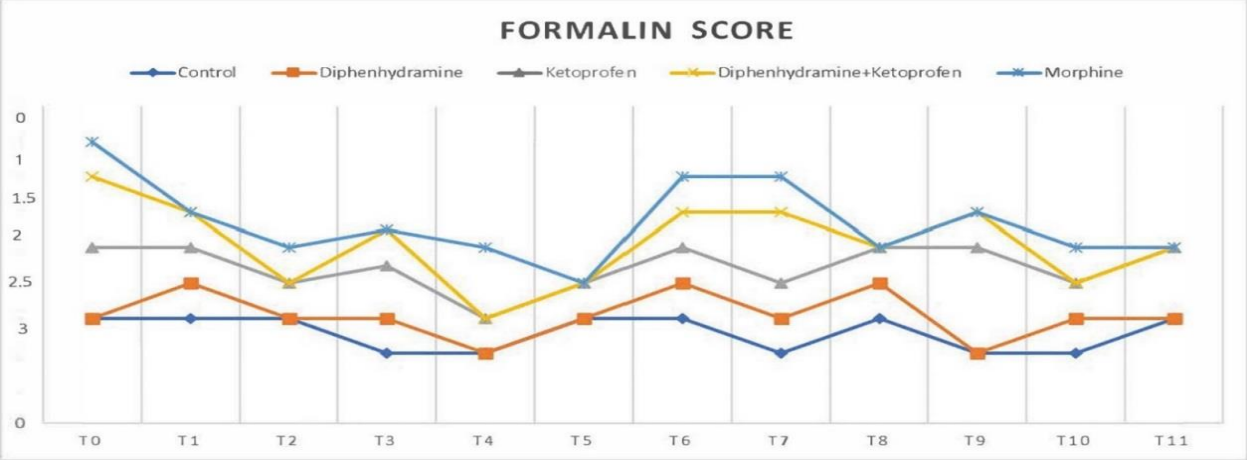

Fig 2: The results of the formalin test at different time points (Twelve 5-minute blocks over 60 minutes) after drug administration.

Results

In the present study, 30 mice were randomly divided into 5 groups of 6, according to the defined groups. After administering the drugs intra-peritoneal, pain assessment for each group was conducted using the hot plate test and formalin test. The results of the hot plate test at different time points (5, 15, 30, 60, and 90 minutes) after drug administration are presented in Figure 1. The results of the hot formalin test at different time points (Twelve 5-minute blocks over 60 minutes) after drug administration are presented in Figures 2 and 3. Based on the results of both diagnostic tests, although the analgesic effect of the combination of two drugs, diphenhydramine and ketoprofen, is less than morphine, it has a significant difference compared to the control group with fewer side effects.

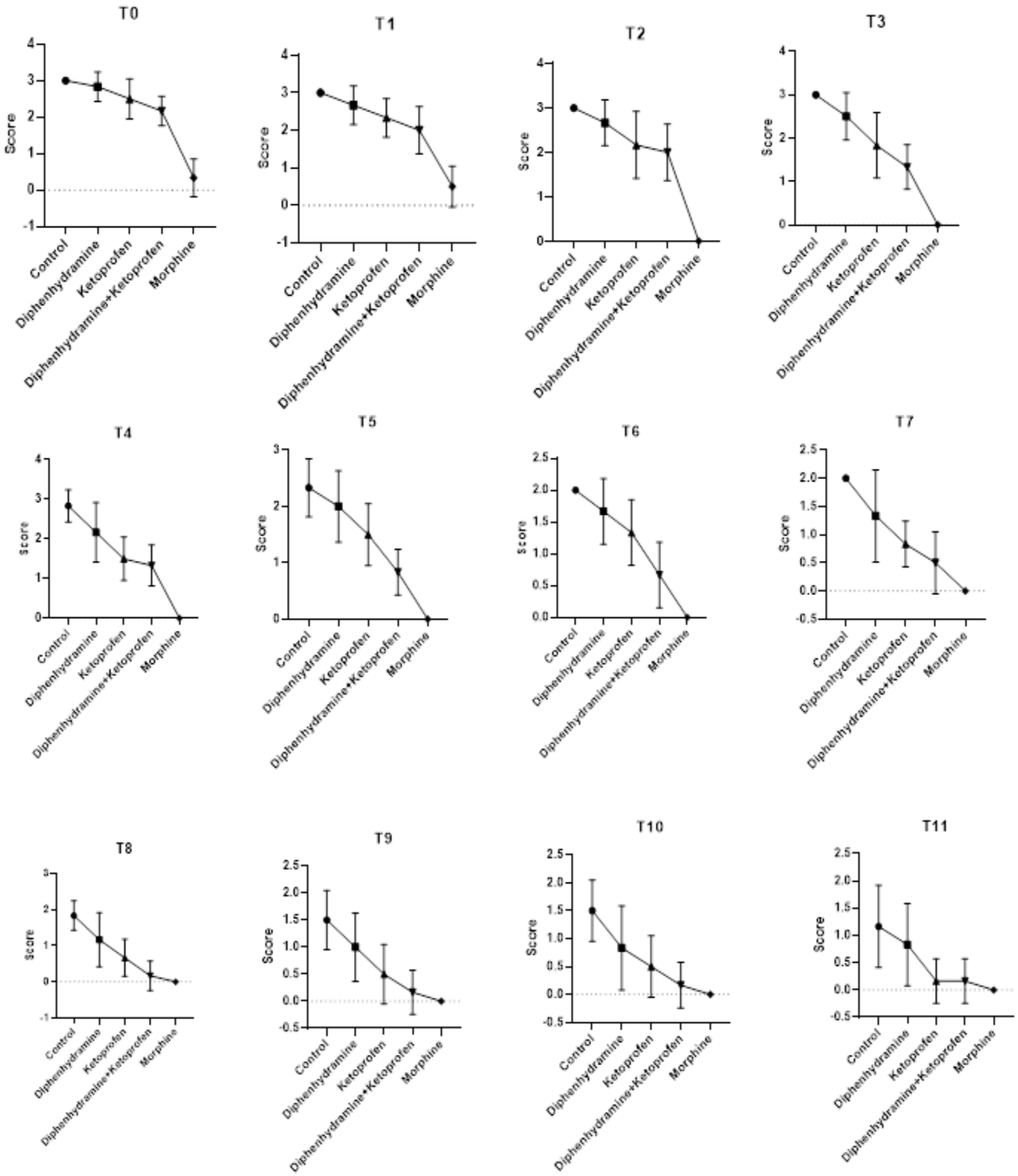

Fig 3: The results of the formalin test at different time points (Twelve 5-minute blocks over 60 minutes) after drug administration.

Discussion

The widespread use of opioids for pain management has long been a fundamental aspect of analgesic treatment. However, the development of tolerance and dependence linked to these medications presents substantial challenges in clinical settings. Morphine, despite its effectiveness, is well-known for its ability to trigger withdrawal symptoms like anxiety, tremors, muscle pain, irritability, and seizures when discontinued [8].

This study aimed to explore alternative analgesics that could mitigate these side effects while providing effective pain relief. The findings from our research indicate that the combined administration of diphenhydramine and ketoprofen produces significant analgesic effects comparable to morphine. This is particularly noteworthy given the growing interest in minimizing opioid use due to their adverse effects and the risk of addiction. The analgesic properties of diphenhydramine, an H_1 histamine receptor antagonist, have been recognized in various contexts, particularly in alleviating allergic symptoms. However, its role in pain modulation is less frequently discussed in the literature.

Recent studies have suggested that histamine plays a critical role in pain perception and modulation. Histamine receptors, particularly H_1 receptors, are involved in the central and peripheral processing of pain signals [9,10]. The involvement of H_1 antagonists like diphenhydramine in enhancing opioid-induced analgesia has been documented, indicating a synergistic effect when used alongside opioids [11]. Our results align with these findings, suggesting that diphenhydramine may not only serve as an antihistamine but also as an effective adjunct analgesic. Moreover, ketoprofen, a non-steroidal anti-inflammatory drug, works by inhibiting cyclooxygenase enzymes to reduce inflammation and pain. The dual action of combining a non-steroidal anti-inflammatory drug with an antihistamine could provide a multifaceted approach to pain management that addresses both inflammatory and neuropathic components of pain. This combination may be particularly beneficial in clinical scenarios involving postoperative pain or chronic pain conditions where inflammation plays a significant role.

Interestingly, previous studies have shown that diphenhydramine can significantly reduce the need for opioids when used prophylactically during surgeries [12]. This suggests that incorporating diphenhydramine into analgesic regimens could lead to lower opioid requirements postoperatively, potentially reducing the risk of opioid-related side effects and dependence. The histaminergic system's influence on pain modulation further emphasizes the importance of exploring non-opioid alternatives. Studies have indicated that repeated doses of H_1 antagonists do not lead to tolerance or reduced efficacy over time. This characteristic is particularly advantageous for long-term pain management strategies where maintaining consistent analgesic effects is crucial [13].

Conclusion

With the significant side effects associated with morphine and opioid medications, there has been a focus on using effective pain relief drugs that have fewer side effects. As indicated in the current study, the simultaneous use of ketoprofen and diphenhydramine shows considerable analgesic effects compared to control group and could potentially serve as alternatives to morphine. However, further studies are necessary to evaluate the side effects of ketoprofen and diphenhydramine.

Acknowledgements

The authors thank to Faculty of Veterinary Medicine, Babol branch, Islamic Azad University, Babol, Iran for providing financial support.

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

The research followed ethical guidelines for working with laboratory animals.

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How to cite this article:
Firouz Faedmaleki, Mohsen Alami Rostami, Mostafa Kalantari, Arya Soneishargh. Evaluation of the Combined Analgesic Effect of Diphenhydramine and Ketoprofen Compared to Morphine in Mice. Veterinary and Comparative Biomedical Research, 2025, 2(1): 29 – 34.
http://doi: 10.22103/vcbr.2025.24547.1032