Evaluation the combination of chlorpheniramine and tramadol at a level of thermal and visceral antinociceptive in a mouse acute pain model

A.I. Thanoon and Gh. A. Faris

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

The possible benefits of employing combination treatment include the ability to increase antinociceptive effects while minimizing the occurrence of unfavorable side effects. As a result, they are combining drugs that provide analgesic synergism allowing for a reduction in needed dosage as well as lowering the occurrence of unwanted side effects. In the current study, we evaluated quantitatively and qualitatively the type of drug interaction between tramadol (a typical opioid analgesic) and chlorpheniramine (an H₁-antagonist) at the level of thermal (hot plate) and visceral (writhing reflex) nociceptive stimuli in mice model. The 50% antinociceptive effective dose (ED₅₀) of intraperitoneal administration for tramadol and chlorpheniramine was 12 and 18.4 mg/kg respectively, using the up and down approach and hot plate apparatus. The treated animals showed signs of sedation and immobility. At 0.5:0.5 and 1:1 ED₅₀ ratios of each, the kind of pharmacological interaction between the two medications was synergism at the level of acute antinociceptive impact, using hot plate apparatus and isobolographic analysis. The reduction in ED₅₀ value was significant for tramadol and chlorpheniramine by 58.8 and 58.8 % at 0.5:0.5 ratio while 53.5 and 53.5 % at 1:1 ratio respectively. The synergistic interaction between the two drugs was also confirmed using the double ED₅₀ dose of each drug as simultaneously i.p. injected of these doses producing a synergism antinociceptive effect at visceral (writhing reflex) test. Which represented as prevent 100% writhing induced by i.p. injection of acetic acid compared to the control group and that with each drug alone at the same double doses. The present results concluded that simultaneous injection of tramadol and chlorpheniramine produced synergism, a potent and safe antinociceptive effect even at low doses which may be clinically useful in treating pain in the veterinary clinic.

Keywords: Antinociceptive, Chlorpheniramine, Hot plate, Tramadol, Writhing reflex

Correspondence: Gh. A. Faris ghadafaris2018@gmail.com

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Introduction

Pain is defined by The International Association for the study of pain, as an unpleasant sensory and emotional experience linked to actual or potential tissue injury (1,2). Pain is divided into three categories nociceptive pain, neuropathic pain, and pain from musculoskeletal disorders, nociceptive pain resulting from tissue damage, neuropathic pain resulting from nerve injury, and neuropathic pain is the third type of pain (2). Tramadol hydrochloride ((+)-trans-2-[(dimethylamino) methyl] -1 -(3-methoxy-phenyl)-cyclohexanol HCl) is a therapeutically effective and centrally acting analgesic (3,4). Used in both human (4) and veterinary medicine (5-7). It can be used to relieve acute, dental, labor, chronic, and cancer pain (1). Tramadol is a racemic combination of two enantiomers, each with a different but complementary mode of action in contrast to other opioid receptor agonists: the (+) enantiomer is a selective μ-receptor agonist that inhibits serotonin reuptake and increases serotonin efflux in the CNS, whereas the (-) enantiomer primarily inhibits norepinephrine reuptake (8,9). Chlorpheniramine is an alkylamine subtype utilized as
an antihistamine H₁ in both humans (10,11) and animals (12,13). It is commonly used to treat the symptoms and diseases of allergy (14-17). It also has analgesic properties by the mechanism which was previously investigated at the visceral (18,19) and cutaneous (20) levels. While their systemic analgesic was excluded (19). Different combinations of tramadol and other analgesics (20), were tested in order to provide good analgesia at low doses, minimize the side effects and extend the analgesia, as a combination of tramadol with naproxen (21), diclofenac (22), acetaminophen, and Ibuprofen (23), ketorolac (24) all of them produce a good analgesic effect. As the hyper nociception can be induced by activating histamine H₁ receptors (25) the antinociceptive impact of antihistamine H₁ receptors has been well documented, both in human and laboratory animals (26). Therefore, the combination of antihistamines with tramadol was also examined, but only to a limited extent (27). Diphenhydramine was one of the combinations combined with tramadol in mice but failed to provide adequate analgesia (28).

The goal of our research was to determine the efficacy of another H₂ antihistamine, Chlorpheniramine, in the antinociceptive effect of tramadol as a combination in mice as well as to assess the kind of pharmacological interaction between the two medicines at the level of visceral and thermal analgesia in order to achieve a novel analgesic combination, which had not been previously evaluated.

Materials and methods

Ethical approval

All mice in this research were handled according to institutional regulations on animal handling and use in research, we obtained official approval for the study protocol from the Committee of Postgraduate Studies at the College of Medicine, University of Mosul, Iraq.

Animals model

Adult (male and female) albino Swiss mice weighed between 22-34 g were utilized in all experiments. The mice were kept in the house of animals which is located in The College of Veterinary Medicine, University of Mosul. The animals were housed as 8 per cage with 12 h of light and 12 h of darkness, at a temperature of 20±2°C. Food and drink were always available to the animals. All experiments were carried out in accordance with the ethical Guide Lines of the Ethical committee from animal Research and the study of pain (29). The scientific committee of the college of veterinary medicine, department of physiology, biochemistry, and pharmacology reviewed and approved the study protocol.

Drugs

The doses of tramadol (100 mg/2ml, DUOPHARMA, Malaysia) and a pure powder of chlorpheniramine (Pioneer pharmaceutical, Iraq) and their combinations were prepared freshly in a solution of physiological saline (Marksans pharma, India) and administered intraperitoneally in a final volume of injection at 5ml/kg. Acetic acid 99.99% (TEDIA, USA) diluted with distal water to 1%.

Detection the ED₅₀ and the type of drug interaction

This experiment was designed according to the up-and-down procedure (30). To calculate the median effective dosage of ED₅₀ of tramadol and chlorpheniramine each alone or as a combination in mice at ratios 1:1 and 0.5:0.5 of ED₅₀ of each one. The kind of pharmacological interaction between two drugs was also discovered in this experimental protocol. All experiments were applied using a hot plate test as a quantitative method for evaluating the antinociceptive effect of drugs induced by thermal pain stimuli. The antinociceptive effect of each drug was evaluated 15 minutes, after intraperitoneal injection of every single dose of tramadol and chlorpheniramine by placing the individual animal on the surface of the hot plate apparatus at a temperature of 56±1°C (28). The first hind paw response as paw withdrawal, licking, and/or jumping was measured as the latency time of response to heat painful. The cutoff point was 20 sec (31).

To calculate the individual ED₅₀ of tramadol and chlorpheniramine, 11 mice used weighting 22-34 g. The first tramadol dose was administered intraperitoneally to the first mouse at 10 mg/kg (initial dose) whereas the initial chlorpheniramine dose was 20 mg/kg, the dose was selected dependent on initial trials and other studies of tramadol (32) and chlorpheniramine (33). The animals were used separately in two separate trials. 15 minutes after injection in each experiment, the mouse was put on the hot plate surface. The latency (in seconds) of response to heat stimuli was recorded using the stopwatch. The decrease and increase in the subsequent doses were at a fixed ratio of 2.5 mg/kg for tramadol and 5 mg/kg for chlorpheniramine which represented 25% of ED₅₀ of each drug. Each animal had a baseline latency time before the injection of an individual dose of each drug and then recorded the latency time 15 minutes after each later dose of each drug. The ED₅₀ value was detected finally by using the following equation ED₅₀=xf+kd (34).

Kind of pharmacological interaction between chlorpheniramine and tramadol

11 mice were used to determine the kind of pharmacological interaction between the two medicines at the level of analgesia. In two separate trials, tramadol and chlorpheniramine were simultaneously injected into the first mice at 12 and 18.4 mg/kg intraperitoneally, respectively which represented the ED₅₀ of each 1:1 ratio. While the first animal in the second trial was injected with tramadol and chlorpheniramine at 6 and 9.2 mg/kg intraperitoneally, respectively as half of the ED₅₀ value of each 0.5:0.5 ratio.
The doses previously obtained in experiment one. 15 minutes after the injection of the dose in each trial the mice were subjected to evaluate the analgesic effect on the hot plate surface as in experiment one (31). The fixed up and down in subsequent ED50 value of tramadol and chlorpheniramine was 3 and 4.6 mg/kg respectively at a 1:1 ratio. While in the 0.5:0.5 ratio trial the doses were at 1.5 and 2.3 mg/kg respectively. The effective 50% dose of each drug at each ratio was detected as in experiment one. To explain the type of interaction between tramadol and chlorpheniramine at 1:1 and 0.5:0.5 ratio of ED50 of each, the isobolographic analysis was used (34,35). Fixing individual ED50 of each drug on a different axis of graphic paper. Then connected the individual ED50 in a straight line. The ED50 of the combination at each ratio was plotted on the graphic paper. If located at the diagonal line, the interaction will be additive (no interaction), while falling above or below the line the interaction is considered as antagonism or synergism (34,35).

The interaction index was also used to obtain the value of Y as an additional confirmation to indicate the interaction between the two drugs, by using the following formula \[ Y = \frac{d_a}{D_a} + \frac{d_b}{D_b} \], if Y value > 1 means pharmacological interaction is antagonism, while if Y ≤ 1 the interaction is synergism and no interaction respectively (35,36). We also obtained the percentage (%) of reduction in ED50 value of each drug at each ratio of combination by using the equation %reduction of ED50 = \frac{\text{individual ED50 - ED50(interaction)}}{\text{individual ED50}} \times 100 (36).

The writhing reflex (visceral pain)

To additional support for the antinociceptive effect by hot plate (central and peripheral pain), tramadol and chlorpheniramine alone or together were evaluated for antinociception activity by writhing reflex test (visceral pain) (37). This procedure is considered as a quantitative method by recording the cumulative number of writhing between 0 and 30 minutes after injection of nociception stimuli (37). The mice were individually divided into four groups of six animals each. Group I was considered as a control, which was intraperitoneally injected with normal saline. Group II was treated with tramadol at 24 mg/kg. Group III was treated with chlorpheniramine at 36.8 mg/kg, intraperitoneally. While the last group (IV) was administrated with tramadol and chlorpheniramine at 24 and 36.8 mg/kg intraperitoneally, respectively. The dose of tramadol and chlorpheniramine was obtained from experiment one as a double dose of ED50 of each, 30 minutes after the injection of drugs each animal in all four groups was intraperitoneally injected with 0.1ml/10g b.w. of 1% acetic acid (37). Immediately after the injection of acetic acid, the mice in each group were observed individually for recording the latency time of the onset of the writhing reflex. The number of writhing reflexes (constriction of abdominal muscle accompanied by the extension of the hind limb) was also recorded for 30 minutes in each group (37). The percentage of reduction in writhing was also calculated in each group is compared with the control group (acetic acid alone) by using the equation.

\[ \text{Writhing inhibition} = \frac{\text{mean No. of control} - \text{mean No. of test/mean No. of control} \times 100} {38} \]

Exploring the antinociceptive effect of a non-analgesic dose of tramadol and chlorpheniramine on hot plate

On the heated plate, the antinociceptive impact of a non-analgesic dose of tramadol and chlorpheniramine each alone or together was determined. We designed the experiment by randomly dividing 20 mice at a weight of 26-35 g into separated four groups of 5 mice each and intraperitoneally injected as follows: group I respected as the control group (normal saline), Group II and III were injected with sedative non-analgesic doses of tramadol and chlorpheniramine at 6 and 9 mg/kg, respectively. While group IV is considered a combination group that received tramadol and chlorpheniramine together at 6 and 9 mg/kg. Each animal in each group had a baseline of latency time and then repeated 15 minutes after injection of each drug. All experiments were performed on the hot plate apparatus. The dose for each drug was obtained from experiment one (50% of ED50) of each drug.

Statistical analysis

The parametric data in experiments three and four were statistically evaluated using One Way Analysis of Variance (ANOVA) via using Sigma plot software version 12.5 and the analysis between different treatment groups were performed by the least significant test (LSD). While Mann Whitney U test was applied to nonparametric data (scores) as the number of writhing (64). All data were presented as mean ± SE. The data were considered statistically significant at a P level less than or equal to 0.05.

Results

Estimation the individual ED50 of tramadol and chlorpheniramine in mice

By using the up and down method on the hot plate apparatus, the ED50 of tramadol and chlorpheniramine each alone were 12 and 18.4 mg/kg b.w respectively. These doses produced a 50% antinociceptive effect 15 minutes after intraperitoneal injection in mice (Table 1). All up and down doses of each drug were combined with signs of sedation, quiet, and immobility (Table 1).

The type of interaction between tramadol and chlorpheniramine

The combination ED50 value, which was determined by simultaneously intraperitoneal injection of tramadol and chlorpheniramine at ratios of 1:1 and 0.5:0.5 of ED50 of each alone was 5.58:8.56 mg/kg and 4.94:7.58 mg/kg, respectively (Table 1). The percentage of reduction in the
value of ED$_{50}$ of tramadol and chlorpheniramine each alone was 53.5 and 53.5% respectively at 1:1 and 58.8 and 58.8% respectively at 0.5:0.5 in comparison with individual ED$_{50}$ of each drug alone (Table 1). The kind of pharmacological interaction between two medications at 1:1 and 0.5:0.5 of individual ED$_{50}$ of each was synergism dependent on isobolographic analysis method. The falling of combination ED$_{50}$ at 1:1 and 0.5:0.5 under the diagonal straight line between the ED$_{50}$ of each drug on graph paper indicated the synergism interaction between the two drugs (Figure 1). The results were confirmed by the estimation of the Y value from the interaction index equation. The Y values were 0.92 and 0.82 at 1:1 and 0.5:0.5 respectively (Table 1). This is considered more indicator of the synergism pharmacological interaction between two medications at the level of central analgesia as Y < 1 (Table 1).

Table 1: Determination of ED$_{50}$ of tramadol and chlorpheniramine each alone or as combination in mice on hot plate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tramadol</th>
<th>Chlorphen</th>
<th>Combination at 0.5:0.5</th>
<th>Combination at 1:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED$_{50}$(mg/kg)</td>
<td>12</td>
<td>18.4</td>
<td>4.94</td>
<td>7.58</td>
</tr>
<tr>
<td>Dose range (mg/kg)</td>
<td>15-10=5</td>
<td>20-15=5</td>
<td>6.4-5=1.5</td>
<td>9.2-6.9=2.3</td>
</tr>
<tr>
<td>First dose (mg/kg)</td>
<td>10</td>
<td>20</td>
<td>6</td>
<td>9.2</td>
</tr>
<tr>
<td>Last dose (mg/kg)</td>
<td>12.5</td>
<td>20</td>
<td>6</td>
<td>9.2</td>
</tr>
<tr>
<td>Dose change (mg/kg)</td>
<td>2.5</td>
<td>5</td>
<td>1.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Number of mice</td>
<td>6(OXXOX)*</td>
<td>5(OXXOX)</td>
<td>5(XOXOX)*</td>
<td>5(XOXOX)*</td>
</tr>
<tr>
<td>% decrease in ED$_{50}$</td>
<td>58.8%</td>
<td>58.8%</td>
<td>53.5%</td>
<td>53.5%</td>
</tr>
<tr>
<td>Y value</td>
<td>0.82</td>
<td>0.82</td>
<td>0.92</td>
<td>0.92</td>
</tr>
</tbody>
</table>

*X: analgesic effect, O: no analgesic effect. The up and down approach used to calculate ED$_{50}$ value.

Figure 1: Isobolographic analysis of tramadol and chlorpheniramine interaction at 0.5:0.5 and 1:1 ratio in mice. The ED$_{50}$ of each drug connected by diagonal line. (circle) 0.5:0.5 and (star) 1:1 point represented the ED$_{50}$ combination of two drugs, fall down under the diagonal line, indicated synergism interaction.

Effect of double ED$_{50}$ dose of tramadol and chlorpheniramine on acetic acid produce writhing reflex (visceral pain)

Intraperitoneal injection of double ED$_{50}$ dose of tramadol at 24 mg/kg and chlorpheniramine at 36.8 mg/kg each alone in mice 30 minutes before the intraperitoneal injection of acetic acid, significantly decreased the number of writhing induced by acetic acid in comparison with the control group (acetic acid alone) (Table 2), while co-administration of tramadol and chlorpheniramine at the same doses prevent the writhing reflex by 100% when compared with tramadol and chlorpheniramine each alone as well as with control group (Table 2 and Figure 2). There was a significant increase in the onset of writhing in the group treated with tramadol or chlorpheniramine alone compared with the control group, while the combination of the two drugs at the same double dose produce a significant increase in the onset time of writhing compared to the control group as well as with the two drugs each alone at the same doses (Table 2).

Figure 2: Effect of tramadol 24 mg/kg i.p and chlorpheniramine 36.8 mg/kg i.p either individually or in combination on mice’s visceral pain (writhing reflex).

Effect of sub analgesic dose of tramadol and chlorpheniramine on the thermal nociception

Sub-analgesic doses of tramadol and chlorpheniramine at 6 and 9 mg/kg intraperitoneally as ED$_{25}$ failed to produce a significant analgesic effect on the hot plate surface (Table 3). Whereas simultaneously intraperitoneal injection of the same sub-analgesic doses of both drugs induced a complete antinociceptive effect 100% in comparison with control, tramadol, and chlorpheniramine each alone (Table 3), with no apparent side effects (as deep sedation).
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antinociceptive effect in acetic acid of pain a double dose of chlorpheniramine 36.8 mg/kg in visceral decreases succeeded in relieving visceral pain by significantly (37) and we revealed that the double chlorpheniramine each alone on visce increased the pain threshold.

Our current study confirmed the findings of previous s regarded as the first detection in our research as no previous mice, chlorpheniramine by intraperitoneal injection of each in apparatus at thermal (hot plate) and visceral (writhing reflex) detected the individual ED₅₀ of tramadol and chlorpheniramine by intraperitoneal injection of each in mice, the doses were 12 and 18.4 mg/kg respectively.

The ED₅₀ of tramadol was in agreement with that in the previous study (40). While ED₅₀ of chlorpheniramine is regarded as the first detection in our research as no previous research referred to or detected using the same method and apparatus in the mice model. There was only one study that referred to the analgesic dose of chlorpheniramine in mice on the hot plate but not as the up-and-down ED₅₀ dose (41). Our current study confirmed the findings of previous studies that referred to the antinociceptive effect of chlorpheniramine (19) and tramadol (40) on the hot plate, which was illustrated by increasing the pain threshold.

We also tested the analgesic action of tramadol and chlorpheniramine each alone on visceral pain (writhing test) (37) and we revealed that the double ED₅₀ of each alone succeeded in relieving visceral pain by significantly decreasing the number of writhing reflexes in mice. We used a double dose of chlorpheniramine 36.8 mg/kg in visceral pain perception, as the previous study showed that the dose of 20 mg/kg i.p. of chlorpheniramine failed in producing an antinociceptive effect in acetic acid-induced visceral pain (42). Our findings in the current study agreed with previous research for tramadol (21) and chlorpheniramine (18) as each of them alone produced a good visceral antinociceptive effect.

Combinations of antinociceptive medications are frequently used in the treatment of pain in order to increase or maintain analgesia, low doses needed as well as to reduce the adverse effects of each drug (43,44). When two or more medications are concomitantly used, they may be exerted a distinct effect producing additive or no interaction. Other combinations may be over or less than anticipated, which produced synergism or even antagonism combinations (44). Many different forms of combinations were introduced to the clinic which related to particular drug combinations and could not be extrapolated to other drug combinations (44). So that we always need to discover a novel combination to explore the mechanism by which these combinations affect. Tramadol as a good analgesic (45) and chlorpheniramine as a specific H₁-receptor antagonist (19) are widely used in clinics. Each of them has a mixed mechanism as analgesic action. In our study, we explored the kind of pharmacological interaction between tramadol and chlorpheniramine at different ED₅₀ of 0.5:0.5 and 1:1 ratio at the level of analgesia using isobolographic analysis (34,35). The synergism was revealed as the decrease in the value of ED₅₀ of tramadol and chlorpheniramine by 58.8 and 58.8% at 0.5:0.5 and 53.5 and 53.5% at 1:1 ratio respectively. We confirmed this result by calculating the Y value (interaction index) which was less than one (34,35). We also used another sensitive test (writhing reflex) as a more confirm the synergism combination, which is regarded as a sensitive tool to assess the anti-inflammatory and analgesic effect of the new drug (46-48). This test also revealed a synergistic interaction between the two drugs in

Table 2: percentage of inhibition for tramadol and chlorpheniramine each alone or as combination by using writhing test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Onset time (minute) of writhing</th>
<th>NO. of writhing (score)/30 min</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (acetic acid)</td>
<td>2.39 ± 0.17</td>
<td>108.8 ±2.7</td>
<td>0 %</td>
</tr>
<tr>
<td>Tramadol 24mg/kg, i.p.</td>
<td>8 ± 0.27*</td>
<td>1.8+a ± 14.6</td>
<td>↓ 86 %</td>
</tr>
<tr>
<td>Chlorpheniramine 36.8mg/kg i.p.</td>
<td>8.32 ± 0.44*</td>
<td>33 ± 4.2*</td>
<td>↓ 69 %</td>
</tr>
<tr>
<td>Tramadol and Chlorpheniramine</td>
<td>0.00± ab ± 0.00</td>
<td>0.00± ab ± 0.00</td>
<td>↓ 100%</td>
</tr>
</tbody>
</table>

Acetic acid was injected intraperitoneally 30 minutes after injection of tramadol and chlorpheniramine in each group. *Significant difference from the control group (P<0.05). a significant difference from Chlorpheniramine 36.8mg/kg group (P<0.05). b significantly differences from Tramadol 24mg/kg group (P<0.05).

Table 3: Effect of tramadol and chlorpheniramine in sub-analgesic doses (low doses) on the thermal pain (hot plate) in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latency time</th>
<th>Latency time After 30 minutes</th>
<th>Analgesia %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal saline 0.9%) i.p.</td>
<td>3.85 ± 0.081</td>
<td>3.64 ± 0.1</td>
<td>0 %</td>
</tr>
<tr>
<td>Tramadol 6 mg/kg i.p.</td>
<td>3.60 ± 0.15</td>
<td>3.40 ± 0.11</td>
<td>0 %</td>
</tr>
<tr>
<td>Chlorpheniramine 9 mg/kg i.p</td>
<td>3.854±0.20</td>
<td>3.71 ± 0.23</td>
<td>0 %</td>
</tr>
<tr>
<td>Tramadol + Chlorpheniramine</td>
<td>3.75 ± 0.13</td>
<td>7.15 ± 0.27* #ab</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Chlorpheniramine was injected directly after tramadol. #: significant to control group at P<0.05. #: significant to Tramadol 6 mg/kg group at P<0.05. b: significant to Chlorpheniramine 9 mg/kg group at P<0.05.

Discussion

We aimed to investigate the antinociceptive activity of chlorpheniramine alone or as a combination with the conventional medication tramadol. This study is considered the first research that evaluated qualitatively and quantitatively the kind of pharmacological interaction between the two medications at the level of acute pain in mice model using two types of nociceptive stimuli, thermal (hot plate) and visceral (writhing reflex), by using hot plate apparatus at 56°C (39) and up and down method (30), we detected the individual ED₅₀ of tramadol and chlorpheniramine by intraperitoneal injection of each in mice, the doses were 12 and 18.4 mg/kg respectively.

Many different forms of combinations were introduced to the clinic which related to particular drug combinations and could not be extrapolated to other drug combinations (44). So that we always need to discover a novel combination to explore the mechanism by which these combinations affect. Tramadol as a good analgesic (45) and chlorpheniramine as a specific H₁-receptor antagonist (19) are widely used in clinics. Each of them has a mixed mechanism as analgesic action. In our study, we explored the kind of pharmacological interaction between tramadol and chlorpheniramine at different ED₅₀ of 0.5:0.5 and 1:1 ratio at the level of analgesia using isobolographic analysis (34,35). The synergism was revealed as the decrease in the value of ED₅₀ of tramadol and chlorpheniramine by 58.8 and 58.8% at 0.5:0.5 and 53.5 and 53.5% at 1:1 ratio respectively. We confirmed this result by calculating the Y value (interaction index) which was less than one (34,35). We also used another sensitive test (writhing reflex) as a more confirm the synergism combination, which is regarded as a sensitive tool to assess the anti-inflammatory and analgesic effect of the new drug (46-48). This test also revealed a synergistic interaction between the two drugs in
the current study. These results are regarded as the first study that explored the kind of interaction between tramadol and chlorpheniramine on the level of visceral antinociceptive effect in mice as no previous study referred to the same effect.

We also examined the competence of the novel combination by simultaneously intraperitoneal injection of sub-analgesic doses (low doses) of each drug, which succeeded in producing a synergistic effect on the hot plate without overt side effects. We used the same procedure in our previous studies to prove the synergism interaction between different analgesics (49).

The mechanism by which the two drugs exerted their synergism interaction related to the mixed mechanism of action for each. Tramadol has a mixed mechanism as an analgesic by their central activation of μ-receptor as well as peripheral (on the gut) (49). The drug has two enantiomers, (+) dexo enantiomer, which acts as μ opioid receptor agonist as well as serotonin reuptake inhibitor. While (-) levo enantiomer acts as a noradrenalin reuptake inhibitor (50,51). The effect of drug on the 5HT3A receptor was also involved in their analgesic effect (52). Related to the previous reports that referred to the critical role of serotonin in the modulation of pain perception (53). As the neurotransmitter (serotonin) activates the 5HT receptors and the presynaptic 5HT1 has a role in the antinociceptive effect (54). The stimulation of the adenosine A1 receptor also has a role in the analgesic effect of tramadol (54). Tramadol also has an anticholinergic effect (anti-muscarinic) (55). As well as it has an H1 receptor antagonist which is involved in its analgesic effect as previous research referred to the critical role of the H1 receptor in pain perception (56) and the opioid-produced analgesic effect via their antagonism to this receptor as well as stimulation of the μ receptor (57).

Chlorpheniramine also produced its analgesic effect as an H1 receptor antagonist (19). As histamine evoked their visceral and somatic pain via activation of the H1 receptor (19). So that our findings agreed with previous research that discovered the role of chlorpheniramine (H1 antagonist) in potentiating the antinociceptive effect of morphine (opioid) in mice using mechanical, thermal, and chemical nociceptive stimuli and related this effect as the two drugs act as H1 receptor antagonist (57). Another study also revealed the role of chlorpheniramine in potentiating the antinociceptive effect of morphine on the visceral writhing reflex test by a mechanism related to their effect as activation of endogenous opioid receptors as well as H1 blockers. So, histamine H1 antagonists produced antinociceptive effects on their own or in combination with opiates (58).

The combination between tramadol (a synthetic opioid) and chlorpheniramine in our study also was synergistic, as the two drugs have an H1 receptor antagonism effect as well as μ receptor activation (57). Chlorpheniramine potentiated the analgesic effect of tramadol may be also via their effect as serotonin reuptake inhibitor as tramadol. This is the characteristic that distinguishes it from most other 1st generation antihistamines (58).

The drug also has a G1 proteins stimulation (59) as inactivation of these proteins blocked the analgesic effect of GABA, and catecholamine and prevented the analgesic effect of tricyclic antidepressants and opioids (60-62). Chlorpheniramine also has an anti-muscarinic effect which may play a role in its analgesic effect (63-65). All these suggested mixed mechanisms of tramadol and chlorpheniramine, which may be involved in the synergism interaction between them at the level of thermal and visceral analgesia in mice in our current study, especially on the level of μ receptor activation, serotonin reuptake inhibitor as well as antagonism ofH1 receptors (pharmacodynamic interaction). In the future, we need further studies on the pharmacokinetic level to explore if the interaction between the two drugs may be related to the effect of one on the kinetic of the other (66-68).

Conclusion

In our current study, we concluded that the interaction between tramadol and chlorpheniramine was synergistic at the level of the thermal and visceral test even using low (sub-analgesic) doses, which evoked a novel safe, and effective analgesic combination that may be useful in clinical practice. The novel combination has super advantages using low doses, producing strong analgesia without overt side effects.

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Conflict of interest

None.

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تأزري باستخدام جرع مضاعفة من قيمة الجرعة المسكنة الوسطية من كل عقار وإعطانهما معا للحيوان نفسه بالحقن داخل الخلب والذي أحدث تأثير مسكن تأزري قوي وأمن حتى عند إعطانهما بجرع واطئة والذي قد يعد مفيدا سريريا في معالجة الألم داخل العيادات البيطرية.

تآثر باستخدام جرع مضاعفة من قيمة الجرعة المسكنة الوسطية من كل عقار وإعطانهما معا للحيوان نفسه بالحقن داخل الخلب والذي أحدث تأثير مسكن تأزري قوي وأمن حتى عند إعطانهما بجرع واطئة والذي قد يعد مفيدا سريريا في معالجة الألم داخل العيادات البيطرية. 

التآثر باستخدام جرع مضاعفة من قيمة الجرعة المسكنة الوسطية من كل عقار وإعطانهما معا للحيوان نفسه بالحقن داخل الخلب والذي أحدث تأثير مسكن تأزري قوي وأمن حتى عند إعطانهما بجرع واطئة والذي قد يعد مفيدا سريريا في معالجة الألم داخل العيادات البيطرية.