

Isobolographic analysis of the antinociceptive interaction between tramadol and diphenhydramine in Mice

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Abstract: Tramadol was administered alone at (10 to 60) mg/kg I.P produced analgesia in a dose dependent manner and the Maximal Possible Effect (MPE) was obtained at 40 minutes after tramadol administrated. The analgesic activity of diphenhydramine, also were evaluated after administration of drug at (0.25 to 8) mg/kg S.C produced an analgesic effect in mice in dose depended manner and the %MPE was obtained after 10 minutes from injected of the drug. This study was observed that the combination of tramadol and diphenhydramine produced antagonist interaction this was evaluated by the isobolographic analysis. Isobolographic analysis found that administration of tramadol (ED₅₀) with diphenhydramine(ED₅₀) in a ratio (1:0.5, 1:1, 1:2, 0.25:1, and 0.5:1) that produced an antagonist interactive between two drugs, this lead to increase the dose of tramadol that needed to produce the analgesic effect. This study concluded that the administration of tramadol with diphenhydramine produce antagonist effects.

Keywords: Analgesic, Antinociceptive, Tramadol, Diphenhydramine, Isobolographic analysis, Hot-plate test, Drug interaction.

INTRODUCTION

Tramadol hydrochloride is a synthetic, centrally acting analgesic, which has been used since 1977 for the relief of moderate to severe acute and chronic pain ⁽¹⁾ and it's also been structurally related to codeine and morphine ⁽²⁾. In addition to its synthetic analgesic, it also has both weak opioid agonist with selectivity for the μ -receptor and non-opioid as a weak inhibitor of monoamine neurotransmitter noradrenaline and serotonin reuptake⁽³⁾. So, these two mechanisms of action are responsible for analgesic activity of tramadol. This dual mechanism of action may be attributed to the differences between the two enantiomer of racemic tramadol. The (+) enantiomer has a higher affinity for the μ -receptor and is a more effective inhibitor of serotonin reuptake, whereas the (-) enantiomer is more effective inhibitor of noradrenaline reuptake and increase its release by auto receptor activation ⁽⁴⁾. This inhibitory effect may also contribute to the analgesic effect of tramadol by inhibitory pain transmission in the central nervous system ⁽⁵⁾.

Diphenhydramine is an ethanolamine, and antagonist of the histamine H₁ receptor and has anticholinergic properties. It is among the oldest H₁- antihistamine drugs and is invented in 1943 by Dr. Gorge Riereschl ⁽⁶⁾. Diphenhydramine is commonly used as anti-allergy medication and also combined with other ingredient for the treatment of colds, allergies and insomnia ⁽⁷⁾. Diphenhydramine works by blocking the effect of histamine at H₁ receptor sites .

Drug pharmacological interaction occurs when one therapeutic agent either alters the concentration of another drug (pharmacokinetics interaction)⁽⁸⁾ or alter biological effect of another agent (Pharma codynamic interaction) ⁽⁹⁾. Sansone et al. (1986) ⁽¹⁰⁾ showed that H₁ antagonists can potentiate the analgesic effect of opioid. H₁ and H₂ antagonists potentiate the anti-nociceptive effects of morphine and fentanyl⁽¹¹⁾. However, the effect of the diphenhydramine on antino- ciceptive effect of tramadol has not been extensively studied. Therefore, our study was done to evaluate the interaction between tramadol and diphenhydramine and investigate the type of interaction.

MATERIALS AND METHODS

Animals Male and female albino mice weighing 20-30gm were used in the study. The animals were approved from animal care housed in the Dentistry Collage of Mosul University in Iraq. Animals were housed in rodent plastic cages (30×20×17)cm with wire mesh covers, at 22±2°C on a 12hr Light/dark cycle, with free access to food and water. Animals were allowed in the new environment for 30minutes prior to start experimental procedure. All animals were used only once .

Experiment 1: Determination the pain reaction time and dose-response curve of tramadol

The animals were randomly divided into seven groups. Each group consisted of seven mice and they were treated with the following doses: Group 1 served as a saline control and was given normal saline 0.9% (1 ml/kg , I.P) , group 2,3,4,5,6,7 received (10,20,30,40,50,60 mg /Kg , I.P) Tramadol Hydrochloride ampoule 5% (Mepha Ltd Aesh-Basel swit-zerland) respectively .Pain reaction time and latency reaction time (licking paw or jumping) was recorded before and (20, 40, 60, 80, 100 and 120 min.) following intraperitoneal administration of tramadol by using hot-plate test (Mice were placed on a hot-plate maintained at $55\pm 1^{\circ}\text{C}$. The reaction time is that between placing the animals on the hot-plate and kick, holding, jumping,licking of the fore or hind paws. Acute off time of 30 seconds is followed to avoid any thermal injury to the paws ⁽¹²⁾. To assess the dose-response and time effective curve. The prolongation of latency times compared with the values of the control was used for comparison. The percentage of antinociceptive Maximal Possible Effect (MPE) was calculated from the formula: ⁽¹³⁾

$$\% \text{ MPE} = (\text{Test latency} - \text{predrug latency} / \text{cut off time} - \text{predrug latency}) \times 100$$

MPE: Percentage of antinociception maximal possible effect.

Test latency: Sec after drug treatment.

Predrug latency: Sec before drug treatment at zero time.

Cut off time: 30 second.

Experiment 2: Determination the pain reaction time and dose-response curve of diphenhydramine

Healthy albino mice of either sex weighing 20-30gm were selected for the study. The animals were randomly divided into seven groups. Each group consisted of seven mice and they were treated with the following doses: Group 1 served as a saline control and was given normal saline 0.9% (1 ml/kg , I.P) , Groups 2,3,4,5,6,7 received (0.25,0.5,1,2,4,8 mg /Kg , S.C) Diphenhydramine HCL ampoule 1%.(The State Company For Drug Industries and Medical Appliances NINAVAH_IRAQ) Pain reaction time and latency reaction time(licking paws or jumping) was recorded before and (10, 20, 30, 40, 50 and 60 min.) following subcutaneous (s.c.) administration of diphenhydramine by using the hot-plate test to assess the dose-response and time effective curve. The duration of latency times compared with zero time and the values of the control that estimated as MPE% according to previous experiment one.

Experiment 3: Isobolographic analysis of the antinociceptive interaction between tramadol and diphenhydramine

To determine whether the interaction between the two drugs was synergistic, additive or antagonistic using isobolographic analysis. It is subjected that the ED₅₀ of both drugs that were estimated from up-and-down method of each drug administered individually, or in combination. A straight line was drawn from the isobolographic analysis between median effective dose (ED₅₀) of tramadol and diphenhydramine given to mice alone. The ED₅₀ point of tramadol was represented on the X axis, while ED₅₀ of diphenhydramine was represented on the Y axis. The interaction index, denoted by Y, was an assessment of the degree of synergism, antagonism or additive effect. The index was defined by the isobolar relation ⁽¹⁴⁾: $Y = a/A + b/B$

A: Dose of drug 1 alone (Tramadol), B: Dose of drug 2 alone (Diphenhydramine). (a, b) combination doses produce the same effect. The quantities in the equation are obtained from the up-and-down method of drug A, B and their combination. If $Y=1$, the interaction is additive. $Y < 1$, the interaction is super-additive (synergy). $Y > 1$, the interaction is sub-additive (antagonism).

The experiment was divided into two parts,

Part 1: In this part of the experiment, the dose of tramadol was fixed and used various doses of diphenhydramine as in following ratio (1:0.5, 1:1, 1:2).

Part 2: In this part, the dose of diphenhydramine was fixed and used various doses of tramadol as in following ratio (0.25:1, 0.5:1).

Statistical analysis

The data were expressed as mean \pm SD, difference between three experimental groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the least significant difference test. The level of significance was at $p < 0.05$. ⁽¹⁵⁾

RESULTS

Experiment 1 :

Determination the pain reaction time and dose-response curve of tramadol: In the hot plate test, the I.P. administration of tramadol at (10, 20, 30, 40, 50, and 60) mg/kg lead to significant increase in pain threshold between pre and post treatment at 20 min after drug administration (Table 1).

Table (1) :The increase in pain reaction time threshold of tramadol in mice.

Time Dose	+20 Minute	+40 Minute	+60 Minute	+80 Minute	+100 Minute	+120 Minute
Control N.S 0.9%mg/kg (I.P.)	0.42±0.2	0.0±0.0	0.42±0.2	0.42±0.2	0.28±0.1	0.28±0.1
Tramadol 10mg/kg (I.P.)	2.8±1.1	5.14±0.9 *	4.4±0.84	3.85±0.96	2.14±0.91	1.4±0.61
Tramadol 20mg/kg (I.P.)	6.42±0.84 *	8.57±0.64 *	7.57±1 *	6.42±1.4 *	5.5±1.5 *	2.7±1.4
Tramadol 30mg/kg (I.P.)	8.42±2.4 *a	9.8±2.48 *	9±1.85 *	7.42±1.06 *	5.8±0.73 *	3.2±0.71
Tramadol 40mg/kg (I.P.)	11.28±1.86 *ab	14.42±1.9 *ab	15.28±2.7 *abc	12.5±2.66 *ab	8.28±1.50 *a	6.14±1.33 *
Tramadol 50mg/kg(I.P.)	15.85±1.81 *abc	19.14±2.7 *abc	17.7±3.15 *abc	16.4±2.8 *abc	12.14±2.48 *abc	9±2.6 *abc
Tramadol 60mg/kg (I.P.)	15.14±1.7 *abc	18±2.02 *abc	16.14±2.6 *abc	14.4±2.4 *abc	12.14±2.8 *abc	8±3.03 *abc

* significant with control were $p \leq 0.05$.

Mean \pm SE for seven groups in second

a: significant with tramadol 10 mg/kg were $p \leq 0.05$.

b: significant with tramadol 20 mg/kg were $p \leq 0.05$.

c: significant with tramadol 30 mg/kg were $p \leq 0.05$.

The time effective curve of different doses of tramadol was shown in (Figure, 1)

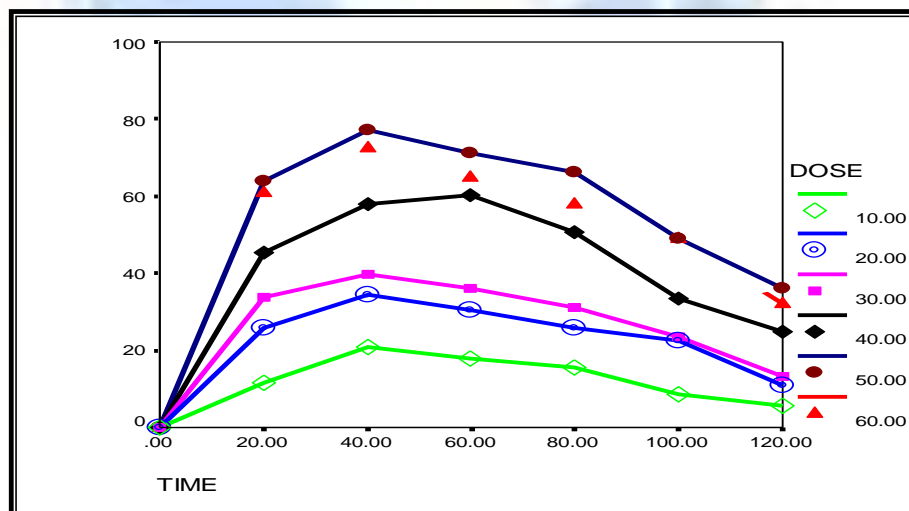


Figure (1) The time effective curve of tramadol

The changes of maximum possible effect (MPE% Antinociceptive %) usually approached the base line (13.19%) in 120 min after the administration of drug except for the two higher doses 50, 60 mg/kg of tramadol (36.2%) (32.1%), respectively.

The maximum effective does not always happen at the same time and the shapes of time effect curve vary with doses, and 20-120minute after injection of tramadol indicated to mean analgesic effect of tramadol, the best time of tramadol action is 40 minute .(Figure ,1)

The dose-responses curve for different doses of tramadol were shown in (Figure, 2).

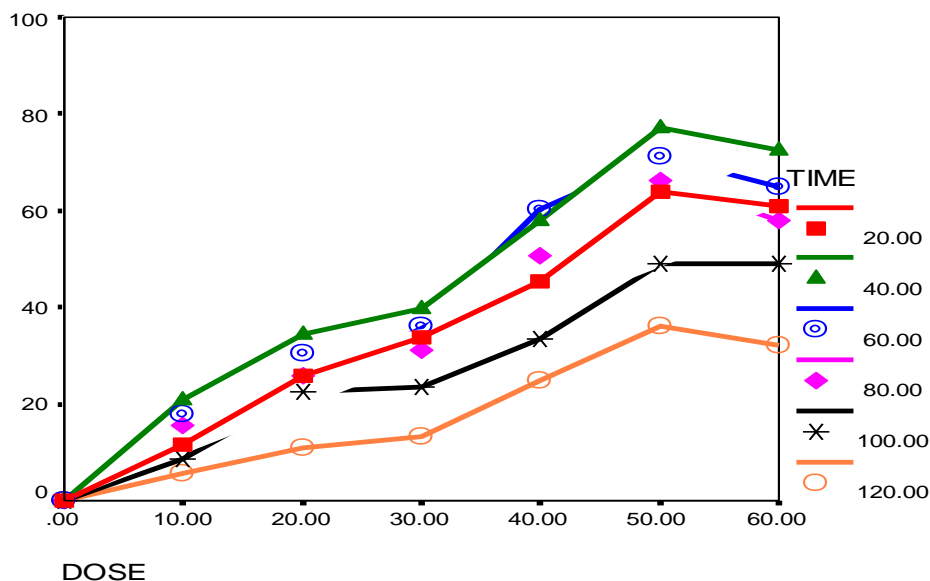


Figure (2) The dose-response curve of tramadol.

Tramadol produced a dose related changes in maximum possible effect (MPE%) in comparison to control. The increase in dose lead to increase in MPE% changes, and the maximum possible effective dose occurring at (50mg/kg) is (76.9%) in 40 minutes.

Experiment 2 :

Determination the pain reaction time and dose-response curve of diphenhydramine: The subcutaneous administration of diphenhydramine in (0.25, 0.5, 1, 2, 4 and 8) mg/kg lead to increase in pain threshold between pre and post treated of mice after 10minute of drug administration (Table, 2).

Table (2): The increase in pain reaction time threshold of diphenhydramine in mice.

Time Dose	+10 Minute	+20 Minute	+30 Minute	+40 Minute	+50 Minute	+60 Minute
Control N.S 0.9%mg/kg (S.C.)	0.42±0.2	0.14±0.26	0.14±0.45	0.4±0.2	0.28±2.8	0.0±0.4
Diphenhydramine 0.25mg/kg (S.C.)	4±1.1	3±0.75	2.1±0.85	1.5±0.7	0.57±0.8	0.14±0.59
Diphenhydramine 0.5mg/kg (S.C.)	4.2±1.3	3.57±1.2 *	2.7±1.2	2±0.89	1.1±0.8	0.42±0.8
Diphenhydramine 1mg/kg (S.C.)	4.4±1.7 *	3.42±1.25 *	3±0.95	2.2±0.94	1.5±0.6	0.5±0.6
Diphenhydramine 2mg/kg (S.C.)	5±1.6 *	4.42±0.86 *	3.42±1.04 *	2.7±1.08	1.7±0.9	0.5±0.6
Diphenhydramine 4mg/kg (S.C.)	5.7±1.3 *	5.14±1.42 *	5.14±1.4 *	4.2±0.99 *	3.2±0.8 *a	2.4±0.7 *a
Diphenhydramine 8mg/kg (S.C.)	6.7±1.6 *	5.8±1.56 *	5.85±1.5 *a	5.2±1.4 *abc	3.5±1.1 *abc	2.7±1.0 *abcd

*

significant with control were $p \leq 0.05$.

Mean \pm SE for seven groups in second

a: significant with diphenhydramine 0.25mg/kg were $p \leq 0.05$

b: significant with diphenhydramine 0.5mg/kg were $p \leq 0.05$

c: significant with diphenhydramine 1mg/kg were $p \leq 0.05$.

d: significant with diphenhydramine 2 mg/kg were $p \leq 0.05$.

The time effective curve of different doses of diphenhydramine were shown in (Figure, 3)

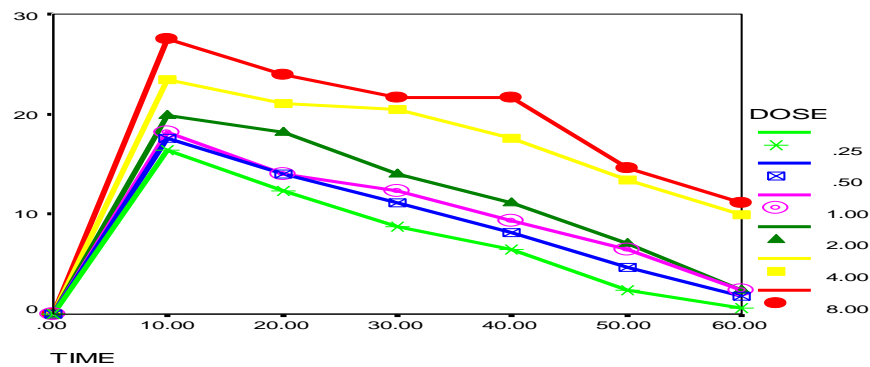


Figure (3) The time effective curve of diphenhydramine.

The changes of MPE% usually reach to the baseline (3%) after 60 min. after the administration of drug except the two higher doses (4,8mg/kg s.c.) is (12%), (13.2%) respectively. The maximum effective doses do not always occur at the same time and the shapes of time effective curve vary with doses, and (10-60) minute, the best time of diphenhydramine action is 10 minutes. After injection of diphenhydramine indicated to mean duration of analgesic effect of diphenhydramine (Figure3).

The dose-response curve for different doses of diphenhydramine shown in (Figure 4).

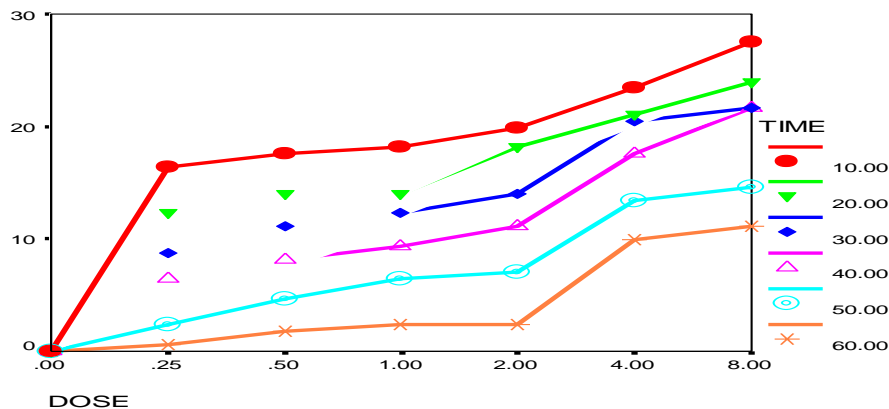
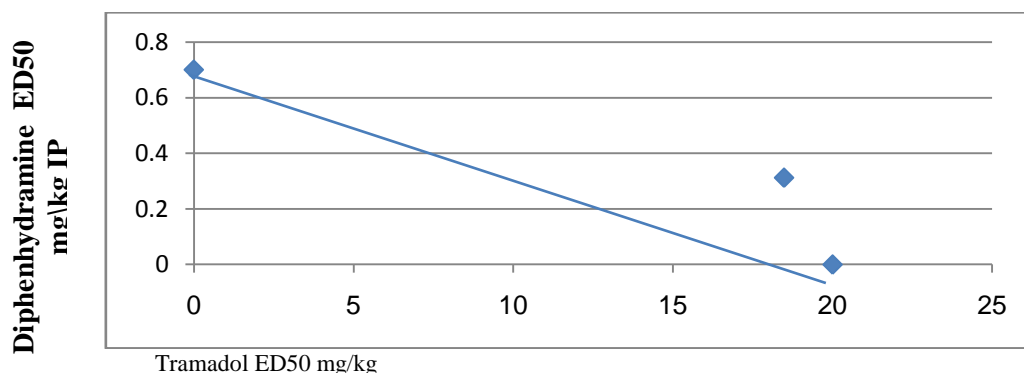


Figure (4) The dose effective curve of diphenhydramine.

Diphenhydramine produced a dose dependent changes in MPE% in comparison to control, the increase in dose lead to increase in MPE% changes. The maximum possible effective MPE% occurring at 8mg/kg is (27.4%) in 10 minute.

Experiment 3: Isobolographic analysis of the antinociceptive interaction between tramadol and diphenhydramine:

Isobolographic analysis of ED50 for both drugs of tramadol and diphenhydramine either alone or in combination indicated that the combined administration of the two drugs has an antagonistic effect on the antinociceptive effect of tramadol in mice as in (Figures 5 a, b, c, d and e). The antagonist effects were established by the location of the point, representing the specific level of response when combined ED50 of tramadol and diphenhydramine above the line that connect between ED50 of tramadol and diphenhydramine when given each one alone (Figure, 5a, b, c, d and e).



Figure(5a):Isobologram showing interaction between tramadol and diphenhydramine in ratio (1:0.5).

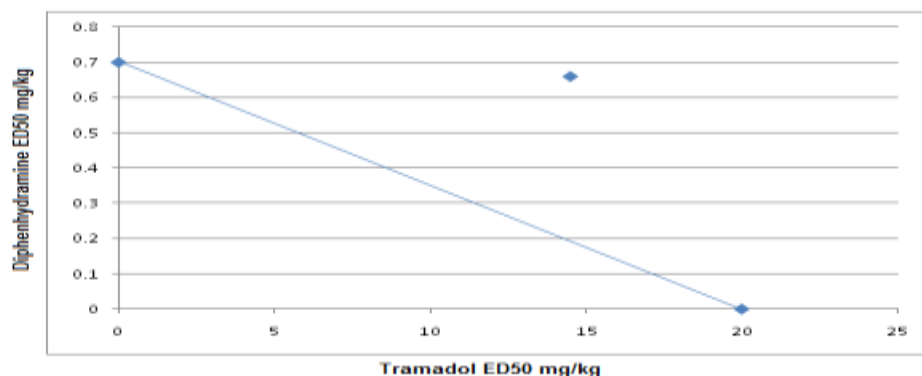


Figure (5 b) Isobologram shows interaction between tramadol and diphenhydramine in ratio (1:1)

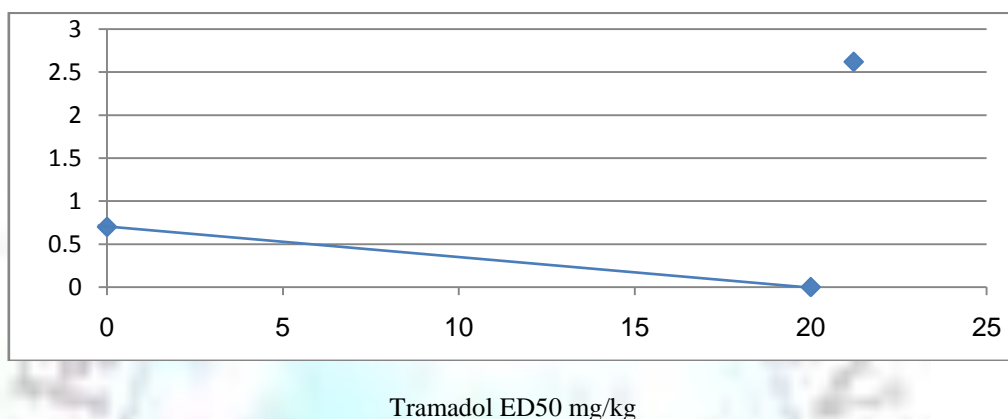


Figure (5 c) Isobologram shows interaction between tramadol and diphenhydramine in ratio (1:2)

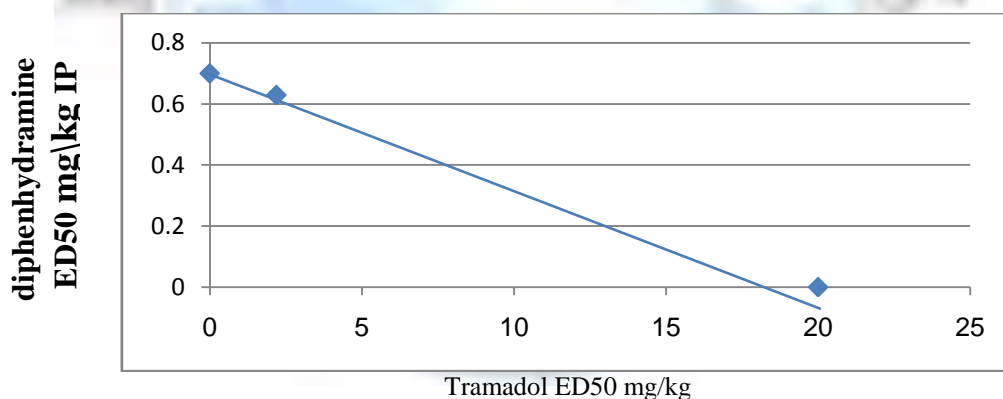


Figure (5,d) Isobologram shows interaction between tramadol and diphenhydramine in ratio (0.25:1).

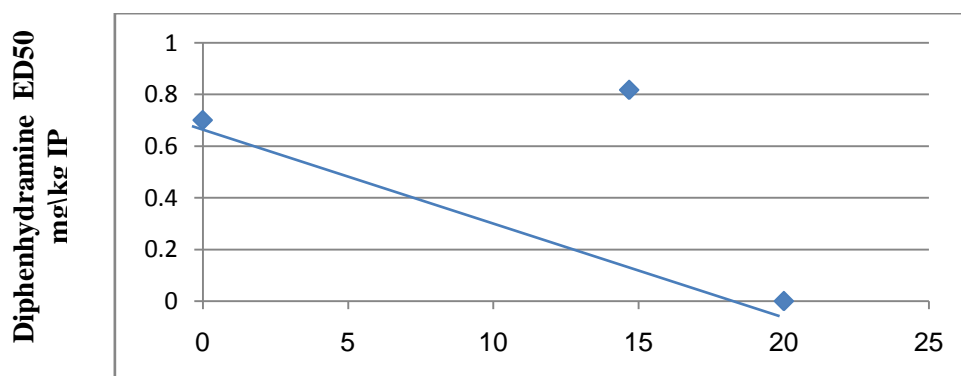


Figure (5, e) Isobologram shows interaction between tramadol and diphenhydramine in ratio (0.5:1)

Furthermore the calculated interaction for antinociceptive between tramadol and diphenhydramine in ratio (1:0.5, 1:1, 1:2) were equal (1.815), (1.667) and (5.081) respectively. Also, when used tramadol and diphenhydramine in the following ratio (25:1 and 0.5:1) were equal (1.336) and (2.634) respectively, this result indicating an antagonistic interaction between tramadol and diphenhydramine an index $Y > 1$ indicate antagonism (Table3).

Table (3) Effect of tramadol and diphenhydramine and their different ratio combined with the hot-plate test.

Tramadol /diphenhydramine Combination ratio	ED50 mg/kg Used dose ratio	ED50 mg/kg Combined dose effect	Y
Tramadol alone	20mg/kg	–	–
Diphenhydramine alone	0.7mg/kg	–	–
1:0.5	20:0.35	18.48:0.312	1.815
1:1	20:0.7	14.5:0.66	1.667
1:2	20:1.4	21.22:2.62	5.081
0.25:1	5:0.7	2.19: 0.629	1.336
0.5:1	10:0.7	14.676:0.8169	2.634

DISCUSSION

Tramadol is considered as centrally acting, safe and effective analgesic. It has been used during the last two decades to treat several types of pain⁽¹⁵⁾. In the present study, the analgesic properties of tramadol was observed by using a hot – plate test in mice, several studies indicated that the thermal painful stimuli are known to be selective to centrally, but not peripherally acting analgesic drug⁽¹⁶⁾.

The present study showed that the antinociceptive effect of tramadol is started after 30 min from drug injection and lasted about two hours after tramadol administration. The maximum effect shown in about 40 minutes after tramadol intraperitoneal administration. These results were in agreement with other studies about analgesic effect of tramadol in humans⁽¹⁷⁻¹⁹⁾ and different species of animals intraoperative antinociceptive and post-operative analgesia⁽²⁰⁾.

The antinociceptive effect of tramadol may be due to the drug is a racemic mixture of two enantiomers which are metabolized in the liver to the active metabolite (M1)⁽²¹⁾. Several studies have established that (+M1) has a higher affinity to μ -opioid receptor than the parent (\pm) tramadol⁽²²⁾. In addition, there is an evidence that non – opioid mechanism are involved in the analgesic properties of tramadol; in particular the inhibition of neuronal reuptake of both norepinephrine and serotonin⁽²³⁾. The ability of tramadol to bind with μ – receptor agonist cause to increase the extracellular concentration of 5 –HT, which enhances the effect of analgesia⁽²⁴⁾.

To explain the role of tramadol mechanism of action on non – opioid receptors or inhibit norepinephrine and serotonin reuptake, we must know that noradrenergic descending and the serotonergic system innervate all levels of the spinal cord and can modulate afferent pain signal this level⁽²⁵⁾ to produce antinociception. This may explain the role of tramadol due to its mechanism of action on opioid and as inhibitor reuptake at the level of the spinal cord to produce antinociceptive effect of tramadol.

Also, this study showed that tramadol dose – dependently increase the percentage of antinociceptive effect in mice. This resulting agreement with other study suggested that increase in the dose of tramadol lead to increase in the duration and potency of tramadol analgesic effect⁽²⁶⁾ and agreement with other studies observed that each of μ -receptor agonist produces analgesia in dose related manner⁽¹⁸⁾.

In this study, the analgesic activity of diphenhydramine was studied and evaluated using the hot – plate test in mice. Data obtained from the present study indicated that diphenhydramine can increase pain threshold reaction time in mice, and it produces an inhibitory effect on the nociceptive response in hot plate test. The analgesic effect is dose dependent, and last for one hour while it reach the maximum analgesic effect after 10 minutes of drug subcutaneous administration. This may be due to the similarity of its properties with lidocaine or other classical local anesthetics which causing blockage to Na – channel⁽²⁷⁾.

Diphenhydramine as some other antihistamine can also block the fast sodium channels and produce local anesthetic and analgesic effect. There is improved by several studies about these drugs generally possess sodium channel blocking properties, and experiments involving animal models of neuropathic pain (rat) that have in fact revealed that the sodium channel blocking agents exhibit analgesic effect⁽²⁸⁾.

Combining drugs with similar or different effect may result in synergistic, additive or antagonistic interaction. The analysis of the interaction was accomplished using isobolograms and interaction indexes, which are well established as valid methods to assess drug – drug interaction when both drugs showed a significant antinociceptive effect when administered individually. The antinociceptive antagonism obtained by combination of tramadol and diphenhydramine does not previously studied.

In the present study, the isobolographic method was used to determine the type of interaction between tramadol and diphenhydramine in mice, this interaction was shown to be as antagonistic analgesic effective.

This antagonist effect may be due to the action of two drugs at the same site of metabolism. The first generation H_1 – antihistamines are classified into different groups according to their chemical structure and all of them are metabolized by cytochrome P450 in the liver⁽²⁹⁾, most classical H_1 antihistamines are metabolized by CYP2D6, and some of them by CYP3A4⁽³⁰⁾. Studies based on the use of diphenhydramine as an example of a first – generation H_1 – antihistamine has shown that these drugs are not only CYP2D6 substrates, but also inhibit this pathway of cytochrome P450^(30, 31).

This should be considered when other drugs that use this metabolic pathway are administered concomitantly such as tricyclic antidepressants, beta – blockers – metoprolol, antipsychotics and tramadol^(30,32). Tramadol is metabolized to M1 by the CYP2D6 isoenzyme of the cytochrome P-450 enzyme system and is a substrate form of CYP2D6 isoenzyme while diphenhydramine is acted as inhibitors for this enzyme and so it inhibits the conversion of tramadol prodrug in to it's active M1 metabolite⁽³³⁻³⁵⁾. So, this explains the result in the present study that diphenhydramine is antagonist for the tramadol analgesic effect. This result is similar to the study in which that diphenhydramine found to be inhibitor of the action of metoprolol due to the interaction of two drugs on the CYP2D6. The hepatic metabolism of the β_1 – selective adrenoceptor antagonist metoprolol depends in part on the genetically determined activity of the cytochrome P – 450 2D6 (CYP2D6) isoenzyme⁽³⁶⁾, and where diphenhydramine is CYP2D6 inhibitor, it can inhibit the metabolism of metoprolol⁽³⁷⁾.

CONCLUSIONS

Both tramadol and diphenhydramine alone have analgesic effect and increase the antinociceptive effect by increasing the dose. The analgesic effect of diphenhydramine is weaker than tramadol but when a combination of these two drugs, causing antagonist interaction and decrease the analgesic activity of tramadol.

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