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EVALUATION THE ANTINOCICEPTIVE EFFECTS OF METOCLOPRAMIDE AND THEIR INTERACTION WITH DIPHENHYDRAMINE IN ACUTE MODEL OF PAIN IN MALE MICE

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ABSTRACT

The aim of this study was to evaluate the analgesic effect of metoclopramide alone or as a combination with diphenhydramine. The type of interaction between two drugs also determined at level of acute pain centrally and peripherally using hot-plate and writhing test in male mice. The individual ED_{50} value of metoclopramide and diphenhydramine for induction of antinociceptive effect was at 30.7 mg/kg,ip and 0.57mg/kg, sc ,respectively. While combined this ED_{50} value of diphenhydramine and metoclopramide at several ratios (1:1, 0.5:1, 0.5:0.5 and 0.25:1) respectively, produced synergism interaction between two drugs at all ratios (except the final ratio, produced antagonism). This combination decreased the ED_{50} of each drug and produced good antinociceptive effect in male mice at centrally level. Concomitant administration of metoclopramide ip and diphenhydramine sc at double doses of ED_{50} for each drug, also produced good analgesic effect at visceral pain level, which induced by acetic acid in writhing test. Avery effective and safe antinociceptive effect and also achieved using combination of metoclopramide and diphenhydramine at sedative doses (not analgesic).The results suggested that synergism (super-additive) interaction of diphenhydramine and metoclopramide combination was a good and safe antinociceptive effect produced by a combination of sedative dose for each drug . The combination of these drugs very important in practice of the veterinary medicine.

INTRODUCTION

Metoclopramide is a benzamide derivatives, gastroprokinitic agent, used as antiemetic in animals (1,2 and 3) and human (4,5), its used also in a cases of reflux esophagitis in dog (6) and man (7). This effect related to their antagonize of dopamine D2-receptors centrally and peripherally (2,7 and 8), inhibition of serotonin receptors 5-HT3 (9,10) and agonist of 5-HT4 receptors (11,12). As well as to indirect cholinergic activity (13) that lead to promotes release the neurotransmitter acetylcholine (11). Metoclopramide has sedative and hypnotic effects alone (14) or with Ketamine in chicken (15), the sedative effect also reported in man (5). The drug has been successes as analgesic in human (16, 17), mice (18, 19) rats (20) and in dogs (21). Many combinations with metoclopramide used to improve the analgesic effect such as metoclopramide with diphenhydramine in human to treat headache (22), decrease emesis and nausea after surgery (23, 24). Metoclopramide also used with tramadol to produce analgesia, that equal to morphine (opioid agent) after surgery in man (25). The mechanism related with the analgesic effect of metoclopramide not understands yet.

Diphenhydramine is an ethanolamine derivative, which cross blood brain barrier (26, 27). It's the oldest first generation H1-receptors antagonist, in human and animals (28, 29). The drug is commonly used as anti-allergy medication for treatment of rhinitis, anaphylaxis, colds, motion sickness, agitation and insomnia (30, 31). It has also anticholinergic properties (29, 27) ant muscarinic, and antiadrenergic in human (32, 33) and animals (34). Used to treat cancer pain (35), and as local analgesic by block sodium channel (32,36). It has anti-inflammatory properties in mice (37). Combination of diphenhydramine with Ibuprofen, produced synergism effects in human by increase analgesia and anti-inflammatory action of Ibuprofen (38). Diphenhydramine also potentiate the analgesic effect of morphine in mice (39). However; the antinociceptive effect of metoclopramide and diphenhydramine combination in mice not examined in previous studies , and the aim and introduction of the current study was evaluated the antinociciption effect of this combination in male mice, as well as detected the type of interaction by using thermal method (hot plate) (40,42 and 66) and chemical method (writhing reflex) (19,41 and 42) .

MATERIALS AND METHODS

In this study we used male albino Swiss mice weighing (20-35) g, housed in animal house appurtenant to Veterinary Medicine of the university of Mosul/Iraq. At standard circumstance of temperature (22± 2c°), ventilation and 10 hr light/14 hr dark. Animals housed in rodent plastic cages (17x20x30) cm, food and water were given *ad libitum* and complete care of mice until reaching 2 months. The doses of metoclopramide and diphenhydramine were prepared by dissolve pure powder of each drugs in physiological saline solution directly before the experiment, the volume of administration of each drugs was at 5ml/kg, body weight in all experiments.

Experiment 1: Determine the individual median analgesic dose (ED50) of metoclopramide (ip) and diphenhydramine (sc) in male mice by up and-down method (43).

6 male mice were used for each drug, weight (19-30) g. The initial dose of metoclopramide (Vaikunth,India) was at 20 mg/kg i.p., while for diphenhydramine (Samanta Organics PVT,India) was at 1 mg/kg s.c., the doses were choices from preliminary experiments and previous studies (19,44), The increase or decrease in the later doses of metoclopramide and diphenhydramine was at 5,0.25 mg/kg, respectively . Analgesic effect was measured using the hot plate test (thermal method) (45), at a temperature of 56 C°. Animals were placed individually on the hot plate (Heidolph Me Hei-standard, Germany) before administration of drug and recorded response as latency time in seconds (first removal of fore or hind paw and licking or/and jumping, shaking) , then recorded latency time 10 min after injection of metoclopramide i.p. or diphenhydramine s.c. by placed the same mice on the hot plate. The cut-off time for analgesia was 20 s (the maximum time was allowed the animal to stay on the hot plate to avoid damage of the paw tissue (19,42,66). The ED50 value was calculated according to the formula (43):

ED50= Xf + Kd

Xf: final dose

K: table value

d: increase or decrease in dose(constant)

Dixon table:

Second part from series	K represent series tests that begin as at					Stander error
	O	OO	OOO	OOOO		
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX	0.61
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO	
XOXO	0.701	0.737	0.741	0.741	OXOX	
XOXX	0.084	0.169	0.181	0.182	OXOO	
XXOO	0.305	0.372	0.380	0.381	OOXX	
XXOX	0.305-	0.169-	0.144-	0.142-	OOXO	
XXXO	1.288	1.500	1.544	1.549	OOOX	
XXXX	0.555	0.897	0.985	1.000	OOOO	
	X	XX	XXX	XXXX	Second part from series	
	- K represent series tests that begin as at					

Experiment 2: Determination the type of drug interaction between metoclopramide and diphenhydramine as a combination for induction antinociceptive effect in male mice: At Ratio of 1:1

7 male mice were used at 2 months age , weighing (20-28) g selected randomly. We tested the mice as same previous experiment on hot pate to determined latency time. The initial doses administrated to the first mice as a combination were the ED50 of metoclopramide and that of diphenhydramine (30.7mg/kg, and 0.57mg/kg) respectively, which detected previously in experiment 1. The increase and decrease in the ED50 value of metoclopramide and diphenhydramine was (7.5 mg/kg and 0.14 mg/kg) respectively. Then the individual ED_{50} of each drug alone and that of a combination of two drugs at ratio 1:1 subjected to isobolographic analysis, to explore the type of interaction between two drugs (46,47,48,51) in producing antinociceptive effect in mice.

Dependent on the Isobolographic analysis, used diagram paper and pointed the individual ED_{50} value of diphenhydramine on the X axis and that of metoclopramide on the Y axis, then drew a straight diagonal line between the individual ED_{50}. The ED_{50} value for the combination then detected as if pointed above (right) or below (left) the line indicate antagonism or synergism interaction respectively , while located on the diagonal line represent additive (no interaction) (15,42,48,49).

For more explore the type of interaction , we used the interaction index from equation : Y= da/Da + db/Db (48), as: Da and Db was individual value of ED_{50} of metoclopramide and diphenhydramine respectively , while da and db was the value of combination the ED50 of both drugs. If Y value equal 1 means no interaction (additive), Y > 1 that means sub additive (antagonism) interaction, while Y < 1 means synergism (super-additive) interaction. The percentage of reduction in the ED50 of both drugs was calculate by following]formula:

% in reducing of ED_{50}= ED_{50} (individual) – ED_{50} (combination)/ED_{50} (individual)

As the same of previous experiment, we injected metoclopramide i.p. with diphenhydramine s.c. as a combination at various ratio : 0.5:1 , 0.5:0.5 , 0.25:1 respectivley and detected the type of interaction for antinociceptive effect at those ratios as previously.

Experiment 3: Effect of metoclopramide and diphenhydramine on the visceral pain in male mice (writhing reflex) chemical method (41,42,45).

Randomly selective 20 male mice weighing (18-30)g which divided in to 4 groups, each group consist of 5 animals : Group 1 (control) injected with 1% Acetic acid ip at (0.1 ml/10 g) which causes visceral pain (writhing reflex), the onset of writhes and the number of writhes recorded during 20 min after injection (19,42). Group 2, 3 individually injected with metoclopramide ip or diphenhydramine sc at double doses of ED_{50} (61.4 mg/kg and 1.14 mg/kg) for each drug, respectively 15 min before injection of acetic acid ip in the same animal. Then recorded the onset of writhes and the number of writhes during 20 min, while the animals in group 4 injected with metoclopramide ip and diphenhydramine sc at (61.4 and 1.14) mg/kg, respectively (as a combination)15 min before injection of acetic acid ip in the same animal , the onset and number of writhes also recorded during 20 min . In order to

calculate the percentage of reduction in the number of writhes in each group used the following formula (40,42):

(N control – N test/ N control) X100

N: number of writhes for each group.

Experiment 4: Effect of metoclopramide and diphenhydramine alone or as a combination at sedative doses (non-analgesic) on the acute pain in male mice.

Divided 20 mice weighing (20-33)g ,selective randomly; in to 4 groups, each group consist of 5 animals treated as follows: Group 1 received normal saline(0.9%) ip at (5 ml/kg) body weight, Group 2,3 were individually received sedative doses (non analgesic doses) of metoclopramide ip at 10 mg/kg or diphenhydramine sc at 0.5 mg/kg (the doses choice according preliminary experiments). While Group 4 received a combination of metoclopramide ip and diphenhydramine sc at non analgesic doses (10 and 0.5) mg/kg , respectively. Each mice was tested in all groups before and 10 min after injection of drug on the hot plate as previously mentioned in experiment 1.

Statistical analysis:

The data in experimental 3 and 4 were analyzed by One Way analysis of variance (ANOVA), then to detect the significant between groups the data subjected to the Least significant test LSD (50). While the score data such as writhing test were statistically analyzed by Mann- whitney U test (50,51,52). The level of significance in all experiments was at ($p < 0.05$).

RESULTS

Experiment 1: Determine the individual median analgesic dose (ED50) of metoclopramide (ip) and diphenhydramine (sc) in male mice by up and-down method. Several doses of metoclopramide and diphenhydramine injected individually in numbers of mice to detect the individual ED50 value for metoclopramide and diphenhydramine explore (30.7 mg/kg and 0.57 mg/kg) respectively (Tab.1). Animals that treated with metoclopramide and diphenhydramine appeared symptom of sedation, quiet and reduce movement.

Table(1).Median analgesic dose of metoclopramide and diphenhydramine individually in male mice by hot plate test:

Variable	Metoclopramide ip	Diphenhydramine SC
ED_{50}	**30.7 mg/kg**	**0.57 mg/kg**
Range of the doses used	**35-20 mg/kg**	**1-0.5 mg/kg**
Initial dose	**20 mg/kg**	**1 mg/kg**
Last dose	**35 mg/kg**	**0.75 mg/kg**
Increase or decrease in the dose	**5 mg/kg**	**o.25 mg/kg**
Number of mice used	**OOXOOX 6**	**XXOXOX 6**

X analgesia O no analgesia

Experiment 2: Determination the type of drug interaction between metoclopramide and diphenhydramine as a combination for induction antinociceptive effect in male mice:

The individual ED50 of metoclopramide and diphenhydramine which induced analgesia in male mice were 30.7 mg/kg and 0.57mg/kg respectively , when combined metoclopramide ip with diphenhydramine sc at different ratio of ED50 value for each drug produce reduction in the ED50 value for each drug at several ratio :1:1, 0.5:1, 0.5:0.5 to (69.3 %, 66.7 %);(67.9% 35 %) and (59.7 %, 68.4 %) respectively, shown its in Tab 2,3,4 and 5 respectively.

Depended on Isobolographic analysis, determined the type of interaction between both drugs at each ratio, that is shown in (figure 1, 2, 3 and 4) respectively. All the value of ED50 for each combined was located under the diagonal line which connected between the individual ED50 value for metoclopramide and that for diphenhydramine , that indicated the synergism interaction between the two drugs at each ratio as shown in Figure (1,2,3), except at ratio 0.25:1 the value of ED50 for combined of the individual ED50 of two drugs located above the diagonal line, therefor the combination of metoclopramide and diphenhydramine at this ratio was antagonist (Figure 4).

Table (2). Determination the median analgesic doses (ED50) of metoclopramide ip and diphenhydramine sc as a combination in male mice at ratio 1:1

Variable	Metoclopramide and Diphenhydramine	
	Metoclopramide	Diphenhydramine
ED_{50}	9.44 mg/kg	0.19 mg/kg
Range of the doses used	30-7.5 mg/kg	0.57-0.15 mg/kg
Initial dose	30 mg/kg	0.57 mg/kg
Last dose	15 mg/kg	0.29 mg/kg
Increase or decrease in the dose	7.5 mg/kg	o.14 mg/kg
Number of mice used	XXXOXOX 7	XXXOXOX 7
% Reduced in ED_{50}	69.3%	66.7%
Y (interaction index)	0.63	0.63

X analgesia **O no analgesia**

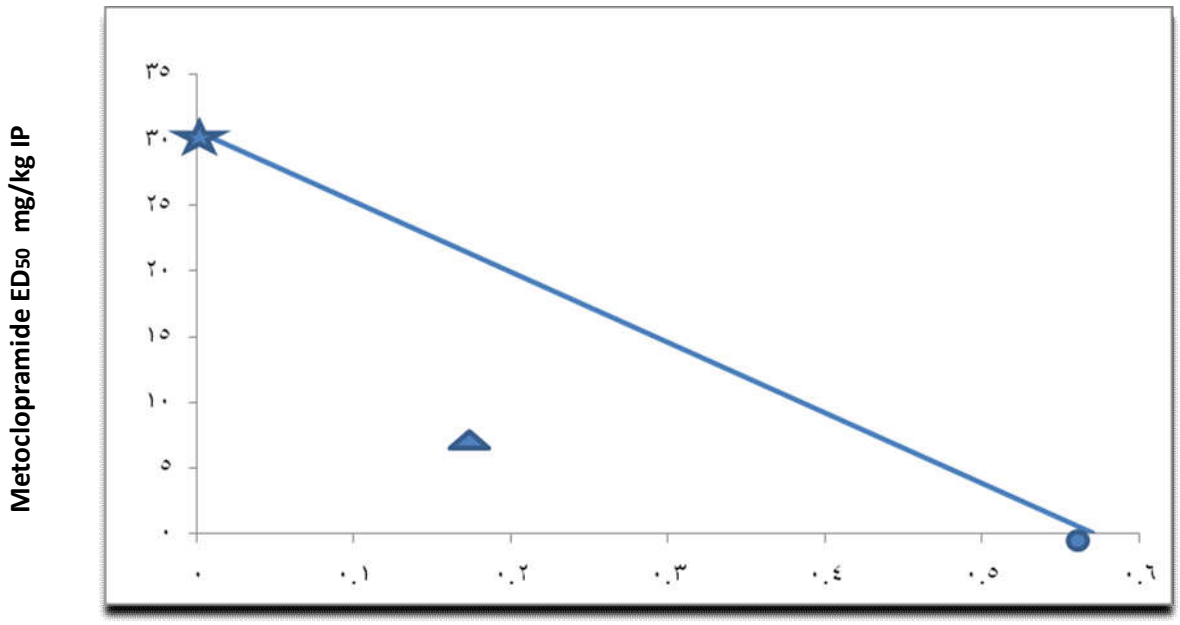

Figure 1: Determination the type of interaction between metoclopramide ip and diphenhydramine sc by Isobolographic analysis at a ratio 1:1. The interaction between two drugs was synergism.

★ ED_{50} of metoclopramide at (30.7) mg/kg , IP

⬭ ED_{50} of diphenhydramine at (o.57) mg/kg , SC

▲ the ED_{50} of metoclopramide ip at (9.44) mg/kg and ED50 of diphenhydramine sc at (0.19) mg/kg at 1:1

Table (3). Determination the median analgesic doses (ED_{50}) of metoclopramide ip and diphenhydramine sc as a combination in male mice at ratio 0.5:1

Variable	Metoclopramide and Diphenhydramine Metoclopramide	Diphenhydramine
ED_{50}	**9.85 mg/kg**	**0.37 mg/kg**
Range of the doses used	**15-7.5 mg/kg**	**0.57-0.29 mg/kg**
Initial dose	**15 mg/kg**	**0.57 mg/kg**
Last dose	**11.25 mg/kg**	**0.43 mg/kg**
Increase or decrease in the dose	**3.75 mg/kg**	**o.14 mg/kg**
Number of mice used	**XXOOXX 6**	**XXOOXX 6**
% Reduced in ED_{50}	**67.9%**	**35%**
Y (interaction index)	**0.96**	**0.96**

X analgesia **O no analgesia**

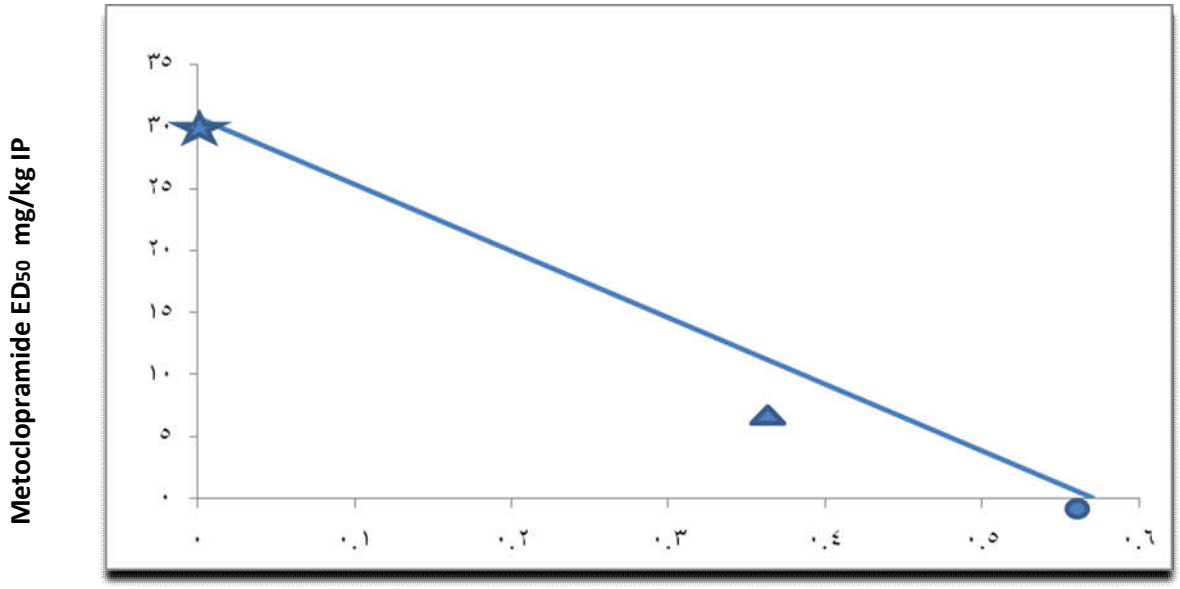

Figure 2: Determination the type of interaction between metoclopramide ip and diphenhydramine sc by Isobolographic analysis at ratio 0.5:1. The interaction between two drugs was synergism.

★ **ED_{50} of metoclopramide at (30.7) mg/kg , IP**

⬭ **ED_{50} of diphenhydramine at (o.57) mg/kg , SC**

▲ **the ED_{50} of metoclopramide ip at (9.85) mg/kg and ED_{50} of diphenhydramine sc at (0.37) mg/kg**

Table(4). Determination the median effected analgesic doses (ED_{50}) of combination for metoclopramide ip and diphenhydramine sc in male mice at ratio 0.5:0.5

Variable	Metoclopramide and Diphenhydramine	
	Metoclopramide	Diphenhydramine
ED_{50}	12.37 mg/kg	0.18 mg/kg
Range of the doses used	15-11.25 mg/kg	0.28-0.14 mg/kg
Initial dose	15 mg/kg	0.28 mg/kg
Last dose	15 mg/kg	0.28 mg/kg
Increase or decrease in the dose	3.75 mg/kg	o.14 mg/kg
Number of mice used	XOXOX 5	XOXOX 5
% Reduced in ED_{50}	59.7%	68.4%
Y (interaction index)	0.72	0.72

X analgesia O no analgesic

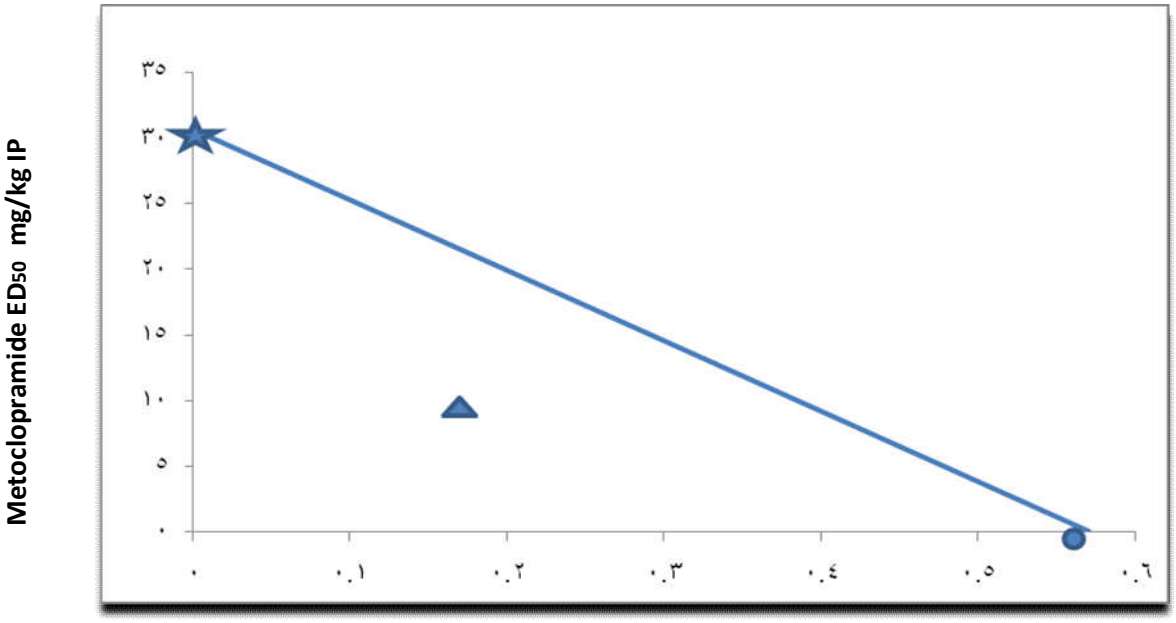

Figure 3: Determination the type of interaction between metoclopramide ip and diphenhydramine sc by Isobolographic analysis at a ratio 0.5:0.5. The interaction between two drugs was synergism.

★ ED_{50} of metoclopramide at (30.7) mg/kg , IP

⬭ ED_{50} of diphenhydramine at (o.57) mg/kg , SC

▲ the ED_{50} of metoclopramide ip at (12.37) mg/kg and ED_{50} of diphenhydramine sc at (0.18) mg/kg

Table (5). Determination the median effected analgesic dose (ED_{50}) of combination for metoclopramide ip and diphenhydramine sc in male mice at ratio 0.25:1

Variable	Metoclopramide and Diphenhydramine	
	Metoclopramide	Diphenhydramine
ED_{50}	8.83 mg/kg	0.67 mg/kg
Range of the doses used	9.4-7.5 mg/kg	0.71-0.57 mg/kg
Initial dose	7.5 mg/kg	0.57 mg/kg
Last dose	7.5 mg/kg	0.57 mg/kg
Increase or decrease in the dose	1.9 mg/kg	o.14 mg/kg
Number of mice used	OXOXO 5	OXOXO 5
Y (interaction index)	1.46	1.46

X analgesia **O no analgesia**

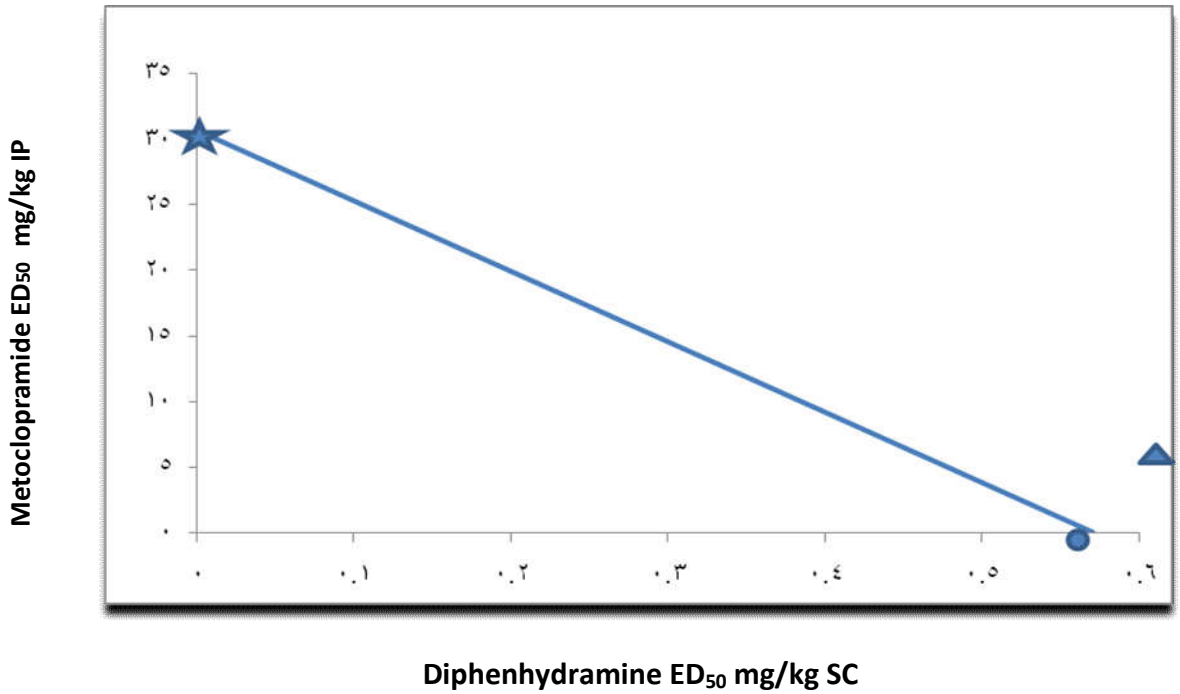

Figure 4: Determination the type of interaction between metoclopramide ip and diphenhydramine sc by Isobolographic analysis at ratio 0.25:1. The interaction between two drugs was antagonism.

★ ED_{50} of metoclopramide at (30.7) mg/kg , IP

⬭ ED_{50} of diphenhydramine at (o.57) mg/kg , SC

- The ED_{50} of metoclopramide ip at (8.83) mg/kg and ED_{50} of diphenhydramine sc at (0.67) mg/kg

Experiment 3: Effect of metoclopramide and diphenhydramine on the visceral pain in male mice (writhing reflex) chemical method.

Injection of metoclopramide alone at (double doses ED_{50}) ip (61.4) mg/kg in male mice significantly increased the time of onset and decreased the writhing number in comparison with the control and diphenhydramine group Table (6). While there was no significant effect of diphenhydramine at dose of (1.14) mg/kg on the onset and number of writhing. While there was a significant increase in the time of onset and decreased the number of writhing in group 4 (combination of two drugs at double doses of ED_{50} (61.4, 1.14) mg/kg respectively by (80%), in comparison with control (0%), diphenhydramine (3%) and metoclopramide (42%), at a level of significant ($p < 0.05$) (Table 6, Figure 5).

Table (6). Analgesic effect of metoclopramide ip and diphenhydramine alone or as a combination on writhing reflex (chemical methoed) in male mice.

Treatments	Latency to Onset of writhing Mean ±SE	Writhing number Mean ±SE	% Reduce in writhing number
Control (1% acetic acid),ip	2.25± 0.47	35.5± 6.27	0%
Metoclopramide 61.4 mg/kg, ip	6.25± 0.25*a	20.5± 3.32*a	42%
Diphenhydramine 1.14mg/kg, sc	2.75± 0.25	34.5± 0.95	3%
Metoclopramide 61.4mg/kg, ip + Diphenhydramine 1.14mg/kg, sc	6.75±0.25* a	7± 1.47*ab	80%

Values are mean ± SE of five mice /group

Diphenhydramine was injected directly after metoclopramide

* : significantly different from the control group at $p<0.05$

a :significantly different from diphenhydramine at dose (1.14) mg/kg at $p<0.05$

b :significantly different from metoclopramide at dose (61.4)mg/kg at $p<0.05$

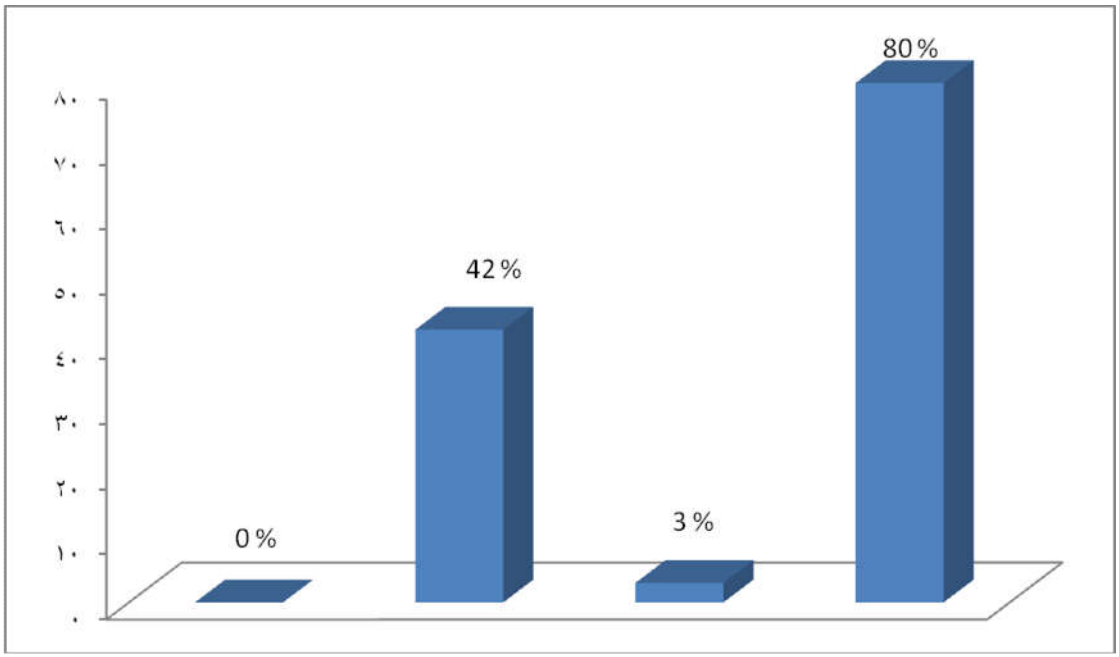

Figure 5. Effect of metoclopramide and diphenhydramine alone or as a combination on the visceral pain in male mice (writhing reflex) chemical method

Experiment 4: Effect of metoclopramide and diphenhydramine alone or as a combination in sedative doses (non-analgesic) on the acute pain in male mice.

Administration of metoclopramide at (10) mg/kg, ip and diphenhydramine at (0.57) mg/kg, sc (sedative doses) each alone produced non-significant antinociceptive effect 30 min after injection, in comparison with control group, , while a combination of metoclopramide ip and diphenhydramine sc (at sedative doses 10, 0.5 mg/kg respectively) produced significant antinociceptive effect at 100% in group 4 in compared with the control group and groups of metoclopramide or diphenhydramine each alone. (Tab. 7).

Table (7). Effect of metoclopramide and diphenhydramine in sedative doses (non-analgesic) on the acute pain, hot plate (thermal method) in male mice.

Treatment	Latency time Mean ±SE (Base line)	Latency time after 30 min Mean ±SE	% of analgesic pain
Control (normal saline 0.9%) ip	5.1 ± 1	4.06 ± 1.03	0%
Metoclopramide 10 mg/kg ip	4.74 ± 0.8	4.2 ± 0.75	0%
Diphenhydramine 0.5mg/kg sc	6 ± 1.7	3.9 ± 0.4	0%
Metoclopramide 10 mg/kg ip + Diphenhydramine 0.5mg/kg sc	4.7 ± 1.06	8 ± 1.15*ab	100%

Values are mean ± SE of five mice /group

Diphenhydramine was injected directly after metoclopramide

* : significantly different from the control group at $p<0.05$

a :significantly different from metoclopramide alone at dose (10) mg/kg, at $p<0.05$

b :significantly different from diphenhydramine at dose (0.5)mg/kg, at $p<0.05$

DISCUSSION

In this work we acted more focus on the analgesic effect of metoclopramide alone or as a combination with diphenhydramine. Whereas metoclopramide is antiemetic drug, but recently many reports have been proved the analgesic effect of metoclopramide alone (17,18) or as a combined with other analgesic drugs such as ketamine (19) or with tramadol (25). In the current study, the analgesic effects of metoclopramide evaluated by using the hot-plate test to exam the centrally pain and chemical test (writhing reflex) to evaluate viscerally pain in mice(19,42,64). As well as determined the analgesic effects of metoclopramide and diphenhydramine as a combination by using ED_{50} value, the type of interaction between them also evaluated using isobolographic analysis and interaction index (Y value)(46,47,48).

Diphenhydramine is H1 receptor antagonist, it's the oldest first generation anti-histamine drug (26,27,28), several study confirmed the analgesic effect of diphenhydramine alone (35,37) or as a combined with other drugs such as with tramadol (44), ibuprophin (38) and morphine (39), However; the present study has been determined the analgesic effect of metoclopramide and diphenhydramine each alone or as a combination in mice, which was not reported in previous study. The combination of drugs may produce antagonism, additive or synergism interaction. In the present study we established the synergism interaction between two drugs at level of antinociceptive effects. The individual ED_{50} value for each drug also detected by using up and down method (43) which was (30.7) mg/kg and (0.57) mg/kg for metoclopramide and diphenhydramine respectively , this data was in accordance with previous study for metoclopramide (19) and diphenhydramine (44) in mice.

By used different ratios of ED_{50} for each drug (1:1, 0.5:1: and 0.5:0.5) , the result was synergism interaction for each ratio and this may be due to each drug act on different receptors (pharmacodynamics) to produce their analgesic effect. As metoclopramide act as analgesic drug by antagonism of D2-receptors which correlated with opioid system without connected with opioid receptors (56). Also the drug has been increased prolactin hormone, which connected with opioid system (57, 58) as well as effect as agonist of serotonin 5-HT4 (61), in addition to alter Ca+ cross through cell membrane (59,60). While the analgesic effect of diphenhydramine is due to block sodium channels, that similar to the mechanism of local anesthetic (36). A good visceral antinociceptive effect of this combination also confirmed in the current study by using writhing test (chemical method induced by acetic acid 1%). This effect similar to the combination effect of metoclopramide with ketamine in mice (19), that related to the central and peripheral analgesic effect of metoclopramide as well as present of diphenhydramine with it as a combination , other suggested mechanism for the analgesic effect of the metoclopramide and diphenhydramine combination that metoclopramide metabolize by CYP450 2D6 iso-form (65), the first generation H1– antihistamine diphenhydramine also metabolize by the same enzyme in the liver, as well as it is CYP2D6 inhibitor(62,66,67), so it can inhibit the metabolism of metoclopramide , this may be resulting the level of metoclopramide and stay for prolong time in the body which produce effective analgesia for longer time, Therefore; we demonstrate that the combination of metoclopramide and diphenhydramine can be use as analgesic agent to control the acute pain centrally through increase threshold of the pain (change the latency time) and peripherally as decrease the numbers for writhing reflex in animals comparison with control group, also we succeed in confirmed the good analgesic effect of metoclopramide and diphenhydramine at sedative doses (non-analgesic doses) for each drug when injected as a combination without any overt side effects on mice except docile, this effects not reported previously.

CONCLUSION

The present study explored the antinociceptive effect of metoclopramide and diphenhydramine each alone; in addition the combination of two drugs produced synergism interaction. Also a combination of sedative doses of each drug produced

effective and safe analgesic effect in male mice which very important in Veterinary practice.

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