Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(1): 1288-1292

Received: 27-11-2017
Accepted: 28-12-2017

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Involvement of biogenic amines in antidepressant effect of tramadol in male mice

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Abstract
Tramadol is a centrally acting analgesic drug which is used for treating moderate to severe pain. It is a synthetic opioid which binds weakly to micro (μ) opioid receptor. Several studies have recommended that both opioid and monoaminergic systems play a role in depression which is the disease that occurring due to deficit of norepinephrine and serotonin. Tramadol inhibits the re uptake of norepinephrine and serotonin in the synaptic cleft. The present study was carried out to evaluate the involvement of the interaction of serotonin and noradrenaline in the antidepressant-like effect of tramadol using tail-suspension test (TST) and forced swim test (FST) in acute and after 15 days of treatments. In acute studies (7 days), animals were divided into six groups and each group comprised of six mice. Group 1 was pretreated with normal saline which served as negative control. Group2 was pretreated with standard antidepressant drug imipramine (positive control) at a dose of 10 mg/kg whereas Group 3 and 4 were pretreated with two different doses (20 and 40 mg/kg) of tramadol. Group 5 was treated with a combination of ondansetron 4 mg/kg + tramadol 40 mg/kg while the Group 6 was pretreated with a combination of terazosin 1 mg/kg + tramadol 40 mg/kg. All these drugs were given intraperitoneally (0.1 ml/10gm) for both acute and after 15 days of treatments. Fifteen-minutes of drug administration, the immobility times (seconds) of treated mice were recorded in TST and FST. In the next stage of study after 15 days of treatment procedures were performed with six different groups of mice each group comprising of six mice and arrangement of groups are the same as that in acute study. The results showed that tramadol at a dose of 40 mg/kg significantly (P<0.05) decreased immobility times in both TST and FST in acute and after 15 days of treatment as compared to control group. There were no significant differences in the antidepressant effect in mice administered with tramadol 40 mg/kg and imipramine 10 mg/kg. Ondansetron and terazosin increase the immobility times in both tests when they were given in combination with tramadol 40 mg/kg.It can be concluded that tramadol has the antidepressant-like effect in standard models of depression and the antidepressant activity of tramadol is mediated through the mechanisms that involved serotonergic and noradrenergic systems.

Keywords: Tramadol, antidepressants, imipramine, serotonergic system, noradrenergic system

1. Introduction
Depression is the most common serious psychiatric disorder worldwide. It is a disorder affecting 10-15 percent of people in some part of their lives [1] and high – income countries about 14.6% of all individuals have presented a major depressive episode at least once in their life time [2]. Depression is defined as depressed mood on a daily basis for a minimum duration of 2 weeks which affects people of any age but its incidence increases with age and it is twice more common in females than in males [3]. It could occur due to genetic factors, neurotransmitter dysfunction or due to chronic illness [4]. It is the most common mental disturbance in almost half of patients with chronic pain which is affecting their activities [5]. It is an important to treat depression to have better living. The commonly involved neurotransmitters in depression are noradrenaline and serotonin. The management of depression is a multistep process and antidepressant drugs are the treatment of choice [6]. Commonly among them are SSRIs (Selective Serotonin Reuptake Inhibitors) and TcAs (Tricyclic Antidepressants) which inhibit the reuptake and increase the availability of the neurotransmitters in the synaptic cleft thus enhance the synaptic transmission and relieve depression, however TcAs produce more side effects such as anticholinergic symptoms drowsiness weight gain sedation and sexual dysfunction [4]. The number of patients failing to respond to antidepressant drugs are increasing [7], so there remains need for alternative drug therapies given the commonness morbidity and mortality of depressive illness beside the incomplete efficacy and undesirable adverse effects of available drugs in many patients.

In view of this there is an intense search to identify novel targets for antidepressant therapy. Opioid peptides and their receptors are potential candidates for the development of novel antidepressant drug. Tramadol (Tramal)® is a synthetic 4-phenylpiperidine analogue of codeine which acts as central analgesic with a low affinity for opioid receptors used parenterally and orally for the treatment of moderate to severe pain [8]. Tramadol is largely prescribed by general practitioners for the treatment of various forms of acute and chronic pain and its prescription is not as restricted as the prescription of other opioid agonists such as buprenorphine or oxycodone and it does not have the major reputation of a major substance of abuse, nor is it a desirable substitute for severe opioid drug addiction [9]. Tramadol inhibits reuptake of norepinephrine and serotonin as do antidepressant drugs [10]. It can function as an antidepressant like venlafaxine [11, 12]. In addition tramadol bears close structural similarity to venlafaxine and thus shares a number of its molecular and pharmacological properties [13]. It is a racemic mixture of two enantiomers each of them display different mechanisms (+) tramadol displays opioid agonist properties and inhibits serotonin reuptake while (-) tramadol inhibits norepinephrine reuptake which is similar to that of antidepressant drugs [14]. In the light of above facts the study was conducted to evaluate the role of tramadol as an antidepressant effect and evaluate the role of serotonergic and noradrenergic systems in the antidepressant effect of tramadol in animal models in acute (7 days) and after 15 days periods of administration.

2. Materials and Methods

2.1 Animals

This study on male mice was performed under the guidelines supervision of Ethical Committee for Lab. animals work in the College of Veterinary Medicine, University of Baghdad. This study was carried out at the Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad. Adult albino male mice (Swiss strain) weighing (25-30 gm) were supplied from the animal house of Iraqi Center of Cancer Research, Baghdad, Iraq. The animals inbred at the animal house of the College of Veterinary Medicine, Baghdad. Mice were housed in clean polypropylene cages, six mice in each cage in a controlled environment conditions (22-25 °C) temp. with a 12 hour light and dark cycle. They were fed with commericial pelleted chow and water *ad libitum*. The mice were allowed to acclimatize for these conditions for one week before the beginning of the experiments.

2.2 Drugs and Solutions

Tramadol (50 mg) tablet (Brawn – Laboratories Limited, India) was used. The test solution of tramadol was prepared by dissolving 50 mg tablet in normal saline to get concentrations of 2 and 4 mg/ml respectively and administered at doses of 20 and 40 mg/kg [15].

Imipramine (25 mg) tablet (NOVARTIS, India) was used. The standard solution of imipramine was prepared by dissolving 25 mg tablet in 25 ml of normal saline to get a concentration of 1 mg/ml and administered at a dose of 10 mg/kg [15].

Ondansetron (8 mg) tablet (Bristol Laboratories LTd, UK) was used. The solution was prepared by dissolving 8 mg tablet in 20 ml of normal saline to get a concentration of 0.4 mg/ml and administered at a dose of 4 mg/kg [6].

Terazosin (5 mg) tablet (ALFARES Pharmaceuticals, India) was used. The solution was prepared by dissolving 5 mg tablet in 50 ml of normal saline to get a concentration of 0.1 mg/ml and administered at a dose of 1 mg/kg [16]. All these drugs were administered intraperitoneally (I/P) in a volume of 0.1 ml/10 gm B.W.

2.3 Experimental design

The experiments were performed during the light phase of the cycle and each animal was used only once. To evaluate the antidepressant effect of tramadol and involvement of serotonergic and noradrenergic systems in the antidepressant like effect of tramadol in acute studies (7 days) of treatment, animals were divided into six groups and each group comprised of six mice. Group 1 (negative control) was pretreated with normal saline. Group 2 was pretreated with standard antidepressant drug imipramine at a dose of 10 mg/kg (positive control). Groups 3 and 4 were pretreated with tramadol at doses of 20 and 40 mg/kg respectively. Group 5 was pretreated with a combination of ondansetron 4 mg/kg + tramadol 40 mg/kg while the group 6 was pretreated with a combination of terazosin 1 mg / kg + tramadol 40 mg / kg. All these drugs were given I/P as a single dose once daily for 7 days. To evaluate the antidepressant effect of tramadol and the involvement of serotonergic and noradrenergic systems in the antidepressant effect of tramadol after 15 days of treatment, other different six groups of mice was used and each group comprised of six mice. The arrangement groups as the same as that in the acute study. The drugs were administered once daily for 15 days and on the 7th (acute study) and 15th days of administration the mice in all groups after 15 minutes of drugs administration were tested in tail suspension (TS) and forced swim (FS) tests.

2.4 Antidepressant Test Models

2.4.1 Tail-Suspension Test (TST)

This test became one of the most widely used model for assessing antidepressant like effect in mice the test based on the fact that animals subjected to the short term in escapable stress of being suspended by their tail will develop an immobile posture, immobility is defined as the absence of initiated movements and include passive swaying.Mice were suspended by tail with tape placed 1 cm from the tip of the tail which connected to hook in the middle of the ceiling of the box, each mouse was given 1 trial that last 6 min. The immobility time was calculated as the main parameter measured [17, 14].

2.4.2 Forced Swim Test (FST)

Forced swim-test was proposed as a model to test antidepressant effect by [18]. Mice were forced to swim individually in a plexiglas cylinder which filled with tap water at 22-23°C to about 10 cm height. After an initial 2 min period each mouse assumed & typical immobileposture. Amouse was considered to be immobile when it remained floating in the water without struggling to make only minimum movements of its limbs necessary to keep its head above the water. The total immobility time was recorded during the next 4 min of the total test duration of 6 min. Each mouse was used only once.

2.5 Statistical Analysis

Data were analyzed by using completely Randomized Design in factorial experimental (One-way) ANOVA SPSS package (2008) was used to calculate the effects of factors on dependent traits. A probability of (P<0.05) was considered as significant differences [19].

3. Results

The study was carried out with tail suspension and forced swim tests of mice standard animal models predictive of antidepressant-like effect. The mice were suspended by their tail or forced to swim in water from which they cannot escape they will adopt a characteristic immobile postures, in such postures they remain suspended or floating in the water making only finer movements to keep their head above the water column. It has suggested that these immobility postures may serve as screening models for antidepressants [20]. Similar studies were done to evaluate the antidepressant like effect of tramadol [21, 22]. In the present study, the results in acute state 7 days tables (1 and 2) revealed that the antidepressant like effects of tramadol were compared with imipramine a commonly used tricyclic in depression, the results showed that tramadol at a dose of 40 mg/kg significantly (P<0.05) decreased the immobility times 98.13±1.52 and 42.25±1.06 seconds in both tests tail suspension and forced swim respectively which compared to negative controls while there were no significant (P<0.05) differences in immobility times between tramadol 40 mg/kg and imipramine 10mg/kg in both tests. The data of our results showed that there were increased in the antidepressant like effects of tramadol with increasing the dosage and there were dose dependent decreased in the immobility times with tramadol. The results in both tables also showed the effect of tramadol in combination with serotonergic blocker and adrenergic blocker on immobility times in both tests. The mean immobility times for (Ondansetron + tramadol) groups were (165.22±1.30) seconds and (96.00±3.00) seconds for both tests respectively while the immobility times of combination of (terazosin + tramadol) were (167.53±1.22) seconds and (98.20±4.30) seconds for both tests respectively and there were significant (P<0.05) differences between tramadol 40mg/kg given alone and tramadol 40mg/kg given in combination with these blockers and the results showed that ondansetronand terazosin could effectively block the antidepressant like effect of tramadol. The results after 15 days of treatment showed that tramadol at a dose of 40mg/kg B.W significantly (P<0.05) decreased the immobility times in both TST and FST tables (3 and 4) which were (62.25±3.35 and 34.42±1.50) seconds respectively as compared to control groups while there were no significant (P<0.05) differences in immobility times in both tests between tramadol at a dose of 40mg/kg and standard drug imipramine 10mg/kg. The results also showed the effect of tramadol when given as in combination with serotonergic and adrenergic blockers on immobility times in both tests which were 158.00±5.33, 160.10±2.80, 90.20±4.00 and 91.20±4.20 seconds for both ondansetron + tramadol and terazosin + tramadol in both tests TS and FS respectively.

Table 1: Effects of acute treatment with tramadol alone and in combination with noradrenergic and serotonergic blockers on immobility time in tail-suspension test.

Groups	Immobility time (second) Mean ± S.E.
Group1: Treated with normal saline served as (negative control).	$170.20±8.80^{C}$
Group2: Treated with imipramine 10mg/kg served as (positive control).	$92.00±1.00^{A}$
Group3: Treated with tramadol 20mg/kg.	$133.62±3.30^{B}$
Group4: Treated with tramadol 40mg/kg.	$98.13±1.52^{A}$
Group5: Treated with ondansetron + tramadol, 4mg/kg + 40mg/kg.	$165.22±1.30^{C}$
Group6: Treated with terazosin + tramadol, 1mg/kg + 40mg/kg.	$167.53±1.22^{C}$

Values are represented as m ± S.E. Different letters denoted to significant differences at (P<0.05) between groups Number=6mice/group.

Table 2: Effects of acute treatment with tramadol alone and in combination with serotonergic and noradrenergic blockers on immobility time in forced swim test.

Groups	Immobility time (second) Mean ± S.E.
Group1: Treated with normal saline served as (negative control).	$100.40±1.66^{C}$
Group2: Treated with imipramine 10mg/kg served as (positive control).	$40.00±0.16^{A}$
Group3: Treated with tramadol 20mg/kg.	$82.30±1.30^{B}$
Group4: Treated with tramadol 40mg/kg.	$42.25±1.06^{A}$
Group5: Treated with ondansetron + tramadol, 4mg/kg + 40mg/kg.	$96.00±3.00^{C}$
Group6: Treated with terazosin + tramadol, 1mg/kg + 40mg/kg.	$98.20±4.30^{C}$

Values are represented as m ± S.E. Different letters denoted to significant differences at (P<0.05) between groups Number=6mice/group.

Table 3: Effects of tramadol given alone and in combination with serotonergic and noradrenergic blockers on immobility time in tail-suspension test after 15 days of treatment.

Groups	Immobility time (second) Mean ± S.E.
Group1: Treated with normal saline served as (negative control).	$166.20±8.20^{C}$
Group2: Treated with imipramine 10mg/kg served as (positive control).	$57.00±2.50^{A}$
Group3: Treated with tramadol 20mg/kg.	$110.20±5.22^{B}$
Group4: Treated with tramadol 40mg/kg.	$62.25±3.35^{A}$
Group5: Treated with ondansetron + tramadol, 4mg/kg + 40mg/kg.	$158.00±5.33^{C}$
Group6: Treated with terazosin + tramadol, 1mg/kg + 40mg/kg.	$160.10±2.80^{C}$

Values are represented as m ± S.E. Different letters denoted to significant differences at (P<0.05) between groups Number=6mice/group.

Table 4: Effects of tramadol given alone and in combination with serotonergic and noradrenergic blockers on immobility time in the forced swim test after 15 days of treatment.

Groups	Immobility time (second) Mean ± S.E.
Group1: Treated with normal saline served as (negative control).	95.00 ± 1.90^{C}
Group2: Treated with imipramine 10mg/kg served as (positive control).	31.21 ± 0.90^{A}
Group3: Treated with tramadol 20mg/kg.	62.00 ± 2.11^{B}
Group4: Treated with tramadol 40mg/kg.	34.42 ± 1.50^{A}
Group5: Treated with ondansetron + tramadol, 4mg/kg + 40mg/kg.	90.20 ± 4.00^{C}
Group6: Treated with terazosin + tramadol, 1mg/kg + 40mg/kg.	91.20 ± 4.20^{C}

Values are represented as m ± S.E. Different letters denoted to significant differences at (P<0.05) between groups Number=6mice/group

4. Discussion

The incidence of depression in the community is very high and is associated with a lot of morbidity, so it is very important to overcome these problems and find effective therapies. On the basis of the clinical observations of depressive disorders and stressful life events, many of the animal models for the evaluation the activity of antidepressant drug which assess the stress precipitated behaviors, the TS and FS tests are the most widely used animal models which are relatively specific to all major classes of antidepressants [23]. In TS and FS tests the immobility reflect states of behavioral despair in mice which is reproduce a condition similar to human depression [24]. It has been revealed that the TST is less stressful and has higher pharmacological sensitivity than FST [25]. In this work the results showed that the administration of tramadol at doses 20 and 40 mg/kg in mice was effective as antidepressant and the effects of tramadol increase with increasing the dosage which increase more after 15 days of treatment than in acute state and there were dose dependent decreased in immobility times with tramadol. Tramadol when given as a combination with ondansetron which acts as serotonin ($5HT_3$) antagonist there was a significant ($P< 0.05$) decrease in the antidepressant like effect of tramadol this indicates that ondansetron declines the antidepressant like effect of tramadol and it gives an evidence that the antidepressant like effect of tramadol is mainly by increasing the availability of serotonin. Ondansetron and terazosin possibly act on $5H_3$ and α-adrenergic receptors on the nerve terminals and inhibit the release or block post neuron binding sites and inhibit the reuptake of 5HT and NA which indicate that tramadol exerts antidepressant like effect by significantly modifying release of 5HT and NA in the terminals [16]. It has been established that the decreasing of immobility times both TST and FST depend on the enhancement of the central 5HT and NA and tramadol mechanisms of antidepressant like effect might be due to the action through serotonergic and adrenergic neurons and increase as the same as that indicated by some earlier studies [15, 22]. Our findings agreed with [15] which revealed that the antidepressant like effect of tramadol was slightly more after 15 days of treatment as compared with acute studies (7 days) of treatment. The antidepressant like effect of tramadol may be explained also as its ability to modulate imidazoline receptors as well as opioid receptors [26, 27]. Jesse *et al*. [28] showed that the acute administration of tramadol produces antidepressant like effect by inhibition of L-arginine-NO oathway while Jesse *et al*. [29] reported that the oral administration of tramadol produces the antidepressant effect in mice by a mechanism that involves the K^+ channels. All these findings need to be confirmed in the future studies to get conclusive evidence regarding the mechanism of antidepressant of tramadol. Our findings agreed with other studies [30, 31] which explained the ability of tramadol to modulate noradrenaline as indicated by some earlier studies, while recent findings [32] suggest that tramadol is believed to inhibit serotonin and noradrenaline transporters in the brain and the inhibition of serotonin reuptake inhibitor in the brain is related to pain reduction of tramadol this inhibition is similar to that shown by selective serotonin reuptake inhibitors. The results of the present study reveal the role of tramadol as antidepressant drug and the involvement of serotonergic and noradrenergic systems in the antidepressant like effect of tramadol when given as combination with tramadol as ondansetron and terazosin caused a reversal of the effects of tramadol on immobility times in both animal models of depression TS and FS tests which indicates that the antidepressant action of tramadol involves serotonergic and alpha adrenergic receptors.

5. Conclusion

From our study, we can conclude that tramadol has significant antidepressant like effect in both acute studies and after 15 days of treatment effect which compared to standard antidepressant drug imipramine and there were dose dependent in increasing these effects of tramadol which exerts the antidepressant like effect by inhibiting reuptake of serotonin and noradrenaline or increase the release of these neurotransmitters in the terminals and enhancement of central serotonin and noradrenaline, these effects of tramadol were reduced by pretreatment of ondansetron and terazosin which indicate the roles of tramadol through the serotonergic and noradrenergic pathways due to the fact that ondansetron and terazosin possibly act as $5H_3$ receptor antagonist and α-adrenergic receptor blocking, respectively. Further studies should be done with a large number of subjects or other species of animals in defferent periods of treatment to potentiate our results and further studies in human may be required to confirm the results of animal studies.

6. Acknowledgment

The authors would like to thank Assist. Prof. Dr. Luma W. Khalil Head of Department of Physiology, Biochemistry and Pharmacology, Veterinary Medicine College, University of Baghdad, Iraq. Prof. Dr. Duraid A. Al-Hadi Abbas and Assist. Prof. Dr. Falah M.K. Al-Rekabi, Veter. Med. College, University of Baghdad, Iraq. for assistance in completing this work.

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