EVALUATION OF ROLE OF NORADRENERGIC SYSTEM IN THE ANTIDEPRESSANT ACTIVITY OF TRAMADOL USING FORCED SWIM TEST IN ALBINO MICE

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Summary

Several studies have suggested that tramadol could play a role in mood improvement. The present study was undertaken to evaluate the role of noradrenergic system in the antidepressant activity of tramadol in a test predictive of antidepressant activity in mice. Animals were divided into five groups, and each group comprised of six mice. Group 1 (control group) was pretreated with normal saline (0.1 ml/10 g). Groups 2, 3 and 4 were pretreated with three different doses (10, 20, and 40 mg/kg) of tramadol. Group 5 was pretreated with imipramine at the dose of 10 mg/kg. All the drugs were given intraperitonially (0.1 ml/10 g). After 15 minutes of drug administration, mice were placed individually in the 5 L glass beakers, filled to a height of 15 cm with water (room temperature) and the duration of immobility is recorded during the last 4 minutes of a 6 minutes test. In the next stage of the study, procedure was repeated with six different groups of animalse, each group comprising of six mice. The groups of animals here included animals pretreated with 40 mg/kg of tramadol, propranolol 5mg/kg, tramadol 40mg/kg + propranolol 5mg/kg, phentolamine 10mg/kg, and phentolamine 10mg/kg + tramadol 40mg/kg. Tramadol at 40mg/kg dose on acute as well as chronic administration for 10 days significantly decreased immobility period as compared to control mice. There was no significant difference in the antidepressant activity in animals administered with tramadol 40 mg/kg and imipramine 10mg/kg. Phentolamine effectively blocked the reduction in immobility duration by tramadol where as propranolol did not have significant effect on the antidepressant like action of tramadol in forced swim test in mice.

Key words: Tramadol, antidepressants, imipramine, noradrenergic system

Introduction

Depression is now known as a complex disorder involving the whole body and the diagnosis of depression is based on a heterogeneous set of symptoms.[1] Approaches to the treatment of depression depend on the severity of the condition and the risks to the patient. Monoamine reuptake inhibitors have been refined over several decades to provide safe and effective pharmacotherapy for depression. Tricyclic antidepressants have long been preferred over MAOIs because of the problem of drug interactions and the need for strict dietary precautions with the latter group. Tricyclics with sedative properties may be more suitable for agitated and anxious patients, whereas those with less sedative properties may be preferred for withdrawn and apathetic patients. However, a substantial number of patients do not respond adequately to antidepressant drugs.[2] There remains a pressing need for alternative drug therapies, given the prevalence, morbidity and mortality of depressive disorders, and the incomplete efficacy and undesirable adverse effects of currently 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 treatment.Tramadol is a synthetic 4-phenyl-piperidine analogue of codeine. It is a central analgesic with a low affinity for opioid receptors. Experimental and clinical data suggest that tramadol may also exert its analgesic effect through direct modulation of central monoaminergic pathways. This drug is a racemic mixture of two enantiomers, each one displaying different mechanisms: (+)tramadol displays opioid agonist properties and inhibits serotonin reuptake while (-)tramadol inhibit preferentially noradrenaline reuptake. The action of tramadol on the monoaminergic reuptake is similar to that of antidepressant drugs. [3-8] Several studies have suggested that tramadol could play a role in mood improvement. The present study was undertaken to evaluate the role of noradrenergic system in the antidepressant activity of tramadol in a test predictive of antidepressant activity in mice.

Materials and Methods

This study was conducted in the Department of Pharmacology, Kasturba Medical College, Mangalore, after getting approval from the Institutional Animal Ethical Committee. Adult Albino Mice (Swiss Strain) of either sex weighing 20-25 grams inbred in the central animal house of Kasturba Medical College were used for the study. Mice were housed in clean polypropylene cages, six mice in each cage, in a controlled environment (26^0-28^0c) with a 12 hour light and dark cycle. They were fed with commercial pelleted chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by VRK Nutritional solutions G-1, Trimurti archade, Opp. Zilla parishad, Sangli-416416, Maharashtra.) and water *ad libitum.* The mice were allowed to acclimatize for these conditions for one week. Experiments were performed during the light phase of the cycle (10:00-17:00). All efforts were made to minimize the animal suffering and the number of animals used. All experiments were carried out between 9AM to 5PM and each animal was used only once.

The following drugs were used:
Inj. Tramadol (Urgendol Winmedicare)
Tablet Imipramine (Torrent pharmaceuticals) at the dose of 10 mg/kg, intraperitonially (0.1ml/10g). 25mg tab dissolved in 25 ml of distilled water (1mg/1ml concentration).
Inj. Propranolol (Sigma)
Inj. Phentolamine (Sigma)

All drugs were administered intraperitoneally in a volume of 10ml/kg body weight.

To confirm the antidepressant like activity of tramadol, animals were divided into five groups, and each group comprised of six mice. Group 1 (control group) was pretreated with normal saline (0.1 ml/10 g). Groups 2, 3 and 4 were pretreated with three different doses (10, 20, and 40 mg/kg) of tramadol. Group 5 was pretreated with imipramine at the dose of 10 mg/kg.
To test the hypothesis that the antidepressant like effects of tramadol are mediated through an interaction with noradrenergic system, animals were divided into six groups, and each group comprised of six mice. Group 1 (control group) was pretreated with normal saline (0.1 ml/10 g). Group 2 was pretreated with 40 mg/kg of tramadol. Group 3 was pretreated with propranolol 5mg/kg, group 4 was pretreated with tramadol 40mg/kg + propranolol 5mg/kg, group 4 was pretreated with phentolamine 10mg/kg, and group 6 was pretreated with phentolamine 10mg/kg + tramadol 40mg/kg.

For acute experiment, drugs were given as single dose 15 minutes before the experiment and the pretreatment period was 15 minutes. For chronic study, drugs were given daily for 10 days and the pretreatment period was 15 minute on the 10^{th} day of drug administration.

Doses and administration schedule were chosen on the basis of previously published literature which confirms the selectivity and efficacy of the doses used in this experiments.[6,7]

The duration of immobility in the forced swim test in male mice is measured to evaluate the antidepressant potential of compounds. After 15 minutes of drug administration, mice were placed individually in the 5 L glass beakers, filled to a height of 15 cm with water (room temperature) and the duration of immobility is recorded during the last 4 minutes of a 6 minutes test. A mouse is considered immobile when floating motionless or making only those movements necessary to keep its head above water surface. The water is changed after each test. Antidepressants decrease the immobility time. The test has been validated by most current types of antidepressants. [9, 10]

Statistical Analysis

Mean duration of immobility for each group is calculated. The data was presented as mean + SD. Comparison between experimental and control group was performed by Kruskal-Wallis one-way ANOVA and Mann-Whitney U tests. A p value less than 0.05 was considered significant.

Results

Mean duration of immobility in forced swim test for the control group was observed to be 68.83.25 ± 30.69 seconds. In the group pretreated with 10mg/kg of tramadol, the immobility period was 61.17±17.75 seconds, 57.33±20.89 seconds for tramadol 20mg/kg group, for tramadol 40mg/kg group was 25.17±6.74 seconds whereas it was 15±16.48 seconds in the group pretreated with single dose of imipramine (figure 1). The differences in the immobility period among different groups was highly significant ($H= 16.49$, $p<0.001$).

Figure 1 also shows the effect of chronic treatment of tramadol and imipramine on duration of immobility in forced swim test. Mean duration of immobility for the control group was 63.5±33.82 seconds. In the groups pretreated with three different doses of tramadol for 10 days, the immobility period was 51.33±23.91 seconds for group 2, 36.67±14.75 seconds for group 3, and 14±10.70 seconds for group 4 whereas it was 6.67±10.35 seconds in the group pretreated with imipramine for 10 days. The differences in the immobility period among different groups was highly significant ($H= 18.32$, $p<0.001$).

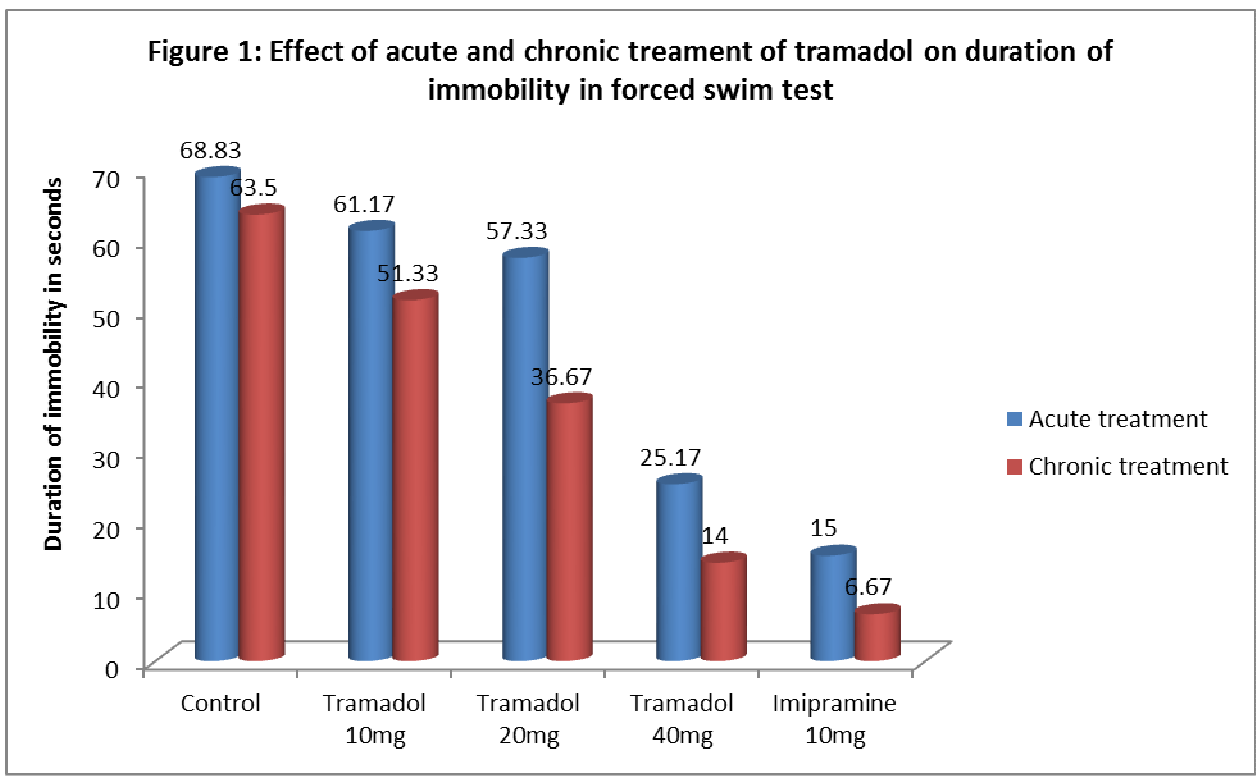

Table 1. shows the group-wise comparison of duration of immobility in forced swim test. The decrease in immobility period in the group pretreated with imipramine as compared to control was highly significant (p=0.02 for acute experiment, p = 0.009 for chronic experiment). Tramadol at 40mg/kg dose on acute as well as chronic administration for 10 days significantly decreased immobility period as compared to control mice (p=0.04 for acute experiment, p =0. 02 for chronic experiment). Decrease in immobility period in the groups pretreated with tramadol (10 and 20 mg/kg) was not found be significant as compared to control group. Duration of immobility was significantly less in tramadol 40mg/kg group when compared to tramadol 10mg/kg and 20mg/kg group. Statistically significant difference was not found in duration of immobility between tramadol 10mg/kg and tramadol 20mg/kg groups. Duration of immobility was significantly more in tramadol 10mg/kg and tramadol 20mg/kg groups when compared to imipramine group.But there was no significant difference in the antidepressant activity in animals administered with tramadol 40 mg/kg and imipramine 10mg/kg.

Table 1: Group-wise comparison of immobility duration in forced swim test

Groups		**Z value**	**p value**
Tramadol 10mg vs normal saline	**Acute**	**-1.28**	**0.2**
	Chronic	**-0.97**	**0.33**
Tramadol 20mg vs normal saline	**Acute**	**-1.28**	**0.2**
	Chronic	**-1.61**	**0.11**
Tramadol 40mg vs normal saline	**Acute**	**-1.94**	**0.04***
	Chronic	**-2.25**	**0.02***
Imipramine 10mg vs normal saline	**Acute**	**-2.41**	**0.02***
	Chronic	**-2.62**	**0.009****
Tramadol 10mg vsTramadol 20mg	**Acute**	**-0.72**	**0.47**
	Chronic	**-0.96**	**0.34**

Tramadol 10mg vsTramadol 40mg	**Acute**	**-2.81**	**0.005****
	Chronic	**-2.73**	**0.006****
Tramadol 20mg vsTramadol 40mg	**Acute**	**-2.81**	**0.005****
	Chronic	**-2.41**	**0.02***
Tramadol 10mg vs imipramine 10mg	**Acute**	**-2.57**	**0.01***
	Chronic	**-2.94**	**0.003****
Tramadol 20mg vs imipramine 10mg	**Acute**	**-2.57**	**0.01***
	Chronic	**-2.69**	**0.007***
Tramadol 40mg vs imipramine 10mg	**Acute**	**-0.8**	**0.42**
	Chronic	**-1.47**	**0.14**

Mann-Whitney U test * Significant ** Highly significant

Figure 2 shows the effect of tramadol in combination with adrenergic blockers on duration of immobility in forced swim test. Mean duration of immobility for the tramadol group was 25.17±6.74 seconds. The immobility period was 53.17±7.55 seconds for group 3 (propranolol), 17.5±20.69 (propranolol+tramadol) seconds for group 4, and 74.17±17.03 (phentolamine) seconds for group 5, and 68.5±61.07 for group 6 (phentolamine+tramadol). The differences in the immobility period among different groups was highly significant (H= 19.94, $p<0.001$). Results showed that phentolamine could effectively block the antidepressant activity of tramadol, but propranolol could not block the antidepressant effects of tramadol.

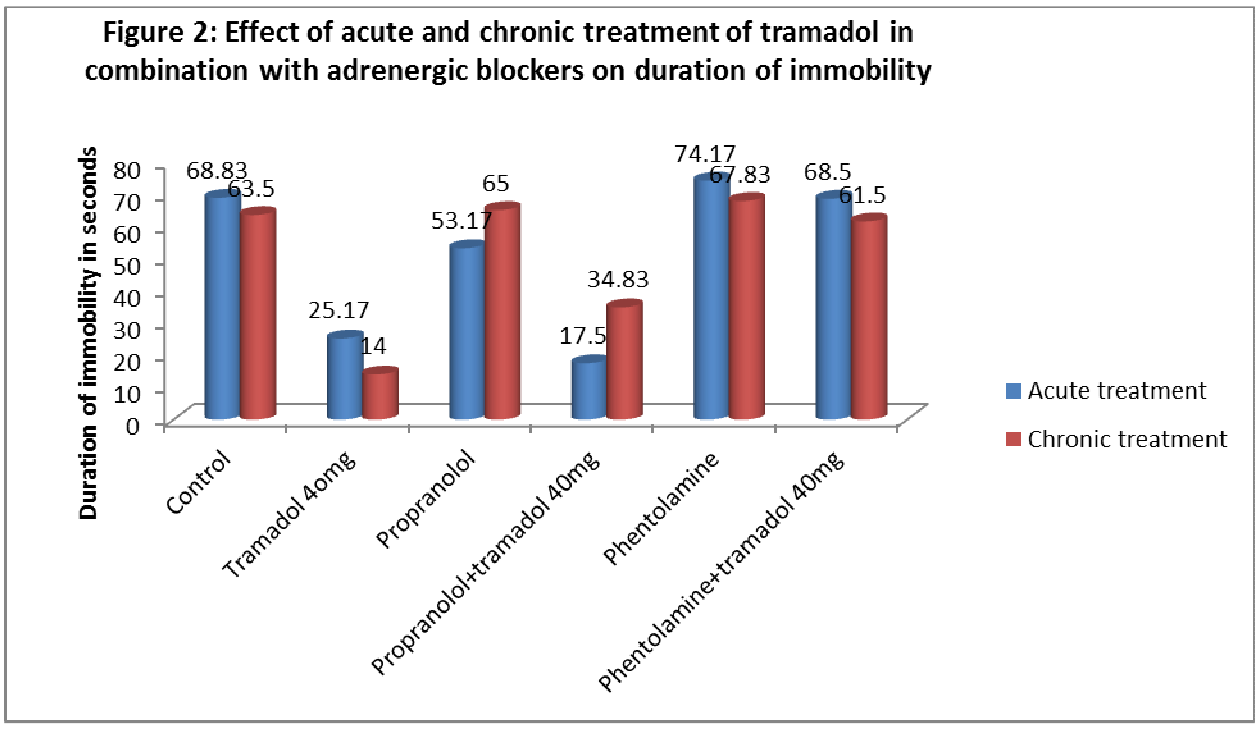

Table 2 shows the group-wise comparison of duration of immobility in forced swim test. The decrease in immobility period in the group pretreated with tramadol as compared to control was significant for chronic treatment ($p=0.02$) and it was also significant for tramadol +propranolol (acute) versus normal saline ($p=0.02$). This test failed to show significant difference in duration of immobility for tramadol versus tramadol+ propranolol or tramadol versus tramadol + phentaolamine.

Figure 2 also shows the effect of chronic treatment of tramadol in combination with adrenergic blockers on duration of immobility in forced swim test. Mean duration of immobility for the tramadol group was 14±10.7 seconds. The immobility period was 65±4.94 seconds for group 3 (propranolol), 34.83±23.94 (propranolol+tramadol) seconds for group 4, and 67.83±21.63(phentolamine) seconds for group 5, and 61.5±57.07 for group 6(phentolamine+tramadol). The differences in the immobility period among different groups was highly significant (H= 20.07, p<0.001). Here also the results show that phentolamine was able to decrease the antidepressant activity of tramadol, but not propranolol.

Table 2: Group-wise comparison of immobility duration in forced swim test

Groups		Z value	p value
Tramadol 40mg vs normal saline	**Acute**	**-1.92**	**0.05**
	Chronic	**-2.25**	**0.02***
Tramadol 40mg+propranolol 5mg vs normal saline	**Acute**	**-2.4**	**0.02***
	Chronic	**-1.29**	**0.2**
Tramadol 40mg+phentolamine 10mg vs normal saline	**Acute**	**-0.64**	**0.52**
	Chronic	**-1.61**	**0.10**
Tramadol 40mg vsTramadol 40mg+propranolol 5mg	**Acute**	**-1.12**	**0.26**
	Chronic	**-1.76**	**0.08**
Tramadol 40mg vsTramadol 40mg+phentolamine10mg	**Acute**	**-1.21**	**0.26**
	Chronic	**-0.56**	**0.57**
Normal saline vs propranolol 5mg	**Acute**	**-1.60**	**0.11**
	Chronic	**-1.93**	**0.05**
Normal saline vs phentolamine 10mg	**Acute**	**-1.12**	**0.26**
	Chronic	**-0.64**	**0.52**

Z=Mann-Whitney U test * Significant

Discussion

The present study was carried out to evaluate the antidepressant activity of tramadol in forced swim test in animals. Since its introduction almost 20 years ago forced swim test was the most widely used models for assessing antidepressant like activity in mice. This model is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Although rodent behavioral models have a good predictive validity for antidepressants and they are sensitive to the acute administration of these compounds, it is widely recognized that the symptoms of depression in patients are only ameliorated after chronic drug treatment. Therefore, we decided to check whether the effects of antidepressants in the forced swim test and tail suspension tests are dependent on the duration of drug treatment. Hence the effect of chronic administration of tramadol was also evaluated in our study.

The present study conclusively shows that tramadol has significant antidepressant activity which was comparable with standard antidepressant drug imipramine. Our results confirm the literature data, by showing that tramadol reduces the immobility time in both the models used. Antidepressant like effect of tramadol in the forced swim test in mice [6,7, 11] and rats[12, 8] has been reported. In addition to the effect on the animal's behavior, case reports and case series

have illustrated a clinically antidepressant effect of tramadol in depressive states, including resistant depression.[7,8,11] In addition, our study extends the literature data by providing convincing data that its effect probably mediated by an interaction with noradrenergic system.[12] In forced swim test model, tramadol has shown significant antidepressant activity at the dose of 40mg/kg which was comparable with imipramine 10mg/kg. Antidepressant activity was slightly more on chronic administration of the drug for 10 days when compared to single dose administration. Tramadol did not show significant antidepressant activity at lower doses. The dose dependent antidepressant activity of tramadol was observed for acute as well as chronic administration of the drug. The results of the present study were comparable with previously published data.[7]

Current pharmacological treatment for depression is based on the use of drugs that act mainly by enhancing brain serotonin and noradrenaline neurotransmission by the blockade of the active reuptake mechanism for these neurotransmitters. The adaptive changes in the noradrenergic system were considered as an important part of antidepressant treatment. In order to investigate the possible involvement of noradrenergic system in the antidepressant like effect of tramadol, phentolamine, a classical nonselective alfa blocker and propranolol, a nonselective beta blocker were used. The alpha-adrenoceptor antagonist phentolamine countered the immobility-reducing action of tramadol. Both these drugs when given alone have did not affect the immobility duration when compared to control animals. The results of the present study indicate the involvement of noradrenergic system as phentolamine caused reversal of the effects of tramadol on immobility duration. Propranolol failed to reverse the antidepressant activity of tramadol which indicates that antidepressant action of tramadol involves alfa adrenergic receptors. The alfa1 and alfa 2 adrenergic receptors have been shown to involved in antidepressant like effects of drugs in behavioural models of depression.[12] These observations give direct support to previous presumptions that tramadol has antidepressant-like effect in mice, probably mediated by the noradrenergic system. Similar observations were noted in the earlier literature.[13] The results may have to be evaluated further in different models of depression and also in different species of animals.

The antidepressant like effect of tramadol may also be explained by its ability to modulate opioid receptors, the serotenergic system, the dopaminergic system.[14,15] Role of these systems was not evaluated in the present study which needs to be investigated in future studies. Imidazoline receptors (I_1 and I_2 receptors) also may be involved in the antidepressant like activity of tramadol in mice.[12] Jesse CR et al showed that the acute administration of tramadol produces antidepressant like effect in the rat forced swim test by a mechanism that involves the inhibition of l-arginine–NO–cGMP pathway.[16] Same investigators also suggested that oral acute administration of tramadol produces antidepressant like effect on the forced swim test in mice by a mechanism that involves the K^+ channels.[12]

All these findings need to be confirmed in the future studies to get conclusive evidence regarding the mechanism of antidepressant activity of tramadol.

In conclusion, the results of present study indicate that tramadol has significant antidepressant like activity which is comparable to imipramine. Pretreatment of mice with phentolamine prevented the antiimmobolity effect of tramadol. The findings also suggest that antidepressant activity of tramadol is mediated through interaction with noradrenergic system, may be increasing the levels of noradrenaline.

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