INTRODUCTION

According to World Health Report approximately 450 million people suffer from mental or behavioral disorder. Among these, depression is the most prevalent disorder characterized by apathy, loss of energy, retardation of thinking and activity, suicidal tendency. Approaches to the treatment of depression depend on severity of condition and the risk to patient. Until date various drugs such as monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors and atypical antidepressant are being successfully used in the treatment of depression. In spite of availability of these antidepressant drugs, depression continues to be a major problem. Hence great interest is being taken in development of innovative antidepressants.

Tramadol (TRM) is a centrally acting opioid agonist which is clinically effective in the treatment of moderate to severe pain. By virtue of its action of inhibiting norepinephrine and serotonin uptake, it can function as an antidepressant like venlafaxine. In addition, TRM bears close structural similarity to antidepressant venlafaxine and thus shares a number of its molecular and pharmacological properties. In the light of above facts we have investigated antidepressant activity of TRM alone and in combination with standard antidepressant fluoxetine (FLX) in animal models of depression.
water *ad libitum*, both being withdrawn 24 hrs before the experiment. They were maintained under standard 12 hrs light and dark cycle. The animals were divided into six groups containing six animals in each.

**Materials**

**Drugs and solutions**

The standard solution of FLX was prepared by dissolving 20 mg of the pure powder form (purchased from Aurobindo Pharma Ltd., A.P. India) in 10 ml of normal saline and administered in dose of 20 mg/kg i.p. The test solution of TRM was prepared by dissolving 20 mg of pure powder form (purchased from HCM Organics Ltd., Maharashtra, India) in 10 ml of normal saline and administered in dose of 20 mg/kg i.p. Normal saline was administered in dose of 0.1 ml/10 g i.p.

**Study design**

The animals were divided into six groups containing six animals in each group. Group I pretreated with normal saline (0.1 ml/10 g i.p.), Group II with FLX (20 mg/kg, i.p.), Group III with TRM (20 mg/kg, i.p.), Group IV with TRM (40 mg/kg, i.p.), Group V with FLX (20 mg/kg, i.p.) + TRM (20 mg/kg, i.p.) and Group VI with FLX (20 mg/kg, i.p.) + TRM (40 mg/kg, i.p.).

For acute study, normal saline, FLX and TRM were administered i.p. for 7 days to respective groups of animals. In chronic study, normal saline, FLX and TRM were administered i.p. for 14 days to respective groups of animals.

**Methods**

**Forced swimming test (FST)**

This animal model is based on the principle that forcing mice to swim in restricted space from which they cannot escape leads to a characteristic behavior of immobility. This test was done in two modes i.e., acute and chronic, along with a habituation session before actual test. For habituation session, 1-day before the experimental study, each mouse was placed in glass cylinder containing water for 15 mins. No scoring of immobility was performed during this session. In acute study, drugs were administered to mice for 7 days, on 7th day test was done after 30 mins of drug administration. Vertical glass cylinder (25 cm × 10 cm) was filled with fresh water, mice were individually forced to swim inside vertical glass cylinder containing water column of 15 cm height and their behavior was observed for 6 mins by video recording camera. The duration of immobility was recorded for 6 mins. Immobility period was counted with time sampling method using blocks of 5 sec. After each test floor of apparatus was cleaned with spirit. In chronic study, normal saline, FLX and TRM were administered i.p. for 14 days to respective groups of animals. On 14th day the test was performed 30 mins after administration of drugs same as that of single dose study.

**Tail suspension test (TST)**

In this test immobility was induced by suspending the mice by the tail. After initially trying to escape by engaging in vigorous movements, mice rapidly become immobile. In experimental room, white ceiling lights (standard lighting) were used. In acute study, drugs were administered to mice for 7 days, on 7th day test was done after 30 mins of drug administration. Animals were suspended 50 cm above the ground by wrapping adhesive tape around the animal’s tail in the constraint position three quarters of the distance from the base of the tail on the tail suspension apparatus. The duration of immobility was measured for 6 mins by video recording camera. Immobility period was counted with time sampling method using blocks of 5 sec. In chronic study, normal saline, FLX and TRM were administered i.p., for 14 days to respective groups of animals. On 14th day the test was performed 30 mins after administration of drugs same as that of single dose study.

**Open field test**

This test utilizes behavioral changes in rodents exposed to novel environment and is used to confirm that the observed antidepressant effect is not due to stimulation of general motor activity. Open field apparatus have been used to test the mice. The open field test was carried out on dark grey floor subdivided into 25 equal parts in a wooden box (100 cm × 100 cm × 30 cm). For acute study, normal saline, FLX and TRM were administered i.p., for 7 days to respective groups of animals. On 7th day the test was performed 30 mins after the drug administration. The animals were individually placed in the corner square of the open field. The following parameters were observed for 5 mins by video recording camera.

- Activity in the center (number of central squares crossed)
- Spontaneous ambulation (number of peripheral squares crossed)
- Rearing (number of times the animal stands on the rear paws)

After each test floor of apparatus was cleaned with spirit. In chronic study, normal saline, FLX and TRM were administered i.p. for 14 days to respective groups of animals. On 14th day the test was performed 30 mins after administration of drugs same as that of acute study and same parameters as above were observed.

**Statistics**

The data were analyzed using one-way ANOVA, followed by Tukey *post-hoc* with using GraphPad Prism software.
RESULTS

The result of FST in acute study (Table 1) showed that duration of immobility was significantly (p<0.05) reduced for all the drug treated groups as compared to control group. The combination groups (TRM 20 mg/kg + FLX 20 mg/kg and TRM 40 mg/kg + FLX 20 mg/kg) showed significantly reduced duration of immobility (p<0.05) as compared to FLX (20 mg/kg group) and TRM (20 and 40 mg/kg).

In chronic study of FST (Table 2), all the treated groups showed statistically significant (p<0.05) reduction in duration of immobility as compared to control group. The combination groups (TRM 20 mg/kg + FLX 20 mg/kg and TRM 40 mg/kg + FLX 20 mg/kg) showed statistically significant antidepressant activity (p<0.05) as compared to FLX (20 mg/kg) and TRM (20 and 40 mg/kg) group. In both acute and chronic study, the decrease in immobility was not statistically significant in group treated with TRM as compared to FLX group (p>0.05).

The results of TST for acute study (Table 3) showed that the duration of immobility observed in group pretreated with TRM (20 and 40 mg/kg) alone and in combination groups was significantly reduced (p<0.05) as compared to control group. Similarly, duration of immobility was significantly reduced (p<0.05) with FLX (20 mg/kg) group as compared to control group. The combination groups showed significantly reduced (p<0.05) duration of immobility as compared to FLX (20 mg/kg) and TRM (20 and 40 mg/kg) groups.

The result of TST for chronic study (Table 4) showed that duration of immobility was significantly reduced (p<0.05) for all the treated groups as compared to control group. The combination groups (TRM 20 mg/kg + FLX 20 mg/kg and TRM 40 mg/kg + FLX 20 mg/kg) showed significantly reduced (p<0.05) duration of immobility as compared to FLX (20 mg/kg) group and TRM (20 and 40 mg/kg) group. In both acute and chronic study the decrease in immobility was not statistically significant in group treated with TRM as compared to FLX group (p>0.05).

| Table 1: FST – Acute study 7 days. |
|-----------------------------------|
| Group | Drugs | Doses | Duration of immobility in seconds (mean±SEM) |
|-------|-------|-------|---------------------------------------------|
| Group I | Normal saline | 0.1 ml/10 g | 158.3±1.05 |
| Group II | FLX | 20 mg/kg | 110.0±2.23* |
| Group III | TRM | 20 mg/kg | 138.3±3.57* |
| Group IV | TRM | 40 mg/kg | 130.8±5.83* |
| Group V | FLX+TRM | 20+20 mg/kg | 89.1±5.54** |
| Group VI | FLX+TRM | 20+40 mg/kg | 83.3±6.14** |

Values expressed as mean±SEM; n=6 in each group, df=5, 30; *p<0.05 when compared to control, †p<0.05 when compared to TRM alone, p<0.05 when compared to FLX alone, TRM: Tramadol, FLX: Fluoxetine, SEM: Standard error of mean, FST: Forced swimming test.

| Table 2: FST – Chronic study 14 days. |
|-----------------------------------|
| Group | Drugs | Doses | Duration of immobility in seconds (mean±SEM) |
|-------|-------|-------|---------------------------------------------|
| Group I | Normal saline | 0.1 ml/10 g | 136.7±1.05 |
| Group II | FLX | 20 mg/kg | 90.0±2.23** |
| Group III | TRM | 20 mg/kg | 116.7±2.78* |
| Group IV | TRM | 40 mg/kg | 110.8±5.83* |
| Group V | FLX+TRM | 20+20 mg/kg | 69.1±5.54** |
| Group VI | FLX+TRM | 20+40 mg/kg | 63.3±6.14** |

Values expressed as mean±SEM; n=6 in each group, df=5, 30; *p<0.05 when compared to control, †p<0.05 when compared to TRM alone, p<0.05 when compared to FLX alone, TRM: Tramadol, FLX: Fluoxetine, SEM: Standard error of mean, FST: Forced swimming test.

| Table 3: TST - Acute study 7 days. |
|-----------------------------------|
| Group | Drugs | Doses | Duration of immobility in seconds (mean±SEM) |
|-------|-------|-------|---------------------------------------------|
| Group I | Normal saline | 0.1 ml/10 g | 146.7±1.05 |
| Group II | FLX | 20 mg/kg | 100.0±2.23** |
| Group III | TRM | 20 mg/kg | 126.7±2.78* |
| Group IV | TRM | 40 mg/kg | 120.8±5.83* |
| Group V | FLX+TRM | 20+20 mg/kg | 79.1±5.54** |
| Group VI | FLX+TRM | 20+40 mg/kg | 73.3±5.54** |

Values expressed as mean±SEM; n=6 in each group, df=5, 30; *p<0.05 when compared to control, †p<0.05 when compared to TRM alone, p<0.05 when compared to FLX alone, TRM: Tramadol, FLX: Fluoxetine, TST: Tail suspension test.

| Table 4: TST - Chronic study 14 days. |
|-----------------------------------|
| Group | Drugs | Doses | Duration of immobility in seconds (mean±SEM) |
|-------|-------|-------|---------------------------------------------|
| Group I | Normal saline | 0.1 ml/10 g | 163.7±1.05 |
| Group II | FLX | 20 mg/kg | 115.0±2.23** |
| Group III | TRM | 20 mg/kg | 143.3±2.78* |
| Group IV | TRM | 40 mg/kg | 135.8±5.83* |
| Group V | FLX+TRM | 20+20 mg/kg | 94.1±5.54** |
| Group VI | FLX+TRM | 20+40 mg/kg | 88.3±6.14** |

Values expressed as mean±SEM; n=6 in each group, df=5, 30; *p<0.05 when compared to control, †p<0.05 when compared to TRM alone, p<0.05 when compared to FLX alone, TRM: Tramadol, FLX: Fluoxetine, TST: Tail suspension test.
The open field test was done in acute study (Table 5) and for chronic study (Table 6) formats with assessment of the number of square crossed (peripheral and central) and number of rearing in these groups. However, the statistical analysis showed non-significant findings (p>0.05) for all the group comparisons.

**DISCUSSION**

Modern day life style leads to numerous stressful conditions among which anxiety and depression are general and widely prevalent neuro logical disorders. Vigorous efforts are underway to find an ideal antidepressant in both preclinical and clinical studies. In case of preclinical studies for assessing antidepressant like activity in small animals, the widely used animal models are FST, TST and open field test.\(^1\) It is expected that immobility that occurs in these tests reflects a state of behavioral despair or inability to adapt the stress as seen in human. For a drug to be labeled as antidepressant, it should decrease the immobility period in these tests.

In the present study, TRM was used alone and in combination with standard antidepressant FLX. The FST was done in both acute and chronic formats. In both FST and TST, the antidepressant effect of TRM (40 mg/kg) group was better than TRM (20 mg/kg) group, but it was statistically non-significant (p>0.05). In combination groups also, antidepressant effect of TRM and FLX (TRM 40 mg/kg+FLX 20 mg/kg) group was better than other (TRM 20 mg/kg+FLX 20 mg/kg) combination group but it was statistically non-significant (p>0.05).

Open field test was carried out to show that the antidepressant effect of drug was not related to stimulation of general locomotor activity. The open field test was done in acute study and for chronic study formats with assessment of the number of square crossed (peripheral and central) and number of rearing in these groups. However, the statistical analysis showed non-significant findings (p>0.05) for all the group comparisons.

A significant result of acute and chronic dose study in FST and TST shows that TRM has significant antidepressant effect as compared to control group. Results of the open field test show that this effect is not related to stimulation of general motor activity.

TRM is a centrally acting opioid agonist which also inhibits norepinephrine and serotonin uptake.\(^3,4\) In addition, TRM bear close structural (chemical structure of both the substance have methoxyphenyl, N,N-dimethylamino and hydroxycyclohexyl group) and metabolic (both are metabolized by cytochrome P450 2D6) similarity to antidepressant venlafaxine.\(^12\) TRM can also modulate the opioid receptor, the serotonergic system and dopaminergic

### Table 5: Open field test - Acute study 7 days.

| Group   | Drugs   | Doses      | Number of central squares crossed | Number of peripheral squares crossed | Total number of rearing |
|---------|---------|------------|-----------------------------------|-------------------------------------|-------------------------|
| Group I | Normal saline | 0.1 ml/10 g | 20.6±2.91                         | 137.5±5.10                          | 25.6±2.10               |
| Group II| FLX     | 20 mg/kg   | 6.8±3.30                          | 107.3±18.17                         | 9.6±4.64                |
| Group III| TRM    | 20 mg/kg   | 15.0±4.96                         | 145.8±13.73                         | 22.6±6.94               |
| Group IV| TRM     | 40 mg/kg   | 12.0±5.65                         | 147.8±12.16                         | 18.0±6.27               |
| Group V | FLX+TRM | 20+20 mg/kg | 7.8±4.96                          | 147.0±13.05                         | 18.0±3.80               |
| Group VI| FLX+TRM | 20+40 mg/kg| 23.6±4.14                         | 154.5±7.27                          | 27.0±3.12               |

Values expressed as mean±SEM, n=6 in each group, df=5, 30; p>0.05 for all intergroup comparisons i.e. non-significant, SEM: Standard error of mean, TRM: Tramadol, FLX: Fluoxetine

### Table 6: Open field test - Chronic study 14 days.

| Group   | FLX drugs  | Doses      | Number of central squares crossed | Number of peripheral squares crossed | Total number of rearing |
|---------|------------|------------|-----------------------------------|-------------------------------------|-------------------------|
| Group I | Normal saline | 0.1 ml/10 g | 28.0±6.74                         | 168.3±11.67                         | 29.3±2.98               |
| Group II| FLX       | 20 mg/kg   | 15.0±6.71                         | 118.7±18.06                         | 13.0±1.69               |
| Group III| TRM     | 20 mg/kg   | 19.0±7.78                         | 131.8±17.51                         | 26.3±7.33               |
| Group IV| TRM      | 40 mg/kg   | 12.1±5.90                         | 142.8±17.87                         | 16.3±2.89               |
| Group V | FLX+TRM  | 20+20 mg/kg | 4.3±1.25                          | 120.0±21.66                         | 15.8±4.19               |
| Group VI| FLX+TRM  | 20+40 mg/kg| 21.0±6.11                         | 115.0±17.03                         | 30.0±6.30               |

Values expressed as mean±SEM, n=6 in each group, df=5, 30; p>0.05 for all intergroup comparisons i.e. non-significant, SEM: Standard error of mean, TRM: Tramadol, FLX: Fluoxetine
system.\textsuperscript{13,14} Imidazoline receptors (I\textsubscript{1} and L) also may involve in antidepressant like activity of TRM in mice.\textsuperscript{15} These factors explain the capacity of TRM to acts as antidepressant.

TRM induces changes in central nervous system also similar to those induced with conventional antidepressants. It decreases the binding of frontocortical \( \beta \) adrenergic receptors, 5-HT\textsubscript{2A} receptors and \( \alpha_{2} \)-adrenoreceptor, but increase the binding of \( \alpha_{1} \)-adrenoreceptor and dopamine D\textsubscript{2} and D\textsubscript{3} receptors. In addition it inhibits locus ceruleus firing activity through \( \alpha_{2} \)-adrenoreceptors mechanism like antidepressant compound.\textsuperscript{16}

The acute administration of TRM produces antidepressant like the activity by a mechanism that involved inhibition of l-arginine-NO-cGMP pathway. The antidepressant activity of TRM also involves the K\textsuperscript{+} channels.\textsuperscript{17}

Chronic treatment with TRM at dose (10-40 mg/kg) increase the density of \( \alpha_{1} \)-adrenergic receptors, decrease the density of \( \alpha_{2} \)-adrenoreceptor and cause up regulation of dopamine D\textsubscript{2} and D\textsubscript{3} receptors in the nucleus accumbens like antidepressant drug.\textsuperscript{2,18,19} These finding can explain observed antidepressant action of TRM in our study.

Previous studies like Kalra et al. (2008), Szkutnik-Fiedler et al. and Kishore et al. have also shown that the opioid analgesic drug TRM has antidepressant effect and it was compared with other drugs like imipramine, venlafaxine, FLX etc. using various animal models of depression. Our results correlate with the findings in above studies showing antidepressant action of TRM.

Finally, TRM, in both doses, produces a greater antidepressant action when combined with the standard antidepressant, i.e., FLX. This antidepressant action of the combination group was far more significant when compared to both TRM and FLX given alone.

The enhanced antidepressant effect observed in our study after a combination of TRM with FLX is probably a result of additive interaction between two drugs, due to the similar mechanism of the inhibiting reuptake of serotonin and norepinephrine.

This finding may also be due to its ability to modulate the opioid receptors and dopaminergic system, which is similar to the conventional antidepressants like venlafaxine, which blocks reuptake and results in enhanced and prolonged serotonergic, norepinephrine and dopaminergic neurotransmission.\textsuperscript{20}

Hence from our study, we propose that TRM is a good antidepressant especially when it is used in combination with a standard antidepressant. Since this is an animal study, we need these results to be confirmed in human studies for the further establishment of the role of TRM as an antidepressant.

CONCLUSION

The present study was designed to investigate the antidepressant activity of the TRM alone and in combination with FLX. In this study, TRM alone (in doses of 20 mg/kg and 40 mg/kg) and in combination with FLX on acute and chronic administration showed significant antidepressant action in mice exposed to both FST and TST. An insignificant result in open field test shows that TRM does not modify general locomotor activity of the animal.

From our study, we conclude that TRM (alone and in combination with FLX) possesses significant antidepressant activity in animal models of depression.

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