

Original Article

Antidepressant and Anxiolytic Effect of Tramadol as Compared to Imipramine, In Acute and Chronic Dosage in Rats

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ABSTRACT

Objective: To assess the anti-depressant and anxiolytic effect of tramadol as compared to imipramine (approved antidepressant) in acute and chronic doses in rats.

Methodology: This observational experimental animal study was carried out from December 2020 to February 2021 at Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro in collaboration with Agricultural University, Tandojam. Forty-eight healthy male rats housed at Animal House Sindh, Agricultural University, Tandojam after due approval from institutional ethical review board. The 48 rats were categorized into 3 equal groups of 16 each: Group A for Normal saline (0.9% NaCl) 15ml/kg, Group B for Imipramine 15 mg/kg and Group C for Tramadol 15 mg/kg. Each group was further subdivided into two groups namely acute A1, B1, C1 and chronic A2, B2, C2 and evaluated for anti-depressant and anxiety activity using forced swim test and elevated plus maze test. The data obtained was analyzed using SPSS. 22.0.

Results Tramadol acted to significantly reduce the mean duration of immobility as compared to the control ($P < 0.001$). Resolution of immobility due to tramadol was insignificant when compared to imipramine. Likewise, the swimming periods in the tramadol and imipramine groups were significantly longer than the control group (< 0.001), but almost equal in both tramadol and imipramine groups, showcasing that tramadol has antidepressant activity at par with imipramine (p value > 0.05).

Conclusion: Tramadol exhibits significant acute and chronic antidepressant and anxiolytic effects in rats when compared to imipramine and controls.

KEYWORDS: Depressive Disorder (Major), Antidepressive Agents, Anti-Anxiety Agents, Tramadol, Imipramine.

INTRODUCTION

Major Depressive Disorder (MDD), a common disorder of the mind, is characterized by a loss of pleasure or interest, depressed mood, feelings of low self-worth and exaggerated guilt, low motivation / energy, disturbances in the appetite and sleep, coupled with a sub-par concentration level.¹ The characteristic symptoms MDD may be experienced by a patient acutely or for extended periods of time (chronicity) and the symptoms may come and go (recurrent). However, one thing that remains constant is the fact that the symptoms adversely affect (invariably) the ability of an individual to deal with day-to-day responsibilities.¹ Over a 121 million individuals are affected by MDD across the globe.²

The World Health Organization (WHO) states that “MDD is the leading cause of disability as measured by Years Lived with Disability (YLDs) and the fourth leading contributor to the global burden of disease”. It was formerly stipulated that by 2020, MDD may attain the 2nd position in the rank of Disability Adjusted Life Years (DALY) calculated for all ages and the stipulation proved correct; as today, MDD has attained the position of the second commonest cause (for individuals aged 15 - 44 years) of DALYs.³ Data derived from epidemiological research hints at a more prevalent distribution of MDD in the Middle East, North Africa, America, and South Asia.⁴ With 4 among the 10 countries with highest prevalence of MDD belonging to South East Asia. 27% of YLDs and 11% of DALYs in the region are attributed to depressive disorders. A review of epidemiological studies on MDD in South Asia concluded that “the prevalence in primary care is from 26.3% to 46.5%”. Additionally, research stemming from Pakistan during the last decade report the prevalence of MDD to range between 22% to 60%.⁵ Despite availability of pharmacologic therapy, the disease morbidity is much.

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In instances wherein the treatment is successful, and is noted; the depressive disorders do not cease to impose a burden. A case with remission is not guaranteed to be free from all symptoms and certain residual symptoms, particularly social dysfunction and cognitive impairment may continue to be a cause of considerable distress.⁶ The risk of relapse is also ever-present, and recurrence is not rare; consequently, the quality of life in such circumstances is sub-par. A recent review reported that “the rate of recurrence of MDD treated in specialized mental health settings was very high (60% after 5 years, 67% after 10 years, and 85% after 15 years)”.⁷ Thus owing to the chronic nature of the condition, incomplete remission and eventual relapse, individuals suffering from MDD see no end to their suffering.⁸ As stated above, because existing therapies are not sufficing, there is a great deal of unmet clinical need.^{9,10} The mechanism of action of antidepressants available for commercial use is such that it brings about an increase in the serotonin levels (primarily), coupled with a hike in the norepinephrine and or dopamine levels. The hike in the levels of said chemicals exerts both acute and chronic effects.¹¹ thereby asserting its influence to counteract depression acutely and for extended periods of time. Nonetheless, the concoctions are not devoid of complications and adverse effects such as “intolerability, delayed therapeutic effects, limited efficacy in milder depression and existence of treatment resistant depression”. This fuels the drive to continually search for safer and more potent drugs.^{12,13} Few of the previous studies evaluated tramadol which exhibited antidepressant activity in mice using different experimental model of depression.^{14,15} If tramadol is to be proposed as a viable adjunct, more detailed animal testing must be done prior to human trials.^[11] This research investigates the antidepressant and anti-anxiety effects of Tramadol and compares it with imipramine (approved / commercially available anti-depressant) in forced swimming and elevated maze test animal models within 24 hours (acute dosing) and 15 days (chronic dosing).

METHODOLOGY

This experimental animal study was the project of Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro. It was carried out from December 2020 to February 2021 in collaboration with Agricultural University, Tandojam. This project was reviewed and approved by Advanced Studies and Research Board (ASRB) of LUMHS (Doc# LUMHS /Reg/ACD-28265/68). Sample of 48 healthy male rats

achievement of remission

were (housed at an ambient humidity and temperature, with a 12-hour light - dark cycles and ample access to standard pellet and water) at Animal House Sindh, Agricultural University, Tandojam after taking due gate keeper permission. Healthy Male rats with normal behavior and activity weighing between 40 – 80 grams with age 3-4 months were taken while those not meeting the criteria or were previously used in other experiments were excluded. The 48 rats were categorized into 3 equal groups of 16 each: Group A for Normal saline (0.9% NaCl) 15ml/kg, Group B for Imipramine 15 mg/kg and Group C for Tramadol 15 mg/kg. Each group was subdivided into two groups namely acute A1, B1, C1 and chronic A2, B2, C2 and evaluated for anti-depressant and anxiety activity using forced swim test and elevated plus maze test.

Forced swim test – standard protocol

Rats are individually forced to swim inside vertical plexiglass cylinder (25×10×25 cm³) filled with a water to a height of 15 cm. After an initial 2-minute period of vigorous activity, each animal assumes a typical immobile posture. The total duration of immobility, swimming and climbing is recorded each 2 min and total of 6 min test. Duration of immobility, swimming and climbing period is compared with those of control and imipramine group. Twenty-three hours after the first exposure, rats are intraperitoneally administered with drugs at a dose of 15 mg/kg at 8:00 AM or 8:00 PM. Control rats are administered Saline. Drugs is administered to the rats 40 min before conducting the study. For Acute effect assessment like immobility, swimming and climbing. One hour after the administration, the FST is carried out. For the analysis of the Chronic effect Rats are exposed to the FST apparatus and the drug treatment is started the next day. The rats are intraperitoneally administered Saline, Tramadol and Imipramine for 15 days. To equalize the number and the timing of the intraperitoneal injections among the groups, all rats are injected twice a day. Two days after the last administration, the rats are administered drugs at a low dose (10 mg/kg) during the early light period, and 1 hour after the administration, the FST and Elevated maze test is carried out. The rats are returned to their home cage after the FST and Elevated maze test.

Elevated maze test – standard protocol:

The elevated maze is a widely used behavioral assay for anxiety behavior of rats. It is easy to use, can be fully automated and valid results can be obtained in a short, 4-min (2 min for open arm and 2 min for

closed) testing period. Performa attached will be filled accordingly.

1. The maze is assembled in an isolated room away from any extraneous interference of noises, scents, or movement.
2. The experimenter is restrained from making any excessive noise or movement during the entire trial and from wearing perfumes, colognes, or any product with a strong smell, since it could act as anxiety stimulus for mice.
3. Illumination in the room must be measured with the aid of a lux meter, kept constant and controlled according to the analysis that is to be performed. Given that low-intensity luminosity reduces open arm avoidance to analyze an anxiogenic effect low-intensity lighting (5-30 Lux) should be preferred, whereas an anxiolytic effect should be analyzed under higher intensity lighting (200-400 Lux or more).
4. After these experimental conditions are adjusted to a standard, the animals will be brought into the experiment room, where they will be left in their home cages for 45 to 60 min to recover from the stress of being moved.
5. Clean the maze with 70% ethanol before starting the test to remove any dirt or smells accumulated on the apparatus.
6. Turn on the video camera and place the first rat in the center square of the maze facing one of the open arms, preferably the one opposite to the experimenter.
7. The experimenter will stand as far away as possible from the maze and out of sight of the test animal, outside of the room if necessary. He must also avoid making unnecessary movement or sounds.
8. After 5 min of free exploration, the rat may be moved out of the maze and back into its home cage.
9. All the urine and fecal boli must be removed and the maze cleaned entirely with 70% ethanol to remove any residual smell from the first rat. Afterwards, the next rat may be submitted to the test.

Statistical Analysis: The data obtained was analyzed using SPSS version 22.0. Shapiro Wilk test was performed to confirm whether the data followed the normal distribution.

Data conforming to a normal distribution used as mean and standard deviation (SD). Means were compared using parametric test ANOVA. Multiple comparisons were done by post hoc test. P value \leq

Following steps for Elevated maze test:

0.05 indicated statistically significant in statistical analysis.

RESULTS

In the acute setting, the mean duration of immobility in the control group was observed to be 206.25 ± 8.0 seconds(sec), whereas it was 83.6 ± 6.5 sec and 96.1 ± 5.5 sec in the group pretreated with imipramine and tramadol (Table1). In the chronic setting, similar result trends were yielded with the mean duration of immobility in the control group being 193.31 ± 8.0 sec, whereas it was 79.2 ± 2.7 sec and 80.1 ± 4.9 sec in the group pretreated with imipramine (Table 2). The decrease in immobility period in the group pretreated with imipramine or tramadol as compared to control was highly significant (P value < 0.001) (Table 1).

Table 1: Mean Time Differences after 24 Hours Administering Injections in acute setting

	A1 Normal Saline	B1 Imipramine	C1 Tramadol	P values
Forced Swim Test				
Mean Immobility Time	206 ± 8.0	83.6 ± 6.5	96.1 ± 5.5	<0.001
Mean Climbing Time	91.82 ± 5.5	88.1 ± 3.9	91.2 ± 4.1	> 0.05
Mean Swimming Time	91.93 ± 4.1	188.3 ± 9.7	172.7 ± 8.3	<0.001
Elevated Maze Plus Test				
Open Arm Time	48.5 ± 2.1	53.2 ± 5.8	53.4 ± 5.6	>0.05
Close Arm Time	71.5 ± 3.9	66.8 ± 4.7	66.6 ± 5.2	>0.05

P value ≤ 0.05 is taken as significant

However, on multiple comparisons no significant difference was obtained between tramadol and imipramine in acute as well as in chronic settings, with p value > 0.05 , showcasing that tramadol has antidepressant activity at par with imipramine (Tables 1 and 2).

Table 2: Mean Time Differences in seconds after 15 Days Administering Injections in Chronic Setting

	A2 Normal Saline	B2 Imipramine	C2 Tramadol	P values
Forced Swim Test				
	Mean \pm SD	Mean \pm SD	Mean \pm SD	P values
Immobility Time	193.3 ± 8.0	79.2 ± 2.7	80.1 ± 4.9	$<.001$
Climbing Time	83.2 ± 3.8	92.5 ± 3.1	98.1 ± 4.2	$> .05$

Swimming Time	83.5 ± 3.1	188.3 ± 7.2	181.8 ± 6.8	<.001
Elevated Maze Plus Test				
Open Arm Time	49.1 ± 2.5	58.3 ± 7.1	57.9 ± 6.9	> .05
Close Arm Time	70.9 ± 2.1	61.7 ± 6.1	62.1 ± 6.2	> .05

P value ≤ 0.05 is taken as significant

Current results also reveal significant increase in mean swimming time in tramadol and antidepressant group as compared to control groups after 24 hours as well as 15 days of injection administration with p value <0.001 (Tables 1 & 2). Likewise, the climbing time in the tramadol and imipramine groups were longer than the control group, but the difference was not found to be statistically significant (p value > 0.05). On the other hand on multiple comparison, both climbing and swimming periods in pretreated imipramine and tramadol groups are almost found to be same with p values > 0.05. In elevated maze plus test, no significant difference observed in open as well as closed arm time in both acute and chronic settings (Tables 1 & 2)

DISCUSSION

In this study, the antidepressant-like effect of tramadol was investigated in the forced swimming test, an animal model predictive of antidepressant activity. In our experimental conditions, tramadol showed a clear inhibition of immobility latencies (antidepressant-type effect). This effect was similar to that obtained with imipramine and tricyclic antidepressant in published evidence.¹⁶

This is endorsed by existing research that examined the neurochemical profile of tramadol and revealed that it binds to opioid receptors in the same concentration range in which it inhibits the uptake of noradrenaline and serotonin. In humans, opiates have been proved be useful in treating some forms of refractory depression. Finally, several studies have documented two components in the efficacy of antidepressants as an adjuvant therapy for chronic pain. One of them is the increase in mood level that is otherwise frequently decreased in chronic pain patients; and the other is a proper anti-nociceptive effect.¹⁷ In fact, monoamines and opioid pathways are implicated both in pain and mood. In this respect, it could be inferred from experimental studies that tramadol might add an affective (positive emotional) component to its analgesic effect. Further preclinical studies are needed to explore the effect of different administration regimes and the efficacy of tramadol in other types of depression tests.^{18, 19}

This research only investigated the effect of tramadol in a standardized singular dosage regimen; however, others have studied the effect at different doses. One such research revealed that tramadol produced significant antidepressant effect at three different doses and that the antidepressant effect of tramadol at doses of 10 and 20 mg/kg was comparable with that of fluoxetine. Published evidence also claims that the antidepressant activity of tramadol (at a dose of 40mg/kg) surpasses that of fluoxetine in animal models.^{20,21} Antidepressants (selective serotonin reuptake inhibitors; Venlafaxine), by virtue of their property of mood elevation and increasing the level of serotonin and consequently causing inhibition of release of transmitters carrying the pain sensation from nerve endings, are efficacious in chronic pain as an adjunctive treatment. Similarly, it could be inferred from our study that tramadol by acting through similar mechanism (inhibition of reuptake of monoamines leading to spinal inhibition of pain) might add mood-elevation component to its analgesic effect. More preclinical studies in different antidepressant models are needed to corroborate our observations.^{22, 23}

Mico[´] and associates (2003) exposed rats to conditions that mimicked a learned helplessness model of depression. Results showed that rats responded more positively to tramadol, as opposed to placebo and/or methadone. The action tramadol has on alpha 2-adrenergic receptors in the brain of rats is implicated in antidepressant action because downregulation of these receptors control 5-HT and growing evidence suggests dysfunction of these receptors in depression.²⁴ Jesse and associates (2010) conducted a study that shows evidence that the noradrenergic system, as well as dopaminergic receptors, is involved in the antidepressant effect of tramadol on mice. Various alpha 2, D1, D2, and D3 receptor antagonists were co-administered with tramadol to examine alterations in the mouse brain. Antagonists of those receptor sites blocked the antidepressant effects of tramadol. In conjunction, Breuer and colleagues (2009) presented data of pramipexole (D3/D2 receptor agonist) and 7-OH-DPAT (D3 receptor agonist), having antidepressant effects in the rat model.²⁵ Inhibition of 5-HT_{2C} promotes release of dopamine while disinhibition reduces dopamine concentrations.²⁶

This study is among the only few attempts made at only gauging the anti-depressant and anxiolytic effect of tramadol but comparing it against an established agent such as imipramine and controls. The statistics yielded are largely novel and may help serve as the basis for future research. In addition to that, it identifies correlates that may serve as reliable

supplemental treatment options in conjunction with present medications to facilitate treatment of depression.

There are, however, some limitations of our study. First, we did not arrange for comparison with more drugs and just resorted to comparison with imipramine, additionally, we chose only one standardized singular dose of tramadol. Furthermore, there is dearth of published evidence-based literature with which this study could be compared again intensively, thus little is known regarding how this study's results fair other experiments. As the extent of this research was limited to a small sample size and just a singular dose of tramadol was employed, in future similar research may be carried out on a larger sample of participants with more tramadol doses being studied to further the investigation in this field.

CONCLUSION

Tramadol exhibits significant acute and chronic antidepressant and anxiolytic effects in rats similar to imipramine and controls.

Conflict of Interest: The authors declare that the research was conducted in absence of any commercial or financial relationship and have no conflict of interest involved in this research

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Ethical Statement Involving Animal Subjects: This study involving animals were reviewed and approved by Advanced Studies and Research Board of Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro.

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Fiza Soomro	Study design, acquisition of data and manuscript writing. Revised and approved the articles. Study design, acquisition of data and manuscript writing. Revised and approved the articles.
Nasreen Kazi	Study design, acquisition of data and manuscript writing. Revised and approved the articles
Aatir H. Rajput	Study design, data analysis and interpretation and write up of results. Revising manuscript critically for important intellectual content.
Sadat Memon	Study design, acquisition of data, data analysis and interpretation, revised and approve the manuscript
	All authors are equally accountable for accuracy, integrity of all aspects of the research work.

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