

Original Article

EVALUATION OF ANTIDEPRESSANT EFFECT OF CHRONIC ADMINISTRATION OF TRAMADOL ALONE AND IN COMBINATION WITH FLUOXETINE IN LOW DOSES IN ALBINO MICE

SIRISHA G, RAHUL PRAKASH B, USHA NS, MADHU DHAKHAYANI K

Department of Pharmacology, Kamineni Institute of Medical Sciences, Narketpally, Andhra Pradesh, India.

Email: dr.sireesha@gmail.com

Received: 06 Mar 2014 Revised and Accepted: 04 Apr 2014

ABSTRACT

Objective: To study the antidepressant effect of chronic administration of Tramadol alone and its combination with Fluoxetine in low doses in albino mice.

Methods: the antidepressant effect was determined by recording the immobility time in Tail Suspension Test (TST) and the time the mice maintained the cataleptic posture in Haloperidol induced Catalepsy (HIC). The mice were randomly divided into seven groups with six mice in each group. Mice belonging to group 1 were given Normal Saline (10 ml/kg) which acts as control. Tramadol was given in graded doses (10, 20 and 40 mg/kg) to mice of groups 2, 3 and 4. Mice of groups 5 and 6 received graded doses of Fluoxetine (10 and 20 mg/kg) which is the standard drug. A combination of low doses of Tramadol (10 mg/kg) and Fluoxetine (10 mg/kg) was given to mice belonging to group 7. All the drugs were given intraperitoneally for a period of seven days and antidepressant activity was evaluated on day 7 of drug administration.

Results: Tramadol and Fluoxetine have shown significant antidepressant activity when compared to the control. There is dose dependant increase in antidepressant activity of Tramadol. The antidepressant of Tramadol 20 mg/kg was comparable to Fluoxetine 20 mg/kg. Tramadol potentiated the antidepressant effect of Fluoxetine in the low dose combination group. The results were comparable in both the models of depression.

Conclusion: The present study indicates the antidepressant activity of Tramadol and potentiation of antidepressant activity when it is combined with Fluoxetine in low doses.

Keywords: Antidepressant, Tramadol, Fluoxetine, Combination, Tail Suspension Test, Haloperidol Induced Catalepsy.

INTRODUCTION

Major depressive disorder (MDD) is characterized by depressed mood most of the time for at least 2 weeks and/or loss of interest or pleasure in most activities. In addition, depression is characterized by disturbances in sleep and appetite as well as deficits in cognition and energy. Thoughts of guilt, worthlessness, and suicide are common. An estimated 5.8% of men and 9.5% of women experience depressive episodes in their lifetime. 60% of death toll due to suicides is with depressive illness[1]. Approximately two-thirds of the depressed patients respond to the currently available treatments (Tricyclic antidepressants, Selective serotonin reuptake inhibitors, etc), but the magnitude of improvement is still disappointing. More over these drugs have unusual side effects. So the search for more effective drugs with fewer side effects is continuing.

Tramadol is a synthetic centrally acting opioid analgesic used mainly for the treatment of moderate-to-severe pain. It produces analgesia because of weak μ opioid receptor agonist and by inhibiting uptake of norepinephrine and serotonin[[2]]. Tramadol causes activation of both systems mainly involved in inhibition of pain, i.e., the opioid and the descending monoaminergic pain-modulating pathways. There is a large body of evidence to suggest that the analgesic action of Tramadol is mainly related to central monoaminergic mechanism rather than opioid receptor pathway[[3]]. It has also been observed that Tramadol-induced analgesia is blocked by α_2 adrenergic receptor antagonist Yohimbine[[4]].

Studies have already shown that Tramadol effectively inhibits reuptake of monoamines. It has also been established that Tramadol inhibits reuptake of serotonin in the raphe nucleus[5] Tricyclic antidepressants mainly act by inhibiting Norepinephrine or Serotonin reuptake; Tramadol by virtue of its property of blocking monoaminergic reuptake may act as an antidepressant. Also, Tramadol bears a close structural similarity to antidepressant Venlafaxine and thus shares a number of its molecular and pharmacological features.

The antidepressant activity of Tramadol is being worked about in recent times in animal models[6,7]. In the present study, the antidepressant activity of combination of low doses of Tramadol and Fluoxetine was evaluated and also compared with varying doses of Tramadol and Fluoxetine.

Epidemiological studies confirm frequent appearance of pain symptoms in depressed patients and a marked prevalence of depression in pain conditions. These observations seem to point at a close intertwining between mood regulation and pain perception. In the pathogenesis of both depression and pain symptoms, an important role has been attributed to disturbances of serotonergic and noradrenergic neurotransmission as well as to neuropeptides such as opioids and substance P.

MATERIALS AND METHODS

The present study was conducted in Department of Pharmacology, Kamineni Institute of Medical Sciences, Narketpally from October 2010 to September 2012. The study was placebo controlled, Randomized, Laboratory-based comparative study on animals with prior permission of Institutional Animal Ethics Committee (IAEC) Swiss albino mice (25-30 grams) of either sex, were used. Animals were procured from Central Animal House of National Institute of Nutrition (NIN), Hyderabad and kept in air conditioned environment in the Central Animal House, KIMS, Narketpally. After procurement, a study gap of one week was given for acclimatization. The animals were housed under standard laboratory conditions, maintained on a 12:12h light dark cycle and had free access to food and water. All the experiments were carried out between 10:00 and 15:00 hr.

Drugs procured from

Tramadol (Zydus Cadila)
Fluoxetine (Sigma Aldrich)
Normal Saline
Haloperidol (RPG Life Sciences Ltd)

Table 1: It shows grouping of mice and doses of drugs administered to mice for seven days

Groups n=6	Drug	Status	Dose (mg / kg) intraperitoneally
1	Normal Saline	Control	10 ml / kg
2	Tramadol	Test	10
3	Tramadol	Test	20
4	Tramadol	Test	40
5	Fluoxetine	Standard	10
6	Fluoxetine	Standard	20
7	Tramadol + Fluoxetine	Combination	10 + 10

Tail suspension test

This animal model for testing antidepressant activity is based on the principle that suspending mice upside down leads to a characteristic behavior of immobility after initial momentary struggle[8]. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.

Test drugs were administered intraperitoneally for 7 days. On day 7, Tail Suspension Test (TST) was conducted after 40 minutes of drug administration. Mice were suspended on the edge of the rod of a stand 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Mouse was considered to be immobile when it did not show any movement of the body and hanged passively. The total duration of immobility was recorded during the last 4 minutes (240 seconds) of total 6 minutes test.

**Fig. 1: It shows mice subjected to tail suspension test****Haloperidol-Induced Catalepsy**

Catalepsy was induced with haloperidol (1.0 mg/kg intraperitoneal) and assessed at 30 minute intervals until 120 minutes by means of a standard bar test. Haloperidol 1 mg/kg was chosen so that it could elicit a moderate degree of catalepsy and thus enable the detection of either attenuation or potentiation of the phenomenon [9]. Catalepsy was assessed in terms of the time for which the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high wooden bar (1 cm diameter).

The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 1100 seconds was applied. If the animal maintained the imposed posture for at least 20 seconds, it was considered to be cataleptic and given one point. For every additional 20 sec that the cataleptic posture was maintained, one extra point was given.

RESULTS**Tail suspension test**

Lesser the immobility time, greater is its antidepressant activity. The immobility time of Tramadol groups is lesser than the control group, which signifies the antidepressant activity of Tramadol. There is dose dependent decrease in immobility time with Tramadol. The combination group of Tramadol and Fluoxetine showed lesser immobility time than either group alone.

Tramadol in all the three doses significantly reduced the immobility time in second in comparison to control group suggesting Tramadol

produced antidepressant activity in tail suspension test. The standard antidepressant drug Fluoxetine, in the doses of 10 mg/kg and 20 mg/kg i.p. significantly reduced the immobility time in seconds in comparison to control group confirming its antidepressant activity.

**Fig. 2: It shows cataleptic posture of mice in the haloperidol induced catalepsy test**

The combination of low doses of Tramadol 10mg/kg and Fluoxetine 10 mg/kg significantly reduced the immobility time in seconds in comparison to Tramadol 10 mg/kg alone or Fluoxetine 10 mg/kg alone suggesting Tramadol can potentiate antidepressant action of

Fluoxetine. Further the immobility time in seconds after Tramadol 20 mg/kg is not significantly different than Fluoxetine 20 mg/kg suggesting antidepressant activity of Tramadol is comparable to Fluoxetine in Tail Suspension Test.

Table 2: It shows the immobility time of mice (Mean ± SE) in Tail Suspension Test

Groups	Mean ± SE
Control(NS)	202.67 ± 4.66
Tramadol 10mg/kg(T10)	137.50 ± 5.45
Tramadol 20mg/kg(T20)	109.67 ± 3.06
Tramadol 40mg/kg(T40)	101.17 ± 2.97
Fluoxetine 10mg/kg(F10)	122.67 ± 2.73
Fluoxetine 20mg/kg(F20)	105.17 ± 2.57
Tramadol 10mg/kg + Fluoxetine 10mg/kg(T10+F10)	107.83 ± 3.32

Table 3: It shows one way ANOVA for comparison between the groups in Tail Suspension Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45993.33	6	7665.56	94.40	0.0001***
Within Groups	2842.00	35	81.200		
Total	48835.33	41			

There is significant difference between the groups ($p < 0.001$) in Tail suspension test

Table 4: It shows comparison of Mean Immobility time between different groups in Tail Suspension Test (post hoc LSD test)

Comparison of groups	Mean difference	P value	Significance
Control vs T10	65.17	0.0001	***
Control vs T20	93.00	0.0001	***
Control vs T40	101.50	0.0001	***
Control vs F10	80.00	0.0001	***
Control vs F20	97.50	0.0001	***
Control vs T10+F10	94.83	0.0001	***
T10 vs T20	27.83	0.0001	***
T10 vs T40	36.33	0.0001	***
T10 vs F10	14.83	0.007	**
T10 vs F20	32.33	0.0001	***
T10 vs T10+F10	29.67	0.0001	***
T20 vs T40	8.50	0.111	Ns
T20 vs F10	-13.00	0.017	*
T20 vs F20	4.50	0.393	Ns
T20 vs T10+F10	1.83	0.727	Ns
T40 vs F10	-21.50	0.0001	***
T40 vs F20	-4.00	0.447	Ns
T40 vs T10+F10	-6.67	0.208	Ns
F10 vs F20	17.50	0.002	**
F10 vs T10+ F10	14.83	0.007	**
F20 vs T10+ F10	-2.67	0.611	Ns

P value <0.001***, <0.01**, <0.05*, ns – not significant

Table 5: It shows Catalepsy score in points (Mean ± SE) in Haloperidol Induced Catalepsy

Group	NS	T10	T20	T40	F10	F20	T10+F10
30 min	17.17 ± 1.47	14.83 ± 1.51	13.33 ± 0.99	11.33 ± 0.17	14.00 ± 1.29	12.83 ± 1.05	13.00 ± 1.10
60 min	24.50 ± 1.93	23.83 ± 2.29	18.00 ± 1.06	16.17 ± 1.14	21.00 ± 1.21	17.00 ± 1.13	17.50 ± 1.15
90 min	36.50 ± 2.17	34.00 ± 2.29	20.50 ± 1.31	18.50 ± 0.99	27.67 ± 1.41	19.67 ± 1.20	20.00 ± 1.13
120 min	51.33 ± 1.43	39.17 ± 3.05	23.83 ± 1.35	19.67 ± 1.09	32.50 ± 0.96	21.50 ± 1.26	22.67 ± 1.48
Average Mean ± SE	32.38 ± 1.09	27.96 ± 1.09	18.92 ± 1.09	16.42 ± 1.09	23.79 ± 1.09	17.75 ± 1.09	18.29 ± 1.09

Table 6: It shows comparison between the groups in Haloperidol induced catalepsy using multiple way ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	82903.71	1	82903.71	2931.93	0.0001***
groups	5244.62	6	874.10	30.91	0.0001***
Error	989.67	35	28.28		

There is significant difference between the groups ($p < 0.001$) in Haloperidol induced catalepsy

Haloperidol Induced Catalepsy

Lesser the cataleptic score in Haloperidol induced catalepsy, greater is the antidepressant activity. The cataleptic score of mice of control group is greater than the catalepsy scores of Tramadol groups, which signifies the antidepressant activity of Tramadol. There is dose dependent decrease in cataleptic score of Tramadol. The cataleptic score of combination group is lesser than either Tramadol or Fluoxetine given alone.

Tramadol in all the three doses significantly reduced the mean catalepsy score in points in comparison to control group suggesting Tramadol produced antidepressant activity in haloperidol induced

catalepsy test. The standard antidepressant drug Fluoxetine, in the doses of 10 mg/kg and 20 mg/kg i.p. significantly reduced the mean catalepsy score in points in comparison to control group confirming its antidepressant activity. The combination of low doses of Tramadol 10mg/kg and Fluoxetine 10 mg/kg significantly reduced the catalepsy score in points in comparison to Tramadol 10 mg/kg alone or Fluoxetine 10 mg/kg alone suggesting Tramadol can potentiate antidepressant action of Fluoxetine. Further the mean catalepsy score in points after Tramadol 20 mg/kg is not significantly different than Fluoxetine 20 mg/kg suggesting antidepressant activity of Tramadol is comparable to Fluoxetine in haloperidol induced catalepsy test.

Table 7: It shows comparison of Mean Immobility time between different groups in Haloperidol induced catalepsy (post hoc LSD test)

Comparison of groups	Mean difference	P value	Significance
Control vs T10	4.42	0.007	**
Control vs T20	13.46	0.0001	***
Control vs T40	15.96	0.0001	***
Control vs F10	8.58	0.0001	***
Control vs F20	14.63	0.0001	***
Control vs T10+F10	14.08	0.0001	***
T10 vs T20	9.04	0.007	**
T10 vs T40	11.54	0.0001	***
T10 vs F10	4.17	0.010	**
T10 vs F20	10.21	0.0001	***
T10 vs T10+F10	9.67	0.0001	***
T20 vs T40	2.50	0.112	Ns
T20 vs F10	-4.88	0.003	***
T20 vs F20	1.17	0.452	Ns
T20 vs T10+F10	0.62	0.686	Ns
T40 vs F10	-7.38	0.0001	***
T40 vs F20	-1.33	0.391	Ns
T40 vs T10+F10	-1.88	0.230	Ns
F10 vs F20	6.04	0.0001	***
F10 vs T10+ F10	5.50	0.001	***
F20 vs T10+F10	-0.54	0.726	Ns

P value <0.001***, <0.01**, <0.05*, ns – not significant

DISCUSSION

The therapeutically used antidepressant drugs like Tricyclic antidepressants, Selective Serotonin Uptake Inhibitors and atypical antidepressants produce effect on brain monoaminergic system. The clinically used analgesic Tramadol apart from having effect on μ type opioid receptors, also have monoaminergic uptake blockade effect. Hence, the present study was carried out to evaluate the antidepressant activity of Tramadol in Tail Suspension Test and Haloperidol Induced Catalepsy, which are conventional animal models for evaluation of antidepressant activity. The results obtained have been compared with the standard antidepressant drug, Fluoxetine. Also, the effect obtained due to combination of low doses of Tramadol 10 mg/kg and Fluoxetine 10 mg/kg determined, so that the toxicity of either drug can be reduced, while increasing their efficacy as antidepressants.

In Tail Suspension Test, mice subjected to a non-solvable aversive situation alternate between agitation and immobility. The reason of agitation is searching to get out of the situation which is highly energy consuming, while the purpose of immobility is energy conservation. Animals after antidepressant treatment struggle more even in desperate situation, and they spend less time with immobility. These tests are quite sensitive and relatively specific to all major classes of antidepressants like Tricyclic Antidepressants, Selective Serotonin Reuptake Inhibitors (SSRI), MAO Inhibitors and atypical antidepressants. It has been argued that Tail Suspension Test is less stressful than the Forced Swim Test and has greater pharmacological sensitivity.

Antidepressants inhibit the cataleptic state induced by Haloperidol. This catalepsy induced by neuroleptics like Haloperidol is due to blockade of post synaptic dopamine receptors. Despite this evidence,

several other neurotransmitters such as serotonin and opioids have also been implicated in the catalepsy induced by Haloperidol.

So these tests have been attempted in the context that few previous studies have explained that the antidepressant activity of Tramadol is due to its ability to modulate opioid receptors, the serotonergic system and the dopaminergic system.

The antidepressant-like effect of Tramadol may be explained on its ability to modulate Noradrenaline as indicated by some earlier studies[10],[11]. Imidazoline receptors as well as opioid receptors[12] may also be involved in the antidepressant-like activity of Tramadol in mice. Jesse et al showed that the acute administration of Tramadol produces antidepressant effect by inhibition of L-arginine- NO- Camp pathway[13]. The same investigators also suggested that oral administration of Tramadol produces antidepressant-like effect in mice by a mechanism that involves the K^+ channels[14]. All these findings need to be confirmed in the future studies to get conclusive evidence regarding the mechanism of antidepressant activity of Tramadol.

CONCLUSION

This study suggests that Tramadol has significant antidepressant activity, which is further potentiated when it is combined with Fluoxetine in low doses. Further studies in humans may be required to confirm the results of animal studies.

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