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Original Article

The Effectiveness of a Combination of Low Dose Citalopram and Tramadol in Reducing Immobility Time in Forced Swimming Test in Mouse Model of Depression

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Abstract

This study was conducted in the pharmacology department of an Indian medical college, with institutional approval. It was undertaken to explore the dose-dependent antidepressant activity of citalopram and tramadol in albino mice using the forced swimming test (FST) and to evaluate if a combination of low doses of the two drugs is equally effective. The FST is a behaviour despair model that has been widely used to test antidepressant activity. Six albino mice in each of the nine groups were administered different doses and combinations of citalopram and tramadol for the study. The ability of the individual drugs and the low-dose combination in reducing immobility in the FST was tested in the mouse model of depression using reserpine in dimethyl sulfoxide (vehicle).

Citalopram and tramadol dose dependently decreased the mean immobility time in FST compared to the saline control and in the reserpine mouse model of depression. Though low doses of citalopram (1.5 mg/kg) and tramadol (10 mg/kg) individually did not alter the duration of immobility, the combination produced significant reduction in duration of immobility in FST compared to the control and in the reserpine mouse model of depression.

There have been reports of serotonin syndrome with higher doses of selective serotonin reuptake inhibitors (SSRI). In general studies have shown that the more powerful the serotonergic agent and the higher the dose, the more serious the symptoms of serotonin syndrome. Over 60% of patients with depression fail to respond to monotherapy. Experiencing pain in depressive disorders can complicate diagnosis and impair

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treatment outcomes. The low dose combination of citalopram and tramadol may be a safer alternative in refractory depression where pain is a symptom. Adding tramadol in low doses may reduce SSRI requirement and may be important in enhancing depression response and remission rates.

Hence we suggest the use of a combination of low dose citalopram and tramadol, which act synergistically in reducing depression-like state in mice. The low doses preclude undue increase in serotonin levels.

Introduction

Depression is a disorder of major public health importance. The report on Global Burden of Disease estimates the point prevalence of unipolar depressive episodes to be 1.9% for men and 3.2% for women, and the one-year prevalence has been estimated to be 5.8% for men and 9.5% for women. It is estimated that by the year 2020 the burden of depression will increase to 5.7% of the total burden of disease and it would be the second leading cause of disability-adjusted life years (DALYs), second only to ischemic heart disease (1).

A large population-based study from South India, which screened more than 24,000 subjects in Chennai reported an overall prevalence of depression to be 15.1% after adjusting for age using the 2001 census data (2). If not treated, approximately 10-15% of individuals with major depression and 25% of bipolar disorders, display suicidal behaviour during their life time and hence it is essential that they be appropriately managed with anti-depressant medication (3).

Depression is a multifactorial disease, commonly occurring due to deficiency of monoamines. Alteration in nor-adrenergic and serotonergic function in the central nervous system has been implicated in the pathophysiology of depression. Many classes of drugs including the, selective serotonin reuptake inhibitors (SSRI), norepinephrine reuptake inhibitors and dual action agents that inhibit uptake of serotonin and norepinephrine have been shown to be effective in treating depression and elicit their therapeutic effects by increasing synaptic concentrations of the monoamines serotonin and/or norepinephrine (4).

Selective Serotonin Reuptake Agents (SSRI) are the main stay of treatment in patients with depressive illness as they are better tolerated than the earlier anti-depressant group of tricyclic antidepressants. Citalopram, is the most selective SSRI. It inhibits reuptake of serotonin immediately and increases synaptic availability of serotonin by inhibiting reuptake in a large number of post-synaptic serotonin receptor types (5). Studies have shown that antidepressant-like effect of citalopramis primarily at the serotonin receptors and persists in norepinephrine-deficient mice while, antidepressant effects of fluoxetine are diminished in these mice (6).

Depression is characterized by a wide range of debilitating emotional and physical symptoms and although complete remission of symptoms is the goal of depression treatment, many patients fail to attain or maintain a long-term, symptom-free status. Recent findings (STAR*D Study) indicate that about 63% of patients with major depressive disorder fail to respond to suitable first-line monotherapy with a selective serotonin reuptake inhibitor (SSRI) (7).

Many residual symptoms are also physical in nature, pain being amongst these. Experiencing pain in depressive disorders can complicate diagnosis and impair treatment outcomes (8). It is necessary to recognise and optimise the management of pain that commonly co-exists with depression and this may be important in enhancing depression response and remission rates (9).

Tramadol is a centrally acting synthetic opioid with analgesic efficacy comparable to codeine which also inhibits the reuptake of norepinephrine and serotonin in the synaptic cleft. The structure of tramadol is closely related to dual action antidepressants

venlafaxine, mirtazapine and duloxetine which inhibits the synaptic reuptake of NE and 5HT and are proven effective antidepressants. This shared monoaminergic action resulted in the research of tramadol as a potential treatment for depression (10).

Researchers have attributed varied phamacodynamics for the anti-depressant effects of tramadol. Rojjas-Corrales et al showed that the action of tramadol on monoaminergic reuptake is similar to that of antidepressant drugs. In a study conducted in mice using the FST experimental model, they showed that tramadol exhibits antidepressant activity (11). Kalra et al found that there was significant reduction in immobility times of drug-treated mice compared to control mice in the FST with doses of 10, 20 and 40 mg/kg of tramadol (12). LourduJafrin et al showed the antidepressant activity of tramadol in male *balb c mice* by FST and also evaluated its mechanism through the serotonergic pathway. They concluded that tramadol has significant antidepressant property, which was reduced with pre-treatment with serotonin antagonist, ondansetron, showing the serotonergic pathway has a role to play in the antidepressant action of tramadol (13). Ostadhadi et al suggested a role for NMDA receptor signalling in the anti-depressant effects of tramadol using the mouse FST (14). Jesse et al tested mice using FST after oral acute administration of tramadol and found it produced antidepressant-like effects by a mechanism that involves the K^+ channels (15). Tramadol may represent an alternative to commonly used antidepressants in select treatment-refractory patients (16).

Berrocoso et al found that the combination of SSRI with sub-therapeutic doses of tramadol produced a large decrease in the immobility time of mice in the FST. Synergistic co-operation of the opioid and serotonergic mechanisms generate superior antidepressant-like effects in mice models of depression without effects on motor behaviour in mice. These data support the hypothesis that a combination of classical serotonergic antidepressants and weak opioid receptor agonists may be a helpful new strategy in the treatment of refractory depression (17).

Regarding pharmacokinetics the SSRI are metabolized by CYP2C19 and also have an inhibitory effect on CYP2D6, which is the enzyme that metabolises tramadol and its metabolite O-desmethyltramadol (18). Keeping in mind the benefits of reducing the dose of SSRIs to reduce the risk of serotonin syndrome, this seems a logical approach to add low dose of tramadol which acts synergistically with SSRI as the combination of sub-therapeutic doses of tramadol with citalopram lead to an accentuated reduction in immobility time in the FST (17).

As adding tramadol for its combined opioid and nor-epinephrine–induced anti-depressant effects to low dose citalopram seems a good option for treating select patients with depression this study was undertaken to find out the effectiveness of a low dose combination of citalopram and tramadol in reducing immobility time in FST in albino mice.

The objectives of the present study were,

1. To assess the reduction in duration of immobility in FST in albino mice with citalopram, tramadol and low dose combination of citalopram and tramadol compared to controls treated with saline.

2. To compare the effectiveness of citalopram, tramadol and low dose combination of these two drugs in reducing duration of immobility in FST in albino mice in the reserpine mouse model of depression.

Materials and Methods

This study was done in the pharmacology department of a medical college in Gujarat with institutional approval. Albino mice were divided into nine groups of six each. The test drugs were administered intra-peritoneally depending on the group as given in Table I. Drugs were administered two hours prior to the test. Mice were individually forced to swim inside the vertical plexi-glass cylinder (height, 25 cm; diameter, 10 cm) containing a water column of 9 cm

TABLE I: Study Group Details.

Group	*Agent used*	*Doses used*	*Number of Mice*
Group 1	Normal saline - control	2 hours prior to the test	6
Group 2	Citalopram	A. 1.5 mg/kg; B. 3 mg/kg; C. 5.7 mg/kg; D. 8.6 mg/kg.	6 in each sub-group
Group 3	Tramadol	A. 10 mg/kg; B. 20 mg/kg; C. 40 mg/kg; D. 80 mg/kg.	6 in each sub-group
Group 4	Citalopram + Tramadol	1.5 mg/kg + 10 mg/kg	6
Group 5	DMSO - control	2 hours prior to the test	6
Group 6	Reserpine	2 mg/kg	6
Group 7	Citalopram + Reserpine	8.6 mg/kg + 2 mg/kg	6
Group 8	Tramadol + Reserpine	80 mg/kg + 2 mg/kg	6
Group 9	Citalopram + Tramadol + Reserpine	Citalopram (1.5 mg/kg), Tramadol (10 mg/kg) and Reserpine 2 mg/kg	6

height at a room temperature of 22±1 degree C for six-minute sessions daily for two days (19).

On the first the day mice were initially highly active in the glass jar, rigorously swimming in circles, trying to climb the wall of glass jar and diving to the bottom. After two to three minutes, the activity began subsiding and was interspersed with phases of immobility of increased length, when the mouse was floating. The mice were judged to be immobile whenever they remained floating passively in the slightly hunched but upright position; their head just above the surface. This constituted the “Pre-test session” Twenty – four hours later, each animal was again forced to swim for a period of six minutes in the ’Test session” Mice remained immobile for approximately 80% of the time in each successive session. During the six-minute session following the FST, depression was indicated by a decrease in functional mobility or immobility.

The solutions of each drug were prepared on the day of the experiment. Powder of citalopram hydrobromide (Sun Pharmaceutical industries, Vadodara) and tramadol hydrochloride (Sarabhai Chemicals, Vadodara) were prepared in saline while reserpine hydrochloride (Loba-chemie, Bombay) was prepared in the solvent dimethyl sulfoxide (DMSO). Six albino mice were administered the drugs in each group/ sub-group.1. Drugs were administered intra-peritoneally at various doses and latency periods and duration of immobility were observed.

The time of immobility and the latency to the initial immobility period of the swim session are the primary dependent measures of the FST. Duration of immobility period was expressed as mean±standard error of mean (S.E.M). For comparison between any of the two groups, student ‘t’ test was employed. Values of probability less than 5% ($p<0.05$) were considered to be statistically significant.

The effects of the vehicles, saline for citalopram and tramadol and dimethyl sulfoxide (DMSO) for reserpine, on the duration of immobility were first studied at various time intervals. Neither normal salinenor DMSO modified the duration of immobility in mice.

Results

I. Immobility Time in the Forced Swimming Test at Various Latencies

There was a significant reduction of duration of immobility in the FST in the mice which received citalopram, tramadol and the low dose combination of citalopram and tramadol compared to the control mice at all the latency periods studied as shown in Table II.

II. Immobility Time in the Forced Swimming Test at Various Doses

Graded doses of citalopram above 3 mg/kg and tramadol above 20 mg/kg produced significant dose dependent decrease in the duration of immobility in mice at a latency of 2 hours compared to the saline treated groups. Though individual doses of 1.5 m/kg of citalopram and 10 mg of tramadol did not produce significant change, the low dose combination of 1.5 mg/kg citalopram and 10 mg tramadol also produced a significant reduction of immobility time (Table III).

TABLE II: Action of Agents on Duration of Immobility in FST at Various Latencies.

Treatment	*Latency Period (hours)*	*Duration of Immobility (seconds)*		*Significance*
		Control (saline)	*Drug*	
Citalopram (8.6 mg/kg)	1/2	213.0±0.73	180.83±5.21	p = < 0.001
	1	212.0±0.91	163.50±4.66	p = < 0.001
	2	213.0±0.79	129.66±3.96	p = < 0.001
	3	213.8±0.72	180.00±7.71	p = < 0.01
Tramadol (80 mg/kg)	½	213.0±0.73	192.66±2.36	p = < 0.001
	1	212.0±0.91	138.60±2.96	p = < 0.001
	2	213.0±0.79	164.50±7.02	p = < 0.001
	3	213.8±0.72	180.30±7.32	p = < 0.01
Citalopram (1.5 mg/kg) + tramadol (10 mg/kg)	2	213.0±0.79	142.67±5.01	p = < 0.001

TABLE III: Action of Agents on Duration of Immobility in FST at Various Doses.

Antidepressant drug/s	*Dose (mg/kg)*	*Immobility Time (Seconds)*	*Significance compared to control*
Citalopram	1.5	211.80±1.57	Not significant
	3	156.8±4.16	p = < 0.001
	5.7	144.33±2.26	p = < 0.001
	8.6	129.66±3.96	p = < 0.001
Tramadol	10	203.16±4.11	Not significant
	20	147.5±6.07	p = < 0.001
	40	145.5±10.71	p = < 0.001
	80	138.6±2.96	p = < 0.001
Citalopram (1.5 mg/kg) + Tramadol (10 mg/kg)		142.67±5.01	p = < 0.001

III. Duration of Immobility in FST in the Reserpine Mouse Model of Depression

Mean immobility time in mice pre-treated with reserpine (2 mg/kg) was significantly higher (330.5±3.52) than the mean immobility time in control mice given only the vehicle (213.6±1.53).

There was a significant decrease in the duration of the mean immobility time in the reserpine-treated mice which were administered 8.6 mg/kg citalopram (231.33±3.42), 80 mg/kg of tramadol (228.60±7.10) and the low-dose combination of 1.5 mg/kg of citalopram and 10 mg of tramadol (264.30±5.52). The clustered bar chart in Fig. 1 illustrates the change in mean immobility time with various agents and controls.

The clustered bar graph shows mean immobility time in FST in Albino mice with various agents and controls. The mean immobility time was significantly reduced by citalopram (8.6 mg/kg), tramadol (80 mg/kg) and low-dose combination of citalopram (1.5 mg/kg) and tramadol (10 mg/kg) compared to saline controls.

In mice pre-treated with reserpine (2 mg/kg), mean immobility time was significantly increased compared to the control group treated with the vehicle.

Mean immobility time in reserpine-treated mice was significantly reduced by citalopram (8.6 mg/kg), tramadol (80 mg/kg) and low-dose combination of citalopram (1.5 mg/kg) and tramadol (10 mg/kg) compared to the control.

Discussion

Various paradigms have been developed to detect the antidepressant like potential of novel compounds in preclinical settings. The models commonly used are diverse and were developed originally based on the behavioural consequences of stress, drug, lesions or genetic manipulations. Among all animal models, the 'Forced Swimming Test' (FST), in miceremains the most used test for assessing antidepressant activity pre-clinically, largely as a result of its ease

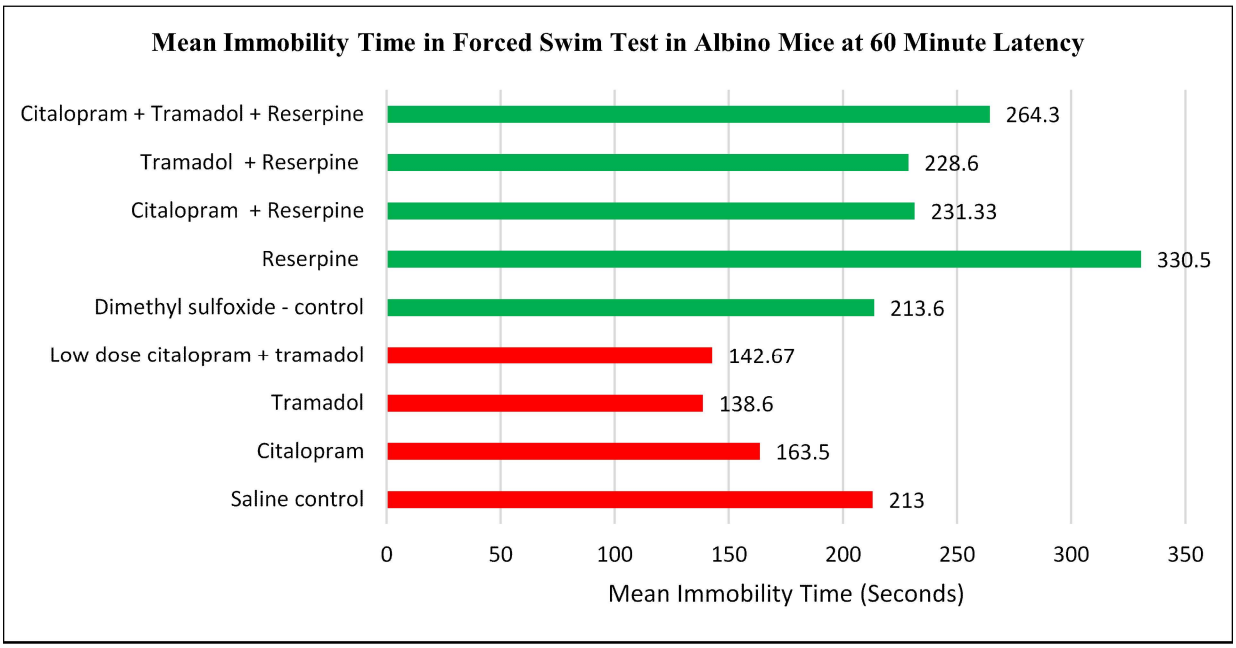

Fig. 1: Immobility Time with Forced Swim Test in Reserpine Mouse Model.

of use, reliability across laboratories and ability to detect a broad spectrum of antidepressant agents. On reviewing the FST, Demouliere et al came to the conclusion that it is a good screening tool for anti-depressants with good reliability and predictive validity (20).

The Forced swimming test (FST) is a rodent behavioural screening method, first proposed by Porsolt (1977). It is a behaviour despair model to test antidepressant activity of various drugs (21). The FST, which is based on the principle that forcing mice to swim in a restricted space from which they cannot escape leads to a characteristic behaviour of immobility. Traditionally, 'floating behaviour' (where the animal remains almost immobile and with its head above water) is used as a parameter to analyse 'hopelessness' and thus serves as a good model for depression-like behaviour. This behaviour reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression. The FST is used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at preventing depressive-like states. The mouse version of the FST is a relatively low cost and less time-consuming behavioural test that requires no training of the mice and can be conducted with minimal equipment (22).

The test is based on the observation that mice, following initial escape-oriented movements, developed an immobile posture when placed in an inescapable cylinder of water. If they are replaced in the testing apparatus, 24 hour later, they resume this posture quickly. The immobility is thought to reflect either a failure or persistence in escape directed behaviour. (i.e. behavioural despair) or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli. If antidepressant treatment is given between the two exposures, the mice will actively persist engaging in escape directed behaviour for longer periods than after vehicle treatment (control). The immobility time in this model is reduced by a variety of drugs which are therapeutically effective in depression (21).

Reserpine was introduced in 1950 for the treatment of hypertension. However, soon studies showed that the chronic use of reserpine has a serious side-effect of depression. This led researchers to use reserpine administered in mice to produce a practical animal model for depression. Reserpine-mediated

depression is thought to be caused by the depletion of monoamines such as norepinephrine in the brain. This model has been used in numerous experiments to examine and compare the symptoms of depression in mice to those of humans, as well as determining the efficacy of certain anti-depressant medications (22).

Citalopram, a selectively serotonin reuptake inhibitor blocks neuronal transport of serotonin immediately and apparently indefinitely, leading to a complex secondary response (4). In the present study, different doses of citalopram, over 3 mg/kg, produced significant dose dependent decrease in mean immobility time in mice compared to the control. At a lower dose, citalopram (1.5 mg/kg) did not alter the mean duration of immobility significantly. The maximum decrease in mean immobility time occurred in a dose of 8.6 mg/kg at 120 minutes. Citalopram also reduced mean immobility time significantly in the reserpine-administered mouse model of depression (23).

A dose-dependent decrease in mean immobility time like in citalopram was also seen with tramadol in FST. At a lower dose of 10 mg/kg of tramadol, there was no significant alteration in the mean duration of immobility at 60 minutes. But with increase in the dose there was a significantly gradual decrease in the duration of immobility. The maximum decrease in the mean immobility time was observed at the dose of 80 mg/kg. Reduction of mean immobility time was also observed in the reserpine-induced depression model.

We observed that, when the lower dose of citalopram (1.5 mg/kg) was used along with lower sub-therapeutic dose of tramadol (10 mg/kg) a significant reduction in mean immobility time was observed whereas the individual drugs in low dose alone were unable to make any significant difference in the mean immobility time. Studies have shown that SSRIs inhibit CYP2D6 resulting in higher blood levels of tramadol. The concurrent use of low-dose tramadol and citalopram have a synergistic serotonergic effect probably by increasing the availability of both NE and 5-HT in CNS (18).

Reserpine, a vesicular reuptake blocker, which depletes catecholamines and lowers norepinephrine turnover in the brain, produces a depression-like syndrome in mice (23). In the reserpine model of depression in mice, we observed an increase in mean immobility period after a latency period of four hours after treatment. Our results are in accordance with the other studies (11, 17). Citalopram (8.6 mg/kg) reversed the reserpine induced behavioural despair in mice at 4 hours and 24 hours after treatment indicating its efficacy in treating depression.

There have been several reports that high doses of SSRIs fluoxetine, paroxetine and sertraline can lead to serotonin syndrome. Serotonin syndrome is due to increase in serotonin in the central nervous system and usually results from concurrent administration of excessive doses of a single serotonergic drug or combinations of high dose of serotonergic drugs like monoamine oxidase inhibitors (18, 25). There have also been reports of high dose citalopram monotherapy leading to serotonin syndrome. Serotonin syndrome is associated with the use of higher doses of serotonergic drugs that influence serotonin uptake, metabolism, synthesis, release, and serotonin receptor activity; also drugs with the ability to interfere with cytochrome P450 metabolism, specifically CYP2D6 and CYP3A4 (18). Birmes et al reviewed several cases of serotonin syndrome and found the more powerful the serotonergic agent and the higher the dose, the more serious the symptoms of serotonin syndrome (25). Mahlberg reported a case with functional polymorphisms in cytochrome P450 enzymes CYP2D6 and CYP2C19, that caused deficient metabolic activity reducing citalopram clearance and resulting in serotonin syndrome (26). However after reviewing several reports Park et al concluded that tramadol is only contra-indicated in combination with MAOIs but not other antidepressants in common use today and concluded that tramadol can be safely combined with SSRIs (27). Habibolahi et al studied 512 patients with tramadol overdose with a median tramadol dose of 1500 mg and looked for serotonin toxicity using the Hunter criteria and found not a single incident of serotonin toxicity (28).

In our study we have shown that low dose combination of citalopram and tramadol is effective in reducing immobility period in mice and reserpine-induced depression, thus minimising the risk of serotonin syndrome. Combination treatment with low doses of citalopram and tramadol may effectively treat depression without unduly increasing serotonin levels.

We can conclude from our study that combining citalopram with tramadol using low, individually sub-therapeutic doses effectively reduces the duration of immobility in the FST thereby indicating effective anti-depressant action. The synergistic action of low doses of both agents enable efficacy in treating refractory depression without the risk of unduly elevating serotonin levels and causing toxicity.

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Conclusions

We can conclude that low dose combination of citalopram and tramadol effectively reduce the duration of immobility in FST in albino mice compared to the saline controls

In mice pre-treated with reserpine there was an increase in duration of immobility in FST, compared with the DMSO control, however the immobility time was significantly reduced with the low dose combination of the citalopram and tramadol.

References

1. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global Burden of Disease and Risk Factors. Washington: The World Bank; 2006. Available at <https://www.ncbi.nlm.nih.gov/books/NBK11812/>
2. Poongothai S, Pradeepa R, Ganesan A, Mohan V. Prevalence of depression in a large urban South Indian population - The Chennai Urban Rural Epidemiology Study (CURES-70) PloS One. 2009; 4: E7185.
3. Baldessarini RJ, Jamison. Effects of Medical Interventions on Suicidal Behavior KR. *J Clin Psychiatry* 1999; 60 (suppl 2): 117–122.
4. Mann JJ. The medical management of depression. *N Engl J Med* 2005; 353: 1819–1834.
5. Mombereau C, Gur1 TL, Onksen J, Blendy JA. Differential effects of acute and repeated citalopram in mouse models of anxiety and depression. *Int J Neuropsychopharmacol* 2010 April; 13(3): 321–334.
6. Cryan JF, O'Leary OF, Jin SH, Friedland JC, Ouyang M, Hirsch BR, et al. Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. *Proc Natl Acad Sci U S A*. 2004; 101(21): 8186–8191.
7. Gaynes B, Rush JA, Trivedi MH, Wisniewski SR, Spencer D, Fava M. The STAR*D study: Treating depression in the real world February 2008 *Cleveland Clinic Journal of Medicine* 75(1): 57–66.
8. Demyttenaere K, Bonnewyn A, Bruffaerts R, Brugha T, De Graaf R, Alonso J. Comorbid painful physical symptoms and depression: prevalence, work loss, and help seeking. *Journal of Affective Disorders* 92: 185–193.
9. Kroenke K, Shen J, Oxman TE, Williams Jr. JW, Dietrich AJ. Impact of pain on the outcomes of depression treatment: results from the RESPECT trial. *Pain* 134, 209–215.
10. Barber J. Examining the use of tramadol hydrochloride as an antidepressant. Experimental and Clinical Psychopharmacology, 2011; 19(2): 123–130.
11. Rojas-Corrales MO, Gibert-Rahola J, Mico JA, Tramadol induces antidepressant-type effects in mice. *Life Sci* 1998; 63: PL175–PL180.
12. Kalra BS, Tayal V, Chawla S. Antidepressant-like activity of tramadol in mice. *Indian J Psychiatry* 2008; 50: 51–53.
13. Lourdu JA, Banupriya R, Meher Ali R Antidepressant Effect of Tramadol on balb-c mice in Animal Models of Depression. *Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS)* 2013 April-June; 4:2: 1438.
14. Ostadhadi S, Norouzi-Javidan A, Chamanara M, Akbarian R, Imran-Khan M, Ghasemi M, Dehpour AR. Involvement of NMDA receptors in the antidepressant-like effect of tramadol in the mouse forced swimming test. *Brain Res Bull* 2017 Sep; 134: 136–141.
15. Jesse CR, Wilhelm EA, Barbosa NBV, Nogueira CW. Involvement of different types of potassium channels in the antidepressant-like effect of tramadol in the mouse forced swimming test. *European Journal of Pharmacology* 2009 June; 613: 1–3:74–78.
16. Shapira NA, Verduin ML, DeGraw JD. Treatment of refractory major depression with tramadol monotherapy. *The Journal of Clinical Psychiatry* 2001; 62(3): 205–206.
17. Berrocoso E, Mico J-A. Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. *International Journal of Neuro-psychopharmacology* 12: 8, 1 September 2009, Pages 1033–1044.
18. Beakley BD, Kaye AM, Kaye AD. Tramadol, Pharmacology, Side Effects, and Serotonin Syndrome: A Review. *Pain Physician* 2015; 18: 395–400.

19. Vogel GH, Vogel WH. Drug discovery and evaluation-pharmacological assays. Spinger Publication; 1997; p. 292–312.
20. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity Review. *Psychopharmacology* 2005; 177: 245–255.
21. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 1977 Oct; 229(2): 327–336.
22. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The Mouse Forced Swim Test. *J Vis Exp* 2012; (59): 3638.
23. Greenwood R, Zhao J, Ludrick B, Golden T, Wu N. A Practical Animal Model for Depression by Reserpine-Administered Mice. *Theranostics Brain Disord* 2018; 3(2): 555609.
24. Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Micó JA. Antidepressant-like effect of tramadol and its enantiomers in reserpinized mice: comparative study with desipramine, fluvoxamine, venlafaxine and opiates. *J Psychopharmacol* 2004 Sep; 18(3): 404–411.
25. Birmes P, Coppin D, Schmitt L, Lauque D. Serotonin syndrome: a brief review. *Canadian Medical Association Journal (CMAJ)* May 27, 2003; 168 (11).
26. Mahlberg R, Kunz D, Sasse J, Kirchheiner J. Serotonin syndrome with tramadol and citalopram. *Am J Psychiatry* 2004 Jun; 161(6): 1129.
27. Park, Susie, Wackernah, Robin, L Stimmel, Glen. (2013). Serotonin Syndrome: Is It a Reason to Avoid the Use of Tramadol With Antidepressants? *Journal of Pharmacy Practice* 27. 10.1177/0897190013504957.
28. Habibollahi P, Garjani A, Samad Shams Vahdati SS, Seyyed-Reza Sadat S-R, Ebrahimi, Parnianfard N. Severe complications of tramadol overdose in Iran, Volume: 41, Article ID: e2019026, 5 pages.