WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 790-801. Research Article ISSN 2277– 7105

EVALUATION OF THE EFFICACY OF COMBINATION THERAPY OF DULOXETINE AND LAMOTRIGINE IN THE TREATMENT OF BIPOLAR DEPRESSION

Nimmy Baby Poovanpallil *and Dr. Kedar Prabhavalkar

Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Gate No.1, Mithibai College Campus, V.M. Road, Vile Parle (West), Mumbai 400 056, India.

Article Received on
25 April 2015,

Revised on 15 May 2015,
Accepted on 02 June 2015

***Correspondence for Author**
Nimmy Baby Poovanpallil
Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Gate No.1, Mithibai College Campus, V.M. Road, Vile Parle (West), Mumbai 400 056, India.

ABSTRACT

Objective: To evaluate the therapeutic effects of lamotrigine, an anti-epileptic mood stabilizer, when used alone and in combination with duloxetine (SNRI) in treating bipolar depression, at different doses of lamotrigine. **Materials and methods:** Mice were divided randomly into six groups. Intra-peritoneal treatment of normal saline (0.9%NaCl), duloxetine (10 mg/kg), lamotrigine (30 mg/kg), three combination groups i.e.,duloxetine (5mg/kg) + lamotrigine (10mg/kg), duloxetine (5mg/kg) + lamotrigine (15mg/kg), duloxetine (5mg/kg) + lamotrigine (20mg/kg), were given to groups I-VI, respectively. The immobility period was analyzed 30 min after the treatment from forced swim and tail suspension tests. The amount of sucrose consumed was estimated using Sucrose preference test. **Results:** The combination of duloxetine and lamotrigine given in the doses of 5mg/kg and 15mg/kg respectively, exhibited antidepressant activity by decreasing immobility time and increasing the amount of sucrose consumption in stressed mice as compared to lamotrigine monotherapy and other combinations used. **Conclusion:** Combining duloxetine (5mg/kg) and lamotrigine (15mg/kg) could be a better treatment option for bipolar depression owing to its greater efficacy over lamotrigine monotherapy and as it shows significant antidepressant-like effects.

KEY WORDS: bipolar depression, duloxetine, lamotrigine.

1. INTRODUCTION

Treatment of bipolar depression is the neglected phase in the management of bipolar disorders, as more focus is being given to the treatment of acute mania, thereby suggesting need for new therapeutic approaches in managing the depressive phase of the bipolar disorder.[1,2] The mood stabilizers that are available currently lack equal efficacy in treating both mania and bipolar depression.[3,4] Conventional antidepressants used for treating acute bipolar depression fail to meet the requirements of depression mood stabilizer because of its increased tendency to cause mood destabilization by precipitating switch to mania or by causing rapid cycling disorder on its long term use, thereby limiting its use in the treatment of bipolar depression.[5,6,] The rate of success (60-70%) shown by the first-line monotherapy was limited which gave more importance to augmentation as the second line treatment.[7] Duloxetine is the inhibitor of both serotonin and norepinephrine.[8-10] It is found to be superior in comparison to other antidepressants like fluoxetine, paroxetine and venlafaxine in terms of safety, efficacy and tolerability with lesser side effects.[11,12] Treatment for less than one week with duloxetine showed significant results in patients.[13] Duloxetine when administered in the dose of 10 mg/kg dose significantly reduced the immobility period in forced swim test.[14,15] Lamotrigine decreased the immobility time dose-dependantly in mice forced swimming test with a statistically significant decrease at 30mg/kg.[16] The efficacy of lamotrigine in the treatment of major depressive disorders including refractory unipolar depression suggested the use of lamotrigine as an augmentation drug for bipolar depression.[17-19] Thus, the present study aims to evaluate and perform a comparative statistical analysis of the therapeutic effects of lamotrigine, an epileptic mood stabilizer, when used alone and in combination with duloxetine in treating bipolar depression at different doses of lamotrigine.

2. MATERIALS AND METHODS

2.1. Animals

Male Swiss Albino mice (20–27 g) were procured from Bharat Serum Ltd, Thane and were group housed in Perspex cage, 6 mice/cage, in the standard conditions of animal room temperature (22–24 °C) and humidity (50–60%) controlled central animal house facility under a 12:12 h light:dark illumination cycle having given free access to standard food and water. Animals were acclimatized for a period of one week prior to exposure to the drugs and animal model studies. The studies were carried out between 9:00 and 17:00. Each experimental group consisted of randomly grouped mice of the same weight, which were used only once. All the experimental procedures were performed in accordance with NIH

Guide for the Care and Use of Laboratory animals and were approved by an Institutional Review Committee for the use of Animal Subjects. All the experimental animals were subjected to minimal suffering and efforts were made to minimize the number of animals used in the study.

2.2 Drug solutions and treatment

The drugs used for the experimental study are Duloxetine. HCl and Lamotrigine, both of which were received as gift samples from Cipla Pharmaceuticals, Mumbai. Control group received 0.25 ml of normal saline ie. 0.9% NaCl. Duloxetine solution was prepared by dissolving in 0.9% of NaCl. Lamotrigine was dissolved in saline with 1% aqueous solution of Tween 80, immediately before use. Each drug or the vehicle (normal saline) was administered intraperitoneally at a constant volume of 0.5 mL/20 g body weight. Experimental animals were divided randomly into 6 groups (n=6 animals/ group) and received treatment intraperitoneally as normal saline (0.9%NaCl), duloxetine (10 mg/kg), lamotrigine (30 mg/kg), duloxetine (5mg/kg)+lamotrigine (10mg/kg), duloxetine(5mg/kg) + lamotrigine(15mg/kg), duloxetine (5mg/kg) + lamotrigine (20mg/kg), respectively.

For testing the hypothesis that the antidepressant-like effect of lamotrigine is potentiated by combining with duloxetine, animals were pretreated with duloxetine (5mg/kg, i.p.) 30 minutes before the administration of lamotrigine (10, 15, 20 mg/kg, i.p.) for groups IV, V and VI respectively. These groups were subjected to forced swimming, tail suspension and sucrose preference tests and the recordings were compared to the animals treated with duloxetine (10mg/kg) and lamotrigine (30mg/kg) alone, in the similar manner.

Selection of the doses and the administration schedule was done on the basis of previously performed pilot studies in our laboratory and the literature data gives confirmation of the selectivity and efficacy of the concentrations and treatments mentioned above for this study.

2.3 Forced Swimming Test

FST was performed by using the method described by Porsolt et al. with certain modifications. In this, the mice were weighed individually and forced to swim in an open glass cylinder (diameter 10 cm, height 25 cm) with water level upto 19 cm at 25±1°C for a period of 6 minutes, out of which the total duration of immobility during the last 5 minutes were evaluated by video recording. The immobility of each mouse was judged only when it

stopped struggling and floated motionless in water by keeping its head above water. Antidepressant-like effect was indicated by decrease in the immobility time.

2.4. Tail Suspension test

The Mice were suspended by their tails with tape, in such a position that it cannot escape or hold on to nearby surfaces. The apparatus used was a set of aluminum stands measuring 58 cm (high) × 30 cm (wide). At 58 cm height of horizontally fixed aluminum rod, adhesive tape was used to suspend each mouse by its tail. It was placed approximately 1 cm from the tip of the tail (Vogel and Vogel, 2008). Mice received treatment 30 min before undergoing the 5 min test session. Video recordings of each animal were evaluated to analyze the immobility period.

2.5. Sucrose Preference test

This is a rewarding behavioral test which was conducted by subjecting the animals to different stress like food and water deprivation, cage tilting, wet bedding, continuous overnight illumination for a period of 14 days followed by measuring the amounts of sucrose consumed, which was compared to the amount of sucrose consumed prior to subjecting to stress. All the 6 groups were subjected to the test 30 minutes after dosing, as per the following protocol.

Table No. 1. 14-day protocol for Sucrose preference test

DAY	TYPE OF STRESS INDUCED	TIME
Day 1	Cleaning of cages followed by no stress	10:30 am
	Food and water deprivation	04:30 pm
Day 2	Food and water provided followed by no stress	10:30 am
	Tilting of cage to 45^0C	04:30 pm
Day 3	Cage brought to normal position followed by wet bedding	10:30 am
	Wet bedding cage subjected to continuous light illumination	04:30 pm
Day 4	Cage cleaning followed by no stress	10:30 am
	Food and water deprivation	04:30 pm
Day 5	Food and water provided followed by tilting of cage to 45^0C	10:30 am
	Cage brought to normal position followed by continuous light illumination	04:30 pm
Day 6	Food and water deprivation	10:30 am
	Food and water provided followed by no stress	04:30 pm
Day 7	Sucrose intake calculated	12:00 pm

6. Statistical analysis

Statistical analysis was performed using the Graphpad InStat for 32 bit Windows version 3.06 (GraphPad Software, Inc). The statistical significance between the experimental and control groups were estimated using one way analysis of variance ANOVA, followed by Tukey's honest significant difference post-hoc test. The experimental results are represented as mean ± SEM values and n = 6 per group.

3. RESULTS

3.1. Forced swimming test

The immobility period was significantly decreased in drug treated groups when compared against control group, except for the duloxetine (5mg/kg)+lamotrigine (20mg/kg) combination treated group. There was no significant decrease in the immobility period of the combination treated group duloxetine (5mg/kg)+lamotrigine (10mg/kg) as compared to the other 'single' treatment groups. Combination treated group duloxetine (5mg/kg)+lamotrigine(15mg/kg) showed significant decrease in immobility period, as compared to 'lamotrigine alone'(10mg/kg) treated group. 'Duloxetine alone' (10mg/kg) treated group showed significant decrease in immobility period, as compared to duloxetine (5mg/kg)+lamotrigine (20mg/kg) treated combination group. Immobiliy time of combination treated group duloxetine (5mg/kg) + lamotrigine (15mg/kg) was decreased significantly as compared to the other 2 combination treated groups.

Table No.2 Effect of Duloxetine, Lamotrigine and its combination in forced swim test.

SR.NO.	TREATMENT	IMMOBILITY TIME (sec)
1.	Control	271 ± 10.003
2.	Duloxetine (10mg/kg)	169.33±16.087***
3.	Lamotrigine (30mg/kg)	202.5±21.834*
4.	Duloxetine (5mg/kg)+Lamotrigine(10mg/kg)	202±18.035*
5.	Duloxetine (5mg/kg)+Lamotrigine(15mg/kg)	133.67±10.544***$^{\#@!!!}$
6.	Duloxetine (5mg/kg)+Lamotrigine(20mg/kg)	243.67±10.654

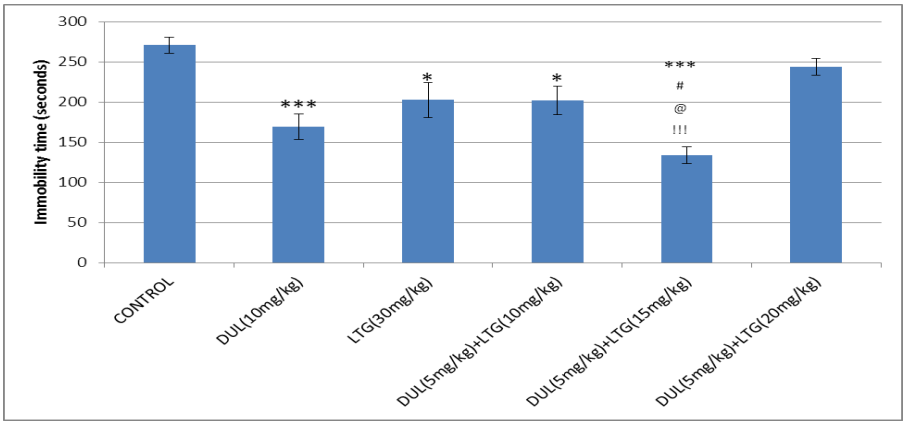

Fig 1.

Forced Swimming test: Significant difference is denoted by ***P<0.001,*P<0.05,*P<0.05,***P<0.001 as compared against control treated group; #P<0.05 as compared to lamotrigine treated group; @P<0.05 as compared to duloxetine (5mg/kg) + lamotrigine (10mg/kg) treated group; !!!P<0.001 as compared to compared toduloxetine (5 mg/kg) + lamotrigine (20mg/kg) treated group.

3.2. Tail Suspension test

The immobility period was significantly decreased in drug treated groups when compared against control group. Combination treated group duloxetine (5mg/kg) + lamotrigine (15mg/kg) showed significant decrease in immobility period, as compared to 'lamotrigine alone' (30mg/kg) treated groups. 'Duloxetine alone' (10mg/kg) treated group showed significant decrease in immobility period, as compared to duloxetine (5mg/kg) + lamotrigine (20mg/kg) treated combination group. Combination of duloxetine (5mg/kg)+lamotrigine(10mg/kg) treated group showed significant decrease in immobility period, as compared to duloxetine (5mg/kg)+lamotrigine(20mg/kg) combination treated group. Immobiliy time of combination treated group duloxetine (5mg/kg)+lamotrigine(15mg/kg) was also decreased significantly as compared to duloxetine (5mg/kg)+lamotrigine(20mg/kg) combination treated group.

Table.No.3 Effect of Duloxetine, Lamotrigine and its combination in tail suspension test.

SR.NO.	TREATMENT	IMMOBILITY TIME (seconds)
1.	Control	179 ± 9.754
2.	Duloxetine (10mg/kg)	107.17±7.989***
3.	Lamotrigine (30mg/kg)	133.83±9.628*
4.	Duloxetine (5mg/kg)+Lamotrigine(10mg/kg)	105.67±12.54***

5.	Duloxetine (5mg/kg)+Lamotrigine(15mg/kg)	66.833±6.887***#@@@
6.	Duloxetine (5mg/kg)+Lamotrigine(20mg/kg)	148.17±5.747

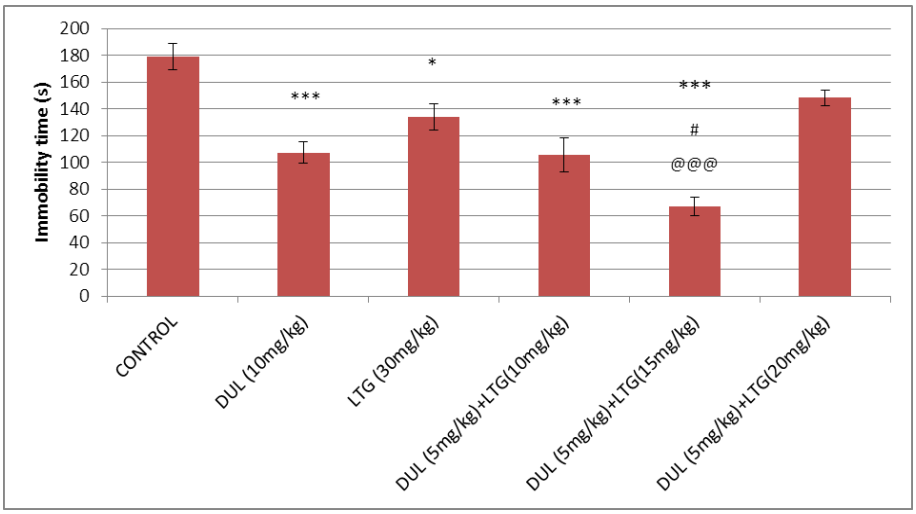

Fig 2.

Tail Suspension test: Significant difference is denoted by ***P<0.001, *P<0.05,***P<0.001,***P<0.001 as compared against Control treated group; $^{\#}$P<0.05 as compared against Duloxetine treated group; $^{@@@}$P<0.001 as compared against Lamotrigine treated group.

3.3. Sucrose Preference test

No statistically significant difference was obtained on the amount of sucrose consumed on 7th day, except for 'duloxetine alone' treated group. On 14th day all drug treated groups showed statistically significant increase in amount of sucrose consumed as compared to control group. Combination of duloxetine (5mg/kg) + lamotrigine (15mg/kg), showed significant increase in amount of sucrose consumed as compared to 'lamotrigine alone' (30mg/kg). The amount of sucrose consumed was significantly increased in duloxetine (5mg/kg) + lamotrigine (15mg/kg) treated group when compared also against other 2 combination treated groups. Combination treated groups duloxetine(5mg/kg)+lamotrigine (10mg/kg) and duloxetine(5mg/kg)+lamotrigine(20mg/kg) did not show any significant increase in amount of sucrose consumed as compared to 'duloxetine alone'(10mg/kg) and lamotrigine alone' (30 mg/kg) treated groups.

Table.No.4. Effect of Duloxetine, Lamotrigine and its combination in sucrose preference test.

SR.NO	TREATMENT	AMOUNT OF SUCROSE CONSUMED (gm)	
		7th DAY	14th DAY
1	Control	0.935±0.2157	1.12±0.2139
2	Duloxetine (10mg/kg)	2.277±0.4016¥	4.048±0.4843***
3	Lamotrigine (30mg/kg)	2.128±0.3249	2.828±0.1818**
4	Duloxetine (5mg/kg)+Lamotrigine(10mg/kg)	1.828±0.1896	2.752±0.1507
5	Duloxetine(5mg/kg)+Lamotrigine(15mg/kg)	2.064±0.2982	4.373±0.4918#@©
6	Duloxetine(5mg/kg)+Lamotrigine(20mg/kg)	1.677±0.2608	2.958±0.1322

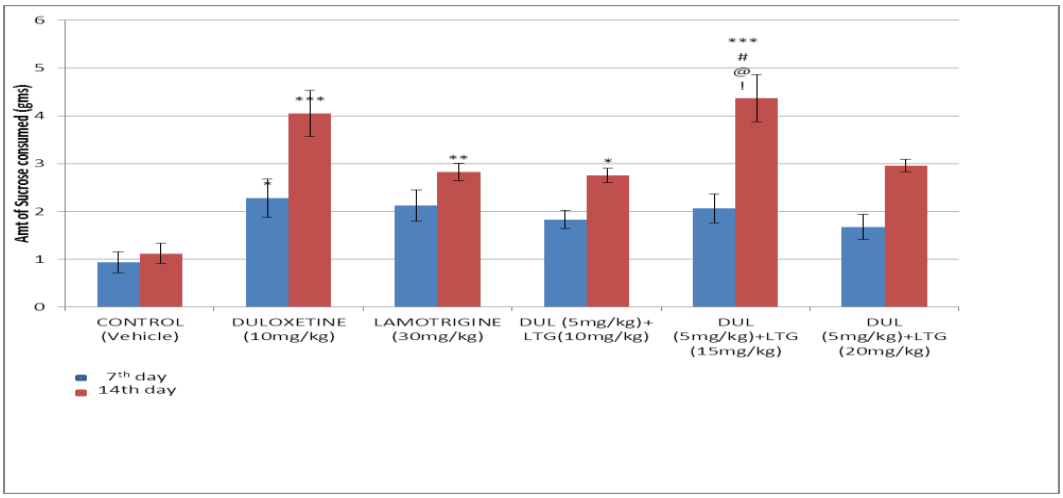

Fig 3.

Sucrose Preference test. Significant difference is denoted by ***P<0.001,**P<0.01,*P<0.05,***P<0.001,**P<0.01 as compared to control treated group; #P<0.05 as compared to lamotrigine treated group; @P<0.05 as compared to duloxetine (5mg/kg)+lamotrigine (10mg/kg) treated group; !P<0.05 as compared to duloxetine (5mg/kg)+lamotrigine (20mg/kg) treated group.

4. DISCUSSION

In forced swim and tail suspension tests, the evaluation of antidepressant activity is expressed on the basis of immobility period produced due to inescapable condition. These models have low degree of construct and/or etiologic validity. These tests, however are of great advantage in being the most predictive and widely models for the purpose of assessing antidepressant activity of mice[20.] In the present study, reduction in immobility period was observed in forced swim and tail suspension tests with duloxetine alone and lamotrigine alone treated groups,

which is in agreement with previously published reports.[21,22] The immobility period was also significantly reduced in the first two combination treated groups, as compared to control groups in both antidepressant models.

The results of the present study demonstrate that lamotrigine as well as the two out of three combinations duloxetine (5mg/kg) + lamotrigine (10mg/kg) and duloxetine (5mg/kg) + lamotrigine (15mg/kg) showed significant anti-depressant-like effects in the FST, TST and sucrose preference test models.

In this study, two behavioral animal models, FST and TST were used, both of which are widely accepted behavioral models for screeing pharmacological antidepressant activity. Characteristic behavior observed in these tests are scored in terms of immobility which reflects behavioral despair as seen in human depression.[20] In the present study, the antidepressant effects of lamotrigine in combination with duloxetine and lamotrigine using both the animal models, respectively was explored. The reduction in immobility period was better with duloxetine(5mg/kg)+lamotrigine(15mg/kg) as compared to the other combination treated groups.

In addition to using TST and FST animal models, another chronic stressor model Sucrose preference test was employed for testing the antidepressant effect of drug treatments and combinations used. In this model, mice are sequentially exposed to a variety of stressors. As the procedure requires subjecting mice to repeated stress, several behavioral and hormonal disturbances were produced, which is similar to a large extent to those found in depressed humans. A critical symptom indicated in this test is reduction of sucrose intake, a symptom which according to Willner et al. is equal to anhedonia, one of the core symptoms of depression as per the definition of the DSM-IV (American Psychiatric Association, 1994). Anhedonia has been defined as decreased responsiveness to rewards, which is now measured by decreased preference of sucrose solution. Amount of sucrose consumed on 7th day did not produce statistically significant results whereas 14 day treatment showed significant change in amount of sucrose consumed. Combination duloxetine(5mg/kg)+lamotrigine(15mg/kg) showed better antidepressant like activity as compared to other combinations in sucrose preference test. However, when compared against the standard antidepressant duloxetine, this combination ie. duloxetine(5mg/kg)+lamotrigine(15mg/kg) did not show any significant effect.

As per the statistical data obtained for each of the three models, it was seen that there was a dose-dependant decrease in the immobility time, shown by the combination treated groups, except for the third combination i.e.duloxetine (5mg/kg) + lamotrigine (20mg/kg). It could be estimated that the combination duloxetine(5mg/kg)+lamotrigine(15mg/kg) showed significant antidepressant-like effect as compared to lamotrigine monotherapy as well with the other combination treated groups. However, this combination did not show more significant effect in comparison to duloxetine treated group.

5. CONCLUSION

In conclusion, the combination of duloxetine and lamotrigine given in the doses of 5mg/kg and 15mg/kg exhibited antidepressant activity by decreasing immobility time and increasing the amount of sucrose consumption in stressed mice as compared to lamotrigine monotherapy, which was shown to be significant in all the 3 animal models in which it was tested. In the tail suspension test, this combination showed greater reduction of the immobility time as compared to duloxetine however, this decrease in the immobility time was not significant. Bipolar depression requires a treatment which helps in mood stabilization without precipitating a switch to mania. Duloxetine used alone focuses only on treating depressive pole of the bipolar disorder, which may induce a back shift to mania. Lamotrigine monotherapy is already reported for its efficacy as maintenance therapy in bipolar disorder as well as in rapid cycling disorder. Thus, the combination of duloxetine (5mg/kg)+lamotrigine(15mg/kg) could be a better treatment option for bipolar depression owing to its greater efficacy over lamotrigine monotherapy and as it shows significant antidepressant-like effects.

ACKNOWLEDGEMENTS

The Authors are grateful to extend special thanks to Dr. Bhanuben Nanavati College of Pharmacy for providing all kind of facilities for the successful completion of this work.

REFERENCES

1. Bowden CL, Mitchell P, Suppes T. Lamotrigine in the treatment of bipolar depression. European Neuropsychopharmacology, 1999; 9: 113–117.
2. Ketter TA, Calabrese JR. Stabilization of mood from below versus above baseline in bipolar disorder: a new nomenclature. J. Clin. Psychiatry, 2002; 63: 146– 151.
3. Henry C, Sorbara F, Lacoste J, Gindre C, Leboyer M. Antidepressant- induced mania in bipolar patients: identification of risk factors. J. Clin. Psychiatry, 2001; 62: 249–255.

4. Post R, Denicoff KD, Leverich GS, Frye MA. Drug-induced switching in bipolar disorder. CNS Drugs, 1997; 8: 352– 365.
5. Mojtabai R. Unmet need for treatment of major depression in the United States. Psychiatr Serv, 2009; 60: 297–305.
6. Richelson E. Multi-modality: a new approach for the treatment of major depressive disorder. Int J Neuropsychopharmacol, 2013; 16: 1433–1442.
7. Thase ME. Augmentation strategies for depression: history and concepts. CNS Spectr, 2007; 12: 3–5.
8. Hunziker ME, Suehs BT, Bettinger TL, Crisrnon Duloxetine ML. hydrochloride: a new dual-acting medication for the treatment of major depressive disorder. Clin Ther, 2005; 27: 1126–1143.
9. Reneman L, Lavalaye J, Schmand B, De Wolff FA, Van Den BW, DenHeeten GJ. Cortical serotonin transporter density and verbal memory in individuals who stopped using 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy"): preliminary findings. Arch Gen Psychiatry, 2001; 58: 901–906.
10. Ressler KJ, Nemeroff CB. Role of norepinephrine in the pathophysiology of neuropsychiatric disorders. CNS Spectr, 2001; 6: 663–670.
11. Stahl SM, Grady MM, Moret C, Briley M. SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. CNS Spectr, 2005; 10: 732–747.
12. Thase ME, Pritchett YL, Ossanna MJ, Swindle RW, Xu J, Detke MJ. Efficacy of duloxetine and selective serotonin reuptake inhibitors: comparisons as assessed by remission rates in patients with major depressive disorder. J Clin Psychopharmacol, 2007; 27: 672–776
13. Brannan SK, Mallinckrodt CH, Detke MJ, Watkin JG, Tollefson GD. Onset of action for duloxetine 60 mg once daily: double-blind, placebo-controlled studies. J Psychiatr Res, 2005; 39: 161–172.
14. Ciulla L, Menezes HS, Bueno BB, Schuh A, Alves RJ, Abegg MP. Antidepressant behavioral effects of duloxetine and fluoxetine in the rat forced swimming test. Acta Cir Bras, 2007; 22: 351–35.
15. Rénéric JP, Lucki I. Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology (Berl), 1998; 136: 190–197.

16. Bourin M, Masse F, Hascoët M. Evidence for the activity of lamotrigine at 5-HT1A receptors in the mouse forced swimming test. J Psychiatry Neurosci, 2005; 4: 30.
17. Rocha FL, Hara C. Lamotrigine augmentation in unipolar depression. Int Clin Psychopharmacol, 2003; 18: 97-9.
18. Barbee JG, Jamhour NJ. Lamotrigine as an augmentation agent in treatment-resistant depression. J Clin Psychiatry, 2002; 63: 737-41.
19. Frye MA, Ketter TA, Kimbrell TA, Dunn RT, Speer AM, Osuch EA. A placebo-controlled study of lamotrigine and gabapentin monotherapy in refractory mood disorders. J Clin Psychopharmacol, 2000; 20: 607-14.
20. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther, 1977; 229: 327–36.
21. Bourin M, Masse F, Hascoët, M. Evidence for the activity of lamotrigine at 5-HT1A receptors in the mouse forced swimming test. J Psychiatry Neurosci, 2005; 4: 30.
22. Zomkowski AD, Engel DM. Cunha NH, Gabilan AL. The role of the NMDA receptors and l-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effect of duloxetine in the forced swimming test. Pharmacol Biochem Behav, 2012; 103: 408–417.