Research in Pharmaceutical Sciences, October 2020; 15(5): 447-453
Received: 29-02-2020
Peer Reviewed: 22-03-2020
Revised: 22-04-2020
Accepted: 23-09-2020
Published: 19-10-2020

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Dextromethorphan improved cyclosporine-induced depression in mice model of despair

Azadeh Mesripour[1,*], Mojgan Golbidi[1], and Valiollah Hajhashemi[1,2]

[1]Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[2]Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Background and purpose: Cyclosporine (Cyc) is a calcineurin inhibitor used in immunosuppressive therapy that may cause psychological problems such as depression. Previous investigations have shown the positive antidepressant effects of dextromethorphan (Dxt). Therefore, the aim of this study was the evaluation of the Dxt effect on Cyc-induced depression in an animal model of despair in two separate cohorts.
Experimental approach: Male albino mice were used, first total activity was evaluated by the locomotor test, and then after that, the immobility time during the forced swimming test was measured as an indicator of depression. Cyc, Dxt, and fluoxetine (the reference antidepressant drug) were all administered IP. Tests were performed either 4 h after injection (cohort 4 h) or in separate groups 24 h after injection (cohort 24 h).
Findings/Results: Cyc reduced total activity measured after 4 h in the locomotor test and it was normalized after 24 h. Immobility time dose-dependently increased during the forced swimming test and remained so after 24 h (cohort 24 h; Cyc 10, 20, and 40 mg/kg, 157 ± 22, 180 ± 8, and 228 ± 4 s, respectively; Cyc 40 mg/kg $P < 0.001$ *vs* control 142 ± 13 s) that indicated Cyc induced depressive-like behavior. Dxt (30 mg/kg) like fluoxetine reduced the immobility time when co-administered with Cyc compared with Cyc and remained effective after 24 h (cohort 24 h; 120 ± 30, $P < 0.001$ *vs* Cyc 40 mg/kg alone).
Conclusion and implications: Dxt was a useful drug for preventing Cyc-induced depression that remained effective for 24 h in mice. Since interpretation from animal studies to humans must be done with caution further clinical studies on the effect of Dxt in patients suffering from psychological side effects of Cyc may be reasonable.

Keywords: Calcineurin; Cyclosporine; Depression; Dextromethorphan.

INTRODUCTION

Cyclosporine (Cyc) is a calcineurin inhibitor that is widely used in immunosuppressive therapy for organ transplantation and autoimmune diseases. These medications may cause neuropsychological problems such as tremor, confusion, anxiety, and depression in patients (1). Calcineurin, the Ca^{2+}-dependent protein phosphatase, has been reported to participate in neurotransmission, memory, and neuronal plasticity (2). In mice it was shown that a high dose of Cyc (60 mg/kg, IP) decreased the release of serotonin and dopamine, thus as a result of dysfunction of the prefrontal cortex, anxiety increased and social behavior was disturbed (3).

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates protein synthesis in synapses (4). On the other hand, the mTOR function is regulated by glutamate receptors, mainly the N-methyl-D-asparate (NMDA) receptor activity (5). It has been revealed that mTOR is involved in the rapid antidepressant effects of ketamine that is an NMDA antagonist (4).

*Corresponding author: A. Mesripour
Tel: +98-3137927089, Fax: +98-3136680011
Email: a_mesripour@pharm.mui.ac.ir

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.297847

Findings show that depressive-like behavior induced by calcineurin inhibitors is mediated by blockade of the mTOR signaling pathway. Therefore, stimulation of specific brain mTOR could be useful to decrease the risk of depressive disorders in patients treated with calcineurin inhibitors (4). In addition, studies have shown a link between dysregulation of mTOR signaling and deficiency in synaptic proteins in major depressive disorder (6).

Dextromethorphan (Dxt) is a commonly used antitussive with various pharmacologic actions in the central nervous system and a good margin of safety. Numerous preclinical and clinical investigations have discovered its positive effects on a variety of psychiatric disorders (7).

Dxt shows pharmacological properties similar with antidepressants and particularly, ketamine. Dxt and ketamine also share pharmacodynamic similarities including the effect on NMDA, μ opiate, sigma-1 receptors, serotonin transporter, and calcium channel (8). They can also both activate mTOR that could be responsible for their rapid-acting antidepressant property. Dxt may activate mTOR through μ, sigma-1 receptors, and the serotonin transporter (8). Recent animal studies reported that the fast antidepressant response of NMDA receptor antagonists is through activation of the mTOR pathway leading to a surge in synaptic signaling proteins and an increased number and function of new synapses in rats' prefrontal cortex (9).

Cyc depression is a result of irregular mTOR signaling and synaptic plasticity (2), Dxt antidepressant effect is related to its NMDA receptor antagonist property and activation of the mTOR pathway (10). Therefore, observing the effect of Dxt on Cyc depressant effect would be a useful and logical aspect. First, mice depressive behavior was evaluated after the administration of Cyc, by a despair model of depression. Then, the effect of Dxt was evaluated following Cyc induced depression. Finally, as Cyc and Dxt both can influence mTOR and synaptic proteins the study was conducted in two independent cohorts of animals evaluating depressive behavior after 4 and 24 h to see if the effects remain after 24 h.

MATERIALS AND METHODS

Animals

Male albino mice weighed 25 ± 3 g were used. They had free access to standard mice chow and tap-water, six animals were maintained in each cage at room temperature (21 ± 2 °C), in a 12-12-h dark/light cycle (lights on at 6 AM). Animals were placed in the experimental room 24 h before the test for acclimatization. All the experiments were performed between 8 AM and 1 PM in the pharmacology laboratory. All animal procedures were performed in accordance with guidelines for the Care and Use of Laboratory Animals issued by The National Ethical Committee of Iran (Ethical No: IR.MUI.REC.1398.377). All the efforts in the experiments were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Drug administrations

The following drugs were applied: Cyc (Sandimmun, 50 mg/mL; Novartis, Switzerland) 10, 20, 40 mg/kg (presented as Cyc10, Cyc20, and Cyc40) (3,11); Dxt hydrobromide (Gift from Amin Industry, Isfahan, I.R. Iran) 15, and 30 mg/kg (presented as Dxt15, and Dxt30) (10); fluoxetine (Flx) hydrochloride (Sigma-Aldrich, India) 20 mg/kg was used as the reference antidepressant drug (12,13), the volume for all injections were 10 mL/kg. All of the drugs were prepared daily by dissolving in normal saline and they were all injected IP only once.

Sixteen groups comprising at least six animals were evaluated as follow: two control groups that received normal saline, one group was tested after 4 h the other one after 24 h; three groups received Cyc10, Cyc20, or Cyc40 and behavior tests were performed after 4 h; three groups received similar treatments but were tested after 24 h; two groups received Dxt15 or Dxt30, they were tested after 4 h; two groups received the same treatments but were tested after 24 h; one group that was injected with Dxt30 following Cyc40 examined after 4 h, another group received the same drugs but were examined after 24 h; two groups that received Flx following Cyc40 injection, one group was tested after 4 h and the other group after 24 h.

Locomotor test

The motor activity of mice was assessed before the forced swimming test in an open arena (Borj Sanat, I.R. Iran), which was divided into 15 zones by red beams. Mice were placed in the corner of the arena and allowed to explore it for 3 min, the number of zone entries was counted automatically by passing through the red beams while rears on hind-legs were recorded manually. The total activity was considered for each animal, which was the sum of zone entries (horizontal exploration) and rears (vertical exploration).

Forced swimming test

This test was performed as an animal model of despair behavior. Mice were forced to swim in 25 °C water in a glass 2-L beaker (diameter 12.5 cm, depth 12 cm) for 6 min. The immobility time defined when no additional activity was observed other than that required to keep the animals' head above the water was measured during the last 4 min of the trial after habituation was considered in the first 2 min. Swimming behavior, defined as horizontal movement throughout the beaker which involved at least two limbs; and climbing behavior, defined as upward movements of the forepaws along the side of the beaker were also recorded (14). The whole experiment was recorded by a camera and analyzed later. After 6 min, the mice were dried carefully to avoid hypothermia and returned to their home cage.

Statistical analysis

Results are conveyed as group mean ± SEM. All results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests. *P* values less than 0.05 were considered significant. The software programs that were used for data analyzing and making graphs were GraphPad Prizm 6 and Excel 2010.

RESULTS

The effect of drugs on locomotor activity

As it is presented in Table 1, Dxt did not cause any noticeable change in the locomotor activity after 4 or 24 h compared with the control group. Cyc20 and Cyc40 unlike Cyc10 significantly reduced the locomotor activity compared to the control group after 4 h ($P < 0.001$), but this change was not observed following 24 h. Dxt30 administration prevented the change in the locomotor activity caused by Cyc40, however by adding Flx to cyc40 the locomotor activity remained lower than control after 4 h.

The effects of drugs on mice behavior during forced swimming test in cohort 4 h

In this cohort, animals were tested 4 h after different treatments were applied (Fig. 1). Dxt15 and Dxt30 reduced the immobility time during the forced swimming test (FST; $P < 0.001$ *vs* the control), Dxt30 reduction in the immobility time (26 ± 11 s) was more than Dxt15 (87 ± 13 s) ($P < 0.01$). The immobility time during FST in Cyc20 and Cyc40 groups was higher than the control but only in a significant manner for Cyc40 (224 ± 8 s, $P < 0.001$). The depressant-like effect of Cyc20 and Cyc40 were significantly higher than Cyc10 (138 ± 11 s). Dxt30 in a similar manner as Flx significantly reduced the immobility time when they were co-administered with Cyc40 compared with Cyc40 alone (122 ± 20 s, $P < 0.001$).

Table 1. Total activity during the locomotor test in two independent cohorts of mice. Total activity was considered as a sum of the horizontal exploration and vertical exploration. Data express the mean ± SEM. *** $P < 0.001$ *vs* the control group.

Groups	Total activity count	
	After 4 h	After 24 h
Control	178 ± 10	183 ± 10
Dxt (15 mg/kg)	211 ± 10	173 ± 7
Dxt (30 mg/kg)	191 ± 14	208 ± 5
Cyc (10 mg/kg)	136 ± 17	193 ± 7
Cyc (20 mg/kg)	118 ± 14***	161 ± 15
Cyc (40 mg/kg)	114 ± 5***	183 ± 9
Cyc (40 mg/kg) + Dxt (30 mg/kg)	134 ± 10	162 ± 14
Cyc (40 mg/kg) + Flx (20 mg/kg)	90 ± 9***	145 ± 7

Dxt, Dextromethorphan; Cyc, cyclosporine; Flx, fluoxetine.

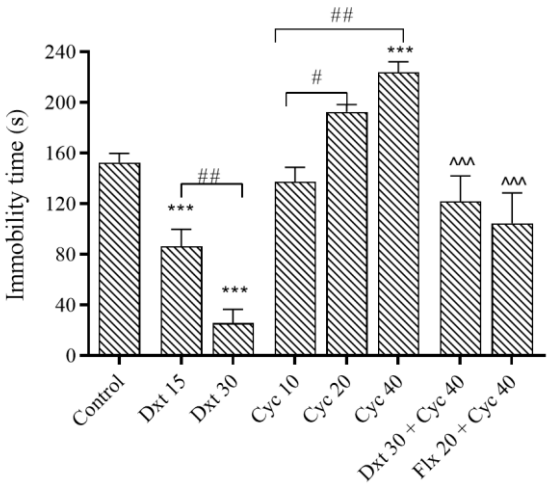

Fig. 1. The immobility time during FST in cohorts 4 h of mice. Dxt (15 and 30 mg/kg), Cyc (10-40 mg/kg), and Flx (20 mg/kg) were injected IP; the control group received normal saline. Number of animals in each group was 6. Data are mean ± SEM. *** $P < 0.001$ indicates significant differences compared with the control group; ^^^ $P < 0.001$ *vs* Cyc40 alone group; and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for specified comparisons. Dxt, Dextromethorphan; Cyc, cyclosporine; Flx, fluoxetine.

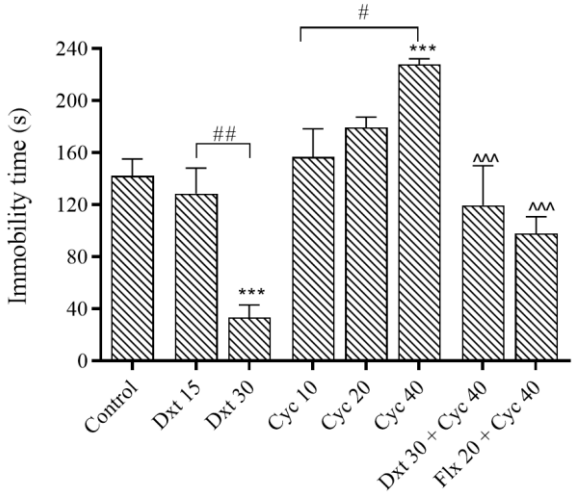

Fig. 2. The immobility time during FST in cohorts 4 h of mice. Dxt (15 and 30 mg/kg), Cyc (10-40 mg/kg), and Flx (20 mg/kg) were injected IP; the control group received normal saline. Number of animals in each group was 6. Data are mean ± SEM. *** $P < 0.001$ indicates significant differences compared with the control group; ^^^ $P < 0.001$ *vs* Cyc40 alone group; and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for specified comparisons. Dxt, Dextromethorphan; Cyc, cyclosporine; Flx, fluoxetine.

Table 2. Different activities during FST in two independent cohorts of mice. Results are expressed as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group, $^{\$}P < 0.05$ *vs* Cyc10 group, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with Cyc40 group.

Groups	After 4 h		After 24 h	
	Swimming (s)	Climbing (s)	Swimming (s)	Climbing (s)
Dxt (15 mg/kg)	75 ± 7	13 ± 4	76 ± 8	15 ± 6
Dxt (30 mg/kg)	124 ± 14**	30 ± 14	100 ± 16	12 ± 5
Cyc (10 mg/kg)	159 ± 9***	56 ± 5**	128 ± 13**	79 ± 14**
Cyc (20 mg/kg)	98 ± 10	4 ± 2	72 ± 17	11 ± 7
Cyc (40 mg/kg)	40 ± 5$^{\$}$	7 ± 3	59 ± 7	1.3 ± 1.1
Cyc (40 mg/kg) + Dxt (30 mg/kg)	16 ± 7**, $^{\$}$	0.5 ± 0.5	12 ± 4**,$^{\$}$	0.3 ± 0.2
Cyc (40 mg/kg) + Flx (20 mg/kg)	95 ± 10$^{\#\#\#}$	23 ± 13	103 ± 23$^{\#\#\#}$	10 ± 6
Dxt (15 mg/kg)	119 ± 20*, $^{\#\#\#}$	17 ± 5.3	91 ± 16$^{\#\#}$	51 ± 8**,$^{\#\#\#}$

Dxt, Dextromethorphan; Cyc, cyclosporine; Flx, fluoxetine.

As shown in Table 2, Dxt30 significantly increased both the swimming and the climbing time in the FST compared to the control group ($P < 0.01$). While Dxt15 only significantly increased the swimming time compared to the control group ($P < 0.01$). Cyc dose-dependently reduced the swimming time, as for Cyc40 it was considerably lower than the control and Cyc10 groups ($P < 0.05$), Cyc20 reduced swimming time that was noticeably lower than Cyc10 ($P < 0.05$). While the climbing time for Cyc was very short but the amounts were not noticeably lower than the control. In a similar way to Flx, adding Dxt30 following Cyc40 injection, significantly increased swimming time compared with Cyc40 alone ($P < 0.001$), although climbing time also increased the change was not considerable.

The effects of drugs on mice behavior during forced swimming test in cohort 24 h

In the next cohort, animals were tested 24 h after the treatments (Fig. 2). While the immobility time of Dxt15 during FST was not different from the control, it remained considerably lower than the control for Dxt30 after 24 h (34 ± 9 s, $P < 0.001$ *vs* control 142 ±13 s). The immobility time for the groups treated with Cyc increased and it was significantly higher than the control for Cyc40 (228 ± 4 s, $P < 0.001$). Like Flx, Dxt30 decreased the immobility time when it was injected with Cyc40 evenly after 24 h compared with Cyc40 alone (120 ± 30 s, $P < 0.001$). According to Table 2 Dxt30 significantly elevated the swimming and climbing time during FST ($P < 0.01$ *vs* control). Cyc dose-dependent effect in reducing the swimming time during FST was also observed after 24 h, Cyc40 swimming time was significantly lower than the control and Cyc10 groups ($P < 0.05$), while climbing duration was not considerably lower than the control. Adding Dxt30 following Cyc40 injection in the same way as Flx increased swimming time compared with Cyc40 alone ($P < 0.001$). The climbing time was higher than the control when Flx was injected concomitantly with Cyc40 ($P < 0.01$).

DISCUSSION

This study for the first time proved that Cyc could induce depression, dose-dependently, which consistently remained after 24 h. Dxt was a useful drug in order to prevent Cyc depressant effects and its antidepressant benefits continued for 24 h. In addition, Dxt antidepressant effects were similar to the reference drug Flx.

FST is a reliable tool that has been used globally not only in industrial settings for screening and discovering new antidepressant substances but also in complementary depression medicine research. During FST by placing mice in the water the animal gradually loses hope to escape the stressful environment, thus the immobility time reflects a measure of depression-associated endophenotypes, ie. behavioral despair (15). In addition to the immobility time, assessing the quantity of different movements, climbing time, and swimming time are useful for predicting the possible neurotransmitters involved, catecholaminergic (higher climbing time), and serotonergic substances (higher swimming time) (14).

The locomotor activity must be evaluated prior to behavioral tasks since deviations in locomotor activity can influence performance in many behavioral tests. Cyc dose-dependently reduced the locomotor activity measured after 4 h. Our findings were in agreement with Sato *et al.* they evaluated mice motor activity every 30 min during the 24 h period in home cages that were monitored by an infrared ray sensor, and they realized that Cyc at 60 mg/kg significantly reduced animal motor activity (3). Nevertheless, locomotor activity increased in forebrain-specific calcineurin knockout mice, i.e. the gene for calcineurin is destroyed (16). It is thus possible to speculate that the influence of calcineurin in control of locomotor activity is different in various brain areas but overall by IP injecting the calcineurin inhibitor, Cyc, locomotor activity declined (Table 1). However, in our experiment, the locomotor activity returned to normal values after 24 h.

Both doses of Dxt reduced the immobility time during FST tested after 4 h, indicating antidepressant-like effects. This was in line with previous studies that evaluated depressive behavior in mice 30 min after injecting Dxt (10). In addition to former studies, we observed that the antidepressant effects remain after 24 h for Dxt30, while Dxt15 was no longer effective (Fig. 1). It is speculated that rapid Dxt antidepressant response is related to dendritic spine amplification (17). Dxt and dextrorphan (i.e. 25% more potent than Dxt) antidepressant effects may also be related to the increased dendritic spine formation, synaptogenesis, and neuronal survival through NMDA antagonism and sigma-1 and mTOR activation (17). Since Dxt15 antidepressant effect faded after 24 h, perhaps Dxt15 did not induce synapse formation. This assumption warrants further investigation. Dxt30 increased swimming and climbing time that was also high in cohort 24 h although swimming was 62% higher than climbing time (Table 2). It was previously advocated that the increased swimming time during FST in mice could be a result of

improvement in the serotonergic system (16). This statement is supported by previous research that showed the pharmacodynamic similarities between Dxt and ketamine regarding their action on serotonin transporters in addition to NMDA receptor antagonist property (8).

Cyc dose-dependently increased the immobility time during FST in cohort 4 h, since the animals' total activity during the locomotor test declined, therefore claiming that Cyc has induced depression after 4 h could not be confident (Table 1). Nevertheless, after 24 h in the Cyc groups, total activity in the locomotor test increased to values near the control group while the immobility time during FST was profoundly higher than normal and clearly showed depressive-like behavior (Fig. 1). Calcineurin inhibition could be responsible, former studies notified the participation of calcineurin in neurotransmission and neuronal excitability (2). Findings have also shown that depressive-like behavior induced by calcineurin inhibition is performed by blockade of the mTOR signaling pathway (4). Activation of mTOR is practically linked with protein synthesis in synapses that results in the creation, maturation, and function of new synapses (18). However, the principal neural mediators that cause depressive-like behavior by inhibition of calcineurin have not yet been recognized. Swimming noticeably remained low after 24 h, while the climbing value that could indicate a scape tendency was near zero (Table 2). This difference could be related to the neurotransmitter changes induced by Cyc that deserve further research. Interestingly, the co-administration of Dxt30 and Cyc40 slightly increased total activity during the locomotor test, so that activity was not noticeably different from the control group in cohort 4 h (Table 1). While this effect was not observed with the co-administration of Flx. Nevertheless, they all became normal after 24 h. Immobility time during FST was significantly lower when Dxt was co-administered with Cyc and it remained so after 24 h, similar results were achieved by Flx (Fig. 1). Thus, we achieved our hypothesis that Dxt could prevent the depressant effects of Cyc, while different mechanisms may be responsible. However, according to the earlier studies, the NMDA antagonist effect of Dxt may have a critical role *via* activation of mTOR signaling pathway. Previously, activation of mTOR signaling caused a fast and sustained rise of synapse related proteins and dendritic spine number in the prefrontal cortex in rats (9). Additionally, it has been indicated that NMDA receptor antagonist ketamine, increased synapse related protein synthesis, and dendritic spine formation *via* activation of mTOR pathway (9), indicating that mTOR signaling is connected to the antidepressant effect. MK-801 which is another NMDA receptor antagonist also increased proteins in the mTOR/p70S6K pathway in the rat frontal cortex (19).

Apart from the side effects of most current antidepressants that appear early in therapy another disadvantage is that their efficacy is not obtained before 2 weeks, thus rapid-acting antidepressants are in great demand. In addition to the possible antidepressant effects of Dxt it has anti-inflammatory and immunomodulatory properties. For instance, Dxt reduced the production of pro-inflammatory factors such as tumor necrosis factor-alpha in the activated microglia or macrophages in the brain and aortic sinuses (20).

CONCLUSION

Dxt was a useful drug in preventing Cyc-induced depression and its antidepressant benefits continued for 24 h in mice. Dxt may be a logical choice for the antidepressant drug in patients suffering from psychological side effects of Cyc, meanwhile, interpretation from animal studies to humans must be done with caution, thus further clinical studies are recommended.

ACKNOWLEDGEMENTS

This work was financially supported through Grant No. 398343 by the Vice-Chancellor of Research of School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this study.

AUTHORS' CONTRIBUTION

A. Mesripour supervised and contributed to the conception, design, and interpretation of the reported study. M. Golbidi contributed to the experiments and design of the study. V. Hajhashemi contributed in the execution and interpretation of the reported study.

REFERENCES

1. Wijdicks EF. Neurotoxicity of immunosuppressive drugs. Liver Transpl. 2001;7:937-942. DOI: 10.1053/jlts.2001.27475.
2. Mansuy IM. Calcineurin in memory and bidirectional plasticity. Biochem Biophys Res Commun. 2003;311(4):1195-1208. DOI: 10.1016/j.bbrc.2003.10.046.
3. Sato Y, Takayanagi Y, Onaka T, Kobayashi E. Impact of cyclosporine upon emotional and social behavior in mice. Transplantation. 2007;83(10):1365-1370. DOI: 10.1097/01.tp.0000263332.65519.1f.
4. Yu JJ, Zhang Y, Wang Y, Wen ZY, Liu XH, Qin J, *et al*. Inhibition of calcineurin in the prefrontal cortex induced depressive-like behavior through mTOR signaling pathway. Psychopharmacology (Berl). 2013;225(2):361-372. DOI: 10.1007/s00213-012-2823-9.
5. Gong R, Park CS, Abbassi NR, Tang SJ. Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. J Biol Chem. 2006;281(27):18802-18815. DOI: 10.1074/jbc.M512524200.
6. Abelaira HM, Réus GZ, Neotti MV, Quevedo J. The role of mTOR in depression and antidepressant responses. Life Sci. 2014;101(1-2):10-14. DOI: 10.1016/j.lfs.2014.02.014.
7. Nguyen L, Thomas KL, Lucke-Wold BP, Cavendish JZ, Crowe MS, Matsumoto RR. Dextromethorphan: an update on its utility for neurological and neuropsychiatric disorders. Pharmacol Ther. 2016;159:1-22. DOI: 10.1016/j.pharmthera.2016.01.016.
8. Lauterbach EC. Dextromethorphan as a potential rapid-acting antidepressant. Med Hypotheses. 2011;76(5):717-719. DOI: 10.1016/j.mehy.2011.02.003.
9. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, *et al*. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959-964. DOI: 10.1126/science.1190287.
10. Mesripour A, Purhasani A, Hajhashemi V. N-methyl-D-aspartate receptor antagonists decrease interferon-alpha induced depressive behavior in mice model of despair. Thai J Pharm Sci. 2019;43(1):8-13.
11. Pacheco-López G, Doenlen R, Krügel U, Arnold M, Wirth T, Riether C, *et al*. Neurobehavioural activation during peripheral immunosuppression. Int J Neuropsychopharmacol. 2013;16(1):137-149. DOI: 10.1017/S1461145711001799.
12. Piras G, Giorgi O, Corda MG. Effects of antidepressants on the performance in the forced swim test of two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. *Psychopharmacology (Berl)*. 2010;211(4):403-414. DOI: 10.1007/s00213-010-1904-x.
13. Mesripour A, Hajhashemi V, Kuchak A. Effect of concomitant administration of three different antidepressants with vitamin B6 on depression and obsessive compulsive disorder in mice models. Res Pharm Sci. 2017;12(1):46-52. DOI: 10.4103/1735-5362.199046.
14. Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol. 1997;8(6-7):523-532. DOI: 10.1097/00008877-199711000-00010.
15. Deussing JM. Animal models of depression. Drug Discov Today Dis Models. 2006;3(4):375-383. DOI: 10.1016/j.ddmod.2006.11.003.
16. Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H, *et al*. Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc Natl Acad Sci U S A. 2003;100(15):8987-8992. DOI: 10.1073/pnas.1432926100.
17. Lauterbach EC. An extension of hypotheses regarding rapid-acting, treatment-refractory, and conventional antidepressant activity of dextromethorphan and dextrorphan. Med Hypotheses. 2012;78(6):693-702. DOI: 10.1016/j.mehy.2012.02.012.
18. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci. 2010;33(2):67-75. DOI: 10.1016/j.tins.2009.11.003.
19. Yoon SC, Seo MS, Kim SH, Jeon WJ, Ahn YM, Kang UG, *et al*. The effect of MK-801 on mTOR/p70S6K and translation-related proteins in rat frontal cortex. Neurosci Lett. 2008;434(1):23-28. DOI: 10.1016/j.neulet.2008.01.020.
20. Liu Y, Qin L, Li G, Zhang W, An L, Liu B, *et al*. Dextromethorphan protects dopaminergic neurons against inflammation-mediated degeneration through inhibition of microglial activation. J Pharmacol Exp Ther. 2003;305(1):212-218. DOI: 10.1124/jpet.102.043166.