VILAZODONE ENHANCES THE ENDURANCE CAPACITY AND IMPROVES THE BIOLOGICAL CHANGES IN TREADMILL TEST IN RATS

Paula Alina Fotache[1,2], Liliana Mititelu-Tartau[2*], Ionela Lăcrămioara Șerban[2], Monica Hăncianu[3], Cornelia Geanina Mircea[3], Ana-Maria Pelin[4], Liliana Lăcrămioara Pavel[5]
"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania
1. Ph.D. Student
Faculty of Medicine
2. Department of Morpho-functional Sciences (II)
Faculty of Pharmacy
3. Department of Pharmaceutical Sciences (II)
"Dunărea de Jos" University Galati, Romania
4. Department of Pharmaceutical Sciences
5. Department of Morphological and Functional Sciences
[*]Corresponding author. E-mail: lylytartau@yahoo.com

VILAZODONE ENHANCES THE ENDURANCE CAPACITY AND IMPROVES THE BIOLOGICAL CHANGES IN TREADMILL TEST IN RATS (Abstract): The **aim** of our study was the evaluation of the vilazodone (VLZ) influence on the endurance capacity and biological modifications in rats subjected to forced exercise. **Material and methods:** For the experiment, 24 white Wistar rats, were randomly assigned into four groups. The substances were administered orally, in a single daily dose, for 14 consecutive days: Group 1 (Negative control): distilled water 0.3 mL/100g body without effort; Group 2 (Positive control): distilled water 0.3 mL/100g body subjected to effort; Group 3 (Mg): 200 mg/kg body magnesium chloride; Group 4 (VLZ): 20 mg/kg body vilazodone. The rats motor behavior and the endurance capacity were evaluated using the treadmill test, and some blood parameters, estimating the stress influence were assessed: The investigation protocol was approved and implemented according to recommendations of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi Committee for Research and Ethical Issues. **Results:** The administration of VLZ resulted in a prolongation in distance traveled, an increase in the number of touches the opposite end of belt and a diminution in the number of delivered shocks. Moreover, it decreased the blood levels of glucose and cortisol, and increased the serum values of b*rain derived neurotrophic factor*. The use of VLZ was accompanied by an intensification of anti-oxidant enzymes activity, respectively a decrease of the oxidative stress marker level in blood. Its effects were less intense, when compared to Mg. **Conclusions:** The treatment with VLZ improved the physical performances and the endurance capacity in this experimentally-induced stress model in rats. The use of this antidepressant drug recovered the biochemical changes and produced antioxidant effects in animals subjected to forced exercise in treadmill test. **Keywords:** VILAZODONE, TREADMILL TEST, RATS, STRESS, BDNF.

Currently, the most commonly prescribed therapy for affective disorders is based on active agents that block the reuptake or degradation of monoamines at

the synapse. The administration of such drugs is associated with a variable response and long period (weeks to months) between the onset of administration and the achievement of clinical results (1). Paradoxically, the neurochemical effects of typical antidepressants are measurable in the days immediately following treatment initiation (2). This discrepancy suggests that, in addition to increasing serotonin and/or noradrenaline levels, classical antidepressants seem to be involved in the modulation of other cellular pathways to achieve the therapeutic effect (3).

A new theory of antidepressant function suggests that selective serotonin reuptake inhibitors create a window for change by increasing brain plasticity and increasing the patient's susceptibility to factors in their environment (4-6). Classical antidepressants, such as selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors, and monoamine oxidase inhibitors, therapeutic agents that participate in the modulation of brain monoamine levels, have limited efficacy and a delayed therapeutic response of several weeks or months (2).

The relatively new antidepressant drug, vilazodone (VLZ) is an indolylalkylamine derivative with a dual mechanism of action consisting of partial 5-HT1A receptor agonist and selective serotonin reuptake inhibitory activity. It does not bind with high affinity to norepinephrine or dopamine reuptake sites (7-9). VLZ is used for the treatment of major depressive disorder in adult patients (10). It also, has been found to improve mental and somatic symptoms in generalized anxiety disorder (7). Due to its unique mechanism of action, VLZ has the following therapeutic benefits: faster onset, higher efficacy, and lower risks of adverse events compared to currently used antidepressants, especially lower sexual side effects (11).

The purpose of our experimental research was the evaluation of the VLZ influence on the endurance capacity and biological modifications in rats subjected to forced exercise.

MATERIAL AND METHODS

Substances

VLZ (≥98% HPLC, molecular weight 477.99 g/mol, catalogue code: SML1098), magnesium chloride (anhydrous, ≥98%, molecular weight 95.21 g/mol, catalogue code: M8266), saline solution (molecular weight 18.02 g/mol, catalogue code: 07-6061) were bought from Sigma-Adrich Chemical Co (Steinheim, Germany). Substances were dissolved in saline solution, prepared proximately before the administration.

Laboratory animals

White Wistar rats (weighting 200-250 g, 6 months), healthy, non-genetically modified, with equal repartition by sexes were procured from the National Medical-Military Institute "Cantacuzino" for Research and Development, Băneasa Resort, Bucharest, Romania through the biobase of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania. The rats were kept in standard laboratory conditions (with constant temperature of 21°C ± 2°C, relative humidity of 50-70% and alternating lighting regime (light/dark ratio = 12 hours/12 hours), with standard food pellets and water *ad libitum*.

Treadmill test

The animals were randomly assigned into 4 groups of 6 rats each, and treated orally (using an esogastric tube), in a single daily dose, for 14 consecutive days, according to the following protocol: Group 1 (Negative control): distilled water 0.3 mL/100 g body without effort; Group 2 (Positive control): distilled water 0.3 mL/100 g body subjected

to effort; Group 3 (Mg): 200 mg/kg body magnesium chloride; Group 4 (VLZ): 20 mg/kg body vilazodone. During the experiment, the normal behavior of the laboratory animals was observed, by monitoring the general condition, posture, spontaneous behavior, movements, orientation ability, hair appearance, breathing, the reaction to various stimuli. All behavioral disturbances, changes in posture or reactions to handling, abnormal movements, stereotypic behaviors (excessive grooming, repeated circular movements), and any other bizarre behavior (self-mutilation, walking backwards) were noticed. On the 14th day of the experiment, 15 minutes after the administration of the substances, the animals from the last three groups were subjected to treadmill test. Magnesium chloride (Mg) was considered a positive control substance, with known effects on this behavioral model in rats.

This experimental model is part of the group of classical behavioral tests, being used to evaluate the motor function of laboratory animals and their ability to endure sustained physical effort. The treadmill for testing laboratory animals is a device that forces them to run on a rotating belt at a constant speed, having the same operating principle, as the treadmill used by humans for to practice physical exercises (12). The device allows the application of additional electrical stimuli (with constant intensity from 0 to 2 mA, called - electric shocks). These shocks push the animal to continue traveling, when it tends to stop, or when it stumbles and falls on the moving (12, 13). For each experimentation session of 8 minutes, the following were monitored: total distance covered; the number of applied electric shocks, the time of application of the electric shocks; the length of time the animal resists until the application of the first electric impulse; the number of touches of the opposite end of the conveyor belt.

Laboratory investigations

To compare the influence on the on the biological constants, we used the negative control group with rats not subjected to physical exercise. The blood samples (0.3 mL) were collected from the lateral tail vein after performing the exercise test (13). For the laboratory investigations the appropriate analyzers and kits to measure the blood level of glucose (Hematology Analyzer 5 DIFF model BF-5180, DIRUI, Istanbul, Turkey), cortisol (by electrochemiluminescence immunochemical method), and b*rain derived neurotrophic factor* (*BDNF*) (by ELISA method – using a specific kit) (15). The oxidative stress was evaluated (using a Shimadzu 1800 spectrophotometer, Kyoto, Japan, using the specific RANSOD kit from RANDOX Laboratories Ltd., Warsaw, Poland) by estimating the activity of: malondialdehyde - MDA (using the thiobarbituric acid method); glutathione peroxidase - GPx (using the dithio-nitrobenzoic acid method). The blood levels of superoxide dismutase - SOD, were assessed according to a method based on inhibiting the reduction of nitro blue tetrazolium with xanthine-xanthine oxidase, used as a superoxide generator.

The obtained results were expressed as arithmetic mean ± the standard deviation (S.D.) of the mean values and statistically analyzed using *SPSS* software for Windows EXCEL program (New York, Unites States) and ANOVA method. The *p* (probability) coefficient values lower than 0.05 were considered statistically significant compared to the control group.

The experimental studies were carried out after approval by the Ethics Committee of the University (Certificate No. 25/14.07.2020), in strict accordance with the international ethical regulations regarding work on laboratory

animals. The research methodology was established in accordance with international and national standards (16).

RESULTS

The treatment with Mg was linked to an increase in distance run (146.17±8.26 meters), statistically significant (**p <0.01) compared to distilled water group subjected to effort (107.17±8.16 meters). The administration of VLZ was accompanied by a substantial (*p <0.05) prolongation in distance traveled (124.83±3.49 meters) when compared to control group, but a little shorter than the one traveled by Mg group, the same time interval in treadmill test (fig. 1a).

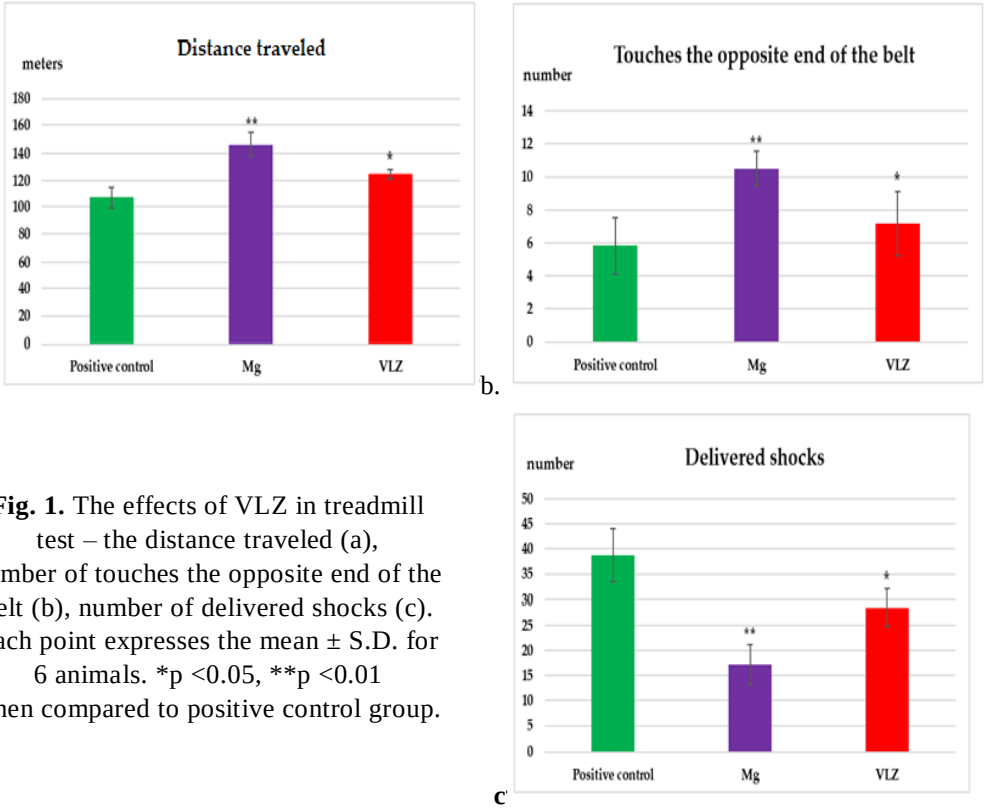

Fig. 1. The effects of VLZ in treadmill test – the distance traveled (a), number of touches the opposite end of the belt (b), number of delivered shocks (c). Each point expresses the mean ± S.D. for 6 animals. *p <0.05, **p <0.01 when compared to positive control group.

The treatment with Mg was correlated with a considerable (**p <0.01) increase in the number of touches the opposite end of the belt (4.83±1.72), compared to positive control animals (10.5±1.05). Oral administration of VLZ for 14 days led to an important (*p <0.05) increase in the number of touches (7.17±1.94) when compared to control group, but its effects were less intense than those of Mg group, the similar session in treadmill test (fig. 1b).

The administration of Mg was associated by a noticeable (**p <0.01) reduction in the number of delivered electric impulses (17.17±4.02), when compared to animals from distilled water group (38.83±5.31). The treatment with VLZ for 2 weeks resulted in an obvious (*p <0.05) decrease in

the number of needed shocks (28.50±3.62) comparing with positive control group, but, lower than those counted for Mg group, the same training session in forced exercise test (fig. 1c).

The use of Mg considerable (**p <0.01) increased the time need to first shock delivery (221 ± 8.65), and markedly shorten the time of providing electric stimuli (17.67 ± 2.80), comparing with positive control (134.33 ± 12.48, respectively 38.17 ± 4.17). The subacute administration of VLZ for 14 days caused an important (*p <0.05) prolongation of the time need to first shock use (184.67 ± 7.66), and a reduction in the time of providing shocks (31.17 ± 4.71), when compared to positive control (134.33 ± 12.48, respectively 38.17 ± 4.17) (tab. I).

TABLE I.

The effects of VLZ in treadmill test – time to first shock delivery, time of providing shocks (seconds). Each point expresses the mean ± S.D. for 6 animals. *p <0.05, **p <0.01 when compared to positive control group.

Groups	Time to first shock delivery	Time of providing shocks
Positive control	134.33 ± 12.48	38.17 ± 4.17
Mg	221 ± 8.65**	17.67 ± 2.80**
VLZ	184.67 ± 7.66*	31.17 ± 4.71*

TABLE II.

The effects of VLZ on blood levels of glucose, cortisol and BDNF in treadmill test in rats. Each point expresses the mean ± S.D. for 6 animals. *p <0.05, **p <0.01 when compared to positive control group, ♦p <0.05, ♦♦p <0.01 versus negative control group.

Groups	Glucose (mg/dL)	Cortisol (pg/mL)	BDNF (pg/mL)
Negative control	88.50 ± 6.24	0.14 ± 0.01	575.67 ± 47.25
Positive control	116.33 ± 1.53♦♦	0.19 ± 0.02♦	477.50 ±33.23♦♦
Mg	93.33 ± 8.02*	0.16 ±0.03	569.50 ±31.82*
VLZ	94.33 ± 3.06*	0.16 ±0.01	560.67 ±12.10*

An increase in blood glucose values was highlighted in the positive control group (116.33±1.53 mg/dL), statistically significant (♦♦p <0.01) compared to the negative control animals (88.50±6.24 mg/dL). The treatment with Mg for 14 days was associated with a significant reduction (*p <0.05) of blood glucose (93.33±8.02 mg/dL) compared to distilled water group subjected to exercise. The use of VLZ produced the relevant decrease (*p <0.05) in blood glucose level (94.33±3.06 mg/dL) compared to positive control, but less intense than noted for Mg group. In rats subjected to exercise stress, a significant increase (♦p <0.05) of the serum cortisol level (0.19±0.02 pg/mL) compared to animals without exercise (0.14±0.01 pg/mL) was detected. Mg caused a slight decrease in the cortisol level (0.16 ± 0.03 pg/mL), but without significance compared to positive control animals. The administration of VLZ led in a slight decrease in the blood value of cortisol (0.16±0.01 pg/mL), non-significant when compared to the positive control group (tab. II). The physical exercise induced a substantial de-

crease (♦♦p <0.01) in blood levels of BDNF (477.50±33.23 pg/mL), compared to the negative control group (575.67±47.25 pg/mL). The administration of Mg determined a relevant increase (**p <0.01) in the value of BDNF in the blood (569.50 ± 31.82 pg/mL), compared to distilled water group subjected to exercise. VLZ use led to an increase in serum BDNF (560.67±12.10 pg/mL), statistically significant (**p <0.01) compared to the positive control group, but weaker than produced by Mg (tab. II).

TABLE III.

The effects of VLZ on the SOD, MDA and GPx activity in treadmill test in rats. Each point expresses the mean ± S.D. of mean, for 6 animals in a group. *p <0.05, **p <0.01 when compared to positive control group, ♦p <0.05, ♦♦p <0.01 versus negative control group.

Groups	SOD (µg/mL)	MDA (nmol/L)	GPx (pg/mL)
Negative control	24.33 ± 2.46	27.83 ± 3.16	321.17 ± 33.27
Positive control	15.00 ± 2.83♦♦	38.50 ± 2.40♦♦	194.00 ± 35.36♦♦
Mg	23.00 ± 1.41*	26.65 ± 3.46**	311.50 ±113.84**
VLZ	20.83 ± 3.37*	29.57 ± 3.54*	307.67 ± 75.65**

A significant (♦♦p <0.01) decrease in SOD activity (15.00±2.83 µg/mL) compared to the negative control group (24.33 ± 2.46 µg/mL) was revealed in positive control animals in the treadmill test. The use of Mg was correlated with an obvious enhancement (*p <0.05) of SOD activity (23.00±1.41 µg/mL), compared to positive control group. The administration of VLZ was followed by marked intensification (*p <0.05) of SOD activity (20.83±3.37 µg/mL), when compared to the positive control group, but with less importance that observed for Mg group (tab. III). The positive control animals showed a considerable increase (♦♦p <0.01) of MDA activity (38.5±2.40 nmol/L), compared to the negative control (27.83 ± 3.16 nmol/L). The treatment with Mg induced an obvious diminution (**p <0.01) in the serum values of MDA (26.65±3.46 nmol/L), comparing with distilled water group with effort. The use of VLZ was accompanied by a notable (*p <0.05) decrease in the blood level of MDA (29.57 ± 3.54 nmol/L), compared to positive control group (tab. III). Subjecting control rats to physical exercise resulted in an obvious decrease (♦♦p <0.01) of GPx values (194.00 35.36 pg/mL), compared to the negative control animals (321.17±33.27 pg/mL) (Table 3). Mg caused a significant increase (**p <0.01) in the serum level of GPx (311.50±113.84 pg/mL), compared to distilled water group subjected to exercise (Table 3). The treatment with VLZ resulted in the increase of GPx values in blood (307.67±75.65 pg/mL), with statistical significance (**p <0.01) compared to positive control animals (tab. 3).

DISCUSSION

The administration of VLZ, during 14 consecutive days was associated by an increase in endurance capacity to forced exercise, objectified by the prolongation the distance traveled by the animal on the treadmill, the increasing in the number of touches the opposite end of belt, and a decreasing in the number of delivered electric stimuli. Biochemical analysis showed

that the treatment with VLZ significantly improved the glycaemia and decreased the serum value of cortisol. The effects of this antidepressant drug on these two parameters were of lower intensity than those induced by Mg in rats.

We investigated the modification of BDNF blood values in rats subjected to forced exercise, and the influence of the administration of VLZ. In our experimental condition the use of VLZ was followed by a substantial increase in the serum level of BDNF, *thus suggest its obvious stress-reducing effects in rats exposed to physical effort. Its effects were less intense when compared to Mg.* BDNF plays a critical role in the development of mental illnesses such as depression and anxiety (17). Previous research has also demonstrated the relationship between stress and BDNF expression in many critical brain regions (18). Clinical studies report that serum BDNF levels are decreased in depressed patients and then increased in response to treatment with typical antidepressants. Reduced BDNF is also observed in post-mortem tissue of untreated depressed individuals (19). Physical activity has been shown to increase BDNF mRNA expression in multiple brain regions, especially in the hippocampus, which is consistent with the delay in the antidepressant behavioral response in experimental rodent models (20). Recent studies have highlighted the fact that the induction of BDNF expression in the hippocampus is important in the manifestation of the effectiveness of antidepressant treatment, and its repression seems to have a determining role in the pathophysiological mechanisms of depression, in various animal models (21). Conversely, sustained induction of BDNF in the nucleus accumbens, which persists for at least one month after chronic stress, mediates depression-like behavioral abnormalities, while reversal of this effect induces an antidepressant response (22-24).

In response to endurance training in rats, free radicals are generated due to oxidative stress. The assessing of blood levels of MDA, GPx and SOD offers the possibility of estimation of VLZ influence on the cellular oxidative processes. In animals exposed to forced exercise training high levels of MDA in blood, as well as a diminution in SOD and GPx activity was detected. The improvement of the changes observed in these parameters, following the use of VLZ, suggests its influence of reducing oxidative stress. *The use of VLZ was accompanied by an intensification of antioxidant enzymes activity, respectively a reduction of the serum value of* MDA. *Its effects were less intense, when compared to magnesium.*

CONCLUSIONS

The treatment with VLZ improved the physical performances and the endurance capacity in this experimentally-induced stress model in rats. *The use of this antidepressant drug recovered the biochemical changes and produced antioxidant effects in animals subjected to forced exercise in treadmill test.*

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

This work is part of Paula Alina Fotache's Ph.D. studies and was co-funded from the European Social Fund - the Human Capital Operational Program, Project / Grant No: POCU/993/6/13/154722.

REFERENCES

1. Harmer CJ, Duman RS, Cowen PJ. How do antidepressants work? New perspectives for refining future treatment approaches. *Lancet Psychiatry* 2017; 4: 409-418.
2. Trivedi MH, Rush AJ, Wisniewski SR, *et al*. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* 2007; 173: 28-40.
3. Nagy C, Vaillancourt K, Turecki G. A role for activity-dependent epigenetics in the development and treatment of major depressive disorder. *Genes Brain Behav* 2018; 17: e12447: 1-9.
4. Uchida S, Yamagata H, Seki T *et al*. Epigenetic mechanisms of major depression: Targeting neuronal plasticity. *Psychiat Clin Neurosci* 2018; 72: 212-227.
5. Dirven BCJ, Homberg JR, Kozicz T, *et al*. Epigenetic programming of the neuroendocrine stress response by adult life stress. *J Mol Endocrinol* 2017; 59: R11-R31.
6. Branchi I. The double-edged sword of neural plasticity: increasing serotonin levels leads to both greater vulnerability to depression and improved capacity to recover. *Psychoneuroendocrinol* 2011; 37: 339-351.
7. Shi L, Wang J, Xu S, Lu Y. Efficacy and tolerability of vilazodone for major depressive disorder: evidence from phase III/IV randomized controlled trials. *Drug Design Develop Ther* 2016; 10: 3899-3907.
8. Sahli Z, Banerjee P, Tarazi F. The preclinical and clinical effects of vilazodone for the treatment of major depressive disorder. *Expert Opin Drug Discov* 2016; 11(5): 515-523.
9. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/022567s012lbl.pdf
10. Papadakis MA, McPhee SJ. Current Medical Diagnosis & Treatment 2015, 54th Edition, McGraw Hill Education, 2015, USA.
11. Dale E, Bang-Andersen B, Sanchez C. Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. *Biochem Pharmacol* 2015; 95: 81-97.
12. Zhang XF, Wu L, Wan DJ, *et al*. Evaluation of the efficacy and safety of vilazodone for treating major depressive disorder. *Neuropsychiatr Dis Treat* 2015; 11: 1957-1965.
13. Wang XQ, Wang GW. Effects of treadmill exercise intensity on spatial working memory and long-term memory in rats. *Life Sci* 2016; 149: 96-103.
14. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010; 1(2): 87-93.
15. Elfving B, Plougmann PH, Wegener G. Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: pitfalls and solutions. *J Neurosci Methods* 2010; 187(1): 73-77.
16. Directive 2010/73/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. http://eur-lex. europa. eu/ legal-content/ EN/TXT/ ?uri=CELEX:32010L0073.
17. McEwen BS. Protective and damaging effects of stress mediators: central role of the brain. Dialogues Clin Neurosci 2007; 8: 377-381.
18. Nakagawa Y, To M, Saruta J, *et al*. Effect of social isolation stress on saliva BDNF in rat. *J Oral Sci* 2019; 61(4): 516-520.
19. Aydemir O, Deveci A, Taneli F. The effect of chronic antidepressant treatment on serum brain-derived neuro-trophic factor levels in depressed patients: a preliminary study. *Prog Neuropsychopharmacol Biol Psychiatry* 2005; 29: 261-265.
20. JiaWen W, Hong S, ShengXiang X, *et al*. Depression- and anxiety-like behavior is related to BDNF/TrkB signaling in a mouse model of psoriasis. *Clin Exp Dermatol* 2018; 43(3): 254-271.
21. Zaletel I, Filipović D, Puškaš N. Hippocampal BDNF in physiological conditions and social isolation. *Rev Neurosci* 2017; 28(7): 775-792.
22. Hing B, Sathyaputri L, Potash JB. A comprehensive review of genetic and epigenetic mechanisms that regulate BDNF expression and function with relevance to major depressive disorder. *Am J Med Genet B Neuropsychiatr Genet* 2018; 177(2): 143-177.
23. Deyama D, Bang E, Kato T, *et al*. Neurotrophic and antidepressant actions of brain-derived neurotrophic factor require vascular endothelial growth factor. Biol Psychiatry 2019; 87(2): 143-152.
24. Bazzari AH, Bazzari FH. BDNF therapeutic mechanisms in neuropsychiatric disorders. *Int J Mol Sci* 2022; 23: 8417.