Original Article

Creatine and α-Lipoic Acid Improved Depressive Behavior Induced by Interferon-α in Mice: Malondialdehyde Level Remained Unchanged

Azadeh Mesripour[1,2], Zahra Karimi[1], Mohsen Minaiyan[1]

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

Abstract

Objective: Interferon-alpha (IFNa) is a cytokine with various biological roles but it may induce psychological adverse effects. Introducing alternative medicine is essential to prevent this side effect. This study was performed to determine the antidepressant effects of creatine (Crt) and α-lipoic acid (ALA). **Materials and Methods:** Female albino mice (6–8 weeks old) were used. IFNa (16×10^5 IU/kg/day, SC), Crt (5 and 10 mg/kg, gavage feeding tube), and ALA (20 and 40 mg/kg, IP) were administered for 6 days. After the locomotor test, behavioral parameters of depression, including immobility during the forced swimming test (FST), and finally serum malondialdehyde (MDA) were measured as factors for oxidative stress. **Results:** There was not important difference regarding the locomotor test. Crt 5 mg/kg and ALA 40 mg/kg when administered alone reduced the immobility time during FST when compared with the control groups (75±15 and 82±16 s, respectively, $P < 0.05$). Crt 5 mg/kg and IFN co-administration reduced the immobility time to 108±23 s, which was lower than that of the IFNa alone group (156±8 s, $P < 0.05$). Administrating ALA 40 mg/kg and IFNa together showed same results (95±11 s vs. IFNa alone group, $P < 0.01$). The results of measuring MDA did not show noticeable difference. **Conclusion:** In general, improvement of behavioral parameters in mice treated with Crt and ALA indicates a clear effect of these two compounds in modulating mood and depressive behaviors. Although MDA level differences were not observed, Crt and ALA modulation in the neurotransmitter system may be involved in their antidepressant effects.

Keywords: *Alpha lipoic acid, creatine, depression, interferon alpha, malondialdehyde*

Introduction

Interferon-alpha (IFNa) belongs to type I IFNs, cytokines with a variety of biological roles in anti-viral, anti-proliferation, anti-tumor, and apoptotic pathways.[1] Evidently, IFNa not only shortens hospitalization period in COVID-19 patients, but also augments viral clearance.[2] IFNa-related depression is an important drawback in medicine and neurology; various studies have introduced alternative and complementary medicines in order to prevent this side effect.[3,4] Various mechanisms are suggested for IFNa inducing psychological effects.[5] For instance, activation of indoleamine 2,3-dioxygenase (IDO) due to a shift in metabolizing tryptophan to quinolinic acid, N-methyl-d-aspartate (NMDA) receptor agonists, results in excitotoxicity and loss of neurons in the hippocampus. Moreover, a decrease in serotonin production has been connected to neurochemical changes related to depression.[6] Evidently, there is a key role for massive Ca 2+ influx through the NMDA channel as a trigger of excitatory neurotransmitter (glutamate) neurotoxicity and neuronal cell death.[7] Results proved that initial mitochondrial impairment plays a critical role in glutamate neurotoxicity.[8] In addition, depression-like behavior in animals is linked to mitochondrial inefficiency in brain regions associated with neural circuitry of depression such as frontal cortex and hippocampus.[9]

Creatine (Crt) is synthesized endogenously from three amino acids, arginine, glycine, and methionine, in the liver, kidney, and brain or it can be obtained from high-protein foods.[10] Crt supplementation has been studied in neurological diseases that may be related to mitochondrial impairment, such as Parkinson's disease.[10] Researches show that Crt by buffering adenosine triphosphate (ATP) levels could protect neuros against neurotoxic substances such as harmful levels of glutamate.[11]

Alpha-lipoic acid (ALA), also known as 1,2-dithiolane-3-pentanoic acid, is a natural essential co-factor for pyruvate and alpha-

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Address for correspondence:
Dr. Azadeh Mesripour, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Hezarjerib Boulevard, Isfahan, Iran.
E-mail: a_mesripour@pharm.mui.ac.ir.

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ketoglutarate dehydrogenase within the mitochondria. Exogenous ALA is used as an antioxidant dietary supplement in some regions including Europe and America and Japan.[12] More than a decade ago, the antidepressant effect of ALA was assumed by Salazar.[13] In addition, it has been proven that ALA ameliorates cognitive dysfunction in memory tasks in rats by lowering age-associated oxidative damage; ALA reverses mitochondrial structural decay and improves mitochondrial function.[14]

Psychiatric side effects and mood changes caused by IFNa are common but generally mild. Therefore, pretreatment with common antidepressant drugs might expose individuals to excessive medications, thus presenting that a harmless complementary medicine would be valuable. Additionally, a major limitation of available monoaminergic-based antidepressant drugs is their long lag period (approaches about 4 weeks) for starting their therapeutic effects. Based on the hypothesis that mitochondria might be involved in the pathophysiology of depression, in the following experiments, an observation study was conducted in order to evaluate the effect of Crt and ALA, following IFNa-induced depressive-like behavior in mice. The serum malondialdehyde (MDA) level was also assessed as a marker of lipid peroxidation.

Materials and Methods

Animals

Female albino mice weighing 24±3 g (6–8 weeks old) were chosen according to the previous studies, suggesting that Crt effects on depressive behavior are sex-dependent, as female rats had shown better antidepressant-like response.[15] Animals were maintained at room temperature 21± 2°C with free access to standard mice chow and tap-water, on a 12–12 h light–dark cycle (lights were on till 6 a.m.). In total, 12 groups of animals consisting of six were studied. Six animals were housed together in each cage, and they were placed in the experimental room 24 h before the test for acclimatization. All the experiments were performed between 8 a.m. and 1 p.m. 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 and approved by Vice-Chancellor in Research Affairs-Medical University of Isfahan (Ethical No. IR.MUI.REC.1399.180). All the efforts in the experiments were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Drug administration

IFNa (PDferon, Pooyesh Darou 3×10^6 IU, Iran) 16×10^5 IU/kg was injected SC.[4] Crt monohydrate (Karen Pharma and Food Supplement, Iran) 5 and 10 mg/kg was administered by a daily gavage feeding tube. ALA (Sigma Aldrich, India) 20 and 40 mg/kg was injected IP (the doses were based on pilot studies). Control groups were injected with normal saline; in the sham group, animals received normal saline by a gavage feeding tube. All treatments were done for 6 consecutive days, and the tests were performed on the following day. In a separate group of mice, the concomitant administration of Crt 5 mg/kg and ALA 20 mg/kg was evaluated on IFNa-induced depression-like behavior.

Locomotor test

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

Forced swimming test (FST)

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 at the first 2 min. Swimming behavior is defined as horizontal movement throughout the beaker which involved at least two limbs; climbing behaviors defined as upward movements of the forepaws along the side of the beaker were also recorded.[16] 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.

Serum MDA level

After the experiments, animals were decapitated and jugular blood samples were collected and maintained for 30 min at room temperature. Following centrifugation (3000 rpm, 20 min), serum was transferred to small-capped vials and stored frozen (−20°C) until analyzed. All the process for evaluating the activity of MDA was performed according to the company instruction of the Nalondi Lipid Peroxidation MDA Assay Kit (Navand Lab Kit, Iran), and absorbance was measured at 532 nm.

Data processing and statistical analysis

Results were expressed 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 used for data analyzing and making graphs were Excel 2010 and GraphPad Prism 8.

Results

The effect of Crt on depressive behavior following IFNa administration

According to [Figure 1A], Crt 5 mg/kg significantly reduced the immobility time (75±15 s compared with

the sham group 136±14 s, $P < 0.05$), which indicated its antidepressant-like effects. Following IFNa injection, immobility time was significantly higher than that of the control (156±8.2 s vs. 107±9.1 s, $P < 0.05$), which showed despair behavior. Following the co-administration of Crt 5 mg/kg and IFNa, the immobility time reduced to 108±23 s that was noticeably lower than that of the IFNa alone group, while adding ALA 20 mg/kg to Crt 5 mg/kg did not show any additive effect on the immobility time. The treatments did not cause important changes in the locomotor activity [Table 1]; this indicates that the change in immobility time during the FST could be considered as depressive-like behavior. Swimming and climbing were also measured during the FST for describing the possible neurotransmitter involved in the depression behavior. Climbing values did not show important difference among the groups [Table 1]. By administrating Crt 10 mg/kg swimming was noticeably higher than control, and by co-administrating Crt with IFNa, the values were significantly higher than those of the IFNa alone group.

The effect of ALA on depressive behavior following IFNa administration

As presented in Figure 1B, the administration of ALA 40 mg/kg significantly reduced the immobility time during the FST (82±16 s vs. control 127±10 s, $P < 0.05$). Co-administrating ALA 40 mg/kg and IFNa induced antidepressant-like effects, since the immobility time was lower (95±11 s) than that of the IFNa alone group (156±8 s, $P < 0.01$); adding Crt to the treatment did not have additive antidepressant effect. At the prescribed doses, the total activity during the locomotor test did not show important differences between groups [Table 2]. The active phase in the FST showed important differences in the swimming time; ALA 40 increased swimming time when it was administered alone compared with vehicle ($P < 0.05$), and together with IFNa ($P < 0.05$ vs. IFNa alone), climbing time was higher than control when ALA 40 mg/kg was injected with IFNa.

Serum MDA concentration

The serum MDA concentrations did not show important differences among different groups [Table 3].

Table 1: The effect of Crt alone and together with IFNa and ALA on the locomotor test and the active stages of FST

Groups (n=6)	Swimming time (s)	Climbing time (s)	Total locomotor activity (number)
Sham	73±14	25±14	162±37
Crt 5 mg/kg	106±8	51±13	175±19
Crt 10 mg/kg	113±9$^{\$}$	8.5±3	150±14
Control	98±14	17±5	156±26
IFNa	53±6*	29±5	110±13
IFNa + Crt 5 mg/kg	127±21$^{\#\#}$	5±2	191±19
IFNa + Crt 10 mg/kg	99±10$^{\#}$	5.5±5	143±27
IFNa + Crt 5 + ALA 20 mg/kg	82±14	35±15	135±22

Total activity during locomotor test= (horizontal +vertical) exploration. The sham and control groups received normal saline (by gavage and SC, respectively). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test

$^{\$}P$<0.05 compared with the sham group

*P<0.05 compared with the control group

$^{\#}P$<0.05, $^{\#\#}P$ < 0.01 compared with the IFNa alone group.

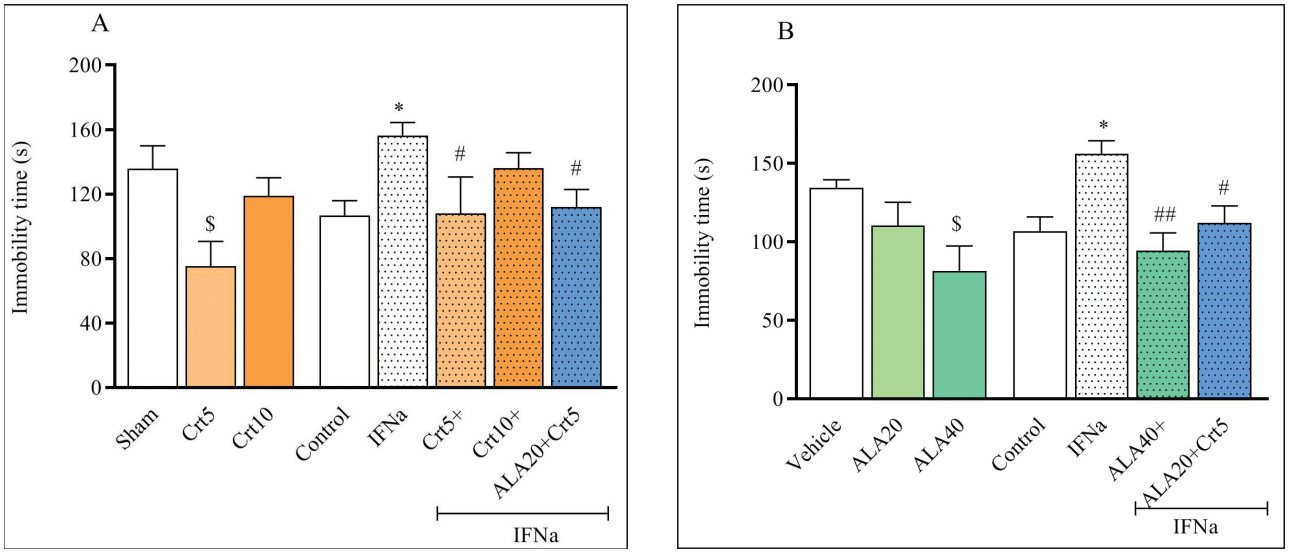

Figure 1: The effect of Crt and ALA on the immobility time during the FST. A. Crt 5 and 10 mg/kg, the sham group received normal saline via gavage tube. B. ALA 20 and 40 mg/kg, the vehicle group received normal saline by IP injection. IFNa 16×105 IU/kg, and the control group received normal saline by SC injection. Results are expressed as group mean ± SEM and analyzed by ANOVA, followed by Tukey's comparison tests (n=6). $^{\$}P$<0.05 compared with the sham or the vehicle group, *P<0.05 compared with the control group, $^{\#}$P<0.05 and $^{\#\#}P$ <0.01 compared with the IFNa alone group

Discussion

The following research clearly showed that IFNa increased the immobility time in the FST, which is an indication for depression-like behavior in mice. Crt and ALA improved depressive behavior caused by IFNa. FST is a principal test used to assess the efficacy of antidepressant substances and the effects of various neurobiological fluctuations preclinically.[15] The locomotor is accompanied prior to the FST test in order to understand the possible sedative or excitatory effects of drugs that could distort the FST results.

IFNa-induced depression-like effect was in agreement with previous findings.[3] It has been shown that a soybean diet as a good source for tryptophan could improve IFNa depression, probably by increasing the brain serotonin level.[4] Several mechanisms are suggested for IFNa-induced depression; one possible mechanism is related to the activation of IDO that results in neurotoxicity due to an increased level of quinolinic acid, an NMDA receptor agonist.[5] Additionally, it was shown that in mouse bone marrow cells, IFNa and IFN-beta mediated apoptosis by mitochondrial apoptotic pathway and reactive oxygen species.[17]

Crt administration reduced the immobility time during FST; it also prevented IFNa from causing depression-like behavior. The main energy source in the brain is ATP, which is tightly coupled to phosphocreatine (PCr). Crt exerts neuroprotective effects as a substrate for Crt kinase, it increases PCr that converts adenosine diphosphate to ATP and prevents ATP depletion.[18] The mitochondria and Crt and PCr establish a system that is critical in energy homeostasis in high energy-demanding organs, such as the brain.[18] Crt by stimulating the rate of ATP synthesis and producing high amounts of PCr may have prevented the IFNa-induced neuron loss and depression-like effect in mice. It has been advocated previously that the buildup of PCr could help neurons endure ATP levels for a prolonged period of time, especially during energy depletion or stress conditions.[18] In addition, Crt effect on the neurotransmitters could be important in improving depressive behavior and should also be considered. A shortcoming of this research was that the neurotransmitter levels were not evaluated and it is suggested in future research. By measuring swimming and climbing time in the FST, the possible neurotransmitters involved could be speculated. The catecholaminergic agents decrease immobility time while increasing the climbing behavior, and serotonin-related compounds such as fluoxetine decrease immobility time while increasing swimming behavior.[15] Crt when administered alone and in combination with IFNa increased swimming time which may be related to the increased serotonin level. This was supported by a previous study which showed that female rats fed with Crt maintained higher swimming activity with no differences in climbing activity.[16] On the contrary, oral Crt has been found to increase brain dopamine synthesis in the substantia nigra of mice by protecting against striatal dopamine depletion; also Crt can enhance tyrosine hydroxylase activation, a rate-limiting enzyme for catecholamine biosynthesis.[18,19] In addition, it was shown that Crt supplementation reduces the tryptophan: tyrosine ratio, suggesting that Crt modulates brain serotonin and dopamine level.[20] Injecting ALA reduced the immobility time during the FST, and this change was pronounced with ALA 40 mg/kg. ALA antidepressant-like effects demonstrated in the FST are in agreement with previous results.[21,22] Oxidative stress has been involved in the pathogenesis of psychiatric illnesses, such as major depression disorder.[23] ALA is a mitochondrial antioxidant and a neuroprotective substance; therefore, the beneficial effects of ALA in improving depression-like behavior support the hypothesis that antioxidants could have antidepressant properties.[22] The swimming time was higher than normal when ALA 40 mg/kg was administered

Table 2: The effect of ALA alone and together with IFNa and Crt on the locomotor test and the active stages of FST

Groups (*n*=60)	Swimming time (s)	Climbing time (s)	Total locomotor activity (number)
Vehicle	91±5	9±3	128±22
ALA 20 mg/kg	120±712	10±4	104±11
ALA 40 mg/kg	141±11$	18±10	147±23
Control	98±14	17±5	156±26
INFa	53±5*	29±4	110±13
INFa + ALA 40 mg/kg	96±14#	49±10*	98±20
INFa + Crt 5 + ALA 20 mg/kg	82±14	35±15	135±22

Total activity during locomotor test = (horizontal +vertical) exploration. The vehicle and control groups received normal saline (by IP and SC, respectively). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison test.
$ $P<0.05$ compared with the vehicle group
* $P<0.05$ compared with the control group
# $P<0.05$ compared with the INFa alone group.

Table 3: The effect of Crt, ALA, and IFNa on serum MDA level

Groups (*n*=5)	MDA (nmol/mL)	Groups (*n*=5)	MDA (nmol/mL)
Sham	1.9±0.1	Control	1.7±0.1
Crt 5 mg/kg	1.8±0.4	IFNa	2.3±0.5
Crt 10 mg/kg	2±0.3	IFNa + Crt 5 mg/kg	2.4±0.3
Vehicle	2.1±0.4	IFNa + Crt 10 mg/kg	2±0.2
ALA 20 mg/kg	3±0.5	INFa + ALA 40 mg/kg	1.9±0.4
ALA 40 mg/kg	2±0.3	IFNa + Crt 5 + ALA 20 mg/kg	3±0.3

The sham, vehicle, and control groups received normal saline (by gavage feeding tube, IP and SC, respectively). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests

alone. While ALA was co-administered with IFNa, both swimming time and climbing time were higher than those with IFNa alone. It has been assumed that ALA by increasing tryptophan availability in the blood may contribute to the treatment of depression.[13] Synthesis of serotonin depends on the concentration of tryptophan (precursor to serotonin) in the blood and the activity of tryptophan hydroxylase, a rate-limiting enzyme in the synthesis of serotonin. Normally, an increase in tryptophan availability results in an increase in serotonin synthesis.[24] Therefore, ALA apart from improving mitochondrial function and preventing mitochondrial decay and oxidative damage[14] has effect on serotonin synthesis that must be carefully considered in the treatment of depression. ALA co-administration with Crt did not show additive antidepressant-like effects.

MDA is a vital biomarker in preclinical and clinical studies.[25,26] However, by different therapies applied in this study, important difference in the serum MDA level was not detected. MDA is a result of polyunsaturated fatty acids peroxidation; elevated levels of MDA have been known in individuals with depression disorder and in patients suffering from recurrent depressive disorders.[27,28] The lack of difference may be related to pretreatment procedures, biological sample preparation, and storage.[25] Another possible reason that a significant change in MDA was not observed could be the short treatment period. Perhaps, 6 days of IFNa injection, although induced behavioral depression-like changes in mice, was not enough to make significant change in MDA. It is suggested to evaluate the MDA level after long-term IFNa, Crt, and ALA administration and to use different assessment protocols.

In conclusion, improvement of behavioral parameters in mice treated with Crt and ALA indicates a clear effect of these two compounds in modulating mood and depressive behaviors. Crt and ALA prevented IFNa depressant-like behavioral effect; therefore, at least part of IFNa-induced depression may be related to mitochondrial dysfunction. Measuring serum MDA level did not show substantial biomedical differences. In addition, Crt and ALA inducing possible changes in the neurotransmitter system may also be involved in their antidepressant benefit effects. In general, although interpreting animal result to human should be vigilant, it can be concluded that Crt and ALA are reasonable alternative medications to prevent IFNa-induced depressive symptoms.

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Conflicts of interest

There are no conflicts of interest.

References

1. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, *et al*. Interferons at age 50: Past, current and future impact on biomedicine. Nat Rev Drug Discov 2007;6:975-90.
2. Wang B, Li D, Liu T, Wang H, Luo F, Liu Y. Subcutaneous injection of IFN alpha-2b for COVID-19: An observational study. BMC Infect Dis 2020;20:723.
3. Mesripour A, Sajadian S, Hajhashemi V. Antidepressant-like effect of vitamin B6 in mice forced swimming test and the possible involvement of the noradrenergic system. J Rep Pharm Sci 2019;8:133-8.
4. Azimi Fashi Y, Mesripour A, Hajhashemi V. Evaluation of the effect of soybean diet on interferon-α-induced depression in male mice. Avicenna J Phytomed 2017;7:436-43.
5. Pinto EF, Andrade C. Interferon-related depression: A primer on mechanisms, treatment, and prevention of a common clinical problem. Curr Neuropharmacol 2016;14:743-8.
6. Zahiu CD, Rimbas M. Neuropsychiatric side-effects of interferon-alpha treatment: Pathophysiology and therapeutic options. Maedica (Bucur) 2014;9:121-6.
7. Kritis AA, Stamoula EG, Paniskaki KA, Vavilis TD. Researching glutamate-induced cytotoxicity in different cell lines: A comparative/collective analysis/study. Front Cell Neurosci 2015;9:91.
8. Schinder AF, Olson EC, Spitzer NC, Montal M. Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. J Neurosci 1996;16:6125-33.
9. Klinedinst NJ, Regenold WT. A mitochondrial bioenergetic basis of depression. J Bioenerg Biomembr 2015;47:155-71.
10. Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in the central nervous system. Brain Res Bull 2008;76:329-43.
11. Brustovetsky N, Brustovetsky T, Dubinsky JM. On the mechanisms of neuroprotection by creatine and phosphocreatine. J Neurochem 2001;76:425-34.
12. Takahashi H, Bungo Y, Mikuni K. The aqueous solubility and thermal stability of α-lipoic acid are enhanced by cyclodextrin. Biosci Biotechnol Biochem 2011;75:633-7.
13. Salazar MR. Alpha lipoic acid: A novel treatment for depression. Med Hypotheses 2000;55:510-2.
14. Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM, *et al*. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R-alpha-lipoic acid. Proc Natl Acad Sci USA 2002;99:2356-61.
15. Allen PJ, D'Anci KE, Kanarek RB, Renshaw PF. Chronic creatine supplementation alters depression-like behavior in rodents in a sex-dependent manner. Neuropsychopharmacology 2010;35:534-46.
16. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: Recent developments and future needs. Trends Pharmacol Sci 2002;23:238-45.
17. Yim HY, Yang Y, Lim JS, Lee MS, Zhang DE, Kim KI. The mitochondrial pathway and reactive oxygen species are critical contributors to interferon-α/β-mediated apoptosis in ubp43-deficient hematopoietic cells. Biochem Biophys Res Commun 2012;423:436-40.
18. Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, *et al*. Creatine and cyclocreatine attenuate MPTP neurotoxicity. Exp Neurol 1999;157:142-9.
19. Klivenyi P, Gardian G, Calingasan NY, Yang L, Beal MF. Additive neuroprotective effects of creatine and a cyclooxygenase 2 inhibitor against dopamine depletion in the 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP) mouse model of Parkinson's disease. J Mol Neurosci 2003;21:191-8.
20. Hadjicharalambous M, Kilduff LP, Pitsiladis YP. Brain serotonin and dopamine modulators, perceptual responses and endurance performance during exercise in the heat following creatine supplementation. J Int Soc Sports Nutr 2008;5:14.
21. de Sousa CN, Meneses LN, Vasconcelos GS, Silva MC, da Silva JC, Macêdo D, *et al*. Reversal of corticosterone-induced BDNF alterations by the natural antioxidant alpha-lipoic acid alone and combined with desvenlafaxine: Emphasis on the neurotrophic hypothesis of depression. Psychiatry Res 2015;230:211-9.
22. Silva MC, de Sousa CN, Gomes PX, de Oliveira GV, Araújo FY, Ximenes NC, *et al*. Evidence for protective effect of lipoic acid and desvenlafaxine on oxidative stress in a model depression in mice. Prog Neuropsychopharmacol Biol Psychiatry 2016;64:142-8.
23. Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: Evidence base and therapeutic implications. Int J Neuropsychopharmacol 2008;11:851-76.
24. Carlsson A, Lindqvist M. The effect of L-tryptophan and some psychotropic drugs on the formation of 5-hydroxytryptophan in the mouse brain *in vivo*. J Neural Transm 1972;33:23-43.
25. Khoubnasab Jafari M, Ansarin K, Jouyban A. Comments on "use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: A review". Iran J Public Health 2015;44:714-5.
26. Safaeian L, Emami R, Hajhashemi V, Haghighatian Z. Antihypertensive and antioxidant effects of protocatechuic acid in deoxycorticosterone acetate-salt hypertensive rats. Biomed Pharmacother 2018;100:147-55.
27. Lopresti AL, Maker GL, Hood SD, Drummond PD. A review of peripheral biomarkers in major depression: The potential of inflammatory and oxidative stress biomarkers. Prog Neuropsychopharmacol Biol Psychiatry 2014;48:102-11.
28. Rybka J, Kędziora-Kornatowska K, Banaś-Leżańska P, Majsterek I, Carvalho LA, Cattaneo A, *et al*. Interplay between the pro-oxidant and antioxidant systems and proinflammatory cytokine levels, in relation to iron metabolism and the erythron in depression. Free Radic Biol Med 2013;63:187-94.