Cell J
Cell Journal (Yakhteh)
Archive of SID
Homepage: https://www.celljournal.org/
Original Article
Vol 25, No 3, March 2023, Pages: 165-175

Distribution of Plasma One-Carbon Metabolism Factors and Amino Acids Profile in Depression State Treated with Paroxetine: A Model Study

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Abstract

Objective: Stress may have an important role in the origin and progress of depression and can impair metabolic homeostasis. The one-carbon cycle (1-CC) metabolism and amino acid (AA) profile are some of the consequences related to stress. In this study, we investigated the Paroxetine treatment effect on the plasma metabolite alterations induced by forced swim stress-induced depression in mice.

Materials and Methods: In this experimental study that was carried out in 2021, thirty male NMRI mice (6-8 weeks age, 30 ± 5 g) were divided into five groups: control, sham, paroxetine treatment only (7 mg/kg BW/day), depression induction, and Paroxetine+depression. Mice were subjected to a forced swim test (FST) to induce depression and then were treated with Paroxetine, for 35 consecutive days. The swimming and immobility times were recorded during the interventions. Then, animals were sacrificed, plasma was prepared and the concentration of 1-CC factors and twenty AAs was measured by spectrophotometry and high-performance liquid chromatography system (HPLC) techniques. Data were analyzed by SPSS, using One-Way ANOVA and Pearson Correlation, and $P<0.05$ was considered significant.

Results: Plasma concentrations of phenylalanine, glutamate, aspartate, arginine, ornithine, citrulline, threonine, histidine, and alanine were significantly reduced in the depression group in comparison with the control group. The Homocysteine (Hcy) plasma level was increased in the Paroxetine group which can be associated with hyperhomocysteinemia. Moreover, vitamin B12, phenylalanine, glutamate, ornithine, citrulline, and glycine plasma levels were significantly reduced in the depression group after Paroxetine treatment.

Conclusion: This study has demonstrated an impairment in the plasma metabolites' homeostasis in depression and normal conditions after Paroxetine treatment, although, further studies are required.

Keywords: Amino Acid, Depression, Mice, Selective Serotonin Reuptake Inhibitor

Citation: **Aghajani R, Naderi N, Sadeghi N, Ani M, Ani S, Nasr-Esfahani MH. Distribution of plasma one-carbon metabolism factors and amino acids profile in depression state treated with paroxetine: a model study. Cell J. 2023; 25(3): 165-175. doi: 10.22074/CELLJ.2023.557498.1056.**

Introduction

Depression, a universal mental disorder, consists different features such as depressed mood, irritability, anhedonia, anergia, poor concentration, sleep disorders, appetite alterations, and declined cognition (1). The percentage of the global population with depression in 2015 is assessed to be 4.4% (2). Clinical depression remains elusive and may be attributable to one-carbon cycle (1-CC) dysregulation, including folate deficiency, hyperhomocysteinemia, and monoamine metabolism disorders (3).

The important regulators of the 1-CC include all essential dietary necessities like methionine, folate, and vitamin B12 (4). One-carbon metabolism, which consists of the folate and methionine (Met) cycles, plays a vital role in the methyl donors' production in the S-adenosylmethionine (SAM) form. It is an essential factor in the 1-CC and the methylation reactions. While, the SAM is involved in various cell membrane functions and the synthesis of nucleic acid, protein, phospholipids, and monoamine neurotransmitters, such as serotonin, noradrenaline, and dopamine, it may play a valuable role in biochemical mechanisms that have been associated with depression (5). Folate and Met cycles are connected by methionine synthase (MS), the rate-limiting enzyme that converts homocysteine (Hcy) to Met using 5-methyltetrahydrofolate (5-mTHF) as a methyl donor and vitamin B12 as a vital cofactor (4).

Received: 10/July/2022, Revised: 16/November/2022, Accepted: 02/January/2023
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Besides 1-CC metabolites, there is a growing acknowledgment of the key roles of amino acids (AAs) in neurotransmitter synthesis, protein construction, signal conduction, and immunoregulation, they have become candidates for the biomarkers of various diseases, such as cancer, metabolic syndrome, and psychiatric disorders (6). Both animal and human clinical studies have suggested that excitatory AA transmission dysfunction within the central nervous system (CNS) is involved in the depression pathophysiology (7). In addition, the reduced serum Met, phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr) are associated with depression (8). Furthermore, the Met is catabolized to SAM, as a key factor in the 1-CC, which is found to be lower in depressed patients (9). Therefore, there is clinical and experimental evidence linking the 1-CC and AA metabolism in depression that needs to be considered.

In recent years, various classes of antidepressant drugs with different action mechanisms have been advanced. Selective serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline reuptake inhibitors (SSRIs and SNRIs) are new classes of antidepressants (10). Paroxetine as an SSRI increases the nerve conduction rate of 5-HT and has promising antidepressant effects. Due to its highly selective inhibitor of the presynaptic serotonin reuptake and low interaction, the Paroxetine has been extensively utilized in the clinical remedy for depression (11). In this line, a previous study has revealed that paroxetine-treated mice respond with less floating in the forced swim test (FST) indicating lower anxiety (12). However, there is little information on the alterations of metabolites according to the medicine with SSRIs. On the other hand, finding alterations of biomarkers in depression increases understandings of the illness and also help the choice of drugs and affect treatment efficacy. So, we studied the changes in the concentration of 1-CC factors and AA profiles in the mouse model for depression and in response to paroxetine treatment.

Materials and Methods

All experimental procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Ethical Committee of the Royan Institute, Esfahan, Iran (IR.ACECR.ROYAN.REC.1399.084).

Animals

In the present study, thirty mature male NMRI mice with weight 30 ± 5 g, and the age range of 6 to 8 weeks were provided by the Animal Biotechnology Institute, Isfahan, Iran. All of them were kept under the standard conditions (12 light/12 dark cycle, temperatures of 22 ± 2°C and, 40-60% humidity), with free access to standard food and water.

Experimental design

After the adaptation of animals, the mice were divided randomly into five equal groups (n=6) consisting of Control group: normal mice without intervention, sham group: normal mice receiving only saline as a vehicle of Paroxetine by gavage, Paroxetine group: normal mice receiving only Paroxetine [7 mg/kg body weight (BW)/day] by gavage, depression group: depressed mice as a result of the FST as described below, and Paroxetine+depression group: depressed mice treated with Paroxetine (7 mg/kg BW/day) by gavage. The experimental period lasted for 35 days. Paroxetine administered in doses of 10, 20, 30, 40 mg/kg BW, which is usually taken daily in the morning. The dose regimen for Paroxetine in this study was selected according to Dunner and Dunbar's study (13). According to the equivalent doses of the drug, male NMRI mice were treated with doses of 3, 5, 7, and 9 mg/kg BW, and the optimal dose of the drug was 7 mg/kg BW.

Forced swim test

The FST is one of the tests for the survey of depressive-like behavior in rodents and the assessment of antidepressant drugs (14). In this method, the animal is held by its tail and slowly placed it in the water tank filled with water at 32 to 34°C to a 13-15 cm depth. During the first trial, mice were left in the water tank for 15 minutes, first for four consecutive days and then once a week for one month. From the fourth swim onwards, the behavior of the mice is recorded by video and the duration of swimming and the immobility of the animals are examined. Treatment with Paroxetine started 24 hours after the fourth swim. Recorded videos of animal behavior were checked to assess the degree of depression in each group and the effectiveness of antidepressants.

Blood analysis

After 35 days, the mice were fasted for 5 hours, and anesthetized with intraperitoneal administration of 200 mg/kg BW Ketamine (Batch No: 10258, Panpharma, Germany)+10 mg/kg BW Xylazine (Batch No: 335826A, Bioveta, Czech Republic), and sacrificed. Blood samples (1 ml) were drawn from the heart using a syringe containing 10 µl of ethylenediaminetetraacetic acid (EDTA, 0.2 mol/L, Cat No: 6381-92-6, Merck, Germany). Separation of plasma was provided by centrifugation (Model No: BH-1200, Behdad, Iran) under 2500 rpm for 5 minutes. The plasma was stored at -80°C until analysis. The plasma concentrations of folate (Folate III, Roche, Switzerland) and vitamin B12 (Vitamin B12 II, Roche, Switzerland) were analyzed using ELISA kits (Elecsys 2010 and Cobas e411 analyzers, Roche, Switzerland) according to manufacturer's protocol, and absorbance was measured by spectrophotometry at 450 nm. And, the plasma levels of Hcy and 20 AAs were then assayed by high-performance liquid chromatography system (HPLC, Kenover, Germany) equipped with a fluorescence detector and C18 capillary column.

Statistical analysis

The Shapiro-Wilk normality test was done to ensure normal data distribution. The results were shown as the mean ± standard error of the mean (SEM), and significance between study groups was specified by the Tukey's post hoc test using SPSS®, version 23.0 statistical software (SPSS Inc. Chicago IL, USA). Pearson's correlation test was used to find out the correlation between the plasma levels of Hcy and AAs. P<0.05 was considered significant. All graphs were represented by Graph Pad Prism 8 (San Diego, California USA).

Results

Immobility in the forced swimming test

There is no significant difference in immobility and swimming time between depressed groups in the absence or presence of Paroxetine treatment (Fig.1A, B).

The plasma levels of one-carbon cycle factors

No significant changes were observed between different experimental groups in the plasma folate levels (Fig.1C). Plasma concentrations of vitamin B12 were significantly decreased after the Paroxetine treatment in the normal (P=0.002) and depressed (P=0.001, Fig.1D) mice in comparison with the control group, however, no significant difference in plasma levels of vitamin B12 was observed between depression and control groups. The Hcy level was remarkably higher in the normal animals treated with the Paroxetine in comparison with the control group (P=0.006, Fig.1E). No significant difference was revealed in the Hcy level between the depressed groups in the absence or presence of Paroxetine and the control group.

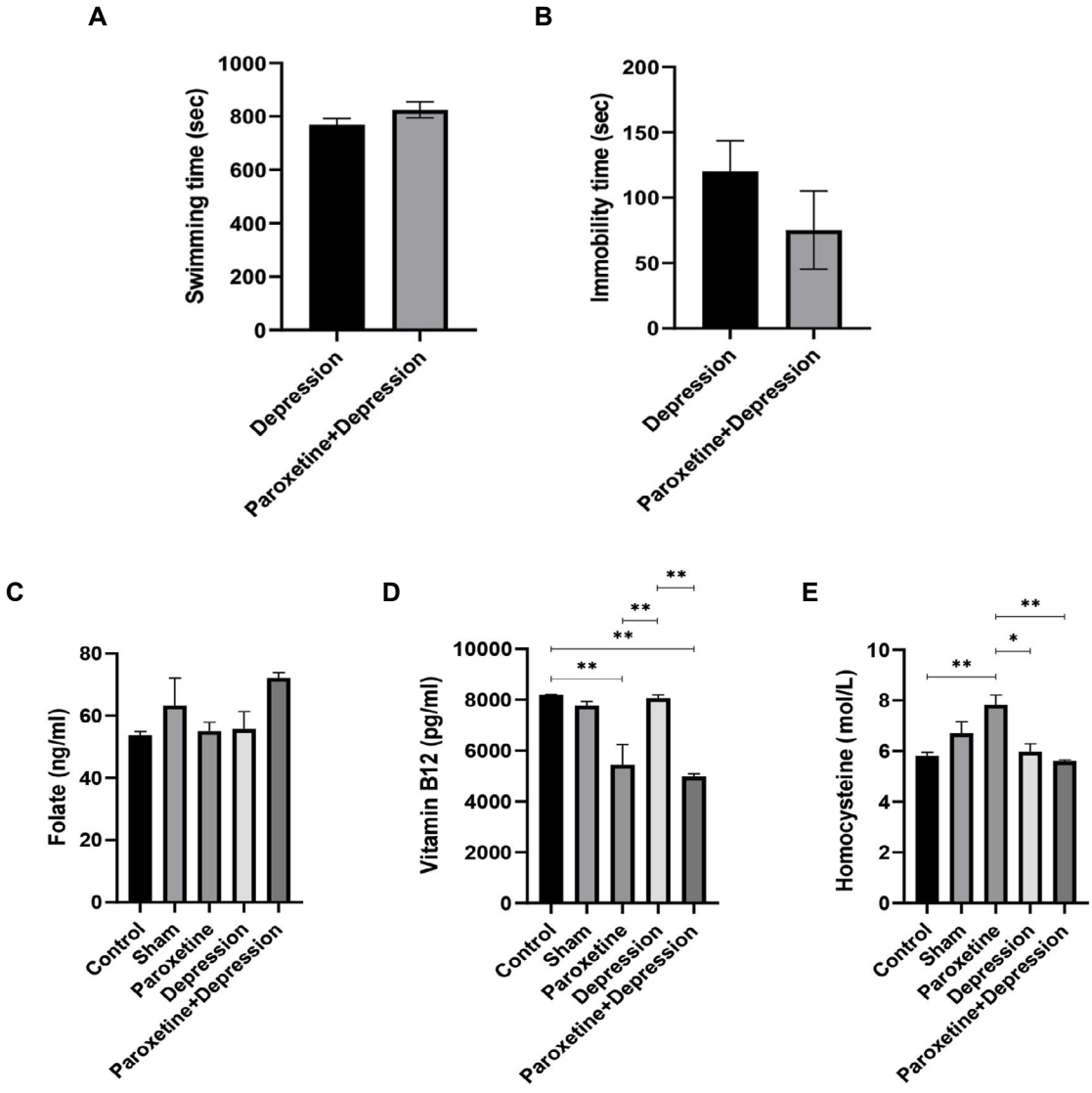

Fig.1: Behavioural performance and one-carbon cycle factors plasma concentrations of NMRI mice in the forced swim test under the basal condition and after treatment with Paroxetine. **A.** Swimming time, **B.** Immobility time, **C.** Folate, **D.** Vitamin B12, and **E.** Homocysteine. Each column represents the means ± standard error of the mean (SEM). *; Shows the significant difference between the two groups at P<0.05, **; Shows the significant difference between the two groups at P<0.01, and sec; Seconds.

The plasma levels of aromatic amino acids

Our results showed that the administration of the Paroxetine to normal mice, who were normal mice that received Paroxetine treatment, was significantly decreased the plasma levels of Phe in comparison with the control group (P=0.000, Fig.2A). Also, there was a significant decline in the Phe level in the depressed mice in comparison with the control group (P=0.000), but the plasma concentration of the Phe was significantly enhanced in the depressed mice with the Paroxetine treatment in comparison with the depressed animals (P=0.000). These results showed that the Paroxetine administration to the normal mice caused a significant increment in the Tyr plasma level in comparison with the control group (P=0.000, Fig.2B). Besides, the Tyr level was significantly elevated in the depressed mice in the absence or presence of the Paroxetine in comparison with the control group (P=0.000). No significant difference in the Trp plasma level was found in our study groups (Fig.2C).

The plasma level of excitatory amino acids

Our results showed that the plasma Gln level was significantly enhanced in the depressed mice in contrast with the control group (P=0.001, Fig.2D). Also, we observed considerable difference in the Gln plasma concentration between the Paroxetine-treated depressed mice and the control group (P=0.002). Plasma level of Glu was significantly declined in the normal mice after oral-gavage administration of the Paroxetine in comparison with the control group (P=0.000, Fig.2E). Also, the plasma Glu level was significantly reduced in the depressed groups in the absence or presence of Paroxetine than in the control group (P=0.000), but no difference was found among depressed groups in the absence or presence of the Paroxetine treatment. These findings showed that Paroxetine treatment in the normal (P=0.002) and depressed (P=0.002) mice caused a considerable increase in the Asn plasma level in contrast with the control group (Fig.2F). No differences were observed in the Asn plasma level between depressed and the control groups. The normal animals after treatment with the Paroxetine showed a significantly lower Asp plasma level in comparison with the control group (P=0.012, Fig.2G). We observed a significant reduction of the Asp plasma level in the depressed group in comparison with the control group (P=0.012), whereas a significant increase in the Asp plasma level was found in the depressed mice treated with the Paroxetine in comparison with the depressed mice (P=0.034), and was close to the control group.

The plasma levels of arginine metabolism biomarkers

The Paroxetine administration to the normal mice led to a significant reduction in the Arg plasma level in comparison with the control group (P=0.005, Fig.2H). The Arg plasma level of the depressed group was also significantly lower than the control group (P=0.01). Also, we observed an increase of the Arg plasma level in the depressed group follow the Paroxetine treatment, and it reached around the control group level. The ornithine (Orn) plasma level was significantly reduced in the depression group in the absence (P=0.038, Fig.2I) and presence (P=0.028) of the Paroxetine administration in comparison with the control group. The citrulline (Cit) plasma level was significantly reduced in the Paroxetine (P=0.002) and depression (P=0.005) groups in comparison with the control group. A significant decrease in the Cit plasma level was detected in the depressed group after receiving the Paroxetine in comparison with the control group (P=0.005, Fig.2J).

The plasma levels of branched-chain amino acids

Our results showed no meaningful differences in the Valine (Val) plasma concentration among our groups (Fig.3A). The Leucine (Leu) plasma level of depressed mice was significantly higher after treatment with the Paroxetine in comparison with the depressed mice (P=0.024, Fig.3B). As shown in Figure 3C, the Paroxetine treatment in normal animals led to a significant increase in the Isoleucine (Ile) plasma levels in comparison with the control group (P=0.003), also a meaningful increase in the plasma concentration of Ile in the Paroxetine-treated depressed mice was observed in comparison with the depressed animals (P=0.011).

The plasma levels of Glycine, Serine, Threonine, and Histidine

As shown in the Figure 3D, a significant decrease in the Glycine (Gly) plasma level in the normal mice after receiving Paroxetine was observed in comparison with the control group (P=0.002). Depression led to a significant increase in the plasma Gly level in comparison with the control group (P=0.002). The Gly concentration was significantly reduced in the Paroxetine-treated depressed group in comparison with the depressed group (P=0.002), which was near to the control group. No meaningful differences in the Serine (Ser) plasma level were found among our groups (Fig.3E). The Threonine (Thr) plasma level was remarkably lower in the normal mice treated with the Paroxetine (P=0.036, Fig.3F) and also in the depressed mice (P=0.000) in comparison with the control group. A significant increase in Thr level was found in the depressed mice follow of receiving Paroxetine in comparison with the depressed group (P=0.003). No changes in the Histidine (His) plasma concentration were observed in the Paroxetine-treated normal animals in comparison with the control group (Fig.3G). The plasma concentration of His was significantly lower in the depressed mice than in the control group (P=0.012), and Paroxetine-treated depressed mice showed a significantly higher level in comparison with the depression group (P=0.000) and control group (P=0.003).

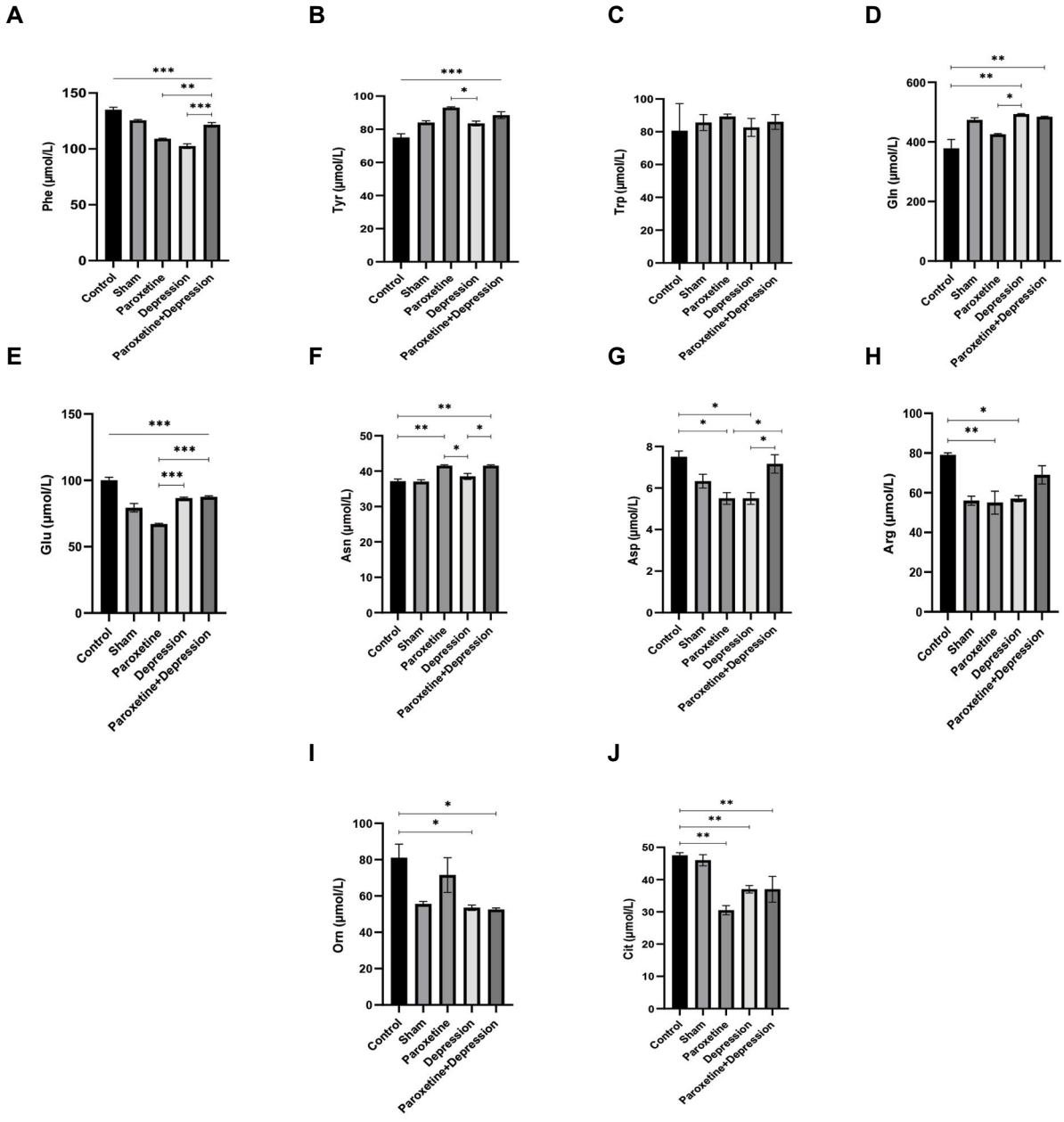

Fig.2: Plasma concentrations of aromatic, excitatory, and arginine metabolism amino acids after the treatment with Paroxetine in normal and depressed NMRI mice. **A.** Phenylalanine (Phe), **B.** Tyrosine (Tyr), **C.** Tryptophan (Trp), **D.** Glutamine (Gln), **E.** Glutamate (Glu), **F.** Asparagine (Asn), **G.** Aspartate (Asp), **H.** Arginine (Arg), **I.** Ornithine (Orn), and **J.** Citrulline (Cit). Each column represents the means ± standard error of the mean (SEM). *; Shows the significant difference between the two groups at P<0.05, **; Shows the significant difference between the two groups at P<0.01, and ***; Shows the significant difference between the two groups at P<0.001.

The plasma levels of Methionine, Lysine, and Alanine

As shown in Figure 3H and I, the concentrations of Met and Lysine (Lys) were not altered in our groups. Also, a significant reduction in the plasma Alanine (Ala) levels of the normal mice after receiving Paroxetine (P=0.002, Fig.3J) and the depression group (P=0.001) was observed in comparison with the control group. Our results also showed that the plasma Ala concentration was significantly higher in the depressed mice treated with Paroxetine in comparison with the depressed group (P=0.000), which was close to the control group.

Correlation between Homocysteine and different amino acids

Pearson's correlation was shown the plasma level of Hcy was inversely related with plasma level of Ser, Val, and Ile in the Paroxetine group, while having a statistically significant positive correlation with the plasma level of Phe, Tyr, Asn, and Lys in this group. The Hcy level showed no statistically significant positive or negative correlation with the same AAs in other experimental groups (Table 1).

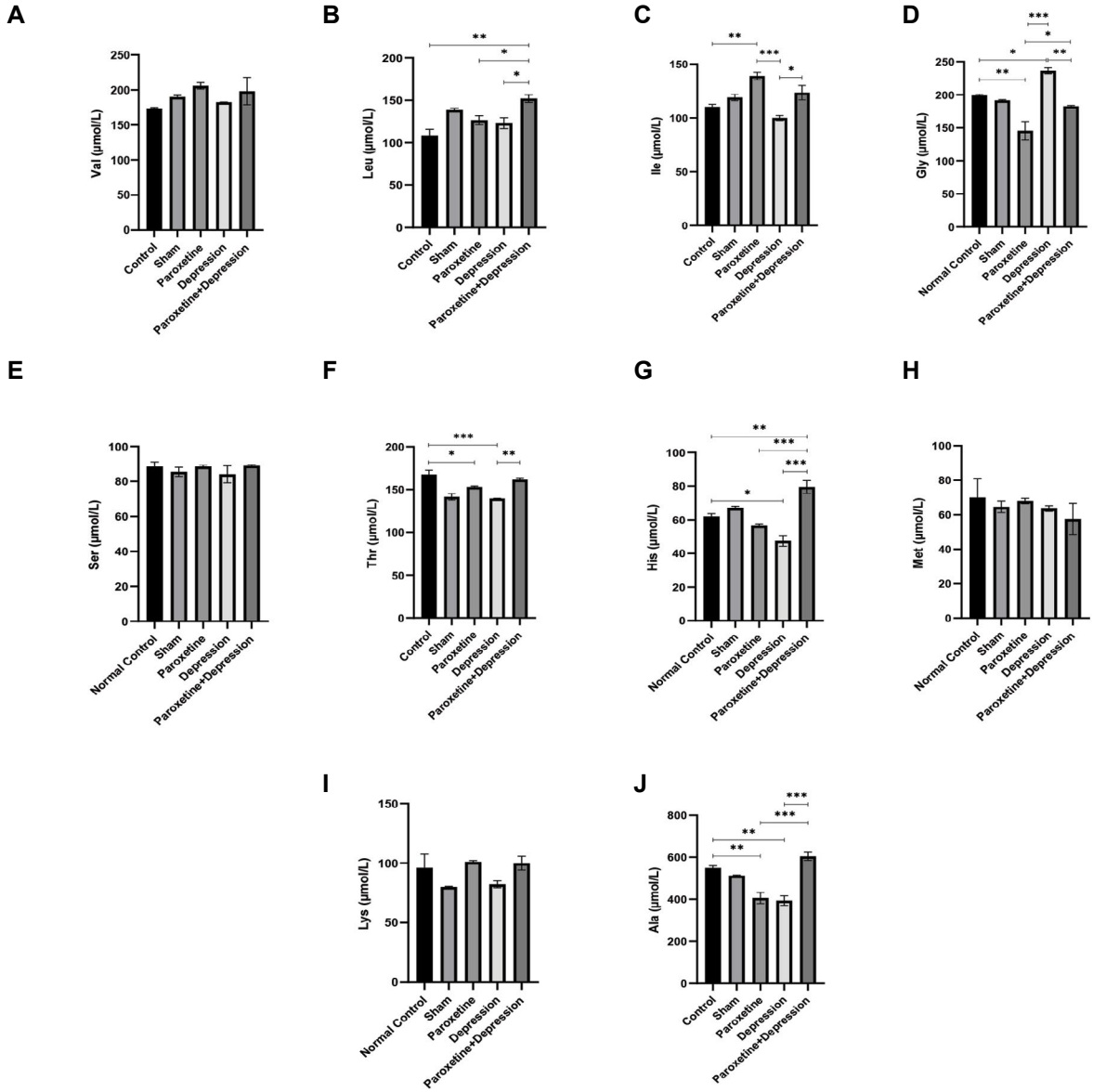

Fig.3: Plasma concentrations of branched-chain and other amino acids after the treatment with Paroxetine in normal and depressed NMRI mice. **A.** Valine (Val), **B.** Leucine (Lue), **C.** Isoleucine (Ile), **D.** Glycine (Gly), **E.** Serine (Ser), **F.** Threonine (Thr), **G.** Histidine (His), **H.** Methionine (Met), **I.** Lysine (Lys), and **J.** Alanine (Ala). Each column represents the means ± standard error of the mean (SEM). *; Shows the significant difference between the two groups at P<0.05, **; Shows the significant difference between the two groups at P<0.01, and ***; Shows the significant difference between the two groups at P<0.001.

Table 1: Pearson correlation coefficients between Hcy and seven amino acids in different experimental groups

Groups		Amino acids						
		Phe	**Tyr**	**Asn**	**Ser**	**Val**	**Ile**	**Lys**
Control	Hcy	-0.189	-0.189	-0.454	0.189	-0.189	-0.888	-0.189
Sham	Hcy	1.000	-0.500	0.500	0.500	-0.500	1.000	-1.000
Paroxetine	Hcy	0.999*	0.999*	0.999*	-0.999*	-0.999*	-0.999*	0.999*
Depression	Hcy	0.545	-0.454	-0.999	-0.877	0.545	0.454	-0.840
Paroxetine+depression	Hcy	-0.500	1.000	-0.500	0.500	0.773	-0.500	0.500

*; Correlation is significant at P<0.05, negative values indicate the opposite correlation. Phe; Phenylalanine, Tyr; Tyrosine, Asn; Asparagine, Ser; Serine, Val; Valine, Ile; Isoleucine, Lys; Lysine, and Hcy; Homocysteine.

To better understand the changes induced by the Paroxetine treatment in the amino acid metabolism, one carbon, urea, and NO cycles in normal and depression conditions, we summarized these pathways in a brief plot (Fig.4).

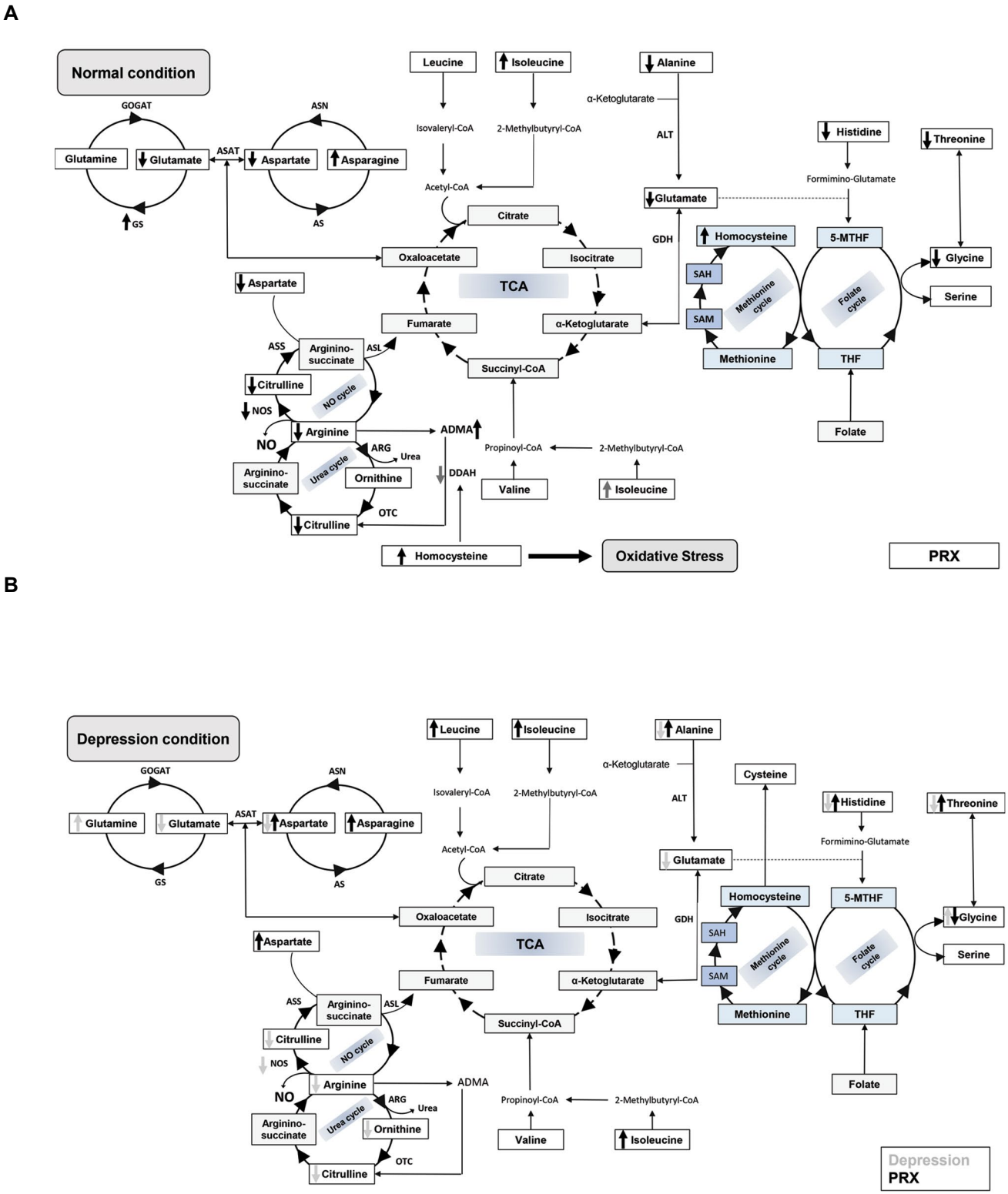

Fig.4: Schematic representation of the changes induced by the Paroxetine (PRX) treatment in the amino acid metabolism, one carbon, urea, and NO cycles in **A.** Normal and **B.** Depression conditions. AS; Asparagine synthetase, ASN; Asparaginase, ASAT; Aspartate aminotransferase, GS; Glutamine synthetase, GOGAT; Glutamate synthase, GDH; Glutamate dehydrogenase, TCA cycle; Tricarboxylic acid cycle, ARG; Arginase, OTC; Ornithine transcarbamoylase, NO; Nitric oxide, NOS; Nitric oxide synthase, ASS; Argininosuccinate synthase, ASL; Argininosuccinate lyase, DDAH; Dimethylarginine dimethylaminohydrolase, ADMA; Asymmetrical dimethylarginine, SAM; S-adenosylmethionine, SAH; S-adenosylhomocysteine, THF; Tetrahydrofolate, and ALT; Alanine aminotransferase.

Discussion

Forced swim-induced depression results in biochemical alterations and is used as a stressor by itself. Therefore, metabolomics studies are a new field of investigations for the identifications of molecular changes in the depression states and after treatment with antidepressants. This is, to our knowledge, the first study identifying metabolite changes in acutely Paroxetine treatment in a forced swim stress-induced depressive-like phenotype in NMRI male mice.

The FST is a behavioral test that used as a rodent model for divining the clinical efficiency of antidepressants. Immobility in the FST has been recommended to represent "behavioral despair," which occurs after recognizing escape is impossible. Consistent with the our findings in NMRI mice, Paroxetine did not influence immobility time in the FST in DBA/2J, DBA/2N, and BALB/c mice (15). The Immobility duration depends on the 5-HT transporter binding levels, which leads to strain variations in immobility time in the FST. Moreover, variations in 5-HT transporter binding may originate differences in the SSRIs' effects on behaviors (16).

In this study, 1-CC biomarkers examination revealed that folate in depressed mice was maintained at the control level, while Carboni et al. (17) reported a high incidence of folate deficiency in patients with depression. Although, different results were obtained depending on the criteria used to describe a folate deficiency. Given that, depression is often accompanied by a decreased appetite and dietary folate intake (18). In the our study, there was no significant alteration in the plasma folate level in the Paroxetine-treated groups, our results were in line with a previous report that no alteration was found in the serum folate level in the Paroxetine treated patients (17).

Our findings indicate that the alteration of plasma vitamin B12 in the depressed group was not significant. Given that present evidence shows an association between vitamin B12 deficiencies and depression. However, it is not necessarily assumed that these results will transfer to all populations, because of the variances in nutrient consumptions (19). Also, the effect of the Paroxetine treatment on the vitamin B12 level in the normal and depressed animals was statistically significant in comparison with the control group, but vitamin B12 level was still in the normal range (normal: > 300 pg/mL) in different study groups. Our results are consistent with the results of Yurdakul et al. that antidepressant drugs like Paroxetine have not adverse effect on serum folate and vitamin B12 levels of patients with major depression (20).

Normal level of Hcy is preserved by the Hcy remethylation that leads to the Met production, while the related enzymes need coenzymes including folic acid and vitamin B12. And also, Hcy catabolism to cysteine occurs with a vitamin B6-dependent enzyme. Elevated Hcy is due to uncommon genetic defects in the metabolism of Met, folate, or vitamin B12, nutritional deficiencies of folate or vitamin B12, or mutations in the gene for MTHFR (21). In the present study, Hcy plasma level significantly increased in the normal animals after treatment with Paroxetine. The Hcy concentration showed a correlation with the levels of seven AAs (Phe, Tyr, Ser, Asn, Val, Ile, and Lys) in the Paroxetine group against the control group. In comparison with the control group, the Paroxetine group showed a lower concentration of Phe, and a high level of Tyr, Asn, and Ile, although there was no difference in the concentrations of Ser, Val, and Lys between the Paroxetine and control groups. Thus, it is suggested that altered these AAs could play a role in the Hcy level increase in the normal animals after Paroxetine administration. The increase in the Hcy level may induce oxidative stress by upregulating protease-activated receptors 4 (PAR-4) and stimulating reactive oxygen species (ROS) generation by increasing NADPH oxidase and diminishing thioredoxin (22), therefore, Paroxetine can lead to the Hcy-induced oxidative stress.

The aromatic AAs (AAA), Phe, Tyr, and Trp, are thought to play a significant role in the pathogenesis of depression (23). According to Islam et al. (8), the serum level of Phe, Tyr, and Trp significantly decrease in the patients with major depressive disorder (MDD). The lower Phe plasma level in the depressed mice observed here is in line with the results from the study by the Ogawa et al. (9). In this study, Paroxetine-treated groups had a significant reduction of the Phe plasma level. This finding is inconsistent with a previous study reported no significant alteration in the Phe plasma level in depressed patients after the antidepressant treatment (24). Since patients with MDD often display hypercortisolemia, thus decreased the Phe plasma level may partially be caused by a high glucocorticoid level, which may be associated with the decrease in the catecholamines in MDD (9).

In this study, the plasma levels of Tyr were increased in depressed mice which is consistent with a previous study (25), but not with the study of Ogawa et al. (9). We also observed an increase of the Tyr plasma level in the normal and depressed mice treated with the Paroxetine , which is inconsistent with the results of Woo et al. (24). The Trp is metabolically converted to metabolites, including serotonin, melatonin, kynurenine, and nicotinamide. Our results revealed no significant difference between study groups for the Trp plasma level and is in agreement with a previous study that observed no difference in the serum levels of Trp and Trp/competing AA ratio between the MDD and control groups (19). Besides, consistent with a previous study (24), Paroxetine did not affect the plasma concentration of Trp in Paroxetine-treated groups.

Our results propose that AAs associated with the glutamatergic pathway are affected by depression states and Paroxetine treatment. The results of our study are consistent with a previous study reporting a significant increased Gln plasma level in the depressed patients. The brain neuron survival may in part be dependent upon Gln plasma level, thus, the increased Gln plasma level may serve as a compensatory response against a likelihood of neurotoxicity in the hippocampus of patients with depression (7). However, other studies have reported no

significant alteration in the Gln plasma level (24). The higher Gln plasma level observed in the after treatment with Paroxetine in depressed mice is in line with the results from the study by Park et al. (12) which reported that Paroxetine enhanced protein expression of glutamine synthetase (GS) both in the Paroxetine-treated long-time floating (PLF) and Paroxetine-treated short-time floating (PSF) mice, that can play a role in increased plasma levels of Gln.

Our results of declining plasma levels of Glu in depressed mice are inconsistent with previous reports (9, 24) while there is a study that reported no significant differences (8). Also, previous study reporting that Glu release decreases with antidepressant treatment (26). Thus, based on the formed abnormalities in the Gln-Glu pathway, decreased Glu plasma levels are not unexpected. Our results of increased the plasma concentration of Asn in Paroxetine-treated groups are inconsistent with a previous report (24). The lower Asp plasma level observed in the depressed mice is in line with Pinto et al. (25) results. Asp plays a significant role either in the energy production of the cell which is an acquisition popularity in the controlling of the Krebs cycle where AAs and biochemicals are produced. Hence, Yang et al. (27) showed that an Asp plasma alteration level is important to the energy variation which is directly related to depression based on the statistics from Human Metabolome Database suggested that Asp might have a tight connection to depression. Also, in depressed mice with Paroxetine treatment, the Asp plasma level was reduced but not significant, while could reduce the Asp plasma level in the normal group. According to a previous study (28), the plasma level of Glu and Asp were reduced in the SSRI treated rats . The results recommend that under conditions like a disturbed homeostasis of a neuronal network, SSRIs may influence excitatory systems in the brain.

A significant reduction of the Arg plasma level in the depressed animals is consistent with Ali-Sisto et al. (29) study that reported its association with MDD. However, Islam et al. (8) observed no significant alterations in the plasma Arg level. Alterations in the levels of Arg and its related catabolic products, including Orn, Cit, and argininosuccinate, might have contributed to abnormal nitric oxide (NO) and urea cycles and can play a role in the MDD pathogenesis (30). Our findings showed a significant decrease in the plasma Orn and Cit levels in the depressed mice, while some studies have reported no significant alterations in the plasma Orn (9, 24) and Cit (24) levels of MDD patients. In line with our findings, it was observed lower plasma level of Cit in MDD patients in comparison with the healthy control group. They suggested that endothelial NO synthase (eNOS) activity level in the depression is low (31). It seems A decrease in the plasma Arg bioavailability in the MDD (29) combination with a decrease production of NO causes a nitrosohomocysteine formation reduction and leads to an increase in the plasma Hcy level (32). Therefore, hyperhomocysteinemia indirectly declines eNOS expression in mRNA and protein levels, via reducing the expression of dimethylarginine dimethylaminohydrolase (DDAH), that leads to asymmetric dimethylarginine (ADMA) accumulation and directly induces ROS formation and oxidative stress, and contributes in the NO bioavailability and Cit level decrease (22).

We also observed changes in the levels of Arg and its related catabolic products in the Paroxetine-treated groups that are in conflict with Woo et al. (24) report, while the reduced plasma Orn and Cit levels in the Paroxetine-treated groups are in line with MahmoudianDehkordi et al. study (33). Clinical trials have shown that antidepressant drugs, including Ketamine, participated in the increase of circulating level of Arg and Cit, and no differences were observed between Arg and Cit after treatment with Ketamine in MDD patients compared with healthy control (34). While Paroxetine was introduced as a new and strong NOS enzyme inhibitor in the *in vitro* (isolated hamster papillary muscles) and *in vivo* (depressed patients) (35). Therefore, the decrease in the plasma Cit level in the depressed mice after treatment with Paroxetine could be the result of NOS activity inhibition. Interestingly, the use of Paroxetine in normal mice also could reduce the plasma Arg and Cit levels, which can be a cause of increased Hcy levels in the Paroxetine group in the present study.

The branched-chain AAs (BCAAs), including Val, Leu, and Ile, might act a critical role in the depression development via activation of the mammalian target of the rapamycin (mTOR) pathway (36). Here, we found that the Val plasma level was not affected through depression, and also the Paroxetine led to a non-significant increase in the Val plasma concentration, these results are in agreement with previous reports of Val in MDD patients without and with SSRIs treatment (9, 24, 25). The results of our study are in agreement with the Pinto et al. (25) study, both research teams observed no significant difference in the plasma Leu and Ile levels in the depressed condition in comparison with the control group. Our study showed that the higher Lue and Ile levels in the normal and depressed mice after treatment with Paroxetine are in line with Webhofer et al. (37), who revealed that the concentrations of Lue and Ile enhanced by 50-70% in mice after Paroxetine treatment.

The finding of this study was shown that the Gly plasma level was significantly enhanced in the depressed mice. This is in contrast to previous study indicating either no significant difference (8). In the depressed mice after the Paroxetine treatment, the Gly plasma level was preserved at the control level and this finding is in agreement with a previous study (24), it is shown there is no difference in the plasma Gly level in depressed patients after treatment with SSRIs. About Ser, our results are consistent with other studies (7, 24) and there were no significant alterations in the plasma Ser level between different study groups. Therefore, when the size of the Met and Ser pool is constant (38), the enzyme activity of β-homocysteine methyltransferase and 5-methyltetrahydrofolate homocysteine methyltransferase greater than that of the cystathionine-β-synthase (CBS),

which can cause interrupts in 1-CC metabolism, specially Met metabolism, by increasing SAM formation and decrease cystathionine formation that lead to methionine-induced hyperhomocysteinemia. The findings of a study recommend that depression and SSRIs utilization may disturb the Gly-Ser metabolism pathways (39). Therefore, from this study, it can be stated that higher plasma level of the Hcy in the normal animals with Paroxetine treatment may result from these disorders.

Moreover, the depressed mice in this study showed a significant decrease in the Thr plasma level, however previous studies reported no significant alterations in Thr level in the depression (7, 9, 24). According to findings of Maes et al. (39), the Thr plasma concentration in the depressed mice after Paroxetine treatment was increased and preserved at the control levels. But Park et al. (40) reported that levels of choline and Thr were altered both in the PLF and PSF groups after treatment with the Paroxetine (5 mg/kg/day). The Threonine can be converted to the Glycine through an aldol cleavage, therefore, the lower level of Thr in the normal mice after treatment with the Paroxetine may be associated with the low Gly plasma level in this group. Our results about plasma His level reduction, an essential brain functional AA, in the depressed mice are supported by Ogawa et al. (9) findings. Although, the higher His level observed in the depressed group after treatment with the Paroxetine is not in line with Woo et al. (24) findings. While, the Histidine is catabolized to provide the formimino group, which is accepted by tetrahydrofolate and converted to different forms of one-carbon unit, such as a 5-CH3-tetrahydrofolate, it is possible that its low level in a depression situation by reducing 5-CH3-tetrahydrofolate-dependent remethylation of Hcy can lead to enhancing the plasma Hcy level (32).

Met is continuously regenerated from the Hcy via one-carbon metabolism, and is the essential precursor of SAM, that play an important role in depression etiology. Our results are in disagreement with those of Ogawa et al. (9), who reported a decrease Met plasma level in MDD patients in comparison with the normal controls, but it is in accordance with the Woo et al. (24) study. Our result about Lys is consistent with previous studies (24, 25). The present study showed a lower Ala plasma level in depressed mice, inconsistent with some studies (24, 25). Also, the Paroxetine treatment increased the Ala plasma level in the depressed mice. Maes et al. (39) suggested that plasma levels of Ala and Asn were increased follow of the Trazodone treatment , while there is not same findings follow of co-treatment with the Trazodone and Fluoxetine or Pindolol .Therefore ,based on the conflicting reports provided ,further studies on alterations in the metabolite homeostasis in depression and assessment of response to treatment with antidepressants are suggested.

Conclusion

This study demonstrates that the oral administration of Paroxetine as a SSRI during the stress-induced depression in the male mice leads to perturbation of AAs profiles especially excitatory AAs, arginine and its catabolic products, and other proteinogenic AAs, which perform an important role in the preservation of tricarboxylic acid (TAC) cycle hemostasis and metabolism of energy, and also use of antidepressants can alter metabolites hemostasis. This is important that the use of Paroxetine in normal animals also could impair the homeostasis of metabolites like homocysteine, and by increasing plasma levels of Hcy may lead to oxidative stress. These results and similar information reveal the potential of the study of metabolites for understanding the mechanisms of depression and have the ability to estimate therapeutic responses in normal and depressed subjects.

Acknowledgments

This research was financially supported by Royan Institute for Biotechnology, Isfahan, Iran. The authors thank Dr. Marziyeh Tavalaee for scientific collaboration in the conception and design of study, and also Dr. Parviz Gharagozloo for drug gift (Paroxetine, CellOxess company). There is no conflict of interest in this study.

Authors' Contributions

M.H.N.-E.; Conceptualization, Supervision, and Validation. R.A., M.H.N.-E.; Data curation. M.H.N.-E., M.A., N.N., S.A.; Formal analysis. R.A., N.S., N.N.; Investigation. R.A., N.S.; Methodology. M.H.N.-E., M.A., N.N.; Writing–original draft. M.H.N.-E., S.A.; Writing–review and editing. All authors read and approved the final manuscript.

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