ISSN 0006-2979, Biochemistry (Moscow), 2023, Vol. 88, No. 8, pp. 1181-1190 © Pleiades Publishing, Ltd., 2023.

REVIEW

Biochemical Mechanisms of Beneficial Effects of Beta-Alanine Supplements on Cognition

Gholam Hossein Meftahi[1,a]* and Gila Pirzad Jahromi[1]

[1] *Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

[a] *e-mail: hossein.meftahi@bmsu.ac.ir; meftahi208@yahoo.com*

Received March 12, 2023
Revised May 20, 2023
Accepted May 23, 2023

Abstract—Using nutritional interventions to cure and manage psychiatric disorders is a promising tool. In this regard, accumulating documents support strong relationships between the diet and brain health throughout the lifespan. Evidence from animal and human studies demonstrated that β-alanine (Beta-alanine; BA), a natural amino acid, provides several benefits in fight against cognitive decline promoting mental health. This review summarizes and reports state-of-the-art evidence on how BA affects cognitive health and argues existence of potential unrevealed biochemical mechanisms and signaling cascades. There is a growing body of evidence showing that BA supplement has a significant role in mental health mediating increase of the cell carnosine and brain-derived neurotrophic factor (BDNF) content. BDNF is one of the most studied neurotrophins in the mammalian brain, which activates several downstream functional cascades via the tropomyosin-related kinase receptor type B (TrkB). Activation of TrkB induces diverse processes, such as programmed cell death and neuronal viability, dendritic branching growth, dendritic spine formation and stabilization, synaptic development, cognitive-related processes, and synaptic plasticity. Carnosine exerts its main effect via its antioxidant properties. This critical antioxidant also scavenges hypochlorous acid (HOCl), another toxic species produced in mammalian cells. Carnosine regulates transcription of hundreds of genes related to antioxidant mechanisms by increasing expression of the nuclear erythroid 2-related factor 2 (Nrf2) and translocating Nrf2 to the nucleus. Another major protective effect of carnosine on the central nervous system (CNS) is related to its anti-glycating, anti-aggregate activities, anti-inflammatory, metal ion chelator activity, and regulation of pro-inflammatory cytokine secretion. These effects could be associated with the carnosine ability to form complexes with metal ions, particularly with zinc (Zn^{2+}). Thus, it seems that BA via BDNF and carnosine mechanisms may improve brain health and cognitive function over the entire human lifespan.

DOI: 10.1134/S0006297923080114

Keywords: β-alanine, cognition, nutritional supplement, BDNF, carnosine, brain health

INTRODUCTION

Currently, nutritional supplements are widely used in population; for example, in the USA, it is estimated that over half of adults take some form of nutritional supplements. Such products as polyunsaturated fatty acids, vitamins, minerals, antioxidants, and amino acids are not only essential for physiological functioning but also have significant effects on body composition, behavior, and mental well-being [1]. In addition, mounting pieces of evidence provide support for the existence of direct relationships between the nutrition and mental functions throughout the lifespan. Improvement of understanding of how nutritional interventions promote and maintain brain fitness could help us to improve wellness management abilities and reduce economic burden associated with brain disorders and cognitive impairment [2].

A non-essential amino acid, β-alanine (beta-alanine) (BA) is synthesized in the liver and it also can be received through the diet, particularly by consuming

Abbreviations: ANAM, automated neuropsychological assessment metrics; BA, Beta-alanine; BDNF, brain derived neurotrophic factor; CNS, central nervous system; NPY, neuropeptide Y; Nrf2, Nuclear factor erythroid 2-related factor 2; PSS, predator-scent stress; SST, serial sevens test; TrkB, tropomyosin-related kinase receptor type B.

* To whom correspondence should be addressed.

poultry, fish, and red meat [3]. This amino acid supplement is supposed to be one of the most favored supplements being used by professional athletes to boost sports performance, but apparently, it does not provide any ergogenic aid by itself. The main goal of BA intake is to elevate carnosine content in skeletal muscle cells through enhancing intracellular buffering magnitude, which enables muscles to contract to a greater extent in anaerobic exercises [4]. In addition, the results of animal and human studies indicate that the elevated carnosine content has additional physiological benefits to the central nervous system (CNS) [5]. Accordingly, in this review, we aim to examine neurological outcomes of BA supplementation and discuss whether it is useful for mental health under stressful situation. We do not present any new data or notions but rather summarize and critically discuss the latest reports in this area and point out related gaps. For this goal, publications in PubMed, Scopus, Web of Science, and Google Scholar were searched without time period limits. The keywords included β-alanine, carnosine, brain-derived neurotrophic factor (BDNF), cognitive function, mental disorders, brain performance, amino acid supplements and dietary interventions in both clinical and preclinical data.

HUMAN STUDIES

Effects of BA intake on human CNS and cognitive function have previously been reported by several researchers. They have shown that the supplements containing this nonessential amino acid may improve emotional, non-cognitive, and cognitive brain functions such as executive functioning, decision-making, short-term memory, and reaction time [6, 7]. Most human studies investigating BA-supplemented diet for the purpose of examination of cognition were carried out after physical exertion, during periods of intense training, or in elderly subjects [7, 8]. For example, Hoffman et al. [8] examined the effect of four weeks of BA uptake (2 g per serving) and three meals per day (total BA intake was 6 g per day) on the cognitive performance of military personnel while fatigued in the advanced military drills. They employed a revised version of the original serial sevens test (SST) to examine cognitive performance. This test is comprised of a two-minute mathematical test in which participants should subtract the number seven from a randomly generated four-digit number, supposed to calculate how rapidly and accurately they can discover an answer to an easy math problem. The authors observed that BA ingestion during twenty-eight days of intensive military training was useful for improving psychomotor behaviors (shooting accuracy) in soldiers, but it was not effective to improve cognition [8]. This finding did not give any evidence in support of the role of BA in enhancing cognition in fatigued soldiers. It is to be expected that the serial subtraction test (SST) performed under normal conditions was not sufficient to establish potential BA effects, in contrast, the subjects who used BA supplements following fatiguing activity show enhanced cognitive performance. Notably, it was shown in the study by Hoffman et al. that although 4 weeks of BA supplementation significantly improved cognitive performance (2-min SST), no difference between the groups was observed in the brain carnosine contents, when compared to those soldiers who were treated with placebo [9]. Although this finding contradicts the previous reports, these differences may be due to how the serial subtraction test was performed. In the former study, subjects were in a quiet location [8] while in the latter one Hoffman et al. performed the test under conditions of continuous noise of firing range, which may have contributed to high level of anxiety in the participants [6].

Solis and colleagues hypothesized that the diets containing insufficient BA such as vegetarian diets may lead to the low and diminishing brain carnosine levels [10]. Hence, they examined four weeks of BA supplementation on brain carnosine content, which was evaluated using proton magnetic resonance spectroscopy (1H-MRS) method in vegans. In another part, they examined the brain carnosine levels (baseline and after an exhaustive exercise) in nineteen trained male cyclists. The trained cyclists undertook four (two pre and two post) BA supplements with multiple cognitive evaluations (Stroop test, Sternberg paradigm, Rapid Visual Information Processing task) being performed before and after exercise on each point. Their findings demonstrated that there was not any significant effect of BA intake on the brain carnosine concentration and cognitive function in both non-vegetarians and vegetarians and in the trained cyclists before or after exercise. Despite the rising numbers of research suggesting that carnosine administration strategies may improve mental health in a variety of populations, they did not confirm benefits of BA supplementation on the behavioral capacities either at rest or post-exercise [10]. It is worth reminding that other parameters such as participant characteristics (e.g. age, health status, and exercise training experience), cognitive tests performed (types of memory, attention, reaction time, etc.), presence of other diseases, which may affect brain function (e.g., neural and psychiatric disorders), the type and different protocols of supplementation (e.g., BA, carnosine, acute and chronic ingestion) could also partly explain conflicting results reported in these investigations.

Furst et al. further investigated the effect of BA supplementation on the executive functioning in a middle-aged human population [4]. They hypothesized that BA supplementation can enhance executive function. In their study, executive functions were examined prior to and after each time-to-exhaustion test by the way of the Stroop test, which was designed to assess working memory.

The subjects included eight men and four women over 50 years old, who were instructed to take three capsules of BA per day for 28 days. The results show that immediately after the exercise session performance in the Stroop test declined in the control group, which was accompanied by the increased time to accomplish the task. The authors observed that BA supplementation eliminated decrease in executive functioning after endurance exercise, as reported in the literature [4]. However, this result appears to be in contradiction with the Hoffman et al. report [6]. Additionally, Solis et al. noticed that the BA intake provided no benefits in the Stroop test performance [10]. Furst and coworkers concluded that this discrepancy may be due to the different physical conditioning level of subjects, which make it difficult to compare the results with the literature findings [4].

Varanoske and colleagues [11] investigated effects of the BA-supplemented diet on cognitive performance during a day of the simulated military operation. They instructed each subject to consume supplements with food and water three times per day (12 g/day) for 2 weeks. Their results showed that BA supplement potentially may have numerous benefits for cognition, considering that subjects consuming BA had better visual reaction time (RT) and lower mistakes during RT testing [11]. These findings were comparable to the previous studies, which reported increasing number of the correct responses in the SST [12], and improving marksmanship while subjects were fatigued [8]. These results imply that BA intake may be beneficial for improving cognitive performance exerted under stressful situations, rather than on the routine tasks. Besides the advantageous properties of the BA-supplemented diet on cognition, it appears that consuming BA does not impact mood patterns, feelings of soreness, and fatigue as compared to the control subjects [11].

Wells and colleagues [13] examined BA supplementation on cognition in the subjects exposed to sustained military operations (SUSOPs), which consisted of several stressors, including continuous physical training, caloric deficit, and sleep deprivation for a day. In addition, soldiers were instructed to take BA (12 grams/3 servings a day) daily with their regular meals for 2 weeks. Cognitive behaviors were assessed by the Automated Neuropsychological Assessment Metrics (ANAM) software, and psychological stress was assessed using the CSI at the start, middle, and end of the SUSOPs. The CSI consisted of 12 symptoms (including headache, dizziness, nausea, fatigue, and balance problems) scored on a seven-point Likert-type scale from zero (non-existent) to six (intense). The authors reported a significant increase in the frequency and severity of signs of psychological stress in the soldiers during the SUSOP, while 14 days of BA supplementation prior to SUSOP did not decrease the degree of psychological stress or cognitive impairment significantly [13]. They conclude that although preliminary examination showed that BA supplementation during stressful conditions may offer some benefits to cognition, whether this could be linked to the increased carnosine content remains unknown. Varanoske et al. [7] further examined effects of BA supplementation on cognitive evaluations in males during simulated military operational stress. In this survey, multiple methods and equipment were used for cognitive evaluation such as SST, ANAM, visuomotor training, and multiple object-tracking devices. These authors indicated that the high-dose BA supplementation (two weeks/12 g per day) before a simulated military operation does not influence cognition, but has some benefits for mood states in the recreationally active young subjects [7]. These findings indicate that BA supplementation may counteract mood disruption during stressful moments, but could not improve mood states and cognitive function greater than the baseline levels in healthy people. It appears that BA consumption provides to the subject an improved ability to cope with stressful situations probably by lessening bad mood states, rather than by increasing baseline mood states. In addition, BA intake may counteract negative mood states connected with stress, even in the healthy and mentally fit subjects. These authors, in agreement with Furst et al. [4] and Solis et al. [10], did not notice any improvement in the rest-state cognitive performance after BA supplementation. Similarly, Solis and colleagues did not report any significant changes in the cognitive function at rest following use of the BA-supplemented diet by the elite cyclists. However, these authors observed that cognitive function after endurance exercise did not decrease significantly [10]. It must be noted that the prolonged physical activity such as endurance cycling represses cognitive functions.

Together, these findings recommend that the BA supplementation could be favorable for developing mental function and behaviors specifically when it is used in the subjects under physiological stress, elevated oxidative stress, and inflammation. A key point of consideration is that BA significantly improved cognitive function after incorporating protocols that induced significant increase in oxidative stress and inflammation such as military operations. On the other hand, the rest of executive functioning was not different among the BA-supplemented individuals.

ANIMAL STUDIES

Several researchers indicate that BA supplementation in rodents increases carnosine content within various brain segments, and this elevation may be associated with the enhanced learning and reduced symptoms of anxiety [14, 15]. For instance, Murakami and Furuse examined whether chronic intake of BA supplements

attenuates mental disorders under stressful conditions [16]. In their study, a BA-containing diet decreased accumulation of 5-hydroxyindoleacetic acid (5-HIAA) in hypothalamus [16]. It is believed that 5-HIAA is a metabolized form of 5-HT, which is a marker of anxiety in animal models [17]. In the Murakami survey, the BA-supplemented mice show significant increase in the percentage of time spent and entries in the open arms in the elevated plus maze test. This result led authors to suggest that chronic intake of BA could have anti-anxiety effects in mice [16].

Hoffman et al. examined effects of BA supplementation on the behavioral responses, which consisted of the elevated plus maze, acoustic startle response, and contextual freezing in the animal model of post-traumatic stress disorder (PTSD) [6]. The authors observed that 30 days of BA consumption attenuates effectively some stress-related behaviors associated with exposure to predator-scent stress (PSS). They also reported that the subjects fed a normal diet and subjected to PSS were significantly less active, when assessed by the elevated maze test, and also, they had a considerable anxiety level, when compared to the supplemented control animals. However, the BA-supplemented diet was ineffective to reduce all of the misbehaviors related to stress exposure. Animals subjected to PSS had significantly higher freezing and startle responses, and lesser startle habituation than the control animals, no matter whether they had BA supplements or not. Noteworthy, rats that were exposed to PSS plus supplemented with BA showed reduced startle (19%) and freezing (15%) responses than the exposed and not supplemented subjects [6].

Oxidative stress and inflammation in the brain have been suggested to contribute to cognitive disorder and neurodegeneration [18, 19]. In addition, neuropeptide Y (NPY) is another candidate for the possible mechanism of BA action in the brain. NPY is associated with learning and memory [20], and provides neuroprotection and neurotrophic effects in various brain parts [21]. Hoffman et al. indicated that four weeks of BA intake after exposure to a low-pressure blast wave (an effective model that simulates mild traumatic brain injuries in animals) were effective in bringing down the rates of mTBI-like responses and increasing resiliency of the subjects [22]. In addition, it appeared that animals supplemented with BA had a reduced inflammatory response regardless of whether the BA intervention was not capable to strengthen the NPY expression [22].

Hoffman and colleagues investigated effects of the 30-days of BA supplementation on the anxiety of young (120 days old) and older (420 days old) male Sprague–Dawley rats [21]. The anxiety-related behavioral assessments consisted of the Morris water maze (evaluates spatial learning and memory), the Elevated plus maze (assesses anxiety index), and the Acoustic startle response (determines percentage habituation). These authors observed that chronic BA intake in both young and older rats effectively attenuates anxiety and increases spatial learning, when compared to the control old and young rats [21]. They also indicated that the young rats in comparison with the older rats had higher capacity for learning and memory, when they were supplemented with BA. It appears that BA supplementation provides ergogenic benefits such as enhancing spatial learning and memory just only for the young subjects. Regardless of age, the rats supplemented with BA had a significantly reduced anxiety index than the control ones, but it did not influence inflammatory markers. Most likely the mechanisms that regulate memory, learning, and anxiety are independent on inflammatory responses [21].

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

One potential mechanism that can explain the effect of BA supplementation on the brain health is overexpression of BDNF [7]. BDNF is a regulatory factor that has been extensively studied in the mammalian brain. This neurotrophin mediates differentiation and survival of neurons, and it serves as an important regulator of synaptic plasticity and neuronal survival [23]. Although it is not clear how BA supplementation enhances the brain BDNF content, there is a positive correlation between the cortex and hypothalamus carnosine content and BDNF expression following daily BA supplementation in animal subjects [24]. For instance, Murakami and Furuse showed that chronic intake of BA significantly increases BDNF amount in the hippocampus in comparison with the control group [16]. They observed that this elevation was related to the reduction of the depression and anxiety signs in the physically stressed mice [16]. In addition, Hoffman et al. demonstrated that although consuming BA and followed brain carnosine synthesis did not raise the BDNF expression in non-stressed rats, it apparently preserved BDNF expression in those animals subjected to PSS [6]. The authors concluded that the enhanced brain carnosine levels were not directly responsible for alteration of the BDNF expression [6]. Furthermore, another investigation indicated that the animals exposed to a low-pressure blast wave plus had a 30-day BA supplementation appeared to have a higher BDNF level in the specific segment of hippocampus compared to the rats that were exposed to the stressor, but did not consume the BA-supplemented diet [22]. In another study, Hoffman and colleagues examined effects of BA supplementation on the emotional responses and expression of BDNF, NPY, and inflammation markers in the young and older male rats [21, 22]. They observed that one month of BA intake was efficient in decreasing anxiety and enhancing BDNF expression in the hippocampus of both young and older

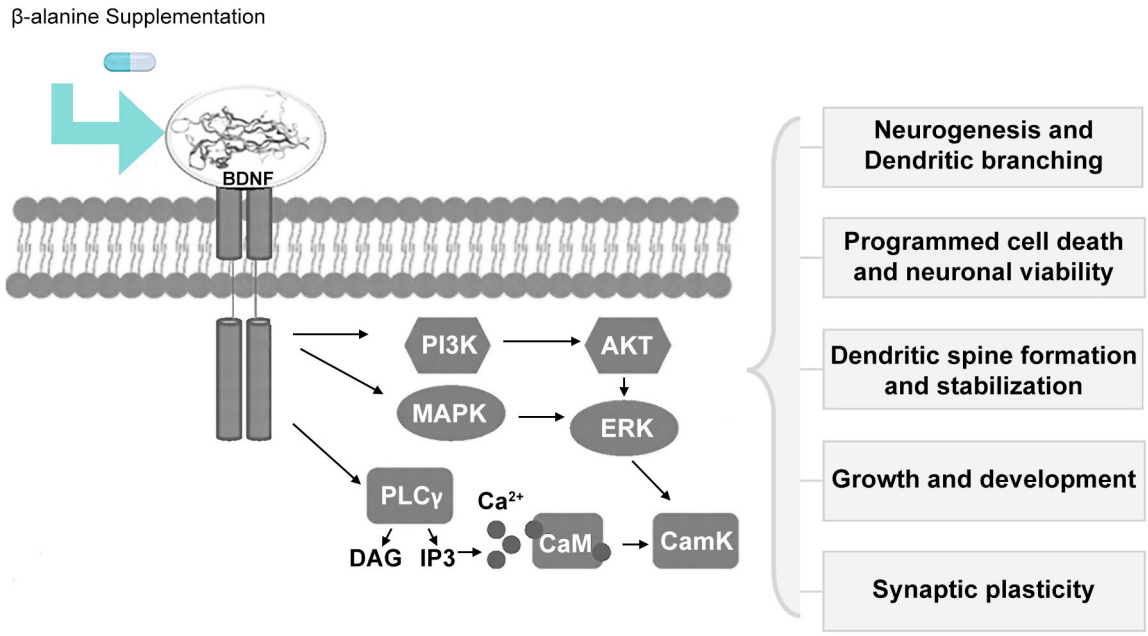

Fig. 1. Overview of the mechanism of action of beta-alanine supplement enhancing brain function via the BDNF mediated processes. BDNF binds to its receptors and activates several downstream cascades. Multiples enzymes were activated by TrkB phosphorylation such as phosphoinositide 3-kinases (PI3K)-AKT (PI3K-AKT), Ras-mitogen-activated protein kinase (Ras-MAPK), which activates ERK 1/2, and CREB cascades, and, finally, phospholipase C-γ (PLC-γ), which leads to activation of protein kinase C (PKC). These enzymes induce diverse processes, such as programmed cell death and neuronal viability, dendritic branching growth, dendritic spine formation and stabilization, synaptic development, and synaptic plasticity [23].

rats [21, 22]. In addition, the young rats that were fed with BA had significantly greater spatial learning ability and enhanced BDNF expressions than the control rats independent of their age [25]. Also, Belity et al. showed that the acute heat stress caused a significant decrease in the number of BDNF-ir cells [15]. On the other hand, they showed that the BA-supplemented diet in the exposed rats maintained BDNF levels in the CA3 and paraventricular hypothalamic nucleus (PVN) subregions [15]. They suggested that the maintained BDNF-ir cell numbers in the CNS could provide a degree of neuroprotection against heat stress. These findings were in agreement with other reports, which demonstrated that the BA treatment in young rats can preserve BDNF levels in the animals subjected to BDNF-reducing situations such as low-pressure blast wave [22], and a predator scent stress [6].

Contrary to the studies that reported BDNF expression in animals can be increased by the BA-supplemented diets, Varanoske et al. demonstrated that 14 days of BA supplementation at high dosage does not effectively change cognition or plasma BDNF concentrations, but it was beneficial for mood states in the soldiers before a simulated military operation, significantly [7]. These authors concluded that it seems that BA supplementation could not enhance circulating BDNF [7]. It is noteworthy to mention that Varanoske and colleagues used blood samples from human subjects for BDNF analysis, while other investigators used rodent brain tissue [6, 16, 22, 25]. Therefore, circulating BDNF concentrations may not accurately reflect BDNF overexpression in different parts of the brain (Fig. 1). Consistent with this notion, Varanoske et al. reported that the individuals treated with BA had lower cognitive decline during the day of simulated military operation as compared to the placebo, on the other side, no differences between the groups were reported with regards plasma BDNF concentrations [11]. It is feasible that BA supplementation may be an effective tool for enhancing brain health, but further research is needed for determining its potential mechanisms. Here we describe some of the important signaling pathways.

BDNF is considered as a mediator for functional and structural plasticity in the CNS, which regulates many different cellular functions on both pre- and post-synaptic target sites (Fig. 1). BDNF isoforms can activate several functional downstream cascades via the tropomyosin-related kinase receptor type B (TrkB) and the p75 neurotrophin receptor (p75NTR) [23]. BDNF binds to TrkB and dimerizes this receptor, then its intracellular tyrosine residues are autophosphorylated. This phosphorylation of TrkB activates several signaling pathways, which initiate various processes such as programmed cell death [26] and neuronal viability [27], dendritic branching growth [28], dendritic spine formation and stabilization, synaptic development [29], cognitive dependent processes on synaptic plasticity [30].

The first enzymes that are activated by TrkB phosphorylation are phosphoinositide 3-kinases (PI3K)-AKT (PI3K-AKT). The PI3K-AKT exerts antiapoptotic activity and mediates survival of neurons and enhances synaptic plasticity through the NMDA receptor regulation [31]. The PI3K/Akt also activates the mammalian target of rapamycin (mTOR) signaling pathway, which enhances protein synthesis for cytoskeleton development, dendritic growth, and branching [32].

The second enzyme is the Ras-mitogen-activated protein kinase (Ras-MAPK), which is activated by TrkB, then it activates ERK 1/2 and CREB cascades [33]. This pathway controls protein synthesis during neuronal growth and development [34]. The MAPK/Ras-signaling cascade is crucial for multiple events such as early response gene expression (e.g., c-Fos), cytoskeleton protein synthesis (e.g., Arc and cypin) [35], as well as shaping and branching dendrite architecture in the hippocampal neurons [36]. The third enzyme that is activated by TrkB phosphorylation is phospholipase C-γ (PLC-γ), which leads to activation of the protein kinase C (PKC). BDNF via activation of PKC regulates neuronal activity and synaptic signal transmission by increasing intracellular Ca^{2+} concentration and glutamate release. This pathway has been also reported to enhance synaptic plasticity [37] (Fig. 1).

BRAIN CARNOSINE CONTENT

Prior investigations in rodents stated that BA supplementation could induce carnosine overexpression in numerous brain regions with this increase being considered to be associated with learning improvement [25], and also is related to the reduced anxiety following acute stress [16]. Although identical rise in the brain carnosine expression was not reported in the human studies after BA supplementation [10, 12], there is strong evidence that shows that BA may improve human cognitive function and mood state [11, 38], which demonstrate that carnosine effects may be involved in cognitive health and illness, however, its underlying mechanism is not well understood. Hence, we discussed physiological multifunctional role of carnosine in the nervous system.

Carnosine is a nitrogen-containing compound, which has been extensively studied during the last few years because of its health benefits. This natural endogenous molecule is a histidine-containing dipeptide that is highly abundant in the mammalian skeletal and cardiac muscles, but is also present in the CNS. Although evidence shows that the carnosine-containing tissues such as brain have active oxidative metabolism, but its physiological roles are poorly understood [39].

Carnosine is formed from β-alanine and L-histidine amino acids obtained from the circulatory system. However, besides the *de novo* synthesis, carnosine also can be obtained from the diet. The main carnosine effects in CNS are associated with its antioxidant properties [39]. The antioxidant actions decrease oxidative stress (OS) caused by overproduction of reactive oxygen species (ROS) [40]. Glutathione (GSH) and superoxide dismutase 1 (SOD1) enzyme are the major cell antioxidants that have vital roles in cellular health. However, in recent years it was found that carnosine is also a very important antioxidant [41]. Indeed, carnosine after oxidative transformation into 2-oxo-carnosine exhibits a stronger antioxidant effect than GSH [42]. Carnosine also deactivates hypochlorous acid (HOCl), another toxic species produced in mammalian cells, and thus can protect brain against the degenerative disorders such as Alzheimer's disease [43].

Furthermore, besides its antioxidant qualities, carnosine may indirectly enhance antioxidant defense through multiple molecular pathways. For example, carnosine attenuates activity of Nrf2 (nuclear factor erythroid 2-related factor 2), which is the main regulator of the cell antioxidant defense [44]. Carnosine indirectly regulates transcription of hundreds of genes related to antioxidant mechanisms such as thioredoxin 1, SOD1, or catalase by increasing Nrf2 expression and translocating Nrf2 to the nucleus in OS [41]. Although the exact mechanism of carnosine triggering the Nrf2 pathway is not clear, a group of researchers demonstrated that carnosine in the diabetic mice could stimulate Nrf2 by activating the PI3K (phosphatidylinositol 3-kinase)/AKT (protein kinase B) pathway [45].

Another major protective effect of carnosine on CNS is related to its anti-glycating and anti-aggregate activities [46]. OS significantly impairs neuronal cells functions through modifications of lipids, DNA, or proteins. They are the main targets of ROS, which play an important role in several CNS disorders by producing carbonyl products from the advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) [47]. In addition, methylglyoxal (MGO) and glyoxal are carbonyl compounds participating in protein glycation and aggregation leading to neuron death [48]. Carnosine can hinder AGEs and ALEs generation by detoxifying reactive carbonyl species via reaction with its imidazole ring. In a similar manner, carnosine inhibits activities of MGO and glyoxal [39]. These effects probably are related to the carnosine-activated increase of the Nrf2 pathway and, therefore, initiate overexpression of several antioxidant enzymes and oxidoreductases that convert carbonyl compounds into less reactive products such as alcohols [41]. There is evidence indicating that carnosine exhibits protease activity in the rat neural cells, which provides therapeutic potential for treating protein aggregation [49].

Anti-inflammatory and metal ion chelator activity is the third mechanism associated with carnosine

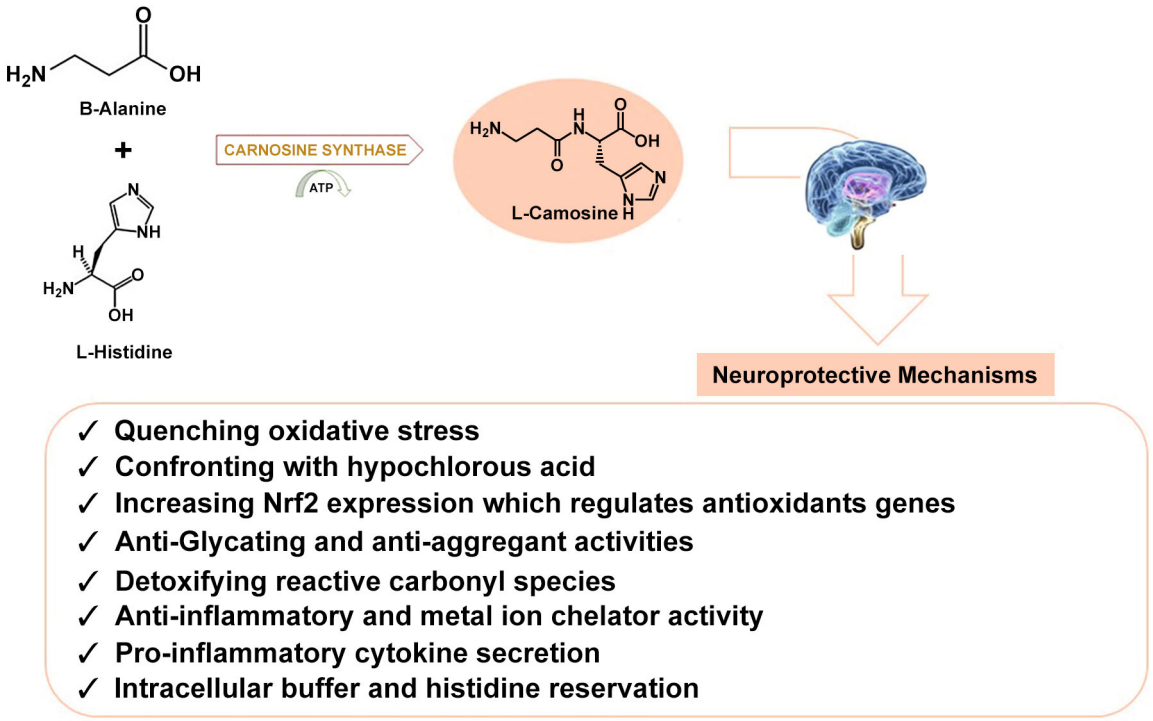

Fig. 2. Summary of neuroprotective effects of beta-alanine in the brain by increasing brain carnosine content. Carnosine is synthesized by an ATP-dependent synthase from β-alanine and l-histidine. In the brain, several neuroprotective actions were attributed to these carnosine-enhanced levels: reducing oxidative stress, counteracting effects of hypochlorous acid, regulating nuclear factor erythroid 2-related factor 2, and anti-glycating and anti-aggregate activities. Anti-inflammatory and metal ion chelator activity are other possible mechanisms [39].

neuroprotective properties. Carnosine attenuates inflammation likely through regulation of the pro-inflammatory cytokines [50]. However, this mechanism is still poorly understood and this anti-inflammatory effect could be related to the carnosine ability to form complexes with metal ions, particularly with zinc (Zn^{2+}) called polaprezinc [49]. Polaprezinc is a molecule that has various beneficial properties, such as inhibition of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling, reduction of the interleukin-8 secretion, and increase of the Hsp72 production [51]. In addition, polaprezinc may reduce inflammation through its chelator activity[52]. Carnosine also directly binds to Zn^{2+} and copper (Cu) and, as a consequence, regulates synaptic impulse and synaptic transissions [53].

In addition to acting as an intracellular buffer in the brain, a possible action of carnosine in the brain is sequestering of histidine, which is a precursor of histamine [42]. It was reported that histidine diminishes brain histamine levels and, subsequently, increases anxiety-related behaviors [43]. It also can lead to the development of stress and depression, which eventually impairs cognitive performance [54]. On the other side, when the histamine level was elevated, the extent of anxiety symptoms was decreased and memory capabilities were preserved significantly [55]. It is likely that the enhanced brain carnosine content may also lead to the improved brain cognitive function (Fig. 2). However, further understanding of the mechanism would provide novel potential therapeutic strategies.

CONCLUSION

The present review implies that BA supplementation is associated with cognitive enhancement in human and animal subjects. Furthermore, potential mechanism that can explain improvement in the brain function and mental health following daily BA intake is elevated BDNF and carnosine content. Summary of the neuroprotective effects of beta-alanine in the brain mediated by the changes in carnosine and BDNF levels is shown in Figs. 1 and 2. However, further research is necessary to shed light on the effects of amino acids on CNS health and function.

Contributions. GHM had the idea for the article; GHM performed the literature research and data analysis; GHM and GPJ wrote the article. All authors have read and agreed to the published version of the manuscript.

Acknowledgments. This study was supported by the Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Ethics declarations. The authors declare no conflict of interest in financial or any other spheres. This article does not contain description of studies involving humans or animals performed by any of the authors.

REFERENCES

1. Muscaritoli, M. (2021) The impact of nutrients on mental health and well-being: insights from the literature, *Front. Nut.*, **97**, 656290, doi: 10.3389/fnut.2021.656290.
2. Olivera-Pueyo, J., and Pelegrín-Valero, C. (2017) Dietary supplements for cognitive impairment, *Actas Espanolas de Psiquiatria*, **45**, 37-47.
3. Woitas, L. R., and Ribas, J. W. (2022) Major clinical findings of β-alanine in sports performance: a systematic review, *Intern. J. Nutrol.*, **15**, 1-6, doi: 10.54448/ijn22205.
4. Furst, T., Massaro, A., Miller, C., Williams, B. T., LaMacchia, Z. M., and Horvath, P. J. (2018) β-Alanine supplementation increased physical performance and improved executive function following endurance exercise in middle aged individuals, *J. Int. Soc. Sports Nutr.*, **15**, 32, doi: 10.1186/s12970-018-0238-7.
5. Ommati, M. M., Heidari, R., Ghanbarinejad, V., Aminian, A., Abdoli, N., and Niknahad, H. (2020) The neuroprotective properties of carnosine in a mouse model of manganism is mediated via mitochondria regulating and antioxidative mechanisms, *Nut. Neurosci.*, **23**, 731-743, doi: 10.1080/1028415X.2018.1552399.
6. Hoffman, J. R., Ostfeld, I., Stout, J. R., Harris, R. C., Kaplan, Z., and Cohen, H. (2015) β-Alanine supplemented diets enhance behavioral resilience to stress exposure in an animal model of PTSD, *Amino Acids*, **47**, 1247-1257, doi: 10.1007/s00726-015-1952-y.
7. Varanoske, A. N., Wells, A. J., Boffey, D., Harat, I., Frosti, C. L., Kozlowski, G. J., Gepner, Y., and Hoffman, J. R. (2021) Effects of high-dose, short-duration β-alanine supplementation on cognitive function, mood, and circulating brain-derived neurotropic factor (BDNF) in recreationally-active males before simulated military operational stress, *J. Diet. Supp.*, **18**, 147-168, doi: 10.1080/19390211.2020.1733730.
8. Hoffman, J. R., Landau, G., Stout, J. R., Dabora, M., Moran, D. S., Sharvit, N., Hoffman, M. W., Ben Moshe, Y., McCormack, W. P., Hirschhorn, G., and Ostfeld, I. (2014) β-Alanine supplementation improves tactical performance but not cognitive function in combat soldiers, *J. Int. Soc. Sports Nutr.*, **11**, 15, doi: 10.1186/1550-2783-11-15.
9. Hoffman, J. R., Landau, G., Stout, J. R., Hoffman, M. W., Shavit, N., Rosen, P., Moran, D. S., Fukuda, D. H., Shelef, I., Carmom, E., and Ostfeld, I. (2015) β-Alanine ingestion increases muscle carnosine content and combat specific performance in soldiers, *Amino Acids*, **47**, 627-636, doi: 10.1007/s00726-014-1896-7.
10. Solis, M. Y., Cooper, S., Hobson, R. M., Artioli, G. G., Otaduy, M. C., Roschel, H., Robertson, J., Martin, D., Painelli, V., Harris, R. C., Gualano, B., and Sale, C. (2015) Effects of beta-alanine supplementation on brain homocarnosine/carnosine signal and cognitive function: an exploratory study, *PLoS One*, **10**, e0123857, doi: 10.1371/journal.pone.0123857.
11. Varanoske, A. N., Wells, A. J., Kozlowski, G. J., Gepner, Y., Frosti, C. L., Boffey, D., Coker, N. A., Harat, I., and Hoffman, J. R. (2018) Effects of β-alanine supplementation on physical performance, cognition, endocrine function, and inflammation during a 24 h simulated military operation, *Physiol. Rep.*, **6**, e13938, doi: 10.14814/phy2.13938.
12. Hoffman, J. R., Stout, J. R., Harris, R. C., and Moran, D. S. (2015) β-Alanine supplementation and military performance, *Amino Acids*, **47**, 2463-2474, doi: 10.1007/s00726-015-2051-9.
13. Wells, A. J., Varanoske, A. N., Coker, N. A., Kozlowski, G. J., Frosti, C. L., Boffey, D., Harat, I., Jahani, S., Gepner, Y., and Hoffman, J. R. (2020) Effect of β-alanine supplementation on monocyte recruitment and cognition during a 24-hour simulated military operation, *J. Strength Cond. Res.*, **34**, 3042-3054, doi: 10.1519/JSC.0000000000003809.
14. Tomonaga, S., Matsumoto, M., and Furuse, M. (2012) β-Alanine enhances brain and muscle carnosine levels in broiler chicks, *J. Poultry Sci.*, **49**, 308-312, doi: 10.2141/jpsa.0110165.
15. Belity, T., Hoffman, J. R., Horowitz, M., Epstein, Y., Bruchim, Y., and Cohen, H. (2022) β-Alanine supplementation attenuates the neurophysiological response in animals exposed to an acute heat stress, *J. Dietary Suppl.*, **19**, 443-458, doi: 10.1080/19390211.2021.1889734.
16. Murakami, T., and Furuse, M. (2010) The impact of taurine-and beta-alanine-supplemented diets on behavioral and neurochemical parameters in mice: antidepressant versus anxiolytic-like effects, *Amino Acids*, **39**, 427-434, doi: 10.1007/s00726-009-0458-x.
17. Haleem, D. J., and Mahmood, K. (2021) Brain serotonin in high-fat diet-induced weight gain, anxiety and spatial memory in rats, *Nutr. Neurosci.*, **24**, 226-235, doi: 10.1080/1028415X.2019.1619983.
18. Hadipour, M., Bahari, Z., Afarinesh, M. R., Jangravi, Z., Shirvani, H., and Meftahi, G. H. (2021) Administering crocin ameliorates anxiety-like behaviours and reduces the inflammatory response in amyloid-beta induced neurotoxicity in rat, *Clin. Exp. Pharmacol. Physiol.*, **48**, 877-889, doi: 10.1111/1440-1681.13494.
19. Meftahi, G. H., Bayat, M., Zarifkar, A. H., Akbari, S., Haghighi, A. B., Naseh, M., Yousefi Nejad, A., and Haghani, M. (2021) Treatment with edaravone improves the structure and functional changes in the hippocampus after chronic cerebral hypoperfusion in rat, *Brain Res. Bull.*, **174**, 122-130, doi: 10.1016/j.brainresbull.2021.06.006.
20. Hoffman, J. R., Zuckerman, A., Ram, O., Sadot, O., Stout, J. R., Ostfeld, I., and Cohen, H. (2017) Behavioral and inflammatory response in animals exposed to a low-pressure blast wave and supplemented with β-alanine, *Amino Acids*, **49**, 871-886, doi: 10.1007/s00726-017-2383-8.
21. Hoffman, J. R., Rathmacher, J. A., Robinson, J., Gepner, Y., and Cohen, H. (2019) Effect of β-alanine

supplementation on carnosine and histidine content in the hippocampus of 14-month-old rats, *Appl. Physiol. Nutr. Met.*, **44**, 1112-1115, doi: 10.1139/apnm-2019-0102.

22. Hoffman, J. R., Gepner, Y., and Cohen, H. (2019) β-Alanine supplementation reduces anxiety and increases neurotrophin expression in both young and older rats, *Nutr. Res.*, **62**, 51-63, doi: 10.1016/j.nutres.2018.11.001.
23. Azman, K. F., and Zakaria, R. (2022) Recent Advances on the role of brain-derived neurotrophic factor (BDNF) in neurodegenerative diseases, *Int. J.Mol. Sci.*, **23**, 6827, doi: 10.3390/ijms23126827.
24. Hoffman, J. R., Varanoske, A., and Stout, J. R. (2018) Effects of β-alanine supplementation on carnosine elevation and physiological performance, *Adv. Food Nutr. Res.*, **84**, 183-206, doi: 10.1016/bs.afnr.2017.12.003.
25. Colucci-D'Amato, L., Speranza, L., and Volpicelli, F. (2020) Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain cancer, *Int. J. Mol. Sci.*, **21**, 7777, doi: 10.3390/ijms21207777.
26. Çelik, H., Kandemir, F. M., Caglayan, C., Özdemir, S., Çomaklı, S., Kucukler, S., and Yardım, A. (2020) Neuroprotective effect of rutin against colistin-induced oxidative stress, inflammation and apoptosis in rat brain associated with the CREB/BDNF expressions, *Mol. Biol. Rep.*, **47**, 2023-2034, doi: 10.1007/s11033-020-05302-z.
27. Miranda-Lourenço, C., Ribeiro-Rodrigues, L., Fonseca-Gomes, J., Tanqueiro, S. R., Belo, R. F., Ferreira, C. B., Rei, N., Ferreira-Manso, M., de Almeida-Borlido, C., Costa-Coelho, T., and Freitas, C. F. (2020) Challenges of BDNF-based therapies: from common to rare diseases, *Pharmacol. Res.*, **162**, 105281, doi: 10.1016/j.phrs.2020.105281.
28. Ravindran, S., Nalavadi, V. C., and Muddashetty, R. S. (2019) BDNF induced translation of limk1 in developing neurons regulates dendrite growth by fine-tuning cofilin1 activity, *Front. Mol. Neurosci.*, **12**, 64, doi: 10.3389/fnmol.2019.00064.
29. Zagrebelsky, M., Tacke, C., and Korte, M. (2020) BDNF signaling during the lifetime of dendritic spines, *Cell Tissue Res.*, **382**, 185-199, doi: 10.1007/s00441-020-03226-5.
30. Aliabadi, N., Sahraei, H., Bahari, Z., and Meftahi, G. H. (2018) Effect of prenatal immobilization stress on spatial memory, anxiety-like behavior and brain BDNF concentration in the F1 generation male mice, *Sci. J. Kurdistan Univ. Medical Sci.*, **23**, 73-84.
31. Ahmed, S., Kwatra, M., Gawali, B., Panda, S. R., and Naidu, V. G. M. (2021) Potential role of TrkB agonist in neuronal survival by promoting CREB/BDNF and PI3K/Akt signaling in vitro and in vivo model of 3-nitropropionic acid (3-NP)-induced neuronal death, *Apoptosis*, **26**, 52-70, doi: 10.1007/s10495-020-01645-x.
32. Kumar, M., and Bansal, N. (2022) Implications of phosphoinositide 3-kinase-Akt (PI3K-Akt) pathway in the pathogenesis of Alzheimer's disease, *Mol. Neurobiol.*, **59**, 354-385, doi: 10.1007/s12035-021-02611-7.
33. Grannonico, M., Brandolini, L., Varrassi, G., Sebastiani, P., Colanardi, A., Paladini, A., Piroli, A., Allegretti, M., and Di Loreto, S. (2020) DF3016A induces increased BDNF transcription in ischemic neuroinflammation injury, *Brain Res.*, **1748**, 147057, doi: 10.1016/j.brainres.2020.147057.
34. Joshi, R., and Salton, S. R. (2022) Neurotrophin crosstalk in the etiology and treatment of neuropsychiatric and neurodegenerative disease, *Front. Mol. Neurosci.*, **15**, 932497, doi: 10.3389/fnmol.2022.932497.
35. Batool, S., Raza, H., Zaidi, J., Riaz, S., Hasan, S., and Syed, N. I. (2019) Synapse formation: from cellular and molecular mechanisms to neurodevelopmental and neurodegenerative disorders, *J. Neurophysiol.*, **121**, 1381-1397, doi: 10.1152/jn.00833.2018.
36. Kwon, M., Fernández, J. R., Zegarek, G. F., Lo, S. B., and Firestein, B. L. (2011) BDNF-promoted increases in proximal dendrites occur via CREB-dependent transcriptional regulation of cypin, *J. Neurosci.*, **31**, 9735-9745, doi: 10.1523/JNEUROSCI.6785-10.2011.
37. Bazzari, A. H., and Bazzari, F. H. (2022) BDNF therapeutic mechanisms in neuropsychiatric disorders, *Int. J. Mol. Sci.*, **23**, 8417, doi: 10.3390/ijms23158417.
38. Baguet, A., Everaert, I., De Naeyer, H., Reyngoudt, H., Stegen, S., Beeckman, S., Achten, E., Vanhee, L., Volkaert, A., Petrovic, M., Taes, Y., and Derave, W. (2011) Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity, *Eur. J. App. Physiol.*, **111**, 2571-2580, doi: 10.1007/s00421-011-1877-4.
39. Solana-Manrique, C., Sanz, F. J., Martínez-Carrión, G., and Paricio, N. (2022) Antioxidant and neuroprotective effects of carnosine: therapeutic implications in neurodegenerative diseases, *Antioxidants*, **11**, 848, doi: 10.3390/antiox11050848.
40. Calabrese, V., Scuto, M., Salinaro, A. T., Dionisio, G., Modafferi, S., Ontario, M. L., Greco, V., Sciuto, S., Schmitt, C. P., Calabrese, E. J., and Peters, V. (2020) Hydrogen sulfide and carnosine: modulation of oxidative stress and inflammation in kidney and brain axis, *Antioxidants*, **9**, 1303, doi: 10.3390/antiox9121303.
41. Aldini, G., de Courten, B., Regazzoni, L., Gilardoni, E., Ferrario, G., Baron, G., Altomare, A., D'Amato, A., Vistoli, G., and Carini, M. (2021) Understanding the antioxidant and carbonyl sequestering activity of carnosine: direct and indirect mechanisms, *Free Radic. Res.*, **55**, 321-330, doi: 10.1080/10715762.2020.1856830.
42. Ihara, H., Kakihana, Y., Yamakage, A., Kai, K., Shibata, T., Nishida, M., Yamada, K.-I., and Uchida, K. (2019) 2-Oxo-histidine–containing dipeptides are functional oxidation products, *J. Biol.Chem.*, **294**, 1279-1289, doi: 10.1074/jbc.RA118.006111.
43. Carroll, L., Karton, A., Radom, L., Davies, M. J., and Pattison, D. I. (2019) Carnosine and carcinine derivatives rapidly react with hypochlorous acid to form chloramines and dichloramines, *Chem. Res. Toxicol.*, **32**, 513-525, doi: 10.1021/acs.chemrestox.8b00363.

44. Caruso, G., Privitera, A., Antunes, B. M., Lazzarino, G., Lunte, S. M., Aldini, G., and Caraci, F. (2022) The therapeutic potential of carnosine as an antidote against drug-induced cardiotoxicity and neurotoxicity: focus on Nrf2 pathway, *Molecules*, **27**, 4452, doi: 10.3390/molecules27144452.
45. Zhao, K., Li, Y., Wang, Z., Han, N., and Wang, Y. (2019) Carnosine protects mouse podocytes from high glucose induced apoptosis through PI3K/AKT and Nrf2 pathways, *Biomed Res. Inter.*, **2019**, 4348973, doi: 10.1155/2019/4348973.
46. Caruso, G., Caraci, F., and Jolivet, R. B. (2019) Pivotal role of carnosine in the modulation of brain cells activity: Multimodal mechanism of action and therapeutic potential in neurodegenerative disorders, *Prog. Neurobiol.*, **175**, 35-53, doi: 10.1016/j.pneurobio.2018.12.004.
47. Ghodsi, R., and Kheirouri, S. (2018) Carnosine and advanced glycation end products: a systematic review, *Amino Acids*, **50**, 1177-1186, doi: 10.1007/s00726-018-2592-9.
48. Hipkiss, A. R. (2018) Glycotoxins: dietary and metabolic origins; possible amelioration of neurotoxicity by carnosine, with special reference to parkinson's disease, *Neurotox. Res.*, **34**, 164-172, doi: 10.1007/s12640-018-9867-5.
49. Boldyrev, A. A., Aldini, G., and Derave, W. (2013) Physiology and pathophysiology of carnosine, *Physiol. Rev.*, **93**, 1803-1845, doi: 10.1152/physrev.00039.2012.
50. Son, D. O., Satsu, H., Kiso, Y., Totsuka, M., and Shimizu, M. (2008) Inhibitory effect of carnosine on interleukin-8 production in intestinal epithelial cells through translational regulation, *Cytokine*, **42**, 265-276, doi: 10.1016/j.cyto.2008.02.011.
51. Odashima, M., Otaka, M., Jin, M., Wada, I., Horikawa, Y., Matsuhashi, T., Ohba, R., Hatakeyama, N., Oyake, J., and Watanabe, S. (2006) Zinc l-carnosine protects colonic mucosal injury through induction of heat shock protein 72 and suppression of NF-κB activation, *Life Sci.*, **79**, 2245-2250, doi: 10.1016/j.lfs.2006.07.032.
52. Li, M., Sun, Z., Zhang, H., and Liu, Z. (2021) Recent advances on polaprezinc for medical use, *Exp. Ther. Med.*, **22**, 1445, doi: 10.3892/etm.2021.10880.
53. Kawahara, M., Tanaka, K. I., and Kato-Negishi, M. (2018) Zinc, carnosine, and neurodegenerative diseases, *Nutrients*, **10**, 147, doi: 10.3390/nu10020147.
54. Caruso, G., Fresta, C. G., Grasso, M., Santangelo, R., Lazzarino, G., Lunte, S. M., and Caraci, F. (2020) Inflammation as the common biological link between depression and cardiovascular diseases: Can carnosine exert a protective role? *Curr. Med. Chem.*, **27**, 1782-1800, doi: 10.2174/0929867326666190712091515.
55. Sureshkumar, K., Durairaj, M., Srinivasan, K., Goh, K. W., Undela, K., Mahalingam, V. T., Ardianto, C., Ming, L. C., and Ganesan, R. M. (2023) Effect of L-carnosine in patients with age-related diseases: a systematic review and meta-analysis, *Front. Biosci. Landmark*, **28**, 18, doi: 10.31083/j.fbl2801018.