Received: 21 October 2023, Accepted: 23 November 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 10, 2118-2126

Ameliorative Effect of L-arginine on Monosodium Glutamate Induced Cognitive Hypofunctions in Male Albino Rats

Hadeer M. Fahmy[1*], Amal M. EL-Nahla[2], Saadia A. Ali[2], Amina A. Dessouki[3], Basant A. Elbaz[4], Samar K. Abdelaziz[2]

[1]Department of Physiology, Faculty of Veterinary Medicine, Arish University, Egypt.

[2]Department of Physiology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

[3]Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

[4]Ph.D. student in Behavior and Management of Animal, Poultry, and Fish Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

***Correspondence**
Corresponding author: Hadeer M. Fahmy
E-mail address: hadeer.mamdoh @vet.aru.edu.eg

Abstract

The present study aimed to investigate the ameliorative effect of L-arginine (L-A) on cognitive hypofunctions induced by monosodium glutamate (MSG). Thirty-six male albino Wistar rats 10 weeks old (180 to 200g) were divided into 4 groups: Group I: received distilled water, group II received L-arginine (L-A) 500 mg/kg, group III received 2 mg/kg monosodium glutamate (MSG), group IV received L-arginine 500 mg/kg and 2 mg/kg monosodium glutamate by oral gavage for 10 days. Cognitive performance was assessed using novel object recognition (NOR) and Y-maze tests. The relative brain weight of experimental rats was recorded. The malondialdehyde (MDA) level in the brain homogenate as oxidative stress biomarkers, antioxidants glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) activities, and dopamine (DA) levels were estimated. Histopathology and immunohistochemistry to serotonin (S-2A) receptors and caspase-3 were performed. Results revealed that MSG can cause a decline in cognitive functions as evidenced by NOR and y-maze tests. Besides, it has a neurotoxic effect as evidenced by increasing MDA level and GPx activity, decreasing SOD and CAT activities, reduced DA level, histopathological alteration in the brain, decreased S-2A receptors, and increased apoptosis as demonstrated by promoted caspase-3. Treatment with L-A 500 mg/Kg BW ameliorated the neurophysiological effects of MSG through improving memory, decreasing MDA level, GPx activity, increasing SOD and CAT activities, increasing DA level, improving histoarchitecture of the brain, improving S-2A receptors and decreasing apoptosis ameliorating caspase-3 protein in the brain.

KEYWORDS
L-arginine, Monosodium glutamate, Cognitive hypofunctions, Oxidative stress, S-2A receptors, Caspase-3

INTRODUCTION

A cognitive hypofunction is any disorder that significantly decreases a cognitive ability to the point where it is difficult to function normally (Dhakal and Bobrin, 2023). According to Zhang *et al*. (2019), cholinergic degeneration, hippocampal lesions, a decline in neurotransmitters, and synaptic damage are all directly related to cognitive impairment and memory loss. In addition, oxidative stress can impair endogenous antioxidant enzymes, and cytotoxicity and neuroinflammatory responses in the central nervous system (CNS) (Wang *et al*., 2021). Furthermore, aging, metabolic health, food, and general quality of life, including stress, have a significant impact on cognitive functions (Novotný *et al*., 2019). Excessive uptake of food additives like sodium benzoate (Khoshnoud *et al*., 2018), and monosodium glutamate (MSG) (Abdel Moneim *et al*., 2018) influence cognitive deficits or hypofunctions.

Monosodium glutamate (MSG), is a common food enhancer (Stańska and Krzeski, 2016; Zanfirescu *et al*., 2019). MSG can be naturally contained in various foods, such as cheese and tomatoes (Tawfik and Al-Badr, 2012). It is used in cooking as a flavor enhancer with an umami taste that intensifies food's meaty, savory flavor (Stańska and Krzeski, 2016).

According to Abu-Elfotuh *et al*. (2022), MSG is a neurotransmitter in the central nervous system and it causes over-activation of glutamate receptors in the brain when given. Its excessive use causes excitotoxicity, which can result in ischemia and traumatic brain injury as well as severe neuronal damage. Rats treated with MSG, in the hippocampus, had 11.5% more cellular degeneration than the control group, these modifications suggested MSG's to-excitotoxic effect, which ultimately affects hippocampus integrity (Kazmi *et al*., 2017). Male albino rats' cerebral cortex was also studied for MSG's harmful effects, and it was discovered that rats given 3 g/kg/day of the substance formed zones of degeneration that were surrounded by granule cells and pyknotic Purkinje (Hashem *et al*., 2012).

L-arginine (L-A) is categorized as an essential amino acid (Virarkar *et al*., 2013). It serves as a substrate for the production of nitric oxide (NO), which is vital for various physiological activities, including neurotransmission, vasorelaxation, cytotoxicity, and immunology (Gad, 2010). Also, it encourages optimal cerebral blood flow, strengthens memory functions, and makes long-term potentiation (Virarkar *et al*., 2013). L-arginine dramatically lowered low-density lipoprotein and total serum cholesterol levels (Fonar *et al*., 2018), improved local tissue blood supply and oxygenation, improved creatine transport (Kurhaluk, 2023), increased energy levels (Szlas *et al*., 2022), antioxidant (Saleh and El-Demerdash, 2005), stimulation of dopamine, adrenaline, and noradrenaline release (Gad, 2010). L-A improvement of wound healing, enhancing immunity, stimulating thymus, and promoting lymphocyte production (Kang *et al*., 2014). It protects gastric ulcers induced by various agents (Usman *et al*., 2014). L-arginine and its derivatives have also been used for the treatment of neurological disorders (Paul and Ekambaram, 2011). According to

ISSN: 2090-6277/2090-6269/

Mahmoud *et al*. (2021), It plays crucial functions in both health and disease. In this study, we aimed to assess the ameliorative effects of L-A against the cognitive hypofunction induced by MSG.

MATERIALS AND METHODS

Ethical statement

All animal experimental procedures were conducted at the Laboratory Animal House, Faculty of Veterinary Medicine, Suez Canal University, Egypt. The procedures of this experiment were approved (No. 2022023) and the guidelines of the Committee of Scientific Research and Biological Ethics for animals used in laboratory experiments in the Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Animals

Thirty-six male albino Wistar rats 10 weeks old with an average body weight ranging from 180 to 200 g were used. Rats were left for 2 weeks to be adapted to the surrounding environment. Nine rats per cage were kept in a room with a sawdust-covered floor and controlled temperature (25.0±2.0°C). The rats were diet and water ad libitum.

Chemicals

Monosodium glutamate was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, United States). L-A was obtained from Puritan's pride Company (Cairo, Egypt).

Experimental Design

The rats were assigned randomly into four groups, each group had nine rats.
Group I (Control): Rats gavaged distilled water.
Group II (L-A): Rats were gavaged 500 mg/kg L-A (25%w/v in distilled water) (Dos Santos *et al*., 2014).
Group III (MSG): Rats were gavaged 2 g/kg MSG (10%w/v in distilled water) (Dief *et al*., 2014).
Group IV (L-A + MSG): Rats were gavaged L-A 500 mg/kg (25%w/v in distilled water) and 2 g/kg MSG (10% w/v distilled water), one hour apart (Mahmoud *et al*., 2021).

Rat gavaged via gastric tube daily days and dose of each drug calculated daily for each animal for a consecutive 10.

Neurobehavioral assessments

At the end of 10 days, the behavior tests were performed for all groups one day after the last dose administration sequentially as described.

The neurobehavioral assessments were mounted with an HD video camera (model NO. B80, Yes-original. co, China) for recording and scoring behaviors, and the cameras were connected to a digital video recording device (model NO. OR 4CH 5IN1, Yes-original, China).

Each neurobehavioral assessment session was analyzed using the Behavioral Observation Research Interactive Software (BORIS, v. 2.95, University of Torino, Torino, Italy.

Novel object recognition (NOR) test

Rats were transferred to the testing apparatus; each rat was placed in the central square for 10 minutes. A total of 9 rats/groups were tested. The light color and intensity of the NOR apparatus were like the home cages for rats.

The test was divided into 3 phases (habituation phase, training phase, and testing phase). During habituation, the animals are allowed to explore an empty arena. Twenty-four hours after habituation (training phase), the animals are exposed to the familiar arena with two identical objects (A and B) placed at an equal distance. The next day (testing phase), the rat is allowed to explore the arena in the presence of the familiar object (B) and a novel object (C) to test long-term recognition memory.

The behavioral activities were observed: (1) preference index (PI) to (object 1, object 2, familiar object, and novel object), (3) Recognition Index (RI), and (3) Discrimination Index (DI). All the previous behavioral elements were according to Antunes and Biala (2012).

Y- maze test

Rats were transferred to the testing apparatus; the rat was placed in the center of the maze for 10 minutes. The animal can freely explore the three arms through multiple arm entries. The number of arm entries and the number of triads are recorded to calculate the percentage of alternation. An entry occurs when all four limbs are within the arm. A total of 9 rats/group were tested. The Y-maze apparatus's light color and intensity were like the rats' home cages. The following behavioral activities were observed: (1) number of entries arms; (2) alteration percentage. All the previous behavioral elements were according to Kraeuter *et al*. (2019).

Samples collection

At the end of the experimental period, the animals were euthanized, and their brains were weighed and divided medially into two halves. One half was immersed in Bouin's solution for histopathology and the other half was subjected to malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and dopamine analysis.

Malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) analysis

The cerebral cortex and hippocampal homogenate MDA contents were assayed using commercial ELISA kit (Cat. No STA-330, Cell Biolabs, Inc company, San Diego, USA) and GPx, SOD and CAT contents, as antioxidant indicators were assayed using commercial ELISA kit (Cat. No 21017, OXISResearchTM company, Portland, U.S.A), (Cat. No STA- 340, Cell Biolabs, Inc company, San Diego, USA) and (Cat. No STA-341, Cell Biolabs, Inc company, San Diego, USA). All steps were carried out according to the manufacturer's protocol.

Dopamine Level

Cerebral cortex homogenate dopamine levels were assayed using specific ELISA kits (Cat. No CSB-E08660r, CUSABIO, Fannin St, Houston, USA). Procedures were followed according to the manufacturer's instructions.

Histopathological examination

Bouin-fixed halves of the brain for histological sections (4–5 µm) and stained by Haematoxylin and Eosin stain (HandE) according to Culling *et al*. (2014). Sections were examined under a

light microscope equipped with a digital camera.

Immunohistochemistry (IHC)

Paraffin-embedded brains were sliced into 5 µm sections on positively charged slides for serotonin (S-2A) receptors and caspase3 immunohistochemistry. The following primary antibodies; (#sc-32538, Santa Cruz Biotechnology, Dallas, TX, USA) in concentration 1:100 (sc-56053) and (#sc-56053Santa Cruz Biotechnology Company, Dallas, TX, USA) in concentration 1:1000. Protocol for IHC will be performed as described by Schacht and Kern (2015) for S-2A receptors and by Al-Arbeed *et al* (2023) for caspase-3. The quantitative analysis of immunoreactive parts percentage (IRP%) was implemented using Image J software. Seven random microscopic fields per slide were subjected to analysis after subtraction of the light background.

Statistical Analysis

Results were assessed for statistical analysis using SPSS 22 software (SPSS Inc., Chicago, IL, USA). Data were represented as mean ± standard error. The differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test. $p < 0.05$ refers to significant differences between groups.

RESULTS AND DISCUSSION

Regarding Novel object recognition, the obtained data revealed that MSG-treated rats induced cognitive deficit and impaired memory, which was indicated by the preference of rats for familiar objects was higher, lower explorative and interactive activity toward novel objects than the control group (Table 1) and decreased in the percentage of an alternation in Y-maze test compared to the control group (Table 2). These results were in line with that of Hazzaa *et al*. (2020), who demonstrated when rats were tested for their ability to retain short-term spatial memories in an open field and a Y-maze, monosodium glutamate significantly decreased their ability to do so. There was a relationship between cellular and structural alterations in the cortex and hippocampus with deteriorated spatial cognitive performance (Begega *et al*., 2012). Moreover, the cognitive and Behavioral deficits and hippocampal degeneration observed in the MSG group could be attributed to the damage implicated by MSG-induced oxidative stress (OS) on brain structure and function. Numerous studies have demonstrated that the use of MSG causes the production of reactive oxygen species (ROS), which is consistent with our findings (Onaolapo and Onaolapo, 2018)

Our results reveal that administration of L-A simultaneously with MSG retrieved memory and cognitive abilities which was indicated by NOR (Table 1) and Y-maze (Table 2) tests as well as mitigated hippocampal and cortical degeneration. L-A was thought to possess antioxidant and anti-lipid peroxidation effects in brain tissue (Hosseini *et al*., 2018). Moreover, L-A could enhance the efficacy of O2 utilization (Bailey *et al*., 2010) and NO production (Hassan *et al*., 2022). The latter is an important neurotransmitter that plays a marginal role in maintaining normal functions of the CNS and boosts cerebral blood flow, neural communications, intracellular signal transduction, and memory (Hamad, 2020). In agreement with our results, many studies reported improved learning and accelerated retrieval of maze tasks (Olusanya *et al*., 2018) and other behavioral tests (Hosseini *et al*., 2018; Mahmoud *et al*., 2021; Dubey *et al*., 2022) following L-A treatment.

In our study, it was revealed that the relative brain weight (Table 3) exhibited non-significant ($P < 0.05$) variation in MSG and control groups, this agrees with Abu-Taweel *et al*. (2014). Due to the well-known phenomena of brain-sparing, when the brain cannot acquire enough substrates to function when oxygen and nutrients are restricted, the vascular system vasodilates its arteries because of boosting blood flow. This makes it possible for the brain to get the nutritional supplements it requires (Benítez-Marín *et al*., 2021). On the other hand, Akataobi (2020) and Abd-Elhaleim El Azazy. (2021) reported that brain weight was found to be decreased significantly following MSG-contained diet intake as MSG can get into the hypothalamus, injuring and frequently killing its neurons. Neuronal injury associated with MSG may be dose-dependent, corroborating other studies that have reported brain injury at high doses of MSG (Leshchenko *et al*., 2012; Onaolapo *et al*., 2013). According to Onaolapo *et al*. (2016), MSG increased mean relative brain weight in rats.

The L-arginine-treated group in our study revealed no signif-

Table 1. Effects of L-arginine (L-A) on novel object recognition (NOR) test in monosodium glutamate (MSG) -treated rats.

Behavioral observation of NOR/Groups	Control	L-A	MSG	L-A + MSG
PI familiar object	38.95±10.84^{b}	34.88±3.44^{b}	50.87±9.87^{a}	15.06±4.48^{c}
PI novel object	61.05±10.84^{b}	65.12±3.44^{b}	49.13±9.87^{c}	84.94±4.48^{a}
RI	0.61±0.11^{b}	0.65±0.04^{b}	0.49±0.10^{c}	0.85±0.04^{a}
DI	22.08±21.67^{b}	30.25±6.88^{b}	1.75±19.74^{c}	69.88±8.97^{a}

Data were represented as mean ± SE. The different superscripts (a, b, c), within the same raw, were significantly differed at $P < 0.05$.

Table 2. Effects of L-arginine (L-A) on Y-maze test in monosodium glutamate (MSG) -treated rats.

Behavioral observation of y-maze/Groups	Control	L-A	MSG	L-A + MSG
Alternation percentage.	72.02±7.08^{ab}	61.15±1.39^{b}	48.29±3.55^{c}	80.70±1.99^{a}
No. of entries arms	22.67±0.44^{a}	21.00±1.04^{a}	20.00±1.50^{ab}	17.67±1.09^{b}

Data were represented as mean ± SE. The different superscripts (a, b, c), within the same raw, were significantly differed at $P < 0.05$.

Table 3. Effects of L-arginine (LA) on relative brain weight in monosodium glutamate (MSG) -treated rats.

Parameter/Groups	Control	L-A	MSG	L-A+MSG
Relative brain weight (%)	0.7482± .02164^{a}	0.8110±.02397^{a}	0.7432±.02652^{a}	0.8126±.01899^{a}

Data were represented as mean ± SE. The different superscripts (a, b, c), within the same raw, were significantly differed at $P < 0.05$.

icant (P < 0.05) effect in relative brain weight as compared to the control group and the L-A+ MSG-treated group exhibited no significant (P < 0.05) effect in relative brain weight as compared to MSG and control groups (Table 3), this means that L-A does not affect brain weight, this agrees with several studies (Mahmoud *et al*., 2021; Hassan *et al*., 2022) and disagree with Maia *et al*. (2009) who reported that L-A hurts brain weight.

Our study revealed an elevation of MDA concentration which is a lipid peroxidation biomarker, an increase GPx activity, and a concomitant decrease in SOD and CAT activities in the brain (Table 4) which was parallel to the finding achieved before by Kesherwani *et al*. (2022). The released free radicals contribute to the excitotoxicity of MSG (Lau and Tymianski, 2010). Released free radicals cause cell membranes and DNA peroxidation, causing cell damage and apoptosis (Colín-González *et al*., 2015).

Our findings were supported by earlier finding (Hassan *et al*., 2022) that showed L-A+MSG supplementation inhibited lipid peroxidation in the brain tissue. This was shown by a decrease in MDA concentration, a reduction in GPx activity, and an increase in SOD and CAT activities (Table 4). L-A was thought to possess antioxidant and anti-lipid peroxidation effects in brain tissue. Daily administration of L-A immediately with MSG retrieved memory and cognitive abilities as well as diminished hippocampal and cortical degeneration (Hosseini *et al*., 2018).

MSG-treated rats displayed a significant decline in the levels of dopamine (Table 5) in the brain tissue, this agrees with Albrakati (2023), who reported significant declines were detected in the levels of DA in the brain cortex of the MSG group. Hussein *et al*. (2017) disagree with our results and found that MSG increased levels of DA in the brain. Çetin Kardeşler and Başkale (2017) reported that DA levels increased in a parallel manner with increasing concentrations of MSG throughout the experiment as the level of dopamine increased.

Administration of L-A with MSG markedly elevated DA concentration (Table 5). These results are coincidental with those of Attia *et al*. (2020) and Mahmoud *et al*. (2021). It was explained that the effect of L-A on monoamines could be attributed to its role as a precursor of NO production (Abdel Baky *et al*., 2010). NO is an important intercellular messenger in the brain and has been found to increase the release of DA in different brain regions (Philippu, 2016; Attia *et al*., 2020).

The control group showed the normal histological structure of the hippocampus (Fig.1 a-d). The L-A-treated group showed the normal structure of the hippocampus; both Cornu Ammonis and dentate gyrus (Fig.2 a-d).

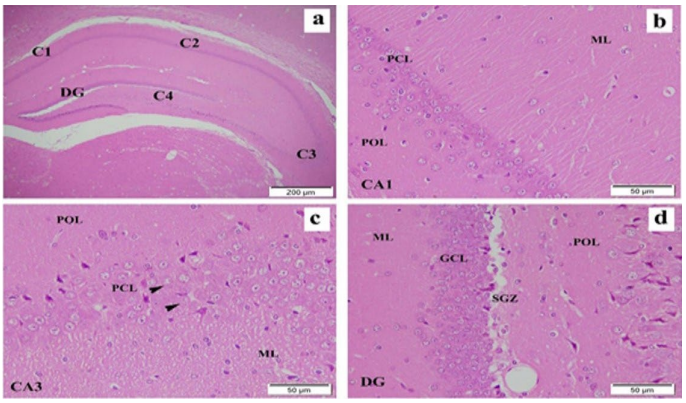

Fig. 1. Histological structure of hippocampus of control group.Control group showing: (a) section through the hippocampus showing C-shaped hippocampal proper (Cornu Ammonis). (b), the CA1 region (c), the CA3 region (d), dentate gyrus consisted of three layers superficial (outer) polymorph layer (POL), granular (middle) cell layer (GCL), and deep (inner) molecular layer (ML). Hematoxylin and Eosin stain.

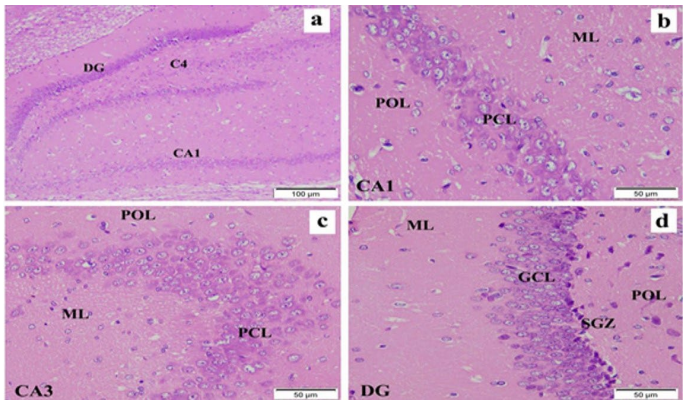

Fig. 2. Histopathological structure of hippocampus of L-A treated group. L-A group showing: (a) section through the hippocampus showing C-shaped hippocampal proper (Cornu Ammonis) with its four regions: CA1, CA2, CA3, and CA4. Notice the dentate gyrus (DG). (b), the CA1 region showing large-sized pyramidal neurons. (c), the CA3 region shows large pyramidal neurons. (d), dentate gyrus consisted of three layers superficial (outer) polymorph layer (POL), granular (middle) cell layer (GCL), and deep (inner) molecular layer (ML). Hematoxylin and Eosin stain.

MSG administration caused degeneration and focal necrosis of neurons and decreased the number of pyramidal cell layers due to neuronal cell death in the CA1 and CA3 areas of the hippocampus (Fig. 3 a,b,c) which are mainly involved in higher-order

Table 4. Effects of L-arginine (LA) on oxidative stress biomarkers and antioxidants in brain tissue in monosodium glutamate (MSG) -treated rats.

Parameters / Groups	Brain	Control	L-A	MSG	L-A+MSG
MDA (μM/mg)	Cortical	1.31 ±0.02 ^{c}	1.26 ±0.02 ^{c}	1.98 ±0.03 ^{a}	1.65 ±0.0^{b}
	Hippocampal	1.07 ±0.01 ^{c}	0.96 ±0.02 ^{c}	1.79 ±0.03 ^{a}	1.38 ±0.01 ^{b}
GPx (mU/mg)	Cortical	2.45± 0.03^{c}	2.54 ± 0.01^{c}	3.72 ± 0.03^{a}	2.83 ± 0.01^{b}
	Hippocampal	1.82 ± 0.02 ^{c}	1.86 ± 0.02 ^{c}	2.90 ± 0.02 ^{a}	2.21 ± 0.01 ^{b}
SOD (U/mg)	Cortical	2.99 ± 0.02 ^{a}	3.08 ± 0.03 ^{a}	2.25 ± 0.02 ^{c}	2.64 ± 0.01 ^{b}
	Hippocampal	2.56 ± 0.02 ^{a}	2.59±0.02 ^{a}	1.84 ± 0.01 ^{c}	2.21 ± 0.01 ^{b}
CAT (U/mg)	Cortical	4.86 ±0.02^{a}	4.97 ±0.01^{a}	3.63±0.02^{c}	4.48 ±0.02^{b}
	Hippocampal	2.92 ±0.01^{a}	2.66 ±0.01^{a}	2.12 ±0.01^{b}	2.52 ±0.01^{ab}

Data were represented as mean ± SE. The different superscripts (a, b, c), within the same raw, were significantly differed at P < 0.05.

Table 5. Effects of L-arginine (LA) on cortical dopamine levels in monosodium glutamate (MSG) -treated rats.

Parameter/Groups	Control	L-A	MSG	L-A+MSG
Cortical dopamine (ng/mg)	1.23 ±0.01 ^{a}	1.24 ±0.01 ^{a}	0.88 ±0.00 ^{c}	1.05 ±0.01 ^{b}

Data were represented as mean ± SE. The different superscripts (a, b, c), within the same raw, were significantly differed at P < 0.05.

spatial memory functions including associative retrieval of the learned task which agree with Madhavadas *et al*., (2016). Astrocytosis was characterized by increased proliferative astrocytes, spaces forming lacunae in the CA1 and CA3 around the nerve cells in the molecular and the pyramidal layers. Encephalomalacea was observed in the pyramidal layers of CA1 and CA3 (Fig. 3 b and c). Severe neuronal degeneration, pyknosis, and marked pericellular edema, in addition, degeneration in some glial cells was noticed. In addition, pyknosis of nuclei with loss of pyramidal cells, leaving empty spaces filled with vacuolations. In addition, the majority of the nerve cells had deformed shapes, and pyramidal cells in the dentate gyrus which is one of the few areas where neurogenesis is still active, the neurogenesis in DG was thought to play an important role in hippocampus-dependent learning and memory (Li *et al*., 2008). The dentate gyrus of the MSG-treated group showed a marked reduction in the number of cells of all three layers with the disarrangement of granular cells (Fig. 3 d). Our findings were in agreement with Rycerz *et al*. (2014), who found that administration of MSG to rats in the postnatal week revealed a loss of 11.5% of pyramidal neurons in the hippocampus. Gudiño-Cabrera *et al*. (2014) indicated that MSG administration induced hypertrophy of astrocytes and microglial cells in the hippocampus, also increasing neuronal apoptosis in CA1 and CA3 areas, leading to cognitive impairment. These histopathological alterations in the brain tissues could be caused by induced apoptosis in the hippocampus which may be attributed to oxidative stress that leads to mitochondrial damage with the release of cytochrome C and activation of caspases, These degenerative effects disrupted the normal flow of information from the dentate gyrus to CA3 through mossy fibers, and also may have affected the flow of information from CA3 to CA1 (Owoeye and Salami, 2017).

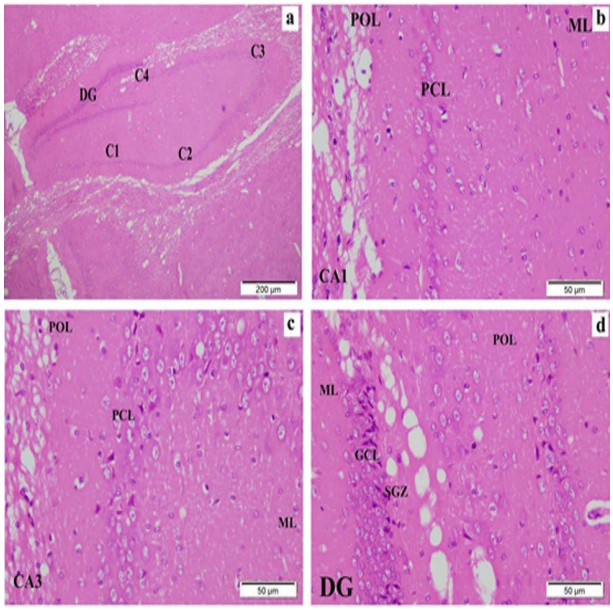

Fig. 3. Histopathological structure of hippocampus of MSG treated group. MSG group showing: (a) focal encephalomalacia and vacuolation in different parts of the hippocampal region. (b) and (c). disarrangement and decreased number of pyramidal layers in CA1 with pyknotic nuclei, intercellular edema, and degeneration of neurons. Empty spaces filled with vacuolations in the surrounding neutrophils. (d). The dentate gyrus showed a marked reduction in the number of cells of all three layers with disarrangement of granular cells and darkly stained nuclei with vacuolations of their cytoplasm along with vacuolation and edema (Hematoxylin and Eosin stain).

In the Normal control group, the cerebral cortex revealed a normal histological structure (Fig.5 a, e, i). The L-A group showed the normal structure of the cerebral cortex (Fig. 5 b, f, j). On the other hand, the MSG group showed a relative reduction in the three cerebral layers mainly in the molecular, granular, and pyramidal layers. Multiple increases in neuronal injury are characterized by numerous degenerations of pyramidal and granule neurons (with vacuolations) (Figure 5 c, g, k). The MSG group also revealed focal microglial infiltration, which surrounded the areas of neuronal damage. Our results were in agreement with the work done by Hamad. (2020) and Abu-Elfotuh *et al*. (2022), this might be explained by excessive glutamate receptor activation, which leads to increased Ca2+ and Na+ inflow, which in turn triggers off a chain reaction of enzymatic activities that destroy neurons, significantly disrupting normal cellular physiology (Zhang *et al*., 2012).

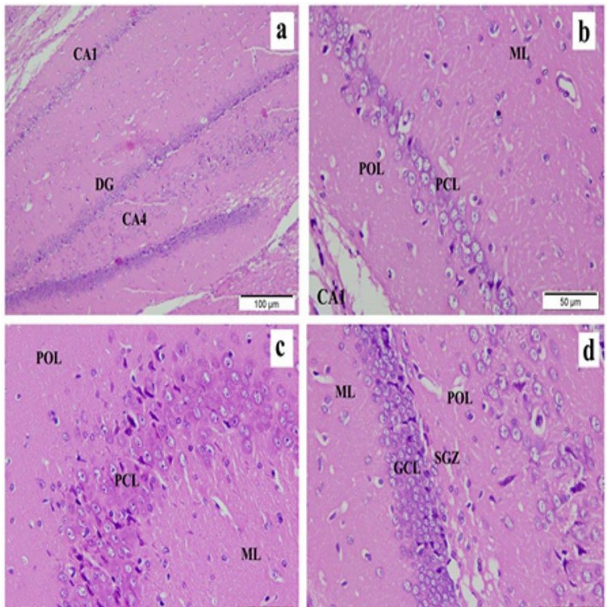

Fig. 4. Histopathological structure of hippocampus of L-A+MSG treated group. MSG+L-A treated group showing: (a) section through the hippocampus showing normal hippocampal region. (b), the CA1 region shows minimal degeneration and vacuolation of some pyramidal and glial cells. (c), the CA3 region shows normal large pyramidal neurons. (d), dentate gyrus showing mild focal vacuolation in sub granular zone (Hematoxylin and Eosin stain).

L-A+MSG treated rats, resulted in a marked amelioration of the histological alterations observed in the brain that were induced by excess MSG administration as evidenced by a decrease of degenerative changes of minimal neuronal cells, mild focal degeneration, vacuolation, and edema of some glial cells were observed (Fig. 4) and mild degenerative changes in the cerebral cortex (Fig. 5). These were confirmed by work done by Hami *et al*. (2015), these observed curative effects of L-A on the neurotoxicity of excess MSG could be attributed to increased anti-oxidative enzymes (Elbassuoni *et al*., 2018) and attenuation of oxidative stress and malonaldehyde in brain tissues (Hami *et al*., 2015) as well as scavenging of free radicals (Lass *et al*., 2002).

The control and L-A groups revealed normal immunoreactivity for S-2A receptors in both the cerebral cortex and hippocampus. At the same time, the MSG-treated group showed negative immunostaining either in the cortex or hippocampus. Moderate positive reactions were observed in both L-A+ MSG groups (Fig. 6). The Immunoreactive parts percentage (IRP%) of S-2A receptors showed no significant ($p < 0.05$) difference in the cerebral cortex and hippocampus of control and L-A groups. The cerebral cortex and hippocampus of MSG-treated rats exhibited a significant ($p < 0.05$) reduction in S-2A IRP% than control. There were significant ($p < 0.05$) improvements in the S-2A IRP% of the L-A+MSG group than MSG- treated group (Fig. 8).

MSG-treated rats exhibited a significant decrease in brain S-2A receptors IRP% (Fig. 8) which is a crucial catecholaminergic neurotransmitter playing a vital role in dominating numerous body functions, it regulates body posture, emotion, behavior, cognition, and motor functions (Meneses and Liy-Salmeron,

2012). The present results agreed with Albrakat, (2023). Our results revealed that the administration of L-A with MSG had an ameliorated effect on serotonin receptors in the brain, these results are in line with Mahmoud *et al*. (2021) while Tran *et al*. (2020) found that L-A did not affect serotonin levels in different regions of the brain of 5-day-old chicks. The explanation of our results is the effect of L-A on serotonin could be attributed to its role as a precursor of NO production (Abdel Baky *et al*., 2010). NO is an important intercellular messenger in the brain and has been found to increase the release of S-2A in different brain regions (Philippu, 2016).

The immunohistochemical detection of caspase-3 in brain tissue of different groups showed that the expression of caspase-3 protein in the cerebral cortex CA1, CA3, and dentate gyrus of hippocampus tissue of the control and L-A groups was mild, and no difference between the two groups. MSG-treated rats showed a strong reaction for caspase-3 protein expression in the cerebral cortex, CA1, CA3, and dentate gyrus. However, treatment of rats with L-A+MSG showed a reduction in caspase-3 protein expression in the cerebral cortex, CA1, CA3, and dentate gyrus (Fig.7). The IRP% of caspase-3 receptors showed no significant ($p < 0.05$) difference in the cerebral cortex and hippocampus of the control and L-A groups. The cerebral cortex and hippocampus of MSG-treated rats exhibited a significant ($p < 0.05$) elevation in caspase-3 IRP% than control. There was a significant ($p < 0.05$) reduction in the caspase-3 IRP% of the L-A+MSG group to the MSG-treated group (Fig.8).

Our result revealed that MSG causes an increase in caspase-3 in the brain and a significant ($p < 0.05$) (Fig.7) and elevation in caspase-3 IRP% (Fig.8), caspase-3 has been identified as a key mediator of apoptosis (Shalini *et al*., 2015), this explanation coincided with apoptosis that was observed in the histopathological section of this study, this result collaborated with John *et al*. (2022), contradicted the study of Mathew and Keerikkattil. (2021) found that insignificant downregulation of caspase-3 after S/C treated rats with MSG for 7 days. Excessive ROS production

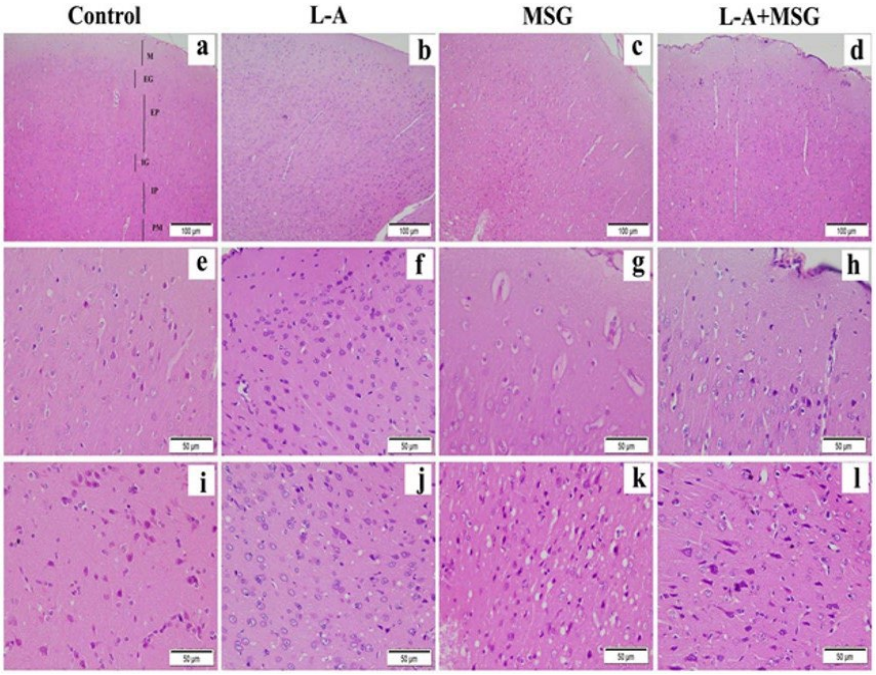

Fig. 5. Histopathological structure of cerebral cortex of control, L-A, MSG, and L-A+MSG -treated groups. The cerebral cortex of control and L-A groups revealed a normal histological structure and cell arrangement of all six cerebellar layers. The MSG group showed numerous degenerations of pyramidal and granule neurons with vacuolations along with focal microglial infiltration. The MSG+L-A group showed mild degrees of degenerative changes with astrocytosis (Hematoxylin and Eosin stain).

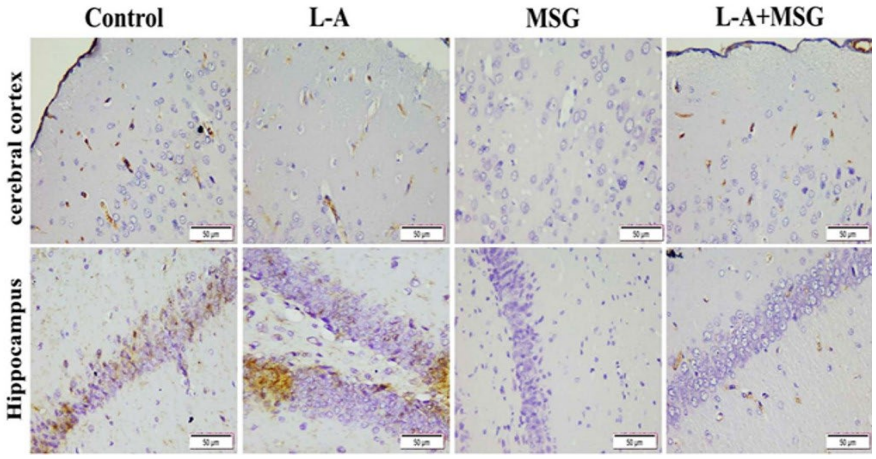

Fig. 6. Immunohistochemical staining of the cerebral cortex and hippocampus with serotonin (S-2A) receptors. Immunohistochemical staining of the cerebral cortex and hippocampus with serotonin (S-2A) receptors. Positive brownish immunoreactive perikarya in control and L-arginine (LA) groups. Negative staining was evident in the Monosodium glutamate (MSG) group. Improvements and promotion of immunostaining were observed in the L-A + MSG-treated group [anti-S-2A × 400].

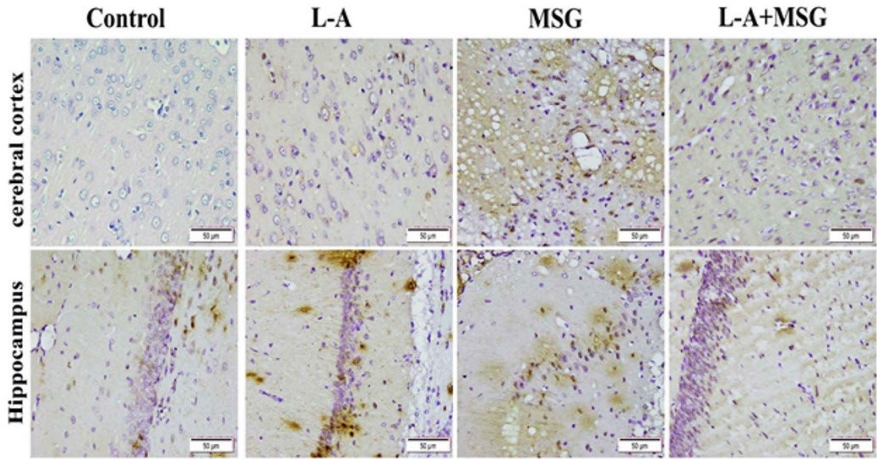

Fig. 7. Immunohistochemical staining of the cerebral cortex and hippocampus with caspase 3. Immunohistochemical staining of the cerebral cortex and hippocampus with caspase 3. The figure shows weak cytoplasmic immune staining reactions in all areas in the control and L-A groups. The MSG group showed a strong positive cytoplasmic immune staining reaction in the cerebral cortex, Cornu Ammonis (CA1, CA3), and the granular and polymorphic layers of the dentate gyrus region of the hippocampus with the appearance of dark brown areas, whereas the L-A +MSG-treated group showed a moderate reaction (caspase-3 X400)

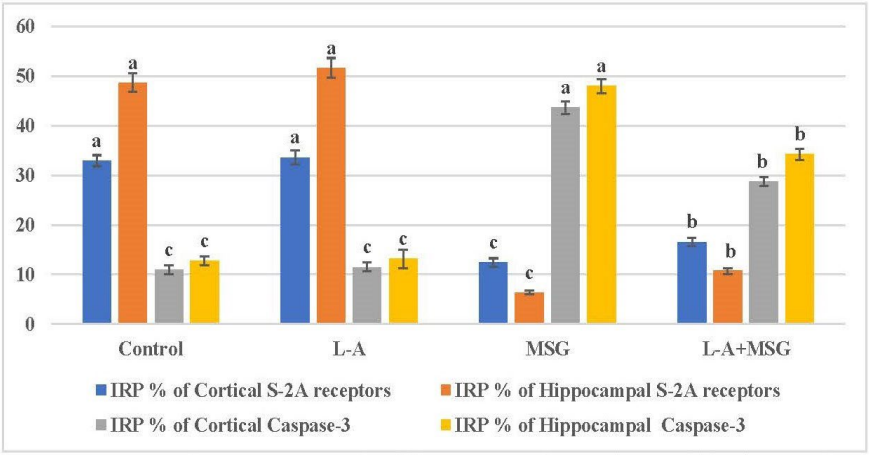

Fig. 8. Immunoreactive parts percentage (IRP%) of cortical and hippocampal S-2A receptors and caspase-3. Data were represented as mean ± SE. The different superscripts (a, b, c), within the same bar, were significantly differed at $P < 0.05$.

and inflammatory response of MSG trigger apoptosis (Xie *et al.*, 2017). Our results revealed that a reduction in caspase-3 protein expression in the cerebral cortex, CA1, CA3, and dentate gyrus and a significant ($p < 0.05$) reduction in the caspase-3 IRP% of the LA+MSG group (Fig.7), this mean L-A decrease apoptosis. The observed anti-apoptotic effects of L-A observed in this study came by those of Attia *et al.* (2020) who reported that pre-treatment with L-A successfully attenuated the abnormalities in caspase-3, BAX, Bcl-2, and BAX/ Bcl-2 ratio reflecting their prophylactic effect against MSG-induced apoptosis. Also via the antioxidant and anti-inflammatory properties of L-A, it enhances neuron integrity and viability that may be hypothesized to be related to diminished neural loss and apoptosis (Mahmoud *et al.*, 2021).

CONCLUSION

Treatment with L-arginine 500 mg/Kg BW -1 ameliorates the neurophysiological effects of MSG through improving memory, decreasing oxidative stress, increasing antioxidant enzymes, increasing dopamine, improving histoarchitecture of the brain, improving serotonin receptors and decreasing apoptosis ameliorating caspase-3 protein in the brain.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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