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Original article

The Possible Protective Effects of Melatonin and/or Vitamin C on Monosodium Glutamate-induced Hippocampal Neurotoxicity in Male Albino Rats: A Histological and Immunohistochemical Study

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Abstract

Background: The hippocampus, a key region for learning and memory, is highly susceptible to excitotoxicity from excessive glutamate receptor activation, leading to neuronal injury or death. Monosodium glutamate (MSG), a common food additive, has been associated with various neurotoxic and neurodegenerative effects. **Materials and Methods:** Adult rats were used to evaluate the protective effects of vitamin C and melatonin against MSG-induced hippocampal damage. Sixty rats were divided into five groups: control, MSG (2 g/kg), VC (MSG + 100 mg/kg vitamin C), ML (MSG + 10 mg/kg melatonin), and ML+VC (MSG + both vitamin C and melatonin). Treatments were administered orally for 28 days. Hippocampal tissues were collected for histological and immunohistochemical analysis of GFAP, TNF-α, and caspase-3 expressions. Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) of Benha University, Egypt. **Results:** MSG exposure significantly increased GFAP, TNF-α, and caspase-3 expression, indicating neuroinflammation, apoptosis, and marked histological alterations in the CA1 region. In the VC group, some pyramidal neurons were distorted with scattered glial cells and vacuolations, whereas in the ML group, pyramidal neurons were more densely packed with minimal glial pyknosis and vacuolations. Combined melatonin and vitamin C treatment largely preserved hippocampal architecture and markedly reduced inflammatory and apoptotic markers. **Conclusion:** MSG exerts pronounced neurotoxic effects in the rat hippocampus. Co-administration of vitamin C and melatonin produced a synergistic neuroprotective effect, highlighting their potential to mitigate MSG-induced hippocampal damage.

Key words: Melatonin, Vitamin C, hippocampal neurotoxicity

Introduction

Certain food additives have been shown to exert adverse effects on brain function, among which monosodium glutamate (MSG)—designated as E621—is one of the most widely used [(1)]. MSG is commonly added to foods such as sauces, soups, puddings, and various mixed condiments to enhance flavor. Although glutamate, a component of MSG, is a nonessential amino acid and the principal excitatory neurotransmitter in the central nervous system, elevated glutamate levels have been linked to the generation of reactive oxygen species (ROS), oxidative stress, and excitotoxic neuronal injury [(2)]. In experimental animals, MSG exposure has been associated with irreversible cell damage, inflammation, and even male reproductive toxicity [(3)], in addition to neurotoxic effects, such as neuropathic pain and retinal degeneration [(4)].

The hippocampus, a brain region vital for learning and memory, is particularly vulnerable to excitotoxicity due to its high density of glutamate receptors. Excessive glutamatergic stimulation in the hippocampus may result in oxidative damage and cell death [(5)]. Excitotoxicity has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), where it contributes to progressive synaptic dysfunction and neuronal loss [(6)].

Vitamin C (ascorbic acid) is a potent water-soluble antioxidant found in fruits and vegetables. It plays a crucial role in scavenging a wide range of reactive species, including superoxide, hydroxyl, and peroxyl radicals [(7)]. Studies have indicated that vitamin C may enhance memory function, reduce cognitive impairment in models of neurodegeneration, and alleviate oxidative damage in neuronal tissues [(8)].

In recent years, interest has grown in the use of antioxidants to counteract glutamate-induced neurotoxicity. Melatonin (N-acetyl-5-methoxytryptamine), a neurohormone secreted by the pineal gland, possesses strong antioxidants, anti-inflammatory, and metal-chelating properties [(9)]. Melatonin has been shown to neutralize free radicals and reduce oxidative stress in brain tissues, thereby offering neuroprotection in various pathological models [(10)].

Despite individual reports on the protective roles of melatonin and vitamin C, few studies have compared their relative neuroprotective effects against MSG-induced hippocampal damage. Therefore, the current study aimed to investigate the histological effects of MSG on the hippocampus and to assess the potential protective roles of melatonin and vitamin C in mitigating MSG-induced neurotoxicity.

Materials and Methods

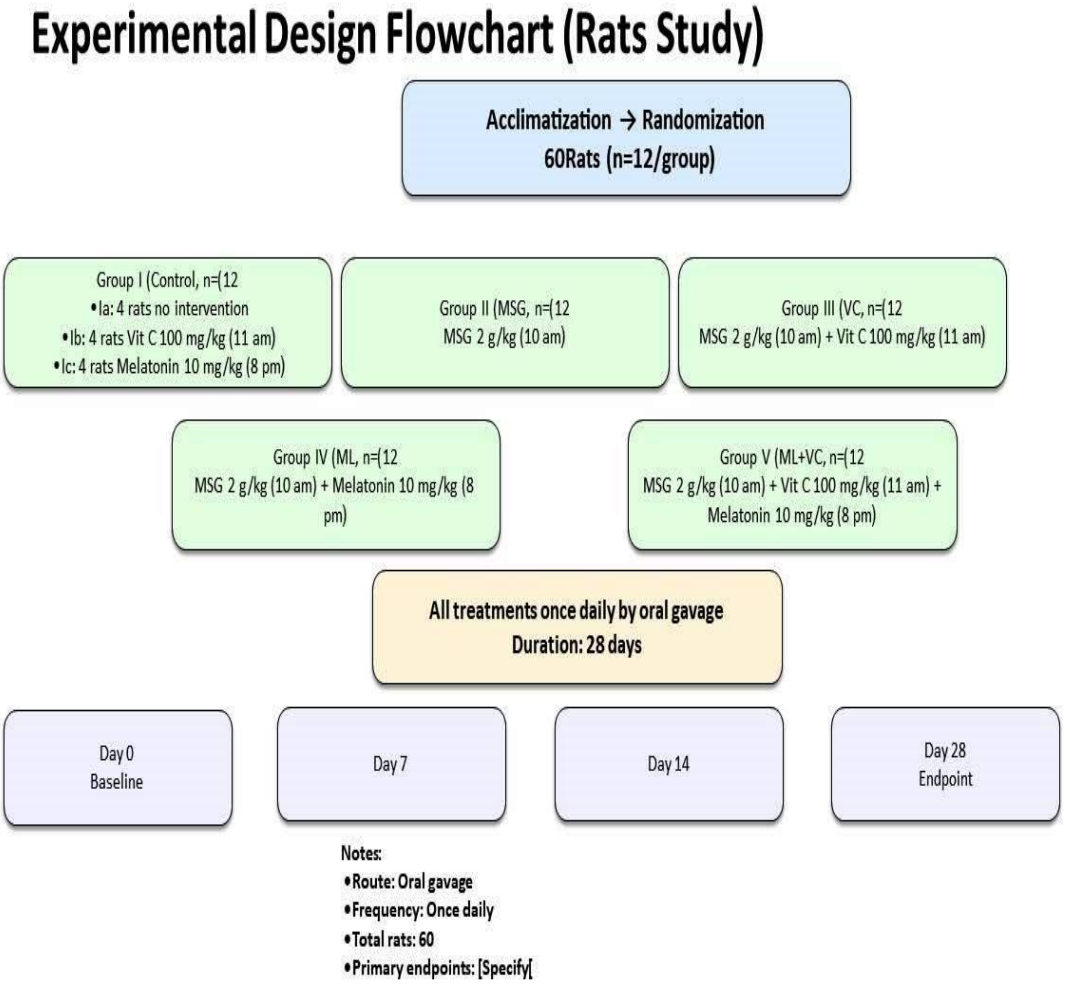
Chemicals

Monosodium glutamate: 99% pure powder (CAS: 6106-04-03; Fisher Scientific Co. LLC, Pittsburgh, PA, USA) was acquired. Distilled water was used to dissolve it.

Vitamin C was purchased from local pharmacy and came in the form of 500 mg

C Retard Ascorbic acid capsules (Hikma Pharmaceuticals Company). Distilled water was used to dissolve each crushed one.

The melatonin has been purchased from Nature's Bounty, USA Company. Just prior to administration, the tablets containing 3 mg of melatonin was crushed and dissolved in 1% ethanol. Numerous investigations have reported that melatonin can be dissolved in 10% ethanol without causing any toxicity [11, 12]. This was an in vivo, controlled laboratory-based experimental study included sixty mature male albino rats (150- 210 grams) which was conducted in September–October 2023. They were obtained from the Animal House-Faculty of Medicine at Cairo University and treated in accordance with the rules after ethics committee permission from Benha University (RC 31-9-2023). To reduce nonspecific stress on the experiment days, all animals were handled for a week before the experiment with standard food and water and housed at room temperature with a 12:12 light: dark cycle.



Rats were randomly assigned to five groups (n = 12) randomly after the acclimatization period. The five groups were:

Group I (Control Group)

Subgroup Ia: four rats that had no intervention.

Subgroup Ib: four rats were received 100 mg/kg b.w. of vitamin C in the morning (11:00 am) ([13].

Subgroup Ic: four rats received that received melatonin 10 mg/kg b.w. body at night (8:00 pm) [14].

Group II (MSG group): twelve rats were administrated MSG (2 g/kg b.w.) in the morning (10:00 am) [15].

VC group (VC group): twelve rats that were given MSG (2 g/kg b.w.) in the morning (10:00 am) [15] and given 100 mg/kg b.w. of vitamin C after one hour [13].

Group IV (ML group): twelve rats that were given MSG (2 g/kg b.w.) in the morning (10:00 am) [15] and received 10 mg/kg b.w. of melatonin at 8:00 pm [14].

Group V (ML+ VC group): twelve rats that were given MSG (2 g/kg b.w.) at 10:00 am [15] and given 100 mg/kg b.w. of vitamin C after one hour [13] and in combination with 10 mg/kg b.w. of melatonin at 8:00 pm [14].

Rats of all experimental groups were received MSG, vitamin C, and melatonin once a day for 28 days by oral gavage.

Histological and Immunohistochemical Analysis

At the end of the experiment (23rd October 2023), rats were given ether anesthesia, and they were sacrificed to harvest their brains before being divided in half along the median sagittal line. According to Suvarna, Layton, and Bancroft [16] the cerebral hemispheres were embedded in paraffin blocks, dried in ethanol at varying percentages, and fixed for 24 hours in 10% neutral buffered formalin. After that, 5 μm thick sections of the brain were cut out and stained with hematoxylin and eosin (H&E).

For immunohistochemical staining, successive 5 μm thick paraffin sections of the cerebral cortex were mounted on positively charged slides and incubated overnight at 37 °C. After deparaffinization, rehydration, and antigen retrieval, the sections were incubated with the primary antibodies against GFAP (Cat. No: MA5-33059, Rockford, USA), TNF-α (Cat. No: BS-10802R, Bioss, Boston, USA), and caspase-3 (Cat. No: BS-2593R, Bioss, Boston, USA) at a dilution of 1:100 overnight at 4 °C according to technique described by Jackson and Blythe [17]. The slides were then rinsed in PBS and incubated with the corresponding biotinylated secondary antibody (1:200) for 1 h at room temperature, followed by visualization with DAB substrate. Positive immune reactions appeared as brown staining of astrocytes for GFAP, and brown cytoplasmic staining for TNF-α and caspase-3.

A light microscope (Olympus CX 41, Japan) and an attached camera (Olympus E 330, Japan) were used to analyze and

record H&E and immunohistochemical sections at Benha University, Egypt's Faculty of Medicine's Anatomy Department. For each group, twelve hippocampal sections were analyzed, spaced at regular intervals to represent the entire hippocampus. All analyses, including histological and immunohistochemical assessments, were performed by investigators blinded to the treatment groups to prevent bias.

Morphometric study and Statistical Analysis

Version 6.0 of the Image-Pro Plus software (Media Cybernetics Inc., Bethesda, Maryland, USA) was utilized to compute the mean area percentage of GFAP, TNF- α and caspase 3 immune expressions in eight images of hippocampus from eight non-overlapping fields of each group rats at 400× magnification.

All data, including the mean area (%) of positive immunohistochemistry expression of GFAP, TNF- α and caspase 3 were displayed as mean±SD for each group using IBM SPSS Statistics software for Windows, Version 22 (IBM Corp., Armonk, NY, USA). Using the Post Hoc LSD test and one-way analysis of variance (ANOVA), the differences between the groups of morphometric findings were examined. Standard deviation (SD) and mean (M) values were represented, and any variation was considered significant at $P< 0.01$.

Results

Hematoxylin and Eosin staining

Control group showed typical hippocampal histology architecture. It is composed of two main parts: the V-shaped dentate gyrus (DG) that encircles CA4 (fig 1a) and the C-shaped hippocampal portion known as CornuAmmonis (CA), which includes CA1, CA2, CA3, and CA4.A higher magnification of the hippocampus CornuAmmonis region revealed three distinct layers: the inner molecular cell (ML) layer displayed branching pyramidal cell apical dendrites and light deeply stained glial cell nuclei; the outer polymorphic layer (PoL) showed small light and deeply stained glial cell nuclei; and the middle pyramidal cell layer (PyL), which is the main layer and displayed rows of spherical, densely packed cell bodies with noticeable nucleoli and big vesicular nuclei. Note the presence of the sporadic blood vessels in the background (fig. 1b).

The PyL layer was disorganized and thinner in Group II, and most pyramidal cells were irregularly shrunken and faintly stained with pericellular haloes, while some had darkly stained nuclei. Throughout the three levels, scattered pyknotic glial cells with pericellular haloes were seen. Additionally, the vacuolated surrounding neuropil showed dilated blood capillaries (fig. 1c).

VC group revealed some more or less normal PyL cell bodies and glial cells while other PyL cell bodies looked distorted, faintly stained and absent in a few places. Pericellular haloes around some glial cells were noticed. Vacuolations and dilated blood capillaries were found (fig 1d).

ML group had normal pyramidal cell bodies. There were glial cells strewn about, some of which were pyknotic. Additionally, a few vacuolations and dilated blood capillaries were found (fig 1e).

Rats in ML+VC group, which received a combination of melatonin and vitamin C, displayed marked improvement and maintained architecture of molecular (ML), pyramidal (PyL), and polymorphic cell layers (PoL) of the CA layers. (fig 1f).

II- Immunostaining results

There were a few tiny, widely spaced GFAP-positive astrocytes with short, thin processes in the control group, Whereas MSG group showed numerous, large sized astrocytes with long thick processes and strong reaction expression.VC group and ML group showed fewer small sized astrocytes with low reaction expression.ML+VC group showed scattered small sized GFAP +ve cells (fig 2a-e). There was a significant increase in mean area % of GFAP immune reaction of MSG group (10.04% ± 0.51) compared with control (0.44% ± 0.04, $p < 0.01$), VC (2.95% ± 0.37, $p < 0.01$), ML (1.20% ± 0.05, $p < 0.01$) and ML+ VC (0.58% ± 0.03, $p < 0.01$) groups. There was also significant increase in the mean area % of GFAP immune reaction of VC group and ML group compared to control group ($p < 0.01$) (table 1, fig 2f).

In hippocampus layers, Control group displayed a weak TNF-α immune stain, while MSG group displayed a strong TNF-α immune stain. Immune reaction was mild in VC and ML groups. Weak immune reactivity was displayed by ML+VC group (fig. 3a-e). The mean area % of TNF-α immune response in MSG group (7.64% ± 0.34) was significantly higher than that of the control (0.17% ± 0.02, $p < 0.01$), VC (1.89% ± 0.06, $p < 0.01$), ML (1.68% ± 0.09, $p < 0.01$), and ML+VC (0.92% ± 0.09, $p < 0.01$) groups.

Additionally, the mean area % of TNF-α immune reactivity in VC, ML+VC groups were significantly higher compared with control group ($p < 0.01$) (table 1, fig. 3f).

In Caspase-3 Immunohistochemical sections, cells of hippocampus exhibited no cytoplasmic immune reactivity in control group, while the cells displayed an elevated level of cytoplasmic immune reactivity for caspase-3 in MSG group. Cytoplasmic immunological reactivity was moderate in groups VC and ML. While mild Caspase-3 Immuno-histochemical staining is observed within the cells of ML+VC group (fig. 4a-e).

The mean area % of Caspase-3 immune response in MSG group (13.22% ± 0.35) increased significantly than that of the control (0.08% ± 0.02, $p < 0.01$), VC (5.85% ± 0.15, $p < 0.01$), ML (3.92% ± 0.16, $p < 0.01$), and ML+VC (0.56% ± 0.03, $p < 0.01$) groups. Additionally, the mean area % of Caspase-3 immune reactivity in VC, ML as well as ML+VC groups showed significantly increased comparing to the control group ($p < 0.01$) (table 1, fig. 4f).

Table 1: displaying the average percentage and standard deviation of GFAP, TNF, and caspase-3 immune response for each group, along with a comparison of each group using the Post Hoc LSD test.

Mean area % ± SD	Control group	MSG group	VC group	ML group	ML+VC group
GFAP immune reaction	0.44% ± 0.04^{b,c,d}	10.04% ± 0.51^{a,c,d,e}	2.95% ± 0.37^{a,b,d,e}	1.20% ± 0.05^{a,b,c,e}	**0.58% ± 0.03^{b,c,d}**
TNF immune reaction	0.17% ± 0.02^{b,c,d,e}	7.64% ± 0.34^{a,c,d,e}	1.89% ± 0.06^{a,b,e}	1.68% ± 0.09^{a,b,e}	**0.92% ± 0.09^{a,b,c,d}**
caspase-3 immune reaction	**0.08% ± 0.02^{b,c,d,e}**	**13.22% ± 0.35^{a,c,d,e}**	**5.85% ± 0.15^{a,b,d,e}**	**3.92% ± 0.16^{a,b,c,e}**	**0.56% ± 0.03^{a,b,c,d}**

Data are represented as mean ±SD ($p < 0.01$)

a Significantly with control group

b Significantly with MSG group

c Significantly with VC group

d Significantly with ML group

e Significantly with ML+VC group

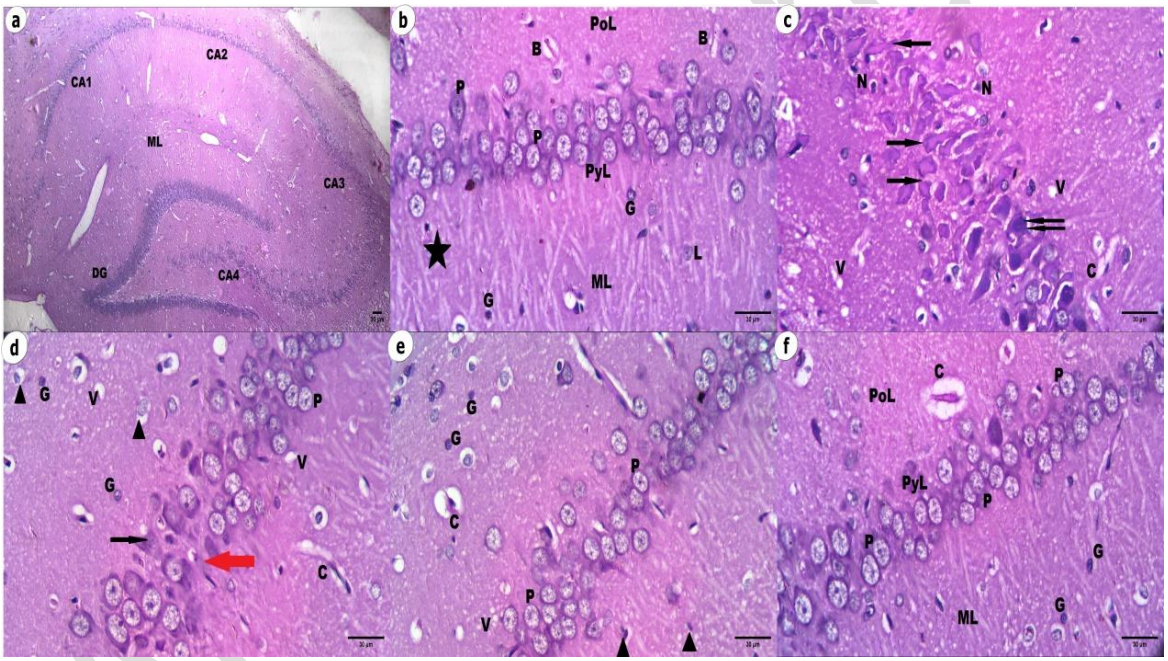


Fig.1: Representative pictures of sections from CA1 of the hippocampus of different studied groups
(A) Control group section showing hippocampus (CA) as CA1, CA2, CA3& CA4 regions and the V-shaped dentate gyrus (DG) around CA4. Notice the Molecular layer (ML) is in between DGand CA **(H&E X100)**.
(b) A Higher magnification of Control group section showing the well-defined appearance of three layers of the CA1: molecular (ML), pyramidal (PyL) and polymorphic cell layers (PoL). PyL shows closely packed rounded cell bodies with scanty basophilic cytoplasm, large vesicular nuclei, and prominent nucleoli (P).The PoL and ML display light (L) and deeply stained nuclei of glial cells (G) with scattered blood capillaries (B) within the pink stained neuropil (☆).
(c) **MSG groupshowing** disarrangement and decreased thickness of the PyL layer . Most of pyramidal cells are distorted , shrunken faint stained with pericellular haloes (↑) while others have darkly stained nuclei (↑↑). Notice, scattered pyknotic glial cells with pericellular haloes (N) and dilated blood capillaries (C) within the vacuolated surrounding neuropil (V).
(d)**VC groupshowing** some PyL cell bodies appears more or less normal (P) while others appear shrunken, irregular and faint stained (↑). Areas of pyramidal cell loss are seen (red ↑). Notice scattered normal glial cells (G) while some are still surrounded with pericellular haloes (►).Dilated blood capillaries (C) and some vacuoles (V) are detected.

(e) **ML groupshowing** somewhat closely packed apparently normal pyramidal cell bodies (P). Scattered normal glial cells (G) with some of them are pyknotic with pericellular haloes (▶). Dilated blood capillaries (C) and few vaculations are detected also (V)
(f) **ML+VC grouprevealing** normal appearance of CA layers; molecular (ML), pyramidal (PyL) and polymorphic layers (PoL). Normal pyramidal cells with vesicular nuclei (P) and glial cells (G) can be seen. Notice, dilated blood capillaries (C).**(H&E X400)**

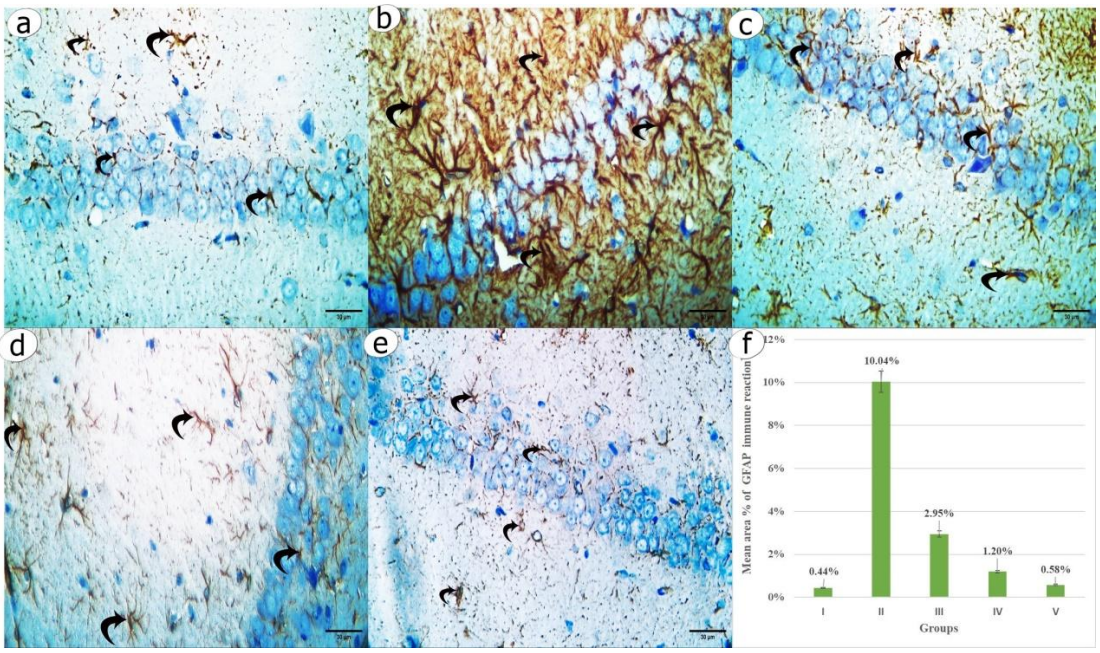


Fig 2: Representative pictures of GFAP immune stained sections of hippocampus ofrats from experimental groups:(a) Control group showing few, small sized astrocytes and their short thin processes (curved arrows).**(b) MSG group showing** strong +ve GFAP hypertrophied astrocytes with multiple elongated processes (curved arrows). **(c) VC group showing** apparently smaller sized astrocytes with less GFAP expression (curved arrows). **(d) ML groupshowing** apparently decreased number and size of astrocytes with low reaction expression (curved arrows). **(e) ML+VC groupshowing** scattered small thin GFAP +ve astrocytes with short processes (curved arrows). **(f)** the mean area % and SD of GFAP immune reaction in different groups. **(GFAP X400)**

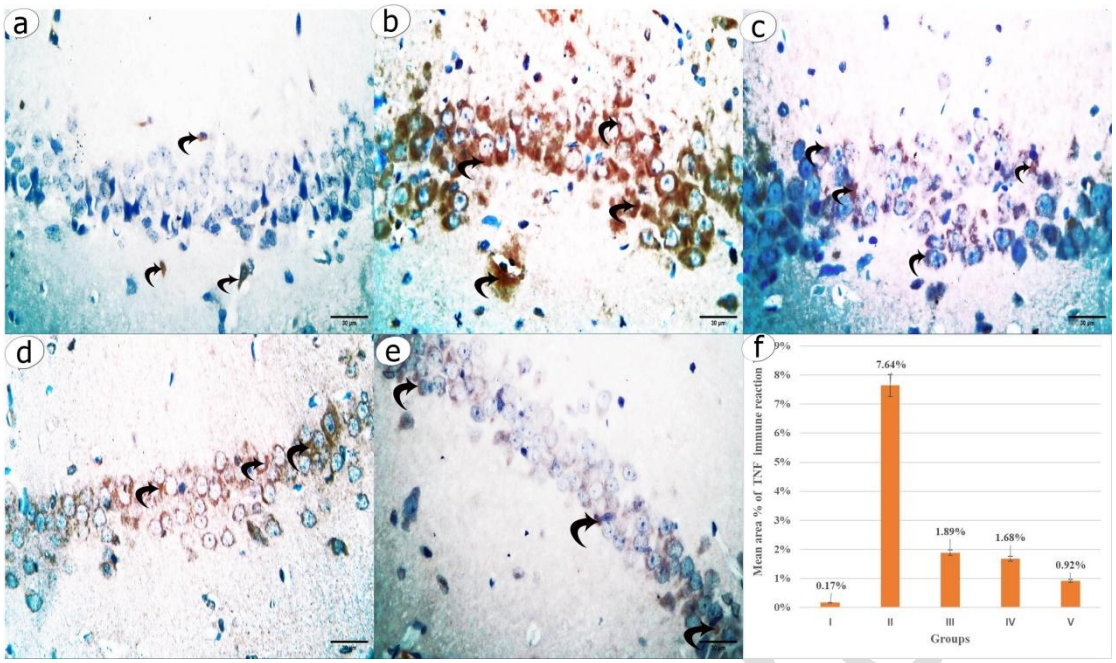


Fig 3: Representative pictures of TNF-α immunostained sections of hippocampus ofrats from experimental groups:**(a) Control group showing** faint immune reactivity in all hippocampus layers (curved arrows). (b) **MSG groupshowing** intense immune reactivity for TNF-α in cells (curved arrows). **(c) VC group showing** mild immune reactivity (curved arrows). (**d) ML group showing** decreased TNF-α expression (curved arrows). **(e) ML+VC group showing** weak immune reactivity (curved arrows). **(f)** The mean area % and SD of TNF-α immune reaction in different groups. **(TNF-α X400)**

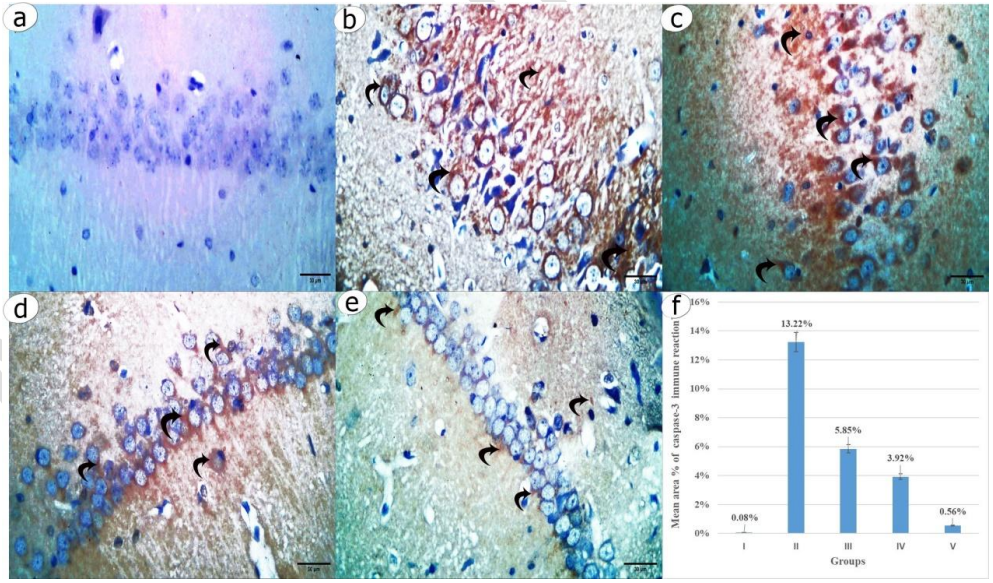


Fig 4: Representative pictures of Caspase-3 immunostained sections of hippocampus of rats from experimental groups:(a) Control group showingexhibited no cytoplasmic immune reactivity in the cells (curved arrow). **(b) MSG groupshowing** strong cytoplasmic immune reactivity for caspase-3 (curved arrow). **(c) VC groupshowing** moderate cytoplasmic immune reactivity (curved arrow). **(d) ML groupshowing**decreased cytoplasmic immune reactivity (curved arrow).**(e) ML+VC groupshowing weak** cytoplasmic immune reactivity for caspase-3 in the cells (curved arrow). **(f)** The mean area % and SD of **Caspase-3** immune reaction in different groups. **(Caspase-3 X400)**

Discussion

Monosodium glutamate activates the central nervous system's ionotropic and metabotropic glutamate receptors. Excitotoxicity and neuronal death had resulted from over activation of these receptors [18]. This study's objective was to assess the potential protective effects of melatonin and vitamin C on the hippocampal region.

It is widely believed that the C-shaped structure, which represents the hippocampus proper, looks like the horns of Ammon [19]. According to Gori H P, et al [20], the current study demonstrated that hippocampus could be separated into four portions, CA1, CA2, CA3, & CA4, depending on the pyramidal cells' size and density. The stratum pyramidal, or CA1, is really made up of four to six layers of big neurons. The most susceptible and vulnerable zone to oxidative stress and other problems is CA1 [21].

Histological analysis of hippocampal slices in MSG group indicated degenerative alterations and disarray in the pyramidal and granule cells of CA1 with pale-staining nuclei and pericellular haloes with agreement with previous studies [21]. The necrosis, death, and shrinking of cells departing pericellular spaces may account for the halo of empty spaces around neuronal cells. Another research revealed a similar finding [22]. Cytoskeletal affection causes pericellular spaces, which are followed by cell shrinkage and process withdrawal. Auer and Sutherland [23] observed vacuolation in the surrounding neuropil because of this. MSG-induced apoptosis may be the cause of cell shrinkage and acidophilic cytoplasm in the same groups. Denatured proteins and degenerated cells accumulated due to uncompensated oxidative stress and the buildup of free radicals caused by the antioxidant systems' failure [15].

Sections of the MSG group stained with GFAP in the current investigation showed increase in star-shaped astrocytes exhibiting strong GFAP immune response. Glial cells maintain the integrity of the central nervous system and shield neurons from harm brought on by stress and enhance neuronal survival under various pathological circumstances. It has been demonstrated that an increase in astrocyte immunoreactivity frequently follows toxic insults because astrocytes share a significant percentage of glutamate homeostasis and are essential for the reuptake of free glutamate and the avoidance of glutamate excitotoxicity [23]. In addition to astrocyte reactivity in the form of glial process hypertrophy, the excitotoxicity phenomenon is accompanied by a rise in astrocytic GFAP staining and astrocytic proliferation [24].

In this work, TNF-α- stained sections of MSG group showed star-shaped astrocytes with strong immune response. TNF-α is a neurotoxic substance that induces NO and free radicals in neuronal cells, which results in both direct and indirect neuronal cell death [25]. Additionally, it has been found that exposure to elevated glutamate raises TNF-α level in intestinal and brain tissues [26]. It has been proposed that inflammation of neural cells breaks down the blood-brain barrier, allowing more MSG to enter and causing more damage and inflammation [27].

Apoptosis is an essential physiological process, and caspase-3 is recognized as a key participant in the apoptotic pathway [28]. However, exposure to neurotoxic

chemicals can have detrimental consequences on neuronal cells, such as increased formation of reactive oxygen species (ROS), which in turn promotes the release of cytochrome C, which in turn activates caspase-3 and gradually increases the death rate of neuronal cells. DNA fragmentation results from the activation of cytoplasmic endonucleases by caspase-3, which breaks down nuclear materials and proteins [29]. According to our research, rats given MSG showed increased caspase-3 immunoreactivity in their hippocampal neuronal cells. This was corroborated by a marked increase in the TNF-α immune reaction which causes neurotoxicity and cellular damage. An analogous outcome has been documented by other authors [25, 30]. Increased caspase-3 expression and worsened apoptosis-induced mortality may result from MSG-induced oxidative stress, inflammation, and DNA damage [31].

Rats given vitamin C together with MSG in our study had improved hippocampal histology when compared to the MSG group. This finding is in line with Hashem H E et al [32], who suggested that after MSG-induced neurotoxicity, vitamin C preserved the structure of the cerebellar cortex histologically, except for a few Purkinje cells with aberrant shapes. Furthermore, Pavlovic V and Sarac M [18] suggest that vitamin C may significantly reduce the oxidative stress and apoptosis that MSG causes in the thymus. According to previous work, vitamin C mitigates thiomersal-induced neurotoxicity in the cerebral cortex by increasing antioxidant levels and decreasing apoptotic cell counts [33].

According to our findings, those that received vitamin C are associated with low GFAP immunoexpression, which was accompanied by neuron preservation. This is consistent with earlier research showing that vitamin C stimulates astrocytes to shield cerebellar tissue from the harmful effects of MSG [34]. This result is consistent with previous observation that ascorbic acid can maintain membrane transport function during a hyperoxic episode and prevent astrocytic cell enlargement [35].

According to our results, vitamin C treated group showed mild to moderate TNF-α and caspase 3 activity. This was consistent with the findings of Ismael Z M and Elsamman W N [36], who found that vitamin C reduced apoptosis and the inflammatory response in renal glomerulus and tubule cells in azithromycin-induced nephrotoxicity. Additionally, preliminary research revealed that vitamin C delivery reverses the effects of MSG therapy in brain tissue by lowering TNF-α expression [37].

The melatonin-treated group demonstrated histological features that were identical to those of the control group. This observation aligns with earlier reports [38] that highlighted the protective effect of melatonin against Al_2O_3 nanoparticle–induced neurotoxicity in the brain and hippocampal regions. Likewise, melatonin administration was shown to markedly reduce hippocampal edema and the number of pyknotic neurons in cisplatin-treated rats [39]. The neuroprotective potential of melatonin is attributed to its strong lipophilic nature, which enables it to readily penetrate subcellular compartments. Its accumulation within the nucleus and mitochondria allows melatonin to safeguard nuclear DNA and cytosolic molecules from free radical–induced damage [9].

The melatonin-treated group in this study exhibited a favorable immunological response to GFAP of star-shaped astrocytes with thin processes and tiny bodies. This is in line with prior research that discovered no statistically significant variations in the mean area % of GFAP reaction between the control group and the group treated with melatonin and tramadol [(40)].

The mean area % of caspase 3 and TNF in the hippocampus significantly reduce in the ML group which is in line with earlier research [(14)]. Melatonin has a neuroprotective effect because of its special interactions with certain receptors that support the upkeep of neuronal cells and by regulating inflammation and apoptosis after various forms of brain damage [(41)].

However, what is particularly novel in our work is the demonstration that their combined administration provided superior protection compared to either therapy alone. Histological analysis revealed that rats treated with both agents maintained hippocampal architecture like that of controls, while molecular analysis showed a more pronounced reduction in TNF-α and caspase-3 expression under the combined regimen. The synergistic effect observed may be attributed to complementary mechanisms as melatonin protects mitochondrial integrity and suppresses apoptosis, while vitamin C scavenges free radicals and reduces oxidative stress. Together, these actions account for the significant reduction in TNF expression and caspase-3 activity in the treated groups.

These findings extend earlier observations of vitamin C and melatonin as protective agents in lung damage induced by sepsis [(42)] and highlight their promise as dual therapy for conditions associated with excitotoxic or oxidative hippocampal damage.

Conclusion

MSG administration induced marked hippocampal damage at both histological and immunohistochemical levels. Treatment with either vitamin C or melatonin alone provided partial neuroprotection, as evidenced by some preservation of hippocampal architecture and reduced expression of apoptotic and inflammatory markers. Notably, combined administration of melatonin and vitamin C offered superior protection, maintaining hippocampal structure while more effectively reducing GFAP, TNF-α, and caspase-3 expression compared to single treatments.

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Author contribution

Each author made an equal contribution to the study.

Conflict of interest

No conflicts of interest are disclosed by the authors.

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