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Study of Impact Monosodium Glutamate on Histology, and Oxidative Stress in Adult Female Rats and The Protective Role of Vitamine B12

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Abstract

Glutamate is one of the most exciting neurotransmitters in the brain. The current study investigates the effect of Monosodium glutamate (MSG) on Serum level of that include malondialdehyde MDA and Monosodium glutamate GSH levels. Histological changes in the brain, and protective effect of vitamin B12. Rats were divided into five equal groups n=10, each group was further divided into two groups (A and B, n=5) and treated for 30 and 60 days, respectively. The 1st group received distilled water orally by gavage, while 2nd and 3rd group received MSG in a dose of 25 and 45 mg/kg body weight orally by gavage. The 4th and 5th groups received MSG and vitamin B12 in a dose of 25 or 45 mg MSG/kg in addition to 0.3m vitamin B12/kg. Monosodium glutamate causes a significant histopathological change in brain tissue. However, MDE, and GSH were not significantly altered. These changes were reversed by treatment with vitamin B12. Degenerative, degeneration and necrosis of the supporting cells of astrocytes. There was an improvement in the levels of MDA and GSH as well as brain tissue.

Key words: Glutathione, Malondialdehyde, and Monosodium Glutamate.

1. Introduction

Monosodium glutamate, a sodium salt of glutamate is widely used as a food additive and Flavors enhancer and can be found in various concentrations in numerous food products [1]. Monosodium glutamate used in many foods such as frozen food, snack chips, canned food, and soup. As a taste enhancer, salad dressing [2].

MSG is used with trade names such as Ajinomoto Vetsin accent and tasting powder. It was made predominantly from wheat gluten but now from bacterial fermentation [3]. In developed nations, the typical daily MSG intake is 300-1000 mg per person. The intake depends on the amount of MSG in the diet and the taste preferences of the individual [4]. MSG is the sodium salt of glutamate (simply

glutamate, water and sodium) [5] and is widely used as a food additive and as flavouring agent to increase appetite [6] due to the presence of sodium ion and the appetizer effect increased by the presence of glutamate ion on gustatory nerve [7].

MSG has been linked to endocrine abnormalities, retinal degeneration, urticarial obesity, brain damage, neuropathic pain, anxiety, amyotrophic lateral sclerosis and Parkinson's disease, according to previous research [8]. Excessive consumption of monosodium glutamate has been reported to cause oxidative stress on the brain, liver and kidneys resulting in increased production of reactive oxygen species (ROS) [9].

MSG also affects mitochondrial lipid peroxidation (LPO) and antioxidant status in the cerebral hemispheres, brain stem, cerebellum, and diencephalon [10]. pathological changes occur in the brain tissue, which are vacuolated cells, low nuclei, low density of neurons, and distorted layers in the brain tissue [11].

Antioxidants are the body's first line of protection against free radical damage and are necessary for good health [12]. Vitamin B12 also known as cobalamin is a vitamin that has an important role in cellular metabolism, especially in DNA synthesis methylation [13]. The current paper determines of oxidative stress and antioxidants glutathione malondialdehyde, as well as examining the histological alteration in brain.

2. Materials and Methods

Animals' collection and experimental design fifty adult female rats weighing 200-250 grams were obtained from the Iraqi Centre for Cancer Research and Medical Genetics at Al-Mustansiriya University. Animals were housed for four weeks before the start of the experiment for adaptation, housed in five rats per cage within a room with temperature and humidity controlled, and were maintained in good health.

Animals were maintained in 12 hours natural light and 12 hours dark cycle and fed a balanced diet for the duration of the experiment. Rats were divided into five group n=10, rats in each group were subdivided into two groups A and B and treated for 30 days, and 60.

Group 1 (Control Group) was treated with 0.3 mL of orally distilled water was obtained by gavage. Group 2 was given MSG 25 mg/kg in 0.5 mL distilled water orally by gavage. Group 3 was given MSG 45 mg/kg in 0.5 mL distilled water orally by gavage. The 4^{th} group was treated with MSG 25 mg/kg and cobalamin 0.3mg/kg in 0.5 mL distilled water orally by gavage. Finally, group 5 treated with MSG 45 mg/kg and cobalamin 0.3 mg/kg in 0.5 mL distilled water orally by gavage. At the end

of experiment for groups A and B rats were sacrificed and blood samples were obtained by cardiac puncture technique.

2.1 Preparation of MSG

Monosodium glutamate was prepared by dissolving the powder in distilled water, to obtain the following concentration: 25 mg and 45 mg per 0.3 mL [14)].

2.2 Preparation of B12

Vitamin B12 powder was prepared by crushing tablets, dissolved in distilled water, and transferring into an opaque glass container. The B12 was prepared doses was 0.2 mg/kg [15].

2.3 Histological Preparation

The tissue brain was fixed in 10 % neutral formalin for two days before being processed in an escalating sequence of ethyl alcohol 70 %, 80 %, 90 %, and 100 %. Cleaned in xylene and embedded in paraffin wax for histological analysis. Haematoxylin and Eosin staining were used on tissue slices with a thickness of 5 mm [16].

2.4 Statistical Analysis

Determination the influence of several variables (Groups and Treatment Time) on research parameters, the Statistical Analysis System- SAS (2012) program was utilized. In this study, the least significant difference LSD test (ANOVA) was utilized to make a meaningful comparison between means [17].

3. Results

3.1 Serum malondialdehyde (MDA) concentration (nmole/mL)

After 30 days, the results in show a non-significant difference ($P > 0.05$) after 30 days in MDA concentration in groups G2, G3, G4, G5 (54.39 ± 3.31, 66.95 ± 14.49, 42.02 ± 3.65, 47.18 ± 6.42) respectively compared with G1 (52.38 ± 0.74) as shown in (table 1).

However, after 60 days, the results in show non-significant difference ($P \leq 0.05$) after 60 days in MDA concentration in groups G2, G3, G4, G5 (54.32 ± 4.28, 64.44 ± 6.17, 38.48 ± 3.21, 42.08 ± 9.72) as compared with control G1 (55.33 ± 9.60) (table 1).

Comparing the mean of MDA concentration between periods 30 days and 60 days, there was a non- significant ($P \leq 0.05$) difference in MDA concentration in groups G2,G3, G4, G5 that was G2 (54.39 ± 3.31), G3 (66.95 ± 14.49), G4 (42.02 ± 3.65), G5(47.18 ± 6.42) after 30 days and became G2 (54.32 ± 4.28), G3 (64.44

± 6.17), G4 (38.48 ± 3.21), G5 (42.08 ± 9.72) after 60 days.

Table1: Effect of monosodium glutamate and vitamin B12 on malondialdehyde (MDA) concentration in female rats.

Group	Mean ± SE of MDA (nmole/mL)		P value
	The first period of 30 days	The second period of 60 days	
G1 = Control	52.38 ± 0.74 ab	55.33 ± 9.60 ab	12.64 NS
G2 = MSG (20) mg	54.39 ± 3.31 ab	54.32 ± 4.28 ab	10.91 NS
G3 = MSG (40) mg	66.95 ± 14.49 a	64.44 ± 6.17 a	12.42 NS
G4 = MSG (20) mg + B12 (0.3) mg	42.02 ± 3.65 b	38.48 ± 3.21 b	9.72 NS
G5 = MSG (40) mg + B12 (0.3) mg	47.18 ± 6.42 ab	42.08 ± 9.72 b	11.37 NS
P value	21.92 *	21.01 *	---
Means having the different letters in same column differed significantly; P ≤ 0.05; Group N = 10; Subgroup N = 5			

3.2 Serum glutathione (GSH) concentration (ng/mL)

After 30 days, the result in show a non-significant (P ≤ 0.05) after concentration in groups G2, G3, G4. G5 (0.746 ± 0.16, 0.836 ± 0.36, 1.012 ± 0.70, 1.266 ± 0.28) in comparison with G1 (1.004 ±0.40) as shown in (table 2).

Moreover, after 60 days, results showed that non-significant (P ≤ 0.05) after 60 days in GSH concentration in groups G2, G3, G3, G4, G5 (0.628 ± 0.19, 0.818 ± 0.22, 0.458 ± 0.11, 0.844 ± 0.31) compared with G1 (1.082 ± 0.36).

Comparing the mean of GSH concentration between period G5 that was G2 (0.746 ± 0.16), G3 (0.836 ± 0.36), G4 (1.012 ± 0.70), G5 (1.266 ± 0.28) after 30 days and become G2 (0.628 ± 0.19), G3 (0.818 ± 0.22), G4 (0.458 ± 0.11), G5 (0.844 ± 0.31) after 60 days. With non-significant (P ≤ 0.05) in GSH concentration in groups G2, G3, G4, as demonstrated in (table 2).

Table 2: Effect of monosodium glutamate and vitamin B12 on Glutathione (GSH) concentration in female rats.

Group	Mean ± SE of GSH (nmole/ml)		P value
	The first period of 30 days	The second period of 60 days	
G1= Control	1.004 ± 0.40	1.082 ± 0.36	0.562 NS
G2 = MSG (20) mg	0.746 ± 0.16	0.628 ± 0.19	0.438 NS
G3 = MSG (40) mg	0.836 ± 0.36	0.818 ± 0.22	0.491 NS
G4 = MSG (20) mg + B12 (0.3) mg	1.012 ± 0.70	0.458 ± 0.11	0.822 NS
G5 = MSG (40) mg + B12 (0.3) mg	1.266 ± 0.28	0.844 ±0.31	0.703 NS
P value	1.25 NS	0.751 NS	---
NS: Non-Significantly; Group N = 10; Subgroup N = 5			

3.3 Histological findings of brain

Histology of cerebral in control group (C1) after 30 days is demonstrated in (figure 1). The sections of cerebral layers showed normal appearance of molecular layer, pyramidal layer, external granular layer ganglionic layer, inner granular layer and multiform layer is shown in (figure 2).

The Cerebral tissue of rats that received 25 mg/kg monosodium glutamate after 30 days showed normal molecular layer is shown in (figure 3). That include cerebral and tissue of rats that received 45 mg/kg monosodium glutamate after 30 days. The sections of cerebral cortex revealed marked moderate vascular

degenerative encephalopathy which characterized by necrosis of 2nd and 3rd cortical cerebral layers cell in displayed (figure 4). The magnified sections have revealed various size cavities with marked shrunken of astrocytes with pyknosis of their nuclei neuron showed marked shrunken their bodies in (figure 5).

Where, cerebral tissue of rats that received 25 mg/kg monosodium glutamate + B12 (0.3 mg\kg) after 30 days. The sections of cerebral layers of the third group were also like those in the control group and showed normal appearance molecular layer, pyramidal layer, external granular layer ganglionic layer, inner granular layer and multiform layer is shown in (figure 6).

The cerebral tissue of rats that received 45mg/kg monosodium glutamate +B12 (0.3 mg\kg) after 30 days. The sections of cerebral layers of the fifth group were also like those in the control group and showed normal appearance of molecular layer, pyramidal layer, external granular layer ganglionic layer, inner granular layer and multiform layer in (figure 7).

Histology of cerebral in control group (C1) after 60 days is shown in (figure 8). While cerebral tissue of rats that received 20 and 45 mg/kg monosodium glutamate after 60 days in comparison with the sections of the control group is displayed in (figure 8). The sections of cerebral cortical layers of the second group showed sever vascular degeneration and necrosis of supporting cells mainly astrocytes are shown in (figure 9) and (figure10).

Meanwhile, sections of the third group revealed furthermore sever case which showed multiple focal tissue depletion are demonstrated in (figure 11). The cerebral tissue of rats that received 25mg/kg monosodium glutamate +B12 (0.3 mg/kg) after 60 days. Have sections of cerebral layers of the fourth group were also like those in the control group and showed normal appearance of molecular layer, pyramidal layer, external granular layer ganglionic layer, inner granular layer and multiform layer in (figure12).

Cerebral tissue of rats that received 45mg/kg monosodium glutamate + B12 (mg/kg) after 60 days. Sections of cerebral layers of five group also similar those in control group and showed normal appearance of molecular layer, pyramidal layer, external granular layer ganglionic layer, inner granular layer and multiform layer in (figure 13).

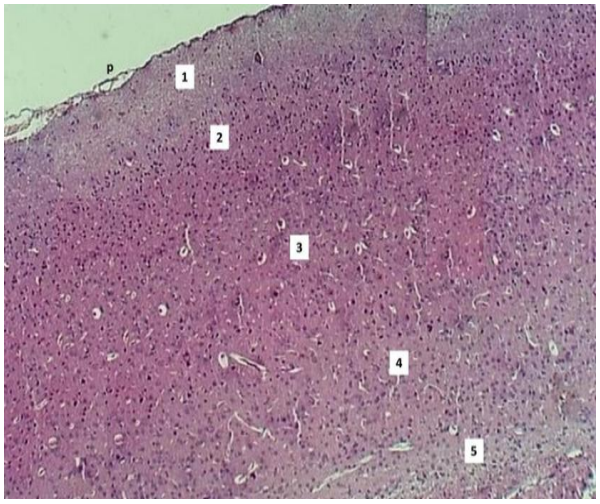

Figure1: Section of cerebral cortex (control 30 days) shows normal molecular layer (1), external granular layer (2), pyramidal layer (3), inner granular layer (4), and ganglionic layer (5) and pia matter (p). H&E stain, 40 ×.

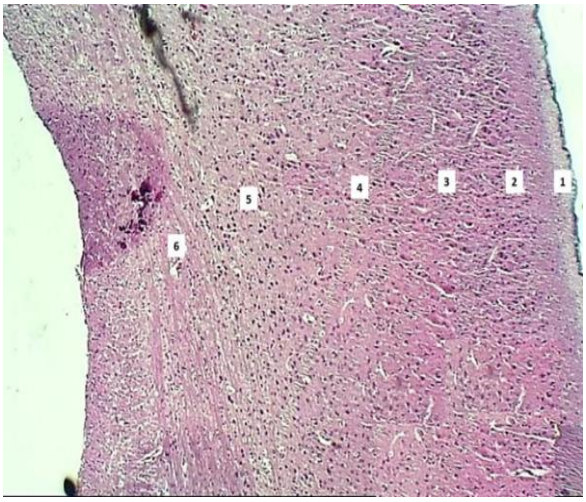

Figure 3: Section of cerebral cortex (group 2-25 mg for 30 days) shows normal molecular layer 1, external granular layer 2, pyramidal layer 3, inner granular layer 4, ganglionic layer 5, and multiform layer 6. H&E stain. 40×.

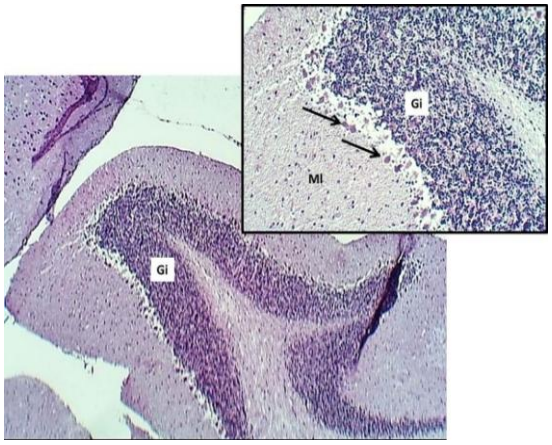

Figure 2: Section of cerebellum (control for 30 days) shows molecular layer (Ml), granular layer (Cl), and purkenji cells layer (arrows). H&E stain,100 and 400 ×.

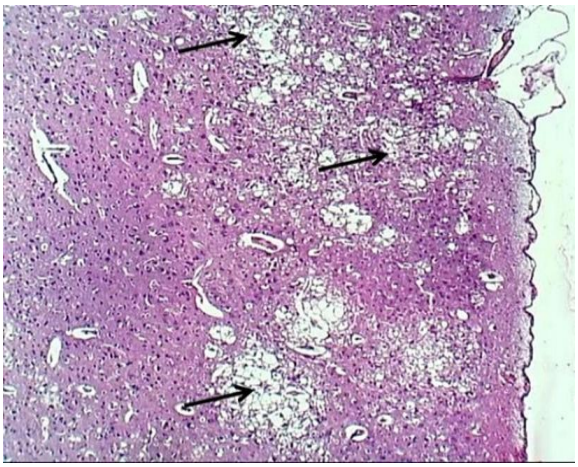

Figure 4: Section of cerebral cortex (group 3-45 mg for 30 days) shows marked focal degeneration and necrosis of 2nd and 3rd layers of cerebral cortex with vacuoles formation (Arrows). H&E stain, 40 ×.

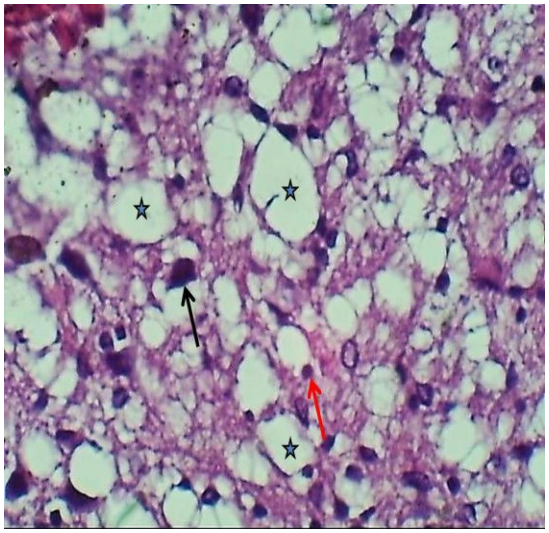

Figure 5: Section of molecular layer of cerebral cortex (group 3-45 mg for 30 days) shows marked degeneration of astrocytes with nuclear pyknosis (red arrow), and degeneration (black arrows) with marked vacuoles formation (Asterisks). H&E stain. 400 ×.

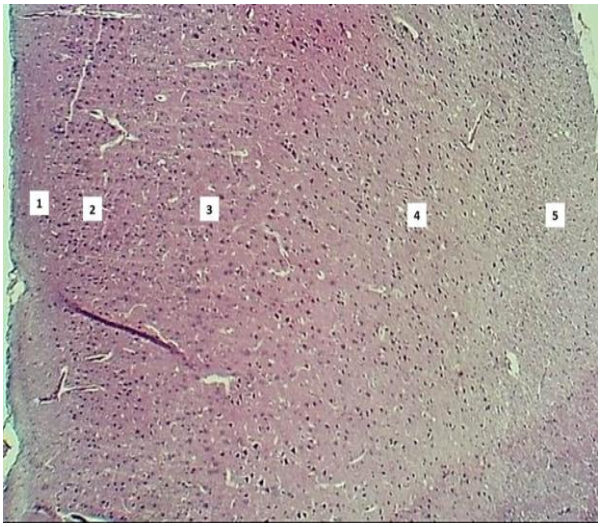

Figure 7: Section of cerebral cortex (group 5-45 mg + B1 for 30 days) shows: normal molecular layer (1), external granular layer (2), pyramidal layer (3), inner granular layer (4), ganglionic layer (5), and multiform layer (6). H&E stain, 40 ×.

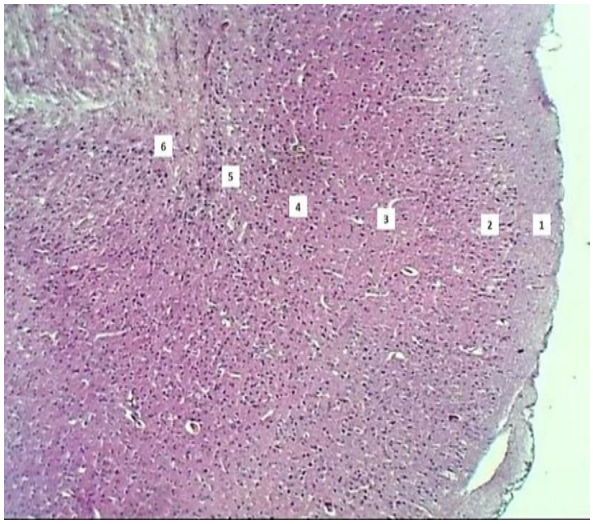

Figure 6: Section of cerebral cortex (group 4-25 mg + B1 for 30 days) shows normal molecular layer (1), external granular layer (2), pyramidal layer (3), inner granular layer (4), ganglionic layer (5), and multiform layer (6). H&E stain, 40 ×.

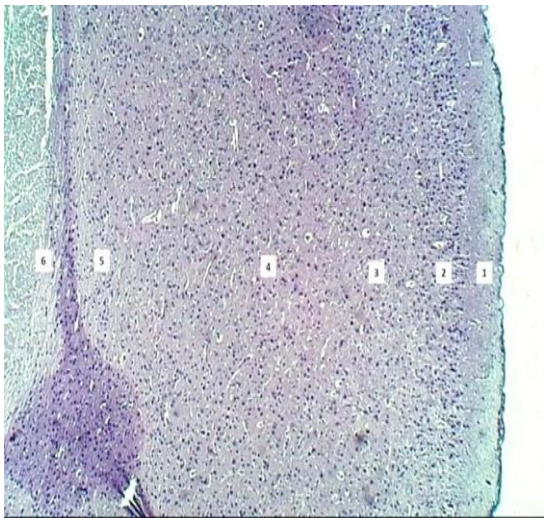

Figure 8: Section of cerebral cortex (control 60 days) shows: normal molecular layer (1), external granular layer (2), pyramidal layer (3), inner granular layer (4), ganglionic layer (5), and pia matter (p). H&E stain, 40 ×.

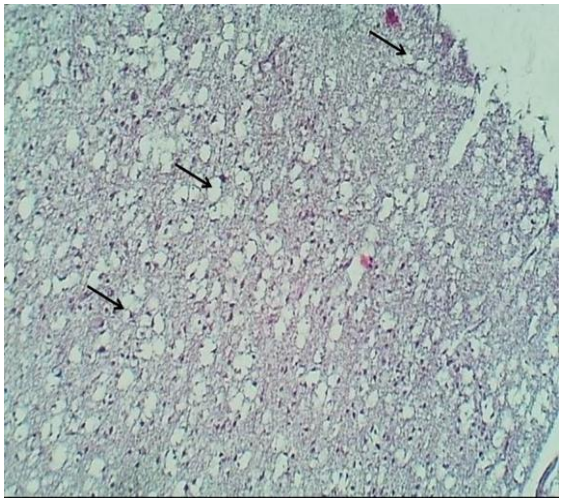

Figure 9: Section of cerebral cortex (group 2-25 mg for 60 days) shows marked degeneration and necrosis of all cortical layers with vacuoles formation (arrows). H&E stain, 40 ×.

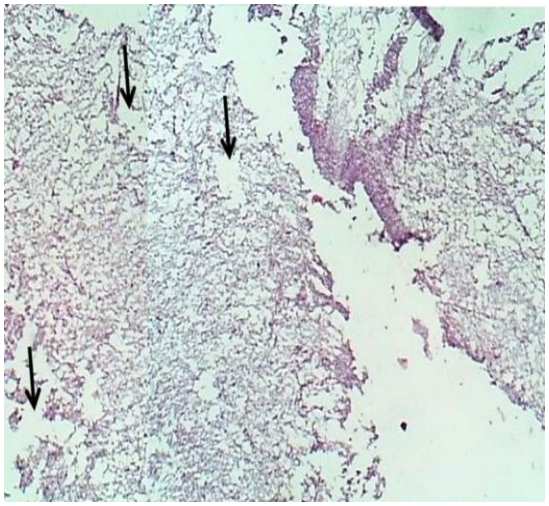

Figure 11: Section of cerebral cortex (group 3-45 mg for 60 days) shows: sever necrosis of all cortical layers with vacuoles formation, and multiple focal tissue depletion (Arrows). H&E stain, 40 ×.

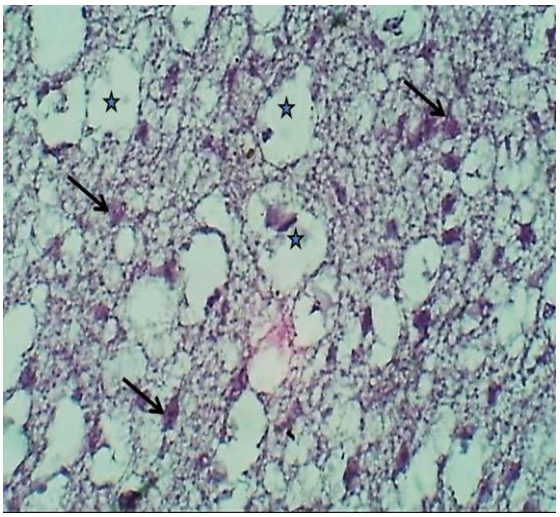

Figure 10: Section of molecular layer of cerebral cortex (group 2-25 mg for 60 days) shows marked degeneration of astrocytes (asterisks), and degeneration of neurons which had eosinophilic cytoplasm (black arrows). H&E stain, 400 ×.

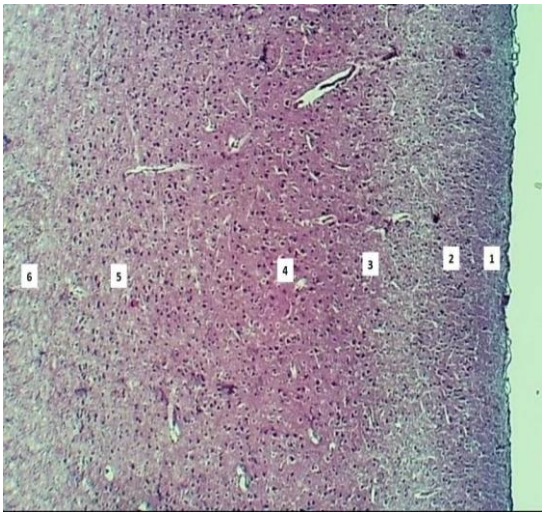

Figure 12: Section of cerebral cortex (group 4-25mg + B1 for 60 days) shows normal molecular layer (1), external granular layer (2), pyramidal layer (3), inner granular layer (4), ganglionic layer (5), and multiform layer (6). H&E stain, 40 ×.

Figure 13: Section of cerebral cortex (group 5-45 mg + B1 for 60 days) shows normal molecular layer (1), external granular layer (2), pyramidal layer (3), inner granular layer (4), ganglionic layer (5), and multiform layer (6). H&E stain, 40×.

4. Discussion

Findings showed a significant decrease in serum serotonin, epinephrine, glutamate and acetylcholinesterase in groups G2, G3, G4 and G5 for to periods. These results are agreement with the result of Hamza R. Z., and co-workers [18]. This can be explained by oxidative stress pathways induced by monosodium glutamate in the cerebral cortex can reduce neurotransmitters when exposed to MSG can increase indices of oxidative stress and alterations in neurotransmitter [19].

Level of oxidative stress due to long term use of MSG causes many neurological disorders due to production of free radicals [20]. That are collected within the brain tissue due to the intake of MSG, which breaks down most proteins, including enzymes [21]. The research results were not consistent with the results of previous studies [22, 23].

Differences may be due to differences in the dosing regimen and possibly to the different periods of exposure and the type and breed of animals used in this study. Oxidative stress is associated with increase in MDA and a decrease in GSH and accumulation of ROS due to their excessive production or insufficient removal that leads to cell damage [24].

An increase in MDA may also be caused by damage to cell membrane tissue after MSG administration [25]. One of the possible causes of brain dysfunctions is changes in neurotransmitter levels and oxidative stress caused by MSG intake [26].

Pathological changes included a degenerative encephalopathy characterized by cell necrosis of the second and third cortical cerebral layers. Results of histological changes showed cavities of different sizes with a noticeable shrinkage of astrocytes with enlargement of their nuclei, and there was severe vascular degeneration and necrosis of mainly supporting cells of the astrocyte in addition to the depletion of multiple focal tissues, while sections of the cerebellum appeared normally.

MSG is known to exert its toxicity through oxidative stress by generating free radicals in some MSG-treated cells which leads to malformation of brain tissue [27, 28]. There was improvement in neurotransmitters levels, but it is not statistically significant, which could be because of MSG on the chemical composition of the hippocampus and activation of neurodegenerative pathways [29]. It may also be attributed to the cumulative effect of MSG, which increases neurotoxicity [30].

It is also possible that the period of treatment with vitamin B12 is not enough. Results also showed improvement in the level of MDA because vitamin B12 reduces oxidative stress, as it acts as an antioxidant, reduces cytotoxic effects and protects them from the production of ROS [31-33].

Results also showed an improvement in tissue that received MSG and then were treated with vitamin B12, which may be due to B12 helps regenerate nerves by stimulating axonal growth and Schwann cell differentiation, which improves functional recovery in injuries [34].

5. Conclusion

Monosodium glutamate administration in rats causes histological changes in brain tissue, including degeneration and necrosis of cells supporting astrocytes. These changes were improved by cobalamin treatment.

6. References

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