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Effect of Monosodium Glutamate on Serum Neurotransmitters and the Protective Role of Vitamin B12 in Adult Female Rats

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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, glutamate, acetylcholinesterase, epinephrine and serotonin 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, the 2nd and 3rd group received MSG in a dose of 20, and 40 mg/kg body weight orally by gavage, respectively, the 4th and 5th groups received MSG and vitamin B12 in a dose of 20 or 40 mg MSG/kg in addition to 0.3 m vitamin B12/kg, respectively. Monosodium glutamate causes a significant reduction in serum level of neurotransmitters. These changes were reversed by treatment with vitamin B12. There was improvement in the levels of neurotransmitters after treatment with vitamin B12.

Key word: Cobalamin, Epinephrine, Glutamate, Monosodium glutamate, Serotonin

1. INTRODUCTION

Monosodium glutamate, a sodium salt of glutamate is widely used as a food additive and flavor enhancer and can be found in various concentrations in numerous food products [1], which is used in many foods such as frozen food, Cubes, 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 is now made mostly 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]. Monosodium glutamate effects many organs and biochemical parameters including neurotransmitters. It is ingestion leads to disturbances in the levels of serotonin, dopamine and adrenaline [5], and thus, leading to clear effects on neurochemical factors, learning memory and motor activities [6].MSG is the sodium salt of glutamate (simply glutamate, water and sodium) [7], and is widely used as a food additive and as flavoring agent to increase appetite [8], due to the presence

of Na ion and the appetizer effect increased by the presence of glutamate ion on gustatory nerve [9]. MSG has been linked to endocrine abnormalities, retinal degeneration, urticaria, obesity, brain damage, addiction,

schizophrenia, neuropathic pain, anxiety, amyotrophic lateral sclerosis and Parkinson's disease, according to previous research [10]. 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) [11]. MSG also affects mitochondrial lipid peroxidation (LPO) and antioxidant status in the cerebral hemispheres, brain stem, cerebellum, and diencephalon [12].

Monosodium glutamate (MSG) is excitatory neurotransmitter in brain, mediating fast synaptic transmission and increased the perception of wetness and saltiness as a taste sensation UNAMI [13].MSG leads to neurotoxicity and thus pathological changes occur in the brain tissue, which are vacuolated cells, low nuclei, low density of neurons, and distorted layers in the brain tissue [14]. Antioxidants are the body's first line of protection against free radical damage and are necessary for good health [15]. Vitamin B12: also known as cobalamin is a vitamin that has an important role in cellular metabolism, especially in DNA synthesis, methylation and mitochondrial metabolism [16].

2. MATERIAL AND METHODS

2-1- Animals' collection and experimental design

Fifty adult female rats weighing 200-250 grams were obtained from the Iraqi Center 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 a 12-h natural light and 12-h 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 groups and 60-day groups Bb as in the following:

Group 1(Control Group): 0.3 ml of orally distilled water was obtained by gavage.

Group 2: Given MSG 20mg/kg in 0.5 ml distilled water orally by gavage.

Group 3: Given MSG 40mg/kg in 0.5 ml distilled water orally by gavage.

Group 4: Given MSG 20mg/kg and cobalamin 0.3 mg/kg in 0.5 ml distilled water orally by gavage.

Group 5: Given MSG 40mg/kg and cobalamin 0.3mg/kg in 0.5 ml distilled water orally by gavage. At the end of experiment (30 and 60 days, for groups A and B, respectively) the rats were sacrificed and blood samples were obtained by cardiac puncture technique.

2-2- Preparation of MSG

Monosodium glutamate (is a chemical compound with the formula C5H8NNaO4, and is found in the form of white crystals) was prepared by dissolving the powder in distilled water. To obtain the following concentration: 20mg and 40mg per 0.3 ml [17].

2-3- Preparation B12

Vitamin B12 powder was prepared by crushing tablets, dissolving them in distilled water, and lowering them into an opaque glass container, ready for use, according to the source. B12 was prepared in doses of 0.3 mg/kg [18].

The chemical composition of this vitamin consists of three sections or parts. The first part is called the planar part, the second is called the nucleotide part, and the third part is called the negative ionic part. The planar part is perpendicular to the nucleotide part when drawing the structural structure of this vitamin. The planar part consists of three pyrrole rings and one pyrrolidine ring connecting them with some (=H) groups in a structure known as a corinne.

2-4- Statistical analysis

To determine 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 [19].

3. RESULT AND DISCUSSION

Biochemical results

1- Serum glutamate concentration

A-After 30 days

The results in table (1) show a significant decrease (P≤0.05) after 30 days in Glutamate concentration in groups G2, G3, G4, G5 (44.46 ±2.83, 21.15 ±4.70, 55.01 ±4.62, 50.14 ±1.72), respectively compared with G1 (444.13 ±39.01).

B- After 60 days.

Significant decrease (P≤0.05) after 60 days in Glutamate concentration in groups G2, G3, G4, G5 (45.49 ±3.97, 16.32 ±1.12, 36.38 ±10.67, 31.29 ±4.31), respectively compared with G1 (434.28 ±31.94)**.**Comparing the mean of Glutamate concentration between periods 30 days and 60 days, there was non- a significant (P≤0.05) in Glutamate concentration in groups G2, G3 that was G2 (44.46 ±2.83), G3 (21.15 ±4.70) after 30 days and become G2 (45.49 ±3.97), G3(16.32 ±1.12) after 60 days, but there was a significant decrease in G4 and G5 that G4 was 55.01 ±4.62 after 30 days and became 36.38 ±10.67 and G5(50.14 ±1.72) after 30 days become (31.29 ±4.31) after 60 days.

Table (1): Effect of monosodium glutamate and vitamin B12 on serum glutamate concentration in female rats

Group	Mean ± SE of GLU (ng/ml)		P value
	The first period 30 day	The second period 60 day	
G1=control	444.13 ±39.01a	434.28 ±31.94 a	14.95 NS
G2=MSG (20)mg	44.46 ±2.83 b	45.49 ±3.97 b	16.37 NS
G3=MSG (40)mg	21.15 ±4.70 b	16.32 ±1.12 b	12.47 NS
G4=MSG (20)mg+B12(0.3)	55.01 ±4.62 b	36.38 ±10.67 b	17.56 *
G5=MSG (40)mg+B12(0.3)	50.14 ±1.72 b	31.29 ±4.31 b	16.98 *
P value	52.38 *	45.11 *	---
Means having with the different letters in same column differed significantly. (P≤0.05), group (N=10) ,sub group (N=5)			

2- Serum acetylcholinesterase concentration (mU/ml)

A-After 30 days

The results in table (2) show a significant decrease (P≤0.05) after 30 days in ACET concentration in groups G2, G3, G4, G5 (6.12 ±0.44, 5.87 ±0.39, 9.22 ±1.14, 7.61 ±0.97) respectively compared with G1 (16.22 ±1.08).

B - After 60 days

That significant decrease (P≤0.05) after 60 days in ACET concentration in groups G2, G3, G4, G5 (5.87 ±0.24, 5.88 ±0.39, 7.68 ±0.85, 6.46 ±0.14) respectively compared with G1 (15.64 ±1.32).Comparing the mean of ACET concentration between periods 30 days and 60 day there was non- a significant (P≤0.05) in GSH concentration in groups

G2, G3, G4, G5 that was G2 (6.12 ±0.44), G3 (5.87 ±0.39), G4 (9.22 ±1.14) ,G5 (7.61±0.97) after 30 days and became G2 (5.87 ±0.24), G3(5.88 ±0.39), G4(7.68 ±0.85), G5(6.46 ±0.14) after 60 days.

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

Group	Mean ± SE of ACET (mU/ml)		P value
	The first period 30 day	The second period 60 day	
G1=control	16.22 ±1.08 a	15.64 ±1.32 a	2.08 NS
G2=MSG (20)mg	6.12 ±0.44 c	5.87 ±0.24 b	1.36 NS
G3=MSG (40)mg	5.87 ±0.39 c	5.88 ±0.39 b	1.29 NS
G4=MSG (20)mg+B12(0.3)	9.22 ±1.14 b	7.68 ±0.85 b	2.42 NS
G5=MSG (40)mg+B12(0.3)	7.61 ±0.97 bc	6.46 ±0.14 b	1.97 NS
P value	2.57 *	2.17 *	---
Means having with the different letters in same column differed significantly. P≤0.05, group N=10 ,sub group N=5			

3- Serum epinephrine concentration (ng/ml)

A-After 30 days

The results in table (3) show a significant decrease (P≤0.05) after 30 days in EPIN concentration in groups G2, G3, G4, G5 (137.76 ±48.32, 106.05 ±24.95, 300.71 ±81.72, 187.85 ±24.03) respectively compared with G1 (567.95 ±42.34).

B- After 60 days

significant decrease (P≤0.05) after 60 days in EPIN concentration in groups G2, G3, G4, G5 (93.81 ±14.15, 96.07 ±20.06, 151.56 ±28.40, 177.18 ±20.83) respectively compared with G1 (559.56 ±64.84). Comparing the mean of EPIN concentration between periods 30 days and 60 days there was non-significant (P≤0.05) difference in EPIN concentration in groups G2, G3, G5 that was G2 (137.76 ±48.32), G3 (106.05 ±24.95), G5 (187.85 ±24.03) after 30 days and become G2 (93.81 ±14.15), G3(96.07 ±20.06), G5(177.18 ±20.83) after 60 days. but there was significant decrease (P≤0.05) between G4(300.71 ±81.72) after 30 days and G4 (151.56 ±28.40) after 60 days.

Table (3): Effect of monosodium glutamate and vitamin B12 on Epinephrine concentration in female rats

Group	Mean ± SE of EPIN (ng/ml)		P value
	The first period 30 day	The second period 60 day	
G1=control	567.95 ±42.34 a	559.56 ±64.84 a	82.19 NS
G2=MSG (20)mg	137.76 ±48.32 c	93.81 ±14.15 b	73.01 NS
G3=MSG (40)mg	106.05 ±24.95 c	96.07 ±20.06 b	76.47 NS
G4=MSG (20)mg+B12(0.3)	300.71 ±81.72 b	151.56 ±28.40 b	116.20 *
G5=MSG (40)mg+B12(0.3)	187.85 ±24.03 bc	177.18 ±20.83 b	62.94 NS
P value	144.56 *	102.61 *	---
Means having with the different letters in same column differed significantly. P≤0.05, group N=10 ,sub group N=5			

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4- Serum serotonin concentration (pg/ml)

A-After 30 days

The results in table (4), was showed that significant decrease (P≤0.05) after 30 days in Serotonin concentration in groups G2, G3, G4, G5 (321.69 ±30.93, 218.42 ±15.92, 407.77 ±28.46, 321.36 ±38.62) respectively compared with G1 (560.77 ±82.69).

B- After 60 days

The results significant decrease (P≤0.05) after 60 days in Serotonin concentration in groups G2, G3, G4, G5 (234.10 ±5.99, 205.64 ±9.66, 339.28 ±37.01, 330.66 ±9.34) respectively compared with G1 (524.12 ±45.91).
Comparing the mean of serotonin concentration between periods 30 days and 60 days, there was non-significant (P≤0.05) difference in serotonin concentration in groups G3, G4, G5 that was G3 (218.42 ±15.92), G4 (407.77 ±28.46), G5 (321.36 ±38.62) after 30 days and become G3 (205.64 ±9.66), G4(96.07 ±20. 06), G5(339.28 ±37.01) after 60 days. However, there was a significant decrease (P≤0.05) between G2 (321.69 ±30.93) after 30 days and G2 (234.10 ±5.99) after 60 days.

Table (4): Effect of monosodium glutamate and vitamin B12 on Serotonin concentration in female rats

Group	Mean ± SE of SERO (pg/ml)		P value
	The first period 30 day	The second period 60 day	
G1=control	560.77 ±82.69 a	524.12 ±45.91 a	72.13 NS
G2=MSG (20)mg	321.69 ±30.93 bc	234.10 ±5.99 c	80.66 *
G3=MSG (40)mg	218.42 ±15.92 c	205.64 ±9.66 c	52.06 NS
G4=MSG (20)mg+B12(0.3)	407.77 ±28.46 b	339.28 ±37.01 b	84.25 NS
G5=MSG (40)mg+B12(0.3)	321.36 ±38.62 bc	330.66 ±9.34 b	67.09 NS
P value	134.22 *	80.19 *	---
Means having with the different letters in same column differed significantly. P≤0.05, group N=10 ,sub group N=5			

The findings showed a significant decrease in serum glutamate, acetylcholinesterase, epinephrine and serotonin in groups G2, G3, G4 and G5 for to periods. These results are agreement with the result of [20]. 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 [21]. The level of oxidative stress due to long term use of MSG causes many neurological disorders due to production of free radicals [22], which are collected within the brain tissue due to the intake of MSG, which breaks down most proteins, including enzymes [23].

Moreover, changes in serotonin levels causes a defect in learning and consolidation of memory [24]. Sodium glutamate administration caused a decrease in the level of epinephrine and serotonin, which may be due to the disturbance of glutamate receptors [25]. MSG is linked to changes in, anxiety-related behaviors, open-field activities, and brain glutamate/glutmine levels; taking it boosts the brain's reward system. [26].

Our results were not consistent with the results of previous studies [27,28], the 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 [29], which leads to cell damage. An increase in MDA may also be caused by damage to cell membrane tissue after MSG administration [30]. One of the possible causes of brain dysfunctions is changes in neurotransmitter levels and oxidative stress caused by MSG intake [31].

There was improvement in neurotransmitters levels but it is not statistically significant, which could be due to the effect of MSG on the chemical composition of the hippocampus and activation of neurodegenerative pathways [32]. It may also be attributed to the cumulative effect of MSG, which increases neurotoxicity [33], it is also possible that the period of treatment with vitamin B12 is not enough.

5. CONCLUSION

Monosodium glutamate administration in rats causes decreases in levels of the neurotransmitter glutamate, acetylcholinesterase, epinephrine, and serotonin. These changes were improved by cobalamin treatment.

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