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Monosodium glutamate affects cognitive functions in male albino rats

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Abstract

Background: Monosodium Glutamate is a silent toxin in our food, especially our kids' food. Some of the products in the market contain MSG in a level exceed the European limit.

Method: Sixty male albino rats divided into three groups, control group, and treated groups, the first with a low dose of MSG (1/20 LD50) and the second with a high dose of MSG (1/10 LD50). All animals examined for cognitive function, serotonin level. The second part in this study, examination of some commercial food for the presence of MSG and its level by HPLC.

Results: MSG affected the cognitive function of treated rats in small and high doses of MSG. The weight of the animals in treated group with a high dose of MSG was significantly increased in comparison with the control group. Also, the cognitive function of the rats administered MSG affected significantly either in low dose and in high dose. Some of the studied commercial food in the market like Kapsa and Indomi contains a higher level of MSG more than the European limit.

Conclusion: MSG has many dangerous effects on health especially kids, so it is so necessary to declare its presence or absence and level on the labels of the products.

Keywords: Monosodium glutamate, Cognitive functions, Serotonin level

Background

Monosodium Glutamate (MSG) has a flavor-enhancing effect in our foods. When it is added to foods, it adds a flavoring function similar to naturally occurring free glutamate (International Food Information Council Foundation IFICE, 2001). It is used to enhance the natural flavors of meats, poultry, seafood, snacks, soups and stews (International Food Information Council Foundation IFICE, 2001). According to the inquiry by the governments of Australia and New Zealand in 2003, Chinese restaurant meal contains between 10 and 1500 mg of MSG per 100 g. A condensed soup typically contains between 0 and 480 mg and packaged sauces or seasonings contain 20 to 1900 mg (Freeman, 2006).

The consumption of MSG has increased all over the world in recent years with an average daily intake from foods reported to be about up to 1 g in Europe, 4 g in Asian countries and 10 g in Germany (Park et al., 2014). MSG

may be present in packaged foods without appearing on the label (Alalwani, 2014).

Many types of food contains MSG for example Frozen dinners and processed meat such as; bacon, pastrami, pepperoni, sausages, luncheon meats, smoked meat products, hamburger, cold cuts and salami. Sauces and dressing such as; ketchup, mayonnaise, barbecue sauce, salad dressing, soy sauce and mustard. Soup bases such as; bouillon cubes and granulated powders. Flavored snacks such as potato chips. Seasoning, spices, gelatin containing substance and bodybuilding protein powder (Anglesey, 1997; Lavine, 2007; Populin et al., 2007). Also, Fast-food restaurants commonly use MSG to enhance the flavor of anything from chicken nuggets, burger, fried chicken to the seasoning used on some French fries (Lavine, 2007).

The use of MSG was linked to Chinese restaurant syndrome (CRS), which was firstly described in 1968, which is characterized by headache, throbbing of the head, dizziness, a feeling of facial pressure, tightness of the jaw, burning or tingling sensations over parts of the body, chest pain and back pain (Park et al., 2014). Also,

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MSG can precipitate an attack of migraine in susceptible individuals; this may occur through an allergic reaction or may be due to change in vascular tone (Millichap & Yee, 2003).

Studies in suckling rodent pups showed severe bodily disturbances and marked obesity after sc injection or oral administration of MSG, some of those animal models presented fasting hyperinsulinemia, hyperleptinemia, adiposity and increase of plasma fatty acids and triacylglycerols (Seiva et al., 2012). The administration of MSG to newborn rats results in distinctive lesions in hypothalamic arcuate nucleus neurons. The neuronal loss impairs insulin and leptin signaling and impacts energy balance as well as pituitary and adrenal activity (Yin et al., 2013). These effects are mostly attributed to immaturity of blood brain barrier in those young animals (Boonnate et al., 2015).

Metabolic syndrome involves insulin resistance and pancreatic changes, a study in adult rats showed that daily MSG dietary consumption was associated with reduced pancreatic β -cell mass and enhanced hemorrhages and fibrosis, but did not affect glucose homeostasis, which suggests that high dietary MSG intake may exert a negative effect on the pancreas and thus have a role in the epidemics of metabolic syndrome (Boonnate et al., 2015). Here, with a mature BBB, the effect of MSG has a different mechanism; MSG-induced cytotoxicity was associated with increased oxidative stress and led to apoptosis and autophagy of pancreatic β -cells (Di Cairano et al., 2011). It has been reported that MSG has neurotoxic effects resulting in brain cell damage, retinal degeneration, endocrine disorders and some pathological conditions such as, stroke, epilepsy, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's chorea, and amyotrophic lateral sclerosis, cited in (Eweka et al., 2013). Also, MSG can cause anxiogenic- and depressive-like behaviors (Porsolt et al., 1979; Onishi & Xavier, 2010; Quines et al., 2014). Some studies revealed that it can affect the cognitive function and learning ability (Abu-Taweel et al., 2014), memory retrieval (Pilgrim et al., 2012; Summers et al., 2003).

The aim of this study is to investigate the following;

1. Effect of MSG on nervous system regarding the cognitive function in male albino rats by;
 - a. Testing their cognitive behavior in a rat maze
 - b. Measuring serotonin level in brain tissue
 - c. Histopathological examination of brain tissue regions that are reported to be responsible for cognitive activities.
2. Quantification of MSG in different food samples, consumed in Assiut city, by High performance liquid chromatography.

Material and methods

The present study was conducted in the period from the 1st of December 2015 to the 10th of April 2016. It has been designed to study the effect of MSG on nervous system regarding the cognitive function in male albino rats and to measure the quantities of MSG in different food samples, consumed in our country Egypt, by HPLC.

Chemicals

L-Glutamic acid monosodium salt (MSG); Phosphate buffer solution (PBS); Serotonin ELISA kit, Formaline 10%, Materials of High performance liquid chromatography (HPLC): All solvents were of HPLC grade, Merck (Darmstadt, Germany). All other materials were of analytical grade. Double distilled water was used for preparing solutions. NBD-Cl (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) was purchased from Sigma Chemical Co. (St. Louis, MO).

Treatment protocol

Ethical consideration for animal use

Ethical approval from the Ethical committee- Assiut university - Faculty of Medicine were taken. All procedures for animals followed the Helsinki guidelines in the care, use of animals and in samples taken (Touitou et al., 2006).

Animals Sixty male albino rats of age range from 5 to 6 weeks were used, obtained from the animal house, Faculty of Medicine, Assiut University. Their weights were between 45 and 70 g at the beginning of the study.

The rats randomly divided into three groups for sub-acute experimental study, as follow:

Control Group (Group I): Twenty male albino rats: was subdivided into two equal groups;

- **C₁ (positive control) (N = 10)** each animal received 3 ml of the distilled water orally by gavage for 30 days.
- **C₂ (negative control)** which was the blank group (N = 10) in which animals received nothing except normal feeding.

Group II: Twenty male albino rats received MSG at a dose of 1.66 g/kg/day (1/10 LD₅₀) dissolved in distilled water given orally by gavage for 30 days.

The LD₅₀ of MSG in rats is 15–18 g/kg body weight according to (Walker & Lupien, 2000).

Group III: Twenty rats received MSG at a dose of 0.83 g/kg body weight/day (1/20 LD₅₀) dissolved in distilled water given orally by gavage for 30 days.

According to the ethics of the experimentation on animals, rats were housed in groups in clean capacious cages (5 per cage) under standard laboratory conditions including good aerated room with suitable temperature, maintained at good light, with alternating 12 h light and dark cycle. Standard rats' food and water were available.

The animals were observed during all days of the experiment and the weight was monitored by weighing every week.

Testing some of the cognitive function After the exposure period for 1 month and according to (Abu-Taweel et al., 2014), the animals subjected to cognitive behavioral tests in the eight-arm radial maze. Two types of memory are assessed in this test: reference memory and working memory. Reference memory is assessed when the rats only visit the arms of the maze that contains the reward. Working memory is assessed when the rats enter each arm a single time (Tarragon et al., 2012).

Biochemical analysis

Serotonin levels in animals brain tissue and serum were determined using rat Serotonin ELISA kits according to the instructions of the manufacturer. Reactions were assessed by measuring the optical density using an automated ELISA reader at 450 nm according to (SunRed Shanghai, 2015).

Sample preparation for serotonin measurement

Tissue samples

- Parts of forebrains from all groups were rapidly frozen with liquid nitrogen after adding Phosphate buffer solution (PH 7.2–7.4). Samples then were melted at 2–8 °C.
- PBS Phosphate buffer solution (pH 7.4) added to samples then homogenized by tissue homogenizer, centrifuged for 20-min at the speed of 2000–3000 r.p.m. and the supernatant was used for assay.

Serum samples

- Blood samples were taken then left to coagulate at room temperature then centrifuged for 20-min at the speed of 2000–3000 r.p.m. and the supernatants were used for assay.

Test principle

- The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the Rat Serotonin Transporter (SERT) in samples.

Quantification of MSG in food samples by HPLC

Method of (Populin et al., 2007) modified by Drug Research Center, Assiut University.

Chromatographic system

The chromatographic system consisted of an Dionex UltiMate 3000 HPLC System (Thermo Scientific, England), which consisted of a HPG-3400SD solvent delivery pump, WPS-3000(T)SL analytical Autosampler

and Dionex UltiMate-3000 RS fluorescence detector. The HPLC system control and data processing were performed by computer integration software (Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS) software).

The separation was achieved using a Luna RP-C18 column (250 × 4.6 mm, 5 μm), (phenomenex, USA) protected with a guard column (Phenomenex, USA) fitted just before the inlet junction of the analytical column.

Mobile phase consisted of a mixture of acetonitrile: 30 mM phosphate buffer (potassium di-hydrogen phosphate and di-potassium hydrogen phosphate, adjusted to pH 3.5) in a ratio of 50:50, v/v. Mobile phase was filtered through a 0.2 mm membrane filter (Phenomenex, USA) using vacuum filtration unit (Phenomenex, USA) and was degassed in an ultrasonic cleaner (Cole-Parmer, Chicago, IL, USA) and delivered at a flow rate of 1 mL/min. The injection sample volume was 20 μL. The fluorescence detector was set at 470 nm as the excitation wavelength and 530 nm as the emission wavelength. The chromatography was performed at room temperature.

Food samples

Three commercial food samples were purchased from supermarkets in Assiut city. Kabsa rice spices., Indomie noodles; chicken flavor and Potato chips; Kabab flavor.

Preparation of solutions

a) Standard and Sample Preparation

A stock solution of sodium glutamate (200 μg/ml) was prepared in water. The working standard solutions were prepared by diluting the stock solutions with water. The six calibration standards of sodium glutamate (final concentrations: 10, 25, 50, 100, 150 and 200 μg mL⁻¹) were prepared independently. All solutions were stored at 4 °C until used.

One gram of each sample was quantitatively transferred to a 100 mL volumetric flask and dissolved in 50 mL water and completed to volume with the same solvent to produce a stock solution of 1% (w/v).

b) NBD-Cl derivatizing reagent

An accurately weighed amount of NBD-Cl (25 mg) was quantitatively transferred to a 50 ml volumetric flask and dissolved in 10 ml methanol; the contents of the flask were diluted to volume with the same solvent to produce a stock solution of 0.05% (w/v). The solution was freshly prepared daily and protected from light during use.

General Derivatization procedure

Sodium glutamate standard solution (1 ml) was transferred to a screw-cap reaction tube by using a micropipet. A volume of 1 ml borate buffer, pH 8.6, and 100 μ L NBD-Cl solution were added. The tube was capped, the contents were swirled, and the tube was left to stand in a thermostatically controlled water bath (Selecta, Spain) at 70 °C for 30 min. The tube was cooled rapidly, and a volume of 100 μ L HCl was added. A 20 μ L aliquot of the resulting solution was injected into the HPLC system.

Calibration curve

Sodium glutamate calibration standard solutions were derivatized and analyzed according to the described procedures. Three injections of each standard solution were made. Peak areas of varying amounts of sodium glutamate versus its corresponding concentrations were used to obtain the calibration curve.

Statistical analysis

Data were analyzed using SPSS software version 16. Differences among experimental groups were determined by One way ANOVA (analysis of variance) and Student t test. Data was expressed as mean \pm Standard deviation (SD).

For all the data the *p* value was considered; not significant if > 0.05 , significant if < 0.05 and highly significant if < 0.001 .

Results

Tabulations

Results of the first part (experimental study)

There was no significant difference between negative (C1) and positive (C2) control groups in all parameters of the study. The results of groups II (high dose) & III (low dose) were compared with the control group, Group I, (average of C1 and C2).

Rats weight

Table 1: Compares the mean body weight \pm SD in the three groups at the beginning and weekly throughout the

study by using one way anova test. There is a statistically significant difference between three groups by the end of each week (*p* value < 0.05). Group III showed statistically significant increase in weight gain in Group III compared to the control groups (*p* < 0.05) in all the study periods. While GII showed a statistically significant increase in the mean (\pm SD) of weight by the end of the first week (*p* < 0.05) compared to the control groups. However, in the second and third weeks the animals in Group II showed a statistically non-significant (*p* > 0.05) decrease in the mean of their body weight, then they started to gain weight in the fourth week. There was no significant difference in weight between group II and control groups in the second, third and fourth weeks (*p* > 0.05).

Figure 1 linear graph; showing the relationship between the mean weight in the three groups during the whole month.

Assessment of some cognitive function

Table 2 and Fig. 2: illustrate the cognitive function (reference and working memory) of the animals in the three groups assessed by the rat maze. Showing that the total time taken by the MSG exposed animals (Group II & III) during the test period to enter the food containing arms was significantly increased (*p* < 0.001) compared to the control groups.

Biochemical analysis

Figure 3: illustrates the effect of MSG in high dose (Group II) and low dose (Group III) on the levels of the neurotransmitter; serotonin (5-HT), in tissue (forebrain) and serum of the male rats. Only the animals exposed to high dose (Group II) MSG were significantly affected (*p* < 0.05). Animals in Group III didn't show a statistically significant decrease in forebrain serotonin levels compared to control groups.

Table 3: Compares the mean serotonin level in forebrain tissue and serum in the three groups at the end of the study using one way ANOVA. There is a statistically significant difference between three groups, with *p* value ≤ 0.001 when comparing the mean serotonin level in forebrain tissue and

Table 1 Weight comparison between rats in the three groups using one way Anova test

Groups	Mean weight \pm SD				
	At-the beginning	1st week	2nd week	3rd week	4th week
Group I (control) <i>n</i> = 20	55 \pm 10	60.91 \pm 13.1	78.21 \pm 17.74	94.74 \pm 27.73	99.05 \pm 26.9
Group II (high dose) <i>n</i> = 20	55 \pm 10	86.17 \pm 15.19	83.33 \pm 16.54	80.66 \pm 17.5	104.83 \pm 4.3
Group III (low dose) <i>n</i> = 20	55 \pm 10	81.17 \pm 8.63	102.08 \pm 15.33	127.67 \pm 20.15	140.33 \pm 20
<i>p</i> value		0.007*	0.059*	0.007*	0.005*

Values in each column followed by * are significantly different (*P* ≤ 0.05), and that followed by ** are highly significantly different (*P* ≤ 0.001)

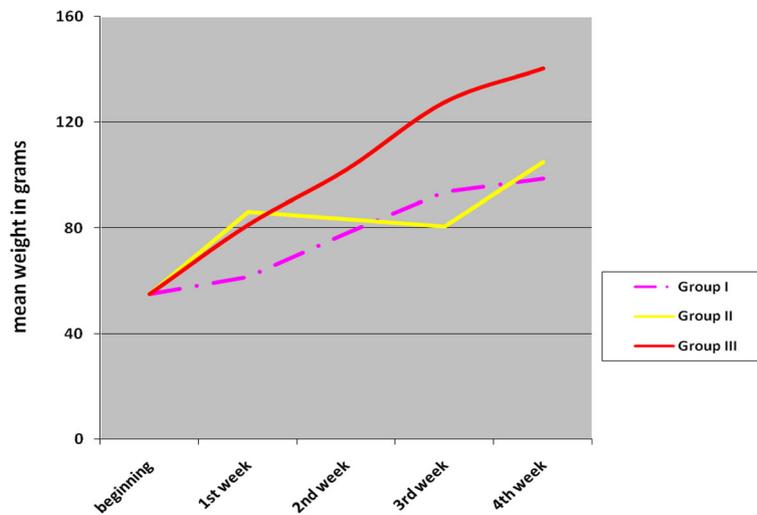


Fig. 1 Linear graph showing the relation between weight gain in rats treated with MSG for one month; Group II (high dose), Group III (low dose) and control groups

p. value ≤ 0.05 when comparing the mean serotonin level in serum.

Results of the second part

This part of the study was performed to measure the quantity of monosodium glutamate in various food stuff and was conducted at Drug Research Center, Assiut University.

Figure 4: shows the calibration curve of monosodium glutamate obtained by plotting the final concentrations versus the peak areas of varying amounts of sodium glutamate .

Table 4 and Fig. 5: Illustrate the different concentrations of MSG in various food samples, the highest concentration (7.34%) was found in Indomie, instant noodles chicken flavour, followed by potato chips Kabab flavour (2.1%) and lastly the kabsa rice speices (1.36%). It is important to note that the instant noodles and potato chips are ready to eat products with no further dilution of the estimated levels of MSG is expected before eating. This is not the case with rice spices where the MSG level is expected to be diluted about 10 times after cooking.

Table 2 The mean time taken by rats in each group in the rat maze to reach food in the maze arms using Student t test

Groups	Group I (Control) <i>n</i> = 20	Group II (High dose) <i>n</i> = 20	Group III (Low dose) <i>n</i> = 20
Mean time \pm SD (in sec)	14 \pm 1.67	46.33 \pm 8.68	23.5 \pm 2.35
t-test		8.952	8.077
<i>p</i> -value		< 0.001**	< 0.001**

Values in each column followed by * are significantly different ($P \leq 0.05$), and that followed by ** are highly significantly different ($P \leq 0.001$)

Discussion

Monosodium Glutamate (MSG) is one of the world’s most extensively used food additives which is ingested as part of commercially processed foods (Husarova & Ostatnikova, 2013).

In 1958 the U.S. Food and Drug Administration (FDA) designated MSG as a Generally Recognized As Safe (GRAS) ingredient, along with many other common food ingredients such as salt, vinegar and baking powder (International Food Information Council Foundation IFICF, 2001).

The glutamate industry often uses flavors in ingredients labeled “flavor,” or “flavoring,” (this preceded by the word “natural”). They use “reaction flavors” as “clean label” alternatives to the use of “monosodium glutamate”. The consumers rarely know that “Clean label” can include MSG (Truth in Labeling, 2007).

The young children are usually at risk from MSG. Their blood brain barrier is not fully developed; and cannot protect against toxins. In addition, glutamic acid can penetrate the placental barrier. Companies use glutamic acid because it is cheap, and since it is neurotoxic, manufacturers continue to go on using it and do not want the public to know that. (NOHA, 2008).

In the current study, rats of group II (high dose) showed a significant increase in their body weights compared to the control group (Group I) in the first week of the study. This was followed by reduction in body weight in the second and third weeks followed by another increase in their weight in the last week. However, there was no significant difference in weight between group II and control by the end of the study ($p > 0.05$). The reduction in body weight that was noted in the second and third week agrees with other studies that also reported reduction in body weight

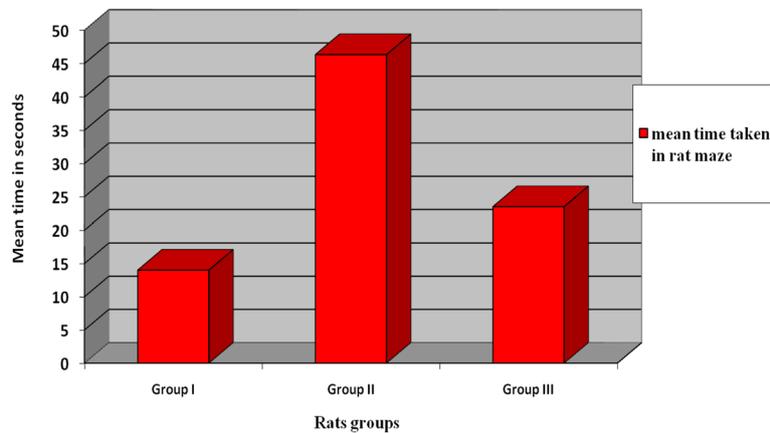


Fig. 2 The mean time taken by rats in each group in the rat maze to reach food in the maze arms

gain in animals treated with MSG (). The increase in weight in the last week can be attributed to the urinary retention (full bladder was noted upon dissecting all the animals of this group (Group II).

Rats in group III (low dose) showed a significant increase in body weight compared to control group ($p < 0.05$). This is concordant with Alalwani, 2014 (Alalwani, 2014) who reported a significant weight gain in MSG treated rats (single daily dose 30 or 60 mg/kg intra-peritoneal injection for 2 months). Another supporting study reported that MSG treated rats (subcutaneous injection of 4 g MSG/ kg body weight /day) presented higher body weights than the control rats, despite the fact that treated rats were found to be hypophagic, presenting lower average daily food consumption (de Oliveira Lazarin et al.,

2011). The different effect of MSG on body weight might be due to the variations in dose regimen and perhaps to different periods or duration of exposure and the type or strain of the animals used in these studies. Although MSG could improve the palatability of foods by exerting a positive influence on the appetite center (Alalwani, 2014). Its main effect on body weight is by increasing mRNA expression of interleukin-6, tumor necrosis factor-alpha, resistin and leptin in visceral adipose tissue, it increased insulin, resistin and leptin levels in serum and it also impaired glucose tolerance. The ingested glutamate also exerts a local effect; its presence in gastrointestinal tract activate both the gastric and the celiac branches of the vagus nerve led to the activation of the insular cortex, limbic system, hypothalamus and nucleus tractus solitaries (Husarova & Ostatnikova, 2013).

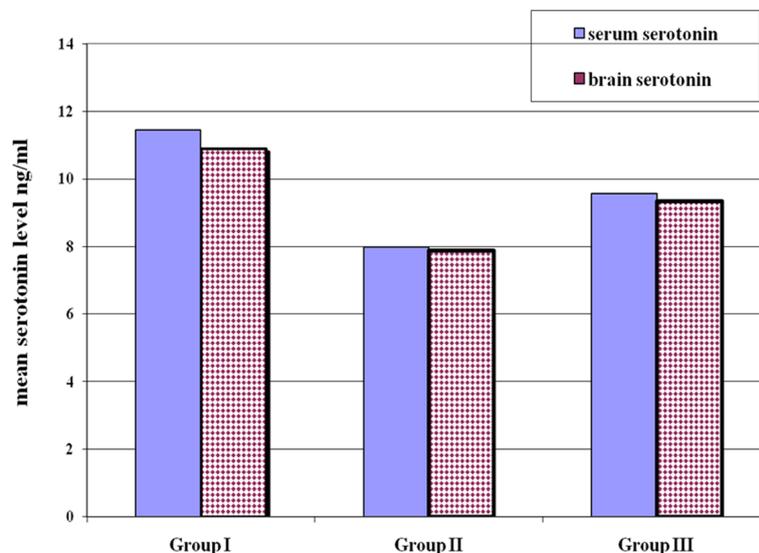


Fig. 3 Relation between mean serotonin levels in both forebrain and serum in all the study groups (Group I (C1 & C2), Group II, Group III)

Table 3 The level of serotonin (mean) in the forebrain and serum in the three groups after treatment using one way ANOVA

Groups	Group I (Control) n = 20	Group II (High dose) n = 20	Group III (Low dose) n = 20	p value
Mean serotonin level in forebrain	10.84	7.87	9.33	0.001**
Mean serotonin level in serum	11.45	7.99	9.57	0.002*

Values in each column followed by * are significantly different ($P \leq 0.05$), and that followed by ** are highly significantly different ($P \leq 0.001$)

Rats exposed to MSG (group II and III) in the present study showed decline in their learning capabilities and short memory as observed from their performance in the radial arm maze test. However the cognitive affection was much more obvious in the group receiving the higher dose. This finding is also supported by earlier studies (Ali et al., 2000; Olvera-Cortes et al., 2005; Porsolt et al., 1979). Onaolapo et al., 2017, concluded that in their study MSG associated with changes in open field activities, anxiety-related behaviors and brain glutamate/glutamine levels; its ingestion leads to a stimulation of the brain reward system (Onaolapo et al., 2017).

The hippocampus in the forebrain plays a major role in controlling memory and regulating learning functions and thinking (Compton, 2004). Thus the decline in cognitive functions in animals exposed to MSG, as observed in the study, could be due to its neurotoxic effect on forebrain neurons including the hippocampus. Decline in the cognitive function can also be explained by alteration in brain neurotransmitters such as serotonin, dopamine, norepinephrine and acetylcholine (Husain & Mehta, 2011).

Among different neurotransmitters, serotonergic alterations have been implicated in cognitive alterations, and low extracellular serotonin levels seem to be associated with impaired learning and memory consolidation (Ramos-Rodriguez et al., 2013). In the present study only the animals exposed to high dose (Group II) MSG showed

significant decrease ($p < 0.05$) in the levels of the neurotransmitter; serotonin in brain tissue as well as in serum. In support of the involvement of serotonergic mechanisms in MSG induced toxicity, a study has tested the MSG induced lethality (MSG; 6–10 g/kg, intra-peritoneal) in mice after adding drugs that depletes serotonin stores, this resulted in an increase in MSG-induced lethality (Kamei et al., 1991). In agreement with that, L-glutamic acid was reported to decrease serotonin synthesis, release in rat rostral and caudal rhombencephalic raphe cells culture and cause an increase in serotonin metabolism (Becquet et al., 1993). In another study; subcutaneous injection of MSG in newborn rats (4 g/kg/day from the 1st to 5th postnatal day) results in an increase in the serotonin uptake in cerebral cortices (Quines et al., 2014).

On the other hand animals in Group III (low dose) didn't show a statistically significant decrease in forebrain serotonin levels compared to control group. The obtained data from Group III are in agreement with the study by (Dawson, 1983) where adult female mice were injected intraperitoneal with MSG (4 mg/g) and decapitated 30 min later, for measuring serotonin levels in their brain tissue, the results showed no significant alteration in the brain level of serotonin compared to control group. MSG administration (at a dose of 8 mg/kg body weight/day, dissolved in drinking water for 1 month) to mice also showed no significant alteration in the brain level of serotonin (Porsolt et al., 1979).

The optimal palatability concentration for MSG is between 0.2–0.8% (w/w) and its use tends to be self-limiting as over-use decreases its palatability (Lölinger, 2000). The limit of added MSG to food products established by European Directive is 10 g/kg (1%) of product or prepared food (European Parliament and Council Directive, 1995).

Hashem et al., 2012 in their study agreed with this study about the dangerous effects of MSG on brain, they concluded that MSG has neurotoxic effect leading to degenerative changes in neurons and astrocytes in cerebellar cortex of albino rats (Hashem et al., 2012).

Also, Umukoro et al., 2015 proved that low dose of MSG given orally to mice did not produce significant impairment in the Y maze test but produce depressive like symptoms in the forced swim test at dose of 500 mg/kg, and increased the levels of malondialdehyde (MDA) and decreased the level of glutathione (GSH) concentration in brain tissue (Umukoro et al., 2015).

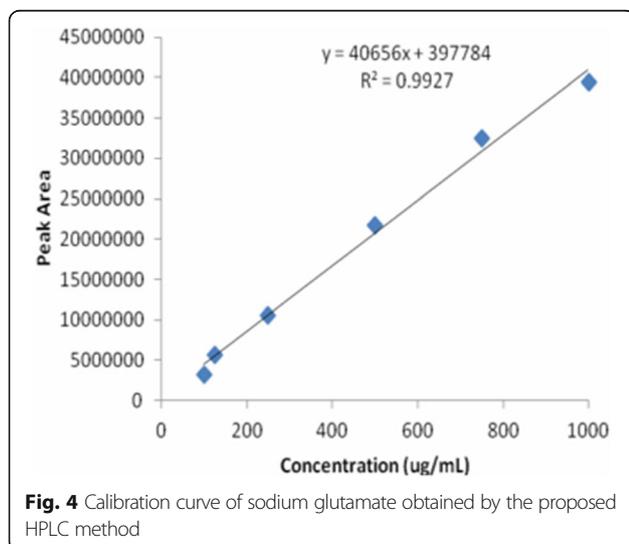


Fig. 4 Calibration curve of sodium glutamate obtained by the proposed HPLC method

Table 4 The concentrations of Monosodium glutamate in food samples

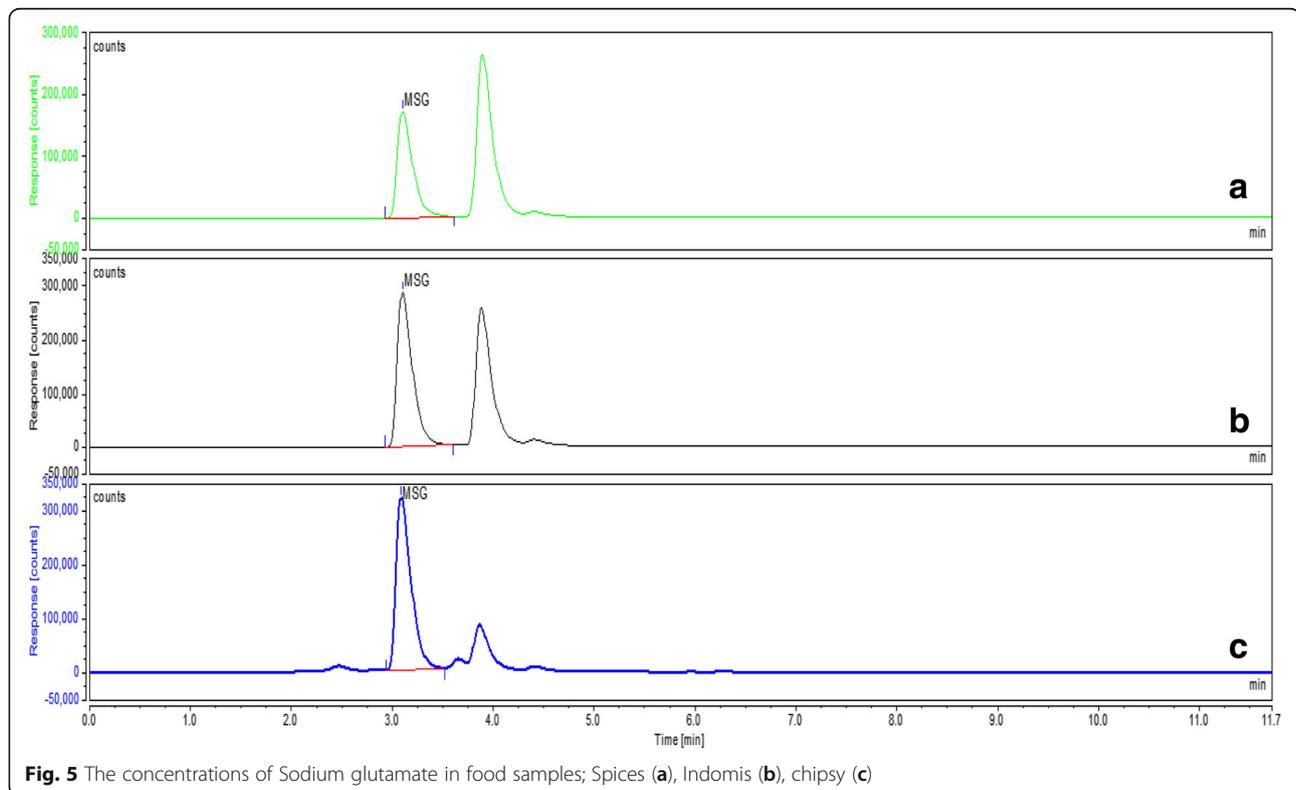
Sample	Concentration	Equivalent to	Equivalent to
Kabsa rice spices	135.5 µg/mL	13.55 g/Kg	1.355 g/100 g (1.355%)
Indomie chicken flavor	733.5 µg/mL	73.35 g/Kg	7.335 g/100 g (7.335%)
Potato chips; Kabab flavor	210.2 µg/mL	21.02 g/Kg	2.102 g/100 g (2.102%)

Prastiwi et al., 2015 found that the administration of MSG at a high dose of 3.5 mg/g body weight, but not at lower dosages, lead to significant decrease of motor coordination and the estimated total number of Purkinje cells of rats and significant correlation between motor coordination and the total number of Purkinje cells (Prastiwi et al., 2015).

This study was conducted to determine the monosodium glutamate content of selected food samples from market in Assiut city. Three different samples were selected these include; Kabsa rice spices, Indomie noodles; chicken flavor and Potato chips (Kabab flavor). Estimation of monosodium glutamate was carried out by modified HPLC (High Performance Liquid Chromatography). The results from laboratory analysis concluded that MSG was present, altogether in the three samples with the following values; 135.5 g/100 g (1.35%), 733.5 g/100 g (7.33%) and 210.2 g/100 g (2.1%), respectively.

In case of spices, they are used to prepare 0.5–1.0 kg meat or rice and so the MSG was diluted 10 times (Lateef et al., 2012), so the estimated percent of MSG decreases to 0.135% in prepared food which lie within the optimal palatability concentration i.e. 0.2–0.8% as suggested by (Lölinger, 2000). However in the other two samples; Indomie (7.33%) & potato chips (2.1%), the estimated MSG levels exceed the allowable limit per serving (1% of product or prepared food as stated by (European Parliament and Council Directive, 1995).

Another studies measured MSG levels in different food staffs using HPLC; in one of the reports, quantification of MSG in hamburgers commercially available in Argentine markets was 0.1–0.2% (w/w) (Rodriguez et al., 2003). In another study, a survey on free glutamic acid content in a variety of foods (broths, soups, sauces and salad dressings), with and without added monosodium glutamate (MSG), broths and soups with added MSG had free glutamic acid contents of 0.266–0.753%. The highest amounts of free glutamic acid in foods with no added MSG were found in products containing hydrolyzed proteins 0.12% (Populin et al., 2007). Lateef et al. (Lateef et al., 2012), used a modified HPLC method to quantify MSG in Pakistani spices using single organic mobile phase. The MSG concentration in different spice samples were was found in the range of 2.7–8.8%. Another study was conducted to determine the monosodium glutamate content of selected traditional meat dishes. Six traditional

**Fig. 5** The concentrations of Sodium glutamate in food samples; Spices (a), Indomis (b), chipsy (c)

meat dishes were selected from five different restaurant of Lahore. The dishes included were chicken karahi, mutton qorma, chicken biryani, seekh kabbab, chicken tikkah, palak gosht, the estimated levels of MSG in all the meat dishes ranges from 0.2–0.8% (Mustafa et al., 2015).

Conclusion

Monosodium Glutamate is a silent toxin present in our food, especially our kids' food. It can cause decline in the cognitive functions and alternations in serum and brain serotonin levels. Also, it can cause degenerative changes in the brain of the male albino rats. At lower doses MSG caused a significant increase in weight; decline in cognitive function, but without alterations in serum or brain serotonin levels or pathological changes in the brain.

Some of the products in our market with added MSG exceeded the European limit of 10 g/kg (1%) of product. Other products may have MSG within the allowable limits, but one can ingest many of these products per day without paying attention to the total amount of MSG ingested. This confirms the necessity to declare on the label the percent of MSG present per product.

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Availability of data and materials

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WAM selected the subject, and revision of the research. HAY completed the statistics of the research, cognitive test and shared in writing. RMA shared in the lab test and writing. NAM shared in the lab test and writing. All authors read and approved the final manuscript.

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Ethics approval

Ethical approval granted by the Ethical Committee Assiut University - Faculty of Medicine. All procedures for animals followed the Helsinki guidelines in the care, use of animals and in samples taken.

Consent for publication

All authors approve the publication in this form.

Competing interests

The authors declare that they have no competing interests.

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