Evaluation of Monosodium Glutamate Induced Neurotoxicity and Nephrotoxicity in Adult Male Albino Rats

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Abstract: Monosodium glutamate (MSG) is a food additive with a wide use in modern nutrition. The Current research concerned with studying the toxic effects of MSG on rats cerebrum and kidneys. Thirty six adult male albino rats were divided into three groups (each containing 12 rats); negative control group, positive control group (received 2 ml saline orally for 28 days) and MSG group (received 830 mg/ Kg. B. wt orally for the same period). Serum creatinine and blood urea nitrogen, urine analysis for urinary albumin excretion and histopathological examination for cerebrum, kidneys were examined in all groups. The results showed marked increase in the serum creatinine, BUN levels in MSG group as compared to control group. These changes were accompanied with a significant increase in the urinary excretion of albumin. These alterations were accompanied by variable histopathological changes of the examined cerebral and renal tissues. There were neurogenerative changes in the form of vacuolization, pyknosis, satellitosis and chroidal plexus congestion in the cerebral cortex. The renal tissue showed swelling in the lining endothelium of the glomeruli associated focal areas of glomerular atrophy. There was also hydropic degeneration of the tubules with tubular dilatation and hyaline casts. The inter-tubular spaces showed dilatation and congestion of the cortical blood vessels with focal hemorrhage between the tubules. Moreover MSG treatment induced up regulation of Bax protein in all examined tissues as compared to control rats indicating that MSG induced apoptosis. In conclusion, the results confirmed the neurotoxic and nephrotoxic effects of MSG, where Bax protein appeared to have a pivotal role in MSG induced apoptosis in these organs.

1. Introduction:

Monosodium glutamate (MSG) is a substance widely used as flavoring agent in the whole world. It is the sodium salts of glutamic acid. It is added to the food either as a purified monosodium salt or as a component of a mixture of amino acids and small peptides resulting from the acid or enzymatic hydrolysis of proteins **(Schwartz, 2004).** When it is added to food in relatively small quantities, the palatability of this food increases **(Vindini *et al.,* 2010).** There is a substantial evidence that the sensory basis for this effect is that MSG stimulates the sense of taste **(Garattini, 2000).**

Monosodium glutamate is absorbed very quickly from gastrointestinal tract and could spike blood plasma level of glutamate **(Schwartz, 2004).**

Glutamate is the most abundant amino acid in the central nervous system where it functions as an excitant neurotransmitter. It is especially highly concentrated in those regions of the brain that are essential in cognitive processes mediation; in the cerebral cortex, hippocampal gyrus dentatus and striatum **(Cekic *et al.,* 2005).**

Glutamate in high doses produce neuroendocrine abnormalities and neuronal degeneration **(Moreno *et al.,* 2005),** and oxidative damage in different organs **(Farmobi and Onyema,** **2006; Pavlovic *et al.,* 2007).** On the other hand, glutamate in high concentrations, in particular in postnatal period, it acts as a neurotoxin (excitotoxin) **(Eweka *et al.,* 2011).**

The exact mechanism of neuronal cell death, induced by excitotoxins, still remains unknown. However, there is accumulating evidence suggesting that glutamate- induced toxicity can be mediated through necrosis and apoptosis **(Ankarcron *et al.,* 1998; Martin *et al.,* 2000).**

Kidneys are quite vulnerable to toxic injury because they are exposed directly to the blood plasma via open fenestrae of their glomerular capillaries. Moreover, it metabolized many toxic substances where the concentration of the toxin any be several hundreds times greater in the kidney than else **(Stine and Brown, 2006).**

Previous scientific investigations aimed at determining the effect of MSG on body organs **(Nwaopara *et al*., 2004; 2007a, b; 2008a, b).** There are some reports of the toxic effects of MSG on the pancreas **(Nwaopara *et al.,* 2004),** liver **(Nwaopara *et al*., 2007b)** and kidney **(Nwaopara *et al*., 2008 a).**

Osfor *et al.* (1997) indicated that kidney, liver, brain, and heart weight were significantly increased in rats treated with MSG. **Diniz *et al.* (2004)** reported that MSG induced alterations in

metabolic rate of glucose utilization and decreased antioxidant defenses. Generation of reactive oxygen species in different body cells is known to induce damage to DNA, lipids and proteins, lipid peroxidation in cellular membrane due to damage of the polyunsaturated fatty acids in the cell membranes. These effects may lead to cellular death by apoptosis.

Researches in recent years have been focused on understanding the molecular targets for toxin induced apoptosis **(Stine and Brown, 2006).** The two main families of apoptotic regulators taken into consideration in mammals are the caspases**,** and the Bcl-2 family members, represented by Bcl-2, Bcl-x_L, Bcl-w, and Mcl-1 (cell death inhibitors) and by Bax, Bcl-x_s, Bad, Bak (cell death promoters) **(Kelekar and Thompson, 1998).**

Hence previous literature regarding the detailed histopathological changes in the brain and kidneys was insufficient especially regarding the exact molecular target for MSG induced cell death, so the aim of the present study is concerned with the possible biochemical, histological and Bax immunohistochemical changes that may occur in rats' cerebrum and kidneys following short term chronic toxicity of MSG.

Material and Methods
Material:
1- Chemical:
The chemical used monosodium glutamate (MSG) (C5H9NO4.Na) from Al-Dawlya Chemicals Co., Egypt with Purity > 98% NT.
2- Kits:
* Biomerieux France kits (Boehringer, Germany) for estimation of blood urea nitrogen (BUN) and serum creatinine.
* ABC Diagnostic Kits (New Damietta, Egypt) for estimation of urinary microalbuminuria .

3- Experimental animals:
Thirty six adult male albino rats were used in the present study. The weights of rats ranged from 180- 200 grams and their ages ranged from 3-4 months and obtained from the Animal Farm, Faculty of Veterinary Medicine, Zagazig University, divided into 3 groups and caged under standardized environmental conditions. Passive preliminaries for seven days in order to adapt themselves to their new environment and to ascertain their physical wellbeing. They were housed in a separate well ventilated cages, under standard conditions, with free access to the standard diet and water ad libitum. The experiment was conducted at the animal house of Faculty of Medicine Zagazig University. The experiment was performed in accordance with the "Guide for the Care

and Use of Laboratory Animals" (**Institute of Laboratory Animal Resources, 1996).**
Methods:
1- Experimental design:
For this study, thirty six (36) adult male albino rats were divided into three groups, each group consisted of twelve rats.
Group I (negative control group): kept with no injection till end of experiment.
Group II (positive control group): each animal of this group received orally 2 ml saline daily, parallel to the chemical treated group
Group III (monosodium glutamate (MSG) group): each rat received daily an oral dose of MSG equals 830 mg/kg b. wt. (1/20 of rat's oral LD50 which equals 16600 mg/kg b. wt.) **(Richard and Lewis, 1990)**.

This experiment lasted for 28 days. On the morning of the last day of the experiment, all rats were subjected for collection of 24 hrs of urine for assessment of urinary albumin excretion then they were weighted for recording the final total body weight and body weight gain percent. Then rats were anaesthetized by ether for collection of blood samples for assessment of kidney function tests then sacrificed and dissected for collection of cerebra and kidneys to be weighted for recording the relative cerebral and kidney weight and then to be subjected for histopathological examination.
2- Blood samples collection:
Retro-orbital blood samples were collected from all rats. The collected blood was incubated at 37ºC until clotted then centrifuged to separate the serum. The serum samples were maintained at (-20°C) to be used for measurement of kidney functions tests (blood urea nitrogen and serum creatinine).
3- Urine samples collection:
On the morning of the last day of the experiment, each rat was housed individually in a metabolic cage and was allowed to access water as these metabolic cages are supplied with drinking bottles. Funnels of suitable size were arranged at the bottom of the metabolic cages for collection of urine and perforated plastic discs were arranged in the funnels to retain fecal matter (**Abd-AlAziz et al., 2008).** The 24 hrs urine sample was collected for each animal in a beaker arranged at the bottom of the funnel. The volumes of the 24 hrs urine samples collected in the beakers were noted individually and used for determination of urinary albumin excretion or microalbuminuria.
4- Assessment of kidney function tests (KFTs):
* Blood urea nitrogen (mg/dL): Biomerieux France kit Estimation of blood urea has been carried out using kit of biomerieux France. It was done as

described by **Kaplan (1965)** according to the pamphlet of Biomerieux France kit by enzymatic colorimetric method**.**

* Serum creatinine (mg/dL): Estimation of serum creatinine has been carried out using kit of Biomerieux France. It was done as described by **Bjurosson (1979)** according to the pamphlet of Biomerieux France kit by colorimetric method**.**

5- Assessment of urinary albumin excretion (μg/ml**):** Urinary albumin excretion or microalbuminuria (μg/ml) was determined by rapid colorimetric method using commercially available kits (ABC Diagnostics, New Damietta, Egypt) and according to manufacture instruction **(Schosinsky et al., 1987)**. Microalbuminuria was expressed as μg/24 hours by multiplying the results by the urine volume (ml) in 24hours.

6- Histopathological Studies:

After scarification and dissection, cerebrum (frontal cortex) and kidneys were removed immediately and fixed in 10% formal saline**.** After fixation, kidneys and cerebra were embedded in paraffin blocks and processed for the preparation of 5 μ thickness sections. These sections were subjected for the following stains; Hematoxylin and Eosin (H& E) as described by **Wilson and Gamble (2002)** and Bax immunohistochemical staining as described by **Zhang et al. (2006).**

Immunohistochemical Methods:

Immunohistochemical staining for Bax was performed by using the Streptavidin/peroxidase method. After dewaxing, the slices were heated and boiled for 30 min in citrate buffer Solution (0.01M, pH6.0) for retrieval antigen. Each section was treated with 3% hydrogen peroxide for 20 min at room temperature to diminish nonspecific staining. After rinsing with PBS, the slices were blocked with 5% normal goat serum in PBS (0.1M, pH 7.4) for 30 min at room temperature. The slices were then incubated with the primary rabbit anti- Bax (1: 200 (Biotechnology, Santa Cruz, USA) overnight at 4 ° C. After rinsing with PBS, the specimens were incubated with biotinylated secondary antibody at room temperature for 30 min, and then re- incubated with peroxidase- labeled streptavidin for 15 min. The immunoreactivity was visualized by 3, 3 - diaminobenzidine tetrahydrochloride solution (DAB-Chromogen-Kit, Carpinteria, CA). The slides were counterstained with hematoxylin. Positive reactions appeared brown. Both cytoplasmic and nuclear staining of the cell was the only pattern of staining considered to be positive for Bax **(Zhang et al., 2006).**

4- Statistical analysis:

Data were represented as means ± SD The differences were compared for statistical significance by student's t test. Differences was considered significant at p < 0.05. All statistical analysis were performed using Epi-Info version 6.1.**(Dean et al., 2000).**

3. Results
1-Total and relative body weight changes:

All treated animals were survived to the end of experimental period. The data obtained from the mean body weights are given in Table (1). There were no statistical significance differences (p>0.05) as regard the mean values of the initial body weight(g), final body weight(g), percent of body weight gain (%), and relative body weights of cerebrum and kidney (mg/g) when comparing both control groups (groups I&II).

A statistical comparison of the mean values of the initial body weight (g) of MSG treated rats (group III) compared to those of the negative control group (group I) showed no statistical significant difference (p>0.05). While the mean values of final body weight (g) and the percent of body weight gain (%) of MSG group (group III) showed a statistically significant increase (P<0.001) when compared with those of the negative control group (Group I).

There was also a non significant difference (p>0.05) as comparing the mean values of the relative cerebral weight (mg/gm) in MSG group (group III) to those of negative control group (group I). In contrast, there was a significant increase (p<0.001) in the mean values of relative kidney weight (mg/ g) of MSG group compared to the negative control group.

2-Results of kidney function test (KFTs) and urinary albumin excretion:

Biochemical analysis of kidney function tests and microalbuminuria are shown in Table (2). There were no statistical significance differences (p>0.05) as regard the mean values of KFTs (BUN & serum creatinine) and urinary albumin excretion when comparing both control groups. Statistical comparison of mean values of KFTs and urinary albumin excretion between the **negative control group** (group I) and **MSG group** (group III) revealed significant increase (p< 0.001).

3- Results of histopathological examination of the cerebrum (frontal cortex):

The control groups (groups I &II) have the same histological and immunohistochemical findings. H&E stained cerebral sections of rats of control groups showed normal picture. Examination of H&E stained cerebral sections of the control groups showed the normal arrangement of the layers of the cerebral frontal cortex, the molecular layer

covered with pia matter, the granular layer, and the pyramidal layer (Figs. 1&2). On the other hand immunoperoxidase stained sections for Bax showed no immunoreactions for both nerve cells and endothelial cells (Fig. 7) .

Table (1): Effects of monosodium glutamate treatment on total body weights, relative cerebral weight (CW/BW) and relative kidney weight (KW/BW) compared to control groups.

Groups	Negative control group	Positive control group	MSG group
Initial Body Weight (g)	188.17 ± 7. 25	188.66± 6.87	189.25± 6.31 .
Final Body Weight (g)	216.33± 8.44	217.67± 10.59	230.58± 3.55*
% of body weight gain	14.97%	15.83%	21.84%*
Relative cerebral weight CW/BW (mg/g)	1.67± 0.08	1.65± 0.07	1.59± 0.09
Relative kidney weight KW/BW(mg/g)	2.72 ±0.16	2.78 ±0.14	4.49 ±0.04*

Values are given as mean ±SD for group of 12 animals, CW/BW: cerebral weight compared to final body weight, KW/BW: kidney weight compared to final body weight .   = non-significant difference compared to negative control; * = significant difference compared to negative control group.

Table (2): Effects of monosodium glutamate treatment on kidney function tests (BUN& S. creatinine) and urinary albumin excretion compared to control groups.

Groups	Negative control group	Positive control group	MSG group
Blood urea nitrogen (mg/dl)	21.00±1.03	22.02± 1.00	38.5± 3.3*
Serum Creatinine (mg/dl)	0.94±0.15	0.92± 0.14	2.18± 0.06*
Urinary albumin excretion (µg/24hrs urine)	100 ±16	98 ±14	409 ±40*

Values are given as mean ±SD for group of 12 animals,   = non-significant difference compared to negative control; * = significant difference compared to negative control group.

On the contrary, MSG treated rats (group III) presented signs of neurodegeneration with pyknosis and vacuolization. Examination of H&E stained cerebral frontal cortex sections of MSG treated rats showed most nerve cells distorted in shape and had deeply stained shrunken pyknotic nuclei. cells are surrounded by unstained areas . there was vacuolization of the neuropile surrounding the affected damaged nerve cells (Figs. 3&4). There was also focal aggregation of glial cells (gliaosis and satellitosis) (Fig. 5) and chroidal plexus congestion (Fig. 6). Immunoperoxidase stained sections for Bax showed positive immunoreactions in most of the nerve cells when compared to the control group (Fig. 8).

3- Results of histopathological examination of the kidneys:

The kidneys of rats' of both control groups (Groups I&II) showed normal histological picture of the kidney. The renal cortex is formed of Malpigian renal corpuscles, which are formed of tuft of capillaries (glomerulus), surrounded by Bowman's capsule. Renal space is preserved. Proximal and distal convoluted tubules were normal (Figs. 9&10).

Microscopic examination of the kidneys specimens of the rats of MSG group (Group III) after 28 days of treatment Showed variable pathological changes in glomeruli and renal convoluted tubules. Such changes exhibited an existence of hydropic degeneration of the tubular epithelium with vacuolization and tubular dilatation with intra-lumenal hyaline casts (Figs. 11&12). The presence of mononuclear inflammatory cellular infiltration in interstitial tissues were noticed (Fig. 12).

Also, they were swelling in the lining endothelium of the glomeruli associated focal areas of glomerular atrophy (Figs. 13&14). Dilatation and hyperemia in the intertubular cortical blood vessels and focal hemorrhage between the tubules were seen clearly (Fig. 14).

Immunolocalization of Bax in all examined kidney specimens of control groups revealed negative expression in both glomerular endothelium and renal tubular epithelium (Fig. 17).
 Twenty eight days of treatment with monosodium glutamate resulted in positive immune reaction of Bax in glomerular endothelium and some renal tubular epithelial cells (Fig.18)

Figure (1): A Photomicrograph of a section in the cerebral cortex of a control rat showing the molecular layer (ML) covered with the pia matter (P), granular layer (GL), pyramidal layer (PL). H&E x 100.
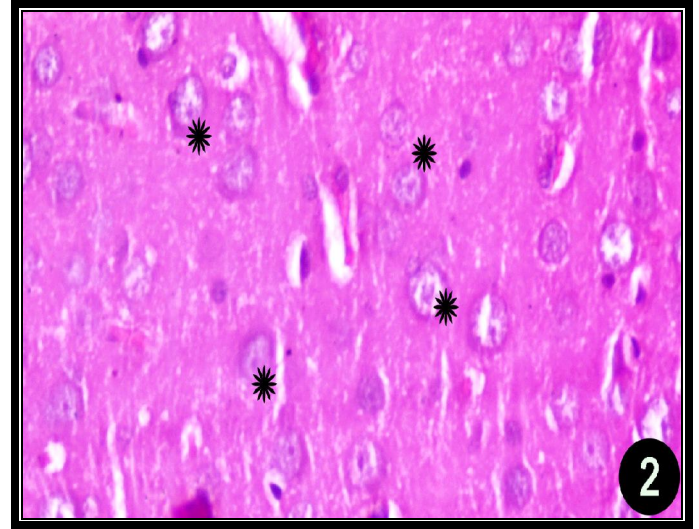

Figure (2): A Photomicrograph of a section in the cerebral cortex of a control rat showing the normal neurons of the granular layer with large vesicular nuclei (*). H&E x 400.
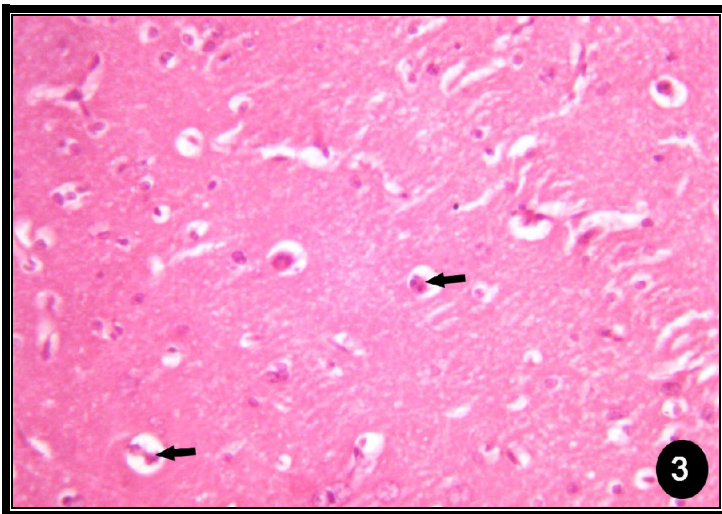

Figure (3): A Photomicrograph of a section in the cerebral cortex of MSG- treated rat showing most neuronal cells shrunken surrounded by spaces (Arrows).H&E x 200.

Figure (4): A Photomicrograph of a section in the cerebral cortex of MSG- treated rat showing most pyramidal cells appear distorted with deeply stained shrunken pyknotic nuclei, these cells are surrounded by unstained areas (*). There are vacuolizations (V) of the neuropile surrounding the affected nerve cells. H&E x 400.
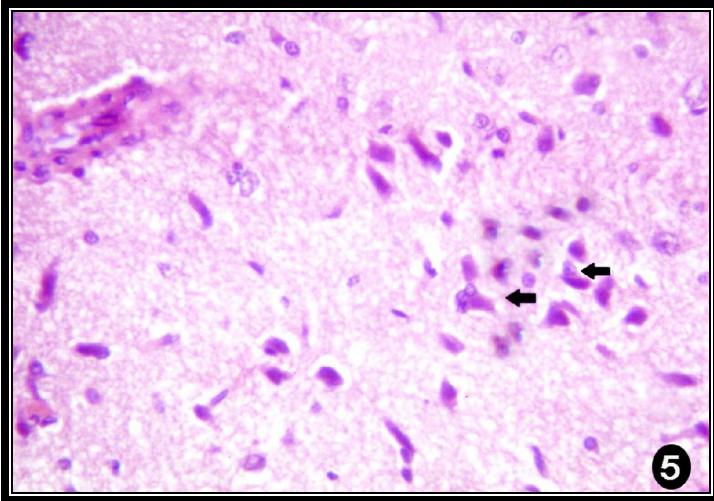
Figure (5): A Photomicrograph of a section in the cerebral cortex of MSG- treated rat showing focal aggregation of glial cells (gliaosis and satellitosis) (Arrows).H&E x 200.
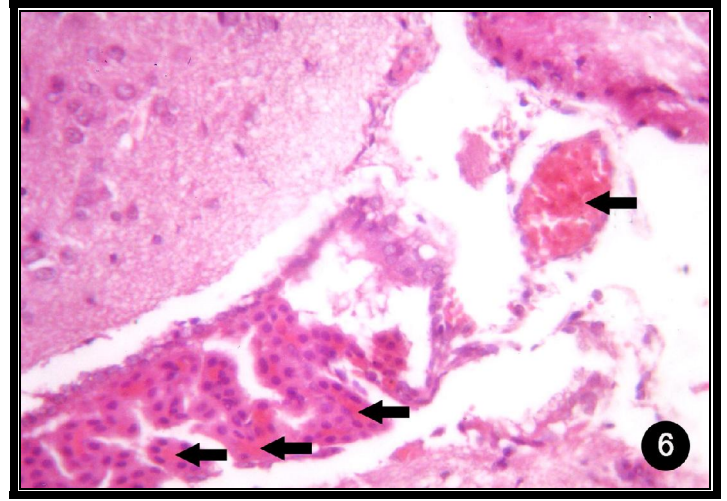

Figure (6): A Photomicrograph from a section in the cerebral cortex of MSG- treated rat showing chroidal plexus congestion (Arrow). H&E x 400
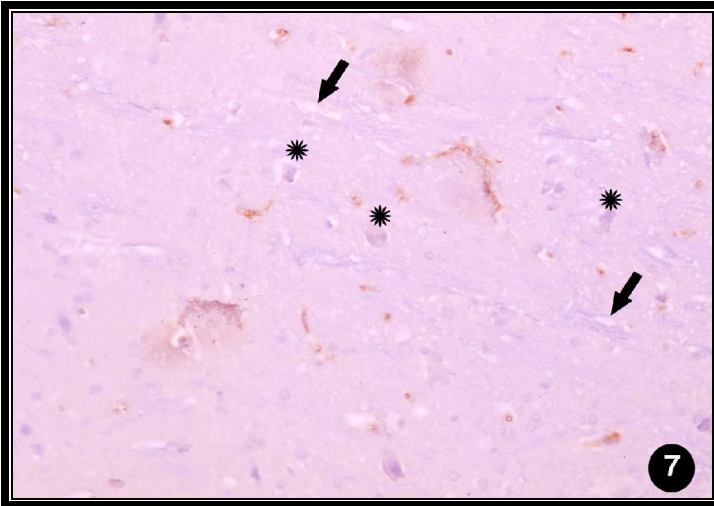

Figure (7): A Photomicrograph of a section in the cerebral cortex of a control rat showing negative immunoreactions for Bax protien in nerve cells (*) and endothelial cells (Arrow). immunoperoxidase for Bax x 400
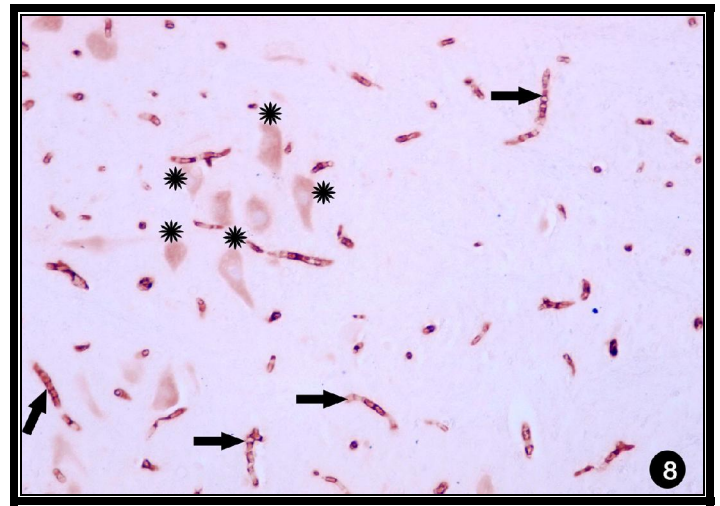

Figure (8): A Photomicrograph from a section in the cerebral cortex of MSG- treated rat showing positive immunoreactions for Bax protein in endothelial cells (Arrows) and weak reaction in nerve cells (*). immunoperoxidase for Bax x 400
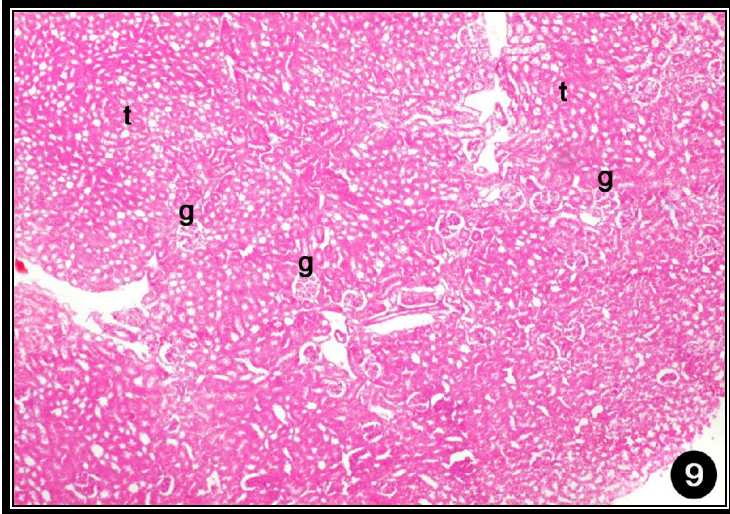
Figure (9): A photomicrograph of a section in a control rat's kidney showing normal glomerular tuft (g) and tubules (t).   (H & E x40)
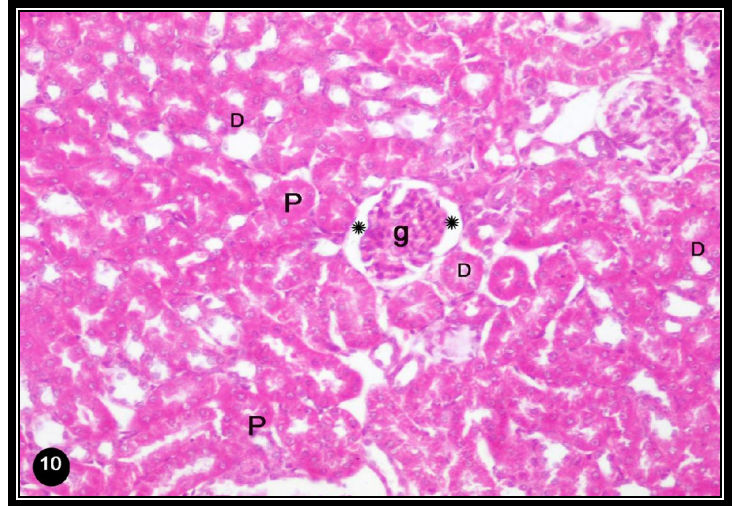

Figure (10): A photomicrograph of a section in the kidney cortex of a control rat showing renal corpuscle formed of glomerulus (g) surrounded with Bowman's space and preserved renal space (*). Note the proximal (P) and distal (D) convoluted tubules. (H & E x100)
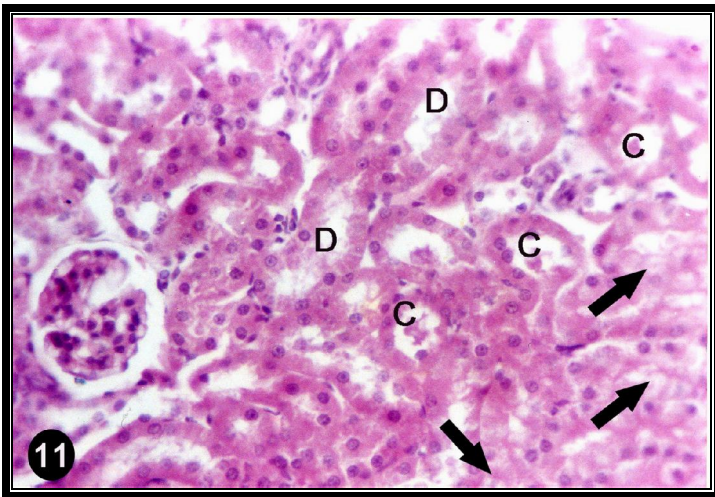

Figure (11): A photomicrograph of a section from the kidney of a rat of MSG group showing hydropic degeneration and vacuolization of the tubules (arrows), tubular dilatation (D) with hyaline casts (C)   (H & E x200)
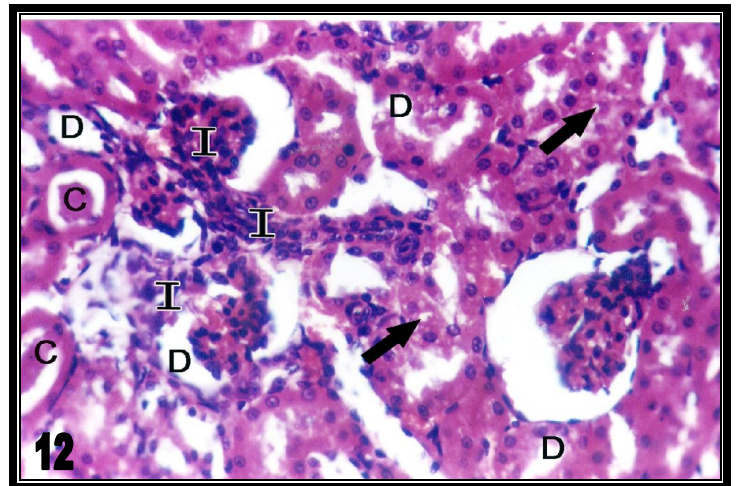

Figure (12): A photomicrograph of a section from the kidney of a rat of MSG group showing monocellular inflammatory cell infiltrates   (I). Tubular dilatation (D), hyaline casts (C) and vacuolization of tubular cells are also observed (arrows) (H & E x200)
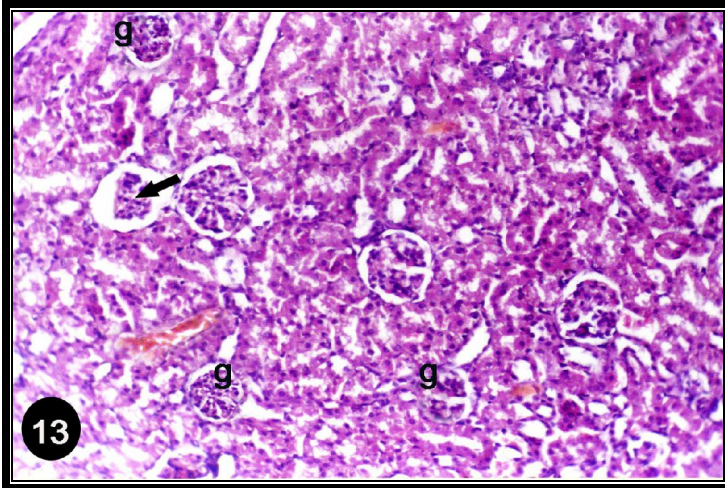
Figure (13): A photomicrograph of a section from the kidney of a rat from MSG group. Some glomeuli showing shrinkage (arrow) and the others showing swelling with partial loss of the Bowman's spaces (g). (H & E x100)
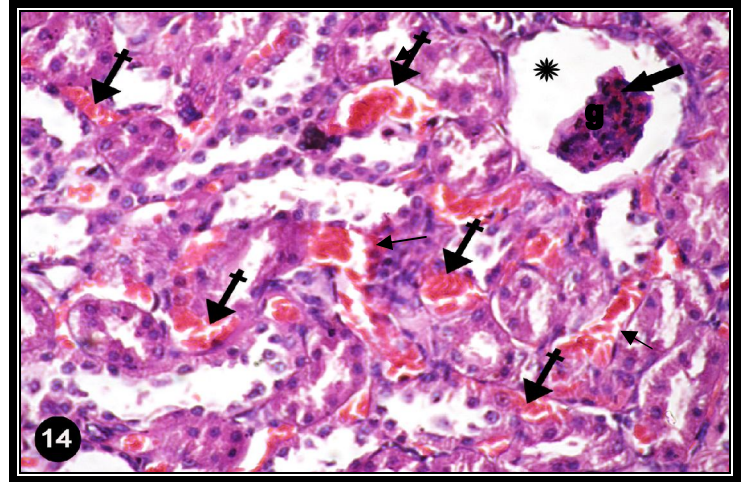

Figure (14): A photomicrograph of kidney section form a rat of MSG group showing severe shrinkage of the glomeulus (arrow) with increased in Bowman's spaces (*). There is also dilated congested interstitial blood vessels and interstitial hemorrhage in the inter tubular spaces (crossed arrows). (H & E x 400).
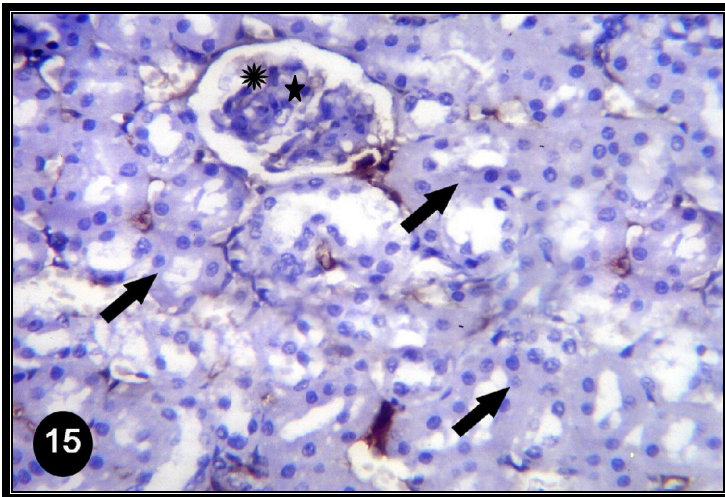

Figure (15): A Photomicrograph from a section in kidney of a control rat showing negative immunoreactions for Bax protien in glomeruli (*) and tubular cells (Arrow). immunoperoxidase for Bax x 400

Figure (16): A Photomicrograph from a section in kidney of a rat of MSG group showing positive immunoreactions for Bax protein in mainly in glomeruli (*) and some tubular cells (Arrows). immunoperoxidase for Bax x 400

4. Discussion

Nowadays, monosodium glutamate (MSG) is frequently used as a flavor enhancer, the fact of which makes it one of the most applied food additives in the modern nutrition allover the world **(Garattini, 2000).**

In 1959, the U.S. Food and Drug Administration (FDA) classified monosodium glutamate as generally recognized as safe. Since that, FDA has sponsored extensive reviews on the safety of monosodium glutamate where most of the experts concluded its safety. They noted that doses as high as 2.1 g per kg for 30 days produced no adverse effects in males when given for a 70 kg male **(Beyreuther et al., 2007).**

But accurate information on the daily intake of specific food additives by individuals is difficult to obtain especially for food additives that considered GRAS (generally recognized as safe) and so studying

their cumulative toxic effects should be a matter of concern (**Fennema, 1987**).

So the aim of the present study is concerned with the possible biochemical, histological and Bax immunohistochemical changes that may occur in rats' cerebra and kidneys following short term chronic toxicity for 28 days of MSG in a dose of 830 mg/kg that equals 1/20 of oral rat LD_{50}.

Results of the present study demonstrated several toxicological consequences in experimental animals secondary MSG treatment. These findings included a significant increase in the final total body weight, in the percent of total body weight gain and relative kidney weight.

These findings were consistent with the previous studies concerned with MSG induced obesity. It was found that monosodium glutamate has been shown to cause obesity in lab rats by down regulating hypothalamic appetite suppression and, thus, increasing the amount of food the lab rats consumed (**Hermanussen *et al.,* 2006**).

The negative effects monosodium glutamate were first observed in newborn mice by Lucas D R and Newhouse J P, 1957 who noted that monosodium glutamate induced elevation of glutamate level in the brain of mice that end in destruction of the neurons in the inner layers of the retina. Later, in 1969, John Olney discovered the phenomenon was not restricted to the retina, but occurred throughout the brain, and coined the term excitotoxicity (**Kandel *et al.,* 2000**).

In the present study monosodium glutamate treatment in a dose of 850 mg/kg has resulted in variable histopathological changes in the rats cerebra. These changes were in the form of neurodegenerative changes with pyknosis and vacuolization. Examination of H&E stained cerebral cortex sections of MSG treated rats showed most nerve cells distorted in shape with deeply stained shrunken pyknotic nuclei surrounded by unstained pale areas with vacuolization of the neuropile surrounding the damaged neurons. There was also chroidal plexus congestion and focal aggregation of glial cells (Gliaosis and satellitosis)**.**

Satellitosis observed here coincided with MSG induced neuronal cell death. As satellitosis means accumulation of neuroglial cells around neurons; seen whenever neurons are damaged (**Vijayan *et al.,* 1993**).

The results of the current study were in consensus with **Martin *et al.* (2000); Ortiz *et al.* (2006); and Pavlovic *et al.* (2007).** They reported treatment with glutamate as monosodium glutamate (MSG) induced severe neurochemical damage and neurotoxic effects on some brain regions.

Excessive accumulation of glutamate in the synaptic cleft has been associated with excitotoxicity

(**Mallick, 2007**). Excitotoxicity is the pathological process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters such as glutamate and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the N-methyl- D- aspartate (NMDA) receptor are over activated.

Excitotoxins like NMDA and kainic acid which bind to these receptors, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium ions (Ca2+) to enter the cell. Ca2+ influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases enzymes. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA (**Fujikawa, 2005**).

Moreover, the production of tumor necrosis factor- á (TNF- á), an inflammatory mediator, was significantly increased in rat's brain following glutamate treatment (**Chaparro- Huerta *et al.,* 2005**).

Additionally, it was also observed in the present study that MSG can induce a change in the pattern of expression of Bax protein in cerebral tissues.

These results are in agreement with **Goa *et al.* (2008).** They found that glutamate treatment for neuronal cells resulted in over expression of Bax protein and neuronal cell death with no effect on Bcl_2 expression. These observations could be attributed to glutamate ability to interact with NMDA receptors which induces mitochondrial calcium increase, free Radical generation, activation of proteases, phospholipases and endonucleases, and the transcriptional activity of apoptotic programmes (**Pelligrini- Giampetro *et al.,* 1997**).

The mitochondria are essential for life. Without them, a cell ceases to respire aerobically and quickly dies, a fact exploited by some apoptotic pathways. Apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out. Mitochondrial proteins are released into the cytosol following an increase in permeability. They binds to inhibitor of apoptosis proteins (IPA) and deactivates them, preventing them from arresting the apoptotic process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of a group of cysteine proteases called caspases, which carry out the degradation of the cell, therefore the actual degradation enzymes can be seen to be indirectly

regulated by mitochondrial permeability (**Fesik and Shi, 2001).**

Mitochondrial permeability is regulated by various proteins, such as those encoded by the mammalian Bcl-2 family of anti-apoptopic genes. *Bcl-2* proteins are able to promote or inhibit apoptosis by direct action on mitochondrial permeability where Bax and/or Bak increase mitochondrial permeability, while Bcl-2, Bcl-xL inhibit it (**Dejean *et al.,* 2006).**

The present study indicated that MSG induced marked biochemical and histopathological alterations in the kidney tissues of rats. There was affection for both the brain and the kidneys. kidney damage usually is associated with functional changes that may have been detrimental to the health status of the experimental animal. The greater the severity of the insults the more rapid the progression of neuronal injury. The principle holds true for toxicological insult to the brain and other organs (**Eweka, 2007).**

It may have been inferred from the present study that prolonged administration and higher doses of MSG resulted in increased toxic effect on the kidney that may play a pivotal role in cerebral damage observed here in the present study.

The observed renal damage was accompanied with impairment of the renal biochemical parameters as there was a significant elevations in the mean values of BUN, serum creatinine and urinary albumin excretion in MSG treated rats as compared to control. These findings indicating impairment of kidney function in rats treated with MSG.

In this context **Attia *et al.* (2008)** found that administration of MSG resulted in impairment of some renal biomarkers reflected by the significant increase in urea and decrease in albumin serum levels.

The significant increase in microalbuminuria measured by the end of the present study reflecting changes in glomerular filtration rate, glomerular impairment and failure of the kidney to retain the plasma albumin (**Abd-Alaziz *et al.,* 2008).**

It is generally believed that the increased urinary albumin excretion in most renal insults is mostly glomerular in origin. This may be due to increased intraglomerular pressure, loss of negatively charged in the basement membrane, and increased basement membrane pore size (**Marshall, 2004).**

Furthermore, the biochemical alterations induced by MSG treatment in the current study were augmented by the observed histopathological changes in the examined renal tissues of rats treated with MSG. These histopathological alterations were in the form of swelling of the lining epithelium of glomeruli, hydropic degeneration and vacuolization of the renal convoluted tubules, blood vessels dilatation and focal hemorrhage between the degenerative renal tubules.

Similar Results have been reported by Mitsumari *et al.* **(1998) and Attia *et al.* (2008).**

Additionally, it was also observed in the present study that MSG can induce a change in the pattern of expression of Bax protein in both glomerular endothelial cells and some tubular epithelial cells.

The changes of Bax and Bcl-2 expression have been described in various experimental renal models; ischemic renal injury, diabetic nephropathy, and glomerulonephritis (**Yang *et al.,* 2001**).

Glomerular swelling observed in the current study could be attributed to a decrease in O_2 levels which causes a drop in aerobic respiration. To maintain ATP levels, the cells must rely more on glycolysis. Glycolysis leads to lactic acid builds up, which causes the intracellular pH to drop. An acidic environment in the cell causes dysfunction of the Na+/ K+ ATPas and consequent cell swelling due to an influx of Na+ and H_2O. In the present investigation, many renal tubules of the rat kidneys showed marked degenerative lesions under the effect of MSG. This is justifiable since the renal tubules are particularly sensitive to toxic influences, in part because they have high oxygen consumption and vulnerable enzyme systems, and in part because they have complicated transport mechanisms that may be used for transport of toxins and maybe damaged by such toxins. Also the tubule come in contact with toxic chemicals during their excretion and elimination by the kidneys (**Tisher and Brenner, 1989).**

The circulating MSG was dissociated into sodium (Na) and L- glutamate. A part of the L-glutamate in the cell conjugates, in order to be eliminated, and another part is transformed into glutamine. When this occurs, the cells try to repair some of the damages by using enzymes that are present in the smooth endoplasmic reticulum but the cell is not able to completely remove the excess glutamine (**Singh *et al.,* 2003).** Probably for this reason, the convoluted tubules showed cloudy swelling.

When L- glutamate arrives in high concentrations through the renal artery, the kidney tries to excrete it. The renal corpuscle receives the L-glutamate through the afferent arteriole, it is absorbed, filtrated and across the membrane damaging the cell (**Attia *et al.,* 2008).**

Alterations in the levels of lipid peroxides and antioxidants such as reduce glutathione, catalase and superoxide dismutase were observed in different organs and systems of adult rat during MSG treatment (**Ahluwalia *et al.,* 1996 and Choudhary *et al.,* 1996).**

In addition, many glutamate receptors have been demonstrated outside the CNS (**Attia et al., 2008**).

Moreover, NMDA receptors (one of glutamate receptors) have been found in extraneuronal tissues, including pancreatic cells, the male lower urogenital tract, kidneys, lymphocytes, and megakaryocyte. There is scant evidence regarding its physiological function in extraneuronal tissues, especially in the kidneys. overstimulation of NMDA receptors can modulate glutamate postsynaptic neurotransmission by generating Ca^{2+} channel openings, and by overloading (**Nagata et al., 1995**) and excessive reactive oxygen species generation (**Conn and Pin, 1997**).

Ischemia, followed by reperfusion, impairs kidneys and contributes to renal dysfunction (**Avshalumov and Rice**, **2002**). Ischemia-reperfusion or hypoxia-reoxygenation injury also evokes burst amounts of reactive oxygen species and Ca^{2+} overload in damaged renal tubules, triggering the entry of these tubular cells into apoptotic and necrotic cell death, and subsequently, to renal dysfunction (**Deng et al., 2002**).

In conclusion, short term administration of MSG to rats induced different toxic effects on both the cerebra and kidneys of these rats. These toxic effects manifested by widespread neurodegenerative changes in the rats's cerebral cortexes, biochemical elevations of BUN, serum creatinine, and urinary albumin excretion, and alterations in renal histopathological architectures. On the other hand, MSG treatment induced upregulation of Bax protein (apoptotic inducer) in these tissues. So, we can concluded that the results of the present study confirmed the neurotoxic and nephrotoxic effects of MSG, where Bax protein appeared to have a pivotal role in MSG induced apoptosis in these organs.

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7/2/2011