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MONOSODIUM GLUTAMATE-INDUCED OXIDATIVE STRESS AND GENOTOXICITY IN THE RAT AS EVIDENCE OF A POTENTIAL RISK IN HUMAN DIETS

BY

Ashoush, I. S.* and Abd-Elaziz, A. M. S.**

* Food Sci. Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.
** Animal Production Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

ABSTRACT

The present investigation was performed to study the effect of daily oral administration of Monosodium glutamate (MSG) as a widely food flavouring used with three dose levels (35, 350 and 3500 mg / kg b.w. /day), for 90 days on albino male rats. The results revealed that the addition MSG caused elevation in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, also significant elevation in malondialdehyde in plasma was occurred after 90 days of the treatment. In addition, MSG caused significant alteration in histological structure in liver and kidney, moreover chromosomal aberration was also found.

Key words: Monosodium glutamate (MSG), oxidative stress, malondialdehyde, genotoxicity, Biochemical analysis and Rats.

INTRODUCTION

Monosodium glutamate (MSG) is commonly used as a flavor enhancer, especially in Chinese, Thai and Japanese foods. MSG is marketed under such trade names as A-One, Ajinomoto and Vedan. A-One (> 99% MSG) is a popular condiment in soups, stew sauces and porridge preparation in several West African countries. However, its use as a flavor has been questioned due to a number of reports describing toxic effects in human adults, as manifested by the Chinese restaurant syndrome. Thus, MSG has been reported to produce symptoms, such as numbness, weakness, flushing, sweating, dizziness and headaches, which begin between 10 min and 2 hours after a meal containing MSG, and last for a period up to 4 hours. In addition to the Chinese restaurant syndrome, MSG is known to elicit other toxic effects. On the other hand, alteration in mitochondria lipid peroxidation and antioxidant status in different regions, namely the cerebral hemispheres, cerebellum, brain stem and diecephalon, is well documented by many investigators, (Schaumberg *et al.* 1969; Geha *et al.* 2000 and Obaseiki-Ebor *et al.* 2003).

Ahluwalia *et al.* (1994) investigated the effect of subcutaneous administration of monosodium glutamate (MSG) to normal adult male mice for 6 days at dose levels of 4 and 8 mg/g body weight. A significant increase in erythrocyte glucose content accompanied by increase in lipid peroxidation. They

also observed an incremental level in the activities of glutathione reductase (GR), glutathione-S-transferase (GST) and glutathione peroxidase (GPX). It was observed that MSG, above 4 mg/g body weight, produced oxidative stress which was counteracted by the body by maintaining the level of glutathione, which was done by increasing the activity of its metabolizing enzymes.

Shibata, *et al.* (1995) showed that no significant increase in proliferative or neoplastic lesion development in the urinary tract were observed in any MSG-treated animals. In addition, the occurrence of neoplastic lesions in other organs did not differ between treated and control groups.

Choudhary, *et al.* (1996) demonstrate that, daily administration of monosodium glutamate (MSG) to adult male mice subcutaneously, for 6 days, at dose levels of 4 and 8 mg/g body weight, significantly increased lipid peroxidation in the hepatic microsomes, as seen 31 days after the last injection. A highly significant increase was observed in the level of hepatic calcium and ascorbic acid. These observations suggested that MSG at dose levels above 4 mg/g body weight induced oxidative stress in hepatic microsomes. Attempts to maintain the redox state of the cell are suggested by increase in the ascorbic acid content and the activities of glutathione dependent enzymes.

Yeda, *et al.* (2004) examined the effects of a hypercaloric diet (HD) on hepatic glucose metabolism of young rats with and without monosodium glutamate (MSG) administration and the association of these treatments with evaluating markers of oxidative stress. Glycogen, hexokinase (HK), glucose-6-phosphatase (G6PH), lipid hydroperoxide, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were determined in liver. HD rats showed hypoglycemia, hyperinsulinemia, high hepatic glycogen and HK with decreased G6PH. MSG and MSG-HD had hyperinsulinemia, hyperglycemia, decreased HK and increased G6PH in hepatic tissue. Hypercaloric diet and monosodium glutamate administration induced alterations in metabolic rate of glucose utilization and decreased antioxidant defenses. Therefore, the hepatic glucose metabolic shifting induced by HD intake and MSG administration were associated with oxidative stress in hepatic tissue.

Farombi and Onyema (2006) examined the effect of continues Monosodium glutamate (MSG) as a flavor enhancer in West African and Asian diets. MSG-induced oxidative damage in the liver, kidney and brain of rats. In addition, genotoxicity of MSG was investigated in a rat bone marrow micronuclei model. MSG administered intraperitoneally at a dose of 4 mg/g body wt markedly increase malondialdehyde (MDA) formation in the liver, the kidney and brain of rats. MSG increased the activities of alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transferase in rats treated with MSG.

Ortiz, *et al.* (2006) evaluate the toxic effects of the monosodium glutamate in liver and kidney after an intra-peritoneal injection. They observed increments in the concentration of alanine aminotransferase an aminotransferase at 30 and 45 min. Also, an increment of the lipid peroxidation products, in

kidney, was exhibited at 15, 30 and 45 min while in liver it was observed at 30 and 45 min as well as degenerative changes were observed (edema-degeneration-necrosis) at 15, 30 and 45 min.

Therefore, the present work was carried out to know if a dose of 35, 350 and 3500 mg MSG per Kg of body weight is toxic when administered systemically to rats. Further studies were made on some biochemical and morphological changes in the liver and kidney; activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the lipid peroxidation marker of membrane damage for free radicals and chromosomal aberrations in bone marrow of rats cells was taken as indications of genotoxic action of monosodium glutamate.

MATERIALS AND METHODS

Chemicals:

Monosodium glutamate (MSG) were purchased from the Sigma Chemical Company, P.O. BOX 4508, St. Louis, U.S.A..

Animals and treatment:

Twenty-four male albino rats (Wister strain), with an initial body weight of 150 g were obtained from Organization of Biological Products and Vaccines (Helwan farm) were randomly distributed into four groups. The rats were allowed to acclimatize for 1 week. Animals were maintained on a 12:12 hour light/dark cycle. The control group dosed with distillated water. The animals were fed normal laboratory chow purchased from Imbaba Feeds and had access drinking water. Rats in groups 2- 4 were treated orally every day by using a round-head needle syringe with 35, 350, 3500 mg/kg BW MSG for 90 days.

Rats were observed daily for the appearance of any symptoms of discomfort that might be related to treatment. Rats fasted overnight before butchery, were slaughtered at the end of the experiment. Blood samples were collected from the retro-orbital plexus from all animals of each group on the day 30, 60 and 90 days into clean, dry and heparinzed labeled tube (10 I.U. /ml). Blood was centrifuged (3500 r.p.m. for 15 min.) to separate plasma, which was tightly kept in sealed aliquot tubes at -18 °C until biochemical assays.

Serum enzyme assay:

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method adopted by Reitman and Frankel (1957), using special Biodiagnostic kits (Egypt).

Determination of malondialdehyde (MDA):

Lipid peroxidation was determined by measuring the thiobarbituric acid reacting substances (TBARS) in plasma at 534 nm according to Ohkawa, *et al.* (1979), using special Biodiagnostic kits (Egypt).

Tissue sampling:

Autopsy samples were taken from the liver and kidney of the sacrificed rats and fixed in 10% formoline saline solution for ten hours at least then washed in tap water for 12 hours. Serial alcohol (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue speciemens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3 micron thickness by slidge microtome. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin stain (Banchroft *et al*; 1996) for histopathological examination by the light microscope.

Preparation of bone marrow smear:

As mentioned before, the animals were treated with MSG at 35, 350 and 3500 mg/kg body weight daily and after 90 days the cytogenic effects of this substance were studied. The technique discussed by Asanami and Shimono (2000) briefly, the femurs of each rat were removed and stripped clean of muscle. A syringe was then introduced into the marrow canal at the epiphyseal end, the marrow was flushed out through the hole at the iliac end with fetal bovine serum. The bone marrow was placed on a slide and mixed to obtain a homogenous mixture and spread as a smear. After the slides were dried, they were fixed in absolute ethanol for 5 min and air died to remove the solvent. The slides were stained in 5% Giemsa for 30 min and rinsed in phosphate buffer pH 7.4 for 30 sec. and distilled water for 2 min and air dried. The slides were coded screened to avoid bias and scored using a compound microscope with the aid of a tally counter for the presence of aberrations.

Statistical analysis:

All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980) and practicing Statistical Analysis System program (SAS, 1997), using Student's t-test.

RESULTS AND DISCUSSION

1. Effects on liver function parameters:

Data in tables (1&2) indicated that MSG flavor enhancer, when orally administrated into albino rats, induced significant elevation in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes after treatment with medium and highest doses (350 & 3500 mg / kg BW) over the experimental period.

The resultes of this study showed high levels of ALT and AST, which indicate that serum concentration of these enzymes fluctuates with the hepatic damage. The localization of ALT and AST in the hepatocyte is cytoplasmatic; the MSG cytotoxic effect induce tissue damage and enzyme release which increase their serum levels. These findings are in agreement with the results obtained by Farombi and Onyema (2006) and Ortiz, *et al.* (2006).

2. Effects on lpid peroxidation:

As seen in table (3) the daily administration of MSG significantly increased the levels of MDA after 90 days, especially in medium and high dose groups (350 & 3500 mg/kg BW). The increase of lipid peroxidation products MDA was the result of

liver and kidney damage. At the same time, there were an increase in MDA accompanied with the increase in the activity of ALT and AST enzymes. The morphology of the damage shows the correlation between the progressive damage and the lipid peroxidation products. In liver the necrobiotic were observed with high levels of MDA. In kidney, very similar responses were exhibited with fibrosis. All of these data could be explained by the excitotoxic role of the glutamate. These findings are on the agreement with those obtained by Ahluwalia *et al.* (1994); Choudhary, *et al.* (1996) and Farombi and Onyema (2006).

Table (1): Aspartate aminotransferase (AST) activity as affected by different concentrations of monosodium glutamate (MSG) in male albino rats.

MSG Concentration mg/kg b.w.	AST activity (U/L) at the indicated post-treatment (days)		
	30	60	90
0	9.34 ± 0.74	10.67 ± 0.79	12.01 ± 0.49
35	11.07±0.49	10.64±0.84	11.77±0.59
350	16.51±1.33*	23.52±1.96**	27.57±1.89**
3500	19.85±1.16**	30.32±2.01**	33.22±1.98**

* Statistical significant differences ($P < 0.05$)
** Statistical significant differences ($P < 0.01$)

Table (2): Alanine aminotransferase (ALT) activity as affected by different concentrations of monosodium glutamate (MSG) in male albino rats.

MSG Concentration mg/kg b.w.	ALT activity (U/L) at the indicated post-treatment (days)		
	30	60	90
0	4.78 ± 0.37	6.44± 1.36	7.44 ± 1.36
35	5.38±0.57	9.39±1.34	15.31±2.16*
350	8.51±1.03*	22.93±3.96*	33.16±2.99**
3500	15.72±1.17**	37.00±2.06**	43.71±2.04**

* Statistical significant differences ($P < 0.05$)
** Statistical significant differences ($P < 0.01$)

Table (3): Effect of different concentrations of MSG on lipid peroxaidation (µmol TBARS formed/min/ml) in male albino rats.

MSG Concentration mg/kg b.w.	lipid peroxaidation (µmol MDA formed/min/ml) after 90 days of treatment
0	0.99 ± 0.04
35	1.29 ± 0.27
350	2.44 ± 0.12**
3500	3.16 ± 0.18**

* Statistical significant differences ($P < 0.05$)
** Statistical significant differences ($P < 0.01$)

3. Effect of MSG on histopathological changes in male albino rats:

Data concerning the histopathological examination in the tissues of some organs of treated rats when orally administered with doses of MSG for 90 days are presented in Micrograph from (1) to (6). The obtained results, revealed the influence of MSG on tested animal tissues. To facilitate the presentation of data, each organ is discussed separately as follows:

3.1. Liver:

In micrograph 1, no histopathological alteration observed, normal histological structure of the central vein (CV) and surrounding hepatocytes (h) are seen. While at the second dose (micrograph 3) hepatocytomegaly (arrow) with double nuclei (d) was observed with some associated mononuclear leucocytes and inflammaory cells infiltration (m) in between.

At a third dose, the mononuclear leucocytes inflammatory cells infiltration (C) with necrobiotic changes in some hepatocytes (arrow) was recorded (Micrograph 5).

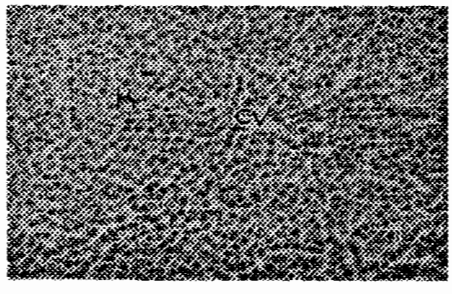
3.2. Kidney:

In MSG–treated group with the first dose, showing no histopathological alteration observed and the normal histological structure of the glomeruli (G) and renal tubules (R), (Micrograph 2).

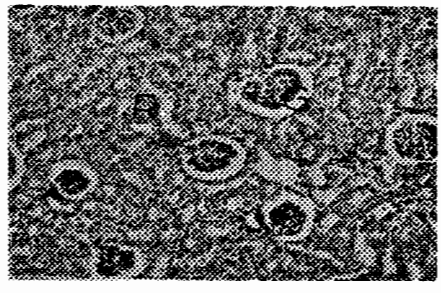
Kidney tissue exposed to second dose showed showing swelling granular and vacuolar degeneration in the cytoplasm of the epithelial cells lining the renal tubules (R) (Micrograph 4).

At the third dose Focal fibrosis (arrow) was noticed in between the degenerated renal tubules (D) (Micrograph 6).

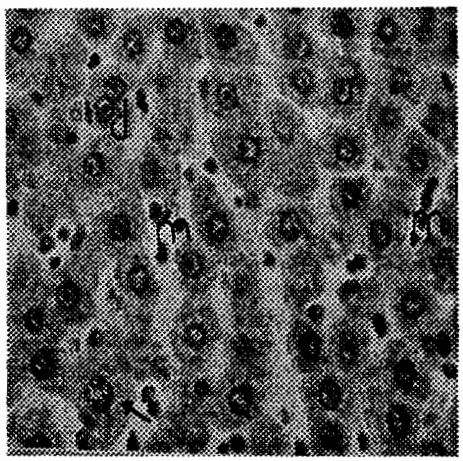
These results in agreement with the results obtained by Ortiz, *et al.* (2006) and Shibata, *et al.* (1995) which reported that, monosodium glutamate-induced damage in both liver and kidney.



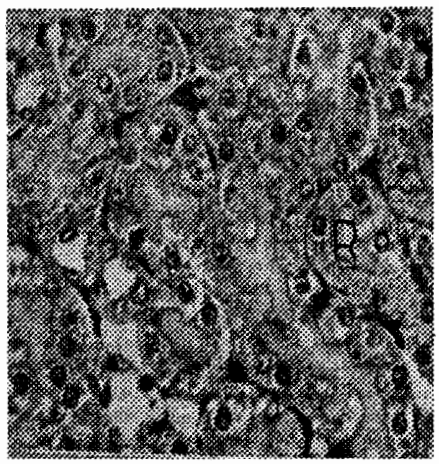
Micrograph (1): Photomicrography section in the Liver of MSG administered rat (1st dose) for 90 days. (H & E, 40X)



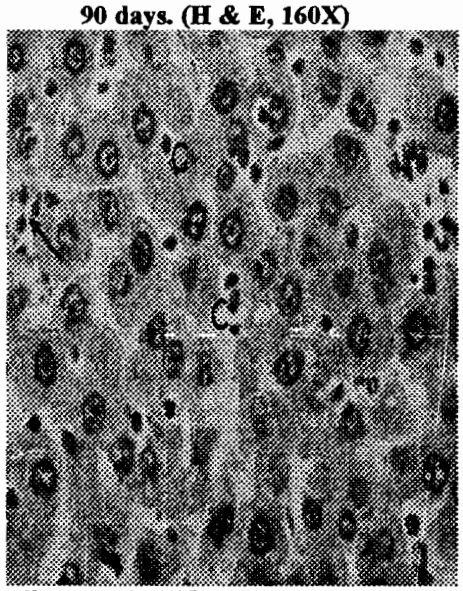
Micrograph (2): Photomicrography section in the Kidney of MSG administered rat (1st dose) for 90 days. (H & E, 40X)



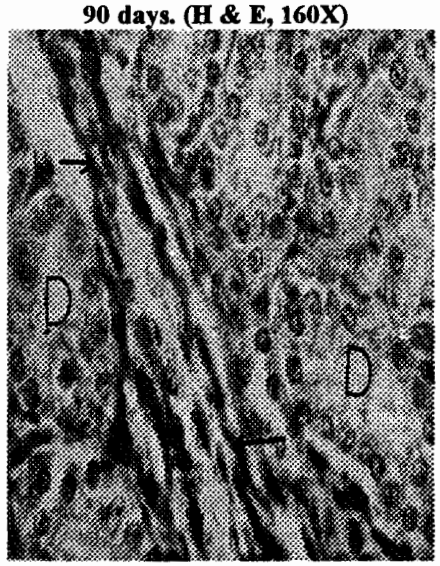
Micrograph (3): Photomicrography section in the Liver of MSG administered rat (2nd dose) for 90 days. (H & E, 160X)



Micrograph (4): Photomicrography section in the Kidney of MSG administered rat (2nd dose) for 90 days. (H & E, 160X)



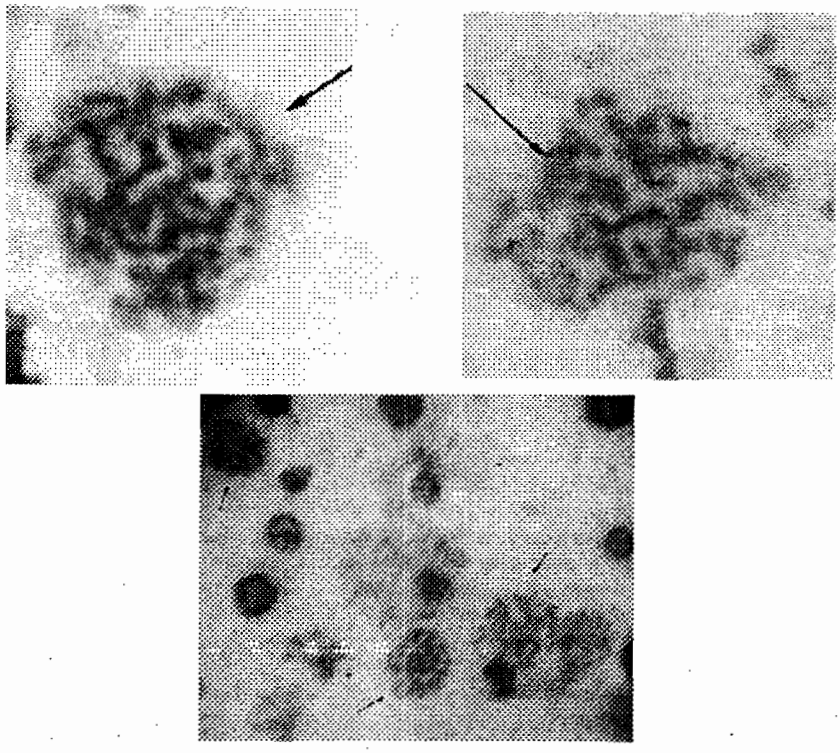
Micrograph (5): Photomicrography section in the Liver of of MSG administered rat (3rd dose) for 90 days (H & E, 160X)



Micrograph (6): Photomicrography section in the the Kidney of MSG administered rat (3rd dose) for 90 days (H & E, 160X)

4. Chromosomal aberrations of monosodium glutamate:

As seen from micrograph (7), the examination of bone marrow of all tested rats (35, 350 & 3500 mg/kg BW) showed imbalance of chromosomal distribution due to sticky chromosomes which may lead to lowered fertility and gametes imbalance. These results supported with the results obtained by (Abd-Elaziz and Ashoush 2006; Pizzi and Barnhart 1979). Furthermore, the results of lipid peroxidation, confirmed the oxidative stress which affect protein-synthesis in cells (Ahluwalia *et al.* 1994 and Farombi and Onyema, 2006).



Micrograph (7): Several Sticky chromosome in metaphase from bone marrow cells of rat's treated with MSG.

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الإجهاد التأكسدى والسمية الوراثية لأحادى جلوتاميت الصوديوم على الفئران كدليل على الخطر الكامن فى أغذية الإنسان

إيهاب صلاح عشوش* ، أحمد محمد سعيد عبد العزيز **

* قسم علوم الأغذية – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر

** قسم الإنتاج الحيوانى – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر

أجريت هذه الدراسه لمعرفة تأثير المعاملة بأحادى جلوتاميت الصوديوم كمادة مكسبة للنكهة وشائعة الإنتشار فى تدعيم نكهة الأغذية بثلاث جرعات (٣٥- ٣٥٠ – ٣٥٠٠ مجم/ كجم من وزن الجسم / يوم) يوميا لمدة ٩٠ يوما على ذكور الفئران البيضاء. وأشارت النتائج إلى أن المعاملة أدت الى زيادة فى نشاط الإنزيمات الناقلة للأمين (ALT&AST) فى بلازما دم الفئران، كما حدث أيضا زيادة مستوى المالونالدهيد فى بلازما الدم. بالإضافة الى ذلك فقد أوضحت الدراسة أيضا أن محسن النكهة المستخدم يؤدى إلى تغيرات هيستولوجية فى أنسجة الكبد والكلى، كما حدث أيضا تغيرات فى كروموسومات خلايا نخاع عظام الفئران المعاملة خاصة عند إستخدام تركيزات مرتفعة من هذه المادة ولذلك ينصح بعدم زيادة تركيزها عن ٣٥ مجم/كجم.