The Effect of Monosodium Glutamate (MSG) On Rat Liver  
And The Ameliorating Effect Of “Guanidino Ethane Sulfonic acid (GES)”  
(Histological, Histochemical and Electron Microscopy Studies)  

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Abstract:

Food additives are chemical substances added intentionally to food stuffs to preserve, color, sweeten and flavor food. Monosodium glutamate (MSG) is used as a flavor enhancer and found in most soups, salad dressing and processed meat. The use of MSG in food is growing. Irrational fear had increased in the last few years due to the adverse reactions and toxicity of MSG.

The present study was designed to investigate the effect of MSG on the rat liver and the ameliorating effect of taurine analog “Guanidinoethane sulfonic acid (GES)”. Sixty albino rats (2-3 months old) were used in the present study. MSG was given orally at a daily dose of 60 mg/1000 g for one month, two months and was given at a daily dose of 100mg/1000gm for one month. The results revealed that the deleterious effects of MSG were dose related and cumulative. In MSG treated rats, the examined sections showed remarkable alterations varied considerably from moderate structural changes to cytoplasmic lysis and signs of degeneration of cellular organelles. The histological changes showed disturbed liver architecture, hemorrhage in the central veins, areas of necrosis, vacuolation and increased inflammatory cells infiltration. The glycogen granules increased as well as the collagen fibers in the liver cells. Ultrastructural changes showed loss of cytoplasmic differentiation, vacuolation, pyknotic nuclei with irregular nuclear membranes and elongated electron dense mitochondria. Conversely, treatment of rats with taurine analog (GES) significantly attenuated the cellular toxicity of MSG.

Introduction

Monosodium glutamate (MSG) is the sodium salt of glutamic acid, a naturally occurring amino acid which is produced in small quantities by the human body. Monosodium glutamate is used as a flavor enhancer in a variety of foods prepared at home, in restaurants and by food processors (Walker and Lupien, 2000).

The safety and toxicity of MSG had become controversial in the last few years because of reports of adverse reactions in people who have eaten foods that contain MSG. Many studies had confirmed the adverse reactions of MSG. Blaylock and his colleagues (1994) found that MSG caused headache, vomiting, diarrhea, irritable bowel syndrome, asthma attacks in asthmatic patients and panic attacks. MSG may kill brain cells and cause neuroendocrine disorders in laboratory animals (Hardikar et al., 2001; Praputpitay and Wililak 2003; Elefferious et al., 2003, Nagata et al., 2006).

The adverse effects of MSG ingestion may be cumulative. Authors have reported that eating of small amounts of MSG once a week without experiencing reactions, while having reactions when the same products were consumed two or three days in a raw. Later studies by Samuels, (1999), indicated that the damage by monosodium glutamate was much more widespread.

Studies with synthetic taurine and taurine analogs as antioxidants suggested that these substances caused a significant reduction of many toxic agents. Taurine had
a neuroprotective function through the regulation of calcium level (Chen et al., 2001, Negata, 2006; Anderheggen, et al., 2006))

There is a lot of irrational fear and concern about the use of MSG as a food additive due to the unsubstantiated claims of health problems blamed on MSG. The aim of the recent study was to investigate the effects of toxicity of MSG on the liver cells of rats. Also the study investigated the ameliorating effect of taurine analog “Guanidinoethane Sulfonic acid (GES) on the liver of rats treated with MSG.

Material and Methods

A sixty albino rats (2-3 months old) were used in the present study. Their weights ranged from 200 - 250 grams. The rats were maintained at a suitable temperature in a well ventilated animal house under natural photoperiod conditions and had free access to food and water during the course of the experiment. The rats were divided into four groups:-

Group I: Included ten rats as a normal group (control)

Group II: Included twenty rats which were subdivided into :-

a- Ten rats that were given a daily oral dose of MSG (60mg/100g) for one month and then they were killed.

b- Ten rats that were given a daily oral dose of MSG (60mg/100gm) for two months and then they were killed.

Group III: Included ten rats that were given a daily oral dose of MSG (100 mg/100gm) for one month and then were killed.

Group IV: Included twenty rats and subdivided into:-

a- Ten rats that were given a daily oral dose of MSG (60 mg/100g) simultaneously with (60 mg/100g) of Guanidinoethane sulfonic acid (GES) for two months and then they were killed.

b- Ten rats that were given a daily oral dose of MSG (60 mg/100g) simultaneously with (120 mg/100 gm) of Guanidinoethane sulfonic acid (GES) for two months and then were killed.

The dose response relationship of MSG showed a maximum effect at about 60-100 mg/100g (Samuels, 1999).

Rapid dissection was done and liver specimens were obtained and divided into two parts for light and electron microscopy.

1- Preparation for Histological and Histochemical microscopy:-

Liver specimens were fixed in 10% formol saline for 24 hours, then washed and dehydrated in ascending grades of alcohols. Liver blocks were cleared in terpinol for 2 days, then embedded in paraffin wax and prepared for sectioning under the usual microtome. The 6 microns thick paraffin sections were stained with hematoxylin and eosin while other sections were subjected to Periodic Acid Schiff (PAS) reaction and Masson’s trichrome stain (Mc Manus and Mowry, 1963).

2- Preparation for electron microscopy:-

Liver specimens were cut into small blocks less than 1mm. They were fixed in 2% glutaraldehyde for 2 hours, rinsed in 0.1 M cacodylate buffer then post-fixed in 1% osmium tetra oxide (pH 7.5) for 2 hours. Dehydration is achieved by transferring the specimens through ascending grades of alcohols. Semi-thin sections, 1um thick were cut and stained with toluidine blue and examined by light microscope for orientation. Cutting of the specimens was done by ultra-micromtome (LBK), followed by double staining of thin sections first in uranyl acetate followed by lead citrate (Hayat, 1989). Ultra-thin sections were examined an Joel 100CX transmission microscope.

Results:

I – Histological Observations:

A- Examination of the liver sections stained with haematoxylin & eosin revealed that:

1- In group I

Control rat liver was observed in fig (1)
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2- In group II (MSG treated group):

Examination of the liver sections after administration of a daily dose of MSG (60mg / 100g) for one month , showing mild disturbance of liver architecture, small necrotic areas with mild vacuolation ,enlarged and congested central vein with disturbed and ruptured endothelial lining which was invaded by lymphocytic infiltration and few inflammatory cells, congested central vein was observed Fig. (2).

liver sections taken from rats after administration of a daily dose of MSG (60mg/100g) for two months showed disturbed liver architecture hemorrhage, areas of necrosis, and increased vacuolation most nuclei are atrophied Fig (.3).

3- In group III:

liver sections taken from rats after administration of a daily dose of MSG (100mg/100g) for one month showed irregular and disturbed liver architecture , , areas of necrosis, congested central vein with highly affected endothelial lining which contained hypertrophied nuclei , highly dilated sinusoidal spaces with thickened tunica media of it and pyknotic cells .

4- In group IV:

Liver sections taken from rats treated with a daily dose of MSG (60 mg/ 100g) & GES (60 mg /100 g) for two months showed preserved liver architecture, slightly congested central vein with irregular wall , and mild vacuolation of hepatocytes , marginal chromatin . Cortical hepatocytes are highly affected Fig. (5)

Liver sections taken from rats after treated with a daily dose of MSG (60mg / 100g) & GES (120 mg /100g) for two months showed more or less a normal liver architecture as shown in , some sinusoidal spaces are dilated ,while the others accepted the normal appearance Fig. (6).

B- Histochemical Observations:-
*Examination of liver sections with PAS reaction:-

1- In the control group using PAS reaction, a strong reaction was seen in the cytoplasm of hepatocytes where glycogen flakes and granules were noticed as shown in Fig (7)

2- In MSG treated group, PAS reaction showed moderate increase of glycogen content in some hepatocytes after giving MSG (60mg/100g) for one month as shown in Fig.(8) and marked increase of glycogen content at the dose of (60mg/100g) of MSG for two months as shown in Fig.(9).

3- In the group treated with 60mg/100gm MSG and 60mg/100g GES for two months, there was a slight increase in glycogen content around the central vein Fig. (10), with higher dose of GES (120mg/100g), glycogen content of the hepatocytes was similar to that of the control group as shown in Fig (11)

** Examination of liver sections stained with Masson’s trichrome:-

1- In the control group, Masson’s trichrome stain was used to demonstrate the liver connective tissue. Thin layer of collagenous fibers was seen around the central vein .no intervening intra-lobular or interlobular fibrous tissue was detected as shown in Fig.(12).

2- In MSG treated group, Masson’s trichrome stain showed moderate increase in connective tissue around the portal area after giving a daily dose of MSG (60mg/100g) for one month as shown in Fig (13). With the same dose of MSG (60mg/100g) for two months, highly disturbed bile canaliculi , bile artery and hepatic portal vein. marked increase of connective tissues was detected around the portal area and central vein as shown in Fig.(14).

3- In the group treated with MSG (60mg/100g) and GES (60mg/100g) for two months, there was mild increase in connective tissues around the highly disturbed portal area as shown in Fig.(15).

With higher dose of GES (120mg/100g) for the same period, a thin layer of collagen fibers was observed around the portal area similar to that of the control group , Fig (16).
II. Electron microscopy observations:

Examination of the liver ultra-thin sections showed the following:

1. In group I (control):
The hepatocytes showed oval nuclei, the cytoplasm contained mitochondria, rough endoplasmic reticulum and glycogen granules as shown in Fig.(17).

2. In MSG treated group:
Examined sections showed a remarkable alterations varied considerably from moderate structural changes to cytoplasmic lysis and signs of degeneration of cellular organelles. After giving a daily dose of MSG (60mg/100g) for one month, the cytoplasm of hepatocytes showed electron dense swollen mitochondria with destructed cristae, increased glycogen, hypertrophied cisternae of endoplasmic reticulum and vacuolation as shown in Figs (18 & 19). With administra-tion of MSG (60mg/100g) for two months, the hepatocytes showed signs of cytoplasmic differentiation, vacuolation, elongated electron dense mitochondria and hyper-chromatic shrunken nuclei with irregular nuclear membrane, Figs. (20, 21).

Pyknotic nuclei with irregular nuclear membranes and fibrosis in the form of increased collagen fibers were also detected as shown in Fig. (21).

3. In MSG & GES treated group:
Examined sections. In treated group with daily does of 60mg/100g MSG and 60mg/100g GES for two months, showed signs of amelioration and recovery that were dose dependant. The cytoplasmic organelles were preserved showing lost of cisternae rough endoplasmic reticulum, electron dense mitochondria and increased fibrous tissue. Elongated nucleus with irregular membrane and large degenerated area represented by destructed cisternae of RER, as shown in Fig. (22).

When the dose of GES increased to120mg/100g, some hepatocytes appear nearly normal. The nucleus was healthy and the cytoplasmic organelles were well defined as shown in Fig.(23).

List of Figs.

Fig 1: A photomicrograph of a section of normal control rat liver showing central vein (cv), liver cords of hepatocytes (h) radiating from the central vein and separated by blood sinusoid (s) with rounded nuclei (arrow). (H&E, X 400)

Fig 2: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) for one month showing mild disturbance of liver architecture, small necrotic areas with mild vacuolation in some cells. Enlarged and congested central vein with disturbed and ruptured endothelial lining which was invaded by lymphocytic infiltration and few inflammatory cells, congested central vein could be observed.

Fig 3: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) for two months showing disturbance of liver architecture, areas of necrosis (n), hemorrhage (hg) increased vacuolation (v) and fatty degeneration. (H&E, X 400)

Fig 4: A photomicrograph of a section of rat liver after administration of MSG (100mg/100g) for one month showing disturbance of liver architecture, areas of necrosis (n), congested central vein (cv) with highly affected endothelial lining which contained hypertrophied nuclei highly dilated sinusoidal spaces with thickened tunica media of it (v) and pyknotic nuclei (arrows) can be seen. (H&E, X 400)

Fig 5: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) & GES (60mg/100g) for two months showing slightly congested and irregular wall of central vein (CV), mild vacuolation (v) marginal chromatin, cortical hepatocytes are highly affected and preserved liver architecture. (H&E, X400)

Fig 6: A photomicrograph of a section of rat liver treated with MSG (60mg/100g) & GES (120mg/100g) for two
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months showing normal liver architecture, central vein (cv), liver cords of hepatocytes (h) radiating from the central vein Some sinusoids are dilated while others accepted the normal pattern (arrow). (H&E, 400)

Fig 7: A photomicrograph of a section of rat liver (control) showing glycogen deposited as granules in the hepatocytes (arrows). (PAS X 400)

Fig 8: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) for one month showing moderate increase of glycogen granules in some hepatocytes (arrows). (PAS X 400)

Fig 9: A photomicrograph of a section of rat liver after administration of MSG (60/100g) for two months showing marked increase of glycogen granules in some hepatocytes (arrows). (PAS X 400)

Fig 10: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) & GES (60mg/100g) for two months showing mild increase of glycogen granules in some hepatocytes (arrows). (PAS X 400)

Fig 11: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) & GES (120mg/100g) for two months showing normal glycogen granules in some hepatocytes while the others are less stained (arrows). (PAS X 400)

Fig 12: A photomicrograph of a section of rat liver (control) showing a thin layer of collagenous fibers around the central vein with minute collagenous material in-between hepatic cord (arrows). (Masson’s trichrome, X 400)

Fig 13: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) for one month showing moderate increase of collagenous fibers around and in the portal area (arrows). (Masson’s trichrome, X 400)

Fig 14: A photomicrograph of a section of rat liver after administration of MSG (60/100g) for two months showing marked increase of collagenous fibers around the portal area and the central vein (arrows). (Masson’s trichrome, X 400)

Fig 15: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) & GES (60mg/100g) for two months showing mild increase of collagenous fibers around and in portal area (arrows). (Masson’s trichrome, X 400)

Fig 16: A photomicrograph of a section of rat liver after administration of MSG (60mg/100g) & GES (120mg/100g) for two months showing a thin layer of collagenous fibers around the highly disturbed portal area (arrows). (Masson’s trichrome, X 400)

Fig 17: An electron micrograph of rat liver (control) showing: Hepatocytes with oval nucleus (n). The cytoplasm contains many organelles; mitochondria (m), rough endoplasmic reticulum (er) and glycogen particles (g). (Original X 10000)

Fig 18: An electron micrograph of rat liver after administration of MSG (60mg/100g) for one month showing: Electron dense mitochondria (m), lysosomal bodies (L), increased glycogen granules (g), irregular nuclear membrane and marginal nucleolus. (original X 2000)

Fig 19: An electron micrograph of rat liver after administration of MSG (60mg/100g) for one month showing: swollen mitochondria (m) with destructed cristae, dilated endoplasmic reticulum (er) and vacuolations (v). (original X 15000)

Fig 20: An electron micrograph of rat liver after administration of MSG (60mg/100g) for two months showing: loss of cytoplasmic differentiation, degeneration (v), elongated and malformed electron dense mitochondria (m) and hyper chromatic shrunken nucleus (n). Ruptured rough endoplasmic reticulum could also be seen. (Original X 6000)

Fig 21: An electron micrograph of rat liver after administration of MSG (60mg/100g) for two months showing: Pyknotic nucleus (n) with irregular nuclear membrane and increased

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collagen fibres (co) some areas are free from cytoplasmic organoids. (Original X 8000)

Fig 22: An electron micrograph of rat liver after administration of MSG (60mg/100gm) and GES (60mg/100g) for two months showing: preserved cytoplasmic organelles; electron dense mitochondria (m), rough endoplasmic reticulum (er), and sphericol vesicular nucleus (n). (Original X 6000)

Fig 23: An electron micrograph of rat liver after administration of MSG (60mg/100g) and GES (120mg/100g) for two months showing: preserved cytoplasmic organelles; endoplasmic reticulum (er), mitochondria (m) with thin cristae membranes and well developed chromatin, glycogen flakes and granules, and enlarged vesicular nuclei (n) with irregular nuclear membrane. (Original X 8000)
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Fig 5

Fig 6

Fig 7

Fig 8
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Fig13

Fig14

Fig15

Fig.16
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Fig. 21

Fig. 22

Fig. 23
Discussion

MSG contaminated food supply is a health problem that impacts every one of us. Geha et al. (2000) mentioned that monosodium glutamate (MSG), a flavor enhancer and food additive is recognized as a neurotoxin. The present study proved that reactions to MSG are dose related drug reactions where the deleterious effects were marked when the dose increased from (60mg/100g) to (100mg/100g) . The safety and toxicity of MSG had become the focus of many researches. The results of the present study revealed that adverse effects of MSG were cumulative as more cell damage occurred after two months than after one month. These results were in agreement with that reported by Samuels (1999) who mentioned that the adverse effects of MSG ingestion might be cumulative. People had reported eating small amounts of MSG once a week without experiencing reactions, while reactions happen when the same product was consumed two or three days in a raw.

The present study revealed that the ingestion of MSG was deleterious causing degeneration, vacuolation, cellular infiltration and fibrosis in the liver. These results were in accordance with that reported by Bopanna et al. (1999) who studied the histopathological changes on rat liver and kidney fed with monosodium contaminated food. They found that there were foci of necrosis, fatty degeneration and micro vascular changes in the liver. In the kidney, patchy tubular necrosis and interstitial infiltrations were present.

Malik and Ahluwalia (1994) studied the administration of MSG subcutaneously to adult mice for 6 days at dose of 2, 4 and 8 mg / g body weight. Dose level above 4mg/g body wt. showed a significant increase in the content of liver glycogen, total lipids, phospholipids, triglycerides and free fatty acids. The results of the present study showed that MSG increased the glycogen content and increased collagen content of the liver. These results were in accordance with those reported by Diniz et al. (2004) who found that administration of MSG was associated with oxidative stress in hepatic tissues. Monosodium glutamate (MSG) increased glycogen content of the liver cells because its administration was associated with hyperglycemia and hyperinsulinemia. Monosodium glutamate induced alteration in metabolic rate of glucose utilization and decreased antioxidant defenses. The oxidative stress induced by MSG intake caused remarkable formation of collagen fibres in the liver cells.

Electron microscopic study revealed that in MSG treated group an obvious alteration varied from moderate structural changes to cytoplasmic lysis and degeneration of the cellular organelles. The cytoplasm of hepatocytes showed electron dense mitochondria and dilated cristae of endoplasmic reticulum. In other sections, the cytoplasm showed swollen mitochondria, disturbed nucleus with irregular nuclear membrane, increased glycogen particles, and vacuolation . Results of the present study were in harmony with that reported by Bopanna et al. (1999) who mentioned that MSG caused degeneration of the liver cells. Similar results were observed in the pancreas of monosodium glutamate treated rats. The pancreatic acinar cells of the rats showed dilatation of rough endoplasmic reticulum, swollen mitochondria, vacuoles and interstitial spaces (Lee and Sheen 1994).

Today scientists know that MSG kills brain cells and causes neuro endocrine disorders in laboratory animals and that it causes adverse reactions in human. They also understand that MSG is simply processed free glutamic acid combined with sodium. Glutamic acid is a neurotransmitter that causes nerves to fire; and when present in excess quantities, causes nerves to fire until they die. So MSG acts as excitotoxin (Samuels, 1999; Praputpitaya and Wililak 2003 ; Elefferious et al., 2003).

Many studies mentioned that taurine and its analogs can reduce the cellular disorders by decreasing oxidative stress and
had a protective effect against drugs that induced liver cirrhosis. (Wingenfeld et al., 1996; You and Chany, 1998; Hardikar et al., 2001).

Heaton et al. (2002) and Ciba et al. (2002) mentioned that taurine had a protective effect against the cytopathological changes in the cells of the kidney and pancreas. They also stated that sustained increase in circulating levels of antioxidants exerted a protective effect by decreasing DNA damage and leading to improved immunological performance.

In the recent study, the administration of taurine analog ameliorated the toxic effect of MSG. The results of the present study were in line with that published by Messina and Dawson (2000) who mentioned that taurine and taurine analogs had cytoprotective actions via different mechanisms. They also stated that taurine at concentrations normally found in cells can inhibit oxidative damage to DNA.

In the present study, Guanidinoethane sulfonic acid (GES) was used as a biological analog of taurine because MSG could decrease taurine level (Messina and Dawson, 2000). The administration of GES had decreased the deleterious effect of MSG and the results of the present study indicated that GES had a protective effect against MSG toxicity by decreasing oxidative stress.

Chen et al. (1999) reported that taurine prevented accumulation of collagen in the liver cells because it prevented the increase of type I, III procollagen mRNA expression. Mankovskaya et al. (2000) suggested that the effect of taurine and its analogs might be due to antiacidotic action and stabilizing the cell membrane activity. Saransaari and Oju (2000) found that taurine released simultaneously with an excess of excitatory amino acids in the hippocampus under ischemic and other neuron damaging conditions may constitute an important protective mechanism against excitotoxicity.

Conclusion

Based on this study, there is considerable evidence to suggest that consumption of MSG is a serious public health problem and taurine and taurine analog are powerful antioxidants that ameliorate the cell toxicity of MSG. Further studies on large scale are recommended.

References

11. Hayat M (1989); Principles and techniques of electron microscopy; biological
تأثير الجلوتاميت أحادى الصوديوم على كبد الجرذ الأبيض والتأثير المحسن لمادة الجوانيدنوياثان سلفونيك اسيد (دراسة هستولوجية وهستوكييميائية ودراسة بالميكروسكوب الإلكتروني)

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تعتبر مكسيات الطعم مواد كيميائية تضاف للطعام من أجل الحفظ والتلوين والتحلية وكذلك لإعطاء الطعام نكهة خاصة. يستخدم الجلوتاميت أحادى الصوديوم كمادة حافظة و لإعطاء الطعام نكهة في معظم أنواع الحساء ومثلات السلاطة والحم المخلل. ويعد هذا الاستخدام في زيادة مستمر. وقد ازدادت المخاوف في الفترة القليلة الماضية لما لهذه المادة من تأثير سمي وتفاعل معناكس. وقد صممت هذه الدراسة لبحث الآثار المترتبة على استخدام الجلوتاميت أحادى الصوديوم على الكبد ومحاولة استخدام مادة الجوانيدنوياثان سلفونيك اسيد كمادة محسنة للأثار المترتبة على الاستخدام. وقد أعطيت مادة الجلوتاميت أحادى الصوديوم على جرعات يومية بمعدل 60 مجم لكل 1000 جم وزن لمادة شهر ثم لمدة شهرين وكذلك بجرعة قدرها 100 مجم لكل 100 جم وزن لمدة شهر.

أظهرت النتائج أن المادة الجلوتاميت أحادى الصوديوم تأثير ضار على الخلي من حيث التركيب الهستولوجي والهستوكييميائي والتركيب الدقيق وقد وضح تحسن لهذه الآثار الضارة إلى حد كبير باستخدام مادة الجوانيدنوياثان سلفونيك.