Effect of Monosodium Glutamate administration on Gastric Mucosa of Adult Male Albino Rat: A Histological, Immuno-histochemical and Histomorphometric Study

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Abstract: Monosodium Glutamate (MSG) is worldwide food additive commonly used as a flavor enhancer. Although its consumption can be regarded as harmless, yet histo-pathological changes in the gastric mucosa were described with prolonged consumption of MSG. The objective of the present study was to evaluate effects of MSG on the gastric mucosa. Thirty adult male albino rats (n=30) were used in this study. The animals were randomly divided into two equal groups (n=15 rats): Group A (Control); each rat daily received 2ml distilled water and Group B (Treated); each rat daily received MSG at a dose of 2mg/kg body weight dissolved in 2 ml distilled water. One set of sections were stained with H&E, Mallory trichrome stain and Periodic Acid Schiff (PAS) stain and another set were immuno-histochemically-stained to detect the proliferating cell nuclear antigen (PCNA). The gastric mucosa of MSG-treated rats revealed atrophic changes with ulcer formation and shedding of surface epithelium into the gastric lumen bases. The area of mucous neck cells showed many cells with a strong brown positive PCNA expression. Quantitatively, as compared to control group, the gastric mucosa revealed reduced thickness, widening of gastric glands and gastric pits and increased PCNA positive cells in MSG-treated group. Therefore, very low doses of MSG could produce histo-pathological changes in the gastric mucosa of rats and its safety as food additive in humans must be reconsidered.

Keywords: Stomach; gastric mucosa; flavor enhancer; monosodium glutamate; food additives.

1. Introduction

Monosodium Glutamate (MSG) is generally used as a flavor enhancer. Its intrinsic taste is different than the four basic tastes; saltiness, sweetness, sourness and bitterness, and corresponds to the Japanese expression ‘umami’ meaning tastiness (Yamaguchi, and Kimizuka, 1979). Umami taste was identified as a fifth taste, as it was well represented in primate taste cortical areas as were other four tastes (Baylis and Rolls, 1991). The characteristic umami taste is imparted by glutamate present naturally in many foods, such as meat, poultry, seafood and vegetables like tomato, green peas, cabbage and spinach (Yamaguchi and Ninomiya, 2000). It was suggested that the gastric wall, intestinal wall and hepatoportal region contain MSG sensors, due to increased afferent activity in the vagal gastric, celiac and hepatic nerves secondary to MSG infusion into the stomach, duodenum and portal vein (Nijjima, 2000).

The intake of larger amounts of MSG by some hypertensive persons can trigger a "Chinese restaurant syndrome", which is characterized by temporary disorders such as drowsiness, headache, stomach ache and stiffening of joints and these disappear after a short time (Beliz et al, 2004). Total intake of glutamate from food in European countries is generally stable and ranged from 5 to 12 g/day. A maximum intake of 6.000 mg/kg body weight is regarded as safe, therefore glutamate salts (monosodium-L-glutamate and others) as food additive can be regarded as harmless for the whole population (Beyreuther et al, 2007).

MSG was also used as a bleaching agent for the removal of stains from clothes and its bleaching properties could be harmful or injurious to the stomach mucosa (Eweka and Adjene, 2007). Its strong bleaching effects were comparable to the actions of hydrogen peroxide on the bones (Huthman et al, 2009). Continuous intake of high amounts of MSG in modern nutrition can increase the oxidative stress and result in cytotoxicity in many organs (Pavlovic and Sarac, 2010). The oral median lethal dose (LD50) for MSG in the mouse and rat is very high, between 10 and 20 g/kg bodyweight and the largest palatable dose of MSG in humans is about 60 mg/kg body weight. Dietary levels of up to 4% in diet did not reveal any adverse effects in rats and mice (Kaitano, 2014).

Non-uniform histopathological changes was induced in the gastric mucosa of albino rat upon consumption of MSG. It might have a causal relationship to the onset of gastric ulcer (Oluwole and Iyortim, 2006). The gastric mucosa of rats that received 3g of MSG revealed increased basophilia with cellular hypertrophy, whereas with intake of 6g of
MSG there was more pronounced degenerative and atrophic changes (Eweka et al, 2007). The prolonged MSG administration caused an initial increase in weight gain followed by terminal suppression, that was explained by induction of gastric mucosal damage (Abd El-Aziz et al, 2014). However, no notable distortion of gastric mucosa occurred following intake of MSG in doses 0.1g/kg, 0.15g/kg and 0.2g/kg three times daily for two weeks (Ilegbedion et al, 2013).

Due to the conflicting reports about effect of MSG intake on the stomach mucosa and the debate about its safety usage as a flavoring agent, the aim of this study was to evaluate the effects of MSG on the gastric mucosa of adult male albino rat by histological, Immuno-histochemical and histomorphometric analyses.

2. Material and Methods

Monosodium Glutamate (MSG):

MSG was purchased from El-Dawlia pharmaceutical chemical company (Cairo, Egypt) in the form of white colored powder which is water soluble.

Experimental animals:

The study was carried out on 30 adult male albino rats weighing 200±20 gram each. The animals were obtained from the animal house, Faculty of medicine, Zagazig University. The rats were acclimatized in the laboratory for one week before the experimental work. Animals were housed in a temperature-controlled and light-controlled room (12-h light/dark cycle), with standard rodent chow and tap water ad libitum.

Experimental design:

All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Faculty of Medicine; Zagazig University.

The animals were randomly divided into two equal groups (n=15 rats): Group A (Control); Each rat daily received 2ml distilled water. Group B (Treated); Each rat daily received MSG at a dose of 2mg/gm body weight dissolved in 2 ml distilled water. The dose of MSG was selected according to previously reported studies employing higher doses successfully (Singh et al, 2003; EL-Kenawy et al, 2013; Hamza and AL-Harbi, 2014).

The amount of MSG administration per animal was as per their respective weight and a gastric cannula was used in oral gavages. After 6 weeks from the start of the experiment, the animals were sacrificed 24-hours after the last dose, under ether anesthesia.

Each rat was placed in a dorsal decubitus position with the 4 limbs fixed to the surgical table. The stomach was dissected out and opened along the greater curvature and gently washed with saline. Small pieces from the body region of the stomach were quickly excised and immersed in 10% neutral buffered formaldehyde pH 7.4 for fixation.

Histological techniques:

One set of sections of 5μm thick were obtained using standard histological techniques and stained with H&E, Mallory trichrome stain and Periodic Acid Schiff (PAS) stain (Suvarna et al, 2013). In another set of sections, immuno-histochemical staining was performed to detect the proliferating cell nuclear antigen (PCNA). The stained slides were examined for the positive brown immunostaining and the fields with the best staining were captured (Picut et al, 2008).

Histomorphometric study:

Sections were quantified using the public domain image-processing software "Image J 1.49v/Java 1.6.0_244 (64-bit)" (National Institutes of Health, USA) in five (5) non-overlapping fields per each slide in 5 slides form each animal. The image analyzer was calibrated for measurements before use to automatically convert the image pixels into actual micrometer units and data were presented as mean ± standard deviation. The level of positivity of PCNA expression was calculated according to the formula: level of positivity = Number of positive cells / number of counted cells multiplied by 100 (Novellino et al, 2003).

Statistical analysis:

Two-paired student's t-test was used for quantitative differences between control and MSG-treated groups. A significant or highly significant difference was present with p-values less than 0.05 or 0.01 respectively.

3. Results

A. Histological and Immuno-histochemical study:

1. Control rats:

The gastric mucosa revealed long tubular gastric glands opening into the stomach lumen by gastric pits. These glands are lined by parietal and chief cells with a lamina propria of connective tissue extending between their bases (Fig.1-a). The luminal surface was lined by columnar mucous-secreting cells and extended to line gastric pits. The mucous neck cells are low columnar cells with basal nuclei lying deep in the bases of gastric pits and continues into the lumina gastric glands. The superficial part of gastric glands are mainly lined by parietal cells (Fig.1-b). Parietal cells appeared as large cells with central nuclei, prominent nucleoli and eosinophilic cytoplasm, whereas chief cells appeared as low columnar cells with rounded, basally located nuclei and basophilic cytoplasm and were mainly seen in the basal part of gastric glands (Fig.1-c). The surface epithelial cells, gastric pits and the mucous neck cells revealed positive PAS reaction (Fig.2-a). Thin collagen fibers
were observed in the thin lamina propria and in between the basal parts of gastric glands (Fig.2-b). The area of mucous neck cells showed faint immuno-histochemical staining with brown positive PCNA expression in only few cells (Fig. 2- c&d).

2. MSG- treated rats:

The gastric mucosa revealed atrophic changes with ulcer formation and shedding of surface epithelium into the gastric lumen (Fig.3-a). The gastric glands, adjacent to the ulcers, were dilated with lymphocytic infiltration in the lamina propria (Fig.3-b). The surface epithelial cells were coalesced together and partly detached from the mucosal surface or completely shedding into the gastric lumen. The dilated pits and glands were lined by irregular degenerated epithelial cells (Fig.3-c). The superficial mucosal capillaries were congested with occasional intra-glandular minute hemorrhage (Fig.3-d). The parietal cells were enlarged and revealed a faintly-stained vacuolated cytoplasm and vesicular nuclei. The chief cells were markedly condensed with dark stained nuclei (Fig.3-e). The gastric glands were markedly disorganized with degeneration of their luminal epithelium (Fig.3-f). The surface epithelial cells, gastric pits and the mucous neck cells revealed a strong positive PAS reaction, with a thin surface film (Fig.4-a). The collagen fibers were massively deposited in the lamina propria and in between the basal parts of gastric glands. The blood capillaries were irregularly-dilated and markedly congested (Fig.4-b). The area of mucous neck cells showed strong immuno-histochemical staining with brown positive PCNA expression in many cells (Fig. 4-c&d).

B. Histomorphometric study:

The mean thickness of gastric mucosa was 878.17 ± 137.43 and 508.12 ± 81.57 microns in control and MSG-treated groups respectively and the higher thickness in control animals was highly significant \[ t= 11.58 (24), p= (< 0.0001) \] (Fig. 5- a and Table 1). The mean width of gastric glands was 22.97 ± 4.10 and 31.79 ± 6.37 microns in control and MSG-treated groups respectively and the higher width in MSG-treated animals was highly significant \[ t= - 5.82 (24), p= (< 0.0001) \] (Fig. 5- b and Table 1). The mean width of gastric pits was 10.52 ± 2.04 and 16.70 ± 4.55 microns in control and MSG-treated groups respectively and the higher width in MSG-treated animals was highly significant \[ t= - 6.20 (24), p= (< 0.0001) \] (Fig. 5- c and Table 1). The mean percent of counted PCNA positive cells was 5.5 ± 2.07 and 8.2 ± 1.4 in control and MSG-treated groups respectively and the higher percent in MSG-treated animals was highly significant \[ t= - 3.42 (9), p= (< 0.0001) \] (Fig. 5- d and Table 1).

4. Discussion

MSG has become a common flavor enhancer in foods of modern societies. The effects of this food additive on the gastric mucosa of adult male albino rat were revealed in the present study. Its oral administration produced atrophic changes in gastric mucosa that was significantly lower than that in control group. The atrophied mucosa was associated with ulcer formation and shedding of surface epithelium into the gastric lumen. This finding is in accordance with Oluwole and Iyortim, (2006) who reported that MSG consumption induced non-uniform histopathological changes in the gastric mucosa of albino rat, with a causal relationship to the onset of gastric ulcer.
Figure 2. A photomicrograph of a section in gastric mucosa of adult male control rat showing: (a) Positive PAS reaction in the surface epithelial cells and within gastric pits extending into mucous neck cells (arrow heads). (PAS stain X 100); (b) Collagen fibers (CF) in lamina propria and between gastric glands (arrow heads). (Mallory's Trichrome stain X 100); (c) Faint brown positive PCNA expression in the area of mucous neck cells. (PCNA stain X 100); (d) Few cells revealed a brown positive PCNA expression. (PCNA stain X 400)

Figure 3. A photomicrograph of a section in gastric mucosa of adult male MSG – treated rat showing: (a) Mucosal atrophy with ulcer formation (inside circle). Surface epithelium (arrows) are shedding into the gastric lumen. (H & E x 100); (b) Dilated glands with degenerated epithelial lining and lymphocytic infiltration (arrows) in lamina propria. (H & E, X 400); (c) Coalesced surface epithelium (inside rectangle), dilated gastric pits (GP), dilated gastric glands (GG) lined by irregular degenerated epithelial cells (inside circle). Surface epithelium (arrows) are shedding into the gastric lumen. (H & E, X 400); (d) Gastric pits (GP) and gastric glands (GG) are widely dilated with congestion of superficial capillaries and intra-glandular minute hemorrhage (arrows) (H&E X 400); (e) Enlarged parietal cells with faintly- stained vacuolated cytoplasm (V) and vesicular nuclei. The chief cells were markedly condensed with dark stained nuclei (arrow). (H & E, X 400); (f) Dilated disorganized gastric glands (GG) with degeneration of their luminal epithelium (arrow heads) and markedly condensed chief cells (CC). (H & E, X 400)
Moreover, Eweka et al (2007) described pronounced degenerative and atrophic changes in the gastric mucosa of rats that received 6g of MSG. In addition, Abd El-Aziz et al (2014) in adult male rats with prolonged administration of MSG, attributed a terminal weight suppression, independent of food consumption, after an initial weight gain, to an induction of gastric mucosal damage. On the other hand, Numan et al (2010) reported that MSG induced statistically-significant increased thickness of gastric mucosa. On contrary, Ilegbedion et al (2013) denied any harmful effects of MSG and described that no notable distortion of gastric mucosa occurred following its intake. As regard the pathogenesis of gastric ulcer, Konturek and Konturek (2014) described that peptic ulceration is a result of an imbalance between factors potentially damaging the gastric mucosa (aggressive) and protective (defense) factors. In the present study it was suggested, that MSG has a potential damaging local effect on the gastric mucosa.

In agreement, Eweka AO and Adjene (2007) reported MSG as a bleaching agent for the removal of stains from clothes with a growing apprehension that its excellent bleaching properties could be injurious to the stomach mucosa when ingested as a flavor enhancer in food. In accordance, Laine et al (2008) described that mucosal injury may occur when noxious factors "overwhelm" an intact mucosal defense or when the mucosal defense is impaired. Moreover, Huthman et al (2009) reported that the strong bleaching effects of MSG competed favorably with the actions of hydrogen peroxide on the bones.

In the present study, several histological changes were observed in the gastric mucosa in response to MSG intake as coalesced surface epithelial cells that were either partly detached from the mucosal surface or completely exfoliated into the gastric lumen. The gastric pits and glands were markedly disorganized, dilated and lined by degenerated irregular epithelial cells. The superficial mucosal capillaries were congested with occasional intra-glandular minute hemorrhage. In agreement, Oluwole and Iyortim (2006) described increasing cell damage, ulceration with edematous lamina propria containing inflammatory cells, vascular congestion in MSG-treated rats and reported that MSG is a potential aggressive factor to gastric mucosa through enhancing the secretagogue-effect of histamine. Moreover, Falalieieva et al (2010) found that rats fed MSG in doses 15 to 30 mg/kg (equivalent to 1 and 2 g/person)
for 10-, 20-, 30-days caused erosive and ulcerative lesions of the gastric mucosa and an increased secretion of hydrochloric acid and an increased body weight. In the present study, the documented cell death and exfoliation of surface epithelial cell into gastric lumen suggested that MSG must have a strong direct damaging of cellular membrane with subsequent fatal permeability defect. In accordance, Raju et al (2009) described a similar mechanism in ethanol-induced gastric ulcer, where the rapid penetration of ethanol into the gastric mucosa caused cell and plasma membrane damage with increased intracellular membrane permeability to sodium and water and massive intracellular accumulation of calcium leading to cell death and exfoliation in the surface epithelium. However, the actual mechanism by which MSG induced the aforementioned degeneration of gastric mucosal epithelium noted in this study requires further investigations.

![Figures and Table 1](http://www.sciencepub.net/nature)
In the present study, in MSG-treated rats, the gastric glands were markedly disorganized and dilated with degeneration of their luminal epithelium; enlarged parietal cells with faintly-stained vacuolated cytoplasm and vesicular nuclei and markedly condensed chief cells with dark stained (pyknotic) nuclei. The associated lamina propria revealed severe congestion of blood capillaries, infiltration of inflammatory cells and massive deposition of collagen fibers. These findings are in accordance with the findings described by Mohamed (2010) in the gastric mucosa of adult albino rats after a single dose of Non-Steroidal Anti-inflammatory Drug (48 mg/kg body weight of Indomethacin). The previous author noted cellular infiltration, superficial and deep erosions in the mucosa with remnants of gastric glands and detached exfoliated cells in the lumen and necrotic changes as vacuolated cytoplasm with pyknotic nuclei in the gastric mucosal cells, completely distorted shape of gastric glands with mononuclear cellular infiltration and dilated blood vessels. In the current study, the documented marked deposition of collagen fibers in the lamina propria of MSG -treated rats, particularly around blood vessels, could be a possible cause of reducing the blood flow to gastric mucosa with subsequent ischemia and induction of ulcerations. According to Damiano et al (1990), triggering fibroblast activation, cell division and collagen deposition occurred with severely damaged epithelial cells.

In the present study, in MSG-treated rats, the surface epithelial cells, gastric pits and the mucous neck cells revealed a strong positive PAS reaction, with a thin surface film. The gastric pits revealed a statistically significant dilatation which could reflect excessive mucous production by mucous neck cells to prevent further mucosal damage. In agreement, Oluwole and Iyortim (2006) described a significant increase in the total gastric mucous secretion in MSG-treated rats. Moreover, Laine et al (2008) showed that Oluwole and Iyortim (2006) described a significant increase in the total gastric mucous secretion in MSG-treated rats. Moreover, Laine et al (2008) showed that mucus strengthens the mucosal barrier and for the healing process, it is desirable that mucus strength be augmented or at least maintained to protect the regenerating gastric epithelium. In this study, the area of mucous neck cells in MSG-treated rats showed a statistically highly significant increase in percent of cells with brown positive expression of PCNA staining. According to Celis and Celis (1985), a strong nuclear staining pattern of PCNA is observed as the cell progress in S-phase. Therefore, the documented strong positive PCNA staining in this study in gastric mucosa, is well-correlated with the cell division and increased proliferative activity that would produce regeneration of damaged epithelial and mucous-producing cells to renew mucous protective layer, prevent further damage by acidic pH and allow for healing of ulcerated areas of mucosa.

Conclusion:
The finding of the current study have shown that very low doses of MSG could produce histopathological changes in the gastric mucosa of rats and its safety as food additive in humans must be reconsidered.

Disclosure:
All authors state that they have did not receive any fund for performing this study and that they have no conflicts of interest.

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References
11. Hamza RZ and AL-Harbi MS. Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. Toxicology Reports 2014; 1: 1037–1045.