EFFECT OF MONOSODIUM GLUTAMATE AND VITAMIN C ON RAT PAROTID GLAND

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ABSTRACT

BACKGROUND: Monosodium glutamate is one of the most frequently applied taste enhancers in modern food industry. Long term consumption of high doses of MSG can elevate the oxidative stress mainly through the production of Reactive Oxygen Species and lead to cytotoxicity in multiple tissues in the body. Vitamin C is a well-known potent antioxidant and has been shown to protect various tissues against oxidative stress induced damage.

OBJECTIVE: to determine the histological effect of Monosodium glutamate and vitamin C on parotid glands of albino rats.

MATERIALS AND METHODS: 30 male adult albino rats were equally divided into 3 groups; *control group* that received 1 ml distilled water orally once daily, *MSG group* that received 30 mg/kg bodyweight of Monosodium Glutamate daily and *MSG+ Vit C group* that received 30 mg/kg bodyweight of MSG followed by 100mg/kg bodyweight of Vitamin C in distilled water daily by oral gavage. After 8 weeks, rats were euthanized, parotid glands were dissected out to be processed for histological examination.

RESULTS: Histological examination of parotid of MSG group revealed acinar cells with signs of atrophy and degeneration demonstrated as cytoplasmic vacuolations, pyknotic or apoptotic nuclei. Secretory striated duct showed luminal dilatation and partial loss of basal striations. MSG+ Vit C group showed more preservation of normal architecture of serous acini and ducts.

CONCLUSION: MSG produced significant degenerative effects on parotid glands of albino rats and co-administration of vitamin C was effective in protecting gland tissue against MSG-induced damage.

KEYWORDS: Monosodium glutamate, Vitamin C, Parotid glands, Albino rats, Antioxidant

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INTRODUCTION

Food additives are substances added to food for a specific purpose. They either enhance taste, texture, color, or durability of food (1). Monosodium Glutamate (MSG) is a popular flavor enhancer frequently used in food industry (2). Aside from being used as a food additive, it is also naturally found in many types of food such as cow milk, eggs, cheese, tomatoes, almonds, apple, onion, potato, carrot, garlic and walnut (3, 4). Now it is added to many food products such as processed meats, canned food, crackers, soups, salad dressings, infant formula, dietary supplements, fast foods and potato chips (4).

The widespread use of monosodium glutamate in modern diets raises safety concern, safety of MSG use is still debated. The Food and Drug Administrations (FDA) declared that limited consumption of MSG is considered safe while increased MSG intake may be linked to several potential side effects. Reports from different studies of human and animal subjects indicated that MSG is toxic specially at high doses (4, 5).

Prolonged glutamate excitotoxicity leads to oxidative stress mainly through upregulation of Reactive Oxygen Species (ROS) generation and decrease in the antioxidant defense pathways which make the cell more vulnerable to damage and death. Recent studies reported that the oxidative stress status induced by MSG consumption may affect multiple organs such as the brain, liver, kidney, and testis and cause their damage (2, 6-8).

Oxidative stress induced by MSG may be prevented by administration of antioxidants

that act as protective agents against oxidative stress related organ toxicity (9-11). Vitamin C (ascorbic acid) is a well-known water-soluble antioxidant that has been shown to protect various tissues against the damage caused by ROS (12).

The parotid gland is a major salivary gland, and its structure may be affected by toxic substances resulting in tissue damage and dysfunction. Therefore, the present study was conducted to highlight the effect of MSG administration on parotid salivary gland tissue of albino rats as well as the possible ameliorative effect of Vitamin C.

MATERIALS AND METHODS

Experimental animals

The study was conducted on 30 adult male albino rats weighing between (150–200) grams. The animals were obtained from the Institute of Medical Research, Alexandria University. The animals were kept under normal laboratory conditions of temperature (22-25°C), good ventilation and standard light/dark cycle (12/12 h). They were supplied a regular diet and drinking tap water throughout the whole experimental period (8 weeks).

The study was conducted after the approval of the Research Ethics Committee, Faculty of Dentistry, Alexandria University.

MATERIALS

1. Monosodium Glutamate (MSG): Purity 99% NT product by Mega Foods Company is sold in most supermarkets in Egypt. The product is packed in the form of crystals that dissolve easily in water.

Dose was calculated and a stock solution was prepared by dissolving 3 grams of MSG crystals each in 100 ml of distilled water to obtain concentrations of 30 mg/ml. The schedule of dose administration was adjusted in a manner that the amount of MSG given per animal was comparable to their respective weight (13).

2. Vitamin C: VITACID C effervescent tablet form, product of Chemical Industries Development (CID) was used in this experiment. Each tablet contains 1 g of ascorbic acid. Solutions of Vitamin C were prepared fresh daily (directly before administration).

Experimental design

Rats were grouped into:

Control group: 10 rats were kept under normal condition and received 1 ml distilled water orally once daily for 8 weeks.

MSG group (Study I): 10 rats received 30 mg/kg bodyweight of Monosodium Glutamate dissolved in distilled water orally once daily for 8 weeks (14).

MSG + Vit. C group (Study II): 10 rats received 30 mg/kg bodyweight of Monosodium glutamate dissolved in distilled water followed by of 100mg/kg bodyweight vitamin C in distilled water by oral gavage daily for 8 weeks (14, 15).

Rats were euthanized after the experimental period (8 weeks) by intravenous injection with a lethal dose (100 mg/kg) of pentobarbital sodium. The rats were decapitated, parotid salivary glands were dissected out. After decapitation of rats, the rests of their bodies were burned out.

Histological procedures

Parotid specimens were fixed in 10% neutral buffered formalin and then dehydrated gradually in ascending concentrations of ethyl alcohol, cleared with xylene, infiltrated in paraffin wax, and finally embedded in paraffin wax blocks. Serial sections of 3-5 microns thickness were cut by rotary microtome and stained with hematoxylin and eosin stain for histological examination (16).

Morphometric measurements

Morphometric analysis was carried out to measure the surface area of the acini in pixels at x400 magnification in different groups by using the Image J software Analyze System.

Statistical analysis

Data of the histomorphometric measurements were analyzed using IBM SPSS software package version 20.0. One-way ANOVA and post hoc Tukey tests were used to compare data. Data were expressed as mean and standard deviation; $P < 0.05$ was considered statistically significant.

RESULTS

Histological result

Control group

The gland showed normal histological structure. Serous acini were spherical in shape and lined with pyramidal-shaped cells surrounding a narrow lumen. Acinar cells contained basally located rounded nuclei and apical basophilic cytoplasm. (Figure 1)

Normal secretory striated ducts with tall columnar cell lining were also apparent. Ductal cells showed large, spherical centrally placed nuclei and eosinophilic cytoplasm with typical basal striations. (Figure 2)

MSG group (Study I)

The gland showed severe histological changes. The serous acini exhibited an atrophic appearance with ill-defined boundaries. The acinar cell showed pyknotic crescent shaped

nuclei and the cytoplasm appeared severely vacuolated. (Figure 3)
Secretory striated ducts are dilated with loss of the basal striations. Dilated blood vessels engorged with RBC's are seen in close proximity to the striated ducts. (Figure 4)

MSG + Vit C group (Study II)

The gland showed almost normal histological architecture. The structure and boundaries of serous acini are well preserved. Only few acinar cells showed milder vacuolations when compared with MSG group. The secretory striated ducts appeared normal. (Figures 5,6)

Histomorphometric results

MSG group measurements showed a highly significant decrease in the mean surface area of serous acini when compared with the *control group* ($P_1 < 0.001$). A significant decrease was also reported on comparing the *control group* to the *MSG + Vit C group* ($P_2 <0.001$). *MSG+ Vit C group* recorded a significant increase in the mean SA of acini when compared to *MSG group* ($P_3 < 0.001$). (Table.1)

Table 1: Comparison between the three studied groups according to surface area of acini.

Surface area of acini in pixels	Control group	MSG group	MSG + Vit C group	F	p
Mean ± SD.	25777 ±2455	12847[a] ± 995.4	20564[ab] ± 1132	458.95*	<0.001*
Sig. bet. grps.	$p_1<0.001^*$, $p_2<0.001^*$, $p_3<0.001^*$				

SD: Standard deviation
F: F for One way ANOVA test, pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: p value for comparing between the three studied groups
p_1: p value for comparing between Control group and MSG group
p_2: p value for comparing between Control group and MSG + Vit C group
p_3: p value for comparing between MSG group and MSG + Vit C group
a: Significant with Control group
b: Significant with MSG group
*: Statistically significant at $p \leq 0.05$

Figure 1: LM, (control group) showing normal histological structure of parotid salivary gland, the serous acini appeared spherical in shape lined with pyramidal cells. Acinar cells showed basal round nuclei and normal cytoplasm, H&E stain x400.

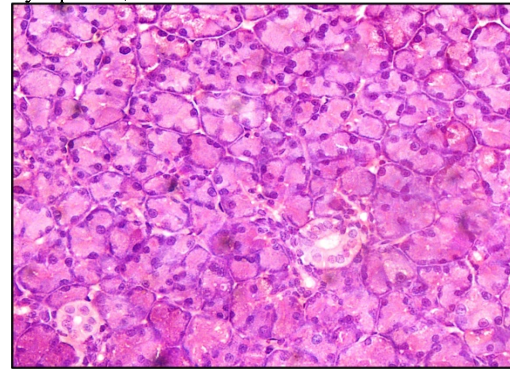


Figure 2: LM, (control group) showing normal intralobular secretory striated ducts lined by tall columnar cells with typical basal striations (arrows), H&E stain x400.

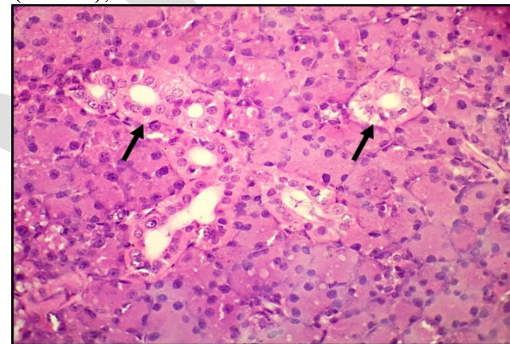


Figure 3: LM, (MSG group) showing degenerative changes in the serous acini demonstrated by severe cytoplasmic vacuolation (short arrows) and crescent shaped nuclei (long arrows) of the acinar cells, H&E stain x400.

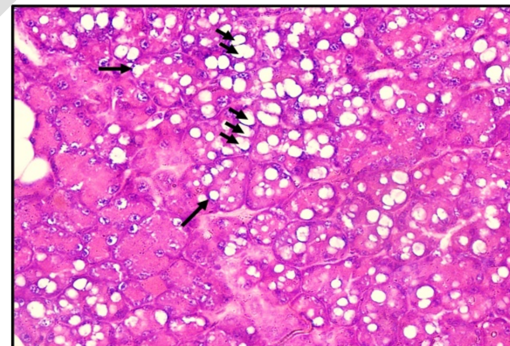


Figure 4: LM, (MSG group) showing abnormal dilatation of secretory striated duct (short arrows) with partial loss of the characteristic basal striations. Note: blood capillaries engorged with RBCs in close proximity to striated duct (long arrows), H&E stain x400.

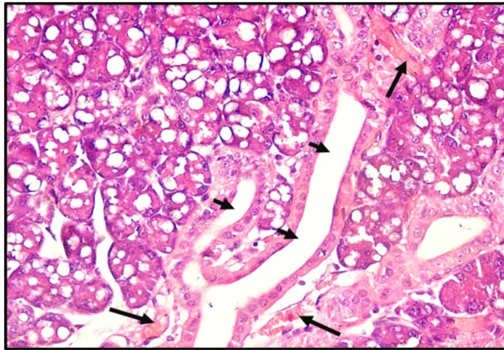


Figure 5: LM, (MSG + Vit C group) showing well preserved serous acini, some of the acinar cells show mild cytoplasmic vacuolations (arrows) with normal spherical basal nuclei, H&E stain x 400.

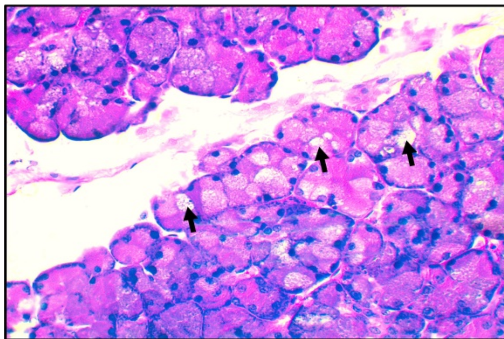
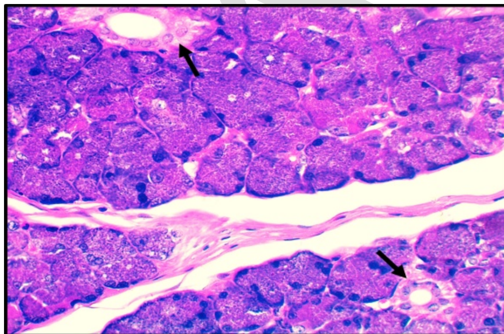


Figure 6: LM, (MSG + Vit C group) showing preservation of the structure of secretory striated ducts with normal luminal width and prominent basal striations (arrows), H&E x 400.



DISCUSSION

Monosodium glutamate is a well-known food additive applied to many food products to enhance flavor. The toxic effects of MSG on various body tissues were extensively studied in human and animal subjects. MSG toxicity is explained by its strong ability to generate Reactive Oxygen Species (ROS) and downregulate the activities of antioxidant enzymes such as Super Oxide Dismutase (SOD), Glutathione peroxidase and Glutathione reductase (17).

In the present study, histological examination of *MSG group* revealed degenerative changes affecting the serous acini. Acinar cells showed ill-defined cell borders, extensive cytoplasmic vacuolations and pyknotic crescent shaped nuclei.

These findings are due to the fact that MSG could induce oxidative stress and subsequent lipid peroxidation, which results in impairment of normal cell function. This leads to increased membrane fluidity, inactivation of membrane-bound receptors and promotion of cytosolic solutes efflux (18).

These observations agreed with Bhattacharya et al. (19), and Moussa (20) who stated that extensive lipid peroxidation is associated with cellular disorganization, disintegration of membrane integrity and nuclear pyknosis.

The vacuolization of acinar cell cytoplasm noticed in the parotid glands of MSG group was previously reported by Al-Mosaibih (21), who studied the effect of Monosodium glutamate on rat hepatic tissue and also reported severe cytoplasmic vacuolations of hepatocytes. This increased number of vacuoles can be explained by the MSG-induced oxidative stress which leads to increase of oxygen consumption, impaired food energy utilization and defective oxidative phosphorylation capacities.

The vacuolations in the cytoplasm of serous acinar cells could also be explained as a kind of cellular defense mechanism against toxic substances. Cheville (22), explained that these vacuoles may be responsible for collecting the toxic injurious elements and preventing them from interfering with the normal biological activities of the affected cells. These vacuoles could displace the nuclei from its normal position forming the observed crescent shape nuclei, other explanation for this shape may be due to the presence of dense areas of chromatin condensation (23).

Another finding in MSG group was the presence of many pyknotic or apoptotic nuclei in acinar cells. This observation was similar to the findings of Pavlovic et al. (24), who explained that MSG administration significantly raised the rate of the programmed cell death (apoptosis) in thymocytes. They demonstrated that oxidative stress induced by MSG causes increase in malondialdehyde level and xanthine oxidase activity in thymocytes. The accumulation of these compounds led to an uncontrolled increase in intracellular calcium concentration which participates in cellular death through various mechanisms.

It is well documented that antioxidants can protect body organs and

tissues from damage caused by oxidative stress. It has been established that vitamin C is a cheap water-soluble antioxidant that has the potential to scavenge free radicals and arrest oxidative stress induced by Monosodium glutamate (25).

In the present study, histological examination of the group received a daily dose of vitamin C (study II) showed much less degeneration of parotid gland acini and ducts in comparison with the group received MSG only. These observations agree with Farombli et al. (10) who also reported that dietary vitamin C has a protective potential against cell death by complete suppression of ROS and had a modulatory effect on MSG in the kidney and liver tissues of rats.

Soujanya et al., reported that vitamin C works both outside and inside the cell to neutralize unstable free radicals by donating electrons thereby suppressing their reactivity. (26). Vitamin C also inhibits lipid peroxidation and production of lipid peroxide either directly (by scavenging Oxygen-free radicals) or indirectly by regenerating other antioxidants such as vitamin E, the major lipid soluble antioxidant. Therefore, it plays an important role in maintaining cell membrane integrity (27).

CONCLUSION

From this study, we concluded that oral administration of Monosodium glutamate in albino rats may result in degenerative changes in parotid gland acini and ducts and co-administration of vitamin C was highly effective in protecting gland tissue against MSG-induced damage.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Al-Agili Z. The effect of food additives (Monosodium Glutamate - MSG) on human Health - A critical review. Journal of AlMaarif University College. 2020;31:362-9.
2. Farhat F. Monosodium glutamate safety, neurotoxicity and some recent studies. Al-Azhar J Pharm Sci. 2021;64:222-43.
3. Henry-Unaeze HN. Update on food safety of monosodium l-glutamate (MSG). Pathophysiology. 2017;24:243-9.
4. Kazmi Z, Fatima I, Perveen S, Malik SS. Monosodium glutamate: Review on clinical reports. Int J Food Prop. 2017;20:1807-15.
5. Walker R, Lupien JR. The safety evaluation of monosodium glutamate. J Nutr. 2000;130:1049S-52S.
6. Singh P, Mann KA, Mangat HK, Kaur G. Prolonged glutamate excitotoxicity: effects on mitochondrial antioxidants and antioxidant enzymes. Mol Cell Biochem. 2003;243:139-45.
7. Singh K, Ahluwalia P. Effect of monosodium glutamate on lipid peroxidation and certain antioxidant enzymes in cardiac tissue of alcoholic adult male mice. J Cardiovasc Dis Res. 2012;3:12-8.
8. Umukoro S, Oluwole GO, Olamijowon HE, Omogbiya AI, Eduviere AT. Effect of monosodium glutamate on behavioral phenotypes, biomarkers of oxidative stress in brain tissues and liver enzymes in mice. World J Neurosci. 2015;5:339-49.
9. Abdul-Hamid M, Galaly SR, Ahmed RR, Hamdalla H. Monosodium glutamate as a food additive: Toxic implications and the protective role of Quercetin. Merit Res J Med Med Sci. 2017;5:384-402.
10. Farombi EO, Onyema OO. Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Toxicol. 2006;25:251-9.
11. Hajihasani MM, Soheili V, Zirak MR, Sahebkar A, Shakeri A. Natural products as safeguards against monosodium glutamate-induced toxicity. Iran J Basic Med Sci. 2020;23:416-30.
12. El Kotb SM, El-ghazouly DE-s, Ameen O. The potential cytoprotective effect of Vitamin C and Vitamin E on monosodium glutamate-induced testicular toxicity in rats. Alex J Med. 2020;56:134-47.
13. Shredah M. Molecular study to the effect of monosodium glutamate on rat gingiva. Tanta Dent J. 2017;14:155-63.
14. El Imam HF, abd El Salam NN. Histological and Ultra structural Study of the Effect of Monosodium Glutamate on the Submandibular Salivary Gland of Adult Albino Rats. Egypt Dent J. 2019;65:319-29.
15. Taghyan SA, Messiry HE, Zainy MAE. Evaluation of the toxic effect of silver nanoparticles and the possible protective effect of ascorbic acid on the parotid glands of albino rats: An in vivo study. Toxicol Ind Health. 2020;36:446-53.

16. Bancroft JD, Lyton C, Suvarna KS. Theory and practice of histological techniques. 7th ed. Sierra Leone: Elsevier; 2012.
17. Soliman A. Extract of Coelatura aegyptiaca, a freshwater clam, ameliorates hepatic oxidative stress induced by monosodium glutamate in rats. Afr J Pharmacy Pharmacol. 2011;5:398-408.
18. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol. 2012;2012:645460.
19. Bhattacharya T, Bhakta A, Ghosh SK. Long term effect of monosodium glutamate in liver of albino mice after neo-natal exposure. Nepal Med Coll J. 2011;13:11-6.
20. Moussa EA, Al Mulhim JAA. Modulating Effect of Nigella sativa on Renal Structural Changes by Monosodium Glutamate in Female Mice. Egypt Acad J Biol Sci. 2013;5:33-45.
21. Al-Mosaibih MA. Effects of Monosodium glutamate and acrylamide on the liver tissue of adult wistar rats. Life Sci J. 2013;10:35-42.
22. Cheville NF. Ultrastructural pathology: The comparative cellular basis of disease. 2nd ed. Ames: Wiley-Blackwell; 2009.
23. Boraks G, Tampelini FS, Pereira KF, Chopard RP. Effect of ionizing radiation on rat parotid gland. Braz Dent J. 2008;19:73-6.
24. Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Jevtovic-Stoimenov T, Cekic S, et al. Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus. Mol Cell Biochem. 2007;303:161-6.
25. Ragab EE. The possible protective effect of vitamin C on monosodium glutamate induced renal toxicity in male albino rats. Egypt J Histol 2018;41:386-97.
26. Soujanya s, Lakshman M, Kumar A, Reddy A. Histopathological and ultrastructural changes induced by imidacloprid in brain and protective role of vitamin C in rats. J Chem Pharma Res. 2012;4:4307-18.
27. Adikwu E, Deo O. Hepatoprotective effect of vitamin C (Ascorbic acid). Pharmacol Pharm. 2013;04:84-92.

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