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EFFECT OF MONOSODIUM GLUTAMATE ON THE SUBLINGUAL SALIVARY GLANDS OF RATS (HISTOLOGICAL AND HISTOCHEMICAL STUDY)

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ABSTRACT

Objective: The aim of the present study was to investigate the effect of two different doses of Monosodium Glutamate (MSG) on the sublingual salivary glands of rats.

Design: Thirty adult male Sprague Dawley rats with an average weight of 200-230 gm were randomly allocated into 3 groups. Group I (control group): received distilled water, Group II: received 15 mg/kg b.w MSG orally and Group III: received 30 mg/kg b.w MSG orally. After 8 weeks, the rats were sacrificed. The sublingual salivary glands were examined histologically, immunohistochemically and histomorphometrically.

Results: Histologically, the striated ducts in group II showed ill-defined borders, pyknotic nuclei and cytoplasmic vacuolations. Examination of group III revealed marked cellular pleomorphism and loss of cell borders of mucous acini, the striated ducts showed distorted striations, while polymorphonuclear leukocytes (PMNL) infiltration and engorged blood vessels were traced in the connective tissue. Immunohistochemical examination showed strong immunoreactivity to caspase-3 in group III and weak to moderate reaction in group II.

Conclusion: Monosodium Glutamate (MSG) causes variable degrees of degenerative changes in the sublingual salivary glands of rats.

KEY WORDS: Monosodium Glutamate**,** sublingual salivary glands**.**

INTRODUCTION

Monosodium Glutamate (MSG) is the world's most extensively used flavor enhancer.[1] Monosodium Glutamate (MSG) is widely distributed and is naturally occurring in various foods including poultry, cheeses, meat broths, seafood and vegetables. It is also present in a wide variety of processed foods including prepared meals, flavored snacks and tuna, marinated meats, canned soups or sauces, fresh sausages, vegetarian burgers, luncheon chicken, turkey and sausages.[2] When added to food, MSG provides a flavoring taste similar to the naturally occurring free glutamate that differs from the four classic

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tastes of sweet, sour, salty and bitter; this unique taste is known as "umami" which improves the quality of food intake.[3]

Despite it increases palatability and food selection in meals; reports indicate that MSG is toxic to human and experimental animals. It has been implicated in male infertility, asthma, atopic dermatitis, ventricular arrhythmia, tightness, flushing, tearing, dizziness, syncope, and facial pressure. It has been also reported to have neurotoxic effects resulting in brain damage, endocrine disorder, in addition to some pathological conditions such as stroke, epilepsy, anxiety, Parkinson's disease and Alzheimer's disease.[4,5] Also, the obesogenic properties of MSG were studied for many years as it was found that it positively influences the appetite, and induces weight gain through stimulation of the orosensory receptors and subsequent improvement of meals palatability.[6]

Oxidative stress is caused by the excessive production of free radicals, or the decreased elimination of these variety of oxidative chain reactions acting on unsaturated fatty acids and proteins of cell membranes, producing lipid peroxides and protein carbonyls respectively causing the destruction of organelles and macromolecules.[7,8]

Nutrition metabolism and other cellular factors such as hormones, cytokines, and detoxification processes play an important role in the oxidative stress. Earlier studies have shown that chronic Monosodium Glutamate (MSG) intake causes oxidative damage and decreased levels of major anti-oxidant enzymes, resulting in impaired in the brain, kidneys, and liver functions.[9] A dose of 4mg Monosodium Glutamate (MSG)/ gm. b.w induces oxidative stress in experimental animals.[10]

Many researches on the effects of Monosodium Glutamate (MSG) on different body organs were conducted but there is little attention to oral tissues especially salivary glands, which is the tissue of our concern in this study.

Indeed, the diversity in manifestations of toxic effects and susceptibility of different species of animals to Monosodium Glutamate (MSG) was such that till today no specific dietary limitations have been recommended. Thus, the aim of this study is to evaluate the effect of two different doses of Monosodium Glutamate (MSG) on the sublingual salivary glands of rats.

MATERIALS AND METHODS

MATERIALS:

Animals: 30 adult male albino rats with average weight of 200g were obtained and kept in the animal house at the Medical Research Institute, Alexandria University. The rats were housed in cages under standard hygienic condition and were fed with rat chow and water ad libitum. All experiments were carried out in accordance with The Research Protocols established by The Animal Care Committee of The National Research Center (Cairo, Egypt), which followed the recommendations of The National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, Revised 1985).

Chemicals: Monosodium Glutamate (MSG) was purchased from BDH laboratory (Poole, UK), the chemical used was Monosodium Glutamate (MSG) ($C_5H_9NO_4$.-Na) Purity 99%. It is sold in the markets in Egypt under the license of Ajinomoto co.INC. Tokyo, Japan.

Experimental Design:

Animals were randomly assigned into three groups (n= 10); Group I served as a control group receiving distilled water orally on daily basis for 8 weeks; Group II was given 15 mg/kg b.w of Monosodium Glutamate (MSG) orally on daily basis for 8 weeks; Group III was given 30 mg/kg b.w of Monosodium Glutamate (MSG) on a daily basis for 8 weeks.[11]

METHODS:

On termination of the experiment, the animals were humanely sacrificed with a lethal dose (150 mg/kg body weight) of sodium thiopental. The sublingual salivary glands were dissected out, and were immediately immersed in 10% neutral formalin for 48 hours, then rinsed in distilled water. The glands on the right side were used for the light microscopic examination forming a total of 30 specimens (10 from each group), while the glands on the left side were used for the immunohistochemical examination forming a total of 30 specimens (10 from each group).

For light microscopic examination: The specimens were dehydrated in ascending grades of alcohol and embedded in paraffin. Serial sections of 5 μm thickness were stained with haematoxylin and eosin according to the conventional method. Histological examination was performed using light microscope.

For immunohistochemical study: All tissue blocks were cut at 5 µm, placed on positive charged slides and subjected to the biotin-streptavidin amplified system. Then they were subjected to caspase-3 IHC to detect cell apoptosis. Caspase-3 labeled cells were identified by brown nuclear-cytoplasmic staining. All steps were performed following the same protocol as Korany and Ezzat.[12]

Histomorphometric analysis:

Sections stained with immunohistochemical reaction were analyzed using LEICA Quin 500 analyzer computer system (LEICA Microsystems, Switzerland). The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the Olympus microscope (CX 41) and controlled by Leica Qwin 500 software. The optical density (OD) of caspase-3 was measured using an objective lens of magnification x40 i.e. of a total magnification of 400. Ten fields were measured for each specimen. After grey calibration, the image was transformed into a grey delineated image to choose areas exhibiting different grades of positive reactivity (minimum, maximum and medium reactivity). Areas of positive reaction were then masked by a blue binary color. Then the mean values and standard deviations were calculated automatically by image analyzer.

Measuring apoptosis affecting the sublingual salivary glands:

Image analysis for apoptotic changes in the sublingual salivary glands was done using Image J22 software. Standard measuring frame per five photomicrographs for each group using a magnification ×400 by light microscopy were transferred to the monitored screen.[13]

Statistical analysis:

The obtained data were processed and analyzed using SPSS version 19.0 for Windows. Means and standard deviations for different groups were calculated as the descriptive statistics for quantitative data. Kruskal-Wallis Test was used to compare more than two means among the different groups. The level of significance was set at $P<0.05$.

RESULTS

Histological results:

Examination of the control group (Group I) showed normal architecture of the sublingual gland. The acini were predominantly mucous, formed of pure mucous acini and mucous acini capped with serous demilunes. The striated ducts consisted of low columnar cells with centrally placed open faced nuclei and basal striations (fig. 1)

Histological examination of group (II) revealed that the acini were similar to those in the control group. The striated ducts showed ill-defined cell borders, some pyknotic nuclei and multiple cytoplasmic vacuoles interrupting the basal striations. Enlarged blood vessels engorged with red blood cells (RBCs) were noticed adjacent to the striated ducts (fig. 2)

In group (III), the serous demilunes showed marked nuclear hypertrophy and hyperchromatism. Loss of cell borders among the mucous acini was evident in some areas, while the striated ducts showed nuclei with different sizes and shapes (polymorphism), ill-defined cellular boundaries and distorted basal striations. The blood vessels were markedly enlarged with extravasated RBCs. Few polymorphonuclear leukocytes (PMNL) cell infiltrations were also noticed in the connective tissue (fig. 3)

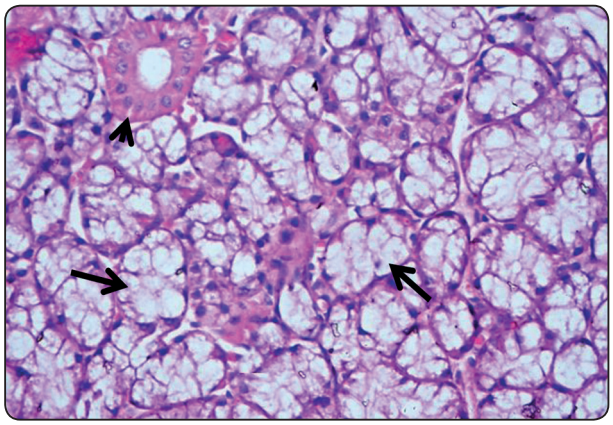

Fig. (1) Photomicrograph of Group I (control group) showing normal glandular structure of the mucous acini (arrows) and striated ducts showing basal striations (arrowhead). (400x)

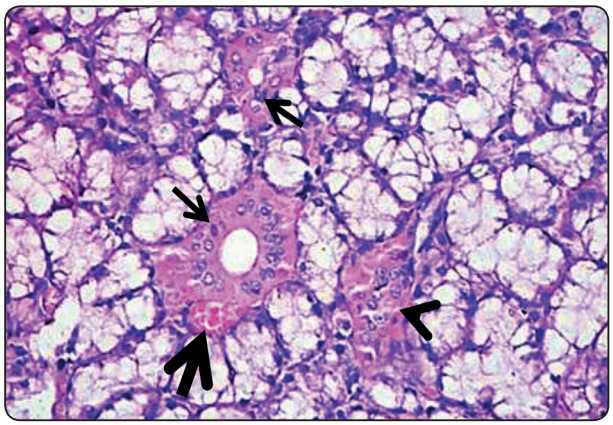

Fig. (2) A photomicrograph of Group II showing: marked disruption of duct cells borders (arrowhead), pyknotic nuclei (Thin black arrows), and enlarged blood vessels engorged with (Bold black arrow). (400x)

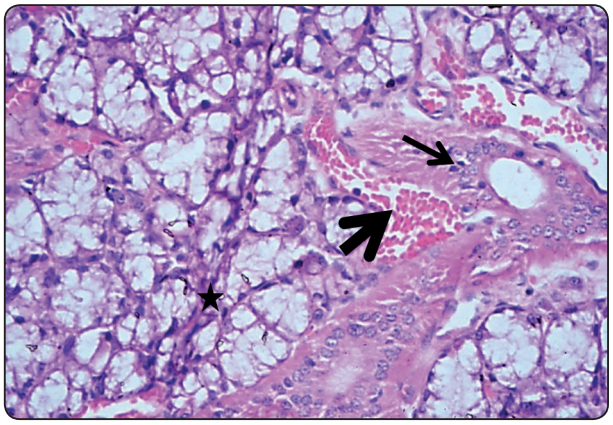

Fig. (3) Photomicrograph of group III showing: polymorphism of duct cells nuclei (Thin black arrow), enlarged blood vessels with extravasated RBCs (Bold black arrow), and few PMNLs infiltration in the interacinar C.T septa (black star). (400x)

Immunohistochemical results:

Immunohistochemical evaluation of the effect of low and high doses of Monosodium Glutamate (MSG) on cellular apoptosis using caspase-3 IHC revealed negative immunoreactivity in the nuclei and cytoplasm of Group (I) fig (4). However, Group (II) receiving 15 mg/kg b.w Monosodium Glutamate (MSG) showed weak to moderate degree of positive immunoreactivity in the nuclei and cytoplasm of both acinar and ductal cells fig (5).While group (III) receiving 30 mg/kg b.w Monosodium Glutamate (MSG) showed strong nuclear and cytoplasmic staining positivity to caspase-3 in most acini and duct cells fig (6).

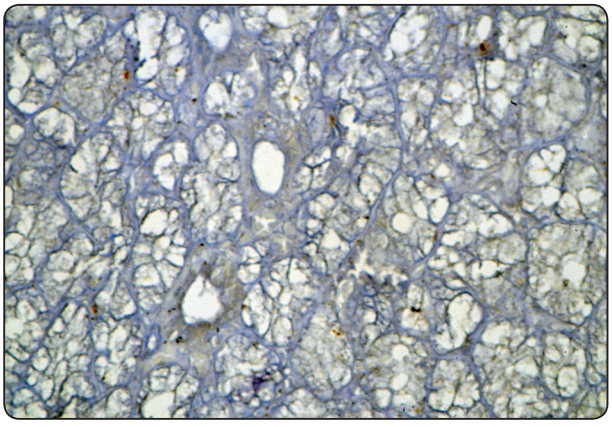

Fig. (4) Photomicrograph of Group I (control group) showing negative immunoreactivity of nuclei and cytoplasm of the acini and ducts. (400x)

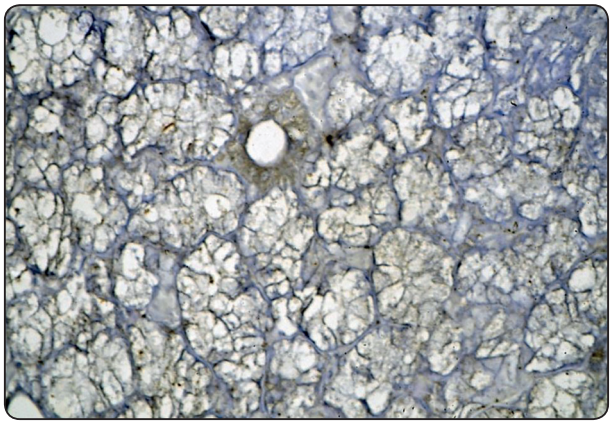

Fig. (5) Photomicrograph of Group II showing weak to moderate nuclear and cytoplasmic reaction. (400x)

TABLE (1): Comparison between the control and studied groups regarding the apoptotic changes affecting the sublingual salivary glands.

Area fraction	Group 1 (control group)	Group 2 group II	Group 3 group III	p
Range	1.999-3.214	19.73-20.39	31.88-33.25	p1=0.001* p2=0.001* p3=0.001*
Mean ±SD	2.51±0.552	20.06±0.28	32.53±0.63	

P = Control group vs. Group II (15 mg/kg b.w of MSG). P2= control vs. Group III (30mg/kg b.w of MSG). P3= Group II vs. Group III. *P≤0.05, significant.

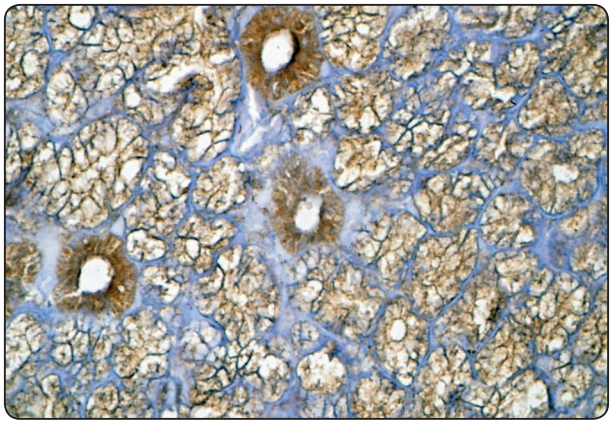

Fig. (6) Photomicrograph of Group III) showing strong nuclear and cytoplasmic immunoreactivity in the acini and striated ducts (400x)

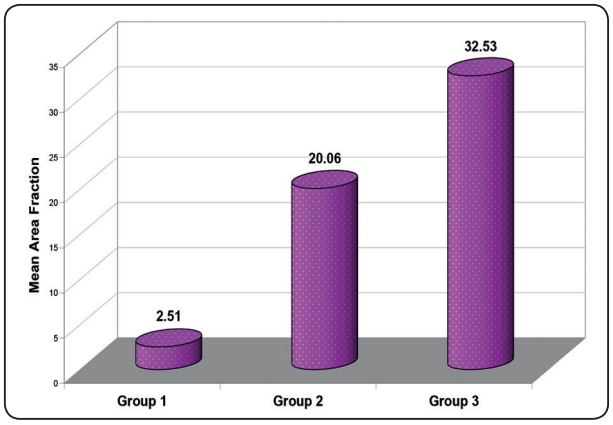

Fig. (7): A histogram showing comparison between Group I (control group=Group 1) , Group II (received 15 mg/kg b.w MSG = Group 2) and Group III (received 30 mg/kg b.w MSG= Group 3) regarding apoptotic changes of the sublingual salivary glands.

Image analysis results:

On measuring the amount of apoptotic changes in the control and experimental groups, significant increase in the amount of apoptosis from the control group to the experimental group II and experimental group III was recorded. Indeed, P value and mean ±SD results revealed significant increase in the degree of apoptosis from the experimental group II to the experimental group III (Table 1 and Fig. 7).

DISCUSSION

Monosodium Glutamate (MSG) is one of the most frequently applied food additives in modern nutrition, with a related decline in overall health of both humans and rats. The toxic effects of Monosodium Glutamate (MSG) were correlated to its potential ability to generate Reactive Oxygen Species (ROS) and significantly decrease the activities of antioxidant enzymes such as Super Oxide Dismutase (SOD), and glutathione

metabolizing enzymes like Glutathione Reductase (GR) and glutathione peroxidases GPx.[14, 15]

In the present study, histological examination of group (II) receiving 15 mg/kg b.w revealed some degenerative changes affecting the striated ducts. The ductal cells showed ill-defined cell borders and vacuolations interrupting the basal striations, pyknotic nuclei, also blood vessels engorged with blood were noticed. However, when the dose was increased to 30 mg/kg b.w, the changes in the sublingual glands were more aggressive as compared to group II. The serous demilunes showed marked nuclear hypertrophy and hyperchromatism. The striated ducts showed abnormal epithelial lining with ill-defined cellular boundaries. The connective tissue showed extravasated RBCs and PMNL cell infiltrations.

These findings may be due to the fact that MSG-induced oxidative stress causes lipid peroxidation, with subsequent impairment of normal cell function. This leads to increased membrane fluidity, inactivation of membrane-bound receptors and promotion of cytosolic solutes efflux.[7] These findings were in agreement with Bhattacharya et al.,[16] and Moussa [17] who stated that extensive lipid peroxidation is correlated with the ultimate disintegration of membrane integrity, nuclear pyknosis and cellular disorganization.

Indeed, the vacuolization of acinar and ductal cell cytoplasm noticed in the sublingual glands was in accordance with the findings of El-Kenawy et al.,[17] and Kumbhare et al.,[18] who studied the effect of MSG on rat hepatocytes and reported increased number of cytoplasmic vacuoles. This vacuolations can be interpreted by the MSG-induced oxidative stress which leads to impaired food energy utilization, increase in oxygen consumption, and defective oxidative phosphorylation capacities. This increase in the levels of oxidative parameters is accompanied by a decrease in antioxidant activity causing oxidative stress damage to the cellular components.[18] Regarding nuclear pleomorphism, this finding is supported by Al-Mosaibih [19] who reported that hepatocytes nuclei of rats injected IP with 30 mg/kg b.w of Monosodium Glutamate (MSG) showed pyknosis and pleomorphism. Alalwani [20] suggested that pyknosis of cell nuclei due to Monosodium Glutamate (MSG) treatment might indicate the loss of functional efficiency of the cells.

MSG-induced oxidative stress results from the chronic intake of Monosodium Glutamate (MSG) and subsequent rise and accumulation of Glutamate in blood [14]. This amino acid acts on multiple receptor types which have been reported to be expressed in peripheral, neuronal, or non-neuronal tissues, such as skin, pancreas and salivary glands.[21] Glutamate-induced oxidative damage in various tissues occurs through the release of certain molecules such as α-ketoglutarate dehydrogenase, glutamate receptors and cystine-glutamate antiporter which were found to contribute to the oxidative stress through different mechanisms. [22, 23]

Interestingly, the immunohistochemical results and histomorphometric data of the present study were in coherence with the histological results. Apoptotic cell death was demonstrated immuno-histochemically using caspase-3 as an apoptotic marker. Strong immunoreactivity was observed in group III receiving 30 mg/kg b.w of Monosodium Glutamate (MSG) in comparison to weak immunostaining in group II receiving 15 mg/kg b.w of Monosodium Glutamate (MSG). This was in agreement with Eweka and Adjene [4] who reported neuronal cell death due to Monosodium Glutamate (MSG), and stated that apoptotic death pathway in brain cells might occur in response to neurotoxins.

Indeed, Pavlovic et al.,[24] explained that Monosodium Glutamate (MSG) administration to animals significantly increased the programmed cell death rate of thymocytes. They suggested that Monosodium Glutamate (MSG)-induced oxidative stress causes significant increase in malondialdehyde (MDA) level and xanthine oxidase (XO) activity in

thymocytes. The accumulation of these compounds led to an uncontrolled rise in intracellular calcium concentration which contributes to cell death by various mechanisms.[25]

Image analysis results revealed significant increase in the apoptotic changes from the control group to experimental groups II and III. The control group recorded a mean value of 2.51±0.552, while experimental group II recorded 20.06±0.28 mean value. Also, experimental group III showed significant increase in the apoptotic changes when compared with experimental group II. Experimental group III recorded 32.53±0.63 mean value. The increase in the amount of apoptosis in both experimental groups of the present study was found to be dose dependent using image analysis.

From this study, we conclude that when Monosodium Glutamate (MSG) was applied in different doses, variable degrees of acinar and ductal degenerative changes were traced in the sublingual salivary glands. We suggest that further studies should be performed on the effect of Monosodium Glutamate (MSG) on the other oral tissues of experimental animals. It is also recommended that excessive consumption of food supplemented with Monosodium Glutamate (MSG) should be avoided to protect the body organs from its reported hazardous effects.

REFERENCES

1. Fárr AM, Jung, A.,Chiopu, A.L. Possible implications of monosodium glutamate in development of obesity and some liver diseases. J of Expl Med & Surg Res 2010: 138 - 40.
2. Abd-Ella E.M., Mohammed A.M. Attenuation of Monosodium Glutamate-Induced Hepatic and Testicular Toxicity in Albino Rats by Annona Muricata Linn. (Annonaceae) Leaf Extract IOSR Journal of Pharmacy and Biological Sciences 2016;11(6):61-69
3. Tawfik M.S A-BN. Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamins C and E Food and Nutrition Sciences 2012;3:651-59
4. Eweka AOaA, J.O. . Histological Studies Of The Effects Of Monosodium Glutamate the medial geniculate body of adult wistar rats. Electron J Biomed. 2007;2:9-13.
5. Geha R. S. BA, Ren C., Patterson R., Gra- mmar L. C., DittoA. M. and Harris K. E. Review of Allergic Reaction to Monosodium Glutamate and Outcome of a Multicenter Double Blind Placebo-Controlled Study. Journal of Nutrition 2001;130:1032S-38S. .
6. Beregova T.V. FTM, Neporada K.S., and Gordienko L.P. Metabolic Changes in Salivary Glands of Rats under Glutamate-Induced Obesity Journal of Dentistry, Oral Disorders & Therapy. Open Access 2014:1-4.
7. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol; 2012: 645460.
8. A.W.A-Z,N.S.H,M.AE-Y.Effect of aflatoxin contaminated diet on major salivary glands and the protective role of Ozone application. Life Sci J 2014;11(8):450-60.
9. Calis I.U. CDT, Saydam F, Kolac U.K., Soyocak A., Kurt H., Gunes H.V.,1 Varol Sahinturk V.,4 Fezan Sahin Mutlu F.S.,5 Zeynep Ozdemir Koroglu Z.O., Degirmenci I. The Effects of Monosodium Glutamate and Tannic Acid on Adult Rats. Iran Red Crescent Med J 2016 18(10).
10. Onyema OO, Farombi EO, Emerole GO, Ukoha AI, Onyeze GO. Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. Indian J Biochem Biophys 2006;43(1):20-4.
11. Leshchenko IV, Shevchuk, V.H., Falalieieva, T.M. & Beregova, T.V. . The influence of long-term monosodium glutamate feeding on the structure of rats pancreas. Fiziol Zh 2012;58(2):59-65.
12. .Korany N.S. EBA. Prophylactic effect of green tea and Nigella sativa extracts against fenitrothion-induced toxicity in rat parotid gland. Arch Oral Biol. 2011;56(11):1339-46.
13. Doube M, Kłosowski MM, Arganda-Carreras I, Cordelières FP, Dougherty RP, Jackson JS, Schmid B, Hutchinson JR, Shefelbine SJ. BoneJ: Free and extensible bone image analysis in ImageJ. Bone. 2010; 47(6).
14. Soliman AM. Extract of Coelatura aegyptiaca, a freshwater clam, ameliorates hepatic oxidative stress induced by monosodium glutamate in rats. African Journal of Pharmacy and Pharmacology 2011;5(3):398-408.
15. Une D, Deb S, Chikazawa G, Kommaraju K, Tsuneyoshi H, Karkhanis R, et al. Cut-off values for transit time flowmetry: are the revision criteria appropriate? J Card Surg;28(1):3-7.

16. Bhattacharya T, Bhakta A, Ghosh SK. Long term effect of monosodium glutamate in liver of albino mice after neonatal exposure. Nepal Med Coll J 2011;13(1):11-6.

17. Moussa EA, Al Mulhim, J.A. (2013): . Modulating effect of nigella sativa on renal structural changes by monosodium glutamate in female mice. . Egypt. Acad. J. Biolog. Sci. 2013; 5(2):33-45.

18. Gu C. SY, Le G. . Effect of Dietary Protein Level and Origin on the Redox Status in the Digestive Tract of Mice. Int J Mol Sci 2008;9:464-75.

19. AL-Mosaibih MA. Effects of monosodium glutamate and acrylamide on the liver tissue of adult Wistar rats. Life Sci J. 2013;10(2):35-32.

20. Alalwani AD. Monosodium glutamate induced testicular lesions in rats (histological study). . Middle East Fertility Society J. 2013;19(4):274-80.

21. Lee B, Lee H, Nam YR, Oh JH, Cho YH, Chang JW. Enhanced expression of glutamate decarboxylase 65 improves symptoms of rat parkinsonian models. Gene Ther 2005;12(15):1215-22.

22. A. S. Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: a mini-review. Journal of Biomedical Science 2015 1-6.

23. Hordiienko LP, Berehova TV, Neporada KS, Falalieieva TM. [Oxidative stress development in the tissues of salivary glands of rats in conditions of monosodium glutamate-induced obesity]. Fiziol Zh;60(4):105-7.

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(1-2):161-6.

25. Narayanan SN, Kumar RS, Paval J, Nayak S. Effect of ascorbic acid on the monosodium glutamate-induced neurobehavioral changes in periadolescent rats. Bratisl Lek Listy;111(5):247-52.