Int J Fertil Steril
International Journal of Fertility & Sterility
Homepage: https://www.ijfs.ir/
Original Article
Vol 17, No 4, October-December 2023, Pages: 281-286

Monosodium Glutamate Effect on The Expression of α7nACHR and α4nACHR Subunits in The Testicular Tissue

Raheleh Baradaran, Ph.D.[1], Nasibeh Ghandy, Ph.D.[2, 3], Nasim Alipour, Ph.D.[2, 3], Fatemeh Rahimi Anbarkeh, Ph.D.[4*]

1. Department of Operating Room, Faculty of Paramedical Sciences, Gonabad University of Medical Sciences, Gonabad, Iran
2. Department of Anatomy and Cell Biology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran

Abstract

Background: Monosodium glutamate (MSG) is a popular food flavor enhancer, and a glutamate subset that induces different toxicities such as hepatotoxicity, neurotoxicity, reproductive toxicity, and nephrotoxicity. This study was conducted to assess the effects of MSG on the α7 and α4 nicotinic acetylcholine receptor (nACHR) protein subunits expression of adult rat testis and the safety role of vitamin C.

Materials and Methods: For this experimental research, 24 rats were haphazardly grouped into four equal groups (n=6) and orally gavaged for 30 days as follows: control group (distilled water gavage), MSG group (3 g/kg/b.w/day), vitamin C group (150 mg/kg/b.w/day), and MSG+vitamin C group (3 g/kg/b.w/day+150 mg/kg/b.w/day, respectively) that rats of all groups on the 30th day were anesthetized, and the left testes were used for of α4 and α7 nACHR protein subunit evaluation by immunohistochemistry (IHC). Statistical computations were performed using Graph Pad Prism software.

Results: The present study revealed a significant reduction in the expression and optical density (OD) of α7 nACHR and α4 nACHR in the seminiferous tubules and intertubular connective tissue in the MSG group compared to the control group. In the MSG+vitamin C group, the expression and OD of α7 nACHR and α4 nACHR increased in the seminiferous tubules and intertubular connective tissue but this improvement was not significant compared to the MSG group.

Conclusion: MSG decreased the expression level of nACHR protein subunits, α7 and α4, in the seminiferous tubules and interstitial testicular tissue. Vitamin C in the MSG+vitamin C group could not significantly improve the expression of α7 and α4 nACHR subunits in testicular tissue. Probably, MSG toxicity can be compensated with higher doses of vitamin C.

Keywords: Ascorbic Acid, Nicotinic Receptors, Sodium Glutamate, Testis

Citation: Baradaran R, Ghandy N, Alipour N, Rahimi Anbarkeh F. Monosodium glutamate effect on the expression of α7nACHR and α4nACHR subunits in the testicular tissue. Int J Fertil Steril. 2023; 17(4): 281-286. doi: 10.22074/IJFS.2023.561854.1368

Introduction

Nicotinic acetylcholine receptors (nACHR) are the main members of ligand-gated ion channels that respond to the acetylcholine binding. In the central nervous system (CNS), nACHRs are composed of various subunits. Subunits such as α7 and α4β2 are the most abundant subunits of nACHRs in the CNS (1). Although, some studies have reported different expressions of nACHRs subunits on germ cells and sertoli cells, they play roles in germ cell differentiation, proliferation, and sperm movement (2, 3).

Today, many food companies use flavor enhancers in their products, including canned vegetables, soups, and processed meats. The monosodium glutamate (MSG) is one of the most common food flavorings (4, 5). While, the glutamic acid is tasteless molecule, its combination with the sodium leads to glutamate receptors activation in the taste receptor cells. It causes a distinct sense of taste by sending signals to specific brain areas (6, 7).

Several pre-clinical studies have shown that the MSG is toxic to different tissue such as the CNS, testis, ovary, uterus, kidney, and liver (8-10). The MSG can decrease the germinal epithelial thickness, testosterone hormone level, leydig cell numbers and

Received: 10/September/2022, Revised: 08/February/2023, Accepted: 15/February/2023
*Corresponding Address: P.O.Box: 93186-14139, Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran
Email: rahimif2@nums.ac.ir

Royan Institute
International Journal of Fertility & Sterility

increase fibrosis, caspase-3, apoptotic cell numbers, and abnormal sperm morphology in the testis of male Wistar rats (5, 6, 11). One of the known mechanisms of these changes is oxidative stress induction follow of free radicals and hydrogen peroxide formation (7, 12). MSG's functional and morphological effects on the male reproductive system can also be due to decreased gonadotropin-releasing hormone (GnRH) secretion (8).

MSG directly influences the spermatogenic tubules glutamate transporter, which makes this molecule toxicity mechanism in the testis (8). Free glutamate derived from the MSG can create excitotoxicity and pathological alterations in peripheral tissues via acting on the glutamate receptors (13). The inevitable use of monosodium molecules in foods and their deleterious effects led to the requirement of using protective substances, especially antioxidants (8, 14). On the other hand, enzymatic and non-enzymatic antioxidants act as a defense mechanism by decreasing oxidative stress, lipid peroxidation, and pathological changes. Among these antioxidants, vitamin C molecules, ascorbic acid, is introduced as a protector of various cells and tissues against oxidative stress (15, 16). This study was conducted to assess the effects of the MSG molecules on the nACHR subunits, α7 and α4, expression of the adult rat testis and the protective role of the vitamin C molecule.

Materials and Methods

This experimental investigation was confirmed (IR.MUMS.fm.REC.1395.611) by the Ethics Committee of Mashhad University of Medical Sciences, Khorasan Razavi, Iran. Working with animals was also carried out on the basis of the guideline for the care and use of laboratory animals (NIH publication no. 86-23).

Animals

Twenty-four adult male wistar rats (body weight: 200-250 g, age: 6-8 week-old) were used for this experimental study. All animals were preserved under controlled conditions, 12 hours light/darkness cycle with free access to water and food. All rats were obtained from laboratory animals of the Mashhad University of Medical Sciences, Khorasan Razavi, Iran.

Experimental design

The animals were distributed into four equal groups (n=6). Administration of MSG ($C_5H_8NNaO_4$, Negin Tejarat Payam Co., Iran under the license of Huifenghe, China, Cat No; 142-47-2) (4, 5), vitamin C (Osve Co., Iran) (14), and distilled water was done with oral gavage for 30 days (once a day). On the 30th day, the rats were anesthetized by chloroform, and heart perfusion (17) was carried out for full formalin penetration. The left testes (12) were used for immunohistochemistry (IHC) evaluation (Table 1).

Tissue preparation

The testes were divided into several parts and fixed in 10% formalin for 5 days and after the tissue processes were embedded in paraffin. Serial sections of the tissue (5 µ thick) were taken by microtome and mounted on poly-L-lysine coated slides for IHC (12).

Table 1: Different experimental groups

Group name	Intervention
Control	Distilled water
MSG	MSG 3 g/kg/b.w/day
Vit. C	Vit. C 150 mg/kg/b.w/day
MSG+Vit. C	MSG 3 g/kg/b.w/day+Vit. C 150 mg/kg/b.w/day

MSG; Monosodium glutamate and Vit C; Vitamin C.

Immunohistochemistry

For immunohistochemical staining, the slides were deparaffinized by xylene (Mojallali, Iran), hydrated using a descending alcohol series (Mojallali, Iran), and washed in phosphate-buffered saline (PBS) for 10 minutes. The slides were kept in boiling PBS for 15 minutes to retrieve the antigen. Then the samples were treated with the bovine serum albumin (BSA, A4503, Sigma Aldrich, USA) at the room temperature. The sections were immersed in Hydrogen peroxide (H_2O_2-3%) for 15 minutes to block endogenous peroxidase and after that, incubated with normal goat serum (ab 7481, abcam, USA) for 25 minutes. In the next step, samples were covered with primary antibodies, rabbit anti-CHRNA4 antibody (Orb11095, Biorbyt, UK), and rabbit anti-CHRNA7 antibody (Orb11096, Biorbyt, UK) overnight at 4°C. After washing the slides, samples were treated with secondary antibody goat anti-rabbit HRP (1:1000, ab6721, Abcam, USA) for 1 hour at room temperature. After washing with PBS, we applied the diaminobenzidine solution (DAB, Sigma-Aldrich, USA) for 15 minutes in darkness. The slides were washed with water and counterstained with hematoxylin (05-M06004, Bio Optica, Italian) for 1 minutes (18).

Photomicrographs were taken by a bright-field microscope (BX51, Olympus, Japan) equipped with a camera (DP12, Olympus, Japan). The amount of brown color indicates the intensity of distribution of nACHR protein subunits, α7 and α4, expression in the testes parenchyma. ImageJ software (version 1.48, National Institutes of Health, Bethesda, MD, USA) was applied to measure the average staining intensity in the sections. The intensity of the brown color was converted into optical density (OD). The following formula was used to calculate OD using average staining intensity. The maximum intensity in RGB (Red-Green-Blue) figures is 255 (18, 19).

$$OD = \log\left(\frac{\text{Max intensity}}{\text{Mean intensity}}\right)$$

Statistical analysis

Statistical computations were performed using Graph Pad Prism software version 8 (GraphPad Prism Software Inc., San Diego, CA, USA). Statistical significance between different groups were compared using non-parametric Kruskal-Wallis test. Results were considered mean ± SEM with the significance level at $P<0.05$.

Results

Expression of the α4 nicotinic acetylcholine receptor in the seminiferous tubules

A significant reduction (P=0.0031) was observed in the expression of α4nACHR protein subunit in the seminiferous tubules in the MSG group in comparison with the control group. In the vitamin C treatment group, the distribution of α4nACHR protein subunit were significantly elevated in comparison with the MSG group (P=0.0080). In the vitamin C+MSG group, the augmented distribution of α4nACHR protein subunit was not remarkably different from the MSG group (P=0.9999).

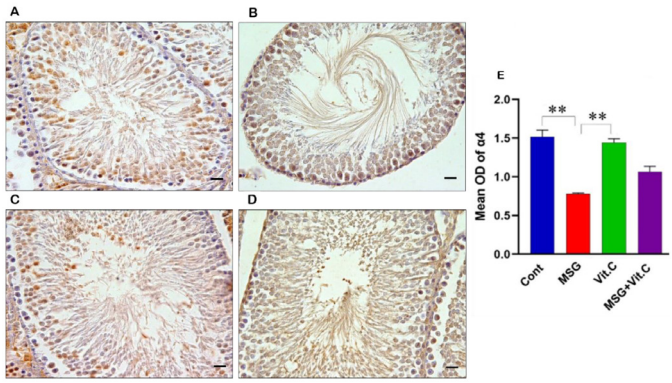

Fig.1: Expression of α4 nACHR in the seminiferous tubules in different groups. The brown color (dots and spots) indicates the immunoreactivity of α4 nACHR protein subunit expression in the seminiferous tubules of **A.** Control, **B.** MSG, **C.** Vit. C, and **D.** MSG+Vit. C groups. **E.** OD of α4 nACHR protein subunit in all groups. All data were considered as mean ± SEM values (Cont vs. MSG, **; P=0.0031, and Vit. C vs. MSG, **; P=0.0080) (scale bar: 100 µm). nACHR; Nicotinic acetylcholine receptor, MSG; Monosodium glutamate, Vit. C; Vitamin C, OD; Optical density, and Cont; Control.

Expression of the α7 nicotinic acetylcholine receptor in the seminiferous tubules

The results obtained in the current study showed that a significant decline in the expression and OD of the α7 nACHR protein subunit was recognized in the MSG group in comparison with the Control group (P=0.0017). In the vitamin C treatment group, expression and OD of the α7 nACHR protein subunit increased considerably in comparison with the MSG group (P=0.0385). In the vitamin C+MSG treatment group, the distribution and OD of α7nACHR protein subunit increased, but there was not a considerable difference between this group and the MSG group (P=0.5851, Fig.2, A-E).

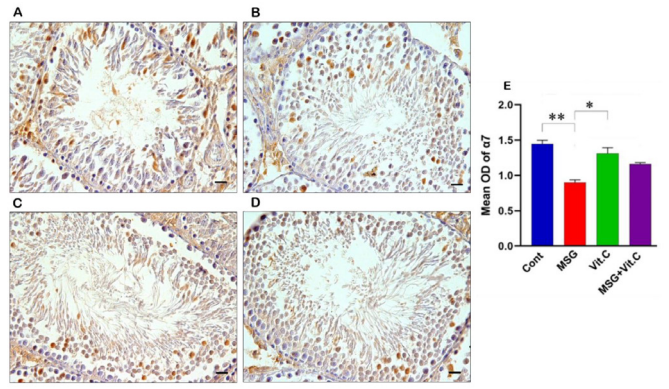

Fig.2: Expression of α7 nACHR in the seminiferous tubules. The brown color (dots and spots) demonstrates the immunoreactivity of α7 nACHR protein subunit expression in the seminiferous tubules of **A.** Control, **B.** MSG, **C.** Vit. C, and **D.** MSG+Vit. C groups. **E.** OD of α7 nACHR protein subunit in all groups. Results are presented as mean ± SEM values (Cont vs. MSG, **; P=0.0017, and Vit. C vs. MSG, *; P=0.0.0385) (scale bar: 100 µm). nACHR; Nicotinic acetylcholine receptor, MSG; Monosodium glutamate, Vit. C; Vitamin C, OD; Optical density, and Cont; Control.

Expression of the α4 nicotinic acetylcholine receptor in the intertubular connective tissue

According to Figure 3, the lowest distribution and OD of α4 nACHR protein subunit were indicated significantly in the MSG group compared to other groups (P=0.0009). In the MSG group, the distribution, and OD of α4 nACHR protein subunit elevated remarkably, and there was a meaningful difference between the vitamin C and MSG groups (P=0.0234). There was not a meaningful difference in the OD of the α4 nACHR protein between the MSG+vitamin C and MSG groups (P=0.9999, Fig.3E).

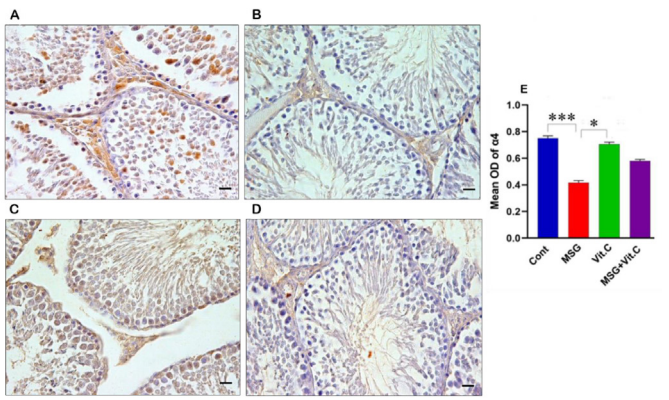

Fig.3: Expression of the α4 nACHR in the intertubular connective tissue. The brown color (dots and spots) between seminiferous tubules indicates the immunoreactivity of the α4 nACHR protein subunit of A. Control, B. MSG, C. Vit. C, and D. MSG+Vit. C groups. E. OD of α4 nACHR protein subunit in all groups. Results are presented as mean ± SEM values. (Cont. vs. MSG, ***; P=0.0009, and Vit. C vs. MSG, *; P=0.0234) (scale bar: 100 µm). nACHR; Nicotinic acetylcholine receptor, MSG; Monosodium glutamate, Vit. C; Vitamin C, OD; Optical density, and Cont; Control.

Expression of the α7 nicotinic acetylcholine receptor in the intertubular connective tissue

The control group had the highest distribution of the α7nACHR protein subunit, and the MSG group had the lowest distribution of the α7nACHR protein subunit (Cont. vs. MSG, P=0.0025). In the vitamin C treatment group, α7nACHR protein subunit expression significantly

increased in comparison with the MSG group (P=0.0097). There was no significant difference in the expression of the α7nACHR protein subunit in the MSG+vitamin C group in comparison with the MSG group (P=0.9999, Fig.4A-E).

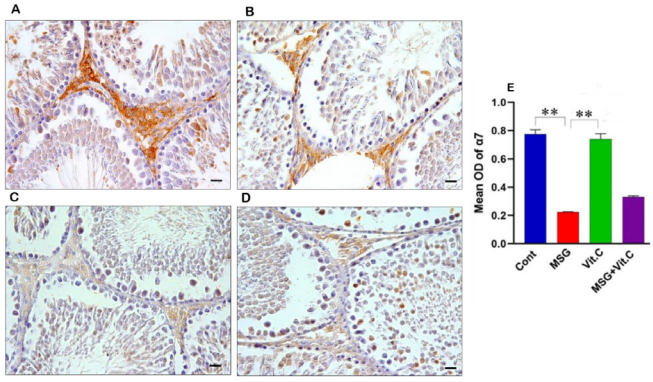

Fig.4: Expression of the α7 nACHR in the intertubular connective tissue. The brown color (dots and spots) between seminiferous tubules shows the immunoreactivity of the α7 nACHR protein subunit of **A.** control, **B.** MSG, **C.** Vit. C, and **D.** MSG+ Vit. C groups. E. α7 nACHR protein subunit in all groups. Results are presented as mean ± SEM values. (Cont. vs. MSG, **; P=0.0025, and Vit. C vs. MSG, **; P=0.0.0097) (scale bar: 100 µm). nACHR; Nicotinic acetylcholine receptor, MSG; Monosodium glutamate, Vit. C; Vitamin C, OD; Optical density, and Cont; Control.

Discussion

The present study evaluated the nACHR subunits, α7 and α4, protein expression in the testis of MSG treated rats, and also the vitamin C protective role. Our results revealed that expression these subunits of the nACHR protein in the seminiferous tubules and interstitial tissue of the MSG group were significantly lower than in our other groups. Also, the vitamin C was able to prevent the decrease in the expression of nACHRs protein subunits in our rat testis parenchyma, but not significantly.

The MSG has been shown to have destructive effects on the testicular tissue. It can reduce the weight of the testis (20, 21) and sperm motility, also induce oligozoospermia, testicular atrophy, and hemorrhage, and change the structure of the testis, sperm morphology, and sperm cell population (12, 21, 22). Also, the germinal epithelial thickness decreases in adult Wistar rats because of using MSG (11, 12, 21, 22). Of course, all of the complications mentioned above depend on the duration, course, dose, induction method of treatment with MSG, and species studied (23). It has been proven that testicular toxicity in the MSG group can be related to Ca^{2+} overloading (24), reactive oxygen species (ROS) production in the testis and decreased antioxidant activities (11, 12, 21, 22), increased malondialdehyde (MDA) and apoptosis (11,12, 25).

As we reported previously, the MSG caused testicular tissue germ cells apoptosis (12). It seems that the MSG probably reduces the number of testicular cells and subsequently the number of nicotinic receptors through apoptosis. In this line, John et al. confirmed that the MSG usage increases Ca^{2+} flow in the neuronal cells, enhances the level of inflammatory cytokines, ROS production, Bax, Bcl-2 and caspase-3 expression, and neuronal apoptosis in the brain tissue, which occurs through glutamate receptors (26). Testicular tissue, like the brain, has many glutamate receptors. Therefore, the MSG can affect the testis by the same mechanisms, apoptosis.

Furthermore, the MSG reduces the scavengers of free radicals, including antioxidant enzymes, glutathione, and ascorbic acid. These scavengers can protect cell membranes from lipid peroxidation and oxidative damages (11). Ismail El-Sawy et al. (27) showed that MSG decreases levels of testosterone and luteinizing hormone (LH) in the serum, and antioxidant activities in the testis, also disturb sperm profiles. Therefore, The destruction of cell membranes, apoptosis, and cells reduction in the seminiferous tubules and interstitial tissue can explain the decrease of nACHRs α7 and α4 subunits in the MSG administrated group.

Another explanation for the reduction of the nACHRs expression level in the MSG group is that it has a protective effect on the spermatogonia cell line and Leydig cells. Administered MSG can hyper-activate the glutamate receptors in spermatogonia cells, increasing intracellular Ca^{2+} flow, which lastly induces apoptosis and sloughing of spermatogenic cells into the seminiferous lumen (28). In such cases as a compensatory mechanism, nACHRs are likely to be reduced to prevent intracellular Ca^{2+} overload and induction of excessive apoptosis.

It is suggested that nACHRs can influence the differentiation, growth, maturation, and morphogenesis process of different types of testis cells (3). The α7 subunit of this protein deficiency in the mouse sperm induces impaired motility and reduced hyperactivation. On the other hand, studies reported that the MSG may alter normal sperm morphology and impair fertility (20, 21).

In this regard, we think that this phenomenon occurs through reducing nACHRs subunits, α7 and α4, expression.

Our study showed that these subunits, α7 and α4, expression reduced in the interstitial testicular tissue of the MSG-treated group. It can be related to apoptosis and reduction of Leydig cells in the interstitial tissue of the testis, as reported in 2019 by Shima (29). Therefore MSG probably through increased ROS and decreased testosterone can be stopped spermatogenic cell maturation and nACHRs subunits, α7 and α4, expression. This issue can be another explanation for the reduction of the mentioned receptors in the interstitial tissue. Inoue et al. showed the MSG intake increases intracellular and mitochondrial Ca^{2+} flow which induces the ROS production. ROS induces a lipid peroxidation process that damages the cell membranes and DNA in the Leydig cells (30). Also, ROS induces disruptions in the electron chain function of mitochondria. Lipid peroxidation process disarranges membrane permeability and ATP production, subsequently destroying the Leydig cells (29), it issue can lead to the reduction of nicotinic receptors in the testicular

interstitial tissue. Also, the loss of Leydig cells results in testosterone secretion decrease in the testis tissue (31). In addition to the testis, the glutamate receptors are abundant in the hypothalamus. ROS production in the hypothalamus decreases GnRH secretion and subsequently the LH secretion. These events disrupt Leydig cells' stimulation for secreting testosterone, and finally, reduce testosterone level (7, 32, 33). It has been proven that a testosterone level decrease can arrest the maturation of spermatogenesis and the protein expression of nACHRs (6, 11). Schirmer et al. (3) suggested the vital role of α4 and α7 subunits in the Leydig cells for androgen secretion.

In the present study, we observed that the vitamin C can prevent the harmful effects of MSG on the testicular tissue and increase nACh receptors, but these changes were not significant. Vitamin C is known as a powerful antioxidant, it is an electron donor, and this property causes all its known functions (34). Its double-bond electrons can transfer to oxidant molecules. Thus, vitamin C can regenerate active species involved in many diseases. It has been proven that MSG reduces the scavengers of free radicals, including ascorbic acid (11). Thus, vitamin C can compensate for this deficiency and reduce MSG complications. In contrast to the present results, Arzuaga et al. (35) and Rahayu et al. (33) stated that the composition of "Marsilea Crenata" can inhibit the MSG's harmful effects and preserve the Leydig and spermatogenic cells. They attributed this property to vitamin C existing in Marsilea Crenata. In our study, vitamin C in the MSG+vitamin C group probably can preserve the Leydig and spermatogenic cells. Thus, the expression of receptors improved in the MSG+vitamin C group (not meaningfully). In addition, vitamin C participates in the synthesis of L-carnitine. This compound plays a critical role in energy output by conveying fatty acids to the mitochondria (35, 36). Koohpeyma et al. (37) concluded that L-carnitine ameliorates MSG's renal damage through its antioxidant and anti-apoptotic effects. However, the results of this project showed that vitamin C had no significant protective effect against the MSG toxicity which is in agreement with Uzun et al. (38) and Zhou et al. (39) studies that reported that vitamin C could not improve testicular toxicity. Reasons such as choosing the correct dose of vitamin, the method of administration, and the route of vitamin absorption can be effective in this result. In addition, there is a possibility that MSG causes toxicity through pathways different from apoptosis and induction of oxidative stress and vitamin C cannot intervene in these pathways. However, these pathways need to be further investigated.

The strengths of this study can be mentioned in the investigation of nicotinic receptors which undergo changes during MSG consumption. One of the limitations of this study is the lack of testing of testosterone and LH serum levels. Also, if the treatment was continued for 64 days, we could check the sperms in the epididymis and get a more complete result. It is hoped that researchers will pay attention to the important role of nACh receptors in future studies.

Conclusion

Summarily, MSG decreased the expression levels of the nACHR subunits, α7 and α4, in the seminiferous tubules and interstitial testicular tissue, while the vitamin C administration increased the expression levels of these subunits in the testis tissue. The results of this study along with the results of other studies suggest being controlled and managing the use of MSG in the food industry.

Acknowledgements

There is no financial support and conflicts of interest in this study.

Authors' Contributions

R.B., N.Gh., N.A., F.R.A.; Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing draft, Review, and Editing. All authors read and approved the final manuscript.

References

1. Dineley KT, Pandya AA, Yakel JL. Nicotinic ACh receptors as therapeutic targets in CNS disorders. Trends Pharmacol Sci. 2015; 36(2): 96-108.
2. Wessler IK, Kirkpatrick CJ. Non-neuronal acetylcholine involved in reproduction in mammals and honeybees. J Neurochem. 2017; 142 Suppl 2: 144-150.
3. Schirmer SU, Eckhardt I, Lau H, Klein J, DeGraaf YC, Lips KS, et al. The cholinergic system in rat testis is of non-neuronal origin. Reproduction. 2011; 142(1): 157-166.
4. Hashem HE, El-Din Safwat MD, Algaidi S. The effect of monosodium glutamate on the cerebellar cortex of male albino rats and the protective role of vitamin C (histological and immunohistochemical study). J Mol Histol. 2012; 43(2): 179-186.
5. Iamsaard S, Sukhorum W, Samrid R, Yimdee J, Kanla P, Chaisiwamongkol K, et al. The sensitivity of male rat reproductive organs to monosodium glutamate. Acta Med Acad. 2014; 43(1): 3-9.
6. Alalwani AD. Monosodium glutamate induced testicular lesions in rats (histological study). Middle East Fertil Soc J. 2014; 19(4): 274-280.
7. Haddad M, Esmail R, Khazali H. Reporting the effects of exposure to monosodium glutamate on the regulatory peptides of the hypothalamic-pituitary-gonadal axis. Int J Fertil Steril. 2021; 15(4): 246-251.
8. Kayode OT, Rotimi DE, Kayode AAA, Olaolu TD, Adeyemi OS. Monosodium glutamate (MSG)-induced male reproductive dysfunction: a mini review. Toxics. 2020; 8(1): 7.
9. Kayode OT, Rotimi DE, Olaolu TD, Adeyemi OS. Ketogenic diet improves and restores redox status and biochemical indices in monosodium glutamate-induced rat testicular toxicity. Biomed Pharmacother. 2020; 127: 110227.
10. Kayode O, Kayode A, Mgbojikwe I, Rotimi D. Effect of ketogenic diet on monosodium glutamate-induced uterine fibroids in female wistar rats. J Babol Univ Med Sci. 2021; 23(1): 1-8.
11. Sarhan N. The ameliorating effect of sodium selenite on the histological changes and expression of caspase-3 in the testis of monosodium glutamate-treated rats: light and electron microscopic study. J Microsc Ultrastruct. 2018; 6(2): 105-115.
12. Anbarkeh FR, Baradaran R, Ghandy N, Jalali M, Nikravesh MR, Soukhtanloo M. Effects of monosodium glutamate on apoptosis of germ cells in testicular tissue of adult rat: An experimental study. Int J Reprod Biomed. 2019; 17(4): 261-270.
13. Gill SS, Mueller RW, McGuire PF, Pulido OM. Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. Toxicol Pathol. 2000; 28(2): 277-284.
14. Husarova V, Ostatnikova D. Monosodium glutamate toxic effects and their implications for human intake: a review. JMED Research. 2013; 1-12.

15. Zhang X, Zhou C, Cheng W, Tao R, Xu H, Liu H. Vitamin C protects early mouse embryos against juglone toxicity. Reprod Toxicol. 2020; 98: 200-208.
16. Shen L, Tang X, Wei Y, Long C, Tan B, Wu S, et al. Vitamin E and vitamin C attenuate Di-(2-ethylhexyl) phthalate-induced blood-testis barrier disruption by p38 MAPK in immature SD rats. Reprod Toxicol. 2018; 81: 17-27.
17. Rahimi Anbarkeh F, Nikravesh MR, Jalali M, Sadeghnia HR, Sargazi Z. The effect of diazinon on cell proliferation and apoptosis in testicular tissue of rats and the protective effect of vitamin E. Int J Fertil Steril. 2019;13(2): 154-160.
18. Baradaran R, Anbarkeh FR, Delavar A, Khorasgani EM, Rahimian N, Abbasi Y, et al. Hippocampal asymmetry and regional dispersal of nAChRs alpha4 and alpha7 subtypes in the adult rat. J Chem Neuroanat. 2021; 116: 101977.
19. Ruifrok AC, Johnston DA. Quantification of histochemical staining by color deconvolution. Anal Quant Cytol Histol. 2001; 23(4): 291-299.
20. Nayanatara AK, Vinodini NA, Domadar G, Ahemed B, Ramaswamy CR, Shabarianth, et al. Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight,sperm morphology and sperm count, in rat testis. J Chinese Clin Med. 2008; 3: 1-5.
21. Nosseir NS, Hassan M, Ali M, Ebaid HM. A histological and morphometric study of monosodium glutamate toxic effect on testicular structure and potentiality of recovery in adult albino rats. Res J Biol. 2012; 2(2): 66-78.
22. Zaheer K, Humayoun Akhtar M. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Crit Rev Food Sci Nutr 2017; 57(6): 1280-1293.
23. Samuels A. Dose dependent toxicity of glutamic acid: a review. Int J Food Prop. 2020; 23(1): 412-419.
24. Bojanić V, Najman S, Veličkov A, Bojanić N, Milenković J. Diltiazem prevents monosodium glutamate toxicity in the rat testes. Acta Medica Median. 2020; 59(3): 20-26.
25. Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. Cell Mol Life Sci. 2020; 77(1): 93-113.
26. John E, Mishra B, Tripathi AS, Aleesha R. Protective effect of Celastrus paniculatus on cognitive function in glutamate-induced brain-injured mice by reducing the intracellular influx of Ca. Int J Dev Neurosci. 2022; 82(4): 331-338.
27. Ismail El-Sawy HB, Soliman MM, El-Shazly SA, Ali HAM. Protective effects of camel milk and Vitamin E against monosodium glutamate induced biochemical and testicular dysfunctions. Prog Nutr. 2018; 20(1): 76-85.
28. Hamza RZ, Al-Harbi MS. Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. Toxicol Rep. 2014; 1: 1037-1045.
29. Shima Y. Development of fetal and adult Leydig cells. Reprod Med Biol. 2019; 18(4): 323-330.
30. Inoue M, Baba T, Morohashi KI. Recent progress in understanding the mechanisms of Leydig cell differentiation. Mol Cell Endocrinol. 2018; 468: 39-46.
31. Ahmed RR, Abdul-Hamid M, Galaly SR, Hamdalla H. Monosodium glutamate-induced liver microscopic and biochemical changes in male rats, and the possible amendment of quercetin. Egypt J Zool. 2019; 71(71): 44-55.
32. von Rintelen K, Arida E, Häuser C. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. Res Ideas Outcomes. 2017; 3: e20860.
33. Rahayu S, Annisa R, Anzila I, Christina YI, Soewondo A, Marhendra APW, et al. Marsilea crenata ethanol extract prevents monosodium glutamate adverse effects on the serum levels of reproductive hormones, sperm quality, and testis histology in male rats. Vet World. 2021; 14(6): 1529-1536.
34. Pehlivan FE. Vitamin C: an antioxidant agent. In: Hamza AH, editor. Vitam C. 2017; 23-35.
35. Arzuaga X, Smith MT, Gibbons CF, Skakkebæk NE, Yost EE, Beverly BEJ, et al. Proposed key characteristics of male reproductive toxicants as an approach for organizing and evaluating mechanistic evidence in human health hazard assessments. Environ Health Perspect. 2019; 127(6): 065001.
36. Foyer CH, Kyndt T, Hancock RD. Vitamin C in plants: novel concepts, new perspectives, and outstanding issues. Antioxid Redox Signal. 2020; 32(7): 463-485.
37. Koohpeyma F, Siri M, Allahyari S, Mahmoodi M, Saki F, Dastghaib S. The effects of L-carnitine on renal function and gene expression of caspase-9 and Bcl-2 in monosodium glutamate-induced rats. BMC Nephrol. 2021; 22(1): 162.
38. Uzun FG, Kalender S, Durak D, Demir F, Kalender Y. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. Food Chem Toxicol. 2009; 47(8): 1903-1908.
39. Zhou J, Sun Q, Yang Z, Zhang J. The hepatotoxicity and testicular toxicity induced by arecoline in mice and protective effects of vitamins C and e. Korean J Physiol Pharmacol. 2014; 18(2): 143-148.