DE GRUYTER OPEN

http://www.jrdiabet.ro
Rom J Diabetes Nutr Metab Dis. **22**(4):375-384
doi: 10.1515/rjdnmd-2015-0044

STUDY OF THE PROTECTIVE EFFECTS OF QUINCE (CYDONIA OBLONGA) LEAF EXTRACT ON FERTILITY ALTERATIONS AND GONADAL DYSFUNCTION INDUCED BY MONOSODIUM GLUTAMATE IN ADULT MALE WISTAR RATS

Davoud Kianifard [1, ✉], ***Gholamreza Vafaei Saiah*** [2], ***Farhad Rezaee*** [3]

[1] Division of Histology & Microscopic Anatomy, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[2] Division of Physiology & Laboratory Animals Sciences, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[3] Department of Gastroenterology and Hepatology, Erasmus MC, University of Rotterdam, Rotterdam, the Netherlands

received: September29, 2015 *accepted*: November 27, 2015
available online: December 15, 2015

Abstract

Background and Aims: *Starting from the cytotoxic effects of monosodium glutamate (MSG), the aim of this study was to evaluate the protective effects of quince leaf extract as natural antioxidant on the reproductive dysfunction induced by monosodium glutamate in rats.* ***Material and methods:*** *Monosodium glutamate was administrated with a dose of 30 and 60 mg/kg and quince leaf extract was administrated with a dose of 500 mg/kg. At the end of study, body and testicular weight measurement, hormonal and epididymal sperm analysis were performed.* ***Results:*** *Follicle stimulating hormone (FSH) and testosterone levels were reduced after administration of monosodium glutamate. The levels of luteinizing hormone (LH) exhibited no significant changes. Treatment with quince leaf extract led to improvement in follicle stimulating hormone and testosterone levels. Epididymal sperm population was reduced after administration of monosodium glutamate and treatment with quince leaf extract. The increased sperm motility rate induced by monosodium glutamate was reduced after treatment with quince leaf extract. Administration of monosodium glutamate led to more body weight gain in comparison to combined administration monosodium glutamate and quince leaf extract.* ***Conclusions:*** *The quince leaf extract can be effective in reduction of functional alterations of reproductive system induced by monosodium glutamate.*

key words: *Gonadal dysfunction, Monosodium glutamate, Quince leaf, Rat.*

Background and aims

Monosodium glutamate (MSG) is a food additive which acts as a preservative or enhancer of palatability [1]. Monosodium glutamate is the sodium salt of the L-form of glutamic acid. Glutamic acid is one of the most widespread amino acids found in natural products [2]. Despite of MSG's role in improvement of taste stimulation, some studies indicated that MSG has toxic effects on human and animal's tissues [3]. In this regard, some neurotoxic alterations

✉ P.O. Box: 5166616471 Tabriz, Iran, Tel/Fax: +98 41 36378743
corresponding author e-mail: davoudkianifard@gmail.com / Kianifard@tabrizu.ac.ir

such as brain damage and endocrine disorders have been seen after administration of monosodium glutamate [4,5]. Male infertility, reduction of body growth, obesity and hypogonadism have been reported following administration of MSG [6,7]. Testicular hemorrhage and alteration of sperm production and morphology are the most reported changes in cases of male infertility after administration of MSG [3,8].

On the other hand, herbal drugs have gained importance because of their efficacy and cost effectiveness in the treatment of several diseases. Flavonoids can inhibit the secretion of inflammatory mediators such as nitric oxide, interleukin-12 (IL-12) and tumor necrosis factor alpha (TNF-α) [9-11]. Some studies mentioned the reduction of oxidative reactions following the administration of flavonoids [10,12]. Moreover, it has been demonstrated that flavonoid compounds from quince have strong antioxidant and immune-regulatory effects [9].

Starting from the various side effects of monosodium glutamate on the reproductive system biology and spermatogenesis, the aim of this study was to evaluate the protective effects of quince leaves extract, as herbal antioxidant, on the functional alterations of testicular tissue mediated by monosodium glutamate.

Material and methods

Animal Procedure

A total of 60 adult male Wistar rats with a body weight of 120±20 g were used in this study. All animals used for testing were housed under a 12 hour light-dark cycle with room temperature of 23-25°C and had access to food and water *ad libitum*. All animal procedures used in this study were approved by the *University of Tabriz* standards for human care and use of laboratory animals, in accordance with the Ethical Research Committee of the Ministry of Health and Medical Education of Iran (adopted in April 17, 2006) based on the Helsinki Protocol (Helsinki, Finland, 1975).

Monosodium glutamate and quince leaves extract preparation

Monosodium glutamate (L-Glutamic acid monosodium salt hydrate, Sigma-Aldrich, St Louis, MO 63178, USA) was administrated with doses of 30 and 60 mg/kg by intraperitoneal (i.p.) injection. The quince leaf extract was prepared according to the protocol of Rodrigues et al. [13].

Experimental design

The animals were divided into six groups, each including ten rats: 1) Control: normal and healthy rats that did not receive any type of treatment; 2) MSG30: the rats from this group received monosodium glutamate (30 mg/kg body weight – i.p.) daily for eight weeks; 3) MSG60: the rats from this group received monosodium glutamate (60 mg/kg body weight – i.p.) daily for eight weeks; 4) MSG30+Extract: in this group, quince leaf extract (500 mg/kg body weight) was administrated daily by oral gavage method to MSG30 treated animals for eight weeks; 5) MSG60+Extract: this group consisted of MSG60 treated animals which received quince leaf extract (500 mg/kg body weight) for eight weeks; 6) Control+Extract: the animals of this group, were treated with quince leaf extract (500 mg/kg body weight) orally for eight weeks. For the same stress conditions in all experimental groups, distilled water was gavaged in groups 1,2,3 and also injected intraperitoneally to the animals of groups 1 and 6 during the period of study.

Measurement of body and testicular weight

At the beginning of the study, the weight of each animal was recorded and then, the body weight of animals was recorded twice a week

during the study. At the end of study, the animals were euthanized and the weight of right and left testes were measured.

Analytic procedures in plasma samples

At the end of study, the animals were euthanized and blood plasma was separated for hormonal analysis. Testosterone levels were measured by an enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (Monobind Inc. USA), blood follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were determined also by ELISA using specific commercial kits (Pishtazteb diagnostics, Iran).

Sperm analyses

For analyses of sperm, the cauda epididymis was separated from testis and cut into small pieces into one milliliter of Ham's F10 culture medium. The epididymal sperm count was evaluated by hemocytometer with light microscope at 400× magnification. Sperm motility was assessed with a phase contrast microscope at 400× magnification. In average 10 microscopic fields were observed and the mean of counted sperms was considered as sperm motility for each rat. To estimate the percentage viability, a volume of 20 µl of sperm suspension was mixed with an equal volume of 0.05% eosin-Y. The prepared slides were viewed by a bright-field microscope at 400× magnification. Two hundred sperms were considered for calculating the indices for the experimental groups [14].

Statistical analyses

The obtained results were analyzed using the GraphPad PRISM® software version 5.04 (GraphPad Software, Inc. USA). All data were reported as mean ± SEM (Standard Errors of Means). The comparison of means between experimental groups was evaluated using the one way analysis of variance (ANOVA). To clarify the significance of differences between experimental groups, *Tukey's* multiple comparison test was performed as post-hoc test after analysis of variance test. Differences were considered to be statistically significant if $p < 0.05$.

Results

Body weight and testicular weight

Table 1 shows the mean of body weight and testicular weight in the experimental groups. The comparison of initial and final body weight for every single group showed a significant difference. As Table 1 show, the difference in the mean testicular weight was not significant between experimental groups.

Table 1. Effect of monosodium glutamate and quince leaf extract on the body weight and testicular weight in the experimental groups.

	Initial body weight (g)	**Final body weight (g)**	**Testicular weight (g)**	**Testis/body weight ratio (%)**
Control	130.4±6.55*	173.2±12.85*	2.29±0.21	1.32
MSG30	115.6±2.78*	156±2.89*	2.15±0.08	1.37
MSG60	117.2±3.32*	166±3.57*	2.05±0.02	1.23
MSG30+Ext	108±1.55*	174.6±5.45*	2.74±0.06	1.56
MSG60+Ext	121.3±2.40*	144±4.16*	2.06±0.13	1.43
Control+Ext	115±3.31*	177±6.13*	2.78±0.12	1.57

Data are presented as mean ± SEM. * Significant difference between initial and final body weight for each group.

Figure 1 shows the changes of mean body weight in experimental groups during the period of study. According to this figure, administration of MSG with higher dose led to more weight gain at the end of study. As well, use of quince leaf extract in Control+Ext and MSG30+Ext groups led to more weight gain in these groups in comparison to control and MSG30 groups. The lowest weight gain was seen in MSG60+Ext group.

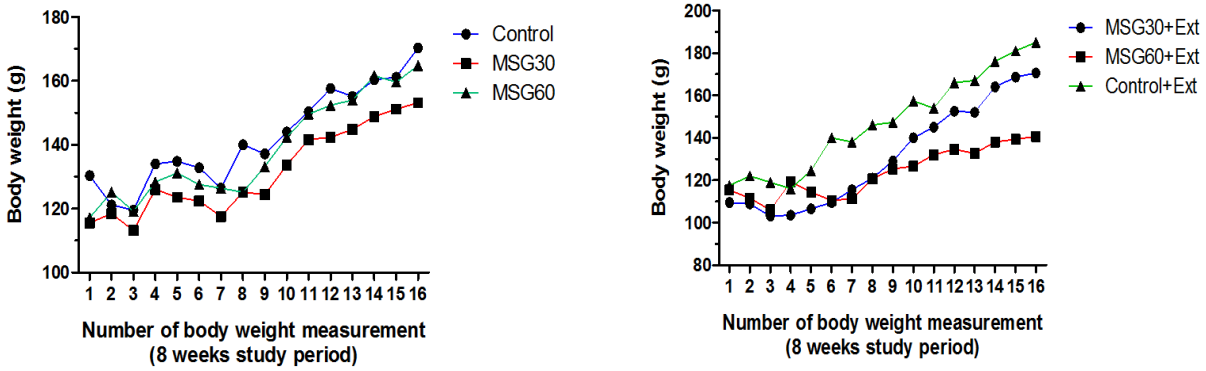

Figure 1. *Diagram of body weight gain during of the study period. The body weight measurement was carried out twice a week.*

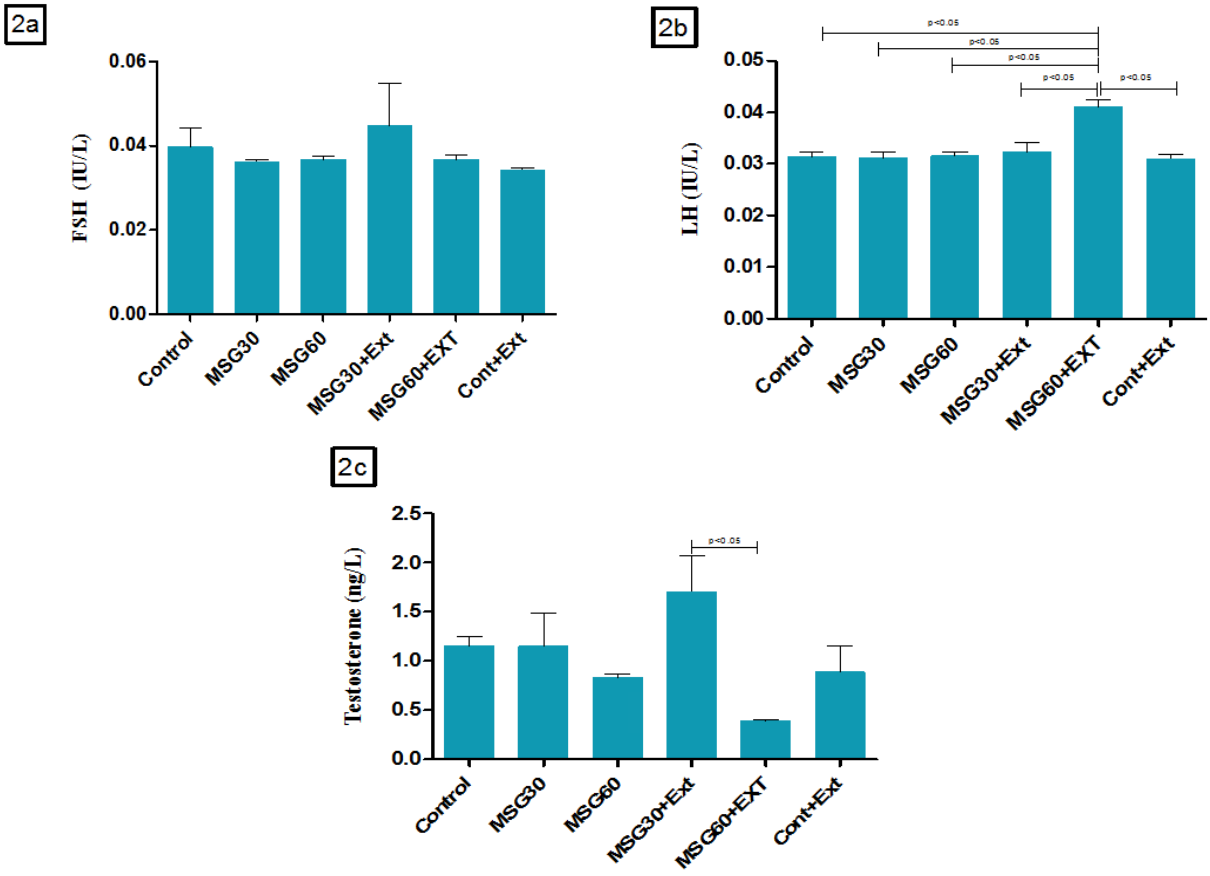

Figure 2. *Blood concentrations of pituitary gonadotropins and testosterone in the experimental groups. Data are presented as mean ± SEM.*

Hormonal assays

Figure 2 shows the blood concentration of pituitary gonadotropins and testosterone in the experimental groups. Administration of MSG led to a decrease of FSH levels in comparison to the control group (Figure 2a). The level of FSH was reduced following treatment of normal rats with quince leaf extract. However, this reduction was not significant in comparison to the control group. In this regard, treatments of MSG groups with quince leaf extract lead to slight and non-significant elevation of FSH in MSG30+Ext group (F ratio=0.783; P value=0.5747). Accordingly, the highest level of LH was seen in MSG60+Ext group (Figure 2b). The plasma level of LH in this group was significantly higher in comparison to the other groups (F ration=9.15; P value=0.0002). Measurement of testosterone levels showed that, the administration of MSG led to a decrease of testosterone levels (Figure 2c). However, treatments of normal rats with quince leaf extract led to a reduction of this hormone in comparison to the control group. In this regard, the lowest level of testosterone was seen in MSG60+Ext group and the highest level was seen in MSG30+Ext group (F ratio=3.50; P value=0.0349).

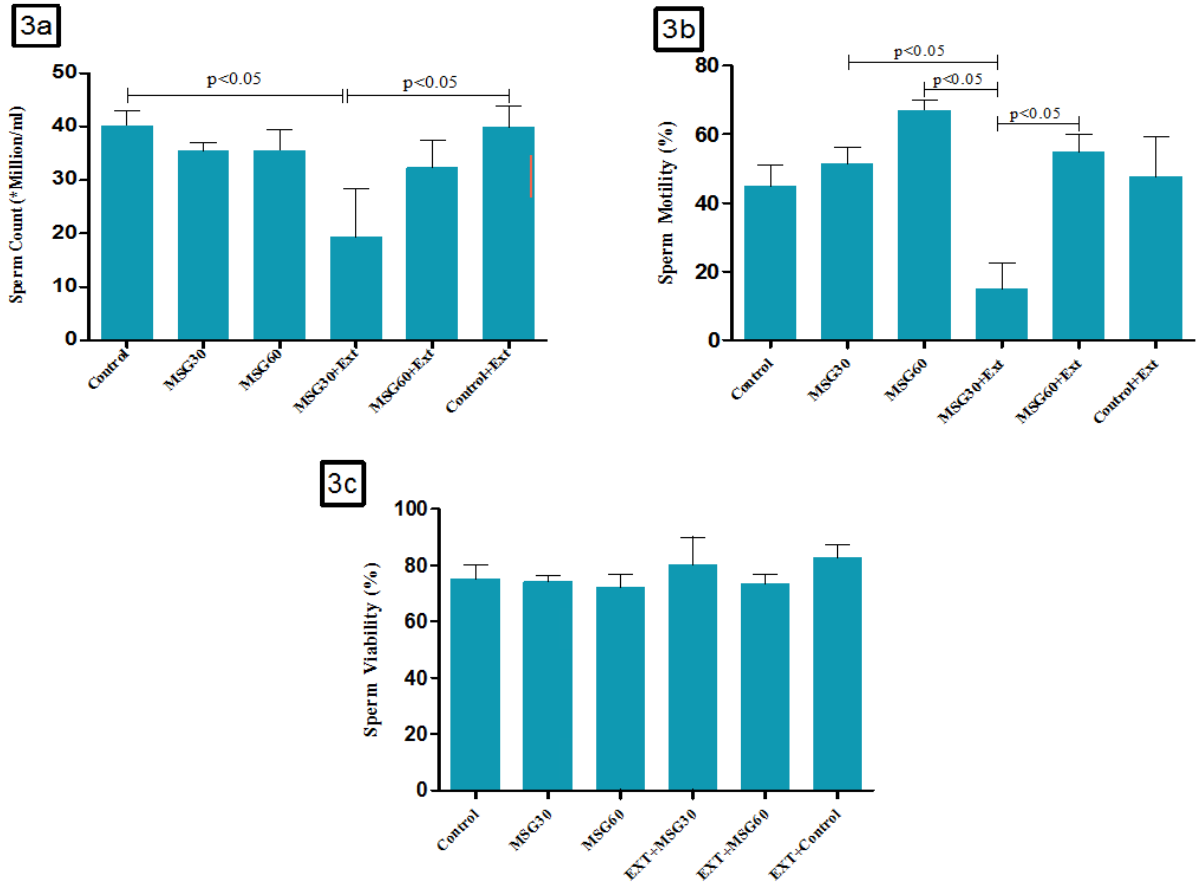

Figure 3. *Epididymal sperm analysis parameters in experimental groups. Data are presented as mean ± SEM.*

Sperm analysis

The results of sperm analysis showed that epididymal sperm count and sperm viability were reduced in the MSG administered groups (Figure 3). Treatment of control group with quince leaf extract led to a non-significant reduction of sperm population (Figure 3a). Moreover, administrations of MSG with quince

leaf extract treatment led to further reduction of epididymal sperm population ($p>0.05$). The reduction of sperm count was significant in the MSG30+Ext group in comparison to control and Control+Ext groups (F ratio=2.78; P value=0.0495). The results of sperm motility showed that this parameter was increased more in the MSG60 group in comparison to the MSG30 group (Figure 3b). Moreover, treatment of normal animals with quince leaf extract had no significant effect on sperm motility in comparison to the control group. In this regard, treatment of MSG administrated groups with quince leaf extract, led to a reduction of sperm motility rate. This reduction was significant in the MSG30+Ext group in comparison to MSG30, MSG60 and MSG60+Ext groups (F ratio=5.09; P value=0.0044). The sperm viability showed a greater reduction in MSG60 group in comparison to MSG30 group (Figure 3c). In this way, treatment of animals with quince leaf extract led to an elevation of sperm viability percentage in comparison to the non-treated groups However, these changes in sperm viability were not significant (F ratio=0.763; P value= 0.5887).

Discussion

Glutamate has several roles in biological systems such as regulation of gene expression, of antioxidant reactions and immune responses [15]. A wide range of adverse effects including muscle pain, sweating, headache, allergic reactions and asthma may occur following administration of monosodium glutamate [16]. Extreme use of monosodium glutamate may induce multiple complications in biological systems such as neurological effects and subsequent tissue and cellular changes [17,18].

It is also known that monosodium glutamate can induce oxidative stress through production of oxygen radicals and hydrogen peroxide which subsequently lead to oxidative DNA damage and cell membrane peroxidation and cellular death [19]. Glutamate receptors have been identified in several tissues and organs including the hypothalamus, spleen, thymus, liver, kidney, endocrine glands and the ovaries. In this regard, some studies demonstrated glutamate receptors in testicular tissue of rats [20,21].

The Quince (*cydonia oblonga*) has a large amount of phenolic acids and flavonoids as antioxidant compounds [22-24]. Reaction of flavonoids with free radicals leads to formation of more stable radicals with lower cytotoxicity. As well, they are capable of chelating iron ions ($Fe2^{+}$), leading to reduction of the side effects of free radicals [9]. Flavonoids can inhibit the secretion of inflammatory mediators such as nitric oxide, IL-12 and TNF-α [9-11]. Some studies mentioned the reduction of oxidative reactions following the administration of flavonoids [10,12]. It has been found that, flavonoid compounds of the quince have strong antioxidant and immune-regulatory effects [9].

According to expression of glutamate receptors in testicular tissue, this organ can be affected by monosodium glutamate. In this regard, it has been shown that treatment of young rats with monosodium glutamate leads to decreased fertility in both sexes during puberty [25]. Administration of monosodium glutamate is associated with testicular tissue alterations such as seminiferous tubules atrophy; spermatogenic cells nuclear pyknosis, germinal epithelium damages, testicular hemorrhage and reduction of cell population [26-28]. One of the possible mechanisms of structural and functional alterations of testicular tissue could be due to the direct effect of monosodium glutamate through glutamate receptors found on the cells of seminiferous tubules. Another mechanism for the spermatogenic alteration (mentioned in some studies) is represented by the neurotoxic effects

of monosodium glutamate on the hypothalamic-pituitary-gonadal system [29,30]. It is known that, the reduction of testicular ascorbic acid levels following administration of monosodium glutamate can lead to oxidative damage of testicular tissue [31].

In this study, the mean testicular weight was reduced in MSG treated groups. The administration of monosodium glutamate is associated with reduction of testicular weight [32]. Moreover, in this study, the parameter of testicular/body weight ratio was reduced following administration of 60 mg/kg of MSG whereas this parameter was increased more in the quince extract treated groups. Consequently, structural alterations of testicular tissue following administration of higher doses of MSG such as tubular atrophy may lead to a reduction of testicular weight.

The administration of monosodium glutamate leads to increase of body weight [33]. In the present study, treatment of control group with quince extract led to more weight gain in comparison to control group. As shown in the Figure 3, the weight gain process has a lower slope in MSG60+Ext group in comparison to the other groups. Accordingly, a higher dose of MSG led to more weight gain in comparison to lower doses of monosodium glutamate. Based on our finding, use of quince extract may have a beneficial effect on weight gain, particularly when administrated in combination with a lower dose of monosodium glutamate.

Spermatogenesis is regulated by pituitary gonadotropins [34,35]. Imbalance of gonadotropins may lead to structural and functional alterations of testicular tissue [36]. Increased amounts of glutamate is associated with augmentation of intracellular calcium levels which may lead to neuronal cell death [37,38]. Hypothalamic neuronal cell death is specified following the administration of MSG to adult mice [39]. Moreover, neuronal cell death of hypothalamic arcuate and preoptic nucleuses has been documented in some studies [40,41]. As a result, hypothalamic-pituitary-gonadal axis dysfunction can lead to testicular functional alterations [31]. The administration of monosodium glutamate is associated with the reduction of LH and testosterone levels [42]. In this study, the blood levels of FSH were also slightly reduced following administration of MSG. Moreover, treatment of control group with quince extracts lead to a decrease of FSH levels. Decrease of FSH levels has a relationship with malfunction of Sertoli cells and subsequently causes some spermatogenic alterations. In this study, the alterations of LH levels were not remarkable. However a slight increment in LH levels was seen in groups that were treated with MSG and quince extract. The evaluation of testosterone levels in this study revealed that the administration of monosodium glutamate and quince extract can be effective in reducing of testosterone levels. This reduction of testosterone levels is more evident if MSG (60 mg/kg) and quince extract administered together. Furthermore, the administration of monosodium glutamate leads to a reduction of Leydig cells population and consequently decrement of testosterone synthesis [43]. The observed hormonal changes in this study are in accordance with the previous reports [43-46].

In this study, the results of sperm analysis indicated that, the administration of monosodium glutamate and quince extract can lead to a reduction of epididymal sperm population. Moreover, simultaneous use of MSG and quince extract has a more pronounced effect on the reduction of sperm population. This is concordant with previous studies which reported the reduction of epididymal sperm population following administration of monosodium glutamate [6,29,31]. The results of this study suggested that, the effect of monosodium glutamate on the sperm motility can be dose

dependent. Likewise, it seems that simultaneous administration of MSG and quince extract leads to further reduction of sperm motility. Consequently, it is possible that the quince leaf extract has some effects on reduction of sperm motility. According to the results of this study it seems, the quince leaf extract may have a constructive effect on the sperm viability. Since the susceptibility of spermatozoid cell membrane to oxidative damages, it can be discussed that, the antioxidant properties of quince leaf extract can prevent the spermatozoids from oxidative damages.

Conclusions

Our study suggested that, dose dependent chronic administration of monosodium glutamate can induce structural and functional alterations of testicular tissue and spermatogenesis and, the use of antioxidant herbs such as quince leaves can be effective in reducing of the adverse effects of cytotoxic compounds on target tissues and organs. However, further studies are needed to better understand the mechanism of action of monosodium glutamate on the structure and the functions of reproductive system.

Acknowledgments. This work was financially supported by research council of *University of Tabriz*.

Duality of interest - no conflicts of interest.

REFERENCES

1. Moore KL. Congenital malformations due to environment. In: *Developing Humans.* WB Saunders co Ltd, Philadelphia, pp 173-183, 2003.

2. Samuels A. The toxicity/safety of processed free glutamic acid (MSG): a study in suppression of information. *Account Res* 6: 259-310, 1999.

3. Eweka A. Histological studies of the effects of monosodium glutamate on the kidney of adult Wistar rats. *Internet J Health* 6: 2, 2006.

4. Miksowiak B, Partyka M. Effects of neonatal treatment with MSG (monosodium glutamate) on hypothalamo-pituitary-thyroid axis in adult male rats. *Histol Histopathol* 8: 731-734, 1993.

5. Mozes S, Sefcíková Z, Lenhardt L, Racek L. Obesity and changes of alkaline phosphatase activity in the small intestine of 40- and 80-day old rats subjected to early postnatal overfeeding of monosodium glutamate. *Physiol Res* 53: 177-186, 2004.

6. Onakewhor JUE, Oforofuo IAO, Singh SP. Chronic administration of monosodium glutamate induces oligozoospermia and glucogen accumulation in Wistar rat testes. *Africa J Reprod Health* 2: 190-197, 1998.

7. Pizzi WJ, Barnhart JE, Unnerstall JR. Reproductive dysfunction in male rats following neonatal administration of monosodium L-glutamate. *Neurobehav Toxicol* 1: 1-4, 1979.

8. Oforofuo IAO, Onakewhor JUE, Idaewor PE. The effect of chronic administration of MSG on the histology of the adult Wistar rat testis. *Bioscience Res Comm* 9: 1-2, 1997.

9. Nijveldt RJ, Van Nood E, Van Hoorn DE, Boelens PG, Van Norren K, Van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nut* 74: 418-425, 2001.

10. Ostrowska J, Skrzydlewska E. The comparison of effect of catechins and green tea extract on oxidative modification of LDL in vitro. *Adv Med Sci* 51: 298-303, 2005.

11. Rao YK, Fang SH, Tzeng YM. Inhibitory effects of the flavonoids isolated from Waltheria indica on the production of NO, TNF-alpha and IL-12 in activated macrophages. *Biol Pharm Bull* 28: 912-915, 2005.

12. Kurin E, Atanasov AG, Donath O, Heiss EH, Dirsch VM, Nagy M. Synergy study of the inhibitory potential of red wine polyphenols on vascular smooth muscle cell proliferation. *Planta Med* 78: 772-778, 2012.

13. Rodrigues AL, da Silva GL, Mateussi AS et al. Involvement of monoaminergic system in the

antidepressant-like effect of the hydroalcoholic extract of Siphocampylus verticillatus. *Life Sci* 70: 1347-1358, 2002.

14. Wyrobek AJ, Gordon LA, Burkhart JG et al. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutat Res* 115: 1-72, 1983.

15. Wu G. Functional amino acids in growth, reproduction, and health. *Adv Nutr* 1: 31-37, 2010.

16. Geha RS, Beiser A, Ren C et al. Multicenter, double-blind, placebo-controlled, multiple-challenge evaluation of reported reactions to monosodium glutamate. *J Allergy Clin Immunol* 106: 973-980, 2000.

17. Beas-Zarate C, Perez-Vega MI, Gonzalez-Burgos I. Neonatal exposure to monosodium L-glutamate induces loss of neurons and cytoarchitectural alterations in hippocampal CA1 pyramidal neurons of adult rats. *Brain Res* 952: 275-281, 2002.

18. Reis HJ, Guatimosim C, Paquet M et al. Neuro-transmitters in the central nervous system & their implication in learning and memory processes. *Curr Med Chem* 16: 796-840, 2009.

19. Ahluwalia P, Tewari K, Choudhary P. Studies on the effects of monosodium glutamate (MSG) on oxidative stress in erythrocytes of adult male mice. *Toxicol Lett* 84: 161-165, 1996.

20. Gill SS, Mueller RW, McGuire PF, Pulido OM. Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. *Toxicol Pathol* 28: 277-284, 2000.

21. Takarada TE, Hinoi E, Balcar VJ, Taniura H, Yoneda Y. Possible expression of functional glutarnate transporters in the rat testis. *J Endocrinol* 181: 233-244, 2004.

22. Magalhaes AS, Silva BM, Pereira JA, Andrade PB, Valentao P, Carvalho M. Protective effect of quince (Cydonia oblonga Miller) fruit against oxidative hemolysis of human erythrocytes. *Food Chem Toxicol* 47: 1372-1377, 2009.

23. Oliveira AP, Pereira JA, Andrade PB, Valentão P, Seabra RM, Silva BM. Organic acids composition of Cydonia oblonga Miller leaf. *Food Chem* 111: 393-399, 2008.

24. Silva BM, Andrade PB, Valentao P, Ferreres F, Seabra RM, Ferreira MA. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and Jam: antioxidant activity. *J Agric Food Chem* 52: 4705-4712, 2004.

25. Pizzi WJ, Barnhart JE, Fanslow DJ. Monosodium glutamate administration to the newborn reduces reproductive ability in female and male mice. *Science* 196 (4288): 452-454, 1977.

26. Das RS, Ghosh SK. Long-term effects of monosodium glutamate on spermatogenesis following neonatal exposure in albino mice - A histological study. *Nepal Med Coll J* 12: 149-153, 2010.

27. Ismail NH. Assessment of DNA damage in testes from young Wistar male rat treated with monosodium glutamate. *Life Sci J* 9: 930-939, 2012.

28. Mohamed IK. The effects of oral dosage of monosodium glutamate applied for short-and long-terms on the histology and ultrastructure of testes of the adult rats. *J Anim Vet Advanc* 11: 124-133, 2012.

29. Giovambattista A, Suescun MO, Nessralla CC, Franca LR, Spinedi E, Calandra RS. Modulatory effects of leptin on Leydig cell function of normal and hyperleptinemic rats. *Neuroendocrinology* 78: 270-279, 2003.

30. Gong SL, Xia FQ, Wei J, Li XY et al. Harmful effects of MSG on function of hypothalamus-pituitary-target gland system. *Biomed Environ Sci* 8: 310-317, 1995.

31. Nayanatara AK, Vinodini NA, Damodar G et al. Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight, sperm morphology and sperm count, in rat testis. *J Chin Clin Med* 3: 1-5, 2008.

32. Olney JW, Sharpe LG, Feigin RD. Glutamate-induced brain damage in infant primates. *J Neuropathol Exp Neurol* 31: 464-488, 1972.

33. Diniz Y, Faine LA, Galhardi CM et al. Monosodium glutamate in standard and high-fiber diets: metabolic syndrome and oxidative stress in rats. *Nutrition* 21: 749-755, 2005.

34. Pakarainen T, Zhang FP, Makela S, Poutanen M, Huhtaniemi I. Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteinizing hormone receptor knockout mice. *Endocrinology* 146: 596-606, 2005.

35. Wang RS, Yeh S, Tzeng CR, Chang C. Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice. *Endocr Rev* 30: 119-132, 2009.

36. Shetty G, Wilson G, Huhtaniemi I, Shuttlesworth GA, Reissmann T, Meistrich ML. Gonadotropin-releasing hormone analogs stimulate and testosterone inhibits the recovery of spermatogenesis in irradiated rats. *Endocrinology* 141:1735-1745, 2000.

37. Frandsen A, Schousboe A. Mobilization of dantrolene-sensitive intracellular calcium pools is involved in the cytotoxicity induced by quisqualate and N-methyl-D-aspartate but not by 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate and kainate in cultured cerebral cortical neurons. *Proc Natl Acad Sci U S A.* 89: 2590-2594, 1992.

38. Gillessen T, Budd SL, Lipton SA. Excitatory amino acid neurotoxicity. In: *Molecular and Cellular Biology of Neuroprotection in the CNS.* Springer US, pp 3-40, 2002.

39. Park CH, Choi SH, Piao Y et al. Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol Lett* 115: 117-125, 2000.

40. Garattini S. Glutamic acid, twenty years later. *J Nutr* 130 [4S Suppl]: 901S-909S, 2000.

41. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 164(3880): 719-721, 1969.

42. Sakr SA, Badawy GM. Protective effect of curcumin on monosodium glutamate-induced reproductive toxicity in male albino rats. *Glob J Pharmacol* 7: 416-422, 2013.

43. Nosseir NS, Ali MHM, 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* 2: 66-78, 2012.

44. Iamsaard S, Sukhorum W, Samrid R et al. The sensitivity of male rat reproductive organs to monosodium glutamate. *Acta Med Acad* 43:3-9, 2014.

45. Igwebuike UM, Ochiogu IS, Ihedinihu BC, Ikokide JE, Idika IK. The effects of oral administration of monosodium glutamate (msg) on the testicular morphology and cauda epididymal sperm reserves of young and adult male rats. *Veterinarski Arhiv* 81: 525-534, 2011.

46. Miśkowiak B, Limanowski A, Partyka M. Effect of perinatal administration of monosodium glutamate (MSG) on the reproductive system of the male rat. *Endokrynol Pol* 44: 497-505, 1993.