



Protective Effects of Morus Alba (M.alba) Extract on the Alteration of Testicular Tissue and Spermatogenesis in Adult Rats Treated with Monosodium Glutamate

Davoud Kianifard

Division of Histology and Microanatomy, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Abstract

Various studies have confirmed toxic effects of monosodium glutamate in different organs. The review of literatures about influence of MSG on the male reproductive system shows the variety of tissue damages. Most of these changes mainly manifested by increased oxidative stress and cytotoxicity. The present study was aimed to investigate the possible protective effects of herbal antioxidants (Morus Alba) on the tissue damages related to monosodium glutamate cytotoxicity. Forty-eight adult Wistar rats were divided into two control and experimental groups. Each group divided into short (14 days) and long term (28 days) group. Experimental group was subdivided into four subgroups. The experimental group was injected with MSG (4 mg/kg BW, i.p.). Morus Alba extract (100 mg/kg BW) was administrated to two MSG treated experimental subgroups. At the end of study, the right testis was processed for histological evaluation and the left one was processed for sperm analysis and morphometric studies. Decrease of spermatogenic indices was accompanied with the changes in histomorphology of seminiferous tubules in MSG treated groups. Moreover, the sperm analysis indices were decreased in MSG treated rats. These changes were more obvious in long term treatments. The administration of Morus Alba extract led to improvement in histologic and spermatogenic indices. The most observable improvement in tissue changes was obtained by long term treatment with Morus Alba extract. The present study suggests that the administration of Morus Alba extract can preserve the positive effects in reduction of testicular tissue alterations in Monosodium glutamate tissue toxicity.

Keywords: Monosodium glutamate, Morus Alba, rat, spermatogenesis, testicular tissue

(Rec.Date: Aug 17, 2014 Accept Date: Sep 18, 2014)

Corresponding Author: Davoud Kianifard, Division of Histology and Microanatomy, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

E-mail: davoudkianifard@gmail.com

Introduction

Monosodium glutamate, MSG, is a food additive which acts as preservative or enhancer of palatability [1]. MSG is sodium salt and is L-form of glutamic acid. Glutamic acid is one of the most widespread amino acids found in natural products [2]. Despite 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 such as brain damage and endocrine disorders have been seen after administration of MSG [4,5]. Male infertility, reduction of body growth, obesity and hypogonadism has 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]. Herbal drugs have gained importance because of their efficacy and cost effectiveness in treatment of several diseases. Morus Alba, M. alba, has long been used in treatment and prevention of many diseases and as a general tonic to enhance health in traditional oriental medicine. The mulberry extract have potent antioxidant activity [9-11]. Mulberry fruit contains high amounts of flavonoids and alkaloids and many of its bioactivities specifications were linked to the presence of these compounds [11-13]. Administration of exitotoxins leads to tissue cytotoxicity and cellular damages by excessive production of reactive oxygen species [14]. According to various antioxidant activities of Morus alba, in present research the protective effects of Morus alba on the structural and functional alterations of spermatogenesis following administration of Monosodium glutamate were evaluated.

Materials and Methods

Animal Procedure

This study was performed on forty-eight adult (age 12 weeks) male Wistar rats with mean body weight 196 ± 11.46 gm. The animals were procured by the Center of Animal Housing and Breeding of the Faculty of Veterinary Medicine, Urmia University. The animals were placed in standard cages under 12:12 h light: dark cycle with 23-25°C room temperatures. Animals were fed on standard laboratory animal's chow and water ad libitum during the whole period of experiment. All animal procedures were approved by the standards for humane care and use of laboratory animals, University of Tabriz in accordance with Research

Ethical Committee of the Ministry of Health and Medical Education of Iran (adopted in April 17, 2006) based on Helsinki Protocol (Helsinki, Finland, 1975).

Experimental design and chemicals

Monosodium glutamate (Roth Company, Germany) was dissolved in distilled water, 1 g of MSG in 1 ml of distilled water, and administrated by oral gavage [15]. The animals were divided into two control and experimental groups. Control group was subdivided into two groups (C1, C2). Experimental group was subdivided into four groups (E1-E4). Each group holding eight rats as described. 1) Group C1: Health age-matched animals that did not receive any treatment but gavaged by distilled water daily and were sacrificed after 14 days; 2) Group C2: All procedures in this group were done similar to group C1 but the animals were scarified after 28 days; 3) Group E1: Animals in this group received daily injection of MSG (4 ml/Kg BW, i.p.) for 14 days and were sacrificed after the last day of injection; 4) Group E2: Animals of this group were treated daily with MSG (4 ml/Kg BW, i.p.) for 28 days and were sacrificed after the last day of injection [16]; 5) Group E3: This group consist of 14 days MSG treated animals that gavaged by M.alba extract (100 ml/Kg BW) in the period of injection; 6) Group E4: In this group M.alba was gavaged (100 ml/Kg BW) to MSG treated animals for 28 days [10].

Morus Alba extract preparation

Fresh Mulberry fruit (*Morus Alba*) was collected from the trees. The collected samples selected to uniformity in shape and color. The fruits were dried at 70 °C in duration of four days and then ground to powder. For water extraction, each 50 gram of powder was mixed with 100 milliliters of boiling distilled water by magnetic stirrer for 15 minutes. The water extract was freeze dried and kept in 4°C.

Histology of testicular tissue

At the end of study, the animals were euthanized with CO₂ inhalation. Histological studies of seminiferous tubules were performed on formaldehyde-fixed and paraffin embedded testicular samples with thickness of 6-7 micrometer stained with Hematoxylin and eosin method. Tissue sections were studied at ×200 magnification. The histological study of tissue samples was performed on the images obtained with digital camera (Olympus DP70, Olympus Europe,

Hamburg, Germany). For each tissue sample, the numbers of twenty tubules were studied in different microscopic fields.

Evaluation of spermatogenesis in testicular tissue

For evaluation of spermatogenesis, three different indices were used. Tubular differentiation index (TDI): the number of seminiferous tubules that have more than three layers of germinal cells derived from type A of spermatogonia; repopulation index (RI): the ratio of active spermatogonia to inactive spermatogonia and spermiation index (SPI): the ratio of the number of seminiferous tubules with spermatozooids to the empty tubules [17].

Sperm analyses

For sperm analysis, the cauda epididymis was separated from testis and cut into small pieces in one milliliter of Ham's F10 culture medium. The epididymal sperm count was evaluated by hemocytometer with light microscope at $\times 400$ magnification. Sperm motility was assessed with phase contrast microscope at $\times 400$ magnification. Ten microscopic fields were observed and the mean of counted sperms was considered as sperm motility for each sample. To estimate the percentage of viability, twenty microliter of sperm suspension was mixed with equal volume of 0.05 percent eosin-Y. The prepared slides were viewed by bright-field microscope at $\times 400$ magnification. Two hundred sperms were considered for calculating the indices for the experimental groups [18].

Statistical analyses

The obtained results were analyzed with the SPSS Statistics software version 19. All data were reported as mean $\pm$ SEM. The comparison of means between experimental groups was evaluated with one way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test. Differences were considered to be statistically significant if $p < 0.05$.

Results

Histology of testicular tissue

Figures 1-5 show the histology of testicular tissue in different groups. In control groups, the seminiferous tubules had normal germinal epithelium with regular cellular junctions (Figure 1). Treatment of animals with monosodium glutamate for 14 days, led to some alteration in microscopic structure of seminiferous tubules. As the figure 2 shows, the alteration in population of spermatogenic cells and derangement of cellular junctions led to

moderate deformity and atrophy of seminiferous tubules. These above mentioned changes were observed in higher intensity after long time administration of monosodium glutamate (Figure 3). As the figure 3 shows, severe tubular atrophy and germinal epithelium depletion was accompanied with derangement of spermatogenic cells in tissue samples of animals which treated with MSG for long time. The administration of M.alba extract, led to decrement of MSG associated tissue changes (Figures 4 and 5).

Microscopic indices of spermatogenesis

The evaluation of tubular differentiation index (TDI) showed that, the administration of monosodium glutamate for 28 days led to significant reduction ($p < 0.05$) of this index in group E2 in comparison to control groups (Table 1). This significant reduction also was seen between group E2 and E1. In this regard, the comparison of TDI percentage showed that, long time administration of M.alba extract led to significant increase of TDI in MSG treated groups. As table 1 show, significant decrement in SPI percentage was seen in group E2 after long time treatment with MSG in comparison to control, E1 and E4 groups ($p < 0.05$). The assessment of repopulation index (RI) showed that, the greatest decrement of this index was observed in group E2 in comparison to other experimental groups (E1, E3, E4) but, these differences were not significant. The results obtained from three indices of spermiation showed that, short time administration of M.alba extract had no protective effect in MSG treated groups.

Sperm analysis

All indices of sperm analysis were reduced in experimental groups after treatment with monosodium glutamate. As table 2 shows, the epididymal sperm count was reduced in all experimental groups in comparison to control groups. This reduction was significant between group E2 and control groups ($p < 0.05$). Accordingly, epididymal sperm count was increased in MSG treated groups which received M.alba extract compared to other experimental groups (E1&E2). The percentage of sperm motility and viability was reduced more in group E2 in comparison to other experimental groups however; there was no significant difference in sperm motility and viability between these groups. In group E2, the percentage of sperm viability was decreased significantly in comparison to control groups ($p < 0.05$).

Table 1. Microscopic indices of spermatogenesis in testicular tissue of experimental groups

Microscopic indices of spermatogenesis			
Group	TDI (%)	SPI (%)	RI (%)
C1	90.46±5.35 ^{*λ}	89.68±6.11 [*]	83.19±6.47
C2	90.12±3.57 ^{*λ}	90.25±6.25 [*]	82.87±5.92
E1	87.37±5.76 [*]	85.22±4.36 [*]	81.39±5.14
E2	81.76±5.17	79.45±3.60	78.62±5.11
E3	85.17±5.27	84.41±8.89	80.69±4.57
E4	86.55±4.14 [*]	85.97±5.40 [*]	81.34±4.54

Values shown are mean ± SEM. (*) significantly different in comparison to group E2. (λ) Significantly different in comparison to group E3. TDI: tubular differentiation index; SPI: spermiation index; RI: repopulation index.

Table 2. Epididymal sperm analysis in experimental groups

	Sperm Count (10 ⁶ /ml)	Sperm Motility (%)	Sperm Viability (%)
C1	87.34±7.33 [*]	85.17±8.19	89.19±5.73 [*]
C2	88.07±6.94 [*]	85.22±7.23	89.27±6.42 [*]
E1	83.37±6.17	83.03±7.21	86.71±7.66
E2	80.24±7.91	81.79±5.51	82.15±6.19
E3	84.11±5.27	82.76±6.17	84.25±8.04
E4	83.93±6.28	82.59±5.46	85.36±6.31

Values shown are mean ± SEM. (*) significantly different in comparison to group E2.

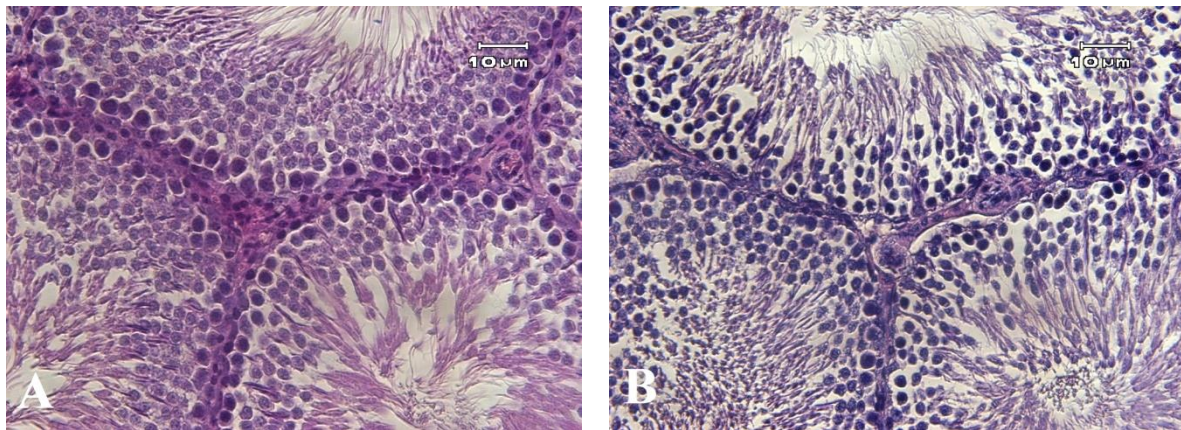


Figure 1. Light micrograph of testicular seminiferous tubules from control groups. **A&B**, Germinal epithelium has normal cell population and arrangement. Note the narrow interstitial connective tissue between seminiferous tubules. (Hematoxylin & eosin stain; $\times 400$).

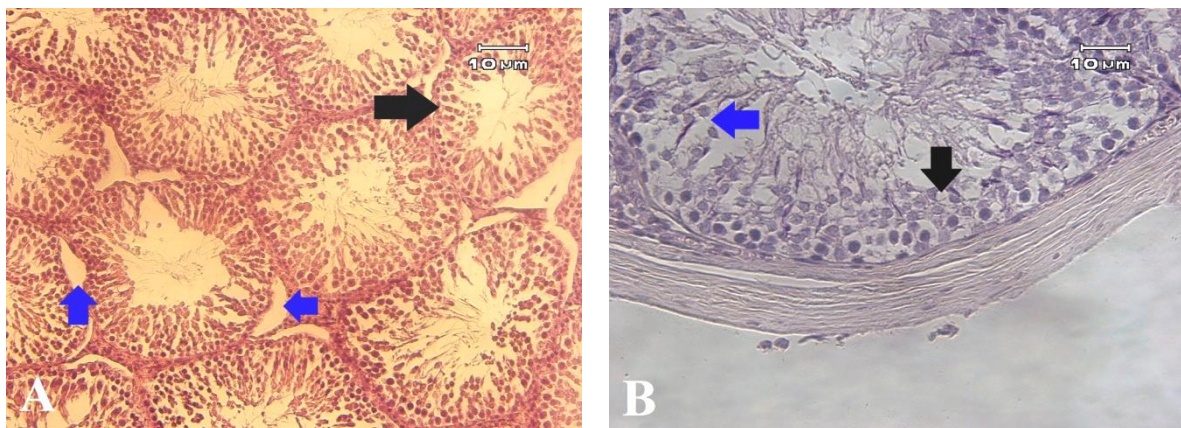


Figure 2. Light micrograph of seminiferous tubules from group (E1). **A**, moderate atrophy is seen in some seminiferous tubules (blue arrows). Derangement of germinal epithelium is seen in some tubules (black arrow). **B**, higher magnification of tubular wall. Decrease of germinal epithelium height due to decrement of cell division (black arrow) and derangement of cellular junctions (blue arrow) is seen. (Hematoxylin & eosin stain, A $\times 200$, B $\times 400$).

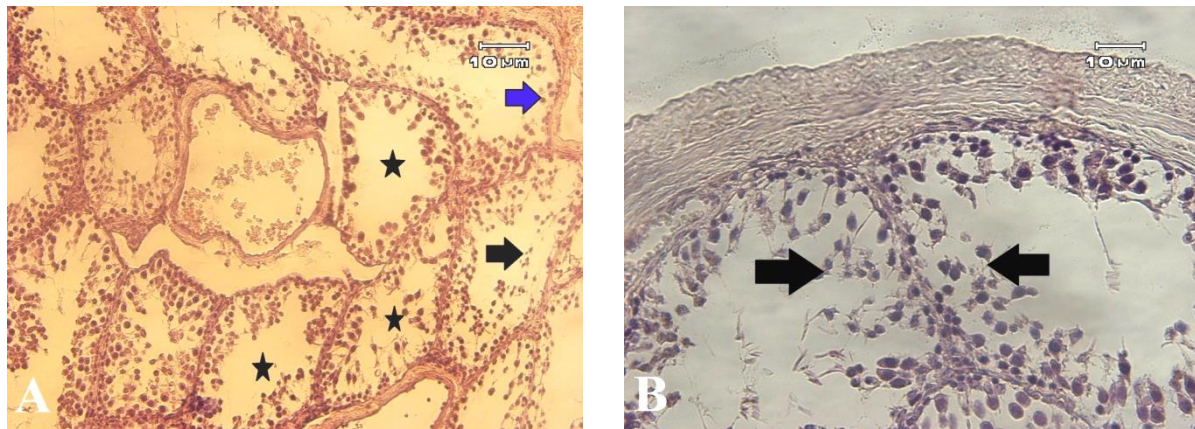


Figure 3. Light micrograph of seminiferous tubules from group (E2). **A**, tubular atrophy is seen in most of seminiferous tubules (black asterisks). Severe depletion of germinal epithelium (blue arrow) and derangement of spermatogenic cells (black arrow) is seen in atrophied tubules. **B**, higher magnification of tubular wall. Most of seminiferous tubules are seen with considerable alteration in germinal epithelium. Appreciable decrement of spermatogenic cells and derangement of cellular junctions (black arrows) is seen. (Hematoxylin & eosin stain, A $\times 200$, B $\times 400$).

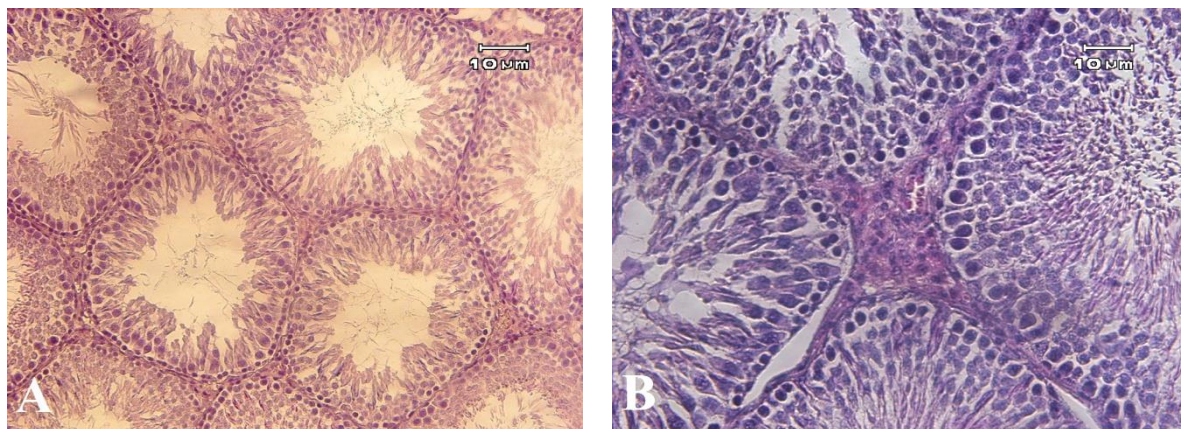


Figure 4. Light micrograph of seminiferous tubules from group (E3). **A**, tubular structure and germinal epithelium appear normal. **B**, higher magnification of tubular wall. Cellular architecture of seminiferous tubules is similar to control samples. (Hematoxylin & eosin stain, A $\times 200$, B $\times 400$).

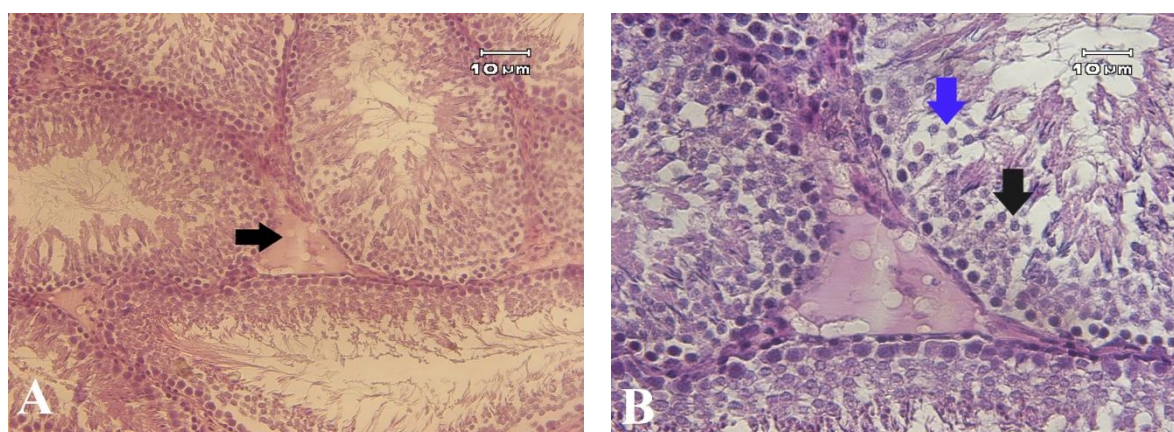


Figure 5. Light micrograph of seminiferous tubules from group (E4). **A**, decrease of tubular atrophy can be observed but, interstitial edema visible between tubules (black arrow). **B**, higher magnification of tubular wall. The number of spermatogenic cells has been increased (black arrow). Derangement of cellular junctions (blue arrow) can be seen in some regions of tubular wall. (Hematoxylin & eosin stain, A $\times 200$, B $\times 400$).

Discussion

The environmental causes represent one of the major factors affecting male fertility [19]. Monosodium glutamate, MSG, is known to affect the structure and function of male reproductive system and showed to be toxic to the testis of human and experimental animals [20, 21]. It has been suggested that, the alteration of testicular tissue structure and reduction of spermatogenic activities due to administration of MSG, mediated with oxidative damage in the testis [14]. Prevention of oxidative damage with natural antioxidants is considered as one of the most effective strategies in control and reduction of target tissues complications. In this way, the use of phenolic rich fruits and medical herbs can reduce the risk of developing chronic diseases by reduction of oxidative stress and inhibition of macromolecular oxidation [22]. The excessive production of reactive oxygen species and microvascular damages are reported to substantially contribute to the occurrence of the structural and functional changes [23-25]. Herbal drugs have gained importance because of their efficacy and cost effectiveness in treatment of several diseases. In this study, the protective effects of *Morus Alba*, as one natural antioxidant, was evaluated. The administration of exitotoxins leads to tissue cytotoxicity and cellular damages by excessive production of reactive oxygen species. The mulberry extract have potent antioxidant activity [9-11]. According to previous studies [3, 8], our results from histological evaluation of testicular tissue showed that, following

administration of MSG, decrement in diameter of STs, depletion in the height of germinal epithelium and atrophy of seminiferous tubules occur in testicular tissue. These histological observations in STs, illustrate the depressed cellular activity of spermatogenic cells. Our study showed that, the administration of M.alba leads to improvement of histology of testicular tissue. The evaluation of microscopic indices of spermatogenesis confirms these histologic results. Diminished tubular differentiation (TDI) and spermiation (SPI) indices in MSG treated rats indicates that, conversion of spermatogonia to primary spermatocytes is reduced. Reduction of repopulation index demonstrates the number of inactive spermatogonia increased. This process cause a decline of the number of primary spermatocytes derived from spermatogonia cells. These alterations in cellular conversion and/or activity lead to reduction in production of spermatozooids. The results from different indices of sperm analysis in this study indicate that, the number, ability of movement and the mortality rate of spermatozooids have a relationship with cellular activity of testicular germinal epithelium. The structure of spermatozooids has large quantities of lipids and the oxidative stress can influence the normal structure of developing spermatozooids due to induction of excessive lipid phosphorylation [26]. Oxidative stress in testicular tissue has a direct relationship with abnormal spermatogenesis due to decrease of glutathione in male germ cells which lead to incomplete functional maturation and capacitation of spermatozoa [26,27]. The results obtained by this study showed that, microscopic changes of testicular tissue following treatment with monosodium glutamate decreased after administration of M.alba extract. In this regard, the protective effects of M.alba in reduction of tissue changes preserve more effective in long term administrations.

Conflict of interests

There is no conflict of interest.

Acknowledgments

All financial resources of this study were supported by the author of manuscript. The author would like to thank Dr. Farhad Rezaee form University Medical Center Groningen (UMCG, the Netherlands) for his assistance in data analysis and thank Imaging Center of the University Medical Center Groningen for their technical support. The author also thanks all staff members of Animal Housing and Breeding Center of Urmia University for their kindly attention in animal care and handling.

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