

PROTECTIVE ROLE OF GINGER ON TESTICULAR TISSUE AND TESTOSTERONE HORMONE OF MALE RATS EXPOSED TO MONO-SODIUM GLUTAMATE

Snoor Jalal Mustafa *, Gulala Ibrahim Qader** and Shler Akram Fage Mahmood ***



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ABSTRACT

Background

Monosodium glutamate (MSG) is commonly used food additive and flavor enhancer and it causes tissue damage. Ginger (*Zingiber officinale*), a medicinal plant that gaining popularity for its variety of biological protection activities.

Objectives

This study was carried out to find out the protective role of ginger in eliminating the toxic effect of MSG on testicular tissue and testosterone hormone in rats.

Materials and Methods

Twenty four adult male rats were divided, randomly, into 3 equal groups. The 1st group was designed as control, the 2nd administered MSG orally at dose 4mg/kg body weight for 14 days and the 3rd group treated with MSG in oral doses of 4 mg/kg body weight plus Ginger extract at dose 100 mg /kg body weight for 14 days.

Results

The study showed that MSG has altered the microarchitecture of testicular tissues, reduced seminiferous tubule diameter and germinal epithelial thickness, and increased the interstitial spaces with reduction in Leydig cells and Testosterone hormone level. In addition Ginger extract has prevented the MSG effect. All the results were significant statistically.

Conclusion

Ginger has protective and improving role against the effects induced by MSG on the rat testicular tissue.

Keywords: *Monosodium glutamate (MSG), rats, testes, seminiferous tubules, Ginger, testosterone.*

* Department of Anatomy, College of Medicine, University of Sulaimani.

Correspondence: snoorm88@gmail.com

** Department of Pharmacology, College of Medicine, University of Sulaimani.

*** Department of Microbiology, College of Medicine, University of Sulaimani.

INTRODUCTION

Infertility is one of the major health problems in human ⁽¹⁾ approximately 30 % of infertilities are due to a male factor ^(2,3). Nutritional status is one of the major factors controlling fertility in human ⁽⁴⁾. Drug treatment, chemotherapy, toxins and environmental factors can have harmful effect on spermatogenesis and normal sperm production⁽¹⁾.

Monosodium glutamate (MSG) is a white crystal-like substance that is used commonly as a food additive and flavor enhancer ⁽⁵⁾. MSG contains 78% of glutamic acid, 22% of sodium and water ⁽⁶⁾. Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides of most tissues. Glutamate is also produced in the body and plays an essential role in human metabolism. It is a major component of many protein-rich food products either in free or bound state of animals such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato ⁽⁷⁾.

Although many Food and Drug Control Agencies have certified MSG to be safe for human consumption without any specified dosage ⁽⁸⁾, experimental findings have linked the intake of MSG with many structural and, perhaps, functional defects. Various studies have shown that Monosodium glutamate is neurotoxic, nephrotoxic, hepatotoxic, and gonadotoxic ^(9,10). The mode of action of MSG through which it causes tissue damage might be related, however, to induction of oxidative stress ^(11,12).

MSG has a toxic effect on the testis by causing a significant oligozoospermia and increases abnormal sperm morphology in a dose-dependent fashion in male Wistar rats ⁽¹³⁾. It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology ⁽¹⁴⁾.

Medicinal plant for the treatment of diseases has a long tradition. The Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China and India. The first written reports was in 600 B.C. with the Caraka Samhita of India and the early notes of the Eastern Zhou dynasty of China that became systematized around 400 B.C. ⁽¹⁵⁾. *Zingiber officinale* commonly called ginger belongs to the family Zingiberaceae. It has been used in traditional medicine to aid digestion and treat stomach upset, diarrhea, nausea, and arthritis for centuries. Today, ginger root is widely used as a digestive aid for mild stomach upset and is commonly

recommended by health care professionals to help prevent or treat nausea and vomiting associated with motion sickness, pregnancy, and cancer chemotherapy ^(16,17).

The important active components of the ginger roots are thought to be volatile oils and pungent phenol Compounds such as gingerols, shagols, zingerone and gingerols ^(18,19). Although the useful result of ginger has been subjugated, its activity on male reproductive functions except a study that reported that *Z. officinale* possesses androgenic property ⁽²⁰⁾.

This study has been carried out to study the protective effects of aqueous extract of ginger on histomorphometric changes testicular and testosterone level of rats treated with Monosodium Glutamate.

MATERIALS AND METHODS

1) Animals

Twenty four adults male rats weighing between 200-230g were maintained in a well-ventilated animal house under same laboratory condition of humidity, temperature and a constant 12 hour light: 12 hour dark lighting schedule, they were allowed to acclimatize for one week prior to the start of the experiment. All animals were maintained on a standard diet and water available ad libitum. They were obtained from the animal house of veterinary college, University of Sulaimani, Kurdistan region, Iraq. The animals were housed in clear polypropylene cages lined with wood chip beddings. The health and reproductive status of the animals were assessed and only healthy animals were selected for the experiment.

2) Preparation of monosodium glutamate

Synthetic monosodium glutamate (MSG) of 98% purity was obtained from Aldrich chemistry, France. A stock solution was prepared by dissolving (1.0 g) MSG granule in (1.0) ml distilled water ⁽²¹⁾.

3) Preparation of the aqueous extracts of Ginger

The rhizomes of ginger were sun-dried and crushed into powdered form using a blender. The dried powder was weighed (1 g) and macerated in 50 ml of distilled water. Final extract concentration obtained is 20 mg/ml. Extract was stored in air-tight container and refrigerated throughout the experiment ⁽²²⁾.

4) Experimental design

The twenty four rats were divided randomly into three groups of eight animals each and maintained under same laboratory conditions. The ginger extract and the MSG were given orally using gavage tube once a day for 14 days⁽²³⁾. They were administered as follows: Control Group I: distilled water Group II: MSG (4.0g/kg B.W) Group III: MSG+Ginger (4.0g/kg B.W +100mg/Kg).

At the end of the experiment; all the rats were sacrificed at day15. Immediately after dissection, the specimens of the testes were placed in a tissue cassette and fixed in 10% formal saline for 24 h after which they were processed using standard histopathological methods⁽²³⁾. The testis was sectioned and processed for routine histological examination; five micrometer sections were prepared, and then stained with hemotoxylin and eosin. The appropriate sections were photographed using photomicroscope (Olympus BX51) and digital camera (Olympus DP70) (Mitani Corporation, Tokyo, Japan). An ocular micrometer used to measure the diameter of seminiferous tubules after calibration with a stage micrometer. Blood was collected by cardiac puncture; serum was prepared by centrifugation and used for testosterone analysis.

The diameter of seminiferous tubules was measured at X200 magnification with assistance of Leica image analyzer (DMLB) and Leica Qwin software, the diameter of seminiferous tubules was measured across the shortest and longest axis, then the mean diameter of the tubules obtained⁽²⁴⁾. The germinal epithelial height of seminiferous tubules were measured from the spermatogenic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules. All data were obtained from 10 random microscopic fields per animal in all groups.

5) Testosterone assay

Prior to sacrificing each rat 2 ml blood was collected by cardiac puncture samples were collected into a test tube, and allowed to clot, the serum was harvested and used for testosterone assay. The assay was carried out using ELISA technique.

Statistical analysis of the data was performed by using SPSS (Version 16), using independent paired t-test.

RESULTS

The cross sections of the seminiferous tubules of the testes of rats in the control group showed normal histological features of stratified epithelium of spermatogenic series, sperm cells in the lumen, the tubules were regular and densely populated with spermatogonia and normal interstitial Leydig cells (Plate 1). The photomicrograph of the testes in the group II that administered MSG 4.0g/kg B.W orally, showed disorganized irregular tubules with appearance of cytoplasmic vacuolation in most of spermatogonia, decreased height of spermatogenic epithelium and increased in intercellular spaces in seminiferous tubules with increased interstitial connective tissue space and Leydig cells were sparsely distributed with reduction in sperm bundles in the lumen of the most of the tubules with vacuolation in Sertoli cells compared to those of the control group (plate 2).

The photomicrograph of testes of animals in the group III that received MSG at dose 4.0g/kg BW and Ginger at dose 100mg/kg B.W showed no histological alterations, regular and well organized tubules with normal interstitial connective tissue and Leydig cells. Most of seminiferous tubules have normal architecture (Plate 3).

The diameter of the seminiferous tubules, the germinal epithelial thickness, the morphometric changes in all animal groups are shown in Table 1, in which the germinal epithelium height has decreased in group treated with MSG compared with control and group MSG+Ginger. The results were significant as the p-value was <0.005 in rats treated with MSG compared with control and those in group treated with MSG plus Ginger.

The mean serum testosterone levels of rats that received MSG in group II, in this study were significantly lower ($P < 0.05$) than that of the control rats. Furthermore the serum testosterone levels in rats in group III that received MSG and Ginger were higher than that in group II and close to those in the control group ($p < 0.05$) (table 2).

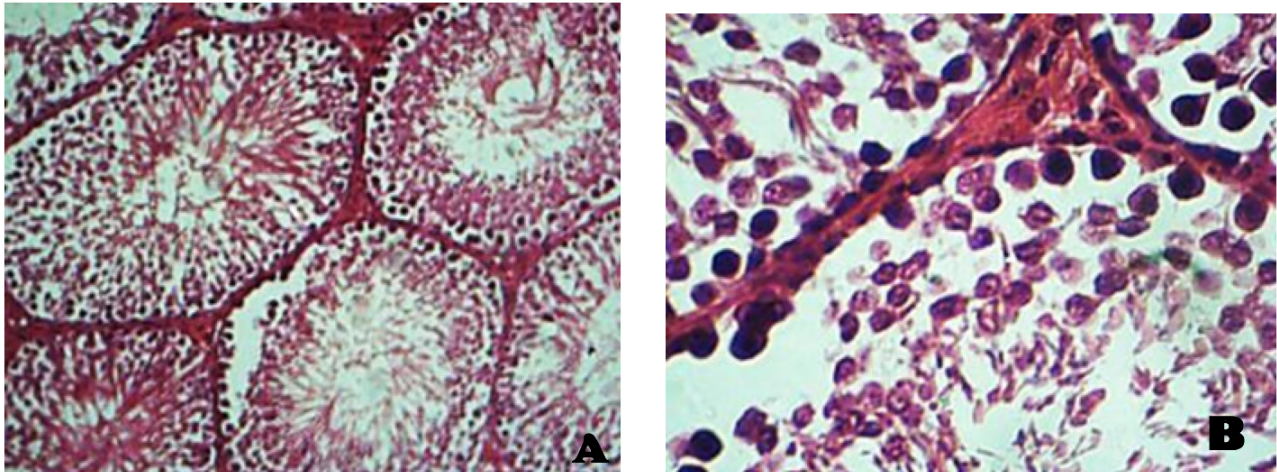


Plate 1. Photomicrograph of rat testis in Control group (A. X100 and B.X400) showing normal seminiferous tubules, germinal epithelium with sperm in their lumen and interstitial space containing Leydig cells (arrow). H&E stain.

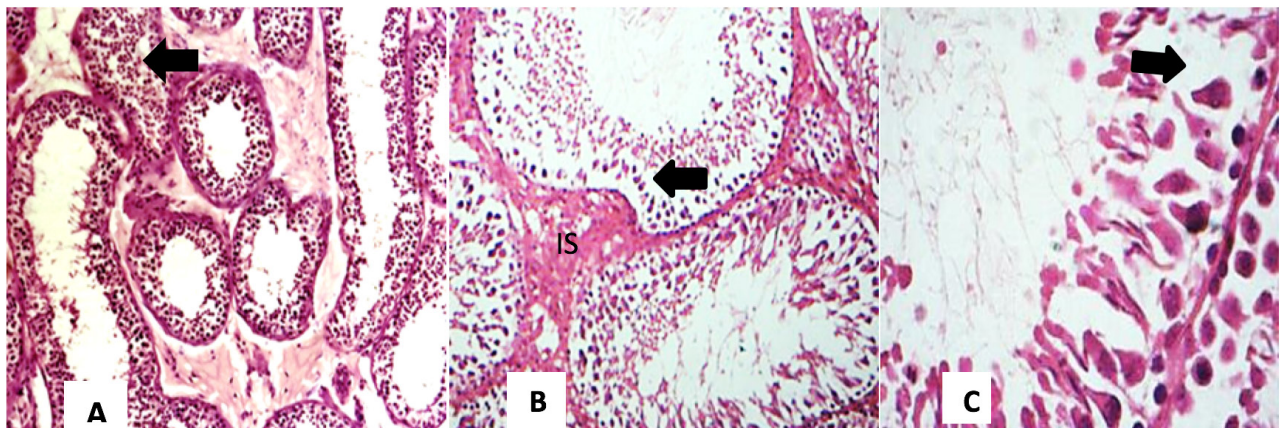


Plate 2. Photomicrograph rat testis in MSG group (A. X40, B.X100, C.X400) A. showing destroyed seminiferous tubules (arrow), B. shows decreased germinal epithelium height, vacuolation of spermatogonia (arrow) with decreased in sperm in their lumens and widening of interstitial spaces (IS), C. shows vacuolated Sertoli cell (arrow). H&E stain.

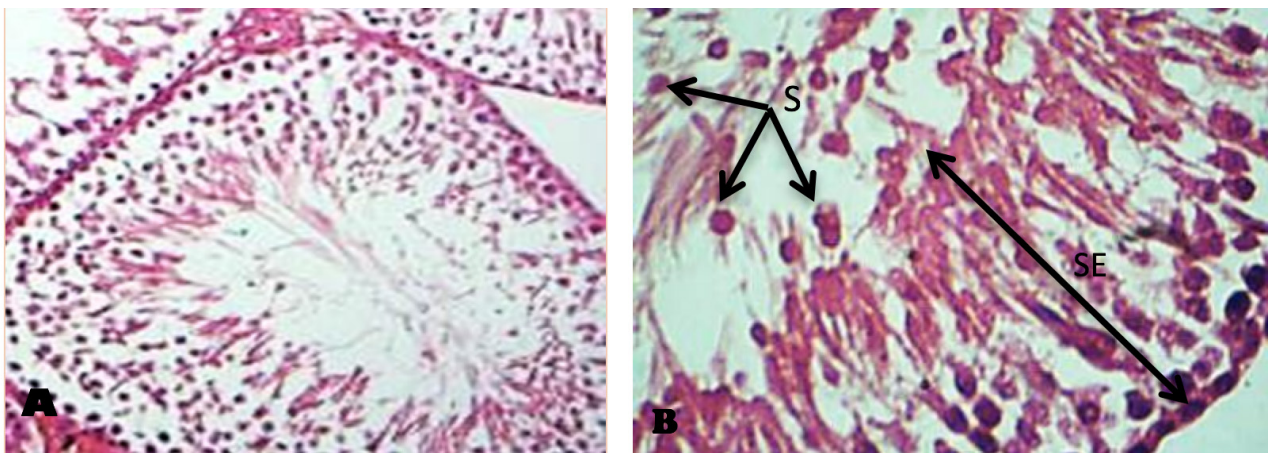


Plate 3. Photomicrograph rat testis in MSG+ Ginger group (A. X100 and B.X400) showing normal seminiferous tubules and normal thickness of germinal epithelium (SE), with many sperm (S) (arrow) in their lumens. H&E stain.

Table 1: Effect of MSG and Ginger on Seminiferous tubule diameter and germinal epithelial thickness (Mean ± SD)

Groups	Tubular Diameter (µm)	Epithelial thickness (µm)
Control	208.60	77.75± 2.85
MSG treated	125.05	60.44± 9.75
MSG+Ginger	206.3	75.09± 1.8

Table 2: Testosterone level in the control, MSG, and MSG plus Ginger groups (Mean +SD)

Groups	Testosterone (ng/ml)
Control	0.5020±0.00735
MSG	0.1300 ±0.01844
MSG+Ginger	1.2660±0.11682

DISCUSSION

This study indicated that MSG induced many histological and histomorphometric changes in testicular tissue of rats. The changes include degeneration in seminiferous tubules epithelium with reduction in sperm bundles⁽²⁵⁾. The changes recorded in the current study are in accordance to the histological studies that were carried out on the testes of different animals treated with MSG.^(26, 27) They found that MSG induced histological alteration in both the germinal epithelium and Leydig cells of the testes of neonatal mice, in another research⁽²⁸⁾ the researcher suggested that these changes may be due to either local effect of the chemical or indirectly caused by imbalance in gonadotrophic hormones. Vacuolation has been appeared in the spermatogenic cells and Sertoli cells in the current work with signs of tubular distortion, these changes have been noticed by other researchers and they referred it to testicular toxicity and cell damage^(29, 30). In addition to mentioned effects, MSG significantly led to reduction in the diameter of seminiferous tubule and germinal epithelial thickness, it also decreased spermatozoa production in testes of rats administered 4.0 g/kg B.Wt. this is because MSG caused degeneration of germ cells and vacuolation of sertoli cells which normally protect and support the spermatogenic cells. On the other hand the conditions that cause cell death by apoptosis in rat testis are low levels of testosterone hormone, and toxic

substances. Apoptosis was probably triggered by the association of testosterone deprivation with oxidative stress for long periods in germ cells. The oxidative stress is determined by hyperglycemia which causes a reduction of endogenous antioxidants and an increasing of superoxide, hydroxyl, and peroxy production with injury of the cellular DNA and proteins⁽³¹⁾.

Group of researchers⁽³²⁾ reported in their work that animals treated with MSG showed changes in the levels of FSH, LH, and Testosterone, these hormones are essential for normal testis function and healthy spermatogenesis, reduction in testosterone level has been noticed in the present study too and this could be either by direct toxic effect on the Leydig cells which are the endocrine cells that synthesize testosterone hormone or because of hypothalamus-pituitary-gonadal axis and the same results has been found by⁽³⁰⁾. Concerning the protective effect of Ginger, The present results indicated that ginger improved, recovered or prevented the histological and morphometric alterations induced by MSG in testes of rats that administered combination of MSG and Ginger. Similarly, researchers^(32, 33) reported that ginger extract improved testicular damage induced by metalaxyl and metiram fungicide in mice. It was reported that the mechanism of protection of ginger is related to its antioxidant properties⁽³⁴⁾, reported that ginger extracts exhibited free radical scavenging, inhibited lipid peroxidation,

DNA protection and reduced power abilities indicating strong antioxidant properties. In the other study ⁽³⁵⁾ ginger significantly increased sperm viability, motility and serum testosterone level also others ⁽³⁶⁾ found that ginger overcame reproductive toxicity of gentamicine and induced spermatogenesis through the elevation of testosterone level their results confirm our result which showed elevation in testosterone level and this is confirmed by results of researchers found out that ginger has a potent androgenic activity ⁽³⁷⁾. Another finding of this research was thickening of interstitial connective tissue of the seminiferous tubules. The literature has described interactions between extracellular matrix, tubular wall and germinative cells as important to their normal development ⁽³²⁾. Some studies have demonstrated an important increase in the amount of collagen resulting in interstitial fibrosis and a thickened lamina propria of the seminiferous tubules which prevents the development of spermatogenic cells ⁽³⁸⁾.

In conclusions, administration of Ginger extract has prevented the toxic effects induced by MSG on the rat testicular tissue and exerted protective effect. The ginger improves fertility status.

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