

Protective Effect of Curcumin on Monosodium Glutamate-Induced Reproductive Toxicity in Male Albino Rats

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Abstract: The present study was carried out to investigate the possible protective effect of curcumin on reproductive toxicity induced by the flavor enhancers, monosodium glutamate (MSG) in male albino rats. Treating animals with MSG caused decrease in testes weights and sperm counts. Several histological alterations were observed in the testis and epididymis. The testes showed deformed Sertoli cells and loss of the spermatogenic cells. The interstitial tissue appeared with different vacuoles, blood hemorrhage and Leydig cells have pyknotic nuclei. The diameter of seminiferous tubules and their epithelial height were significantly decreased in MSG treated animals in compared with controls. Moreover, testosterone and LH levels decreased significantly in rats treated with MSG. Histological examination of the epididymis revealed deformed ductus epididymis and their epithelial cells appeared with marked vacuolization and decrease of characteristic stereocilia in addition to hyperplasia. Co-administration of curcumin to MSG-treated rats improved the histopathological alterations induced by MSG in testis and epididymis and increased the sperm count. It also significantly increased the serum testosterone and LH.

Key words: Monosodium Glutamate • Curcumin • Testis • Epididymis • Histology

INTRODUCTION

Monosodium glutamate (MSG) is one of the flavor enhancers widely used in many countries and is found in different canned and packed food. MSG is also a food additive in restaurants (particularly mixed in noodles, soups etc.), packaged food industries (e.g. instant meals) and household kitchens. It is produced through a fermentation process of molasses from sugar cane or sugar beets, as well as starch and corn sugar [1]. The adverse effects of MSG on experimental animals were evaluated by many studies. Burde *et al.* [2] demonstrated that both subcutaneous injection and oral administration of MSG to immature rats and mice resulted in neuronal losses in the hypothalamus. Oral administration of MSG caused significant electrophysiological and histological changes in retina of rabbits [3]. Samuels *et al.* [4] reported that MSG is a neurotoxic agent leading to endocrine disorders and renal damage. Moore [5] reported that MSG affects the structure and function of male reproductive system and

showed to be toxic to the testis of human and experimental animals. Boodnard *et al.* [6] mentioned that administration of MSG to rats led to atrophic changes in the testis and destruction of Sertoli cells and Leydig cells. Nayatara *et al.* [7] recorded MSG reduction in testicular weight and decrease in the sperm count in rats treated with MSG. Treating rats with MSG caused decrease in testicular weight, decrease in tubular diameter, reduction in germinal epithelium height, decrease in the spermatid count and abnormalities of sperms morphology [8].

Medical plants play an important role in the management of different diseases. Curcumin is an important constituent of rhizomes of the plant *Curcuma longa* which is a member of the family (Zingiberaceae). It is used as a spice to give specific flavor and yellow color to Curry [9]. Curcumin was found to exhibit a variety of biological activities including antitumor [10], antioxidant [11], anti-inflammatory properties and antiviral activities [12]. The protective effects of curcumin against hazardous chemicals were studied in different animals [13-15]. The present study aimed to evaluate the

protective effect of curcumin against monosodium glutamate induced reproductive damage in male albino rats.

MATERIAL AND METHODS

Monosodium Glutamate: Monosodium glutamate (MSG) was obtained from El Dawlia for Medical Equipments and Chemicals Co. Egypt. It was dissolved in distilled water before use.

Curcumin Extract: Dry turmeric rhizomes of the plant *Curcuma longa* were purchased from a local market at Shebin El-kom, Menufia, Egypt. They were crashed into powder, dissolved in distilled water and orally given at a dose level of 150 mg/kg body weight daily for eight weeks [16].

Animals and Treatments: Sexually mature male Sprague Dawley rats with initial body weight 140 ± 5 g were used. Animals were housed in metal cages and kept in the laboratory under constant conditions of temperature ($24 \pm 2^\circ\text{C}$) and ($50 \pm 5\%$) humidity for at least one week before and throughout the experimental work. They provided with rodent pellet and water was available *ad libitum*. All the experiments were done in compliance with the guide for the care and use of laboratory. Animals were divided into 4 groups:

Group 1: Animals (10 rats) were fed on the standard diet and were served as a control group.

Group II: Rats (15 animals) were orally administrated with curcumin at a dose level of 150 mg/kg body weight.

Group III: Animals (20 rats) were treated with monosodium glutamate at a dose level of 4mg/kg b.wt, daily for 4 weeks.

Group IV: Animals (20 rats) were given monosodium glutamate together with curcumin (same doses) daily for 4 weeks.

Histological Study: After 4 weeks of experimental period, animals were sacrificed via decapitation, then they were dissected, testes and epididymis were removed, weighed and fixed in 10% neutral formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 μ thicknesses were cut using rotary microtome and mounted on clean slides.

For histological examination, sections were stained with Ehrlich's hematoxylin and counterstained with eosin. The numbers of seminiferous tubules were calculated in 10 random microscopic fields of 1mm^2 at a magnification of X100 using square ocular micrometer. Seminiferous tubules diameter and germinal epithelial height 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.

Epididymal Sperm Concentration: The left epididymis of each rat was used for the determination of epididymal sperm concentration using the Neubauer haemocytometer.

Biochemical Assays: For enzymes determination, blood samples were collected from the inferior vena cava and then centrifuged. Sera were obtained by centrifugation of the blood sample and stored at -20°C . Testosterone and LH were determined using radioimmunoassay kits supplied by Diagnostic Products Corp. (Los Angeles, CA, USA) according to Maruyama *et al.* [17].

Statistical Analysis: Data were expressed as mean values and standard deviations and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

Testes Weights and Sperm Count: Results in Table 1 revealed that rats treated with MSG showed significant decrease in the testes weights after 4 weeks of treatment. Treatment with curcumin caused apparent increase in testes weights. Epididymal sperm concentration in the MSG-treated rats was significantly lower ($p < 0.05$) than those of control. Rats treated with curcumin and MSG revealed an increase in sperm count (Table1).

Table 1: Change in mean value of testes weights in rats and sperm count in different groups.

Animal group	Testes weights(g)	Sperm count($10^6/\text{ml}$)
Control	2.30 ± 0.3	5.9 ± 0.1
Curcumin	2.22 ± 0.2	5.7 ± 0.2
MSG	$1.44 \pm 0.2^*$	$3.3 \pm 0.4^*$
Curcumin+MSG	1.70 ± 0.1	4.8 ± 0.3

(*). Significant at $p < 0.05$

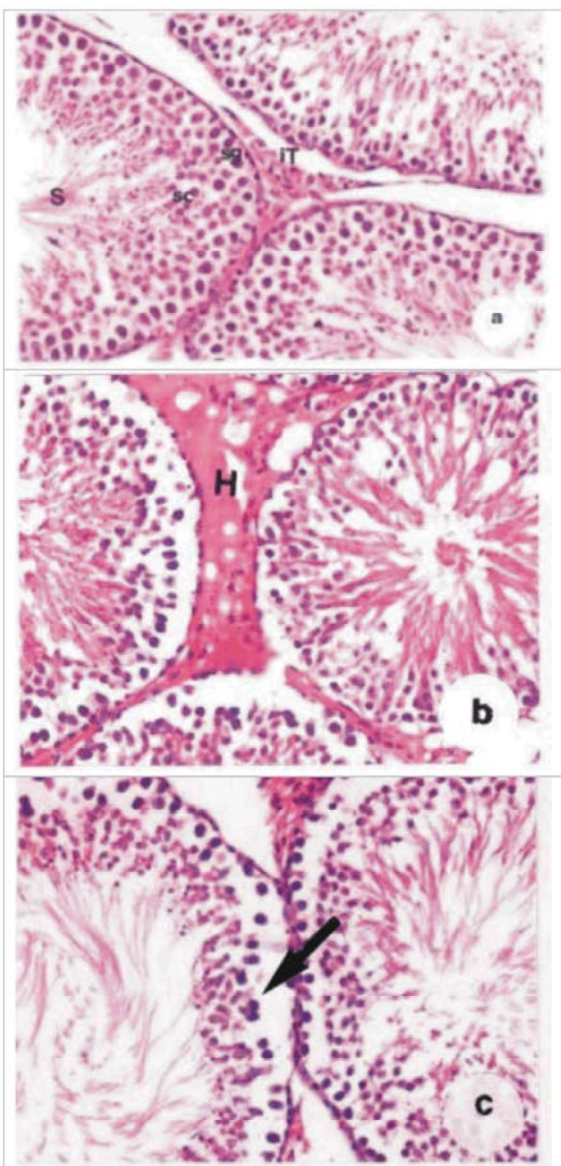


Fig. 1: (a):Section in testis of a control rat showing spermatogonia (Sg), Spermatocytes (SP), Sperm (S) and interstitial cells (IT); (b). Testis of MSG-treated rat showing intertubular hemorrhage (H); (c). detached of germ cells from the irregular basal lamina, (Arrow) X300.

Histological Observations: Histological sections of testes of control rats showed the normal structure of seminiferous tubules and interstitial tissue (Fig.1a). The germ cells (spermatogonia, primary and secondary spermatocytes, spermatides and spermatozoa) and Sertoli cells within the seminiferous tubules were normal. No histological alterations were observed in animals treated with curcumin. Testes of rats treated with MSG

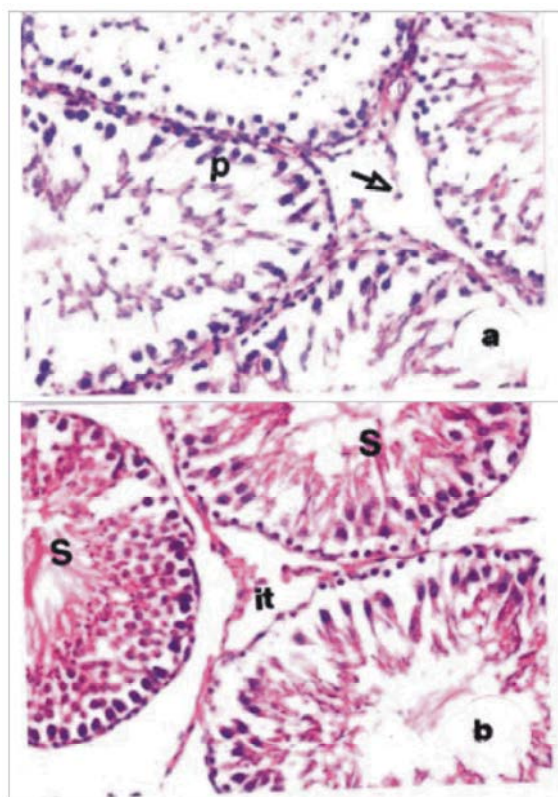


Fig. 2: (a): Testis of MSG-treated rat showing degenerated interstitial tissue (arrow) and deformed germ cells with pyknotic nuclei (P); (b). Testis of a rat treated with MSG+ Curcumin showing increase of sperm (S) and improved interstitial tissue (it), X 300.

displayed variable degree of histopathological alterations. The interstitial tissue appeared with different vacuoles, blood hemorrhage and Leydig cells have pyknotic nuclei (Fig.1b). The seminiferous tubules showed deformed germ cells as well as Sertoli cells being detached from the irregular basal lamina (Fig.1c). Many seminiferous tubules were severely damaged and had few Sertoli cells and spermatogonia with pyknotic nuclei (Fig.2a). Spermatocytes and early spermatides were lost from most of the tubules. Animals treated with MSG and curcumin showed an improvement of seminiferous tubules and an increase in the number of germ cells (Fig.2b).

Sections of caput epididymis of control rats showed numerous ductus epididymis surrounded by a myoconnective tissue sheath. The duct had a wide lumen in which sperms were stored. The entire ductus epididymis was lined with a pseudostratified stereociliated columnar epithelium. There are four cell types: principal, basal, apical and migratory cells (Fig. 3a).

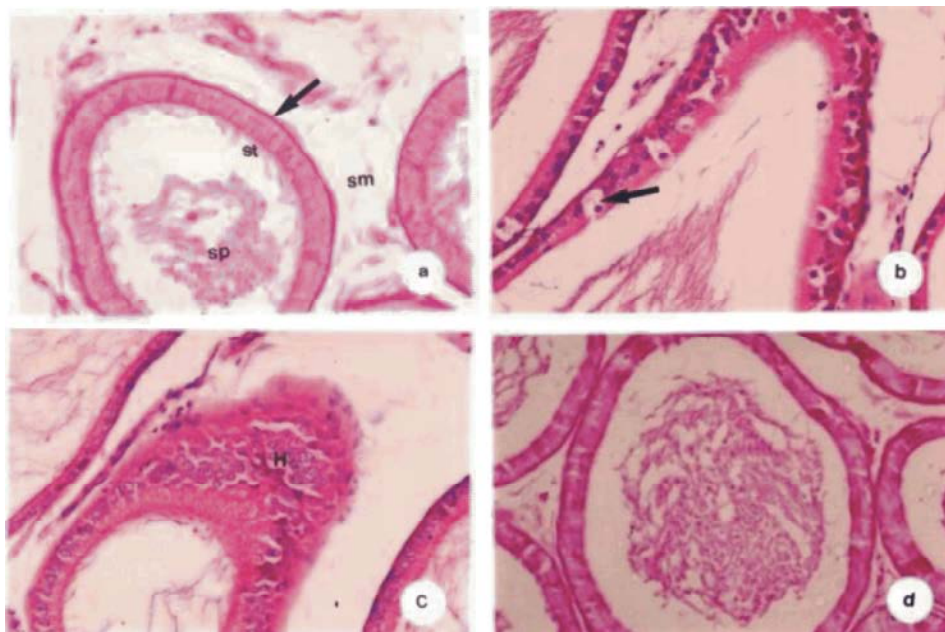


Fig. 3: (a): Section in epididymus of a control rat showing ductus epididymus, Basal membrane (arrow), stereocilia (st), sperm (sp) and stroma (sm); (b). Ductus epididymus of a rat treated with MSG showing vacuolated cells (arrow); (c). Ductus epididymus with hyperplasia (H); (d). Ductus epididymus of a rat treated with MSG+curcumin showing increase of sperm and normal structure, X200.

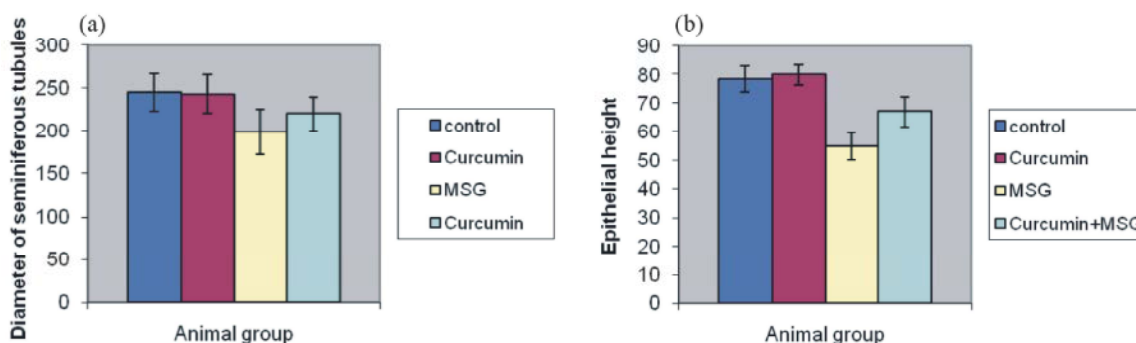


Fig. 4: Change in diameter of seminiferous tubules (a) and their epithelial heights (b) in different animal groups.

Treating rats with MSG showed that the ductus epididymis were deformed and lost their normal shape and the epithelial cells appeared with marked vacuolization and decrease of characteristic stereocilia (Fig. 3b). Marked hyperplasia was observed (Figs. 3c). The sperm bundles were degenerated in some of the ductus and completely absent in the others. Examination of epididymis of rats treated with MSG and curcumin revealed less prominent histopathological changes when compared with MSG group. In these specimens, the ductus epididymis showed normal epithelial cells with increase in stereocilia and there was an increase in the sperm bundles in their lumens (Fig. 3d).

Morphometric Results: The diameter of seminiferous tubules was significantly decreased ($199 \pm 26 \mu\text{m}$) in MSG treated animals in compared with controls ($225 \pm 22 \mu\text{m}$) (Fig. 4a). A decrease in germ cell height of seminiferous tubules was recorded in compare with control ones. The mean epithelial height was $78.5 \pm 4.5 \mu\text{m}$ and $55 \pm 4.8 \mu\text{m}$ in controls and MSG groups, respectively (Fig. 4b). Treating animals with curcumin and MSG showed an improvement in the mean tubular diameter and in germ cell height in comparison with MSG-treated animals. Animals given curcumin showed seminiferous tubules with normal diameters and epithelial heights.

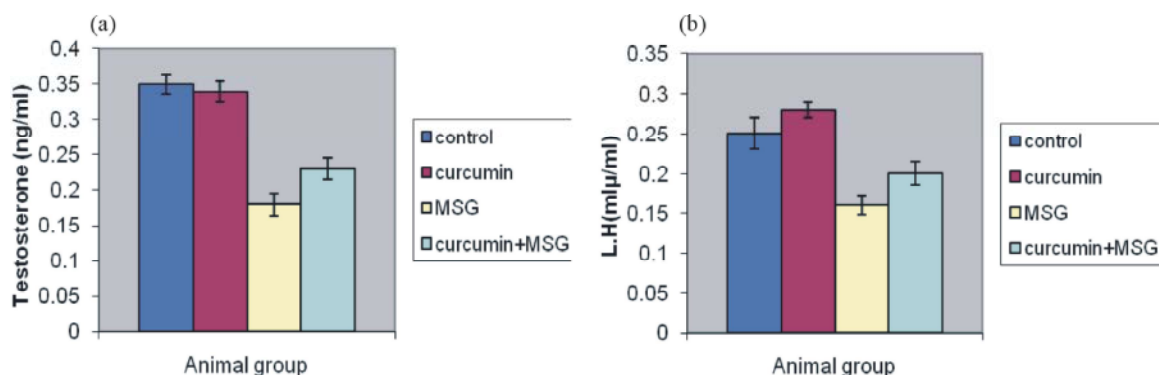


Fig. 5: Change in testosterone (a) and L.H (b) in sera of different animal groups.

Biochemical Results: Testosterone levels decreased significantly in rats treated with MSG alone compared with the control group ($P < 0.05$); but co-administration of curcumin to MSG-treated rats significantly increased the serum testosterone levels compared with MSG (Fig. 5a). Similarly, LH was significantly lower than those in control group ($P < 0.05$). Animals treated with MSG and curcumin showed a significant elevation in LH (Fig. 5b).

DISCUSSION

The present results showed that treating rats with MSG caused a decrease in the testis weight and sperm count. Moreover, histological results revealed damage of the seminiferous tubules together with degeneration of Leydig cells and inhibition of spermatogenesis. The epididymis showed many histological alterations. These results are in consistent with findings of other studies on the effect of MSG. Nayatara *et al.* [7] reported that treating rats with MSG reduced the sperm count and increase the incidences of abnormal sperm. Igwebuike *et al.* [18] showed that a reduction of caudal epididymal sperm counts was observed in the MSG-treated rats. Das and Ghosh [19] observed loss of spermatogenic cells in mice injected with MSG. Treating rats with MSG at short-term exhibited slight to moderate damaged seminiferous tubules, including cytoplasmic vacuolization of spermatogonia and loss of late spermatids. Long-term treatment caused severe damage of germ [20]. Ekaluo *et al.* [21] reported that MSG-treatment caused reduction of testes and epididymis weight, sperm count and increase in sperm abnormalities.

Our results demonstrated that serum testosterone and LH levels were reduced in rats treated with MSG. Similarly, Franc *et al.* [22] reported that the central nervous system of MSG-treated rats showed neurogenic functional changes in the hypothalamus that induced a reduction in levels of LH, FSH and testosterone.

Igwebuike *et al.* [18] recorded reduction in serum testosterone in rats given MSG. It was reported that MSG destroyed neurons of the hypothalamus in rats and mice [2]. Such neuronal losses in the hypothalamus can result in disruption of the hypothalamic-pituitary-testis regulatory axis that controls the steroidogenesis of testicular Leydig cells [23]. This will lead to decrease of serum testosterone levels recorded in the present work. Testosterone and LH hormones are essential for normal testes function and healthy spermatogenesis. These two hormones decreased in MSG-treated rats, such decrease may adversely affect the reproductive capacity of the affected animals.

The obtained results revealed that the variable histological and morphometrical changes as well as change in testosterone and LH induced by MSG in the testes were significantly improved after treatment with curcumin suggesting that curcumin treatment caused improvement of spermatogenesis impairment and MSG toxic effect on the testis. These results are in accordance with the observations of Sakr *et al.* [16] who reported that administration of curcumin to fluoxetine-treated rats was shown to ameliorate the testicular toxicity of fluoxetine and caused significant increase in LH and testosterone. Salama and El-Bahr [24] observed that the use of curcumin attenuated the damaged effects of cadmium on reproduction of male rats, improved its spermatogenic damage, decreased sperm count, increased testosterone level and induced antioxidant defense. Ilbey *et al.* [25] reported that treating rats with curcumin improved the cisplatin-induced testicular injury. A significant increase in plasma testosterone levels, GSH levels and GSH-Px activity and a decrease in MDA and NO levels in testicular tissue were observed with cisplatin plus curcumin compared with that with cisplatin alone. Sharaf *et al.* [26] reported that the treatment of rats with curcumin prior to exposure to ultraviolet rays led to protection against the testicular damage of ultraviolet irradiation.

Antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes leading to cell damage. Many studies showed that curcumin possesses antioxidant activity. Farombi *et al.* [14] indicated that curcumin protected against testicular oxidative damage induced by di-n-butylphthalate. Mathuria and Verma [15] showed that curcumin ameliorated aflatoxin-induced lipid peroxidation in liver, kidney and testis of mice. Srinivasan [27] reported that curcumin inhibited lipid peroxidation by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes, SOD, CAT, glutathione peroxidase and glutathio-s-transferase. Manikandana *et al.* [28] reported that curcumin significantly decreased the levels of free radicals and this protective effect was attributed to its free radical scavenging activity, induction of detoxification enzymes and providing protection against degenerative diseases. Chan and Yu [29] stated that curcumin exerted a good ability to scavenge oxygen free radicals and could protect DNA from UV-induced damage.

It was reported that MSG was associated with the production of oxygen free radicals and oxidative stress in different tissues of experimental animals [30, 31]. The obtained histological and biochemical disturbances appeared as a result of the oxidative stress induced by MSG. The results showed that curcumin exerted a protective effect on MSG- induced testicular damage and this is probably due to its antioxidants properties.

In conclusion, the present study showed that curcumin has ameliorative effect on the testicular toxicity of fluoxetine. This may be explained by the fact that it prevents cellular damage occurring as a result of oxidative stress in spermatogenic cells and Leydig cells.

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