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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

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Abstract: The present study aims to pinpoint the effects of the flavour enhancer Monosodium Glutamate; MSG on the histology and ultrastructure of the testes. Such effects were monitored after giving 10 (Short-term) and 30 (Long-term) oral doses each was 4 g kg^{-1} body weight; a third of LD_{50}, of MSG to adult white rats *Rattus norvegicus*. Their testes were routinely processed for examination with the light and electron microscopes. The seminiferous tubules of rats treated with MSG for short-term exhibited slight to moderate damage. The slight damage of the tubules included appearance of vacuoles and loss of late spermatids. The vacuoles were found inside the cytoplasm of the spermatogonia, the primary spermatocytes and Sertoli cells. Some seminiferous tubules displayed sloughing of impaired early spermatids into their lumina. Other tubules showed complete loss of early and late spermatids. Due to shrinkage of many tubules the intertubular spaces widened. By using the electron microscope, the primary spermatocytes revealed loss of cristae of many of their mitochondria, great enlargement of Golgi apparatus and disruption of the nuclear envelope as well. The sloughed early spermatids possessed irregular head caps, dilated endoplasmic reticulum, mitochondria with fused cristae and few cytoplasmic vacuoles. Also, few mitochondria of these cells underwent disorganization of their distribution. Mitochondria of Sertoli cells exhibited loss of some or all of the cristae. In case of long-term treatment with MSG, the testes of rats contained severely damaged tubules. Such seminiferous tubules were characterized by containing only Sertoli cells almost arranged in a ring together with occasional occurrence of ruined spermatogenic cells and appearance of masses of necrotic germ cells. Many mitochondria with disintegrated cristae were noticed in the severely perished primary spermatocytes. Sertoli cells showed extensive vacuolation, disintegration of the cytoplasm and highly harmed mitochondria with electron-dense matrices. The structural changes of testes of the MSG-treated rats were found to be dosage-duration-dependent and ranged from slight to moderate damage in case of the short-term treatment however, severe damage was recorded in the case of the long-term treatment. Thus, it is important to reconsider the usage of MSG as a flavour enhancer.

Key words: Rats, testis, monosodium glutamate, food additives, spermatogenesis, Egypt

INTRODUCTION

Now a days, monosodium glutamate is one of the most flavour enhancers commonly used all over the world by millions of people in their food. Monosodium glutamate is found in a wide variety of canned and packaged food. MSG is also a food additive in restaurants, hospitals, retirement homes and cafeterias (John, 2006). Many Egyptians consume MSG in both ready and home-made food, almost daily (Swelim, 2004a). Recently, MSG is commonly sold in the Egyptian supermarkets. Many studies were carried out on the effect of MSG on different body organs of experimental animals. Gong *et al*. (1995) recorded harmful effects of MSG on function of hypothalamus-pituitary-target gland system in rats. This resulted in decrease in the weights of the body, pituitary gland and testes. Also, there was a marked reduction in the serum levels of some hormones such as LH, FSH, TSH, GH and TS. Oforofuo *et al*. (1997) found that rats under MSG chronic treatment revealed changes in the morphology of their sperms.

According to Ebling *et al*. (1998) lesions were induced in the arcuate nucleus of male hamsters that were treated with 4 mg g^{-1} b.wt. of MSG. Such lesions were accompanied by significant reduction in their testicular growth.

Onakewhor *et al*. (1998) found that chronic administration of MSG caused a reduction in the spermatozoa production in rats. MSG-treated adult male rats displayed a significant hypogonadism (Giovambattista *et al*., 2003).

Other studies mentioned that brain lesions (Swelim, 2004a) and degeneration of the eye retinal cells (Swelim, 2004b) were induced by MSG in adult and neonatal mice. The administration of MSG to neonatal mice led to a reduction in weights of testes and seminal vesicles

(Kaledin *et al*., 2005). Franca *et al*. (2006) proved that adult MSG-treated rats exhibited significant low plasma levels of LH and FSH. Histological studies of the effects of MSG on the medial geniculate body of adult Wistar rats revealed the presence of some cellular degenerative changes, autophagic vacuoles and some degree of neuronal hypertrophy (Eweka and Adjene, 2007). It was reported that there was a decrease in the testicular ascorbic acid level and the weight of testes of rats received MSG (Nayanatara *et al*., 2008; Vinodini *et al*., 2008). Also, a reduction in sperm count and increased incidences of abnormal sperms were recorded in rats treated with MSG (Nayanatara *et al*., 2008). An earlier study by Ciric *et al*. (2009) concluded that the neonatal rats treated with MSG acquired a decrease in the relative lengths of the bone, body and tail. Their relative absolute testicular mass showed reduction as well. It was found that MSG-treated mice had an increase in the number of the pachytene stage of the primary spermatocytes (Das and Ghosh, 2010).

Since, the testes are the organs for spermatogenesis, the present study was designed to determine the testicular damage in the white rats *Rattus norvegicus* following short and long-terms of treatments with daily oral doses of MSG.

MATERIALS AND METHODS

Used animals: In the current study, 24 adult male Sprague-Dawley white rats, *Rattus norvegicus* each weighs between 180 and 190 g were used. The animals were obtained from Helwan farm for experimental animals, Cairo, Egypt. Cleaned and good aerated animal plastic cages were used to keep the rats under laboratory conditions of room temperature (25±1°C), humidity (50±5%) and at a 12 h day and night cycle in the animal house of the Zoology Department, Faculty of Science, Ain-Shams University, Cairo, Egypt. The rats were fed standard food pellets (Purchased from abu-Zaabal Co., Cairo, Egypt) and drinking water *ad libitum*. All animal-related protocols were approved by the Institutional Animal Care and Use Committee (Ain-Shams University, Cairo, Egypt) and according to the Guide for Care and Use of Laboratory Animals (Bashandi, 2003).

Monosodium glutamate and its administration: In the present study, a daily dose of 4 g kg^{-1} body weight of MSG (Dissolved in 2 mL physiological saline) was administered orally to the rats via a stomach tube. This chosen dosage represented a third of the oral Lethal Dose (LD_{50}) as a preliminary investigation was undergone to determine the oral LD_{50} of the MSG to the used rats. Monosodium Glutamate (MSG) and all other chemicals were purchased from Merck, Darmstadt, Germany.

Experimental design: The 24 rats were divided randomly into four groups (Number of rats n = 6 for each group): These are the control groups IA and IB and the MSG-treated groups IIA and IIB.

Group IA (Control group): The rats were orally given 2 mL day^{-1} physiological saline for 10 days.

Group IIA (Short-term MSG-treated group): The rats orally received a dose of 4 g kg^{-1} body weight/day of MSG, dissolved in 2 mL physiological saline for 10 days.

Group IB (Control group): The rats were orally given 2 mL day^{-1} physiological saline for 30 days.

Group IIB (Long-term MSG-treated group): The rats were treated with an oral dose of 4 g kg^{-1} body weight/day of MSG, dissolved in 2 mL physiological saline for 30 days. The rats of groups IA and IIA were sacrificed on day 11th while rats of groups IB and IIB were sacrificed on day 31st of the treatment. The testes of both control and treated rats were dissected out for histological and ultrastructural examinations.

Tissue processing for light microscopy: The testes were carefully dissected out and cut into small pieces that were immediately fixed in aquous Bouin's solution. Then, dehydration was carried out in ascending grades of ethyl alcohol. The pieces of the testes were cleared in chloroform and then embedded in paraplast. An American Optical microtome (AO-821, USA) was used for cutting the testes into 5 μm thick sections. The deparaffinised sections were routinely stained with haematoxylin and eosin.

Assessment of histological damage: The resulted damages in the seminiferous tubules were graded into three categories; slight, moderate and severe.

Tissue processing for transmission electron microscopy: The exposed dispersed seminiferous tubules from the decapsulated testes were cut into small pieces and rapidly fixed in 0.1 M cacodylate buffered 3% glutaraldehyde for 4 h at 4°C then washed in the 0.1 M cacodylate buffer overnight before they were post-fixed in cacodylate buffered 1% osmium tetroxide for 2 h. Dehydration was carried out in an ascending series of ethyl alcohol cleared

in propylene oxide and embedded in epoxy resin in an oven at 60°C for 20 h to produce a firm block. The tissues were cut with glass knives and by using ultratome of American Optical Corporation-USA, into 1 μm thick semithin sections to be stained with toluidine blue. Then, 80 nm thick ultrathin sections were obtained, mounted on perforated copper grids (Plano, Wetzlar, Germany) and finally, stained with uranyl acetate and lead citrate. Examination and making film-electron micrographs have been done by using the transmission electron microscope (JEOL, 1200 EXII, Tokyo, Japan) at 80 kV acceleration voltage.

RESULTS AND DISCUSSION

Histology and ultrastructure of the testes of the control groups: Histological cross sections of testes of the rats of the control groups (IA and IB) of the current study (Fig. 1) revealed normal architecture of all the seminiferous tubules. Each tubule is surrounded with boundary tissue, inner to which is the basal lamina. Myoid cells form the outer layer of the boundary tissue. The tubules are lined with spermatogenic epithelium and Sertoli cells that rest on the basal lamina. The germ cells include different spermatogenic cells that represent different stages of the spermatogenesis. Such spermatogenic cells could be easily differentiated from each other. The spermatogonia have oval or rounded nuclei. They are located on or near the basal lamina. The primary spermatocytes are rounded cells with large spherical nuclei. The secondary spermatocytes are about half the volume of the primary spermatocytes but can not be easily seen because they pass this stage readily. Many layers of different stages of early spermatids are arranged towards the lumina of the tubules. The early spermatids are small spherical or polygonal cells. The central lumina could easily be delineated in almost all the tubules and the majority of them are occupied by the late spermatids that acquire darkly stained elongated heads and long tails. On the basal lamina of the tubules, Sertoli cells were clearly identified with characteristic oval, indented nuclei that possess obvious nucleoli. Small aggregations of Leydig cells were monitored within the intertubular connective tissue between the seminiferous tubules.

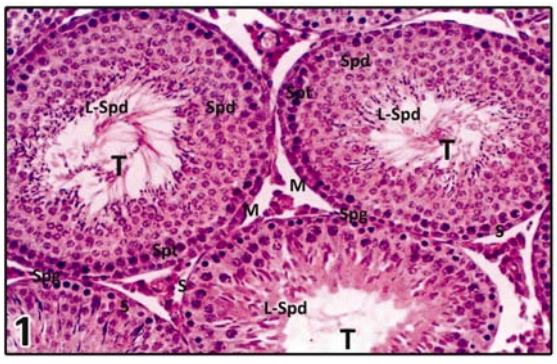

Fig. 1: A photomicrograph of a cross-section of a testis of a control rat showing normal spermatogenic progression. The seminiferous Tubules (T) have healthy spermatogenic cells; Spermatogonia (Spg), primary Spermatocytes (Spt), early Spermatids (Spd), Late Spermatids (L-Spd). Notice: Myoid cells (M) and Sertoli cells (S). H and E, x250

Ultrastructurally, each cell type of the seminiferous tubules has its own characteristics. The nuclei of type-A spermatogonia are flattened or oval whereas the nuclei of type-B spermatogonia are spherical. The largest spermatogenic cells are the primary spermatocytes; each has a dark nucleus with a characteristic sex vesicle. The most conspicuous character of the early spermatids is the presence of many mitochondria that are located in one layer subjacent to the cell-membrane. Also, the early spermatids develop acrosomic system with head caps. The late spermatid has a very small dark head and long tail. Sertoli cells are characterized with their oval, indented nuclei containing prominent nucleoli. The extensive cytoplasm of Sertoli cells extends and branches throughout the seminiferous epithelium to enclose all the spermatogenic cells.

Histopathological and ultrastructural findings

Short-term MSG-treated rats (Group IIA): Sections of testes of rats given a daily dose of 4 g kg^{-1} body weight of MSG for 10 days depicted slight and moderate damage of the seminiferous tubules. The following microscopical changes are considered as characteristic signs for the slightly damaged tubules. The majority of the seminiferous tubules showed a complete loss of the late spermatids but few heads were present in some tubules. Some vacuoles were easily noticed close to the periphery of the tubules (Fig. 2). Few small vacuoles were seen inside the B-type spermatogonia. Other large vacuoles were present inside the cytoplasm of Sertoli cells that contained their characteristic lobulated nuclei. Mitochondria of Sertoli cells and the primary spermatocytes exhibited different degrees of damage such as loss of some or all their cristae. The head caps of some early spermatids gained a slight irregular outlines but their mitochondria were still arranged as one row close to the cell membrane (Fig. 3).

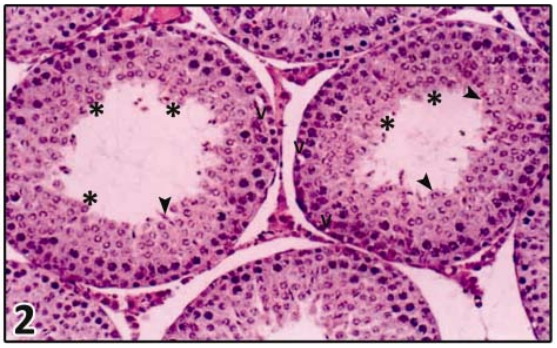

Fig. 2: A photomicrograph of a cross-section of a testis of a rat given a daily dose of 4 g kg^{-1} b.wt. of MSG for 10 days. Note the loss of late spermatids (Asterisks) from the seminiferous tubules and the presence of few small Vacuoles (V) in the tubules. Few heads of late spermatids (Arrow heads) are still present in the seminiferous tubules. H and E, x250

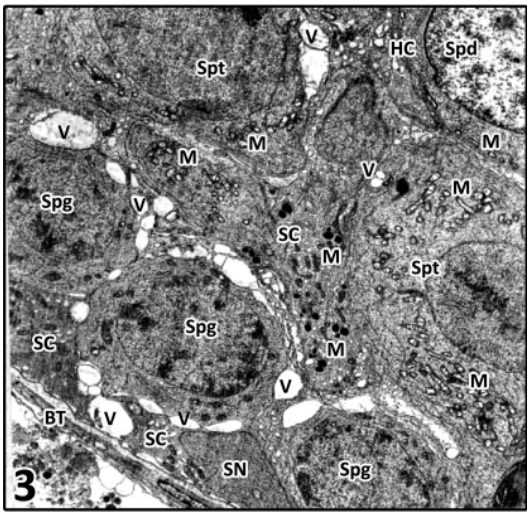

Fig. 3: A low power electron micrograph of a part of a seminiferous tubule of a rat given a daily dose of 4 g kg^{-1} b.wt. of MSG for 10 days showing marked Vacuolation (V). Note that the Mitochondria (M) of Sertoli cells and the primary spermatocytes show different degrees of damage. The early spermatid has an irregular Head Cap (HC) but its mitochondria are still arranged as one row close to the cell membrane; BT: Boundary Tissue; SC: Sertoli Cell Cytoplasm; SN: Sertoli cell Nucleus; Spg: B-type spermatogonium; Spt: primary Spermatocyte; Spd: early Spermatid, x3800

Many seminiferous tubules of rats of group IIA manifested changes of the moderate damage that included

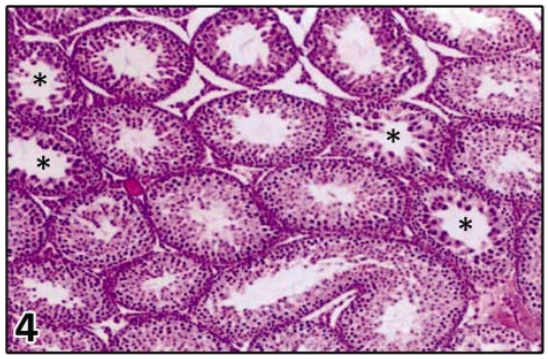

Fig. 4: A Low power photomicrograph of a cross-section of a testis of a rat received a daily dose of 4 g kg^{-1} b.wt. of MSG for 10 days showing a complete loss of late spermatids from almost all the seminiferous tubules. Some tubules contain only the spermatogenic epithelium without early and late spermatids (Asterisks). H and E, x100

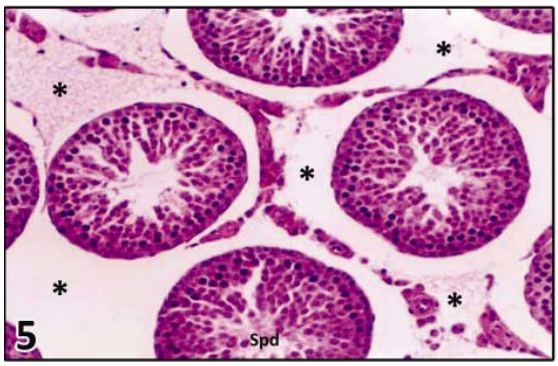

Fig. 5: A photomicrograph of a cross-section of a testis of a rat treated with a daily dose of 4 g kg^{-1} b.wt. of MSG for 10 days showing some seminiferous tubules with marked shrinkage due to loss of early spermatids. This shrinkage of the tubules and their boundary tissue leads into enlargement of the intertubular space (Asterisks) around the tubules. Note that few early Spermatids (Spd) tend to be exfoliated in the centre of the tubules. H and E, x200

the following abnormalities. The majority of the testicular tubules lost the early and late spermatids. Other tubules possessed only the spermatogonia, sloughed early spermatids and Sertoli cells (Fig. 4 and 5). Loss of many of the early spermatids led into marked shrinkage of some of the seminiferous tubules. As a result of shrinkage of these tubules and their boundary tissue, the spaces between the seminiferous tubules were widened. Some

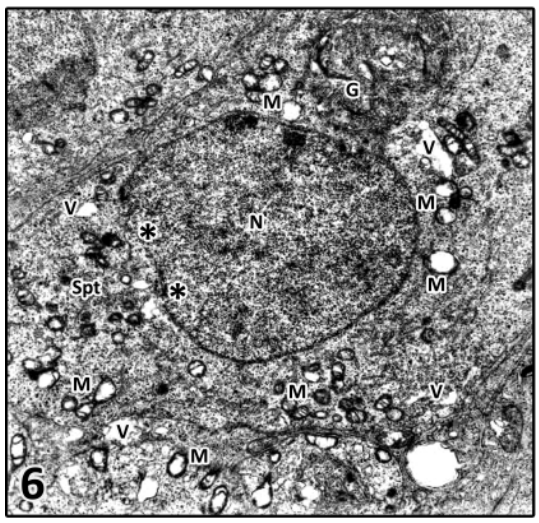

Fig. 6: An electron micrograph of a primary Spermatocyte (Spt) from a seminiferous tubule of a rat received a daily dose of 4 g kg^{-1} b.wt. of MSG for 10 days. Note that the Golgi apparatus (G) is greatly enlarged. Cristae of some Mitochondria (M) are lost. Some intercellular Vacuoles (V) are shown. The nuclear envelope is disrupted (asterisks). N: Nucleus, x6300

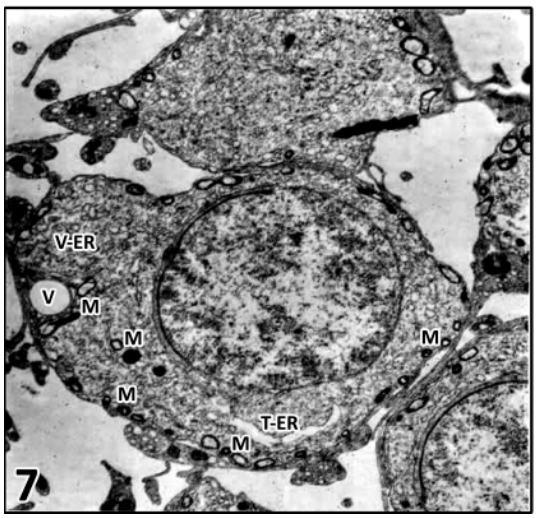

Fig. 7: An electron micrograph of an exfoliated early spermatid from a seminiferous tubule of a rat treated with a daily dose of 4 g kg^{-1} b.wt. of MSG for 10 days showing appearance of a cytoplasmic Vacuole (V). The Tubular (T-ER) and the Vesicular (V-ER) elements of the endoplasmic reticulum exhibit marked dilation. Few Mitochondria (M) have fused cristae and are displaced from their peripheral localization under the cell membrane to be deeply located inside the spermatid. The acrosomic system appears normal in shape, x6300

tubules had markedly perished primary spermatocytes as they contained few cytoplasmic vacuoles. Their Golgi apparatus displayed great enlargement. Many of their mitochondria became harmed and lost their cristae. Also, the nuclear envelope was disrupted in certain areas as well (Fig. 6).

Focusing on exfoliated early spermatids in some moderately damaged seminiferous tubules proved the presence of many signs of deterioration of these cells. The changes included appearance of few vacuoles inside their cytoplasm. Dilation of both tubular and vesicular elements of the endoplasmic reticulum was clearly observed. Few mitochondria exhibited fused cristae and were displaced from their peripheral localization close to the cell membrane to be deeply located (Fig. 7).

Long-term MSG-treated rats (Group IIB): Testes of rats received a daily dose of 4 g kg^{-1} body weight of MSG for 30 days contained severely damaged tubules. Only few primary spermatocytes were occasionally seen; they displayed marked elongation. The late spermatids were destroyed or lost (Fig. 8). Most of these tubules had only a large number of Sertoli cells that are almost arranged in a ring at the periphery of the tubules (Fig. 8 and 9). Also,

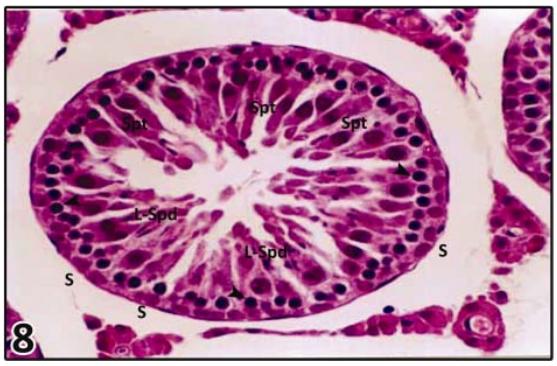

Fig. 8: A photomicrograph of a cross-section of a seminiferous tubule of a testis of a rat given a daily dose of 4 g kg^{-1} b.wt. of MSG for 30 days showing a remarkable damage of B-type spermatogonia (Arrow heads) and marked elongation of primary Spermatocytes (Spt). Note the presence of large number of Sertoli cells (S) that are located at the periphery of the tubule. Few damaged Late Spermatids (L-Spd) with darkly-stained elongated heads are still present in the tubule. But the majority of late spermatids are lost. H and E, x300

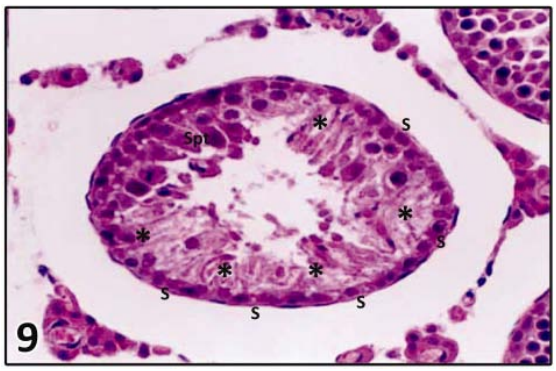

Fig. 9: A photomicrograph of a cross-section of a seminiferous tubule of a testis of a rat received a daily dose of 4 g kg^{-1} b.wt. of MSG for 30 days. Note the presence of a severely damaged seminiferous tubule. Few primary Spermatocytes (Spt) are still seen. Large areas of necrotic spermatogenic cells are easily noticed (Asterisks). S: Sertoli cells. H and E, x250

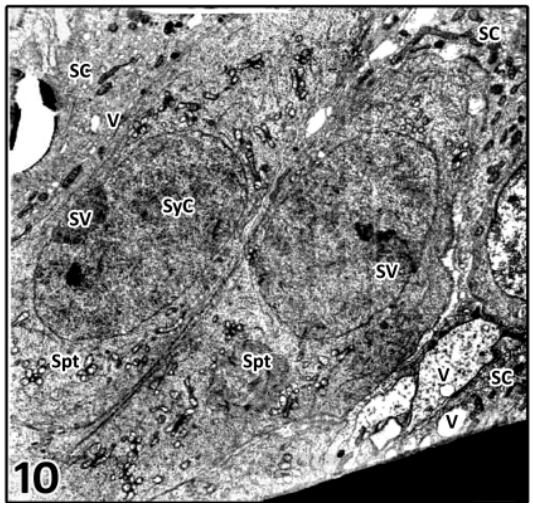

Fig. 10: An electron micrograph of two markedly elongated primary Spermatocytes (Spt) of a seminiferous tubule of a testis of a rat treated with a daily dose of 4 g kg^{-1} b.wt. of MSG for 30 days. Note the Vacuolated (V) Sertoli Cell cytoplasm (SC). SV: Sex Vesicle; SyC: Synaptonemal Complex, x5000

a large mass of necrotic spermatogenic cells was easily noticed in the majority of the tubules (Fig. 9). By using the electron microscope, many markedly elongated primary spermatocytes were easily recognized by the sex vesicle and the synaptonemal complex in their nuclei (Fig. 10). Other primary spermatocytes were highly spoiled as they

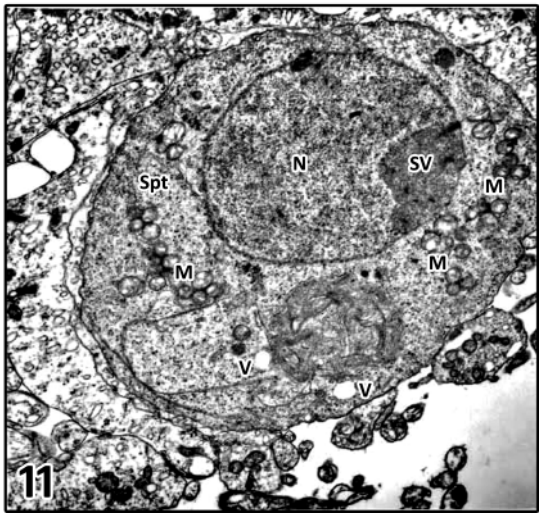

Fig. 11: An electron micrograph of a highly damaged primary Spermatocyte (Spt) of a seminiferous tubule of a testis of a rat given a daily dose of 4 g kg^{-1} b.wt. of MSG for 30 days. Notice the highly damaged Mitochondria (M) with almost complete disintegration of their cristae. The characteristic Sex Vesicle (SV) of the primary spermatocyte is clearly seen in its Nucleus (N). V: Vacuoles, x8000

possessed strongly devastated mitochondria with almost complete disintegration of their cristae (Fig. 11). Sertoli cells of these severely damaged tubules acquired prominent changes that were easily noticed. Appearance of many vacuoles in the cytoplasm of Sertoli cells was one of such obvious changes (Fig. 10). In many of these tubules, the cytoplasm of Sertoli cells underwent a complete disintegration. Also, their mitochondria became highly injured and contained electron-dense matrices (Fig. 12).

From the above-mentioned results, it was clear that the histological and ultrastructural effects of long-term treatment with MSG on rats' testes (Group IIB) were greater than those shown in short-term treated rats (group IIA). The current study has been the first to the best of researchers knowledge to describe the histopathological and ultrastructural changes of the rat testes that resulted from daily oral treatment with MSG for short and long-term periods.

Previous studies found that animals treated with MSG revealed a significant increase in their body weight and a significant decrease in their testes weight as compared to their controls (Miskowiak *et al*., 1993; Giovambattista *et al*., 2003; Kaledin *et al*., 2005; Kuznetsova *et al*., 2005; Nayanatara *et al*., 2008; Vinodini *et al*., 2008; Ciric *et al*., 2009). Such observations are parallel to incidentally observed slight increase in the

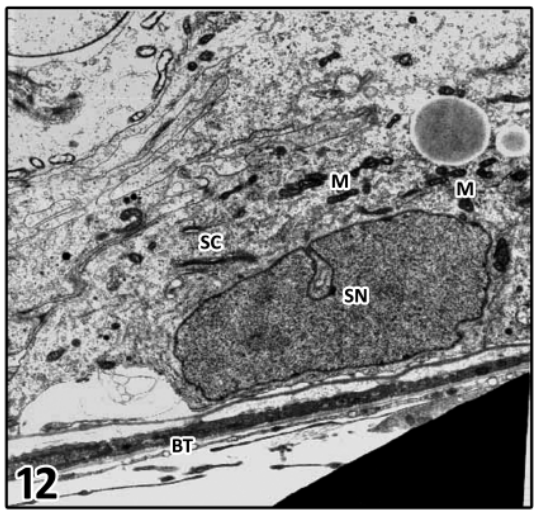

Fig. 12: An electron micrograph showing a part of severely damaged seminiferous tubule from a rat received a daily dose of 4 g kg^{-1} b.wt. of MSG for 30 days. Only Sertoli cell Nucleus (SN) is shown within a completely disintegrated cytoplasm (SC). Mitochondria (M) with electron dense matrices are noticed in Sertoli cell cytoplasm. BT: Boundary Tissue, x8000

body weight and moderate decrease in the testes weight of the rats treated with MSG for long-term as compared to the rats of the control and short-term treated groups in the present study. Such effects could be explained on the basis of the findings of the earlier study carried out by Hamaoka and Kusunoki (1986) on MSG-treated mice. They suggested that upon treatment of mice with MSG most of the important organs with major physiological functions (Such as heart, lungs, spleen, pancreas, kidneys, testes, brain and submandibular glands) became hypoplastic and had decreased weights. This resulted in low energy expenditure by these organs. Moreover, the resulted hypertrophy and hyperplasia of the mucosal cells of the intestine of MSG-treated mice gave a possible acceleration of the absorptive function for more digested food. Thus, the net result was a relatively excessive energy supply that led to obesity of the MSG-treated mice.

In the present study, the seminiferous tubules of MSG-treated rats for 10 days (Group IIA) exhibited slight to moderate damage. The most conspicuous histological alterations in the slightly damaged tubules included appearance of vacuoles and loss of late spermatids. While the signs in the moderately damaged tubules were loss of early and late spermatids marked shrinkage of some of the seminiferous tubules, widening of the spaces between the tubules and exfoliation of some early spermatids. Some tubules possessed only the spermatogonia, exfoliated early spermatids and Sertoli cells.

The current results indicated severely damaged tubules in testes of rats received a daily dose of 4 g kg^{-1} body weight of MSG for 30 days. They contained only Sertoli cells almost arranged in a ring, together with occasional occurrence of damaged germ cells such as B-type spermatogonia and elongated primary spermatocytes. The late spermatids were impaired or lost from these testicular tubules. Large masses of necrotic cells were present in many tubules.

Some changes of the present investigation are in accordance with results of the few histological studies that were carried out on the testes of different animals treated with MSG. Absence of spermatids in seminiferous tubules of hamster treated with MSG was noticed by Lamperti and Blaha (1980). Subcutaneous injection of MSG into neonatal mice for a long-term exposure caused loss of spermatogenic cells (Das and Ghosh, 2010).

Earlier comparable histological changes in testes of rats under the effect of different treatments were recorded. Complete loss of germ cells was observed in the testes of rats irradiated with caesium-137 (Sawada and Esaki, 2003). Vacuolation of seminiferous tubules of rats was considered as a toxicological effect of treatment with diesel fuel additives (Poon *et al*., 2004). Testicular histopathological changes were produced in rats by acute cadmium toxicity (Yadav *et al*., 2005). Testicular toxicity of rats exposed to 1-bromopropane was represented by persistent depletion of spermatogenic cells (Banu *et al*., 2007). It was reported by Issam *et al*. (2009) that hypospermatogenesis and degenerated spermatogenic cells were pathologic features in testes of rats treated with deltamethrin pyrethroid insecticide. Decreased cell numbers of seminiferous epithelia was due to exposure of rats to estradiol benzoate (Li *et al*., 2009). Degeneration, necrosis and exfoliation of spermatogenic cells of testes were observed in rats exposed to hypobaric hypoxia (Liao *et al*., 2010). Similar findings were obtained in rats treated orally with melatonin that caused loss of early and late spermatids, shrinkage of seminiferous tubules and severe damage of many testicular tubules (Rashed *et al*., 2010). In another study, Di-n-butylphathalate, an environmental pollutant, caused shrinkage of seminiferous tubules, disintegration and shedding of seminiferous epithelial cells in testes of adult rats (Zhou *et al*., 2010). Testicular necrotic germ cells and sloughing of apoptotic spermatogenic cells were characteristic deleterious effects of long-term treatment of rats with microwave radiation (Chen *et al*., 2011).

Ultrastructural examination of the present seminiferous tubules of MSG-treated rats revealed marked changes in the spermatogenic cells and Sertoli cells as previously illustrated in the results. However, it was difficult to find earlier literature dealing with structural features of the testes of MSG-treated animals therefore, comparison of the present microscopical abnormalities with previous studies dealt with the effects of other toxicological agents on testes would be traced.

In this regard, it was reported that spermatogonia of rat testes had dilation of the endoplasmic reticulum after exposure to electromagnetics (Zeng *et al*., 2003). Marked ultrastructural anomalies of spermatids were observed in seminiferous tubules of rats treated with either lithium carbonate (Zarnescu and Zamfirescu, 2006) or indenopyridine CDB-4022 (Hild *et al*., 2007). In another study by Farag Allah *et al*. (2009) on effect of cisplatin on testes of rats, the spermatogenic cells showed dilated endoplasmic reticulum and devastated mitochondria. Similar effects were obtained but in the case of testicular torsion/detorsion-induced ischemia-reperfusion injury of rats (Kanter, 2010). Treatment of rats with melatonin resulted in other effects that included disintegration of mitochondrial cristae of spermatogenic cells, dilation of the subacrosomal space, disorganization of the distribution of mitochondria and discontinuity of the nuclear envelopes in the early spermatids (Rashed *et al*., 2010), disruption of Golgi apparatus, fragmentation of head caps in early spermatids (Rashed *et al*., 2011).

The alterations occurred in Sertoli cells in the current study were comparable to those resulted in other studies on testes of rats treated with cadmium (Bizarro *et al*., 2003; Ismail and Ibrahim, 2010; De Souza Predes *et al*., 2011), cimetidine (Sasso-Cerri and Cerri, 2008), estradiol benzoate (Li *et al*., 2009), perfluorononanoic acid PFNA (Feng *et al*., 2010), anabolic-androgenic steroids (Naraghi *et al*., 2010), melatonin (Rashed *et al*., 2010) and chronic crude garlic (Abdelmalik, 2011).

The mechanisms by which MSG inhibited the spermatogenesis in the current experiments could be explained on the basis of earlier findings of previous researchers.

Earlier studies proved presence of functional glutamate transporters and receptors in testes of rat (Gill *et al*., 2000; Takarada *et al*., 2004) and mice (Hu *et al*., 2004). Therefore, testes are considered as target organ for MSG. So, one of the mechanisms may be a direct effect of MSG via glutamate receptors and transporters of the epithelial cells of the seminiferous tubules thus, the changes recorded in the present investigation occurred.

The second mechanism was proved by other researchers (Gong *et al*., 1995; Giovambattista *et al*., 2003; Franca *et al*., 2006); it stipulates that there were neurotoxic effects of MSG on function of hypothalamus-pituitary-gonadal system. Gong *et al*. (1995) and Franca *et al*. (2006) reported that the central nervous system of MSG-treated animals showed neurogenic functional changes in the hypothalamus that induced a reduction in the levels of FSH, LH and testosterone. These hormones are essential for normal testes function and healthy spermatogenesis. Therefore, the present study suggested that the spermatogenic epithelium was affected indirectly via the hypothalamic lesions.

The third mechanism reported that exposure to MSG resulted in a decrease in the testicular ascorbic acid level that could lead to oxidative damage of rat testes (Nayanatara *et al*., 2008; Vinodini *et al*., 2008).

From the aforementioned mechanisms, it is clearly obvious that MSG may induce testicular damage mediated through not only one of the previous three mechanisms but may be via all these mechanisms together.

CONCLUSION

The structural changes of testes of the MSG-treated rats were found to be dosage-duration-dependent and ranged from slight to moderate damage in case of the short-term treatment however, severe damage was recorded in the case of the long-term treatment. The long-term daily oral doses of MSG revealed more deleterious effects on the spermatogenic cells and Sertoli cells than those induced by the short-term treatment. However, both kinds of treatment may lead into infertility problem in rats. Accordingly, using MSG as flavour enhancer should be reconsidered and it is the time to stop the slow poisoning of mankind via such flavour enhancers.

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