Assessment of DNA Damage in Testes from Young Wistar Male Rat Treated with Monosodium Glutamate

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Abstract: Monosodium glutamate (MSG), the sodium salt of glutamate, is a flavor enhancing food additive that may be present in packaged food without appearing on the label. This could lead to inadvertent consumption of monosodium glutamate in high concentrations. The study investigated the effects of MSG on testes of young male Wistar rats, by daily oral exposure to 8g /kg b.wt. of monosodium glutamate for 90 days. Wistar rats (n=24) of average weight of 65- 80g were randomly assigned into two groups A, and B in each group (n=12). The control group (A) received distilled water. The treatment group (B) were given oral doses each was 8g/kg body weight of monosodium glutamate by gavage on daily doses for 90 days. Rats were sacrificed on day 90 of the experiment. The testes were carefully dissected out and quickly fixed in Bouin`s for routine histological procedures. Results :The histological changes in the testes of rats due to the administrated MSG were mainly alterations of the seminiferous tubules which included atrophied malformed profiles ,appearance of hyaline material in the lumina of the seminiferous tubules and interstitial connective tissues .In addition sloughing and exfloliation of spermatocytes , spermatids, and immature germ cells appeared into the lumen of the seminiferous tubules .Many cells of the different types of spermatogenesis appeared with pyknotic nuclei and necrotic cells ,and dilated congested inter tubular blood vessels. Vacuolations were also observed between the inner cells of seminiferous tubules in the treated group. Increase in sperm shape abnormality was recorded . Furthermore, the alkaline Comet Assay showed significantly increased tail moment in testes cells of animals treated with MSG compared to control group. Conclusion: MSG may have some deleterious effects on the testes of Wister rats and by extension may contribute to the causes of male infertility. Thus, it is important to reconsider the usage of MSG as a flavor enhancer.

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1. Introduction

Monosodium glutamate, MSG, is a widely used flavor enhancing food additive that may be present in packaged foods without appearing on the label .This flavour enhancer, not very long ago, was isolated in the laboratory, and identified Monosodium Glutamate (MSG). At a later stage this flavour gained immense popularity worldwide. Since then it has been in use, widely in restaurants (particularly mixed in noodles, soups etc.), packaged food industries (e.g. instant meals) and household kitchens. Modern commercial MSG is produced by fermentation of starch, sugar, beet sugarcane or molasses **(Walker and, Lupien , 2000)**. This is particularly disturbing given the reported cases of MSG-induced adverse effect in animals **(Belluardo *et al.*,1990, Gonzalez-Burgos *et al.*,2004, Mozes *et al.*,2004)**, even at a relatively lower concentration **(Egbuonu *et al.*,2009)**. Although, MSG could improve the palatability of foods by exerting a positive influence on the appetite centre, it increased body weight **(Rogers and Blundell, 1990, Egbuonu *et al.*,2010). Fallarino *et al.* (2010)** demonstrated that Glutamate might affect neuroinflammation via effects on immune cells. However, in humans, adverse effects of MSG appear to manifest in MSG-sensitive individuals suggesting that some people may have an MSG intolerance that causes MSG symptom complex, with symptoms such as headaches, or migraine in some individuals. In addition, MSG gives rise to a characteristic taste called umami **(Yamaguchi and Ninomiya, 1998)**, which is one of the five taste qualities detected by mammals. Umami is not palatable in itself; nevertheless, it makes a variety of foods delectable **(Yamaguchi, 1998).**

Previous scientific investigations aimed at determining the effect of MSG on testes **(Das and Ghosh, 2010; Igwebuike, *et al.*,2011;Ihab, 2012)** .MSG caused a reduction in the sperm count (**Onakewhor *et al.*,1998; Giovambattista *et al.*, 2003;Nayanatara *et al.*, 2008)** . However, it couldn't get any literature regarding the histological studies of the testes and DNA damage in testes treatment of animals with MSG. So the present study was designed to investigate the effects of monosodium glutamate on the testes and cauda epididymal sperm reserves of young male rats. and DNA damage in testes.

2. Material And Methods

(a) Experimental animals

This study was performed on 24 young male Wistar rats, weighing about 65-80 g b.wt.. The animals were bred and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to food and water in the animal house. They were acclimatized for one week prior to the experiment and caged six per cage in a fully ventilated room at room temperature. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care.

(b) Tested compound

The chemical used monosodium glutamate (MSG) (C5H9NO4.Na) from Al-Dawlya Chemicals Co., Egypt with Purity > 98% NT., it was dissolved in distilled water, 1g of MSG in 1 ml of distilled water **(Nayanatara *et al.*,2008).** A stock solution was prepared by dissolving 8g of MSG crystals in 100 ml of distilled water (D.W.). The dose schedule was so adjusted that the amount of MSG administration per animal as per their respective weight.

(c) Experimental design

The animals were divided randomly into 2 groups; each included 12 rats.

Group 1(Control group): The rats were orally given distilled water /day for 90 days.

Group 2(Treated group): The rats were orally given 8g /kg b.wt /day for 90 days.

d) Histological procedures

At the end of the experimental period, the animals were sacrificed by ether overdose. The testes were excised, rinsed in physiological saline and fixed by immersion in Bouin's fluid for 24 hours. Later, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. The sections were cut at 5 µm thick, mounted on glass slides, and stained with hematoxylin and eosin and mounted with (DPX). Sections of testes were examined by light microscope.

e) Sperm shape abnormality assay

Caudal epididymis was collected from the rats, then sperm-shape abnormality was made according to the technique described by **Wyrobek and Bruce (1978).** The extracted caudal epididymis from rats were placed in a Petri-plate containing 1 ml of saline solution (0.9%Nacl) at room temperature. The epididymis was cut into small portions to allow the sperms to swim out. After that, smears were prepared using 3-4 drops of the solution, air dried overnight, fixed with absolute methanol for 15 min and stained with haematoxylin and eosin. 5 hundred sperm per animal were examined to determine the morphological abnormalities under oil immersion.

f) Comet Assay:

The comet assay was performed as described by **Singh *et al.* (1988)** with minor modifications. Regular agarose (RA) and low melting point agarose (LMPA) were prepared at 0.75% and 0.5% respectively in Ca^{++} and Mg^{++} free PBS, 110 µL of RA were added to fully frosted microscope slides. 75 µL of LMPA containing 105 cells were added. Finally, a top layer of 75 µL LMPA was added. Slides were immersed in lysis solution for at least 1 h at 4°C, and were then left in alkaline buffer for 15 minutes to allow the expression of alkali-labile sites and DNA unwinding. The slides were electrophoresed at 25V and 300 mA for 20 minutes, washed with neutralizing buffer and stained with ethidium bromide (2g/ml in distilled water).

Observations were made at 400X magnification using a fluorescent microscope (Olympus) equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. When possible, fifty cells per animal were analyzed for DNA migration. The tail length was measured from the trailing edge of the nucleus to the leading edge of the tail, using a calibrated scale in the ocular. The severity of DNA damage was measured comparing comet tail lengths (µm) with the diameter of the nucleus of undamaged cells observed in the same field. To determine the effect of malnutrition on the extent of DNA damage, two parameters were evaluated: the mean tail length of DNA migration, (compared by student t test) and the proportion of damaged cells, compared by the nonparametric Mann Whitney two samples test.

Statistical analysis

Data from control and treated animals were analyzed statistically to assess the significant differences using student's t-test. **(Fowler *et al.*, 1998)** was used for comparing the level of significance in the results between the MSG treated group and the untreated control.

3. Results

Histological examination of the testes:

Sections of testes of rats given a daily dose of MSG 8g/kg body weight for 90 days depicted severely damaged of seminiferous tubules .The following microscopical changes were considered as characteristic signs for the severely damaged tubules. The majority of somniferous tubules exhibited, severely atrophied, malformed somniferous tubules, and hyaline material in intertubular connective tissues. At the same time exfoliated spermatids appeared in the lumena due to cellular debris in some tubules and sloughing in

other tubules (Fig.2).Some seminiferous tubules manifested grade damage that included disorganization of spermatogenesis cells, hyaline material involved in between somniferous tubules, and the damaged germ cells lifting off the basal lamina(Fig.3). Abnormality shaped seminiferous tubules ,with hyalinizated in intertubular connective tissues were also manifested(Fig.4). Also some seminiferous tubules appeared with maturation arrest. Other tubules displayed marked elongated forms with hyaline material involved in their lumena. Many spematocyte appeared with pyknotic nuclei ,which acquired deeply basophilic stain ability. Also destruction of most spermatogenesis' layers with absence of spermatozoa was clearly recognized in other seminiferous tubule (Fig.5).Daily doses of MSG for 90 days revealed degenerative alteration in seminiferous tubules, maturation arrest in early and late stages of spermatids and numerous vacuoles of variable sizes in both the seminiferous tubules and interstitial connective tissues. Additionally, degenerated spermatogonia and spematocyte were common features (Fig.6--7).At the same time the nuclei of spermatognia and spematocytes exhibited signs of pyknosis and necrosis(Fig.6).Dilated congested blood vessels were also detected(Fig.7).

Sperm shape abnormality assay:

From the results obtained, it was clear that MSG caused highly significant ($P < 0.0001$) increase in the average of total sperm abnormalities,head,tail and head&tail as compared with control as shown in (Table1).Figs.(8&9,a-l) showed various morphological sperm-shape abnormalities in control and treated group. Tail abnormalities were included as disconnected tail (Fig.8). Head abnormalities were represented as banana, hookless, triangular, amorphous and double head (Fig. 9).

Comet Assay:

Comet assay revealed that, MSG induced statistically significant ($P<0.05$) increase in the average of the tail moment from (6.66±0.56) in control group to (13.01±3.9) in treated group, (Table 2).

Figure 10& table 2 show the distribution pattern of nuclear DNA expressed in the present cells as five comet classes from type 1 (undamaged) to type 5 (maximally damaged) in testes of rats treated with MSG. The results in Table 2 indicate that the administration of MSG decreased the level of undamaged cells when compared to control, average reached 30 ±1.58 & 50 ±2.52 respectively, and increased levels in type 2 and 3 (55.2 ±1.3 & 8.8±1.92) compared with control (45.8 ±1.92&4.2±1.7).Some cells nuclei showed high levels of DNA damaged (Category 4) the average reach 4 ± 0.7 and the other show maximally damaged (Categgory 5) recorded 2 ± 0.7.

Table (1): Showing sperm abnormalities in rats administered MSG for 90 .

Groups	Total abnormalities (M ± SD)	Average of abnormally spems/500		
		Head (M ± SD) %	Tail (M ± SD) %	H&T (M ± SD) %
Control	7.4 ±1	3.6 ± 0.5	2.4 ± 0.5	1.4 ± 0.6
Treated	21.8 ±2.6	9.2 ± 0.8	9.2 ± 0.9	3.4 ± 0.6
Percentage	208.1	155.6	323.8	142.9

Values are mean (±SD) of 5 rat.

Table (2): Showing tail moment and types of damaged DNA in rats administered MSG for 90 days.

Groups	Tail moment (M±SD)	Average of types of damaged DNA				
		Type 1	Type 2	Type 3	Type 4	Type 5
Control	6.66 ± 0. 6	50 ± 2.5	45.8± 1.9	4.2 ± 1.7	non	non
Treated	13.01 ± 3.9***	30 ±1.6***	55.2±81.3***	8.8 ± 1.9***	4 ± 0.7***	2 ± 0.7***
Percentage	95.43	- 40	20.5	11		

*** Significant at P value < 0.001

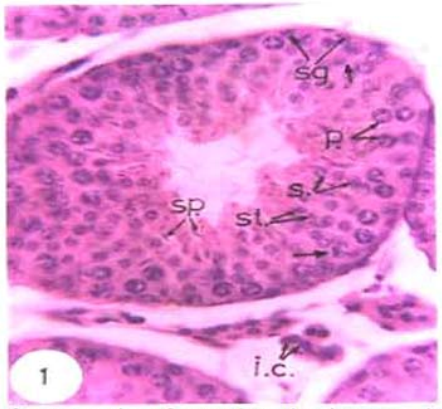
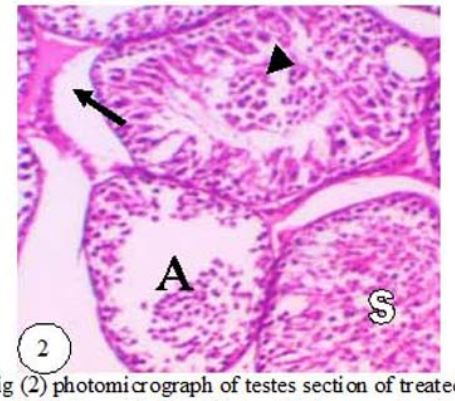


Fig (1) photomicrograph of testes section of control rat showing normal spermatogenesis progresstion, and interstitial cells. H.E X 200



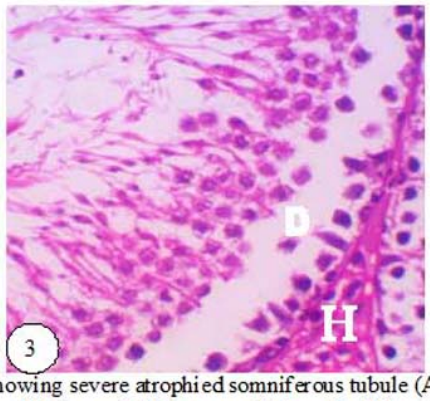
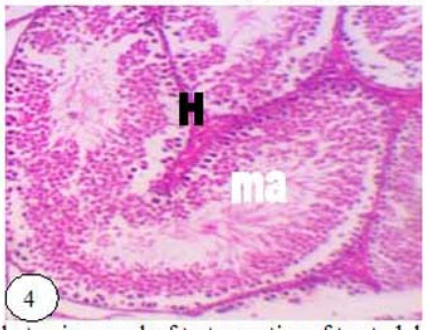


Fig (2) photomicrograph of testes section of treated showing severe atrophied somniferous tubule (A) hyalinization with loss of interstitial connective tissue cells (arrow).Also accumulation of cellular dipris within the lumina of some somniferous tubules (head) another appeared sloughed(s) (X200
Fig(3) photomicrograph of testes section of treated showing disorganization of spermatogenic cells an between the somniferous tubules(H)damaged germ cells are lifting off the basal lamina (D) (X400)



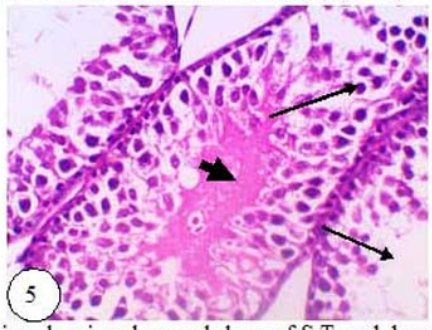


Fig (4photomicrograph of testes section of treated showing showing abnormal shape of S.T. and damag Interstitial cells with hyalinization (H).and S.T. showed maturation arrest (ma) (X100)
(5) Showing elongated, with hyalinization in lumina and marked reduction of spermatocytes (head) and Pyknosis of nuclei (arrow), destruction of most spermatogenesis layers and absence of spermatozoa oth (Short arrow). X400

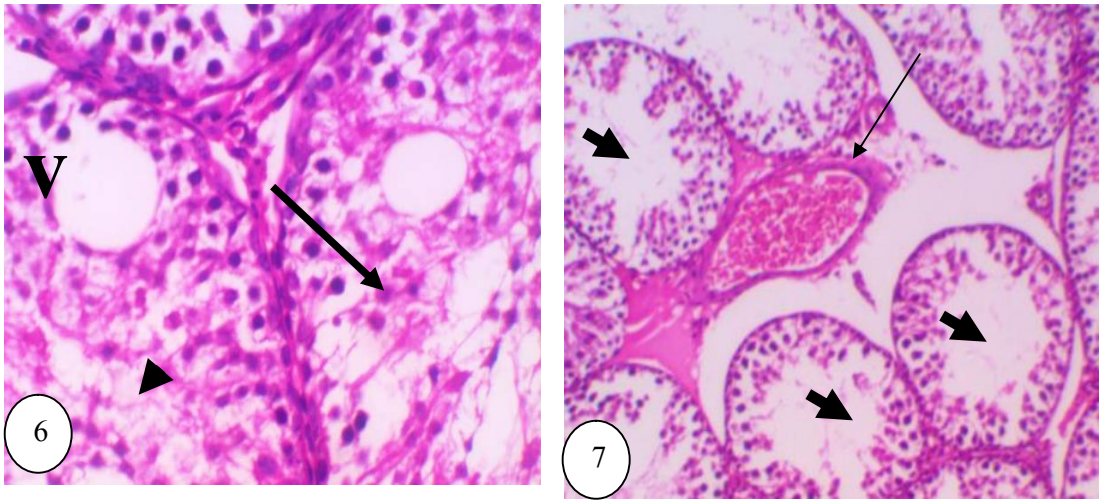


Fig (6): Photomicrograph of testes section of treated showing vaculation (v) in between the spermatogenic cells, reduction of spermatocytes with pyknotic nuclei (arrow) also absence of sperms (head). (x400)

Fig.(7):Photomicrograph of testes of treated showing dilated congested blood vessel. (Long arrow) loss of spermatids (x200)

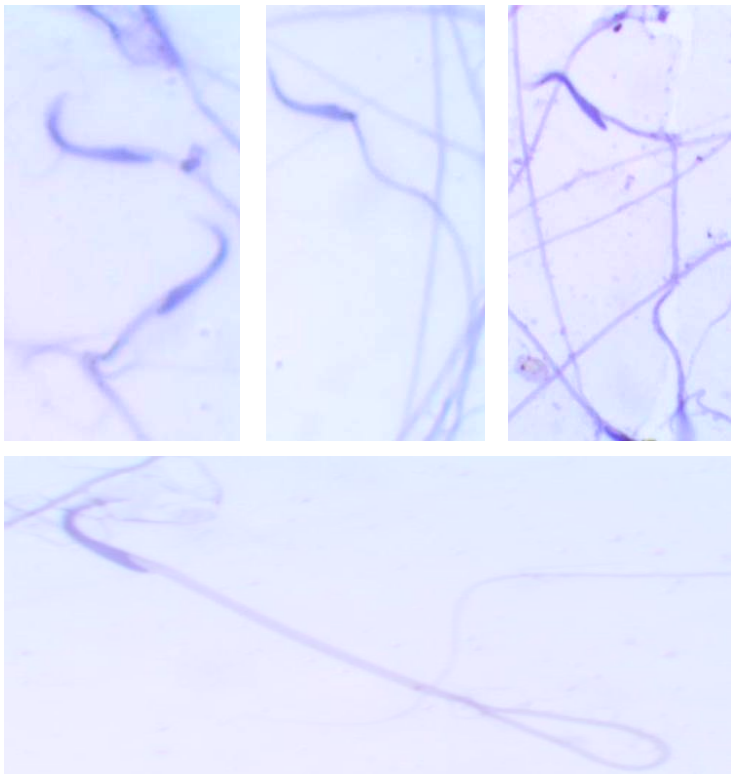


Fig. (8): Showing abnormal tail of sperms. X100

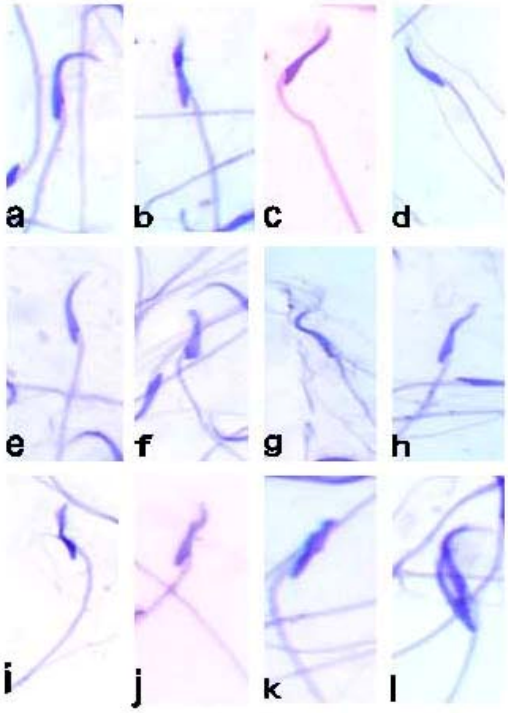


Fig. (9): Showing normal and abnormal head of sperms.(a) normal,(b,c &d))banana shape, (e) hook less, (f)triangular, (g,h,i,j&k)amorphous and (l) double headed . X1000

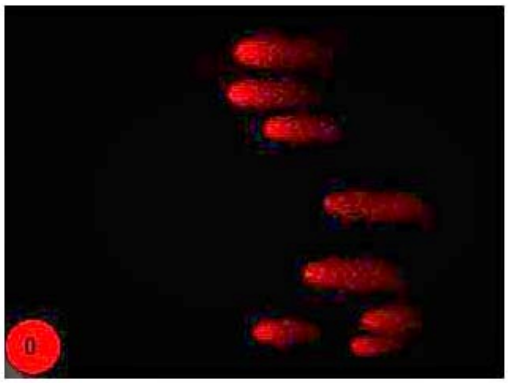


Fig. (10) Photograph comet essay of testes showing D.N.A damage induced after treatment of MSG for 90 days.

4. Discussion

Monosodium glutamate (MSG) is a widely used flavor enhancing food additive that may be present in packaged foods without appearing on the label. This could lead to inadvertent consumption of MSG above the average daily intake of 1.0 g in enlightened societies **(Marshal, 1994).** However, inadvertent abuse of this food additive may occur because of its abundance, mostly without labeling, in many food ingredients **(Egbuonu *et al.*, 2009).**

The histological changes evoked in the testes by the administrated MSG were mainly alterations of the seminiferous tubules which included atrophied seminiferous tubules ,appearance of

hyaline materiel in the lumena of seminiferous tubules and interstitial connective tissues. In addition sloughing and exfoliation of spermatocytes, spermatids, and immature germ cells appeared into the lumen of the somniferous tubules .Many nuclei of different types of spermatogenesis appeared pyknotic and necrotic ,and dilated congested inter tubular blood vessels, and vacuolations were also observed between the inner cells of seminiferous tubules in the treated group, accompanied by maturation arrest in others .

A great deel of changes recorded in the current investigation are in accordance to the histological studies that were carried out on the testes of defirent animals treated with MSG, ***Das and Ghosh*** **(2010)** found that the MSG induced histological changes in the testes of neonatal mice showed that both the germinal epithelium and Leydig cells were affected. **Mohamed (2012)** reported that the treatment with MSG at short-term exhibited slight to moderate damaged seminiferous tubules, included vacuoles were found inside the cytoplasm of spermatogonia and loss of late spermatids, shrinkage, widening of the spaces between the tubules . Long–terme treatment caused severe damage of germ cells and lage masses of necrotic cells were present in many tubules. Focusing on exfoliated, sloughing early spermatids and vacuolation in some damage of seminiferous tubules proved the presence of many signs of deterioration of these cells of tubules.

Present results were more or less in accordance with those encountered in the testicular tissue of rats after treatment with a toxic substance nitroso-dimethylamine (NDMA). **Attalla(1966)** suggested that these histological changes may be due to either local effect of the chemical or indirectly caused by imbalance in gonadotrophic hormones. **Balasubramanian *et al.* (1980)** explained the congestion of blood vessels as being due to the inhibition of prostaglandins synsthesis, since these compounds are known to be involved in regulation of testicular blood flow.**El-Deeb *et al.*(2000)** stated that vacuolation and exfoliation might be a sign of testicular toxicity and cell degeneration. The maturation arrest observed in the present study was explained by **(El-Zayat, 1988 , El-Wessemy,2007)** who correlated this arrest to the testosterone inhibition which caused stopping of spermatogenesis .

Previous research's have explained the mechanisms by which MSG inhibited the spermatogenesis in the current experiment. Glutamate receptors are present in different tissues: hypothalamus, spleen, thymus, liver, kidneys, endocrine system, ovaries, etc. (**Gill and Pulido, 2005; Gill *et al.,* 2008**).Earlier studies proved the presence of functional glutamate transporters and receptors in testes of rat (**Gill *et al.,* 2000. Takarada *et al.,* 2004) and (Hu *et al.,* 2004**) in mice. 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 . The second mechanism was proved by other researchers (**Gong *et al.,* 1995; Giovabattisa *et al.,* 2003**); that stipulates that there are neurotoxin effects of MSG on function of hypothalamus-pitutary-gonadal system. The effects of such toxicants on male reproduction may be anatomical or only functional, depending on whether they produce structural changes in the reproductive system, or merely affect the functions of the reproductive organs (**Witorsch, 1995**). **Franc *et al.* (2006)** ,reported that the central nervous system of MSG-treated animals showed neurogenic function in the levels of FSH,LH and testosterone .These hormones are essential for normal testes function and healthy spermatogenesis. In mammals, spermatogenesis is totally dependent upon testosterone (**Pakarainen *et al.,* 2005; Wang *et al.,* 2009**). Glutamate is a predominant excitatory neurotransmitter in the mammalians central nervous system (**Schlett, 2006; Greenwood and Connolly, 2007; Liguz-Lecznar and Skangiel-Kramska, 2007**). Such excessive activation of glutamate receptors and overloading with intracellular calcium can induce neural death (**Gil-Loyzaga *et al.,* 1993**). Therefore, the present study suggested that spermatogenesis was affected indirectly via the hypothalamic lesions. The ability of monosodium glutamate to damage nerve cells of the hypothalamus is a pointer to the fact that it may alter the neural control of reproductive hormone secretion via the hypothalamic-pituitary-gonadal regulatory axis. Such alterations in reproductive hormone secretion may adversely affect the reproductive capacity of the affected animals.

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.,* 2010),** and oxidative damage in different organ **(Moreno *et al.*, 2005; Farmobi and Onyemia, 2006; Pavlovic *et al.*, 2007)**

There was a significant reduction in the caudal epididymal sperm reserves ($P<0.05$) of the rats that received monosodium glutamate relative to the control rats. Present results agree with, previous studies found that animals treated with MSG revealed a reduction in sperm count and increased incidences of abnormal sperm (**Giovambattista *et al.,* 2003; Nayanatara *et al.,* 2008). Igwebuike *et al.*(2011)** showed that the indication is that the reduced caudal epididymal sperm counts observed in the MSG-treatment rats may be the end result of a considerable decline in the influence of testosterone on spermatogenesis in these rats.

Egbuonu *et al.* (2011) reported that exposure to high dose of monosodium glutamate (15 g/kg), such as through its inadvertent abuse, may alter lipid status in animals by damaging high metabolic organs, such as the liver.

From the above mentioned results, it was clear that the sperm shape abnormality assay and comet assay effected of treatment with MSG on rat testes the adverse effects of MSG could have been due to oxidative damage induced by MSG on this tissue. previous studies showed that administration of MSG induces oxidative damage and changes in the level of lipid peroxide (**Moreno *et al.*, 2005 Farmobi and Onyemia, 2006; Pavlovic *et al.*, 2007; Nayanatara *et al.*, 2008; Vinodini *et al.*, 2010**) .Lipid peroxidation (LP) is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenesis of many carcinogens. Free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce LP which considered a key process in many pathological events and is one of the reactions induced by oxidative stress (**Schinella *et al.*, 2002**).

Damage of DNA of the present study are similar to that obtained by some investigators using different treatments were recorded. **Dobrzynska (2007)** found that X-rays enhanced DNA damage in somatic and germ cells caused by acrylamide. Such damage in somatic cells, if not repaired or if repaired incorrectly can lead to mutation, cancer transformation or cell death in bone marrow, spleen, liver, kidney, lungs and testes . Damage in germ cells can affect the process of fertilization and lead to spontaneous abortion, congenital malformations and in heritable diseases, including cancer in the offspring (**Olsham *et al.*, 1993,Wyrobck *et al.*, 1997**). In particular, the integrity of germ cell DNA plays an important part in the transmission of genetic information to the offspring. DNA damage measured using the comet assay in human spermatozoa has been shown to be associated with infertility (Irvine et al.,2000).MSG has a toxic effect on many body organs by altering ionic permeability of neural membrane and induces persistent depolarization (**Robinson, 2006**).

The current study has been the first to the best of researchers' knowledge to describe the histological, sperm shape abnormality assay and comet assay of the rat testes that resulted from daily oral treatment with MSG for 90 days.However, treatment may lead into infertility problem in rats. Accordingly, using MSG as flavor enhancer should be reconsidered and it is the time to stop the slow poisoning of mankind via such flavors enhancers.

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