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Effects of Monosodium Glutamate on Semen Quality and the Cytoarchitecture of the Testis of Adult Wistar Rats

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ABSTRACT: Monosodium glutamate (MSG) is a widely used food additive and flavour enhancer. This study was conducted to determine the effects of monosodium glutamate on the micro-architecture of the testis and semen parameters of male Wistar rats. Thirty adult male Wistar rats weighing between 126-200g were randomly divided into five groups and varying doses of MSG solution was administered to the animals daily for 14 days: Group A: Control, received distilled water; Group B: received 250 mg/ml MSG; Group C: received 500 mg/ml MSG; Group D: received 1g/2ml MSG; and Group E: received 2 g/2ml MSG.
Results from the study revealed various degrees of alterations in both semen characteristics and testes of the treated adult Wistar rats. Abnormalities of semen characteristics which follow the consumption of monosodium glutamate is capable of causing impairment in male reproductive functions, and may propably be implicated in infertility. Hence, caution should be taken in the consumption of Monosodium glutamate.

Keywords: Monosodium glutamate; Semen parameters; Cytoarchitecture; Testis.

Introduction

Monosodium glutamate (sodium glutamate, or MSG**)** is a white crystal-like substance that is used commonly as a food additive and flavor enhancer, and it does seem to bring out more of the food's natural essences (Leung and Foster, 2003). It has also been used as a component of fertilizers, fungicides as well as cosmetics. The bacteria that aid the fermentation process that leads to the production of MSG have the ability to synthesize glutamic acid outside of their cell membranes and excrete it into the medium to accumulate there (Samuels,1999). When MSG is added to food, it provides a flavoring function similar to the naturally occurring free glutamate: which differs from the four classic tastes - sweet, sour, salty and bitter (Eweka and O'Iniabohs, 2008). Commercial production of monosodium glutamate requires large vast of harmless bacteria to convert glutamate from sugars or starches into glutamic acid. This acid is then allowed to evaporate, and the remaining brownish white or white crystals are sold as pure monosodium glutamate.

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Monosodium glutamate contains 78% of glutamic acid, 22% of sodium and water (Samuels,1999). The optimal palatability concentration for MSG is between 0.2 – 0.8% and its use tends to be self-limiting, as over-use decreases palatability; the largest palatable dose for human is about 60mg/kg body weight (Walker and Lupien 2000). Although many Food and Drug Control Agencies have certified MSG to be safe for human consumption without any specified dosage (Eweka and O'Iniabohs, 2008), 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 (Kuldip and Ahluwalia, 2002; Eweka, 2007; Vinodini et al, 2010). The mode of action of MSG through which it causes tissue damage might be related, however, to induction of oxidative stress (Farombi, 2006; Vinodini et al, 2010).

Monosodium glutamate is associated with disorders of the central nervous system, vascular diseases, and a number of neurodegenerative and neurobehavioural changes (Samuels,1999). Some of the adverse effects on the male gonad and reproduction include, oligozoospermia, increased percentage of abnormally shaped sperm cells, testicular haemorrhage and alteration in sperm count (Onakewhor et al, 1998; Oforofuo et al, 1997). Its effects on the testis is however dose-dependent. Furthermore, studies on the brain revealed that neonatal exposure to Monosodium glutamate results in impairment of learning ability (Olvera-Cortés et al, 2005), neuronal cell death and dendritic hypotrophy (González-Burgos et al, 2001).

The current study was aimed at knowing the effect of MSG on both semen parameters and the microarchitecture of the testis, with a view of determining a safe dose.

Materials and Methods

Monosodium Glutamate

The monosodium glutamate (Aji-no-moto®) was purchased at an area in Ilorin, Nigeria. Different weights of the monosodium glutamate was measured using the sensitive analytical weighing balance (Gallenkamp FA 2104A, England), and then dissolved in distilled water to make appropriate solution.

Experimental Design

Thirty rats were purchased from Homemade Research Institute, Ilorin, Nigeria. They were housed in different cages in the Animal House of the Anatomy Department, University of Ilorin, and allowed to acclimatize for two weeks. The environmental conditions for the breeding of the rats were kept at a relatively constant condition and normal room temperature, and maintained under a 12 h light/ 12 h dark cycle, with feeds and water available *ad libitum*.

The animals which weighed between 126-200g were randomly grouped into five, each group made up of four rats:

- Group A: Control, given 1 ml of distilled water
- Group B: treated with 250 mg of Monosodium glutamate solution
- Group C: treated with 500 mg of Monosodium glutamate solution
- Group D: treated with 1 g of Monosodium glutamate in 2 ml of solution
- Group E: treated with 2 g of Monosodium glutamate in 2 ml of solution.

Monosodium glutamate solution was administered orally to the animals using size 6 feeding tube and calibrated syringe (2.0 ml). Administration was carried out prior to feeding in the morning, and lasted for 14 days.

Animal Sacrifice

The animals were sacrificed by cervical dislocation 24 h after the last treatment. Dissection was through an anteromedian plane to expose the thorax, abdomen and pelvic region. The testes were excised and weighed. One

testis from each animal was fixed in 10% formal saline for about 48 hours prior to tissue processing, while the second testis was used for semen analysis.

Semen Analysis

The total number of spermatozoa was counted using the new improved Neuber's counting chamber (haemocytometer), expressed as number of sperm cells in millions/ml. The fluid from the caudal epididymis was diluted with Tris buffer solution to 0.5 ml, in order to determine sperm motility, which was expressed in percentage (%). Abnormal features of sperm morphology were observed and categorized as tail defects, neck and middle piece defects, and head defects; and the findings were expressed as percentage (%) of morphologically abnormal sperm.

Progressivity was graded as follows:

A- Excellent forward directional movement (EFDM)

B- Good forward directional movement(GFDM)

C- Fair forward directional movement(FFDM)

D- Poor forward directional movement(PFDM)

Statistical Analysis

The results were analysed statistically by application of Student's t-test, and presented as mean±SEM with determination of level of significance.

Results

Physical Observation

The treatment groups were more aggressive than the control group, and this was more in the animals given the highest dose of MSG solution. The animals in the treatment groups showed noticeable increase in food and water consumption compared to the control animals. This however did not translate directly into increased body weight of the treatment groups. The Treatment Groups had significantly reduced growth rate compared to animals in the Control Group, with the Group that received the highest concentration of the MSG solution having the least growth rate (Table 1). There were no gross morphological changes on physical examination of the testes.

Only slight variations was recorded in the sperm concentration of all the groups (Table 2). There was decreased sperm motility in all the treatment groups, with Group C given 500 mg/ml MSG solution having the least % motility (44.67±9.69; $p<0.05$). This same group had the highest number of dead sperm cells, as revealed by the lowest Life-Death ratio of 46.13±4.85. All the treatment groups however had increased in cell death compared to the Control, but not as much as Group C animals administered with 500 mg/ml MSG solution. Progressivity reduced equally with all the treatment groups having Fair Forward Directional Movement, compared to the Control which had an Excellent Forward Directional Movement.

Morphological observations showed different abnormal head shapes (curved, rounded) and tail lengths (short , long, double) especially in the group E that received the highest dose, which also had the highest percentage of abnormal morphology (40.00±0.64; $p<0.05$). Other treatment groups (B: 33.48±1.25; C: 35.50±1.71; D: 38.63±0.72) also had high percentages of abnormally shaped sperm cells which were statistically significant ($p<0.05$) compared to the Control group.

Table 1: Weights of the Experimental Animals

Groups	Final Weight (g)	Initial Weight (g)	Weight Difference (g)*	Testis weight (g)**	Testis-Body weight Ratio
A	171.50±3.97	153.00±3.90	18.50	1.257±0.184	0.00733
B	179.33±1.67	168.00±2.38	11.33	1.142±0.102	0.00635*
C	197.60±4.70*[1]	180.00±1.58	17.60	1.216±0.951	0.00621**
D	191.83±2.07*[1]	186.00±1.31	5.83	1.228±0.453	0.00637**
E	205.83±2.29*[1,2]	201.00±2.68	4.83	1.245±0.122	0.00583*

*p<0.05 (compared with Grp A: Control). [1]p<0.05(compared with Grp B). [2]p<0.05 (compared with Grp D)
**p>0.05 (compared with Grp A: Control). Mean±SEM.

Table 2: Semen Parameters

Groups	Sperm Motility (%)	Sperm Count ($x10^6$/ml)	Sperm Morphology (%)	Life-Death Ratio	Progressivity[++]
A	85.30±1.41	49.90±6.64	16.70±0.63	89.40±1.27	A
B	69.88±2.48**	46.10±3.72	33.48±1.25*	73.08±2.66	C
C	44.67±9.69*	49.20±2.34	35.50±1.71*	46.13±4.85	C
D	67.25±3.07**	52.45±1.55	38.63±0.72*	71.98±2.31	C
E	71.30±4.59**	51.00±3.10	40.00±0.64*	74.77±3.12	C

*p<0.05; **p>0.05 (compared with the Control Group A). [++]Progressivity: A- Excellent forward directional movement C- Fair forward directional movement

Histological Observation

Photomicrograph of the testes of animals in Group A, which is the Control, showed a normal cross section of the seminiferous tubules which clearly showed the stratified epithelium consisting of sperm cells and spermatogenic cells. It also showed a normal stroma consisting of interstitial Leydig cells. The microarchitectural organization of cells is well preserved. The tubules were regular and densely populated with spermatogonia (Figure 1).

Photomicrograph of the testes of Treatment Group B animals that received 250 mg/ml MSG solution showed slight distortion of tissue structures. The cells of the seminiferous tubules were disorganized and were closely adhered to each other. There was mild reduction in spermatogenic cells, stroma of the interstitial cells, and the luminal cells, when compared to the Control Group A. The Leydig cells were sparsely populated compared to those of the Control (Figure 2).

The animals treated with 500 mg/ml MSG solution (Group C) showed distortions in their testicular tissue also. There was disorganization of cells of the seminiferous tubules, with closer adherence to each other compared to the Control and Group B rats. The seminiferous tubules also revealed a moderate decrease in the number of spermatogonia and matured sperm cells when compared to other groups. The Leydig cells were visible but more sparsely populated compared to those of the Group B rats (Figure 3).

The photomicrograph of testes of animals in Group D that received 1g of MSG showed seminiferous tubules that were irregular and reduced in size, with a corresponding increase in interstitial connective tissue space. There was moderate decrease in the number of sperm cells in the lumen of the tubules, and further reduction in the population of Leydig cells compared to those of Group C rats (Figure 4).

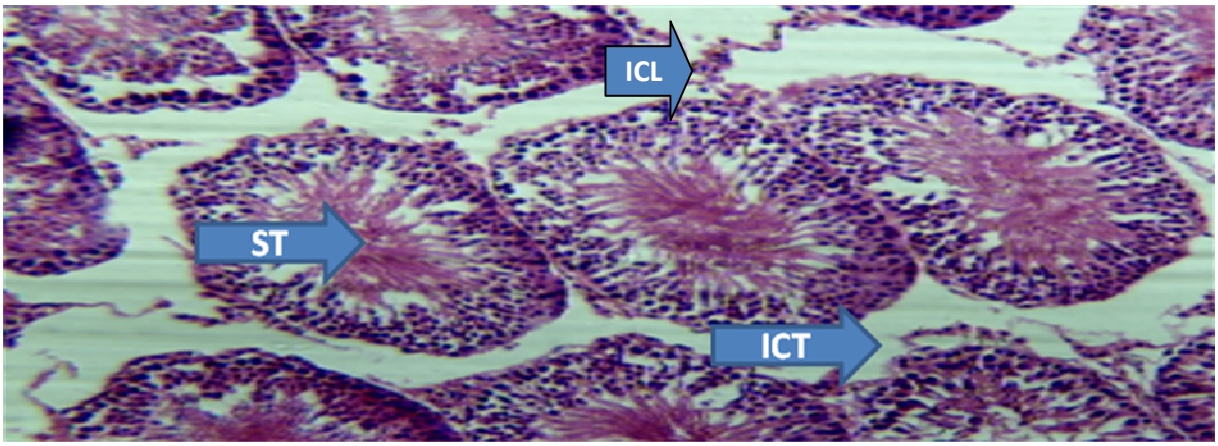

Figure 1: Photomicrograph of the transverse section of the testis of the Control Group showing normal cross section of the seminiferous tubules (ST), with stratified epithelium consisting of spermatogenic and non-spermatogenic cells; normal interstitial connective tissue (ICT) present consisting of interstitial Leydig cells (ICL). H&E ×100.

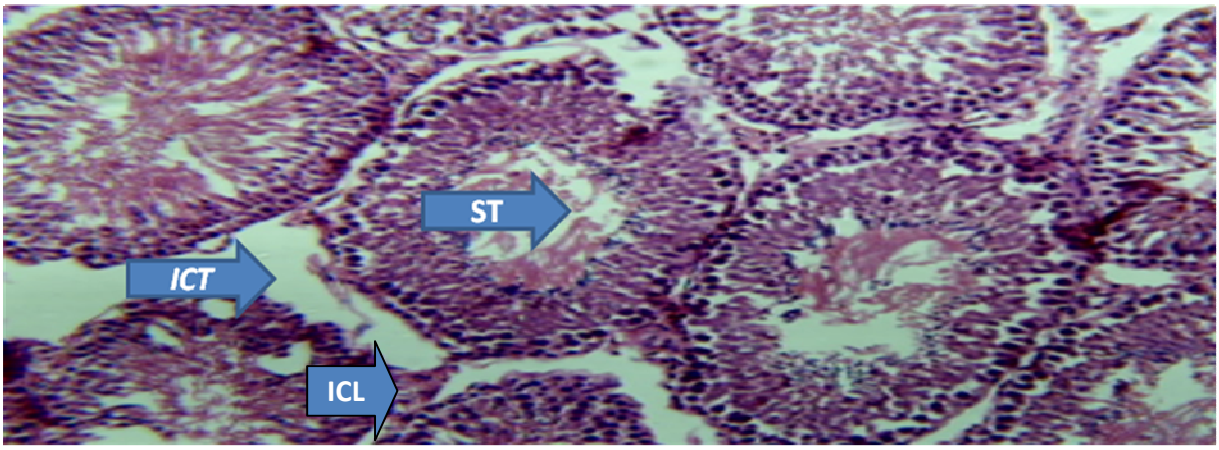

Figure 2: A transverse section through the testis of Treatment Group B given 250 mg/ml showing closely packed seminiferous tubules, mild reduction in spermatogenic cells, stroma of the interstitial cells and the luminal cells compared to the Control. The Interstitial cells of Leydig (ICL) were few compared to those of the Control (H&E ×100) **.**

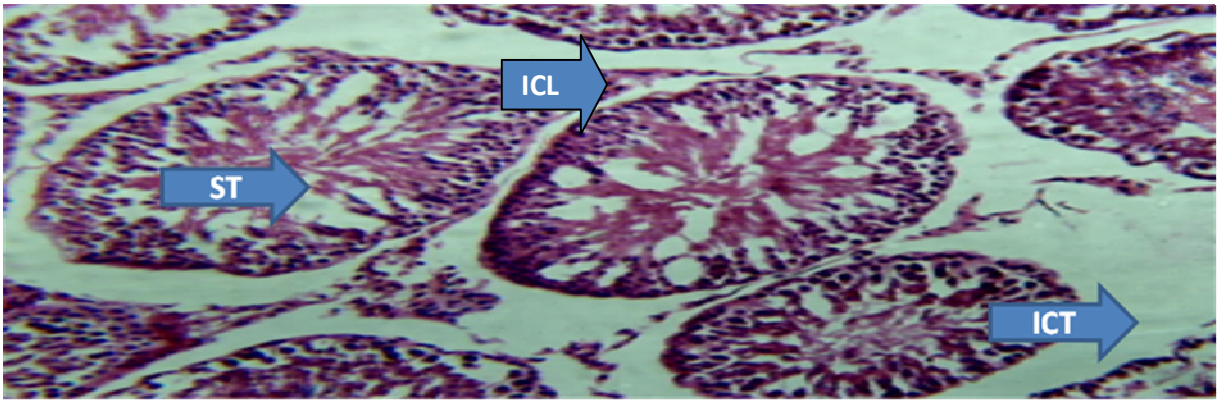

Figure 3: Photomicrograph of the transverse section of the testis of Treatment Group C animals treated with 500 mg/ml MSG showing seminiferous tubules with moderate decrease in the number of spermatogonia and matured sperm cells when compared to other groups; Interstitial cells of Leydig (ICL) were reduced compared to those of Group B rats (H&E ×100).

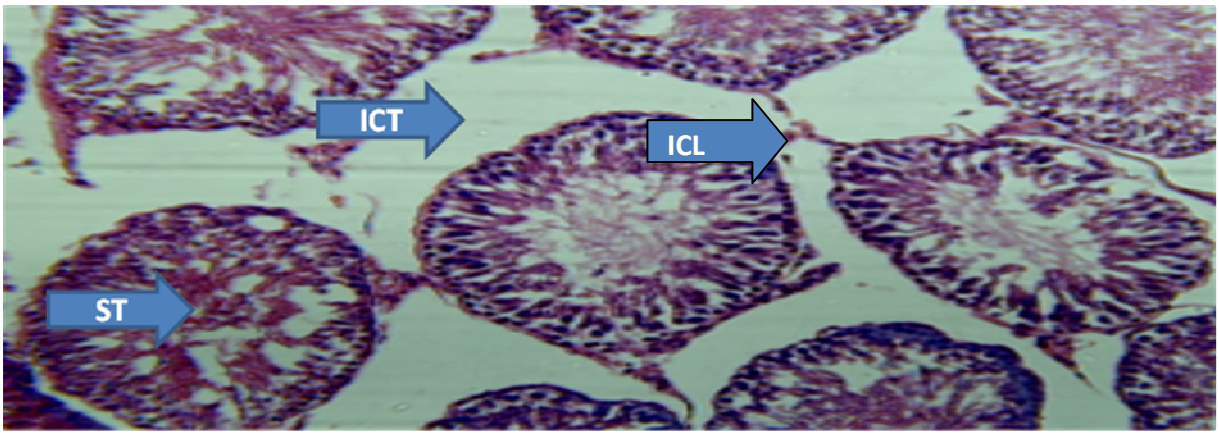

Figure 4: Photomicrograph of the transverse section of the testis of Treatment Group D animals treated 1 g/2ml MSG showing distortion of microarchitecture, reduction in size of seminiferous tubules and corresponding increase in interstitial connective tissue space (ICT). There is decrease in the number of sperm cells, and less population of Leydig cells (H&E x100).

The Photomicrograph of testes of Wistar rats in Group E given the highest dose of MSG (2 mg) revealed loss of connective tissue, irregularity of the seminiferous tubules, and marked reduction in the population of the sperm cells and spermatogonia in the tubules. The Leydig cells were visible but not as populated as those of the Control and other Treatment Groups (Figure 5).

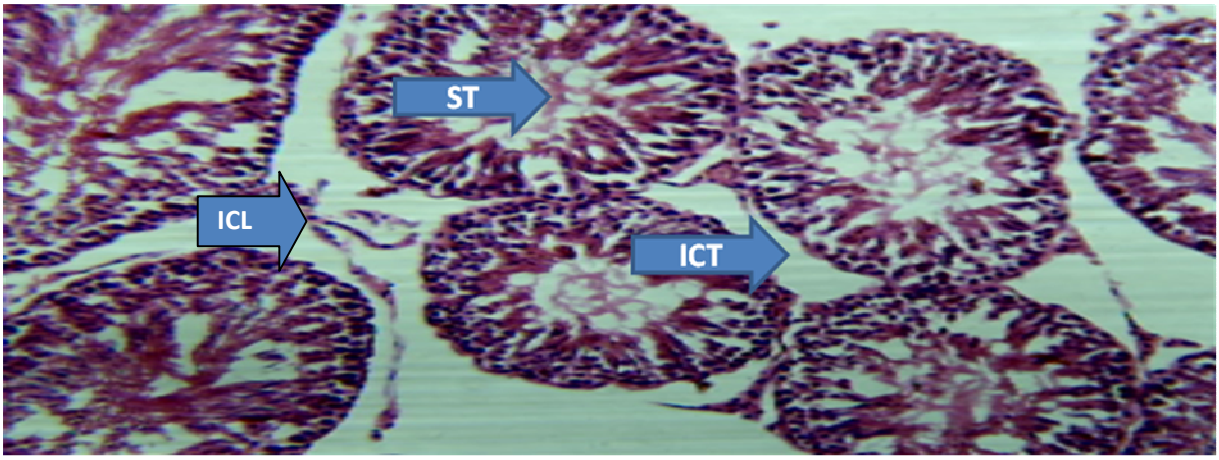

Figure 5: A transverse section through the testis of Treatment Group E given 2 g/2ml MSG showing loss of connective tissue and marked reduction in the cell population (H&E ×100)**.**

Discussion

Monosodium glutamate has been used as a food additive for decades and it is commonly marketed as a flavor enhancer. Through its stimulation of the orosensory receptors and by improving the palatability of meals, Monosodium glutamate influences the appetite positively, and induces weight gain (Rogers and Blundell,1990). In the current studies, the animals treated with Monosodium glutamate were noticed to have increased appetite, although this did not translate to increase in body weight, as the animals all had reduction in weight difference compared to the Control.

The photomicrograph of the control sections of the testes revealed normal histological features with the cross section of the highly convoluted seminiferous tubules showing stratified epithelium which consists of two distinct populations of clearly organized cells: the spermatogenic cells and the Sertoli cells. The Leydig cells within the supporting tissues in the interstitial spaces between the tubules were all visible.

The treatment sections of the testes showed some histological changes that were at variance with those obtained in the control. There was reduced concentration of the sperm cell population. It also showed a less convoluted seminiferous tubule, and various degrees of disorganization of the cells of the seminiferous tubules compared to the control group.

The use of Monosodium glutamate has been associated with various abnormalities in the microarchitecture of the testis as well as semen characteristics. Just as it was noted to cause marked distortion in the cytoarchitecture of cortical structure in the kidney and some degree of cellular necrosis, thereby affecting renal functions adversely (Eweka, 2007), consumption of Monosodium glutamate causes oligozoospermia, increased abnormal sperm morphology, and various degenerative changes (Onakewhor et al, 1998). It has deleterious effects on the Sertoli cells and Leydig cells of the testis, and consequently, adversely affects spermatogenesis, spermiogenesis and testosterone production in adult male Wistar rats (Oforofuo et al, 1997).

Consumption of high dose of monosodium glutamate resulted in damage to the testes. The viability and efficiency of the sperm also reduced due to distortion of the sperm characteristics and this can be a major cause of infertility in males. The actual mechanism by which MSG induced cellular degenerative and atrophic changes is not well understood, but induction of oxidative stress has been suggested (Farombi, 2006; Vinodini et al, 2010).

Degenerative changes caused by the administration of monosodium glutamate have been reported to result in cell death, by apoptosis and necrotic cell death; these two types of cell death differ morphologically and biochemically (Wyllie, 1980).

In conclusion, consumption of Monosodium glutamate could affect male fertility, due to its dose-dependent effects on sperm cells.

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