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Exposure of male rats to monosodium l-glutamate from prenatal life to adulthood: the effects on serum testosterone, cholesterol and proteins, serum enzymes, epididymal sperm reserves and testes

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ABSTRACT

This study investigated the effects of exposure of male rats to monosodium l-glutamate (MSG) from prenatal life to adulthood on the serum levels of testosterone, cholesterol and proteins, serum enzymes, epididymal sperm reserves and testicular allometric weights. Forty-eight albino rats (40 females and 8 males) were used as starting animals for the study. Being a generational study, the 48 mature albino rats eventually gave birth to the 64 male offspring which were used to conclude the study. Initially, the 40 starting females were randomly assigned into four groups (A, B, C and D) of 10 female rats each, while the 8 males were assigned to the four groups (2 for each) for mating. Females of groups A, B, C and D received 0.0 %, 0.5 %, 1.0 % and 2.0 % of MSG respectively in their drinking water. The treatment with MSG for the starting females, which was on a daily basis, started four weeks prior to the introduction of the mating males, and lasted throughout the mating period, pregnancy and delivery, and ended three weeks post-partum. The mating males did not receive MSG as the starting females had access to drinking water containing MSG during the day, and to the mating males during the night. Outside this mating period, the starting females had access to MSG in drinking water for 24 hours daily until 3 weeks post-partum. At the point of weaning (3 weeks post-partum), 64 male offspring (16 each from each of the four groups) were randomly assigned into 16 sub-groups of 4 male offspring each. The 16 male offspring from group A were divided into four sub-groups (A^1, A^2, A^3 and A^4), those of B were divided

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into four sub-groups (B^1, B^2, B^3 and B^4), those of C were also divided into four sub-groups (C^1, C^2, C^3 and C^4) and those of D were divided into four sub-groups (D^1, D^2, D^3 and D^4), all of 4 offspring per sub-group. From that point of weaning, the A^1, B^1, C^1 and D^1 sub-groups started receiving 0.0 % of MSG, A^2, B^2, C^2 and D^2 sub-groups started receiving 0.5 % of MSG, A^3, B^3, C^3 and D^3 sub-groups started receiving 1.0 % of MSG, and A^4, B^4, C^4 and D^4 sub-groups started receiving 2.0 % of MSG, all in their drinking water, until adulthood at 16 weeks of age. At 16 weeks of age, the serum levels of testosterone, cholesterol and total protein, serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed. Caudal epididymal sperm reserves and testicular allometric weights were also determined. The testes were dissected out for histomorphological studies. Results showed that only the sub-groups that were exposed to MSG from weaning age to adulthood (A^2, A^3 and A^4) had mean testosterone levels that were significantly ($P<0.05$) lower than that of the untreated control (A^1). The mean serum cholesterol levels of some of the treated sub-groups were significantly ($P<0.05$) lower than that of the untreated control. The mean caudal epididymal sperm reserves, testicular allometric weights, and serum ALT and AST activities of all the treatment sub-groups were significantly ($P<0.05$) lower than those of the untreated control (A^1). The mean serum total protein levels of groups A^3, A^4, B^1, B^2, C^1, C^4, D^1 and D^2 were significantly ($P<0.05$) higher than that of the untreated control (A^1). Although the mean serum total cholesterol levels of all the treatment sub-groups were lower than that of the untreated control (A^1), it was only the values of sub-groups B^2, C^2, C^3, C^4, D^1, D^2, D^3 and D^4 that showed a significant ($P<0.05$) decrease. No obvious lesions were observed on the testes of any of the sub-groups. It was concluded that exposure of rats to MSG at the doses used for the study from prenatal life to adulthood led to a significant reduction in serum testosterone and cholesterol levels, mean testicular allometric weights, cauda epididymal sperm reserves, serum activities of ALT and AST, and an increase in serum total protein.

Key words: monosodium l-glutamate, testosterone, cholesterol, proteins, rats

Introduction

Monosodium l-glutamate (MSG) is the sodium salt of the non-essential amino acid (glutamic acid) which is one of the most widely used food additives, especially in Asian and West African cuisine where it is incorporated to enhance flavor (EGBUONU et al., 2009). No sooner had MSG earned worldwide popularity than it also began to gain a poor reputation (GHOSH and DAS, 2010). It has been reported that its administration provokes hormonal alterations (MAGA, 1983; WALKER and LUPIEN, 2000; XU et al., 2005; OCHIOGU et al., 2015). Adult mice and rats treated neonatally with MSG have been reported to exhibit several endocrine and metabolic abnormalities caused by selective destruction of neurons in the arcuate nuclei of the hypothalamus (REDDING et al., 1971). However, there are conflicting reports in the literature concerning the effects of MSG on the reproductive axis (LAMPERTI and BLAHA, 1976). The placenta is considered virtually impermeable to glutamate (BATTAGLIA, 2000) since studies with both sheep and humans have shown that the placenta removes glutamate from fetal circulation, while concurrently supplying glutamine into the fetal circulation in very large amounts (LEMONS et al., 1976; HAYASHI et al., 1978). On the other hand, breast milk concentrations of glutamate are normally quite high, and are further influenced by ingestion of MSG (STEGINK et al., 1972). On this basis, there is concern about the safety of MSG when added to foods ingested by pregnant women and infants.

Developmental toxicology has been evolving as a discipline for decades, with only initial modest recognition despite the early knowledge that an excess of certain nutrients, such as vitamin A (SZABO, 1989) or administration of various chemicals, could cause developmental defects in various animal species (GILMAN et al., 1948). It took the revelation in the early 1960s that thalidomide - a drug promoted as a relatively innocuous sedative and antiemetic - was a potent human teratogen, to arouse interest in testing for potential developmental toxicants (LENZ, 1988).

The development of vertebrates proceeds in a sequence of carefully timed events that progress from the cellular level to the formation of tissues, organ systems, and morphological structures. Disturbances in any of the normal developmental processes can potentially alter subsequent growth and morphogenesis and result in congenital malformations. Adverse fetal outcomes from exposure to developmental toxicants are not limited to congenital malformations, but also manifest as functional deficits, growth retardation, and death (FAWCETT and BRENT, 2006). A series of three protocols has been designed to evaluate test agents for their effects on developing mammals, with the intent of protecting humans and animals exposed to pharmaceuticals, food additives, pesticides, workplace chemicals, and environmental pollutants. These protocols were developed for the purpose of assessing: (1) the outcomes on the conceptus of maternal exposures, beginning prior to mating and ending prior to implantation, (2) exposures during major organogenesis, and (3) exposures during late gestation, parturition, and lactation (HOOD, 2006).

The conflicting reports in the literature concerning the effects of MSG on the reproductive axis (LAMPERTI and BLAHA, 1976) are probably due to differences in the experimental designs. Despite the importance of MSG as a food additive and a substance naturally present in many foods, and the very large amount of research associated with it (RAITEN et al., 1995), relatively little is known about its effects on individuals who are exposed to it from prenatal to adult life. This study was therefore designed to evaluate the effects of prenatal to adulthood exposure to MSG on the levels of serum testosterone, cholesterol, proteins, liver enzymes activities, epididymal sperm reserves and testicular allometric weights.

Materials and methods

Experimental animals and experimental design. Forty-eight albino rats (40 females and 8 males) were used as starting animals in this study. Since this was a prenatal to adulthood study, these starting animals eventually gave birth (in the course of the study) to the 64 males that were the primary focus of the study. The starting animals were acquired from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Both the initial rats and their off-spring were housed at the

same location where they were acquired during the course of the study. Throughout the duration of the study, they were kept in standard, clean rat cages and were fed *ad libitum* with pelletized feed (Grand Cereals and Oil Mills Limited, Jos-Nigeria), containing 16 % crude protein. They were also provided with drinking water that contained specific concentrations of MSG.

Initially, the 40 starting females were randomly assigned to four groups (A, B, C and D) of 10 rats each. The 8 males were also randomly assigned to four groups of 2 rats each for mating with the females of groups A, B, C and D. Group A females, which served as the untreated control, were not given MSG (0.0 % administration). Females of groups B, C and D received in their drinking water 0.5 %, 1.0 % and 2.0 % of MSG, respectively. Treatment with MSG for the starting females, which was on a daily basis, started 4 weeks prior to the introduction of the mating males, and lasted throughout the mating period, pregnancy and delivery, and ended 3 weeks post-partum (the weaning period). None of the mating males received MSG, either prior to or during the mating period. They had access to the females for mating between 6.00 pm and 6.00 am each day as the mating period lasted. Within this period, drinking water that contained the MSG was withdrawn at 6.00 pm, and re-introduced at 6.00 am after the withdrawal of the mating males. In summary, the starting females had access to MSG during the day and to the mating males during the night. Outside this mating period, the starting females had access to drinking water containing varied doses of MSG for 24 hours in the day during the 4 weeks prior to the mating period, and during pregnancy and delivery, and until 3 weeks post-partum.

At the point of weaning (3 weeks post-partum), 16 male offspring were randomly selected from the offspring of each of the four groups of females (A, B, C and D). The 16 selected male offspring from each of the four groups were further randomly assigned into four sub-groups, whereby from group A females we had sub-groups A^1, A^2, A^3 and A^4 of male offspring; from group B females there were sub-groups B^1, B^2, B^3 and B^4 of male offspring; from group C females there were sub-groups C^1, C^2, C^3 and C^4 of male offspring; and from group D females there were sub-groups D^1, D^2, D^3 and D^4 of male offspring. The sub-groups A^1, B^1, C^1 and D^1 male offspring were not given MSG (0.0 % administration) from the point of weaning to adulthood. The sub-groups A^2, B^2, C^2 and D^2 male offspring were given 0.5 % MSG in drinking water from the point of weaning to adulthood, while the sub-groups A^3, B^3, C^3 and D^3 male offspring were given 1.0 % MSG in drinking water from the point of weaning to adulthood. Finally, the sub-groups A^4, B^4, C^4 and D^4 male offspring were given 2.0 % MSG in drinking water from the point of weaning to adulthood.

The 64 male offspring that made up the sub-groups continued to receive their sub-group-specific concentrations of MSG in drinking water *ad libitum* starting from 3 weeks weaning age until 16 weeks, that is adulthood. At 16 weeks of age, the serum

levels of testosterone, total cholesterol and total protein, and serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated. The caudal epididymal sperm reserves and allometric weight of the testes (testicular weight to body weight ratio) were also determined. Finally, the testicular organs were dissected out and routinely processed for histomorphological studies.

The test compound used in this study was the Vedan® brand of MSG ($C_5H_8NNaO_4.H_2O$), containing 99 % MSG, manufactured by the Vedan Enterprise Corporation, Taiwan. The MSG was dissolved in drinking water at the rate of 0.5, 1.0 and 2.0 g, made up to 100 mL volume of drinking water for groups B, C and D respectively. The group A females were not given MSG (0.0 % administration) as the untreated controls.

The serum testosterone levels were assayed using the Accu-Bind testosterone test kit (Monobind Inc., Lake Forest, USA) based on the enzyme-linked immunosorbent assay (ELISA) technique (EKINS, 1998), while the serum levels of total cholesterol were evaluated using the Quimica Clinica Applicada (QCA) serum total cholesterol test kit (QCA, S. A. Spain) based on the enzymatic colorimetric method (ALLAIN et al., 1974). Also, the serum activities of ALT and AST were evaluated following the Reitman and Frankel colorimetric method (REITMAN and FRANKEL, 1957) for *in vitro* determination of ALT and AST in serum, using a Quimica Clinica Applicada (QCA) test kit (Quimica Clinica Applicada, Spain). The serum total protein levels were determined following the direct Biuret method (LUBRAN, 1978) using Quimica Clinica Applicada (QCA) Total Protein test kit (QCA, S. A. Spain).

The cauda epididymal sperm reserves were determined following the standard hemocytometric method (AMMAN and ALMQUIST, 1961). The body weights and testicular weights of the rats were determined using a weighing balance, and testicular allometric weights were calculated by dividing the testicular weight of each rat by its body mass. The dissected testes from representatives of each of the 16 sub-groups of the male offspring were fixed by immersion in Bouin's fluid for 48 hours. Later, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Sections were cut, five micrometers thick, mounted on glass slides, and stained with hematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd. 1999-2004).

The housing, handling and welfare of the rats used for the study were in accordance with the Ethics and Regulations guiding the use of research animals, as approved by the University of Nigeria, Nsukka.

Data generated from the study were subjected to one-way analysis of variance (ANOVA). Variant means were separated using the least significant difference (LSD) method. Significance was accepted at a probability level less than 0.05.

Results

The mean serum testosterone levels of rats in sub-groups A^2, A^3 and A^4 were significantly ($P<0.05$) lower than those in rats in sub-group A^1 (untreated control), while the mean serum cholesterol levels of rats in sub-groups B^2, C^2, C^3, C^4, D^1, D^2, D^3 and D^4 were significantly ($P<0.05$) lower than those in rats in sub-group A^1 (Table 1). The mean cauda epididymal sperm reserves of all the treated sub-groups were significantly ($P<0.05$) lower than in sub-group A^1 (untreated control), with the A^2, A^3 and A^4 sub-groups having the lowest sperm reserves (four times lower than that of A^1) among the treated sub-groups (Table 1). The mean testicular allometric weights of all the treated sub-groups (except sub-group D^1) were also significantly ($P<0.05$) lower than those of sub-group A^1 (Table 1).

The mean serum activities of ALT and AST of all the treated sub-groups were significantly ($P<0.05$) lower than those of the sub-group A^1, but the mean serum total protein levels of rats in sub-groups A^3, A^4, B^1, B^2, B^3, B^4, C^1, C^4, D^1 and D^2 were significantly ($P<0.05$) higher than sub-group A^1 (Table 2).

The histological sections of the testes of the male rats sub-groups (treated and untreated) showed no obvious lesions; they had normal seminiferous tubules and interstices (Figs 1, 2 and 3). There were no differences between the treatment groups in the histological appearance of the sections of their testes.

Table 1. Serum levels of testosterone and cholesterol, epididymal sperm reserves and testicular allometric weights of rats exposed to monosodium L-glutamate from prenatal life to adulthood.

Sub-groups and their treatments	Means of parameters ± standard error			
	Serum testosterone (ng/mL)	Serum total cholesterol (mg/dL)	Epididymal sperm reserves (10^6)	Testicular allometric weight (10^{-2})
SG A^1 (Untreated Control, 0.0 % MSG both PN and PW)	4.96 ± 1.04^{a}	108.34 ± 4.81^{a}	95.00 ± 4.99^{a}	1.46 ± 0.04^{a}
SG A^2 (0.0 % MSG PN, 0.5 % MSG PW)	2.68 ± 0.39^{bc}	87.50 ± 4.17^{ab}	22.00 ± 3.69^{de}	1.22 ± 0.08^{bcd}
SG A^3 (0.0 % MSG PN, 1.0 % MSG PW)	2.27 ± 0.30^{c}	91.67 ± 4.81^{ab}	22.00 ± 4.75^{de}	1.17 ± 0.06^{bcd}
SG A^4 (0.0 % MSG PN, 2.0 % MSG PW)	2.16 ± 0.39^{c}	95.67 ± 7.84^{ab}	20.00 ± 3.61^{e}	1.05 ± 0.03^{cde}
SG B^1 (0.5 % MSG PN, 0.0 % MSG PW)	4.88 ± 1.07^{a}	95.83 ± 4.17^{ab}	46.00 ± 3.53^{bc}	1.20 ± 0.06^{bcd}
SG B^2 (0.5 % MSG PN, 0.5 % MSG PW)	4.49 ± 0.91^{ab}	79.17 ± 7.97^{b}	47.00 ± 9.70^{bc}	1.05 ± 0.05^{cde}
SG B^3 (0.5 % MSG PN, 1.0 % MSG PW)	4.02 ± 0.09^{abc}	87.50 ± 4.17^{ab}	35.00 ± 5.80^{bcde}	0.97 ± 0.04^{e}
SG B^4 (0.5 % MSG PN, 2.0 % MSG PW)	3.76 ± 1.19^{abc}	87.50 ± 7.98^{ab}	38.00 ± 7.01^{bcd}	1.07 ± 0.10^{cde}
SG C^1 (1.0 % MSG PN, 0.0 % MSG PW)	3.83 ± 0.38^{abc}	91.67 ± 4.81^{ab}	49.00 ± 8.41^{b}	1.24 ± 0.10^{bc}
SG C^2 (1.0 % MSG PN, 0.5 % MSG PW)	3.62 ± 0.64^{abc}	79.17 ± 12.03^{b}	32.00 ± 4.61^{bcde}	1.10 ± 0.04^{cde}
SG C^3 (1.0 % MSG PN, 1.0 % MSG PW)	3.39 ± 0.61^{abc}	83.33 ± 6.80^{b}	38.00 ± 11.00^{bcd}	1.03 ± 0.07^{de}
SG C^4 (1.0 % MSG PN, 2.0 % MSG PW)	3.38 ± 0.46^{abc}	83.43 ± 6.72^{b}	33.00 ± 6.13^{bcde}	1.06 ± 0.07^{cde}
SG D^1 (2.0 % MSG PN, 0.0 % MSG PW)	3.15 ± 0.39^{abc}	83.34 ± 9.62^{b}	46.00 ± 5.95^{bc}	1.32 ± 0.07^{ab}
SG D^2 (2.0 % MSG PN, 0.5 % MSG PW)	3.17 ± 0.60^{abc}	85.42 ± 12.90^{b}	39.00 ± 4.93^{bcd}	1.08 ± 0.11^{cde}
SG D^3 (2.0 % MSG PN, 1.0 % MSG PW)	3.90 ± 0.47^{abc}	83.52 ± 6.65^{b}	40.00 ± 3.89^{bc}	1.12 ± 0.10^{cde}
SG D^4 (2.0 % MSG PN, 2.0 % MSG PW)	3.25 ± 0.42^{abc}	79.17 ± 10.49^{b}	30.00 ± 4.05^{cde}	1.04 ± 0.06^{cde}

$^{a, b, c, d, e}$ Different alphabetical superscripts in a column indicate significant differences between the means, $P<0.05$. MSG = Monosodium L-glutamate; PN = Prenatal exposure; PW = Post-weaning exposure; SG = Sub-group.

Table 2. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and total protein of rats exposed to monosodium L-glutamate from prenatal life to adulthood.

Sub-groups and their treatments	Means of parameters ± standard error		
	Serum ALT (IU/L)	Serum AST (IU/L)	Serum total protein (mg/dL)
SG A[1] (Untreated Control, 0.0 % MSG both PN and PW)	67.27 ± 2.85[a]	93.53 ± 0.17[a]	6.14 ± 0.14[a]
SG A[2] (0.0 % MSG PN, 0.5 % MSG PW)	56.20 ± 1.93[bc]	85.60 ± 1.50[b]	6.25 ± 0.18[ab]
SG A[3] (0.0 % MSG PN, 1.0 % MSG PW)	54.19 ± 1.93[bc]	84.31 ± 1.30[bc]	6.68 ± 0.11[cd]
SG A[4] (0.0 % MSG PN, 2.0 % MSG PW)	53.19 ± 3.38[c]	85.67 ± 1.46[b]	6.57 ± 0.25[bcde]
SG B[1] (0.5 % MSG PN, 0.0 % MSG PW)	54.22 ± 1.81[bc]	85.62 ± 1.48[b]	6.48 ± 0.10[bcde]
SG B[2] (0.5 % MSG PN, 0.5 % MSG PW)	55.19 ± 1.64[bc]	84.31 ± 1.30[bc]	6.55 ± 0.16[bcde]
SG B[3] (0.5 % MSG PN, 1.0 % MSG PW)	55.15 ± 1.54[bc]	77.82 ± 3.00[c]	6.50 ± 0.40[abcd]
SG B[4] (0.5 % MSG PN, 2.0 % MSG PW)	54.69 ± 2.65[bc]	83.01 ± 3.67[bc]	6.43 ± 0.25[abcd]
SG C[1] (1.0 % MSG PN, 0.0 % MSG PW)	57.21 ± 1.16[bc]	85.60 ± 2.59[b]	6.64 ± 0.40[bcde]
SG C[2] (1.0 % MSG PN, 0.5 % MSG PW)	53.18 ± 2.01[c]	81.73 ± 2.48[bc]	6.03 ± 0.11[a]
SG C[3] (1.0 % MSG PN, 1.0 % MSG PW)	51.67 ± 1.27[c]	81.71 ± 1.30[bc]	6.18 ± 0.21[ab]
SG C[4] (1.0 % MSG PN, 2.0 % MSG PW)	51.53 ± 1.29[c]	81.77 ± 3.26[bc]	6.86 ± 0.08[cd]
SG D[1] (2.0 % MSG PN, 0.0 % MSG PW)	60.23 ± 1.93[b]	83.01 ± 0.00[bc]	6.95 ± 0.09[c]
SG D[2] (2.0 % MSG PN, 0.5 % MSG PW)	53.69 ± 2.23[c]	81.69 ± 1.33[bc]	6.96 ± 0.14[c]
SG D[3] (2.0 % MSG PN, 1.0 % MSG PW)	57.21 ± 3.86[bc]	80.41 ± 4.97[bc]	5.97 ± 0.10[ab]
SG D[4] (2.0 % MSG PN, 2.0 % MSG PW)	52.18 ± 1.01[c]	79.12 ± 3.26[bc]	6.00 ± 0.17[ab]

[a, b, c, d] Different alphabetical superscripts in a column indicate significant differences between the means, $P<0.05$.
[MSG = Monosodium L-glutamate; PN = Prenatal exposure; PW = Post-weaning exposure; SG = Sub-group].

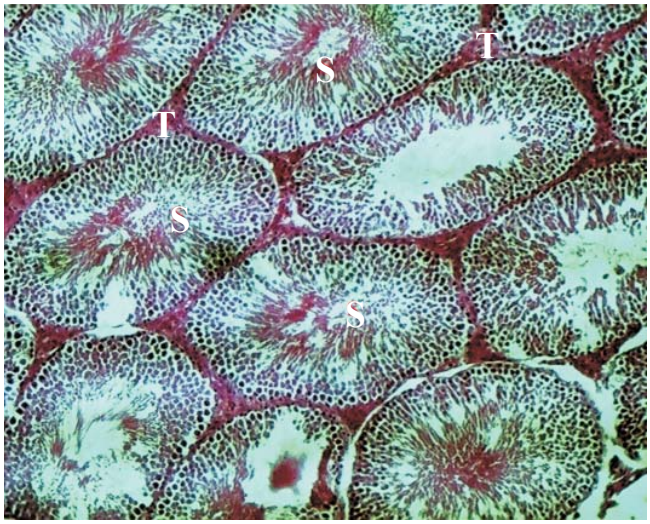

Fig. 1. A representative histological section of the testes of albino rats in sub-groups B*, showing no obvious lesions. Note active seminiferous tubules (**S**) and interstitial spaces (**T**) (H&E; ×100).
* Sub-groups B rats were the off-spring of mother-rats that received 0.50 % of MSG.

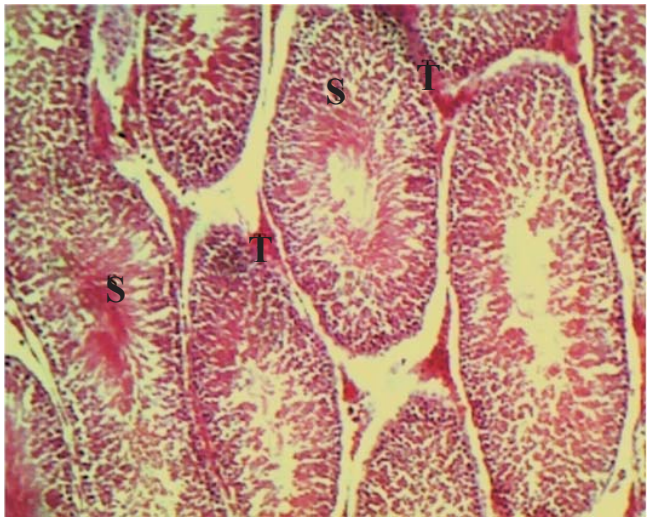

Fig. 2. A representative histological section of the testes of albino rats in sub-groups C*, showing no obvious lesions. Note active seminiferous tubules (**S**) and interstitial spaces (**T**) (H&E; ×100).
*Sub-groups C rats were the off-spring of mother-rats that received 1.00 % of MSG.

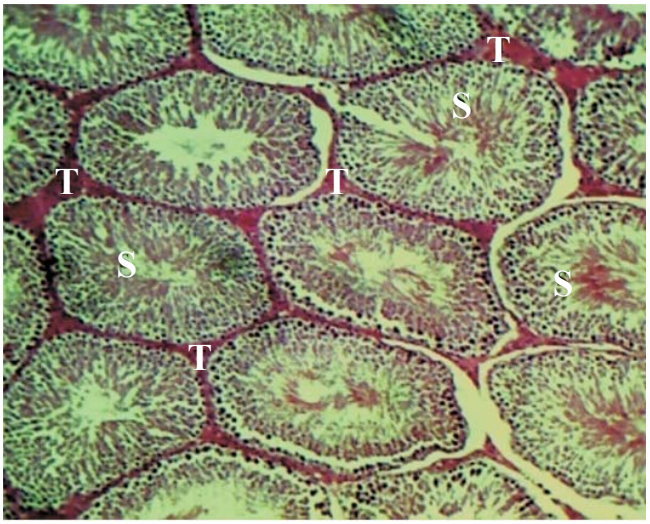

Fig. 3. A representative histological section of the testes of albino rats in sub-groups D*, showing no obvious lesions. Note active seminiferous tubules (**S**) and interstitial spaces (**T**) (H&E; ×100).
* Sub-groups D rats were the off-spring of mother-rats that received 2.00 % of MSG.

Discussion

The effect of MSG administration on the serum testosterone levels of the rats was noteworthy as only rats in sub-groups A^2, A^3 and A^4, which were only exposed to MSG from 3 weeks of post-natal life, had a significant reduction of their serum testosterone levels, which is in contrast with other sub-groups (the offspring of groups B, C and D females) which were exposed to varied doses of MSG from prenatal life. These sub-groups of groups B, C and D female offspring did not show any significant variations in their serum testosterone levels when compared with the untreated control group (A^1). The reduced serum testosterone levels in sub-groups A^2, A^3 and A^4 is in agreement with our previous reports (IGWEBUIKE et al., 2011; OCHIOGU et al., 2015), whereas the non-reduction in the serum testosterone levels of all the offspring of groups B, C and D contradicts these previous reports. Withdrawal of MSG exposure at 3 weeks weaning age from groups B^1, C^1 and D^1 did also not cause significant variations in their serum testosterone levels when compared with the other groups that continued to receive the treatment up until adulthood. It thus seems that following the early and long exposure of these rats to MSG, they became less responsive to its effects, thereby resulting in their testosterone levels being not significantly different from those of the untreated control group (A^1). It is also possible that in these rats in sub-groups from female groups B, C and D, whose offsprings' serum testosterone levels were not significantly affected, there may have been a compensatory mechanism from other nuclei of the hypothalamus, such as

ventral medial and pre-optic nuclei, which, apart from the arcuate nucleus reported to be destroyed by MSG, can produce GnRH (PALKOVITZ et al., 1974; WHEATON et al., 1975). Since GnRH is also produced in these other hypothalamic nuclei, LH and invariably testosterone release may not have been adversely inhibited. This is in agreement with the report of JECFA (1988). It is therefore worthy of note from this study that both the timing and duration of exposure to MSG may be responsible for the conflicting reports associated with MSG administration (LAMPERTI and BLAHA, 1976).

The significantly lower levels of serum cholesterol recorded for some of the treated sub-groups in this present study are in agreement with the reports of OCHIOGU et al. (2015). The lower serum levels of cholesterol correlated with the lower levels of serum testosterone in these treated sub-groups. It is believed that the lowered levels of serum cholesterol contributed to the lowered levels of serum testosterone, as cholesterol is a precursor of testosterone (STOCCO, 1998). This is further in consonance with the fact that the arcuate nucleus of the hypothalamus functions in a regulatory manner towards fat metabolism (AHLUWALIA and MALIK, 1989). This decreased serum cholesterol is in agreement with the report of BAZZANO and OLSON (1969).

The relatively higher severity of reduction in caudal epididymal sperm reserves in the treated sub-groups of group A (A^2, A^3, and A^4), which were not exposed prenatally to MSG when compared with other male offspring sub-groups of groups B, C and D that were exposed prenatally, suggests that prenatal exposure may have triggered compensatory resistance to the adverse effects of MSG on spermatogenesis in these sub-groups that manifested in higher spermatogenic output, and invariably the relatively higher cauda epididymal sperm reserves. This line of thought is in agreement with the records of their near normal testosterone levels which were not significantly different from those of the untreated control. The near normal testosterone levels in these rat sub-groups prenatally exposed to MSG was however not enough to quantitatively restore full spermatogenesis in these rat groups, without GnRH treatment, which is needed to stimulate endogenous gonadotrophins - LH and FSH (FINKEL et al., 1985; WEINBAUER and NIESCHLAG, 1990) as full quantitative restoration of spermatogenesis is usually accomplished by these gonadotrophins.

Another important finding of this study, which appears to be the most interesting, is that the mean testicular allometric weights of all the rat sub-groups (except sub-group D^1) exposed to MSG, either prenatally or from weaning age, were significantly lower than those of the untreated control (A^1). This was a manifestation of hypogonadism and as such additionally explains why the mean cauda epididymal sperm reserves of those sub-groups were significantly reduced. This observed hypogonadism corroborates the report of ADAMO and RATNER (1970) who made the same observation on the ovaries of rats exposed to MSG. Also EBLING et al. (1998) reported that lesions induced in the arcuate nucleus of hamsters treated with 4 g/kg body weight of MSG were accompanied by a

significant reduction in their testicular growth. The observed reduced testicular allometric weights in this study are also in agreement with the report of KALEDIN et al. (2005) that MSG administration to neonatal mice led to a reduction in the weights of testes and seminal vesicles. Also GIOVAMBATTISTA et al. (2003) and MOHAMED (2012) reported that MSG-treated adult male rats displayed significant hypogonadism, while PIZZI et al. (1977) recorded reduced testicular weight that caused MSG-treated male mice not to impregnate control females as readily as the control males did. The significant decrease in testicular allometric weights is thought to be accounted for by changes in hypothalamo-pituitary function. Although in the present study quantitative and qualitative estimates of the damage to the neurons in the arcuate neurons were not attempted, PAULL (1975) reported that about 80 percent of the arcuate nucleus was affected in mice.

The absence of any overt pathological lesions in the histology of the testes of the rats in this present study suggests that MSG did not have any direct toxic effect on the testes. The inference is that MSG may have impacted spermatogenesis through disruption of the hypothalamic-pituitary-testis regulatory axis (IGWEBUIKE et al., 2011; OCHIOGU et al., 2015), and not through any direct toxic effect on the testis.

The findings in this present study tend to contrast with previous reports (LEMONS et al., 1976; HAYASHI et al., 1978; BATTAGLIA, 2000) that stated that the placenta is considered virtually impermeable to glutamate, and that breast milk is only modestly influenced by the ingestion of MSG (STEGINK et al., 1972). This is because the rat sub-groups (B^1, C^1 and D^1) that were exposed to MSG from prenatal life up until weaning age, without continual exposure to adulthood, were as much affected as the rat groups that continued to be exposed to MSG up to adulthood. The implication is that the substance must have reached them either through the placenta or breast milk or both.

The mean serum activities of AST and ALT in all the rat sub-groups exposed to MSG, either from prenatal life or from weaning age, were significantly lower than those in the untreated controls (A^1). Since AST and ALT are organ-specific enzymes, used as indicators of liver and muscle damage (KAPLAN et al., 1988), the significant decreases in serum AST and ALT activities recorded in the MSG-treated rat sub-groups suggest that MSG, as used in this study, may have played a membrane stabilizing or protective role for the liver and muscles. This finding of the possible membrane stabilizing effects of MSG as used in this study is in agreement with the report of OCHIOGU et al. (2014) on goats. It, however, contrasts with reports by ONYEMA et al. (2006) and THOMAS et al. (2009).

The higher serum total protein levels recorded in some of the treated sub-groups is believed to be due to the fact that MSG contains the amino acid glutamate, which is a building block for protein synthesis, and it may have actively contributed to the synthesis of more protein in some of the treated sub-groups than in the untreated control (A^1). The higher serum total protein recorded for some of the treated sub-groups in this present study is in agreement with the findings of OCHIOGU et al. (2014) in goats given MSG.

On the basis of the findings in this study, it was concluded that administration of MSG as used in the study led to significantly lower serum levels of testosterone in rat sub-groups administered MSG from weaning age of three weeks to adulthood, significantly lower cholesterol levels in some of the rat sub-groups exposed to MSG during the prenatal and post-weaning period, and significantly lower epididymal sperm reserves and testicular allometric weight in all rat sub-groups exposed to MSG during both prenatal and post-weaning periods. Administration of MSG, as used in this study, also led to significant reduction in serum activities of ALT and AST, and higher serum total protein levels in some of the treated sub-group.

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OCHIOGU, I. S., D. OGWU, C. N. UCHENDU, C. N. OKOYE, J. I. IHEDIOHA, E. C. MBEGBU: Izloženost štakora mononatrijevu l-glutamatu od prenatalne do odrasle dobi: učinci na razine testosterona, kolesterola, proteine i aktivnost enzima u serumu te na testise i zalihu spermija u epididimisu. Vet. arhiv 86, 421-436, 2016.

SAŽETAK

Istraženi su učinci mononatrijeva l-glutamata (MNG) na razine testosterona, kolesterola, proteina i aktivnost enzima u serumu te na testise, zalihu spermija u epididimisu kao i alometrijske težine testisa u štakora od prenatalne do odrasle dobi. Istraživanje je započelo odabirom 40 albino štakorica i 8 štakora. S obzirom na to da je riječ o istraživanju učinka na potomcima, od navedenog broja ženki i mužjaka za istraživanje su uzeta 64 muška potomka. Na početku pokusa 40 ženki bilo je nasumce raspoređeno u četiri skupine (A, B, C i D). U svaku skupinu od 10 ženki dodana su i dva mužjaka za parenje. Štakoricama skupine A nije bio primijenjen MNG, onima skupine B bio je primijenjen u koncentraciji 0,5 %, skupini C od 1,0 % i skupini D u koncentraciji od 2,0 % u vodi za piće. Dnevna primjena MNG početnim skupinama ženki započela je četiri tjedna prije uvođenja mužjaka u njihove skupine te je trajala kroz razdoblje parenja, skotnosti i dok su se kotile, a završila je tri tjedna nakon što su se okotile. Mužjacima s kojima su se parile nije bio primijenjen MNG budući da su početne ženke imale pristup pitkoj vodi koja ga je sadržavala samo tijekom dana, a mužjaci tijekom noći. Izvan razdoblja parenja početne ženke imale su pristup pitkoj vodi s MNG tijekom 24 sata sve do trećeg tjedna nakon koćenja. U trenutku zalučenja (tri tjedna nakon koćenja), 64 muška potomka (po 16 iz svake od četiri skupine) bili su nasumce razvrstani u 16 podskupina, po četiri mužjaka u svakoj. Tako je 16 muških potomaka skupine A bilo podijeljeno u 4 podskupine (A^1, A^2, A^3 i A^4), skupine B u četiri (B^1, B^2, B^3 i B^4), skupine C u četiri (C^1, C^2, C^3 i C^4) te one skupine D također u četiri podskupine (D^1, D^2, D^3 i D^4) pa su se tako po četiri potomka nalazila u svakoj podskupini. Od trenutka odbijanja od sise podskupine A^1, B^1, C^1 i D^1 bile su kontrolne i nisu dobivale MNG. Podskupine A^2, B^2, C^2 i D^2 počele su dobivati 0,5 % MNG, podskupine A^3, B^3, C^3 i D^3 počele su dobivati 1,0 % MNG, a podskupine A^4, B^4, C^4 i D^4 2,0 % MNG u vodi za piće sve do odrasle dobi odnosno do dobi od 16 tjedana. U toj dobi svi su bili pretraženi na razine testosterona, kolesterola i ukupnih proteina u serumu te na aktivnost alanin-aminotransferaze (ALT) i aspartat-aminotransferaze (AST). Također su bile određene rezerve sperme u kaudalnom dijelu epididimisa kao i alometrijske težine testisa. Tkivo testisa bilo je uzeto i za histološku pretragu. Rezultati su pokazali da su samo štakori podskupina koje su bile izložene MNG od trenutka odbijanja od sise do odrasle dobi (A^2, A^3 i A^4) imali srednje razine testosterona značajno manje ($P<0,05$) nego oni u kontrolnoj skupini (A^1). Srednje razine kolesterola u nekih pokusnih podskupina bile su značajno niže ($P<0,05$) nego u onih kontrolne skupine. Srednje razine zaliha sperme u epididimisu, alometrijske težine testisa, te aktivnosti ALT i AST u svih pokusnih podskupina bile su značajno ($P<0,05$) niže nego u kontrolne skupine (A^1). Srednje razine ukupnih serumskih proteina skupina A^3, A^4, B^1, B^2, C^1, C^4, D^1 i D^2 bile su značajno ($P<0,05$) više od onih u kontrolnoj skupini (A^1). Iako su srednje vrijednosti razina kolesterola u serumu svih pokusnih podskupina bile niže nego u kontrola (A^1), samo su vrijednosti u podskupinama B^2, C^2, C^3, C^4, D^1, D^2, D^3 i D^4 bile značajno smanjene ($P<0,05$). Oštećenja testisa nisu bila primijećene ni u jednoj podskupini. Zaključuje se da izloženost štakora primijenjenim dozama MNG-a od prenatalne do odrasle dobi dovodi do značajnog smanjenja razina testosterona i kolesterola u serumu, do samnjenja alometrijskih težina testisa, smanjenja zaliha sperme u epididimisu, aktivnosti ALT i AST u serumu te povećanja ukupnih serumskih proteina.

Ključne riječi: mononatrijev l-glutamat, štakor, testosteron, kolesterol, enzimi, epididimis, zaliha sperme