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EFFECTS OF MONOSODIUM L-GLUTAMATE ADMINISTRATION ON SERUM LEVELS OF REPRODUCTIVE HORMONES AND CHOLESTEROL, EPIDIDYMAL SPERM RESERVES AND TESTICULAR HISTOMORPHOLOGY OF MALE ALBINO RATS

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This study investigated the effects of administration of monosodium L-glutamate (MSG) on serum gonadotrophin-releasing hormone (GnRH), luteinising hormone (LH), testosterone and total cholesterol (TC), cauda epididymal sperm reserves (CESR) and testicular histomorphology of adult male albino rats. Eighty-four rats, randomly assigned to 7 groups of 12 rats each, were used for the study. Varying low doses (0.25, 0.50 or 1.00 g/kg body weight) of MSG were administered orally or subcutaneously at 48-h intervals for six weeks. Serum GnRH, LH, testosterone and TC, and CESR were evaluated on days 14, 28 and 42 of MSG administration. Testicular histomorphology was evaluated on day 42. The results showed that the mean serum GnRH, LH and testosterone levels, and the CESR of all the treated groups were significantly ($P < 0.05$) lower than those of the untreated control on days 14, 28 and 42 of MSG administration. The mean serum TC levels of all the treated groups were also significantly ($P < 0.05$) lower than those of the control group on days 14 and 28. No lesions were observed on sections of the testes. It was concluded that MSG administration for 14, 28 and 42 days led to significantly lower serum levels of GnRH, LH, testosterone and TC, and significantly lower CESR.

Key words: Monosodium L-glutamate, albino rats, reproductive hormones, cholesterol, epididymal sperm reserves, testicular histomorphology

Monosodium glutamate (MSG) is the sodium salt of glutamate, which is used worldwide as a flavour-enhancing food additive (FSANZ – Food Standards Australia New Zealand, 2003). As of 2009, the total world production of MSG

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was estimated to be 2 million tonnes per year (Sahelian, 2014). The consumption of MSG had been reported to be associated with numerous adverse effects (Raiten et al., 1995; FSANZ, 2003). Apart from the well-known Chinese restaurant syndrome consisting of diarrhoea, vomiting, migraine, weakness, stomach ache and tightness of chest, MSG consumption and/or experimental administration had been associated with stunted skeletal development, obesity, hepatotoxicity, brain damage, deoxyribonucleic acid (DNA) damage, and neuroendocrine, reproductive, haematological and metabolic disorders among other things (JECFA – The Joint FAO/WHO Expert Committee on Food Additives, 1988; FASEB – Federation of American Societies for Experimental Biology, 1995; Gong et al., 1995; Bhattacharya et al., 2011; Igwebuike et al., 2011; Ismail, 2012; Meraiyebu et al., 2012).

There had been specific reports of adverse effects of MSG consumption or administration on the male reproductive system and hormones, mostly when very high doses of MSG are acutely administered parenterally (Ismail, 2012; Mohamed, 2012; Nosseir et al., 2012). In order to simulate possible adverse effects that may be associated with normal consumption of MSG, the present study utilised lower doses and both oral and parenteral routes of administration – the oral representing the major route of consumption by humans. The albino rat was chosen as the model for this study because of its strengths and versatility as an animal model, its physiological similarity to humans and its appropriateness for repro-toxicity studies (Gallagher, 2003). The objectives of this study were therefore to evaluate the effects of oral and subcutaneous administration of varying low doses of MSG on serum gonadotrophin-releasing hormone (GnRH), luteinising hormone (LH), testosterone and total cholesterol (TC), cauda epididymal sperm reserves (CESR) and testicular histomorphology of adult male albino rats.

Materials and methods

Experimental animals

Eighty-four adult male albino rats procured from and housed in the Laboratory Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for this study. Their body weight was 144.7–208.2 g and their age 12–15 weeks at the commencement of the study. Throughout the duration of the study, the rats were kept in standard clean rat cages and were fed *ad libitum* with pelleted feed (Grand Cereals and Oil Mills Limited, Jos-Nigeria) containing 16% crude protein. They were also provided freely with clean drinking water throughout the study.

Monosodium L-glutamate

The test compound used was Vedan® (99% MSG) brand of MSG ($C_5H_8NNaO_4 \cdot H_2O$), manufactured by Vedan Enterprise Corporation, Taiwan. It was dissolved in distilled water before use (Nayanatara et al., 2008). A stock solution was prepared by dissolving a 454-g sachet of MSG crystals in distilled water and made up to a volume of 1620 ml. Therefore, for groups B^O and B^S that received 0.25 g of MSG/kg body weight (bw), 0.9 ml/kg bw was given; for groups C^O and C^S that received 0.5 g of MSG/kg bw, 1.8 ml/kg bw was given; and for groups D^O and D^S that received 1.0 g of MSG/kg bw, 3.6 ml/kg bw was given. The dose schedule was so adjusted that the amount of MSG administered per animal corresponded to their body weight.

Experimental design

The 84 male albino rats were randomly assigned into seven groups (A, B^O, B^S, C^O, C^S, D^O and D^S) of 12 each. Group A served as the untreated control and therefore did not receive MSG. Groups B^O and B^S received 0.25 g of MSG/kg bw orally and subcutaneously (O/SC), respectively. Groups C^O and C^S received 0.5 g of MSG/kg bw O/SC, respectively. Groups D^O and D^S received 1 g of MSG/kg bw O/SC, respectively. The MSG administration (MSGA) was 48 hourly for a period of six weeks. The serum levels of gonadotrophin releasing hormone (GnRH), luteinising hormone (LH), testosterone and total cholesterol; and testicular allometric weights (testicular weights to body weights ratios) and epididymal sperm reserves were evaluated on days 14, 28 and 42 of MSGA. Body weights were determined on days 0, 14, 28 and 42 of MSGA. Testicular histomorphology was evaluated on day 42 of MSGA.

Assay of serum levels of GnRH, LH and testosterone

Enzyme-linked immunosorbent assay (ELISA) was used for the assay of GnRH, LH and testosterone (Ekins, 1998) on days 14, 28 and 42 of MSGA. The LH and testosterone test kits were of Accu-Bind brand manufactured by Monobind Inc. (Lake Forest, CA 92630, USA), while the GnRH test kit was of NovaTeinBio brand manufactured by NovaTeinBio, Inc. (701 Concord Avenue, Cambridge, MA 02138, USA). The kits' reagents were stored at the refrigeration temperature of 2–8 °C before use.

Evaluation of serum total cholesterol

Serum total cholesterol was determined on days 14, 28 and 42 of MSGA following the standard enzymatic colorimetric method (Allain et al., 1974), using Quimica Clinica Applicada (QCA) Cholesterol test kit (QCA, S. A., Spain).

Evaluation of epididymal sperm reserves

The epididymal sperm reserves of male rats treated with MSG together with the controls were evaluated on days 14, 28 and 42 of MSGA. The sperm reserve of the cauda epididymides was assessed by employing the standard haemocytometric method (Amman and Almquist, 1961).

Determination of body weights and testicular allometric weights

The body weights of male rats exposed to MSG together with the controls were determined on days 0, 14, 28 and 42 of MSGA, while the testicular allometric weights were determined on days 14, 28 and 42 of MSGA. A weighing balance of Ohaus brand (manufactured by Ohaus, USA) was used for weighing whole animals (determination of body weights). After this, the animals were humanely sacrificed and the testes dissected out. A more sensitive Mettler weighing balance (manufactured by Mettler Toledo, Switzerland) was used for weighing the testes. The testicular weights were then divided by the respective body weights to get the testicular allometric weights.

Histomorphological evaluation of the testis

The testes were dissected from the male albino rats on day 42 of MSGA. They were fixed by immersion in Bouin's solution for 48 h. Later, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Five-micrometre thick sections were cut, mounted on glass slides, and stained with haematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd. 1999–2004).

Data analysis

Data generated 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 ($P < 0.05$). Histological pictures were analysed using a descriptive method. The results were then presented as tables and figures.

Ethics

The housing, handling and welfare of the albino rats used for the study were duly observed in accordance with the Ethics and Regulations Guiding the Use of Research Animals as approved by the University of Nigeria, Nsukka.

Results

The mean serum levels of GnRH and LH of all the treatment groups were significantly ($P < 0.05$) lower than those of the control group on days 14, 28 and 42 of MSGA (Tables 1 and 2). However, there were no significant ($P > 0.05$) variations in the mean serum GnRH and LH levels within the treatment groups on days 14, 28 and 42. The mean serum levels of testosterone of all the treatment groups were significantly ($P < 0.05$) lower than those of the control group on days 14, 28 and 42 of MSGA, with the exception of that of Group B^S which did not significantly ($P > 0.05$) differ from that of the control group and other treatment groups on day 28 (Table 3). Within the treatment groups, however, there were no significant ($P > 0.05$) variations in the mean serum testosterone levels on days 14, 28 and 42 of MSGA (Table 3).

On day 14 of MSGA, the serum total cholesterol levels of rats in the treatment groups (except Group C^O) were significantly ($P < 0.05$) lower than that of the untreated control, but on day 28 of MSGA the serum total cholesterol levels of all the treatment groups (with no exception) were significantly ($P < 0.05$) lower than that of the untreated control (Table 4). However, on day 42 of MSGA, only the mean serum cholesterol levels of the groups treated through the subcutaneous route (B^S, C^S and D^S) were found to be significantly ($P < 0.05$) lower than that of the control (Table 4).

The mean cauda epididymal sperm reserves (CESR) of all the treated groups were significantly ($P < 0.05$) lower than those of the untreated control group on days 14, 28 and 42 of MSGA (Table 5). Within the treated groups, the groups given 1.0 g/kg bw of MSG subcutaneously (Groups D^S) and the group treated orally with 1.0 g/kg bw of MSG (Group D^O) had the lowest mean CESR on days 14 and 28 of MSGA, respectively (Table 5).

There were no significant ($P > 0.05$) variations between the mean body weights of any of the rat groups on days 0, 14, 28 and 42 of MSGA (Table 6). There were no significant ($P > 0.05$) variations between the mean testicular allometric weights of any of the rat groups on days 14, 28 and 42 of MSGA (Table 7).

Sections of the testes of the albino rats that received varying doses of MSG orally or subcutaneously for six weeks showed normal seminiferous tubules and interstices (Figs 1, 2 and 3). The morphology of the testes of all the male albino rat groups that were given MSG did not show any obvious lesions and was not different from that of the untreated control (Figs 1, 2 and 3).

Discussion

The significantly lower serum GnRH recorded in this study on days 14, 28 and 42 of MSGA is thought to be due to neurological lesions (focal necrosis) of the arcuate nucleus of the hypothalamus reported to be associated with MSG treatment

Table 1

Serum gonadotrophin-releasing hormone (GnRH) levels of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error (pg/ml)		
	Day 14	Day 28	Day 42
Group A (untreated control)	23.33 ± 2.98[a]	16.74 ± 3.04[a]	17.61 ± 2.37[a]
Group B^O (0.25 g/kg bw MSG, per os)	9.41 ± 3.33[b]	5.08 ± 0.76[b]	4.07 ± 0.87[b]
Group B^S (0.25 g/kg bw MSG, SC)	8.57 ± 2.83[b]	4.69 ± 0.98[b]	3.28 ± 0.79[b]
Group C^O (0.5 g/kg bw MSG, per os)	8.71 ± 1.86[b]	5.14 ± 1.01[b]	4.22 ± 2.08[b]
Group C^S (0.5 g/kg bw MSG, SC)	7.18 ± 4.88[b]	2.97 ± 0.94[b]	4.14 ± 1.17[b]
Group D^O (1.0 g/kg bw MSG, per os)	5.95 ± 3.76[b]	2.72 ± 0.41[b]	2.63 ± 0.73[b]
Group D^S (1.0 g/kg bw MSG, SC)	6.58 ± 4.55[b]	2.65 ± 4.55[b]	2.54 ± 0.38[b]

[ab]Different superscripts within a column indicate a significant difference between the means ($P < 0.05$)

Table 2

Serum luteinising hormone (LH) levels of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error ($\times 10^{-2}$) (mIU/ml)		
	Day 14	Day 28	Day 42
Group A (untreated control)	51.90 ± 13.60[a]	71.70 ± 7.60[a]	72.50 ± 4.80[a]
Group B^O (0.25 g/kg bw MSG, per os)	8.40 ± 1.80[b]	2.00 ± 0.20[b]	10.30 ± 3.80[b]
Group B^S (0.25 g/kg bw MSG, SC)	6.40 ± 0.20[b]	1.50 ± 0.20[b]	10.30 ± 3.00[b]
Group C^O (0.5 g/kg bw MSG, per os)	5.80 ± 1.60[b]	1.80 ± 0.30[b]	9.00 ± 1.90[b]
Group C^S (0.5 g/kg bw MSG, SC)	9.10 ± 4.70[b]	1.70 ± 0.20[b]	5.40 ± 1.80[b]
Group D^O (1.0 g/kg bw MSG, per os)	2.20 ± 0.10[b]	2.00 ± 0.30[b]	5.60 ± 1.30[b]
Group D^S (1.0 g/kg bw MSG, SC)	2.70 ± 1.10[b]	1.50 ± 0.20[b]	7.80 ± 2.00[b]

[ab]Different superscripts within a column indicate a significant difference between the means ($P < 0.05$)

Table 3

Serum testosterone levels of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error (ng/ml)		
	Day 14	Day 28	Day 42
Group A (untreated control)	7.50 ± 1.14^{a}	4.93 ± 1.18^{a}	6.01 ± 0.29^{a}
Group B^{O} (0.25 g/kg bw MSG, per os)	2.41 ± 0.93^{b}	1.62 ± 0.50^{b}	2.97 ± 0.53^{b}
Group B^{S} (0.25 g/kg bw MSG, SC)	2.20 ± 0.65^{b}	2.62 ± 0.10^{ab}	2.97 ± 0.33^{b}
Group C^{O} (0.5 g/kg bw MSG, per os)	1.36 ± 0.37^{b}	1.15 ± 0.51^{b}	2.45 ± 0.44^{b}
Group C^{S} (0.5 g/kg bw MSG, SC)	1.23 ± 0.58^{b}	1.56 ± 0.61^{b}	2.54 ± 0.36^{b}
Group D^{O} (1.0 g/kg bw MSG, per os)	1.69 ± 0.79^{b}	2.01 ± 1.28^{b}	2.48 ± 0.38^{b}
Group D^{S} (1.0 g/kg bw MSG, SC)	1.21 ± 0.40^{b}	1.05 ± 0.45^{b}	2.21 ± 0.61^{b}

^{ab}Different superscripts within a column indicate a significant difference between the means ($P < 0.05$)

Table 4

Serum total cholesterol levels of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error (mg/dl)		
	Day 14	Day 28	Day 42
Group A (untreated control)	117.18 ± 1.24^{a}	103.00 ± 4.44^{a}	111.04 ± 3.70^{a}
Group B^{O} (0.25 g/kg bw MSG, per os)	97.14 ± 6.10^{bc}	71.25 ± 4.07^{bc}	100.43 ± 2.26^{ab}
Group B^{S} (0.25 g/kg bw MSG, SC)	91.65 ± 3.70^{bcd}	62.00 ± 0.82^{cd}	76.61 ± 12.70^{c}
Group C^{O} (0.5 g/kg bw MSG, per os)	102.38 ± 8.39^{ab}	77.00 ± 2.65^{b}	99.02 ± 1.46^{ab}
Group C^{S} (0.5 g/kg bw MSG, SC)	81.17 ± 7.57^{cd}	57.25 ± 8.62^{d}	85.85 ± 5.22^{bc}
Group D^{O} (1.0 g/kg bw MSG, per os)	99.31 ± 6.18^{b}	60.50 ± 0.96^{cd}	101.40 ± 2.34^{ab}
Group D^{S} (1.0 g/kg bw MSG, SC)	79.08 ± 1.24^{d}	55.50 ± 4.35^{d}	93.60 ± 6.90^{abc}

^{abcd}Different superscripts within a column indicate a significant difference between the means ($P < 0.05$)

Table 5

Cauda epididymal sperm reserves of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error ($\times 10^6$)		
	Day 14	Day 28	Day 42
Group A (untreated control)	110.00 ± 13.00[a]	75.00 ± 4.67[a]	87.00 ± 15.00[a]
Group B^O (0.25 g/kg bw MSG, per os)	40.00 ± 5.24[bc]	25.00 ± 5.37[bc]	31.00 ± 8.49[b]
Group B^S (0.25 g/kg bw MSG, SC)	42.00 ± 12.00[bc]	16.00 ± 7.71[c]	39.00 ± 2.64[b]
Group C^O (0.5 g/kg bw MSG, per os)	62.00 ± 11.00[b]	36.00 ± 7.92[b]	34.00 ± 4.77[b]
Group C^S (0.5 g/kg bw MSG, SC)	51.00 ± 8.21[bc]	36.00 ± 7.34[b]	46.00 ± 7.10[b]
Group D^O (1.0 g/kg bw MSG, per os)	49.00 ± 5.91[bc]	14.00 ± 3.18[c]	43.00 ± 5.66[b]
Group D^S (1.0 g/kg bw MSG, SC)	30.00 ± 9.63[c]	20.00 ± 2.84[bc]	35.00 ± 10.00[b]

[abc]Different superscripts within a column indicate a significant difference between the means ($P < 0.05$)

Table 6

Body weights of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error (g)			
	Day 0 (n = 12)	Day 14 (n = 12)	Day 28 (n = 8)	Day 42 (n = 4)
Group A (untreated control)	177.60 ± 4.74	210.23 ± 5.24	235.84 ± 8.55	239.00 ± 8.56
Group B^O (0.25 g/kg bw MSG, per os)	177.93 ± 5.28	206.57 ± 5.81	229.36 ± 7.38	240.88 ± 3.21
Group B^S (0.25 g/kg bw MSG, SC)	176.66 ± 5.69	198.60 ± 6.23	224.03 ± 7.66	228.65 ± 9.27
Group C^O (0.5 g/kg bw MSG, per os)	176.83 ± 4.22	200.78 ± 4.89	215.88 ± 4.12	235.50 ± 0.81
Group C^S (0.5 g/kg bw MSG, SC)	178.88 ± 4.29	199.01 ± 4.64	226.55 ± 9.10	230.98 ± 3.43
Group D^O (1.0 g/kg bw MSG, per os)	180.12 ± 4.07	201.00 ± 4.22	217.24 ± 6.33	249.58 ± 8.90
Group D^S (1.0 g/kg bw MSG, SC)	179.60 ± 2.36	200.83 ± 2.86	232.63 ± 2.92	241.63 ± 9.64

There was no significant difference between the means of different groups within a column ($P > 0.05$)

Table 7

Testicular allometric weights of male albino rats given varied doses of monosodium L-glutamate (MSG) per os or via the subcutaneous (SC) route for 42 days

Groups, with treatments and route where applicable	Means ± standard error ($\times 10^{-2}$)		
	Day 14	Day 28	Day 42
Group A (untreated control)	1.21 ± 0.07	1.15 ± 0.06	1.31 ± 0.10
Group B^O (0.25 g/kg bw MSG, per os)	1.16 ± 0.08	1.20 ± 0.06	1.12 ± 0.07
Group B^S (0.25 g/kg bw MSG, SC)	1.08 ± 0.08	1.09 ± 0.07	1.16 ± 0.09
Group C^O (0.5 g/kg bw MSG, per os)	1.21 ± 0.06	1.14 ± 0.05	1.15 ± 0.06
Group C^S (0.5 g/kg bw MSG, SC)	1.13 ± 0.04	1.22 ± 0.06	1.24 ± 0.09
Group D^O (1.0 g/kg bw MSG, per os)	1.17 ± 0.06	1.22 ± 0.10	1.09 ± 0.07
Group D^S (1.0 g/kg bw MSG, SC)	1.16 ± 0.07	1.19 ± 0.07	1.18 ± 0.06

There was no significant difference between the means of different groups within a column ($P > 0.05$)

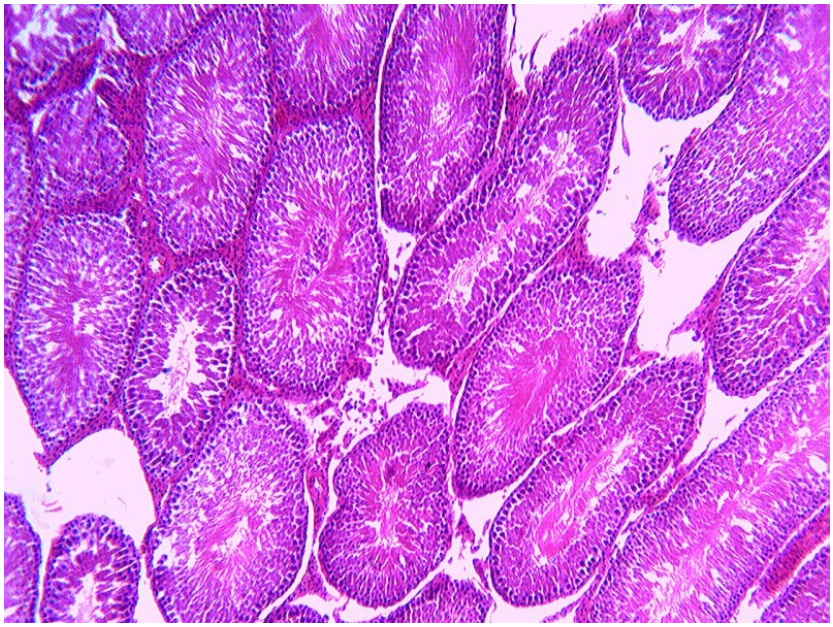

Fig. 1. Histological appearance of the testis of an albino rat given 0.5 g/kg body weight of MSG subcutaneously for 6 weeks, showing no obvious lesions. Haematoxylin and eosin (HE), × 100

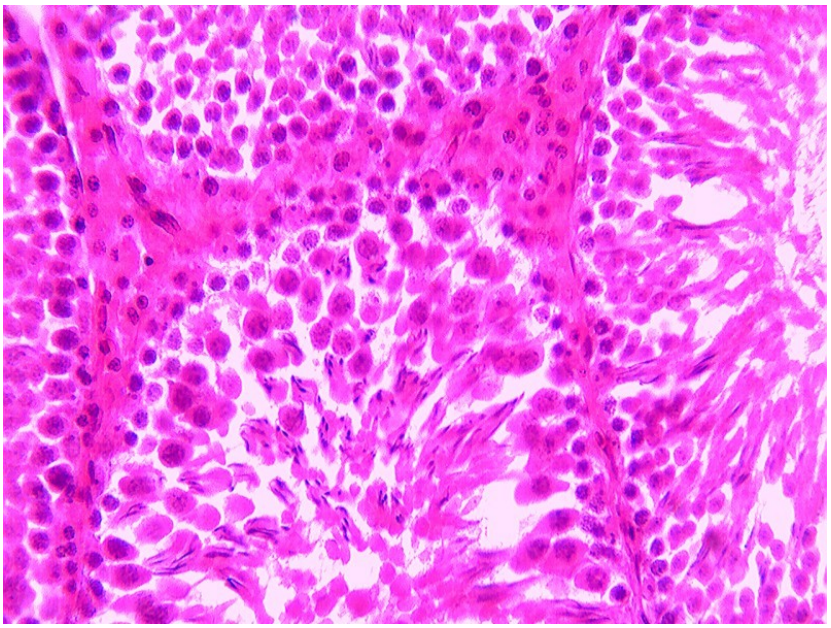

Fig. 2. Histological appearance of the testis of an albino rat given 1.0 g/kg body weight of MSG orally for 6 weeks, showing no obvious lesions. HE, × 400

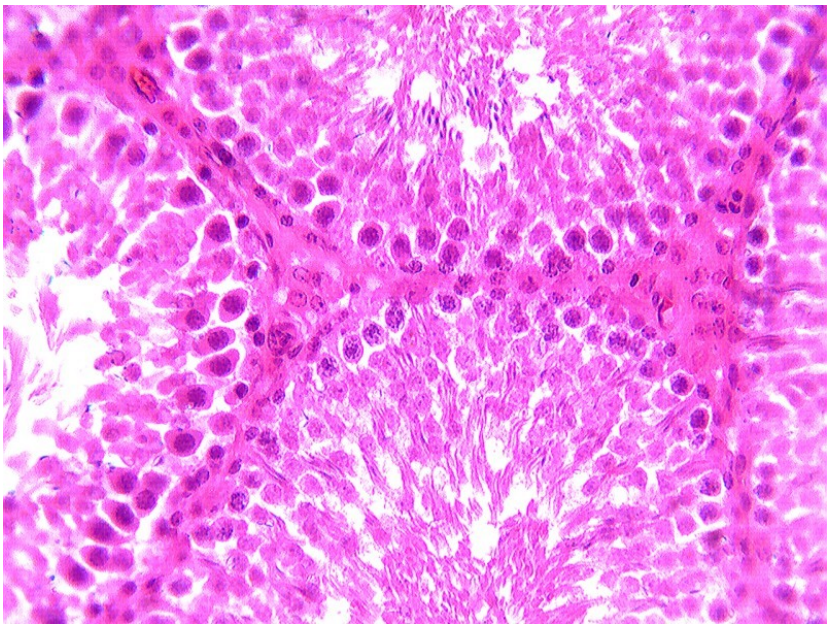

Fig. 3. Histological appearance of the testis of an albino rat given 1.0 g/kg body weight of MSG subcutaneously for 6 weeks, showing no obvious lesions. HE, × 400

in mice, rats and hamsters (Palkovits et al., 1974; Tafelski and Lamperti, 1977), as the arcuate nucleus of the hypothalamus secretes GnRH. The significantly lower serum LH recorded for the treated rats in this study is attributable to the significantly lower serum GnRH, as it is known that GnRH regulates the ability of the anterior pituitary gland to produce LH (Norris, 1997). The significantly lower serum LH reported in this study is in agreement with the findings of Gong et al. (1995).

As cholesterol is known to be a precursor of steroid hormones (Stocco, 1998; Hu et al., 2010), it is thought that the significantly lower serum testosterone levels recorded for the rats treated with MSG in this study may be partly due to the lowered serum cholesterol levels. The significantly lower serum testosterone may also be partly attributed to a disruption in the hypothalamic–pituitary–testis regulatory axis (Nemeroff et al., 1981; Bodnár et al., 2001) mediated by the significantly lower serum levels of GnRH and LH recorded in the study. Luteinising hormone specifically had been reported to be responsible for the stimulation of testosterone production by the Leydig cells (McLachlan et al., 1996).

The significantly lower serum cholesterol recorded for the rats treated with MSG in this study is thought to be related to the reported damage to the arcuate nucleus of the hypothalamus in animals given MSG as the arcuate nucleus of the hypothalamus had been reported to partly function in the regulation of fat me-

tabolism (Dieguez et al., 2011). This lowered serum cholesterol is believed to be partly responsible for the significantly lower serum testosterone recorded for the MSG-treated rats, as testosterone is one of the steroid hormones synthesised from cholesterol (Stocco, 1998; Hu et al., 2010). The significantly lower serum cholesterol recorded for rats given MSG in this study is in agreement with the report of Bazzano et al. (1970) on humans and gerbils, but contrasts with the reports of Ahluwalia and Malik (1989) and Inyang et al. (2012).

It is believed that the significantly lower mean cauda epididymal sperm reserves recorded for the rat groups treated with MSG are directly related to their significantly lower serum testosterone levels as spermatogenesis is dependent on testosterone (Pakarainen et al., 2005; Wang et al., 2009; Walker, 2011). The findings of a significantly lower mean CESR in rats given MSG in this study is in agreement with the reports of Igwebuike et al. (2011) and Fernandes et al. (2012).

The experimental rats were equitably distributed into various groups, which accounted for no significant variations in their mean body weights across all the groups on day 0 of MSGA. Being growing adult rats, their body weights continued to increase during the course of the study. However, this increase in body weights occurred in all the groups at the same rate, thereby accounting for no significant variations in mean body weights in all the groups on days 14, 28 and 42 of MSGA. This is in agreement with the report of Tordoff et al. (2012) who opined that previous studies that reported either significant increase or decrease in body weight associated with MSG involved administration of MSG to immature rodents, and consequently may not be relevant for understanding human obesity.

The non-significant variations in the testicular allometric weights of all the rat groups show the absence of either hypogonadism or hypergonadism. This observation is probably due to the fact that the animals used in this study were adult rats. This finding is contrary to the reports of both testicular and ovarian hypogonadism in male and female mice following a subcutaneous administration of MSG to neonatal mice at the dosage between 2.2 and 4.2 g per kg body weight (Pizzi et al., 1977). Also, Ebling et al. (1998), who made the same observation of hypogonadism of testes in hamsters, used a high dose of 4 g/kg body weight of MSG in their study in neonatal hamsters. Both contrary reports made use of higher doses of MSG and neonatal animals. These may have accounted for the differences between the observations made in this study and theirs.

The non-observation of lesions in the sections of the testes of rats treated with MSG in this study is thought to be due to the relatively lower doses of MSG used for the study, which were meant to simulate intakes in humans. The findings of no lesions in the testes of rats given MSG in this study are in agreement with the reports of Igwebuike et al. (2011) who used doses similar to the ones used in this study, but contrasts with those of Ismail (2012) and Nosseir et al. (2012) who administered higher doses at more regular intervals.

The choice of 0.25 g, 0.5 g and 1.0 g MSG per kg body weight for both oral and subcutaneous routes was made in a bid to as much as possible simulate the quantity of MSG taken by humans as a food additive. Data from the United Kingdom indicate an average intake of 590 mg MSG per day, with extreme users consuming 2,330 mg MSG per day (Rhodes et al., 1991). However, in a highly seasoned restaurant meal intakes as high as 5,000 mg MSG per day may be possible (Yang et al., 1997). The highest dose employed in this study is equal to the lowest dose used by Reynolds et al. (1971). They used 1, 2 and 4 g/kg, while Olney and Sharpe (1969) used a single dose of 2.7 g/kg that produced reproductive effects. The range of dosages producing an effect in the report of Allen et al. (1987) was 0.5 g to 2.5 g MSG. Other workers had used doses as high as 4–8 g or more MSG per kg body weight (Pizzi et al., 1977; Tafelski and Lamperti, 1977). Their choice of high doses of MSG may have been prompted by the fact that MSG has a very low acute toxicity, as the LD_{50} of MSG for rats and mice is about 15 g/kg and 18 g/kg body weight, respectively (JECFA, 1988). However, human consumption levels are not usually based on results of acute toxicity studies or LD_{50} calculations.

In summary, administration of MSG to adult rats in three graded doses (0.25, 0.50 and 1.00 g/kg bw) and through two routes of administration (oral and subcutaneous routes) was associated with a significant lowering of the serum levels of GnRH, LH, testosterone and TC on days 14, 28 and 42 of administration. Rats treated with MSG also had a significantly lower CESR on days 14, 28 and 42 of MSG administration.

References

Ahluwalia, P. and Malik, V. B. T. (1989): Effects of monosodium glutamate (MSG) on lipids, blood glucose and cholesterol in adult male mice. Toxicol. Lett. **45,** 195–198.

Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W. and Fu, P. C. (1974): Enzymatic determination of total cholesterol. Clin. Chem. **20,** 470–475.

Allen, D. H., Delohery, J. and Baker, G. (1987): Monosodium L-glutamate-induced asthma. J. Allergy Clin. Immunol. **80,** 530–537.

Amman, R. P. and Almquist, J. O. (1961): Reproductive capacity of dairy bulls. 1. Technique for direct measurement of gonadal and extragonadal sperm reserves. J. Dairy Sci. **44,** 1537–1539.

Bhattacharya, T., Bhakta, A. and Ghosh, S. K. (2011): Long term effect of monosodium glutamate to liver of albino mice after neonatal exposure. Nepal Med. Coll. J. **13,** 11–16.

Bazzano, G., D'Elia, J. A. and Olson, R. E. (1970): Monosodium glutamate: Feeding of large amounts to man and gerbils. Science **169,** 1208–1209.

Bodnár, I., Göőz, P., Okamura, H., Tóth, B. E., Vecsernyés, M., Halász, B. and Nagy, G. M. (2001): Effect of neonatal treatment with monosodium glutamate on dopaminergic and L-DOPA-ergic neurons of the medial basal hypothalamus and on prolactin and MSH secretion of rats. Brain Res. Bull. **55,** 767–774.

Dieguez, C., Vozguez, M. J., Roweto, A., Lopez, M. and Nogueiras, R. (2011): Hypothalamic control of lipid metabolism: focus on leptin, ghrelin and melanocortins. Neuroendocrinology **93,** 1–11.

Ebling, F. J., Arthurs, O. J., Turney, B. W. and Cronins, A. S. (1998): Seasonal endocrine rhythms in the male Siberian hamster persist after monosodium glutamate-induced lesions of the arcuate nucleus in the neonatal period. J. Endocrinol. **10,** 701–712.

Ekins, R. P. (1998): Ligand assays: from electrophoresis to miniaturized microarrays. Clin. Chem. **44,** 2015–2030.

FASEB (1995): Analysis of Adverse Reactions to Monosodium Glutamate (MSG) – Report. Life Sciences Research Office, Federation of American Societies for Experimental Biology (FASEB), Washington, D.C.

Fernandes, G. S., Arena, A. C., Campos, K. E., Volpato, G. T., Anselmo-Franci, J. A., Damasceno, D. C. and Kempinos, W. G. (2012): Glutamate induced obesity leads to decreased sperm reserves and acceleration of transit time in the epididymis of adult male rats. Reprod. Biol. Endocrinol. **10,** 105–111.

FSANZ (Food Standards Australia New Zealand) (2003): Monosodium glutamate: A safety assessment. Technical Report Series No. 20, FSANZ, Canberra.

Gallagher, R. (2003): Animal research is for human welfare. Scientist **17,** 1–3.

Gong, S. L., Xia, F. Q., Wei, J., Li, X. Y., Sun, T. H., Lu, Z. and Liu, S. Z. (1995): Harmful effects of MSG on function of hypothalamus–pituitary–target gland system. Biomed. Environ. Sci. **8,** 310–317.

Hu, J., Zhang, Z., Shen, W. J. and Azhar, S. (2010): Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutr. Metab. **7,** 47–72.

Igwebuike, U. M., Ochiogu, I. S., Ihedinihu, B. C., Ikokide, J. E. and Idika, I. K. (2011): The effects of oral administration of monosodium glutamate (MSG) on the testicular morphology and cauda epididymal sperm reserves of young and adult male rats. Vet. Arhiv **81,** 525–534.

Inyang, B., Ojewunmi, O. and Ebuehi, O. (2012): Toxicological effects of monosodium glutamate on the liver enzyme markers and lipid profile of adult Wistar rats. Asian J. Biol. Pharmaceut. Res. **3,** 266–273.

Ismail, N. H. (2012): Assessment of DNA damage in testes from young Wistar male rats treated with monosodium glutamate. Life Sci. J. **9,** 930–939.

JECFA (The Joint FAO/WHO Expert Committee on Food Additives) (1988): L-glutamic acid and its ammonium, calcium, monosodium and potassium salts. In: Toxicological Evaluation of Certain Food Additives and Contaminants. Cambridge University Press, New York. pp. 97–161.

McLachlan, R. I., Wreford, N. G., O'Donnell, L., De Kretser, D. M. and Robertson, D. M. (1996): The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH. J. Endocrinol. **148,** 1–9.

Meraiyebu, A., Akintayo, C. O., Uzoechi, A. C. and Okere, S. (2012): The effects of orally administered monosodium glutamate (MSG) on blood thrombocyte, blood coagulation and bleeding in rats. J. Pharm. Biol. Sci. **4,** 4–8.

Mohamed, I. K. (2012): The effects of oral dosage of monosodium glutamate applied for short and long terms on the histology and ultrastructure of testes of adult rats. J. Anim. Vet. Adv. **11,** 124–133.

Nayanatara, A., Vinodini, N. A., Damodar, C., Ahemed, B., Ramaswamy, C. R., Shabarinath, M. and Bhat, M. R. (2008): Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight, sperm morphology and sperm count in rat testis. J. Chinese Clin. Med. **3,** 1–5.

Nemeroff, C. B., Lamartiniere, C. A., Mason, G. A., Squibb, R. E., Hong, J. S. and Bondy, S. C. (1981): Marked reduction of gonadal hormone level in rats treated neonatally with monosodium L-glutamate: further evidence for disruption of hypothalamic–pituitary–gonadal axis regulation. Neuroendocrinology **33,** 2265–2267.

Norris, D. O. (1997): Vertebrate Endocrinology. Academic Press, San Diego, CA, USA.

Nosseir, N. S., Ali, N. H. M. and Ebaid, N. (2012): A histological and morphometric study of monosodium glutamate toxic effect on testicular structure and potentiality of recovery in adult albino rats. Res. J. Biol. **2,** 66–78.

Olney, J. W. and Sharpe, L. G. (1969): Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Science **166,** 386–388.

Pakarainen, T., Zhang, F., Makela, S., Poutane, M. and Huhtaniemi, I. (2005): Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteinizing hormone receptor knockout mice. Endocrinology **146,** 596–606.

Palkovits, M., Arimura, A., Brownstein, M., Schally, A. V. and Saavedra, J. M. (1974): Luteinizing hormone-releasing hormone (LH-RH) content of the hypothalamic nuclei in rat. Endocrinology **96,** 554–558.

Pizzi, W. J., Barnhart, J. E. and Fanslow, D. J. (1977): Monosodium glutamate administration to the newborn reduces reproductive ability in female and male mice. Science **196,** 452–454.

Raiten, D. J., Talbot, J. M. and Fisher, K. D. (eds) (1995): Executive summary from the report: analysis of adverse reactions to monosodium glutamate (MSG). J. Nutr. **125,** 2892S–2906S.

Reynolds, W. A., Lemkey-Johnston, N., Filer, L. J. Jr. and Pitkin, R. M. (1971): Monosodium glutamate: absence of hypothalamic lesions after ingestion by newborn primates. Science **172,** 1342–1344.

Rhodes, J., Titherley, A. C., Norman, J. A., Wood, R. and Lord, D. W. (1991): A survey of the monosodium glutamate content of foods and an estimation of the dietary intake of monosodium glutamate. Food Addit. Contam. **8,** 265–274.

Sahelian, R. (2014): Glutamate benefit and side effects, risk and danger. www.raysahelian.com/glutamate.html. Accessed 25 March 2013.

Stocco, D. M. (1998): Testosterone biosynthesis. In: Neill, J. D. and Knobil, E. (eds) Encyclopedia of Reproduction. Volume 4. Academic Press, New York. pp. 784–789.

Tafelski, T. J. and Lamperti, A. A. (1977): The effects of a single injection of monosodium glutamate on the reproductive neuroendocrine axis of the female hamster. Biol. Reprod. **17,** 404–411.

Tordoff, M. G., Aleman, T. R. and Murphy, M. C. (2012): No effects of monosodium glutamate consumption on the body weight or composition of adult rats and mice. Physiol. Behav. **107,** 338–345.

Walker, W. H. (2011): Testosterone signaling and the regulation of spermatogenesis. Spermatogenesis **1,** 116–120.

Wang, R., Yeh, S., Tzeng, C. and Chang, C. (2009): Androgen receptor roles in spermatogenesis and fertility: Lessons from testicular cell-specific androgen receptor knockout mice. Endocr. Rev. **30,** 119–132.

Yang, W. H., Drouin, M. A., Herbert, M., Mao, Y. and Karsh, J. (1997): The monosodium glutamate symptom complex: assessment in a double-blind, placebo-controlled, randomized study. J. Allergy Clin. Immunol. **99,** 757–762.