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The Role of l-arginine in prevention of testicular function toxicity induced by monosodium glutamate burden in Wistar rats

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ABSTRACT

Background: Monosodium glutamate, MSG, is a widely consumed and inadvertently abused glutamate-based flavour enhancer that could impair male fertility. L-arginine, is a fatherhood amino acid that could improve male reproductive apparatus. Possibility of simultaneous consumption of MSG and l-arginine exists with unknown outcomes on testicular function. This study aimed to evaluate the role of l-arginine in prevention of testicular function toxicity induced by monosodium glutamate burden in Wistar rats.

Methods: The study involved five rat groups (n = 5). Group 1 rats, normal control, received distilled water. Groups 2,3,4 and 5 rats, respectively received MSG (8000 mg/kg), l-arginine (60 mg/kg), MSG (8000 mg/kg) plus l-arginine (60 mg/kg) and MSG (8000 mg/kg) plus l-arginine (120 mg/kg). Exposure was by oral intubation for 28 consecutive days. Testicular function indicators studied included testosterone activity (in the rats' testes homogenate and serum), sperm quality (sperm count, sperm motility, sperm volume and sperm morphology) and testes histomorphology.

Results: Results revealed significant ($P<0.05$) and marked diminution of testosterone activity (in the testes and serum), sperm quality and spermatogenic cells of the testes in the rats that received MSG compared to rats in normal control and other groups, notably group 5 that received MSG and high dose of l-arginine together.

Conclusion: The study demonstrated that MSG burden caused testicular function toxicity in the rats, demonstrated by the altered testes histomorphology, diminished testosterone activity and compromised sperm quality. There was a significant reversal by l-arginine notably at 120 mg/kg of the testicular function toxicity caused by MSG burden in the rats *via* probable modulation of the compromised indicators as determined in the rats. Thus, l-arginine could play a significant role in the prevention of testicular function toxicity induced by MSG burden in rats.

1. Introduction

Monosodium glutamate (MSG) is a flavour enhancing food additive with potential toxicity. Previous studies reported that MSG altered endocrine functions [1,2] and caused significant damage to the male reproductive system [3]. Earlier studies suggested that MSG could exert overall male infertility *via* induction of testicular haemorrhage, compromised quality and quantity of produced sperm [4] and prostate pathologies [5]. Diminished prostate function could for instance diminish the production of prostatic fluid that aids sperm motility and nourishment. MSG could hamper male fertility *via* alteration of endocrine functions as it could exert excitatory effect on the neurons *via* its amino acid backbone, glutamate. Excitatory action of MSG destroys the hypothalamic neurons and ultimately disrupts the hypothalamic-pituitary-testis regulatory axis which regulates steroidogenesis of testicular Leydig cells responsible for testosterone synthesis [4].

Generally regarded as fatherhood amino acid, l-arginine as other amino acids participates in protein

synthesis, cell division and immune function. L-arginine could improve important physio-functions including reproductive functions *via* its sole metabolic product nitric oxide, NO [6,7]. The possibility of inadvertent abuse of MSG in diets even with concomitant consumption of l-arginine in diets and drugs exists and, to the knowledge of the authors, with unknown or unreported biochemical consequences on male testicular functions and histology. This study hypothesized that MSG burden could exert a toxic influence on the testicular function and that l-arginine could mitigate the MSG effect. This study aimed to assess the role of l-arginine in prevention of testicular function toxicity induced by monosodium glutamate burden in Wistar rats' model.

The specific objectives set to achieve the study aim included provision of answer to the research question: What is the effect of concomitant exposure of MSG burden and l-arginine on semen quality, testosterone activity and testes histology of rats? Semen quality was assessed through the determination of sperm motility, sperm count, sperm volume and sperm morphology namely normal morphology, abnormal head and abnormal tail. Testosterone activity was determined in the testes homogenate and serum of rats. Testes histology was examined and captured in a photomicrograph. Reference to sperm quality indicators for instance served as acceptable indicator of testicular function-related reproductive function of healthy men [8].

2. Materials and methods

2.1 Materials

Monosodium glutamate, MSG (Ajinomoto®, 99 % purity) was purchased from a regular food stuff market at Aba, Abia State. The chemicals used, including l-arginine, l-ARG (99 % purity) were of analytical grade by Sigma-Aldrich chemical, St Louis. Diagnostic kits used in this study were products of Randox kit, UK and procured from competent sources.

2.2 Experimental design and treatment

A total of twenty-five (25) male Wistar rats weighing 85.20 g were purchased from the animal farm of the Department of Veterinary Medicine, University of Nigeria Nsukka. After a week of acclimatization, they were randomized to five treatment groups (sample size, n = 5) using a completely randomized blocked design of five treatment groups replicated thrice with each replicate having two rats.

Group1, the normal control rats received distilled water. Group 2 rats received MSG (8000 mg/kg) [9,10]. Group 3 rats received l-ARG (60 mg/kg) [11]. Group 4 rats received MSG (8000 mg/kg + l-ARG (60 mg/kg) while group 5 rats received MSG (8000 mg/kg + l-ARG (120 mg/kg). Rats in each group were allowed free access to portable water and feed. Administration was by oral intubation for 28 days. Ethical guidelines of the National Research Council, USA [12] were strictly adhered to throughout the animal experimentation.

2.3 Sample collection and preparation

At the end of the exposure, 15 hours after the last feeding, 2 ml of blood was collected from each rat by cardiac puncture technique, allowed to clot and centrifuged for 10 minutes at 3,000 g under room temperature. The harvested serum was stored in deep freezer maintained at minus 20 °C until used for the determination of testosterone activity. Testis sample of each rat was excised and shared into three. One part for homogenization was washed with distilled water to remove blood and fat contents and then homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4) containing 0.1mM ethylene di-amine tetra acetic acid (EDTA). The supernatant was separated by means of centrifugation at 500 g for 20 minutes at 4 °C and stored in a deep freezer maintained at minus 20 °C for use in the determination of testes homogenate testosterone activity.

The second part for histomorphologic examination was immediately fixed in 10 % formaldehyde buffered saline (formal saline)**.** The third part of the testis for semen quality analysis (sperm motility, sperm morphology, sperm count and sperm volume) was carefully exposed, removed and trimmed free of the epididymis and adjoining tissues. From the respectively separated epididymis, the caudal parts were removed and placed in a beaker containing 1 ml of physiological (normal) saline solution (0.9 %), promptly macerated with a pair of sharp scissors and then left for a few minutes to liberate its spermatozoa content into the physiological saline solution *prior* to semen quality analysis.

2.4 Estimation of testosterone activity in the rats' testes homogenate and in the serum

Testosterone activity (IU/L) was determined in either sample by direct testosterone immunoassay method as described earlier by Handelsman *et al.* [13].

2.5 Estimation of semen quality

Sperm motility was estimated by the method of

Maree and Vander-Horst [14]. Sperm morphology was estimated by the protocol involving drying and staining of the sperm smears as described by Vander-Horst and Maree [15]. Sperm count was determined by the method described by Badkoobeh *et al.* [16] while sperm volume was determined by the method described by Yeung *et al.* [17].

2.6 Rats' testes tissue preparation and histomorphological examination

The part of testes fixed in 10% formaldehyde buffered saline (formal saline) was subsequently sectioned and prepared for histomorphological assessment as described earlier [18]. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at magnification of x 160.

2.7 Statistical analysis

Numeric data were subjected to one way analysis of variance by *post-hoc* multiple comparison to determine the level of significance between test and control. Statistical significance was set at $P<0.05$. The results were presented as mean ± standard deviation (S.D.).

3. Results

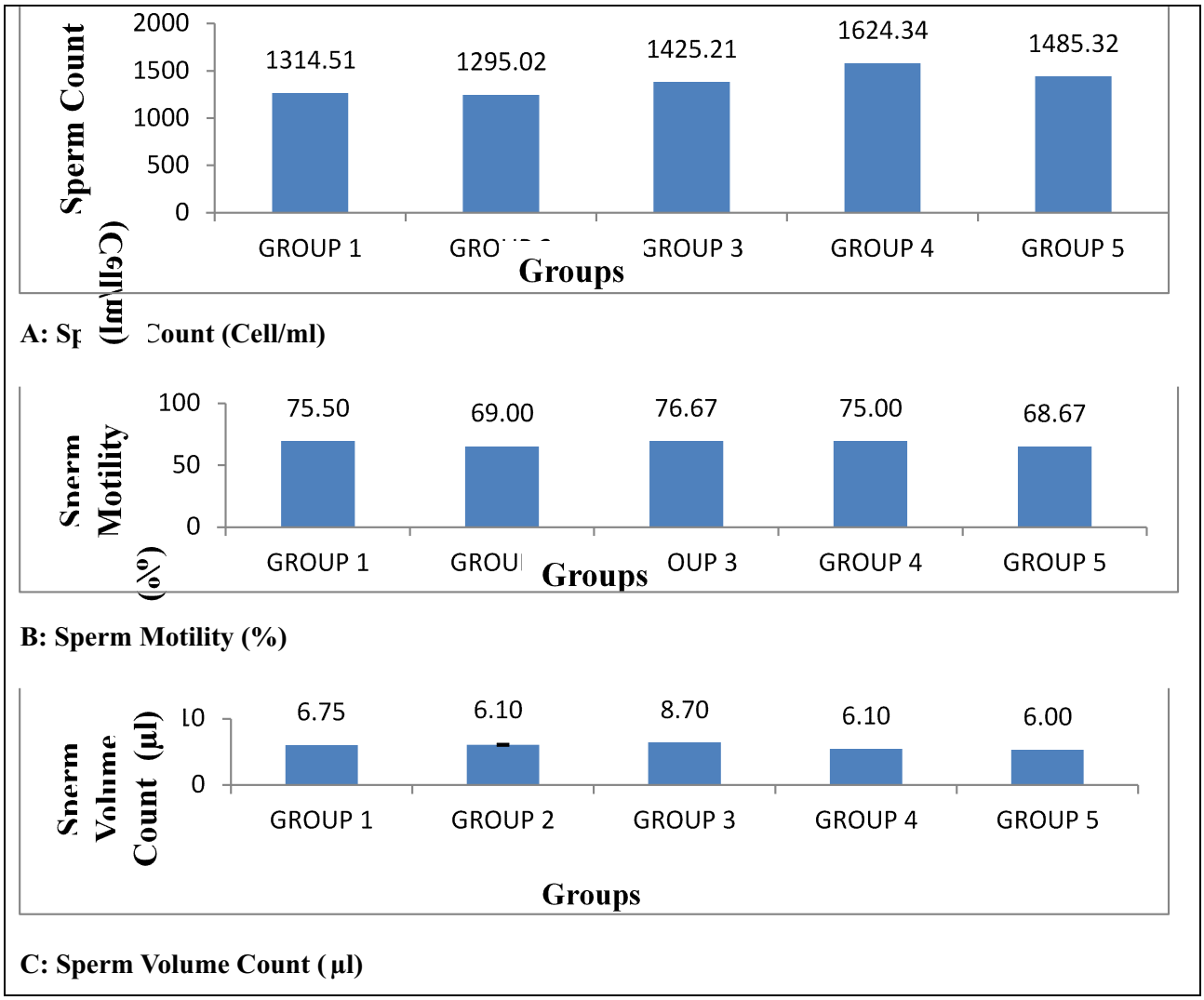
The testosterone activity (IU/L) in the MSG-burdened rats testes homogenate (4.74±3.96) and serum (8.08±1.50) was significantly lower ($P<0.05$) compared to control and other treatments. The testosterone activity (IU/L) in the l-arginine-exposed rats testes homogenate (11.52±6.17) and serum (30.87±1.80) was significantly higher ($P<0.05$) compared to control and other treatments. The testosterone activity (IU/L) in the testes homogenate and serum respectively of low l-arginine co-treated group (6.76±0.49, 29.14±1.05) and high l-arginine co-treated group (5.29±2.54, 28.32±1.04) lowered compared to that of MSG-burdened rats (Table 1).

Table 1: Effect of l-arginine on testes homogenate and serum testosterone activity (IU/L) in monosodium glutamate-burdened Wistar rats

Groups	Testes homogenate testosterone activity (IU/L)		Serum testosterone activity (IU/L)	
	Mean	S.D	Mean	S.D
1	8.36	1.27 ^{d}	19.63	1.16 ^{b}
2	4.74	3.96 ^{a}	18.08	1.50 ^{a}
3	11.52	6.17 ^{e}	30.87	1.80 ^{e}
4	6.76	0.49 ^{c}	29.14	1.05 ^{d}
5	5.29	2.54 ^{b}	28.32	1.04 ^{c}

Results=mean±standarddeviation,SD.Samplesize,n=5rats.Differentsuperscriptmeansaresignificantlydifferentat$P<0.05$.

Similarly, sperm count (Cells/ml) and sperm motility (%) were lower ($P < 0.05$) in MSG-burdened rats but higher ($P<0.05$) in l-arginine-exposed and dose dependently higher ($P<0.05$) in l-arginine co-consumed rats (Figure 1). Also, sperm morphology (%) with highest ($P<0.05$) normal morphology, least ($P<0.05$) abnormal head and tail and highest ($P<0.05$) sperm volume (µl), were recorded in l-arginine-exposed rats as against those of MSG-burdened rats and others (Table 2 and Figure 1).



Results=mean±standarddeviation,SD.Samplesize,n=5rats.MeansaresignificantlydifferentatP<0.05.

Figure 1: Effect of l-arginine on A: Sperm count (Cells/ml), B: Sperm motility (%) and C: Sperm volume count (µl) in monosodium glutamate-burdened Wistar rats

Table 2: Effect of l-arginine on sperm morphology (%) in monosodium glutamate-burdened Wistar rats

Groups	Sperm morphology (%)		
	Normal morphology	Abnormal head	Abnormal tail
1	76.67 ± 2.89^{c}	15.00 ± 7.07^{b}	12.50 ± 3.54^{c}
2	72.50 ± 3.54^{a}	16.67 ± 5.77^{c}	13.33 ± 2.89^{d}
3	80.00 ± 0.01^{e}	10.00 ± 0.03^{a}	5.00 ± 0.05^{a}
4	77.50 ± 10.61^{d}	15.00 ± 0.10^{b}	12.50 ±10.61^{c}
5	73.33 ± 10.41^{b}	15.00 ± 10.00^{b}	11.67± 2.88^{b}

Results=mean±standarddeviation,SD.Samplesize,n=5rats.Differentsuperscriptmeansaresignificantlydifferentat$P<0.05$.

As shown in Figure 2, the markedly decreased spermatogenic cells in rats that received MSG (Group 2) and in those that received low l-arginine plus MSG together (Group 4) were relatively normal with normal seminiferous tubules (white arrow) in rats that received l-arginine (Group 3) and in those that received high l-arginine plus MSG together (Group 5).

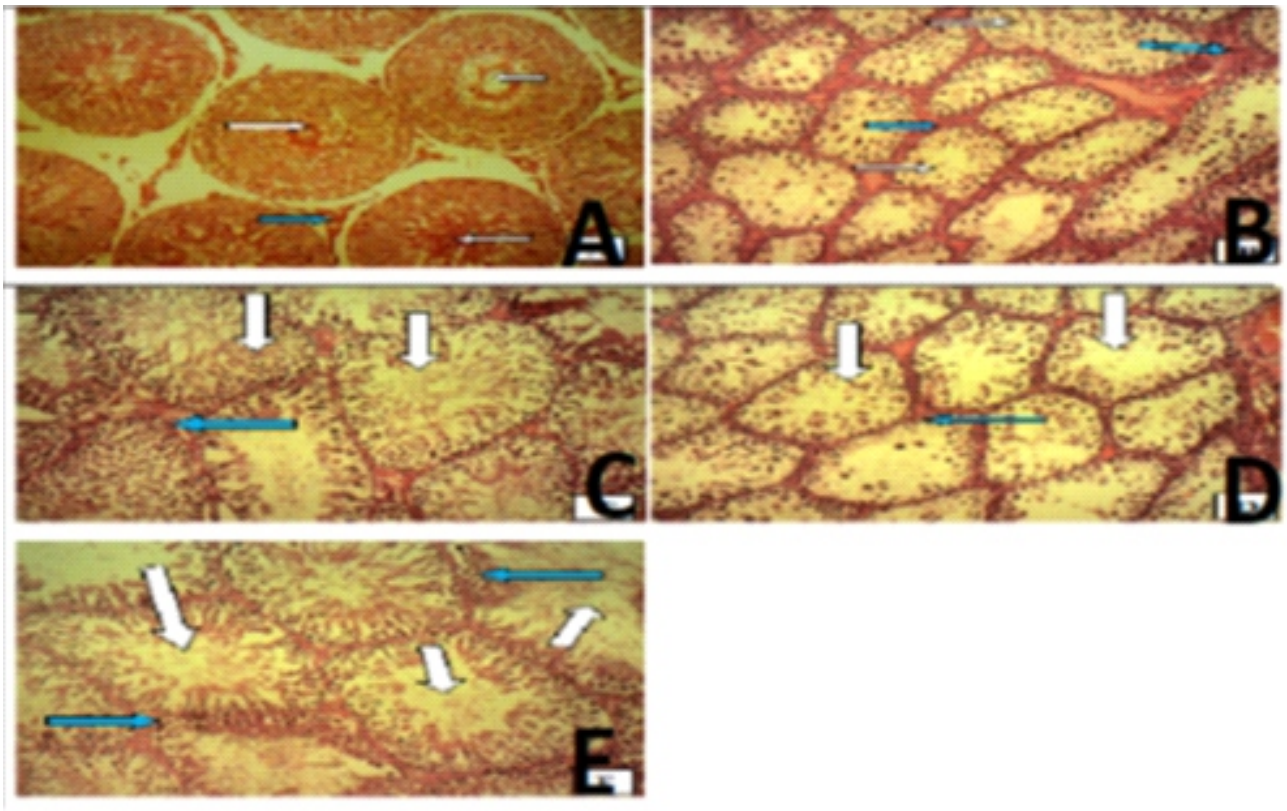


Figure 2: Effect of l-arginine on testes histology in monosodium glutamate-burdened Wistar rats (H & E × 160)

Notes:

A: Group 1 (normal control) rats' testes sections showing normal testicular histoarchitecture with normal seminiferous tubules (white arrow) and highly vascularized testicular interstitium (blue arrow).

B: Group 2 (MSG treated) rats' testes sections showing markedly decreased spermatogenic cells on the epithelia lining of the seminiferous tubules (white arrow) and less vascularized testicular interstitium (blue arrow).

C: Group 3 (l-arginine fed) rats' testes sections showing relatively normal testicular histoarchitecture with spermatogenic cells on the epithelial lining of the seminiferous tubules (white arrow) and moderately vascularized testicular interstitium (blue arrow).

D: Group 4 (MSG burdened + low l-arginine fed) rats' testes sections showing mildly decreased spermatogenic cells on the epithelial lining of the seminiferous tubules (white arrow).

E: Group 5 (MSG burdened + high l-arginine fed) rats' testes sections showing relatively normal testicular histology and spermatogenic cells on the epithelial lining of the seminiferous tubules (white arrow).

1. Discussion

The possibility of simultaneous consumption of MSG and l-arginine exists with unknown outcomes on testicular function. This study hypothesized that MSG burden could exert toxic influence on the testicular function and that l-arginine could mitigate the MSG effect. Thus, this study evaluated the role of l-arginine in prevention of testicular function toxicity induced by monosodium glutamate burden in Wistar rats. The results showed that testes homogenate and serum testosterone levels were markedly reduced in rats that were treated with MSG. This demonstrated that MSG burden down-regulated testosterone synthesis and expression in the rats' testes. Generally, MSG-related excitatory actions could disrupt the rats' hypothalamic neurons and consequently endocrine functions associated with the testosterone expression. Earlier, administration of MSG altered endocrine functions [1,2] by destroying the hypothalamic-pituitary-testis regulatory axis that controls the steroidogenesis of testicular Leydig cells [4] leading to the noted decrease in testosterone levels. Thus, the study further demonstrated that l-arginine could up regulate testosterone expression in the rats' testes and could significantly mitigate the effect induced by MSG. Testosterone expression is fundamental to testicular functions particularly spermatogenesis [19]. In agreement with the result of this study, Kianifard *et al.* [20] had reported adverse effect of monosodium glutamate on testosterone expression. The l-arginine-related benefits and mitigation potential on the testosterone activity recorded in this study could be by way of improved neurons in the endocrine organs responsible for optimal testosterone expression and improved overall sperm quality [21,22].

Lowered sperm count as observed in this study in rats that received MSG indicated adverse influence on the normal process of spermatogenesis which depends largely on testosterone [4]. The present observation on the assessed sperm quality indicators of the rats further demonstrated MSG-related adversity, l-arginine-related benefit and mitigation potential against MSG effect on the sperm quality analytic indicators of rats' testicular functions. The outcome was in line with the results of the testes homogenate and serum testosterone activity reported herein. The reductions in sperm count, sperm motility, sperm volume and sperm with normal morphology in MSG-burdened rats indicated testicular function toxicity following apparent adverse alteration in the normal process of spermatogenesis in the rats as a consequence of MSG-related decrease in testosterone activity reported in this study. In support of the present results and propositions thereto, MSG exposure compromised quality sperm production and morphology in earlier studies [3,20,23].

The histological outcomes of the rats were in line with the above results and attendant proposition of adverse alteration of spermatogenesis in MSG-burdened rats. Diminished testosterone expression could lead to diminished spermatogenic cells [4] as noted in this study in the testes histology of MSG burdened rats. Earlier, MSG exposure altered testes histology and compromised quality sperm production and morphology [3,20,23]. Thus, the observed semen quality analytic, serum and testes homogenate chemistry changes significantly supported the compromised testes histomorphology of MSG-burdened rats while co-administration with l-arginine, notably at 120 mg.kg offered a potential preventive role on the effect induced by MSG on the testes histology of rats. Previously, glutamate resulting from the glutamate moiety of MSG impaired reproductive organ function [24]. In a recent study, Jubaidi *et al.* [3] surmised that MSG altered the testis histology by spiking the circulatory glutamate concentration to hyper-activate the glutamate receptors in spermatogenic cells. Glutamate concentration was not determined in the present study to collaborate this hence is a notable limitation of the present study that warrants incorporation in further studies.

Generally, in rats as in other mammals, spermatogenesis wholly depends on normal testosterone expression (synthesis and activity) [25]. The presented results and propositions thereto agree with the report of Akhigbe and Ajayi [26] of a dose-response relationship between the testis histology or the expression of testicular function components and the overall testicular functions in animals' testes. Pertinently, MSG could adversely affect sperm motility by way of diminished prostate function-related production of prostatic fluid that aids sperm motility and nourishment [5]. Although not accessed in this study, reproductive organs accessories as prostatic fluid and prostate function are important determinants of reproductive organ health [27], warranting probing studies in that direction. On the other hand, reported inconsistent l-arginine benefits attributable to its multiple metabolic fates suggested the need for optimal l-arginine-mediated synthesis of nitric oxide for persistent l-arginine-mediated benefits and mitigation roles [6,18]. Thus, further studies to investigate the sustainability of the mitigation role of L-arginine at 120 mg/kg against MSG-burdened adversity in the rats are warranted hence recommended.

2. Conclusion

The study demonstrated that MSG burden caused testicular function toxicity in the rats demonstrated by the altered testes histomorphology, diminished testosterone activity and compromised sperm quality in the rats that received MSG. This was *via* probable modulation of the compromised indicators as determined in the rats.

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