https://rijournals.com/scientific-and-experimental-sciences/

RESEARCH INVENTION JOURNAL OF SCIENTIFIC AND EXPERIMENTAL SCIENCES 3(3):57-64, 2024
©RIJSES Publications ISSN: 1597-2917

Testicular and Scrotal Antioxidant and Structural Changes Associated with Monosodium Glutamate in Male Wistar Rats

Allende Caleb Ololade

Faculty of Pharmacy Kampala International University Western Campus Uganda

ABSTRACT

MSG is a common food additive particularly in West African and Asian dishes, generally accepted as a safe requiring no specified average daily intake or an upper limit intake. However, inadvertent abuse of this food additive may occur because of its abundance, mostly without the labelling of many food ingredients. The study aimed to assess the role of monosodium glutamate (MSG) in modulating testicular and scrotal tissue calcium levels, antioxidant activity, and histopathological changes in male Wistar rats. The experimental study involved 30 adult 7-week-old male Wistar rats and collected quantitative and qualitative data. The study found that MSG administration did not affect calcium levels in the scrotum and testis, which had previously been associated with high levels of calcium in urine. The study also found no significant differences in calcium concentrations from the no MSG control group, indicating that the role of MSG in propagating cellular calcium dyshomeostasis in the urogenital system is not justified. Antioxidant (catalase) and malondialdehyde (MDA) were equally not affected by the MSG concentrations and treatment routes used in both scrotum and testicular tissue. This study failed to reproduce toxic effects reported in previous studies and concluded that MSG is safe for human consumption under internationally recommended concentrations, and more studies to show its ability to modulate gene expression remain to be established.

Keywords: Mono sodium glutamate, Calcium, Antioxidant, Urogenital system, Wistar rats

INTRODUCTION

Mono sodiurn glutamate, a white crystalline powder, is the sodium salt of a naturally occurring non-essential amino acid, glutamic acid [1]. MSG is commonly marketed as a flavour enhancer and is used as a food additive, particularly in West African and Asian dishes [2]. Generally, MSG is accepted as a safe food additive that needs no specified average daily intake or an upper limit intake. However, inadvertent abuse of this food additive may occur because of its abundance, mostly without the labelling of many food ingredients [3]. Modern commercial MSG is produced by the fermentation of starch, sugar, beet sugarcane, or molasses [4]. Some reports have reported that MSG is toxic to humans and experimental animals. It could produce symptoms such as numbness, weakness, flushing, sweating, dizziness, and headaches. In addition, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy, and abdominal discomfort [5]. MSG has a toxic effect on the testis by causing significant oligozoospermia and increasing abnormal sperm morphology in a dose-dependent fashion in male Wistar rats. It has been implicated in male infertility by causing testicular haemorrhage, degeneration, and alteration of sperm cell population and morphology [6]. Increased expression of the glutamate transporters in the tissue has been reported, and mitochondrial fractions play an important role in this regard [7]. Moreover, a previous finding has shown that the metabotropic glutamate (mGlu) receptors that are also in humans have been found to be crucial in this regard [8]. In the testis, glutamate decarboxylase (GAD), which is a synthetic enzyme for gamma-aminobutyric acid (GABA), has been shown [9], thus demonstrating its relevance to reproductive organs. Increased expression of glutamate receptors leads to increased carbohydrate metabolism and the build-up of free oxygen radical species (ROS) [10]. Moreover, it is thought that MSG adversely affects testicular histology and decreases its antioxidant activity [11]. Moreover, a recent study has shown that MSG leads to improved antioxidant activity [12]. Oxidative damage primarily occurs via the production of reactive oxygen species such as superoxide anion and peroxide, and it can damage lipids, proteins, and DNA. Therefore, it may cause a loss of enzymatic activity and the structural integrity of enzymes and activate inflammatory processes. It is suggested

that the toxic effects of MSG lead to alterations in the structural integrity of the mitochondrial inner membrane, resulting in the depletion of mitochondrial GSH levels and increased formation of hydrogen peroxide by the mitochondrial electron transport chain [11]. Oxidative stress plays an important role in the aetiology of defective sperm formation, function, sperm count profile, and male infertility, thus showing the need to assess its effect on testicular tissue. ln humans. Decreased antioxidant activity has been reported in dermal fibroblasts of glutamate-depressed patients, thus showing the role of glutamate in the skin [13]. In rabbits, peripheral glutamate receptors in the skin play a crucial role in heat and mechanical sensation accompanying pain [14]. Furthermore, following skin trauma, antioxidant activity has been associated with poor healing [15]. The testis uses glutamate for the production of energy required for normal function [16], and mitochondrial respiration plays a crucial role in the production of ATP [7], through the activity of glutamate decarboxylase A lot of conflicting reports on the role of glutamate have shown that it leads to increased oxidative stress (OS) [11], and improved OS [12], thus showing the need to assess its effect in testicular tissue. In the skin, glutamate has been shown to play a crucial role in nociception and wound healing. [13,14]. Increased antioxidant activity has been associated with regard to wound healing [15], the role of glutamate in altering antioxidant activity still remains to be established. Since the testis are covered by the scrotal sac, it's important to assess the role of glutamate in the scrotum to identify any effects on the health status of the testis. The Study was to assess the role of monosodium glutamate in modulating testicular and scrotal tissue calcium levels, antioxidant activity, and histopathological changes in male Wistar rats.

METHODOLOGY

Study Design

This was an experimental study in which quantitative and qualitative data were collected from T2DM Wistar rats. It involved assessing the structural changes, Ca^{2+} levels as well as antioxidants in heart and lung tissue were studied.

Monosodium Glutamate (MSG)

This was procured from Ajinomoto Co. Inc, Japan through the Institute of Innovation, and Laboratory of Dr. Sakai-Ryosai (DVM, PhD). The study was an experimental study involving 30 adult 7-week-old male Wistar rats. Rats were kept for a total of 30 days and sacrificed at the end.

Experimental Animals

Table 1 showing summary on study design.

1	Experimental Groups on Supplementation	Group description	No of Rats	Av. Wgt (g)
A	Regular feed*+ MSG Sub-cut at 6glkglday	MSG *sic*	6	120
B	Powdered feed*+ 5% MSG in feed	MSG alone in feed	6	120
C	Powdered feed*+ 2% MSG in feed	Low MSG in feed	6	120
D	Regular feed*+ MSG Sub-cut 60mglkglday	MSG *sic*	6	120
E	Regular feed*+ water	Control	6	120

feeds were provided adlib.

Organ collection for laboratory analysis

Rats were euthanized using sodium pentobarbitone injected intraperitoneally. The testis and scrotum were harvested from each rat and placed in sterile sample bottles. Samples were divided into duplicates for biochemical and histological analysis. Samples for biochemical analysis weresubsequently homogenized in 50 mL of L-1. Phosphate buffer (at pH = 7.0) was added at a ratio of I:100 (v/v) and centrifuged at 3000 rpm for 5 min. The filtrate was collected into sterile Eppendorf tubes, which were stored in a refrigerator at -20°C for biochemical analysis. Samples for histological analysis were placed in 10% buffered formalin.

Biochemical Analysis

Calcium Determination

Calcium concentrations in the experimental diets and tissues were determined using atomic absorption spectroscopy (AAS). The AAS (Perkin-Elmer, model GBC932AA, USA) was set up according to the manufacturer's recommendations, and an equation from the standard curve (absorbance = 450 nm) was used to determine calcium concentrations from each sample.

Catalase Determination

Catalase activity was determined according to method by Johansson and Borg [17] for small animal samples. This was based on the principle that dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide, which formed chromic acid as an unstable intermediate. The chromic acetate produced was measured calorimetrically at wavelengths of 570–610 nm. Since dichromate had no absorbance in this region, the presence of the compound in the assay mixture did not interfere at all with the spectrometric determination of chromic acetate. The 0.2 M hydrogen peroxide was diluted to different concentrations of standards. Different concentrations of H_2O_2, ranging from 10 to 160 µM, were pipetted into Eppendorf tubes (2.5 ml) and 0.5 ml of dichromate/acetic acid (prepared by adding a 5% solution of potassium dichromate ($K2Cr_2O_7$) with glacial acetic acid (I:3) by volume) was added to each. The addition of the reagent to H_2O_2 instantaneously produced an unstable blue precipitate of perchromic acid. On subsequent heating for 5 minutes in a boiling water bath, the colour of the solution changed from yellow to stable green due to the formation of chromic acetate. After cooling to room temperature, the volume of solution was transferred into cuvettes, and the optical density was then obtained at 580 nm using a spectrometer while using a blank solution. The catalase preparation was stopped at a particular time (1 minute) by the addition of the dichromate/acetic acid mixture, and the remaining hydrogen peroxide was determined by measuring chromic acetate calorimetrically after heating the reaction mixture. A standard curve at an absorbance of 580 nm, absorbance = 0.0013 (concentration) + 0.0521, R^2= *0.9719*, which was in the form of y = mx.+c was obtained.

Determination of malondialdehyde in animal tissues

The determination of malondialdehyde (MDA) was done by the nonspecific thiobarbituric acid reactive substances (TBARS) measurement as previously described ([18]. In particular, the concentration was determined by using the equation: Concentration = absorbance / (molar extinction coefficient x 1L) x 10^6 nm, where an extinction coefficient of 156 was used.

Histopathology Analysis

Sections of the testis and scrotum tissue blocks of each rat were analyzed according to a systematic random embedding, random sectioning, and sampling method. Microscopic changes were assessed using light microscopy and described descriptively.

Data Analysis

Quantitative data was analyzed using Graph Pad Prism version 6 and expressed as mean ± SD. Multiple comparisons using a Tukey's test were used to determine significance, and for all tests, a P 0.05 was considered significant, and different superscripts were used to represent the presence of significant differences. Information was presented in the form of tables, graphs, and photographs. Qualitative data from the histological analysis was descriptively analyzed and presented in paragraphs.

Ethical Considerations

The animals were kept under consideration of the National Institute of Health's (NIH) guidelines regarding experimental animals [19]. The rats will be kept in the Animal House of the College of Veterinary Medicine Animal Resources and Biosecurity (CoVAB) after acquiring institutional ethical clearance. The number of animals was reduced to the minimum required for the study purpose, and the techniques used to achieve the objectives were carefully handled. In brief, the provision of sufficient space, adequate fresh air, 12-hour daylight exposure, and comfortable bedding materials (sawdust) provided and housed in a raised structure will protect them from predators and secondary sources of infection. Water was provided ad libitum, and the feed ration was provided. After completion of the study, the carcass was properly disposed of.

RESULTS

Calcium concentrations in the scrotum and testis in rats after MSG administration.

The calcium level was 1.27 mmol in the scrotum following high dosage treatment through the subcutaneous route, and it was 1.21 mmol following feed supplementation, and no significant differences were found in the scrotal tissue. In the testis, calcium levels were 1.16 mmol in the subcutaneous treatment groups, while in feed supplementation, they were 1.21 mmol in the highest feed supplementation group, as shown below.

Table 2: Scrotal and Testicular Calcium Levels

Experimental Groups	N	Scrotal	Testis
		Mean ± SD Calcium Concentration (nMol/l)	
6g/kg s.c.	5	1.27±0.12[a]	1.16±0.34[a]
5% MSG in feed	6	1.21±0.10[a]	1.21±0.18[a]
2% MSG in feed	6	1.22±0.10[a]	1.16±0.16[a]
60 mg/kg/day MSG	6	1.17±0.31[a]	1.06±0.07[a]
Control	6	0.97±0.19[a]	1.05±0.15[a]

KEY: Tukey's multiple comparison group between groups i.e. (a, a = P > 0.05).

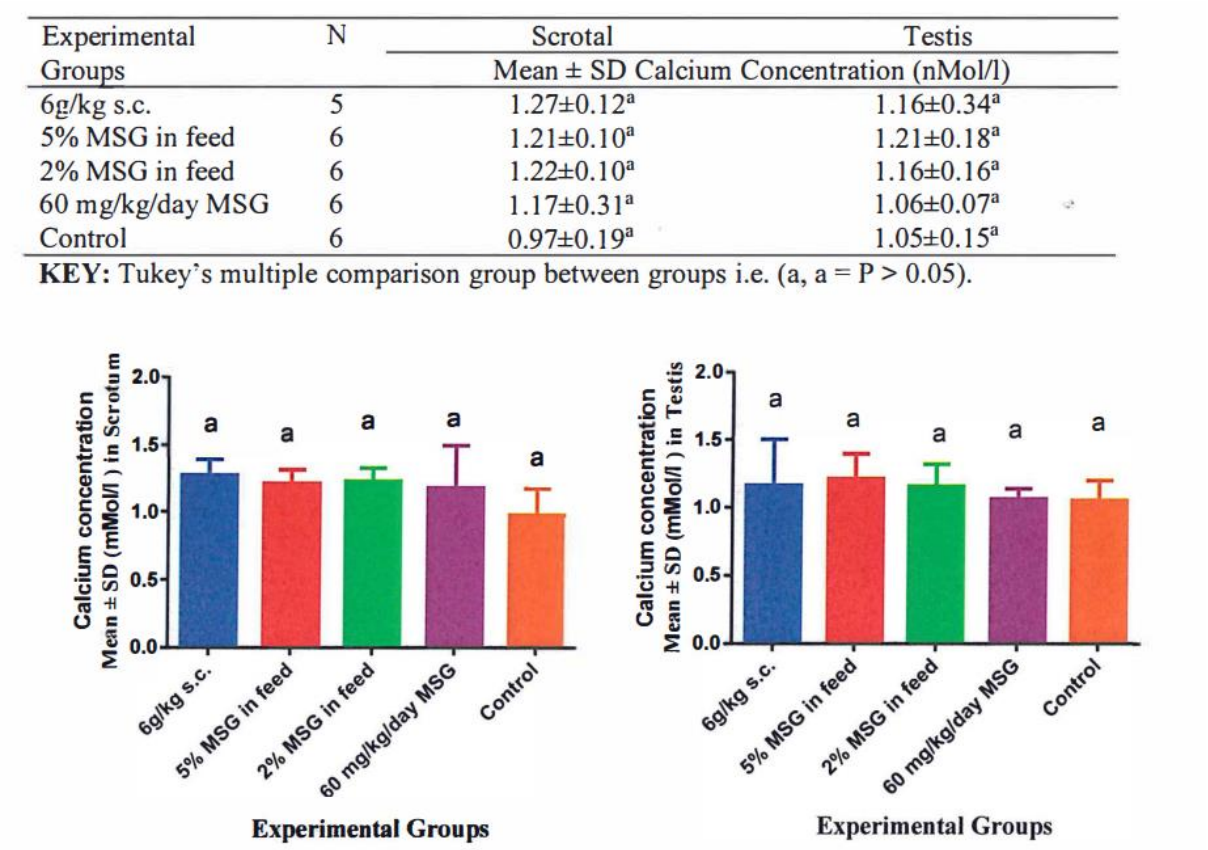

Figure1: Calcium concentrations in scrotum and testis of Rats after MSG administration.

Antioxidant activity in the scrotum and testis in rats after MSG

The study showed that catalase levels in the scrotum were relatively the same (P > 0.05), and similar observations were found in testicular tissue. In addition, MDA levels in the testis were not affected, while no differences were found in the experimental treatment groups, except for the control in scrotal tissue, as shown below.

Table 3: Antioxidant Variations in Testicular and Scrotal Tissues of Rats on MSG

Experimental Groups	N	Scrotum		Testis	
		CAT (nMol/1)	MDA (nMol/2rotein)	CAT (nMol/1)	MDA (nMol/2rotein)
		Concentrations ± SD			
6g/kg s.c.	5	128.80±5.77[3]	557.70±48.61[3]	133.00±7.45[3]	1325.00±622.60[3]
5% MSG in feed	6	128.80±4.15[3]	589.70±89.51[3]	132.40±4.09[3]	1250.00±525.00[3]
2% MSG in feed	6	129.80±5.96[3]	568.90±48.08[3]	132.80±5.80[3]	1100.00±259.10[3]
60 mg/kg MSG	6	129.30±8.75[3]	630.30±48.96[3]	138.40±8.75[3]	1298.00±430.60[3]
Control	6	133.90±3.95[3]	812.00±80.66[b]	137.30±3.80[3]	1474.00±198.90[3]

KEY: CAT = Catalase activity. Tukey's multiple comparison test carried out and different superscripts (a, b) show that $P < 0.05$.

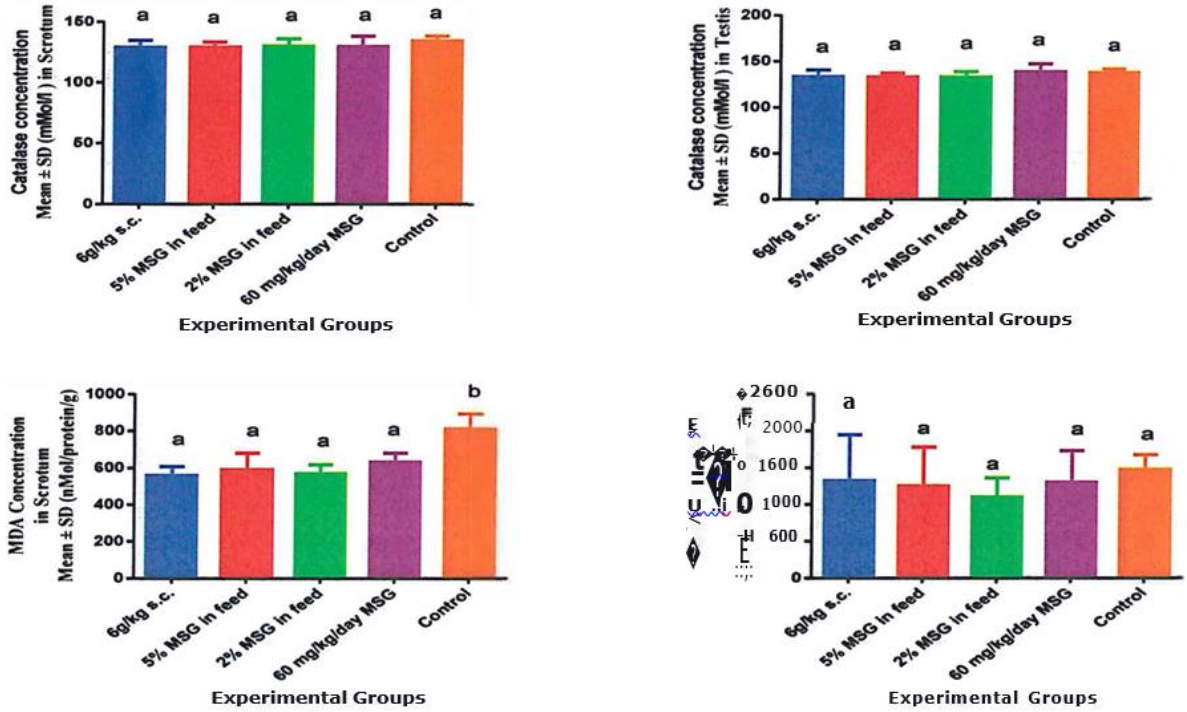

Figure 2 Catalase and MDA variations in Rats on MSG.

MSG Histopathology Effects

No significant changes were seen in both the scrotum and testis of rats after MSG exposure.

Histopathological Changes in the Scrotum

The hypodermis and hypodermis layers were consistently laid out in all groups, and there was no evidence of systemic injury on the scrotal tissue, as shown in the figure below.

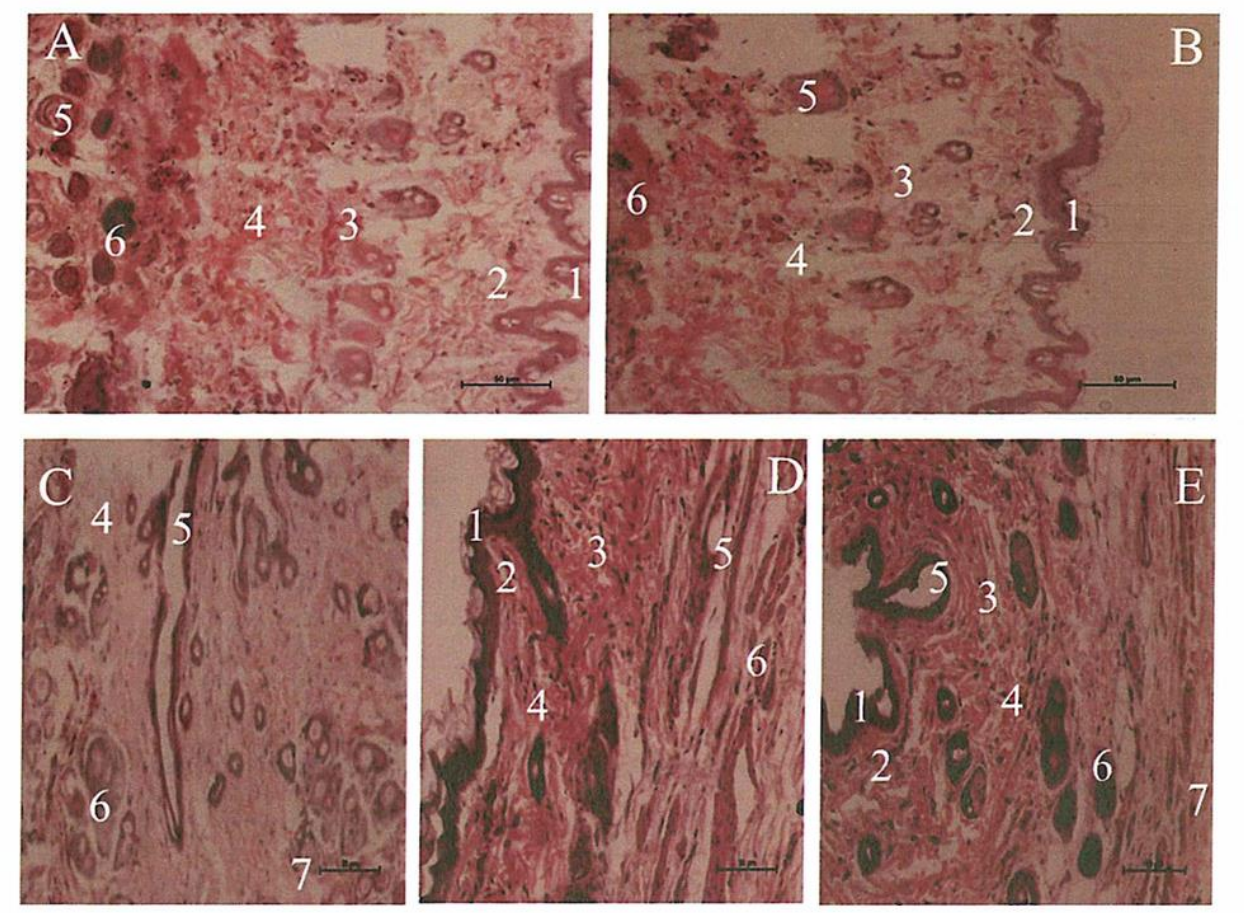

Figure 3: Scrotum in Rats after MSG Exposure

Key: Group A = rats treated with MSG at 6 g/kg/day s.c.; B = 5% MSG in feed; C = 2% MSG in feed; D = 60 mg/kg/day MSG s.c.; E = no MSG. In the photomicrographs, 1 = stratum corneum, 2 = stratum granulosum, and 3 = stratum basale. 4 = reticular dermis, 5 = Hair Follicle, 6= rector pili muscle; 7 = hypodermis.

DISCUSSION

Calcium levels in the scrotum and testis were not affected by MSG administration. Previously, high levels of calcium in urine had been associated with intraperitoneal administration of MSG at 2 mg/kg [20]; however, we demonstrate that at 60 mg/kg, those observations are not reproducible. This would have been due to the shorter duration of our study or minor modifications since we used the subcutaneous route. In addition, the administration of MSG intraperitoneally has no direct correlation to human consumption models. We observed that rats offered MSG at 5% and 2% showed no significant differences in calcium concentrations from the no MSG control group, thus showing a lack of evidence to justify the role of MSG in propagating cellular calcium dyshomeostasis in the urogenital system. In addition, the skin continues to serve as a mechanical barrier, thus offering crucial protection to the body. Calcium dysregulation has been associated with ageing and the appearance of several skin diseases [21]. Bearing in mind that the observations in the study showed no significant alterations in skin calcium, evidence that MSG is involved in skin pathology would be unfounded [22,23]. Antioxidant (catalase) (CAT) and malondialdehyde (MDA) were equally not affected by the MSG concentrations and treatment routes used in the current study in both scrotum and testicular tissue. These observations are in agreement with findings from Ibegbulem et al. [24] who showed that MSG at low concentrations is not associated with toxic effects. This subsequently led to an absence of toxicological effects in both scrotal and testicular tissue. Administration of MSG at a dosage of 60 g/kg (10 times above LD50) has been associated with toxicological effects [25], although this is of no benefit for human use since the dosage used and routes are not practical for daily usage in men. The ability to maintain a conducive environment for testicular function relies on the ability of the testis to be kept in a physiological environment since the tight junctions that form the testicular blood barrier (TBB) are highly effective under minimal stress conditions [26].

CONCLUSION

The study showed that administration of MSG at low concentrations did not interfere with basic biochemical and physiological functions as well as histomorphological nature, thus re-validating the usage of MSG at low concentrations in humans. The inability of the study to reproduce toxic effects reported in previous studies shows that a lot remains to be done. MSG is safe for human consumption under internationally recommended concentrations, and more studies to show its ability to modulate gene expression remain to be established.

REFERENCES

1. First, P., & Stehle, P. What are the essential elements needed for the determination of amino acid requirements in humans? *The Journal of Nutrition*, 2004;134(6), 1558–1565.
2. Farombi, E. 0., & Onyema, 0. 0. Monosodium glutamate-induced oxidative damageand genotoxicity in the rat: modulatory role of vitamin C, vitamin E, and quercetin. *Human & Experimental Toxicology*, 2006;25(2006), 251-259. http://doi.org/10.1191/0960327106ht621oa
3. Egbuonu, A. C. C., Obidoa, 0., Ezeokonkwo, C. A., Ezeanyika, L. U.S., & Ejikeme, P. M. Hepatotoxic effects of low-dose oral administration of monosodium glutamate in male albino rats. *African Journal of Biotechnology*, 2009;8(13), 3031–3035.
4. Walker, R., & Lupien, J. R. Glutamate Safety in the Food Supply: The Safety Evaluation of Monosodium Glutamate. *The Journal of Nutrition*, 2000; 130, 1049–1052.
5. Geha, R. S., Beiser, A., Ren, C., Patterson, R., Greenberger, P. A., Grammer, L. C.,... Saxon, A. Review of the alleged reaction to monosodium glutamate and outcome of a multicenter, double-blind placebo-controlled study. *The Journal of Nutrition*, 2000;130(4S), 1058–1062.
6. Gupta, A. Das, Dhundasi, S. A., Ambekar, J. G., & Das, K. K. Effect of I-ascorbic acid on the antioxidant defence system in the testes of albino rats exposed to nickel sulfate. *Journal of Basic and Clinical Physiology and Pharmacology*, 2007;18(4), 255–66. http://doi.org/1O. 515/JBCPP.2007.18.4.255
7. Takarada, T., Hinoi, E., Balcar, V. J., Taniura, H., & Yoneda, Y. Possible expression of functional glutamate transporters in the rat testis. *Journal of Endocrinology, 2004;181(2)*, 233–244. http://doi.org/ l 0. I 677/joe.0. l 810233.
8. Storto, M., Sallese, M., Salvatore, L., Poulet, R., Condorelli, D. F., Dell'Albani, P.,... De Blasi. Expression of metabotropic glutamate receptors in the rat and human testes *The Journal of Endocrinology*, J. 2001;70(1), 71–8. http://doi.org/l 0. l 677/joe.0.1700071
9. Tillakaratne, N. J., Erlander, M. G., Collard, M. W., Greif, K. F., & Tobin, A. J. Glutamate decarboxylases in nonneural cells of rat testis and oviduct: differential expression of GAD65 and GAD67. *The Journal of Neurochemistry*, 1992;58(2), 618–627.
10. Coyle, J. T., & Puttfarcken, P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science (New York, NY)*, 1993;262(5134): 689–95. http://doi.org/10.1126/science.7901908
11. Hamza RZ, Al-Harbi MS. Monosodium glutamate induced testicular toxicity and the possible ameliorative role of vitamin E or selenium in male rats. *Toxicology Reports*. 2014;1:1037-1045. doi: 10.1016/j.toxrep.2014.10.002.
12. Yin J, Liu M, Ren W, Duan J, Yang G, Zhao Y, Fang R, Chen L, Li T, Yin Y. Effects of dietary supplementation with glutamate and aspartate on diquat-induced oxidative stress in piglets. *PLoS One*. 2015;10(4):e0122893. doi: 10.1371/journal.pone.0122893.
13. Gibson, S. A., Korade, Z., & Shelton, R. C. Oxidative stress and glutathione response in tissue cultures from people with major depression. *Journal of Psychiatric Research,2012;46 (*Π 0), 1326–1332. http://doi.org/10.1016/j.jpsychires.2012.06.008
14. Du, J., Zhou, S., & Carlton, S. M. Kainate-induced excitation and sensitization of nociceptors in normal and inflamed rat glabrous skin. *Neuroscience, 2006;137(3), 999–1013*. http://doi.org/10.1016/j .neuroscience.2005.10.008.
15. Benderitter, M., Isoir, M., Buard, V., Durand, V., Linard, C., Vozenin-Brotons, M. C., Gourmelon, P. Collapse of skin antioxidant status during the subacute period of cutaneous radiation syndrome: a case report. *Radial Research, 2007;167(1), 43–50*. http://cloi.org/1O.l 667/RR0577.1.
16. Lee, A., Anderson, A. R., Barnett, A. C., Chan, A., & Pow, D. V. Expression of multiple glutamate transporter splice variants in the rodent testis. *Asian Journal of Andrology*, 2011; I3(2), 254–265. http://doi.org/l 0.1038/aja.2010.99
17. Johansson, L. H., & Borg, L. A. A spectrophotometric method for the determination of catalase activity in small tissue samples. *Analytical Biochemistry*, 1988; 174(1), 331-6. http://doi.org/10.1016/0003-2697(88)90554-4

18. Zeb, Alam & Ullah, Fareed. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances (TBARS) in fried fast foods. *Journal of Analytical Methods in Chemistry*. 2016. 10.1155/2016/9412767.
19. National Institute of Health. *Guide for the Care and Use of Laboratory Animals* (8th Edition) http://doi.org/10.1163/1573-3912_islam_DUM_3825 2006
20. Sharma, A., Prasongwattana, V., Cha'on, U., Selmi, C., Hipkaeo, W., Boonnate, P., Reungjui. Monosodium glutamate (MSG) Consumption Is Associated with Urolithiasis and Urinary Tract Obstruction in Rats. *PLoS ONE, 2013*;8(9). http://doi.org/10.1371/journal.pone.0075546.
21. Rinnerthaler, M., Streubel, M. K., Bischof, J., & Richter, K. Skin ageing, gene expression, and calcium. *Experimental Gerontology, 2015; 68, 59–65.* http://doi.org/10.1016/j.exger.2014.09.015
22. Lansdown AB. Calcium: a potential central regulator in wound healing in the skin. *Wound Repair and Regeneration*. 2002;10(5):271-85. doi: 10.1046/j.1524-475x.2002.10502.x.
23. Simon, R. A. Additive-induced urticaria: experience with monosodium glutamate (MSG). *The Journal of Nutrition, 2000; 130 (4S* Suppl), 1063S–6S.
24. Ibegbulern, C. 0., Chikezie, P. C., Ukoha, A. I., & Opara, C. N. Effects of diet containing monosodium glutamate on organ weights, acute blood steroidal sex hormone levels, lipid profile, and erythrocyte antioxidant enzyme activities of rats. *Journal of acute Disease, 2016; 5(5),* 402-407. http://doi.org/10.1016/j.joad.2016.08.007
25. Alalwani, A. D. Monosodium glutamate-induced testicular lesions in rats (histological study). *Middle East Fertility Society Journal*, 2014;19(4), 274–280. http://doi.org/10.1016/j.mefs.2013.09.003.
26. Khavkin J, Ellis DA. Aging skin: histology, physiology, and pathology. *Facial Plastic Surgery Clinics of North America*. 2011;19(2):229-34. doi: 10.1016/j.fsc.2011.04.003.

CITE AS: Allende Caleb Ololade (2024). Testicular and Scrotal Antioxidant and Structural Changes Associated with Monosodium Glutamate in Male Wistar Rats. RESEARCH INVENTION JOURNAL OF SCIENTIFIC AND EXPERIMENTAL SCIENCES *3(3)*:57-64.