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Research Article

Antioxidant Outcomes of L-arginine on Monosodium Glutamate-induced Oxidative Stress in Rats' Testes

Anthony C. C. Egbuonu [1], Prince O. Alaebo [1], George C. Njoku [1], Paschal Ugwu [1]

[1]*Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria*

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CORRESPONDENCE
Egbuonu, A.C.C.
tonycemalukegbuonu@yahoo.com
egbuonu.anthony@mouau.edu.ng
+23480-3636-6565

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ABSTRACT

Oxidative stress was implicated in monosodium glutamate-related adversity in male reproductive organs. This study examined the possible antioxidant outcomes of L-arginine on oxidative stress induced by monosodium glutamate burden (8000 mg/kg of body weight) in rats' testes after oral intubation for 28 days. Exposure to monosodium glutamate significantly ($p < 0.05$) increased malondialdehyde (3.84±0.34 mg/dl) level but diminished the level of reduced glutathione (9.60 ± 0.28 mg/dL), catalase (5.51 ± 0.35 IU/L) and superoxide dismutase (1.74 ± 0.20 IU/L) in the rats' testes homogenate compared to control and other groups. L-arginine treatment alone or l-arginine co-treatments with monosodium glutamate significantly ($p < 0.05$) decreased the level of malondialdehyde but increased that of reduced glutathione, catalase and superoxide dismutase in the rats' testes compared to control and monosodium glutamate treated groups. Thus, the oxidant effects induced by monosodium glutamate at a dose of 8000 mg/kg in the rats' testes were significantly mitigated by L-arginine. This portends the antioxidative role of L-arginine in oxidative stress related testes dysfunctions by probable deregulation in malondialdehyde but up-regulation in reduced glutathione, catalase, and superoxide dismutase expression and functions

Keywords: *Superoxide dismutase, malondialdehyde, reduced glutathione, catalase*

INTRODUCTION

L-arginine is generally regarded as a fatherhood amino acid owing to its capacity to improve sperm quality and libido (Chen and Li, 2018). It mitigates oxidative stress possibly by acting as an antioxidant *via* its sole metabolite, nitric oxide (Ezeanyika and Egbuonu, 2011; Otasevic *et al.*, 2013). Monosodium glutamate is a widely used flavor enhancer. It is a potential excitotoxin (*via* glutamate) and endocrine function disruptor known to cause oxidative stress-related damage in the male reproductive system (Phaniendra *et al.*, 2015; Jubaidi *et al.*, 2019; Abdul-Hamid *et al.*, 2021). Oxidative stress is fundamental to agent-related toxicity mechanisms and many ailments. It is indicated by significant alteration in the level of malondialdehyde (MDA), reduced glutathione (GSH) catalase (CAT), and superoxide dismutase (SOD) (Joseph *et al.*, 2015; Malik *et al.*, 2016; Egbuonu and Ejike 2017).

Co-consuming L-arginine with a high concentration of monosodium glutamate is likely and could alter the oxidant *cum* antioxidant function balance in male testes. It is necessary to study the possible effects of concomitant use of L-arginine and monosodium glutamate to establish whether arginine could aggravate, mitigate or fail to alter the potential oxidant effects of monosodium glutamate on rats' testes antioxidant status. The testes like other vital organs employ well-developed multiple antioxidant defense systems for protection against free radicals attack and consequent oxidative stress (Poljak and Fink 2014). Therefore, this study aimed to examine the possible interactive effects, and the possible antioxidant responses, of l-arginine on monosodium glutamate burden-induced oxidant effects in rats' testes. Thus, antioxidant status indicators (malondialdehyde (MDA), Reduced Glutathione

(GSH), catalase (CAT), and superoxide dismutase (S0D)) were determined in the rat's testes homogenate.

MATERIALS AND METHODS

Chemicals and drug

L-arginine was obtained from Sigma Aldrich Chemical Company, St. Louis, MO. The USA. Monosodium glutamate, MSG (99 % purity) was purchased from a regular local foodstuff market. Diagnostic kits used in this study were products of Randox kit, UK. Other chemicals were of certified analytical grade and procured from the Department of Biochemistry laboratory, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria.

Animals and treatments

Twenty-five (25) Wistar rats were purchased from the animal farm of the Department of Veterinary Medicine, University of Nigeria Nsukka. The animals were assigned to five groups of five rats (average weight of 85.20 g). This was after 1 week of acclimatization in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria. Group 1 (control) rats were given distilled water (1 ml/kg). Group 2 rats were fed monosodium glutamate (8000 mg/kg). Group 3 rats were fed L-arginine (60 mg/kg) alone. Group 4 rats were fed monosodium glutamate (8000 mg/kg) with L-arginine (60 mg/kg). Group 5 rats were fed monosodium glutamate (8000 mg/kg) with L-arginine (120 mg/kg). Treatment was done by daily oral intubation for 28 days. The rats were housed in cleaned stainless steel cages at room temperature (28±2 ^{0}C); 12 h light/dark cycle and humid tropical conditions. Animals were freely provided with rat feed (Vital Feed Growers Marsh containing 20 % crude protein and 280 kcal 100^{-1} g metabolizable energy, manufactured by Vital Feed Industries Limited, Nigeria) and portable (tap) water for the duration of the experiment. The animals were handled in strict adherence to the guidelines provided by the National Research Council, USA (NRC, 2011) as adopted by the Postgraduate Committee of the Department of Biochemistry of the Michael Okpara University of Agriculture Umudike, Nigeria (no ethical clearance number).

Testes tissue collection and preparation

Testes samples of the rats sacrificed following mild anesthesia 24 h after 28 days of treatment were excised individually and homogenized to obtain supernatant for biochemical evaluation. Ten (10) % testes tissue homogenate was obtained by separately grinding a 0.5 g of respective testes sample in 5 ml of phosphate buffer saline (pH 7.4), using a mortar and pestle. The supernatant was removed by centrifugation at 500 g for 20 minutes at 4 ^{o}C, collected individually, and stored in a deep freezer maintained at minus 20 ^{o}C for determination of testes homogenate GSH, CAT, MDA, and SOD. The DNA damage to the animal testes was not determined. The testes histology was determined but already presented in a part of this study under publication.

Determination of antioxidant status indicators in the rats' testes homogenate

The reduced glutathione (GSH) concentration was estimated by the method described by Sedlak and Linsay (1968) based on a slight modification of the method of Ellman (1959). The determination of malondialdehyde concentration was according to the method described by Wallin et al. (1993) while catalase activity was determined by the method described by Johansson and Borg (1998). The determination of superoxide dismutase (SOD) activity was by the spectrophotometric method as described by Madesh and Balasubramanian (1998).

Statistical analysis

All analyses were performed by one-way analysis of variance (ANOVA), using the statistical package for social sciences (SPSS) for windows version 16.0. Dunnett's test was used for the posthoc multiple comparisons of means. Mean differences were considered significant at a $p < 0.05$ level of significance. The results were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Result

Monosodium glutamate treatment significantly ($p < 0.05$) and markedly increased malondialdehyde concentration (0.87 ± 0.29 mg/dl) in the rats' testes as compared to control and other treatment groups. L-arginine treatment alone or combined in two graded doses with monosodium glutamate significantly and dose-dependently decreased malondialdehyde in the rats' testes compared to the control and monosodium glutamate treated group (Figure 1).

Compared to control and others, monosodium glutamate treatment significantly ($p < 0.05$) diminished reduced glutathione concentration (9.60 ± 0.28 mg/dl) in the rats' testes. L-arginine either mono-treatment or co-treatments with monosodium glutamate significantly and dose-dependently increased the reduced glutathione concentration as compared to monosodium glutamate treated rats (Figure 2). Monosodium glutamate treatment significantly ($p < 0.05$) diminished catalase (5.51 ± 0.35 IU/L) and superoxide dismutase (1.74 ± 0.20 IU/L) activities in the rats' testes in comparison with the control and other groups.

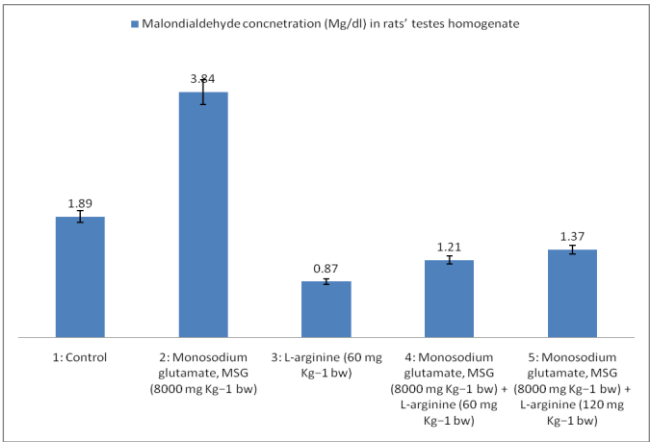

Figure 1. L-arginine and monosodium glutamate + L-arginine outcomes on malondialdehyde (MDA) level (mg/dl) in rats' testes homogenate.

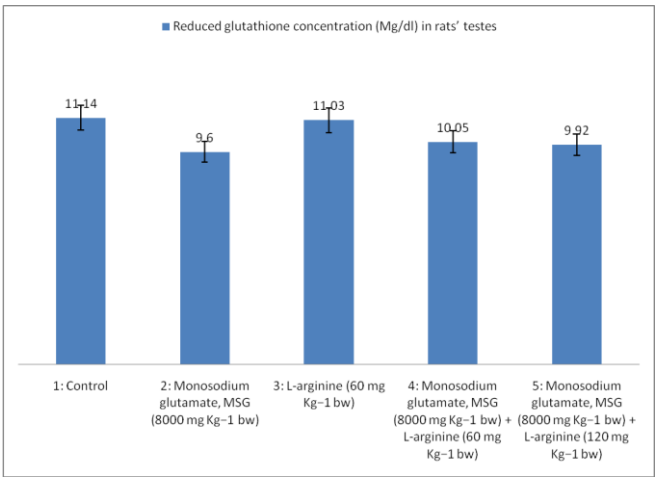

Figure 2. L-arginine and monosodium glutamate + L-arginine outcomes on reduced glutathione level (mg/dl) in rats' testes homogenate

L-arginine exposure alone or together in two graded doses with monosodium glutamate significantly and dose-dependently increased catalase and superoxide dismutase activities in the rats' testes compared to the monosodium glutamate treated rats group (Table 1).

Discussion

This study evaluated the possible antioxidant outcomes of L-arginine on oxidative stress induced by monosodium glutamate burden in rats' testes. Markedly increased ($p < 0.05$) malondialdehyde concentration but diminished ($p < 0.05$) level of reduced glutathione, catalase and superoxide dismutase in monosodium glutamate-treated rats' testes indicated induction of oxidative stress in consistency with previous reports (Malik *et al.*, 2016; Ilaria *et al.*, 2017; Qiu *et al.* 2019; Zhang *et al.*, 2019).

Table 1. L-arginine and Monosodium glutamate + L-arginine outcomes on catalase (CAT), and superoxide dismutase (SOD) enzymes levels in rats' testes homogenate

Group/Treatment (mg/kg)	Catalase (IU/L)	Superoxide dismutase (IU/L)
Control	8.06 ± 0.59^{b}	3.53 ± 0.48^{c}
Monosodium glu\tamate, MSG (8000 mg kg^{-1} bw)	5.51 ± 0.35^{c}	1.74 ± 0.20^{d}
L-arginine (60 mg kg^{-1} BW)	8.44 ± 1.17^{b}	5.48 ± 0.48^{a}
Monosodium glutamate, MSG (8000 mg kg^{-1} BW) + L-arginine (60 mg kg^{-1} BW)	8.80 ± 0.97^{b}	4.44 ± 0.16^{b}
Monosodium glutamate, MSG (8000 mg kg^{-1} BW) + L-arginine (120 mg kg^{-1} BW)	11.43 ± 0.76^{a}	4.66 ± 0.48^{ab}

Induction of oxidative stress results in a collapse in the antioxidant response mechanisms in the rats' testes and the observations herein. Increased MDA concentration together with decreased SOD activity as recorded in the present study strongly indicated oxidative stress status in animals (Mandal *et al.*, 2016). The antioxidant defense system comprises balanced SOD, CAT and GSH expression and functions (Demir *et al.*, 2011). This study revealed that MSG treatment concomitantly decreased these notable antioxidant defense apparatus which confirms oxidative stress in the rats' testes in line with the report by Fabio *et al.* (2012). In particular, diminished CAT and SOD activities and GSH concentration as observed in this study may have resulted following active participation of CAT and SOD enzymes in antioxidant response against oxidative stress in the rats' testes. Ighodaro and Akinloye (2018) reported concerted defense by GSH, CAT, and SOD as the first line of antioxidant response to oxidative stress in animals.

Conversely, a significant and dose-dependent decrease in malondialdehyde increased the level of reduced glutathione, catalase, and superoxide dismutase in the rats' testes following L-arginine exposure either alone or when co-treated with monosodium glutamate was reported herein. This indicated L-arginine-related benefits on the rats' antioxidant response system and apparent potential to mitigate oxidative stress in the rats' testes in conformity with previous study reports (Liang *et al.*, 2018; Qiu *et al.*

2019). The present outcomes demonstrate a significant and dose-dependent mitigation response of L-arginine against oxidative stress dysfunctions induced in the rats' testes following monosodium glutamate burden. These were *via* probable deregulation in malondialdehyde but upregulation in reduced glutathione, catalase, and superoxide dismutase expression and functions in the rats' testes.

The biochemical implication of co-administering l-arginine and monosodium glutamate could account for the present observations and suggestions thereto. Diverse biochemically important metabolic products of l-arginine, including nitric oxide and spermin, probably interacted to mitigate the monosodium glutamate-induced oxidative stress in the rats' testes. Interaction between l-arginine and monosodium glutamate could lead to the dissociation of sodium ion moiety to release the non-toxic deprotonated glutamate moiety into the amino acid pool. These seem to be supported by the rats' testes histology results (data not shown). A detailed DNA damage study could enhance further clarification of the present observations. However, the DNA damage to the animal testes was not determined herein. This is a notable shortcoming that needs to be incorporated into the design of future similar studies.

CONCLUSION

The present study demonstrated MSG-burden-related induction of oxidative stress in the rats' testes. It also demonstrated L-arginine-related apparent benefit and capacity to significantly mitigate the MSG-burden-related oxidative stress in the rats' testes. Thus, the oxidant effects induced by monosodium glutamate at a dose of 8000 mg/Kg in the rats' testes were significantly mitigated by L-arginine. This portends antioxidative role of L-arginine in oxidative stress related testes dysfunctions by probable deregulation in malondialdehyde but up regulation in reduced glutathione, catalase and superoxide dismutase expression and functions.

AUTHORS' CONTRIBUTIONS

ACCE got the concept, design, supervision, corrections of the manuscript. POA did the proofreading, corrections and critical revision of the manuscript. GCN made the drafting and interpretation of results obtained. PU participated in the drafting and interpretation of results of the study. All gave a final approval of the revised version and agreed to the publication.

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CONFLICT OF INTEREST

The author declared that there is no conflict of interest

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