ANTIOXIDANT AND HAEMATOLOGICAL RESPONSES OF L-ARGININE ON MONOSODIUM GLUTAMATE-INDUCED OXIDATIVE STRESS IN RATS’ SERUM

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ABSTRACT

This study aimed to examine the possible antioxidant and haematological responses of L-arginine on oxidative stress induced by monosodium glutamate burden in rats’ serum. Thus, monosodium glutamate, at a dose of 8000 mg/Kg of body weight was administered to male Wistar rats by oral intubation daily for 28 days. Monosodium glutamate treatment significantly increased malondialdehyde but diminished reduced glutathione concentrations, catalase and superoxide dismutase activities in the rats’ serum. Monosodium glutamate treatment significantly decreased haemoglobin and packed cell volume but non-significantly decreased white blood cells contents in the rats’ serum. L-arginine either mono-treatment or co-treatments with monosodium glutamate significantly decreased malondialdehyde but increased reduced glutathione concentrations catalase and superoxide dismutase activities in the rats’ serum compared to control and monosodium glutamate treatment. L-arginine either mono-treatment or co-treatments with monosodium glutamate significantly increased haemoglobin, packed cell volume and white blood cells contents compared to control and monosodium glutamate treatment. Results revealed that these effects induced by monosodium glutamate at a dose of 8000 mg/Kg in the rats’ serum were significantly mitigated by L-arginine. The antioxidant effects by L-arginine were notably significant at 120 mg/Kg in the rats’ serum and accompanied by probable up-regulation in the rats’ haematological expression and functions.

Keywords: Haemoglobin, White blood cells, Superoxide dismutase, Reduced glutathione, Catalase.

INTRODUCTION

L-arginine is a semi-essential basic amino acid. It is common in natural foods and participates in overall animal immunity. [1] It exerts potential benefits in animal health, and could mitigate oxidative stress by acting as an antioxidant via its sole metabolic product and a conditional antioxidant, nitric oxide, NO. [2,3,4] L-arginine is intentionally added to diet and drug-based supplements and may be co-consumed with monosodium glutamate (MSG) - a widely used flavor enhancer. Monosodium glutamate is potentially toxic to animals especially when consumed at a high concentration or by MSG-intolerant individuals. Monosodium glutamate acts as an excitotoxin via glutamate to cause significant damage in animals. [5,6]

Earlier studies in animals indicated that treatment for consecutive 10 days had a significant alteration in the level of lipid peroxidation and in the activities of antioxidant enzymes, including catalase (CAT), and superoxide dismutase (SOD) that were indicative of induction of oxidative stress. [7] Oxidative stress is fundamental to agent-related toxicity mechanism and consequently many ailments. [8,9] It results from oxidant actions of free radicals in excess of antioxidant protective capacity of the animal. Free radicals could be deleterious by attacking and transforming cellular biomolecules into other free radicals, including hydrogen peroxide, and hydroxyl radicals. [10] Animals employ well-developed multiple antioxidant defense systems for protection against free radicals attack and consequent oxidative stress. [11] Hydrogen
peroxisome and hydroxyl radicals are usually scavenged by antioxidant defense system involving catalase and superoxide dismutase enzymes.

Co-consuming L-arginine with monosodium glutamate could elicit unknown consequences on the antioxidant defense in animals and even animals’ immunity via impaired haematological functions. 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 (and related effects on the haematological functions) of monosodium glutamate in rats. Therefore, this study was aimed at examining the potential interactive effects of arginine, commonly used in diets and drugs, on monosodium glutamate-induced oxidant effects. The intent was to elucidate the possible antioxidant and haematological responses of L-arginine on serum oxidative stress and on haematologic dysfunction induced by monosodium glutamate burden in rats. Antioxidant status indicators (malondialdehyde (MDA), reduced Glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) and haematological parameters - haemoglobin (HGB), packed cell volume (PCV), and white blood cells (WBC) were determined in the rats’ serum.

**MATERIALS AND METHODS**

**Chemicals and drug**

L-arginine was obtained from Sigma Aldrich Chemical Company, St. Louis, MO. USA. Monosodium glutamate, MSG (99 % purity) was purchased from a regular food stuff market at Aba, Abia State. Diagnostic kits used in this study were products of Randox kit, UK. Other chemicals were of certified analytical grade. These were sourced and purchased from reputable chemical stores at Ariaria market, Aba, Abia State and Department of Biochemistry laboratory, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria.

**Animals and treatments**

Twenty-five (25) Wistar rats weighing 85.20 g were purchased from the animal farm of the Department of Veterinary Medicine, University of Nigeria Nsukka. They were acclimatized for a week in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria. The animals were randomly assigned to five groups of five rats (average weight 85.20 g). Group 1(control) were given distilled water (1 ml/Kg). Group 2 rats were fed monosodium glutamate (8000 mg/Kg of body weight) alone, whereas group 3 rats were fed L-arginine (60 mg/Kg) alone. Groups 4 and 5 rats were fed monosodium glutamate (8000 mg/Kg of body weight) with L-arginine (60 mg/Kg) and (120 mg/Kg), respectively. Treatment was by daily oral intubation for 28 days. The rats were housed in cleaned stainless steel cages at room temperature (28±2 °C); 12 h light/dark cycle and humid tropical conditions. Animals were provided with rat feed (Vital Feed Growers Marsh containing 20 % crude protein and 280 kcal/100 g metabolizable energy, manufactured by Vital Feed Industries Limited, Nigeria) and portable (tap) water ad libitum for the duration of the experiment. The MSG-burdened dose (8000 mg/Kg) was as in earlier studies. [12,13] The L-arginine doses (60 mg/kg and 120 mg/kg) were based on earlier study. [14] The animal experimentation strictly followed the Ethical guidelines of the National Research Council, USA. [15]

**Blood and liver tissue collection and preparation**

Blood samples of the rats sacrificed following mild anesthesia 24 h after 28 days treatment were collected individually with sterile capillary tubes into properly labeled plain polystyrene centrifuge tubes by cardiac puncture technique. The blood samples thus collected were allowed to clot. Then, the
serum was removed by centrifugation under room temperature at 3000 rpm for 10 minutes, collected individually and stored in a deep freezer maintained at minus 20 °C for determination of the serum malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) superoxide dismutase (SOD), haemoglobin (HGB), packed cell volume (PCV) and white blood cells (WBC).

**Determination of antioxidant status indicators in the rats’ serum**

The determination of malondialdehyde concentration was according to the method described by Wallin et al. [16]. The determination of catalase activity was by the method described by Johansson and Borg. [17]

The reduced glutathione (GSH) concentration was estimated by the method described by Sedlak and Lindsay. [18] Reduced glutathione (GSH) level was determined using the method described by Ellman [19] with slight modifications. The determination of superoxide dismutase (SOD) activity was the spectrophotometric method as described by Madesh and Balasubramanian. [20]

**Determination of haematological parameters in the rats’ serum**

The haemoglobin (HGB) content was determined by the technique described by Ochei and Kolhatkar. [21] The determination of white blood cells (WBC) and was according to the method described by Cheesbrough. [22] The packed cell volume (PCV) was determined by the microhematocrit centrifuge method. [22]

**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. The Dunnett’s test was used for the post-hoc multiple comparison of means. Differences in mean were considered significant at p < 0.05 level of significance. The results were presented as mean ± standard deviation (SD).

**RESULTS AND DISCUSSION**

This study evaluated the possible antioxidant and haematological responses of L-arginine on oxidative stress and related haematologic dysfunction induced by monosodium glutamate burden in rats. From this study, monosodium glutamate treatment significantly increased malondialdehyde but diminished reduced glutathione concentrations, catalase and superoxide dismutase activities in the rats’ serum. These indicated induction of oxidative stress following apparent collapse in the antioxidant response mechanisms in the rats (Figure 1 and Table 1).

![Figure 1. Response of L-arginine and Monosodium glutamate + L-arginine on malondialdehyde (MDA) levels in rats’ serum](image-url)

This is in consistence with previous reports that associated induction of oxidative stress or compromised antioxidant response mechanisms in animals with particularly increased MDA concentration. [23,24] Monosodium glutamate increased MDA concentration in oxidative-stressed animals. [7]
Also, increase in serum MDA in response to oxidative stress was documented. [25] Also, in support with the present study, increased MDA concentration but decreased SOD activity indicated oxidative stress status. [1]

Table 1. Response of L-arginine and Monosodium glutamate + L-arginine on reduced glutathione (GSH), catalase enzyme, and superoxide dismutase enzyme (SOD) levels in rats’ serum

<table>
<thead>
<tr>
<th>Group/Treatment (mg/Kg)</th>
<th>Reduced glutathione concentration (Mg/dl)</th>
<th>Catalase enzyme activity (IU/L)</th>
<th>Superoxide dismutase enzyme activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Control</td>
<td>8.20±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.94±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.92±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2: Monosodium glutamate, MSG (8000 mg/Kg)</td>
<td>3.73±0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.68±0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.14±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3: L-arginine (60 mg/Kg)</td>
<td>4.69±0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.69±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.47±0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4: Monosodium glutamate, MSG (8000 mg/Kg) + L-arginine (60 mg/Kg)</td>
<td>3.83±0.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.94±0.49&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.27±0.15&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5: Monosodium glutamate, MSG (8000 mg/Kg) + L-arginine (120 mg/Kg)</td>
<td>6.80±0.44&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.80±0.58&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11.20±0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: The results are mean ± standard deviation, SD for five rats in each group. a,b,c,d,e,f Means on a column with different superscript letters (arranged from a = least to f = highest) are significantly different at p < 0.05.

Antioxidant defense system consists of SOD, CAT and GSH. [26] In this study, MSG treatment decreased SOD, CAT and GSH in the rats’ serum in line with previous report. [27] Concerted defense by GSH, CAT and SOD as the first line of antioxidant response to oxidative stress in animals have been reported. [28] Diminished CAT and SOD 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. Monosodium glutamate treatment significantly decreased haemoglobin and packed cell volume but non-significantly decreased white blood cells contents in the rats’ serum (Table2).

Table 2. Response of L-arginine and Monosodium glutamate + L-arginine on haemoglobin (HGB), packed cell volume (PCV), and white blood cells (WBC) contents in rats’ serum

<table>
<thead>
<tr>
<th>Group/Treatment (mg/Kg)</th>
<th>Haemoglobin content (g/dl)</th>
<th>Packed volume content (%)</th>
<th>White blood cells content (×10&lt;sup&gt;3&lt;/sup&gt;/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Control</td>
<td>19.07±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.33±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.94±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2: Monosodium glutamate, MSG (8000 mg/Kg)</td>
<td>18.23±2.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.66±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3: L-arginine (60 mg/Kg)</td>
<td>22.43±0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.66±3.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.80±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4: Monosodium glutamate, MSG (8000 mg/Kg) + L-arginine (60 mg/Kg)</td>
<td>20.10±0.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>46.33±2.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.08±0.34&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5: Monosodium glutamate, MSG (8000 mg/Kg) + L-arginine (120 mg/Kg)</td>
<td>23.03±0.66&lt;sup&gt;e&lt;/sup&gt;</td>
<td>48.00±2.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.66±0.30&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: The results are mean ± standard deviation. SD for five rats in each group. a,b,c,d,e,f Means on a column with different superscript letters (arranged from a = least to f = highest) are significantly different at p < 0.05.

These confirmed successful induction of hematologic dysfunction in the monosodium glutamate-fed rats. This is consistent with previous study outcome that associated concomitant decrease in these haematological parameters with haematological dysfunction, notably anaemia. [29] It further suggests that haematological dysfunctions may accompany oxidative stress in rats induced by monosodium glutamate burden. L-arginine either mono-treatment or co-treatments with monosodium glutamate significantly increased haemoglobin, packed cell volume and white blood cells contents compared to control and monosodium glutamate treatment. This indicates L-arginine-related benefits on the rats’ haematological response accompanying monosodium glutamate-related oxidative stress in the rats. It further indicates that the effects by L-arginine against monosodium glutamate-related oxidative stress in the rats were accompanied by probable up-regulation in the rats’ haematological expression and functions.
Generally, haemoglobin could act as an antioxidant by participating in the transport of oxygen and other gases involved in antioxidant response mediation while the Packed Cell Volume (PCV) indicates the volume percentage (%) of erythrocytes in blood. White blood cells or leucocytes cells are responsible for animals’ immunity. [1] The increase in HGB, PCV and WBC contents as observed in this study suggests improved antioxidant and haematological responses in L-arginine mono-treated and L-arginine plus MSG co- treated rats. The modulating effect of L-arginine on blood HGB and PCV may contribute to its potential anti-oxidative stress due to its possible antioxidant potential. This observation and suggestion compared with the work of Tarumoto et al. [30] who reported that L-arginine administration modulated haemoglobin level. Results revealed that these effects induced by monosodium glutamate at a dose of 8000 mg/Kg in the rats’ serum were mitigated by L-arginine irrespective of dose. These effects by L-arginine were notably significant at 120 mg/Kg in the rats’ serum. Serum outcome represents that contributed by the whole animal system. The present study demonstrated MSG-burden-related induction of oxidative stress and haematologic dysfunction in the rats. It demonstrated L-arginine-related benefit and capacity (notably at 120 mg/Kg) to significantly mitigate the MSG-burden-related adversity on the rats’ serum antioxidant and haematological functions.

CONCLUSION

Thus, the oxidative stress induced by monosodium glutamate at a dose of 8000 mg/Kg in the rats was significantly mitigated by L-arginine. The antioxidant effects by L-arginine were notably significant at 120 mg/Kg in the rats’ serum and accompanied by probable up-regulation in the rats’ haematological expression and functions.

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