Biochemical Changes in the Malnourished Rats Serum and Liver Exposed to Dietary Monosodium Glutamate

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Authors' contributions

This work was carried out in collaboration among all authors. Author BAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BAA and GAO managed the analyses of the study. Author GAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Monosodium glutamate (MSG) is a flavor enhancer. Its toxicity in a malnourished state appears not to have been fully investigated. This study was carried out to determine the effects of MSG on malnourished rats. Rats were randomly assigned into four groups of five rats/group. Group 1 rats were fed with malnourished feed; Group 2 rats received malnourished feed with dosed 1.6 mg/g MSG per body weight; Group 3 rats were fed with normal feed and dosed 1.6 mg/g MSG per body weight and Group 4 rats served as the control group (normal healthy rats) and were fed with normal feed for 28 days. After 28 days, the rats were sacrificed with the liver harvested and blood samples collected. Results from the study showed that malnourished rats had significantly lower levels of oxidative stress biomarkers including, anti-oxidants compared with the control. The levels of malondialdehyde concentration and xanthine oxidase activity were high in malnourished fed...
rats. Aspartate aminotransferase and alanine transaminase levels of malnourished and normal rats administered MSG were significantly low compared to the normal healthy suggesting that labialization occurs in liver leading to leakage of these enzymes from the liver to the serum. Malnourished rats showed significant decrease in body weight losing 48 grams after 28 days compared to malnourished and normal rats fed with MSG which recorded significant increase in body weight after 28 days adding 26 g and 42 g respectively.

Keywords: Monosodium glutamate (MSG); malnutrition; antioxidant; oxidative stress.

1. INTRODUCTION

Eating is considered a primordial factor in humans daily routine, not only for being a basic necessity, but mainly because incorrect eating habits are a public health problem, either because of excesses or deficiencies [1]. The lack of food or incorrect eating can induce a nutritional deficit or malnutrition condition, which is characterized as a pathological condition resulting from lack of energy, proteins and/or nutrients in varied proportions [2]. Some years ago malnutrition was associated with poverty, low educational standards, large numbers of people living in the same house, poor housing and sanitation, and maternity at less than 20 years old [3]. The World Health Organization (WHO) estimates that malnutrition contributes to more than a third of all infant casualties in the world, although it is rarely listed as a direct cause of mortality [4]. Even moderate levels of nutritional deficiency, detected by biochemical and/or clinical tests, can cause serious losses to human health [5].

Aiming at a better understanding of the effects and consequences of malnutrition, studies using experimental models have emerged as important sources of information [6]. Wistar rats submitted to protein deficit, showed that the main changes observed in the stomach are: atrophy of the gastric mucosa with obvious papillomatosis, and decreased secretion of hydrochloric acid and pepsin, causing weakening of the gastric barrier to bacteria. Physical changes in this experimental model have also been reported, as weight loss and changes in body and organ growth [7]. Besides, the development of certain illnesses such as hepatic steatosis [8] and steatohepatitis in undernourished Wistar rats, with effects similar to those of alcoholic steatohepatitis [9] among others, has been related to malnutrition. Biochemical parameters are also negatively affected by malnutrition [10]. Hypoinsulinemia and discrete hypoglycemia were detected in adult Wistar rats, due to protein restriction during lactation. Significant decreases in total protein concentration in mice when subjected to protein-energy malnutrition had been observed [11-13]. Also, changes in blood sugar, insulinemia and leptinemia in Wistar rats, caused by protein restriction during gestation and/or lactation, can be passed transgenerationally to the second generation of pups [14], besides, the hematological parameters are affected, as demonstrated [15,16] in situations of food restriction and protein restriction respectively. In these studies, significant drop of total leukocyte concentration was detected in Wistar rats, characterizing leucopenia [17].

Monosodium glutamate (MSG), a white crystalline powder, is the sodium salt of a naturally occurring non-essential amino acid, glutamic acid [18]. Monosodium Glutamate (MSG) is one of the world's most extensively used food additives which is ingested as part of commercially processed foods. MSG is commonly marketed as a flavour enhancer and is used as a food additive particularly in West African and Asian dishes [19].

When MSG is added to food, it provides a flavoring function similar to the naturally occurring free glutamate which differ from the four classic tastes of sweet, sour, salty and bitter [20]. As food additive, MSG is described and listed on food labels as a Flavouring or hydrolysed vegetable protein. Through its stimulation of the orosensory receptors and by improving the palatability of meals, MSG influences the appetite positively, and induces weight gain. Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals [21]. MSG could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches. In addition to these MSG symptom complex, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic
dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort [22,23]. Monosodium glutamate can be used to reduce salt intake (sodium), which predisposes to hypertension, heart diseases and stroke [24]. This is because of its flavour enhancing property which results from its interaction with sodium chloride salt, and other umami substances such as nucleotides. Even with a 30% salt reduction, the taste of low-salt foods improves with MSG inclusion. The sodium content of MSG (12%) is roughly a third of the amount of that in sodium chloride (39%). Other salts of glutamate have been used in low-salt soups, but with a lower palatability than MSG [25]. However, when enhancing palatability it is important to ensure that the food additive will not cause harm to the consumer.

Considering the discrepancies in the literature and growing safety concern for the use of MSG, there is need for further study on this important food additive [26].

2. MATERIALS AND METHODS

2.1 Source of Food Materials

A pack of Ajinomoto (a brand of monosodium glutamate manufactured by Ajinomoto Co., Inc. Tokyo, Japan) was obtained from wazo market, Ogbomoso, Oyo state, Nigeria, stored and kept away from direct sunlight.

2.2 Chemical and Reagent

Reagents used were of analytical grades obtained from Sigma Aldrich Louis, USA and Randox laboratories, limited, United Kingdom. Distilled water was obtained at LAUTECH Research Laboratory Ogbomoso.

2.3 Experimental Animals

Healthy female albino rats (Rattus novergicus) between 150-250 g were obtained from Physiology Department, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. They were presumed healthy as none showed any sign of stress or infection. They were housed in plastic cages. Their weights were determined prior to feeding and dosing. Acclimatization was done for two weeks in well ventilated environment. Food and water were supplied Ad libitum.

2.4 Induction of Malnutrition

Malnutrition was induced in rats feed by reducing the protein content to six percent (6%) while the normal feed contained nineteen percent(19%) [17], which was prepared by FMG Feeds and Feed Mills, Ogbomoso, Oyo State.

2.5 Experimental Design

Twenty female albino rats were divided (based on their weights) into four groups, with each group comprising of five rats each. Group 1 rats were fed with malnourished feed only. Group 2 rats received malnourished feed and dosed 1.6 mg/g MSG per body weight. Group 3 rats were fed with normal feed and dosed 1.6 mg/g MSG per body weight. Group 4 rats served as the control group (normal healthy rats) and were fed with normal feed only. MSG was mixed with the animal diets every day for 28 days. The rats in the control group were handled in the same manner as the test groups. All the experimental animals had free access to food (pellets) and clean water throughout the period of the experiment. The animals individual weights were recorded before and after dosing.

2.6 Collection of Sample

No mortality was recorded during the period of the administration. At the end of the 28 days of the administration, the rats were anaesthetized using chloroform. Blood was drawn by heart puncture into sterile, dry vacutainers. The blood was allowed to clot, spun and the supernatant serum collected. Each clotted blood sample was centrifuged at 3000xg for 25 min to obtain the serum. The supernatant serum was siphoned using micropipette. The livers were quickly removed, rinsed with 1.15% KCl and stored in chilled 0.25 M sucrose solution

2.7 Tissue Homogenates

After rinsing the liver free of the blood and connective tissue, the liver was cut into small pieces and homogenized in equal volumes of chilled 10 mM Tris/HCl buffer pH 7.4 and 0.25 M sucrose solution in a mortar and pestle. The homogenates were used for the biochemical analyses.
2.8 Assay Methods

2.8.1 Determination of total protein

Protein concentration was determined based on the principle of Folin-Lowry method [27]. Suitable dilutions were made containing 0.300 µg Bovine serum albumin (BSA) in water. A total volume of 1.2 ml was taken from each of the test-tubes and 6 ml of alkaline copper reagent were added and mixed thoroughly. The solution was allowed to stand for 10 min, 0.3 ml of Folin-ciocalteau reagent was added to 0.2 ml of the sample and mixed thoroughly. The solution was allowed to stand for 30 min. Absorbance of the sample was taken at 500 nm.

2.8.2 Determination of Alanine Transaminase activity (ALT) and Aspartate Transaminase activity (AST)

The activity of AST and ALT was determined using commercially prepared reagent kit produced by RANDOX laboratories limited. 0.1 ml of the sample was pipette into test-tubes and 0.5 ml of reagent 1 was then added, then incubated for 30 min at 37ºC, 0.5 ml of reagent 2 was added and the mixture was allowed to stand for exactly 20 min at 20ºC to 25ºC, 5 ml of 0.4 M of NaOH was added. The absorbance was taken after five minutes at 546 nm.

2.8.3 Determination of xanthine oxidase activity

Xanthine oxidase was determined according to the method of Roussos [28]. The assay mixture in final volume of 3 ml consists of 0.3 ml Trisglycine buffer 50 mM, pH 7.4, 0.3 ml CuSO$_4$ (10 mM), 0.05 ml xanthine buffer, 0.1 ml serum and distilled water to make up the volume. Change in absorbance was recorded at 290 nm at 15 secs interval for 1 min. One unit of activity was defined as change in absorbance at 290 nm in one minute by 1ml enzyme preparation.

2.8.4 Determination of superoxide dismutase (SOD) activity

SOD activity was determined according to the method of Misra [29]. One millilitre of sample was diluted with 9 ml of distilled water to make 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer pH 10.2 to equilibrate in the spectrophotometer and the reaction started by addition of 0.3 ml of adrenaline. Absorbance of the sample was taken at 480 nm against blank.

Calculation:

Increase in absorbance per minute = \( \frac{A_3 - A_0}{2.5} \)

Where,

\( A_0 = \) absorbance at time \( t = 0 \)

\( A_3 = \) absorbance at time \( t = 150 \) seconds

% inhibition = 100 - 100/ (change in absorbance at 480nm inhibited) / (change in Absorbance at 480 nm uninhibited)

Activity = % inhibition x dilution factor/ 50% inhibition

Specific activity = (Activity / Protein concentration) = (Units of enzymes / mg/ml protein)

2.8.5 Assessment of lipid peroxidation

This was carried out according to the method of Varshney [30], 0.4 ml of the reaction mixture i.e. sample already quenched with 0.5 ml of 30% trichloroacetic acid were added to 1.6 ml of Tris HCl. Addition of 0.5 ml TBA and incubation for 45 min at 80ºC produced pink coloured reaction mixtures which were centrifuged at 14000 revolution for 15 min. The absorbance of the clear pink supernatant was then read at 532 nm.

Calculation:

MDA (units/mg protein) = \( \frac{\text{absorbance x volume of the mixture}}{\text{E}_{532} \times \text{volume of the sample} \times \text{mg of the protein}} \)

where,

\( \text{E}_{532} \) is molar absorbitivity at 532 nm is \( 1.56 \times 10^{-5} +10^{-3} = 1.56 \times 10^{-2} \)

2.8.6 Determination of Glucose Oxidase activity in the serum

The activity of glucose oxidase was determined using commercially prepared reagent kit produced by RANDOX laboratories limited. Serum glucose levels were determined using Randox Kit principle. Glucose is oxidized to gluconic acid in the reaction catalyzed by glucose oxidase. Hydrogen peroxide released in
the process reacts with phenol and 4-aminophenazone to form a red quinoneimine in the reaction catalyzed by peroxidase. The concentration of red violet quinoneimine formed, which can be determined spectrophotometrically is proportional to the glucose level of the serum sample. Twenty millitres of the reagent were added to 0.02 ml of the test sample and also to standard glucose, mixed and incubated for 25 min at 15-25°C. The absorbance was measured at 500 nm against the reagent blank within 60 min. Glucose level of the sample was determined by using the formula

\[
\text{Glucose concentration (nm/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100
\]

2.8.7 Estimation of Glutathione (GSH)

Glutathione (GSH) was measured according to the method of Beutler [31]. 0.9 ml of distilled water were added to 0.1 ml of sample. About 1.5 ml of precipitating agent were added (3.34 g metaphosphoric acid, 0.4 g of EDTA and 60 g sodium chloride). Tubes were shaken and allowed to stand for 5 min at room temperature (25±1°C). The mixture was then centrifuge for 15 min at 4000 revolution per min. In 1.0 ml supernatant, 4 ml of phosphate (0.3 M disodium hydrogen phosphate) and 0.5 ml 5-50 dithiobis-(2-nitrobenzoic acid) (DTNB) (80 mg in 1% sodium citrate) were added. The development of yellow colour complex was read immediately at 412 nm on a spectrophotometer. GSH concentration was calculated and expressed as µmol of GSH/mg protein.

2.8.8 Statistical analyses

Data were subjected to analysis of variance using SPSS (IBM version. 20.0, SPSS Inc., Quarry Bay, Hong Kong) and presented as means (SEM). Comparisons between different groups were done using Analysis of Variance (ANOVA) and Duncan’s Multiple Range Test (DMRT). Values of p < 0.05 were considered as statistically significant as described [32].

3. RESULTS AND DISCUSSION

3.1 Body Weight

The difference of the final body weight with the initial body weight among the Rats revealed a significant (p<0.05) difference Table 1. Rats fed with malnourished feed had a significant weight decrease losing 48 g (22.42%) of body weight after 28 days. However, rats fed with malnourished and normal feed with 1.6 mg/g MSG per body weight recorded a significant increase in body weight after 28 days adding 26 g (11.30%) and 42 g (22.10%) respectively while the normal healthy rats had a higher weights increase of 66 g (33.00%) compared to sample MM (Malnourished + MSG) and NM (Normal + MSG). It was observed that rats dosed with 1.6 mg/g MSG per body weight had significant increase in weight along with the feed that was administered with it thus, much intake of MSG could lead to accumulation of body weight, thereby resulting into high BMI (Body Mass Index) as previously reported in [33]. This finding agrees with the result of Tawfik and Al-Badr [23] who reported an increase of 34.6 - 44.5% for animals treated with MSG.

3.2 Hepatic Superoxide Dismutase (SOD) Activity in MSG Fed Rats

A significant (p<0.05) decrease in SOD activity was observed in MM (0.26 Umol/mg protein) and NM (0.18 Umol/mg protein) compared to NH (0.91 Umol/mg protein) Fig. 1. Normal and malnourished rats fed with MSG showed significant (p<0.05) decrease in SOD activity when compared with sample NH (Normal healthy rats). However, a slight decrease in the SOD activity was observed in rats fed with normal, malnourished feed dosed with MSG compared to the rats fed with normal healthy feed. The present study showed that malnourished rats have significantly lower levels of anti-oxidants when compared with NH (normal healthy rat). It is well established that any alterations in the oxidant and anti-oxidant balance results in oxidative stress. Results obtained in this study are in agreement with the results obtained by various authors [34,35]. Moreover, various studies reported low anti-oxidant status in malnourished rats [36]. The reason for decreased anti-oxidant capacity in malnourished rats compared to normal health rat could be as a result of increased generation of reactive oxygen species (ROS). As anti-oxidants are involved in fighting against oxidative stress, some amount might be exhausted, also, low SOD activity observed may be due to low level of copper and zinc which are co-factors for SOD.
3.3 Glutathione (GSH) Concentration in MSG Fed Rats

Monosodium glutamate, one of the most abundant naturally occurring amino acids, is frequently added as a flavor enhancer. MSG is known to have some adverse effects in humans and experimental animals [37]. Monosodium glutamate concentration revealed in Fig. 2. Showed a significant (p<0.05) increase in sample MO (4.32 µmol/mg) compared to MM (2.83 µmol/mg). It was observed that sample NM had the highest (4.81 µmol/mg) GSH concentration while the normal healthy had the least (2.64 µmol/mg). Monosodium glutamate was able to increase the GSH concentration in malnourished only and normal + MSG fed rats. Although, malnourished only rats recorded a slight increase in the GSH activity when compared with the normal rats fed with MSG. Significant (p<0.05) decrease was observed in malnourished fed MSG rats compared to the normal rats fed with MSG. Thus, sample NM with the highest GSH concentration could be able to prevent damage to important cellular components caused by ROSs such as free radicals, lipid peroxide and heavy metals. According to El-Ezaby, et al. [38] the consumption of the tested food additives induced harmful changes in the antioxidant status of the rat. So, its consumption should be restricted.

3.4 Malondialdehyde (MDA) Concentration and Xanthine Oxidase (XO) Activity in MSG Fed Rats

The MDA concentration revealed in Fig. 3 showed that there was no significant (p>0.05) difference in sample MM (0.77 nmol/mg protein), NM (0.74 nmol/mg protein) and NH (0.74 nmol/mg protein) that is, no significant (p>0.05) increase was recorded for the MDA concentration across the normal rats fed with MSG and the malnourished rats fed with MSG compared with the normal healthy rats but a significant (p<) increase was observed in the MDA concentration of malnourished only rats compared with the normal healthy rats. While for the XO activity showed in Fig. 4. A significant increase was recorded for the malnourished rats (6.39 U/ml) only compared to the normal healthy rats (1.8 U/ml). However, no increase was observed in normal and malnourished rats fed with MSG. The trace element Zn plays an important role as nutritional co-factor. However, results of this study found that MDA concentration and XO activity in malnourished rats were not significantly high compared to the control, high MDA concentration and XO activity was observed. Various authors [39] also found increased lipid peroxidation in malnourished rats. These results indicate that the decreased defense status of blood may result in increased lipid peroxidation of all membrane lipids and enhanced concentration of lipid peroxidation products like MDA. They also provided evidence that there are two important pathophysiological changes, a weakened antioxidant defense system and increased lipid peroxidation, occurring in PEM. The decreased levels of antioxidants found in this study, could be attributed to the depletion in blood anti-oxidant stores due to their consumption during free radical scavenging in response to ROSs production.

3.5 Serum Glucose concentration in Rats Fed MSG

The glucose concentration result (Fig. 5) revealed that the glucose concentration of rats fed with MSG ranged from 82 - 96 mg/dL while a significant (p<0.05) decrease was observed for sample MO (68 mg/dL). Comparatively, the result obtained for sample MM compares well with the control (Normal healthy) 98 mg/dL. It was observed that MSG was able to alleviate the glucose concentration in sample NM. Furthermore, glucose concentration in the serum was not significantly low across all groups compared to the control (normal healthy) suggesting that MSG do not have glucose altering activity. The values obtained in this findings are low compared to the results of Oluwajuyitan and Ijarotimi, [40] who reported a glucose concentration ranging from 106-130 mg/dL for rats fed on improved dough meal.

3.6 Liver Functions

3.6.1 Aspartate amino transferase (AST) and Alanine aminotransferase (ALT)

The AST result (Fig. 6) revealed that no significant (p>0.05) decrease was observed in normal rats fed with MSG (80.3 U/L) compared to the normal healthy rats (107.5 U/L). However, a slight increase was recorded in the malnourished rats (112.1 U/L) compared to the normal healthy rats (107.7 U/L). A significant (p<0.05) decrease was observed in malnourished rats fed with MSG (87.2 U/L) compared to the normal healthy fed rats (107.6 U/L). A decrease was observed in
normal rats fed with MSG compared (80.3 U/L) with the malnourished only rats (84 U/L), but, no significant decrease was observed in normal rats fed with MSG compared with the malnourished rats fed with MSG. While for ALT, significant (p < 0.05) increase in the serum alanine aminotransferase was observed in MO (59.6 U/L) and normal healthy (57.2 U/L) rats compared to the MM (33.4 U/L) and NM (31.5 U/L). The ALT enzyme is a sensitive marker of liver damage [41]. Therefore, the increase in the serum ALT activity might perhaps be an indication of liver damage. This increase could also be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage [42]. Monosodium glutamate, malnourished and nourished feed resulted to a significant reduction in the serum level of ALT enzyme at 1.6 mg/g MSG per body weight. The use of these combinations found to be more responsive in improving the serum level of ALT enzyme especially at lower dose of MSG (1.6 mg/g body weight) as recorded in sample MM (33.4 U/L) and NM (31.5 U/L).

Fig. 1. Hepatic SOD activity in MSG fed rats
MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy

Fig. 2. GSH concentration in difference groups of rats
MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy
Table 1. Change in body weights of rats fed with malnourished and nourished feed dosed with monosodium glutamate (MSG)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight gain/loss (g)</th>
<th>Percentage difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO</td>
<td>212 ± 4.90\textsuperscript{a}</td>
<td>164 ± 5.78\textsuperscript{b}</td>
<td>-48\textsuperscript{d}</td>
<td>22.64</td>
</tr>
<tr>
<td>MM</td>
<td>230 ± 56.57\textsuperscript{a}</td>
<td>256 ± 8.12\textsuperscript{a}</td>
<td>26\textsuperscript{c}</td>
<td>11.30</td>
</tr>
<tr>
<td>NM</td>
<td>190 ± 12.25\textsuperscript{c}</td>
<td>232 ± 4.90\textsuperscript{c}</td>
<td>42\textsuperscript{b}</td>
<td>22.10</td>
</tr>
<tr>
<td>NH</td>
<td>200 ± 0.00\textsuperscript{a}</td>
<td>266 ± 6.00\textsuperscript{b}</td>
<td>66\textsuperscript{a}</td>
<td>33.00</td>
</tr>
</tbody>
</table>

Means (±SEM) with different superscripts in the same column are significantly different (P<0.05)

MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy

Fig. 3. MDA concentration in difference groups of rats

MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy

Fig. 4. Hepatic XO activity rats fed MSG

MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy
Fig. 5. Serum glucose concentration in rats fed MSG
MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy

Fig. 6. Serum AST activity in rats fed MSG
MO: Malnourished only; MM: Malnourished + MSG; NM: Normal + MSG; NH: Normal healthy
4. CONCLUSION

Malnourished rats showed significant decrease in body weight losing 48 grams after 28 days compared to malnourished and normal rats fed with MSG which recorded significant increase in body weight after 28 days adding 26 g and 42 g respectively. The increase in the serum ALT activity might be an indication of liver damage in the rats. This study showed that MSG have potentials of increasing the anti-oxidant defense mechanism especially in malnourished conditions but its liver damaging activity makes it unhealthy for consumption.

ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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