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OXIDATIVE STRESS AND MORPHOLOGICAL ASSESSMENT OF BONE MARROW IN MONOSODIUM GLUTAMATE-TREATED RAT

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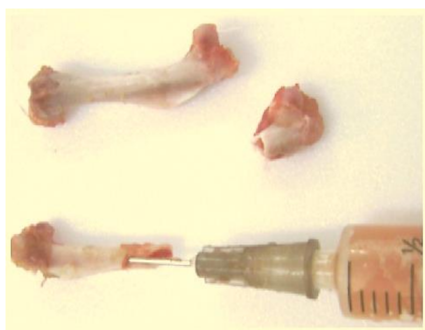
Graphical abstract



Abstract

Excess consumption of monosodium glutamate (MSG) was reported to cause oxidative stress on brain, liver and renal and altered haematological parameters. Therefore, this study was aimed to investigate the effect of MSG on oxidative stress status on bone marrow of rats. Male Sprague-Dawley rats (n=24) weighing between 160-200 g were divided randomly into three groups: Control which was given distilled water (1 mg/kg), MSG 60 and MSG 120 which were given 60 mg/kg MSG and 120 mg/kg MSG, respectively. All substances were oral force fed for 28 days consecutively. At the end of the study, bone marrow cells were isolated by flushing technique for measurement of the oxidative stress status and bone marrow smear observation. Results showed that the superoxide dismutase activity and protein carbonyl level were significantly increased in MSG 120 group than to control and MSG 60 groups ($p<0.05$). Conversely, glutathione level had declined significantly in both MSG groups as compared to control group ($p<0.05$). The malondialdehyde level was not significantly affected in MSG groups than to control group. Bone marrow smear indicated no evidence of morphological alteration in all groups. In conclusion, MSG at both doses caused oxidative stress on bone marrow after 28 days of exposure.

Keywords: Oxidative stress, antioxidant, bone marrow, monosodium glutamate, morphology

Abstrak

Pengambilan monosodium glutamate (MSG) secara berlebihan telah dilaporkan menyebabkan tekanan oksidatif pada organ otak, hepar dan renal serta merubah parameter hematologi. Kajian ini dijalankan untuk mengkaji kesan MSG terhadap status tekanan oksidatif sum-sum tulang tikus. Tikus jantan Sprague-Dawley (n=24) dengan berat antara 160-200 g telah dibahagikan secara rawak kepada tiga kumpulan: Kawalan yang menerima air suling (1 ml/kg), MSG 60 dan MSG 120, masing-masing menerima 60 mg/kg MSG dan 120 mg/kg MSG. Kesemua bahan telah diberikan secara paksaan oral selama 28 hari berturut-turut. Pada akhir kajian, sum-sum tulang diambil menggunakan teknik *flushing* bagi pengukuran status tekanan oksidatif dan pemerhatian apusan sum-sum tulang. Hasil kajian menunjukkan aktiviti superoksida dismutase dan aras protein karbonil meningkat secara signifikan pada kumpulan MSG 120 berbanding kumpulan Kawalan dan MSG 60 ($p<0.05$). Sebaliknya, penurunan yang signifikan dapat dilihat pada aras glutation bagi kumpulan MSG berbanding Kawalan ($p<0.05$). Aras malondialdehid pada kumpulan MSG tidak dipengaruhi secara signifikan berbanding kumpulan Kawalan. Apusan sum-sum tulang menunjukkan tiadanya sebarang perubahan morfologi antara semua

kumpulan. Kesimpulannya, MSG pada kedua-dua dos telah menyebabkan tekanan oksidatif kepada sum-sum tulang selepas 28 hari pendedahannya.

Kata kunci: Tekanan oksidatif, antioksidan, sum-sum tulang, monosodium glutamat, morfologi

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1.0 INTRODUCTION

Monosodium glutamate (MSG) is one of the food additives in which its safety usage has generated much controversy worldwide. MSG is naturally occurring sodium salt contains non-essential amino acid known as L-glutamic acid and is used as a flavor enhancer in food industries [1]. MSG is widely sold under the trade name AJINOMOTO with the main constituents is glutamic acid (78%) [2]. It has a unique umami taste which is a savory or meaty like taste and once dissolves in aqueous solution, it will dissociate to form sodium and free glutamate [3].

Glutamate is found naturally in protein-rich foods such as fish and meat, dairy products such as milk and cheese and also vegetables like tomatoes and mushrooms. It is the most abundant excitatory neurotransmitter in the brain that is released into the synapses in the central nervous system [4]. According to Shivasharan *et al*. [5], the excessive intake of MSG causes neurotoxicity due to glutamate action. Generally, MSG has been categorized as a safe food enhancer in which its consumption has increased globally with human average daily intake of approximately 10 g/day [6]. Therefore, this food additive has been inadvertently abusing in the food industry because of its abundance and improper labeling in many food ingredients [7]. Previous studies had found that MSG causes oxidative stress to the mammalian organ such as brain [8], renal [9] and testis [10].

Oxidative stress is defined as the imbalance between the formation of reactive oxygen species (ROS) and the protective mechanisms of antioxidant [11] that causes bio-molecule damage in an organism. The excessive amount of ROS induced cellular damage and apoptosis of stem cells and its precursor [12]. Bone marrow is the soft tissue residing in the bone cavity housing important precursor cells for living organisms [13]. Bone marrow is the primary site of hematopoiesis and requires a suitable microenvironment for the growth of cells. The homeostasis regulation of redox status on hematopoietic tissue is important to maintain the normal hematopoiesis as the hematopoietic tissue is susceptible to oxidative stress [14].

Interestingly, MSG is reported to cause a toxic effect on the hematological system in animal studies. According to Ashaolu *et al*. [15], MSG has a direct toxic effect on blood cells that leads to anemia and reduction of immune status which is characterized by a decrease in a number of neutrophils. MSG also causes the formation of micronucleus on bone marrow [16] that is linked to the risk of cancer [17]. However, to date, no study has been done to investigate the effect of MSG towards bone marrow cells through oxidative stress mechanism. Therefore, this research is conducted to study the effect of MSG administration on the bone marrow for 28 days through oxidative stress mechanism.

2.0 METHODOLOGY

2.1 Chemicals

MSG was obtained from local market. All other chemicals were purchased from Sigma-Aldrich, United States of America except for thiobarbituric acid was purchased from ICN Biomedicals, United States of America.

2.2 Animals and Treatment

Male Sprague-Dawley rats (n=24), weighing between 160 and 200 g were obtained a week earlier from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM) Kuala Lumpur for adaptation purposes. The rats were kept in plastic cages, exposed under suitable environmental condition (room temperature) with 12-h light and dark cycle throughout the study period. All rats were provided with pellet diet and water *ad libitum*. The study was conducted after obtaining approval from UKM Animal Ethics Committee (UKMAEC), resolution number: FSK/2016/IZATUS/23-NOV./807-NOV.-2016-FEB.-2019.

The rats were divided randomly into three groups of eight rats per group. Group 1 (Control) received 1 ml/kg distilled water while, Group 2 (MSG 60) and 3 (MSG120) received MSG at dose 60 mg/kg and 120 mg/kg, respectively. Based on WHO [18], the Acceptable Daily Intake (ADI) limit of MSG is 120

mg/kg of body weight. The MSG was given orally for 28 consecutive days between 9.00 to 10.00 am to standardize the daily effects of MSG. The weight gain of rats, food and water intake were measured throughout the study period.

2.3 Bone Marrow Cells Isolation and Smear Preparation

At the end of the study, the bone marrow cells were obtained from tibia and femur by using flushing technique. The cells were flushed out with cold phosphate buffer saline (pH 7.4), filtered through a cell strainer and centrifuged at 2500 rpm for 7 minutes. The pellet was collected for bone marrow smear and cell lysate. Pellet was dropped onto microscope slides for bone marrow smear, air-dried and stained with Wright stain for microscopic observation. Lysis buffer was added to the pellet for cell lysate and centrifuged at 2500 rpm for 7 minutes. The supernatant was collected and kept at -20°C until further biochemical analysis was conducted.

2.4 Determination of Antioxidant Status and Oxidative Damage Biomarkers

For determination of antioxidant status, assessment of superoxide dismutase (SOD) activity and glutathione (GSH) level using cell lysates were employed following methods of Beyer and Fridovich [19] and Ellman [20], respectively. In principle, the reaction of superoxide with SOD leads to nitro blue tetrazolium (NBT) reduction, generating a purple coloured product which is detectable at 560 nm. The SOD activity was expressed as U/mg protein. Meanwhile, the GSH level was measured based on the reaction of acid 5, 5'dithiobis [2-nitrobenzoic] (DTNB) and GSH molecule to form a 5-thionitrobenzoic acid (TNB) and GS-TNB. The formation of a yellow coloured product was then measured at 412 nm. The GSH level was expressed as mmol/mg protein.

Meanwhile for determination of oxidative damage biomarkers, the level of malondialdehyde (MDA) and protein carbonyl (PC) were measured to indicate lipid peroxidation and protein oxidation; respectively. The MDA was assessed based on the reaction between thiobarbituric acid and MDA which presence in the sample. The formation of coloured compounds was then measured at 532 nm [21] and the MDA level was expressed as nmol/mg protein. The PC level was assessed according to method as described [22]. Protein pellets were dissolved in guanidine hydrochloride and the supernatant was measured at 366 nm. The PC level was expressed as nmol/mg protein.

2.5 Statistical Analysis

All data were analysed using Statistical Package for Social Sciences (SPSS) version 22. The data were tested for normality and analysed with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All the results were expressed as mean ± SEM with the significant value was $p<0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Body Weight Gain and Food and Water Intake

Table 1 shows the body weight gain and food and water intake of the experimental rats after 28 days exposure. The body weight gain of MSG-treated rats showed insignificant increment as compared to control group. In contrast with the previous study, MSG which was given orally at the dose of 200 mg/kg had significantly increased the weight gain of treated rats [23]. However, in our study, we used 120 mg/kg as the highest dose. This might explain the insignificantly increased of weight gain in MSG-treated rats in the present study. Furthermore, the slight increase in the food and water intake correlates with the slight increase in weight gain of treated animals as observed throughout the study period; which the finding is in line to the previous study [24].

Table 1 Body Weight Gain and Food and Water Intake of Experimental Rats. Data represent the means ± SEM (n=8).

Groups	Body Weight Gain (%)	Food (g)	Water (ml)
Control	72.59 ± 4.25	215.28 ± 29.06	631.04 ± 14.53
MSG 60	79.44 ± 6.69	255.44 ± 36.73	656.89 ± 18.36
MSG 120	79.35 ± 5.29	254.64 ± 43.86	661.71 ± 21.93

3.2 Antioxidants Status

Figure 1 shows the activity of SOD in experimental rats. The SOD activity was significantly increased in MSG 120 group when compared to MSG 60 and control groups ($p<0.05$). The rise in SOD activity may occur as a compensatory mechanism in the enzymatic antioxidant reaction [8, 25] as SOD is the first line of antioxidant defence mechanism [26]. However, this result contradicts with several studies that showed a decreasing activity of SOD in MSG-treated rats [4, 10, 27] due to the MSG dose used in the previous studies were more concentrated compared to the present study.

Conversely, GSH level significantly decreased ($p<0.05$) in the both MSG-treated groups compared to control group as shown in Figure 2 which also in line with previous studies [5, 28]. As SOD increases in the current study, the formation of hydrogen peroxide (H_2O_2) will also increase. GSH is needed in detoxifying H_2O_2 [29]. Therefore, the reduction of GSH levels in the current study could be explained by the full utilization of GSH for detoxification of the H_2O_2. Another mechanism that may cause depletion of GSH is due to the disruption of cysteine-glutamate antiporter that function is to carry in a cysteine to intracellular for GSH synthesis [30]. Excess glutamate

formation from MSG intake may inhibit GSH synthesis due to the inhibition of cysteine intracellular uptake that will lead to oxidative stress [31].

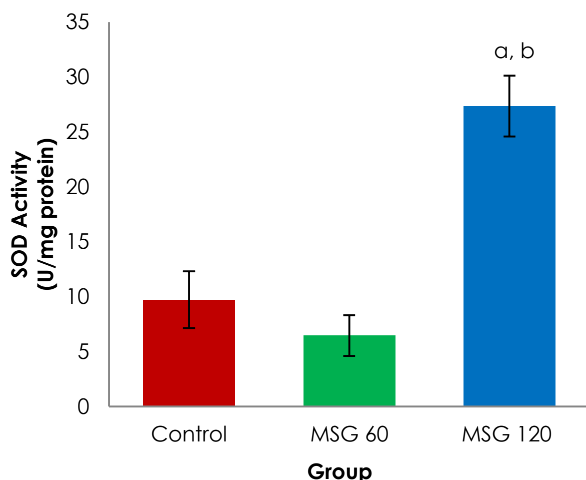


Figure 1 The SOD activity of experimental groups. a is significantly compared to control group ($p<0.05$); b is significantly compared to MSG60 group ($p<0.05$). Data represent the means ± SEM (n=8)

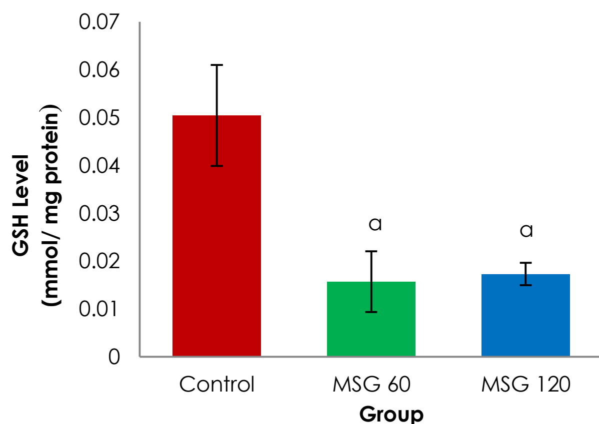


Figure 2 The GSH level of experimental groups. a is significantly compared to control group ($p<0.05$). Data represent the means ± SEM (n=8)

3.3 Oxidative Damage Biomarkers

The level of MDA of bone marrow cells was shown in Figure 3. Administration of MSG showed insignificantly increase of MDA level compared to control group. The previous study also showed an increased level of MDA in serum [32], brain [5] and renal [33] of MSG-treated rats, indicating the oxidative damage had occurred. The level of MDA rises as a product of lipid peroxidation which occurs from ROS action on lipid membrane [34]. In the present study, the insignificant increase of MDA levels in MSG-treated rats might be due to the act of first line antioxidants defence mechanism which involved the utilization of SOD and GSH to counter back the ROS formations; which confer protection to the lipid membrane from peroxidation.

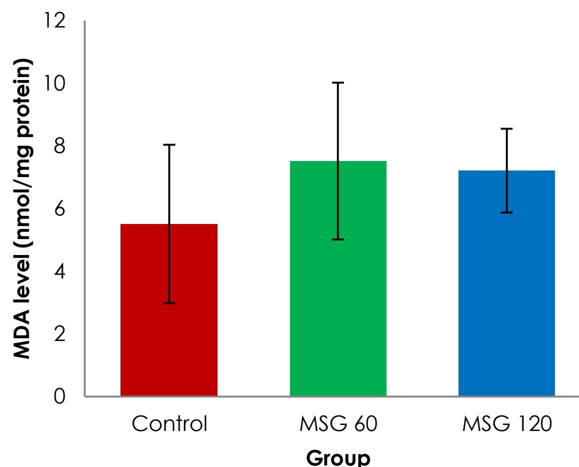


Figure 3 The MDA level of experimental groups. Data represent the means ± SEM (n=8)

However, when compared to MDA, MSG administration had caused significantly increased in PC level ($p<0.05$) compared to control group as shown in Figure 4. Several chemicals such as isoflurane anaesthetic [35], lead acetate an industrial chemical [34] and iron oxide nanoparticles [36] had been found to cause a rise in the level of PC in bone marrow. Protein oxidation might have a direct effect on cells by altering cell signalling, cell structure and enzymatic process such as metabolism as some proteins are more susceptible to oxidation when compared to other proteins [37]. This might also explain the increase of PC levels in bone marrow of MSG-treated rats.

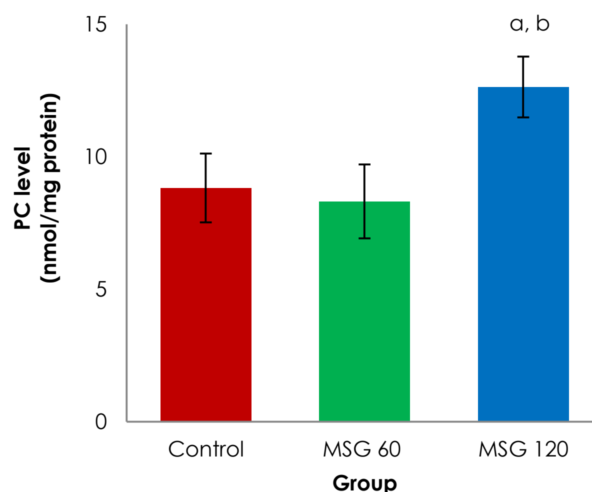
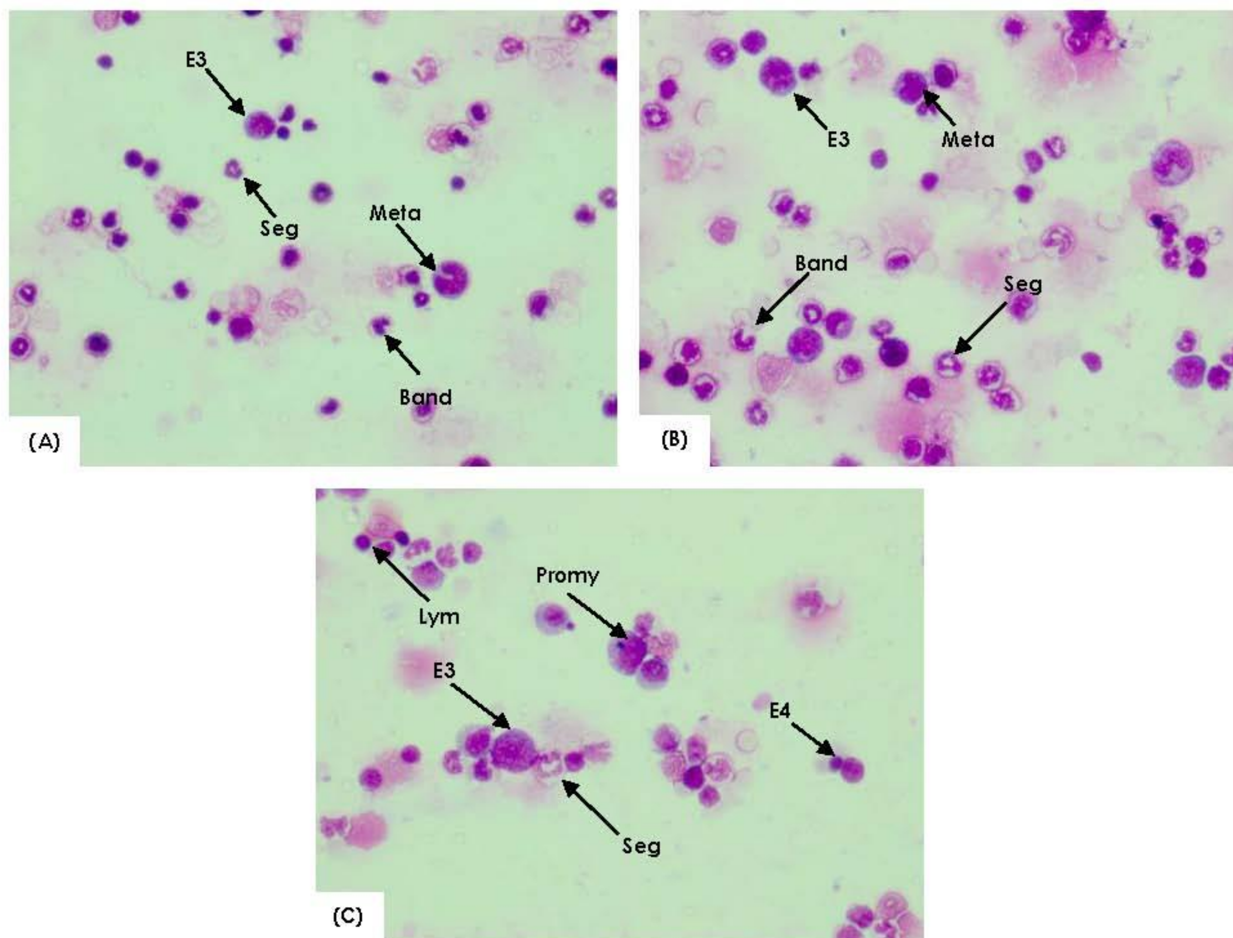


Figure 4 The PC level of experimental groups. a is significantly compared to control group ($p<0.05$); b is significantly compared to MSG60 group ($p<0.05$). Data represent the means ± SEM (n=8)

3.4 Bone Marrow Smear

Figure 5 shows the bone marrow smear of experimental rats. MSG did not cause any morphological alterations in bone marrow of rats. The identified erythroid and granulocyte cells are appeared in various stages of maturation. One of the food additives, aluminum acetate had been found to increase in micronucleated of bone marrow in experimental rats through its oxidative stress mechanism [16]. However, the researchers found that only following repeated exposure at highest dose of aluminum acetate lead to this morphological defect.

The previous study reported a reduction in peripheral blood neutrophils [15, 38] and erythrocyte counts [23] following exposure to MSG, which emphasized the possible role of oxidative stress in mediating MSG-induced hematopoiesis alteration and bone marrow dysfunction. However, the MSG doses used in the previous studies are much higher than the dose used in the current study. Thus, the differential in experimental dosage may explain for no evidence of morphological abnormalities was noted from bone marrow smear analysis in the current study.



4.0 CONCLUSION

In conclusion, administration of MSG for 28 days consecutively towards experimental rats able to induced oxidative stress as shown by the changes of enzymatic and non-enzymatic antioxidants and caused protein oxidation. No morphological alterations were observed on bone marrow cells smear. The morphological observation using bone marrow smear alone was insufficient to determine the type of cell distribution. Thus, further study on cell counting and histological analysis of bone marrow could assist in the future investigation of the MSG effect targeting the integrity of bone marrow tissue.

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