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Artikel Asli/*Original Articles*

Biochemical and Histological Effects of Low Dose of Monosodium Glutamate on the Liver of Adult Male *Sprague-Dawley* Rats

(Kesan Biokimia dan Histologi Dos Rendah Monosodium Glutamat ke Atas Hepar Tikus Jantan *Sprague-Dawley*)

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ABSTRACT

Monosodium glutamate (MSG) is widely used as an additive in food. Excess consumption of MSG was reported to cause oxidative stress on brain, liver and renal resulted in increased production of reactive oxygen species (ROS). This study aims to determine the biochemical and histological effects of low dose MSG on the liver of adult male Sprague-Dawley rats. Animals (n = 6 per group) were randomly divided into three groups with two treatment groups: 60 mg/kg (MSG60) and 120 mg/kg (MSG120), and one control group (distilled water). The substances were administered to the rats via force feeding for 28 consecutive days. On day 29, all rats were killed, and liver tissues were biopsied for the biochemical (total protein, liver enzymes, and the status of oxidative stress) and histological analysis. The total protein appeared significantly decreased ($p < 0.05$) while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) demonstrated a significant increased ($p < 0.05$) in the MSG120 treatment group as compared to the control group. Malondialdehyde (MDA) levels and the antioxidant levels of superoxide dismutase (SOD) were significantly increase ($p < 0.05$) in the MSG120 group as compared to the MSG60 and control groups. The histological findings revealed changes to normal liver architecture and accumulation of red blood cells in the central veins in both MSG groups. This study indicates that the MSG consumption at a dose of 120 mg/kg may ALTer the biochemical and histological parameters of the liver.

Keywords: Monosodium glutamate; oxidative stress; liver; MSG; liver damage

ABSTRAK

Monosodium glutamat (MSG) digunakan secara meluas sebagai bahan perisa tambahan dalam makanan. Penggunaan MSG secara berterusan dilaporkan dapat menyebabkan penghasilan tekanan oksidatif pada otak, hepar dan ginjal yang mengakibatkan peningkatan spesies oksigen reaktif (ROS). Kajian ini bertujuan untuk menentukan kesan biokimia dan histologi MSG dos rendah pada hepar tikus Sprague-Dawley jantan dewasa. Kesemua tikus dibahagikan secara rawak kepada tiga kumpulan (n-6 setiap kumpulan) dengan dua kumpulan rawatan: 60 mg/kg (MSG60) dan 120 mg/kg (MSG120), dan satu kumpulan kawalan (air suling). Rawatan diberikan kepada tikus melalui paksaan oral selama 28 hari berturut-turut. Pada hari ke-29, semua tikus kajian dikorbankan, dan tisu hepar dibiopsi untuk analisis biokimia (jumlah protein, enzim hepar, dan status tekanan oksidatif) dan histologi. Jumlah protein berkurangan secara signifikan ($p < 0.05$), manakala enzim alanin aminotransferase (ALT) dan aspartat aminotransferase (AST) menunjukkan peningkatan yang signifikan ($p < 0.05$) dalam kumpulan rawatan MSG120 berbanding kumpulan kawalan. Aras malondialdehid (MDA) dan aras antioksidan superoksid dismutase (SOD) meningkat dengan signifikan ($p < 0.05$) dalam kumpulan MSG120 berbanding kumpulan MSG60 dan kawalan. Pemerhatian histologi hepar mempamerkan perubahan pada struktur morfologi hepar dan terdapat pengumpulan sel-sel darah merah di bahagian lumen vena dalam kedua-dua kumpulan rawatan MSG. Kajian ini menunjukkan bahawa penggunaan MSG pada dos 120 mg/kg berupaya mengubah parameter biokimia dan histologi pada organ hepar.

Kata kunci: Monosodium glutamate; tekanan oksidatif; hepar; MSG; kerosakan hepar

INTRODUCTION

The use of monosodium glutamate (MSG) as additive is to increase the ingenuity of food. Food additives are produced commercially and can be found widely in processed food and snacks (Rangan & Barceloux 2009). Though there are many types of food additives, yet MSG which is under the AJINOMOTO's brand is the most frequently used and marketed (Walker & Lupien 2000). MSG is a sodium salt of L-glutamic amino acid (GLU) that is added to food to enhance the taste (Insawang et al. 2012).

The use of MSG in high doses has led to widespread speculation on the adverse effects of this seasoning. The detrimental effect of MSG has caused high concern among

consumers around the world (Bera et al. 2019). A study from Tawfik and Al-Badr (2012) has reported that high doses or recurring doses of MSG may cause several deleterious effects in human and animal studies. Increased lipid content has been reported in China as a result of continuous intake of MSG (He et al. 2011). Moreover, MSG administration in high doses to rats has led to the accretion of oxidative stress levels which may cause extensive cellular damage and alter the antioxidant defence mechanism including superoxide dismutase (SOD) and glutathione (GSH) (Abdel-Reheim et al. 2014).

The liver is an organ that plays a significant role in metabolism and has various functions in the body. As the liver is rich in oxidizable substances, thus, it is highly sensitive to oxidative damages (Abdel-Reheim et al. 2014) which may lead to an increase in liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (El-Khayat et al. 2009). Previous studies have demonstrated that usage of MSG at high doses of between 600 and 4000 mg/kg of body weight for 10 days resulted in oxidative damage to some essential organs such as the brain (Farombi & Onyema 2006), liver (Onyema et al. 2006), and testis (Husarova & Ostatnikova 2013). However, the effect of low dose of MSG at 60 and 120 mg/kg in the liver has yet to be determined. This study was conducted by using the two low doses of MSG to determine the biochemical and histological effects in the liver of *Sprague-Dawley* rats.

EXPERIMENTAL METHODS

CHEMICALS

Monosodium glutamate (MSG) was purchased from local market in Kuala Lumpur (Ajinomoto, Malaysia).

ANIMALS

A total of 18 male *Sprague-Dawley* rats weighing between 170 to 200 g was obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. The rats were placed in a plastic cage with 12h light and dark cycles under room temperature. Mouse pellet and water *ad libitum* were provided to the rats throughout the study period. All animal procedures were approved by the Animal Ethics Committee (UKMAEC) with an approval number: FSK/2016/IZATUS/23-NOV/807-NOV-2016-FEB-2019.

Animals were randomly divided into three groups with two treatment group: MSG60 (60mg/kg of MSG) and MSG120 (120 mg/kg of MSG), and one vehicle control group which received distilled water. The MSG dose was chosen based on the Acceptable Daily Intake (ADI) of MSG intake which is 120 mg/kg of body weight (WHO 1971). The treatment was given through oral gavage for 28 consecutive days. At the end of the study period, all rats were killed by using 50 mg/kg of ketamine/xylazine cocktails. The liver from all groups were removed and divided into two tissue parts. The first tissue part was stored in 10% buffered formalin for histological analysis while the second tissue part was homogenized in cold phosphate buffered saline (PBS) with a ratio of 10 g/ml (w/v) and centrifuged at 8000 rpm for 20 minutes in 4°C. The homogenate was then kept at -20°C for biochemical analysis.

MEASUREMENT OF TOTAL PROTEIN AND LIVER ENZYMES

The liver tissue homogenate was used for the determination of total protein by using the Lowry method (Lowry et al. 1951). Both liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the method by Reitman and Frankel (1957).

MEASUREMENT OF OXIDATIVE STRESS STATUS

Antioxidant status was determined in liver tissue homogenate through superoxide dismutase (SOD) and reduced glutathione (GSH) level assessments (Beyer & Fridovich 1987, Ellman 1959). Lipid peroxidation indicative of oxidative stress status was assessed through malondialdehyde (MDA) level measurement according to the method of Stocks and Dormandy (1971).

HISTOLOGICAL ANALYSIS

The formalin fixed liver tissue was processed by an automated tissue processor Leica EG1160 (Equipnet, USA) before embedded in paraffin, sectioned at 4μm of thickness and stained with haematoxylin and eosin (H&E) dyes. The liver histology was observed under the light microscope at 10x and 40x magnifications.

STATISTICAL ANALYSIS

All data were analysed by using the Statistical Package for Social Sciences (SPSS) version 22. Normality test was performed and then followed by the statistical analysis with one-way analysis of variance (ANOVA) and Tukey's post hoc tests. All data were expressed in mean±SEM with the significance value of $p < 0.05$.

RESULTS

ANALYSIS OF TOTAL PROTEIN AND LIVER ENZYMES

Figure 1 shows the total protein content upon treatment with low doses of MSG in the experimental rats. The concentration of total protein was significantly decreased ($p < 0.05$) in MSG120 (12.35 ± 1.21 mg/ml) as compared to MSG60 (14.13 ± 0.70 mg/ml) and control (16.68 ± 1.04 mg/ml) groups. Figure 2 shows the effect of the low doses of MSG on the level of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Figure 2a and 2b). Data revealed a slight

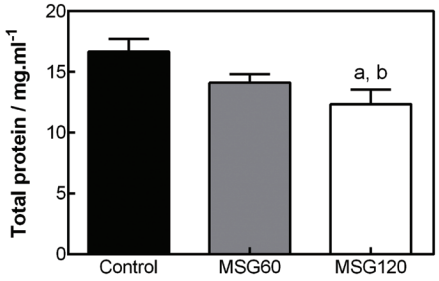

FIGURE 1. The concentration of total protein in treatment (MSG60 and MSG120) and control groups ([a,b] significant decrease compared to the control and MSG60 groups, $p < 0.05$). Data was represented in mean ± SEM with the significance value of $p < 0.05$

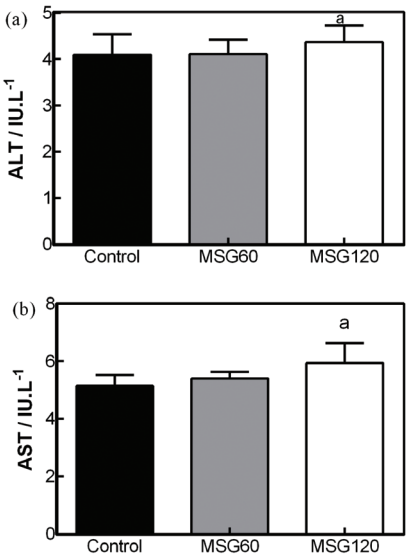

FIGURE 2. (a) The level of ALT and (b) the level of AST enzymes in treatment (MSG60 and MSG120) and control groups ([a] significant increase compared to the control group, $p < 0.05$). Data was represented in mean ± SEM with the significance value of $p < 0.05$

increase in the level of ALT and AST enzymes in the MSG120 group with significant differences ($p < 0.05$) in ALT (4.37 ± 0.36 IU/L) and AST (5.94 ± 0.70 IU/L) enzymes compared to the control group (4.06 ± 0.41 IU/L and 5.10 ± 0.38 IU/L, respectively).

ANTIOXIDANT ACTIVITIES

Figure 3 and 4 depicted a contrast pattern of antioxidant SOD and GSH activities in both treatment and control groups. The level of SOD activity was significantly increased ($p < 0.05$) in MSG120 group (1.32 ± 0.21 U/mg) as compared to MSG60 (0.88 ± 0.13 U/mg) and control (0.72 ± 0.06 U/mg) groups (Figure 3). The GSH activity showed a significant decrease ($p < 0.05$) in MSG120 group (0.36 ± 0.09 nmol/mg) when compared to both MSG60 (0.47 ± 0.03 nmol/mg) and control (0.49 ± 0.06 nmol/mg) groups (Figure 4). The level of MDA that indicates the oxidative stress status is shown in Figure 5. Data revealed a significant increase ($p < 0.05$) of MDA activity in the MSG120 group (21.00 ± 1.69 nmol/mg) compared to both MSG60 (17.63 ± 0.72 nmol/mg) and control (15.24 ± 0.82 nmol/mg) groups.

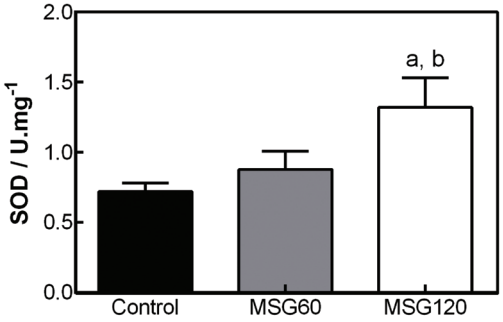

FIGURE 3. The antioxidant SOD activity in both treatment (MSG60 and MSG120) and control groups ([a,b] significant increase compared to the control and MSG60 groups, $p < 0.05$). Data was represented in mean ± SEM with the significance value of $p < 0.05$

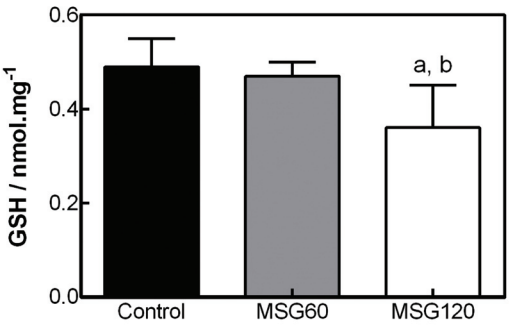

FIGURE 4. The antioxidant GSH activity in both treatment (MSG60 and MSG120) and control groups ([a,b] significant decrease compared to the control and MSG60 groups, $p < 0.05$). Data was represented in mean ± SEM with the significance value of $p < 0.05$

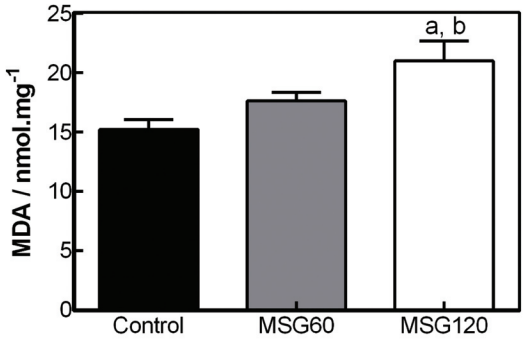

FIGURE 5. The level of MDA in both treatment (MSG60 and MSG120) and control groups ([a,b] significant increase compared to the control and MSG60 groups, $p < 0.05$). Data was represented in mean ± SEM with the significance value of $p < 0.05$

HISTOLOGICAL ANALYSIS

Histological observation of the liver tissues stained with H&E staining in all groups is shown in Figure 6. Figure 6a and 6b showed that the liver tissues remain normal with an intact hepatocytes structure and clear lumen of the central vein in control group. Both liver tissues in MSG60 (Figure 6c,d) and MSG120 (Figure 6e,f) groups display hepatocytes swelling and accumulation of red blood cells in the lumen of central vein. However, only liver tissues in MSG120 group show vacuolization and fat deposition presented in the central vein (Figure 6f, arrow).

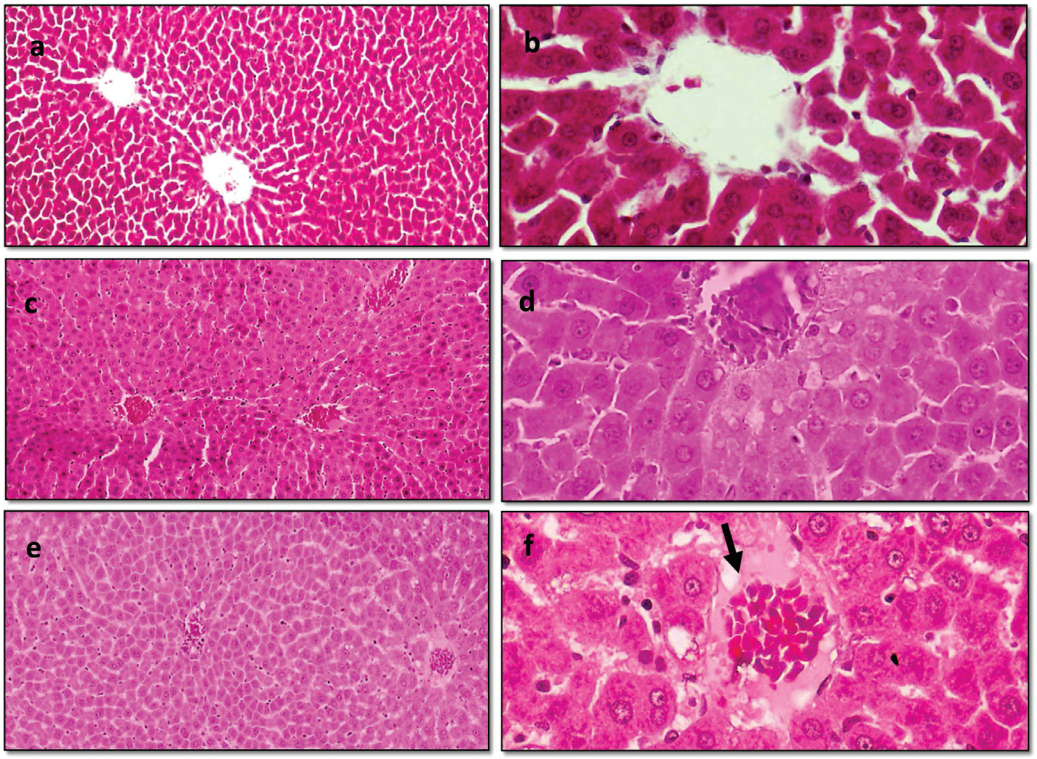

FIGURE 6. The histology of liver tissues stained with H&E in control (a, b); MSG60 (c, d); and MSG120 (e, f) groups. Hepatocytes remained intact with clear lumen in the control group (a, b). MSG60 group shows hepatocytes swelling and the lumen of central vein is constricted and filled with red blood cells (c, d). MSG120 group (e, f) also shows hepatocytes swelling, accumulation of red blood cells in the central vein and the appearance of vacuolization (arrow) (all images were viewed under ×10 and ×40 magnifications)

DISCUSSION

The liver is one of the critical primary organs for protein synthesis in the body. The significant decrease of total protein content in this study may be due to alteration in protein synthesis due to induction of oxidative stress upon continuous treatment with a low dose of MSG (120 mg/kg). This finding is in accordance with the past study (Tawfik & Al-Badr 2012) which demonstrated a significant reduction in the level of total protein in rats that were administered 600 mg/kg and 1600 mg/kg of MSG. The production of oxidative stress may damage the liver, thus altering its function in protein production. Moreover, it is reported that MSG treatment (at 600 mg/kg and 1600 mg/kg) for two weeks may result in the occurrence of hepatotoxicity leading to the livers inability in synthesizing protein (Kazmi et al. 2017).

It is known that both AST and ALT enzymes are released in addition to liver damage (Al-Mamary et al. 2002). Data in this study has revealed a significant increase in the level of AST and ALT enzymes after treatment with 120 mg/kg of MSG. The low dose of MSG at 120 mg/kg may have damaged the hepatocytes, thus, degenerated the membrane and caused the AST and ALT enzymes to be released. Indeed, this finding is associated with the histological changes to the liver tissue architecture in this study. Although previous studies have shown the effect of MSG treatment at high doses (200, 600, and 4000 mg/kg) to cause the increment of AST and ALT enzymes in rats (Onyema et al. 2006; Onyema & Farombi 2006), yet the present work appeared to be the first to indicate the significance released of both hepatic enzymes after treatment with a low dose of MSG (at 120 mg/kg).

Alterations to the protein synthesis and both AST and ALT hepatic enzymes that may reflect liver damage showed a synergistic effect to the significant increase of MDA level at 120 mg/kg of MSG. It is transpired that the action of reactive oxygen species (ROS) on lipid membrane may

induce the level of MDA as a product of lipid peroxidation process (Selvakumar et al. 2006). The continuous intake of MSG at 120 mg/kg may have led to an increase of glutamate level, which could have resulted in the increment influx of intracellular calcium (Jaiswal et al. 2009). The rising influx of calcium into the mitochondria may trigger the production of ROS (Szydlowska & Tymiansk 2010), and this condition could explain the significant increase of MDA in the present study.

Antioxidants are required in the body as a shield against ROS by physiological and pathological processes. SOD is known as an enzymatic antioxidant which acts in the breakage of superoxide anion into hydrogen peroxide (H_2O_2) and oxygen (O_2) (Zelko et al. 2002), while GSH is a non-enzymatic antioxidant which dissociates H_2O_2 into water and oxygen (O_2) (Pompella et al. 2003). In this study, the high activity of SOD rather than GSH upon treatment with 120 mg/kg of MSG may be due to its role as the first line defence antioxidant against the oxidative stress (Hanipah et al. 2018). Also, the high level of SOD may be accounted as a protection against the increment of lipid peroxidation as shown by MDA level in the MSG120 group.

In our work, the concentration of MSG at 60 and 120 mg/kg exhibited changes in the liver histology that may be due to direct damage of MSG to hepatocytes (Egbuonu et al. 2010). It is possible by the high level of lipid peroxidation which is the pivotal factor that contributes to liver inflammation leading to cells damage (Guéraud et al. 2010). The present finding is in agreement with previous study in which treatment of MSG (600 mg/kg) showed alteration in liver architecture and congestion of central vein with red blood cells (Waer & Edress 2006). Histological changes to the liver in the MSG120 group have similar outcome to the data by Shrestha et al. (2018), where vacuolated hepatocytes and congested central veins with blood cells were observed after given MSG at 600 mg/kg. Moreover, a study by Carmiel-Haggai et al. (2005) reported that free radicals' production could cause lipid deposition in the liver via lipid peroxide activity.

CONCLUSION

Based on this study, there are evidence that low doses of MSG could lead to liver damages. The low doses of MSG have shown significant alteration in the concentration of total protein and liver enzymes. MSG also caused an increase of MDA levels, indicating an induction of oxidative stress that lead to deterioration of hepatic membrane and distortion of cellular architectures as supported through biochemical and histological observations. Besides that, changes in the antioxidant defence system of the liver organ upon MSG treatment at 120 mg/kg can be seen in the increment of SOD activity and reduction of GSH activity. Therefore, MSG administration in dose as low as 120 mg/kg did manifest with classic liver injury with elevation of liver biochemical and oxidative markers as well as histological changes, and further additional studies are needed to support the present findings.

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