Effects of chronic oral monosodium glutamate on body weight and liver histology in Wistar rats.

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ABSTRACT

Tala’a AA, Hussin AM., Effects of chronic oral monosodium glutamate on body weight and liver histology in Wistar rats, Onl J Vet Res., 23 (5):414-424, 2019. Groups of 8 mature Wistar male rats were given daily 15mg oral monosodium glutamate (MSG) 30 or 75 days. Eight controls were given water. Rats were sacrificed at days 30 and 75 for liver tissue, microscopy, cytology and mitotic index, and blood for serum AST and ALT. Compared with controls, body weights increased ~28-37% and liver weights ~8-14% (p < 0.05). Hepatocytes were 24-26% more numerous and hyperplastic (11-19%), nuclei 41-44%, and binucleated cells 55-62% more frequent in rats given GMS. Apoptotic and Kupffer cells increased 37-57% and serum AST 47% and ALT 60%. Our findings suggest that chronic intake of high doses of GMS may affect rat livers.

Keywords: MSG, liver, rat, weight. Raw data provided after Reference list

INTRODUCTION

Monosodium glutamate (MSG) sodium salt of glutamic acid is a naturally occurring amino acid produced in small quantities in mammalians used widely as food additive (Legbedion et al., 2013; Savcheniuk et al., 2014). However, chronic over consumption can induce toxicity and obesity (Hassan et al, 2014). MSG affects livers in adult and causing pathological liver changes (Farr et al., 2010) and neonate mice (Bhattacharya, 2011) and is associated with elevated serum ALT and AST in rats (Giffen et al, 2002). We report effects of chronic oral monosodium glutamate on body weight and liver histology in Wistar rats.
MATERIALS AND METHODS

Groups of 8 mature Wistar male rats were given daily 15mg oral monosodium glutamate (MSG) 30 or 75 days by gavage intubation. Eight controls were given water. Rats were sacrificed at days 30 and 75 for liver tissue, microscopy, cytology and mitotic index, and blood for serum AST and ALT. Body weights were recorded before and end to determine gain. Liver tissue was preserved in 10% formalin for 48-72 hr for routine HE staining (Michael and Wojciech, 2006). For hepatocytes, we determined number, diameter, binucleated, nuclei, apoptotic and kupffer cells by using optika digital microscopic camera (Italy, optika view7 software). Serum AST and ALT were determined colorimetrically with a Kit (GESAN INC).To determine differences between means we used one way ANOVA (SPSS version 2000).

RESULTS

Results are shown in Tables 1 to 5 and figures 1-7 below.

Table 1. Mean ± SE body and liver weights (Grams) from groups of 8 rats each gavaged 15mg/kg MSG daily for 30 or 75 days. Controls were given water

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body (g)</th>
<th>Liver (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n=8</td>
<td>201.5±2.7</td>
<td>9.93±0.8</td>
</tr>
<tr>
<td>30 days n=8</td>
<td>279.8±6.8 B (28%)</td>
<td>10.8±0.6 B (8%)</td>
</tr>
<tr>
<td>75 days n=8</td>
<td>321.8±4.96 A (37%)</td>
<td>11.6±0.95 A (14%)</td>
</tr>
</tbody>
</table>

Capital letters denote significant (p<0.05) differences between groups

Table 2 ) Mean ± SE hepatocyte count, nuclei and binucleated cells, diameter of hepatocytes, nuclei and nuclei to cell ratio in groups of 8 rats each gavaged 15mg/kg MSG daily for 30 or 75 days. Controls were given water.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatocytes (n)</th>
<th>Diameter of hepatocytes (µ)</th>
<th>Nuclei (n)</th>
<th>Diameter of nuclei (µ)</th>
<th>Binucleated (n)</th>
<th>Nucleus/cell ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>111±6B</td>
<td>21.5±0.02A</td>
<td>115.4±6.41B</td>
<td>8.0±0.02A</td>
<td>4.26±0.9B</td>
<td>1.03±0.0088</td>
</tr>
<tr>
<td>30 days</td>
<td>146±4.4A (24%)</td>
<td>19.5±0.05B (-11%)</td>
<td>156±5.5A (41%)</td>
<td>8.6±0.02A</td>
<td>9.6±0.7A(55%)</td>
<td>1.07±0.006A</td>
</tr>
<tr>
<td>75 days</td>
<td>151±3 A (26%)</td>
<td>17.4±0.02B (-19%)</td>
<td>159±2.6A (44%)</td>
<td>8.9±0.03A</td>
<td>11.2±1.5A(62%)</td>
<td>1.07±0.008A</td>
</tr>
</tbody>
</table>

Capital letters denote significant (p<0.05) differences between groups

Table (3) Mean ± SE subjective cell and nuclei membrane prominence (+ * ) in groups of 8 rats each gavaged 15mg/kg MSG daily for 30 or 75 days. Controls were given water.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cell membrane</th>
<th>Nucleolus/cell (n)</th>
<th>Nucleolus</th>
<th>Nuclear membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.33±0.06B</td>
<td>1.18±0.03 B</td>
<td>1.42±0.07 B</td>
<td>1.75±0.09 A</td>
</tr>
<tr>
<td>30 days</td>
<td>1.4±0.11B</td>
<td>1.35±0.02 A</td>
<td>1.78±0.07 A</td>
<td>1.84±0.08 A</td>
</tr>
<tr>
<td>75 days</td>
<td>2.46±0.13A</td>
<td>1.39±0.08 A</td>
<td>1.86±0.05 A</td>
<td>1.83±0.04 A</td>
</tr>
</tbody>
</table>

Capital letters denote significant (p<0.05) differences between groups *1= (+) , 2 = (++) , 3= (+++)

Table (4) Mean ± SE number of apoptotic and kupffer cells per field in groups of 8 rats each gavaged 15mg/kg MSG daily for 30 or 75 days. Controls were given water.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Apoptotic cells (n)</th>
<th>Kupffer cells (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>2.43±0.48 B</td>
<td>3.53±0.21 C</td>
</tr>
<tr>
<td>30 days</td>
<td>3.43±0.03 A</td>
<td>5.8±0.30 B</td>
</tr>
<tr>
<td>75 days</td>
<td>3.86±0.21 A(37%)</td>
<td>8.16±0.46 A(57%)</td>
</tr>
</tbody>
</table>

Capital letters denote significant (p<0.05) differences between groups

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Table (5) Mean ± SE serum AST (IU/L) and ALT (IU/L) levels in groups of 8 rats each gavaged 15mg/kg MSG daily for 30 or 75 days. Controls were given water.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>113.95 ±2.16 C</td>
<td>20.57 ±3.87 C</td>
</tr>
<tr>
<td>30 days</td>
<td>180.48 ±4.33 B</td>
<td>38.96 ±2.55B</td>
</tr>
<tr>
<td>75 days</td>
<td>213.17 ±1.67 A (47%)</td>
<td>53.09 ±7.54A (60%)</td>
</tr>
</tbody>
</table>

Capital letters denote significant (p<0.05) differences between groups.

Figure (1) Liver of control rat with normal polygonal hepatocytes and mixed euchromatic and heterochromatic nuclei. Sinusoids are normal width. H and E stain left (100X), right (400X).

Figure (2) Liver of rat gavaged 15mg/kg MSG daily for 75 days with hepatocellular atrophy (Black arrows) widened sinusoids (blue stars), apoptotic cell (arrowhead). Cytoplasm appeared profuse, granular with vesicular euchromatic nuclei, dispersed chromatin and obvious nucleoli (Blue arrow). We found increased number of enlarged Kupffer cells (yellow arrows). Some hepatocytes (red arrow) appeared necrotized. H and E stain (400X).
Figure (3) Liver of control rat after 30 days with few binucleated cells (blue arrow). H and E stain (400X).

Figure (4) Liver of rat gavaged 15mg/kg after 30 days with increased apoptotic cells (black arrows) and few binucleated cells (blue arrows). H and E stain (400X).
Figure (5) Liver of rat gavaged 15mg/kg MSG after 75 days with increased binucleated cells (short black arrows) and chromatin lysis in hepatocytes (blue arrows). The central vein appears infiltrate by inflammatory cells, mainly lymphocytes (red arrows). Large Kupffer hyperchromatic cells were present (long black arrow) with and vascular degeneration (yellow arrow) H and E stain (400X).

Figure (6). Liver of rat gavaged daily 15mg/kg MSG at 30 days with increased number of apoptotic cells (black arrows), severe vacuolar degeneration, tissue lysis (blue arrows) and inflammatory cell aggregation with edematous fluid (blue star) H and E stain (400X).
The safe use of MSG has generated much controversy regarding weight gain and health effects (El-Aziz et al., 2014). Compared with controls, we found increased body weight 28-37% in rats gavaged MSG and sacrificed at day 30 and 75, respectively (Table 1). Iwase et al., (2000), Hermanussen and Tresguerres, 2003; and Hermanussen et al., (2006) suggested a possible link between MSG and obesity in experimental animals whereas others found no effect (Boutry et al., 2011). In fact, some have shown a decline in weight, fat and leptin in rats given MSG probably due to increased energy expenditure (Kondoh and Torii, 2008, 2009). Chronic MSG intake might affect arcuate nuclei and disrupt hypothalamic signaling cascade for leptin inducing leptin resistance and obesity (Hermanussen and Tresguerres, 2003; Hermanussen and Tresguerres, 2005). Weight gain associated with MSG intake might be due to destruction of several brain regions (including the hypothalamus) involved in appetite and energy metabolism (Monno et al., 1995; Kondoh and Torii, 2008; Kondoh et al., 2009).

In our study, liver weights increased 8 to 14% in rats given MSG, not as much as body weight (Table 1). Malik et al., (1994) reported increases in lipids, phospholipids, triglycerides and free fatty acids in liver of adult male mice 31 days after injection of MSG. Yoshida et al., (1995) found that mice given MSG treated (2mg/g SC) became obese 9 weeks after birth with higher blood levels of glucose, total cholesterol, HDL - cholesterol, GPT and cholinesterase along with greater triglyceride content of liver relative to control mice. Also, Shrestha et al., 2018 concluded that MSG can cause increase in liver weight as compared with control group. Our findings appear to confirm previous studies.
Results suggested increased hepatocytes and nuclei in rats given MSG (Table 2) but no change in nucleus diameter (Table 2 and Figures 1-6). However the diameter of hepatocytes was reduced. MSG may cause hepatotoxicity (Chung et al., 2003; AL-Mosaibih, 2013). Our findings are similar to Bhattacharya et al. (2011) who showed that the diameter of nuclei appeared normal in MSG treated rats but contradict Shrestha et al., 2018 who reported that there were decreases in nuclei diameter of hepatocytes in MSG treated group at a dose 1.6 mg/g body weight.

Our results suggest a dose-time effect of MSG similar to Kumbhare et al., 2015 and Waer & Edress, (2006) who found effects dose related and cumulative. We found increased binucleated cells observed as a sort of hyperplasia usually seen in regenerating cells (Gerlyng et al., 2008). (Waer & Edress, 2006; Devi, 2016). Reported that chronic MSG ingestion may lead to cell division with binucleated cells, condensed nuclei, unipolar anaphase. We found increased Kupffer cells (Table 4) (Figures 2 and 5) in rats given MSG. Bhattacharya et al. (2011) found similar results. Kupffer cells are in fact macrophages predominant in chronic inflammation that can become activated by particulates (Cheville, 2009). There were increased apoptotic cells per field in MSG treated rats (Table 4) (Figures 4 and 6).

In serum we found increased AST and ALT in rats given MSG (Table 6). The elevation of serum ALT and AST levels are used routinely as biomarkers of liver injury (Giffen et al, 2002). Moreover, previous studies have shown that MSG impaired liver function and elevated serum ALT and AST levels in rats (Onyema et al, 2006, Eweka et al, 2011, Tawfik and Al-Badr, 2012). Also, Inuwa et al. (2011) concluded that the elevated level of ALT at 200, 300 and 400 mg/mg body weight could be an indication of hepatocellular damage induced by MSG.

REFERENCES


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