Histological Study of Effect of Oral Administration of Monosodium Glutamate (MSG) on Duodenum of Wistar Rats

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Abstrak

Monosodium glutamat (MSG) adalah penguat rasa yang dipasarkan secara luas dan dikonsumsi secara rutin. MSG bersifat toksik bagi beberapa organ termasuk usus halus. Penelitian sebelumnya telah melaporkan efek dari MSG dosis tinggi; namun, temuan ini tidak dapat diaplikasikan secara langsung pada intake manusia yang ekuivalen. Penelitian ini bertujuan untuk menyelidiki efek MSG pada duodenum pada tikus yang diberikan asupan harian rata-rata manusia (*average daily intake* (ADI). Dua puluh empat tikus Wistar jantan dewasa dengan berat 150-250 g pada usia 8-12 minggu dibagi menjadi empat kelompok eksperimen (n = 6): kelompok kontrol normal (C), kelompok 1 (0,378 mg / g bb), kelompok 2 (0,756 mg / g bb), dan kelompok 3 (1,512 mg / g bb) MSG per oral per hari selama 21 hari. Jaringan duodenum didiseksi dan diwarnai dengan hematoksilin dan eosin (HE) untuk pemeriksaan histologis. Tinggi rata-rata villus dihitung untuk setiap kelompok: kontrol (1047,40 ± 158,0 µm), Kelompok 1 (850,68 ± 202,38 µm), Kelompok 2 (906,29 ± 416,63 µm), dan Kelompok 3 (877,55 ± 154,65 µm). Terdapat perbedaan tinggi vili di antara semua kelompok; namun, tidak ada perbedaan yang signifikan secara statistik dibandingkan dengan kelompok kontrol (p = 0,384). Kelompok kontrol menunjukkan tampilan mukosa duodenum yang normal tanpa sel-sel inflamasi. Sebaliknya, kelompok perlakuan menunjukkan infiltrasi sel inflamasi dan tidak memiliki struktur *brush-border* yang jelas. Penelitian ini menunjukkan bahwa pemberian MSG dengan dosis yang mendekati dosis ADI manusia mengakibatkan kerusakan histologis pada duodenum tikus.

Kata Kunci: monosodium glutamat; MSG; vili duodenum; studi histologi

Abstract

Monosodium glutamate (MSG) is a widely marketed flavor enhancer that is regularly consumed. MSG is toxic to several organs including the small intestine. Previous studies have reported the effects of high doses of MSG; however, these findings are not directly applicable to equivalent human intakes. This study aimed to investigate the effect of MSG on the duodenum in rats administered with human average daily intake (ADI). Twenty-four adult male Wistar rats weighing 150–250 g at the age of 8-12 weeks were divided into four experimental groups (n = 6): normal control group (C), group 1 (0.378 mg/g b.wt), group 2 (0.756 mg/g b.wt), and group 3 (1.512 mg/g b.wt) oral MSG per day for 21 days. Duodenal tissue was dissected and stained with hematoxylin and eosin (HE) for histological examination. The average height of the villus was calculated for each group: control (1047.40 ± 158.0 µm), Group 1 (850.68 ± 202.38 µm), Group 2 (906.29 ± 416.63 µm), and Group 3 (877.55 ± 154.65 µm). There was a difference in the height of the villi between all groups; however, there was no statistically significant difference compared with the control group (p = 0.384). The control group showed a normal appearance of the duodenal mucosa without inflammatory cells. In contrast, the treatment group showed inflammatory cell infiltration and lacked distinct brush-border structures. Our study showed that administration of MSG at an estimated dose of human ADI resulted in histological damage to the duodenum.

Keywords: monosodium glutamate; MSG; duodenal villi; histological study

Introduction

Monosodium glutamate (MSG) is one of the most widely used food additives worldwide (1). The Food and Drug Administration (FDA) and the European Food Safety Association (EFSA) have defined the acceptable daily intake (ADI) of MSG as 0-120 mg/kg (2). Most previous studies on MSG toxicity in rats have involved the administration of extremely high doses ranging from 2000 to 8000 mg/kg of body weight. Based on the allometric conversion developed by Shin et al., this dose corresponds to 120 mg/kg of body weight in rats (3).

The small intestine processes two other amino acids: glutamate-aspartate and glutamine. Glutamate, present in 20-40% of most proteins, is a substrate for protein synthesis. The main active glutamate and glutamine transporters in the intestinal tract are excitatory amino acid carrier 1 (EAAC1), glutamate/aspartate transporter 1 (GLAST1), and glutamate transporter (GLT1) in the stomach (4). Glutamate can be converted to free amino acids within the intestine to facilitate metabolism (5).

Glutamate undergoes oxidation within the gastrointestinal tract and is metabolized within the intestinal enterocytes, as demonstrated in both human and animal studies (6). Several reports have demonstrated that MSG negatively affects the small intestine and liver (6–8). The negative impact of excessive monosodium glutamate (MSG) accumulation on the duodenum has been reported. Specifically, this can result in an increase in basophils and atrophy of the small intestinal cells in both the duodenum and jejunum. Furthermore, it may lead to loss of the brush border structure, shallowening of the duodenal crypts, and reduction in the height of the villus. It is important to note that these effects have been observed in various studies (9–11).

Previous studies that examined the acute toxicity of high doses of MSG (3 g and 6 g) in rats did not explore the potential chronic toxicity of supposedly safe low doses of MSG in humans (7, 12). Previous studies have revealed that the oral administration of MSG can result in toxicity in rats. However, the high doses used in these studies make it challenging to establish a direct correlation between the toxic effects of MSG in rats and its human consumption. Therefore, we examined the effect of orally administered MSG at a stratified moderate dose in rats, which is equivalent to the acceptable daily intake (ADI) for humans.

Materials and Methods

Twenty-four adult male Wistar rats, aged 8-12 weeks and weighing 150-250 grams, were

obtained and housed in the animal laboratory facility of the Faculty of Veterinary Medicine of the Universitas Syiah Kuala. Rats were housed in an animal storage chamber and subjected to a 12-hour light/dark cycle. They were also allowed to acclimate for seven days. In the treatment groups, MSG was administered at a stratified dose corresponding to the acceptable daily dietary intake for humans (120 mg/kg) (13, 14). The rats were randomly assigned to four groups (n=6 each): normal control, MSG at 0.378 mg/g body weight (Group 1), MSG at 0.756 mg/g body weight (Group 2), and MSG at 1.512 mg/g body weight (Group 3). Normal control rats received vehicle (distilled water). The rats were provided free access to water and food throughout the study period. After 21 days, the rats were anesthetized and sacrificed and the duodenum was collected for analysis. The experimental design was approved by the Ethics Committee of the Syiah Kuala University School of Medicine (approval number: 255/EA/FK-RSUDZA/2020, October 21, 2020).

The duodenum was dissected and fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, cut into 4-5 μm thick sections, and stained with hematoxylin and eosin (HE) for histological assessment. The height of the duodenal villi was determined in five fields of view using a 400x magnification light microscope. OptiLab Viewer software and the TOUPView program were used to calculate the percentage of duodenal villus heights. Photomicrographs were obtained using a digital research photography microscope at the Department of Anatomical Pathology, Universitas Malikussaleh.

Data analysis was performed using a computer software. The Kruskal-Wallis test was used to compare the differences between groups. The results are presented as mean ± standard deviation (SD). Statistical significance was defined as $p < 0.05$.

Results

The heights of the duodenal villi in the experimental groups are shown in Figure 1. Treatment with MSG at 0.378, 0.756, or 1.512 mg/kg body weight decreased the height of the duodenal villus (850.6 ± 202.3, 906.30 ± 416.4, and 877.5 ± 154.6 μm, respectively); however, the height of the duodenal villus did not differ significantly from that of the control group (1047.4 ± 158.0 μm) ($p = 0.384$). Histological examination of the duodenal sections is shown in Figure 2. In the normal control group, the duodenal mucosa appeared normal with no signs of inflammatory cells. In contrast, the MSG-treated group exhibited inflammatory cell infiltration

and lacked evident brush border structures.

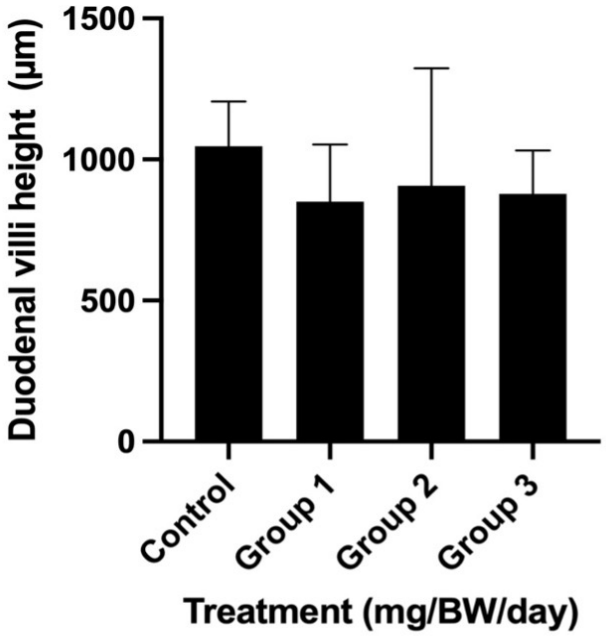

Figure 1. **Height of duodenal villi in different groups. Control; Group 1 (MSG 0.378 mg/g b.wt) Group 1 (MSG 0.756 mg/g b.wt), Group 3 (MSG 1.512 mg/g b.wt)**

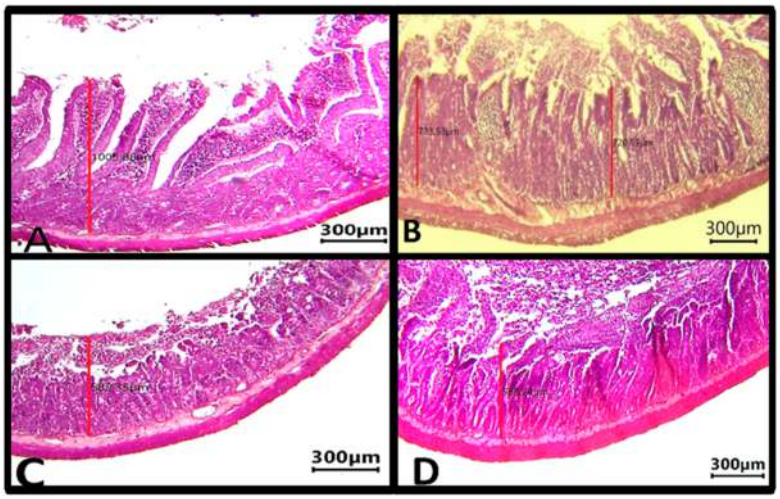

Figure 2. Photomicrographs of the rat duodenum (hematoxylin and eosin stained, 400x). The groups were as follows: (A) control, (B) MSG 0.378 mg/g b.wt, (C) MSG 0.756 mg/g b.wt, and (D) MSG 1.512 mg/g b.wt.

Discussion

Monosodium glutamate is a food additive that is commonly used as a flavor enhancer and

is widely considered safe with no established daily consumption limits. However, excessive consumption of MSG has become prevalent due to the high prevalence of processed foods that are not labeled adequately (15). MSG has been shown to affect several organs including the gastrointestinal tract (7, 12, 16, 17), kidneys (18, 19), liver (19, 20), reproductive system (21), and obesity (22).

Although a specific dose of MSG has not yet been established, it has been suggested that at least 1 g of MSG per serving should be used. Studies in animals and humans have shown that even the lowest dose of MSG can exert toxic effects. The typical daily consumption of MSG is estimated to range from 300 to 1000 mg/kg (23). The challenge of extrapolating the effects of dietary MSG from animal studies to humans is primarily due to the differences in the doses administered to animals and those typically consumed by humans. Therefore, our study used a stratified dose of daily MSG intake of 60 mg/kg (0.378 mg/g body weight), 120 mg/kg (0.756 mg/g body weight), and 240 mg/kg (1.512 mg/g body weight), directly extrapolated from daily human intake, as described by Nair et al. (24).

Our findings revealed a decrease in the height of villi in the MSG-treated group; however, this difference did not prove statistically significant when compared to the control group ($p = 0.384$). A study by Vincent et al. (2015) demonstrated a significant reduction in intestinal villi length and depth of crypts after MSG administration at a dosage of 5 mg/g body weight/day for 28 days, which is equivalent to 120 mg/kg body weight/day or 8.4 g/day. Furthermore, it was discovered that providing MSG in excess of the approved daily intake reduced the villi-to-crypt ratio from 5:1 to 3:1 (10). A recent study also reported a significant reduction in the height of the duodenal villus after administration of 72 mg/200 g body weight/day of MSG (10).

Furthermore, there was an increase in stomach acid secretion, decreased NaHCO3 levels, and microvascular complications. Ishan et al. (2018) also documented a reduction in villus height and duodenal crypt depth on the first day after administration of MSG (4 g/kg bwt/day and 6 g/kg bwt/day for 28 days) compared to the control group (11). A recent study aligned with our research on MSG doses of 60 and 120 mg/kg indicated detrimental effects of MSG on the reproductive system (21).

In the present study, the control group showed a typical brush border structure, whereas the treatment group showed an abnormal brush border structure or indications of inflammatory cell infiltration. These observations indicate a disoriented brush border, implying an

inflammatory process in the duodenal mucosa. The results of this study are consistent with those of previous studies that demonstrated various changes in the small intestine after MSG administration for 14 days, including increased cellular hypertrophy and proliferation in the small intestine (7, 12). Similarly, Vincent et al. (2015) reported disorientation and loss of brush border structures in the duodenal mucosa (9).

The potential harm resulting from MSG in the duodenal mucosa may be indirectly related to stimulation of gastric acid secretion. Furthermore, oxidative stress can lead to increased alkaline phosphate levels and decreased sodium bicarbonate levels. Sodium bicarbonate helps neutralize stomach acids that enter the duodenum; however, a deficiency in this chemical can result in increased acidity within the stomach. Exposure to gastric acid that is not fully neutralized can trigger an inflammatory response and damage the duodenal mucosa, consequently leading to a reduction in villus height (25).

Epithelial regeneration in the duodenum was observed every 4-5 days, with the lower crypt containing 8-14 stem cells that facilitated this process. According to Eweka et al., histological changes were observed after the administration of two high doses of MSG (3 and 6 g). The damaged tissue was replaced by immature cells, thereby preventing injury to the duodenal villi. Administration of 3 g MSG resulted in elevated levels of basophilia and cellular hypertrophy in animals, which corresponded to a daily intake of approximately 0.04 g/kg. In contrast, those who received 6 g of the substance exhibited more pronounced degenerative and atrophic changes, with daily intake of approximately 0.08 g/kg. These results indicate that higher doses of monosodium glutamate may have detrimental effects on the microanatomy of the small intestine in adult Wistar rats (12).

Conclusion

Our study concluded that administering MSG at a dose stratified according to the ADI could result in structural damage to the duodenum of rats. It is crucial to re-evaluate the long-term use of MSG as a flavor enhancer.

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