Original Research Report

HISTOLOGICAL STUDY OF MICE (Mus musculus Linn.) DIGESTIVE ORGANS INDUCED BY MONOSODIUM GLUTAMATE AFTER CONSUMING ROSELLE (Hibiscus sabdariffa Linn.) TEA

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ABSTRACT

Monosodium glutamate (MSG) is one of the most frequently used synthetic additive for food flavoring ingredient. Excessive use of MSG can cause cytotoxic effects which impacted the levels of oxidative stress and free radicals in the body, especially in the human digestive system. Roselle (Hibiscus sabdariffa) is a plant that is known to has red flower petals, and has lots of beneficial active compounds such as polyphenols, flavonoids, anthocyanins, and other compounds which are antioxidants that function as free radical antidotes. This research aims to determine consumption effect of administration roselle tea, as well as its optimal dose for digestive organs restoration on MSG-induced mice. This research applied the completely randomized method, using 25 mice. Mice were divided into 5 treatments, consisting of K- (0.3 ml aquades), K+ (given 4 mg/g bw MSG), P1 (given 4 mg/g bw MSG and 2.6 mg/g bw Roselle), P2 (given 4 mg/g bw MSG and 3.9 mg/g bw Roselle), P3 (given 4 mg/g bw MSG and 5.2 mg/g bw Roselle). Treatment was administered orally using a gavage for 30 days. Histopathological examination of the stomach, duodenum, and liver was made by using the paraffin method and Hematoxylin-Eosin (HE) staining. Parameters observed in the stomach and duodenum were necrosis, inflammatory cell infiltration, villous erosion and epithelial desquamation, while in the hepatic organ parameters observed were necrosis, inflammatory cell infiltration and cell degeneration. The results of the parameters were statistically analyzed using the SPSS for windows version 22 application program. Normal and homogeneous data distribution was analyzed using the Kolmogorov-Smirnov normality test, analyzed by One-Way ANOVA test, followed by Duncan Post-Hoc test if the data were normally distributed and there were significant differences (P < 0.05) between groups. In conclusion, the infusion of roselle tea (H. sabdariffa) with graded doses were able to recover histological damage in the stomach, duodenum, and liver of mice (Mus musculus) that had been induced by MSG.

Keywords: Digestive organs; healthy lifestyle; Hibiscus sabdariffa Linn; MSG

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Highlights:
1. This original study examined the antioxidants compounds derived from naturally sourced Hibiscus sabdariffa calyx
2. Hibiscus sabdariffa tea was able to maintain histological damage such as necrosis, degeneration cell, villous erosion, and epithelial desquamation of mice digestive organs after induced by MSG

INTRODUCTION

Indonesian these days are currently preferring fast food for everyday meal because it is easy to obtain, serves quickly and practically, and has a delicious taste. Delicious taste of fast food comes from flavour enhancer (Pamela, 2018). Monosodium glutamate (MSG) is a synthetic flavour enhancer derived from glutamic acid. MSG is formed from L- acid glutamate which ionizes with sodium, thus forming L- acid glutamate salt sodium which is able to strengthen the taste of food. The chemical structure of MSG has no different from glutamic acid, except that one of the carboxyl groups that containing hydrogen is substituted with sodium. Carboxyl groups that have ionized with sodium can activate stimulation in the taste buds. This is what cause MSG as a flavour enhancer able to give a savory taste (umami) to food (Kurtanty et al., 2018). Glutamate contained in food will be metabolized quickly to become source of energy. Glutamate in MSG is absorbed in the small intestine through an active transport system specific for the amino acid. During absorption in the small intestine, glutamate levels in the blood plasma will increase. When large amounts of glutamate are consumed, the level of glutamate in the body will increase. Increased levels of glutamate in the body can cause increased metabolism of glutamate in the liver, causing the release of glucose, lactate, glutamine, and other amino acids in the body’s metabolism (Information, 2021). MSG in the body is absorbed in the small intestine, and occurs mostly in epithelial cells that lining intestinal mucosa, distributed throughout the body until it has metabolic processes in the liver, then excreted by the kidneys through feces or urine (Zulfi et al., 2013; Airaodion et al., 2019). MSG
metabolism in the liver will be converted into another metabolites, hence if the giving of glutamate exceeds the ability of the liver’s capacity to metabolize glutamate, it will cause an increase in glutamate levels (Onaolapo et al., 2016).

In Indonesia, MSG consumption is estimated around 0.6 gram per days, which is undetermined because consumers often consume food where the exact amount of MSG in the food is unknown, so the number of MSG consumption per day may increase. Therefore, the use of MSG is limited by the Food Drug Administration (FDA) and World Health Organization (WHO) to 120 mg/kg bw per day (Yonata & Iswara, 2016). According to some studies, MSG in a certain amount is still considered safe. In addition, several other countries have set the MSG consumption limit at 0.3 – 1 gram per day (Sulastri, 2017). Consumption of MSG that exceeds the suggested limit continuously over a long period of time, can cause side effects such as increased glutamate levels in the blood, thus affecting the metabolism of the body. Research on the toxic effects of consuming too much glutamate can affect the central nervous system which can interfere with autonomic function and body metabolism, cause obesity, disrupt reproductive system hormones, hepatotoxicity, and nephrotoxicity caused by oxidative stress (Airaodion et al., 2019).

Roselle (Hibiscus sabdariffa L.) has been used as an antioxidant, hypcholesterolemic, antiobesity, insulin resistant activity reduction, antihypertensive, diuretic, and antimicrobial agent (Gheller et al., 2017). Roselle contains an active compound such as gossypetin, anthocyanin, and hibisci glucoside which have protective effect against degenerative disease. The ethanol extract of roselle contains alkaloid, flavonoid, anthocyanin, phenolic, steroid, terpenoid, saponin and tannin compounds (Aryati & Pratiwi, 2020). Anthocyanins are flavonoid compound that provide benefits to human health by protecting cell damage caused by free radical compounds that enter the body. Wahyuni (2017) stated the administration of 0.45 ml of dried roselle petals per day consumed twice per day can reduce cholesterol levels in hypercholesterolemic mice. Other studies have shown that roselle leaf extract is able to provide hepatoprotection effects to overcome damage of the mice’s liver induced by natrium nitrite (NaNO₂) (Sabri, 2020). Therefore, this study aims to determine the effect of administration roselle tea, and its optimal dose in repairing histological damage to the digestive organs of mice.

MATERIALS AND METHODS

This study was an experimental investigation, and the research methodology employed was a completely randomized design. The experimental animals used were male mice aged 2 months, with healthy conditions, and body weight between 20-30 grams. The total number of mice were 25, divided into 5 treatment groups with 5 mice in each treatment group. This research was conducted at Animal Physiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Udayana University, Jimbaran, Bali.

The treatment groups consisted of a negative control group, a positive control group, and a treatment group administered with three doses of roselle tea. The MSG used is brand “X” found in a market, and the dried petals of roselle processed into tea are brand “XX” sold in the supermarkets. The dried petals of roselle were locally grown in Kediri, East Java. The negative control group was only given 0.3 ml of aquades. The positive control group dose refers to the research of Maulida et al. (2013) which is given 4 mg/g bw dissolved in 0.3 ml aquades. Dried roselle petals of the “XX” brand that have been prepared are divided into 3 dose treatment groups that have been determined based on the result of converting human doses to mice, so that the optimal doses to be given are 2.6 mg/day, 3.9 mg/day, and 5.2 mg/day. Each dose was brewed in the same volume of water i.e. 0.5 ml of water, and in the same time of brewing i.e. 5 minutes.

Before treatment, mice were acclimatized for 7 days, placed in a plastic tub cage measuring 40x30x18 cm covered with woven wire at the top, and padded with rice husks. During acclimatization, all the treatment groups were given standard mice feed (i.e., chicken pellets, ad libitum water) and were placed in a room temperature of 25°C with adequate air ventilation (Nugroho, 2018). Treatment was started after acclimatization for 7 days and lasted for 30 days. The treatment was administered using the gavage method, i.e., giving the treatment material orally using a sonde. 25 mice were divided into 5 groups. Groups (K-) was given 0.3 ml aquades, group (K+) was given 4 mg/g bw MSG dissolved in 0.3 ml of aquades, group (P1) was given 4 mg/g bw MSG and 2.6 mg per day of roselle tea brew, group (P2) was given 4 mg/g bw MSG and 3.9 mg per day of roselle tea brew, group (P3) was given 4 mg/g bw MSG and 5.2 mg per day of roselle tea brew.

Treatment was given after the mice had been fasted for 14-18 hours. Treatment of the control groups were started after acclimatization for 7 days and the treatments lasted for 30 days. P1, P2, and P3 groups were given MSG orally first for 7 days, then on the 8th day the mice were given MSG orally one hour before being given roselle tea brew. On the 31st day, all mice were anesthetized with ketamine, sacrificed by dislocating the neck, then stomach, duodenum, and liver organs were collected. The organs were
washed in 0.9% NaCl solution and fixed with 10% NBF solution (Maulida et al., 2013). The preparatory work was made at the Veterinary Center, Denpasar, Indonesia. This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University (No. B/60/UN14.2.9/PT.01.04/2023 on 28/03/2023).

Observation of histological preparations of stomach, duodenum, and liver organs were observed using a microscope at 100x and 400x magnification, photographed with an Optilab digital microscope camera, and observed with the Optilab Viewer application (Yogini et al., 2021). The number of damaged cells were counted from 5 fields of view using the Image Raster application. Parameters observed in the stomach and duodenum were necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. In the hepatic organs observed were necrosis, inflammatory cell infiltration and cell degeneration. The results of the data obtained were statistically analyzed using the SPSS for windows version 22 application program. Normal and homogeneous data distribution was analyzed using the Kolmogorov-Smirnov normality test, analyzed by one-way ANOVA test, followed by Duncan Post-Hoc test if the data were normally distributed and there were significant differences (P < 0.05). If the data distribution is not normal, it is analyzed with the Kruskal-Wallis test and continued with the Mann-Whitney test if the results are significantly different (P<0.05) (Masnunah et al., 2020).

RESULTS

Histopathology of the Stomach Organ

The observation results of histological sections in the stomach revealed the presence of normal and damaged cells in the form of necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation after administered MSG at a dose of 4 mg/g bw per day. Data from the calculation of the average stomach cells damage were tested for normality using the Kolmogorov-Smirnov test. The results of the normality test of stomach histology showed a normal distribution of data (P>0.05). Normally distributed data were continued with homogeneity test and continued with One-Way ANOVA test. The test results obtained (P < 0.05) between treatments with control, then continued with the Duncan test (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>K-</th>
<th>K+</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis (cell)</td>
<td></td>
<td>1.96±1.29</td>
<td>24.93±1.62</td>
<td>11.68±2.76</td>
<td>6.91±1.33</td>
<td>6.82±2.85</td>
</tr>
<tr>
<td>Inflammatory cell infiltration (%)</td>
<td></td>
<td>1.23±0.19</td>
<td>24.62±1.28</td>
<td>12.41±2.43</td>
<td>6.45±0.92</td>
<td>5.76±1.04</td>
</tr>
<tr>
<td>Villous erosion (%)</td>
<td></td>
<td>7.20±5.74</td>
<td>33.60±7.07</td>
<td>18.24±3.32</td>
<td>13.28±2.81</td>
<td>12.6±3.2</td>
</tr>
<tr>
<td>Epithelial desquamation (%)</td>
<td></td>
<td>7.68±5.02</td>
<td>18.56±3.98</td>
<td>14.56±3.98</td>
<td>9.76±6.51</td>
<td>6.24±6.80</td>
</tr>
</tbody>
</table>

Description: different superscript letter behind the numbers in the same row indicate significant differences between treatments (P<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

Further test results with Duncan's test on Table 1 showed a significant difference (P < 0.05) between the control and treatment groups. K(-) was significantly different from K(+) The K(+) group had the highest average damage value. This shows that MSG induction at a dose of 4 mg/g bw given orally per day caused damage to the histology of the stomach mucosa in the form of necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation.

Data showed that for all stomach histological damage, there were significant differences (P < 0.05) between K (+) with P1, P2, and P3. Significant differences (P < 0.05) on necrosis and inflammatory cell infiltration in P1 and P2, P1 and P3. However, P2 was not significantly different (P > 0.05) with P3. The decrease in villous erosion between treatment groups P1, P2, and P3 were not significantly different (P > 0.05). The decrease in epithelial desquamation damage P1 (P < 0.05) compared with P3, but the P2 group was not significantly different (P > 0.05) compared with the P1 and P3 groups. This shows that the administration of roselle tea brew with graded doses were able to reduce stomach histological damage in mice. Stomach histological damage such as necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation each group can be seen in the picture (Figure 1) below.
Histopathology of the Duodenum Organ

The observation results of histological sections in the duodenum revealed the presence of normal and damaged cells in the form of necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation after administered MSG at a dose of 4 mg/g bw per day. Data from the calculation of the average duodenal cells damage were tested for normality using the Kolmogorov-Smirnov test. The results of the normality test of duodenal histology showed a normal distribution of data (P > 0.05). Normally distributed data continued with homogeneity test and continued with One-Way ANOVA test. The test results obtained (P < 0.05) between treatments with control, then continued with the Duncan test (Table 2).

Table 2. Results of duodenum histopathology after administered with MSG and roselle tea

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>K-</th>
<th>K+</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis (cell)</td>
<td></td>
<td>0.99±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.42±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.80±0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.35±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.37±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inflammatory cell infiltration (%)</td>
<td></td>
<td>0.63±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21±1.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.47±0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.95±0.76&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.23±0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Villous erosion (%)</td>
<td></td>
<td>6.08±6.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.00±7.24&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>18.72±4.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.72±3.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.12±5.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epithelial desquamation (%)</td>
<td></td>
<td>9.92±8.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.28±5.95&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13.92±2.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.36±6.36&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.96±4.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Description: different superscript letter behind the numbers in the same row indicate significant differences between treatments (P<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).
Further test results with Duncan's test on Table 2 showed a significant difference (P < 0.05) between the control and treatment groups. K(-) was significantly different from K(+). The K(+) control group had the highest average damage value. This indicates that administration of MSG at a dose 4 mg/g bw orally per day caused histological damage to the duodenal mucosa in the form of necrosis, inflammatory cell infiltration, villous erosion and epithelial desquamation. For all duodenal histological damage, there were significant differences (P < 0.05) between K(+) with P1, P2, and P3, P1 and P3, but there were no significant differences (P > 0.05) between P1 and P2. P2 (P < 0.05) P3 on necrosis and villous erosion, but P2 (P > 0.05) with P3 on epithelial desquamation and inflammatory cell infiltration. This shows that the administration of roselle tea brew with graded doses were able to reduce damage to duodenum histology in mice. Duodenum histological damage such as necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation each group can be seen in the picture (Figure 2) below.

**Histopathology of the Liver Organ**

The observation results of histological sections in the liver revealed the presence of normal and damaged cells in the form of necrosis, inflammatory cell infiltration, and cell degeneration after administered MSG at a dose of 4 mg/g bw per day. Data from the calculation of the average hepatic cell damage were tested for normality using the Kolmogorov-Smirnov test. The results of the normality test of liver histology showed a normal distribution of data (P > 0.05). Normally distributed data were continued with homogeneity test and continued with One-Way ANOVA test. The test results obtained (P < 0.05) between treatments with control, then continued with the Duncan test (Table 3).

Further test results with Duncan's test on Table 3 showed a significant difference (P < 0.05) between the control group and the treatment group. K(-) was significantly different from K(+). The K(+) control group had the highest average damage value. This indicates that MSG administration at a dose of 4 mg/g bw orally per day caused histological damage to hepatic tissue in the form of necrosis, inflammatory cell infiltration, parenchymal degeneration, hydropic degeneration, and fatty degeneration. Significant differences (P < 0.05) in necrosis cells were found in the K(+) group with P1, P2, and P3, between P1 and P2 and P3. Significant differences (P < 0.05) on inflammatory cell infiltration were found in the K(+) group with P1 and P2, P1 and P3, but not significantly different (P > 0.05) between the P2 and P3 groups. The K(+) and P1 groups were not significantly different (P >
0.05) on the damage of parenchymal degeneration and hydropic degeneration. Groups P1 and P2, P2 and P3 were not significantly different (P > 0.05) on damage to hydropic degeneration and fatty degeneration. This shows that the administration of roselle tea brew with graded doses was able to reduce damage to mice hepatocytes. Liver histological damage such as necrosis, inflammatory cell infiltration, parenchymal degeneration, hydropic degeneration, and fatty degeneration can be seen in the picture (Figure 3) below.

<table>
<thead>
<tr>
<th>Variable</th>
<th>K-</th>
<th>K+</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis (cell)</td>
<td>1.64±0.89a</td>
<td>15.92±3.78d</td>
<td>10.10±1.27c</td>
<td>6.86±2.24b</td>
<td>5.47±1.58b</td>
</tr>
<tr>
<td>Inflammatory cell infiltration (%)</td>
<td>2.61±1.97a</td>
<td>11.81±1.86d</td>
<td>7.98±3.12c</td>
<td>6.15±1.57bc</td>
<td>4.73±1.90b</td>
</tr>
<tr>
<td>Parenchymal degeneration (%)</td>
<td>2.93±1.49a</td>
<td>9.80±1.87d</td>
<td>7.76±2.23cd</td>
<td>6.78±2.23bc</td>
<td>4.75±2.03b</td>
</tr>
<tr>
<td>Hydropic degeneration (%)</td>
<td>1.21±0.81a</td>
<td>7.54±2.47d</td>
<td>5.43±1.54ad</td>
<td>3.82±2.71bc</td>
<td>2.18±0.85bc</td>
</tr>
<tr>
<td>Fatty degeneration (%)</td>
<td>0.80±0.84a</td>
<td>8.82±2.03d</td>
<td>5.82±1.28c</td>
<td>3.32±1.43b</td>
<td>0.64±0.58a</td>
</tr>
</tbody>
</table>

Description: different superscript letter behind the numbers in the same row indicate significant differences between treatments (P<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

Figure 3. Liver histological observation with HE staining (400x magnification)
Description: a) Normal cell, b) Hydropic degeneration, c) Parenchymal degeneration, d) Fatty degeneration, e) Necrosis, f) Inflammatory cell infiltration; 1) Sinusoid.
K- (aquades 0.3 ml), K+ (MSG 4 mg/g bw), P1 (roselle 2.6 mg/g bw), P2 (roselle 3.9 mg/g bw), P3 (roselle 5.2 mg/g bw)
DISCUSSION

Histopathology of the Stomach Organ

The results obtained from histopathological observations of mice stomach organs showed that the K(-) group which was only given aquades had stomach mucosal damage with the lowest average value (Table 1) (Figure 1). The presence of necrosis in the K(-) group is normal if it has a low value, because necrosis is a pathological cell death. Inflammatory cell infiltration found in the K(-) group may indicate a cell regeneration process. The presence of epithelial cell desquamation and villous erosion is one of the stomach mucosal responses due to decreased mucus secretion (cytoprotective). The decrease in cytoprotection can be caused by stress factors obtained from environmental conditions such as adaptation sites, and responses received by experimental animals due to mechanical trauma to the stomach when given treatment by sonde every day (Jahra & Muhartono, 2019). The observation results of the K (+) group showed that the giving of MSG at a dose of 4 mg/g bw per day was able to cause damage to the stomach mucosa with the highest average value (Table 1). This damage is caused by high levels of glutamic acid in the body produced from MSG. Increased levels of glutamic acid can cause free radicals in the body (Simon et al., 2013). High levels of free radicals in the body will damage the weak polyunsaturated acids in the cell membrane, making the cell membrane fragile and causing free radicals to enter the cytoplasm and damage the cell nucleus (Chua et al., 2013). Damage to the cell membrane causes extracellular fluid to enter the cell causing degeneration, and if the cell is exposed to toxic substances for a long time, cell death or necrosis will occur. This is in accordance with research conducted by (Yogini et al., 2021) that MSG can causes damage to the MSG-induced stomach.

The results of observations in groups P1, P2, and P3 showed that roselle tea brew influenced reducing necrosis damage and inflammatory cell infiltration, but there was no significant difference in reducing damage to villous erosion and epithelial desquamation (Table 1). Epithelial desquamation damage and villous erosion that occurred in groups P1, P2, and P3 resulted from the continuous administration of MSG, which would increase free radicals (Figure 1). Free radicals can accelerate the inflammatory process and cause oxidative stress, resulting in cell damage. Oxidative stress affects HCL secretion, decreases the production of prostaglandin hormones that function in the stomach mucosal barrier, induces ulcers in the mucosa and disrupts gastric motility due to an increase in the inflammatory mediator histamine and leukotriene (Riong, 2022).

The administration of roselle tea brew to the P1 group at a dose of 2.5 mg/g bw showed little improvement in stomach mucosal damage due to MSG administration (Table 1). The administration of group P2 with a dose of 3.9 mg/g bw showed significant improvement in stomach mucosal damage compared to group P1, whereas group P3 with a dose of 5.2 mg/g bw showed less significant improvement in stomach mucosal damage when compared to group P2 (Table 1). This indicates that the dose of group P2 has been able to repair stomach mucosal damage, however if continued with the dose of group P3 does not cause further damage to the gastric mucosa, and the average value of damage becomes lower (Table 1). The administration of the P3 group dose also gave the lowest average value of epithelial desquamation damage compared to the K(-) control group, due to the presence of roselle tea antioxidant compounds that can repair stomach cells (Figure 1). Group P3 showed the lowest average value of all stomach mucosal cell damage due to MSG administration in this study, with the results of the average value close to the control group K (-). Therefore, the dose of rosela tea 5.2 mg/g bw can be considered as the optimal dose that has the potential to reduce stomach mucosal damage.

Roselle tea has an effect to reduces necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation, because it contains flavonoids that can ward off free radicals. Flavonoid is an antioxidant compound that can protect the body from free radicals by giving one of its electrons to inhibit free radical activity (Nursheha dan Febriani, 2015). Flavonoids contained in roselle petals are able to increase stomach mucus fluid that serves to protect the stomach mucosa from the influence of acid and pepsin, as well as other damaging substances such as MSG, by reducing histamine secretion from mast cells through the mechanism of inhibiting the work of the enzyme histidic decarboxylase. Other antioxidants contained in roselle, such as saponins, function as gastroprotective agent by activating the protection system of the stomach mucous membrane. This is in accordance with research conducted by Riong (2022) regarding the flavonoid, saponin and tannin compounds of widuri leaf extracts that can reduce stomach histology damage in rats that have been induced by aspirin.

Histopathology of the Duodenum Organ

The results obtained from histopathological observations of the duodenum organs of mice showed that in K (-) control group which was only...
given aquades had the lowest average value of duodenum mucosa damage (Table 2) (Figure 2). Necrosis damage in the K(-) treatment is normal in cell damage pathology if it is at a low value. Inflammatory cell infiltration in the K(-) group indicates the process of duodenal cell regeneration. Damage to epithelial cell desquamation and villous erosion is one of the duodenal mucosal responses due to decreased mucus secretion as a cytoprotective agent (Jahra & Muhartono, 2019). The results of observations in the K (+) group showed that the administration of MSG at a dose of 4 mg/g bw per day was able to cause damage with the highest average value (Table 2). This result is in accordance with Vincent et al (2015) research finding that MSG can cause damage to the duodenal mucosa. The mechanism of duodenal mucosa damage begins with epithelial desquamation. Epithelial desquamation is the detachment of epithelial cells from the tissue surface and is also a protective function in the duodenum as a tissue defense response from irritants to prevent further damage (Alfina et al., 2022). This damage is caused by the high levels of glutamic acid in the body produced by MSG. The mechanism of damage caused by MSG can be obtained from free glutamate which increases stomach acid secretion. Villous erosion is an advanced damages experienced by the duodenum in the form of partial loss of epithelium in the mucosal layer of the duodenum (Sulastri et al., 2018). Villous erosion occurs because Brunner's glands do not produce alkaline mucus that can protect the duodenal wall from gastric acid secretion (Putra et al., 2021).

The results of observations in groups P1, P2, and P3 showed that roselle tea brew can reduce necrosis damage, inflammatory cell infiltration, villous erosion, and epithelial desquamation (Table 2). Damage to epithelial desquamation and villous erosion in groups P1, P2, and P3 resulted from the continuous giving of MSG which will increase free radicals (Figure 2). Highly reactive free radical molecules continuously form other free radicals and cause cell damage, resulting in oxidative stress. Stress in the digestive tract can reduce mucosal blood flow, thus disrupting mucosal barrier integrity by inhibiting Brunner's glands (Putra et al., 2021). Duodenal mucosa damage can be caused by the induction of gastric acid secretion in excessive amounts over a long period of time, causing an inflammatory process that can damage the structure of the duodenal mucosa. The administration of roselle tea brew in the P1 treatment with a dose of 2.5 mg /g bw, and P2 with a dose of 3.9 mg /g bw was able to provide improvements in duodenal mucosal damage, but the P3 treatment with a dose of 5.2 mg /g bw was able to provide significant improvement due to MSG administration, with the results of the average value close to the control group K (-) (Table 2). Group P3 showed the lowest average value of all duodenal mucosal cell damage in this study, so the dose of rosela tea 5.2 mg /g bw can be considered as the optimal dose that has the potential to reduce duodenal mucosal damage (Figure 2). Duodenal mucosa damage has the lowest average value of overall damage compared to damage to the stomach. This is because MSG is digested first in the stomach with the help of gastric digestive sap, so the stomach receives a lot of toxic substances from MSG. The toxic substances are then passed on and absorbed by the duodenum in smaller amounts (Vincent et al., 2015).

Roselle tea is a natural antioxidant that contains phenolic compounds such as anthocyanins, gossypetin, vitamin C, vitamin B, vitamin D. Rosela also contains polyphenol and flavonoid compounds as antioxidants that function in binding free radicals so as not to damage cells and prevent lipid peroxidation. According to Hardiningtyas et al (2014), flavonoid antioxidant works by taking in and preventing the regeneration of ROS, as well as indirectly increasing the activity of cellular antioxidant enzymes. Roselle also contains anthocyanins and polyphenols that improve the mucosal barrier of the digestive tract by multiplying digestive microflora bacteria such as Lactobacillus spp. and Bacillus spp. (Amer et al., 2022).

**Histopathology of the Liver Organ**

The results obtained from histopathological observations of mice hepatic organs showed that there were normal and damaged cells in the negative control group K(-) (Table 3) (Figure 3). Normal hepatocytes have characteristics such as hepatocytes that appear regular with a polyhedral shape, round and oval cell shapes with hepatocyte plates, bright red cytoplasm, white sinusoids and appear intact (Anggraeny & Ducha, 2014). In addition to normal cells, the K(-) group also had cells that were necrosis, infiltration of inflammatory cells, and degeneration, with a smaller amount when compared to the group administered MSG. This is due to the pathology process, where every cell in the body will experience cell death due to toxic substances or certain factors, followed by a cell regeneration process that produces new cells. Hepatocyte damage with the highest average value was found in the positive control group K(+) with a MSG dose of 4 mg /g bw (Table 3). The results of statistical analysis showed that many cells had degeneration and necrosis (Figure 3). Hepatocyte damage due to continuous administration of MSG can occur due to the presence of radical compounds produced.
from the side metabolism of glutamic acid which will produce hydrogen peroxide. Hydrogen peroxide can react with compounds in the body and form reactive hydroxyl radicals. Hydroxyl radicals cause lipid peroxides which disrupt the integrity of the cell membrane; therefore, cell structure becomes abnormal and damage (Ayala et al., 2014).

Hepatic tissue damage due to continuous administration of MSG begins with the process of cell degeneration. MSG in large numbers can cause large amounts of extracellular fluid to enter in the cytosol, resulting in swelling of the hepatocytes (Maulida et al., 2013). Continuous giving of MSG can cause the accumulation of MSG in the hepatic organ which has the function of filtering toxic substances that enter the body, so that the amount of accumulation can damage hepatocytes due to the effects of free radicals caused by MSG (Anggraency & Ducha, 2014). Once the cell membrane is damaged, the effects of the toxicant can reach and damage the nucleus causing the cell structure becomes abnormal and necrosis. Cell death or necrosis occurs due to continuous degeneration that damages the cell membrane system causing the cell to lyse and die (Wijaya et al., 2014). Cell membranes damage due to free radicals also causes an inflammatory response in the form of inflammatory cell infiltration. The process of liver inflammation that occurs in this study is a form of the body’s defense process due to the toxic effects of MSG. However, hepatocytes can regenerate quickly enough if the damage caused by toxic effects can be reversed by antioxidants (Adikara et al., 2013).

The administration of roselle tea brew to the P1 group at a dose of 2.5 mg/g bw was unable to repair hepatocyte damage (Table 3). This is indicated by the damage value of parenchymal degeneration and hydropic degeneration in group P1 not significantly different from K (+). Group P2 with a dose of 3.9 mg/g bw showed improvement in hepatocyte damage, but was not significantly different compared with group P1 in terms of damage to parenchymal degeneration, hydropic degeneration, and inflammatory cell infiltration (Table 3). Group P3 with a dose of 5.2 mg/g bw showed improvement in hepatocyte damage, which was not significantly different compared with group P2, but significantly different from group P1 (Table 3). This showed that the P2 group dose was able to repair hepatocyte damage, but when continued with the P3 group dose did not cause hepatocyte damage, and the average value of damage decreased (Figure 3). The dose of P3 group also gave the lowest average value of fatty degeneration damage compared with the K(-) control group, due to the presence of roselle tea antioxidant compounds that are able to repair hepatocytes (Table 3) (Figure 3). The P3 group showed the lowest average value of all hepatocyte damage due to MSG administration in this study, with the average value close to the Kr(-) control group (Figure 3). Therefore, the dose of 5.2 mg/g bw roselle tea can be considered as the optimal dose that has the potential to reduce the weakening of the liver and overall hepatocyte damage.

Roselle tea is a natural antioxidant whose petals contain phenolic compounds such as flavonoids, anthocyanins, and gossypetin. The content of polyphenol and flavonoid compounds as antioxidants that function in binding free radicals so as not to damage cells and prevent lipid peroxidation. Flavonoids contained in roselle can prevent free radical damage by stabilizing ROS (reactive oxygen species) so that the effect of ROS is less reactive. Water-soluble extracts of roselle petals contain protocatechuic acid and anthocyanins, which can protect against liver damage. This is in accordance with Adeyemi et al. (2014) arguing that administration of roselle extract is effective in amelioration the liver fibrosis of diabetic rats induced by streptozotocin. The effectiveness of roselle extract could be partly related to its antioxidant activity such as protocatechuic acid, anthocyanins and flavonoids which prevents peroxidative liver damage. Another study by Zuraida et al. (2015) regarding the administration of roselle extract on MDA levels and catalase activity of rats exposed to carbon tetrachloride (CCL4) revealed that the administration of roselle with higher doses causes the higher the antioxidant activity of ascorbic acid contained within it, the more able it is to inhibit lipid peroxidation caused by free radicals, resulting in a decrease in MDA levels and liver necrosis.

**Strength and limitations**

This study explains the effect of roselle tea antioxidant compounds in repairing histological damage to digestive organs due to MSG administration. Regular consumption of roselle tea is expected to protect the body from the effects of free radicals from other chemicals. However, due to the short duration of this study, the maximum recommended dose of serving roselle tea for humans who have stomach acid is still unknown, because the taste of roselle tea is sour. Therefore, further research needs to be done with different doses, longer duration of administration, and need to examine the side effects of roselle tea.
CONCLUSION

The administration of roselle tea (Hibiscus sabdariffa L.) at graded doses is able to repair histological damage to the stomach, duodenum, and liver of mice (Mus musculus) induced by MSG, especially at the highest dose from this study can provide optimal amelioration results on histological damage to the stomach, duodenum, and liver of mice (Mus musculus). Further comprehensive study is required to investigate the potential impact of administering roselle tea brew on histological damage to organs associated with the administration of other substances. The consideration of roselle tea as a herbal medication is taken into account.

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Conflict of interest

None.

Ethical consideration

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University with number No. B/60/UN14.2.9/PT.01.04/2023 on 28/03/2023.

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Author contribution

KAS conceptualized the study, analyzed and interpreted the data, wrote the article draft, provided the study materials, statistical expertise, administrative support, and collected and assembled data. NIW conceptualized the study, developed the methodology, reviewed and edited the manuscript, critical revision of the article, final approval of the article, statistical expertise, technical and logistic support. AASAS provided validation for the study and statistical expertise. All authors have read and approved the final version of the manuscript for publication.

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