Original Research Report

HISTOLOGICAL STUDY OF THE RESTORATIVE EFFECT OF ROSELLE (*Hibiscus sabdariffa* Linn.) TEA ON THE DIGESTIVE ORGANS OF MONOSODIUM GLUTAMATE-INDUCED MICE (*Mus musculus* Linn.)

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ABSTRACT

Monosodium glutamate (MSG) is a commonly used synthetic additive for enhancing food flavor. Excessive use of MSG can cause cytotoxic effects, which disrupt the balance of oxidative stress and free radicals in the body, particularly in the human digestive system. Roselle (*Hibiscus sabdariffa*) is a plant with red petals renowned for its abundance of beneficial compounds, including polyphenols, flavonoids, anthocyanins, and other antioxidants that function as free radical antidotes. This study aimed to investigate the effect of administering roselle tea and determine the optimal dose for restoring the digestive organs of MSG-induced mice (*Mus musculus*). The research employed a completely randomized design with a random sampling method. A total of 25 mice were divided into five groups: a negative control group (K-) that received 0.3 mL of distilled water, a positive control group (K+) given 4 mg/g bw of MSG, and three treatment groups (P1, P2, and P3) administered with 4 mg/g bw of MSG along with varying doses of roselle tea (2.6 mg/g bw, 3.9 mg/g bw, and 5.2 mg/g bw, respectively). The treatment was orally administered via gavage for 30 days. The stomach, duodenum, and liver underwent histopathological examination using the paraffin method and hematoxylin-eosin staining. The observed parameters in the stomach and duodenum included necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. Meanwhile, the parameters examined in the hepatic organs were necrosis, inflammatory cell infiltration, and cell degeneration. The Kolmogorov-Smirnov normality test and one-way analysis of variance (ANOVA) were employed to assess the normal distribution and homogeneity of the data. If the data exhibited a normal distribution, Duncan's post-hoc test was conducted. The results revealed significant differences among the groups ($p<0.05$). In conclusion, the administration of roselle tea effectively recovered the histological damage in the stomach, duodenum, and liver of MSG-induced mice.

Keywords: Digestive organs; healthy lifestyle; *Hibiscus sabdariffa* Linn.; monosodium glutamate (MSG)

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Highlights:

1. This original study examined the antioxidant compounds derived from naturally sourced Hibiscus sabdariffa calyx.
2. The findings demonstrated that roselle tea offers a viable and cost-effective solution for repairing histological damage to the digestive organs induced by monosodium glutamate.

INTRODUCTION

Presently, Indonesians have a preference for fast food as their everyday meal because it is easy to obtain, quickly served, convenient, and delicious. The delectable taste of fast food comes from the use of flavor enhancers (Pamelia 2018). Monosodium glutamate (MSG) is a synthetic flavor enhancer derived from glutamic acid. It is formed of L-glutamic acid that ionizes with sodium, resulting in the formation of L-glutamate sodium salt. This salt has the ability to enhance the flavor of food. The chemical structure of MSG is identical to that of glutamic acid. The only exception is that sodium substitutes one of the carboxyl groups, which typically contains hydrogen. Sodium-ionized carboxyl groups can stimulate the taste buds. This is what makes MSG, as a flavor enhancer, able to give a savory taste (umami) to food (Kurtanty et al. 2018).

Glutamate contained in food is rapidly metabolized to become a source of energy. The absorption of glutamate from MSG occurs 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 increase. Glutamate consumption in large amounts leads to an increase in the level of glutamate in the body. This increase can cause heightened glutamate metabolism in the liver, which triggers the release of glucose, lactate, glutamine, and other amino acids (National Center for Biotechnology Information 2024). MSG is primarily absorbed in the small intestine by epithelial cells that line the intestinal mucosa. It is then dispersed throughout the body and undergoes metabolic processes in the liver. Finally, it is excreted by the kidneys through feces or urine (Zulfi et al. 2013, Airaodion 2019). The metabolism of MSG in the liver results in the production of a metabolite. Therefore, when the intake of glutamate exceeds the capacity of the liver to metabolize it, there will be a significant rise in glutamate levels (Onaolapo et al. 2016).

The estimated daily MSG consumption in Indonesia is approximately 0.6 g. However, the exact amount remains uncertain as consumers often indulge in foods with unknown MSG content, potentially leading to an increase in daily MSG consumption. While MSG consumption in moderate amounts is still considered safe, it is important to be aware of the permissible intake to avoid overconsumption. The Food and Drug Administration (FDA) of the United States and the World Health Organization (WHO) have established the maximum permissible limit of MSG at 120 mg/kg bw per day. Furthermore, several countries have set the maximum permissible consumption of MSG at 0.3–1 g per day (Yonata & Iswara 2016, Sulastri 2017). Prolonged and excessive consumption of MSG can lead to various side effects, such as increased glutamate levels in the blood, which may negatively affect the metabolism of the body. A high glutamate level also poses toxic effects on the central nervous system, leading to the interference of autonomic function and body metabolism, obesity, the disruption of reproductive system hormones, hepatotoxicity, and nephrotoxicity resulting from oxidative stress (Airaodion 2019).

Roselle (*Hibiscus sabdariffa* L.) has been used for its antioxidant, hypocholesterolemic, antiobesity, insulin-resistant activity reduction, antihypertensive, diuretic, and antimicrobial properties. It contains active compounds such as gossypetin, anthocyanin, and hibiscus glucoside, which have protective effects against degenerative diseases. Roselle ethanol extract comprises alkaloid, flavonoid, anthocyanin, phenolic, steroid, terpenoid, saponin, and tannin compounds (Gheller et al. 2017, Aryati & Rohadi 2020). Anthocyanins are flavonoid compounds that provide benefits to human health by protecting cells from damage caused by free radicals that enter the body. A previous study showed that administering 0.45 mL of dried roselle petal infusion twice per day effectively reduced cholesterol levels in hypercholesterolemic mice (Wahyuni 2015). A separate study investigated the potency of roselle leaf extract on the damaged liver of mice. The study found that the extract showed hepatoprotection effects capable of repairing the damage induced by natrium nitrite (NaNO2) (Sabri 2020). The detrimental effects of consuming excessive amounts of MSG become particularly evident in the digestive organs, which play a crucial role in processing food before it is converted into energy through metabolism. Hence, this study aimed to examine the effect of administering roselle tea and ascertain the most effective dose for repairing histological damage to the digestive organs of MSG-induced mice.

MATERIALS AND METHODS

This study was an experimental investigation using a completely randomized design and random sampling. The experimental animals used in the experiment were male mice aged 2 months with healthy conditions and a body weight of 20–30 grams. The total number of mice was 25, distributed evenly among five groups, with each group containing five mice (Wahid et al. 2017). The five groups consisted of a negative control group (K-), a positive control group (K+), and three treatment groups (P1, P2, and P3). The research was conducted at the Animal Physiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Badung, Indonesia.

The negative control group (K-) was only given 0.3 mL of distilled water. The positive control group (K+) received 4 mg/g bw of MSG dissolved in 0.3 mL of distilled water, according to the instructions provided in a study by Maulida et al. (2013). The three treatment groups (P1, P2, and P3) were administered dried petals that had been prepared into roselle tea at varying doses. The dried petals used for infusion in the roselle tea were 2.6 mg/g bw, 3.9 mg/g bw, and 5.2 mg/g bw for groups P1, P2, and P3, respectively. The doses administered to the mice were determined by converting the amounts intended for human doses. Each dose of dried roselle petals was brewed in the same volume of water (0.5 mL) for the same duration of time (5 minutes). The experiment used MSG from brand "X" that was purchased at a local market. Dried roselle petals from brand "XX" were acquired from a supermarket in Denpasar, Indonesia (8°41'04.1"S 115°12'56.7"E). These dried petals were processed to produce roselle tea for the treatment. The roselle plants were locally grown in Kediri, Indonesia (7°49'55.2"S 111°51'24.3"E).

Before administering the treatment, the mice underwent a seven-day acclimatization. They were housed in a plastic tub cage measuring 40x30x18 cm, with a top cover made of woven wire and a base paddded with rice husks. During acclimatization, all groups were provided standard feed (i.e., chicken pellets and ad libitum water). The room was maintained at a temperature of 25ºC with adequate air ventilation (Nugroho 2018). Following a seven-day period of acclimatization, the treatment started and continued for 30 days. The treatment was administered using the gavage technique, wherein the substance was orally fed through a feeding tube.

The administration of the treatment occurred following a fasting period of 14–18 hours. During the first week of the experiment, following a seven-day period of acclimatization, the mice in Groups P1, P2, and P3 were only given MSG. Starting from day 8, the mice in the treatment groups were administered roselle tea one hour after receiving a dose of MSG. On day 31, all the mice were anesthetized using ketamine and sacrificed by dislocating their necks. Afterwards, the stomach, duodenum, and liver organs were collected. The organs were washed in a 0.9% sodium chloride (NaCl) solution and fixed using a 10% neutral buffered formalin (NBF) solution (Maulida et al. 2013). The preparatory work was conducted at Denpasar Veterinary Center, Indonesia. This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Udayana, Denpasar, Indonesia (No. B/60/UN14.2.9/PT.01.04/2023 on 28/03/2023).

By referring to the study by Yogini et al. (2021), the histological preparations of stomach, duodenum, and liver organs were observed at 100X and 400X magnifications using OptiLab Iris-2 Binocular Biological Microscope (Miconos, Indonesia) and OptiLab Advance V2 Viewer Software for Windows, version 2.21 (Miconos, Sleman, DIY, Indonesia). The paraffin method and hematoxylin-eosin (HE) staining were used in the histopathological examination. The number of damaged cells was counted from five fields of view using the Image Raster, a built-in application from OptiLab Advance V2 Viewer Software for Windows, version 2.21 (Miconos, Sleman, DIY, Indonesia). Parameters observed in the stomach and duodenum were necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. In the hepatic organs, the parameters observed were necrosis, inflammatory cell infiltration, and cell degeneration. The data obtained were statistically analyzed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). The normal distribution and homogeneity of the data were examined using the Kolmogorov-Smirnov normality test and one-way analysis of variance (ANOVA). It was followed by Duncan's post-hoc test if there were a normal distribution and significant differences ($p<0.05$). If the data distribution was not normal, the Kruskal-Wallis test was used. If the results were found to be significantly different ($p<0.05$), the analysis proceeded with the Mann-Whitney test (Masnunah et al. 2020).

RESULTS

Histopathological examination of the stomach organs

After administering MSG at a dose of 4 mg/g bw per day, histological examination of the stomach sections showed the presence of both normal and damaged cells, characterized by necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. The normality of the data on the average number of damaged cells in the stomach was assessed using the Kolmogorov-Smirnov test. The stomach histology normality test yielded a normal distribution of the data ($p>0.05$). The normally distributed data were then subjected to a homogeneity test and a one-way ANOVA. The test results indicated statistically significant differences ($p<0.05$) among the treatment groups and control groups. Afterwards, the Duncan test was performed (Table 1).

The Duncan's post-hoc test results showed a significant difference ($p<0.05$) between the control and treatment groups. K- exhibited significant differences in comparison with K+. It was shown that K+ had the highest average count of damaged cells. This indicated that oral feeding of MSG at a dose of 4 mg/g bw per day resulted in stomach mucosal damage in the mice, as observed through histological examination. The damage was characterized by necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation.

The analysis of the stomach histological damage data showed statistically significant differences ($p<0.005$) between the positive control group (K+) and all the treatment groups (P1, P2, and P3). Statistically significant differences ($p<0.05$) were found in the levels of necrosis and inflammatory cell infiltration when comparing P1 to both P2 and P3.

However, there was no statistically significant difference between P2 and P3 (p>0.05).

Table 1. Results of the histopathological examination of the stomach after the administration of MSG and roselle tea.

Variables	Groups				
	K-	K+	P1	P2	P3
Necrosis (cells)	1.96±1.29^{a}	24.93±1.62^{d}	11.68±2.76^{c}	6.91±1.33^{b}	6.82±2.85^{b}
Inflammatory cell infiltration (%)	1.23±0.19^{a}	24.62±1.28^{d}	12.41±2.43^{c}	6.45±0.92^{b}	5.76±1.04^{b}
Villous erosion (%)	7.20±5.74^{a}	33.60±7.07^{c}	18.24±3.32^{b}	13.28±2.81^{ab}	12±3.62^{ab}
Ephitelial desquamation (%)	7.68±5.02^{ab}	18.56±3.98^{c}	14.56±3.98^{bc}	9.76±6.51^{ab}	6.24±6.80^{a}

Notes: The different superscripts behind the numbers in the same row indicate significant differences among the groups (p<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

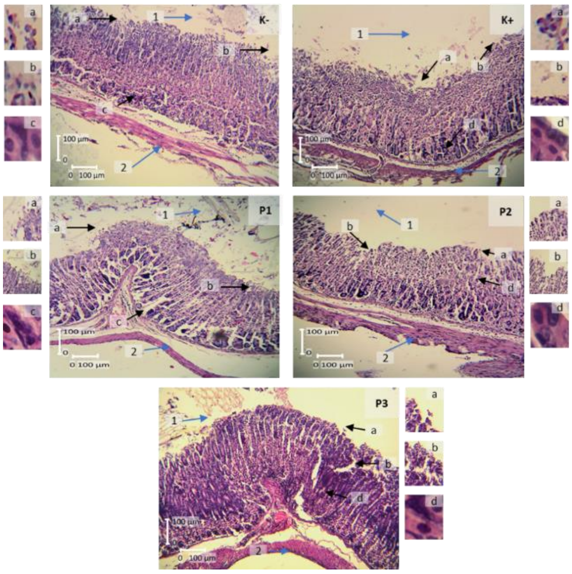

Figure 1. Stomach histological observation by HE staining (100X magnification).
Notes: a) Necrosis, b) inflammatory cell infiltration, c) villous erosion, d) epithelium desquamation; 1) lumen, 2) membrane basal. K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

There was no significant difference in the decrease of villous erosion among P1, P2, and P3 (p>0.05). The decrease in epithelial desquamation damage was significantly different between P1 and P3 (p<0.05), however, there was no significant difference between P2 and either P1 or P3 (p>0.05). These results demonstrated that the administration of roselle tea in varying doses effectively mitigated stomach histological damage in mice. The histological damage to the stomach in each group is shown in Figure 1.

Histopathological examination of the duodenal organs

The histological examination of the duodenal sections showed a simultaneous presence of normal and damaged cells, characterized by necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. This finding resembled the examination of the stomach organs following the ingestion of MSG at a dose of 4 mg/g bw per day. The data obtained from calculating the average number of damaged cells in the duodenum were examined for the normal distribution using the Kolmogorov-Smirnov test. The normality test revealed that the data on the histological duodenal sections had a normal distribution (p>0.05). The normally distributed data were evaluated using a homogeneity test and then proceeded with a one-way ANOVA. The test results yielded a statistically significant value of p<0.05 when comparing the treatment and control groups. Subsequently, the analysis continued with the Duncan test.

Table 2. Histopathological findings from the duodenum following the administration of MSG and roselle tea.

Variables	Groups				
	K-	K+	P1	P2	P3
Necrosis (cells)	0.99±0.41^{a}	6.42±1.02^{c}	3.80±0.90^{b}	3.35±1.02^{b}	1.37±0.76^{a}
Inflammatory cell infiltration (%)	0.63±0.39^{a}	4.21±1.56^{d}	2.47±0.60^{c}	1.95±0.76^{bc}	1.23±0.32^{ab}
Villous erosion (%)	6.08±6.76^{a}	36.00±7.24^{c}	18.72±4.85^{b}	14.72±3.74^{b}	5.12±5.20^{a}
Epithelial desquamation (%)	9.92±8.05^{ab}	29.28±5.95^{c}	13.92±2.48^{b}	7.36±6.36^{ab}	4.96±4.75^{a}

Notes: The different superscripts behind the numbers in the same row indicate significant differences among the groups (p<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

As shown in Table 2, the results of the Duncan's post-hoc test showed a significant difference (p<0.05) between the control and treatment groups. There was a significant difference between K- and K+. Regarding the level of organ damage, K+ exhibited the most severity compared to all the other groups. This indicated that orally administering MSG at a dose of 4 mg/g bw per day led to histological damage to the duodenal mucosa. Necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation were among the observed damages.

According to the analysis of the duodenal histological damage data, there were significant differences (p<0.05) between K+ and all the treatment groups (P1, P2, and P3), as well as between P1 and P3. However, there was no significant difference between P1 and P2 (p>0.05). The comparison between P2 and P3 revealed statistically significant differences (p<0.05) in relation to necrosis and villous erosion, while no significant differences were found in terms of epithelial desquamation and inflammatory cell infiltration (p>0.05). Overall, these results demonstrated the effectivity of roselle tea in graded doses in reducing histological damage in the duodenum of mice. Figure 2 displays the histological damage observed in the duodenum, including necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation.

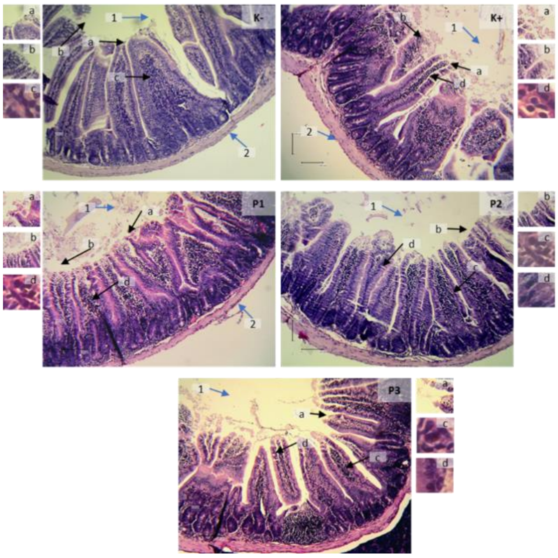

Figure 2. Histological observation of the duodenum using HE staining (100X magnification).
Notes: a) Necrosis, b) inflammatory cell infiltration, c) villous erosion, d) epithelium desquamation; 1) lumen, 2) membrane basal. K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

Histopathological examination of the liver organs

The histological observation results revealed that both normal and damaged cells were present in the liver sections. The mice exhibited necrosis, inflammatory cell infiltration, and cell degeneration after receiving MSG at a dose of 4 mg/g bw per day. The data on the average count of damaged hepatic cells were analyzed using the Kolmogorov-Smirnov test. The results showed that the data had a normal distribution (p>0.05). The normally distributed data were then subjected to a homogeneity test and a one-way ANOVA. The result obtained was p<0.05, indicating a need for further analysis using the Duncan test (Table 3).

Table 3. Results of the liver histopathological examination following the administration of MSG and roselle tea.

Variables	Groups				
	K-	K+	P1	P2	P3
Necrosis (cells)	1.64±0.89^{a}	15.92±3.78^{d}	10.10±1.27^{c}	6.86±2.24^{b}	5.47±1.58^{b}
Inflammatory cell infiltration (%)	2.61±1.97^{a}	11.81±1.86^{d}	7.98±3.12^{c}	6.15±1.57^{bc}	4.73±1.90^{ab}
Villous erosion (%)	2.93±1.49^{a}	9.80±1.87^{d}	7.76±2.23^{cd}	6.78±2.23^{bc}	4.75±2.03^{ab}
Epithelial desquamation (%)	1.21±0.81^{a}	7.54±2.47^{d}	5.43±1.54^{cd}	3.82±2.71^{bc}	2.18±0.85^{ab}

Notes: The different superscripts behind the numbers in the same row indicate significant differences among the groups (p<0.05). K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

The Duncan's post-hoc test results showed a significant difference (p<0.05) between the control and treatment groups. In addition, a significant difference was found between K- and K+. Similar to the results of the stomach and duodenal observations, K+ was found to suffer the highest damage across all groups. The oral administration of MSG at a dose of 4 mg/g bw per day caused histological damage to the hepatic tissue, as evidenced by the presence of necrosis, inflammatory cell infiltration, parenchymal degeneration, hydropic degeneration, and fatty degeneration.

The examination of the liver sections revealed significant differences (p<0.05) in the necrosis cell count between K+ and all the treatment groups (P1, P2, and P3), as well as between P1 and the other treatment groups (P2 and P3). Significant differences in inflammatory cell infiltration were observed between K+ and both P1 and P2, as well as between P1 and P3 (p<0.05). However, there was no significant difference between P2 and P3 (p>0.05). There were no significant differences observed in terms of parenchymal degeneration and hydropic degeneration between K+ and P1 (p>0.05). Additionally, there were no significant differences in hydropic degeneration and fatty degeneration between P1 and P2, as well as between P2 and P3 (p>0.05). Although there were few noticeable differences, the administration of roselle tea demonstrated its capacity to restore damage to the hepatocytes of the mice. The liver exhibited histological impairments such as necrosis, inflammatory cell infiltration, parenchymal degeneration, hydropic degeneration, and fatty degeneration, which can be seen in Figure 3.

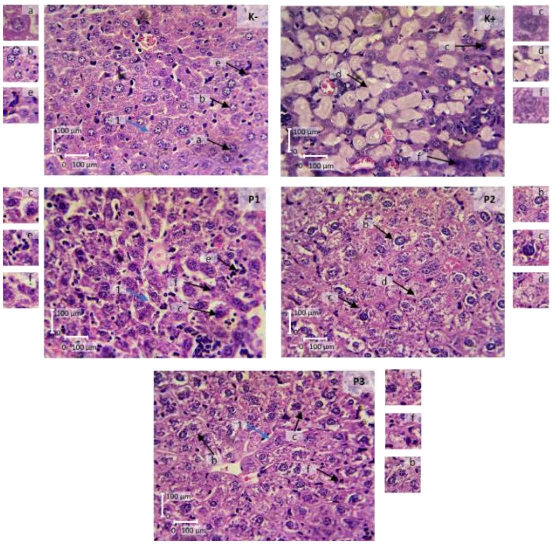

Figure 3. Liver histological observation by HE staining (400X magnification)
Description: a) Normal cells, b) hydropic degeneration, c) parenchymal degeneration, d) fatty degeneration, e) necrosis, f) inflammatory cell infiltration; 1) sinusoid. K- (negative control), K+ (positive control), P1 (treatment 1), P2 (treatment 2), P3 (treatment 3).

DISCUSSION

Histopathology of the stomach organs

The findings of this study demonstrated that the negative control group (K-), which solely received distilled water, exhibited the lowest average level of stomach mucosal damage. Although this group exhibited necrosis at a low level, it was deemed normal, as necrosis is a form of pathological cell death. The presence of inflammatory cell infiltration in this group indicated a cellular regeneration process. In addition, the presence of epithelial cell desquamation and villous erosion might be a stomach mucosal response due to a decrease in cytoprotective mucus secretion. The decrease in cytoprotection could be attributed to stressors arising from environmental conditions, such as adaptation sites, as well as the responses received by the experimental animals due to mechanical trauma to the stomach during daily administration of treatment via a feeding tube (Jahra et al. 2019).

The observation additionally demonstrated that the positive control group (K+) exhibited the highest average level of damage in the stomach mucosa following the administration of MSG at a dose of 4 mg/g bw per day. This damage was caused by high levels of glutamic acid in the body, which is derived from MSG. Increased levels of glutamic acid can produce free radicals in the body. Furthermore, high levels of glutamate affect the trycarbocylic acid (TCA) system, leading to an augmentation in alpha-ketoglutarate dehydrogenase activity. It can eventually stimulate the production of reactive oxygen species (ROS), hence amplifying oxidative stress (Jubaidi et al. 2019). Elevated concentrations of free radicals within the body will impair the weak polyunsaturated acids present in the cell membrane. This condition will cause the cell membrane to be fragile as well as allow the free radicals to enter the cytoplasm and damage the cell nucleus. Free radicals can change physiological functions in the body and trigger the inflammation reaction (Azab et al. 2019). Meanwhile, the influx of extracellular fluids into the cell can result in degeneration. If the cell is exposed to toxic substances for a long time, cell death or necrosis will occur. This is consistent with a previous study that demonstrated the potential harm of MSG consumption to the stomach organ (Yogini et al. 2021).

The findings from the treatment groups (P1, P2, and P3) suggest that roselle tea exerted a significant effect on the restoration of many forms of damage, including necrosis and inflammatory cell infiltration. However, roselle tea failed to show its effectiveness in repairing villous erosion and epithelial desquamation. Epithelial desquamation and villous erosion that occurred in the treatment groups resulted from the continuous administration of MSG. These impairments occurred simultaneously with the increase in free radicals, which can accelerate the inflammatory process and cause cell damage induced by oxidative stress. The heightened oxidative stress affects hydrochloric acid (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 inflammatory mediators such as histamine and leukotriene (Riong 2022).

The administration of roselle tea at a dose of 2.5 mg/g bw resulted in minimal improvement in stomach mucosal damage in treatment group 1 (P1). A roselle tea dose of 3.9 mg/g bw produced a notable improvement in stomach mucosal damage in P2 compared to P1. However, with a roselle tea dose of 5.2 mg/g bw, P3 had less significant differences in terms of improvement in stomach mucosal damage compared to P2. This suggests that the moderate dose was quite effective in repairing stomach mucosal damage. Yet, when the higher dose was administered, it resulted in even greater restoration of the damage to the gastric mucosa, as seen by a decrease in the average level of damage. Furthermore, it also resulted in the lowest average level of epithelial desquamation. This might be due to the presence of antioxidant compounds in roselle tea, which can restore stomach cells (Amer et al. 2022). In this study, P3 exhibited the most minimal average level of stomach mucosal cell damage caused by MSG administration, with results comparable to those of the negative control group. Therefore, the dose of 5.2 mg/g bw was considered the optimal dose of roselle tea with the capability to mitigate stomach mucosal damage.

Roselle tea offers a beneficial effect on reducing necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation due to its flavonoid content, which can counteract free radicals. Flavonoid is an antioxidant compound that protects the body by releasing one of its electrons to inhibit free radical activity (Nursheha & Febrianti 2015). The flavonoids contained in roselle petals are able to enhance the production of stomach mucus fluid. This fluid serves as a protective barrier for the stomach mucosa, shielding it from the harmful effects of acid, pepsin, and other substances such as MSG. This function is achieved by reducing histamine secretion from mast cells through the inhibition of the enzyme histidic decarboxylase. Roselle contains other antioxidants, such as saponins, which act as gastroprotective agents by activating the protection system of the stomach mucous membrane. A study conducted previously demonstrated that the thistle leaf extract also contains flavonoid, saponin, and tannin compounds, which have the ability to alleviate stomach histological damage in aspirin-induced rats (Riong 2022).

Histopathology of the duodenal organs

The histopathological examination of the duodenal organs showed that the negative control group (K-) had the lowest average level of mucosal damage. Necrosis damage in this group was within normal levels, as low levels of necrosis might indicate pathological cell damage. Inflammatory cell infiltration in this group might also indicate the process of duodenal cell regeneration. The presence of epithelial cell desquamation and villous erosion was seen as duodenal mucosal responses due to decreased mucus secretion as a cytoprotective mechanism (Jahra et al. 2019). On the other hand, the positive control group (K+) had the highest average level of damage following the administration of MSG.

The results of this study align with those of a study by Vincent et al. (2015), wherein MSG was found to induce damage to the duodenal mucosa. The mechanism of duodenal mucosal damage begins with epithelial desquamation, which refers to the detachment of epithelial cells from the tissue surface. In the duodenum, this process serves as a defense mechanism in response to irritants, aiming to prevent additional harm (Alfina et al. 2022). The elevated concentrations of glutamic acid in the body, which are generated by MSG, are responsible for this damage. The mechanism of damage caused by MSG can be attributed to the presence of free glutamate, which stimulates stomach acid secretion. Meanwhile, villous erosion is an advanced form of damage when the duodenum experiences partial loss of epithelium in its mucosal layer (Sulastri et al., 2018). It occurs because Brunner's glands do not produce alkaline mucus that can protect the duodenal wall against gastric acid secretion (Putra et al. 2021).

The results from all the treatment groups (P1, P2, and P3) demonstrated that roselle tea effectively decreased necrosis, inflammatory cell infiltration, villous erosion, and epithelial desquamation. The continued administration of MSG in these groups led to damage characterized by epithelium desquamation and villous erosion, which in turn caused an increase in free radicals. Oxidative stress occurs when highly reactive free radical molecules continuously generate free radicals, leading to cellular damage. Stress in the digestive tract can reduce mucosal blood flow, which can disrupt the integrity of the mucosal barrier by inhibiting Brunner's glands (Putra et al. 2021). The duodenal mucosa can be damaged by prolonged and excessive release of gastric acid, leading to an inflammatory process that can harm the structure of the duodenal mucosa.

The administration of roselle tea at doses of 2.5 mg/g bw and 3.9 mg/g bw had a positive effect on improving duodenal mucosal conditions. However, the administration of roselle tea at a dose of 5.2 mg/g bw provided the most significant improvement in the damage caused by MSG, with results comparable to those of the negative control group. The administration of roselle tea at a higher dose resulted in the most minimal damage to the duodenal mucosal cells. Therefore, a roselle tea dose of 5.2 mg/g bw was determined to be the optimal dose that has the potential to reduce duodenal mucosal damage. On a side note, it is noteworthy that the duodenal mucosal damage was less severe in comparison to the damage observed in the stomach. MSG is primarily processed in the stomach with the help of gastric digestive sap, making the stomach exposed to a significant quantity of toxic substances. The toxic substances are then transferred and absorbed by the duodenum in smaller amounts (Vincent et al. 2014).

Roselle tea is rich in natural antioxidants, including phenolic compounds such as anthocyanins, gossypetin, vitamin C, vitamin B, and vitamin D. Additionally, it has polyphenol and flavonoid compounds, which act as antioxidants by binding to free radicals, therefore protecting cells and preventing lipid peroxidation. Hardiningtyas et al. (2014) suggest that flavonoid antioxidants function by absorbing and inhibiting the regeneration of ROS while also indirectly enhancing the activity of cellular antioxidant enzymes. Roselle possesses anthocyanins and polyphenols that enhance the mucosal barrier of the digestive tract by proliferating beneficial digestive microflora bacteria, such as Lactobacillus spp. and Bacillus spp. (Amer et al. 2022).

Histopathology of the liver organs

The histopathological observation of the hepatic organs revealed that the negative control group (K-) exhibited a mixture of normal and damaged cells. Normal hepatocytes typically have polyhedral, round, or oval shapes with hepatocyte plates. Other characteristics of normal hepatocytes are bright red cytoplasm, white sinusoids, and an intact appearance (Anggraeny 2014). In this study, the negative control group displayed necrosis, inflammatory cell infiltration, and degeneration, but to a lesser extent than the positive control group and the treatment groups. The observed organ damage might be attributed to the pathological process, when all cells in the body undergo cell death as a result of toxic substances or certain factors, followed by a regenerative phase that produces new cells.

The positive control group (K+) had the highest average level of hepatocyte damage. The statistical analysis revealed a significant prevalence of cell degeneration and necrosis. The hepatocyte damage resulted from the repeated administration of MSG, which heightened the amount of radical chemicals generated by the secondary metabolism of glutamic acid, leading to the production of hydrogen peroxide. Hydrogen peroxide can react with chemicals present in the body and form reactive hydroxyl radicals. The formation of hydroxyl radicals induces the production of lipid peroxides, which in turn disrupt the integrity of the cell membrane. Consequently, the cell structure becomes abnormal and impaired (Ayala et al. 2014).

Hepatic tissue damage due to continuous administration of MSG begins with the process of cell degeneration. Excessive MSG consumption can lead to the influx of a significant volume of extracellular fluid into the cytosol, causing hepatocytes to swell (Maulida et al., 2013). Prolonged MSG consumption can also accumulate the substance in the hepatic organ, which is responsible for filtering toxic substances that enter the body. This accumulation can harm hepatocytes due to the effects of free radicals caused by MSG (Anggraeny 2014). Once the cell membrane is damaged, the effects of the toxic substance can extend to the nucleus, resulting in structural abnormalities and the onset of necrosis. Cell death, also known as necrosis, is the result of persistent degeneration that harms the cell membrane system, causing the cell to lyse and perish (Wijaya et al., 2014). Free radicals can lead to cell membrane damage, which in turn triggers an inflammatory response characterized by inflammatory cell infiltration. The liver inflammation observed in this study was an immune response triggered by the toxic properties of MSG. However, hepatocytes could regenerate rapidly because the damage caused by the toxic substance was reversed with the administration of antioxidants that possess hepatoprotective agents, such as flavonoids, polyphenols, saponins, and alkaloids (Gebremedhin et al. 2020).

The administration of roselle tea at a dose of 2.5 mg/g bw was unable to repair hepatocyte damage. This was indicated by the damage levels of parenchymal degeneration and hydropic degeneration in treatment group 1 (P1) that were found to be similar to those of the positive control group (K+). Conversely, the administration of roselle tea at a dose of 3.9 mg/g bw resulted in an improvement in hepatocyte damage. However, there was no significant difference observed compared to the administration of the lowest dose in terms of parenchymal degeneration, hydropic degeneration, and inflammatory cell infiltration. Overall, the administration of roselle tea at a dose of 5.2 mg/g bw generated an improvement in hepatocyte damage, which was not significantly different compared to the moderate dose but significantly different than the lowest dose. This dose also resulted in the lowest average level of damage, such as fatty degeneration, because it contains antioxidants that can restore hepatocytes (Amer et al. 2022). Additionally, the results of this dose administration were similar to those of the negative control group. Hence, it was concluded that a roselle tea dose of 5.2 mg/g bw was the most effective in mitigating liver deterioration and hepatocyte damage.

Water-soluble extracts of roselle petals contain protocatechuic acid and anthocyanins, which can protect the liver from damage. A study conducted by (Adeyemi et al. 2014) has provided support for this concept. The study demonstrated that the administration of roselle extract effectively improved the liver fibrosis in diabetic rats induced by streptozotocin. The observed effectivity of roselle extract might be partly related to its antioxidant properties, including protocatechuic acid, anthocyanins, and flavonoids, which help prevent peroxidative liver damage. In a study conducted by (Zuraida et al. 2015), it was found that giving rats higher doses of roselle extract was more effective in enhancing the antioxidant activity of ascorbic acid compared to the use of lower doses. This increased antioxidant activity was beneficial for inhibiting lipid peroxidation caused by free radicals, leading to a decrease in malondialdehyde (MDA) levels and liver necrosis in rats exposed to carbon tetrachloride (CCL4).

Strength and limitations

The administration of roselle tea (*Hibiscus sabdariffa* Linn.) at graded doses can effectively restore histological damage in the stomach, duodenum, and liver of mice (*Mus musculus*) induced by monosodium glutamate (MSG). A high dose provides the most optimal outcomes in terms of ameliorating digestive organ damage. Further comprehensive study is required to investigate the potential effect of administering roselle tea on the histological organ damage associated with the administration of substances other than MSG. The evaluation of roselle tea as an herbal remedy shall be considered.

CONCLUSION

The administration of roselle tea (*Hibiscus sabdariffa* Linn.) at graded doses effectively restored histological damage in the stomach, duodenum, and liver of mice (*Mus musculus*) induced by monosodium glutamate (MSG). A high dose provided the most optimal outcomes in terms of ameliorating digestive organ damage. A further comprehensive study is required to investigate the potential effect of administering roselle tea on the histological organ damage associated with the administration of substances other than MSG. The evaluation of roselle tea as an herbal remedy shall be considered.

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Conflict of interest

None.

Ethical consideration

This study received ethical clearance from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Udayana, Denpasar, Indonesia, with registration 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, drafted the article, provided the study materials, statistical expertise, and administrative support, and collected and assembled the data. NIW conceptualized the study, developed the methodology, critically revised the article for important intellectual content, provided statistical expertise, and provided technical and logistic support. AASAS provided validation for the study and provided statistical expertise. All authors have read and approved of the final version of the article for publication.

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