The Effect of Manilkara Zapota L on The Histopathological Gastric Induced by Absolute Ethanol

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KEYWORDS

Manilkara zapota, gastric ulcer, ethanol-induced injury, gastroprotection, inflammatory response

ABSTRACT:

Background: Gastric ulcers are a significant health issue caused by various factors, including excessive alcohol consumption. Manilkara zapota (M. zapota) has been reported to exhibit anti-inflammatory and antioxidant properties, which may contribute to its gastroprotective effects.

Objective: This study aimed to evaluate the differences in the number of ulcers, ulcer depth, lesion area, and inflammatory cell density in the stomachs of Wistar rats induced by absolute ethanol and treated with M.

Methods: This experimental study used a post-test-only control group design with Wistar rats as subjects.

Results: Gastric ulcer lesions were observed with varying depths and inflammatory cell densities. The Kruskal-Wallis test showed a significant difference in the median number of ulcer lesions among the treatment groups ($p = 0.0182$). Post-hoc analysis confirmed significant variations between groups.

Conclusion: M. zapota fruit extract demonstrated protective effects against ethanol-induced gastric injury, reducing ulcer number, depth, lesion area, and inflammatory cell density. Its gastroprotective efficacy was comparable to C. burmanni, indicating its potential as a natural therapeutic agent for gastric protection. Further research is needed to elucidate the underlying mechanisms and clinical applications.

1. Introduction

Gastric hyperacidity and gastroduodenal ulcers are a problem serious global today. The disease is found in many people around the world and is often caused by excessive alcohol consumption, prolonged use of nonsteroidal anti-inflammatory drugs.The occurrence of gastric ulcers is found when damage is found to the mucosal layer to the mucosal muscular that is round or oval in shape with a diameter of 0.3 cm – 0.6 cm, due to the discontinuity of the integrity of the gastric mucosa. However, in mice, the diameter of the lesion was found to be smaller, about 0.2 cm [1]. Likewise, preliminary research has been carried out on ulcer models that are seen under a microscope of about 190 µm. Ethanol not only directly damages the cells of the gastric mucosa but also stimulates the gastric mucosa to produce stomach acid excessively. In addition, mast cells in the gastric tissue will release large amounts of histamine which in turn will stimulate histamine receptors in parietal cells to produce acid excessively [2]. Alcohol also induces inflammation while reducing antioxidant activity and protective mucus. The imbalance of aggressive and defensive factors is the basis for gastric damage as shown in Figure 1. Inflammatory mediators and Reactive Oxygen Species (ROS), is an important factor in the pathogenesis of acute gastric mucosal lesions induced by ethanol [3]. The accumulation of ROS induces oxidative stress and causes inflammation of the stomach, gastric ulcers and perforations. This

will trigger the expression of pro-inflammatory cytokines regulated by Nuclear Factor Kappa B (NF-κB) and Protein activator 1 (AP-1). Interleukin (IL)-1b, IL-6 and Tumor Necrosis Factor Alpha (TNFα) are the two main cytokines involved in the inflammatory response [4]. The formation of ROS during the inflammatory process, whereas ROS not only directly damages the cell structure but also increases the production of pro-inflammatory factors. During the progression of gastric ulcers, pro-inflammatory cytokines and ROS synergize with each other. Therefore, anti-oxidants and anti-inflammatory play an important role in protecting the gastric mucosa of the mucosa against injury [5].

Indonesian people are rich in natural ingredients and traditional medicines that have been used by most people for generations. Before chemical drugs developed in modern times, our ancestors generally used medicines derived from fruits or plants to overcome their health problems because they were believed to have advantages, namely having small side effects compared to treatment, one of the fruits that has the potential to be used as a medicine is savoy manila or M. Zapota [6]. Investigations of plant extracts that have active pharmacological content, are safe and have gastroprotective properties continue to be developed, especially, plants that have antioxidant content as the main component for the treatment of gastric ulcer disease [7]. Cinnamomum burmanni, known as cinnamon in Indonesia, is beneficial in the treatment of gastric ulcers. Its bioactive compounds such as cynamaldehid, saponins, and flavonoids as well as essential oils rich in saphrols and citrals. The compound has antimicrobial, antioxidant, and anti-inflammatory properties that help protect the gastric mucosa from irritants such as ethanol and hydrochloric acid. C [8]. burmanni works by reducing oxidative stress and inflammation and regulating levels of nitrogen monoxide and malondialdehyde, which play a role in the pathogenesis of ulcers. In addition, bioactive fractions DLBS2411 proven to have a gastropprotective effect by lowering ulcer index and accelerating tissue recovery [9]. The results of preclinical and clinical studies show its effectiveness in reducing ulcer severity, improving the integrity of the gastric mucosa, as well as supporting its use as a potential natural therapy and candidate in ulcer therapy [10].

2. Objective

To evaluate the differences in the number of ulcers, ulcer depth, lesion area, and inflammatory cell density in the stomachs of Wistar rats induced by absolute ethanol and treated with Manilkara zapota fruit extract at doses of 100 mg/kg BW and 300 mg/kg BW, Na-CMC 0.5%, and Cinnamomum burmanni on days 1, 3, and 5. This study aims to assess the potential gastroprotective effects of M. zapota and its comparative efficacy against C. burmanni in mitigating ethanol-induced gastric damage.

3. Methods

This study is an experimental research Post Test only Control Group Design, using rats (Wistar) as the research subject. The research was conducted at the Animal Laboratory of the Faculty of Pharmacy of Hasanuddin University, the Laboratory of Anatomical Pathology of the Faculty of Medicine, Hasanuddin University Hospital, and the Laboratory of Anatomical Pathology of Ibnu Sina Hospital Makassar in February-April 2024. The population and research samples are animals classified as rats obtained from the Animal Laboratory of the Faculty of Pharmacy, Hasanuddin University, Makassar. For 2 days, the mice in the positive control group and the treatment group were induced with 80% ethanol 1 ml/200 grBB using a nasogastric tube. Before being inducted, it is fasted for 6 hours to accelerate the absorption of ethanol. The rats were fed and drunk ad libitum. This induction model has gone through the preliminary stage.

Three mice as a Healthy Group (KS) without euthanasia treatment by injecting ketamine + xyla at an excessive dose. Nine mice as a group that had been induced 80% ethanol 1ml/200grBB for the next two days were only given 0.5% Na-CMC solvent and then euthanized by injecting ketamine + xyla at an excess dose, as a negative control group (KK-), for days 1, 3 and 5 respectively. Nine mice as a group that had been induced with 80% ethanol 1ml/200grBB for the next two days were given cinnamon extract (C. Burmanii) at a dose of 250 mg/KgBB and then euthanized by injecting ketamine + xyla at an excess dose, as the Positive Control Group (KK+), for days 1, 3 and 5 respectively.

Eighteen rats as a group that had been induced with 80% ethanol 1ml/200grBB for the next two days were given M. zapota fruit extract at a dose of 100 mg/KgBB and a dose of 300 mg/KgBB and then euthanized by injecting ketamine + xyla at an excess dose, as a Treatment Control Group (KKP), for days 1, 3 and 5 respectively.

4. Results

The results of microscopic observations are presented in Figure 1 below which shows microscopic gastric ulcer lesions in the histopathology (H&E) column as well as the expression of TNF-α and TGF-β1.

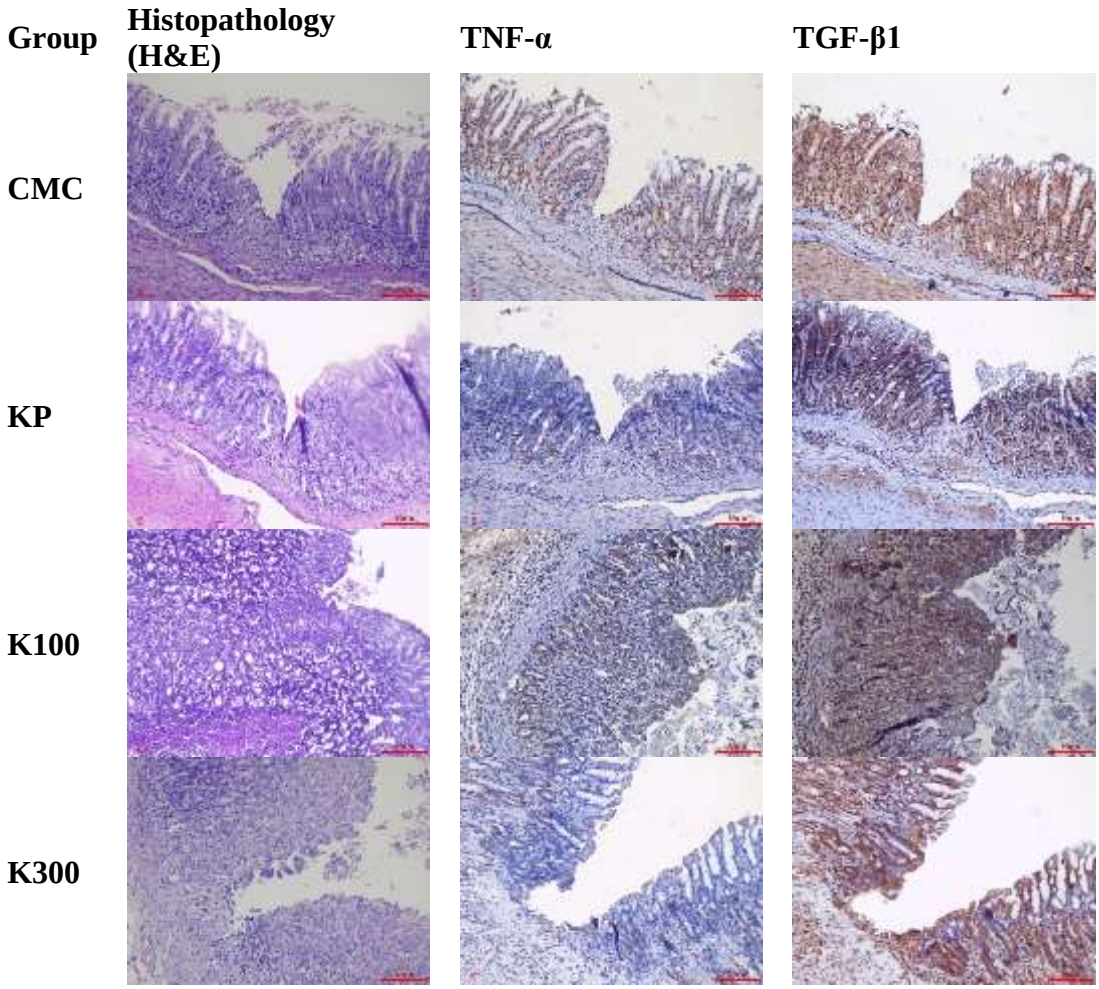

Figure 1. Histopathological picture and expression of TNF-α and TGF-β1 in each treatment and control group.

Figure 1. shows that gastric ulcer lesions appear with varying depths and densities of inflammatory cells. There was also the expression of TNF-α and TGF-β1 with weak to strong intensity. Abbreviations: (CMC: Carboxymethyl Cellulose, KP: Positive Control Cinnamomun burmanni, K100: Manilkara zapota 100mg/kgBB, k300: Manilkara zapota 300mg/kgBB). Olympus CX-45 Microscope, 20x Objective Magnification, Scalebar 50µm.

This study evaluated the number of ulcer lesions in four treatment groups (CMC(1), KP(1), K100(1), and K300(1)) using the Kruskal-Wallis test and Dunn's post-hoc multiple comparisons to determine significant differences between groups. The Kruskal-Wallis test showed a p-value of 0.0182 ($p < 0.05$), indicating a significant difference in the median number of ulcer lesions between the

treatment groups. The Kruskal-Wallis statistical value of 11.00 supports this result, indicating that there is significant variation in the data compared.

Table 1. Dunn's post-hoc test was used to determine which pairs of groups showed significant differences.

Dunn's multiple comparisons test	*Mean rank diff,*	*Significant?*	*Summary*	*P Value*
CMC(1) vs. KP(1)	6,000	Yes	*	0,0406
CMC(1) vs. K100(1)	6,000	Yes	*	0,0406
CMC(1) vs. K300(1)	6,000	Yes	*	0,0406
KP(1) vs. K100(1)	0,000	No	ns	>0,9999
KP(1) vs. K300(1)	0,000	No	ns	>0,9999
K100(1) vs. K300(1)	0,000	No	ns	>0,9999

Table 1. shows that the CMC(1) group had a significantly different number of ulcer lesions compared to the KP(1), K100(1), and K300(1) groups. However, there was no significant difference among the KP(1), K100(1), and K300(1) groups. This indicates that the treatment in the KP, K100, and K300 groups may have a protective effect on ulcer lesions compared to the CMC(1) group, which largely served as a negative control. These results provide evidence that the treatment in the KP(1), K100(1), and K300(1) groups is effective in reducing the number of ulcer lesions compared to the control. Further research is needed to identify the mechanisms underlying these treatment effects. Description and Data Analysis of the Number of Ulcer Lesions on Day 3. This study evaluated the number of ulcer lesions in four treatment groups (CMC(3), KP(3), K100(3), and K300(3)) using ANOVA and Holm-Sidak post-hoc tests to compare differences between groups. The following are the results of the analysis. The results of the ANOVA test showed that there was a significant difference between the average number of ulcer lesions in several groups, as indicated by the F value. With a p value <0.05, this indicates that at least one group is significantly different from the other groups. The R-squared value indicates how much variation in the data can be explained by the treatment.

This study evaluated the number of ulcer lesions in four treatment groups (CMC(3), KP(3), K100(3), and K300(3)) using the ANOVA and post-hoc Holm-Sidak tests to compare the differences between the groups. Here are the results of the analysis. The results of the ANOVA test showed that there was a significant difference between the mean number of ulcer lesions in some groups, as indicated by the F-value. With a p-< value of 0.05, this indicates that at least one group differed significantly from the other. The R-squared value indicates how much variation in the data can be explained by the treatment.

Table 2. The Holm-Sidak test is used to determine group pairs that show significant differences.

Multiple comparisons test	*Mean Diff,*	*Significant?*	*Summary*	*P Value*
CMC(3) vs. KP(3)	1,667	It	Ns	0,0650
CMC(3) vs. K100(3)	2,000	Yes	*	0,0312
CMC(3) vs. K300(3)	0,3333	It	Ns	0,7927
KP(3) vs. K100(3)	0,3333	It	Ns	0,7927
KP(3) vs. K300(3)	-1,333	It	Ns	0,1021
K100(3) vs. K300(3)	-1,667	It	Ns	0,0650

Table 2. present the results showed that the K100(3) group was significantly more effective in reducing the number of ulcer lesions compared to the CMC(3) group. However, there were no significant differences between other groups, such as KP (3), K300 (3), or other pairs compared. Treatment in the K100(3) group showed greater protective potential against ulcer lesions than the control group (CMC(3)). Further research is needed to explore the protection mechanism in this group.

This analysis evaluated the number of ulcer lesions on day 5 of four treatment groups, namely CMC(5), KP(5), K100(5), and K300(5). The statistical test used was Kruskal-Wallis to determine whether there were significant differences between groups, followed by Dunn's multiple comparisons test to see pairs of significantly different groups. The results of the analysis using the Kruskal-Wallis test showed a p value of 0.0406, which indicated a significant difference in the number of ulcer lesions between the treatment groups ($p < 0.05$). This analysis involved four groups tested, namely CMC(5), KP(5), K100(5), and K300(5). Although the Kruskal-Wallis statistical value is not specifically mentioned, the results of this test support a significant difference between groups based on a p-value smaller than 0.05.

Table 3. The Dunn test was carried out to identify pairs of groups that had significant differences

Multiple comparisons test	*Mean rank diff,*	*Significant?*	*Summary*	*P Value*
CMC(5) vs. KP(5)	6,000	Yes	*	0,0406
CMC(5) vs. K100(5)	6,000	Yes	*	0,0406
CMC(5) vs. K300(5)	6,000	Yes	*	0,0406
KP(5) vs. K100(5)	0,000	It	Ns	>0.9999
KP(5) vs. K300(5)	0,000	It	Ns	>0.9999
K100(5) vs. K300(5)	0,000	It	Ns	>0.9999

Table 3. present showed that the treatment group (KP(5), K100(5), and K300(5)) significantly reduced the number of ulcer lesions compared to the control group (CMC(5)) on day 5. However, there was no significant difference between the treatment groups (KP(5), K100(5), and K300(5). Treatment in the KP(5), K100(5), and K300(5) groups showed effectiveness in reducing the number of ulcer lesions compared to control (CMC(5)). This indicates that the treatment in all three groups has a similar protective effect on ulcer lesions. Further research can be conducted to identify the mechanism behind this effect.

The study evaluated ulcer depth (μm) on the first day after treatment, comparing four groups: CMC(1), KP(1), K100(1), and K300(1). Statistical analysis was carried out using ANOVA to see the differences between groups, with Tukey's multiple comparisons test to evaluate the differences between group pairs. The results of the ANOVA test showed that the F value was 10.27, which indicated that there was a considerable variation in the depth of the ulcer between the treatment groups. A p-value of 0.0041 ($p < 0.05$) indicates that the observed differences between the tested groups are statistically significant. In addition, an R^2 value of 0.7939 indicates that 79.39% of the variability in ulcer depth can be explained by the difference in treatment given to each group. This shows that the treatment factor contributes greatly to the difference in the depth of the ulcer observed.

Table 4. Tukey's further test was used to compare specific group pairs

Tukey's multiple comparisons test	*Mean Diff,*	*95,00% CI of diff,*	*Significant?*	*Summary*	*P Value*
CMC(1) vs. KP(1)	129,4	15,37 to 243,5	Yes	*	0,0274
CMC(1) vs. K100(1)	192,1	78.05 to 306.2	Yes	**	0,0029
CMC(1) vs. K300(1)	128,9	14.83 to 242.9	Yes	*	0,0280
KP(1) vs. K100(1)	62,68	-51,37 to 176,7	It	Ns	0,3566
KP(1) vs. K300(1)	-0,5400	-114,6 to 113,5	It	Ns	>0.9999
K100(1) vs. K300(1)	-63,22	-177,3 to 50,83	It	Ns	0,3501

Table 4. Show shows that the CMC(1) group has the highest ulcer depth compared to the other groups, with a value close to 300 μm. Meanwhile, the KP(1), K100(1), and K300(1) groups had lower ulcer depth, with an average value close to 100-150 μm. This indicates that the treatment group (KP, K100, and K300) was able to significantly reduce ulcer depth compared to the CMC control group(1). The results of the statistical test showed that the treatment in the KP(1), K100(1), and K300(1) groups was able to significantly reduce the depth of ulcers compared to the CMC(1) group. However, there was no significant difference between the KP(1), K100(1), and K300(1) groups, suggesting that these three treatments had similar protective effects in reducing ulcer depth. Further research can be conducted to evaluate the effectiveness of treatments over a longer period of time.

This study evaluated ulcer depth (μm) on day 3 after treatment, comparing four groups: CMC(3), KP(3), K100(3), and K300(3). Statistical analysis was carried out using ANOVA to see the differences between groups, with Tukey's multiple comparisons test to evaluate the differences between group pairs. The results of the ANOVA test showed that the F value was 7.154, which indicated that there was a considerable variation in the depth of ulcers between the treatment groups. A p-value of 0.0118 ($p < 0.05$) indicates that the observed differences between the groups are statistically significant. In addition, an R^2 value of 0.7285 indicates that 72.85% of the variability in ulcer depth can be explained by the difference in treatment given to each group. This shows that the treatment factor has a considerable contribution to the observed changes in ulcer depth.

Table 5. Tukey's further test was used to compare specific group pairs

Multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	P Value
CMC(3) vs. KP(3)	14,84	-71,54 to 101,2	It	Ns	0,9439
CMC(3) vs. K100(3)	-0,03000	-86,41 to 86,35	It	Ns	>0.9999
CMC(3) vs. K300(3)	-96,13	-182.5 to -9,752	Yes	*	0,0302
KP(3) vs. K100(3)	-14,87	-101.3 to 71.51	It	Ns	0,9436
KP(3) vs. K300(3)	-111,0	-197.4 to -24.59	Yes	*	0,0143
K100(3) vs. K300(3)	-96,10	-182.5 to -9,722	Yes	*	0,0302

Table 5. shows that the K300(3) group has the highest ulcer depth compared to the other groups, with values close to 250 μm. Meanwhile, the CMC(3), KP(3), and K100(3) groups had lower and relatively similar ulcer depths, ranging at 100 μm. Based on statistical tests, it was found that the depth of ulcers in the K300(3) group was significantly higher compared to CMC(3), KP(3), and K100(3). However, there were no significant differences between the CMC(3), KP(3), and K100(3) groups, suggesting that the treatment in the three groups had a similar effect in reducing the depth of the ulcer. The results of the statistical test showed that the K300(3) group had a significantly larger ulcer depth compared to the other groups, which may indicate that the treatment in this group was less effective in reducing ulcer depth compared to CMC(3), KP(3), and K100(3). Meanwhile, there was no significant difference between the CMC(3), KP(3), and K100(3) groups, suggesting that these three groups had a similar effect in reducing ulcer depth on day 3. Further research can be done to understand the factors that cause these differences.

This study analyzed the depth of ulcers (μm) on the 5th day after treatment, comparing four groups: CMC(5), KP(5), K100(5), and K300(5). Statistical analysis was carried out using ANOVA to determine whether there were significant differences between groups, as well as Tukey's multiple comparisons test to evaluate differences between group pairs. The results of the ANOVA test showed that the F value was 0.3032, which indicates that the variation in ulcer depth between groups is relatively small. A p-value of 0.8225 ($p > 0.05$) indicated that there was no significant difference

between the treatment groups, so the effect of the treatment on ulcer depth could not be statistically confirmed. In addition, an R^2 value of 0.1021 indicates that only 10.21% of the variability in ulcer depth can be explained by the difference in treatment, so the impact of treatment on ulcer depth variation is relatively low and may be influenced by other factors that have not yet been identified.

Table 6. Tukey's further test was carried out to compare pairs of groups specifically

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	P Value
CMC(5) vs. KP(5)	7,280	-151.6 to 166.2	It	Ns	0,9988
CMC(5) vs. K100(5)	32,98	-125.9 to 191.9	It	Ns	0,9075
CMC(5) vs. K300(5)	39,74	-119,2 to 198,6	It	Ns	0,8523
KP(5) vs. K100(5)	25,70	-133.2 to 184.6	It	Ns	0,9524
KP(5) vs. K300(5)	32,46	-126.4 to 191.4	It	Ns	0,9112
K100(5) vs. K300(5)	6,763	-152,1 to 165,7	It	Ns	0,9990

Table 6. Show the the graph shows that the mean depth of ulcers in the CMC(5), KP(5), K100(5), and K300(5) groups is relatively similar, with no significant differences between the groups. All groups had ulcer depth values ranging from 100-175 μm, with a fairly large standard deviation, indicating that there was a variation in data in each group. Based on the results of the statistical test, no significant difference in the depth of ulcers between the treatment groups was found on the 5th day. A high p-value indicates that the effect of treatment on ulcer depth is no longer clearly visible at the time of this observation. In addition, a low R^2 value indicates that the treatment provided does not have a strong influence on the variability of ulcer depth. Thus, these results indicate that on day 5, all groups showed a relatively similar ulcer condition, and it is likely that tissue repair or stabilization had occurred in the healing phase.

Inflammatory cell density analysis was carried out using the Kruskal-Wallis test to determine significant differences between four treatment groups, namely CMC(1), KP(1), K100(1), and K300(1). The results of the analysis showed a p-value of 0.1273, which was greater than 0.05, so no significant difference was found in the median density of inflammatory cells between the tested groups. The Kruskal-Wallis statistic of 5,418 supports the result that there are no significant differences between the groups. Post-hoc tests using Dunn's multiple comparisons test were also conducted to compare the density of inflammatory cells between groups in pairs.

Table 7. The results of the analysis showed that there was no significant difference in all comparisons between the groups

Dunn's multiple comparisons test	Mean rank diff,	Significant?	Summary	P Value
CMC(1) vs. KP(1)	-4,000	It	Ns	0,6304
CMC(1) vs. K100(1)	1,500	It	Ns	>0.9999
CMC(1) vs. K300(1)	-1,500	It	Ns	>0.9999
KP(1) vs. K100(1)	5,500	It	Ns	0,1551
KP(1) vs. K300(1)	2,500	It	Ns	>0.9999
K100(1) vs. K300(1)	-3,000	It	Ns	>0.9999

Table 7. Show the average density of inflammatory cells in each group is relatively similar, with slight variation. The KP(3) group was seen to have a higher average inflammatory cell density than the other groups, but this difference was not statistically significant. In addition, the sizable standard deviation across all groups shows a fairly wide variation in the data. Based on the results of the Kruskal-Wallis test and Dunn's multiple comparisons test, no significant difference in the density of inflammatory cells between the treatment groups was found on day 3. This shows that the treatment given has not shown a real effect on the density of inflammatory cells at this time of observation. Further evaluation the next day or with other methods of analysis may be necessary to identify more obvious change.

5. Discussion

Stomach ulcers remain a significant health problem, mainly caused by infections Helicobacter pylori, excessive use of NSAIDs, and damage to the gastric mucosa due to oxidative stress [10]. The inflammatory process plays an important role in the formation and healing of ulcers, by regulating cytokines such as tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) [11]. Manilkara zapota, known as savo, has attracted attention due to its diverse phytochemical composition, including flavonoids, polyphenols, alkaloids, and saponins. This bioactive compound has powerful antioxidant and anti-inflammatory properties, making Manilkara zapota potential candidates for the management of gastric ulcers [11],[12]. Recent studies have shown that this plant extract can modulate inflammatory cytokines, thereby promoting gastric mucosal healing and reducing ulcer severity [13].

The inflammatory response in gastric ulcers is largely mediated by proinflammatory cytokines, specifically TNF-α, which are known to induce apoptosis and interfere with the integrity of the gastric mucosa. Increased levels of TNF-α worsen gastric injury by stimulating inflammatory pathways such as nuclear factor-kappa B (NF-κB) and Mitogen-activated protein kinase (MAPK), which causes increased oxidative stress and tissue damage [14],[15, pp. 2005–2014]. In contrast, TGF-β plays an important role in tissue regeneration by modulating fibroblast activation and extracellular matrix deposition, which are essential in the wound healing process. The balance between TNF-α and TGF-β determines the degree of gastric damage and recovery [16]. Previous studies have shown that the extract Manilkara zapota can decrease TNF-α expression while increasing TGF-β activity, thereby accelerating mucosal repair and reducing inflammation [17],[18]. Growing evidence supports immunomodulatory effects Manilkara zapota, which shows its potential in controlling inflammatory and fibrotic diseases. The presence of flavonoids and polyphenols in this extract is very important [19], as these compounds have been shown to inhibit TNF-α signaling and enhance tissue repair mediated by TGF-β through modulation of NF-κB and Smad pathways [20],[21]. In addition, research shows that bioactive components Manilkara zapota has antimicrobial properties against H. pylori, which further strengthens its therapeutic relevance in the management of ulcers [22].

The findings of this study show that the extract Manilkara zapota at doses of K100 and K300 had a significant gastroprotective effect, which was indicated by a decrease in ulcer count, ulcer depth, and inflammatory cell density at various time points (Days 1, 3, and 5). Histopathological analysis showed a marked reduction in ulcer severity in the group treated with K100 and K300 compared to control (CMC) [23]. These results are in line with previous studies that highlighted the anti-inflammatory and antioxidative properties of flavonoids and tannins in M. zapota, which contributes to the healing of ulcers [24]. One of the main mechanisms behind this protective effect appears to be the modulation of cytokine levels, especially TNF-α and TGF-β. TNF-α is a pro-inflammatory cytokine that plays a role in gastric mucosal injury, while TGF-β has a dual role in tissue repair and fibrosis regulation [25]. Decreased TNF-α levels in the group treated with M. zapota shows an anti-inflammatory response that reduces the progression of gastric ulcers. In contrast, the observed regulation of TGF-β may indicate its involvement in accelerating mucosal healing while preventing excessive fibrosis, which is important for tissue regeneration [26].

In comparing the effects of K100 and K300, K300 doses showed a stronger impact in reducing ulcers, which suggests a dose-dependent effect [27]. Deeper ulcer healing observed in samples treated with K300 showed increased phytochemical effectiveness M. zapota at higher concentrations [28]. These results are consistent with previous studies that have shown that polyphenolic compounds in M. zapota has an anti-ulcer effect by reducing oxidative stress and mediating inflammation [29].

To further contextualize the therapeutic potential M. zapota, its effectiveness compared to Cinnamomum burmannii, a well-documented gastroprotective agent. Bioactive fraction DLBS2411 of C [30]. burmannii It has been reported to significantly reduce ulcer formation through modulation of inflammatory cytokines and improved mucosal defense mechanisms. This study shows that both M. zapota And C [31]. burmannii has a protective effect, but C. burmannii showed a slightly higher effectiveness in reducing the number of ulcers on Day 3. But M. zapota seems superior in reducing the depth of ulcers on Day 5, which indicates a stronger role in tissue regeneration in the late stages [32]. Histopathological analysis showed that the density of inflammatory cells in the group treated with M. zapota significantly lower compared to CMC controls but comparable to the group treated with DLBS2411 [33]. This supports the hypothesis that M. zapota works through an anti-inflammatory . The reduction in neutrophil infiltration and macrophage activity is in line with the finding that flavonoids in M. zapota modulates the immune response by inhibiting the NF-κB and MAPK signaling pathways [34].

6. Conclution

The findings of this study indicate that Manilkara zapota fruit extract has a protective effect against gastric damage induced by absolute ethanol in Wistar rats. Administration of M. zapota at doses of 100 mg/kg BW and 300 mg/kg BW significantly reduced the number of ulcers, ulcer depth, lesion area, and inflammatory cell density compared to the control group. The gastroprotective effects of M. zapota were comparable to those of Cinnamomum burmanni, suggesting its potential as a natural therapeutic agent for preventing ethanol-induced gastric injury. Further studies are needed to explore the underlying mechanisms and its possible clinical applications.

7. Acknowledgment

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8. Conflict of Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

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