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THE EFFECT OF COMBINATION OF TURMERIC EXTRACT (*Curcuma domestica* Val.) AND HONEY ON GASTRIC ULCERS IN MICE (*Mus musculus*) DUE TO KETOPROFEN ADMINISTRATION MICROSCOPICALLY

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Abstract

Gastric ulcers are one of the common side effects that occur as a result of using non-steroidal anti-inflammatory drugs (NSAIDS) such as ketoprofen. The combination of turmeric extract (*Curcuma domestica* Val) and honey has the potential as an alternative therapy due to its antioxidant, anti-inflammatory, and cytoprotective properties. This research aims to evaluate the effects of the combination of these two substances on gastric ulcers in mice induced by ketoprofen microscopically. To determine the effect of a combination of turmeric extract and honey on the histopathological profile of stomach ulcers in mice (*Mus musculus*) induced by ketoprofen. This study used an experimental method with a completely randomized design. Mice were divided into several groups, namely the negative control group (without treatment), positive control (ketoprofen without therapy), and the treatment group that was given a combination of turmeric extract and honey with varying doses. Gastric ulcers are induced by administration of ketoprofen, followed by microscopic observation of gastric mucosal damage. The data was analyzed statistically to see significant differences between groups. Histopathological observations show that the group given a combination of turmeric extract and honey experienced a significant reduction in the level of gastric mucosal damage compared to the positive control group. The combination is capable of reducing inflammation, necrosis, and accelerating tissue regeneration. The combination of turmeric extract and honey is effective in reducing gastric ulcers in mice induced by ketoprofen microscopically. This research supports the potential use of both natural substances as adjuvant therapy to prevent or address gastric damage caused by NSAIDS.

Keywords: *Curcuma domestica* Val*,* Histopathology, Honey, Mice (*Mus musculus*), Gastric ulcer.

INTRODUCTION

A gastric ulcer is a condition characterized by the presence of sores or ulcers on the stomach lining that can cause various symptoms such as stomach pain, nausea, and even bleeding. According to the World Health Organization (WHO), stomach ulcers are one of the biggest health problems in the world, with increasing prevalence (A. Sari et al., 2022). The main causes of stomach ulcers include infection with the Helicobacter pylori bacteria, the use of non-steroidal anti-inflammatory drugs (NSAIDS) such as

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ketoprofen, and lifestyle factors such as stress, an unhealthy diet, and alcohol consumption (Narayanan et al., 2021). Ketoprofen is one of the most commonly used non-steroidal anti-inflammatory drugs (NSAIDS), effective in relieving pain and inflammation, but long-term use of ketoprofen can damage the stomach lining (D. K. Sari & Utami, 2021). Therefore, along with the development of science in recent years, there is a need for alternative treatments based on natural ingredients, especially in the treatment of gastric ulcers.

Turmeric (*Curcuma domestica* Val) is one of the spices that has long been used in traditional medicine in Indonesia. Turmeric contains active ingredients, especially curcumin, which is known to have anti-inflammatory, antioxidant, and healing properties. According to research (Widiastuti & Rahmawati, 2022), it shows that the curcumin content in turmeric can help repair damage to the stomach lining and reduce inflammation, making it potentially an effective therapy for gastric ulcers. In addition to turmeric, honey is also known to have various natural substances that are good for health, including antimicrobial and healing properties. Honey can speed up the wound healing process, reduce inflammation, and have a positive effect on stomach health. Research (Halim & Setiawan, 2023) shows that honey has a positive effect in speeding up the healing of stomach wounds and reducing inflammation.

Considering the benefits of turmeric and honey that are very good for stomach health, the combination of turmeric and honey extract is expected to provide a synergistic effect in addressing gastric ulcers induced by ketoprofen. By using these two natural ingredients, it is hoped that there will be a safer and more effective treatment alternative than consuming conventional medicine. The combination of these two natural ingredients is expected to provide a synergistic effect to accelerate the healing of gastric ulcers and improve patient health. Recent research has shown the potential of turmeric and honey separately in healing gastric ulcers, but studies on the combined effects of both are still limited, especially in the context of ulcers induced by ketoprofen (Almasaudi et al., 2020).

Based on the background and previous research, this study is expected to contribute scientifically to the development of alternative treatments for stomach ulcers that are safer and more effective using natural ingredients that are already available in Indonesia. Therefore, the aim of this research is to find the microscopic picture of gastric ulcers in mice induced by ketoprofen administration and to analyze the effect of the combination of turmeric extract (*Curcuma domestica* Val) and honey on the healing of gastric ulcers in mice induced by ketoprofen microscopically.

RESEARCH METHODS

Time and Place of Research

This research will be conducted over a period of 3 months, starting from October to December 2024. The research location is the Microbiology Laboratory at Manado State University.

Tools and Material

The tools used in this research include a digital scale to measure materials accurately, an oral dosage measuring device such as a syringe or gavage for precise solution administration, as well as petri

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dishes as sample preparation containers. In the surgical procedure, surgical scissors, a sterile scalpel, tweezers, and a surgical tray are used to ensure aseptic conditions. Histopathological observations were made using a light microscope with preparations mounted on glass slides and cover glasses, while a dropper was used to add reagents or liquid samples. For protection during the experiment, sterile gloves and masks were used. The separation of active compounds was carried out with the help of a rotary evaporator, while a gastric probe was used for controlled ulcer induction.

The materials in this research used 25 male Swiss Webster mice aged 2-3 months as test animals. The main materials used included turmeric extract (*Curcuma domestica* Val.) with dose variations of 50-200 mg/kg body weight, pure honey (2 mL/kg body weight), and ketoprofen (0.3 mg/kg body weight) as an inducer of gastric ulcers. The solvent used in the extraction process was 70% ethanol and aquades. For histopathological preparation, graded alcohols (70%, 80%, 90%, and 100%), 0.9% NaCl solution, xylene, and hematoxylin and eosin (H&E) staining were used for microscopic analysis of gastric tissue.

Research Procedure

This research is a type of experimental laboratory study with a completely randomized design to test the effects of a combination of turmeric extract and honey on ketoprofen-induced gastric ulcers in mice microscopically. The following are the stages of the research conducted. Preparation of Test Animals, Preparing 25 male Swiss Webster strain mice (*Mus musculus*) aged 2-3 months to be used as test animals. The mice underwent a 7-day acclimatization period in individual cages with controlled room temperature conditions, specifically 22-25°C.

Preparation of Test Materials, turmeric extract (*Curcuma domestica* Val.) is prepared using the maceration method, starting with washing fresh turmeric rhizomes (*Curcuma domestica* Val.). Induction of Gastric Ulcer, Ketoprofen was administered intragastrically (orally) at a dose of 0.3 mg/kg body weight to all groups of mice, except for the positive control group which did not receive treatment**.** The administration of a combination of turmeric extract and honey, the treatment group received a combination of turmeric extract (2 mL/kg body weight) and honey (2 mL/kg body weight) given through a gastric tube for 7 days, with an administration interval of 1 hour after the induction of ketoprofen each day.

Sampling, taking of Dieutanasia mice using the cervical dislocation method, with surgery using scissors or scalpel and tweezers to open the abdominal cavity. The stomach that has been taken is then cut into small pieces (about 0.5 cm) and washed**.**

Tissue cutting, gastric tissue is sliced as thin as possible using a surgical knife so that it can be clearly observed under a microscope. The tissue preservation process involves placing tissue slices in a graded alcohol solution (70%, 80%, 95%, and 100%) for 1-2 hours at each stage to remove water from the tissue. Preparation of the specimen, place the stained tissue on the glass slide and cover it with a cover glass.

Microscopic observation, the staining results of gastric tissue were observed under a light microscope with appropriate magnification to identify signs of ulcers.

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Data Analysis

Data analysis was carried out by counting the number of ulcers, the area of ulcers, and the degree of severity based on histopathological observations. Then, analysis of variance (ANOVA) was conducted to compare the mean number of ulcers in each treatment group. Decision-making was based on the following hypotheses.

a. Zero hypothesis (H0): There is no significant difference between the treatment groups.
b. Alternative hypothesis (H1): There is a significant difference between the treatment groups.

The representation of the research is assessed from the ANOVA results which show significant differences, followed by Tukey's test to determine which groups are significantly different at a significance level set at $p < 0.05$. The analysis results are presented in the form of a table to facilitate understanding (Table X). The discussion of the results includes the interpretation of the findings, their relation to previous literature, and the clinical implications of administering a combination of turmeric extract (*Curcuma domestica* Val) and honey on gastric ulcers in mice induced by ketoprofen.

RESULTS AND DISCUSSION

Observation Results

In evaluating the protective effects of turmeric extract, honey, and their combination against ketoprofen-induced gastric ulcers in mice, by observing the microscopic appearance of the gastric mucosa as well as the number, area, and severity of the ulcers. The following are the results obtained from the microscopic observations of the gastric mucosa.

Control Group (without treatment)

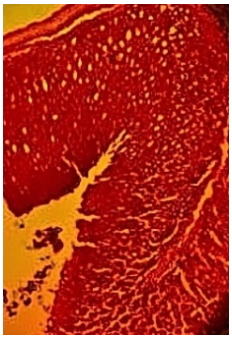

Figure 1. Results of Observations of Stomach Tissue from the control group (without treatment)
Source: Results of Observations in the Microbiology Laboratory of Manado State University

From Figure 1 above, it is noted that the location of gastric ulcers is not found, the gastric tissue appears intact and normal, with no signs of erosion, ulceration, or tissue damage. This observation reflects a normal stomach condition without disturbances, with a score of 0 in tissue damage assessment. This serves as an important comparison for other treatment groups (such as Ketoprofen, Ketoprofen + Turmeric, etc.) in assessing the level of protection against gastric mucosal damage.

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Ketoprofen Control Group

(Score 3) **(Score 2)** **(Score 1)**

Figure 2. Results of Stomach Tissue Observation in the Ketoprofen Control Group.

Source: Results from Observations in the Microbiology Laboratory of State University of Manado.

Based on the observations shown in Figure 2 above, the representation of the results can be explained as follows.

Table 1. Representation of Research Observation Results of the Ketoprofen Control Group

Criteria	Location of Gastric Ulcers	Characteristics
Score 3	A pale yellow area in the middle of the network that penetrates deeply (indicated by the yellow arrows)	• Mucosal damage is very severe. • The damage penetrates to the muscular layer (muscularis). • There is a noticeable loss of mucosal structure and submucosal layer. • The tissue appears destroyed and disorganized.
Score 2	Right at the tip of the yellow arrow, there is an area of erosion extending downward.	• The damage reaches the submucosal layer. • The mucosa is no longer intact, and there is infiltration of tissue underneath. • The size of the lesion is moderate, not as wide as score 3 but deeper than score 1.
Score 1	Location of the gastric ulcer: At the tip of the yellow arrow, slight erosion is observed on the surface of the mucosa.	• Damage is only on the superficial mucosa (surface only). • The structure of the mucosa is mostly intact. • There is no penetration into the submucosa or muscle layer.

Source: Results of Observations in the Microbiology Laboratory of Manado State University.

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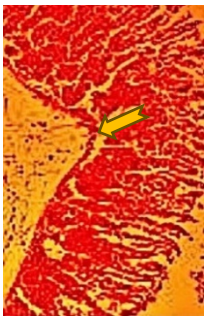
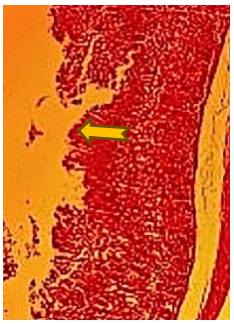
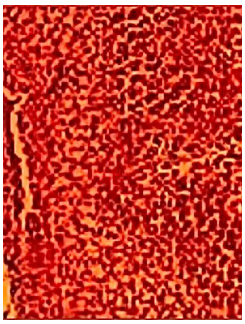
Ketoprofen Group + Turmeric Extract

(Score 2) **(Score 1)** **(Score 0)**

Figure 3. Observation Results of Stomach Tissue of the Ketoprofen + Turmeric Extract Control Group

Source: Observation Results in the Microbiology Laboratory of Manado State University

Based on the observations from Figure 3 above, the representation of the results can be explained in Table 2 as follows.

Table 2. Representation of the Research Observation Results of the Ketoprofen + Turmeric Extract Group

Criteria	Location of Gastric Ulcers	Characteristics
Score 2	Right in the area indicated by the yellow arrow, damage to the tissue is visible from the mucosa penetrating into the submucosa.	• Deep erosion is seen, the mucosa is not intact. • The submucosal structure is beginning to be exposed, indicating that the damage is already quite serious. • However, it has not yet reached the smooth muscle (muscularis).
Score 1	At the tip of the yellow arrow shows superficial mucosal damage.	• The mucosal surface shows mild abrasion. • The structure of the mucosa still appears relatively intact, although not perfect. • There is no penetration into deeper layers such as the submucosa.
Score 0	Not found - the network appears intact and normal.	• The mucosa appears homogeneous, well-organized, without signs of erosion or necrosis. • The even red color indicates healthy tissue. • There is no damage to the mucosa, submucosa, or muscle.

Source: Results of Observations in the Microbiology Laboratory of Manado State University.

The effect of turmeric extract shows increasingly better protection against gastric ulcers. The lower

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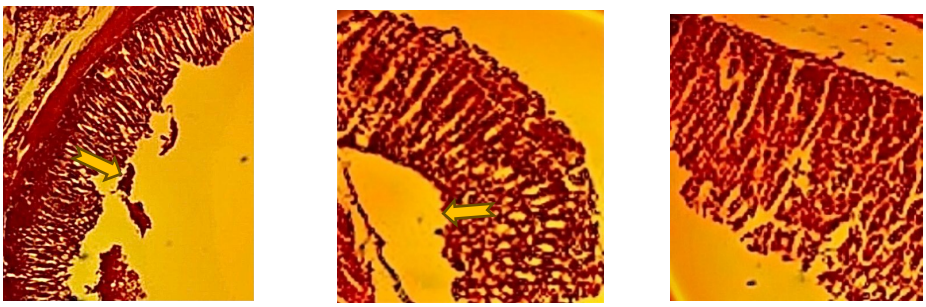
the score, the better the condition of the gastric mucosa. A score of 0 indicates successful protection of the gastric mucosa from damage due to ketoprofen.

Ketoprofen Group + Honey



(Score 2) **(Score 1)** **(Score 0)**

Figure 4. Results of Observations on the Stomach Tissue of the Ketoprofen + Honey Group

Based on the observations shown in Figure 4 above, the representation of the results can be explained as follows.

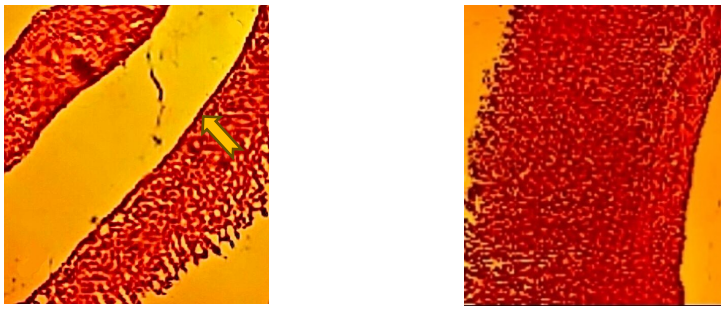
Table 3. Representation of Research Observation Results for the Ketoprofen + Honey Group

Criteria	Location of Gastric Ulcers	Characteristics
Score 2	In the area indicated by the yellow arrow, there appears to be mucosal damage that penetrates down to the submucosal layer.	• The mucosa shows deep damage, not intact. • The submucosal layer appears exposed, indicating moderate damage. • Lighter color in the damaged areas indicates loss of mucosal structure.
Score 1	At the bottom, the arrow shows mild damage to the superficial mucosa..	• The mucosa appears to be still quite neat, but its surface is uneven. • There is mild abrasion or erosion on the surface layer. • No damage is visible down to the submucosa.
Score 0	No erosion found indicates that the tissue is intact and healthy.	• The mucosa appears compact, layered, and intact. • There are no signs of erosion, bleeding, or infiltration of inflammatory cells. • The tissue has normal morphology, indicating the absence of damage..

Source: Results of Observations in the Microbiology Laboratory of Manado State University.

The Ketoprofen + Honey group shows protective effects on the stomach, evidenced by a decrease in the level of mucosal damage. A score of 0 reflects that honey is capable of preventing stomach erosion caused by ketoprofen.

Combination Group of Turmeric Extract & Honey



(Scor 1) **(Scor 0)**

Figure 5. Observation Results of Gastric Tissue in the Combination Group of Turmeric Extract & Honey

Based on the observations shown in Figure 5 above, the representation of the results can be explained as follows.

Table 4. Representation of Research Observation Results for the Ketoprofen + Honey Group

Criteria	Location of Gastric Ulcers	Characteristics
Score 1	In the area indicated by the arrow, there is superficial mucosal damage.	• Light abrasion is visible on the mucosal surface. • The mucosa is mostly still well-structured, but there are minor disruptions on the surface. • There is no penetration into the submucosa or muscle layer. • This indicates mild damage, usually due to mild stress or irritation from ketoprofen that has been significantly mitigated by a combination of turmeric and honey.
Score 0	Not found.	• Intact mucosa, neatly layered, and shows no signs of erosion. • No evidence of inflammatory cell infiltration, bleeding, or necrosis. • Indicates a healthy stomach condition, without damaging effects from ketoprofen. • A combination of turmeric extract and honey is very effective as a gastric mucosal protective agent.

Source: Results of Observations in the Microbiology Laboratory of Manado State University.

The combination group of Turmeric + Honey showed the best effects in protecting the gastric mucosa, as evidenced by the dominance of score 0 and only a small amount of tissue still experiencing superficial abrasion (score 1). This supports the synergistic role of the anti-inflammatory and antioxidant properties of both ingredients.

Based on the results of microscopic observations in each of the groups above, the total scores of the microscopic observations can be presented as follows.

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Table 5. Results of Microscopic Observations.

Group	Mice	Mucosal Erosion Score	Bleeding Score	Inflammation Score
I (Normal Control)	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
II (Negative Control)	1	3	2	3
	2	2	1	2
	3	3	3	3
	4	2	2	2
	5	3	2	3
III (Turmeric)	1	1	0	1
	2	1	0	1
	3	2	1	2
	4	1	0	1
	5	2	1	2
IV (Honey)	1	1	0	1
	2	1	0	1
	3	2	1	2
	4	1	0	1
	5	2	1	2
V (Turmeric + Honey)	1	0	0	0
	2	0	0	0
	3	1	0	1
	4	0	0	0
	5	1	0	1

Source: Results of Microscopic Observation

Based on Table 5, the microscopic observation results show that group I (normal control) has an intact gastric mucosa without signs of erosion, bleeding, or inflammation. Group II (negative control with ketoprofen induction) shows significant mucosal damage in the form of erosion, bleeding, and infiltration of inflammatory cells. Group III (turmeric extract) shows improvement in the mucosa with milder erosion and inflammation. Group IV (honey) showed similar results to the turmeric group, namely improvement of the mucosa with minimal erosion and bleeding. Group V (combination of turmeric and honey extract) showed the best condition, with the majority of mice experiencing no erosion or inflammation, indicating a stronger synergistic protective effect against gastric ulcers caused by ketoprofen.

Description of Observational Data

The data description in Table 6 concludes that group I (normal control) shows mice stomachs in intact condition without ulcers. Group II (negative control with ketoprofen) experienced significant ulcers with a number of lesions 2–3, area 3–6 mm², and a moderate to severe degree. Groups III (turmeric extract) and IV (honey) showed improvement, with lighter ulcers (1–2 lesions, area 1–2 mm², mild to moderate severity). Group V (combination of turmeric and honey) showed the best results, with three mice

free of ulcers and two others only experiencing mild ulcers, indicating a stronger synergistic protective effect compared to single treatment.

Table 6. Data Description

Group	Mice	Number of Ulcers	Area of Ulcer (mm²)	Severity Level of Ulcers
I (Normal Control)	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
II (Negative Control)	1	3	5	3
	2	2	3	2
	3	3	6	3
	4	2	4	2
	5	3	5	3
III (Turmeric)	1	1	1	1
	2	1	1	1
	3	2	2	2
	4	1	1	1
	5	2	2	2
IV (Honey)	1	1	1	1
	2	1	1	1
	3	2	2	2
	4	1	1	1
	5	2	2	2
V (Turmeric + Honey)	1	0	0	0
	2	0	0	0
	3	1	1	1
	4	0	0	0
	5	1	1	1

Source: Results of Microscopic Observation

Statistical Test Results

Table 7. ANOVA Test

ANOVA
Results of the Number of Ulcers

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	20.560	4	5.140	21.417	.000
Within Groups	4.800	20	.240		
Total	25.360	24			

Source: Results of Data Processing SPSS Version 25

Based on table 4.2.3 above, which is the ANOVA results, the figure 0.000 < 0.05, thus Ha is accepted. This indicates that the combination of turmeric extract (*Curcuma domestica* Val) and honey has a significant impact on the healing of gastric ulcers induced by ketoprofen in mice.

To better determine the accuracy of the results, which treatment group has the most significant effect will be tested using Tukey's HSD post-hoc test. Below are the results of the Tukey HSD test in this study.

Table 8. Tukey HSD Test

Result of the Total Ulcers

Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Normal Control	5	.00		
Turmeric and Honey	5	.40		
Turmeric	5		1.40	
Honey	5		1.40	
Negative Control	5			2.60
Sig.		.699	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Source: Results of SPSS Data Processing

Based on Table 4, the results of the Tukey HSD post-hoc test show that the normal control group with an average ulcer count of 0.00 is significantly different from all treatment groups ($p < 0.05$). The negative control group with an average of 2.60 is significantly different from the other treatment groups, while the turmeric and honey groups with averages of 1.40 each do not show significant differences from each other ($p > 0.05$), but both are significantly different from the normal control. The combination of turmeric and honey with an average of 0.40 shows the strongest protective effect, significantly different from the negative control ($p < 0.05$), but not significantly different from the turmeric or honey alone groups ($p > 0.05$), indicating that the combination provides maximum protection against gastric ulcers caused by ketoprofen.

Discussion of Research Results

The results of this study showed that the administration of ketoprofen in mice significantly caused gastric ulcers characterized by mucosal erosion, bleeding, and inflammation observed under a microscope. This is consistent with the literature, which indicates that ketoprofen, as a non-steroidal anti-inflammatory drug (NSAID), has side effects that can damage the gastric lining. The administration of turmeric and honey extract, either alone or in combination, has shown protective effects against ketoprofen-induced gastric ulcers. The results of the analysis of variance (ANOVA) obtained in this study showed a significance value (Sig.) of 0.000, which is much lower than 0.05. This indicates that there is a significant difference in the effects of administering a combination of turmeric extract (*Curcuma domestica* Val) and honey on the healing of ketoprofen-induced gastric ulcers in mice.

This is in line with research by (Sumarni et al., 2019), which shows that a combination of turmeric extract (100 mg/kg body weight) and honey (1 g/kg body weight) provides better healing effects than a single application of each ingredient. This research is also supported by (Putri & Sari, 2021), which

provides evidence that administering honey at a dose of 2 g/kg body weight for 14 days can accelerate the healing of indomethacin-induced gastric ulcers. Another study by (Rahmi et al., 2016) shows that ethanol extract of turmeric at a dose of 100 mg/kg body weight has a significant gastroprotective effect in reducing the severity of aspirin-induced gastric ulcers. The post-hoc Tukey HSD test results also show that the normal control group that did not receive treatment had an average ulcer count of 0.00, which is significantly different from all other treatment groups ($p < 0.05$). This confirms that ketoprofen induction causes gastric ulcer formation in the negative control group.

Overall, the results of this study support the hypothesis that the combination of turmeric and honey extract has a significant protective effect against ketoprofen-induced gastric ulcers in mice. This combination appears to offer maximum protection, although turmeric and honey extracts also show significant protective effects separately. Turmeric extract is known to contain curcumin, which has anti-inflammatory and antioxidant properties. Curcumin can protect the stomach lining by reducing the production of pro-inflammatory cytokines and increasing mucus production. Honey, on the other hand, has antibacterial and anti-inflammatory properties and can accelerate wound healing. The combination of turmeric extract and honey shows a synergistic effect in protecting the stomach lining and speeding up ulcer healing.

CONCLUSION

Based on the discussion and research results, it can be concluded that the microscopic observations indicate that ketoprofen induction causes significant damage to the gastric mucosa in mice, characterized by erosion, bleeding, and inflammation. The administration of turmeric extract and honey, either separately or in combination, significantly improved the condition of the gastric mucosa. The combination of turmeric extract and honey provides the most optimal protective effect, as most mice do not show signs of ulcers, indicating a synergistic effect in healing gastric ulcers. Furthermore, based on the analysis results, there is a significant difference in the effects of the combination of turmeric extract (*Curcuma domestica* Val) and honey on the healing of gastric ulcers induced by ketoprofen in mice.

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