

## Original Research Article

# Effect of Binahong (*Anredera cordifolia*) Leaf Extract on Gastric Histopathology White Rats (*Rattus norvegicus*) Induced by Diclofenac Sodium

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**Abstract:** **Introduction:** Many people easily access information about medications to treat their symptoms, despite not knowing the side effects of these drugs. One of the frequently used medication options is NSAIDs, such as Diclofenac Sodium. The irrational use of Diclofenac Sodium can cause side effects such as gastritis. Measures that can be taken to reduce inflammation and damage to the gastric mucosa are by administering medications and herbs. One of the herbal plants that can be used is Binahong Leaf. **Aims:** To identify the effect of Binahong Leaf extract on gastric histopathology of white rats induced by Diclofenac Sodium. **Method:** The research is a laboratory experiment with a true experimental design posttest only with control group approach in the treatment and control groups. The experimental animal samples were divided into 5 groups, namely the negative control, positive control, treatment 1 (extract dose 100 mg/kgBW), treatment 2 (extract dose 150 mg/kgBW), and treatment 3 (extract dose 200 mg/kgBW). Assessment of gastric mucosal epithelium using Barthel Manja Scoring. Data analysis using One Way Anova Test and Post Hoc Fisher's LSD Test. **Results:** The test results using One Way Anova showed a significant difference in the histopathology of the stomach of white rats given Binahong Leaf extract with a value of  $p = 0.000$  ( $p < 0.05$ ). **Conclusion:** There is an effect of Binahong Leaf extract on gastric histopathology of white rats induced by Diclofenac Sodium with the most effective dose of 200 mg/kgBW. **Keywords:** Binahong Leaf, Gastritis, Diclofenac Sodium, Gastric Histopathology, White Rats (*Rattus Norvegicus*), Herbal Gastroprotection.

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## INTRODUCTION

Technology has developed rapidly in the modern era. Many people can easily access information about medications to relieve symptoms such as fever, swelling, and inflammation, despite having limited knowledge of the potential side effects of these drugs. One of the most commonly used treatment options for such complaints is Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), which are used by more than 30 million people worldwide every day. (Marifah & Tjandra, 2022) According to the *Model List of Essential Medicines* issued by the World Health Organization (WHO), NSAIDs are classified as widely used essential medicines due to their well-established effectiveness. (Bindu *et al.*, 2020)

Over the past three decades, the use of NSAIDs has increased in both developed and developing countries, with approximately 67% of patients reporting NSAID use without a physician's prescription. (Asiri *et al.*, 2020) In Indonesia, a total of 24,496 NSAID products have been reported to be stored in 20,516 households. (Soleha *et al.*, 2018) The most commonly used NSAIDs are diclofenac sodium and ibuprofen, which together account for nearly 40% of global NSAID sales. (Marifah & Tjandra, 2022) The *Essential Medicines Lists* (EMLs) also report that diclofenac is used in 74 out of 100 published countries. (Fokunang, 2018)

Although NSAIDs are known to be effective in treating various conditions, irrational use may lead to undesirable responses that can potentially harm the body,

known as Adverse Drug Reactions (ADRs). A study by Rahmawati *et al.*, (2008) showed that 56.7% of ADRs associated with NSAID use involved gastrointestinal disorders, including dyspepsia, diarrhea, constipation, nausea, vomiting, and gastritis. (Idacahyati *et al.*, 2019) Diclofenac sodium is also associated with gastrointestinal side effects, such as abdominal pain or cramps, constipation, diarrhea, and gastritis. (Oektavia, 2020)

Gastritis is a condition characterized by inflammation of the gastric mucosa and is diagnosed based on histological examination of the gastric mucosa. The term gastritis is widely used to describe the presence of clinical symptoms in the epigastric region. The global prevalence of gastritis reaches 50.8%, while in Indonesia, the prevalence of acute gastritis is reported at 16.9% and chronic gastritis at 36.3%. (Feyisa & Woldeamanuel, 2021; Miftahussurur *et al.*, 2019) Although NSAID-induced gastritis is a detrimental side effect, certain conditions require patients to continue using NSAIDs despite this risk. Measures to reduce gastric mucosal damage include the administration of medications such as Proton Pump Inhibitors (PPIs), H<sub>2</sub> receptor antagonists (H<sub>2</sub>RAs), and misoprostol. In addition to pharmacological therapy, herbal medicines with gastroprotective properties may also be used. Medicinal plants can be processed through extraction, and the resulting extracts may be utilized as therapeutic agents. (Sarno, 2019) Data from the 2018 Indonesian Basic Health Research (*Riset Kesehatan Dasar*; Riskesdas) indicate that 31.4% of households in Indonesia utilize traditional health services. (Kementerian Kesehatan RI, 2018)

One medicinal plant that may be used is binahong leaf (*Anredera cordifolia*), which contains high levels of antioxidants. (Sa'diyah *et al.*, 2020) Studies on the oral administration of binahong leaf ethanol extract for 10 days at doses of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW in Sprague Dawley rats, two hours after induction with 50% ethanol, demonstrated a significant inhibitory effect on gastric mucosal damage. (Badan Pengawas Obat dan Makanan, 2016) Previous research conducted by Horng *et al.*, (2012) also showed a reduction in the gastric ulcer index following administration of binahong leaf extract powder at doses of 250 mg/kgBW, 500 mg/kgBW, and 1,250 mg/kgBW. (Horng *et al.*, 2012) Perwirayuda (2018) reported a decrease in the macroscopic severity of peptic ulceration after administration of binahong leaf ethanol extract at doses of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW. (Perwirayuda, 2018) These findings are consistent with a study by Praswanto (2016), which demonstrated that administration of binahong leaf ethanol extract at the same doses reduced microscopic gastric mucosal lesion damage in indomethacin-induced Wistar rats. (Praswanto, 2016)

Based on these findings, binahong leaf exhibits a protective effect on the gastric mucosa. Therefore, this study aims to investigate the effect of binahong leaf extract on gastric damage in white rats induced by diclofenac sodium.

## METHODS

### Preliminary Phase

The study on the effect of Binahong leaf extract (*Anredera cordifolia*) on the gastric histopathology of white rats (*Rattus norvegicus*) was conducted after completing a preliminary study. The preliminary study was carried out using three rat samples divided into three groups: a negative control group (K<sup>-</sup>) that received no treatment and was provided only with BR-1 feed and distilled water; a positive control group (K<sup>+</sup>) that was administered diclofenac sodium at a dose of 10 mg/kg body weight; and a treatment group that received diclofenac sodium at a dose of 10 mg/kg body weight combined with Binahong leaf extract at a dose of 150 mg/kg body weight. However, the experimental animal in the treatment group died during the preliminary study.

This preliminary study was conducted to learn the proper technique for oral gavage administration and to determine the duration of diclofenac sodium administration required to induce damage to the gastric mucosal epithelium. The results of the preliminary study demonstrated histopathological changes in the gastric mucosal epithelium of the positive control group (K<sup>+</sup>), characterized by erosion, while no findings of desquamation, erosion, or ulceration were observed in the negative control group (K<sup>-</sup>).

### Research Design and Ethical Approval

This study was conducted from September 20 to October 24, 2023, after obtaining ethical clearance from the Health Research Ethics Committee of the Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana (approval letter number 84/UN15.16/KEPK/2023). The study employed a laboratory experimental design with a true experimental posttest-only control group approach involving both control and treatment groups. The sample size was determined using Federer's formula, resulting in five rats per group; to account for potential dropouts, the number of samples was increased to six rats per group. The samples consisted of Wistar strain white rats divided into five groups: a negative control group, a positive control group, treatment group 1, treatment group 2, and treatment group 3. Data analysis was performed using SPSS 25 with One-Way Analysis of Variance (One-Way ANOVA), a parametric test applied due to the normal distribution of the data, followed by Fisher's Least Significant Difference (LSD) post hoc test to determine statistically significant differences among the study groups.

**Inclusion, Exclusion, and Drop-Out Criteria**

The inclusion criteria were Wistar strain white rats (*Rattus norvegicus*), male, aged 8–12 weeks, weighing 150–250 g, and in healthy condition as indicated by clean white fur, absence of hair loss, active movement, and no discharge from the eyes or ears. The exclusion criteria included rats that experienced a body weight loss of  $\geq 10\%$  during the adaptation period and rats that had previously been used in other studies. The drop-out criterion was experimental animals that died during the study period.

**Adaptation of White Rats**

Prior to treatment, the experimental animals were weighed to determine whether there was a body weight loss of more than 10% during the adaptation period. A physical health examination was also performed to assess the general health status of the animals. Healthy rats were defined by the absence of hair loss, clean and white fur, good appetite, and no discharge from the ears or eyes. The animals were then housed in cages for 7 days to allow adaptation to the new environment and to minimize stress. Feeding was carried out routinely twice daily using brailler-I pellet feed (BR-I), while drinking water in the form of distilled water was provided ad libitum using drinking bottles. Cage cleaning was performed once daily to prevent stress and disease in the animals. These procedures were conducted to ensure that the rats remained healthy throughout the study period. (Widiartini *et al.*, 2015)

**Experimental Procedure**

After the adaptation period, rats in the positive control group (K+) and treatment groups (P1, P2, and P3) were administered diclofenac sodium orally at a dose of 10 mg/kg body weight for seven consecutive days (days 8–14). The negative control group (K-) received only standard feed and distilled water. Following diclofenac sodium administration, rats in the treatment groups received Binahong (*Anredera cordifolia*) leaf extract

orally for seven days (days 15–21) at doses of 100 mg/kg body weight for P1, 150 mg/kg body weight for P2, and 200 mg/kg body weight for P3. The positive control group was terminated after diclofenac sodium administration, while the negative control group continued without intervention until termination. (Tandoh *et al.*, 2021; Serliandi, 2018)

**Anesthesia, Surgery, and Organ Collection**

On the scheduled termination day, rats were anesthetized using inhalational ether. Each animal was placed in a closed container containing cotton soaked with 0.2 mL ether until immobile. The anesthetized rats were positioned supine, and all four limbs were fixed. Surgical procedures were performed using standard surgical instruments to harvest the stomach.

**Histological Preparation and Staining**

Gastric tissues were fixed in 10% Neutral Buffered Formalin for at least 24 hours. Tissue processing was performed using the paraffin embedding method, including dehydration through graded alcohol concentrations, clearing with xylol, and embedding in paraffin. Paraffin blocks were sectioned at 4–5  $\mu\text{m}$  thickness using a microtome, and sections were mounted on glass slides. Histological staining was carried out using the Hematoxylin and Eosin (H&E) method, including deparaffinization, rehydration, hematoxylin staining, differentiation, eosin counterstaining, dehydration, clearing, and mounting with Permount. (Riong, 2022)

**Histopathological Assessment**

Histopathological evaluation was conducted to assess gastric mucosal epithelial damage and improvement following Binahong leaf extract administration after diclofenac sodium induction. The assessment was performed using the Barthel–Manja scoring system. (Riong, 2022)

**Table 1 : Barthel-Manja Gastric Histopathology Scoring System**

Destruction Level	Interpretation	Score
Not Found	No desquamation, erosion, or ulceration was observed.	0
Desquamation of Epithelial Mucosa	Mild damage to the gastric mucosal epithelium was observed, extending up to one-third of the mucosal layer.	1
Erosion of Epithelial Mucosa	Epithelial damage was observed in nearly half or more of the mucosal layer but did not extend to the muscularis mucosae.	2
Ulceration of Epithelial Mucosa	Epithelial damage involved the entire mucosal layer and penetrated into the muscularis mucosae.	3

**RESULTS**

**Binahong Leaf Extraction**

A total of 7 kg of red-stem binahong leaves (*Anredera cordifolia*) were washed under clean running water. The leaves were then drained by spreading them evenly on a container and allowed to air-dry at room temperature for 24 hours. The binahong leaves were subsequently processed into simplicia by oven-drying at

50°C for 22 hours. (Ristanti & Mulyani, 2019) From this process, 4 kg of binahong leaf simplicia were obtained and further ground using a blender, then sieved through a 20-mesh sieve to obtain a finer powder. The final yield of fine binahong leaf simplicia (*Anredera cordifolia*) was 841 g.

A total of 841 g of the fine binahong leaf simplicia was macerated for 3 × 24 hours using 70%

ethanol at a volume of 8.41 L, based on a 1:10 ratio, with stirring performed every 24 hours. (Asworo & Widwastuti, 2023) The macerate was filtered using filter paper, yielding 6.1 L of liquid extract, which was subsequently evaporated using a rotary evaporator at 40°C to obtain 200 g of concentrated binahong leaf extract.


The concentrated binahong leaf extract was weighed and adjusted according to the dose administered to each experimental animal in the respective treatment groups (P1, P2, and P3). The weighed extract was dissolved in 3 mL of distilled water (aquadest) to ensure that the administered volume did not exceed the

maximum oral gavage volume of 5 mL per 200 g body weight of the rats. (Astuti, 2020) The dissolved extract was then drawn into a 3 mL syringe and administered to each experimental animal via oral gavage.

**Ethanol Free Testing**

The ethanol-free test of the binahong leaf extract was conducted to confirm that the ethanol used as a solvent during the maceration process had been completely removed, thereby ensuring that a pure binahong leaf extract free from contamination was obtained. Residual ethanol present in the extract could bias the treatment outcomes, as ethanol is toxic to the gastric mucosa. (Wijayanti, 2022)

**Table 2 : Ethanol Free Test**

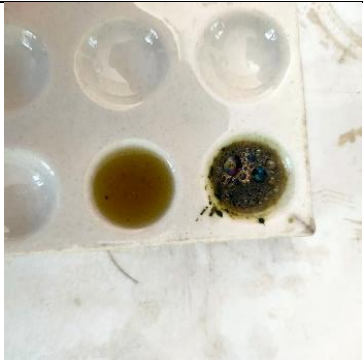

Testing Method	Results	Interpretation
One milliliter of binahong leaf extract was placed into a test tube, followed by the addition of five drops of sulfuric acid (H <sub>2</sub> SO <sub>4</sub> ) and 2 mL of potassium dichromate (K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ).		The extract was confirmed to be ethanol-free, as no color change from the initial color to blue was observed.




**Phytochemical Screening**

The presence of secondary metabolites in binahong leaf extract can be confirmed through phytochemical screening. The metabolites identified in the binahong leaf extract include flavonoids, alkaloids,

saponins, steroids, and tannins. These compounds possess antioxidant properties that may contribute to the repair of damage to the gastric mucosal epithelium. (Wijayanti, 2022)

**Table 3 : Phytochemical Screening**

No.	Compound Class	Results	Interpretation
1.	Flavonoid		Positive (+) for Flavonoids The test produced a reddish-brown coloration.
2.	Alkaloid Dragendorff Meyer Wagner		Positive (+) for Alkaloids a. An orange-red coloration was observed. b. A white precipitate was formed. c. A reddish-brown coloration was observed.

3.	Saponin		Positive (+) for Saponins. The formation of stable foam was observed.
4.	Steroid		Positive (+) for Steroids. The test produced a green or blue coloration.
5.	Tanin		Positive (+) for tannins. The test yielded a greenish-black coloration.

**Interpretation and Results**

This study began with the initial weighing of the rats upon arrival, followed by a 7-day acclimatization period during which the animals were provided with BR-1 standard feed and distilled water. The second body weight measurement was performed after the acclimatization period, on day 7, and was used to calculate the appropriate dosage of diclofenac sodium. On day 8, rats in the positive control group (K<sup>+</sup>) and the

treatment groups (P1, P2, and P3) were administered diclofenac sodium at a dose of 10 mg/kg body weight via oral gavage. Diclofenac sodium was administered once daily for seven consecutive days, prior to feeding. On day 15, a third body weight measurement was conducted, and the results were used to determine the dosage of binahong leaf extract to be administered.

**Table 4 : Changes in the Degree of Gastric Mucosal Epithelial Damage**

Experimental Animal Groups	Total Sample	Changes in Each Sample			
		Not Found	Desquamation	Erosion	Ulcer
Control (-)	5	4	1	-	-
Control (+)	6	-	2	3	1
Treatment 1	5	-	1	3	1
Treatment 2	5	-	5	-	-
Treatment 3	5	1	4	-	-

The occurrence of damage and repair of the gastric mucosal epithelium was influenced by the treatment received in each group. The negative control group (K<sup>-</sup>), which did not receive any treatment and served as a comparison group, showed one sample with a desquamation score and four samples with scores

indicating the absence of desquamation, erosion, and ulceration of the gastric mucosal epithelium. However, other histopathological features, including hyperplasia and hyperkeratosis of the gastric mucosa, were observed.

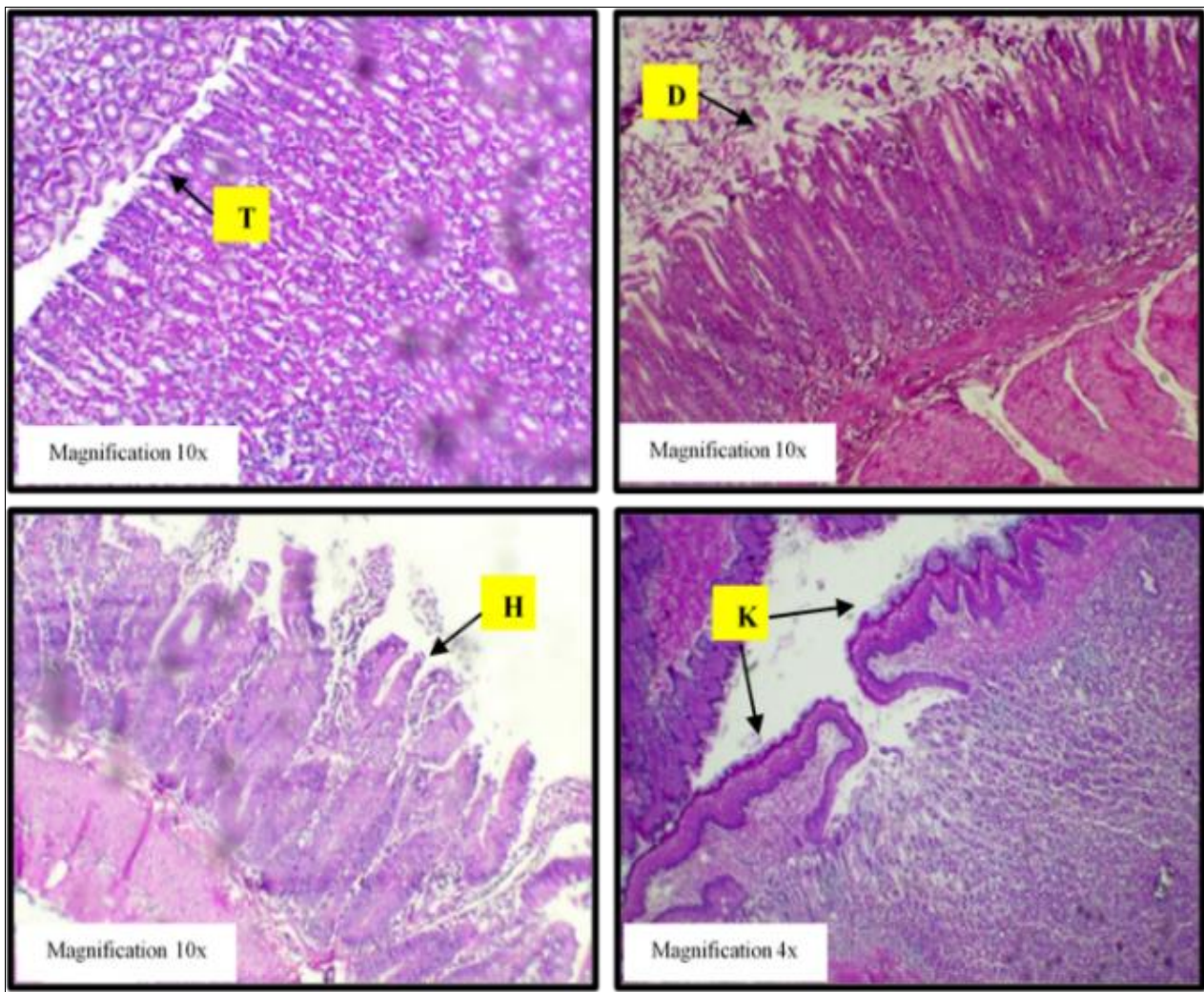
In the positive control group (K+), which received diclofenac sodium at a dose of 10 mg/kg body weight, one sample exhibited ulceration accompanied by hyperkeratosis and mucosal atrophy. Three samples showed erosion accompanied by atrophy, hyperkeratosis, and hyperplasia, while two samples demonstrated desquamation accompanied by atrophy and polyp formation.

Treatment group 1 (P1), which received diclofenac sodium at a dose of 10 mg/kg body weight and binahong leaf extract at a dose of 100 mg/kg body weight, showed three out of five samples with erosion accompanied by hyperplasia and atrophy. One sample exhibited desquamation accompanied by hyperplasia,

and one sample showed ulceration accompanied by hyperplasia.

Treatment group 2 (P2), which received diclofenac sodium at a dose of 10 mg/kg body weight and binahong leaf extract at a dose of 150 mg/kg body weight, demonstrated desquamation in all five samples, accompanied by hyperplasia and atrophy.

Treatment group 3 (P3), which received diclofenac sodium at a dose of 10 mg/kg body weight and binahong leaf extract at a dose of 200 mg/kg body weight, showed four out of five samples with desquamation accompanied by hyperplasia and atrophy, while one sample showed only hyperplasia and atrophy.

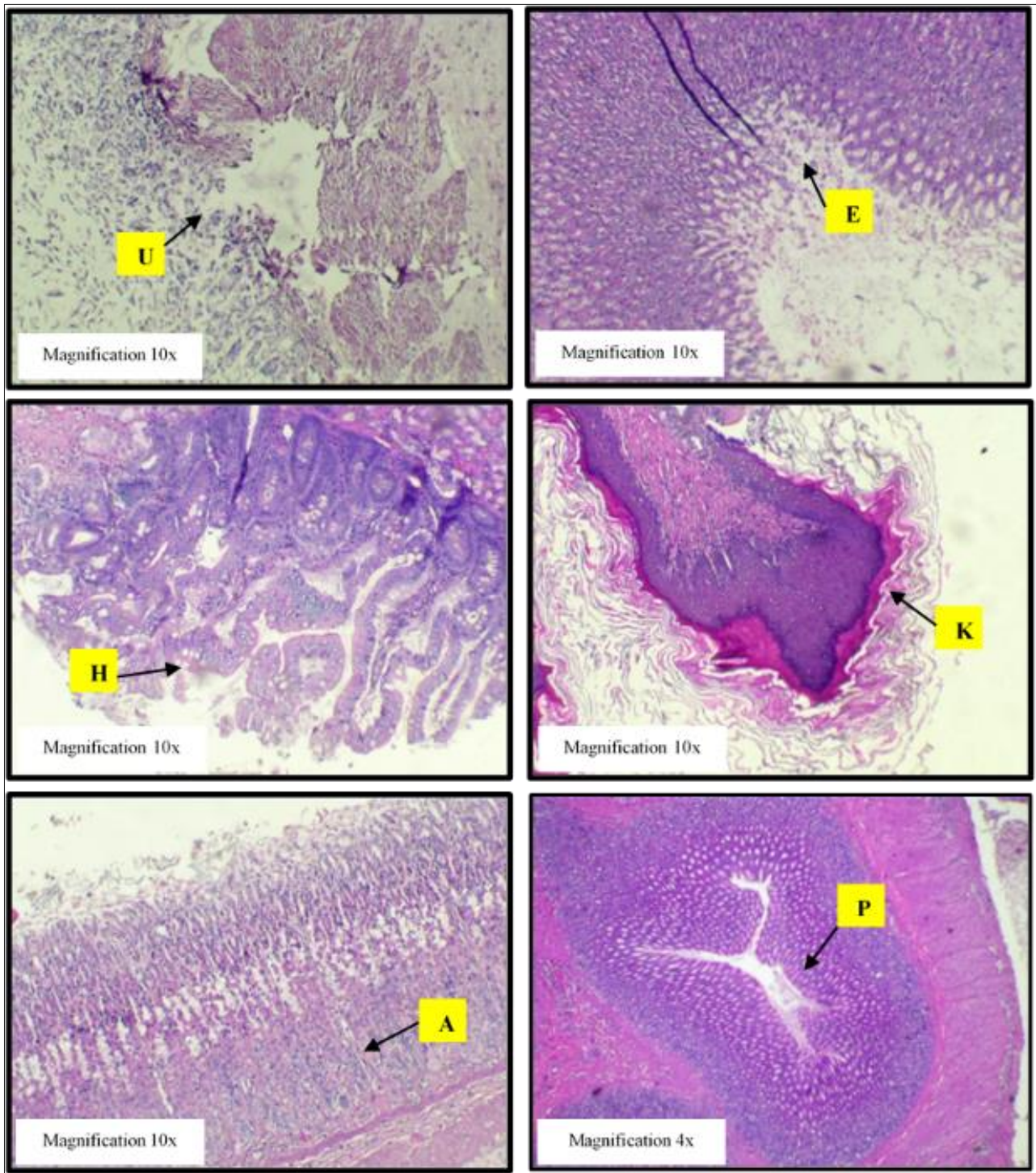


**Figure 1 : Gastric Histopathology Negative Control Group**

**Notes:** T = no desquamation, erosion, or ulceration; D = desquamation; H = hyperplasia; K = hyperkeratosis.

The negative control group (K-) consisted of animals that received only BR-1 standard feed and distilled water throughout the study. Microscopic examination of the gastric mucosal epithelium at magnifications of 4x, 10x, and 40x revealed no evidence of desquamation, erosion, or ulceration in several samples. However, some samples exhibited

desquamation accompanied by hyperplasia (H). Hyperplasia of the gastric mucosal epithelium was characterized by an increased number of foveolar cells [58]. Thickening of the keratin layer in epithelial cells lining the non-glandular anterior gastric lumen in rats, referred to as hyperkeratosis (K), was also observed. (Uehara *et al.*, 2018)

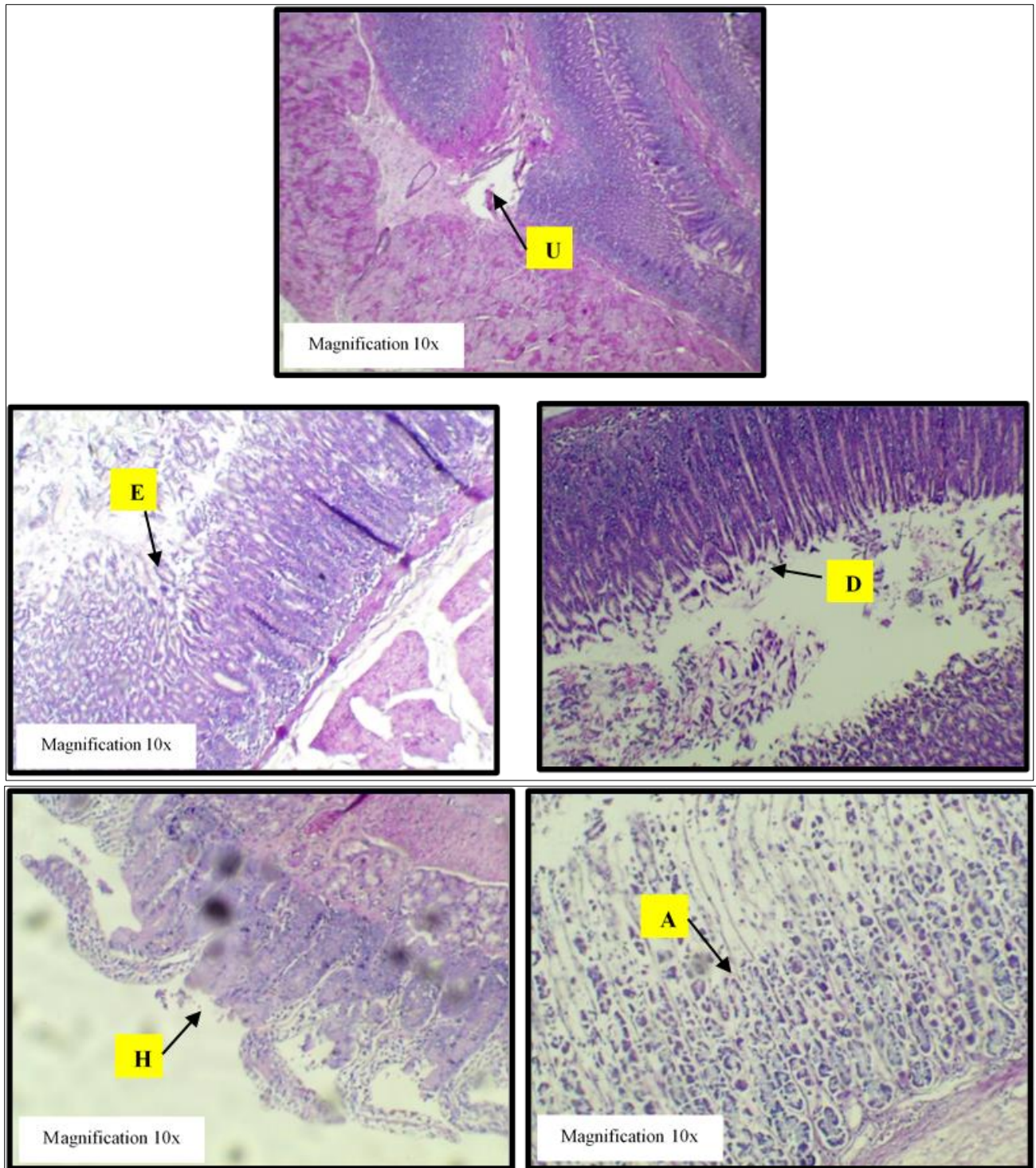


**Figure 2 : Gastric Histopathology Positive Control Group**

**Notes:** U = ulceration; E = erosion; H = hyperplasia; K = hyperkeratosis; A = atrophy; P = polyp.

The positive control group (K+) received diclofenac sodium alone for seven days at a dose of 10 mg/kg body weight, without administration of binahong leaf extract. Histopathological examination revealed ulceration (U), characterized by disruption of the gastric mucosal epithelium extending from the mucosal layer to the serosal layer. Erosion (E) was also observed, indicated by epithelial damage involving nearly half or

more of the mucosal layer. (Niamah, 2021) Additional histopathological findings in this group included hyperplasia (H), hyperkeratosis (K), atrophy (A), and polyp formation (P). Polyps are protrusions of the mucosa or submucosa into the gastric lumen, characterized by irregular and elongated foveolar cells. (Kumar & Abbas, 2015)

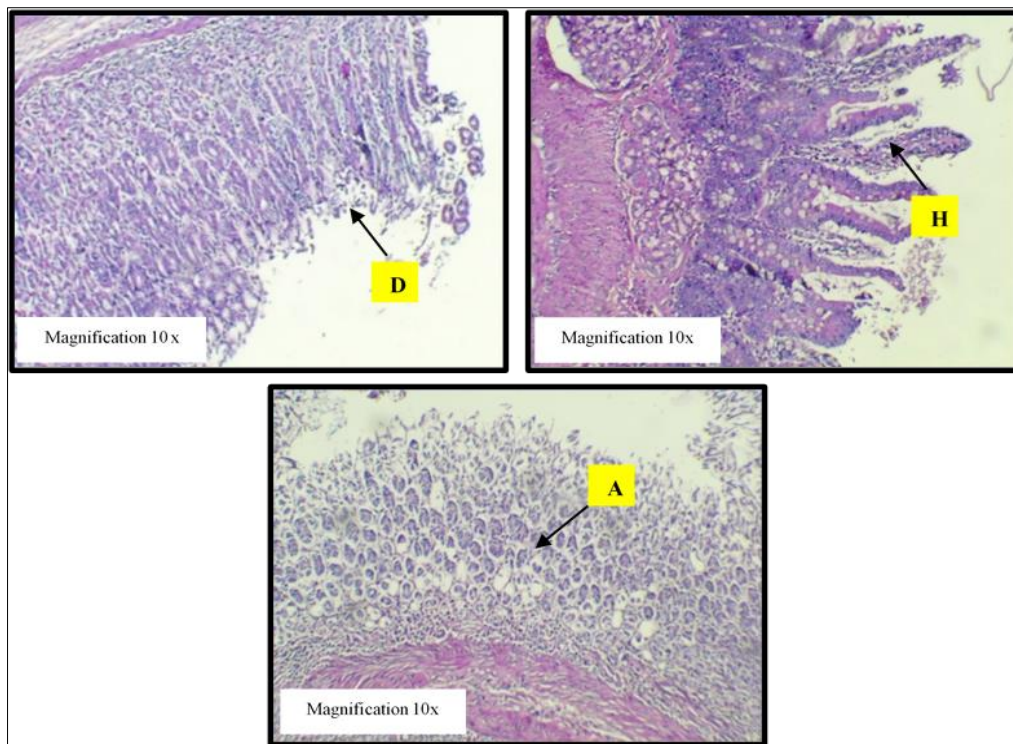


**Figure 3 : Gastric Histopathology Treatment Group 1**

**Notes:** U = ulceration; E = erosion; D = desquamation; H = hyperplasia; A = atrophy

Treatment group 1 (P1), which received diclofenac sodium at a dose of 10 mg/kg body weight and binahong leaf extract at a dose of 100 mg/kg body weight, showed histopathological changes in the gastric mucosal epithelium compared with the positive control group. Microscopic observation revealed persistent ulceration (U) and erosion (E). Desquamation (D) was

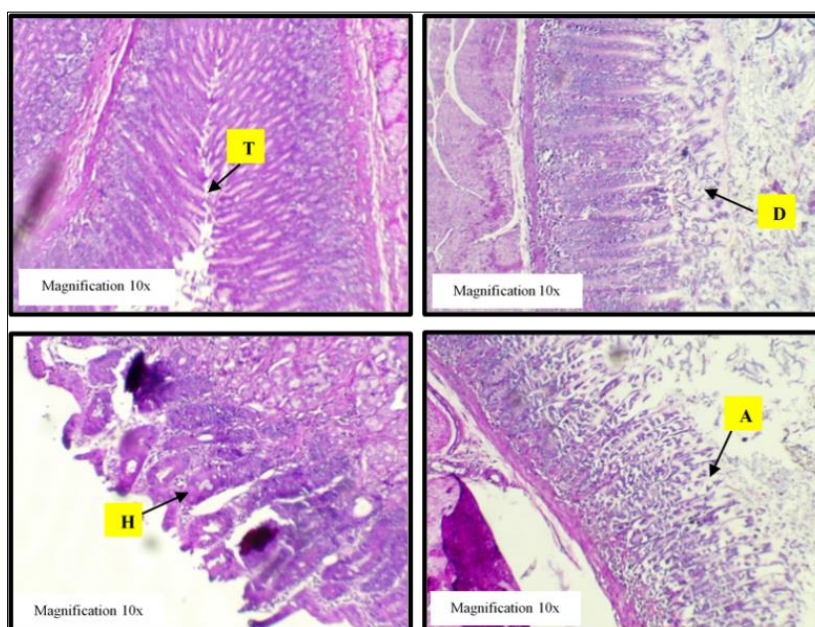
also observed, characterized by mild epithelial exfoliation of the gastric mucosa. Additional findings included hyperplasia (H) and atrophy (A) of the gastric mucosa. Atrophy was indicated by a reduction in cellular volume of the gastric mucosa and loss of gastric glands. (Geraldine *et al.*, 2020)



**Figure 4 : Gastric Histopathology Treatment Group 2**  
**Notes:** D = desquamation; H = hyperplasia; A = atrophy

Observations in treatment group 2 (P2), which received diclofenac sodium at a dose of 10 mg/kg body weight and binahong leaf extract at a dose of 150 mg/kg body weight, demonstrated further improvement compared with treatment group 1 (P1). In this group, no

ulceration or erosion was observed. Histopathological findings included desquamation (D), characterized by exfoliation of epithelial cells on the gastric mucosal surface, accompanied by hyperplasia (H) and atrophy (A).



**Figure 5 : Gastric Histopathology Treatment Group 3**  
**Notes:** T = no desquamation, erosion, or ulceration; D = desquamation; H = hyperplasia; A = Atrophy.

Treatment group 3 (P3), which received diclofenac sodium at a dose of 10 mg/kg body weight and binahong leaf extract at a dose of 200 mg/kg body

weight, exhibited gastric mucosal epithelial features resembling those observed in the negative control group (K-). No erosion or ulceration (T) was detected,

although mild desquamation (D) was observed in one sample, accompanied by hyperplasia (H) and atrophy (A) of the gastric mucosa. These findings indicate that binahong leaf extract at a dose of 200 mg/kg body weight possesses more optimal antioxidant properties for repairing gastric mucosal epithelial damage compared with the lower doses. (Sa'diyah *et al.*, 2020)

**Data Analysis**

The normality test of the data in this study was performed using the Shapiro–Wilk test. The Shapiro–Wilk test was selected because the sample size used in this study was small (less than 50 samples). The results of the Shapiro–Wilk test indicated that the data were normally distributed, with a significance value of  $p > 0.05$ . (Putra *et al.*, 2019)

**Table 5: Results of the Normality Test of Data Distribution on the Effect of Binahong Leaf Extract Administration on the Gastric Histopathology of White Rats**

Test Group	P Value	Data Distribution
Control (-)	0,201*	Normal
Control (+)	0,941*	Normal
Treatment 1 (100 mg/kgBB)	0,940*	Normal
Treatment 2 (150 mg/kgBB)	0,777*	Normal
Treatment 3 (200 mg/kgBB)	0,967*	Normal

Levene’s Test was conducted to determine the homogeneity of the samples used, specifically to assess whether the data had homogeneous or heterogeneous variances. The results of Levene’s Test showed a

significance value of  $p = 0.237$  ( $p > 0.05$ ), indicating that the data groups originated from homogeneous variances. (Putra *et al.*, 2019)

**Table 6: Results of the Homogeneity Test of Binahong Leaf Extract Administration on the Gastric Histopathology of White Rats**

	P Value	Notes
Degree of Gastric Histopathological Damage	0,237*	Homogene

\*Notes:  $P > 0.05$ : The data groups have homogeneous variances.

The selected data analysis was a parametric statistical test, namely One-Way Analysis of Variance (One-Way ANOVA), as the data were normally distributed based on the normality test results. The One-Way ANOVA analysis showed a statistically significant result with a p-value of  $p < 0.05$ , specifically  $p = 0.000$ ,

indicating that  $H_0$  was rejected and  $H_1$  was accepted. (Faradiba, 2020) These findings demonstrate that the administration of Binahong Leaf extract had a significant effect on the gastric histopathology of white rats.

**Table 7: Results of the Parametric Analysis of Binahong Leaf Extract Administration on the Gastric Histopathology of White Rats**

	P Value	Notes
Degree of Gastric Histopathological Damage	0,000*	Significant

\*Notes:  $P > 0.05$ : Significant

The Post Hoc Fisher’s Least Significant Difference (LSD) test is a follow-up analysis performed when a significant result is obtained from the One-Way

ANOVA. This test is used to determine whether there are statistically significant differences between each pair of experimental groups.

**Table 8 : Results of the Post Hoc Fisher’s Least Significant Difference (LSD) Test on the Effect of Binahong Leaf Extract Administration on the Gastric Histopathology of White Rats**

Testing Group I	Testing Group II				
	Control (-)	Control (+)	Treatment 1	Treatment 2	Treatment 3
Kontrol Negatif	-	0,000*	0,000*	0,024*	0,076
Kontrol Positif	0,000*	-	0,675	0,006*	0,002*
Perlakuan 1	0,000*	0,675	-	0,003*	0,001*
Perlakuan 2	0,024*	0,006*	0,003*	-	0,572
Perlakuan 3	0,076	0,002*	0,001*	0,572	-

Based on the results of the Post Hoc Fisher’s Least Significant Difference (LSD) test analyzed using the Statistical Package for the Social Sciences (SPSS) as presented in the table above, the following conclusions can be drawn:

1. The negative control group (K–), which received only BR-1 feed and distilled water, showed significant differences compared to the positive control group (K+) that received sodium diclofenac at a dose of 10 mg/kg body

weight (BW), treatment group 1 (P1) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 100 mg/kgBW, and treatment group 2 (P2) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 150 mg/kgBW. However, no significant difference was observed between the negative control group (K<sup>-</sup>) and treatment group 3 (P3) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 200 mg/kgBW.

2. The positive control group (K<sup>+</sup>), which received sodium diclofenac at a dose of 10 mg/kgBW, demonstrated significant differences compared to the negative control group (K<sup>-</sup>), treatment group 2 (P2) that received sodium diclofenac 10 mg/kgBW and 150 mg/kgBW Binahong leaf extract, and treatment group 3 (P3) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 200 mg/kgBW. However, no significant difference was found between the positive control group (K<sup>+</sup>) and treatment group 1 (P1) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 100 mg/kgBW.
3. Treatment group 1 (P1), which received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 100 mg/kgBW, showed significant differences compared to the negative control group (K<sup>-</sup>), treatment group 2 (P2) that received sodium diclofenac 10 mg/kgBW and 150 mg/kgBW Binahong leaf extract, and treatment group 3 (P3) that received sodium diclofenac 10 mg/kgBW and 200 mg/kgBW Binahong leaf extract. However, no significant difference was observed between treatment group 1 (P1) and the positive control group (K<sup>+</sup>), which received only sodium diclofenac at a dose of 10 mg/kgBW without Binahong leaf extract.
4. Treatment group 2 (P2), which received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 150 mg/kgBW, showed significant differences compared to the negative control group (K<sup>-</sup>), the positive control group (K<sup>+</sup>) that received sodium diclofenac 10 mg/kgBW, and treatment group 1 (P1) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 100 mg/kgBW. However, no significant difference was found between treatment group 2 (P2) and treatment group 3 (P3) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 200 mg/kgBW.
5. Treatment group 3 (P3), which received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 200 mg/kgBW, showed significant differences compared to the positive

control group (K<sup>+</sup>) that received only sodium diclofenac 10 mg/kgBW and treatment group 1 (P1) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 100 mg/kgBW. However, no significant difference was observed between treatment group 3 (P3) and the negative control group (K<sup>-</sup>) or treatment group 2 (P2) that received sodium diclofenac 10 mg/kgBW and Binahong leaf extract at a dose of 150 mg/kgBW.

## DISCUSSION

This study utilized Binahong leaf extract (*Anredera cordifolia*) with the expectation that its administration would exert a beneficial effect in the form of improvement in the gastric histopathology of Wistar strain white rats (*Rattus norvegicus*) that had been induced with sodium diclofenac at a dose of 10 mg/kg body weight (BW).

The negative control group (K<sup>-</sup>), which received only BR-1 feed and distilled water, demonstrated an intact gastric wall architecture consisting of the mucosa, submucosa, muscularis, and serosa layers. These layers were lined by simple columnar epithelium that invaginated into the lamina propria with variable sizes, forming gastric pits resembling branched and elongated tubular glands lining the lamina propria. (Mescher, 2018) However, desquamation of the simple columnar epithelium lining the gastric mucosa was observed in this group. The presence of desquamation in samples not exposed to toxic substances may be attributed to stress-related factors, which stimulate parietal cells and activate G cells to secrete gastrin, leading to increased gastric acid production.

Additionally, elevated cortisol levels may further enhance gastric acid secretion and inhibit prostaglandin production. (Kabrahamun *et al.*, 2022) Other histological findings observed in this group included hyperplasia and hyperkeratosis. Hyperplasia may also result from the habit of consuming large amounts of water in a short period immediately after meals, which can reduce gastric acid concentration and increase gastric pH. The body compensates for this condition by increasing the number of parietal cells, resulting in gastrin cell hyperplasia. (Dong & Min, 2022)

The positive control group (K<sup>+</sup>), which received BR-1 feed, distilled water, and sodium diclofenac at a dose of 10 mg/kgBW, exhibited the most severe epithelial damage, characterized by ulceration, accounting for 16.7% of the total samples in this group. Ulceration was indicated by epithelial damage extending through the entire thickness of the mucosa to the muscularis mucosae, accompanied by inflammatory cell infiltration. Erosion, defined as damage involving approximately half or more of the gastric mucosal layer, was observed in 50% of the samples in this group.

(Niamah, 2021) Additional histopathological features included hyperplasia, hyperkeratosis, and polyps.

Hyperplasia and hyperkeratosis in the positive control group may be attributed to diffuse gastric mucosal inflammation resulting from exposure to sodium diclofenac, a non-steroidal anti-inflammatory drug (NSAID). (Orthop, 2022; Wang, 2022) Polyp formation in this group is also presumed to result from inhibition of cyclooxygenase-2 (COX-2) and reduced prostaglandin synthesis induced by sodium diclofenac. (Shah, 2018)

Histological analysis revealed a significant difference between the positive control group (K+) and the negative control group (K-), with a p-value of 0.000 ( $p < 0.05$ ), confirming the occurrence of gastric damage due to sodium diclofenac administration. This finding is consistent with a study by Tandoh *et al.*, (2021) entitled "Protective Effects of Andrographolide Against Diclofenac-Induced Gastric Damage", which reported that a dose of 10 mg/kgBW of sodium diclofenac is sufficient to induce gastric mucosal injury. (Tandoh *et al.*, 2021)

Sodium diclofenac exerts its effects by inhibiting COX-1, which is involved in prostaglandin production, and COX-2, which plays a role in inflammatory processes. Inhibition of prostaglandin synthesis leads to an imbalance of free radicals. When the body is unable to neutralize excessive free radicals due to insufficient antioxidant capacity, oxidative stress occurs. Reactive Oxygen Species (ROS), which are highly unstable due to unpaired electrons, can damage cellular components such as DNA, proteins, and lipids, ultimately leading to apoptosis. This damage results in functional and structural alterations of the gastric mucosal epithelium, causing erosion and desquamation, and prolonged exposure may lead to ulcer formation. (Ikrima *et al.*, 2019; Nurhidayati, 2018)

The treatment group 1 (P1), which received sodium diclofenac at 10 mg/kgBW and Binahong leaf extract at 100 mg/kgBW, showed histopathological differences compared to the negative control group (K-). Findings included desquamation as well as persistent ulceration and erosion similar to those observed in the positive control group (K+). Erosion of the mucosal cells may occur as a result of continuous epithelial desquamation. (Rahayu *et al.*, 2019)

Hyperplasia and atrophy were also observed in the gastric mucosa. Gastric mucosal atrophy may result from prolonged drug use. (Wang *et al.*, 2022) Statistical analysis between treatment group 1 (P1) and the negative control group (K-) revealed a significant difference ( $p = 0.000$ ;  $p < 0.05$ ). Based on these findings, it can be inferred that Binahong leaf extract at a dose of 100 mg/kgBW may not be sufficient to effectively repair the gastric mucosal epithelium under gastritis conditions

compared to higher doses. This may be influenced by the dose-dependent efficacy of Binahong leaf extract. (Anugerah & Rahman, 2023; Bahtiar *et al.*, 2021)

Based on the test results, treatment group 2 (P2), which received sodium diclofenac at 10 mg/kgBW and Binahong leaf extract at 150 mg/kgBW, showed significant differences compared to the positive control group (K+) ( $p = 0.006$ ;  $p < 0.05$ ) and the negative control group (K-) ( $p = 0.024$ ;  $p < 0.05$ ). This indicates an improvement in the gastric mucosal epithelial appearance compared to the positive control group, although it had not yet reached the condition observed in the negative control group.

Histological examination revealed an average score of desquamation accompanied by hyperplasia and atrophy. Desquamation occurs due to oxidative stress induced by sodium diclofenac, which stimulates gastric epithelial cells to reduce mucus secretion, thereby disrupting gastric cytoprotection and leading to epithelial exfoliation. (Rahayu *et al.*, 2019) Therefore, the presence of antioxidants is essential. Antioxidants can terminate chain oxidation reactions by donating electrons to free radicals, preventing oxidative stress and protecting the gastric mucosal epithelium from damage. (Supriyanto, 2021)

Microscopic examination of treatment group 3 (P3), which received Binahong leaf extract at a dose of 200 mg/kgBW, demonstrated a more significant improvement in the gastric mucosal epithelium. No erosion or ulceration was observed, and desquamation was rarely detected. Histologically, the gastric mucosa in this group closely resembled that of the negative control group (K-), which was supported by the Post Hoc Fisher's LSD test, showing no significant difference ( $p = 0.076$ ).

These findings indicate that Binahong leaf extract at a dose of 200 mg/kgBW has a more optimal effect in repairing gastric mucosal epithelium under gastritis conditions compared to lower doses. This result is consistent with a previous study by Perwirayuda (2018), which reported that Binahong leaf extract at a dose of 200 mg/kgBW was more effective in repairing gastric mucosa, as evidenced by a reduction in macroscopic gastric lesions in indomethacin-induced rats compared to doses of 50 mg/kgBW and 100 mg/kgBW. (Perwirayuda, 2018)

Hypothesis testing using SPSS revealed a significant result ( $p = 0.000$ ;  $p < 0.05$ ), indicating that Binahong leaf extract (*Anredera cordifolia*) significantly affects the gastric histopathology of white rats (*Rattus norvegicus*) induced with sodium diclofenac. The ability of Binahong leaf extract to repair gastric mucosal epithelium is attributed to its content of secondary metabolites, including flavonoids, saponins, tannins,

steroids, and alkaloids, which possess antioxidant and anti-inflammatory properties.

According to a study by Serafim *et al.*, (2020), flavonoids protect the gastric mucosa through multiple mechanisms: acting as cytoprotective agents by increasing mucus production, functioning as antioxidants by enhancing superoxide dismutase (SOD) activity and glutathione levels, serving as immunoregulators by reducing pro-inflammatory cytokines and increasing anti-inflammatory cytokines, and acting as antisecretory agents by reducing H<sup>+</sup> secretion. (Serafim *et al.*, 2020) A study by Shi *et al.*, (2022) also reported that saponins accelerate gastric ulcer healing by reducing the expression of pro-inflammatory factors such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. (Shi *et al.*, 2022) Other compounds, including tannins, steroids, and alkaloids, also contribute to the repair of gastric mucosal damage through their antioxidant mechanisms. (Demarque *et al.*, 2018)

Based on this analysis, it can be concluded that gastric mucosal epithelial damage can be repaired by secondary metabolites contained in Binahong leaf extract (*Anredera cordifolia*), particularly flavonoids, saponins, tannins, steroids, and alkaloids, which play important roles as antioxidant compounds.

## CONCLUSION

Based on the findings of this study investigating the effects of Binahong leaf extract (*Anredera cordifolia*) on gastric histopathological changes in white rats (*Rattus norvegicus*) induced by sodium diclofenac, it can be concluded that the administration of Binahong leaf extract resulted in a significant improvement in the gastric mucosal epithelium in the treatment groups compared with the positive control group that received sodium diclofenac at a dose of 10 mg/kg body weight. This improvement was evidenced by the absence of ulceration and erosion in the gastric mucosa following extract administration.

Furthermore, the results demonstrated distinct differences in the degree of gastric mucosal epithelial damage among rats induced with sodium diclofenac, indicating that diclofenac administration leads to varying histopathological alterations in the gastric mucosa.

Among the tested doses, Binahong leaf extract at a dose of 200 mg/kg body weight was identified as the most effective in improving gastric histopathology in sodium diclofenac-induced rats, suggesting a dose-dependent protective and restorative effect of the extract on the gastric mucosal epithelium.

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