

THE GASTROPROTECTIVE EFFECT OF *Bidens pilosa* L. ETHANOL EXTRACT ON ALCOHOL-INDUCED GASTRIC ULCERS IN RATS: A HISTOPATHOLOGICAL STUDY

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ABSTRACT

The primary objective of this research was to assess the gastroprotective properties of *Bidens pilosa* L. leaf ethanol extract by analyzing gastric histopathology in an alcohol-induced ulcer model in albino rats (*Rattus norvegicus*). The research was conducted experimentally using a completely randomized design involving five groups. The rats in K- group received 0.5% carboxymethyl cellulose (CMC), while the rats in the K+ group were given 30% alcohol. Groups P1, P2, and P3 received 30% alcohol combined with ethanol extract of *Bidens pilosa* at doses of 250, 500, and 750 mg/kg BW, respectively. Alcohol was administered orally at a dosage of 10 mL/kg BW for 43 consecutive days. The observed parameters included histopathological features of the gastric tissue, specifically mucosal erosion, inflammatory cell infiltration, and necrosis. The results demonstrated that the extract significantly reduced alcohol induced gastric tissue damage, as indicated by lower erosion scores and inflammatory cell infiltration ($P \leq 0.05$). Necrosis was not observed in any group, including in the K+ group. In conclusion, *Bidens pilosa* extract possesses potential gastroprotective properties, likely mediated by anti-inflammatory and cytoprotective mechanisms.

Key words: alcohol, *Bidens pilosa*, gastric histopathology, gastroprotective, mucosal erosion

ABSTRAK

Penelitian ini bertujuan mengevaluasi aktivitas gastroprotektif ekstrak etanol daun ketul (*Bidens pilosa* L.) berdasarkan gambaran histopatologi lambung tikus putih (*Rattus norvegicus*) yang diinduksi alkohol. Penelitian dilakukan secara eksperimental menggunakan rancangan acak lengkap dengan lima kelompok. Tikus pada kelompok K- menerima karboksimetil selulosa 0,5 %, tikus pada kelompok K+ diberi alkohol 30%. Kelompok P1, P2, dan P3 masing-masing menerima alkohol 30% yang dikombinasikan dengan ekstrak etanol *Bidens pilosa* pada dosis 250, 500, dan 750 mg/kg BB. Alkohol diberikan dengan dosis 10 ml/kg BB secara oral selama 43 hari. Parameter yang diamati meliputi gambaran histopatologi lambung berupa erosi mukosa, infiltrasi sel radang, dan nekrosis. Hasil penelitian menunjukkan bahwa pemberian ekstrak etanol daun ketul mampu menurunkan tingkat kerusakan jaringan lambung akibat alkohol, ditunjukkan dengan penurunan signifikan pada skor erosi dan infiltrasi sel radang ($P \leq 0,05$). Nekrosis tidak teramati pada seluruh kelompok, termasuk kontrol positif. Temuan ini menunjukkan bahwa ekstrak *B. pilosa* memiliki potensi sebagai agen gastroprotektif melalui mekanisme antiinflamasi dan sitoprotektif.

Kata kunci: alkohol, *Bidens pilosa*; histopatologi lambung, gastroprotektif, erosi mukosa

INTRODUCTION

Alcohol exhibits corrosive properties and can impair the gastric mucosa through several mechanisms such as decreasing the secretion of protective mucus, increasing gastric acid levels, and promoting the buildup of free radicals that induce oxidative stress. These conditions result in gastritis, an inflammation of the gastric mucosa characterized by mucosal erosion, inflammatory cell infiltration, and tissue necrosis. Without adequate treatment, gastritis may progress to more serious disorders, including peptic ulcers, gastrointestinal hemorrhage, or gastric cancer, thereby elevating the risk of mortality.

The global incidence of gastritis is estimated at 1.8 to 2.1 million annual cases, with prevalence varying significantly worldwide. Reported national rates range from a high of 35% in Canada to a low of 14.5% in Japan, with the United Kingdom, France, and China reporting 22%, 29.5%, and 31%, respectively. In Southeast Asia, the incidence is reported to reach 583,635 cases per year, including 274,396 cases in Indonesia out of a population of 238,452,952 (Putra and Wardhani 2023).

Epidemiological studies show that gastritis remains widespread globally and also prevalent in Indonesia, with alcohol recognized as a key contributing factor.

Approximately 10% of ingested alcohol is absorbed in the stomach, while the remainder is absorbed in the small intestine (Rohmani *et al.* 2024). Alcohol is metabolized into acetaldehyde and acetate, leading to the generation of Reactive Oxygen Species (ROS). The increased ROS levels compromise the gastric mucosal defense, trigger cell death, and cause structural damage such as mitochondrial swelling and cytochrome c release. Alcohol induced damage can be assessed histologically through the presence of mucosal erosion, inflammation, and necrosis.

Protective agents are required to restore the balance between aggressive and defensive factors in the gastrointestinal system. One emerging approach involves the use of herbal plants with gastroprotective properties. *Bidens pilosa* L. (*B. pilosa*), known as ketul or ajeran, is a member of the Asteraceae family that has long been used in traditional medicine for treating digestive disorders. This plant has been widely used in herbal medicine, with all parts demonstrating effectiveness in treating various conditions such as malaria, influenza, cancer, inflammation, wounds, metabolic syndrome, immunological disorders, digestive issues, and infections. Due to its antioxidant activity, *B. pilosa* is recognized globally as a valuable phytotherapeutic agent (Angelini *et al.* 2021).

Phytochemical analyses have identified various bioactive compounds in this plant, including polyacetylenes, polyacetylene glycosides, auronones, aurone glycosides, p-coumaric acid derivatives, caffeoylquinic acid derivatives, flavonoids, and flavonoid glycosides, sesquiterpenes, acetylacetone, phenylheptadiynol, phenylpropanoid glycosides, pheophytin, and diterpenes (Wahyuddin *et al.* 2020). It is believed that plants with high antioxidant content can effectively combat free radicals and protect the stomach lining from oxidative damage. A study by Salem *et al.* (2023) demonstrated that *B. pilosa* extract ameliorated gastric mucosal damage induced by indomethacin, as evidenced by enhanced tissue regeneration and reduced inflammatory cell infiltration. Another study by Tan *et al.* (2000) further confirmed the strong anti-ulcerogenic activity of methanol extract of *B. pilosa* leaves against HCl/ethanol-induced ulcer models.

Despite the established presence of bioactive constituents in *B. pilosa*, the gastroprotective efficacy of its ethanolic leaf extract against alcohol-induced gastritis remains unexplored. Therefore, this research investigates the gastroprotective activity of the ethanol extract of *B. Pilosa* by evaluating alcohol-induced histological changes in rat gastric tissue. The findings of this study are expected to contribute scientifically to the development of *B. pilosa* as a promising natural phytotherapeutic agent for protecting the gastric mucosa from alcohol-induced toxicity.

MATERIALS AND METHODS

Experimental Animals and Study Design

This study used 25 male Wistar rats (*Rattus norvegicus*) which were obtained from the Pharmacy Laboratory of Universitas Sumatera Utara. Eligible rats (2-3 months old, 150-200 g) were randomly assigned to one of five treatment groups. The rats in K- group received 0.5% carboxymethyl cellulose (CMC), while the rats in the K+ group were given 30% alcohol. Groups P1, P2, and P3 received 30% alcohol combined with ethanol extract of *B. pilosa* at doses of 250, 500, and 750 mg/kg BW, respectively.

The ethanol extract of *B. pilosa* was administered orally once daily for 43 consecutive days, one hour after alcohol administration. On the 44th day, the rats were euthanized. The animals were anesthetized with chloroform in accordance with institutional animal care guidelines. The stomach was surgically excised, rinsed with 0.9% NaCl solution, blotted dry, and weighed using a digital balance to obtain its absolute weight. The tissues were immediately fixed in 10% Buffered Neutral Formalin (BNF) for histopathological evaluation.

Histopathological Examination

The stomach tissues were fixed in 10% NBF, processed using the paraffin-embedding technique, stained with Hematoxylin-Eosin (HE), and observed under a light microscope. Tissue damage was assessed based on mucosal erosion, inflammatory cell infiltration, and necrosis at 400× magnification across

five fields of view. Damage scoring was performed using semi-quantitative criteria. Mucosal erosion was evaluated according to the Barthel (2003) gastric mucosal damage scoring system. The scoring criteria for inflammatory cell infiltration and necrosis were as follows: 0= no observable change; 1= focal changes; 2= multifocal changes; and 3= diffuse changes.

Data Analysis

The histopathological scores were tabulated and analyzed using one-way Analysis of Variance (ANOVA). If significant differences were detected ($P \leq 0.05$), post-hoc analysis was performed using Duncan's Multiple Range Test (DMRT) at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The administration of ethanol extract of *B. pilosa* aimed to evaluate its gastroprotective activity based on histological changes in the gastric tissue of white rats. Histopathological observations across the treatment groups revealed cellular alterations, including mucosal erosion, inflammatory cell infiltration, and necrosis.

Observations in the normal control group (K-) revealed normal histological architecture of the gastric mucosa, with intact surface epithelium and no signs of damage such as desquamation, erosion, or ulceration. In contrast, the K+ group showed marked structural alterations, including desquamation, mucosal erosion, and ulceration, indicating significant alcohol-induced injury. In the treatment groups (P1, P2, and P3), varying degrees of mucosal damage were observed across several microscopic fields. Among these, group P2 exhibited the mildest erosion compared to P1 and P3.

A post-hoc analysis using DMRT was then conducted to identify which groups showed statistically significant differences in gastric mucosal erosion scores. The results showed that the K+ exhibited the highest mean erosion score (1.66 ± 0.39), which was significantly different from the normal control group (K-) (0.64 ± 0.47 ; $P \leq 0.05$), indicating the successful induction of gastric mucosal injury by 30% alcohol (Table 1).

Treatment groups P1 (250 mg/kg BW) and P3 (750 mg/kg BW) showed relatively high erosion scores (1.46 ± 0.47 and 1.34 ± 0.58 , respectively), with no statistically significant difference compared to the K+ group, suggesting that these doses did not provide optimal gastroprotection. In contrast, group P2 (500 mg/kg BW) demonstrated a substantial reduction in erosion score (0.76 ± 0.42), which was not significantly different from the normal control but differed significantly from the K+ group. This finding indicates a protective effect at the 500 mg/kg BW dose.

Gastric mucosal erosion represents an early stage of gastric injury, characterized by the loss of epithelial integrity. This condition is typically induced by exposure to ethanol, nonsteroidal anti-inflammatory drugs (NSAIDs), or oxidative stress. According to Usman (2016), erosion involves the disruption of the

epithelial lining of the gastric mucosa, whereas ulcers represent more severe tissue damage involving the epithelium, lamina propria, and muscularis mucosae, sometimes extending to the muscularis externa. Ethanol acts as a gastric irritant by disrupting the lipid bilayer and damaging epithelial membrane proteins, thereby triggering superficial necrosis and ulceration (Kim *et al.* 2020).

The highest erosion score observed in group K+ supports the effectiveness of the alcohol-induced ulcer model. This finding is consistent with a study by Pratama (2021), which demonstrated that administration of 14% ethanol at a dose of 2.4 mL for 14 days caused gastric mucosal damage, including epithelial desquamation, erosion, ulceration, and inflammatory infiltration. Similarly, Rohmani *et al.* (2024) reported that administration of 40% ethanol for 30 days resulted in epithelial erosion, ulceration, and desquamation.

The high erosion scores in groups P1 and P3, which were not significantly different from K+, indicate that the 250 mg/kg BW and 750 mg/kg BW doses of *B. pilosa* extract were ineffective in providing mucosal protection. In Contrast, the 500 mg/kg BW dose (P2) yielded a lower erosion score (0.76 ± 0.42), similar to K-,

indicating relatively intact mucosal structure. This protective effect is likely attributable to the presence of bioactive compounds in *B. pilosa*, particularly flavonoids and phenolic compounds with antioxidant properties.

These findings corroborate those of Meutia (2018), who emphasized that the efficacy of gastroprotective agents is significantly influenced by both dosage and the bioactivity of their constituent compounds. A low dose (P1) may be insufficient to elicit optimal protective effects, whereas a high dose (P3) could potentially trigger adverse outcomes due to metabolic imbalance. Calabrese (2008) describes this phenomenon as hormesis, where low doses of bioactive or toxic substances exert beneficial effects, while higher doses may become detrimental. In the context of bioactive compounds, optimal doses can protect cells and support health, whereas excessive doses may become pro-oxidative and induce oxidative damage.

Salem *et al.* (2023) also demonstrated that *B. pilosa* extract significantly reduced the area and depth of gastric erosion induced by indomethacin in a rat model, primarily by enhancing mucosal thickness and reducing epithelial permeability. According to Khanal *et al.*

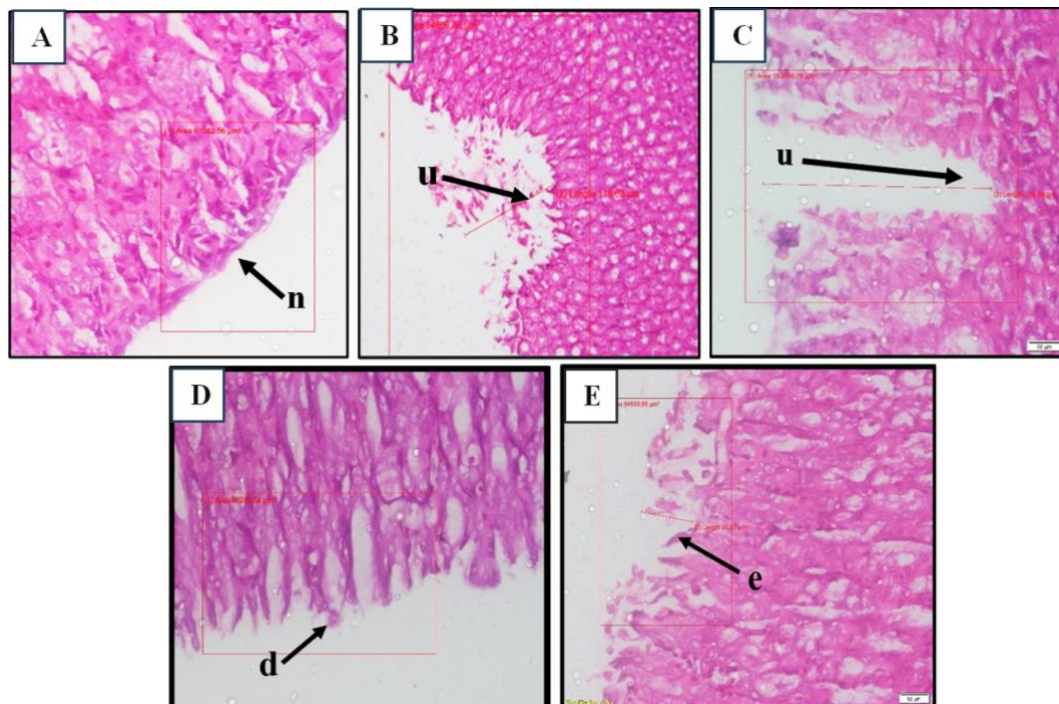


Figure 1. Histopathological features of gastric mucosa erosion in experimental rats (HE 400 $\times$). A= Normal control group (K-) showing intact mucosa (n= Normal); B= K+ group induced with alcohol showing severe ulceration and epithelial damage (u= Ulceration); C= Treatment group P1 (alcohol + 250 mg/kgBW *Bidens pilosa* extract) showing prominent ulceration (u= Ulceration); D= Treatment group P2 (alcohol + 500 mg/kgBW *Bidens pilosa* extract) showing mild epithelial desquamation (d= Desquamation); E= Treatment group P3 (alcohol + 750 mg/kgBW *Bidens pilosa* extract) showing moderate mucosal erosion (e= Erosion)

Table 1. Mean score of gastric mucosal erosion ($\pm$ SD) in different treatment groups of alcohol-induced white rats (*Rattus norvegicus*)

Group	Mean $\pm$ SD
K- (Normal control group)	0.64 $\pm$ 0.47 ^a
K+ (Induced with alcohol)	1.66 $\pm$ 0.39 ^c
P1 (Alcohol + 250 mg/kg BW <i>Bidens pilosa</i> extract)	1.46 $\pm$ 0.47 ^c
P2 (Alcohol + 500 mg/kg BW <i>Bidens pilosa</i> extract)	0.76 $\pm$ 0.42 ^{ab}
P3 (Alcohol + 750 mg/kg BW <i>Bidens pilosa</i> extract)	1.34 $\pm$ 0.58 ^{bc}

^{a, b, c} Different superscripts within the same column indicate significant differences (P<0.05)

(2019), *B. pilosa* contains flavonoids, alkaloids, tannins, terpenoids, and saponins. Flavonoids act as cytoprotective agents through various mechanisms, such as scavenging free radicals, enhancing mucus production, and exerting antisecretory activity (Widyaningsih *et al.* 2018). Alkaloids reduce gastric acid secretion, promote mucus and bicarbonate secretion, and improve mucosal blood flow to support healing and prevent ulcers (Susanto *et al.* 2021). Saponins contribute to mucosal protection by stabilizing epithelial cell membranes and suppressing lesion formation. Additionally, alkaloids inhibit gastric acid secretion, enhance mucus and bicarbonate secretion, and improve gastric mucosal blood flow, thereby facilitating healing and preventing peptic ulcers induced by irritation (Nofianti *et al.* 2024).

Inflammatory Cell Infiltration and Necrosis

Inflammatory cell infiltration is a local immune response to cellular damage and oxidative stress induced by toxic agents. Ethanol acts as an irritant capable of stimulating the release of pro-inflammatory cytokines such as TNF- α and IL-1 β , and activating the NF- κ B signaling pathway, which promotes the accumulation of macrophages, lymphocytes, and neutrophils in the gastric mucosa (Zhu *et al.* 2018).

Histopathological evaluation of inflammatory cell infiltration was based on the presence of inflammatory cells within the gastric tissue. In the normal control group (K-), no inflammatory cell infiltration was observed, indicating the absence of inflammatory responses. In contrast, the K+ group exhibited diffuse infiltration of inflammatory cells in the mucosa and submucosa, representing a clear response to alcohol-induced irritation, although necrosis was not observed.

In treatment group P1, infiltration appeared in both multifocal and diffuse patterns, suggesting that the inflammatory process was still active. Group P2 showed limited, focal infiltration with most tissue appearing histologically normal, indicating an optimal protective effect of the extract at this dose. Group P3 still exhibited focal and multifocal infiltration, though less severe than K+, but not as effectively reduced as in P2. Notably, no necrosis was detected in any of the groups, suggesting that the extent of tissue injury remained within reversible limits. These findings are illustrated in Figure 2. The observation and assessment of inflammatory cell infiltration and necrosis were conducted using a damage scoring system adapted from Oktaviandari (2020). The histopathological findings of the gastric tissues are presented in Table 2.

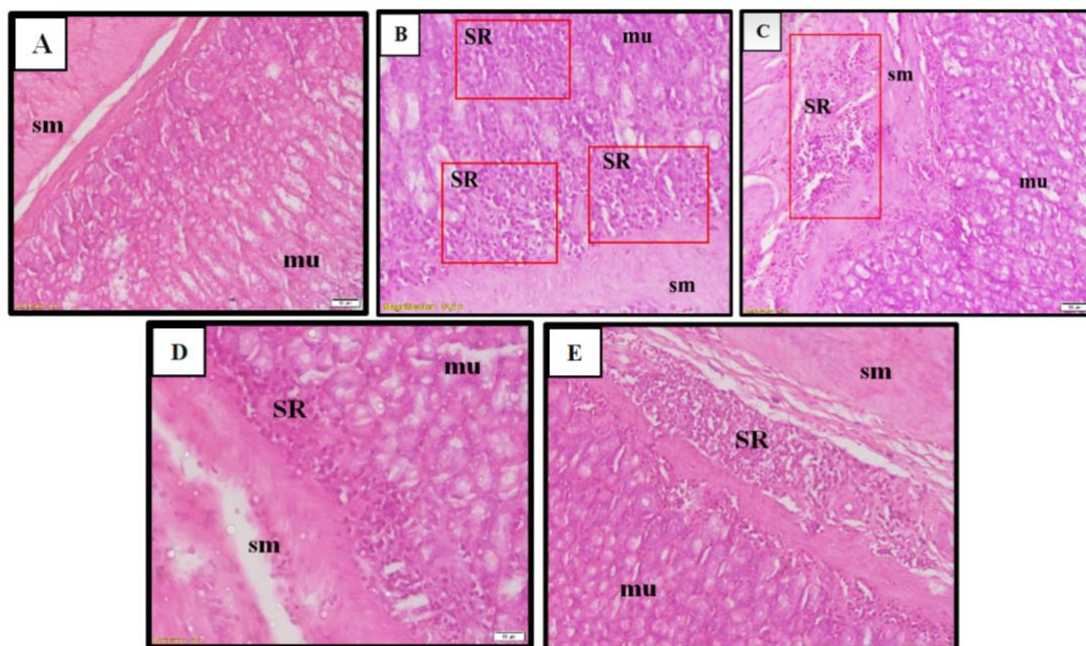


Figure 2. Histopathological appearance of gastric mucosal erosion in white rats (*Rattus norvegicus*) across treatment groups (HE 400 $\times$). A= Normal control group (K-); B= K+ group induced with 30% alcohol showing marked mucosal damage and infiltration of inflammatory cells (SR); C= Group P1 (alcohol + 250 mg/kg BW *B. pilosa* extract); D= Group P2 (alcohol + 500 mg/kg BW *B. pilosa* extract); E= Group P3 (alcohol + 750 mg/kg BW *B. pilosa* extract); mu= Mucosa; sm= Submucosa; SR= Inflammatory cells

Table 2. Mean scores of gastric inflammatory cell infiltration and necrosis ($\pm$ SD) in different treatment groups of alcohol-induced white rats (*Rattus norvegicus*)

Group	Inflammatory cell infiltration $\pm$ SD	Necrosis $\pm$ SD
K- (Normal control group)	0.48 $\pm$ 0.33 ^a	0.00 $\pm$ 0.00
K+ (Induced with alcohol)	1.92 $\pm$ 0.53 ^c	0.00 $\pm$ 0.00
P1 (Alcohol + 250 mg/kg BW <i>Bidens pilosa</i> extract)	1.22 $\pm$ 0.58 ^b	0.00 $\pm$ 0.00
P2 (Alcohol + 500 mg/kg BW <i>Bidens pilosa</i> extract)	0.50 $\pm$ 0.31 ^a	0.00 $\pm$ 0.00
P3 (Alcohol + 750 mg/kg BW <i>Bidens pilosa</i> extract)	1.18 $\pm$ 0.51 ^b	0.00 $\pm$ 0.00

^{a, b, c} Different superscripts within the same column indicate significant differences (P<0.05)

The highest infiltration score was found in the K+ group, with a mean of 1.92 ± 0.53 , which was significantly different from all other groups ($P \leq 0.05$). Histological analysis revealed diffuse infiltration of inflammatory cells within the mucosa and submucosa, indicating severe inflammation induced by alcohol exposure. P1 and P3 group exhibit moderate infiltration scores of 1.22 ± 0.58 and 1.18 ± 0.51 , respectively. The infiltration pattern was predominantly multifocal but was less severe than in K+, suggesting a partial anti-inflammatory effect of *B. pilosa* extract at these doses, although not optimal.

The most prominent protective effect was observed in group P2, which showed an infiltration score of 0.50 ± 0.31 and statistically comparable to the normal control group (K-) with a score of 0.48 ± 0.33 . This suggests that *B. pilosa* extract at this dose was effective in suppressing inflammatory responses in the gastric mucosa. Previously, Silitonga *et al.* (2024) demonstrated that *B. pilosa* extract improved liver histopathology and lipid profiles in alcohol-induced rats. *B. pilosa* contains major flavonoids, including quercetin, luteolin, and apigenin, which exhibit potent antioxidant and anti-inflammatory activities, while cytopiloyne functions as an immunomodulator that supports tissue regeneration (Windah *et al.* 2025).

Flavonoids are known to inhibit inflammation by suppressing the activity of *Cyclooxygenase-2* (COX-2) and *Lipoxygenase* (LOX), which are involved in the synthesis of prostaglandins and leukotrienes, thereby reducing the levels of pro-inflammatory mediator prostaglandin E2 (PGE2) (Al-Khayri *et al.* 2022). Saponins and alkaloids act by downregulating the transcription factor NF- κ B (*nuclear factor kappa-light-chain-enhancer of activated B cells*). NF- κ B activation leads to increased expression of pro-inflammatory cytokines such as TNF- α and IL-6. Alkaloids have also been shown to reduce IL-1 β and IL-6 levels, suggesting their potential role as anti-inflammatory agents through immune response modulation (Zhu *et al.* 2018).

Phenolic compounds contribute to anti-inflammatory activity by reducing free radical production and inhibiting inflammatory signaling pathways such as the Mitogen-Activated Protein Kinase (MAPK) pathway. This pathway regulates cellular responses including proliferation, differentiation, and apoptosis. By blocking MAPK activation, phenolics can suppress pro-inflammatory cytokines while promoting the expression of anti-inflammatory mediators (Liu *et al.* 2023).

Necrosis is an indicator of severe tissue damage, characterized by irreversible cell death resulting from unregulated inflammation or oxidative stress. In the present study, necrosis was not observed in any of the groups, including the K+ as shown in Table 2. This suggests that the duration and dose of alcohol exposure in this model were insufficient to induce irreversible cellular injury. The absence of necrosis may also reflect the preservation of structural tissue integrity and the ongoing regenerative processes in the gastric mucosa.

Necrotic cells typically exhibit significant morphological changes, particularly in the nucleus and

cytoplasm. The nucleus undergoes condensation and appears dark blue (*pyknosis*) due to chromatin clumping, followed by fragmentation (*karyorrhexis*) or complete dissolution (*karyolysis*) (Riong 2022).

The absence of necrosis in all groups, including the K+ group, suggests that alcohol exposure in this study caused only reversible tissue damage. This is likely due to the gastric mucosa's inherent capacity to repair mild to moderate injury, a finding consistent with that of Kololu *et al.* (2014). Similarly, the lack of necrosis in *B. pilosa* treated groups indicates its low cytotoxicity and safety, as necrosis signifies irreversible damage, aligning with Oktaviandari *et al.* (2020).

CONCLUSION

The ethanol extract of *Bidens pilosa* L. demonstrates significant gastroprotective efficacy against alcohol-induced damage. A dose of 500 mg/kg BW was identified as optimal dose, significantly reducing gastric mucosal erosion and inflammation.

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