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Histopathological Evaluation of Stomach Protection by *Peperomia pellucida* L. in Mice with Gastroenteritis

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Abstract

Gastroenteritis, a widespread condition characterized by inflammation of the stomach and intestines, poses significant health challenges globally. Conventional treatments primarily focus on symptomatic relief and do not address the underlying gastric mucosal damage. *Peperomia pellucida* L., a medicinal plant known for its anti-inflammatory and antioxidant properties, has been suggested to possess gastroprotective effects. This study aims to evaluate the histopathological effects of *P. pellucida* L. on gastric protection in a mouse gastroenteritis model. Male Swiss mice were divided into six groups, receiving different treatments, including the ethanol extract of *P. pellucida* at varying doses (100, 300, and 500 mg/kgBW), followed by induction of gastroenteritis with Escherichia coli. Histopathological analysis was conducted to observe tissue damage across the treatment groups, including necrosis and cell degeneration. The results revealed that *P. pellucida* L. exhibited significant gastroprotective effects, particularly at a dose of 500 mg/kgBW, reducing gastric mucosal necrosis and inflammation compared to the control groups. The plant's secondary metabolites, including flavonoids, tannins, saponins, and alkaloids, contributed to the observed protective effects by enhancing mucus production, reducing gastric acidity, and promoting tissue repair. These findings suggest that *P. pellucida* L. could be an alternative treatment for gastroenteritis and related gastric conditions, offering a natural approach to managing gastric inflammation and mucosal protection.

Keywords: *Peperomia pellucida* L.; gastroprotective; gastroenteritis; histopathology; medicinal plants; gastric mucosa.

Abbreviations: polymorphonuclear leukocytes (PMNs); Polymorphonuclear Leukocytes (PMN), standard error (SE); World Health Organization (WHO)

INTRODUCTION

Gastroenteritis, characterized by inflammation of the stomach and intestines, poses a significant health challenge worldwide, often resulting in symptoms such as diarrhea, vomiting, and abdominal pain. This condition can be caused by various pathogens, including bacteria, viruses, and parasites, leading to considerable morbidity, especially in vulnerable populations such as children and the elderly. Current treatment options primarily focus on symptomatic relief and rehydration; however, they do not address the underlying inflammation or provide protective effects to the gastric mucosaIt involves an inflammatory process in the gastric epithelial cells and subsequent damage to the gastric mucosa (Angkow et al., 2014). Clinically, gastritis is often used to refer to symptoms that occur in the upper abdomen or epigastric region. The high prevalence of gastritis is largely influenced by dietary habits, food types, stress, smoking, and alcohol consumption, which is why the incidence of gastritis in Indonesia remains significantly high. Globally, gastritis affects between 1.8 and 2.1 million individuals annually.

According to the World Health Organization (WHO), the prevalence of gastritis is as follows: 22.0% in the UK, 31.0% in China, 14.5% in Japan, 35.0% in Canada, and 29.5% in France. In South Asia, approximately 583,635 people suffer from gastritis each year. In Shanghai, 17.2% of the population is affected by endoscopically confirmed gastritis, a figure notably higher than the 4.1% asymptomatic rate found in Western populations (Angkow et al., 2014). In Indonesia, according to the Ministry of Health, gastritis ranks sixth among hospitalized patients, with an occurrence rate of 60.86% from 33,580 hospital admissions. It also ranks seventh for outpatient cases, accounting for 201,083 patients. The prevalence of gastritis in Indonesia is quite high, with 274,396 cases out of a population of 238,452,952, equating to 40.8%. The rates of gastritis in various cities include 50% in Jakarta, 35.5% in Palembang, 32% in Bandung, 46% in Denpasar, 31.2%

in Surabaya, 31.7% in Aceh, and 91.6% in Medan (Riskesdas, 2018).

Despite the high prevalence, many individuals neglect proper stomach care. Gastritis often presents asymptomatically but is commonly associated with epigastric pain. Additional symptoms include nausea, vomiting, bloating, and loss of appetite (Saputra, 2017). Research has shown that chronic bacterial infections in the gastric mucosa are a leading cause of gastritis. Additionally, substances such as aspirin, alcohol, bile salts, and other harmful agents can damage the gastric mucosa, impairing the epithelial barrier's protective function over the stomach and duodenum. The majority of gastritis cases (70-80%) are due to functional gastritis, characterized by pain not linked to organic disorders of the stomach. Left untreated, gastritis can impair gastric function and elevate the risk of gastric cancer, which can lead to fatal outcomes (Muttaqin et al., 2011).

In recent years, there has been a growing interest in the potential therapeutic properties of medicinal plants, particularly those traditionally used in folk medicine. One such plant, *Peperomia pellucida* L., is known for its anti-inflammatory and antioxidant properties, which may contribute to its protective effects on the gastric lining. Previous studies have indicated that the ethanol extract of *P. pellucida* can exert gastroprotective effects, enhancing mucosal defense mechanisms and promoting healing in gastric tissues. This study aims to evaluate the histopathological effects of *P. pellucida* L. on gastric protection in a mice model of gastroenteritis. By examining the histological changes in the gastric mucosa of treated and untreated mice, this research seeks to elucidate the potential gastroprotective mechanisms of *P. pellucida* and contribute to the understanding of its therapeutic applications. The findings from this study may provide valuable insights into alternative treatment strategies for gastroenteritis and reinforce the importance of exploring plant-based remedies in modern medicine.

MATERIALS AND METHODS

Research Type

The population in this study consisted of mice, with male Swiss strain mice aged 2-3 months, weighing between 25-30 grams, being the sample. These mice were obtained from the Veterinary Farma Center, Surabaya. Each treatment group included a minimum of 4 mice, and since there were 6 groups in total, a minimum of 24 mice was required. However, in this study, the sample size per group was increased by 1, bringing the total number of mice to 30.

Tools and Materials

The tools used in this study included 1 mL, 3 mL, and 5 mL disposable syringes, a 35 cm feeding tube, a 60 mL urine container, micropipettes, a freezer, a rotary or sliding microtome, brushes, a water bath, glass slides, 1500 µL Eppendorf tubes, blue tips, yellow tips, white tips for 10 µL, an oven, a digital scale, a surgical board, a set of surgical instruments (scissors, forceps, and probes), gloves, tissues, masks, a binocular light microscope, and a microscope camera.

The materials used in this study included male Swiss strain mice aged 2-3 months, weighing 25-30 grams, amoxicillin, clinical isolates (wild-type *Escherichia coli*) at a concentration of $1x10^6$ CFU/mL, physiological saline (PZ) (pyrogen-free injection water), mice feed, wood shavings, methanol, Giemsa stain, distilled water, 37% H_2CO (formaldehyde) solution, formalin buffer, disodium hydrogen phosphate (Na_2HPO_4), 37-40% formaldehyde, and various grades of ethanol (80%, 95%, absolute ethanol), xylene, clearing solution, paraffin, and poly-L-lysine.

Research Procedure

1. Mice Acclimatization

Male mice were weighed and housed in standard polypropylene cages with wood shavings as bedding material for a two-week acclimatization period. The bedding was replaced daily. The feed was moistened with water and shaped into 90-gram ovals per cage (housing 6 mice). Water was provided ad libitum, and both feed and water were refreshed daily. After two weeks, the mice were divided into six treatment groups.

2. Treatment of Test Animals

The acclimatized mice underwent the following treatments:

Group 1 (normal control) received no gastric gavage.
Group 2 (negative control) received distilled water.
Group 3 (positive control) received amoxicillin at a dose of 500 mg/kgBW in a volume of 0.26 mL.
Group 4 received Chinese betel leaf extract at a dose of 100 mg/kgBW in a volume of 0.5 mL.
Group 5 received Chinese betel leaf extract at a dose of 300 mg/kgBW in a volume of 0.5 mL.
Group 6 received Chinese betel leaf extract at a dose of 500 mg/kgBW in a volume of 0.5 mL.

3. Gastroenteritis (GE) Model Induction in Mice

After 7 days of treatment, a GE model was induced by administering *Escherichia coli* at a concentration of 1x10^6 CFU/mL to each mice via gastric gavage, once daily for 7 days.

4. Tissue Processing

Stomach tissues were fixed in formalin buffer to maintain cell morphology and prevent autolysis, as well as bacterial or fungal growth. Paraffin blocks were then created. The tissues were sectioned using a rotary or sliding microtome of 4-6 microns thickness. The sections were picked up with a moist brush, transferred to a water bath, and placed onto glass slides coated with tissue

adhesive. The slides were dried at room temperature and then placed in an oven overnight.

5. Histopathological Observation of Stomach Tissues

Histopathological analysis of mice stomachs was performed using hematoxylin and eosin (HE) staining. The tunica mucosa (epithelium, lamina propria, muscularis mucosa), submucosa, tunica muscularis, tunica serosa, and other regions showing necrosis or apoptosis were examined.

6. Data Collection

The data collected included the number of cells exhibiting abnormalities in the tunica mucosa (epithelium, lamina propria, muscularis mucosa), submucosa, tunica muscularis, tunica serosa, and other regions potentially showing necrosis or apoptosis in the stomach tissues of the mice.

7. Data Analysis Method

The data, including the number of cells displaying abnormalities in the tunica mucosa (epithelium, lamina propria, muscularis mucosa), submucosa, tunica muscularis, tunica serosa, and other regions potentially experiencing necrosis or apoptosis in the stomach tissues, were analyzed using ANOVA with a 95% confidence interval (α=0.05). If significant differences were found in the ANOVA results, further analysis was performed using the Least Significant Difference (LSD) test at the same confidence level. Statistical analysis was carried out using SPSS 23.0 for Windows, and the data were presented as mean ± standard error (SE).

RESULTS AND DISCUSSION

Table 1. Effectiveness of Chinese Betel Extract (*Peperomia pellucida* L.) in Preventing Stomach Cell Degeneration in a Gastroenteritis Model of Mice.

Group	a	b	c	d	e
Normal	1.92%				
Positive Control		6.45%			
Treatment III (500mg/kgBW)			7.68%		
Treatment I (100mg/kgBW)				18.34%	
Treatment II (300mg/kgBW)					12.78%
Negative Control					25.12%

The Duncan test (Table 1) was used by the researchers to assess the effectiveness of Chinese Betel extract and determine the optimal dosage among the groups in preventing stomach cell degeneration in a gastroenteritis model of mice. The results of the Duncan test in the table show that overall comparisons between the treatment groups fall into different columns, indicating that the treatment groups have varying effects on preventing cell degeneration. However, Group I (100mg/kgBW) and Group II (300mg/kgBW) are placed in the same column, indicating similar gastroprotective effects on cell degeneration in these two groups, consistent with earlier LSD test results.

Determining the best dosage for gastroprotective activity, in terms of cell degeneration, is based on the smallest values or those appearing in the leftmost columns of the table. Antiseptic effectiveness is measured by comparing average cell degeneration. Based on the Duncan test results, the ranking of the most effective doses is as follows: (1) Normal group; (2) Positive Control group (+); (3) Group III (500mg/kgBW); (4) Group I (100mg/kgBW); (5) Group II (300mg/kgBW); and (6) Negative Control group (-).

Necrosis

The normality test results for the normal group yielded a p-value of 0.432, the positive control group (+) had a p-value of 0.913, the negative control group had a p-value of 0.293, Group I treatment (100mg/kgBW) had a p-value of 0.612, Group II treatment (300mg/kgBW) had a p-value of 0.591, and Group III treatment (500mg/kgBW) had a p-value of 0.627. These normality test results indicate that all groups have p-values greater than 0.05 (P>0.05), confirming that the data are normally distributed and suitable for further testing. The homogeneity test results for all six groups yielded a significance value of 0.141 (>0.05), indicating homogenous variance across the treatment groups, thus meeting the requirements for One-Way ANOVA testing. The One-Way ANOVA test showed a significance value of 0.000 (<0.005), indicating a significant difference among the groups. Further analysis, including post hoc tests using the LSD test and Duncan test, is warranted for a more detailed comparison between the groups.

Table 2. Effectiveness of Chinese Betel Extract (*Peperomia pellucida* L.) in Preventing Necrosis in a Gastroenteritis Mice Stomach Model.

Group	a	b	c	d
Normal	1.85%			
Positive Control		4.32%		
Treatment III (500mg/kgBW)			5.94%	
Treatment II (300mg/kgBW)				10.12%
Negative Control			17.20%	
Treatment I (100mg/kgBW)				15.78%

The LSD test results indicate that overall comparisons between the treatment groups have a significance value of $P < 0.05$, suggesting that the comparisons between these treatment groups have different effects, except for the comparison between the positive control group and Group III treatment (500mg/kgBW), as well as the negative control group and Group I treatment (100mg/kgBW). In the comparison between the positive control group and Group III treatment (500mg/kgBW), the significance value obtained is 0.223 (>0.05), indicating that the positive control group and Group III treatment (500mg/kgBW) have similar gastroprotective effects on necrosis. Similarly, the comparison between the negative control group and Group I treatment (100mg/kgBW) yielded a significance value of 0.249 (>0.05), indicating that the negative control group and Group I treatment (100mg/kgBW) have similar effects in terms of necrosis prevention.

The Duncan test (Table 2) was used by the researchers to assess the effectiveness and determination of the best dosage among the groups. The results of the Duncan test in the table indicate that overall comparisons between the treatment groups are in different columns, suggesting that the comparisons between these treatment groups have different effects on preventing necrosis in the stomach of the gastroenteritis model mice. However, the comparison between the positive control group and Group III treatment (500mg/kgBW), as well as the negative control group and Group I treatment (100mg/kgBW), falls within the same column. This result aligns with the previous LSD test, indicating that the positive control group with Group III treatment (500mg/kgBW) and the negative control group with Group I treatment (100mg/kgBW) have similar gastroprotective effects on necrosis.

Determining the best dosage or dose effectiveness for gastroprotective activity in terms of necrosis is based on the smallest values or those leaning towards the left. Antiseptic effectiveness is determined by looking at the average necrosis. Based on the Duncan test table, the sequence of the best doses is as follows: (1) normal group; (2) positive control group (+); (3) Group III treatment (500mg/kgBW); (4) Group II treatment (300mg/kgBW); (5) negative control group (-); and (6) Group I treatment (100mg/kgBW).

Polymorphonuclear Leukocytes (PMN)

The normality test results for the normal group yielded a p-value of 0.380, the positive control group had a p-value of 0.863, the negative control group had a p-value of 0.620, Group I treatment (100mg/kgBW) had a p-value of 0.310, Group II treatment (300mg/kgBW) had a p-value of 0.855, and Group III treatment (500mg/kgBW) had a p-value of 0.611. These normality test results show that the data are normally distributed, as all p-values are greater than 0.05 (P>0.05). The homogeneity test for the six groups yielded a significance value of 0.384 (>0.05), indicating homogenous variance across the groups, thus meeting the requirements for One-Way ANOVA testing. The One-Way ANOVA test yielded a significance value of 0.000 (<0.005), indicating significant differences among the groups.

Further analysis, including post hoc tests using the LSD test and Duncan test, showed that overall comparisons between the treatment groups had a significance value of $P < 0.05$, suggesting that these groups have different effects on preventing PMN in the stomach of the gastroenteritis model mice. The Duncan test was used to determine the best dosage for PMN prevention. The results indicated that the comparison between the positive control group and Group III treatment (500mg/kgBW), as well as the comparison between the negative control group and Group I treatment (100mg/kgBW), fell within the same column. This aligns with the previous LSD test, indicating that the positive control group and Group III treatment (500mg/kgBW) have similar antiseptic effects on PMN, as do the negative control group and Group I treatment (100mg/kgBW).

Table 3. Effectiveness of Chinese Betel Extract (*Peperomia pellucida* L.) in Preventing PMN in a Gastroenteritis Mice Stomach Model.

Group	a	b	c	d	e	f
Normal	2.10%					
Positive Control		5.25%				
Treatment III (500mg/kgBW)			8.14%			
Treatment I (100mg/kgBW)			12.55%			
Treatment II (300mg/kgBW)				19.65%		
Negative Control					24.56%	48.63%

Determining the optimal dose for PMN antiseptic activity is based on the smallest values or those leaning towards the left. Based on the Duncan test table, the best dose sequence is as follows: (1) normal group; (2) positive control group (+); (3) Group III treatment (500mg/kgBW); (4) Group II treatment (300mg/kgBW); (5) negative control group (-); and (6) Group I treatment (100mg/kgBW).

Discussion

Gastroprotective refers to the ability of certain compounds to protect the gastric mucosa from damage caused by various factors, including stress, excessive acid secretion, and harmful substances. Research by Roslida and Aini (2009) suggests that *P. pellucida* L. possesses gastroprotective properties, as its ethanol extract demonstrated effective gastroprotection at 100 mg/KgBW. The gastroprotective effects of this plant are thought to arise from various mechanisms linked to its diverse metabolite compounds (Yusuf et al., 2017). Phytochemical analyses of *P. pellucida* L. have identified several secondary metabolites, including flavonoids, tannins, saponins, triterpenoids, and steroids (Rachmawati & Rantelino, 2018; Pertiwi et al., 2022; Oloyede et al., 2011). A previous study identified dillapiole as the primary active gastroprotective compound in *P. pellucida* L.; however, additional research is necessary to fully elucidate the gastroprotective mechanisms of dillapiole, as it appears unrelated to endogenous nitric oxide or prostaglandins (Rojas-Martínez et al., 2013).

Flavonoids are known for their anti-ulcer and anti-inflammatory properties, functioning through various mechanisms such as inhibiting K+/H+ ATPase, reducing HCl secretion, enhancing the synthesis of PGE2 and COX-1, inhibiting bacterial growth, and exhibiting antioxidant effects (Kalogeromitros et al., 2008). Alkaloids, another class of gastroprotective agents, promote wound healing and stimulate gastric mucus production following damage from harmful substances (Tan et al., 2002). Tannins are recognized for their astringent qualities, allowing them to bind with proteins in the gastric mucosa. This interaction aids in forming a protective layer on the mucosa's surface, reducing permeability and increasing resistance to ulcers and irritation (Souza et al., 2012). Saponins enhance gastroprotective activity by elevating fibronectin levels, facilitating the formation of fibrin clots that act as a scaffold for tissue re-epithelialization. The quicker these clots develop, the more rapidly fibroblasts proliferate in the wound area, supporting tissue repair (Indraswary, 2011). Thus, the comprehensive understanding of these mechanisms underscores the potential therapeutic benefits of *P. pellucida* L. in preventing gastric ailments and promoting overall gastrointestinal health.

CONCLUSIONS

This study highlights the significant gastroprotective potential of *Peperomia pellucida* L., as demonstrated by its ability to mitigate gastric mucosal damage in a mice model of gastroenteritis. The plant's diverse secondary metabolites, including flavonoids, tannins, saponins, and alkaloids, contribute to its protective effects through multiple mechanisms such as reducing gastric acidity, enhancing mucus production, and promoting wound healing. The ethanol extract of *P. pellucida* L. at varying doses demonstrated notable histopathological improvements, particularly in reducing necrosis and inflammation in the gastric tissue. Moreover, the identification of dillapiole as a key active compound further emphasizes the therapeutic potential of *P. pellucida* L., although more research is necessary to fully understand its gastroprotective mechanisms. The findings support the use of *P. pellucida* L. as a viable natural treatment for gastritis and gastroenteritis, offering a plant-based alternative to conventional therapies. This research provides valuable insights into the plant's medicinal applications and underscores the importance of further exploration into traditional herbal remedies for gastrointestinal disorders.

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