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Immunomodulatory Activity of Chinese Betel (*Peperomia pellucida* L.) Extract on the Spleen Histopathology in a Murine Model of Gastroenteritis

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Abstract

Gastroenteritis, a prevalent digestive disorder caused by various pathogens including Escherichia coli, remains a global health challenge with significant morbidity and mortality, particularly in low-income countries. The spleen, as a critical immune organ, is often compromised during systemic infections. Peperomia pellucida (L.) , a traditional medicinal herb, is known for its antimicrobial, anti-inflammatory, and antioxidant properties. This study investigates the histopathological effects of *P. pellucida* leaf ethanol extract on spleen tissue in mice induced with *E. coli* to model bacterial gastroenteritis. Thirty male Swiss mice were divided into six groups (n=5): normal control, negative control (aquades), positive control (Yakult), and three treatment groups receiving *P. pellucida* extract at 100, 300, and 500 mg/kg BW. After seven days of pretreatment, mice were orally infected with *E. coli* (1×10^6 CFU/mL) for another seven days. Spleen tissues were harvested, fixed, and stained with hematoxylin-eosin for histopathological evaluation focusing on degeneration, necrosis, and PMN infiltration. Statistical analysis was performed using ANOVA followed by LSD tests. The highest dose of *P. pellucida* extract (500 mg/kg BW) significantly reduced spleen tissue damage, showing decreased degeneration (9.08%), necrosis (6.05%), and PMN infiltration (18.45%) compared to lower doses. The effect was comparable to the positive control. The ethanol extract of *P. pellucida* demonstrates a dose-dependent protective effect on spleen histopathology in *E. coli*-induced gastroenteritis in mice, supporting its potential as a natural antiseptic agent.

Keywords: *Peperomia pellucida*; gastroenteritis; *Escherichia coli*; spleen histopathology; antiseptic agent.

Abbreviations: Analysis of Variance (ANOVA); Colony Forming Unit (CFU); *Escherichia coli* (E. coli); Least Significant Difference; (LSD); Polymorphonuclear cells (PMN); *Peperomia pellucida* (P. pellucida); Standard Error (SE); World Health Organization (WHO).

INTRODUCTION

Gastroenteritis, often referred to as stomach flu, is a condition marked by digestive tract inflammation, leading to symptoms such as vomiting, diarrhea, stomach cramps, and sometimes fever (Guerrant et al., 2011). It can be triggered by various infectious organisms, including viruses, bacteria, and parasites, and is most commonly spread through contaminated food or water, or via direct contact with an infected person (Koo et al., 2010).

This illness poses a major global public health challenge, affecting individuals across all age groups and socioeconomic levels. The World Health Organization (WHO) reports that diarrheal diseases affect approximately 1.7 billion people yearly, with gastroenteritis being a leading contributor (World Health Organization, 2017). In high-income countries, viral gastroenteritis accounts for millions of doctor visits and hospital admissions annually, resulting in significant healthcare expenses (Payne et al., 2013). In contrast, in low- and middle-income countries, gastroenteritis remains a top cause of illness and death, especially in children under the age of five (Kotloff et al., 2013).

Gastroenteritis can be caused by various infectious agents, including viruses, bacteria, and parasites. The most common viral causes of gastroenteritis are noroviruses, rotaviruses, adenoviruses, and astroviruses (Glass et al., 2009). Bacterial pathogens associated with gastroenteritis include Campylobacter, Salmonella, Shigella, *Escherichia coli* (particularly enterotoxigenic and Shiga toxin-producing strains), and *Vibrio cholerae*

(Guerrant, et al., 2001). Parasitic agents, such as *Giardia lamblia*, *Cryptosporidium parvum*, and *Entamoeba histolytica*, can also cause gastroenteritis, although their prevalence varies across different geographic regions (Checkley et al., 2015).

The epidemiological patterns of gastroenteritis are influenced by various risk factors and modes of transmission. Person-to-person transmission, particularly through the fecal-oral route, is a common mode of spread for viral and bacterial gastroenteritis (Lopman et al., 2012). Foodborne transmission is also a significant route, with contaminated food and water as vehicles for infectious agents (Newell et al., 2010). Specific risk factors include poor sanitation, inadequate access to safe drinking water, crowded living conditions, and compromised immune systems (Kotloff, 2017).

The geographical distribution of gastroenteritis is global, but the prevalence and predominant causative agents vary across regions. In developed countries, viral gastroenteritis is more common, with noroviruses being the leading cause of outbreaks in healthcare facilities, schools, and cruise ships (Becker et al., 2000). In developing countries, bacterial and parasitic agents are more prevalent, contributing to a substantial disease burden, particularly among young children (Troeger et al., 2018).

The pathogenesis of gastroenteritis varies depending on the causative agent but involves disrupting the normal physiological functions of the gastrointestinal tract. Viral pathogens, such as noroviruses and rotaviruses, primarily target and damage the epithelial cells lining the small intestine (Ramig,, 2004). These viruses can bind to and enter these cells, leading to their destruction and the subsequent malabsorption of fluids and nutrients. The resulting osmotic imbalance and loss of absorptive surface area contribute to diarrhea and vomiting (Bok & Green, 2012).

Bacterial pathogens, like Campylobacter, Salmonella, and Shigella, can cause gastroenteritis through several mechanisms, including toxins, invasion and disruption of the intestinal epithelium, and inflammation. For example, enterotoxigenic *E. coli* (ETEC) secretes heat-labile and heat-stable toxins that disrupt fluid and electrolyte balance, leading to watery diarrhea (Guerrant et al., 2001).

The clinical manifestations of gastroenteritis typically include diarrhea, vomiting, abdominal cramps, and, in some cases, fever. The severity of symptoms can range from mild to severe, depending on the causative agent, the individual's immune status, and other factors (Fleckenstein et al., 2010). Complications of gastroenteritis may include dehydration, electrolyte imbalances, and, in severe cases, sepsis or organ failure (Guerrant et al., 2001).

Many digestive system disorders—such as peptic ulcers, chronic gastritis, upper gastrointestinal bleeding, duodenitis, pseudomembranous enteritis, acute enteritis, intestinal tuberculosis, ulcerative colitis, jaundice, diarrhea, liver cirrhosis, drug-induced liver diseases, chronic and acute cholecystitis, acute pancreatitis, and gallstones—are primarily triggered by external damp-heat, internal pathogenic invasion, or unhealthy dietary habits that promote damp-heat accumulation. Clinical symptoms often include nausea, a feeling of fullness in the chest and upper abdomen, diarrhea, bloating, or foul-smelling loose stools, along with a bitter taste in the mouth, excessive mucus, poor appetite, increased salivation, a tight pulse (which may indicate pain or blood stagnation), and a greasy tongue coating. Treatment generally focuses on clearing damp-heat from the body (Lao, 2008).

The gut microbiota, as a complex micro-ecosystem, relies on mutual balance and regulation among its microorganisms. Internal dampness caused by endogenous factors can disrupt this balance, impairing the function of the intestinal flora. When the Yang energy of the spleen and stomach is weakened and internal cold dominates, it leads to water and dampness stagnation, disturbing the microbial harmony. Therapy aimed at warming the middle region of the body, removing dampness, and strengthening spleen energy can help restore a balanced intestinal flora structure and alleviate signs of internal dampness (Shen, 2004).

In this context, *Peperomia pellucida* (L.), belonging to the Piperaceae family, emerges as a potential therapeutic agent. This plant, commonly utilized as a food source and in traditional medicine (Tablang et al., 2020), is known for its ability to address various health conditions. Locally in Indonesia, it is known as sirih cina or suruhan and grows abundantly in moist areas. Widely consumed by ethnic groups like the Sundanese, it is typically eaten fresh as lalaban or prepared through stir-frying. Rich in essential minerals such as potassium, calcium, and iron, *P. pellucida* is beneficial for enhancing bone strength and aiding recovery (Ooi et al., 2012; Florence et al., 2017). Beyond its nutritional value, it has been employed in traditional healing practices to treat conditions such as headaches, kidney disorders, fever, high blood pressure, and even external issues like wounds and acne (Saputri et al., 2021; Hartati et al., 2015). These medicinal properties may support the restoration of balance in the body, potentially alleviating symptoms related to internal dampness.

MATERIALS AND METHODS

This study used male Swiss strain mice aged 2–3 months and weighing 25–30 g from the Veterinary Pharmacy Center, Surabaya. The sample size was determined using the Federer formula: $(n-1)(t-1) \geq 15$, where $t = 6$ groups, resulting in a minimum of 4 mice per group. An additional mouse was added to each group, totaling 30 mice.

Equipment and Materials

Tools included syringes, feeding tubes, surgical sets, microscopes, microtome, water baths, and glassware. Materials included male mice, *E. coli*, *P. pellucida* extract, amoxicillin, formalin, alcohol series, xylol, paraffin, Giemsa stain, and other histology reagents.

Experimental Design

Mice underwent a two-week acclimatization period and were randomly assigned to six groups: (1) normal control, (2) negative control (aquades), (3) positive control (0.5 mL Yakult), (4–6) *P. pellucida* extract at 100, 300, and 500 mg/kgBW. All treatments were given via oral gavage. After 7 days of treatment, gastroenteritis was induced with *E. coli* (1×10^6 CFU/mL daily for 7 days).

Tissue Processing and Histopathology

Spleen tissues were fixed in buffered formalin, embedded in paraffin, sectioned at 4–6 µm, and stained with hematoxylin-eosin. Observations focused on capsule, trabecula, red pulp, white pulp, and signs of necrosis or apoptosis.

Data Collection and Analysis

Histological data were collected based on abnormal spleen cells. Statistical analysis was performed using one-way ANOVA at a 95% confidence level ($\alpha = 0.05$), followed by the Least Significant Difference (LSD) test if significant. Results were expressed as mean ± standard error (SE) using SPSS 23.0 for Windows.

RESULTS AND DISCUSSION

Result

This study evaluated the antiseptic potential of *Peperomia pellucida* leaf extract by observing histopathological changes in the spleen of mice induced with *Escherichia coli* to model gastroenteritis. Six groups were used, each consisting of four mice. Group I (Figure 1) served as the normal control without treatment, Group II (Figure 2) as the negative control (treated with distilled water), and Group III (Figure 3) as the positive control (treated with ciprofloxacin). Groups IV–VI received *P. pellucida* leaf extract at 100, 300, and 500 mg/kg body weight, respectively, prior to *E. coli* infection.

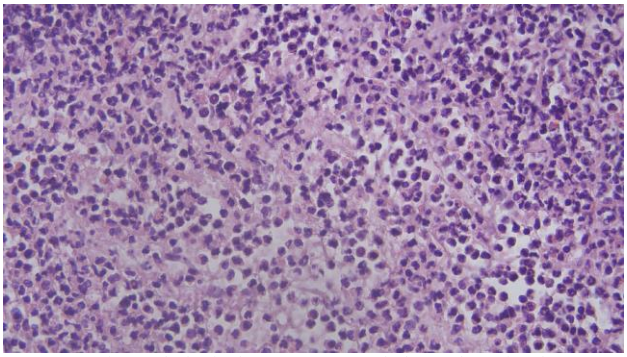

Figure 1. Histopathological Appearance of the Spleen in the Normal Group at 100x Magnification.

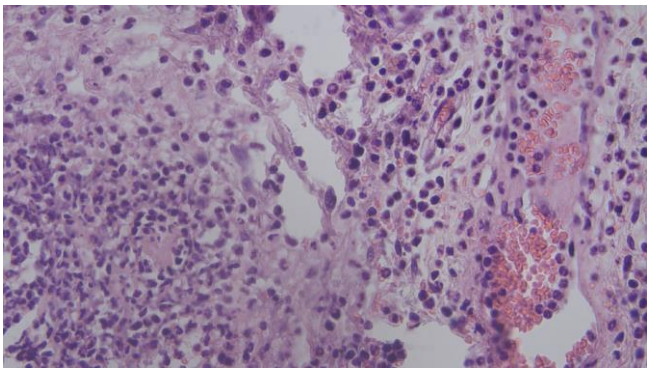

Figure 2. Histopathological Appearance of the Spleen in the Negative Control Group at 100x Magnification.

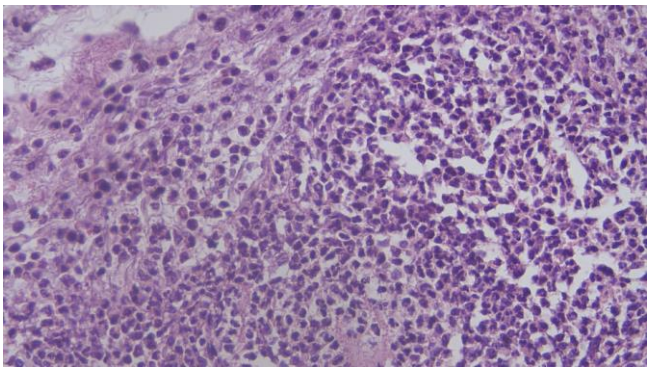

Figure 3. Histopathological Appearance of the Spleen in the Positive Control Group at 100x Magnification.

Histopathological Evaluation of the Spleen

Histopathological examination focused on cell degeneration, necrosis, and polymorphonuclear cell (PMN) infiltration. Table 1 shows that Group I (normal control) had the lowest average percentages for degeneration (2.21±0.02%), necrosis (2.44±0.02%), and PMN infiltration (1.62±0.02%). Group III (positive control) followed, showing lower damage levels than the negative control and treatment groups.

Among the extract-treated groups, Group VI (500 mg/kg BW) demonstrated the most notable reduction in tissue damage, with degeneration (9.08±0.02%), necrosis (6.05±0.02%), and PMN infiltration (18.45±0.03%), indicating its potential antiseptic efficacy.

Statistical Analysis

One-way ANOVA results showed significant differences among groups ($p < 0.05$) for all three histological parameters. LSD and Duncan's post hoc tests confirmed that Group VI (Figure 4) (500 mg/kg BW) had significantly lower damage than Groups V (Figure 5) and IV (Figure 6), while no significant difference was observed between Group IV (100 mg/kg BW) and Group V (300 mg/kg BW) for cell degeneration, suggesting similar antiseptic activity at these doses.

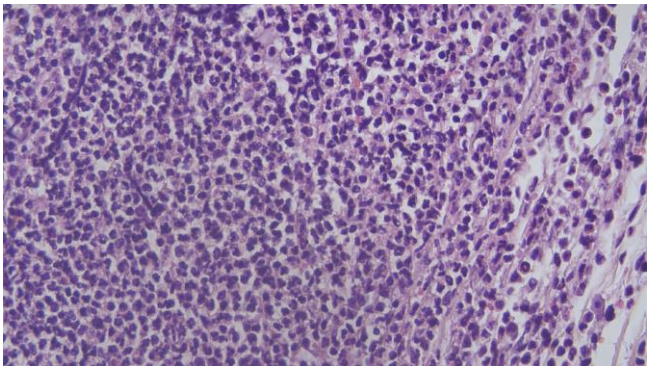

Figure 4. Histopathological Appearance of the Spleen in the *Paederia foetida* Extrect 500 mg/kg BW Group at 100x Magnification.

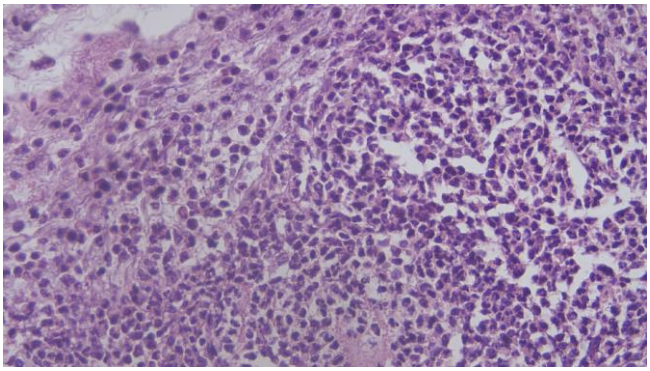

Figure 5. Histopathological Appearance of the Spleen in the *Paederia foetida* Extrect 300 mg/kg BW Group at 100x Magnification.

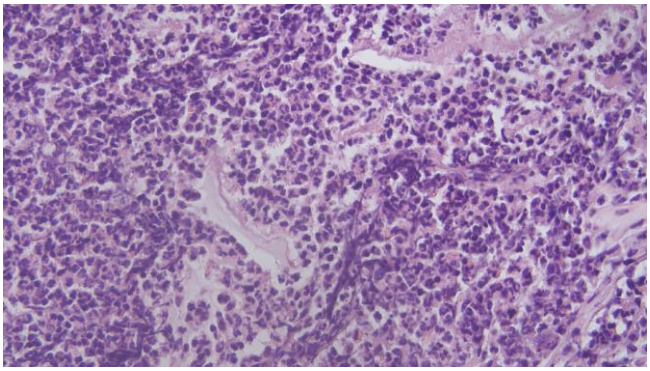

Figure 6. Histopathological Appearance of the Spleen in the *Paederia foetida* Extrect 100 mg/kg BW Group at 100x Magnification.

Discussion

The findings indicate that *P. pellucida* leaf extract can reduce spleen tissue damage caused by *E. coli*-induced gastroenteritis. The reduction in cellular degeneration and necrosis in treatment groups is likely attributed to bioactive compounds such as flavonoids, saponins, and tannins in *P. pellucida*. Flavonoids are known for their strong antioxidant properties, which help mitigate oxidative stress and reduce inflammation by scavenging free radicals and inhibiting proinflammatory cytokines (Middleton et al., 2000; González-Gallego et al., 2007).

Saponins contribute to immune modulation and have anti-inflammatory effects by enhancing the activity of antioxidant enzymes and regulating cytokine production (Shi et al., 2014). Moreover, saponins may support the spleen's detoxification processes, potentially mitigating bacterial damage. Tannins have antimicrobial activity and may inhibit bacterial adherence and colonization in host tissues (Scalbert, 1991).

The results demonstrate that the 500 mg/kg BW dose of *P. pellucida* extract had comparable efficacy to ciprofloxacin, suggesting it may be a potential natural antiseptic agent against bacterial-induced gastroenteritis. However, further investigation into the specific mechanisms and active components is recommended.

The protective effect observed in Group VI (500 mg/kg BW) suggests that higher concentrations of Peperomia pellucida extract confer greater histological protection to spleen tissue following bacterial insult. This aligns with previous studies indicating that the pharmacological activity of medicinal plants often increases with dose, up to a point of biological saturation or toxicity (Rates, 2001). In the context of this study, the increasing dose-dependent efficacy supports the therapeutic relevance of *P. pellucida* in treating infections associated with systemic inflammation.

The spleen plays a key role in the immune response, filtering blood and responding to systemic bacterial invasion. In the presence of *E. coli*, the observed damage in untreated or insufficiently treated groups reflects a strong inflammatory response marked by high PMN infiltration and tissue necrosis. The observed suppression of PMN infiltration in the high-dose treatment group suggests that the extract may possess immunomodulatory properties, potentially by modulating cytokine expression pathways such as NF-κB and MAPK, which are known to regulate neutrophil activation and chemotaxis (Liu et al., 2017; Chen et al., 2018).

The bioactive constituents of *P. pellucida*, especially flavonoids and tannins, may exert synergistic effects. Flavonoids such as quercetin and apigenin—identified in related species—have been shown to inhibit the production of TNF-α and IL-6, key mediators in acute inflammation (Li et al., 2016). Tannins may further inhibit bacterial adhesion to epithelial cells and neutralize endotoxins like lipopolysaccharides (LPS), contributing significantly to septic-like damage in organs including the spleen (Okuda, 2005).

Furthermore, studies have reported the antioxidant and hepatoprotective activities of *P. pellucida*, indicating its systemic benefits beyond the gastrointestinal tract (Almagboul et al., 1985). The spleen, being highly vascularized and sensitive to oxidative stress, benefits from the scavenging activity of antioxidants, which may explain the lower necrosis levels observed in the high-dose extract group.

In contrast, the lower doses (100 and 300 mg/kg BW) showed moderate protection, suggesting that while these

concentrations do confer some degree of benefit, they may not be sufficient to counteract the full inflammatory burden induced by *E. coli*. This highlights the need for optimization of dosage in potential therapeutic applications. From a pharmacognostic perspective, the findings contribute to a growing body of evidence supporting the traditional use of *P. pellucida* in treating gastrointestinal and inflammatory disorders in ethnomedicine across Asia and South America (Gonzaga et al., 2005). However, further studies involving phytochemical isolation, molecular assays, and toxicological profiling are necessary to validate safety and efficacy before clinical use.

CONCLUSIONS

The results of this study demonstrate that ethanol extract of Peperomia pellucida exerts a dose-dependent protective effect on spleen histology in mice infected with Escherichia coli. Histopathological improvements—marked by reduced necrosis and polymorphonuclear (PMN) infiltration—were most pronounced at the highest administered dose of 500 mg/kg BW. These findings suggest that *P. pellucida* possesses significant anti-inflammatory and potential immunomodulatory properties, likely attributed to its rich content of flavonoids and tannins. The extract's ability to mitigate tissue damage in response to bacterial infection supports its traditional use and highlights its potential as a complementary therapeutic agent. However, further research is necessary to isolate specific bioactive compounds, elucidate molecular mechanisms, and assess long-term safety for potential clinical applications.

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