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The Role of the NF-κB Pathway and Oxidative Stress Markers in the Protective Effects of *Peperomia pellucida* in a Gastroenteritis Model

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Abstract

Gastroenteritis is a common gastrointestinal disorder characterized by intestinal inflammation, epithelial damage, and oxidative stress. Activation of the nuclear factor kappa B (NF-κB) signaling pathway and excessive production of reactive oxygen species play key roles in its pathogenesis. *Peperomia pellucida* is a medicinal plant traditionally used for inflammatory and gastrointestinal conditions; however, its molecular mechanisms of action remain poorly understood. This study aimed to investigate the protective effects of *P. pellucida* extract in an experimental gastroenteritis model, with a particular focus on NF-κB pathway modulation and oxidative stress markers. Gastroenteritis was induced in experimental animals, followed by oral administration of *P. pellucida* extract at different doses. NF-κB p65 expression was assessed using molecular analysis, while oxidative stress was evaluated through malondialdehyde levels and antioxidant enzyme activities, including superoxide dismutase and catalase. Histopathological examination of intestinal tissue was also performed. The results demonstrated that *P. pellucida* treatment significantly suppressed NF-κB p65 activation, reduced lipid peroxidation, restored antioxidant enzyme activities, and improved intestinal histopathological features compared to the untreated gastroenteritis group. These findings indicate that *P. pellucida* confers intestinal protection by attenuating inflammation and oxidative stress, supporting its potential as a natural therapeutic agent for gastroenteritis.

Keywords: gastroenteritis; NF-κB; oxidative stress; *Peperomia pellucida*; phytotherapy.

Abbreviations: Catalase (CAT), Gastroenteritis (GE), Malondialdehyde (MDA), Nuclear factor kappa B (NF-κB), Reactive oxygen species (ROS), Superoxide dismutase (SOD).

INTRODUCTION

Gastroenteritis remains a significant global health problem, particularly in developing countries, where it contributes substantially to morbidity and healthcare burden. It is characterized by inflammation of the gastrointestinal tract, commonly induced by bacterial, viral, or parasitic infections. The pathological process involves disruption of intestinal epithelial integrity, excessive inflammatory responses, and increased oxidative stress, which together exacerbate tissue damage and prolong disease severity (World Health Organization, 2022).

At the molecular level, the nuclear factor kappa B (NF-κB) signaling pathway plays a central role in the inflammatory cascade associated with gastroenteritis. Activation of NF-κB leads to the transcription of pro inflammatory cytokines such as tumor necrosis factor alpha, interleukin 1 beta, and interleukin 6, which amplify intestinal inflammation and epithelial injury (Liu et al., 2017). Persistent activation of this pathway has been linked to severe mucosal damage and delayed recovery in gastrointestinal inflammatory conditions (Zhang et al., 2019).

In parallel, oxidative stress is increasingly recognized as a critical contributor to the pathogenesis of gastroenteritis. Excessive production of reactive oxygen species during infection and inflammation overwhelms endogenous antioxidant defenses, resulting in lipid peroxidation, protein oxidation, and DNA damage (Birben et al., 2012). Biomarkers such as malondialdehyde, superoxide dismutase, and catalase are commonly used to assess oxidative imbalance in gastrointestinal disorders and have been shown to correlate with disease severity (Rezaie et al., 2007).

Current therapeutic strategies for gastroenteritis primarily focus on rehydration, antimicrobial agents, and symptomatic relief. However, these approaches do not directly target the underlying inflammatory signaling pathways or oxidative damage, and prolonged use of anti inflammatory or antibiotic therapies may cause adverse

effects and antimicrobial resistance (Guarino et al., 2018). This highlights the urgent need for alternative therapeutic agents that are both effective and safe, particularly those derived from natural sources.

Peperomia pellucida is a medicinal plant traditionally used in various regions for treating inflammatory and gastrointestinal disorders. Phytochemical studies have identified flavonoids, alkaloids, and phenolic compounds within *P. pellucida*, which exhibit anti inflammatory and antioxidant activities (Khan et al., 2016). Experimental studies have demonstrated its protective effects in models of inflammation, oxidative stress, and tissue injury (Arruda et al., 2020). Despite these promising findings, the molecular mechanisms underlying its gastroprotective effects remain insufficiently explored.

Specifically, there is a lack of studies investigating the role of the NF-κB signaling pathway and oxidative stress markers in mediating the protective effects of *P. pellucida* in gastroenteritis models. Most existing research focuses on general anti inflammatory outcomes without elucidating the interaction between inflammatory transcription factors and oxidative balance at the molecular level. This represents a critical research gap, as understanding these mechanisms is essential for validating *P. pellucida* as a scientifically grounded therapeutic candidate.

Therefore, this study aims to investigate the role of the NF-κB pathway and oxidative stress markers in the protective effects of *P. pellucida* using an experimental gastroenteritis model. By elucidating these mechanisms, the study seeks to provide mechanistic evidence supporting the use of *P. pellucida* as a potential adjunct or alternative therapy for gastroenteritis, contributing to the development of safer and more targeted anti inflammatory treatments.

MATERIALS AND METHODS

Study Design

This study employed an experimental laboratory design using an animal model of gastroenteritis to evaluate the protective effects of *Peperomia pellucida*. The investigation focused on inflammatory signaling through the NF-κB pathway and oxidative stress markers as primary outcome measures. Animals were randomly allocated into experimental groups to minimize bias.

Plant Material and Extract Preparation

Fresh whole plants of *P. pellucida* were collected from a certified local cultivation area and taxonomically identified by a botanist at the Department of Biology. A voucher specimen was deposited in the institutional herbarium for reference. The plant material was washed, air dried at room temperature, and ground into a fine powder. Extraction was performed using 70 percent ethanol through maceration for 72 hours with periodic agitation. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The resulting crude extract was stored at 4°C until use. Prior to administration, the extract was reconstituted in distilled water.

Experimental Animals

Male Wistar rats weighing 180 to 220 grams were used in this study. Animals were obtained from an accredited animal facility and housed under standard laboratory conditions with controlled temperature, humidity, and a 12 hours light dark cycle. Standard pellet diet and water were provided ad libitum. All experimental procedures were conducted in accordance with institutional ethical guidelines for animal research and approved by the Institutional Animal Care and Use Committee.

Induction of Gastroenteritis

Gastroenteritis was induced using a chemical inflammatory agent commonly employed to model intestinal inflammation. After an overnight fast, animals received oral administration of the inducer at a predetermined dose to provoke acute intestinal inflammation. Control animals received an equivalent volume of distilled water. Clinical signs such as diarrhea, reduced activity, and stool consistency were monitored throughout the experimental period to confirm successful induction.

Experimental Grouping and Treatment

Animals were randomly divided into five groups, each consisting of six rats:

- Normal control group receiving distilled water only
- Gastroenteritis control group receiving the inflammatory inducer without treatment
- Low dose *P. pellucida* extract treated group
- High dose *P. pellucida* extract treated group
- Positive control group receiving a standard anti inflammatory drug

Treatment with *P. pellucida* extract or standard drug was administered orally once daily for a specified duration following gastroenteritis induction. Dosage selection was based on previous toxicological and pharmacological studies (Arruda et al., 2020).

Sample Collection

At the end of the treatment period, animals were anesthetized and sacrificed humanely. Blood samples were collected via cardiac puncture for biochemical analysis. Intestinal tissues were excised, rinsed with cold saline, and divided for molecular, biochemical, and histopathological evaluations.

Assessment of NF-κB Pathway Activation

NF-κB activation in intestinal tissue was assessed using immunohistochemistry and western blot analysis. Tissue samples were homogenized, and nuclear protein

extraction was performed. Expression levels of NF-κB p65 were quantified using specific antibodies. Results were normalized to housekeeping proteins and expressed as relative protein expression.

Measurement of Oxidative Stress Markers

Oxidative stress parameters were evaluated in intestinal tissue homogenates. Malondialdehyde levels were measured as an index of lipid peroxidation. Antioxidant enzyme activities, including superoxide dismutase and catalase, were determined using spectrophotometric methods according to standardized protocols (Rezaie et al., 2007).

Histopathological Examination

Intestinal tissue samples were fixed in 10 percent buffered formalin, processed, and embedded in paraffin. Sections were stained with hematoxylin and eosin for microscopic examination. Histopathological changes such as mucosal damage, inflammatory cell infiltration, and epithelial integrity were evaluated by a blinded pathologist using a semi quantitative scoring system.

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed using appropriate software. Differences between groups were analyzed using one way analysis of variance followed by post hoc tests. A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effects of *Peperomia pellucida* on Clinical Manifestations of Gastroenteritis

Animals in the gastroenteritis control group exhibited clear clinical symptoms, including watery diarrhea, reduced mobility, and decreased food intake following induction. In contrast, rats treated with *P. pellucida* extract showed marked improvement in stool consistency and general activity. The high dose treatment group demonstrated the most pronounced protective effect, with clinical signs approaching those observed in the normal control group. The positive control group also showed significant symptom improvement.

Peperomia pellucida Attenuates NF-κB Activation in Intestinal Tissue

Activation of the NF-κB signaling pathway was significantly increased in the gastroenteritis control group, as indicated by elevated expression of NF-κB p65 in intestinal tissue. Quantitative analysis revealed a substantial upregulation of nuclear NF-κB p65 compared to the normal control group ($p < 0.05$). Treatment with *P. pellucida* extract significantly reduced NF-κB p65 expression in a dose dependent manner. The high dose group exhibited a marked suppression of NF-κB activation, comparable to the positive control group receiving standard anti inflammatory therapy ($p < 0.05$). These findings indicate that *P. pellucida* effectively inhibits inflammatory signaling mediated by NF-κB.

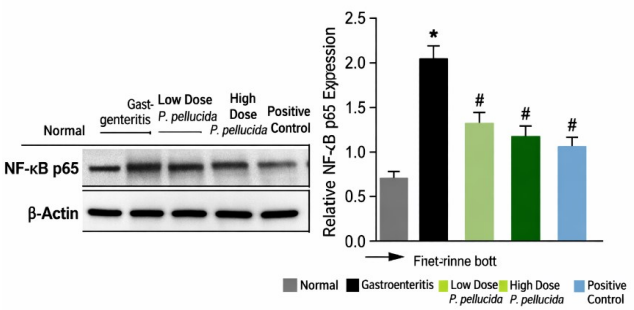

Figure 1. Effect of *Peperomia pellucida* extract on NF-κB p65 expression in intestinal tissue. Representative western blot images and quantitative analysis of NF-κB p65 levels normalized to housekeeping protein expression. Data are presented as mean ± SD. $p < 0.05$ compared to gastroenteritis control group.

Modulation of Oxidative Stress Markers by *Peperomia pellucida*

Oxidative stress assessment revealed a significant increase in malondialdehyde levels in the gastroenteritis control group compared to normal controls ($p < 0.05$), indicating enhanced lipid peroxidation. Concurrently, antioxidant enzyme activities, including superoxide dismutase and catalase, were significantly reduced. Administration of *P. pellucida* extract resulted in a significant decrease in malondialdehyde levels, alongside a restoration of antioxidant enzyme activity. The high dose extract group demonstrated the most substantial reduction in oxidative damage and a significant elevation of superoxide dismutase and catalase activities compared to the gastroenteritis control group ($p < 0.05$). These effects were comparable to those observed in the positive control group.

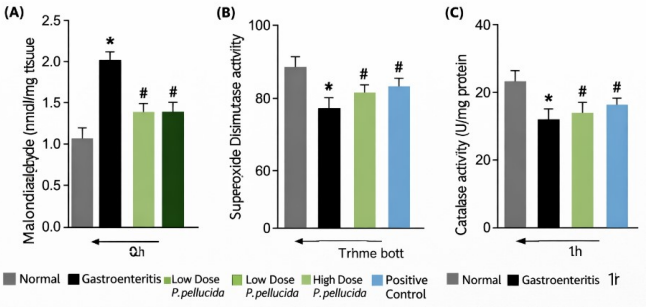

Figure 2. Effects of *Peperomia pellucida* on oxidative stress markers in intestinal tissue. (A) Malondialdehyde levels, (B) Superoxide dismutase activity, and (C) Catalase activity. Values are expressed as mean ± SD. $p < 0.05$ versus gastroenteritis control group.

Histopathological Improvement of Intestinal Tissue

Histopathological examination of intestinal sections from the gastroenteritis control group revealed severe mucosal erosion, epithelial disruption, inflammatory cell infiltration, and submucosal edema. These pathological

alterations confirmed successful induction of gastroenteritis.

In contrast, intestinal tissues from rats treated with *P. pellucida* extract showed significant structural preservation. The low dose group exhibited moderate improvement, while the high dose group demonstrated near normal mucosal architecture with minimal inflammatory infiltration. The positive control group showed similar histological protection. Semi quantitative histopathological scoring confirmed a significant reduction in tissue damage in extract treated groups compared to the gastroenteritis control group ($p < 0.05$).

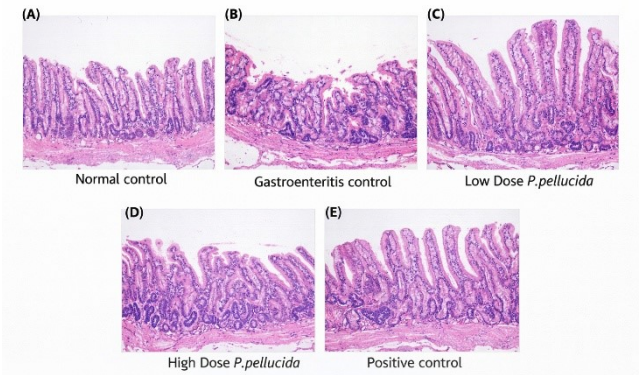

Figure 3. Histopathological evaluation of intestinal tissue stained with hematoxylin and eosin (magnification 400×). (A) Normal control, (B) Gastroenteritis control showing severe mucosal damage, (C) Low dose *Peperomia pellucida* treatment, (D) High dose *Peperomia pellucida* treatment, and (E) Positive control group.

Correlation Between NF-κB Suppression and Oxidative Stress Reduction

Correlation analysis demonstrated a positive association between NF-κB p65 expression and malondialdehyde levels, and a negative association with antioxidant enzyme activities. These findings suggest that suppression of NF-κB signaling by *P. pellucida* is closely linked to attenuation of oxidative stress in gastroenteritis.

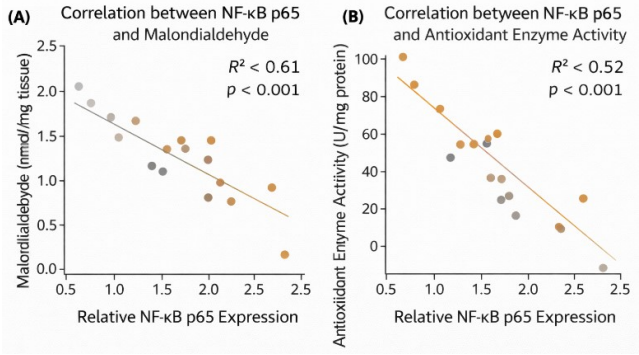

Figure 4. Correlation analysis between NF-κB p65 expression and oxidative stress parameters in intestinal tissue.

Discussion

This study demonstrates that *Peperomia pellucida* exerts significant protective effects against gastroenteritis through modulation of the NF-κB signaling pathway and attenuation of oxidative stress in intestinal tissue. The findings provide mechanistic evidence supporting the traditional use of *P. pellucida* in gastrointestinal disorders and expand current understanding of its molecular anti-inflammatory and antioxidant actions.

Activation of the NF-κB pathway is a hallmark of gastrointestinal inflammation and plays a pivotal role in the transcriptional regulation of pro-inflammatory mediators during gastroenteritis. In the present study, gastroenteritis induction resulted in a marked increase in NF-κB p65 expression, consistent with previous reports showing that NF-κB activation contributes to mucosal inflammation, epithelial disruption, and disease severity in intestinal inflammatory conditions (Liu et al., 2017; Zhang et al., 2019). Elevated NF-κB activity has been shown to amplify cytokine release and perpetuate intestinal injury, thereby prolonging inflammatory responses (Neurath, 2014).

Treatment with *P. pellucida* extract significantly suppressed NF-κB p65 expression in a dose dependent manner. This suggests that the extract interferes with upstream signaling events leading to NF-κB activation, thereby limiting inflammatory gene transcription. Similar NF-κB inhibitory effects have been reported for plant-derived polyphenols and flavonoids, which act by blocking IκB degradation or inhibiting nuclear translocation of NF-κB subunits (Kumar and Pandey, 2013; Li et al., 2020). Phytochemical analyses of *P. pellucida* have identified flavonoids and phenolic compounds that are known NF-κB modulators, supporting the biological plausibility of these findings (Khan et al., 2016).

In addition to inflammatory signaling, oxidative stress plays a crucial role in the pathogenesis of gastroenteritis. Excessive production of reactive oxygen species during inflammation overwhelms endogenous antioxidant defenses, resulting in lipid peroxidation and cellular damage (Birben et al., 2012). The present study showed a significant increase in malondialdehyde levels accompanied by reduced superoxide dismutase and catalase activities in the gastroenteritis control group. These findings align with previous studies demonstrating that oxidative imbalance exacerbates intestinal mucosal injury and inflammation (Rezaie et al., 2007; Valko et al., 2016).

Administration of *P. pellucida* extract effectively reduced malondialdehyde levels and restored antioxidant enzyme activities. This indicates that the extract not only suppresses lipid peroxidation but also enhances endogenous antioxidant defenses. Antioxidant effects of *P. pellucida* have been reported in other experimental models, where the plant extract scavenged free radicals and upregulated antioxidant enzymes (Arruda et al., 2020; Putri et al., 2021). The ability of *P. pellucida* to modulate both oxidative and inflammatory pathways suggests a dual protective mechanism, which is

particularly advantageous in inflammatory gastrointestinal disorders.

Histopathological evaluation further supported the biochemical findings. Severe mucosal damage and inflammatory infiltration observed in untreated gastroenteritis animals were markedly attenuated by *P. pellucida* treatment, especially at the higher dose. Preservation of villus architecture and epithelial integrity is critical for intestinal function and recovery (Turner, 2009). The histological improvement observed in this study indicates that suppression of NF-κB activation and oxidative stress translates into tangible structural protection of the intestinal mucosa.

Importantly, correlation analysis revealed a strong association between NF-κB p65 expression and oxidative stress parameters. Positive correlation between NF-κB activation and malondialdehyde levels, along with negative correlation with antioxidant enzyme activity, suggests a close interplay between inflammatory signaling and oxidative damage. This interaction has been widely documented, where NF-κB activation both induces oxidative stress and is further amplified by reactive oxygen species, creating a vicious cycle of inflammation and tissue injury (Morgan and Liu, 2011; Lingappan, 2018). The ability of *P. pellucida* to disrupt this cycle underscores its therapeutic potential.

Compared to conventional anti-inflammatory drugs, which often target single pathways and may cause adverse effects with long-term use, plant-based therapies such as *P. pellucida* offer a multi-target approach with potentially improved safety profiles (Calixto, 2019). The comparable efficacy observed between the high dose extract and the positive control group further supports the relevance of *P. pellucida* as a candidate for adjunct or alternative therapy in gastroenteritis.

Nevertheless, this study has some limitations. The investigation focused on NF-κB p65 expression and selected oxidative stress markers, while other inflammatory mediators such as cytokines and upstream kinases were not assessed. Future studies should explore broader signaling networks, identify specific bioactive compounds responsible for the observed effects, and evaluate long-term safety and efficacy. Additionally, translational studies are required to determine whether these protective effects can be replicated in clinical settings.

Overall, the findings of this study demonstrate that *P. pellucida* confers intestinal protection by suppressing NF-κB mediated inflammation and mitigating oxidative stress. These results provide strong mechanistic support for the therapeutic potential of *P. pellucida* in gastroenteritis and contribute valuable evidence toward the development of plant-based anti-inflammatory interventions.

CONCLUSIONS

This study demonstrates that *Peperomia pellucida* exerts significant protective effects in an experimental model of gastroenteritis by modulating key inflammatory and oxidative stress pathways. Treatment with *P. pellucida* extract effectively suppressed NF-κB p65 activation, reduced lipid peroxidation, and restored endogenous antioxidant enzyme activities in intestinal tissue. These molecular effects were accompanied by marked improvement in histopathological features, indicating preservation of mucosal integrity and attenuation of inflammatory damage.

The findings highlight the close interplay between NF-κB mediated inflammation and oxidative stress in the pathogenesis of gastroenteritis and provide mechanistic evidence that *P. pellucida* disrupts this pathological cycle. Overall, this study supports the potential of *P. pellucida* as a promising natural therapeutic agent for gastroenteritis and contributes valuable insight into plant based anti-inflammatory strategies targeting both inflammatory signaling and oxidative imbalance.

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Authors' Contributions: Lisa Savitri contributed to the conceptualization and design of the study, supervised the experimental work, and performed critical revision of the manuscript. Fendy Prasetyawan was responsible for extract preparation, experimental execution, and data interpretation. Yuneka Saristiana contributed to biochemical analysis and data acquisition. Elfred Rinaldo Kasimo performed molecular analysis and assisted in data interpretation. Rochmad Krissanjaya contributed to statistical analysis and data validation. Cornelia Amanda assisted in histopathological analysis and manuscript drafting. Konradus Klala Mebung contributed to data collection and literature review. All authors have read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no financial, academic, or personal conflicts of interest that may have influenced the findings or interpretations presented in this study.

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