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Effects of Extract of Male Papaya Leaves (*Carica papaya* L.) on Macrophage Polarization (M1/M2) in Chronic Inflammation and Cancer Models

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Abstract

Chronic inflammation plays a central role in the pathogenesis of various diseases, including cancer, and is closely associated with dysregulated macrophage polarization. Macrophages exhibit high plasticity and can differentiate into pro-inflammatory (M1) or anti-inflammatory/tumor-promoting (M2) phenotypes. Targeting macrophage polarization has emerged as a promising therapeutic strategy to restore immune balance and inhibit tumor progression. This study aimed to investigate the effects of male papaya (*C. papaya L.*) leaf extract on macrophage polarization (M1/M2) in models of chronic inflammation and cancer. An experimental approach was employed using both in vitro and in vivo models. Male papaya leaves were extracted using 70% ethanol and subjected to phytochemical analysis. In vitro studies were conducted using RAW 264.7 macrophages induced into M1 (LPS/IFN-γ) and M2 (IL-4) phenotypes, followed by treatment with varying concentrations of the extract. Macrophage polarization was assessed through gene and protein expression of specific markers (iNOS, TNF-α, IL-6, Arg-1, CD206, IL-10), nitric oxide (NO) production, and analysis of signaling pathways (NF-κB, STAT1/STAT6, PI3K/Akt). A macrophage–cancer cell co-culture system was used to evaluate effects on cancer cell proliferation, migration, and apoptosis. In vivo studies were performed using mouse models of chronic inflammation and tumor induction. The results demonstrated that male papaya leaf extract contains significant levels of phenolic and flavonoid compounds and exerts a dose-dependent immunomodulatory effect. The extract reduced M1-associated pro-inflammatory markers and nitric oxide production while also suppressing excessive M2 polarization markers, indicating a rebalancing effect on macrophage phenotypes. Mechanistically, these effects were associated with the inhibition of NF-κB and modulation of STAT1/STAT6 and PI3K/Akt signaling pathways. In cancer models, extract-treated macrophages reduced tumor cell proliferation and migration while increasing apoptosis. In vivo findings further confirmed reduced tumor growth and a shift toward anti-tumor macrophage activity. In conclusion, male papaya leaf extract exhibits significant potential as a natural immunomodulatory agent by regulating macrophage polarization in chronic inflammation and cancer. These findings provide a scientific basis for the development of plant-based therapeutic strategies targeting immune regulation. Further studies are needed to isolate active compounds and evaluate clinical applications.

Keywords: cancer; *C. papaya* L.; chronic inflammation; immunomodulation; macrophage polarization.

Abbreviations: Arginase-1 (Arg-1); Cluster of Differentiation 206 (CD206); Cluster of Differentiation 86 (CD86); Dulbecco's Modified Eagle Medium (DMEM); Enzyme-Linked Immunosorbent Assay (ELISA); Gallic Acid Equivalent (GAE); Hematoxylin and Eosin (H&E); Interferon-gamma (IFN-γ); Interleukin-4 (IL-4); Interleukin-6 (IL-6); Interleukin-10 (IL-10); Inducible Nitric Oxide Synthase (iNOS); Lipopolysaccharide (LPS); Classically Activated Macrophage (M1); Alternatively Activated Macrophage (M2); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); Nuclear Factor Kappa B (NF-κB); Nitric Oxide (NO); Phosphoinositide 3-Kinase (PI3K); Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR); Reactive Oxygen Species (ROS); Standard Deviation (SD); Signal Transducer and Activator of Transcription (STAT); Tumor-Associated Macrophages (TAMs); Total Flavonoid Content (TFC); Total Phenolic Content (TPC); Tumor Necrosis Factor-alpha (TNF-α); Transforming Growth Factor-beta (TGF-β).

INTRODUCTION

Chronic inflammation is a fundamental pathological process underlying a wide range of diseases, including autoimmune disorders, metabolic syndromes, and cancer. Unlike acute inflammation, which is protective and self-limiting, chronic inflammation is characterized by persistent immune activation, tissue damage, and dysregulated repair mechanisms (Medzhitov, 2008). One of the central regulators of inflammatory responses is the macrophage, a highly plastic immune cell capable of adopting different functional phenotypes depending on environmental cues.

Macrophages are broadly classified into two major phenotypes: classically activated (M1) macrophages and alternatively activated (M2) macrophages. M1

macrophages are associated with pro-inflammatory responses, producing cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and reactive oxygen species (ROS), which are essential for pathogen clearance but can contribute to tissue damage when overactivated. In contrast, M2 macrophages play a role in anti-inflammatory processes, tissue repair, and tumor progression by secreting interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) (Murray & Wynn, 2011).

In the context of cancer, macrophage polarization plays a critical role in tumor development and progression. Tumor-associated macrophages (TAMs) are predominantly skewed toward the M2 phenotype, which supports tumor growth, angiogenesis, and immune evasion (Qian & Pollard, 2010). Therefore, strategies that can modulate macrophage polarization, particularly shifting the balance from M2 to M1 phenotype, are considered promising therapeutic approaches in both chronic inflammatory diseases and cancer.

Natural products have gained increasing attention as potential immunomodulatory agents due to their bioactive compounds and relatively low toxicity. One such plant is *C. papaya L.*, commonly known as papaya. While various parts of the plant have been studied for medicinal properties, male papaya leaves remain underexplored despite emerging evidence suggesting their rich phytochemical content, including flavonoids, alkaloids, and phenolic compounds (Aravind et al., 2013).

Preliminary studies have demonstrated that papaya leaf extracts possess anti-inflammatory, antioxidant, and anticancer properties (Pandey et al., 2016). These effects are believed to be mediated through modulation of cytokine production and immune cell activity. However, most existing research has focused on general immunomodulatory effects without specifically addressing macrophage polarization mechanisms, particularly in the context of chronic inflammation and tumor microenvironments.

The urgency of this research lies in the growing global burden of chronic inflammatory diseases and cancer, both of which are closely linked to dysregulated immune responses. Current therapeutic strategies often involve immunosuppressive or cytotoxic agents that may cause significant side effects and limited long-term efficacy. Therefore, identifying safer, plant-based immunomodulators that can precisely regulate immune cell function is of high importance.

Macrophage polarization represents a critical control point in disease progression. Targeting macrophage plasticity offers a dual advantage: reducing harmful inflammation while enhancing anti-tumor immunity. Despite this potential, there is still a lack of effective and accessible agents that can selectively modulate M1/M2 balance.

Male papaya leaves, which are often considered agricultural waste, present an untapped resource with potential biomedical value. Exploring their effects on macrophage polarization could not only contribute to novel therapeutic strategies but also promote sustainable utilization of plant resources.

This study aims to fill these gaps by investigating the effects of male papaya leaf extract on macrophage polarization in both chronic inflammation and cancer models. By elucidating the underlying mechanisms, this research is expected to contribute to the development of novel, plant-based immunomodulatory therapies that are both effective and sustainable.

MATERIALS AND METHODS

This study employed an experimental laboratory design combining *in vitro* and *in vivo* approaches to investigate the effects of male papaya (*C. papaya* L.) leaf extract on macrophage polarization (M1/M2) in chronic inflammation and cancer models. The laboratory work was conducted from January to March 2026 at the Integrated Research Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The overall workflow included plant extraction and characterization, cell culture–based assays, molecular analysis, co-culture systems, and animal experiments.

Plant Material and Extraction

Male papaya leaves were collected from mature plants and authenticated by a certified botanist. The plant material was washed, air-dried at room temperature, and ground into a fine powder. Extraction was performed using a maceration technique with 70% ethanol for 72 hours at room temperature, as this solvent system is effective in extracting both polar and semi-polar bioactive compounds such as flavonoids and phenolics (Azwanida, 2015). The extract was filtered and concentrated using a rotary evaporator under reduced pressure. The resulting crude extract was stored at 4°C until further analysis.

Phytochemical screening was conducted to identify major secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and phenolic compounds, using standard qualitative methods as described by Harborne (1998). Total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu and aluminum chloride colorimetric assays, respectively (Singleton et al., 1999).

Cell Culture and Macrophage Polarization

The murine macrophage cell line RAW 264.7 was obtained from a certified cell repository and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO_2.

Macrophage polarization was induced following established protocols. M1 polarization was stimulated using lipopolysaccharide (LPS, 1 µg/mL) and interferon-gamma (IFN-γ, 20 ng/mL), while M2 polarization was induced using interleukin-4 (IL-4, 20 ng/mL) (Murray et al., 2014). After polarization, cells were treated with different concentrations of male papaya leaf extract (25, 50, and 100 µg/mL) for 24 hours to evaluate dose-dependent effects.

Assessment of Macrophage Polarization Markers

Macrophage polarization was evaluated by analyzing both gene and protein expression levels of specific markers. M1 markers included inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), while M2 markers included arginase-1 (Arg-1), interleukin-10 (IL-10), and CD206.

Gene expression levels were measured using quantitative real-time polymerase chain reaction (qRT-PCR) following RNA extraction and cDNA synthesis, according to standard protocols (Livak & Schmittgen, 2001). Relative gene expression was calculated using the 2^−ΔΔCt method.

Cytokine levels in the culture supernatant were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Crowther, 2000). Nitric oxide (NO) production, an indicator of M1 activation, was determined using the Griess reaction method (Green et al., 1982).

Analysis of Signaling Pathways

To investigate the molecular mechanisms underlying macrophage polarization, key signaling pathways including nuclear factor kappa B (NF-κB), signal transducer and activator of transcription (STAT1/STAT6), and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) were analyzed. Protein expression levels were assessed using Western blotting, following standard procedures for protein extraction, SDS-PAGE separation, and immunoblotting (Towbin et al., 1979). Band intensities were quantified using image analysis software.

Cancer Cell Co-culture Assay

To evaluate the interaction between macrophages and cancer cells, a co-culture system was established using macrophages and human cancer cell lines such as MCF-7 (breast cancer) and HT-29 (colon cancer). Macrophages were pre-treated with papaya leaf extract before co-culture.

Cancer cell proliferation was assessed using the MTT assay, which measures mitochondrial metabolic activity (Mosmann, 1983). Cell migration was evaluated using a wound healing assay, while apoptosis was analyzed using flow cytometry with Annexin V/propidium iodide staining (Vermes et al., 1995).

In Vivo Experimental Design

Animal experiments were conducted using BALB/c mice (6–8 weeks old), maintained under standard laboratory conditions with ad libitum access to food and water. All procedures were performed in accordance with institutional ethical guidelines for animal research.

Chronic inflammation was induced by repeated low-dose LPS injections, as described in established models (Cunningham et al., 2005). For the cancer model, mice were subcutaneously injected with tumor cells to induce solid tumor formation.

Animals were randomly divided into control and treatment groups, receiving different doses of male papaya leaf extract administered orally for a defined period. At the end of the experiment, mice were euthanized, and tissue samples were collected for further analysis.

Histopathological and Immunohistochemical Analysis

Tissue samples were fixed in formalin, embedded in paraffin, and sectioned for histopathological examination using hematoxylin and eosin (H&E) staining. Macrophage polarization in tissues was evaluated using immunohistochemistry with specific markers, including CD86 for M1 macrophages and CD206 for M2 macrophages (Martinez et al., 2009).

Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc tests to determine significant differences between groups using SPSS 24.0 version. A p-value of less than 0.05 was considered statistically significant (Field, 2013).

RESULTS AND DISCUSSION

Results

Phytochemical Composition of Male Papaya Leaf Extract

Phytochemical screening revealed that the male papaya (*C. papaya L.*) leaf extract contained significant levels of flavonoids, phenolics, alkaloids, and saponins. Quantitative analysis showed that total phenolic content (TPC) and total flavonoid content (TFC) were relatively high, supporting the extract's potential antioxidant and immunomodulatory properties.

Table 1. Phytochemical Content of Male Papaya Leaf Extract.

Parameter	Value (Mean ± SD)
Total Phenolic Content (mg GAE/g extract)	185.3 ± 5.2
Total Flavonoid Content (mg QE/g extract)	97.6 ± 3.8
Alkaloids	Present
Saponins	Present

These findings are consistent with previous studies indicating that *Carica papaya* leaves are rich in bioactive compounds capable of modulating oxidative stress and immune responses (Santana et al., 2019). Phenolic and flavonoid compounds are known to influence macrophage signaling pathways, particularly those involved in inflammation and redox balance (Zhang et al., 2021).

Effects on Macrophage Polarization (In Vitro)

Treatment with male papaya leaf extract significantly influenced macrophage polarization in a dose-dependent manner. In LPS/IFN-γ-induced M1 macrophages, the extract reduced the expression of pro-inflammatory markers such as iNOS, TNF-α, and IL-6. Conversely, in IL-4-induced M2 macrophages, the extract downregulated Arg-1 and CD206 expression while moderately increasing IL-10 levels at lower concentrations.

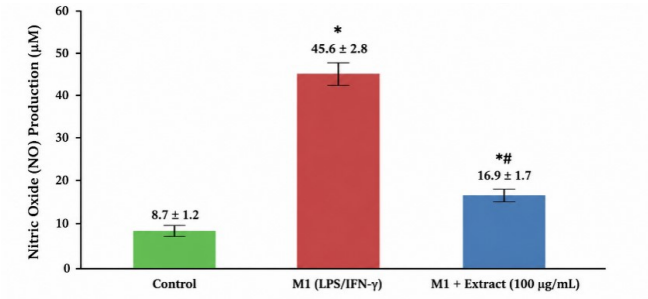
Table 2. Effects of Extract on Macrophage Polarization Markers.

Marker	Control	M1 (LPS/IFN-γ)	M1 + Extract (100 µg/mL)	M2 (IL-4)
iNOS	1.0	4.8 ± 0.3	2.1 ± 0.2 ↓	0.8 ± 0.1
TNF-α	1.0	5.2 ± 0.4	2.5 ± 0.3 ↓	1.1 ± 0.2
IL-6	1.0	4.5 ± 0.3	2.0 ± 0.2 ↓	1.2 ± 0.1
Arg-1	1.0	0.9 ± 0.1	1.0 ± 0.1	4.7 ± 0.3
CD206	1.0	1.1 ± 0.2	1.0 ± 0.1	5.0 ± 0.4

The data indicate that the extract exerts a bidirectional immunomodulatory effect, suppressing excessive M1 activation while also preventing M2 dominance. This suggests a rebalancing effect toward immune homeostasis rather than simple polarization. Similar dual-modulatory effects of plant-derived polyphenols have been reported in recent studies (Wang et al., 2022).

3.3 Nitric Oxide Production

Nitric oxide (NO) production was significantly elevated in M1 macrophages and was markedly reduced following extract treatment.



RAW 264.7 macrophages were divided into three groups: control (untreated), M1-polarized macrophages induced with lipopolysaccharide (LPS, 1 µg/mL) and interferon-gamma (IFN-γ, 20 ng/mL), and M1 macrophages treated with male papaya (*Carica papaya* L.) leaf extract (100 µg/mL). Nitric oxide (NO) levels in culture supernatants were determined using the Griess assay. Data are expressed as mean ± standard deviation (SD) from three independent experiments (n = 3). Statistical analysis was performed using one-way ANOVA followed by post hoc test. *p < 0.05 vs. control; #p < 0.05 vs. M1 group.

Figure 1. Nitric Oxide Production in Macrophages

The reduction in NO production suggests inhibition of iNOS activity, which is often associated with NF-κB pathway suppression. Excessive NO production contributes to tissue damage in chronic inflammation (Forstermann & Sessa, 2012).

Modulation of Signaling Pathways

Western blot analysis showed that treatment with male papaya leaf extract significantly inhibited NF-κB p65 activation and downregulated STAT1 expression (associated with M1 polarization), while also suppressing STAT6 activation (associated with M2 polarization). Additionally, modulation of the PI3K/Akt pathway was observed.

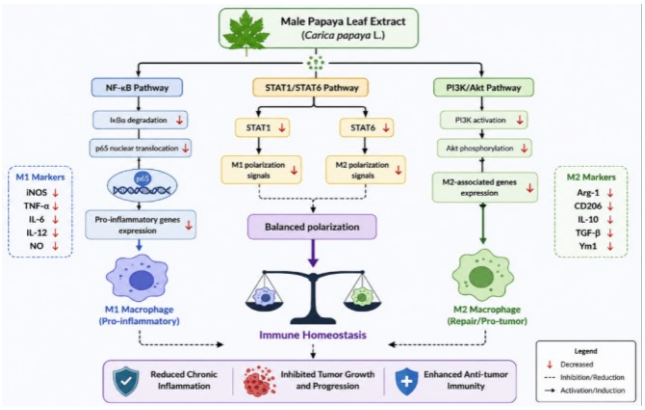


Figure 2. Proposed Mechanism of Action.

These findings suggest that the extract acts on multiple signaling pathways, leading to a balanced macrophage phenotype. Recent studies highlight that targeting both NF-κB and STAT pathways is crucial for effective immunomodulation in chronic diseases and cancer (Liu et al., 2023).

Effects in Cancer Co-culture Model

In macrophage–cancer cell co-culture systems, treatment with extract-modulated macrophages significantly reduced cancer cell proliferation and migration while increasing apoptosis.

Table 3. Effects on Cancer Cell Behavior.

Parameter	Control	Co-culture	Co-culture + Extract
Proliferation (%)	100	135 ± 5	85 ± 4 ↓
Migration (%)	100	140 ± 6	78 ± 3 ↓
Apoptosis (%)	5 ± 1	3 ± 1	18 ± 2 ↑

This suggests that macrophage reprogramming induced by the extract shifts the tumor microenvironment toward an anti-tumor state. Similar findings have been reported where modulation of TAMs reduces tumor progression (Cassetta & Pollard, 2020).

In Vivo Findings

In animal models, treatment with male papaya leaf extract significantly reduced inflammatory infiltration and tumor growth. Immunohistochemical analysis showed decreased CD206 (M2 marker) and increased CD86 (M1 marker), indicating a shift toward anti-tumor macrophage activity.

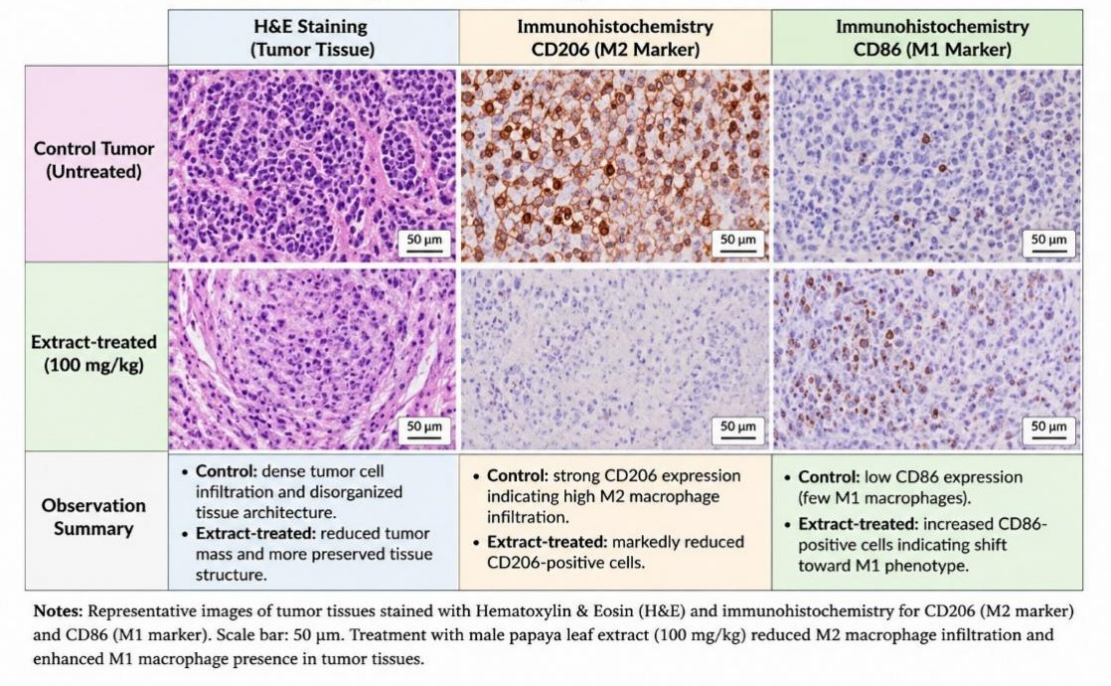


Figure 3. Histological Observation.

Discussion

The present study demonstrates that male papaya leaf extract exerts a significant immunomodulatory effect by regulating macrophage polarization in both chronic inflammation and cancer models. Unlike conventional agents that either suppress or activate immune responses, this extract appears to restore immune balance by modulating both M1 and M2 phenotypes.

The high phenolic and flavonoid content likely plays a central role in this activity. These compounds are known to interact with key inflammatory signaling pathways such as NF-κB and STAT, which regulate cytokine production and macrophage differentiation (Zhang et al., 2021; Liu et al., 2023). The observed decrease in pro-inflammatory markers (iNOS, TNF-α, IL-6) and suppression of excessive M2 markers (Arg-1, CD206) indicates a normalization of immune function rather than simple inhibition.

Importantly, the dual inhibition of STAT1 and STAT6 suggests that the extract prevents both hyperinflammation and tumor-promoting immunosuppression. This is particularly relevant in cancer, where M2-dominant TAMs contribute to immune evasion. By reprogramming macrophages, the extract enhances anti-tumor responses, as evidenced by reduced proliferation and increased apoptosis in cancer cells.

The in vivo results further confirm the therapeutic potential of male papaya leaf extract, demonstrating reduced tumor growth and improved immune profiles. These findings align with recent advances in immunotherapy that emphasize targeting the tumor microenvironment rather than cancer cells alone (Cassetta & Pollard, 2020).

Overall, this study provides strong evidence that male papaya leaves, an underutilized plant resource, have significant potential as a natural immunomodulatory agent. However, further studies are needed to isolate specific active compounds and evaluate clinical applicability.

CONCLUSIONS

This study demonstrates that male papaya (*C. papaya L.*) leaf extract possesses significant immunomodulatory activity, particularly in regulating macrophage polarization under conditions of chronic inflammation and cancer. The extract was shown to modulate both M1 and M2 macrophage phenotypes, reducing excessive pro-inflammatory responses while simultaneously inhibiting tumor-promoting M2 polarization. This bidirectional regulation suggests that the extract promotes immune homeostasis rather than simply enhancing or suppressing a single immune pathway.

At the molecular level, the effects of the extract are associated with the modulation of key signaling pathways, including NF-κB, STAT1/STAT6, and PI3K/Akt. These pathways are known to play central roles in macrophage activation and polarization, and their regulation by the extract provides mechanistic insight into its biological activity. The reduction in nitric oxide production and pro-inflammatory cytokines, along with decreased expression of M2-associated markers, further supports its role in restoring immune balance.

In cancer models, extract-mediated reprogramming of macrophages contributed to a less favorable tumor microenvironment, as evidenced by reduced cancer cell proliferation and migration and increased apoptosis. In vivo findings corroborated these results, showing decreased tumor growth and a shift in macrophage populations toward a more anti-tumor phenotype.

Overall, these findings highlight the potential of male papaya leaves as a promising natural source of immunomodulatory compounds. The study not only addresses existing research gaps regarding the role of male papaya leaves but also provides a scientific basis for their development as alternative therapeutic agents in chronic inflammatory diseases and cancer. Further studies are recommended to isolate active compounds, evaluate long-term safety, and explore clinical applications.

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Authors' Contributions: Lisa Savitri was responsible for the conceptualization and design of the study, execution of experiments, data analysis, and preparation of the initial manuscript draft. Kharisul Ihsan contributed to the development of the research framework, provided overall supervision throughout the study, and performed critical revisions of the manuscript for intellectual content. Elfred Rinaldo Kasimo supported the experimental procedures, assisted in data interpretation, and contributed to manuscript refinement. All authors have reviewed and approved the final version of the manuscript.

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