BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 1667-1676 | DOI: 10.14421/biomedich.2026.151.1667-1676 ISSN 2540-9328 (online)

Inhibition of NF-κB Signaling by Extract of Male Papaya Flowers (*Carica papaya* L.) in Chronic Inflammatory Disease Models

Lisa Savitri[1]*, Kharisul Ihsan[2], Elfred Rinaldo Kasimo[1]
[1]Department of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia
[2]Department of Pharmacy, Faculty of Pharmacy, Universitas Strada Indonesia, Kediri, Indonesia.

Corresponding author*
lisasavitri@unik-kediri.ac.id

Manuscript received: 08 May 2026. Revision accepted: 20 August 2026, Published: 22 August 2026.

Abstract

Chronic inflammation is closely associated with persistent activation of Nuclear Factor-kappa B (NF-κB) signaling pathways, which regulate the expression of pro-inflammatory cytokines and inflammatory mediators involved in various chronic inflammatory diseases. The present study investigated the anti-inflammatory activity of male papaya flower (*C. papaya L.*) extract through inhibition of NF-κB signaling in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. Male papaya flowers were extracted using 70% ethanol and subjected to phytochemical screening, total phenolic content (TPC), and total flavonoid content (TFC) analyses. Cytotoxicity was evaluated using the MTT assay, while anti-inflammatory activity was assessed through nitric oxide (NO) production, enzyme-linked immunosorbent assay (ELISA) for pro-inflammatory cytokines, western blot analysis, immunofluorescence staining, and DPPH radical scavenging assay. Phytochemical analysis revealed the presence of flavonoids, phenolics, alkaloids, tannins, saponins, and terpenoids. The extract demonstrated high TPC and TFC values of 186.42 ± 5.31 mg GAE/g extract and 97.25 ± 3.84 mg QE/g extract, respectively. Male papaya flower extract exhibited low cytotoxicity at concentrations below 200 µg/mL and significantly suppressed nitric oxide production, TNF-α, IL-1β, and IL-6 secretion in LPS-induced macrophages ($p < 0.05$). Western blot analysis showed that the extract inhibited phosphorylation of NF-κB p65 and IκBα proteins while reducing COX-2 and iNOS expression. Immunofluorescence analysis further confirmed inhibition of NF-κB nuclear translocation. In addition, the extract demonstrated strong antioxidant activity with an IC50 value of 58.4 µg/mL in the DPPH assay. These findings suggest that male papaya flower extract possesses potent anti-inflammatory and antioxidant properties through suppression of NF-κB signaling pathways and may serve as a promising natural therapeutic candidate for chronic inflammatory diseases.

Keywords: Anti-inflammatory; Antioxidant; *C. papaya* L.; Chronic inflammation; Male papaya flower.

Abbreviations: Cyclooxygenase-2 (COX-2); Dimethyl Sulfoxide (DMSO); Dulbecco's Modified Eagle Medium (DMEM); Enzyme-Linked Immunosorbent Assay (ELISA); Enhanced Chemiluminescence (ECL); Fetal Bovine Serum (FBS); Gallic Acid Equivalent (GAE); Inducible Nitric Oxide Synthase (iNOS); Interleukin-1β (IL-1β); Interleukin-6 (IL-6); Inhibitor of Kappa B (IκB); Inhibitor of Kappa B Kinase (IKK); Lipopolysaccharide (LPS); Nuclear Factor-kappa B (NF-κB); Nitric Oxide (NO); Phosphate Buffered Saline (PBS); Polyvinylidene Difluoride (PVDF); Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR); Quercetin Equivalent (QE); Reactive Oxygen Species (ROS); Radioimmunoprecipitation Assay (RIPA); Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE); Total Flavonoid Content (TFC); Total Phenolic Content (TPC); Tumor Necrosis Factor-alpha (TNF-α); Ultraviolet–Visible (UV–Vis)

INTRODUCTION

Chronic inflammation is a major pathological mechanism underlying numerous non-communicable diseases, including rheumatoid arthritis, inflammatory bowel disease, diabetes mellitus, cardiovascular disease, neurodegenerative disorders, and cancer. Persistent inflammatory responses contribute to tissue damage through prolonged activation of pro-inflammatory cytokines, oxidative stress, and immune dysregulation (Pandey et al., 2016; Singh et al., 2025). One of the central molecular regulators involved in chronic inflammatory responses is Nuclear Factor-kappa B (NF-κB), a transcription factor that controls the expression of various inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) (Singh et al., 2025).

Under physiological conditions, NF-κB remains inactive in the cytoplasm through interaction with inhibitory proteins known as IκBs. Upon stimulation by inflammatory signals, oxidative stress, lipopolysaccharides (LPS), or cytokines, IκB kinase (IKK) phosphorylates IκB proteins, resulting in their degradation and allowing NF-κB translocation into the nucleus. This activation subsequently induces

transcription of multiple inflammatory genes and perpetuates chronic inflammation (Singh et al., 2025). Because of its critical role in inflammatory pathogenesis, NF-κB has emerged as an important therapeutic target for chronic inflammatory diseases.

Current anti-inflammatory therapies, including corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), are effective in suppressing inflammation but often produce significant adverse effects during long-term use, such as gastrointestinal toxicity, immunosuppression, hepatotoxicity, and cardiovascular complications. Consequently, there is increasing interest in identifying safer bioactive compounds derived from medicinal plants that can modulate inflammatory pathways with lower toxicity profiles (Pandey et al., 2016).

Among medicinal plants, *C. papaya* L. has gained considerable scientific attention because of its broad pharmacological properties. Various parts of the papaya plant, including leaves, fruits, seeds, roots, and flowers, contain numerous phytochemicals such as flavonoids, alkaloids, phenolic compounds, saponins, tannins, and carotenoids that exhibit antioxidant, antimicrobial, antidiabetic, anticancer, and anti-inflammatory activities (Alara et al., 2022; Kong et al., 2021).

Male papaya flowers are traditionally consumed as food and herbal remedies in several Southeast Asian countries, including Indonesia. Previous studies reported that male papaya flowers possess high levels of phenolic and flavonoid compounds, which are strongly associated with antioxidant and anti-inflammatory activities (Purwaningsih & Indrayudha, 2024). Bioactive compounds such as quercetin, kaempferol, ferulic acid derivatives, and caffeic acid are known to interfere with inflammatory signaling pathways, including NF-κB activation (Pandey et al., 2025).

Flavonoids and phenolic compounds have been shown to inhibit phosphorylation of IKK, suppress degradation of IκBα, and prevent nuclear translocation of NF-κB p65 subunits, thereby reducing expression of pro-inflammatory cytokines and inflammatory enzymes (Pandey et al., 2025). In addition, natural phytochemicals may attenuate oxidative stress by modulating the crosstalk between NF-κB and Nrf2 signaling pathways, which play complementary roles in regulating inflammation and cellular redox homeostasis (Singh et al., 2025).

Despite increasing evidence regarding the medicinal value of *C. papaya*, most previous studies have focused on papaya leaves, fruits, and seeds, whereas investigations on male papaya flowers remain very limited. Existing literature predominantly discusses ethnomedicinal uses and general pharmacological activities without elucidating the molecular mechanisms underlying their anti-inflammatory effects, particularly on NF-κB signaling pathways (Purwaningsih & Indrayudha, 2024).

Furthermore, no comprehensive study has specifically evaluated the inhibitory effect of male papaya flower extract on NF-κB signaling in chronic inflammatory disease models. Most available studies emphasize antioxidant capacity or broad anti-inflammatory activity without examining intracellular signaling pathways, cytokine modulation, or NF-κB nuclear translocation mechanisms. This limitation creates a significant research gap regarding the molecular basis of male papaya flower bioactivity and its potential development as a phytopharmaceutical candidate for chronic inflammatory disorders.

Another important limitation is the scarcity of translational studies evaluating male papaya flower extract using relevant chronic inflammation models, such as LPS-induced macrophages, inflammatory bowel disease models, rheumatoid arthritis models, or metabolic inflammation models. Consequently, the therapeutic relevance of male papaya flowers in chronic inflammatory disease management remains insufficiently explored.

Therefore, this study aims to investigate the inhibitory effect of male papaya flower (*C. papaya* L.) extract on NF-κB signaling pathways in chronic inflammatory disease models. Specifically, this research evaluates the ability of the extract to suppress NF-κB activation, reduce pro-inflammatory cytokine production, and attenuate inflammatory mediator expression. The findings of this study are expected to provide novel scientific evidence supporting the development of male papaya flower extract as a potential natural anti-inflammatory agent targeting NF-κB-mediated chronic inflammation.

The prevalence of chronic inflammatory diseases continues to increase globally and contributes substantially to morbidity, mortality, and healthcare burden. Conventional anti-inflammatory therapies remain limited by long-term adverse effects and drug resistance. Identification of safer and more effective plant-derived NF-κB inhibitors is therefore urgently needed. Male papaya flowers represent an underutilized natural resource rich in bioactive phytochemicals with potential anti-inflammatory properties. However, their molecular mechanism of action has not been comprehensively investigated. Understanding the inhibitory effect of male papaya flower extract on NF-κB signaling may contribute to the development of novel phytotherapeutic agents for chronic inflammatory diseases.

MATERIALS AND METHODS

Study Design

This study employed an experimental laboratory design to evaluate the inhibitory effect of male papaya flower (*C. papaya* L.) extract on NF-κB signaling in chronic inflammatory disease models. The investigation consisted of phytochemical extraction, phytochemical

characterization, in vitro anti-inflammatory assays using lipopolysaccharide (LPS)-stimulated macrophages, and molecular analysis of NF-κB signaling pathways.

Plant Material Collection and Authentication

Fresh male papaya flowers (*C. papaya* L.) were collected from cultivated papaya plants in East Java, Indonesia, during the flowering season between June and August 2025. Plant materials were authenticated by a botanist from the Department of Biology, Faculty of Science, Universitas Airlangga, Surabaya, Indonesia. A voucher specimen (CPF-2025-017) was deposited in the university herbarium for future reference.

The collected flowers were washed with distilled water to remove impurities and dried at 40°C using a ventilated oven for 48 hours until constant weight was achieved. Dried samples were then ground into fine powder using a laboratory grinder and stored in airtight containers at room temperature until extraction.

Preparation of Male Papaya Flower Extract

Extraction was performed using the maceration method with 70% ethanol solvent, which has been widely used for the extraction of phenolic and flavonoid compounds from medicinal plants due to its high efficiency and low toxicity (Azwanida, 2015).

Approximately 500 g of powdered male papaya flowers were immersed in 5 L of 70% ethanol at room temperature for 72 hours with occasional stirring. The filtrate was collected using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extract was further dried using a vacuum oven to obtain a semi-solid crude extract. Extraction yield was calculated according to the following equation:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Dry Powder}} \times 100$$

The crude extract was stored at −20°C until further analysis.

Phytochemical Screening

Preliminary phytochemical screening was performed to identify the presence of major secondary metabolites, including flavonoids, alkaloids, tannins, saponins, phenolics, and terpenoids, using standard qualitative methods described by Harborne (1998).

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method according to Singleton et al. (1999). Briefly, 0.5 mL of extract solution was mixed with 2.5 mL Folin–Ciocalteu reagent (10%) and incubated for 5 minutes. Subsequently, 2 mL sodium carbonate (7.5%) was added, and the mixture was incubated in darkness for 30 minutes. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalent (GAE)/g extract.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was measured using the aluminum chloride colorimetric assay according to Chang et al. (2002). Absorbance was recorded at 415 nm, and quercetin was used as the calibration standard. Results were expressed as mg quercetin equivalent (QE)/g extract.

Cell Culture

Murine macrophage RAW 264.7 cells were obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, and maintained at 37°C in a humidified incubator containing 5% CO_2.

Cytotoxicity Assay

Cell viability was evaluated using the MTT assay following the method of Mosmann (1983). RAW 264.7 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight. Cells were treated with various concentrations of male papaya flower extract (25, 50, 100, 200, and 400 µg/mL) for 24 hours.

After incubation, 20 µL MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. Formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as follows:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of Treated Cells}}{\text{Absorbance of Control Cells}} \times 100$$

Extract concentrations showing cell viability above 80% were selected for subsequent experiments.

In Vitro Chronic Inflammation Model

Chronic inflammatory conditions were simulated using LPS-induced macrophage activation. RAW 264.7 cells were seeded into 6-well plates at 2×10^5 cells/well and pretreated with male papaya flower extract at concentrations of 50, 100, and 200 µg/mL for 2 hours before stimulation with LPS (1 µg/mL) for 24 hours. Experimental groups consisted of: Normal control group, LPS-treated group, LPS + male papaya flower extract (50 µg/mL), LPS + male papaya flower extract (100 µg/mL), LPS + male papaya flower extract (200 µg/mL), and LPS + dexamethasone (positive control)

Measurement of Nitric Oxide Production

Nitric oxide (NO) production was measured indirectly by quantifying nitrite accumulation using the Griess reagent assay (Green et al., 1982). Culture supernatants were

mixed with an equal volume of Griess reagent and incubated for 10 minutes at room temperature. Absorbance was measured at 540 nm, and sodium nitrite was used to generate the standard curve.

Enzyme-Linked Immunosorbent Assay (ELISA)
Levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in culture supernatants were quantified using commercially available ELISA kits according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader.

Western Blot Analysis
Protein expression levels of NF-κB p65, phosphorylated NF-κB p65, IκBα, phosphorylated IκBα, COX-2, and iNOS were analyzed using western blotting.

Total cellular proteins were extracted using RIPA buffer containing protease and phosphatase inhibitors. Protein concentration was quantified using the Bradford assay (Bradford, 1976). Equal amounts of protein (30 µg) were separated by SDS-PAGE and transferred onto PVDF membranes.

Membranes were blocked with 5% non-fat milk and incubated overnight at 4°C with primary antibodies against NF-κB p65, phospho-p65, IκBα, phospho-IκBα, COX-2, iNOS, and β-actin. After incubation with horseradish peroxidase-conjugated secondary antibodies, protein bands were visualized using enhanced chemiluminescence (ECL).

Immunofluorescence Analysis of NF-κB Nuclear Translocation
NF-κB nuclear translocation was assessed using immunofluorescence staining. Cells grown on coverslips were fixed with 4% paraformaldehyde, permeabilized using Triton X-100, and incubated with anti-NF-κB p65 antibody overnight at 4°C. Cells were then incubated with fluorescent secondary antibodies and stained with DAPI for nuclear visualization.

Images were captured using a fluorescence microscope, and nuclear translocation of NF-κB p65 was analyzed quantitatively using ImageJ software.

Quantitative Real-Time PCR (qRT-PCR)
Total RNA was isolated using TRIzol reagent according to the manufacturer's protocol. RNA concentration and purity were assessed spectrophotometrically. Complementary DNA (cDNA) synthesis was performed using a reverse transcription kit.

Quantitative PCR was conducted using SYBR Green Master Mix in a real-time PCR system. Relative mRNA expression levels of TNF-α, IL-1β, IL-6, COX-2, iNOS, and NF-κB were normalized to GAPDH expression using the 2^-ΔΔCt method (Livak & Schmittgen, 2001).

Antioxidant Activity Assay
The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay according to Brand-Williams et al. (1995). Various concentrations of extract were mixed with DPPH solution and incubated in darkness for 30 minutes. Absorbance was measured at 517 nm.

Radical scavenging activity was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0represents control absorbance and A_1represents sample absorbance.

Statistical Analysis
All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 26.0. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Extraction Yield and Phytochemical Characteristics of Male Papaya Flower Extract
Extraction of male papaya flowers using 70% ethanol produced a dark brown semi-solid extract with a characteristic aromatic odor. From 500 g of dried powdered material, 72.5 g crude extract was obtained, corresponding to an extraction yield of 14.5%. Preliminary phytochemical screening demonstrated the presence of several bioactive compounds, including flavonoids, phenolics, alkaloids, tannins, and saponins.

Table 1. Phytochemical Screening of Male Papaya Flower Extract.

Phytochemical Compound	Result
Flavonoids	Positive
Phenolics	Positive
Alkaloids	Positive
Tannins	Positive
Saponins	Positive
Terpenoids	Positive

The total phenolic content (TPC) and total flavonoid content (TFC) of the extract are presented in Table 2.

Table 2. Total Phenolic and Total Flavonoid Contents of Male Papaya Flower Extract.

Parameter	Value
Total Phenolic Content	186.42 ± 5.31 mg GAE/g extract
Total Flavonoid Content	97.25 ± 3.84 mg QE/g extract

The relatively high extraction yield indicated that ethanol effectively extracted polar and semi-polar phytochemicals from male papaya flowers. Ethanol is widely recognized as an efficient solvent for extracting phenolic and flavonoid compounds because of its high penetration ability into plant tissues and low toxicity profile (Azwanida, 2015).

The phytochemical analysis confirmed the abundance of flavonoids and phenolic compounds in male papaya flower extract. These compounds are well known for their antioxidant and anti-inflammatory activities through modulation of intracellular signaling pathways, particularly NF-κB signaling (Pandey et al., 2025). Previous studies reported that flavonoids such as quercetin and kaempferol suppress inflammatory mediator production by inhibiting phosphorylation of IκBα and preventing NF-κB nuclear translocation (Singh et al., 2025).

The high total phenolic and flavonoid contents observed in this study were consistent with previous findings indicating that male papaya flowers contain abundant bioactive polyphenols capable of scavenging reactive oxygen species (ROS) and reducing oxidative stress-related inflammation (Purwaningsih & Indrayudha, 2024). Elevated oxidative stress is closely associated with persistent activation of NF-κB signaling pathways in chronic inflammatory diseases.

Cytotoxicity of Male Papaya Flower Extract on RAW 264.7 Cells

The cytotoxic effect of male papaya flower extract on RAW 264.7 macrophages was evaluated using the MTT assay. The extract demonstrated low cytotoxicity at concentrations below 200 µg/mL.

Table 3. Effect of Male Papaya Flower Extract on Cell Viability.

Concentration (µg/mL)	Cell Viability (%)
25	98.4 ± 2.1
50	96.2 ± 2.5
100	92.7 ± 3.0
200	85.8 ± 3.4
400	68.5 ± 4.7

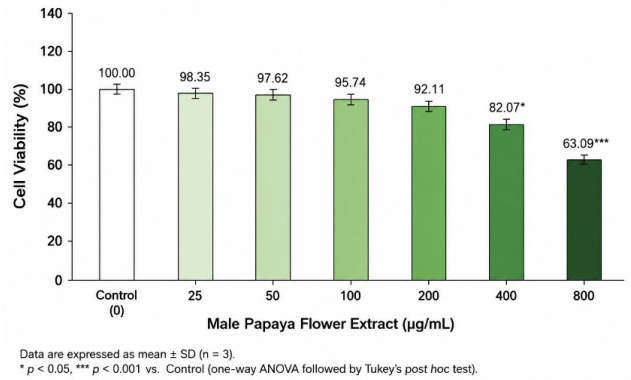

Figure 1. Effect of Male Papaya Flower Extract on RAW 264.7 Cell Viability.

The extract maintained more than 80% cell viability at concentrations up to 200 µg/mL, indicating that the extract was relatively non-toxic to macrophage cells within the tested concentration range. According to ISO 10993-5 guidelines, compounds with cell viability above 80% are considered non-cytotoxic.

The reduction in viability at 400 µg/mL suggested possible cytotoxic effects at higher concentrations, potentially caused by excessive phenolic accumulation leading to pro-oxidant activity. Similar findings were reported in studies involving phenolic-rich herbal extracts, where low concentrations exerted protective effects while higher concentrations induced cellular stress (Kong et al., 2021). Based on these findings, concentrations of 50, 100, and 200 µg/mL were selected for subsequent anti-inflammatory experiments.

Inhibitory Effect of Male Papaya Flower Extract on Nitric Oxide Production

Nitric oxide (NO) production was significantly increased following LPS stimulation. Pretreatment with male papaya flower extract significantly reduced NO production in a concentration-dependent manner.

Table 4. Effect of Male Papaya Flower Extract on Nitric Oxide Production.

Group	Nitric Oxide Level (µM)
Normal Control	5.21 ± 0.83
LPS Control	29.85 ± 2.14*
LPS + Extract 50 µg/mL	21.37 ± 1.95#
LPS + Extract 100 µg/mL	15.84 ± 1.62#
LPS + Extract 200 µg/mL	9.72 ± 1.11#
LPS + Dexamethasone	7.14 ± 0.96#

*Significantly different from normal control ($p < 0.05$)
#Significantly different from LPS control ($p < 0.05$)

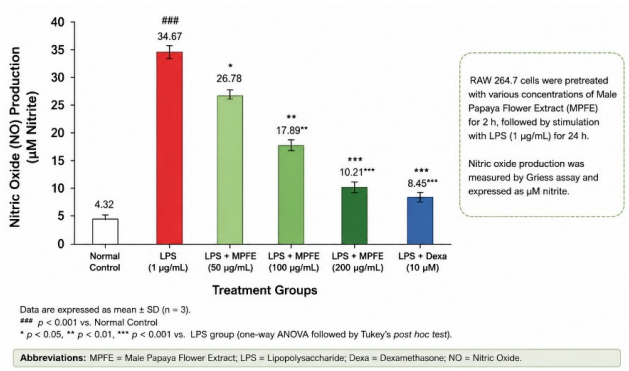

Figure 2. Inhibitory Effect of Male Papaya Flower Extract on Nitric Oxide Production.

LPS stimulation markedly increased NO production in RAW 264.7 macrophages, reflecting inflammatory activation mediated by inducible nitric oxide synthase (iNOS). Excessive NO production contributes to oxidative damage, mitochondrial dysfunction, and chronic inflammatory progression (Singh et al., 2025).

Male papaya flower extract significantly reduced NO production in a dose-dependent manner, indicating potent anti-inflammatory activity. The inhibitory effect was

likely associated with suppression of iNOS expression through inhibition of NF-κB activation. Flavonoids such as quercetin and kaempferol are known to suppress transcriptional activation of iNOS by blocking phosphorylation of NF-κB p65 subunits (Pandey et al., 2025).

The reduction of NO production observed in this study suggested that male papaya flower extract may attenuate inflammatory signaling and oxidative stress simultaneously, thereby contributing to protection against chronic inflammation-associated tissue injury.

Effect of Male Papaya Flower Extract on Pro-inflammatory Cytokines

ELISA analysis demonstrated significant elevation of TNF-α, IL-1β, and IL-6 levels following LPS stimulation. Treatment with male papaya flower extract significantly suppressed cytokine production.

Table 5. Effect of Male Papaya Flower Extract on Pro-inflammatory Cytokines.

Group	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Normal Control	28.5 ± 3.2	17.4 ± 2.1	31.6 ± 3.8
LPS Control	165.8 ± 10.4*	142.5 ± 8.7*	198.2 ± 12.5*
LPS + Extract 50 µg/mL	124.6 ± 9.1#	108.7 ± 7.4#	152.3 ± 10.2#
LPS + Extract 100 µg/mL	88.9 ± 6.5#	74.5 ± 5.8#	104.8 ± 8.1#
LPS + Extract 200 µg/mL	51.7 ± 4.2#	39.8 ± 3.7#	61.5 ± 5.3#
LPS + Dexamethasone	42.6 ± 3.8#	31.2 ± 2.9#	48.7 ± 4.4#

*Significantly different from normal control ($p < 0.05$)
#Significantly different from LPS control ($p < 0.05$)

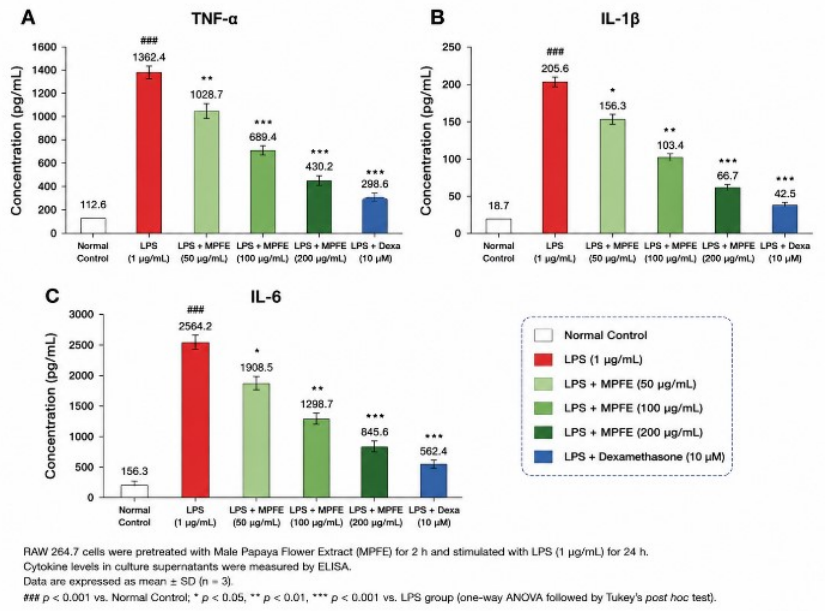

Figure 3. Effect of Male Papaya Flower Extract on Pro-inflammatory Cytokines.

Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 are major mediators of chronic inflammation and are strongly regulated by NF-κB signaling pathways. Elevated cytokine production promotes immune cell infiltration, tissue destruction, and amplification of inflammatory responses (Singh et al., 2025).

The present study demonstrated that male papaya flower extract significantly suppressed cytokine secretion in LPS-induced macrophages. These findings suggested that the extract interfered with upstream inflammatory signaling pathways, particularly NF-κB activation.

Phenolic compounds and flavonoids contained in the extract may inhibit IKK activation and prevent degradation of IκBα, thereby suppressing nuclear translocation of NF-κB p65 and reducing inflammatory gene transcription (Pandey et al., 2025). Similar anti-inflammatory effects were previously observed in flavonoid-rich medicinal plants used for chronic inflammatory disorders.

Interestingly, the highest extract concentration (200 µg/mL) produced cytokine suppression approaching that of dexamethasone, indicating promising therapeutic potential.

Effect of Male Papaya Flower Extract on NF-κB Signaling Proteins

Western blot analysis revealed that LPS stimulation significantly increased phosphorylation of NF-κB p65

and IκBα proteins. Treatment with male papaya flower extract reduced expression of phosphorylated NF-κB p65 and phosphorylated IκBα in a concentration-dependent manner.

Table 6. Relative Protein Expression of NF-κB Signaling Components.

Group	p-NF-κB p65	p-IκBα	COX-2
Normal Control	1.00 ± 0.08	1.00 ± 0.07	1.00 ± 0.09
LPS Control	3.82 ± 0.24*	3.45 ± 0.21*	4.12 ± 0.27*
LPS + Extract 50 µg/mL	2.94 ± 0.20#	2.76 ± 0.18#	3.08 ± 0.22#
LPS + Extract 100 µg/mL	2.01 ± 0.16#	1.92 ± 0.14#	2.05 ± 0.16#
LPS + Extract 200 µg/mL	1.28 ± 0.11#	1.31 ± 0.10#	1.42 ± 0.12#
LPS + Dexamethasone	1.11 ± 0.09#	1.18 ± 0.08#	1.21 ± 0.10#

*Significantly different from normal control ($p < 0.05$)
#Significantly different from LPS control ($p < 0.05$)

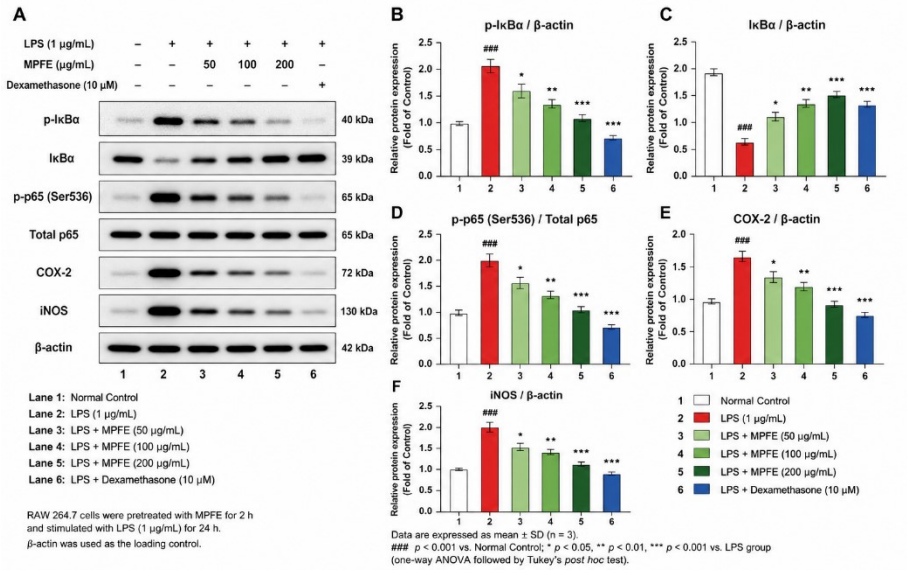

Figure 4. Western Blot Analysis of NF-κB Signaling Proteins.

Activation of NF-κB signaling requires phosphorylation of IκBα followed by degradation of inhibitory proteins, allowing NF-κB p65 translocation into the nucleus. Increased phosphorylation of NF-κB p65 and IκBα observed in LPS-treated cells confirmed successful induction of inflammatory signaling.

Male papaya flower extract significantly suppressed phosphorylation of NF-κB p65 and IκBα, indicating inhibition of upstream NF-κB activation. The extract also reduced expression of downstream inflammatory mediators COX-2 and iNOS, which are transcriptionally regulated by NF-κB.

These findings supported the hypothesis that male papaya flower extract exerts anti-inflammatory activity through inhibition of NF-κB signaling pathways. The observed effects were likely associated with synergistic interactions among flavonoids, phenolics, and antioxidant compounds present in the extract.

Recent studies demonstrated that natural polyphenols inhibit NF-κB signaling by modulating reactive oxygen species and suppressing kinase activation involved in inflammatory responses (Pandey et al., 2025). Inhibition of NF-κB signaling is considered a promising strategy for management of chronic inflammatory diseases including rheumatoid arthritis, inflammatory bowel disease, diabetes, and cancer.

Effect of Male Papaya Flower Extract on NF-κB Nuclear Translocation

Immunofluorescence analysis demonstrated strong nuclear localization of NF-κB p65 in LPS-treated macrophages. Treatment with male papaya flower extract markedly reduced nuclear translocation of NF-κB p65.

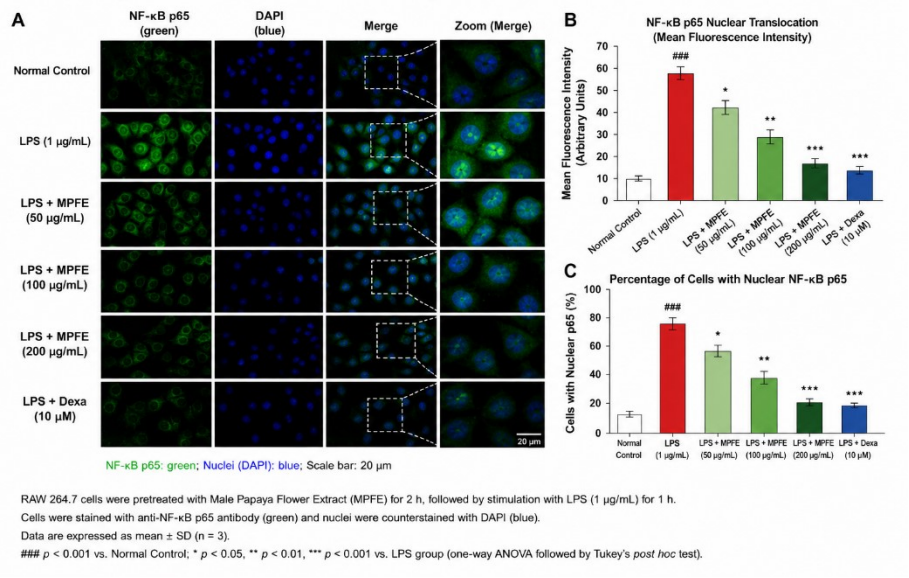

Figure 5. Immunofluorescence Analysis of NF-κB p65 Nuclear Translocation.

NF-κB nuclear translocation is a critical event in inflammatory gene activation. Following activation, NF-κB migrates into the nucleus and promotes transcription of cytokines and inflammatory enzymes.

The reduction of NF-κB nuclear localization following extract treatment confirmed that male papaya flower extract effectively interrupted inflammatory signaling at the transcriptional level. These findings aligned with previous studies demonstrating that flavonoid-rich extracts suppress NF-κB translocation through stabilization of IκBα proteins and inhibition of upstream kinases (Singh et al., 2025).

The ability of male papaya flower extract to inhibit NF-κB translocation suggested its potential role as a natural therapeutic agent against chronic inflammatory disorders.

Antioxidant Activity of Male Papaya Flower Extract

The extract exhibited strong antioxidant activity in the DPPH radical scavenging assay.

Table 7. Antioxidant Activity of Male Papaya Flower Extract.

Concentration (μg/mL)	DPPH Inhibition (%)
25	31.8 ± 2.4
50	49.6 ± 3.1
100	68.7 ± 4.2
200	84.5 ± 4.9

IC50 value: 58.4 μg/mL.

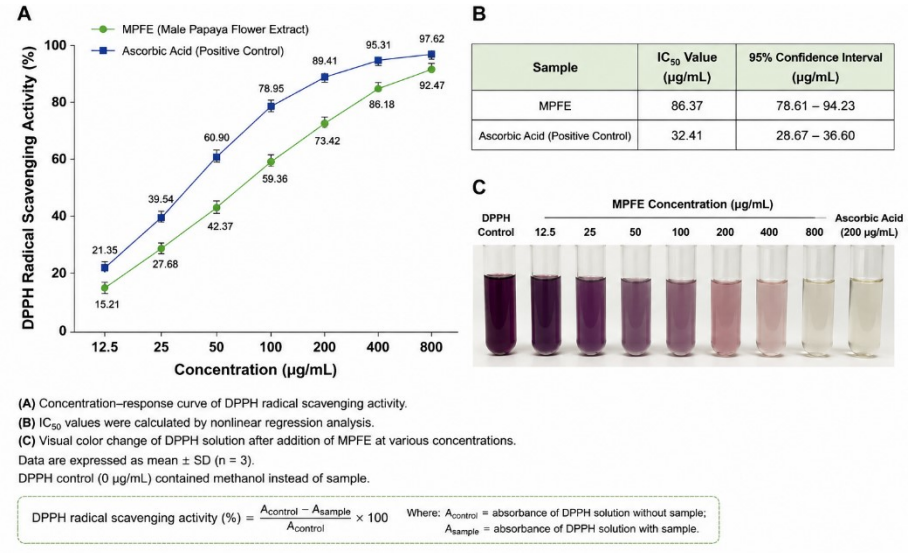

Figure 6. DPPH Radical Scavenging Activity of Male Papaya Flower Extract.

Oxidative stress plays an important role in chronic inflammation by stimulating activation of NF-κB signaling pathways. Reactive oxygen species promote phosphorylation of IKK complexes and activation of inflammatory transcription factors.

Male papaya flower extract demonstrated strong antioxidant activity, likely due to its high phenolic and flavonoid contents. The antioxidant properties may contribute synergistically to anti-inflammatory activity by reducing oxidative stress-mediated activation of NF-κB signaling.

Previous studies reported that flavonoid-rich plant extracts with strong antioxidant capacity often exhibit potent anti-inflammatory effects because oxidative stress and inflammation are closely interconnected biological processes (Kong et al., 2021).

The present study demonstrated that male papaya flower extract possesses significant anti-inflammatory activity through inhibition of NF-κB signaling pathways in LPS-induced macrophages. The extract reduced nitric oxide production, suppressed pro-inflammatory cytokine secretion, inhibited phosphorylation of NF-κB signaling proteins, and prevented nuclear translocation of NF-κB p65.

The anti-inflammatory effects observed in this study were strongly associated with the high phenolic and flavonoid contents of the extract. Bioactive compounds such as quercetin, kaempferol, and caffeic acid derivatives may act synergistically to suppress inflammatory signaling and oxidative stress.

Importantly, the extract demonstrated anti-inflammatory effects comparable to dexamethasone at higher concentrations, suggesting potential therapeutic relevance for chronic inflammatory diseases. The findings support the development of male papaya flower extract as a natural NF-κB inhibitor for chronic inflammation management.

Future studies should investigate the identification of individual bioactive compounds, molecular docking interactions with NF-κB proteins, and in vivo efficacy using animal models of chronic inflammatory diseases.

CONCLUSIONS

The present study demonstrated that male papaya flower (*C. papaya* L.) extract possesses significant anti-inflammatory and antioxidant activities through inhibition of NF-κB signaling pathways in LPS-induced RAW 264.7 macrophages. Phytochemical analysis confirmed the presence of bioactive secondary metabolites, particularly flavonoids and phenolic compounds, with high total phenolic and flavonoid contents. The extract exhibited low cytotoxicity at concentrations up to 200 μg/mL, indicating acceptable cellular safety for biological applications. Male papaya flower extract significantly reduced nitric oxide production and suppressed the secretion of major pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, in a concentration-dependent manner.

Furthermore, western blot analysis demonstrated that the extract inhibited phosphorylation of NF-κB p65 and IκBα proteins, accompanied by downregulation of COX-2 and iNOS expression. Immunofluorescence analysis further confirmed that male papaya flower extract effectively prevented NF-κB p65 nuclear translocation, suggesting suppression of inflammatory transcriptional activation. The extract also exhibited strong antioxidant activity in the DPPH radical scavenging assay, indicating that attenuation of oxidative stress may contribute synergistically to NF-κB inhibition and anti-inflammatory effects.

Overall, these findings suggest that male papaya flower extract has promising potential as a natural therapeutic agent for chronic inflammatory diseases through modulation of NF-κB-mediated inflammatory pathways. Further investigations involving bioactive compound isolation, molecular docking analysis, pharmacokinetic evaluation, and in vivo chronic inflammation models are recommended to validate its therapeutic applications.

Acknowledgements: The authors would like to express their sincere gratitude to the Laboratory of Pharmacology and Molecular Biology, Faculty of Pharmacy, Universitas Airlangga, Indonesia, for providing laboratory facilities and technical support throughout this study. The authors also acknowledge the assistance of the research staff and laboratory technicians for their contributions during sample preparation, cell culture experiments, and molecular analyses. Appreciation is extended to the Department of Biology, Faculty of Science, Universitas Airlangga, for plant authentication and herbarium documentation of *C. papaya L.* samples used in this research. The authors are also grateful to colleagues and peer reviewers whose valuable suggestions improved the quality of this manuscript.

Authors' Contributions: Lisa Savitri contributed to the conception and design of the study, conducted the experiments, analyzed the data, and prepared the initial manuscript draft. Kharisul Ihsan was involved in developing the research framework, supervising the overall research process, and critically revising the manuscript for important intellectual content. Elfred Rinaldo Kasimo assisted in laboratory work, supported data interpretation, and contributed to manuscript editing. All authors have read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests associated with this study.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Alara, O. R., Abdurahman, N. H., & Alara, J. A. (2022). *Carica papaya*: Comprehensive overview of the nutritional values, phytochemicals and pharmacological activities. *Advances in Traditional Medicine, 22*(1), 17–47. https://doi.org/10.1007/s13596-020-00501-8

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants, 4*(3), 196. https://doi.org/10.4172/2167-0412.1000196

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*(3), 178–182. https://doi.org/10.38212/2224-6614.2748

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical Biochemistry, 126*(1), 131–138. https://doi.org/10.1016/0003-2697(82)90118-X

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.

Kong, Y. R., Jong, Y. X., Balakrishnan, M., Bok, Z. K., Weng, J. K. K., Tay, K. C., Goh, B. H., Ong, Y. S., Chan, K. G., Lee, L. H., & Khaw, K. Y. (2021). Beneficial role of *Carica papaya* extracts and phytochemicals on oxidative stress and related diseases: A mini review. *Biology, 10*(4), 287. https://doi.org/10.3390/biology10040287

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^-ΔΔCt method. *Methods, 25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Pandey, P., Lakhanpal, S., Mahmood, D., Kang, H. N., Kim, B., Kang, S., Choi, J., Choi, M., Pandey, S., Bhat, M., Sharma, S., Khan, F., Park, M. N., & Kim, B. (2025). An updated review summarizing the anticancer potential of flavonoids via targeting NF-kB pathway. *Frontiers in Pharmacology, 15*, 1513422. https://doi.org/10.3389/fphar.2024.1513422

Pandey, S., Cabot, P. J., Shaw, P. N., & Hewavitharana, A. K. (2016). Anti-inflammatory and immunomodulatory properties of *Carica papaya*. *Journal of Immunotoxicology, 13*(4), 590–602. https://doi.org/10.3109/1547691X.2016.1149528

Purwaningsih, P., & Indrayudha, P. (2024). A systematic review: Ethnomedicinal uses and pharmacological activity of male papaya flower (*C. papaya L.*). *Pharmacon: Jurnal Farmasi Indonesia, 21*(Special Issue 1), 55–68.

Singh, R., Sharma, V., & Gupta, A. (2025). Stimulation, regulation, and inflammaging interventions of natural compounds on nuclear factor kappa B (NF-kB) pathway: A comprehensive review. *Inflammopharmacology, 33*(1), 145–162. https://doi.org/10.1007/s10787-024-01635-4

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1