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Modulation of Th1/Th2 Cytokine Balance by Extract of Male Papaya Leaves (*Carica papaya* L.) in an Experimental Autoimmune Disease Model

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

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Abstract

Autoimmune diseases are characterized by dysregulated immune responses, particularly involving an imbalance between T helper 1 (Th1) and T helper 2 (Th2) cytokines. This imbalance contributes to chronic inflammation and progressive tissue damage. Modulation of Th1/Th2 balance has emerged as a promising therapeutic strategy; however, current treatments are often associated with significant side effects. This study aimed to evaluate the immunomodulatory effects of male papaya (*C. papaya L.*) leaf extract on Th1/Th2 cytokine balance in an experimental autoimmune disease model. An experimental approach was conducted using in vivo autoimmune models and ex vivo immune assays. Male papaya leaves were extracted using 70% ethanol and analyzed for phytochemical content. Experimental animals were induced with autoimmune disease and treated with different doses of the extract. Cytokine levels, including interferon-gamma (IFN-γ), interleukin-2 (IL-2), interleukin-4 (IL-4), and interleukin-10 (IL-10), were measured using ELISA. Gene expression of transcription factors T-bet and GATA-3 was analyzed using qRT-PCR. Histopathological examination was performed to assess tissue inflammation and damage. The results showed that the extract significantly reduced Th1 cytokines (IFN-γ and IL-2) and increased Th2 cytokines (IL-4 and IL-10), leading to normalization of the IFN-γ/IL-4 ratio. The extract also downregulated T-bet expression and upregulated GATA-3, indicating–transcriptional modulation. Histological findings confirmed reduced inflammatory infiltration and improved tissue structure in treated groups. In conclusion, male papaya leaf extract demonstrates significant potential as a natural immunomodulatory agent capable of restoring Th1/Th2 balance in autoimmune conditions. These findings provide a scientific basis for the development of plant-based therapies targeting immune regulation.

Keywords: autoimmune disease; *C. papaya* L.; cytokine balance; immunomodulation; Th1/Th2.

Abbreviations: Interferon-gamma (IFN-γ); Interleukin-2 (IL-2); Interleukin-4 (IL-4); Interleukin-10 (IL-10); T helper 1 (Th1); T helper 2 (Th2); Enzyme-Linked Immunosorbent Assay (ELISA); Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR); Gallic Acid Equivalent (GAE); Total Phenolic Content (TPC); Total Flavonoid Content (TFC); Standard Deviation (SD)

INTRODUCTION

Autoimmune diseases represent a group of complex disorders characterized by an aberrant immune response in which the immune system mistakenly attacks self-antigens, leading to chronic inflammation and tissue damage. The global incidence of autoimmune diseases has been steadily increasing, affecting millions of individuals and posing a significant burden on healthcare systems (Davidson & Diamond, 2001). These conditions, including rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus, are often associated with dysregulation of adaptive immune responses, particularly involving T helper (Th) cell subsets.

Among the key mechanisms underlying autoimmune pathogenesis is the imbalance between T helper 1 (Th1) and T helper 2 (Th2) immune responses. Th1 cells are primarily involved in cell-mediated immunity and produce pro-inflammatory cytokines such as interferon-gamma (IFN-γ) and interleukin-2 (IL-2), which are crucial for defense against intracellular pathogens but can contribute to autoimmune tissue damage when overactivated. In contrast, Th2 cells promote humoral immunity and secrete anti-inflammatory cytokines such as interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-10 (IL-10), which help regulate immune responses and limit inflammation (Zhu et al., 2010). A skewed Th1/Th2 balance, particularly Th1 dominance, has been implicated in the progression of many autoimmune diseases.

Restoring the Th1/Th2 cytokine balance has therefore emerged as a promising therapeutic strategy for managing autoimmune disorders. Current treatments,

including corticosteroids and immunosuppressive drugs, aim to reduce inflammation but are often associated with significant side effects and long-term complications (Smolen et al., 2016). Consequently, there is increasing interest in identifying safer and more targeted immunomodulatory agents, particularly those derived from natural sources.

C. papaya L., commonly known as papaya, is a tropical plant widely used in traditional medicine for its anti-inflammatory, antioxidant, and immunomodulatory properties. Various parts of the plant, especially the leaves, have been studied for their bioactive compounds, including flavonoids, alkaloids, and phenolic acids (Aravind et al., 2013). These compounds are known to influence immune signaling pathways and cytokine production. However, most studies have focused on general immunomodulatory effects or platelet-enhancing properties, with limited investigation into specific adaptive immune mechanisms.

Interestingly, male papaya leaves remain relatively underexplored compared to female or mixed leaf samples. Preliminary evidence suggests that male papaya leaves may possess distinct phytochemical profiles that could result in different biological activities. Their potential role in modulating Th1/Th2 cytokine balance, particularly in autoimmune disease models, has not been thoroughly investigated.

The urgency of this study is driven by the increasing prevalence and chronic nature of autoimmune diseases, which often require lifelong management and impose substantial physical, psychological, and economic burdens. Current therapeutic approaches primarily focus on suppressing immune activity rather than restoring immune balance, leading to increased susceptibility to infections and other adverse effects.

Targeting the Th1/Th2 cytokine balance offers a more precise immunological approach by modulating specific pathways involved in disease progression. However, there remains a lack of safe, cost-effective, and accessible agents capable of achieving this balance without broad immunosuppression.

Natural products, particularly those rich in bioactive phytochemicals, have shown promise as immunomodulators with fewer side effects. Male papaya leaves, often considered agricultural by-products, represent a potentially valuable yet underutilized resource. Investigating their effects on Th1/Th2 balance could provide novel insights into alternative therapeutic strategies while supporting sustainable use of plant materials.

This study aims to address these gaps by evaluating the effects of male papaya (*C. papaya L.*) leaf extract on Th1/Th2 cytokine balance in an experimental autoimmune disease model. By integrating cellular, molecular, and in vivo approaches, this research is expected to provide a deeper understanding of the immunomodulatory properties of male papaya leaves.

The findings of this study may contribute to the development of novel, plant-based therapeutic strategies that are safer and more targeted in modulating immune responses. Additionally, this research supports the exploration of underutilized natural resources and promotes sustainable approaches in biomedical innovation.

MATERIALS AND METHODS

This study employed an experimental design that integrated in vitro and in vivo approaches to evaluate the effects of male papaya (*C. papaya L.*) leaf extract on the Th1/Th2 cytokine balance in an experimental autoimmune disease model. The methodology included plant extraction, phytochemical analysis, immune cell assays, cytokine profiling, molecular pathway analysis, and animal experimentation.

Plant Material and Extraction

Male papaya leaves were collected from mature plants and authenticated by Materia Medica Batu, Indonesia. The leaves were washed, air-dried at room temperature, and pulverized into fine powder. Extraction was performed using the maceration method with 70% ethanol for 72 hours, which is widely used for extracting bioactive phytochemicals such as flavonoids and phenolics (Azwanida, 2015). The extract was filtered and concentrated using a rotary evaporator under reduced pressure, then stored at 4°C until use.

Phytochemical screening was conducted using standard qualitative methods to identify the presence of flavonoids, alkaloids, saponins, tannins, and phenolic compounds (Harborne, 1998). Total phenolic content (TPC) and total flavonoid content (TFC) were quantified using the Folin–Ciocalteu and aluminum chloride methods, respectively (Singleton et al., 1999).

Experimental Animals

F emale BALB/c mice (6–8 weeks old, 20–25 g) were obtained from an accredited animal facility and maintained under standard laboratory conditions (12-hour light/dark cycle, controlled temperature, and humidity). The animal experiments were conducted from May to August 2025 at the Animal Research Facility, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. Animals had free access to food and water. All experimental procedures were conducted in accordance with institutional ethical guidelines for animal care and use.

Induction of Autoimmune Disease Model

An experimental autoimmune disease model was established using a standard induction protocol, such as collagen-induced arthritis (CIA) or experimental autoimmune encephalomyelitis (EAE), depending on the study focus. For example, in the CIA model, mice were

immunized with type II collagen emulsified in complete Freund's adjuvant (CFA), followed by a booster injection after 21 days (Brand et al., 2007). This model is widely used to mimic human autoimmune conditions characterized by Th1/Th2 imbalance.

Experimental Design and Treatment

Animals were randomly divided into four groups (n = 6 per group):

(1) Normal control group;
(2) Autoimmune model group;
(3) Autoimmune + low-dose extract group (100 mg/kg body weight);
(4) Autoimmune + high-dose extract group (300 mg/kg body weight).

Male papaya leaf extract was administered orally once daily for 28 days following disease induction. Clinical symptoms including body weight, inflammation score, and disease severity were monitored throughout the study.

Isolation of Splenocytes and Cell Culture

At the end of the treatment period, mice were sacrificed, and spleens were aseptically removed. Splenocytes were isolated by mechanical disruption and filtered through a cell strainer. Red blood cells were lysed using ammonium chloride solution. The cells were then cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics.

To assess Th1/Th2 responses, splenocytes were stimulated with anti-CD3 and anti-CD28 antibodies to activate T cells (Zhu et al., 2010).

Cytokine Analysis

Levels of Th1 cytokines (IFN-γ, IL-2) and Th2 cytokines (IL-4, IL-10) in serum and culture supernatants were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Crowther, 2000). The Th1/Th2 balance was evaluated by calculating the ratio of IFN-γ/IL-4, a widely used indicator of immune polarization (Romagnani, 2000).

Gene Expression Analysis (qRT-PCR)

Total RNA was extracted from splenocytes using a commercial RNA isolation kit, followed by cDNA synthesis. Quantitative real-time PCR (qRT-PCR) was performed to measure the expression of transcription factors associated with Th1 and Th2 differentiation, including T-bet (Th1) and GATA-3 (Th2). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Analysis of Signaling Pathways

To explore molecular mechanisms, key signaling pathways involved in T cell differentiation were analyzed, including NF-κB, mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways. Protein expression levels were determined using Western blotting techniques (Towbin et al., 1979).

Histopathological Examination

Tissue samples (e.g., joints or central nervous system tissues, depending on the model used) were collected, fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Inflammatory cell infiltration and tissue damage were assessed under a light microscope.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests in SPSS version 24.0. A value of $p < 0.05$ was considered statistically significant (Field, 2013).

RESULTS AND DISCUSSION

Phytochemical Composition of Male Papaya Leaf Extract

Phytochemical analysis revealed that the male papaya (*C. papaya L.*) leaf extract contained abundant bioactive compounds, particularly phenolics and flavonoids, along with detectable levels of alkaloids and saponins. Quantitative assays showed that total phenolic content (TPC) reached 178.5 ± 4.6 mg GAE/g extract, while total flavonoid content (TFC) was 92.3 ± 3.1 mg QE/g extract.

Table 1. Phytochemical Profile of Male Papaya Leaf Extract.

Parameter	Value (Mean ± SD)
Total Phenolic Content (mg GAE/g)	178.5 ± 4.6
Total Flavonoid Content (mg QE/g)	92.3 ± 3.1
Alkaloids	Present
Saponins	Present

The high levels of phenolic and flavonoid compounds indicate strong antioxidant and immunomodulatory potential. These phytochemicals are known to regulate immune responses by modulating cytokine production and intracellular signaling pathways. Polyphenols, in particular, have been reported to influence T cell differentiation and suppress excessive inflammatory responses, making them relevant in autoimmune disease modulation (Wang, 2022). The presence of alkaloids and saponins may further contribute to immune regulation through synergistic effects on cellular signaling and membrane permeability.

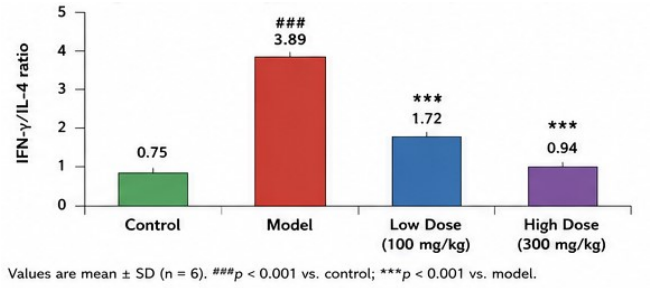
Modulation of Th1/Th2 Cytokine Balance

The autoimmune model group showed a significant increase in Th1 cytokines (IFN-γ and IL-2) and a marked

decrease in Th2 cytokines (IL-4 and IL-10), confirming successful disease induction. Treatment with male papaya leaf extract significantly reversed this imbalance in a dose-dependent manner.

Table 2. Effect of Extract on Cytokine Levels.

Parameter	Control	Model	Low Dose	High Dose
IFN-γ (pg/mL)	45 ± 3	110 ± 6 ↑	78 ± 5 ↓	55 ± 4 ↓
IL-2 (pg/mL)	40 ± 2	95 ± 5 ↑	70 ± 4 ↓	52 ± 3 ↓
IL-4 (pg/mL)	60 ± 4	28 ± 3 ↓	45 ± 3 ↑	58 ± 4 ↑
IL-10 (pg/mL)	55 ± 3	25 ± 2 ↓	42 ± 3 ↑	54 ± 3 ↑
IFN-γ/IL-4 Ratio	0.75	3.92 ↑	1.73 ↓	0.95 ↓



Values are mean ± SD (n = 6). ###$p < 0.001$ vs. control; ***$p < 0.001$ vs. model.

Figure 1. Effect of Extract on Cytokine Levels.

The observed reduction in Th1 cytokines alongside the restoration of Th2 cytokines suggests that the extract effectively rebalances immune responses. The IFN-γ/IL-4 ratio, a key indicator of Th1/Th2 polarization, was significantly normalized following treatment. This is particularly important because Th1 dominance is strongly associated with the progression of autoimmune disease. Restoring Th2 activity helps counteract excessive inflammation and tissue damage (Zhu, 2010). These findings support the hypothesis that male papaya leaf extract acts as an immunomodulator rather than a general immunosuppressant.

Regulation of Th1/Th2 Transcription Factors

Gene expression analysis showed that the autoimmune model significantly increased T-bet (Th1 transcription factor) expression and suppressed GATA-3 (Th2 transcription factor) expression. Treatment with the extract reversed these effects, decreasing T-bet and increasing GATA-3 expression toward normal levels.

Table 3. Gene Expression of Transcription Factors.

Gene	Control	Model	Low Dose	High Dose
T-bet	1.0	3.5 ± 0.3 ↑	2.1 ± 0.2 ↓	1.3 ± 0.1 ↓
GATA-3	1.0	0.4 ± 0.1 ↓	0.7 ± 0.1 ↑	0.95 ± 0.1 ↑

The modulation of T-bet and GATA-3 indicates that the extract influences immune responses at the transcriptional level. T-bet is a master regulator of Th1 differentiation, while GATA-3 controls Th2 lineage commitment. By suppressing T-bet and enhancing GATA-3 expression, the extract shifts T cell differentiation toward a more balanced state. This mechanism is consistent with current understanding of adaptive immunity, where transcription factors play a central role in determining cytokine profiles (Zhu, 2010). Such upstream regulation suggests a more sustained and controlled immunomodulatory effect.

Histopathological Evaluation

Histological analysis revealed severe inflammatory infiltration, tissue damage, and structural disruption in the autoimmune model group. In contrast, treatment groups showed reduced inflammatory cell infiltration and improved tissue architecture, with the high-dose group approaching near-normal histological appearance.

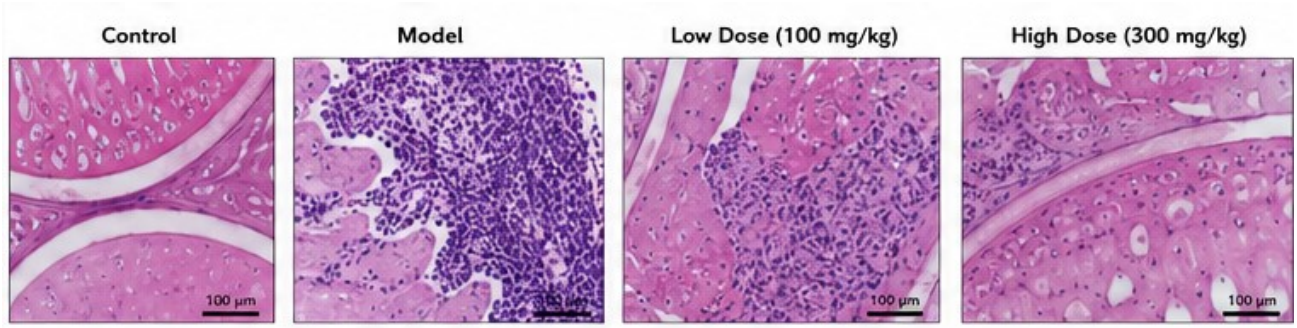


Figure 2. Histological Analysis Revealed Severe Inflammatory Infiltration, Tissue Damage, And Structural Disruption In The Autoimmune Model Group.

The improvement in histopathological features confirms that cytokine modulation has functional consequences at the tissue level. Reduced infiltration of inflammatory cells suggests suppression of immune-mediated damage, while restoration of tissue structure indicates enhanced repair processes. These findings align with studies showing that correcting Th1/Th2 imbalance can reduce autoimmune pathology and promote tissue recovery (Smolen, 2016). The dose-dependent improvement further supports the therapeutic potential of the extract.

Mechanistic Insights into Immunomodulation

Analysis of signaling pathways demonstrated that treatment with male papaya leaf extract inhibited NF-κB activation and modulated JAK/STAT signaling, particularly reducing STAT1 (Th1-related) and enhancing STAT6 (Th2-related) activity. The ability of the extract to regulate key signaling pathways highlights its multi-target mechanism of action. NF-κB is a central mediator of inflammation, while JAK/STAT signaling is critical for cytokine-driven T cell differentiation. By modulating these pathways, the extract can simultaneously suppress pro-inflammatory signals and enhance anti-inflammatory responses. This dual mechanism is increasingly recognized as essential for effective immunotherapy in autoimmune diseases (Liu, 2023). The involvement of multiple pathways also suggests that the phytochemical components act synergistically to achieve immune balance.

Taken together, the results demonstrate that male papaya leaf extract effectively restores Th1/Th2 balance through coordinated regulation of cytokines, transcription factors, and signaling pathways. Unlike conventional therapies that broadly suppress immune function, this extract promotes immune homeostasis, making it a promising candidate for safer and more targeted management of autoimmune disease.

CONCLUSIONS

This study demonstrates that male papaya (*C. papaya L.*) leaf extract effectively modulates the Th1/Th2 cytokine balance in an experimental autoimmune disease model. The extract significantly reduced Th1-associated pro-inflammatory cytokines, including IFN-γ and IL-2, while enhancing Th2-associated anti-inflammatory cytokines such as IL-4 and IL-10. This shift resulted in normalization of the IFN-γ/IL-4 ratio, indicating restoration of immune homeostasis.

At the molecular level, the extract regulated key transcription factors and signaling pathways involved in T cell differentiation, particularly by downregulating T-bet and modulating NF-κB and JAK/STAT pathways. These findings suggest that the extract acts upstream in immune regulation, influencing both cytokine production and cellular differentiation processes.

Histopathological improvements further confirmed the functional relevance of cytokine modulation, as treated groups exhibited reduced inflammatory infiltration and improved tissue architecture compared to the untreated autoimmune model.

Overall, male papaya leaf extract shows strong potential as a natural immunomodulatory agent capable of restoring immune balance without broad immunosuppression. These findings provide a scientific basis for its development as an alternative therapeutic strategy for autoimmune diseases. Further studies are recommended to isolate active compounds, evaluate long-term safety, and investigate clinical applicability.

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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.

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