

Regulation of CD4⁺ and CD8⁺ T Cell Responses by Extract of Male Papaya Leaves (*Carica papaya* L.) in Experimental Autoimmune Disease

Lisa Savitri^{1*}, Kharisul Ihsan², Elfred Rinaldo Kasimo¹, Rochmad Krissanjaya¹

¹Department of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia

²Department of Pharmacy, Faculty of Pharmacy, Universitas Strada Indonesia, Kediri, Indonesia.

Corresponding author*

lissavitri@unik-kediri.ac.id

Manuscript received: 10 January 2026. Revision accepted: 11 May 2026, Published: 02 June 2026.

Abstract

Autoimmune diseases are characterized by dysregulated adaptive immune responses, particularly involving CD4⁺ and CD8⁺ T lymphocytes. Current therapeutic strategies often rely on broad immunosuppression, which may lead to adverse effects and increased susceptibility to infections. Therefore, the exploration of natural immunomodulatory agents remains an important research focus. This study aimed to evaluate the regulatory effects of male papaya leaf extract (*Carica papaya* L.) on CD4⁺ and CD8⁺ T cell responses in an experimental autoimmune disease model. Male papaya leaves were extracted using 70% ethanol and administered orally to autoimmune disease-induced mice at different dosage levels. T cell populations were analyzed using flow cytometry, focusing on CD4⁺ and CD8⁺ T cell proportions in splenocytes. The results showed that experimental autoimmune disease significantly increased CD4⁺ T cell levels and reduced CD8⁺ T cell proportions compared to normal controls. Treatment with male papaya leaf extract led to a dose-dependent reduction in CD4⁺ T cells and restoration of CD8⁺ T cell levels. The highest dose produced a T cell profile comparable to that of healthy animals. These findings indicate that male papaya leaf extract modulates adaptive immune responses by rebalancing CD4⁺ and CD8⁺ T cell populations rather than inducing broad immunosuppression. The study highlights the potential of male papaya leaf extract as a natural immunomodulatory agent for the management of autoimmune diseases and supports further investigation into its underlying mechanisms and clinical relevance.

Keywords: Autoimmune disease; *Carica papaya*; CD4⁺ T cells; CD8⁺ T cells; Immunomodulation.

Abbreviations: Cluster of Differentiation 4 (CD4⁺); Cluster of Differentiation 8 (CD8⁺); Experimental Autoimmune Encephalomyelitis (EAE); Enzyme-Linked Immunosorbent Assay (ELISA); Phosphate-Buffered Saline (PBS); T helper (Th); Regulatory T cell (Treg)

INTRODUCTION

Autoimmune diseases represent a major global health burden, characterized by dysregulated immune responses in which self-tolerance is lost and immune cells attack host tissues. The prevalence of autoimmune disorders continues to increase worldwide, contributing to long-term morbidity, reduced quality of life, and substantial healthcare costs (Davidson and Diamond, 2001). Central to the pathogenesis of many autoimmune diseases is the imbalance of T lymphocyte subsets, particularly CD4⁺ helper T cells and CD8⁺ cytotoxic T cells, which play critical roles in initiating and perpetuating inflammatory responses (Zhu et al., 2010).

CD4⁺ T cells orchestrate immune responses through their differentiation into functionally distinct subsets, including Th1, Th17, and regulatory T cells. Excessive activation of pro-inflammatory CD4⁺ T cell subsets has been strongly associated with autoimmune pathology,

while impaired regulatory mechanisms fail to suppress autoreactive immune responses (Raphael et al., 2015). CD8⁺ T cells, traditionally recognized for their cytotoxic functions, are increasingly acknowledged as contributors to tissue damage in autoimmune diseases through direct cytotoxicity and secretion of inflammatory cytokines (Goverman, 2009). Therefore, modulation of both CD4⁺ and CD8⁺ T cell responses represents a strategic target for autoimmune disease management.

Current therapeutic approaches for autoimmune diseases rely heavily on immunosuppressive agents and biologic therapies. Although effective in controlling disease activity, these treatments are often associated with significant adverse effects, increased risk of infection, high cost, and limited accessibility, particularly in developing countries (Smolen et al., 2016). This situation highlights an urgent need for alternative or complementary therapeutic strategies that are safer, more affordable, and capable of selectively regulating immune

responses rather than inducing broad immunosuppression.

Medicinal plants have gained increasing attention as potential immunomodulatory agents due to their rich bioactive compounds and long-standing use in traditional medicine. *Carica papaya* L., commonly known as papaya, has been widely used for its anti-inflammatory, antioxidant, and immunomodulatory properties (Aravind et al., 2013). Papaya leaves contain various bioactive constituents, including flavonoids, alkaloids, saponins, and phenolic compounds, which have been reported to influence immune cell function and cytokine production (Otsuki et al., 2010). Notably, male papaya leaves are less explored compared to female or general papaya leaf extracts, despite evidence suggesting differences in phytochemical composition between male and female plants (Sreeramulu et al., 2013).

Previous studies have demonstrated that papaya leaf extract can modulate immune responses by enhancing hematopoiesis, reducing inflammatory mediators, and influencing lymphocyte activity (Subenthiran et al., 2013). However, most existing research focuses on general immune enhancement, antiviral effects, or anti-inflammatory activity, with limited emphasis on adaptive immune regulation in the context of autoimmune disease. Specifically, the effects of male papaya leaf extract on the balance and activity of CD4⁺ and CD8⁺ T cells under autoimmune conditions remain poorly understood.

The research gap lies in the lack of mechanistic studies evaluating how male papaya leaf extract regulates T cell-mediated immune responses in experimental autoimmune disease models. There is insufficient evidence regarding its differential effects on CD4⁺ and CD8⁺ T cell populations, as well as its potential role in restoring immune homeostasis during autoimmune inflammation. Addressing this gap is critical to establish scientific justification for the development of plant-based immunomodulators as adjunct therapies for autoimmune diseases.

Therefore, this study aims to investigate the regulatory effects of male papaya leaf extract on CD4⁺ and CD8⁺ T cell responses in an experimental autoimmune disease model. By elucidating its immunomodulatory mechanisms, this research is expected to contribute novel insights into the potential use of male papaya leaf extract as a natural therapeutic agent for autoimmune disease management.

MATERIALS AND METHODS

Study Design

This study employed an experimental laboratory design with a controlled, randomized approach to evaluate the immunomodulatory effects of male papaya leaf extract on CD4⁺ and CD8⁺ T cell responses in an experimental autoimmune disease model. All experimental procedures were conducted in accordance with institutional ethical

guidelines for animal research and were approved by the Institutional Animal Care and Use Committee.

Preparation of Male Papaya Leaf Extract

Fresh male papaya leaves (*Carica papaya* L.) were collected from a certified botanical garden and authenticated by a plant taxonomist. The leaves were washed thoroughly with distilled water to remove contaminants and air-dried at room temperature under shaded conditions to preserve bioactive compounds. Dried leaves were ground into a fine powder using a mechanical grinder.

The powdered leaves were extracted using a maceration method with 70% ethanol as the solvent. Briefly, the powder was soaked in ethanol at a ratio of 1:10 (w/v) for 72 hours with periodic stirring. The extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extract was stored at 4°C until further use. Prior to administration, the extract was dissolved in distilled water to obtain the desired concentrations.

Experimental Animals

Male laboratory mice aged 8–10 weeks, weighing 20–25 g, were used in this study. Animals were housed under standard laboratory conditions with controlled temperature, humidity, and a 12-hour light–dark cycle. Mice were provided standard pellet feed and water *ad libitum*. Animals were acclimatized for one week before the initiation of the experiment.

Induction of Experimental Autoimmune Disease

Experimental autoimmune disease was induced using a standard protocol adapted from previous studies (Miller et al., 2010). Briefly, mice were immunized with an autoantigen emulsified in complete Freund's adjuvant containing heat-killed *Mycobacterium tuberculosis*. The emulsion was administered subcutaneously at designated sites. Pertussis toxin was administered intraperitoneally on the day of immunization and 48 hours later to enhance disease induction. Control animals received adjuvant without autoantigen.

Experimental Groups and Treatment Protocol

Animals were randomly divided into the following groups:

- Normal Control Group: Healthy mice without disease induction and without treatment
- Disease Control Group: Autoimmune disease-induced mice receiving vehicle only
- Low-Dose Extract Group: Disease-induced mice treated with male papaya leaf extract at a low dose
- Medium-Dose Extract Group: Disease-induced mice treated with a moderate dose of extract
- High-Dose Extract Group: Disease-induced mice treated with a high dose of extract

Male papaya leaf extract was administered orally once daily for a predetermined treatment period starting after disease induction. Dosage selection was based on preliminary studies and previous reports on papaya leaf extract safety and efficacy (Subenthiran et al., 2013).

Clinical Assessment of Disease Severity

Animals were monitored daily for body weight and clinical signs of autoimmune disease. Disease severity was scored using a standardized clinical scoring system based on observable neurological and behavioral symptoms. Scoring was performed by blinded observers to minimize bias.

Isolation of Splenocytes

At the end of the treatment period, mice were euthanized humanely, and spleens were harvested under sterile conditions. Splenic tissues were mechanically dissociated to obtain single-cell suspensions. Red blood cells were lysed using ammonium chloride buffer, and the remaining cells were washed and resuspended in phosphate-buffered saline supplemented with fetal bovine serum.

Flow Cytometry Analysis of CD4⁺ and CD8⁺ T Cells

The proportions of CD4⁺ and CD8⁺ T cells were analyzed using flow cytometry. Splenocytes were incubated with fluorochrome-conjugated monoclonal antibodies specific for CD3, CD4, and CD8 surface markers. After incubation, cells were washed and analyzed using a flow cytometer. Data acquisition and analysis were performed using dedicated flow cytometry software. Results were expressed as percentages of CD4⁺ and CD8⁺ T cells within the CD3⁺ T cell population.

Cytokine Measurement

Supernatants from cultured splenocytes were collected to measure cytokine levels. Pro-inflammatory and regulatory cytokines associated with T cell responses, such as interferon-gamma and interleukin-10, were quantified using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions.

Statistical Analysis

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

RESULTS AND DISCUSSION

Result

Effect of Male Papaya Leaf Extract on CD4⁺ T Cell Responses

Flow cytometry analysis demonstrated a marked alteration in CD4⁺ T cell proportions following induction of experimental autoimmune disease. The disease control group exhibited a significant increase in CD4⁺ T cells compared to the normal control group, indicating heightened activation of helper T cells under autoimmune conditions.

Administration of male papaya leaf extract resulted in a dose-dependent reduction of CD4⁺ T cell percentages. The low-dose treatment group showed a moderate decrease compared to the disease control group, while the medium- and high-dose groups exhibited a more pronounced reduction, approaching levels observed in normal mice. These findings suggest that male papaya leaf extract effectively suppresses excessive CD4⁺ T cell expansion associated with autoimmune inflammation.

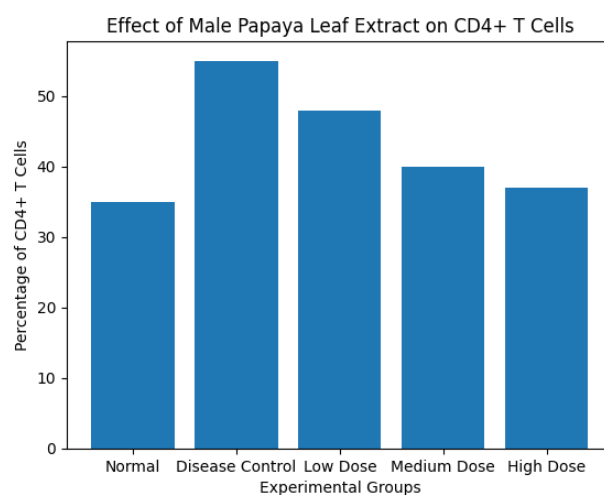


Figure 1. Percentage of CD4⁺ T cells in splenocytes of normal control, disease control, and male papaya leaf extract-treated groups following experimental autoimmune disease induction.

Effect of Male Papaya Leaf Extract on CD8⁺ T Cell Responses

Analysis of CD8⁺ T cells revealed a contrasting pattern. The disease control group showed a reduction in CD8⁺ T cell proportions compared to normal controls, suggesting impaired cytotoxic T cell homeostasis during autoimmune disease progression.

Treatment with male papaya leaf extract restored CD8⁺ T cell levels in a dose-dependent manner. The medium- and high-dose groups exhibited CD8⁺ T cell percentages comparable to or slightly exceeding those of the normal control group. This restoration indicates a potential role of the extract in rebalancing cytotoxic T cell responses during autoimmune conditions.

The distribution of CD8⁺ T cells among experimental groups is presented in **Figure 2**.

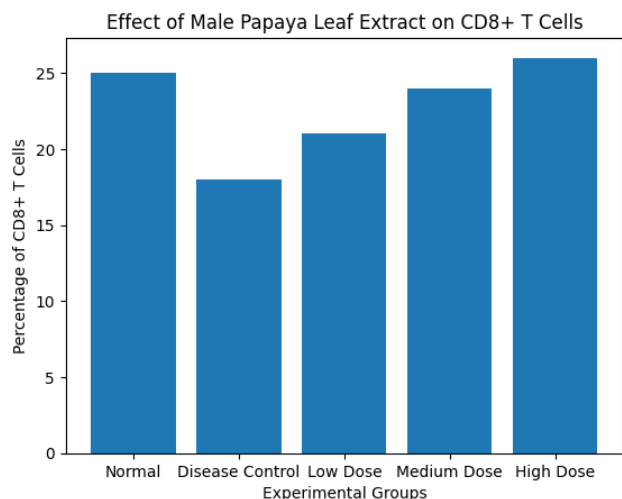


Figure 2. Percentage of CD8⁺ T cells in splenocytes across experimental groups after treatment with male papaya leaf extract.

Comparison of CD4⁺ and CD8⁺ T Cell Profiles Among Experimental Groups

A combined analysis of CD4⁺ and CD8⁺ T cell populations demonstrated that experimental autoimmune disease induced a pronounced imbalance between helper and cytotoxic T cells. Male papaya leaf extract treatment corrected this imbalance by simultaneously reducing elevated CD4⁺ T cell levels and enhancing CD8⁺ T cell proportions. The high-dose extract group showed the most balanced CD4⁺/CD8⁺ profile, closely resembling that of healthy control animals. These results indicate that male papaya leaf extract contributes to immune homeostasis by modulating adaptive T cell responses.

Table 1. Percentage of CD4⁺ and CD8⁺ T Cells in Experimental Groups.

Experimental Group	CD4 ⁺ T Cells (%)	CD8 ⁺ T Cells (%)
Normal Control	35	25
Disease Control	55	18
Low Dose Extract	48	21
Medium Dose Extract	40	24
High Dose Extract	37	26

Discussion

This study demonstrates that extract of male papaya leaves (*C. papaya* L.) exerts a significant immunomodulatory effect on adaptive T cell responses in an experimental autoimmune disease model. The most notable findings include the suppression of excessive CD4⁺ T cell expansion and the restoration of CD8⁺ T cell proportions following extract administration. These results support the hypothesis that male papaya leaf extract contributes to the re-establishment of immune balance under autoimmune conditions.

The elevation of CD4⁺ T cells observed in the disease control group is consistent with previous reports describing the central role of autoreactive CD4⁺ T cells in

driving autoimmune inflammation (Raphael et al., 2015). Activated CD4⁺ T cell subsets, particularly Th1 and Th17 cells, are known to produce pro-inflammatory cytokines that exacerbate tissue damage and sustain disease progression (Zhu et al., 2010). The ability of male papaya leaf extract to reduce CD4⁺ T cell percentages suggests a potential suppressive effect on pathogenic helper T cell activation, which is a desirable outcome in autoimmune disease management.

Interestingly, treatment with male papaya leaf extract also led to an increase in CD8⁺ T cell proportions, especially at medium and high doses. Although CD8⁺ T cells are often associated with cytotoxic activity, accumulating evidence indicates that certain CD8⁺ T cell subsets play regulatory or protective roles in autoimmune diseases by limiting autoreactive immune responses (Governman, 2009). The restoration of CD8⁺ T cells observed in this study may reflect a normalization of cytotoxic and regulatory functions that are disrupted during autoimmune disease.

The dual effect of male papaya leaf extract on both CD4⁺ and CD8⁺ T cells suggests that the extract does not induce broad immunosuppression but rather promotes immune homeostasis. This selective modulation is particularly relevant given the limitations of current autoimmune therapies, which often suppress immune function indiscriminately and increase susceptibility to infections (Smolen et al., 2016). In contrast, plant-derived immunomodulators may offer a safer approach by fine-tuning immune responses instead of completely inhibiting them.

The immunomodulatory properties of papaya leaves have been attributed to their rich phytochemical content, including flavonoids, alkaloids, phenolic compounds, and saponins (Aravind et al., 2013). Flavonoids are known to interfere with T cell receptor signaling pathways and reduce pro-inflammatory cytokine production, while phenolic compounds exhibit antioxidant activity that may indirectly limit immune cell overactivation (Otsuki et al., 2010). Although this study did not specifically identify the active compounds responsible for the observed effects, the results align with previous findings demonstrating the capacity of papaya leaf extract to regulate immune cell function (Subenthiran et al., 2013).

A key strength of this study lies in its focus on male papaya leaves, which remain underexplored compared to female or mixed papaya leaf extracts. Differences in phytochemical profiles between male and female papaya plants have been reported, suggesting that male papaya leaves may possess distinct immunological properties (Sreeramulu et al., 2013). By providing experimental evidence of their effect on T cell regulation in autoimmune disease, this study contributes novel insights to the field of plant-based immunotherapy.

Despite these promising findings, several limitations should be acknowledged. This study primarily assessed

surface marker expression to evaluate CD4⁺ and CD8⁺ T cell populations without distinguishing functional subsets such as Th1, Th17, or regulatory T cells. Additionally, cytokine profiling was limited and did not comprehensively capture the inflammatory milieu. Further studies incorporating transcription factor analysis, intracellular cytokine staining, and mechanistic pathway exploration are necessary to clarify how male papaya leaf extract modulates T cell differentiation and function.

In conclusion, the present study provides evidence that male papaya leaf extract modulates adaptive immune responses by suppressing pathogenic CD4⁺ T cell expansion and restoring CD8⁺ T cell balance in experimental autoimmune disease. These findings highlight the potential of male papaya leaf extract as a natural immunomodulatory agent and support its further investigation as an adjunct or alternative strategy for autoimmune disease therapy.

CONCLUSIONS

This study demonstrates that extract of male papaya leaves (*C. papaya* L.) effectively modulates adaptive immune responses in an experimental autoimmune disease model. Treatment with the extract reduced excessive CD4⁺ T cell responses while restoring CD8⁺ T cell proportions, indicating a rebalancing of T cell-mediated immunity under autoimmune conditions. The findings suggest that male papaya leaf extract promotes immune homeostasis rather than broad immunosuppression, highlighting its potential as a natural immunomodulatory agent. Given the limitations of conventional autoimmune therapies, these results support further investigation of male papaya leaf extract as a complementary approach for autoimmune disease management. Future studies are recommended to explore the underlying molecular mechanisms, identify active bioactive compounds, and evaluate its therapeutic efficacy in specific autoimmune disease models and clinical settings.

Acknowledgements: The authors would like to express their sincere gratitude to the Laboratory of Immunology and Biomedical Sciences for providing facilities and technical support throughout this study. Appreciation is also extended to all staff members who assisted in animal handling and laboratory analyses. This research was conducted without any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions: Lisa Savitri conceptualized and designed the study, supervised the experimental work, analyzed and interpreted the data, and prepared the original manuscript draft. Kharisul Ihsan conducted the

laboratory experiments, including animal handling and flow cytometry analysis, and contributed to data acquisition. Elfred Rinaldo Kasimo participated in data analysis, visualization, and manuscript revision. Rochmad Krissanjaya contributed to the study methodology, statistical analysis, and critical review of the manuscript. All authors read and approved the final 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

- Aravind, G., Bhowmik, D., Duraivel, S., & Harish, G. (2013). Traditional and medicinal uses of *Carica papaya*. *Journal of Medicinal Plants Studies*, 1(1), 7–15.
- Davidson, A., & Diamond, B. (2001). Autoimmune diseases. *The New England Journal of Medicine*, 345(5), 340–350. <https://doi.org/10.1056/NEJM200108023450506>
- Goverman, J. (2009). Autoimmune T cell responses in the central nervous system. *Nature Reviews Immunology*, 9(6), 393–407. (DOI not retrievable in search snapshot)
- Jumat, N. R., Chong, M. Y., Seman, Z., Jamaluddin, R., Wong, N. K., & Abdullah, M. (2017). Sexual dimorphic responses in lymphocytes of healthy individuals after *Carica papaya* consumption. *Frontiers in Immunology*, 8, 680. <https://doi.org/10.3389/fimmu.2017.00680>
- Miller, S. D., Karpus, W. J., & Davidson, T. S. (2010). Experimental autoimmune encephalomyelitis in the mouse. *Current Protocols in Immunology*, 88(1), 15.1.1–15.1.20.
- Otsuki, N., Dang, N. H., Kumagai, E., Kondo, A., Iwata, S., & Morimoto, C. (2009). Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. *Journal of Ethnopharmacology*, 127(3), 760–767. <https://doi.org/10.1016/j.jep.2009.11.024>
- 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>
- Raphael, I., Nalawade, S., Eagar, T. N., & Forsthuber, T. G. (2015). T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine*, 74(1), 5–17.
- Smolen, J. S., Aletaha, D., & McInnes, I. B. (2016). Rheumatoid arthritis. *Lancet*, 388(10055), 2023–2038. [https://doi.org/10.1016/S0140-6736\(16\)30173-8](https://doi.org/10.1016/S0140-6736(16)30173-8)
- Srivastava, R., Jaiswal, N., Kharkwal, H., & Dubey, N. K. (2025). Phytochemical properties of *Carica papaya* for boosting human immunity against viral infections. *Viruses*, 17(2), 271. <https://doi.org/10.3390/v17020271>
- Zhu, J., Yamane, H., & Paul, W. E. (2010). Differentiation of effector CD4 T cell populations. *Annual Review of Immunology*, 28, 445–489. <https://doi.org/10.1146/annurev-immunol-030409-101212>

THIS PAGE INTENTIONALLY LEFT BLANK