

Anti-Inflammatory Activity of *Dolichos lablab* Beans: A Perspective Based on T Lymphocytes Profiles

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ABSTRACT

Dolichos lablab has several known therapeutic properties, but further exploration is needed to clarify its exact anti-inflammatory activity. Since excessive inflammation might worsen many pathological conditions, profiling immune cell regulation, such as T lymphocytes, is critical. This study evaluated the anti-inflammatory activity of *Dolichos lablab* extract (DLE) based on CD4 and CD8 cell populations. Fifteen male BALB/c mice were divided into five groups: untreated (N), injected with *Escherichia coli* (1×10^6 CFU/mouse, i.p., 250 μ L) (I), treated with different doses of DLE (250, 500, and 1,000 mg/kg BW), followed by *E. coli* injection defined as group D1, D2, and D3 respectively. The splenocytes were collected for immunostaining, and CD4 and CD8 lymphocytes were quantified by flow cytometry (FCM). FCM data were analyzed using BDFacs Flow and one-way ANOVA with LSD post-hoc test. DLE treatment suppressed the total number of cells and activated the CD4 and CD8 populations, even after *E. coli* injection. The low dose of DLE (250 mg/kg BW) kept lymphocyte levels near normal, with higher doses showing no significant difference. DLE also decreased the CD4/CD8 ratio. In conclusion, DLE demonstrated measurable anti-inflammatory activity in mice, suggesting its potential as a candidate for further preclinical evaluation.



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1. Introduction

Dolichos lablab (L.), commonly referred to as lablab bean, is an under-utilized legume cultivated across tropical and subtropical regions of Asia and Africa. Beyond its role as an inexpensive source of dietary protein, fiber, and micronutrients, the seed, leaf, and pod fractions have yielded a spectrum of phytochemicals, notably flavonoids, phenolic acids, terpenoids, and lectins, that underpin diverse bioactivities (Purwanti

et al. 2022; Zhang *et al.* 2025; Chander *et al.* 2025). Recent experimental and *in silico* investigations have documented antioxidant, antiviral, antidiabetic, and antimicrobial properties of lablab bean preparations, highlighting their potential contribution to food and pharmaceutical innovation (Minde *et al.* 2020; Purwanti *et al.* 2021; Sipahli *et al.* 2021). Preliminary evidence further indicates that lablab extracts can attenuate inflammatory processes, possibly through protease inhibition and suppression of the nuclear factor- κ B (NF- κ B) signaling axis (Momin *et al.* 2012; Purwanti *et al.* 2021). Nevertheless, the cellular targets

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and dose-dependent consequences of lablab-derived constituents on the mammalian immune system remain insufficiently characterized.

Inflammation is an evolutionarily conserved defense mechanism that eliminates pathogens and initiates tissue repair; however, its persistence or dysregulation precipitates chronic pathologies such as rheumatoid arthritis, inflammatory bowel disease, and cardiometabolic disorders (Medzhitov 2008; Andersen *et al.* 2021). Current anti-inflammatory drugs achieve symptomatic relief, yet adverse effects, high costs, and incomplete efficacy stimulate the search for safer, plant-based modulators. Legume phytochemicals are particularly attractive because they are accessible within the human diet and exhibit multi-target capabilities, including scavenging of reactive oxygen species, interference with pro-inflammatory transcription factors, and modulation of cytokine networks. Establishing mechanistic links between specific legume extracts and discrete immune pathways is therefore essential for rational development of functional foods or phytotherapeutics.

Among the cellular mediators of inflammation, T lymphocytes occupy a central position in orchestrating the transition from innate to adaptive immunity. CD4⁺ helper T cells differentiate into effector or regulatory subsets that either amplify (Th1, Th17) or attenuate (Treg) inflammatory cascades, whereas CD8⁺ cytotoxic T cells directly eliminate infected or transformed cells and secrete pro-inflammatory cytokines (Zhu & Zhu 2020). The absolute and relative abundance of these subsets, often summarized by the CD4/CD8 ratio, serve as sensitive indicators of immune equilibrium. Aberrant expansion of either population correlates with tissue damage and disease progression, and restoration of a balanced ratio constitutes a recognized therapeutic goal (McBride and Striker 2017). Consequently, flow-cytometric profiling of CD4⁺ and CD8⁺ compartments and their activation status has become a robust platform for evaluating the immunomodulatory efficacy of natural products (Kusnul *et al.* 2017; Djati *et al.* 2017).

Despite promising *in vitro* observations (Zhang *et al.* 2023; Zhang *et al.* 2024; Zhang *et al.* 2025), no *in vivo* study has yet delineated how lablab bean extract influences adaptive immune parameters during an acute bacterial challenge. The present investigation addresses this knowledge gap by quantifying CD4⁺ and CD8⁺ T-cell populations and their activation status in BALB/c mice infected with *Escherichia coli* and treated with graded doses of ethanolic *Dolichos lablab*

extract. By integrating established inflammation markers with rigorous statistical analysis, the study aims to elucidate the dose–response relationship between lablab supplementation and immune homeostasis. Findings from this work are expected to refine the understanding of lablab bean as a functional anti-inflammatory agent and to provide a scientific basis for its incorporation into dietary or pharmaceutical interventions targeting immune-mediated diseases.

2.2. Materials and Methods

2.1. Materials and Extraction

Beans were collected from Sumenep, Madura Island, East Java, Indonesia, during the dry season, and their detailed profiles and locations are reported in a previous study (Purwanti *et al.* 2019). The beans were stored at 4°C until use and were processed according to a previously described method (Purwanti *et al.* 2022). Briefly, the beans were ground and soaked in 96% ethanol (3:1 volume-to-weight) for 24 hours. The mixture was then filtered, rotary evaporated, and freeze-dried to obtain the DL bean extract (DLE).

2.2. Animals, Inflammation Model, and DLE Treatment

This study was approved by the Ethical Committee of Universitas Brawijaya (Certificate no. 012-KEP-UB-2023). Fifteen male BALB/c mice (8-10 weeks old weighing 20-25 g) were divided into five treatment groups, i.e., normal mice without treatment (N), mice injected with *E. coli* (I), mice treated with three different doses of DLE (250, 500, and 1,000 mg/kg of body weight (BW)) and injected with *E. coli* (D1, D2, and D3, respectively, n=3 mice per group). After acclimatisation, the mice were treated with the aforementioned oral doses of DLE once a day for 7 days (maximum 0.2 mL/25 g of the body weight of mice) by oral gavage; DLE was freshly reconstituted in sterile Phosphate Buffer Saline (PBS), and the N and I groups received the same vehicle volume (PBS). One hour after the last extract administration, the mice were injected intraperitoneally (i.p.) with *E. coli* (10⁶ CFU/mouse) at an injection volume of 250 µL (Ali *et al.* 2018). After injection, the mice were fasted for 12 hours, and the spleens were harvested for lymphocyte isolation.

2.3. Lymphocyte Isolation

The spleens were washed twice with PBS and homogenized using a syringe plunger in a clockwise

direction until a homogenous mixture was achieved. The homogenate was collected using a micropipette and transferred to a 15 mL tube, then the volume was adjusted to 6 mL with PBS. The tubes were centrifuged at 2,500 rpm at 10°C for 5 minutes, and the cell pellets were resuspended in 1 mL of PBS (Hermanto *et al.* 2020).

2.4. Antibody Staining and Flow Cytometry

The cell suspension (50 μ L) was transferred into a 1.5 mL microtube and incubated with the extracellular antibodies, consisting of FITC anti-mouse CD4, PE anti-mouse CD8, and PE/Cy5 anti-mouse CD62L (BioLegend, USA), in the dark at 4°C for 30 minutes. The cells were resuspended in 400 μ L of PBS and analysed on a flow cytometer (BD FACSCalibur, USA) (Hermanto *et al.* 2020).

Lymphocytes were gated based on forward- and side-scatter (FSC/SSC) characteristics, followed by quadrant analysis for CD4 and CD8 populations; activated T cells were defined as CD62L-negative subsets (CD4⁺CD62L⁻ and CD8⁺CD62L⁻). Where applicable, doublets/debris were excluded using FSC parameters. A minimum of 10,000 events within the lymphocyte gate were acquired per sample. The reported “relative number” represents the percentage of each subset within the gated lymphocyte population, and the CD4/CD8 ratio was calculated from the corresponding CD4⁺ and CD8⁺ percentages.

2.5. Data Analysis

The flow cytometry data were analysed using BDFacs Flow and subjected to one-way ANOVA followed by Least Significant Difference (LSD) post-hoc analysis to determine significant differences between groups. Normality and homogeneity of variance were assessed prior to ANOVA (Shapiro–Wilk and Levene’s tests, respectively). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. CD4⁺ and CD8⁺ T Lymphocyte Populations

Administration of *E. coli* intraperitoneally produced a pronounced expansion of splenic T-lymphocyte subsets. Total CD4⁺ cells in the inflammation group rose significantly above the untreated control ($p = 0.002$), paralleled by a similar increase in total CD8⁺ cells ($p = 0.01$, Figure 1A). Oral supplementation with DLE for seven consecutive days reduced this response across all doses tested. The lowest dose (250 mg/kg BW) lowered

both CD4⁺ and CD8⁺ relative numbers to values that were statistically indistinguishable from the control group ($p > 0.05$), whereas intermediate (500 mg/kg BW) and high (1,000 mg/kg BW) doses produced similar reductions (Figure 1B).

3.2. CD4⁺ to CD8⁺ Ratio

The CD4⁺/CD8⁺ ratio captured alterations in the proportional balance of the two main T-cell subsets. *E. coli* challenge increased this ratio relative to basal conditions ($p = 0.010$), reflecting the greater rise of CD4⁺ cells. Pretreatment with DLE shifted the ratio toward the control group ($p > 0.05$); the effect was most evident at 250 mg/kg BW, where the ratio did not differ significantly from the control. A similar pattern was also observed in 500 mg/kg BW. At 1,000 mg/kg BW, the ratio fell below that measured in the inflammation group ($p = 0.001$, Figure 1B).

3.3. Activated CD4⁺ (CD4⁺CD62L⁻) Lymphocytes

Flow cytometric double staining for CD62L identified activated (CD62L⁻) helper T cells. *E. coli* injection elevated the proportion of CD4⁺CD62L⁻ lymphocytes compared with control mice ($p < 0.05$). All DLE doses significantly reduced this activated population, with no statistical separation among the three treatment levels—the proportions observed after DLE supplementation were comparable to those in the untreated control (Figure 2).

3.4. Activated CD8⁺ (CD8⁺CD62L⁻) Lymphocytes

A comparable pattern was observed for activated cytotoxic T cells. The inflammation group exhibited an increased frequency of CD8⁺CD62L⁻ lymphocytes ($p = 0.003$). DLE administration reduced these cells; the 250 and 500 mg/kg BW doses achieved levels equivalent to the control ($p = 0.9$ and $p = 0.935$, respectively), whereas 1000 mg/kg BW showed a further decrease that was not statistically significant ($p > 0.05$) compared with the inflammation group (Figure 3).

4. Discussion

The DLE curtailed the exuberant expansion of splenic CD4⁺ and CD8⁺ T-cell compartments elicited by acute *E. coli* challenge, signaling an interference with early adaptive-immune amplification that sustains harmful inflammation. By returning the CD4/CD8 ratio toward the physiological range, DLE appears to recalibrate helper-to-cytotoxic balance, a metric closely linked to clinical outcome in chronic inflammatory

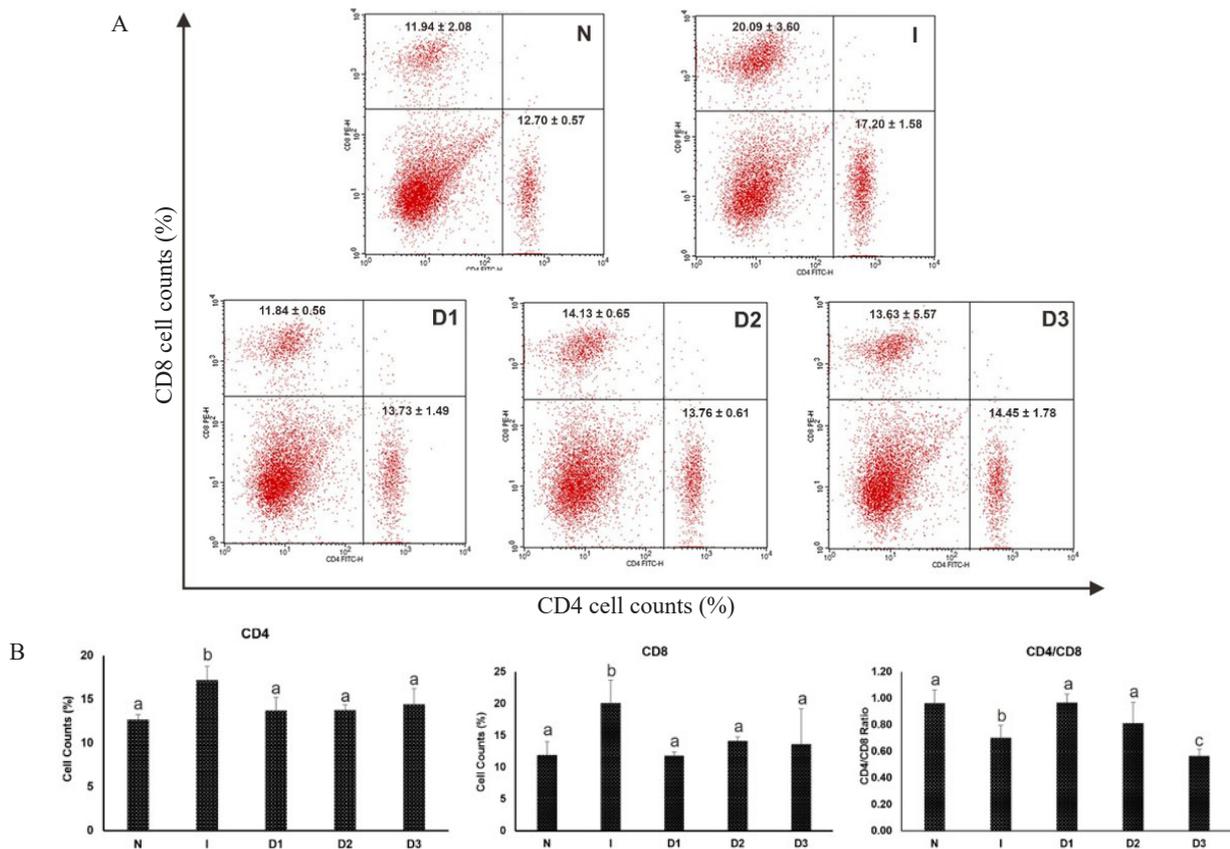


Figure 1. Relative number of CD4, CD8, and the ratio of CD4/CD8 T lymphocytes in a quadrant plot (A) and barplot (B). The data are presented in mean ± standard deviation. Different notations indicate a significant difference $p < 0.05$ ($n = 3$ per group). N = Normal, I = Inflammation, D1 = dose 1 (250 mg/kg BW), D2 = dose 2 (500 mg/kg BW), and D3 = dose 3 (1,000 mg/kg BW)

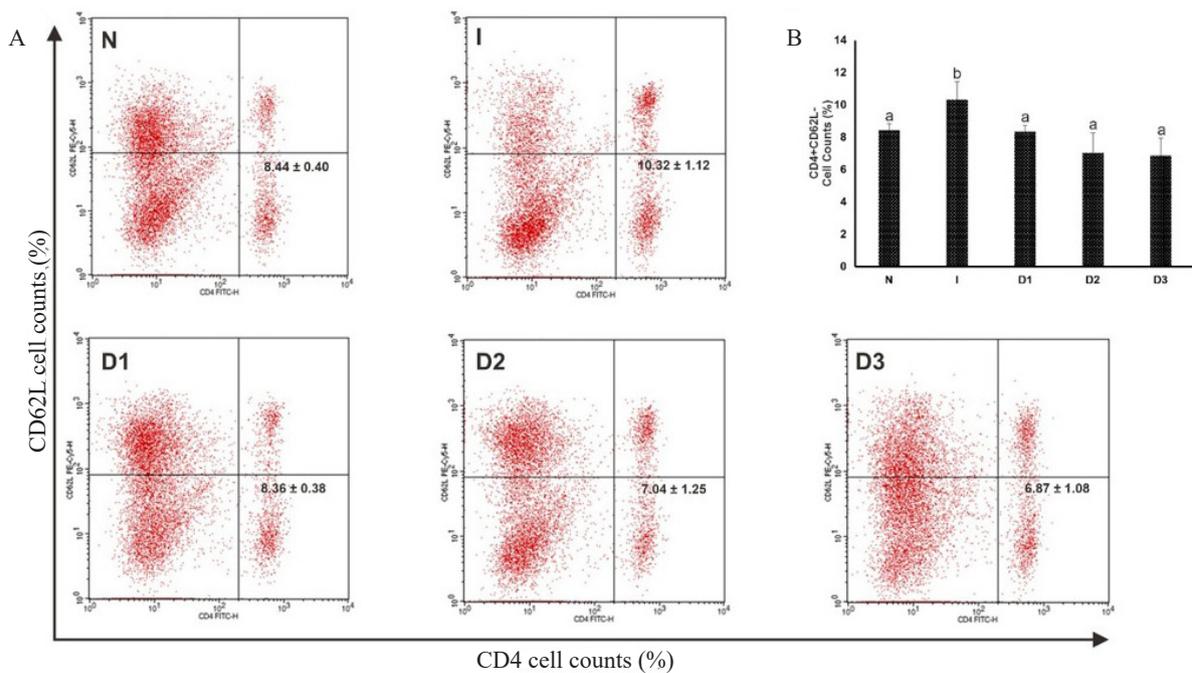


Figure 2. The relative number of activated CD4 T lymphocytes ($CD4^+CD62L^-$) in a quadrant plot (A) and barplot (B). The data are presented as mean ± standard deviation. Different notations indicate a significant difference $p < 0.05$ ($n = 3$ per group). N = Normal, I = Inflammation, D1 = dose 1 (250 mg/kg BW), D2 = dose 2 (500 mg/kg BW), and D3 = dose 3 (1,000 mg/kg BW)

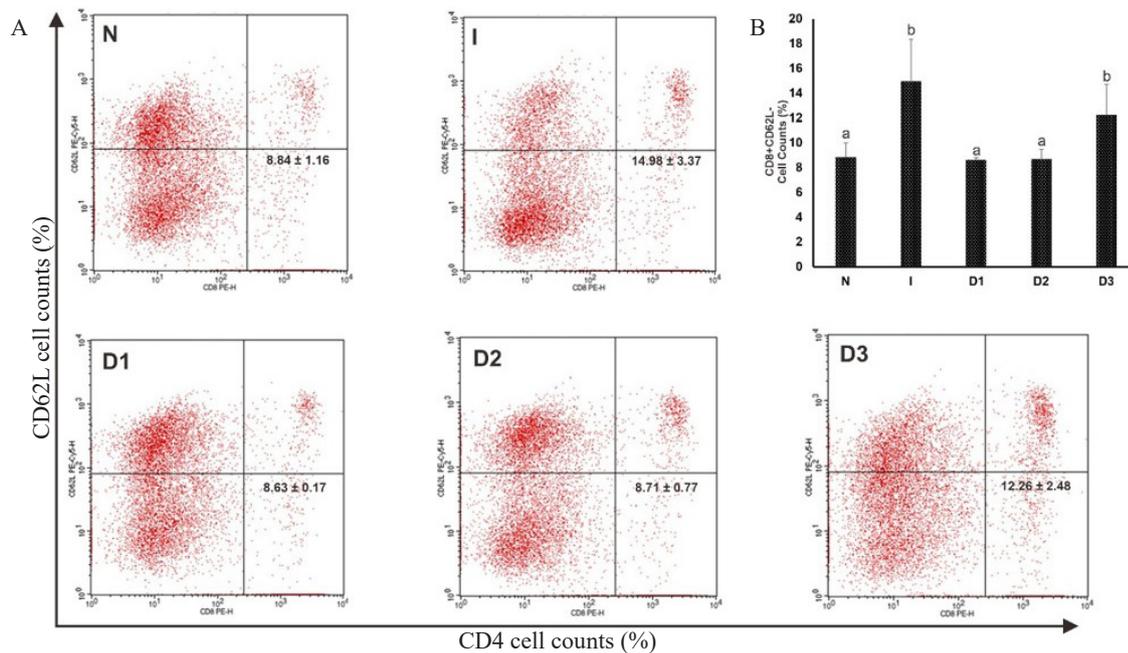


Figure 3. The relative number of activated CD8 T lymphocytes (CD8+CD62L-) in a quadrant plot (A) and barplot (B). The data are presented as mean \pm standard deviation. Different notations indicated a significant difference $p < 0.05$ ($n = 3$ per group). N = Normal, I = Inflammation, D1 = dose 1 (250 mg/kg BW), D2 = dose 2 (500 mg/kg BW), and D3 = dose 3 (1,000 mg/kg BW)

and infectious diseases (McBride & Striker 2017). The concomitant contraction of CD4⁺CD62L⁻ and CD8⁺CD62L⁻ subsets indicates that the extract limits activation and tissue-homing of both effector lineages, an especially relevant effect because excessive recruitment of activated T cells drives cytokine-mediated tissue damage in rheumatoid arthritis, inflammatory bowel disease, and infection-related cardiometabolic complications (Garduño & Däbritz 2021; Andersen *et al.* 2021).

These immunophenotypic changes are coherent with the phytochemical profile of *Dolichos lablab*. The bean is enriched in phenolic acids such as ferulic acid and flavonoids such as quercetin (Purwanti *et al.* 2021, 2022, 2023), molecules that inhibit Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and Mitogen-Activated Protein Kinase (MAPK) signaling and suppress T-cell-receptor-dependent activation (Michalski *et al.* 2020; Shi *et al.* 2023). Methanolic lablab extracts were previously shown to attenuate protease activity and block NF- κ B translocation in macrophages (Momin *et al.* 2012), while *in silico* studies predicted stable binding of lablab's metabolites to inflammatory protein targets (Purwanti *et al.* 2021). The present *in vivo* data therefore provide functional confirmation that these constituents

translate into measurable immunomodulation of adaptive lymphocytes.

The dose-response profile suggests that *Dolichos lablab* bioactive compounds regulate T cell activity, although the effect was not strictly linear across the tested doses. At 250 mg/kg BW, both total and activated T cell counts were restored to normal levels, while 500 mg/kg BW produced an insignificant outcome compared to the lowest dose. At 1,000 mg/kg BW, total and activated CD8⁺ cells remained reduced, while the CD4/CD8 ratio decreased below that of the inflammation group, indicating a more substantial shift in subset balance rather than increased cytotoxic T cell activation. This pattern suggests that different concentrations of the compounds may alter T cell receptor signaling and costimulatory pathways in distinct ways.

Mechanistically, several *Dolichos lablab* compounds, particularly oleanolic acid and ursolic acid, act by preventing NF- κ B from binding to DNA, thereby inhibiting the transcription of pro-inflammatory genes (Purwanti *et al.* 2021). Moreover, dolilabphenosides A, B1, and B3 specifically reduce macrophage secretion of IL-1 β (Zhang *et al.* 2023), whereas saponins and terpenoid glycosides suppress the production of TNF- α , IL-6, and IL-1 β and concurrently attenuate macrophage activation (Zhang *et al.* 2024; Zhang *et al.* 2025).

Macrophage activation, NF- κ B signaling, and pro-inflammatory cytokine expression (including IL-1 β , IL-6, and TNF- α) are critical for antigen presentation, co-stimulation, and effective T-cell priming (Lawrence 2014; Guerriero 2018). Inhibition of these pathways dampens T-cell activation by reducing IL-2 production and, consequently, limiting the proliferation of both CD4⁺ and CD8⁺ T-cell subsets (Kasaian & Biron 1990; Barbour *et al.* 2016). Therefore, the normalization of T cell parameters at lower doses and the suppression observed at higher doses can be explained by *Dolichos lablab*'s compounds inhibiting NF- κ B signaling and cytokine-driven T cell activation, although cytokines and NF- κ B activity were not directly measured in this study.

This study has several limitations that should be considered when interpreting the findings. First, although the bacterial concentration and injection volume were reported, the inflammation model was only assessed at a single post-injection time point, and the *E. coli* strain/source and clinical scoring were not determined, limiting model characterization and generalizability. Second, the sample size was small (n = 3 per group) and only male BALB/c mice were used, which may have reduced statistical power and limited applicability across sexes and strains. Third, the immunological readouts were limited to flow-cytometric profiling of CD4⁺, CD8⁺, and CD62L-defined activation, reported as relative proportions rather than absolute counts, and we did not directly measure inflammatory mediators (e.g., TNF- α , IL-1 β , IL-6), NF- κ B/MAPK activity, bacterial burden, or histopathological changes; therefore, mechanistic explanations remain inferential. Collectively, these limitations underscore the need for future studies that incorporate improved infection-model definitions, expanded immunoinflammatory endpoints, and larger, sex-balanced cohorts.

In summary, the data show that oral DLE attenuates both the magnitude and activation status of key T cell subsets (CD4⁺, CD8⁺, CD4⁺CD62L⁻, and CD8⁺CD62L⁻) in an acute inflammation model, and the 250 mg/kg BW dose maintained these parameters closest to those of the untreated control. Because the DLE composition and the infection model were not fully characterized, these findings extend previous *in vitro* and *in silico* observations and support further targeted preclinical evaluation of the crude extract. Rigorous optimization of dosing and validation in chronic disease models are

warranted to strengthen mechanistic interpretation and reproducibility.

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