Original Article

In Vivo High-dose Epigallocatechin-3-gallate Modulates Splenic IL-2 Expression and Attenuates CD4+ and CD8+ T-cell Populations in Mice

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ABSTRACT

Background: Epigallocatechin-3-gallate (EGCG), a major flavonoid in green tea, possesses potent antioxidant, antiviral, and anticancer properties. However, excessive EGCG exposure has been associated with mitochondrial dysfunction.

Objectives: This study aimed to evaluate the effects of high-dose EGCG on interleukin-2 (IL-2) expression and T-cell subsets in mice.

Methods: For immunohistochemical analysis of splenic IL-2, BAX, and BCL-2 expression, the animals were assigned to two groups: control and EGCG (750 mg/kg for three days). For flow cytometric evaluation of splenic CD4+ and CD8+ T-cells, the animals were divided into four groups receiving 0, 250, 500, or 750 mg/kg EGCG for five days.

Results: High-dose EGCG (750 mg/kg) induced a non-significant decrease in IL-2 expression ($P>0.05$), a non-significant increase in BAX expression ($P>0.05$), and a significant elevation in BCL-2 expression ($P<0.05$). Flow cytometric analysis revealed a dose-dependent decline in CD4+ and CD8+ T-cell proportions, with significant reductions observed only at 750 mg/kg ($P<0.05$). Correlation and regression analyses confirmed a significant negative association between EGCG dose and both T-cell subsets.

Conclusion: These findings suggest that high-dose EGCG modulates IL-2–mediated activation and attenuates CD4+ and CD8+ populations. Collectively, these results indicate that excessive EGCG exposure alters T-cell homeostasis and survival-associated signaling.

Keywords: CD4+, CD8+, Epigallocatechin-3-gallate (EGCG), Interleukin-2 (IL-2), T-cells

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Introduction

Epigallocatechin-3-gallate (EGCG) is a major flavonoid compound found in green tea. EGCG has been widely used in traditional and complementary medicine because of its diverse pharmacological activities (Oliveira et al., 2023). Among its documented biological effects, EGCG exhibits anticancer properties by downregulating the signal transducer and activator of transcription 3 (STAT3) pathway, thereby suppressing tumor cell proliferation (Luo et al., 2024), and exerts antiviral activity by enhancing type I interferon responses that inhibit viral gene expression and assembly (Zhu et al., 2021).

However, the use of EGCG does not consistently yield favourable health outcomes. Between 1999 and 2008, thirty-four cases of green tea–associated hepatotoxicity were reported, demonstrating a clear link between excessive EGCG consumption and hepatic injury (Mazzanti et al., 2009). In vivo investigations have shown that administering EGCG at 750 mg/kg body weight to mice induces hepatic necrosis (Lambert et al., 2010). Such necrotic lesions are typically associated with cell membrane damage arising from lipid peroxidation. This has been confirmed by increased malondialdehyde levels in mice treated with high-dose EGCG (James et al., 2018).

Cellular injury caused by high-dose EGCG may trigger activation of the innate immune system, resulting in an inflammatory response. Inflammation represents an immediate defensive mechanism of innate immunity that eliminates damaged or infected cells and restores tissue integrity (Murphy & Casey, 2017). Our previous study demonstrated that administration of EGCG at 750 mg/kg significantly upregulated interleukin-6 (IL-7) and tumor necrosis factor alpha (TNF-α) expression but did not significantly alter IL-1β levels (Airlangga et al., 2024).

The formation of the proinflammatory cytokine IL-1β is regulated by inflammasome activation, particularly that of the NLRP3 inflammasome. This cytoplasmic multiprotein complex facilitates the maturation of IL-1β into its bioactive form. Mitochondria play a crucial role in this process by generating mitochondrial reactive oxygen species (mROS) that serve as upstream activators of the inflammasome (Billingham et al., 2022). The absence of significant IL-1β induction following high-dose EGCG exposure may therefore result from suppressed mitochondrial activity. Supporting this notion, James et al. (2018) reported that high-dose EGCG downregulated the expression of mitochondrial inner-membrane complexes I and III, as well as the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx), both of which regulate mROS production. During the initial phase of EGCG-induced tissue injury, activation of innate immune mechanisms generates an inflammatory response (Punt et al., 2018). However, persistent injury may subsequently engage adaptive immune responses. Given that high-dose EGCG induces hepatic injury and modulates proinflammatory cytokines, it is plausible that it may also affect the adaptive immune system, particularly T-cells.

T-cells play a central role in adaptive immunity, orchestrating cellular responses through activation, proliferation, and differentiation into effector subsets. Their proliferation and differentiation are tightly regulated by cytokines, such as IL-2 (Omar et al., 2025). Dysregulation of these signalling pathways can compromise T-cell function and impair adaptive immune responses (Abbas et al., 2020). A previous study demonstrated that high-dose EGCG inhibits T-cell proliferation following antigenic stimulation (Wu et al., 2009). Although high concentrations of EGCG generate hydrogen peroxide—one component of mitochondrial ROS—the study found that H_2O_2 was not the principal mediator of the T-cell proliferation inhibition. Interestingly, IL-2 protein levels remained elevated after stimulation, peaking on day three, while IL-2 receptor expression was reduced. Examination of IL-2 and IL-2R mRNA levels revealed no significant changes (Wu et al., 2009). These findings raise an important unresolved question: Do IL-2 dynamics under EGCG exposure differ in nonactivated, physiologically resting T-cells? This aspect has not been thoroughly examined.

Although HO_2 was not identified as the dominant suppressor of proliferation, its generation indicates that EGCG can still perturb redox-dependent signaling within T-cells and potentially disrupt the differentiation of T-cell subsets. ROS function as signaling molecules in T-cells by modulating key intracellular pathways, and imbalances in redox homeostasis can alter T-cell signaling and fate decisions (Sena et al., 2013). Disruption of redox signaling and mitochondrial ROS dynamics, therefore, has the potential to interfere with normal T-cell differentiation, which may contribute to systemic effects in vivo.

Nevertheless, most of these findings have been derived from in vitro experiments, which may not represent the complex physiological interactions present in vivo. In living organisms, immune modulation involves dynamic crosstalk among lymphocytes, cytokines, and tissue microenvironments that collectively shape immune respons-

es. Therefore, it remains essential to determine whether high-dose EGCG modulates IL-2 and attenuates T-cell populations under physiological conditions. Accordingly, the present study aimed to evaluate the effects of high-dose EGCG administration in mice on IL-2 expression and the relative proportions of $CD4^+$ and $CD8^+$ T-cells.

Materials and Methods

Animals

Male mice (balb/c) aged 12 weeks, with an average body weight of 20–25 g, were obtained from the Laboratory of Experimental Animal, Faculty of Veterinary Medicine, Universitas Brawijaya. The animals were randomly assigned to experimental groups using simple randomization and maintained under standard laboratory conditions (temperature: 26±2 ˚C, relative humidity: 50–60%, and a 12:12 h light/dark cycle), with unrestricted access to pellet feed and water ad libitum. Prior to treatment, all animals underwent a seven-day acclimatization period. For the immunohistochemical analysis of IL-2, BAX and BCL-2 expression, 32 mice were used and housed in two cages (16 animals per cage). This design was chosen to capture changes in IL-2, which a previous in vitro study showed peak on day three and to evaluate apoptotic markers at the same time point (Wu et al., 2009). For the flow cytometric analysis of $CD4^+$ and $CD8^+$ T-cell proportions, 28 mice were used and housed in four cages (seven animals per cage). This setup allows assessment of dose-dependent effects on T-cell populations over five days, consistent with previous in vivo observations of EGCG-induced mortality by day five (Lambert et al., 2010). All animal procedures were conducted in accordance with institutional ethical standards and approved by the Animal Care and Use Committee, Institut Biosains, Universitas Brawijaya.

Experimental design

Following a seven-day acclimatization period, EGCG administration commenced on day 8. EGCG (Sigma-Aldrich, Catalog No. E4143) was administered orally once daily. For the IL-2, BAX, and BCL-2 expression analysis, mice received EGCG at a dose of 750 mg/kg body weight for three consecutive days, as our previous study indicated that this dose effectively modulates cytokine levels. For the assessment of $CD4^+$ and $CD8^+$ T-cell proportions, EGCG was administered at doses of 250, 500, or 750 mg/kg body weight for five consecutive days to explore potential dose-dependent effects and capture trends from low to high concentrations. At the end of each treatment period, animals were euthanized by cervical dislocation under humane conditions. For immunohistochemical evaluation of IL-2, BAX and BCL-2 expression, spleens were harvested and fixed in 10% neutral-buffered formalin. For flow cytometric analysis of $CD4^+$ and $CD8^+$ T-cells, spleens were collected and placed in phosphate-buffered saline (PBS) for immediate processing.

Histopathological preparation

Spleen tissues were processed for histopathological examination through sequential steps of fixation, trimming, dehydration, clearing, paraffin embedding, and sectioning. Samples were fixed in 10% neutral-buffered formalin for 24 hours and trimmed to approximately 0.5 cm in thickness. Dehydration was performed using graded ethanol series (70%, 80%, 85%, 90%, and 95%), each for at least one hour, followed by three immersions in absolute ethanol for one hour each. Tissues were then cleared in xylene three times for 5, 5, and 10 minutes, respectively, and subsequently infiltrated with molten paraffin three times at 60 ˚C for 30–60 minutes per cycle. The paraffin-embedded tissues were moulded into blocks and sectioned at a thickness of 5 µm using a rotary microtome. Sections were floated in a warm water bath, mounted on glass slides, and prepared for subsequent staining (Khan et al., 2020).

Immunohistochemical staining

Immunohistochemical staining was performed through sequential steps of deparaffinization, rehydration, antigen retrieval, blocking, antibody incubation, chromogen development, counterstaining, and mounting. Deparaffinization was achieved by immersing slides in xylene three times for 5–10 minutes each, followed by rehydration through graded ethanol (100%, 90%, 80%, and 70%) for 15 minutes per step. Slides were rinsed in PBS (pH 7.4) for 9 minutes. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 minutes, and nonspecific binding was blocked with 5% BSA in PBS for an additional 10 minutes. Following PBS washes, sections were incubated with 100–200 µL of primary antibodies against IL-2 (Elabscience, E-AB-40275, 1:100), BAX (Santa Cruz Biotechnology, sc-7480, 1:100), and BCL-2 (Santa Cruz Biotechnology, sc-7382, 1:100) for 60 minutes. After rinsing, IL-2 immunoreactivity was detected using an HRP-conjugated species-specific secondary antibody (Elabscience, E-AB-1157), while BAX and BCL-2 signals were detected using a mouse IgG κ light chain-binding HRP reagent (Santa Cruz Biotechnology, sc-525409), each incubated for 60 minutes according to the manufacturer's instructions (Elabscience, 2024a,

Elabscienc, 2024b; Santa Cruz Biotechnology, 2024a, Santa Cruz Biotechnology, 2024b, Santa Cruz Biotechnology, 2024c). Enzyme labeling was performed using streptavidin–horseradish peroxidase (SA–HRP) for 40 minutes at room temperature, and color development was achieved with diaminobenzidine (DAB) substrate for 10 minutes. Counterstaining was carried out using Mayer's hematoxylin, followed by rinsing in PBS. Finally, slides were mounted with Entellan medium, covered with glass coverslips, and air-dried for 24 hours before microscopic evaluation. Immunohistochemical staining was analyzed using IHC Profiler software, by summing the percentage area of expression distributed across high positive and positive categories in each field of view (Khan et al., 2020).

Flow cytometry analysis

Fresh spleen samples obtained immediately after necropsy were processed for flow cytometric analysis of $CD4^+$ and $CD8^+$ T-cells. The procedure was conducted at the Laboratory of Animal Physiology, Structure, and Development, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Flow cytometric analysis was performed using a BD FACSCalibur (BD Biosciences, USA). Flow cytometric preparation involved two main steps: cell isolation and antibody staining. For cell isolation, spleens were gently homogenized using the blunt end of a sterile syringe and filtered through a fine wire mesh to obtain a single-cell suspension. The filtrate was collected into polypropylene tubes containing 12 mL of PBS and centrifuged at 2500 rpm for 5 minutes at 4 °C. After discarding the supernatant, the cell pellet was resuspended in 1 mL of PBS. A 50 µL aliquot of this suspension was diluted with 500 µL of PBS and centrifuged again under the same conditions. The resulting pellet was used for antibody staining. Cells were incubated with PE-conjugated anti-mouse $CD4^+$ (BioLegend, 100407; 0.25 µg/test) and FITC-conjugated anti-mouse $CD8^+$ (BioLegend, 100705; 0.25 µg/test) antibodies in a total staining volume of 50 µL for 30 minutes in the dark. Following incubation, 400 µL of PBS was added, and the samples were transferred to flow cytometry tubes for analysis (Pradana et al., 2013).

Data analysis

All statistical analyses were performed using RStudio (version 4.2025.09.1+401). Data are presented as Mean±SD. Normality of data was assessed using the Shapiro–Wilk test, and homogeneity of variances among the groups was tested with Levene's test. For variables that met both assumptions, one-way ANOVA followed by Tukey's post hoc test was used to compare multiple groups. If data did not meet normality assumptions, the non-parametric Kruskal–Wallis test with Dunn's post hoc test was applied. Pairwise comparisons between the control and EGCG 750 mg/kg groups (IL-2, BAX, BCL-2) were performed using independent two-sample t-tests when assumptions were met, or Mann–Whitney U tests when normality was violated.

Relationships between EGCG dose and T-cell subsets ($CD4^+$ and $CD8^+$) were assessed using correlation analysis. Pearson's correlation analysis was used for normally distributed data, whereas Spearman's rank correlation analysis was used for data that did not meet the normality assumptions. Dose–response effects were further evaluated with simple linear regression, with EGCG dose as the independent variable and T-cell counts as the dependent variable. Regression assumptions—residual normality, equal variance (homoscedasticity), and linearity—were checked, and robust standard errors were applied if needed. Regression results are presented as unstandardized coefficients (β), R^2, and 95% confidence intervals (CI). Statistical significance was set at $P<0.05$.

Results

IL-2 expression

The normality of IL-2 and BAX expression data in each group was evaluated using the Shapiro–Wilk test, which indicated no significant deviation from normality ($P>0.05$ for all groups). Homogeneity of variances was further confirmed by Levene's test for IL-2 ($P=0.370$) and BAX ($P=0.467$). Independent two-sample t-tests were subsequently applied to compare the control and EGCG 750 mg/kg groups. IL-2 expression exhibited a non-significant decrease ($P=0.117$), whereas BAX expression showed a non-significant increase ($P=0.616$) in the EGCG-treated group relative to the control group (Figure 1A and B). In contrast, BCL-2 data did not conform to a normal distribution in either the control or EGCG 750 mg/kg group (Shapiro–Wilk test, $P<0.05$). Therefore, the Mann–Whitney U test was employed, revealing a significant elevation in BCL-2 relative expression in the EGCG 750 mg/kg group compared to the control group ($P<0.001$) (Figure 1C).

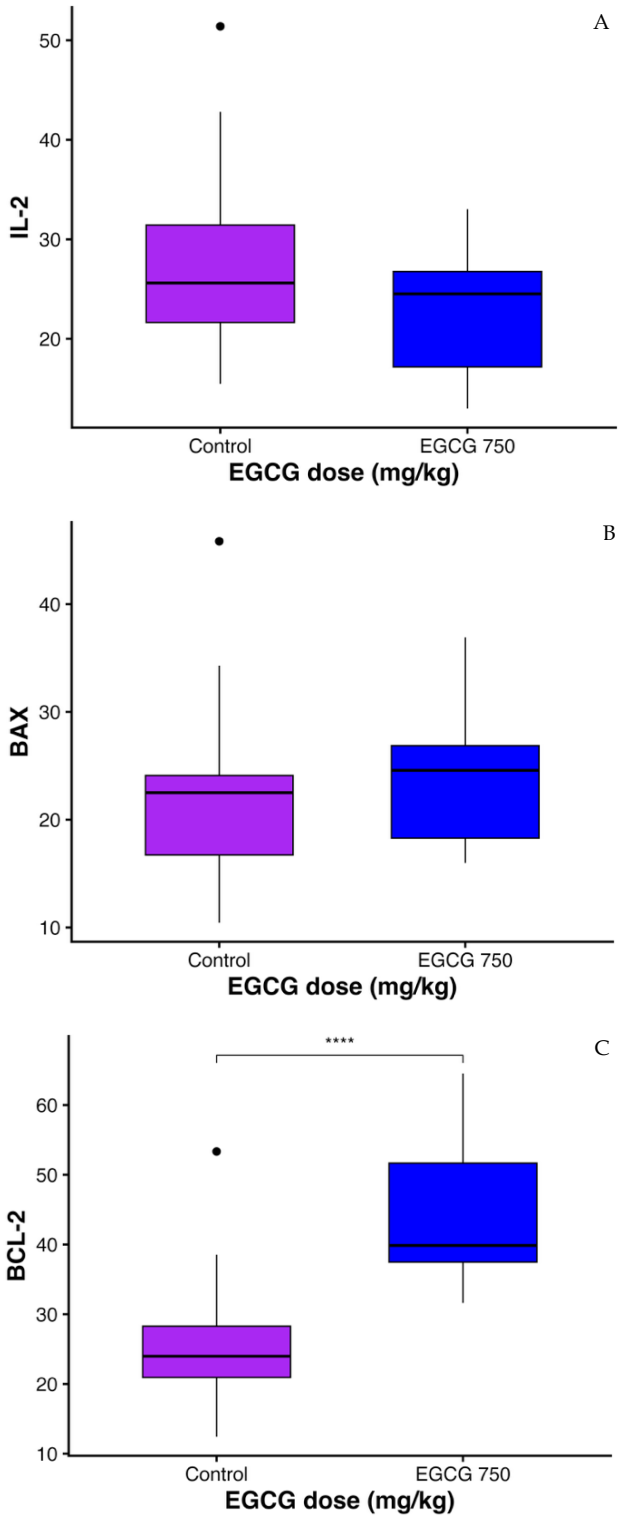


Figure 1. Expression of IL-2, BAX, and BCL-2 in the spleen tissue of mice treated with epigallocatechin-3-gallate (EGCG) (750 mg/kg)

A) IL-2 expression showed no significant difference between the control and EGCG groups (P=0.117), B) BAX expression showed no significant difference between the control and EGCG groups (P=0.616), C) BCL-2 expression was significantly higher in the EGCG group compared with the control group (P<0.001)

Note: Each box plot represents the distribution of expression levels, including the median and interquartile range; individual points represent each observation. Asterisks indicate statistically significant differences.

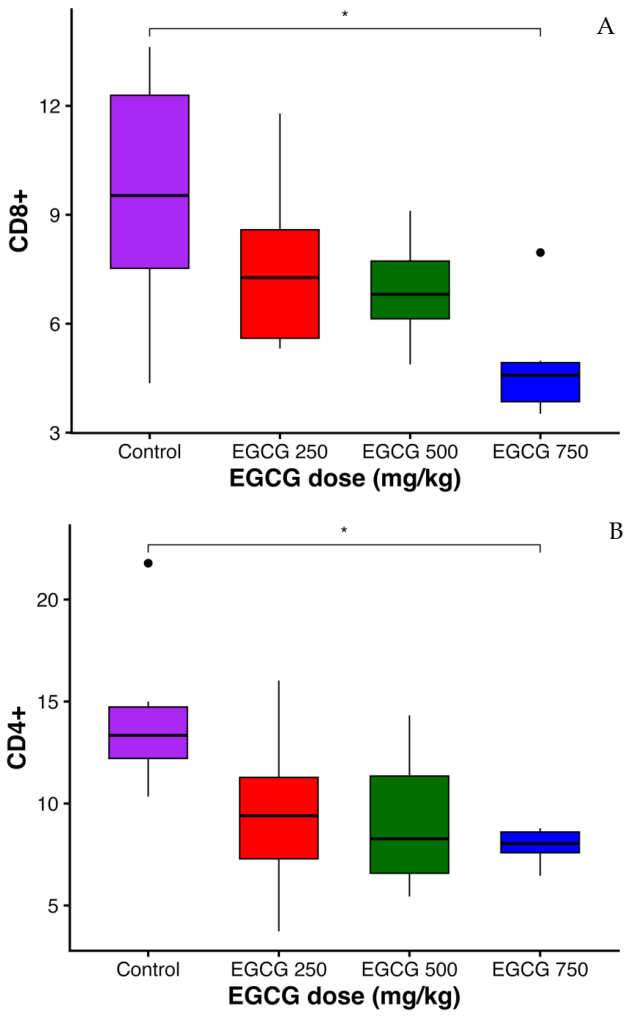


Figure 2. Relative proportions of CD4⁺ and CD8⁺ T-cells in the spleen of mice treated with epigallocatechin-3-gallate (EGCG

A) CD4⁺T-cell proportions in control and EGCG-treated groups (250, 500, and 750 mg/kg): EGCG at 750 mg/kg significantly reduced CD4⁺T-cells compared with the control group (P=0.020, Tukey's post-hoc test); B) CD8⁺T-cell proportions in the same treatment group: EGCG at 750 mg/kg significantly reduced CD8⁺ T-cells compared with the control group (P=0.017, Tukey's post-hoc test).

Note: Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was assessed using Levene's test. Data are expressed as Mean±SD, and error bars indicate standard deviations.

Relative proportions of CD4⁺ and CD8⁺ T-cells

The distribution of relative proportions of CD4⁺ and CD8⁺ T-cells across all groups was first evaluated for normality using the Shapiro–Wilk test, which showed no significant deviation from normality in any group (P>0.05). Homogeneity of variances among treatment groups was further confirmed by Levene's test for both CD4⁺ (P=0.308) and CD8⁺ (P=0.112) T-cells. One-way ANOVA revealed significant overall effects of EGCG treatment on the proportions of CD4⁺ (P=0.021) and CD8⁺ (P=0.028) T-cells. Post-hoc comparison using Tukey's HSD test demonstrated that the highest EGCG dose (750 mg/kg) significantly reduced the relative proportions of both CD4⁺ (P=0.020) and CD8⁺ (P=0.017) T-cells compared with the control group, whereas the lower doses (250 mg/kg and 500 mg/kg) did not produce significant changes in either subset (Figure 2). Representative flow cytometry plots for the control and the three EGCG dose groups are shown in Figure 3.

Spearman's rank correlation analysis demonstrated a significant negative association between EGCG dose (mg/kg) and the relative proportions of CD4⁺ (ρ= –0.576 and P=0.003, respectively) and CD8⁺ (ρ= –0.571 and P=0.004, respectively) T-cells. No significant correlation was observed between CD4⁺ and CD8⁺ T-cells (ρ=0.103 and P=0.629, respectively). Linear regression

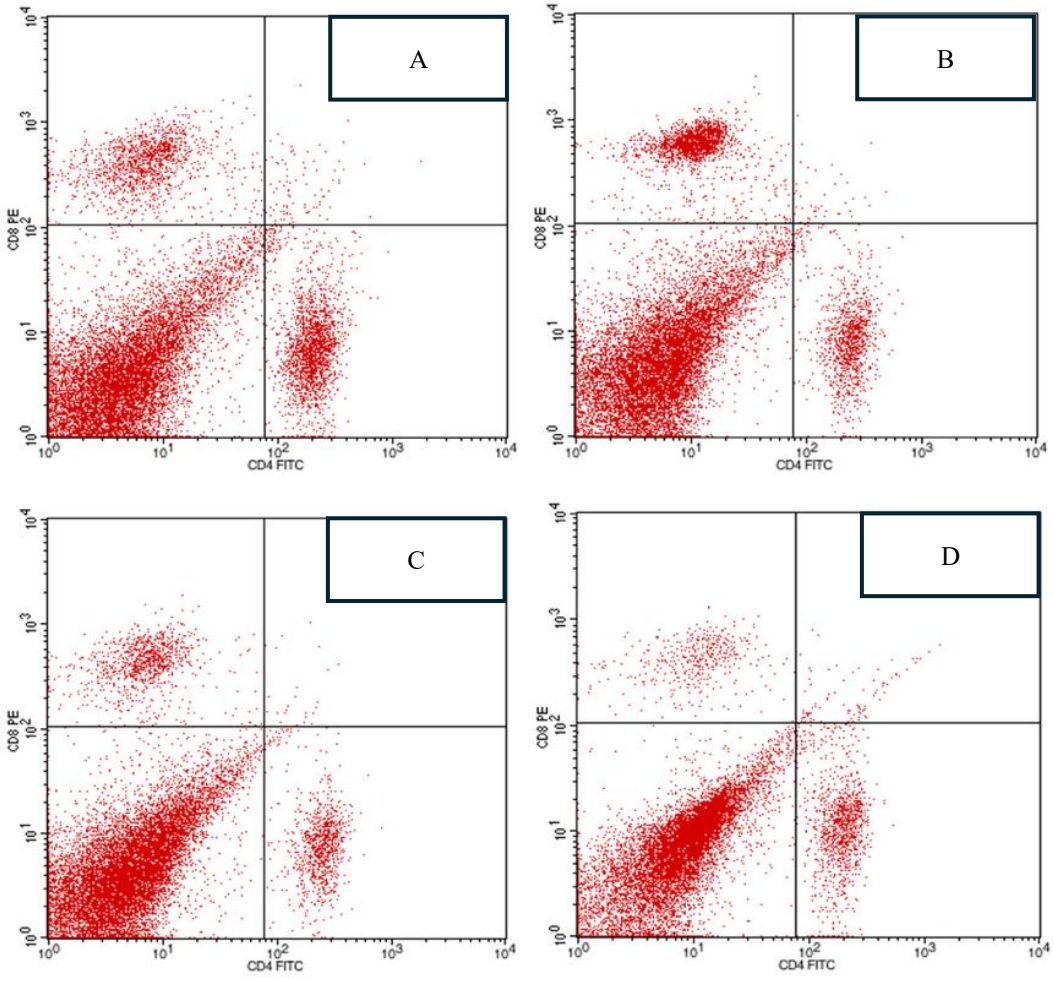


Figure 3. Relative proportions of $CD4^+$ and $CD8^+$ T-cells in the control (A), EGCG 250 mg/kg (B), EGCG 500 mg/kg (C), and EGCG 750 mg/kg (D) groups

Note: The quadrants denote $CD8^+$ single-positive (upper left) and $CD4^+$ single-positive (lower right) subsets.

analysis further confirmed these associations, showing that increasing EGCG dose was significantly associated with a reduction in $CD4^+$ ($\beta = -0.0078$ and P=0.005, respectively) and $CD8^+$ ($\beta = -0.0058$ and P=0.005, respectively) T-cells (Figure 4). Model diagnostics indicated that residuals were normally distributed (Shapiro–Wilk test: $CD4^+$: P=0.747; $CD8^+$: P=0.677), with no evidence of model misspecification (Ramsey RESET test, P>0.3 for both models). Homoscedasticity was confirmed for $CD4^+$ (Breusch–Pagan test, P=0.146), while mild heteroscedasticity detected in the $CD8^+$ model (P=0.019) was corrected using robust standard errors.

Discussion

EGCG is one of the major bioactive flavonoids present in green tea and has been extensively studied for its potential health-promoting properties. Numerous investigations have reported its beneficial biological effects, including antiviral and anticancer activities (Liu et al., 2024; Sehgal et al., 2023; Wang et al., 2025). However, high-dose EGCG exposure has been associated with adverse physiological outcomes, particularly hepatotoxicity. Experimental studies in mice have demonstrated that high doses of EGCG can induce hepatic injury. In particular, molecular analyses revealed that EGCG administered at a dose of 750 mg/kg significantly downregulated the mRNA expression of mitochondrial inner membrane complex I and III proteins and reduced the expression of the antioxidant enzymes superoxide dismutase and glutathione peroxidase (James et al., 2018).

Immunohistochemical analysis revealed that IL-2 expression was lower in the EGCG-treated group (750 mg/kg) compared with the control group, although the difference was not statistically significant. BAX expression increased but not significantly, whereas BCL-2 expression was significantly elevated following EGCG admin-

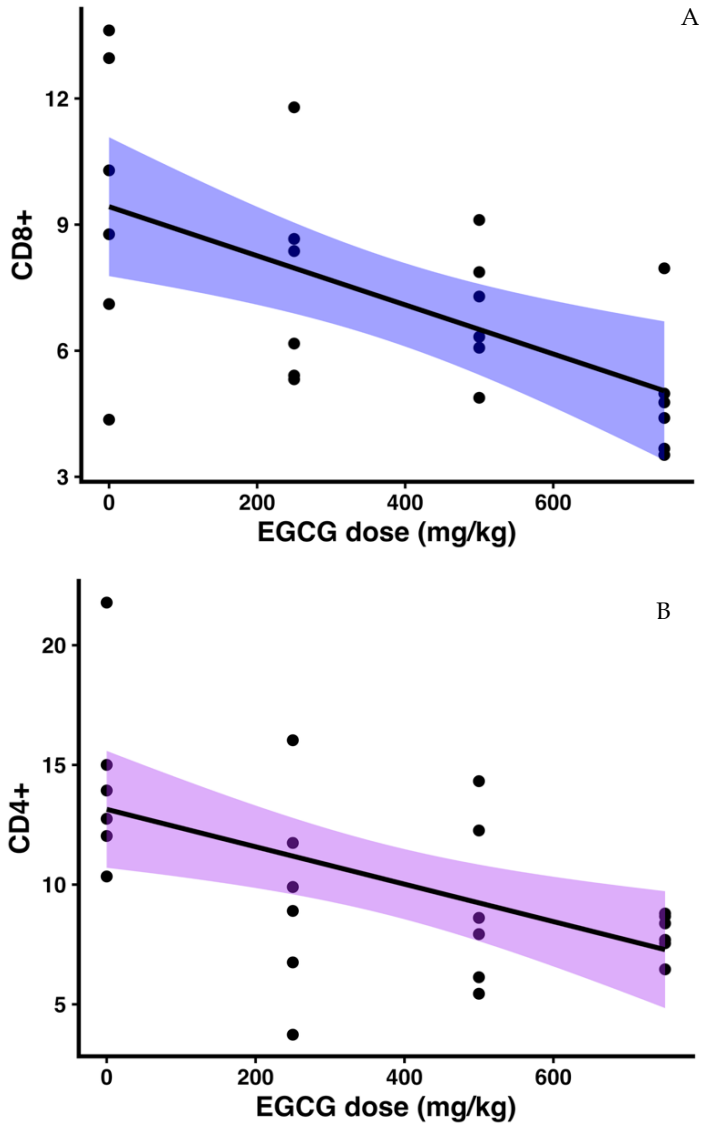


Figure 4. Linear regression analysis of EGCG dose (mg/kg) versus the relative proportions of $CD4^+$ and $CD8^+$ T-cells in the mouse spleen

A) A significant negative association was observed between EGCG dose and $CD4^+$T-cells (β = –0.0078 and P=0.005, respectively); B) A significant negative association was also found for $CD8^+$ T-cells (β = –0.0058and P=0.002, respectively)

Note: The solid black lines represent the fitted regression lines, and the shaded grey areas indicate 95% CI. Increasing EGCG doses were associated with dose-dependent reductions in both $CD4^+$ and $CD8^+$T-cell populations.

istration. Flow cytometric analysis further demonstrated a dose-dependent decline in the relative proportions of $CD4^+$ and $CD8^+$ T-cells, with statistically significant reductions observed only at the highest dose (750 mg/kg). Spearman's correlation analysis revealed a significant negative association between EGCG dose and both $CD4^+$ and $CD8^+$ T-cells. Linear regression analysis further supported these findings, showing that increasing EGCG dose was significantly associated with reduced $CD4^+$ and $CD8^+$ T-cells.

In the present study, IL-2 expression did not differ significantly between the control and EGCG-treated groups. Because the groups were not subjected to antigenic stimulation, the reduction in IL-2 is unlikely to be caused by impaired T-cell receptor–mediated activation. Instead, this finding may reflect a direct effect of EGCG on basal IL-2 expression under resting conditions. Previous studies have shown that the generation of mROS is necessary for the activation of nuclear factor of activated T-cells (NFAT), a key transcription factor regulating IL-2 expression (Sena et al., 2013; Hogan et al., 2017;

Weinberg et al., 2025). Based on these observations, it is possible that EGCG-induced mitochondrial impairment reduces mROS availability, leading to attenuated NFAT activation and lower basal IL-2 expression.

This interpretation is consistent with previous reports showing that high-dose EGCG can disrupt mitochondrial protein complexes (James et al., 2018). However, this explanation remains weak and represents a limitation of the present study, as mROS levels and NFAT activation were not directly measured. In addition, the absence of antigenic stimulation limits conclusions regarding the effects of EGCG on activation-dependent IL-2 production. Further studies assessing mitochondrial redox signaling and NFAT activity, particularly under stimulated conditions, are needed to clarify the mechanism by which EGCG modulates IL-2 expression under resting conditions.

The increased expression of BAX observed in this study is most likely associated with oxidative stress induced by high-dose EGCG. EGCG has been shown to act as a pro-oxidant, leading to excessive hydrogen peroxide (HO_2) generation (Wu et al., 2009). HO_2 serves as a potent intracellular stress signal capable of activating the mitochondrial intrinsic apoptotic pathway (Chidawanyika & Supattapone, 2021). Oxidative stress promotes the activation of BH3-only proteins, particularly BCL-2–interacting mediator of cell death (BIM), which subsequently recruits the pro-apoptotic proteins BAX and BAK to the mitochondrial outer membrane (Westphal et al., 2013; Savitskaya & Onishchenko., 2015). This process facilitates mitochondrial outer membrane permeabilization and the release of cytochrome c into the cytoplasm, initiating apoptosome formation through the interaction of cytochrome c with apoptotic protease activating factor-1 (Apaf-1). The apoptosome then activates caspase-9, which triggers downstream effector caspases and promotes apoptotic signaling (Wu et al., 2014)

In the present study, the increase in BAX expression did not reach statistical significance. The modest but detectable increase in BAX expression may reflect a cellular response to EGCG-associated stress. However, since downstream apoptotic events, such as cytochrome c release or caspase activation were not directly assessed, the observed increase in BAX should be interpreted as indicative of apoptotic pressure rather than definitive evidence of ongoing apoptosis.

In contrast to BAX, BCL-2 expression was significantly increased in the high-dose EGCG group. BCL-2 is a key anti-apoptotic protein that preserves mitochondrial outer membrane integrity by neutralizing pro-apoptotic molecules, such as BIM, thereby preventing BAX and BAK activation (Czabotar & García-Sáez, 2023). In the context of this study, the upregulation of BCL-2 is most consistent with a compensatory survival response maintained by basal homeostatic signaling rather than activation-induced cytokine signaling. Unlike IL-2, IL-7 signaling in resting T-cells is a well-established inducer of BCL-2 expression during T cell homeostasis (Crawley et al., 2013). Thus, even in the presence of EGCG-induced oxidative stress and modest BAX induction, preserved IL-7–dependent survival signaling could sustain BCL-2 expression (Kimura et al., 2012). The concurrent presence of modest pro-apoptotic signaling (BAX) and enhanced anti-apoptotic signaling (BCL-2) suggests that T-cells in this setting exist in a state of cellular stress equilibrium, in which survival and death signals are simultaneously engaged.

The reduction in $CD4^+$ and $CD8^+$ T-cell populations observed at the highest EGCG dose may, at least in part, be explained by altered homeostatic IL-7 signaling. EGCG has been shown to interfere with signaling through the common γ chain (γc), a shared component of the IL-2 and IL-7 receptor complexes (Wang et al., 2012). Disruption of γc-dependent signaling can dysregulate IL-7 signaling, favoring prolonged or poorly terminated IL-7 exposure at the cellular level. Previous studies have demonstrated that persistent IL-7 signaling can induce IFN-γ expression and trigger cytokine-induced cell death (CICD) during T-cell homeostasis (Kimura et al., 2012). In this context, EGCG-mediated perturbation of the γc subunit may shift IL-7 from a survival-supporting signal toward T-cell reduction. Importantly, this interpretation does not imply that CICD was directly measured in the present study but provides a biologically plausible framework consistent with established homeostatic mechanisms.

In addition to the homeostatic state, maintenance of naïve T cell numbers depends on low-level homeostatic proliferation. EGCG has been reported to induce cell-cycle arrest in lymphocytes through upregulation of the CDK inhibitor p27kip1, which constrains progression through the G0/G1 phase (Wu et al., 2009) Increased p27kip1 expression would be expected to limit basal proliferative renewal of naïve $CD4^+$ and $CD8^+$ T-cells, even in the absence of antigenic stimulation. Under physiological conditions, such homeostatic proliferation compensates for spontaneous cell loss and is essential for preserving a stable lymphocyte pool size (Abbas et al., 2022). Suppression of this process, combined with prolonged IL-7 induced IFN gamma expression, would therefore favor a net decline in T-cell numbers. Thus, the observed reduction in $CD4^+$ and $CD8^+$ T-cell populations is most consistent with impaired homeostatic maintenance.

This study has several limitations that should be considered when interpreting the findings. First, the proposed involvement of mitochondrial dysfunction and downstream mROS–NFAT signaling in IL-2 regulation is based on prior literature because mitochondrial activity, mROS levels, and NFAT activation were not directly measured in this study. Second, although changes in BAX and BCL-2 expression suggest alterations in apoptotic regulation, downstream apoptotic events, such as cytochrome c release or caspase activation were not assessed, limiting conclusions regarding actual apoptosis. Additionally, because IL-2 expression and T-cell populations were assessed at different time points (day 3 and day 5, respectively), the ability to draw a direct causal link between these outcomes is limited.

Conclusion

In conclusion, this study demonstrates that high-dose EGCG modulates splenic IL-2 expression and is associated with a significant reduction in $CD4^+$ and $CD8^+$ T-cell populations in vivo. These effects occur in the absence of antigenic stimulation and are accompanied by modest alterations in apoptotic regulatory markers, characterized by non-significant changes in BAX and a significant increase in BCL-2 expression. The findings are consistent with perturbations in mitochondrial and homeostatic regulatory processes that influence T-cell maintenance under resting conditions. Collectively, these results indicate that excessive EGCG exposure alters T-cell homeostasis and survival-associated signaling, providing a mechanistic framework for the observed attenuation of T-cell populations in vivo.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were conducted in accordance with the ethical standards of the Animal Care and Use Committee, Institut Biosains, Universitas Brawijaya Malang, Indonesia (Code: 019-KEP-UB-2024).

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Authors' contributions

Conceptualization, methodology, and writing the original draft: Gegana Wimaldy Airlangga; Formal analysis: Erlienda Novitasari; Data curation: Gegana Wimaldy Airlangga and Erlienda Novitasari; Review, editing, and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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