

COORDINATED IMMUNOMODULATORY AND  
PROLIFERATIVE EFFECTS OF A  
POLYPHENOL–BROMELAIN COMBINATION IN J774  
MACROPHAGES

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**Abstract**

Polyphenols are a diverse family of plant secondary metabolites with well-documented antioxidant and anti-inflammatory activities. Bromelain is a pineapple-derived cysteine protease with documented anti-inflammatory activity. This study aimed to comprehensively evaluate the immunomodulatory, anti-inflammatory, and proliferative effects of a plant-derived polyphenol–bromelain (PB) combination (SammyVit, Turkiye) on the murine macrophage cell line J774. Cells were cultured in RPMI-1640 medium and treated with PB at 1%, 2.5%, 5%, 10%, and 20%. Inflammatory responses were assessed by measuring interleukin-6 (IL-6) and interleukin-10 (IL-10) levels; oxidative balance was evaluated using total antioxidant capacity (TAC). Cell-cycle dynamics were analyzed by flow cytometry. PB significantly reduced IL-6, increased IL-10 and TAC, and elevated the proportion of cells in the S and G2/M phases, supporting enhanced proliferative activity. Collectively, the results indicate coordinated anti-inflammatory, antioxidant, and pro-proliferative actions consistent with suppression of redox-sensitive pathways and reinforcement of a repair-compatible macrophage phenotype. The data support further mechanistic dissection and motivate translational testing in primary human macrophages and relevant in vivo models.

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**Key words:** polyphenol, bromelain, macrophage, IL-6, IL-10, total antioxidant capacity, cell cycle

**Introduction.** Polyphenols constitute a broad class of plant-derived secondary metabolites. These molecules are distinguished in biological systems by their antioxidant, anti-inflammatory, and immunomodulatory properties [1,2]. At the cellular level, polyphenols neutralize reactive oxygen species (ROS), thereby reducing oxidative stress, preventing cellular injury, and tempering inflammatory responses. Owing to these features, polyphenols can modulate immune-cell activity and regulate cytokine production.

Bromelain is a proteolytic enzyme complex obtained from *Ananas comosus* and exhibits anti-inflammatory, antithrombotic, and immunomodulatory effects [3]. It has been reported to decrease the production of inflammatory cytokines, enhance the phagocytic capacity of immune cells, and accelerate wound healing. Recent work further suggests that combinations of polyphenols and bromelain may exert combined effects on redox homeostasis, epigenetic regulation, and cell proliferation [4]. Although each class of molecules is active on its own, combining polyphenols with bromelain could, in principle, couple redox control to protease-driven remodelling of signalling pathways, yielding broader immunomodulatory effects than either component alone.

Macrophages are key effector cells of the immune system, responsible for inflammatory cytokine production, phagocytosis, antigen presentation, and maintenance of redox balance. Consequently, examining the actions of natural compounds on macrophages is essential for elucidating their therapeutic potential. In addition to defensive roles, macrophages contribute to angiogenesis and extracellular-matrix turnover, and their phenotype is tightly linked to cellular metabolism and redox balance. The murine J774 line is widely used to interrogate macrophage biology; while convenient and reproducible, it exhibits lineage-specific features and immortalization-related rewiring that should be considered in interpretation [5,6].

Against this backdrop, we investigated whether a commercially available polyphenol–bromelain (PB) (SammyVit, Turkiye) formulation can simultaneously rebalance cytokine production, reinforce antioxidant capacity, and promote proliferation-compatible cell-cycle progression in J774 macrophages.

**Materials and methods. Cell culture and treatments.** J774 macrophages (ATCC TIB-67) were maintained in RPMI-1640 (Gibco™, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, and L-glutamine at 37 °C in a humidified incubator with 5% CO<sub>2</sub>. Cells were seeded at 1×10<sup>5</sup> cells per well in 96-well plates and allowed 24 h for adherence. Cultures were then exposed for 48 h to a PB formulation at final concentrations of 1%, 2.5%, 5%, 10%, or 20%. All assays were conducted in three independent biological replicates.

**Cytokine quantification.** Post-treatment supernatants were harvested and analyzed for IL-6 and IL-10 using commercial ELISA kits (BT-LAB/Shanghai Korain Biotech, China) according to the manufacturer's instructions. Absorbance was recorded at 450 nm on a microplate reader [7]. Cytokine concentrations were interpolated from standard curves and expressed in pg/mL.

**Total antioxidant capacity.** TAC in culture supernatants was measured according to the supplier's protocol [7] using a colourimetric ABTS•+ radical cation decolourization method (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)). In this assay, antioxidants present in the samples reduce the ABTS•+ radical, resulting in a decrease in absorbance measured spectrophotometrically. TAC values were calculated based on a Trolox standard curve and expressed as Trolox equivalents (mmol/L). As TAC integrates the combined contributions of low-molecular-weight antioxidants and enzyme-dependent antioxidant activity, it provides an overall indicator of redox homeostasis under the applied treatment conditions.

**Cell viability.** Metabolic activity and relative viability were assessed by the MTT assay. Cells received 20 µL of MTT solution (5 mg/mL) and were incubated for 4 h. Supernatants were discarded, formazan crystals were solubilized in dimethyl sulphoxide, and absorbance was read at 590 nm. Viability was calculated as a percentage of the untreated control group [8].

**Cell-cycle analysis.** For DNA-content analysis, cells were detached, washed, fixed, and stained using the NutriCulture Cell Cycle Analysis Kit (Ecotech, Turkey). Samples were acquired on a Beckman Coulter Navios flow cytometer (Indianapolis, USA; software v2.1).

**Statistics.** Data are presented as mean  $\pm$  standard deviation from three independent experiments. Group differences were tested by one-way analysis of variance. A  $p$  value  $< 0.05$  was considered statistically significant. Statistical analyses were performed with GraphPad Prism v9.1.1 (San Diego, CA, USA).

**Results. Cytokine modulation.** Exposure to the PB combination decreased IL-6 and increased IL-10 relative to untreated controls, with the most pronounced and statistically significant changes observed at 5% and 10% ( $p < 0.05$ ) (Fig. 1). Reductions in IL-6, a pleiotropic cytokine implicated in chronic inflammatory states, are compatible with attenuation of NF- $\kappa$ B-dependent transcription. Conversely, the elevation of IL-10, a canonical anti-inflammatory cytokine associated with resolution and matrix remodelling, indicates a shift toward an inflammation-resolving programme.

**Antioxidant capacity.** TAC rose significantly across the tested range and peaked at 5% ( $p < 0.05$ ), indicating reinforcement of antioxidant defenses. Increased TAC is consistent with direct radical-scavenging capacity of polyphenols and potential induction of endogenous antioxidant enzymes. Diminished oxidant burden would be expected to dampen ROS-sensitive pro-inflammatory signalling, providing a biochemical rationale for the observed cytokine reprogramming.

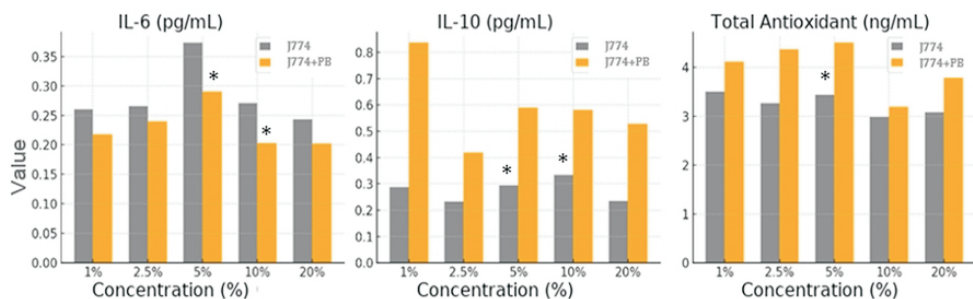


Fig. 1. Concentration-dependent changes in IL-6, IL-10, and TAC in supernatants from J774 macrophages treated with PB. \* $p < 0.05$  (one-way analysis of variance)

**Viability and metabolic activity.** MTT analysis showed minimal change at 1%, significant increases in viability at 2.5% and 5% ( $p < 0.05$ ), and plateauing at 10–20% (Fig. 2). This hormetic-like pattern is typical of bioactive phytochemicals: moderate doses enhance anabolic metabolism and mitochondrial function, whereas higher doses trigger adaptive constraints that limit additional gains. The viability boost at intermediate concentrations aligns with the cytokine and redox data, together indicating a state supportive of repair.

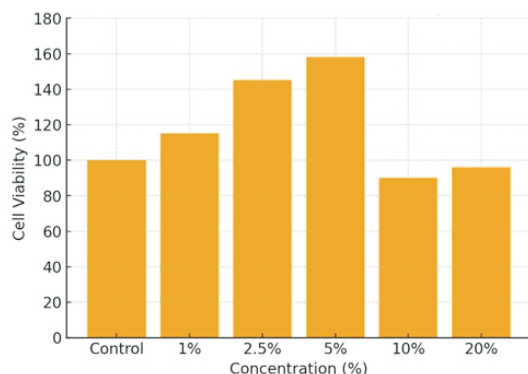


Fig. 2. Concentration-dependent changes in proliferation of J774 macrophages treated with PB

**Cell-cycle dynamics.** Flow cytometry revealed a redistribution from G0/G1 toward S and G2/M in the 5% group. In untreated controls, 57.7% of cells were in G0/G1, 15.9% in S, and 27.6% in G2/M. Following 5% treatment, G0/G1 decreased to 51.5%, S increased to 18.8%, and G2/M rose to 30.4% ( $p < 0.05$ ) (Fig. 3).

**Discussion.** This study demonstrates that the PB combination exerts multifaceted biological effects in macrophage cells. The decrease in IL-6 accompanied by an increase in IL-10 supports the anti-inflammatory potential of the formula-

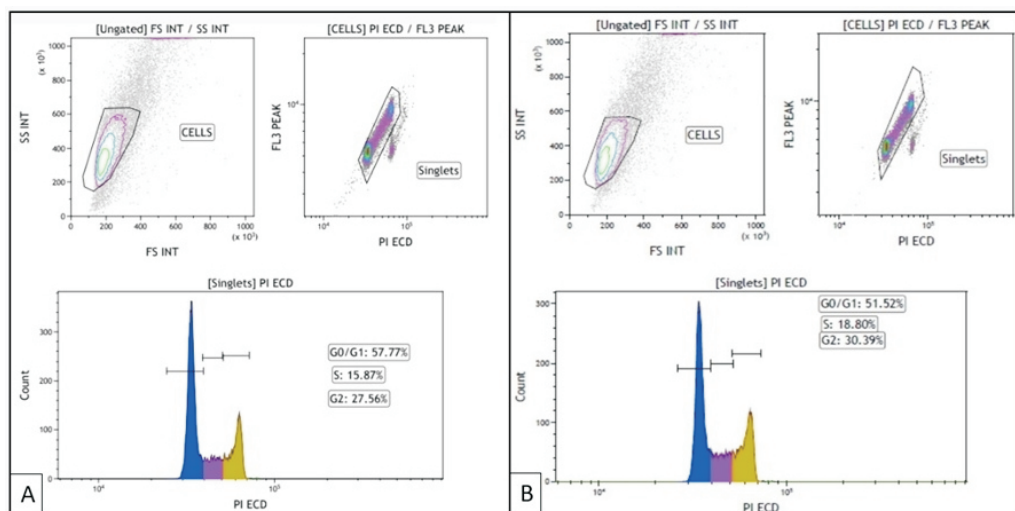


Fig. 3. Cell-cycle analysis by flow cytometry in control (A) and in cells treated with 5% PB (B)

tion [9,10], while the rise in total antioxidant capacity (TAC) indicates reinforcement of cellular defense mechanisms [11]. Moreover, the increased proportion of cells in the S and G2/M phases suggests support for DNA synthesis and mitotic progression.

In J774 macrophages, the reduction in IL-6 together with elevated IL-10 reflects a re-balancing of inflammatory signalling. IL-6 is a pleiotropic cytokine whose sustained overproduction contributes to pathological inflammation [9], and its downregulation is consistent with attenuation of NF- $\kappa$ B/MAPK-dependent pathways. In this context, bromelain has been shown to suppress LPS-induced NF- $\kappa$ B and MAPK activation in murine macrophages, leading to reduced pro-inflammatory mediator release [10,12]. The concomitant increase in IL-10 further aligns with a resolution-oriented, M2-like functional state, in agreement with reports showing similar cytokine modulation by polyphenol-rich extracts in the J774 model [2,5].

The observed increase in TAC provides a biochemical basis for the improved cytokine profile. Polyphenols contribute to redox homeostasis through free-radical scavenging, metal chelation, and modulation of intracellular signalling [1,2]. The dose-dependent enhancement of TAC suggests a reduction in intracellular ROS burden and subsequent downregulation of ROS-sensitive NF- $\kappa$ B/MAPK pathways, consistent with previous reports in macrophages [13]. The use of the ABTS $\bullet$ + decolourization assay further supports the biological relevance of the antioxidant changes detected [11].

Third, the MTT assay revealed a pronounced increase in viability/metabolic activity within the 2.5–5% range, suggesting a mitogenic window in macrophages. Polyphenols can modulate PI3K/Akt and ERK1/2 signalling in a context- and

dose-dependent manner, while bromelain may temper pro-inflammatory flux via NF- $\kappa$ B/MAPK inhibition to support reparative processes [2, 10, 12, 13]. The plateau at higher doses is consistent with feedback mechanisms in immune cells and the hormetic nature of the antioxidant–pro-oxidant balance [2].

Fourth, the increased fractions of cells in S and G2/M phases indicate facilitated DNA synthesis and mitotic entry. Polyphenols can reshape transcriptional programmes via epigenetic regulators such as DNMTs and HDACs, potentially culminating in functional changes along the cyclin/CDK axis [4, 14]. Interpretation should also consider the specific features and limitations of macrophage subtypes and immortalized lines such as J774 [6].

Taken together, our findings reveal a complementary suite of effects – suppression along the pro-inflammatory axis (IL-6 $\downarrow$ ), reinforcement of anti-inflammatory signalling (IL-10 $\uparrow$ ), augmented antioxidant defenses (TAC $\uparrow$ ), and advancement of cell-cycle progression (S/G2/M $\uparrow$ ). This integrated profile is concordant with contemporary preliminary evidence on the immunomodulatory potential of natural compounds [4, 5].

The combined use of bromelain and polyphenols may exert combined actions across key processes, including mitigation of oxidative stress, regulation of intracellular signalling, and re-balancing of immune responses. Recent studies report that bromelain diminishes inflammation through suppression of NF- $\kappa$ B and MAPK pathways, while polyphenols may reinforce these effects via epigenetic regulation [9, 13, 14]. Collectively, these data support the therapeutic potential of natural compounds for immunomodulation and control of gene expression programmes.

**Limitations.** The effects of the PB combination were evaluated relative to untreated controls, without assessing the individual contributions of each component, limiting formal distinction between combined and additive effects. Macrophage polarization was not directly examined after 48 h of treatment, although the IL-6 decrease and IL-10 increase are consistent with a resolution-oriented response. As the study was performed in the immortalized murine J774 macrophage cell line, validation in primary murine or human macrophages would enhance translational relevance. Finally, cytokine analysis was limited to IL-6 and IL-10; inclusion of additional mediators such as TNF- $\alpha$ , IL-1 $\beta$ , or IL-1Ra would enable a broader characterization.

**Conclusion.** A PB combination exerts coordinated anti-inflammatory, antioxidant, and pro-proliferative actions in J774 macrophages. The formulation suppresses IL-6, elevates IL-10, increases total antioxidant capacity, enhances viability at intermediate concentrations, and drives redistribution from G0/G1 into S and G2/M phases. Collectively, these effects are consistent with modulation of redox-sensitive and polarization-linked pathways and justify deeper mechanistic studies alongside translational testing in primary human macrophages and relevant in vivo models. Future dose–response studies will refine therapeutic windows

and optimize schedules for macrophage-targeted interventions in diverse contexts.

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