# Garlic Extracts and Probiotics in a Lipopolysaccharide-Stimulated Intestinal Multicellular Model

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## Introduction

Garlic (*Allium sativum* L.) is widely recognized not only for its therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory and cardiometabolic benefits. These effects are mainly associated with its bioactive composition, particularly organosulfur compounds such as alliin and its derivatives, as well as phenolics and flavonoids[1]. Garlic has also demonstrated anti-inflammatory activity through modulation of key mediators such as TNF-α, IL-6 and IL-1β via inhibition of the NF-κB signaling pathway[2].

Interest has expanded toward black garlic (BG), an aged product obtained by controlled thermal processing of white garlic (WG), in which Maillard reactions and phenolic oxidation lead to the formation of compounds such as S-allyl-cysteine and melanoidins, enhancing its antioxidant and biological activity[3]. Compared with WG, BG exhibits reduced pungency, a sweeter flavor, improved stability, and increased antioxidant capacity, making it a promising ingredient for functional foods and nutraceutical applications[4]. Despite growing evidence on the health benefits of garlic-derived compounds, the comparative effects of WG and BG on intestinal barrier function, inflammatory responses and host-microbiota interactions remain poorly understood.

## Aim of study

**To evaluate the effects of WG and BG extracts on intestinal homeostasis by assessing their impact on LPS-induced inflammation and oxidative stress in Caco-2/HT29 cells, epithelial barrier permeability and interactions with probiotic bacteria.**

## Materials and methods

**White and black garlic preparation:** WG (*Allium sativum*) was thermally aged at 70º C for 30 days to obtain BG. Both samples were freeze-dried, milled and powdered for further analyses.

**Probiotic sensitivity:** the antimicrobial activity of garlic extracts against *L. paracasei* (LP) and *E. coli* Nissle 1917 (EcN) was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

**Cell culture and intestinal model:** Caco-2 and HT29 cells were used to evaluate cytocompatibility and metabolic activity. A Caco-2/HT29 co-culture (9:1) was established to mimic the intestinal epithelium and induce inflammation using lipopolysaccharides (LPS).

**Barrier function and inflammation:** transepithelial electrical resistance (TEER), Lucifer Yellow permeability, ZO-1 immunostaining, mucin production and cytokine expression were measured to assess intestinal barrier integrity and inflammatory response. Simulated gastrointestinal digestion of garlic extracts was performed before treatment (**Fig. 1**).

**Statistical analysis:** Data were analyzed by ANOVA with Tukey's test ($p < 0.05$).

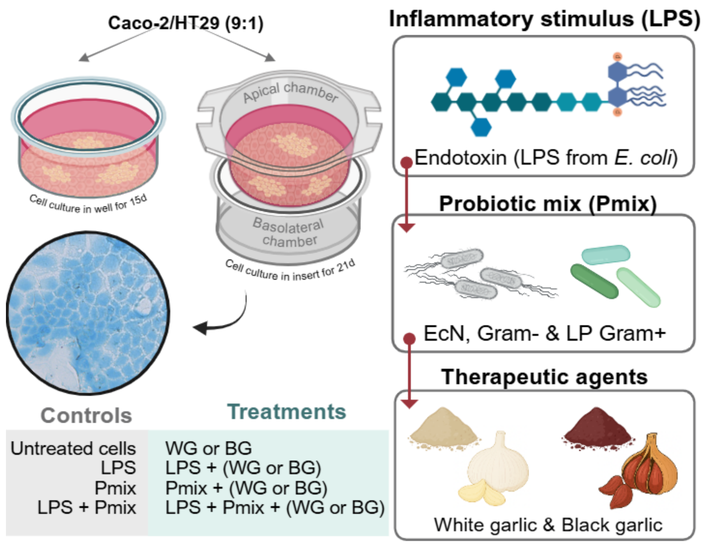



**Fig. 1**. Workflow for co-culture establishment and experimental groups (controls and treatments)

## Conclusion

Thermal aging transforms garlic into a functionally distinct food. Black garlic combines strong anti-inflammatory and barrier-protective effects with improved compatibility with beneficial microbes, highlighting its potential as a dietary immunonutrient for supporting intestinal homeostasis.

## References

[1] Sasmaz, H. K. et al., 2025. *Sci Rep*, 15(1), 23427.

[2] Hernández-Cruz, E. Y. et al., 2022. *Eur J Pain*, 26(5), 947.

[3] Liang, T. et al., 2015. J. *Agric Food Chem*, 63(2), 683.

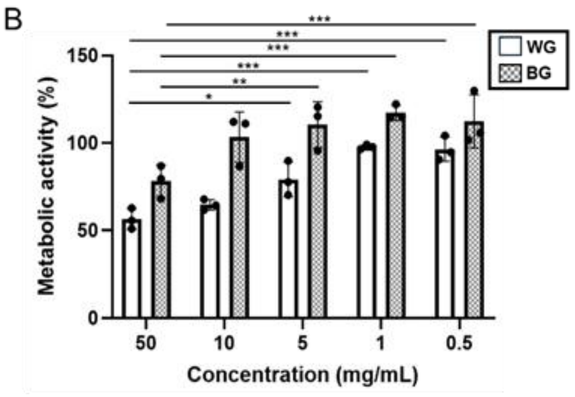
[4] Vecchi, V. et al., 2025. *J Agric Food Chem*., 73(44), 27933.

## Results and Discussion

### Antimicrobial susceptibility and cell cytocompatibility of garlic extracts

A

| Strain | Treatment | MIC range (mg/mL) | MBC range (mg/mL) | Microbial response trend |
|---|---|---|---|---|
| *E. coli* Nissle 1917 | WG | 3.1-12.5 | 6.3-25.0 | Moderate growth modulation; mild inhibitory effect at higher doses |
| | BG | ≥50 | - | Tolerant; minimal inhibition even at high concentrations |
| *L. paracasei* | WG | 1.6-12.5 | 3.1-25.0 | Moderate modulation; transient inhibition with recovery potential |
| | BG | 50.0 | 50.0 | High tolerance; negligible growth inhibition |



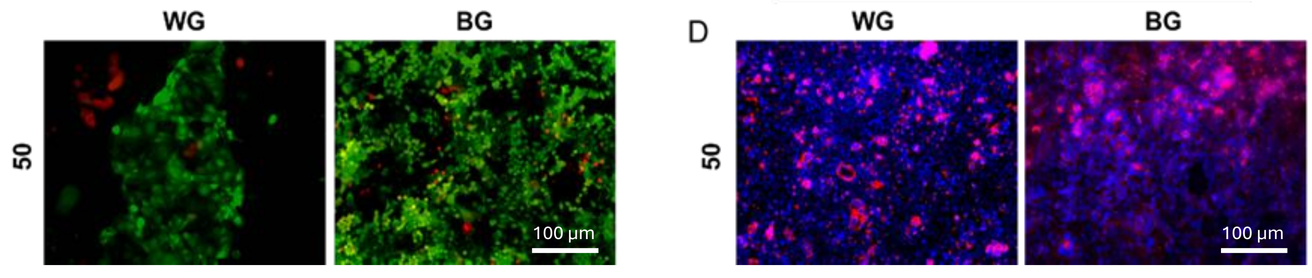



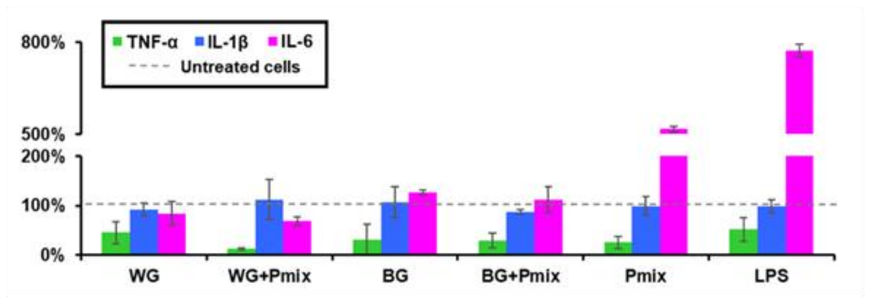

**Fig. 2.** (A) Concentration ranges for MIC and MBC. (B) Caco-2 metabolic activity (PrestoBlue™ assay) after exposure to WG or BG extracts (50 mg/mL). (C) Live/dead staining: live (green), dead (red) cells. (D) Fluorescence staining nuclei (blue) and cytoskeleton (red).

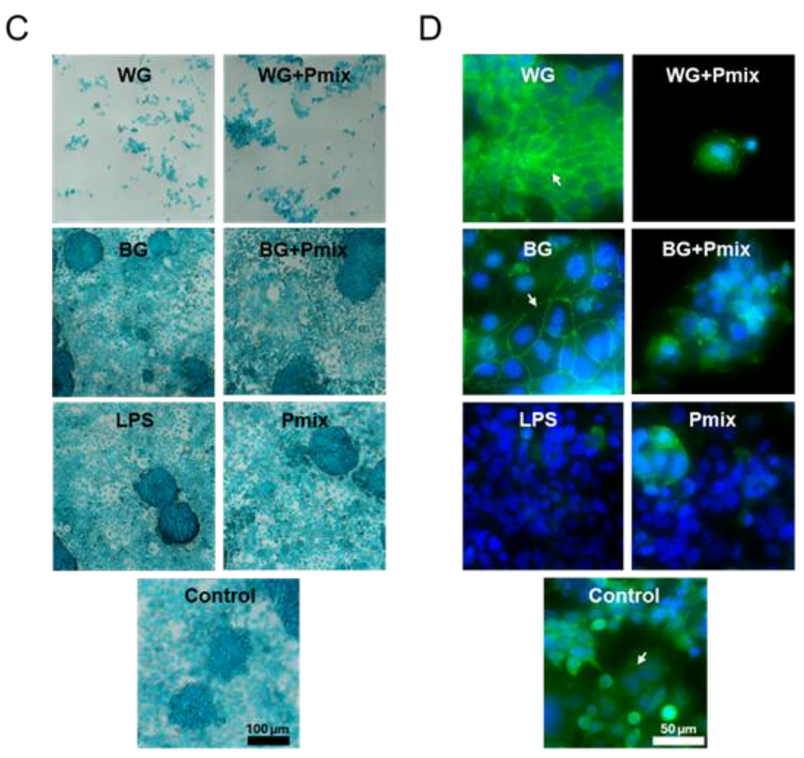
Thermal aging alters garlic metabolism, leading to distinct biological effects. BG showed low MIC of EcN and LP, whereas WG retained stronger antimicrobial activity. In LPS-stimulated models, both extracts reduced cytokine expression, but BG showed stronger anti-inflammatory effects, significantly lowering IL-1β, IL-6, and TNF-α (**Fig. 2**).

### Influence of garlic extracts on *in vitro* gut-like epithelial tissue under pathological conditions

A

| Day | TEER (Ω·cm²) |
|---|---|
| 5 | 340.3 ± 56.2[a] |
| 10 | 395.1 ± 78.1[b] |
| 15 | 423.9 ± 96.7[c] |
| 20 | 478.3 ± 58.3[c] |



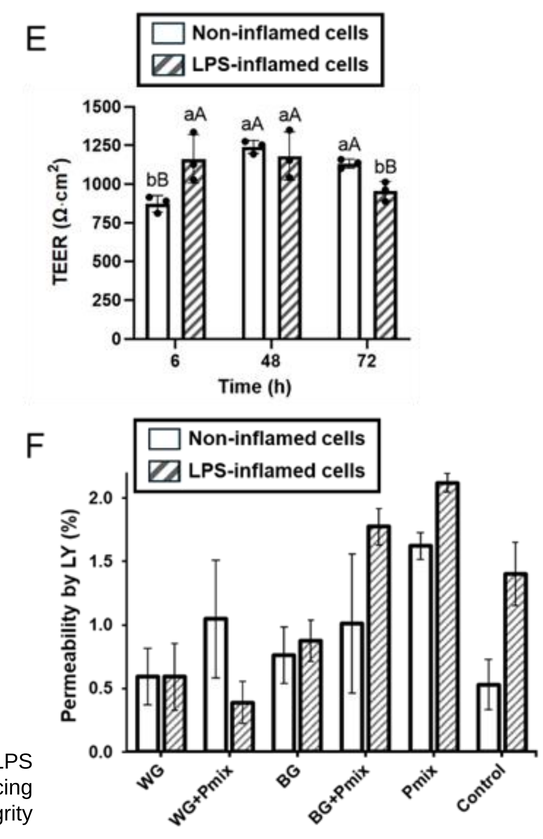




**Fig. 3.** (A) TEER of gut epithelial tissue. (B) Inflammatory cytokine expression after LPS stimulation with or without WG, BG, or Pmix. (C) Alcian Blue staining of mucin-producing regions. (D) ZO-1 tight junctions (green) and nuclei (blue). (E) TEER indicating barrier integrity after treatments. (F) Lucifer Yellow permeability across the epithelial layer.

BG preserved epithelial barrier integrity, maintaining TEER, reducing paracellular permeability, and preserving ZO-1 organization, while remaining compatible with probiotic viability **(Fig. 3).**

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