



## Saponins from *Astragalus membranaceus* and *Panax notoginseng* improve intestinal barrier function and probiotic adhesion in human intestinal Caco-2 cells

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### ABSTRACT

**Background:** Intestinal barrier function is critically important for human health. Despite the promising biological properties of herbal extracts, limited research has addressed the effects of *Astragalus membranaceus* and *Panax notoginseng* saponin (APS) extracts on intestinal barrier function and probiotic adhesion, particularly under conditions of inflammatory stress. In the present study, we investigated the effects of APS on Caco-2 cell monolayers challenged with LPS as an in vitro model of intestinal inflammation.

**Objective:** This study aimed to evaluate the anti-inflammatory effects of *Astragalus membranaceus* and *Panax notoginseng* saponin (APS) extracts in preserving the intestinal epithelial barrier in a lipopolysaccharide (LPS)-stimulated human Caco-2 cell monolayer model.

**Methods:** Caco-2 monolayers were exposed to LPS to induce barrier dysfunction. Subsequently, the monolayers were treated with APS. Barrier function was evaluated by measuring transepithelial electrical resistance (TEER), FITC-dextran flux permeability, tight junction protein (Claudin-1, Occludin, ZO-1) expression, and cellular ATP levels. In addition, the

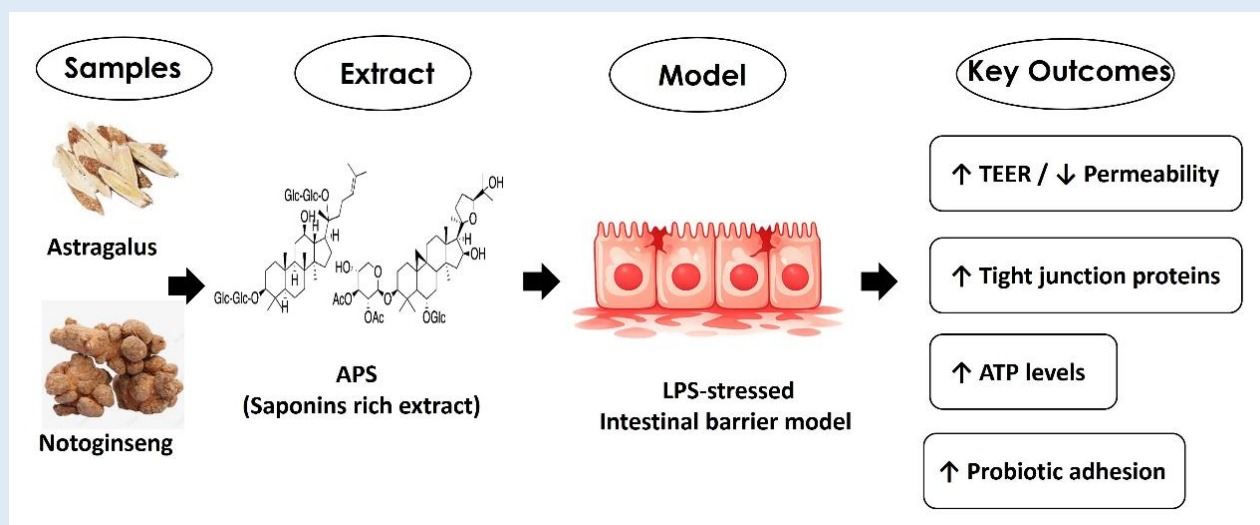
growth and adhesion of *Lactobacillus rhamnosus* to Caco-2 cell monolayers were assessed.

**Results:** APS treatment significantly increased TEER values, decreased FITC-dextran permeability, restored the expression of tight junction proteins (Claudin-1, Occludin, and ZO-1), and recovered intracellular ATP levels compared to the LPS group. Furthermore, APS significantly enhanced the growth and adhesion of *Lactobacillus rhamnosus* to Caco-2 cell monolayers, even under inflammatory conditions.

**Novelty:** This study identifies a previously uncharacterized synergistic role of *Astragalus membranaceus* and *Panax notoginseng* saponins in coordinating intestinal barrier protection and probiotic adhesion under inflammatory stress. These findings provide new mechanistic insight into the integrated actions of combined botanical saponins in maintaining intestinal homeostasis.

**Conclusion:** These findings suggest that APS exerts protective effects on intestinal barrier function and promotes probiotic adherence against LPS-induced intestinal injury in human epithelial cells. APS may be considered a valuable nutritional and therapeutic supplement for maintaining gastrointestinal health.

**Keywords:** *Astragalus membranaceus* and *Panax notoginseng* extracts; Inflammatory bowel disease; Intestinal probiotics; Tight junction proteins.



**Graphic Abstract:** APS Protects Intestinal Barrier and Probiotic Adhesion under LPS Stress

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## INTRODUCTION

The human gut is a dynamic and complex system where microbiota, nutrients, and host cells interact extensively

[1-2]. At the forefront of this interface, the intestinal epithelial layer forms both a physical and functional barrier that separates our body from the external

environment [3-4]. This layer, composed of a monolayer of polarized intestinal epithelial cells (IECs), is crucial for defending against the luminal invasion of pathogenic bacteria and serves as a selectively permeable filter for water, electrolytes, and nutrient absorption [2, 5]. The integrity of the intestinal barrier is therefore critical for maintaining intestinal and systemic health [6-7]. When this epithelial barrier is disrupted, intestinal permeability increases, enabling pathogens and foreign antigens to cross and thereby promote mucosal inflammation [8]. The regulation of this permeability is largely governed by tight junctions (TJs), which seal the paracellular space to prevent unrestricted leakage and function as a fence to maintain cell polarity by preventing membrane proteins and lipids from mixing between the apical and basolateral sides of the intestinal epithelial cells [3, 8]. Recent studies have also shown that TJs can recruit signaling proteins to regulate epithelial cell proliferation and differentiation, adding another layer of complexity to their functional importance [2, 7].

Tight junctions are complex structures composed of transmembrane proteins such as claudins, occludin, and junctional adhesion molecules (JAMs), which are vital for cellular adhesion and barrier formation [3, 7]. These transmembrane components interact with cytoplasmic plaque proteins, such as the zonula occludens (ZO) family, which serve as scaffolds for protein-protein interactions and connect the junctional complex to the actin cytoskeleton, forming a network essential for the regulation of permeability. Disruption of TJs and their associated proteins is now known to impact the progression of inflammatory intestinal and systemic diseases [4]. Previous studies have demonstrated that upregulation of TJ proteins like ZO-1 and occludin can inhibit the increase in intestinal permeability, whereas their decreased expression is associated with enhanced permeability, as observed in clinical cases of inflammatory bowel disease (IBD), Crohn's disease, and extrahepatic cholestasis [9]. Thus, the maintenance of TJ

protein integrity is widely considered a cornerstone for therapeutic strategies targeting intestinal disorders [9-10].

A growing body of evidence has implicated lipopolysaccharide (LPS), a potent pro-inflammatory component of the outer membrane of Gram-negative bacteria, as a key risk factor for both intestinal and systemic inflammation [11-12]. LPS is extensively utilized as a model agent in both in vivo and in vitro research to mimic inflammatory conditions. Mechanistically, LPS induces barrier dysfunction by activating Toll-like receptor-4 (TLR4) and downstream signaling pathways, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), resulting in the release of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and in the alteration of TJ protein expression and localization [12-13]. The pathogenesis of IBD is strongly associated with dysbiosis and an exaggerated immune response to LPS [11, 14]. LPS reduces microbiota diversity, diminishes regulatory T cell (Treg) populations, and thus impairs the mechanisms that maintain immune tolerance in the gut [15-16].

Gut microbiota equilibrium is central to supporting a tolerant and healthy immune system [17-18]. Probiotics, such as *Lactobacillus rhamnosus*, have been shown to provide a wide range of benefits for the host, including strengthening the epithelial barrier, inhibiting pathogen adhesion, improving digestive function, and modulating immune responses [17-18]. Multiple studies confirm that probiotics can modulate the immune response and provide protection against gut inflammatory diseases. Conversely, dysregulation of the microbial ecosystem can fuel chronic inflammation and disease progression, underscoring the importance of maintaining microbial balance for gut health [17, 19-20].

There is growing interest in natural extracts as potential therapeutics for gut barrier dysfunction [21-22]. Various compounds, such as quercetin, grape seed proanthocyanidins, and polysaccharides from

*Ganoderma lucidum*, have demonstrated the ability to ameliorate LPS-induced intestinal inflammation, restore mitochondrial function, enhance TJ protein expression, and reduce oxidative stress in cellular models [21-22]. Among traditional Chinese herbs, *Panax notoginseng* and *Astragalus membranaceus* have attracted significant interest. The principal bioactive components of *Panax notoginseng* are ginsenosides, which have been shown to increase intestinal probiotics, repair mucosal injury, and exert anti-inflammatory effects in animal models [23-24]. Likewise, *Astragalus membranaceus* is rich in astragalosides and is recognized for its immunomodulatory, antioxidant, and anti-inflammatory properties [21, 24-25].

In this study, we specifically evaluated functional biomarkers of intestinal barrier integrity, including transepithelial electrical resistance (TEER), paracellular permeability, and tight junction protein expression. Furthermore, we examined the impact of APS on the growth and adhesion of *Lactobacillus rhamnosus* to Caco-2 cell monolayers, aiming to clarify its potential in supporting gut barrier integrity and promoting a healthy gut microbiome.

## MATERIALS AND METHODS

**Materials:** APS is composed of dried extracts from the roots of *Astragalus membranaceus* (10:1 hydroethanolic extract) and *Panax notoginseng* (50:1 aqueous extract), combined with maltodextrin as an excipient. Its production adheres to current Good Manufacturing Practice (cGMP) standards. The final product is a beige to light yellow powder, standardized to contain at least 1.5% saponins. In this study, APS was sourced from NuLiv Science USA, Inc. (Brea, CA, USA) [24]. Additional materials utilized included LPS (Sigma-Aldrich, St. Louis, MO, USA).

**Cell Culture:** The human intestinal epithelial cell line Caco-2, a widely used model for studying the intestinal

barrier, permeability, and wound healing, was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g/L glucose, 0.584 g/L glutamine, 10% fetal bovine serum (FBS), 3.7% sodium bicarbonate, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 1% nonessential amino acids. APS was dissolved in DMSO for stock solutions, then diluted 1:1000 in culture medium to final concentrations of 10, 1, and 0.1µg/mL (final DMSO 0.1%). APS-containing medium was filter sterilized before use. Control cells were treated with 0.1% DMSO-containing medium, which did not show cytotoxicity versus untreated cells. For differentiation, Caco-2 cells were seeded onto 6 cm culture dishes at a density of  $1 \times 10^6$  cells/dish for 14 days (for western blot and qPCR), or onto 24-well transwell inserts (Corning 3379, MA, USA) at  $5 \times 10^4$  cells/well for 14 days (for barrier function assays). The development of tight junctions and monolayer integrity was verified by measuring transepithelial electrical resistance (TEER; Millicell ERS, Millipore, Bedford, MA, USA). Only monolayers with TEER values of 400–600 Ω·cm<sup>2</sup> were used.

**Experimental Design and Interventions:** Four experimental groups were established in this study, (1) Control group: the cells were treated with 0.1% DMSO-containing medium. (2) APS group: the cells were treated with APS (1 µg/mL) for 24 h. (3) LPS group: the cells were treated with vehicle for 24 h, then exposed to LPS (10 ng/mL) for 4 h. (4) APS+LPS group: the cells were pre-treated with APS (1µg/mL) for 24 h, then exposed to LPS (10 ng/mL) for 4 h.

For some assays, different APS concentrations were tested. For probiotic experiments, *Lactobacillus rhamnosus* ( $1 \times 10^7$  CFU/mL) was used.

**Cell Viability Assay:** Cell viability was evaluated using a Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Briefly, Caco-2 cells ( $5 \times 10^3$  cells/well) were seeded into 96-well plates and incubated in serum-free medium containing different concentrations of APS for 48 hr. After incubation, the medium was discarded and 10  $\mu$ L of CCK-8 solution in 90  $\mu$ L of PBS were added to each well and incubated for a further 2 hr. Absorbance was then recorded at 450 nm with a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Cell viability was expressed relative to untreated control cells.

**Measurement of Intestinal Barrier Integrity:** The TEER of the differentiated Caco-2 monolayer was measured with a Millicell ERS voltohmmeter (Millipore). Only monolayers with TEER of 400–600  $\Omega$ -cm<sup>2</sup> before treatment were used. To measure paracellular permeability, Caco-2 cells on 24-well transwells were treated as above. FITC-dextran (FD-4, 4 kDa, Sigma) was added apically, and after 2 h, samples were taken basolaterally. Fluorescence was measured (excitation 490 nm, emission 520 nm; POLARstar Galaxy, BMG Labtech). Permeability was calculated as the percentage of control.

**RNA Isolation and Real-Time Quantitative PCR (qPCR):** RNA was extracted from Caco-2 cells using TRIzol (Invitrogen). cDNA was synthesized with high-capacity cDNA kits (Applied Biosystems). Real-time PCR (Applied Biosystems 7500) was performed with SYBR Green master mix and primers:

ZO-1: F 5'-CGGGACTGTTGGTATTGGCTAGA-3',

R 5'-GGCCAGGGCCATAGTAAAGTTTG-3'

Claudin-1: F 5'-GCACATACCTTCATGTGGCTCAG-3',

R 5'-TGGAACAGAGCACAACATGTCA-3'

Occludin: F 5'-TCCTATAAATCCACGCCGGTTC-3',

R 5'-CTCAAAGTTACCACCGCTGCTG-3'

GAPDH: F 5'-TGGTATCGTGAAGGACTCA-3',

R 5'-AGTGGGTGCTGCTGTTGAAG-3'

Relative mRNA levels were normalized to GAPDH and

analyzed using the comparative Ct method. Three independent triplicate experiments were performed.

**Western Blot Analysis:** Cells were lysed in RIPA buffer, and proteins separated by 10% SDS-PAGE, transferred to PVDF membranes (Millipore). Immunoblotting used primary antibodies: ZO-1 (21773-1-AP, Proteintech), Claudin-1 (ab180158, Abcam), Occludin (ab216327, Abcam), and GAPDH (60004-1-Ig, Proteintech). Detection was by enhanced chemiluminescence (Amersham Biosciences) and X-ray film. Quantification used densitometry.

**Measurement of Intracellular ATP Levels:** After treatment, ATP was measured using a luminescent ATP detection kit (PerkinElmer) according to the manufacturer's instructions. Luminescence was quantified and normalized to cell number.

**Probiotic Growth and Adhesion Assays:** *Lactobacillus rhamnosus* was cultured in MRS broth with or without APS (1  $\mu$ g/mL) for 24 h at 37°C; growth was assessed by OD600. For adhesion assays, after treatments, Caco-2 monolayers were incubated with *Lactobacillus rhamnosus* ( $1 \times 10^7$  CFU/mL) for 1 h at 37°C. Non-adherent bacteria were washed off, and adherent bacteria were released with Triton X-100, diluted, and plated on MRS agar for colony counts after 48 h.

**Statistical Analysis:** Data are expressed as mean  $\pm$  SEM. Statistical significance between groups was determined by one-way ANOVA followed by Scheffé's post hoc test (SPSS v22; IBM, Armonk, NY, USA). A value of  $p < 0.05$  was considered statistically significant.

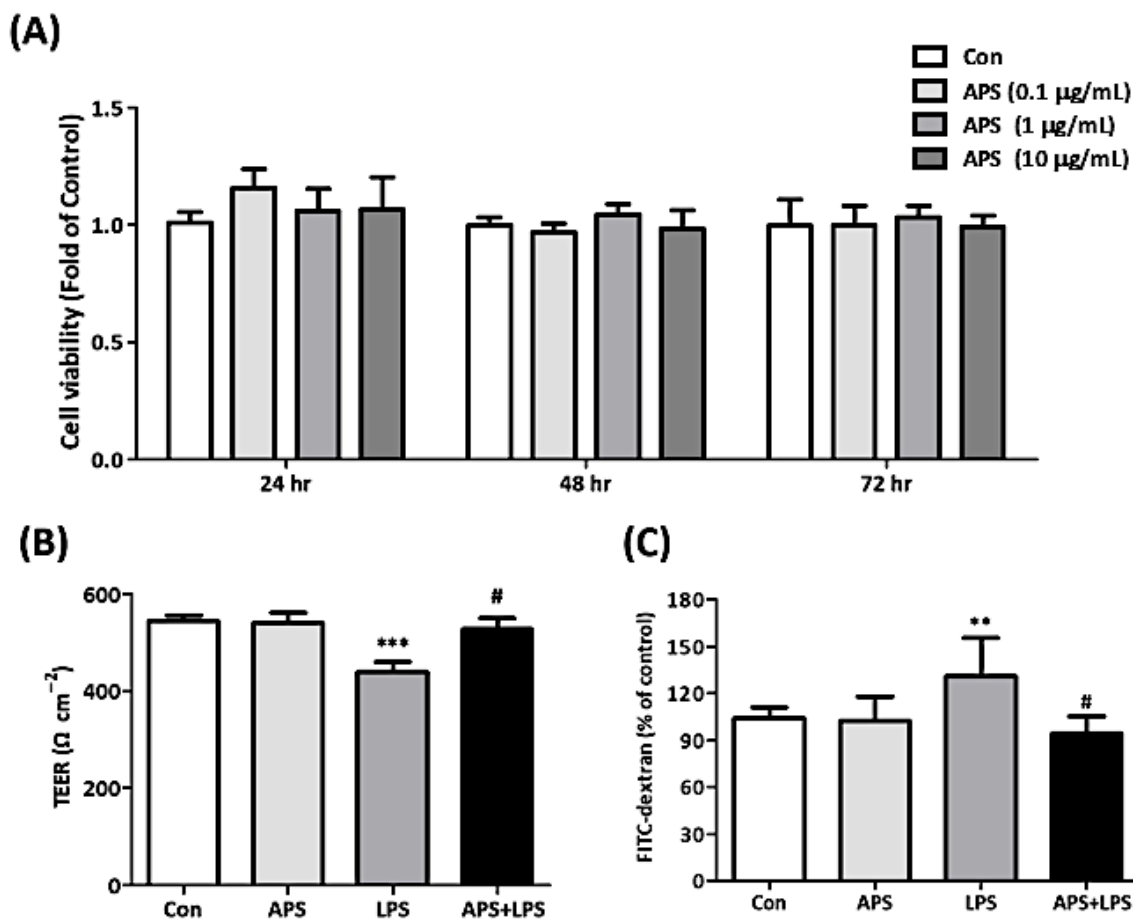
## RESULTS

### Cytotoxicity of APS on Differentiated Caco-2 Monolayer

**Cells:** To evaluate the cytotoxicity of APS, differentiated Caco-2 cells were exposed to increasing APS

concentrations. Cell viability was assessed at 24, 48, and 96 hours. As shown in Figure 1A, APS treatment did not reduce cell viability at any concentration or time point examined, indicating that APS is non-cytotoxic to Caco-2

monolayers under the experimental conditions. Based on these results, a concentration of 1 µg/mL was selected for subsequent experiments.



**Figure 1.** Effect of APS on the viability and barrier function of differentiated Caco-2 monolayer cells. (A) APS showed no cytotoxicity on Caco-2 cells. (B) APS significantly increased the TEER value in LPS-treated cells, indicating improved epithelial barrier integrity. (C) APS suppressed FITC-dextran permeability across Caco-2 monolayers. Statistical analysis was performed by one-way ANOVA followed by Scheffé’s post-hoc test using SPSS software. Results are expressed as mean ± SD. \*\*\*p < 0.001, \*\*p < 0.01 compared to untreated cells; #p < 0.05 compared to LPS-treated cells.

**Effects of APS on Intestinal Mucosal Barrier Function:**

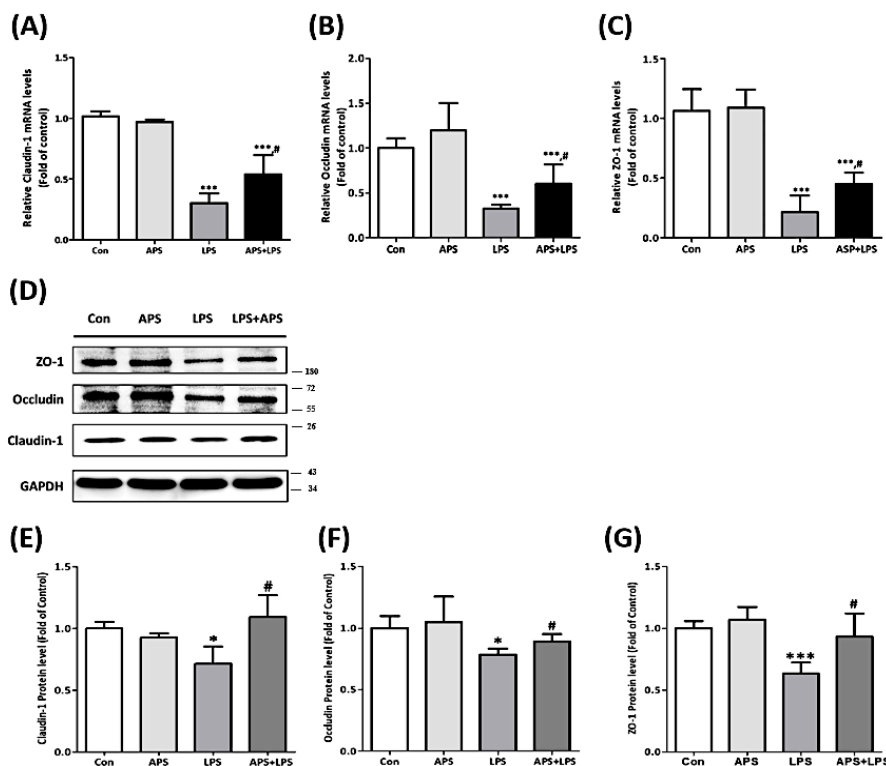
The integrity of the intestinal epithelial barrier was assessed by TEER measurements. TEER value reflects the ionic conductance of the paracellular pathway in the epithelial monolayer. TEER is a widely accepted surrogate for assessing cellular barrier integrity and tight junction dynamics. As shown in Figure 1B, APS treatment alone had no significant effect on TEER values. However, exposure to LPS for 4 hours reduced TEER by

approximately 19% compared to the control group (from 544 to 440 Ω·cm<sup>2</sup>, p < 0.001), indicating compromised barrier function. Notably, pretreatment with APS for 24 hours prior to LPS exposure significantly restored TEER by 20% relative to LPS-treated cells (from 440 to 528 Ω·cm<sup>2</sup>, p < 0.05; Figure 1B), suggesting the protective effect of APS on intestinal epithelial integrity

To further evaluate barrier function, FITC-dextran flux across Caco-2 monolayers was measured. As shown

in Figure 1C, while APS alone had no significant effect, LPS treatment increased paracellular permeability by 24% (from  $100.0 \pm 4.9\%$  to  $123.9 \pm 9.5\%$ ,  $p < 0.01$  vs. control). APS pretreatment significantly reduced LPS-induced

FITC-dextran leakage by 24% (from  $123.9 \pm 9.5\%$  to  $93.9 \pm 11.0\%$ ,  $p < 0.05$  vs. LPS group; Figure 1C). Together, these findings demonstrate that APS prevents inflammation-induced disruption of the intestinal barrier.



**Figure 2.** APS effects on tight junction gene and protein expression in LPS-treated differentiated Caco-2 monolayer cells. Caco-2 cells were incubated with or without APS and/or LPS. (A–C) mRNA expression levels of Claudin-1 (A), Occludin (B), and ZO-1 (C). (D) Representative western blot images. (E–G) Quantification of protein expression for Claudin-1 (E), Occludin (F), and ZO-1 (G). Statistical analysis was conducted using one-way ANOVA with Scheffé’s post-hoc test. Data represent mean  $\pm$  SEM ( $n = 3$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. untreated control; # $p < 0.05$  vs. LPS-treated cells.

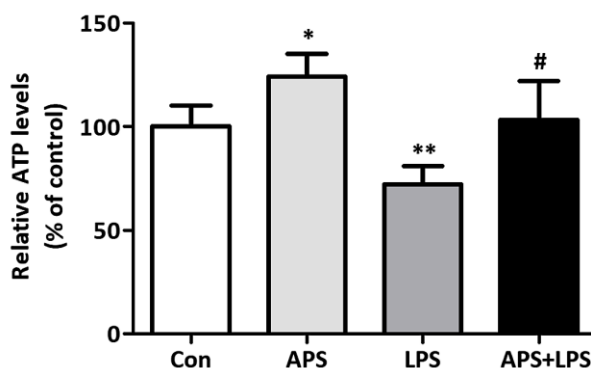
**APS Enhances Tight Junction Protein Expression:** To investigate the mechanism underlying the protective effects of APS, the TJ proteins expression was evaluated at both the mRNA and protein levels. As illustrated in Figure 2A, 2B, and 2C, LPS exposure markedly downregulated the mRNA expression of Claudin-1 (by 72%, from  $100 \pm 3.7\%$  to  $28.3 \pm 8.7\%$ ,  $p < 0.001$ ), Occludin (by 68%, from  $100 \pm 10.8\%$  to  $32.5 \pm 4.3\%$ ,  $p < 0.001$ ), and ZO-1 (by 80%, from  $100 \pm 17.0\%$  to  $19.8 \pm 13.2\%$ ,  $p < 0.001$ ) compared to control. However, APS pretreatment significantly restored the expression of Claudin-1 (+87%, from  $28.3 \pm 8.7\%$  to  $52.9 \pm 15.8\%$ ,  $p < 0.05$ ), Occludin

(+86%, from  $32.5 \pm 4.3\%$  to  $60.3 \pm 15.8\%$ ,  $p < 0.05$ ), and ZO-1 (+115%, from  $19.8 \pm 13.2\%$  to  $42.5 \pm 9.1\%$ ,  $p < 0.05$ ) in LPS-exposed cells (Figure 2A–C).

Since the TJ proteins are crucial for maintaining intestinal barrier integrity and function, their expression at the protein level was also examined using western blot analysis (Figure 2D) and the results were quantified in Figure 2E, 2F, and 2G. While APS alone did not alter TJ protein levels, LPS treatment decreased Claudin-1 by 29% (from  $100 \pm 5.2\%$  to  $71.2 \pm 14.1\%$ ,  $p < 0.05$ ), Occludin by 21% (from  $100 \pm 9.9\%$  to  $78.5 \pm 5.9\%$ ,  $p < 0.001$ ), and ZO-1 by 36% (from  $100 \pm 6.0\%$  to  $63.5 \pm 8.9\%$ ,  $p < 0.001$ )

compared to control. Remarkably, APS pretreatment significantly reversed these effects, restoring Claudin-1 (+53.2%, from  $71.2 \pm 14.1\%$  to  $109.1 \pm 17.8\%$ ,  $p < 0.05$ ), Occludin (+13.7%, from  $78.5 \pm 5.9\%$  to  $89.2 \pm 6.0\%$ ,  $p < 0.05$ ), and ZO-1 (+47%, from  $63.5 \pm 8.9\%$  to  $93.3 \pm 18.8\%$ ,

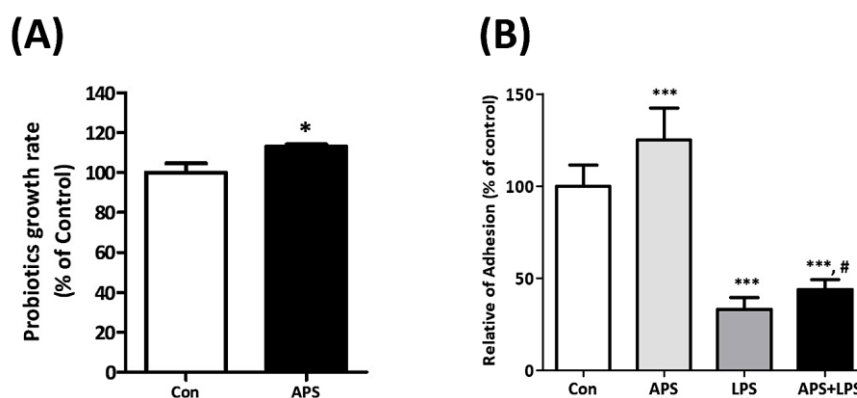
$p < 0.05$ ) levels in the LPS group (Figure 2D–G). These results indicate that APS helps maintain tight junction integrity at both the transcriptional and protein levels under inflammatory stress.



**Figure 3.** APS effects on intracellular ATP levels in differentiated Caco-2 monolayer cells. APS significantly increased ATP levels, especially in the context of LPS-induced injury. Statistical analysis was performed by one-way ANOVA and Scheffé’s post-hoc test. Results are mean  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$  vs. untreated cells; # $p < 0.05$  vs. LPS-treated cells.

**APS Restores Cellular Energy Levels:** Given the importance of ATP in maintaining epithelial integrity, intracellular ATP levels were measured. As illustrated in Figure 3, APS alone led to a notable increased in ATP content by 24% compared to control (from  $100.0 \pm 9.9\%$

to  $124.1 \pm 10.6\%$ ,  $p < 0.05$ , relative to control cells). Conversely, LPS exposure significantly reduced ATP levels by 28% (to  $72.4 \pm 8.9\%$ ,  $p < 0.05$ ), while APS pretreatment restored ATP by 42% in LPS-exposed cells (from  $72.4 \pm 8.9\%$  to  $103.1 \pm 18.8\%$ ,  $p < 0.05$ ; Figure 3).



**Figure 4.** APS effects on *Lactobacillus rhamnosus* growth and adhesion to Caco-2 cells. (A) APS significantly increased the in vitro growth of *Lactobacillus rhamnosus*. (B) APS significantly enhanced the *Lactobacillus rhamnosus* adhesion to differentiated intestinal Caco-2 cells. Statistical analysis was performed by one-way ANOVA with Scheffé’s post-hoc test. Results are mean  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$  vs. untreated cells.

**Effects of APS on Probiotic Growth and Adhesion:** To assess the impact of APS on beneficial bacteria, the growth of *Lactobacillus rhamnosus* was evaluated. As

shown in Figure 4A, APS treatment significantly increased the growth rate by 13% compared to the untreated control (from  $100.0 \pm 4.6\%$  to  $113.0 \pm 1.0\%$ ;  $p < 0.05$ ).

The ability of APS to enhance probiotic adhesion was evaluated by measuring the adherence of *L. rhamnosus* to Caco-2 monolayers. As shown in Figure 4B, APS alone increased adhesion by 26% (from  $100.0 \pm 11.8\%$  to  $125.5 \pm 17.3\%$ ,  $p < 0.001$  vs. control). Conversely, LPS exposure reduced adhesion by 67% (to  $33.1 \pm 6.7\%$ ,  $p < 0.001$ ), but APS pretreatment significantly mitigated this effect, increasing adhesion by 33% compared to LPS-treated cells (from  $33.1 \pm 6.7\%$  to  $44.1 \pm 5.4\%$ ,  $p < 0.001$ ; Figure 4B). These findings suggest that the LPS-induced inflammatory intestinal environment is harmful to probiotic adhesion, whereas APS effectively protects intestinal cells from inflammatory damage.

## DISCUSSION

This study demonstrates that APS protects and enhances intestinal barrier function and promotes probiotic adhesion in human intestinal Caco-2 cell monolayers, especially under inflammatory conditions induced by LPS. First, APS significantly increased TEER values and reduced FITC-dextran permeability in LPS-challenged cells, indicating improved epithelial integrity. Second, APS restored the expression of TJ proteins (Claudin-1, Occludin, and ZO-1) at both the mRNA and protein levels, which were otherwise downregulated by LPS. Third, APS increased ATP production in epithelial cells and promoted the growth and adhesion of *Lactobacillus rhamnosus* to Caco-2 monolayers, even in the context of inflammation. These results suggest that APS exerts a multifaceted protective effect on gut barrier function and microbiota–host interactions.

TJs are well-recognized as gatekeepers of intestinal permeability. Their dysfunction is implicated in a variety of disorders, including IBD, Crohn's disease, and metabolic syndrome [8-10]. The ability of APS to restore and enhance TEER values after LPS-induced barrier disruption supports the concept that saponin extracts can directly reinforce TJ integrity and protect against

inflammatory insult. The APS's effect on tight junction proteins Claudin-1, Occludin, and ZO-1 is particularly noteworthy. Downregulation of these proteins is a hallmark of intestinal inflammation, leading to increased paracellular permeability and barrier dysfunction [22]. Previous studies have shown that APS, as well as its major constituents, including astragalosides and ginsenosides, can upregulate tight junction protein expression and suppress inflammation [22, 24-25]. Our study aligns with and strengthens these findings, providing direct molecular evidence of APS's ability to mitigate LPS-induced loss of TJ proteins in vitro.

The significant reduction in FITC-dextran paracellular flux observed in the APS plus LPS group also supports the hypothesis that APS restores functional barrier properties [26-27]. LPS is well-established as a model inducer of gut barrier dysfunction via activation of TLR4 and downstream NF- $\kappa$ B signaling, resulting in pro-inflammatory cytokine release and TJ disruption [12-13]. Our data indicate that APS protects structural TJ components and functionally reduces intestinal barrier leakage, a key clinical target for many gut disorders [11, 28]. In addition, energy metabolism is tightly linked to epithelial health, as ATP is necessary for cytoskeletal remodeling, TJ maintenance, and epithelial restitution after injury [21]. We observed that APS increased cellular ATP levels in both control and LPS-challenged Caco-2 cells. This finding is consistent with previous reports showing that *Astragalus* and *Panax* saponins enhance mitochondrial function and energy production, supporting mucosal healing and barrier function [24, 29].

APS's capacity to promote probiotic growth and adhesion adds an important new dimension to its potential benefits. The adhesion of probiotics such as *Lactobacillus rhamnosus* to the gut epithelium is critical for colonization, competitive exclusion of pathogens, and immune modulation [30-31]. Pro-inflammatory conditions, as simulated by LPS in our model, are known to impair probiotic adhesion by altering mucin and

surface protein expression on epithelial cells [11, 26]. Our results show that APS stimulates *Lactobacillus rhamnosus* proliferation in vitro and significantly rescues adhesion ability in inflamed Caco-2 cells. This observation is corroborated by studies on dietary oligosaccharides and plant extracts, which have shown that they enhance probiotic adhesion and support a healthy microbiome [32]. Importantly, APS may act as a supportive factor for probiotics, fostering both barrier function and beneficial bacterial colonization [30-31].

Comparing our results to the published literature, there are several points of agreement and novelty in our study. First, our demonstration that APS restores barrier integrity and tight junction protein expression under inflammatory stress aligns with previous in vivo and in vitro research on *Astragalus* and *Panax* extracts [30-31, 33-34]. Second, our observation of enhanced ATP production supports reports that botanical saponins act as signaling molecules and as metabolic enhancers for epithelial repair [31, 34]. Third, the effect of APS on probiotic growth and adhesion, particularly in a model simulating inflammation-induced dysbiosis, represents a relatively underexplored but highly relevant therapeutic target in gut health research [30]. In addition, the combination of saponin extracts from *Astragalus membranaceus* and *Panax notoginseng* in this study may exhibit synergistic effects that are not observed with single extracts, as both herbs contain complementary bioactive compounds [22, 31]. The broader literature also highlights that the gut barrier is regulated by complex, dynamic interactions between host cells, immune mediators, microbiota, and dietary components [33-34]. Further studies are warranted to elucidate the signaling pathways and underlying mechanisms of intestinal protective efficacy of APS.

From a scientific perspective, this study's combined evaluation of saponins from *Astragalus membranaceus* and *Panax notoginseng* using an inflammatory intestinal model constitutes a novel and significant contribution,

particularly in the context of gut barrier modulation and probiotic adhesion. While both herbs have been individually studied, their combined application under LPS-induced stress conditions is innovative. These findings provide new mechanistic insight into the integrated actions of combined botanical saponins in maintaining intestinal homeostasis. Unlike previous studies that primarily examined individual herbs or focused solely on barrier protection in non-inflammatory settings, the present work highlights a dual host-microbiota modulatory role of APS in an LPS-induced intestinal model. This integrative perspective distinguishes the current findings from prior literature and supports the potential of combined botanical saponins as multi-target modulators of gut barrier function.

According to the Functional Food Center's 17-step Functional Food Product Development Model, the present study supports key stages of functional food development, including the goal establishment, identification of relevant bioactive compounds, evaluation of functional mechanisms, and assessment of established biomarkers in a preclinical setting [35-36]. Specifically, the combined saponins from *Astragalus membranaceus* and *Panax notoginseng* were shown to modulate intestinal barrier integrity, cellular energy status, and probiotic adhesion using validated biomarkers such as TEER, tight junction protein expression, and ATP levels. As an in vitro study, these findings provide preclinical evidence supporting the functional potential of APS for gut health applications.

It should be noted that the present findings were derived from an in vitro Caco-2 intestinal epithelial model, which is suitable for mechanistic and functional evaluation but cannot fully reflect the complexity of the in vivo intestinal environment. Therefore, further validation in appropriate animal models and well-designed human clinical studies is required to confirm the

physiological relevance, efficacy, and translational applicability of APS.

In summary, our data show that APS possesses the ability to maintain epithelial barrier integrity, restore TJ protein expression, support cellular energy, and enhance probiotic–host interactions, especially under inflammatory conditions relevant to IBD, leaky gut, and related disorders. In practical applications, the results underscore APS as a promising candidate for use as a functional food ingredient or therapeutic supplement targeting chronic inflammation, probiotic enhancement, and gut health regulation. While APS holds considerable promise as a gut health supplement, further work is required to translate these findings into clinical practice and to explore its utility in specific disease settings such as IBD, metabolic syndrome, and other inflammation-associated conditions.

## CONCLUSION

This study demonstrates that APS effectively enhances intestinal barrier function and promotes probiotic adhesion in human Caco-2 cell monolayers, particularly under inflammatory conditions induced by LPS. APS was shown to restore tight junction protein expression, increase cellular ATP levels, and support the growth and adhesion of *Lactobacillus rhamnosus*. These findings suggest that APS may serve as a promising multi-target intervention for maintaining gut barrier integrity and modulating the gut microbiome, highlighting its potential for future development as a therapeutic or dietary supplement to support intestinal health.

**Abbreviations:** APS: Astragalus membranaceus and Panax notoginseng saponin; ATCC: American type culture collection; ATP: adenosine triphosphate; CCK-8; cell counting kit-8; DMSO: dimethyl sulfoxide; DMEM: Dulbecco's Modified Eagle's Medium; cGMP: current Good Manufacturing Practice; FBS: fetal bovine serum; FITC: fluorescein isothiocyanate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPS:

lipopolysaccharide; TEER: transepithelial electrical resistance; IBD: inflammatory bowel disease; IECs: intestinal epithelial cells; IL6: Interleukin-6; JAMs: junction adhesion molecules; MRS: de Man, Rogosa and Sharpe medium; TJ: tight junction; TLR4: toll-like receptor-4

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**Conflict of Interest:** The authors declare no conflict of interest.

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