



RESEARCH ARTICLE

Epimedium brevicornu Maxim Prevents LPS-Induced Intestinal Inflammation and Protects Intestinal Barrier Through Modulation of Gut Microbiota and Short Chain Fatty Acids

Sarang Mazhar Abro¹, Xubin Du¹, Yanke Shan¹, Muhammad Azhar Memon², Teerath Kumar Suthar³, Muhammad Mubashir⁴, Hongbing Gui⁵, Shanfeng Wang⁵, Moolchand Malhi⁶, Jamila Soomro⁶ and Fei Liu^{1*}

¹Joint International Research Laboratory of Animal Health and Food Safety of Ministry of Education & Single Molecule Nanometry Laboratory, Nanjing Agricultural University, Nanjing, PR China; ²MOE Joint International Research, College of Veterinary Medicine, Nanjing Agricultural University 210095, China; ³University of Chinese Academy of Sciences, Beijing 100049, China; ⁴College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China; ⁵School of Animal Husbandry and Veterinary Medicine, Jiangsu Vocational College of Agriculture and Forestry, Jurong, Jiangsu 212400, China; ⁶Department of Veterinary Physiology & Biochemistry, Sindh Agriculture University, 70060, Tandojam

*Corresponding author: feiliu24@njau.edu.cn

ARTICLE HISTORY (26-364)

Received: April 11, 2026
Revised: July 25, 2026
Accepted: August 03, 2026
Published online: September 15, 2026

Key words:

Epimedium brevicornu
Maxim
Gut barrier integrity
Gut microbiota
Intestinal inflammation
Short chain fatty acids

ABSTRACT

Epimedium brevicornu Maxim (EBM) plant is traditionally used as a therapeutic agent against many diseases, yet its protective role against LPS-induced chronic inflammation and gut barrier impairment remains unexplored. This study aimed to investigate whether EBM can protect against Lipopolysaccharide (LPS)-induced chronic inflammation and gut barrier impairment by modulating gut microbiota composition and short chain fatty acids (SCFAs) levels in a murine model. For this purpose, mice were randomly assigned into six groups receiving saline, LPS, and LPS combined with low or high doses of EBM for 28 days. After sacrificing the mice, histopathological findings confirmed LPS-triggered inflammatory injury accompanied by epithelial barrier dysfunction by marked inflammatory infiltration. Molecular analyses further supported these findings as LPS challenge elevated IL-6 and TNF- α at both mRNA and protein levels, and disrupted gut barrier integrity by decreasing zonula occludin (ZO-1), claudin-1, and occludin. These changes were accompanied by a reduction in beneficial gut bacteria mainly: *Firmicutes*, *Ligilactobacillus*, *Lactobacillus* and *Ruminococcus*, and an elevation in important harmful bacteria including *Proteobacteria*, *Deferribacterota*, *Desulfovibrio* and *Bilophila*, along with a sharp decline in fecal SCFAs. Whereas, co-administration of EBM protected against these changes in a dose dependent manner. It preserved gut barrier integrity at histological level, reduced inflammatory cytokines and preserved barrier proteins while counteracting these shifts by enriching beneficial taxa, suppressing inflammation-associated genera, and maintaining SCFA production, collectively demonstrating its protective role against LPS-induced chronic inflammation and barrier impairment.

To Cite This Article: Abro SM, Du X, Shan Y, Memon MA, Suthar TK, Mubashir M, Gui H, Wang S, Malhi M, Soomro J and Liu F, 2026. *Epimedium brevicornu* maxim prevents LPS-induced intestinal inflammation and protects intestinal barrier through modulation of gut microbiota and short chain fatty acids. Pak Vet J, 46(9): 2205-2219. <http://dx.doi.org/10.29261/pakvetj/2026.209>

INTRODUCTION

Chronic intestinal inflammation is characterized by persistent inflammatory responses, tissue injury, and disruption of intestinal homeostasis (Okumura and Takeda, 2018). The intestinal epithelial barrier is maintained by tight junctions (TJ) that forms a frontline boundary between the host and gut lumen (Roca Rubio *et al.*, 2021).

These junctions are composed mainly of zonula occludens-1 (ZO-1), claudin-1 and occludin proteins which being selective in nature, allows absorption of nutrients while blocking the entry of toxins and pathogens (Li *et al.*, 2019). However, during chronic inflammatory conditions, this barrier is damaged, and TJ proteins decline and permeability increases (Zhao *et al.*, 2025). Consequently, endotoxins and pathogens are translocated in systemic

circulation triggering widespread inflammation which leads to multiple organ injuries (He *et al.*, 2023). Importantly, gut microbiota is considered to influence intestinal inflammation and integrity of this epithelial barrier (Wu *et al.*, 2018), and to maintain TJ proteins through SCFA production (Koh *et al.*, 2016). Therefore, protection of TJ proteins is crucial for maintaining intestinal health and preventing inflammatory stress (Zhu *et al.*, 2020).

Epimedium brevicornu Maxim (EBM) is a well-known plant in traditional Chinese medicine that has been used for centuries (Cui *et al.*, 2024). EBM is mainly composed of flavonoids, isoflavonoids, phenols, tannins and other bioactive compounds. However, icariin, Epimedin A, B, C, icaraside I, and Baohuoside I and metabolites of icariin including icaritin are considered the principal bioactive compounds which are responsible for pharmacological activities of EBM (Wang *et al.*, 2016). EBM has been employed for treatment of a broad spectrum of chronic conditions including sexual dysfunctions, osteoporosis, diseases of cardiovascular system, and immune deficiencies (Ma *et al.*, 2011). Modern studies have clarified the biological basis of these effects showing that EBM possesses potent anti-oxidant (Gao *et al.*, 2022) and anti-inflammatory (Huang *et al.*, 2018) properties. In a similar way, flavonoids in EBM have been shown to reduce intestinal inflammatory responses by selectively modulating gut microbiota (Murota *et al.*, 2018). Supporting this, dietary supplementation in normal conditions with these flavonoids has been reported to increase fecal SCFA levels including acetate, propionate, and butyrate. Besides, it also enhances TJ proteins in intestines, including ZO-1, claudin-1 and occludin (Zhang *et al.*, 2023).

Lipopolysaccharide (LPS) has been demonstrated to induce inflammatory responses and disrupt gut barrier function via impairment of TJ proteins (Bhatia *et al.*, 2022). Despite the evidences which have mostly evaluated therapeutic effects of EBM after inflammatory damage has been established, no study has yet evaluated whether EBM administration during prolonged inflammatory trigger can protect gut barrier integrity remains unclear. Therefore, this study aimed to evaluate whether preventive administration of EBM during repeated LPS challenge could protect against intestinal inflammatory responses, maintain tight junction integrity, and maintain gut microbiota and SCFA production under inflammatory conditions.

MATERIALS AND METHODS

UPLC-HRMS/MS-based phytochemical profiling of EBM extract: The crude ethanolic extract of EBM used in this study was supplied by Xi'an Dongfeng Biotechnology Co., Ltd. (Xi'an, China). The phytochemical profiling of EBM extract was carried out using ultra-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UPLC-Q Exactive HFX Orbitrap-MS/MS). Chromatographic separation was achieved by employing a Waters HSS T3 column, followed by detection using a Q Exactive HFX Hybrid Quadrupole-Orbitrap mass spectrometer equipped with a heated electrospray ionization source (Thermo Fisher Scientific). Compound annotation was achieved using accurate mass,

MS/MS fragmentation patterns, and comparison with available chemical databases.

Animals and ethical approval: Sixty specific pathogen-free (SPF) female C57BL/6 mice (age of 4 weeks, 18-20 g) were procured from the Laboratory Animal Center of Yangzhou University (Yangzhou, China). Animals were housed under standard SPF conditions ($25 \pm 1^\circ\text{C}$, 50-60% relative humidity, 12-hours light/dark cycle) and were provided with free access to water and feed during the entire experimental period. The approval for all experimental procedures and animal welfare was granted by the Experimental Animal Welfare and Ethics Committee, Nanjing Agricultural University (Permission Number: NJAULLSC2024086).

Preparation of LPS and EBM extract doses: LPS solution was prepared by dissolving 10 mg of LPS powder, purchased from Coolaber Science & Technology Co., Ltd., China, in 10 mL of sterile normal saline. This yielded a stock solution of a concentration of 1 mg/mL. For administration, EBM extract was freshly dissolved in normal saline. Two doses of 40 mg/kg and 80 mg/kg body weight were formulated as low and high dose treatments.

Grouping and induction of chronic intestinal inflammation: After a one-week acclimatization period, mice were randomly distributed into six groups (n = 10 in each group):

- **Con-N (saline control):** normal saline by intragastric gavage (i.g.) and intraperitoneal injection (i.p.);
- **Con-L (control + low dose):** EBM extract (40 mg/kg, i.g.) and normal saline (i.p.);
- **Con-H (control + high dose):** EBM extract (80 mg/kg, i.g.) and normal saline (i.p.);
- **LPS-N (LPS only):** lipopolysaccharide (LPS; 0.5 mg/kg body weight, i.p.) and saline (i.g.)
- **LPS-L (LPS + low dose):** LPS (i.p.) and EBM extract (40 mg/kg, i.g.)
- **LPS-H (LPS + high dose):** LPS (i.p.) and EBM extract (80 mg/kg, i.g.)

Mice administered with the LPS to induce inflammation were given extract treatments simultaneously and continued once daily for 28 days. Following co-treatment approach similar to previously published models (Xu *et al.*, 2024).

Samples collection: At the completion of the trial all animals were sacrificed. Euthanization of all animals was carried out by cervical dislocation. Colon tissues, colonic contents, and other organs were taken and promptly kept at -80°C to be used for proceeding analyses.

Histopathological examination: Representative colon tissues from each animal were excised and fixed using 10% formalin. Subsequently, the tissues were embedded in paraffin, sectioned and subjected to hematoxylin and eosin staining for histopathological examination. Histological changes were examined with an optical microscope (Nikon Eclipse E100) equipped with a digital camera system (NIKON DS-U3). Representative sections from each group were evaluated based on the basis of morphological alterations.

RNA isolation and RT-qPCR analysis: RNA from colon tissues was extracted with Trizol (Invitrogen, USA), chloroform, isopropanol (Shanghai Lingfeng Chemical Reagent Co., LTD) and 75% ethanol (Sinopharm, China). Residual DNA was removed using DNaseI (Vazyme Biotech, China). The RNA concentration was determined on NanoDrop 2000 (Nanodrop Technologies, USA). cDNA synthesis was carried out using Prime Script RT Master Mix (TaKaRa, China) following the kit protocol. Quantitative PCR was performed using ChamQ Blue Universal SYBR qPCR Master Mix (Vazyme Biotech, China) on Light Cycler96 system (Roche, Basel, Switzerland). The cycle consisted of an initial 2-min denaturation step, followed by 40 amplification cycles comprising of 95 °C for 30 s (denaturation), 65 °C for 34 s (annealing), and 72 °C for 30 s (extension). The specific primers used were designed using NCBI primer-BLAST and some were selected from previous references (Table 1). Normalization of relative gene expression level was performed using *GAPDH* as housekeeping gene. All samples were tested in technical triplicates. Relative gene expression was quantified using $2^{-\Delta\Delta C_t}$ (Khan *et al.*, 2025).

Table. 1: Primer sequences used in RT-qPCR

Gene	Primers	
<i>IL-6</i>	F- TGTTCCTCTGGGAAATCGTGGA R- GCAAGTGCATCATCGTTGTTCA	(Humbert-Claude <i>et al.</i> , 2016)
<i>TNF-α</i>	F-CCTCTTCTCATTCTGCTTGTTGG R-GGCCATTTGGGAACCTCTCATC	(Humbert-Claude <i>et al.</i> , 2016)
<i>ZO-1</i>	F- GAGACAAGATGTCCGCCAGG R- AAATCCAAACCCAGGAGCCC	(Yin <i>et al.</i> , 2021)
<i>Claudin-1</i>	F-GGCTTCTCTGGGATGGATCG R-TTTGCGAAACGCAGGACATC	(Yin <i>et al.</i> , 2021)
<i>Occludin</i>	F-ACCGTCTAATCAATCTTTG R-AACTCCTGAACCGCACTC	(Wan <i>et al.</i> , 2022)
<i>GAPDH</i>	F: CCCAGCTTAGGTTTCATCAGGT R: GTTCACACCGACCTTCACCA	NCBI BLAST

Note: F: Forward primer, R: Reverse primer

Western blotting: Total protein was extracted from colon tissues using Radio immunoprecipitation (RIPA) buffer (Vazyme Biotech, China) and phenylmethyl sulfonyl fluoride (PMSF) (Beyotime, Beijing, China). Proteins concentrations were measured with a BCA assay kit (Vazyme Biotech, China). Equal volume of proteins was separated on 12% SDS-PAGE (Vazyme Biotech, China) gels and were deposited onto PVDF (polyvinylidene fluoride) membranes (Millipore, Bedford, MA, USA) and then blocked with 5% skim milk solution for 2 hours at room temperature. Subsequent to washing PBST solution the membranes were incubated with ZO-1, claudin-1, occludin, TNF- α , IL-6 and β -actin (Aladdin, Shanghai, China) primary antibodies at 4°C overnight after dilution 1:1000 in PBST. Secondary antibodies employed were horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody and anti-mouse IgG diluted 1:5000 diluted (Aladdin, Shanghai, China). β -actin expression was marked as a control in loading to normalize total protein levels. The blots formed were visualized by using ECL chemiluminescence detection kit (NCM Biotech, Suzhou, China) under MiniChemi Chemiluminiscent imaging system (General Electric Company, USA). The relative density of bands was quantified with ImageJ Analysis Software (National Institute of Health, Bethesda, MD, USA).

Analysis of gut microbiota via 16S rDNA sequencing and statistical analysis: Fecal DNA was isolated using the TGuide S96 Magnetic Universal DNA Kit (Tiangen, DP812). The V3-V4 region of the bacterial 16S rRNA gene was amplified using standard primers (Forward: ACTCCTACGGGAGGCAGCA; Reverse: GGACTACHVGGGTWTCTAAT). Purified amplicons were sequenced using Illumina NovaSeq 6000 (paired-end). and processed using QIIME2 (v2020.6). For denoising and ASV generation DADA2 was used. SILVA 138 database was used for taxonomic assignment. α -diversity (Chao1, ACE, Shannon, Simpson indices and Whole PD Tree) and relative abundances at taxa level were evaluated from the ASV table while β -diversity assessment was conducted with Bray-Curtis and weighted/unweighted UniFrac distances and visualized by PCoA/PCA. Group differences in community composition were tested by PERMANOVA (Adonis, vegan R package), and microbial differences at taxa level evaluated by LEfSe ($P < 0.05$, LDA score ($\log_{10}$) > 4.0).

Short chain fatty acid analysis: SCFAs profile in fecal samples was examined using gas chromatography-tandem mass spectrometry (GC-MS/MS). Fecal samples were acidified and extracted using a standard MTBE-based protocol. The resulting supernatant was injected onto DB-FFAP capillary column for chromatographic separation and SCFAs were quantified in multiple reaction monitoring (MRM) mode. All procedures followed established SCFA analytical protocols.

Statistical analyses: Experimental data were tested for normality and homogeneity of variance through Shapiro-Wilk and Levene's tests prior to statistical analysis. Data were subjected to one-way ANOVA followed by Tukey's multiple comparisons test for post hoc analysis. Comparisons between two independent groups were conducted using a two-tailed unpaired Student's t-test. The data are reported as mean and standard deviation (SD). Statistical significance was defined as $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$. Statistical comparisons were carried out based on the assigned experimental groups. All analyses were performed using IBM SPSS Statistics 26 and GraphPad Prism 10.17 (GraphPad software). Bar and line chart representations were made using GraphPad Prism 10.17 (GraphPad software) and OriginPro 2024.

Correlation analysis between SCFAs and gut microbiota: Spearman's rank correlation was used to evaluate associations between gut microbial genera and short-chain fatty acids (SCFAs). P-values were adjusted for multiple comparisons using the Benjamini-Hochberg FDR method. Correlation patterns were visualized as hierarchical clustered heatmaps, highlighting both all associations and statistically significant correlations ($FDR < 0.05$). Analyses were performed in R (version 4.x), enabling identification of microbial taxa potentially linked to SCFA production.

RESULTS

UPLC-Q Exactive HFX Orbitrap-MS/MS profiling revealed flavonoid diversity in EBM extract: UPLC-Orbitrap-MS/MS analysis revealed the identification of

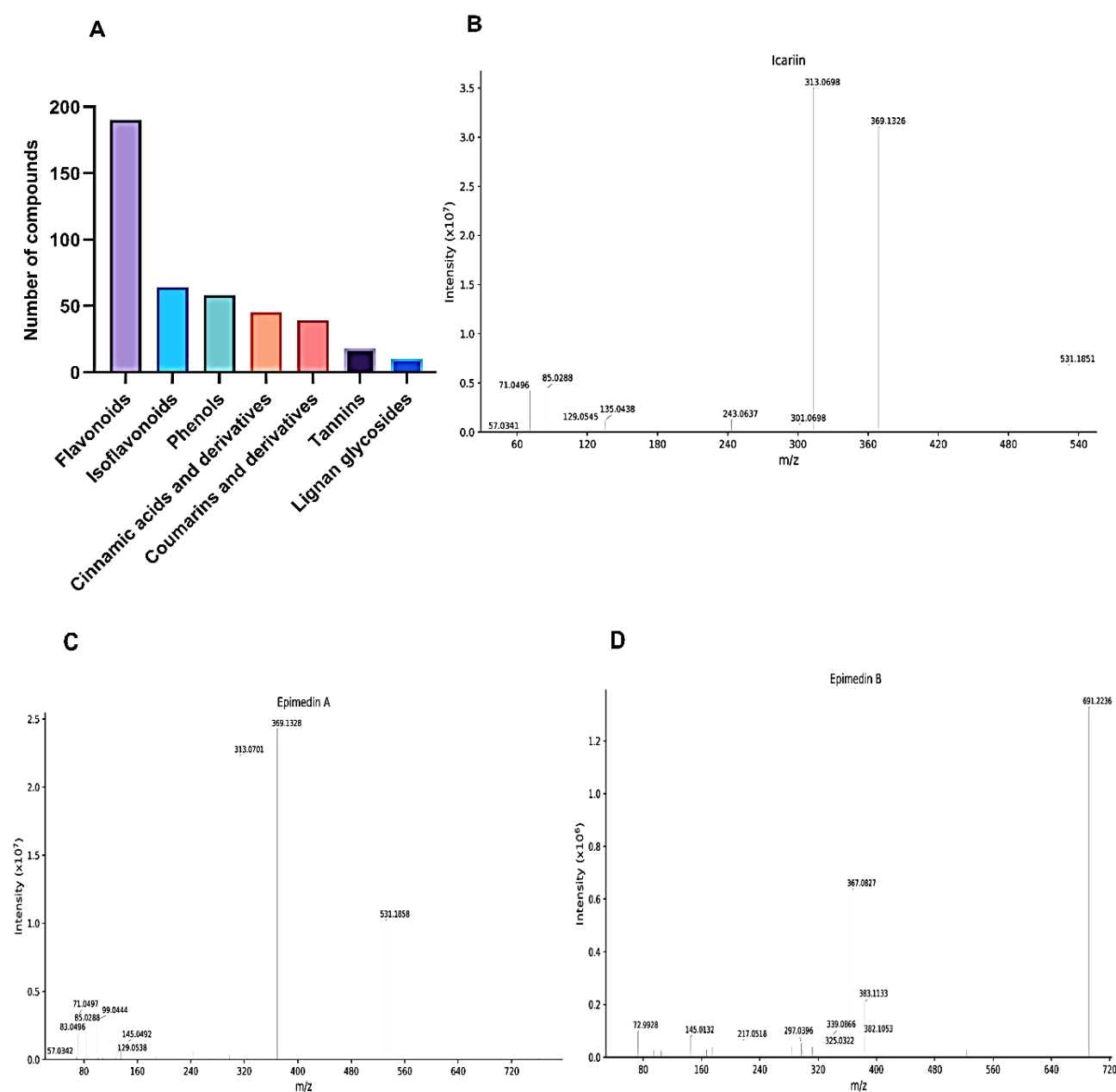
multiple characteristic bioactive phytochemicals in EBM extract (Fig. 1 B-H), including icariin, epimedin A, epimedin B, epimedin C, icaritin, icariside I, and Baohuoside I, respectively. In addition to flavonoids, chemical profiling of the extract identified isoflavonoids and phenols as the second and third most abundant constituents, respectively, followed by cinnamic acid derivatives, coumarins, tannins, and lignin (Fig. 1 A). Collectively, UPLC-HRMS/MS profiling confirmed the presence of multiple flavonoid, phenols and other classes in EBM extract.

EBM preserved the colon against pathological changes:

Representative colon sections stained with H&E (scale bar = 100 μ m) showed that Con-N, Con-L and Con-H groups generally maintained an intact epithelial structure with no visible infiltration of inflammatory cells in lamina propria (Fig. 2). In contrast, LPS-N group (Fig. 2) displayed a notable inflammatory infiltration, consistent with injury to epithelium and barrier disruption. In contrast, the co-administration of EBM in LPS-L and LPS-H groups

showed less infiltration, demonstrating preservation of gut barrier.

EBM counteracts LPS-induced transcriptional upregulation of inflammatory cytokines: RT-qPCR analysis showed a significant upregulation of mRNA expression of pro-inflammatory cytokines *IL-6* ($P < 0.01$) and *TNF- α* ($P < 0.05$) upon LPS exposure (LPS-N), in comparison to control group (Fig. 3 A-B). In contrast, co-administration of EBM markedly prevented this elevation of cytokines in a dose dependent trend. Specifically, the high dose of EBM (LPS-H) displayed a highly significant restrain ($P < 0.01$) in the upregulation of both the *IL-6* and *TNF- α* in comparison with LPS-N group. Likewise, the low dose of EBM (LPS-L) also showed a significant prevention in expression of *TNF- α* ($P < 0.05$) and marked attenuation in *IL-6* as compared to LPS-N group as shown in (Fig. 3 A-B). These findings revealed that preventive administration of EBM limits the transcriptional activation of pro-inflammatory cytokines under the LPS challenge.



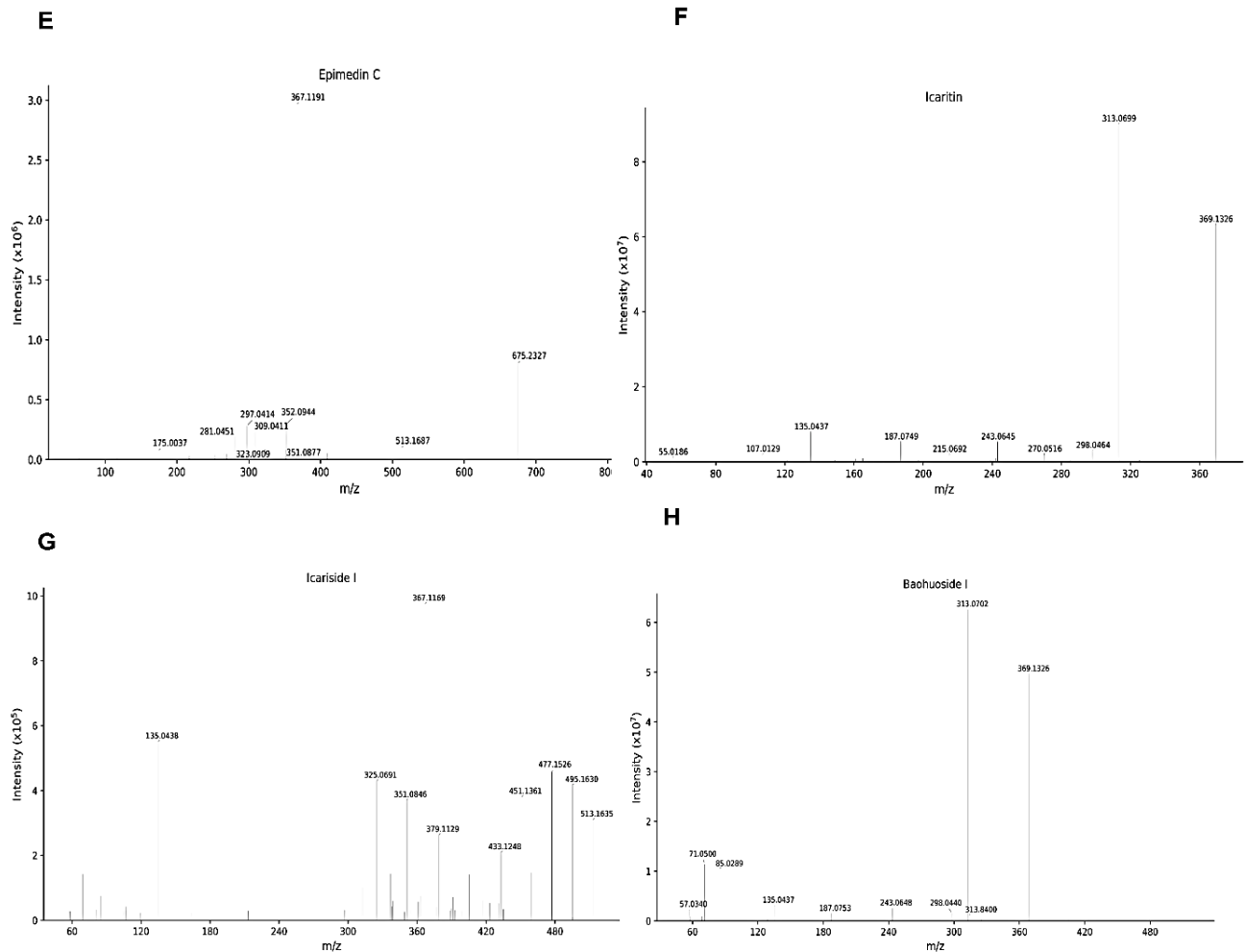


Fig. 1: Phytochemical profiling of *Epimedium brevicornum* Maxim. (EBM) ethanolic extract by UPLC-Q Exactive HFX Orbitrap-MS/MS. (A) Number of compounds identified per chemical class. (B-H) MS/MS spectra of the characteristic flavonoids in EBM: (B) icariin, (C) Epimedin A, (D) Epimedin B, (E) Epimedin C, (F) icaritin (G) icariside I (H) Baohuoside I, with precursor and fragment ion m/z values annotated. Compounds were annotated based on accurate mass, MS/MS fragmentation, and database matching.

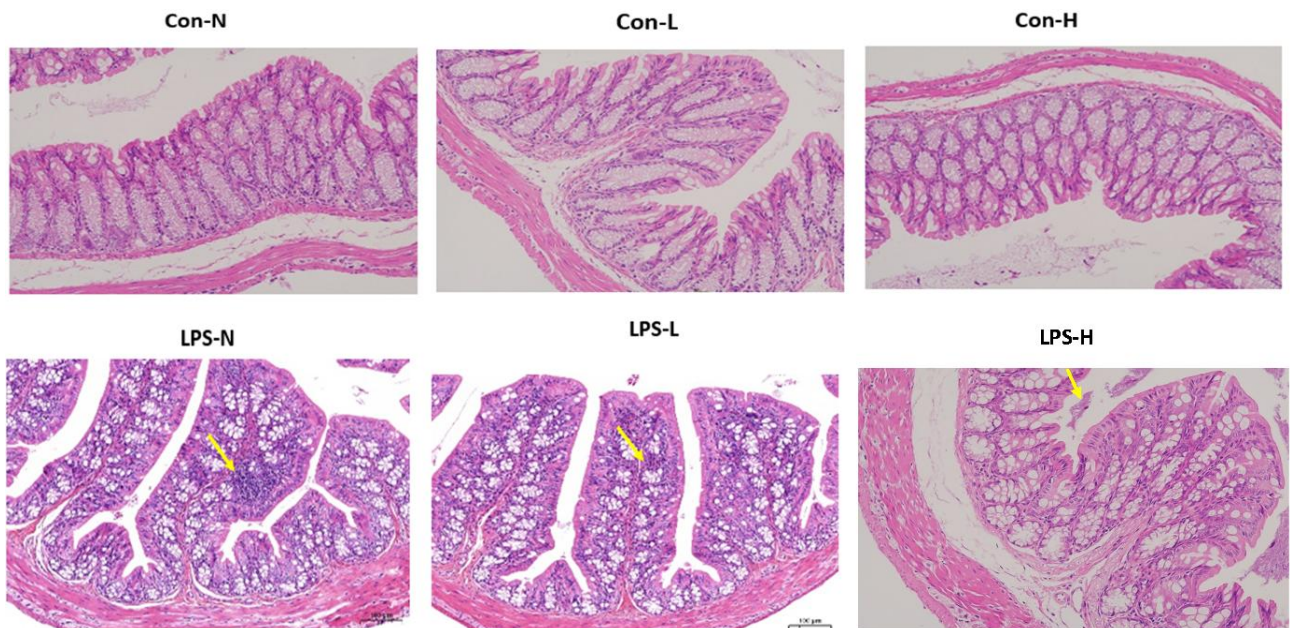


Fig. 2: Hematoxylin and eosin-stained colonic sections from six experimental groups. Con-N shows intact mucosal architecture with no inflammatory infiltration. Con-L and Con-H display normal structural organization comparable to Con-N. LPS-N exhibits severe mucosal disruption and inflammatory infiltration. LPS-L shows improvement with reduced inflammatory infiltration. LPS-H demonstrates marked protection of mucosal architecture with minimal infiltration, indicating efficacy of EBM. Scale bar: 100 μ m.

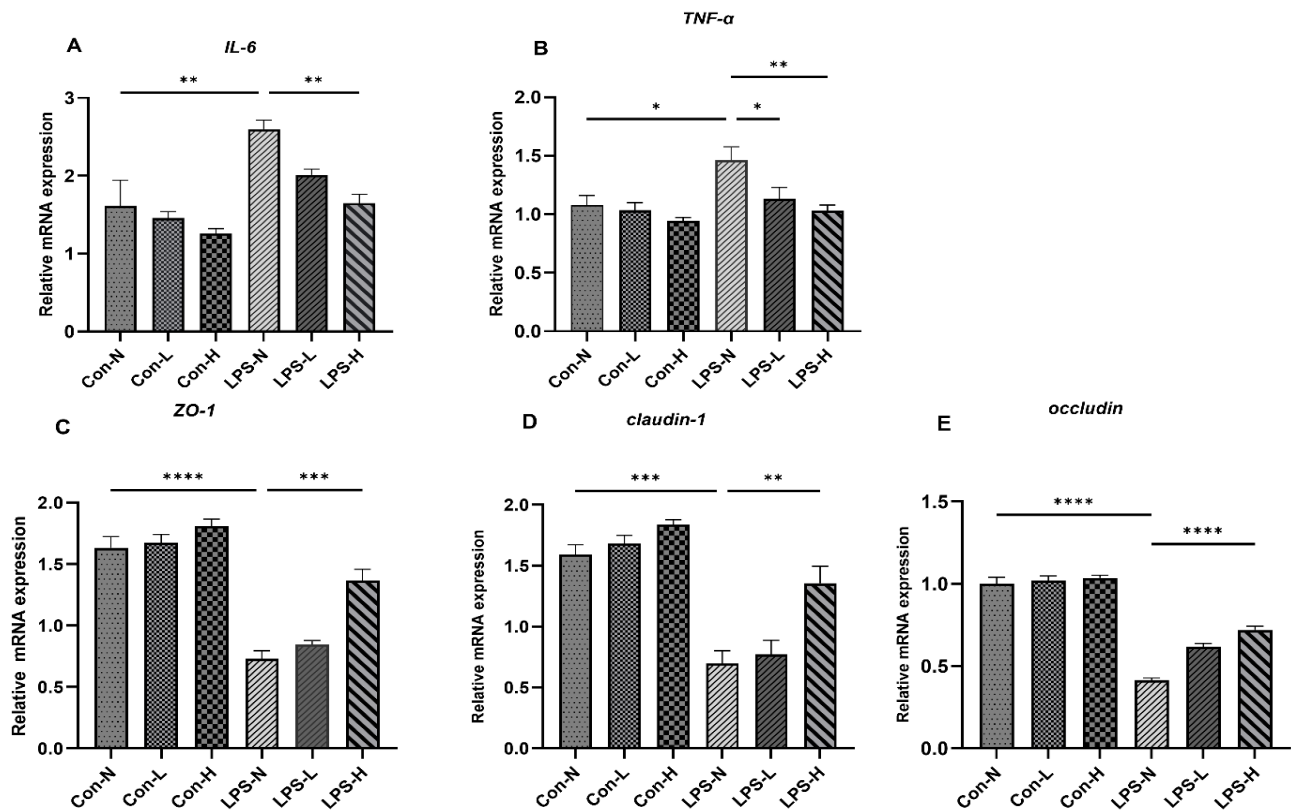


Fig. 3: Quantitative PCR analysis of intestinal mRNA expression for inflammatory markers (TNF- α , IL-6) and tight junction proteins (ZO-1, claudin-1, occludin) in mice colon. Relative expression levels of mRNA were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Data are expressed as mean $\pm$ SD (n = 5 per group). Each biological sample was run in technical triplicates. Statistical significance was determined: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. EBM treatment attenuated LPS-induced inflammatory responses and preserved tight junction integrity in a dose-dependent manner.

EBM prevents downregulation of tight junction protein mRNA: Analysis of qPCR outcomes showed that the LPS trigger and EBM administration resulted into significant alterations in the expression levels of mRNA of ZO-1, claudin-1 and occludin. LPS exposure significantly decreased the expression levels of ZO-1 (P<0.0001) claudin-1 (P<0.001) and occludin (P<0.0001) genes compared against the control group (Fig. 3C-E). Conversely, EBM co-administration significantly prevented their reduction in expressions in a dose dependent trend. In both the low (LPS-L) and high (LPS-H) dose groups of EBM the higher levels of mRNA of ZO-1 (P<0.001), claudin-1 (P<0.01) and occludin (P<0.0001) were seen as compared against the LPS-N group. These findings showed that EBM prevents the reductions in TJ protein mRNA expression in a dose responsive manner.

Preventive effects of EBM on pro-inflammatory cytokines TNF- α and IL-6: The Western blot analysis (Fig. 3 A) showed that LPS challenge significantly elevated the protein expression of TNF- α (P<0.05) and IL-6 (P<0.0001) (Fig. 4 A1-A2) in colon tissues compared to control group. Conversely, co-administration of EBM markedly prevented this LPS-induced elevation of these proteins dose dependently. Notably, the high dose of EBM (LPS-H) demonstrated a highly significant prevention in IL-6 (P<0.0001) and a marked suppression in the TNF- α (P<0.05) protein expression relative to the LPS-N group, while the low dose (LPS-L) exhibited a highly significant suppression in IL-6 (P<0.001) and a notable declining trend in TNF- α levels.

EBM maintains the expression levels of tight junction proteins: Analysis through the Western blot (Fig. 4B) revealed a highly significant reduction in the protein expressions of ZO-1 (P<0.0001), claudin-1 (P<0.001) and occludin (P<0.0001) (Fig. 4B1-B3) in LPS-N group relative to control group, revealing that LPS profoundly decreased the expression of TJ proteins. However, co-administration of EBM effectively attenuated LPS-induced reduction in proteins in a dose-dependent trend. At the low dose (LPS-L) EBM significantly preserved the expression levels of ZO-1 and occludin proteins (P<0.0001), whereas, the high dose of EBM (LPS-H) significantly prevented the decline of ZO-1 (P<0.0001), claudin-1 (P<0.01) and occludin (P<0.0001).

16S RNA sequencing quality evaluation and ASV generation: A total of 2,543,886 paired-end reads were acquired from all fecal samples. After quality filtering using Trimmomatic and Cutadapt, followed by DADA2-based denoising, paired-end merging, and removing chimera in QIIME2 (v2020.6), 2,356,050 clean reads were kept, with an average sequencing depth of 65,446 reads per sample (range: 38,789-68,250 reads). A total of 19,996 ASVs were identified across all samples. Good's coverage values ranged from 0.9991 to 0.9999, showing sufficient sequencing depth for analysis of microbial diversity.

Positive effects of EBM on microbial composition: The overall analyses profile of the gut microbiota is presented in Fig. 5. At the phylum level, among the top dominant

phyla identified (Fig. 5A) are *Bacteroidota*, *Firmicutes*, *Desulfobacterota*, *Actinobacteriota*, *Proteobacteria*, *Campylobacterota*, *Patescibacteria*, *Deferribacterota*, and *Cyanobacteria*, along with unclassified *Bacteria* and others. In comparison to control (Con-N), LPS group (LPS-N) exhibited a marked decrease in the relative abundance of *Firmicutes* (Fig. 5 A1) and a concomitant significant enrichment of *Desulfobacterota* ($P < 0.05$) (Fig. 5 A2). Besides, a numerically high elevation in *Proteobacteria* (Fig. 5 A3) and *Deferribacterota* (Fig. 5 A4) was seen, reflecting the alterations caused by LPS administration. Conversely, co-administration of low (LPS-L) and high dose of EBM (LPS-H) attenuated this LPS-associated dysbiosis as revealed by increased the *Firmicutes* ($P < 0.05$) and decreased *Proteobacteria* and specifically *Deferribacterota* with highly significant ($P < 0.0001$) levels toward control levels in a dose dependent trend. In order to further investigate the microbial differences, the analysis focused on the important taxa relevant to gut health at the genus level. Several functionally important genera, including *Lactobacillus*, *Ligilactobacillus*, *Bilophila*, *Desulfovibrio*, *Alistipes* and *Ruminococcus* showed notable variations among the groups. LPS exposure in LPS-N group significantly increased *Desulfovibrio* ($P < 0.05$) (Fig. 5 B1), *Bilophila* ($P < 0.05$) (Fig. 5 B2) and enriched *Alistipes* (Fig. 5 B5) levels, while decreasing trends were seen in *Lactobacillus* (Fig. 5 B3) and *Ligilactobacillus* (Fig. 5 B4) and *Ruminococcus* (Fig. 5 B6) in comparison to control group. In contrast, co-administration of EBM significantly prevented elevation of *Desulfovibrio* in both LPS-L and LPS-H groups ($P < 0.05$) in a dose dependent manner, while reducing *Bilophila* and *Alistipes* in the same manner, showing the potential of EBM to counteract imbalance in microbiota

under the inflammatory stimuli. Furthermore, high dose of EBM in control group (Con-H) displayed a significant elevation ($P < 0.05$) in *Ligilactobacillus* and numerically profound increase in *Lactobacillus* and *Ruminococcus* as compared to Con-N. Overall, consistent with the variations seen at the phylum level, LPS challenge and EBM administration also produced notable shifts in gut microflora composition at genus level.

α -diversity was assessed for the evaluation of microbial community richness and diversity. Shannon index showed changes in gut microbial α -diversity across all groups (Fig. 5 C1). The highest diversity was displayed by Con-N group, whereas LPS exposure (LPS-N) shifted microbial diversity, reflecting the reconstruction of the gut microbiota. Notably, a significant distinction ($P < 0.05$) was observed between Con-N and LPS-H groups, demonstrating that high dose of EBM markedly shifted the overall microbial diversity toward the normal ecosystem after the alterations were caused by LPS, while the Simpson index (Fig. 5 C2) displayed a marked difference between LPS-H and Con-N groups ($P < 0.05$) indicating that EBM high dose under LPS challenge produced a difference in community evenness without altering overall diversity. No significant difference was seen in Chao1, Ace Index and PD whole tree. Moreover, beta (β)-diversity was measured using Principal Coordinates Analysis (PCoA) and Principal Component Analysis (PCA) based on Bray-Curtis dissimilarity. PCoA (Fig. 5 D1) showed significant differentiation among groups, PERMANOVA ($P = 0.003$), suggesting that microbial community structures are meaningfully different between groups. Similarly, PCA (Fig. 5 D2) demonstrated stronger separation among groups supported by ANOSIM ($R = 0.199$, $P < 0.001$).

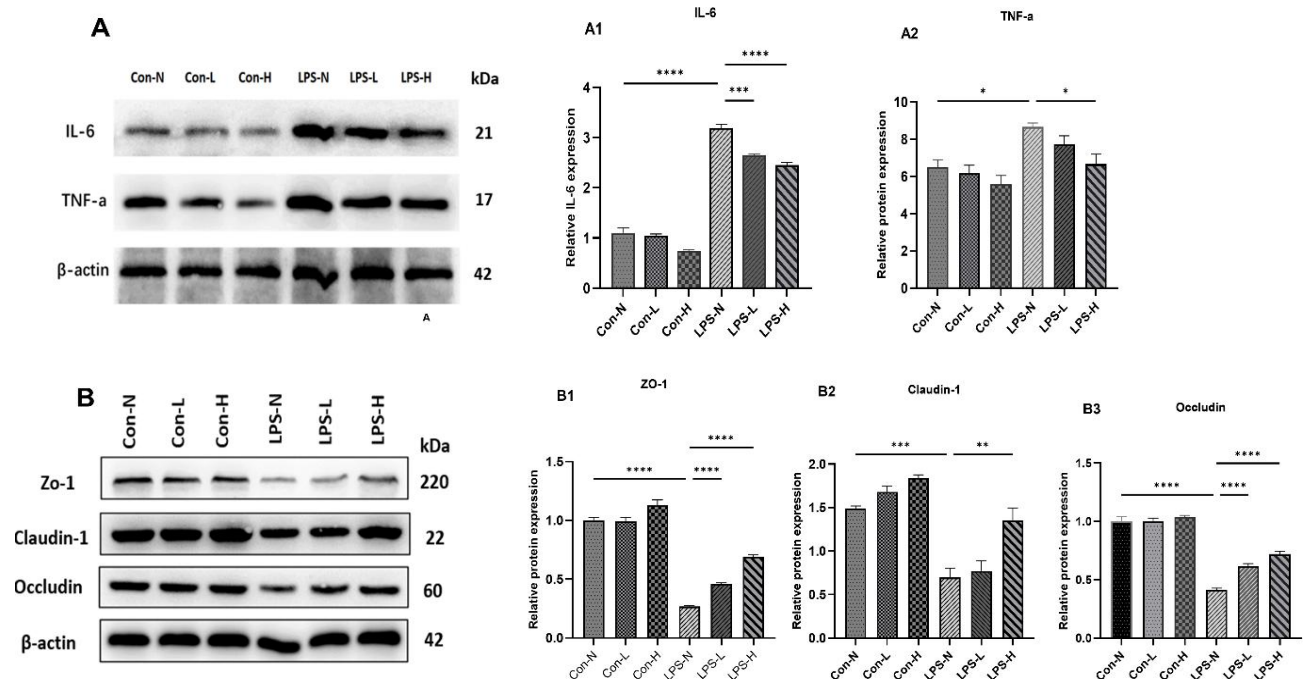
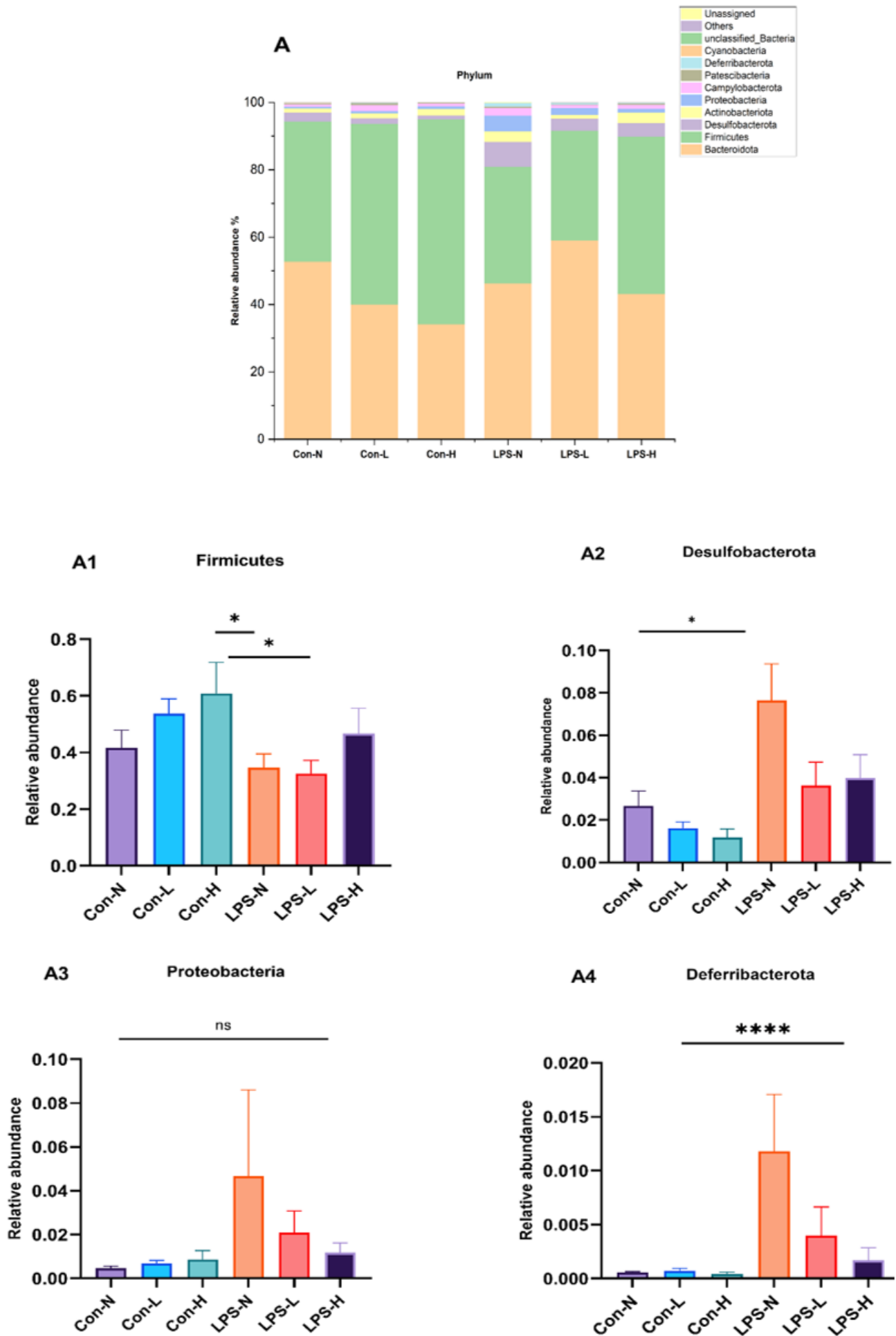
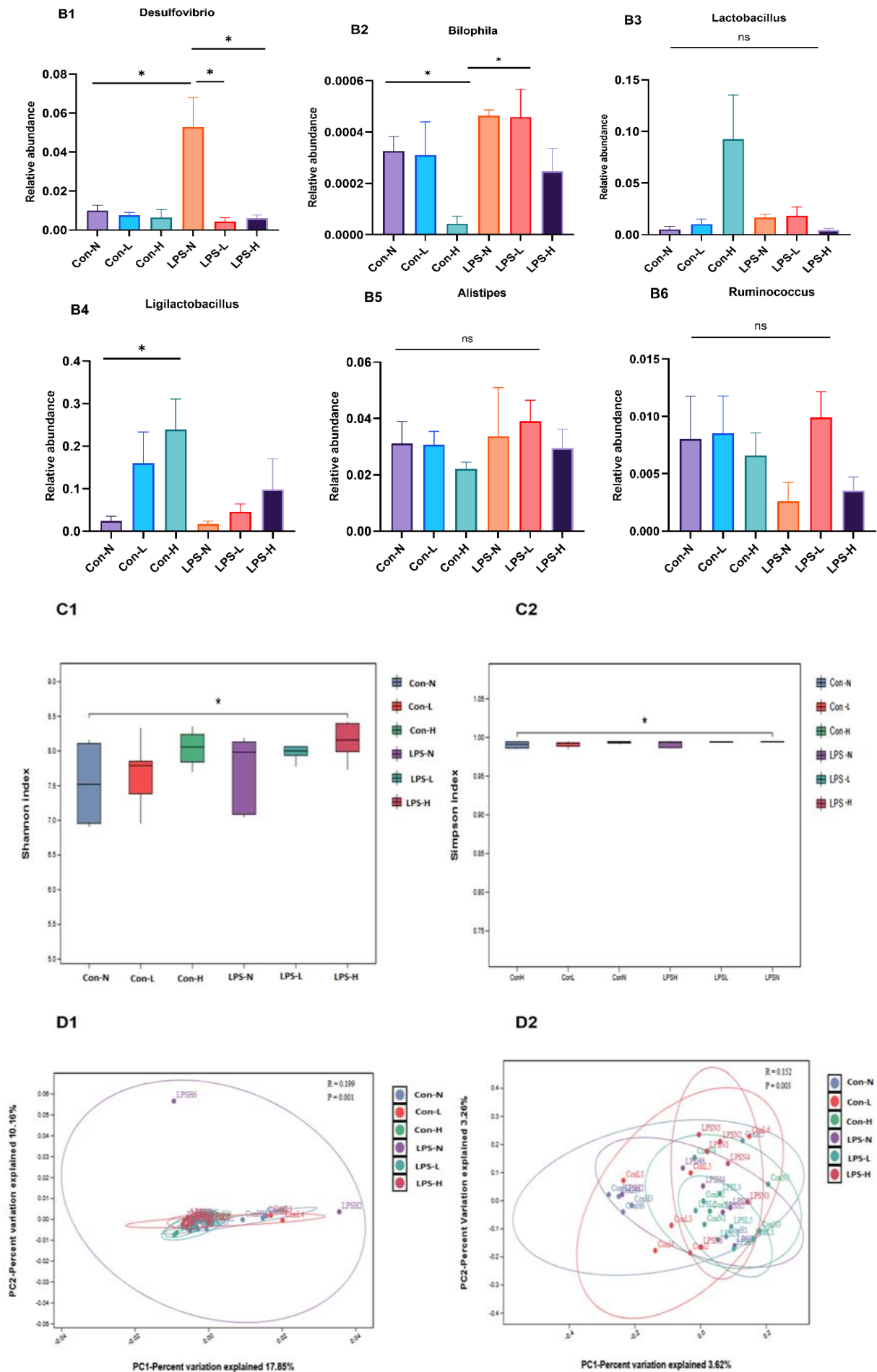


Fig. 4. Protein expression of pro-inflammatory cytokines (IL-6, TNF- α) and tight junction (TJ) proteins (ZO-1, claudin-1, occludin) through Western blot. Representative immunoblot bands (A, B) are shown alongside densitometric quantification of each protein, normalized to the corresponding loading control. EBM prevented LPS-induced IL-6 and TNF- α (A1, A2) upregulation and preserved tight junction proteins (B1, B2, B3) in a dose-dependent trend. Statistical significance was determined: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data are presented mean $\pm$ SD ($n = 3$ independent experiments).





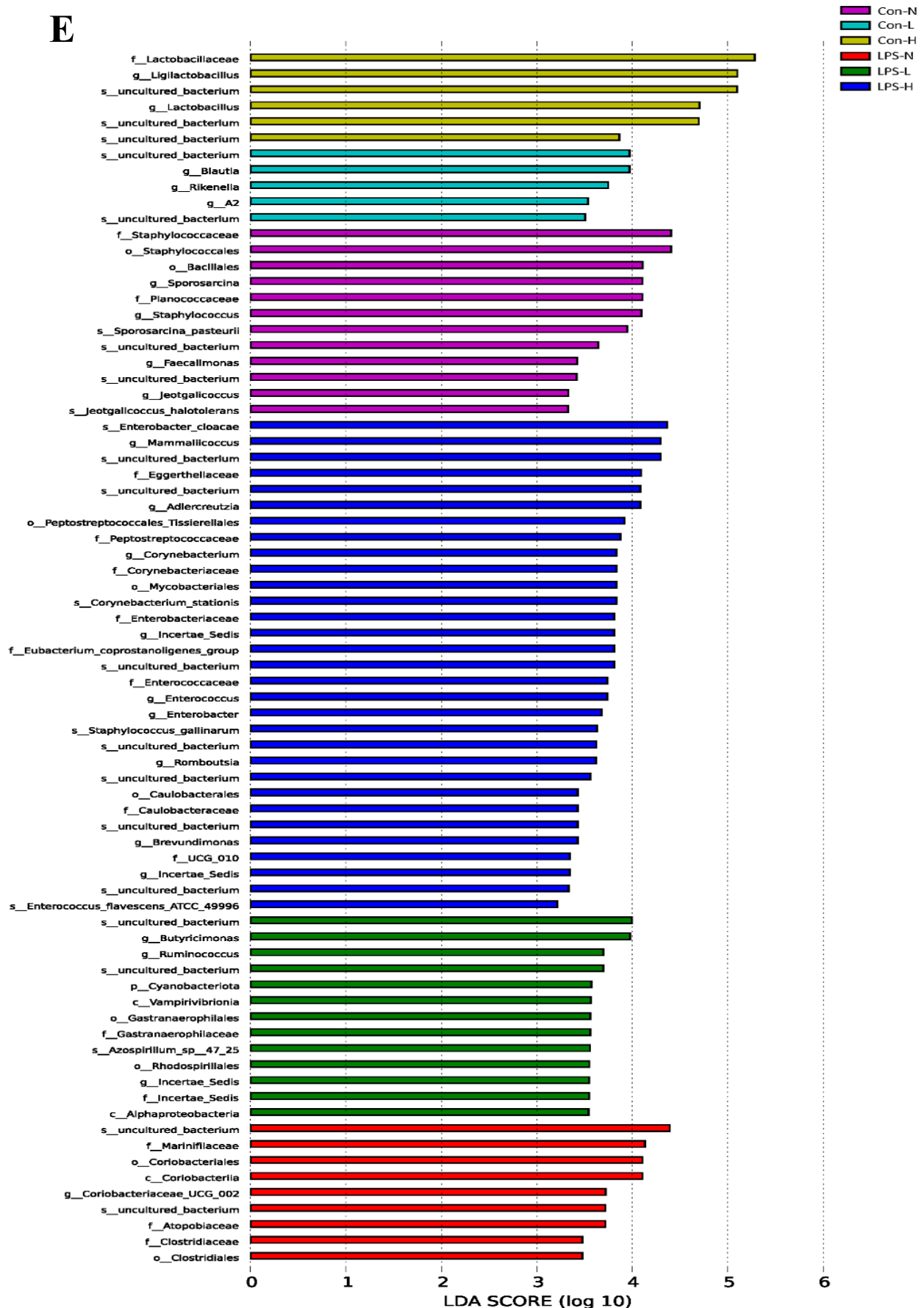


Fig. 5: EBM affects gut microbiota at the (A) Phylum level and major genera level (B1-B6). Alpha diversity indices (Shannon and Simpson) of the gut microbiota (C1-C2). Beta diversity analysis based on Bray–Curtis distances demonstrated by PCoA and PCA (D1-D2). Taxonomic cladogram of linear discriminant analysis effect size (LEfSe) analysis (E). Con-N, control without LPS; Con-L, low dose of EBM without LPS; Con-H, high dose of EBM without LPS; LPS-N, LPS administered without EBM; LPS-L, LPS-induced with low dose EBM; LPS-H, LPS-challenged given high dose of EBM. Values are presented as mean $\pm$ SD (n=6 per group). * P<0.05, *** P<0.0001.

LEfSe analysis (Fig.5 E) indicated distinct enrichment trends. The LPS group was enriched with taxa such as *Clostridiales*, *Clostridiaceae*, *Coriobacteriaceae*, and *Atopobiaceae*, which are often associated with intestinal inflammation and dysbiosis. Conversely, the LPS group receiving high dose of EBM was enriched with taxa primarily affiliated with the *Firmicutes* phylum, including *Lactobacillaceae*, *Ligilactobacillus*, and *Lactobacillus*. Intermediate groups showed distinct enrichments of genera such as *Ruminococcus*, *Butyricimonas*, *Alloprevotella*, and *Muribaculum*.

Modulation of Short chain fatty acids (SCFAs): To assess the impact of LPS and EBM on gut microbial

metabolism, SCFAs content including concentrations of acetic, butyric and propionic acids were measured (Fig. 6 A-C). One-way ANOVA revealed significant overall treatment effects for acetic acid and butyric acid (both $P < 0.0001$), whereas propionic acid showed no significant overall difference among groups. Whereas, post hoc comparisons showed that LPS significantly decreased acetic and butyric acid levels in LPS-N in comparison to Con-N ($P < 0.05$). In contrast co-administration of EBM in LPS-H group resulted in marked prevention ($P < 0.05$) in the reductions of these two acids relative to LPS-N. Furthermore, no pairwise significant difference was seen in propionic acid.

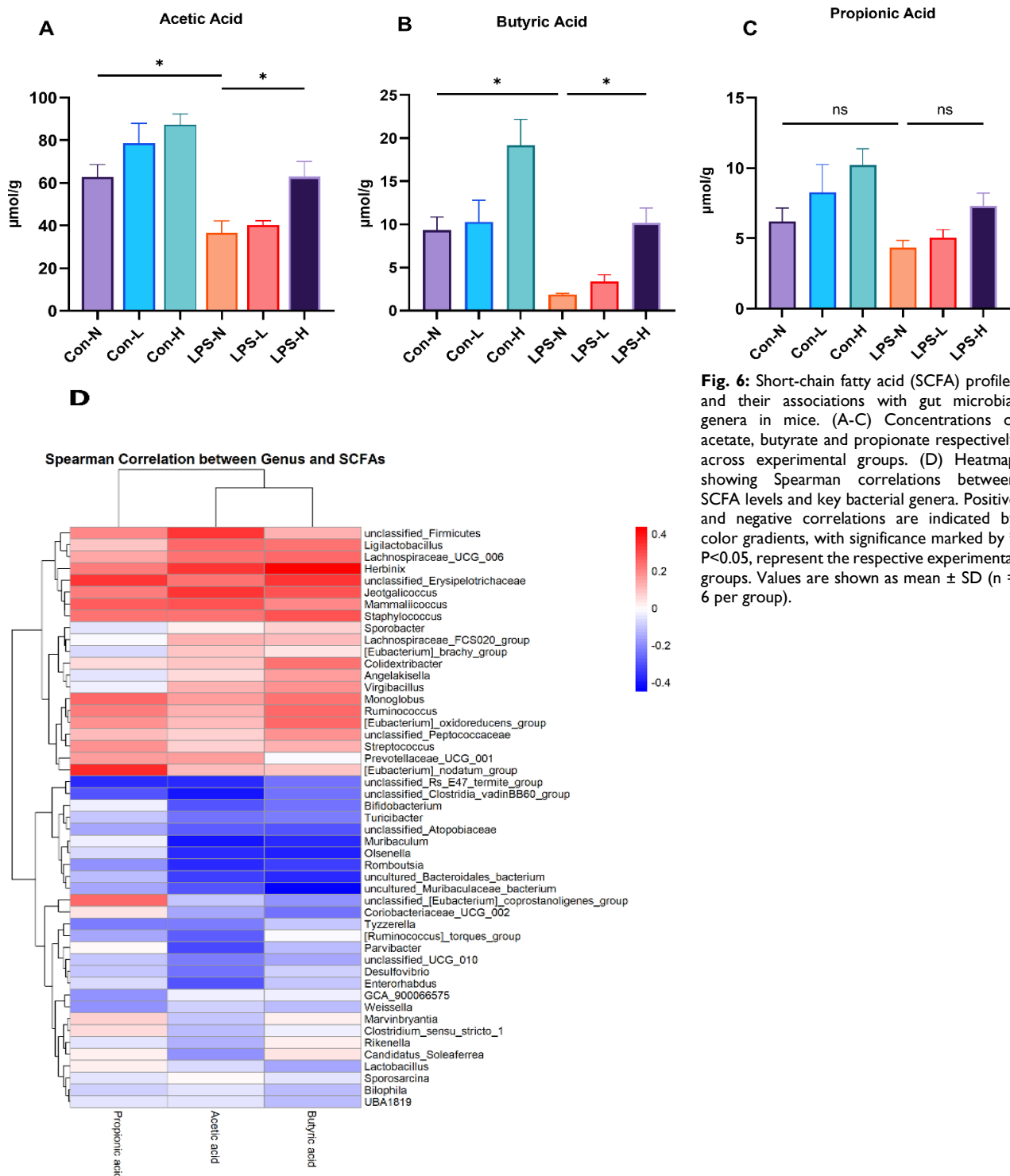


Fig. 6: Short-chain fatty acid (SCFA) profiles and their associations with gut microbial genera in mice. (A-C) Concentrations of acetate, butyrate and propionate respectively across experimental groups. (D) Heatmap showing Spearman correlations between SCFA levels and key bacterial genera. Positive and negative correlations are indicated by color gradients, with significance marked by * $P < 0.05$. Values are shown as mean $\pm$ SD (n = 6 per group).

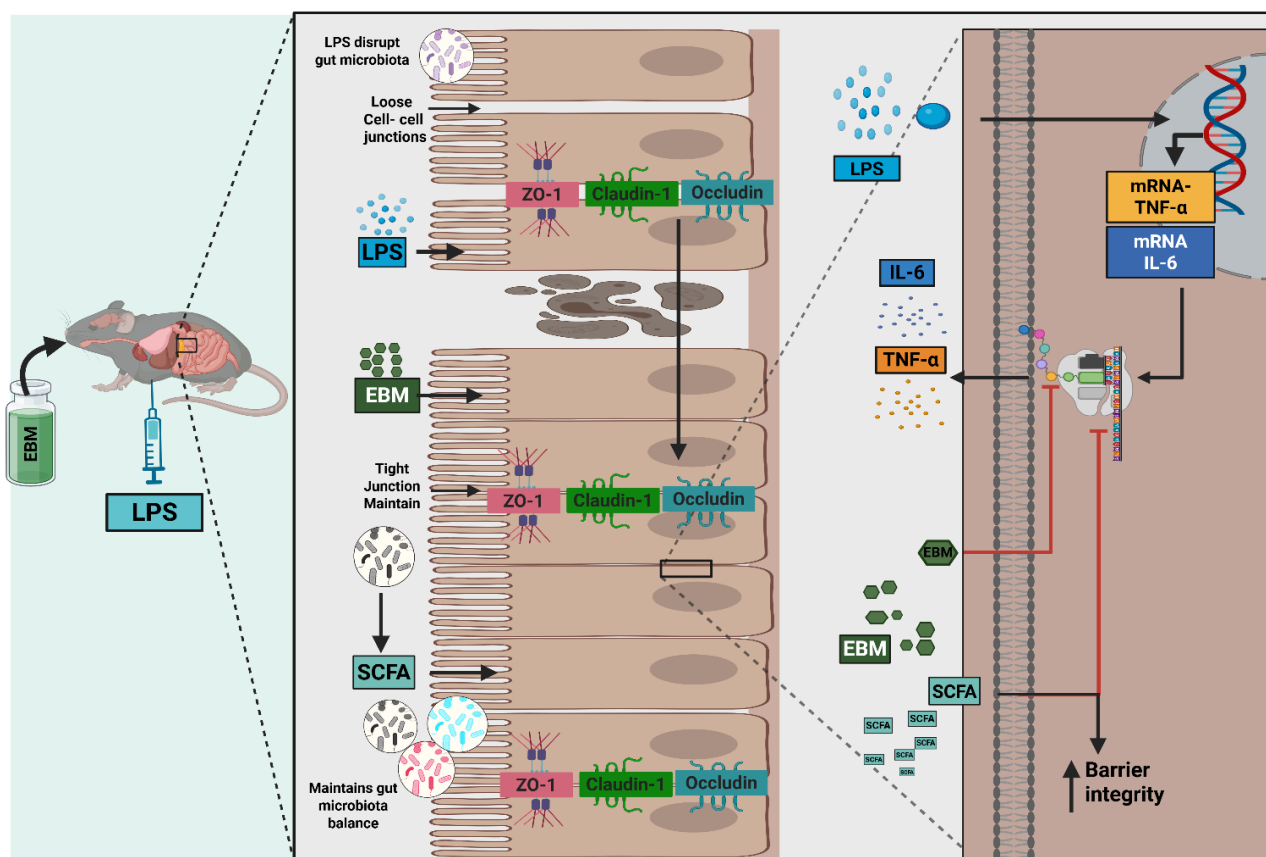


Fig. 7: Schematic illustration of the protective effects of EBM against LPS induced chronic intestinal inflammation and gut barrier dysfunction. EBM suppresses inflammatory signaling and cytokine gene expression and preserves epithelial tight junctions (ZO-1, claudin-1, occludin) through maintaining microbiota balance and SCFA production.

Correlation between gut bacterial genera and SCFAs: Spearman correlation test (Fig. 6 D) was conducted to evaluate the relationships between the microbiota genera and SCFAs levels. Overall, several genera showed notable positive or negative correlations with SCFA levels, indicating their potential roles in SCFA production in gut.

Among the taxa, *Ligilactobacillus*, *Herbinix*, *Jeotgalicoccus*, *unclassified Firmicutes*, *unclassified Erysipelotrichaceae* and *Lachnospiraceae* UCG-006 showed moderate positive correlations ($p \approx 0.25-0.40$), with acetic, propionic, and butyric acids, indicating their potential roles in SCFA production. In addition, moderate positive associations were also seen for *Jeotgalicoccus* and *Mammaliococcus* ($p \approx 0.20-0.30$), indicating their supportive role in SCFA production. In contrast, bacterial taxa including *Bilophila*, *Rikenella*, *Sporosarcina*, *Desulfovibrio*, *Tyzerella*, and *Romboutsia* showed negative correlations with SCFAs.

DISCUSSION

The biological properties of medicinal plants are mostly evaluated by their phytochemical profiling to understand their pharmacological activities (Yu *et al.*, 2021). UPLC-HRMS/MS has been extensively used for phytochemical characterization because of its high sensitivity and ability to differentiate structurally similar constituents (Yao *et al.*, 2026). In the current study, UPLC-Orbitrap-MS/MS confirmed that EBM has a flavonoid-rich phytochemical composition, with the identification of characteristic constituents such as icariin, icarisiide I,

icaritin, epimedins and their derivatives, accompanied by numerous phenolic compounds. These findings are in consistency with previous reports describing flavonoids as the major bioactive constituents of EBM (Ma *et al.*, 2011; Qin *et al.*, 2022).

The administration of LPS is a well-established method to induce intestinal inflammation in experimental mice models (Biasizzo *et al.*, 2021), characterized by compromised intestinal integrity (Zhao *et al.*, 2021). Mechanistically, LPS impairs colon epithelial barrier by activation of TLR4 on epithelial and immune cells, triggering NF-κB signaling and releasing pro-inflammatory cytokines. This cascade results into an increased permeability and leukocyte infiltration into lamina propria and submucosa which can clearly be seen in H&E staining (Guo *et al.*, 2013; Zou *et al.*, 2022). The histopathology seen in our LPS challenged mice is in alignment with these mechanisms, confirming successful induction of intestinal inflammation and epithelial barrier injury. Conversely, co-administration of EBM prevented the histopathological changes induced by LPS. EBM administration preserved the architecture of the colon characterized by an intact epithelial lining, reduced infiltration of leukocytes in a dose dependent trend. The histological protection seen in our findings aligns with study by Zhao *et al.* (2025) who presented that EBM protects histopathology and mucosal integrity in colitis. Similarly, icariin has been seen to inhibit TLR4–NF-κB activation, thereby inhibiting LPS induced gut barrier damage, and preserve barrier function (Xiong *et al.*, 2019).

Key cytokines involved in inflammation, namely TNF- α and IL-6, are the markers of LPS-triggered intestinal inflammatory conditions (Cavaillon, 2001; Sun *et al.*, 2021). Prior studies have evaluated EBM or their constituents mostly as therapeutic interventions where they consistently reduced TNF- α and IL-6 (Yuk *et al.*, 2010; Huang *et al.*, 2018). Building upon this framework, we designed this study to evaluate the preventive potential of EBM under persistent inflammatory challenge. In consistency with the previous studies, LPS exposure significantly elevated TNF- α and IL-6 mRNA levels. In contrast, co-administration of EBM profoundly suppressed this transcriptional elevation dose dependently. The high dose markedly prevented the elevation of both cytokines, while the low dose also notably suppressed TNF- α expression and demonstrated a declining trend in IL-6 levels, indicating the protective potential of EBM against LPS-induced cytokine activation. These results were further validated by Western blot analysis, where LPS exposure markedly elevated the protein expressions of TNF- α and IL-6, and EBM co-administration was linked to a dose-dependent prevention in the expression levels of these proteins. High dose exhibited the highest reductions in TNF- α and IL-6.

The integrity of gut barrier is preserved by TJs which are the key structural components of the intestinal epithelium (Tian *et al.*, 2026). These TJs are mainly composed of ZO-1, claudin-1 and occludin proteins (Wang *et al.*, 2021). LPS induced inflammatory stimulations impairs TJ proteins through TNF- α and IL-6 mediated upregulation of MLCK (myosin light chain kinase). This kinase phosphorylates MLC, leading to actomyosin contraction. Consequently, ZO-1 claudin-1, and occludin dislodge from the membrane (Turner, 2009; Li *et al.*, 2021a), and subsequent impairment of intestinal barrier (Pastorelli *et al.*, 2013). In alignment with earlier studies, our study demonstrated that exposure of LPS markedly declined the mRNA and protein concentrations of all three TJ proteins. EBM co-administration prevented this downregulation in gene expression in a clear dose dependent trend, with the high dose displaying the strongest effect ZO-1 ($P < 0.001$), claudin-1 ($P < 0.01$), and occludin ($P < 0.0001$). Moreover, these transcriptional patterns followed the same pattern at the protein expression level, where high dose of EBM co-administration produced the strongest rescue followed by low dose of EBM (LPS-L). To our knowledge, no previous study has investigated the preservation of TJ proteins under chronic inflammatory conditions. However, flavonoids specifically icariin has demonstrated the enhancement of TJ proteins expression and barrier integrity, supporting our findings. In this regard, it was seen that icariin improved gut barrier integrity by upregulating expressions of TJ proteins namely ZO-1, claudin-1 and occludin (Zhang *et al.*, 2023). Another, study demonstrated that icariin from EBM alleviates LPS-induced gut barrier impairment by upregulating these TJ proteins (Xiong *et al.*, 2020; Li *et al.*, 2022). Together, these findings suggest that EBM exerts gut barrier protective effects that are associated with preservation of TJ protein expression during inflammatory stress.

The gut microbiota is crucial for modulating inflammatory cytokines and maintaining TJ proteins (Yadav and Jha, 2019). Anti-inflammatory and gut barrier

protecting taxa such as *Lactobacillus*, *Ligilactobacillus*, *Alistipes* and *Ruminococcus*, together with broader phylum *Firmicutes* suppress TNF- α and IL-6 and preserve TJ proteins by potentially suppressing TLR4–NF- κ B signaling through their metabolites (Yan and Polk, 2020; Lin *et al.*, 2025; Yu *et al.*, 2025). In contrast, LPS disrupts microbial balance by promoting the expansion of phyla such as *Proteobacteria* and *Deferribacterota* and by increasing genera such as *Desulfovibrio* and *Bilophila*, which aggravate inflammation and damage barrier integrity (Feng *et al.*, 2017; Zhang *et al.*, 2023b). This change is accompanied by a notable loss of beneficial *Firmicutes*, *Lactobacillus* and *Ruminococcus*, a typical pattern of LPS-associated inflammatory dysbiosis (Xie *et al.*, 2019). Our findings displayed the same microbial dysbiosis, confirming successful establishment of LPS-linked inflammatory microbial imbalance. Conversely, co-administration of EBM prevented these LPS-associated alterations by restraining the enrichment of inflammation-linked taxa while maintaining the beneficial populations in a dose dependent manner. High dose of EBM most effectively restricted the expansion of *Proteobacteria*, *Deferribacterota*, and the genera *Desulfovibrio* and *Bilophila*, while concurrently increasing *Firmicutes*, *Alistipes* and *Ligilactobacillus*. Whereas, EBM low dose also mitigated dysbiosis, particularly increasing abundances of *Lactobacillus* and *Ruminococcus* and preventing the expansion of *Desulfovibrio*. These dose dependent trends are in alignment with the biphasic responses usually seen with polyphenolic extracts (Louis *et al.*, 2014). Although, studies of EBM on gut microbiota remains limited, yet there are evidences from its constituents. *Epimedium folium* which refers to the same plant material, show results which are in alignment with our study context. Notably, *Epimedium folium* increased *Lactobacillus* while reducing *Proteobacteria* (Ma *et al.*, 2024). Additionally, icariin demonstrated similar results with increased the *Firmicutes* (Wang *et al.*, 2022) and decreased *Desulfovibrio* and *Bilophila* (Li *et al.*, 2021b). Moreover, icariside I, demonstrated the same gut modulatory properties by elevating the *Lactobacillus* and decreasing the harmful bacteria (Chen *et al.*, 2021). Furthermore, the significant differences at community level (α and β diversity) analysis validated the findings that LPS restructured the microbial ecosystem and high dose of EBM maintained the structure toward that seen in control group. The β -diversity analysis also strengthened these findings where clear separation among groups in both PCoA and PCA shows that EBM produces shifts in overall community structure, not only changes in individual taxa.

SCFAs such as acetate, butyrate and propionate are the dominant fermentation products of *Firmicutes* and other saccharolytic taxa (Tan *et al.*, 2014; Javier *et al.*, 2016). These metabolites suppress cytokines through activation of the GPR41/GPR43 receptors located on intestinal epithelial cells, which have been reported to modulate NF- κ B signaling and support TJ proteins integrity (Kim *et al.*, 2013). LPS-induced inflammatory conditions disrupt the fermentative bacterial communities, which results in a sharp decline in SCFA output (Kroon *et al.*, 2025). In consistency with these reports, we also found that all three SCFAs were markedly reduced under the LPS exposure, parallel to reduction in fermentative bacteria as discussed

earlier. EBM prevented this LPS-triggered reduction in a clear dose dependent trend. The high dose not only maintained SCFAs but also exceeded baseline levels, demonstrating enhanced fermentation potential despite inflammatory stress. This effect is in consistency with the known properties of EBM flavonoids such as ICA and icaraside I, which increase SCFAs through the enrichment of saccharolytic bacteria (Zhang *et al.*, 2023a; Luo *et al.*, 2025). Our correlation analysis between bacterial community structure and SCFA further validated this interpretation. Genera with well-known fermentative activity such as *Ligilactobacillus*, *Ruminococcus* and members of *Lachnospiraceae* displayed positive correlations with all three SCFAs. Furthermore, positive correlations for *Jeotgalicoccus* and *Mammaliicoccus* indicate potential auxiliary contributions. On the other hand, inflammation-linked taxa, including *Desulfovibrio* and *Bilophila*, were negatively correlated. These patterns indicate that EBM preserves SCFA production by maintaining a microbiota composition that favors fermentation rather than inflammation.

Conclusions: In this study we found that EBM has preventive effects against LPS induced intestinal inflammatory injury and barrier impairment. Preventive EBM administration suppressed inflammatory response and preserved tight junction proteins under sustained inflammatory challenge. These protective effects are linked to its ability to maintain gut microbiota composition and sustain SCFAs production, both of which are key regulators of cytokine signaling and barrier integrity. Taken together, our findings demonstrate that EBM effectively protects intestinal homeostasis under inflammatory conditions during under inflammatory stress.

Data availability statement: Data will be made available on request.

Acknowledgment: The funds for this study were provided by the Fundamental Research Funds for the Central Universities, Grant No: KJJO2025021 to FL.

Authors Contribution: Sarang Mazhar Abro: Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Formal analysis, Data curation. Xubin Du: Writing – review & editing, Investigation. Yanke Shan: Investigation. Muhammad Azhar Memon: Visualization, Software. Teerath Kumar Suthar: Visualization, Validation. Muhammad Mubashir: Data curation. Hongbing Gui: Formal analysis, Validation. Shanfeng Wang: Visualization, Validation. Moolchand Malhi: Investigation, Formal analysis. Jamila Soomro: Investigation, Formal analysis. Fei Liu: Supervision, Project administration, Writing – review & editing, Funding acquisition, Formal analysis.

REFERENCES

- Bhatia R, Sharma S, Bhadada SK, *et al.*, 2022. Lactic acid bacterial supplementation ameliorated the lipopolysaccharide-induced gut inflammation and dysbiosis in mice. *Frontiers in Microbiology* 13:930928.
- Biasizzo M, Trstenjak-Prebenda M, Dolinar K, *et al.*, 2021. Cystatin C deficiency increases LPS- induced sepsis and NLRP3 inflammasome activation in mice. *Cells* 10(8):207
- Cavaillon JM, 2001. Pro- versus anti-inflammatory cytokines: myth or reality. *Cellular and Molecular Biology* 47(4):695-702.
- Chen G, Cao Z, Shi Z, *et al.*, 2021. Microbiome analysis combined with targeted metabolomics reveal immunological anti-tumor activity of icaraside I in a melanoma mouse model. *Biomedicine & Pharmacotherapy* 140:111542.
- Cui J, Lin L, Hao F, *et al.*, 2024. Comprehensive review of the traditional uses and the potential benefits of epimedium folium. *Frontiers in Pharmacology* 15:1415265.
- Feng Z, Long W, Hao B, *et al.*, 2017. A human stool-derived *Bifidobacterium* strain caused systemic inflammation in specific-pathogen-free mice. *Gut Pathogens* 9:59.
- Gao Y, Shi W, Tu C, *et al.*, 2022. Immunostimulatory activity and structure-activity relationship of epimedin B from *Epimedium brevicornu* Maxim. *Frontiers in Pharmacology* 13:1015846.
- Guo S, Al-Sadi R, Said HM, *et al.*, 2013. Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *American Journal of Pathology* 182(2):375-387.
- He Y, Wang D, Liu K, *et al.*, 2023. Sodium humate alleviates LPS-induced intestinal barrier injury by improving intestinal immune function and regulating gut microbiota. *Molecular Immunology* 161:61-73.
- Huang S, Meng N, Chang B, *et al.*, 2018. Anti-inflammatory activity of *Epimedium brevicornu* Maxim ethanol extract. *Journal of Medicinal Food* 21(7):726-733.
- Humbert-Claude M, Duc D, Dwir D, *et al.*, 2016. Tollip, an early regulator of the acute inflammatory response in the substantia nigra. *Journal of Neuroinflammation* 13(1):303.
- Javier F, Saúl RB, Ignacio G-d-R, *et al.*, 2016. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: A review. *Journal of Functional Foods* 25:511-522.
- Kim MH, Kang SG, Park JH, *et al.*, 2013. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* 145(2):396-406.
- Khan A, Khan IA, Huang C, *et al.*, 2025. Infectious bronchitis virus like QX strain transmission, pathogenesis, replication and host miRNA biogenesis pathway hijacking mechanism. *Frontiers in Cellular and Infection Microbiology* 15:1645086.
- Kroon S, Malcic D, Weidert L, *et al.*, 2025. Sublethal systemic LPS in mice enables gut-luminal pathogens to bloom through oxygen species-mediated microbiota inhibition. *Nature Communications* 16(1):2760.
- Koh A, De Vadder F, Kovatcheva-Datchary P, *et al.*, 2016. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell* 165(6):1332-1345.
- Li Y, Liu J, Pongkorpsakol P, *et al.*, 2022. Relief effects of icariin on inflammation-induced decrease of tight junctions in intestinal epithelial cells. *Frontiers in Pharmacology* 13:903762.
- Li X, Mao M, Zhang Y, *et al.*, 2019. Succinate modulates intestinal barrier function and inflammation response in pigs. *Biomolecules* 9(9):486.
- Li H, Shang Z, Liu X, *et al.*, 2021a. *Clostridium butyricum* alleviates enterotoxigenic *Escherichia coli* K88-induced oxidative damage through regulating the p62-Keap1-Nrf2 signaling pathway and remodeling the cecal microbial community. *Frontiers in Immunology* 12:771826.
- Li X, Khan I, Xia W, *et al.*, 2021b. Icariin enhances youth-like features by attenuating the declined gut microbiota in the aged mice. *Pharmacological Research* 168:105587.
- Lin X, Xu M, Lan R, *et al.*, 2025. Gut commensal *Alistipes shahii* improves experimental colitis in mice with reduced intestinal epithelial damage and cytokine secretion. *mSystems* 10(3).
- Luo YM, Dong L, Li YL, *et al.*, 2025. Icaraside II ameliorates depression induced by high-fat diet via the microbiota-gut-brain axis in mice. *Phytotherapy Research* 39(11):5159-5173.
- Louis P, Hold GL and Flint HJ, 2014. The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews Microbiology* 12(10):661-672.
- Ma F, Zhang W, Zhou G, *et al.*, 2024. Epimedium Folium decoction ameliorates osteoporosis in mice through NLRP3/caspase-1/IL-1 β signalling pathway and gut-bone axis. *International Immunopharmacology* 137:112472.
- Ma H, He X, Yang Y, *et al.*, 2011. The genus *Epimedium*: An ethnopharmacological and phytochemical review. *Journal of Ethnopharmacology* 134(3):519-541.

- Murota K, Nakamura Y and Uehara M, 2018. Flavonoid metabolism: The interaction of metabolites and gut microbiota. *Bioscience, Biotechnology, and Biochemistry* 82(4):600-610.
- Okumura R and Takeda K, 2018. Maintenance of intestinal homeostasis by mucosal barriers. *Inflammation and Regeneration* 38:5.
- Pastorelli L, De Salvo C, Mercado JR, et al., 2013. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: Lessons learned from animal models and human genetics. *Frontiers in Immunology* 4:280.
- Qin W, Yang Y, Wang Y, et al., 2022. Transcriptomic and metabolomic analysis reveals the difference between large and small flower taxa of *Herba Epimedii* during flavonoid accumulation. *Scientific Reports* 12(1):2762.
- Roca Rubio MF, Eriksson U, Brummer RJ, et al., 2021. Sauna dehydration as a new physiological challenge model for intestinal barrier function. *Scientific Reports* 11(1):15514.
- Sun X, Cui Y, Su Y, et al., 2021. Dietary fiber ameliorates lipopolysaccharide-induced intestinal barrier function damage in piglets by modulation of intestinal microbiome. *mSystems* 6(2):e01374-20.
- Tan J, McKenzie C, Potamitis M, et al., 2014. The role of short-chain fatty acids in health and disease. *Advances in Immunology* 121:91-119.
- Tian K, Yuan H, Qiu SX, et al., 2026. Microbial metabolic shifts and inflammation-related gene expression underlie protective effects of slow digestible starch on rumen epithelium of finishing lambs. *Pakistan Veterinary Journal* 46(5): 1324-1332.
- Turner JR, 2009. Intestinal mucosal barrier function in health and disease. *Nature Reviews Immunology* 9(11):799-809.
- Wan F, Wang M, Zhong R, et al., 2022. Supplementation with Chinese medicinal plant extracts from *Lonicera hypoglauca* and *Scutellaria baicalensis* mitigates colonic inflammation by regulating oxidative stress and gut microbiota in a colitis mouse model. *Frontiers in Cellular and Infection Microbiology* 11:798052.
- Wang S, Wang S, Wang X, et al., 2022. Effects of icariin on modulating gut microbiota and regulating metabolite alterations to prevent bone loss in ovariectomized rat model. *Frontiers in Endocrinology* 13:874849.
- Wang D, Du Y, Wang S, et al., 2021. Effects of sodium humate and glutamine combined supplementation on growth performance, diarrhea incidence, blood parameters, and intestinal microflora of weaned calves. *Animal Science Journal* 92(1):e13584.
- Wang L, Li Y, Guo Y, et al., 2016. *Herba Epimedii*: An ancient Chinese herbal medicine in the prevention and treatment of osteoporosis. *Current Pharmaceutical Design* 22(3):328-349.
- Wu H, Ye L, Lu X, et al., 2018. *Lactobacillus acidophilus* alleviated *Salmonella*-induced goblet cells loss and colitis by Notch pathway. *Molecular Nutrition & Food Research* 62(22):e1800552.
- Xie S, Zhao S, Jiang L, et al., 2019. *Lactobacillus reuteri* stimulates intestinal epithelial proliferation and induces differentiation into goblet cells in young chickens. *Journal of Agricultural and Food Chemistry* 67(49):13758-13766.
- Xiong W, Huang J, Li X, et al., 2020. Icariin and its phosphorylated derivatives alleviate intestinal epithelial barrier disruption caused by enterotoxigenic *Escherichia coli* through modulating p38 MAPK in vivo and in vitro. *FASEB Journal* 34(1):1783-1801.
- Xiong W, Ma H, Zhang Z, et al., 2019. The protective effect of icariin and phosphorylated icariin against LPS-induced intestinal epithelial cells injury. *Biomedicine & Pharmacotherapy* 118:109246.
- Xu C, Ali M, Sun J, et al., 2024. Protective effects of *Abrus cantoniensis* Hance against liver injury through modulation of intestinal microbiota and liver metabolites. *Ecotoxicology and Environmental Safety* 279:116495.
- Yan F and Polk DB, 2020. Probiotics and probiotic-derived functional factors-mechanistic insights into applications for intestinal homeostasis. *Frontiers in Immunology* 11:1428.
- Yadav S and Jha R, 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal of Animal Science and Biotechnology* 10:2.
- Yao S, Long L, Zhu Y, et al., 2026. Integrated GC-MS and UPLC-Q-Orbitrap MS untargeted metabolomics for the comprehensive identification of metabolites and characterization of metabolic pathways in key tissues of *Cinnamomum cassia*. *Food Chemistry: X* 103716.
- Yin S, Sun C, Ji Y, et al., 2021. Herbal medicine WangShiBaoChiWan improves gastrointestinal health in mice via modulation of intestinal tight junctions and gut microbiota and inhibition of inflammation. *Biomedicine & Pharmacotherapy* 138:111426.
- Yu H, Rahman A, Yu L, et al., 2025. Effect of graded levels of dietary L-methionine on growth performance, body composition, amino acid requirements, serum biochemical parameters and gut microbiota in coho salmon (*Oncorhynchus kisutch*) alevins cultured in freshwater. *Pakistan Veterinary Journal* 45: 651-661.
- Yu M, Gouveias I, Rocha J, et al., 2021. Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific Reports* 11(1):10041.
- Yuk SS, Lim EM, Lee JY, et al., 2010. Anti-inflammatory effects of *EPimedium brevicornu* water extract on lipopolysaccharide-activated RAW264.7 macrophages. *Phytotherapy Research* 24(12):1781-1787.
- Zhang J, Yu H, Zhang H, et al., 2023a. Dietary *EPimedium* extract supplementation improves intestinal functions and alters gut microbiota in broilers. *Journal of Animal Science and Biotechnology* 14(1):14.
- Zhang K, Qin X, Qiu J, et al., 2023b. *Desulfovibrio desulfuricans* aggravates atherosclerosis by enhancing intestinal permeability and endothelial TLR4/NF- κ B pathway in Apoe (-/-) mice. *Genes & Diseases* 10(1):239-253.
- Zhao Z, Liu X, Zhang R, et al., 2025. Intestinal barrier in inflammatory bowel disease: Mechanisms and treatment. *Journal of Translational Gastroenterology* 3(2):62-73.
- Zhao Z, Li F, Ning J, et al., 2021. Novel compound FLZ alleviates rotenone-induced PD mouse model by suppressing TLR4/MyD88/NF- κ B pathway through microbiota-gut-brain axis. *Acta Pharmaceutica Sinica B* 11(9):2859-2879.
- Zhu L, Shen H, Gu PQ, et al., 2020. Baicalin alleviates TNBS-induced colitis by inhibiting PI3K/AKT pathway activation. *Experimental and Therapeutic Medicine* 20(1):581-590.
- Zou P, Yang F, Ding Y, et al., 2022. Lipopolysaccharide downregulates the expression of ZO-1 protein through the Akt pathway. *BMC Infectious Diseases* 22(1):774.