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Investigating the Effect of Probiotic and Postbiotic Mixture (Lactobacillus casei, Lactobacillus rhamnosus and Lactobacillus helveticus) on Liver and Small Intestine Tissue Infected with Escherichia coli in Male Wistar Rats

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TITLE PAGE

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Running Title: Probiotic and Postbiotic Effects on *E. coli*-Infected Tissues

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ABSTRACT

Foodborne pathogens, including *Escherichia coli*, are responsible for foodborne illnesses and subsequent tissue damage. This study examined the effects of a probiotic and postbiotic blend (*Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Lactobacillus helveticus*) on liver and small intestine tissues infected with *E. coli* in male Wistar rats. Twenty-eight adult male Wistar rats were divided into four groups: control, *E. coli*-infected (10^8 CFU/mL), infected with probiotic supplementation (10^9 CFU/mL), and infected with postbiotic supplementation (10^9 CFU/mL). Probiotic and postbiotic supplementation were administered by gavage for 35 days, and infection was induced over three doses across three days. Liver enzyme levels were measured from cardiac blood samples. Liver and intestine tissues were collected for gene expression analysis, total antioxidant capacity (TAC) enzyme assays, and histopathological examination. Histological analysis revealed significant epithelial destruction, reduced villi height, and inflammatory cell infiltration in the *E. coli*-infected group, while treatment groups showed relative tissue repair. Liver enzyme levels (AST, ALT, ALP) and TAC levels were significantly elevated in the *E. coli* group compared to controls ($p<0.05$). Treatment groups demonstrated significant reductions in enzyme levels compared to the infected group ($p<0.05$). *TNF-α*, IL-6, and *Bax* gene expression were significantly elevated in infected animals, while *Bcl-2* expression was increased in treatment groups ($p<0.05$). The probiotic and postbiotic blend shows promise as a therapeutic agent against *E. coli*-induced tissue damage.

Key words: Probiotic; Postbiotic; Inflammatory factor; Gene expression; *Escherichia coli*; Rat

INTRODUCTION

Infections of the digestive system represent a common global health concern. *Salmonella enterica* serovar Typhimurium, *Yersinia enterocolitica*, *Campylobacter jejuni*, and various pathogenic strains of *Escherichia coli* are frequent bacterial pathogens in these infections. The widespread and indiscriminate use of antibiotics has resulted in the emergence of antibiotic-resistant bacterial strains, creating treatment challenges, adverse patient effects, and economic ramifications. *E. coli*, Gram-negative bacilli belonging to the Enterobacteriaceae family, are among the major causative agents (Makvana & Krilov, 2015; Kai et al., 2010).

Dysbiosis of the intestinal microbiota has been linked to inflammatory bowel disease (Nell et al., 2010; Strober et al., 2007). Specifically, the spread of adherent and invasive *E. coli* strains has been connected to Crohn's disease (Lapaquette et al., 2010; Darfeuille-Michaud et al., 2004). Understanding the microbiome is essential for developing methods to effectively manage and prevent disease (Frank & Pace, 2008; Stecher & Hardt, 2008).

The gut-liver axis allows bacterial byproducts to enter the liver and facilitates the entry of harmful bacterial metabolites into the intestine (Chu et al., 2018). Liver illnesses have been linked to gut microorganism translocation, with *Enterococcus gallinarum* transfer to the liver reported in autoimmune hepatitis (Kwiat et al., 2022). Bacterial infections serve as a main cause of liver failure and significantly influence outcomes of hospitalized patients with cirrhosis (Piano et al., 2019; Fernández et al., 2018). Infection frequency in hospitalized cirrhotic patients is believed to be 25-35%, with Gram-negative bacteria accounting for the majority of identified pathogens (Ekpanyapong & Reddy, 2019; Giannelli et al., 2014).

Maintaining gut microbiota balance is critical for protecting health against the negative consequences of antibiotic overuse, stress, irregular eating habits, surgery, and environmental toxins. Consuming functional foods, such as probiotics, can help achieve this (Agarwal et al., 2008). The concept of probiotics originated in the early 1900s when Elie Metchnikoff associated Bulgarian longevity with dairy product consumption (Ramos et al., 2017). Contemporary evidence advocates for probiotics in balanced human diets to prevent microbial infections (Dudek-Wicher et al., 2020; Liu et al., 2018). Postbiotics, or metabiotics, consist of metabolites from living bacteria that stimulate positive immunological and anti-inflammatory responses, with short-chain fatty acids recognized for their antioxidant, anti-inflammatory, and anti-apoptotic properties (Martyniak et al., 2021).

Probiotic bacteria provide protection against gastrointestinal disorders through inhibitory chemical production, competition for binding sites and nutrients, and immune system modulation (Abel et al., 2018). Bacterial cells stimulate inflammatory cytokine production, including TNF-alpha and interleukin-6 (Wu et al., 2020).

The increasing incidence of infectious diseases, rising antibiotic resistance, and known benefits of probiotics and postbiotics in reducing inflammation necessitate further research. No prior studies have investigated the impact of a probiotic and postbiotic blend comprising *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Lactobacillus helveticus* on liver and intestinal tissue infected with *E. coli*. Therefore, this study assessed the effects of this combination on various liver and intestinal segments afflicted with *E. coli* infection in male rats.

MATERIAL AND METHOD

Animal Control and Care

This experimentation followed institutional ethical guidelines and laboratory animal care and use protocols (Ethics Code: IR.IAU.CTB.REC.1401.118). Twenty-eight adult male Wistar rats (200-250 g) were purchased from the Pasteur Institute of Iran and acclimatized for one week at the animal house of Central Tehran Branch, Islamic Azad University. Animals were maintained under standard conditions: 12-hour light/dark cycle, 22±3°C temperature, 70% humidity, with unrestricted access to water and food.

Bacterial Cultures and Growth

Escherichia coli O157:H7 (PTCC 1330) was obtained from the Center of Genetic Resources of Iran and cultured on MacConkey agar and Eosin Methylene Blue (Merck, Germany) at 37°C for 24 hours. Colonies were examined microscopically, macroscopically, and biochemically. Five lactose-positive and two lactose-negative colonies were selected and cultured separately on TSI, Simmons Citrate, and MR-VP media (Sigma-Aldrich, Germany) at 37°C for 24 hours. Confirmed bacteria were cultured in liquid TSB at 37°C for 24 hours, centrifuged (Behdad, Iran) at 2000 rpm for 15-20 minutes, and washed three times with PBS. Sediments were dissolved in 0.5 mL PBS, and absorption was measured at 630 nm with a spectrophotometer (Cecil, UK). OD_{630}=0.5 nm corresponded to 1×10^8 CFU/mL. Bacterial solution was dispensed into 2 mL ampoules, and 1 cc was administered to each rat for three consecutive days (Law, 2000).

Probiotic and Postbiotic Preparation

Probiotic bacteria mixture containing *L. rhamnosus* IBRC-M11322, *L. helveticus* TG-35, and *L. casei* IBC-M10784, and postbiotic mixture containing probiotic bacteria metabolites, were obtained in powder form (Takgen Company, Iran) at 10^9 CFU/mL. One gram of each mixture was dissolved in 9 cc distilled water, and 1 cc was gavaged to each rat for 35 days.

Grouping

Twenty-eight male rats were randomly divided into four groups (n=7):

1. Control group: received water and food daily
2. *E. coli*-infected group: 10^8 CFU/mL
3. *E. coli* + probiotic group: 10^8 CFU/mL + 10^9 CFU/mL probiotics
4. *E. coli* + postbiotic group: 10^8 CFU/mL + 10^9 CFU/mL postbiotics

After 35 days, rats were anesthetized with ketamine and xylazine. Blood sampling from the heart and autopsy of liver and small intestine tissues were performed for histological studies, gene expression, and enzyme assays.

Measurement of Liver Enzymes

Blood samples were centrifuged at 4000 rpm for 10 minutes for serum separation. Liver enzymes (ALT, AST, ALP) were measured using an ADit biochemistry kit and Hitachi autoanalyzer (Campos-Murguía et al., 2020).

Pathological Study of Liver and Small Intestine

Liver and small intestine samples were washed with physiological serum, fixed in 10% formalin, and embedded in paraffin. Tissues were sectioned at 5 µm thickness. Intestinal tissue was stained with Periodic Acid-Schiff (PAS), while liver tissue was stained with Trichrome Mason staining. Sections were examined using a microscope (LAMBOMED, USA) (Scripcariu et al., 2020).

Total Antioxidant Capacity (TAC) Assay

TAC levels were evaluated using a Total Antioxidant Capacity Assay kit (Naxifer, Iran). Approximately 50-100 mg of tissues were weighed, and Lysing Buffer was added at 10 times the tissue weight. Samples were homogenized and centrifuged at 10000 rpm for 10 minutes. Supernatants were separated and analyzed according to the kit protocol. Optical absorption was measured at 593 nm (Pang et al., 2024).

Inflammatory and Apoptotic Gene Expression Analysis

RNA was extracted using the RNX-plus kit (SinaClon, Iran). Total RNA (1000 ng) was reverse transcribed using the Revert AidTM First Strand cDNA Synthesis Kit (Fermentas, USA). Real-time PCR was performed with SYBR green (Amplicon, Denmark) using GAPDH as internal control (Duary et al., 2014). Primer sequences are shown in Table 1. The 2^-ΔΔCT method was used to calculate relative mRNA expression.

Table 1. Primer sequences used in this experiment

Gene	Primer Sequence
TNF-α	Forward: 5'-ACTGAACTTCGGGGTGATTG-3' Reverse: 5'-GCTTGGTGGTTTGCTACGAC-3'
IL-6	Forward: 5'-TGATGGATGCTTCCAAACTG-3' Reverse: 5'-GAGCATTGGAAGTTGGGGTA-3'
BAX	Forward: 5'-CGGCGAATTGGAGATGAACTGG-3' Reverse: 5'-CTAGCAAAGTAGAAGAGGGCAACC-3'
BCL2	Forward: 5'-TGTGGATGACTGACTACCTGAACC-3' Reverse: 5'-CAGCCAGGAGAAATCAAACAGAGG-3'
GAPDH	Forward: 5'-AAGATCCTGACCGAGCGTGG-3' Reverse: 5'-CAGCACTGTGTTGGCATAGAGG-3'

Statistical Analysis

Statistical analysis was performed using SPSS-26 software. One-way ANOVA with Tukey's HSD post-hoc test was used for gene expression comparisons. Results are presented as mean ± standard deviation (SD). Antioxidant and liver enzyme data were analyzed using the Kolmogorov-Smirnov test. Statistical significance was set at $p<0.05$.

RESULTS

Biochemical Identification of *Escherichia coli*

In the TSI medium, glucose and gas production were positive while hydrogen sulfide was negative. Simon citrate and VP tests were negative, while MR was positive, confirming *E. coli* identification.

Histological Observation

Small Intestine: The control group showed no abnormalities in intestinal epithelium or muscle (Figures 1A, 2A). The *E. coli*-infected group demonstrated epithelial destruction, reduced villi height, and inflammatory cell accumulation (Figures 1B, 2B). The probiotic (Figures 1C, 2C) and postbiotic (Figures 1D, 2D) treatment groups showed relative damage repair (Table 2).

Table 2. Comparison of Parameters in Small Intestine Tissue

Parameter	**Control**	***E. coli***	***E. coli* + Probiotic**	***E. coli* + Postbiotic**
Villus Height Change	Normal	Very Severe	Severe	Severe
Muscle Layer Change	Normal	Very Severe	Severe	Severe
Vacuolization	Normal	Very Severe	Severe	Severe
Epithelial Destruction	Normal	Very Severe	Severe	Severe
Inflammatory Cell Accumulation	Normal	Very Severe	Severe	Severe

Liver: The control group showed normal cell structure (Figures 3A, 4A). The *E. coli*-infected group exhibited severe hyperemia, necrosis, vacuolization, and inflammatory cell accumulation compared to treatment groups (Figures 3B-D, 4B-D; Table 3).

Table 3. Comparison of Parameters in Liver Tissue

Parameter	**Control**	***E. coli***	***E. coli* + Probiotic**	***E. coli* + Postbiotic**
Hyperemia	Normal	Very Severe	Severe	Severe
Necrotization	Normal	Very Severe	Severe	Severe
Vacuolization	Normal	Very Severe	Severe	Severe
Inflammatory Cell Accumulation	Normal	Very Severe	Severe	Severe

Liver Enzyme Assay

AST, ALT, and ALP levels were significantly elevated in the *E. coli* group compared to controls ($p<0.001$). Treatment groups showed significant reductions compared to the infected group ($p<0.05$; Figure 5).

Total Antioxidant Capacity (TAC)

Liver TAC significantly decreased in the *E. coli* group ($p<0.001$) and *E. coli* + probiotic group ($p<0.05$) compared to controls. The *E. coli* + postbiotic group showed significant increase compared to controls ($p<0.05$). Both treatment groups showed significant increases compared to the infected group ($p<0.001$). Similar results were observed in small intestine tissues (Figure 6).

Gene Expression Analysis

Inflammatory Genes: *TNF-α* and IL-6 expression significantly increased in the *E. coli* group compared to controls ($p<0.001$), with significant reductions in treatment groups ($p<0.05$-0.01; Figure 7). Similar patterns were observed in both liver and small intestine tissues.

Apoptotic Genes: *Bax* expression significantly increased in the *E. coli* group ($p<0.001$) with significant reductions in treatment groups ($p<0.001$). *Bcl2* expression significantly increased in treatment groups compared to both controls and the infected group ($p<0.01$-0.001; Figure 8).

DISCUSSION

Bacterial infections are a major cause of mortality in clinical settings, exacerbated by emerging resistant strains. While antibiotic treatments aim to cure diseases, they may disrupt beneficial microbial communities and promote resistant strain maintenance. Bacteremia and septicemia carry high mortality rates (Gutiérrez et al., 2016). Although most *E. coli* strains are benign in the intestinal environment, they can cause extraintestinal infections (Yamamoto, 2007).

This study evaluated probiotic (*L. casei*, *L. rhamnosus*, *L. helveticus*) and postbiotic mixtures against *E. coli*-induced damage in male Wistar rats. Histological examination revealed significant liver and intestinal tissue damage in infected animals, with lymphocyte accumulation and microvilli destruction. Treatment groups demonstrated marked improvement.

Liver enzymes (AST, ALT, ALP) were significantly elevated in the *E. coli* group, consistent with previous reports (Shi et al., 2022; Elbaz et al., 2020). TAC levels were significantly reduced in infected tissues, indicating oxidative stress (Portolés et al., 1993). Gene expression analysis revealed elevated inflammatory (*TNF-α*, IL-6) and apoptotic (*Bax*) markers in infected animals, with increased anti-apoptotic (*Bcl2*) expression in treatment groups.

These findings align with previous studies demonstrating *E. coli* pathogenicity through lipopolysaccharide-mediated liver damage (Shi et al., 2022), intestinal barrier disruption (Jia et al., 2018), and inflammatory responses (Mehaisen et al., 2016). Our results support the therapeutic potential of probiotics (Marteau et al., 2002; Gareau et al., 2010; Resta-Lenert &

Barrett, 2003) and postbiotics (Mayorgas et al., 2021) in mitigating tissue damage through antioxidant activity, immune modulation, and regulation of apoptosis.

Probiotic mixtures have shown efficacy in reducing inflammatory markers (Yeşilova et al., 2012) and modulating apoptotic gene expression (Ali Asgari et al., 2017). Postbiotics demonstrate similar benefits while offering improved safety profiles (Mayorgas et al., 2021). The observed tissue protection likely involves multiple mechanisms: antioxidant function, free radical scavenging, immune system modulation, and suppression of inflammatory mediators.

CONCLUSION

This study demonstrates that probiotic and postbiotic combinations protect liver and small intestinal tissues against *E. coli*-induced damage in male rats through multiple mechanisms, including antioxidant activity, immune modulation, and regulation of inflammatory and apoptotic pathways. Further research should investigate specific strain effects, optimal dosages, and treatment durations.

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Data availability

Data and material provide a standardised format.

Ethics approval

The study was approved by the Ethics Committee of the Islamic Azad University of Central Tehran Branch in Iran (Ethics Code: IR.IAU.CTB.REC.1401.118).

Consent for publication

All authors consent for publication.

Acknowledgements

No specific acknowledgements are declared.

Declaration

Conflict of interest

The authors declare no competing interest.

AUTHOR CONTRIBUTIONS

Z.K. and S.H. contributed to conceptualization. Z.K., S.H., and S.S. performed methodology and software. Z.K. curated data and wrote the original draft. Z.K. and S.H. reviewed and edited the manuscript. All authors read and approved the final manuscript.

REFERENCES

Abel, A. M., Yang, C., Thakar, M. S., & Malarkannan, S. (2018). Natural killer cells: development, maturation, and clinical utilization. *Frontiers in Immunology*, 9, 1869.

Agarwal, A., Makker, K., & Sharma, R. (2008). Clinical relevance of oxidative stress in male factor infertility: an update. *American Journal of Reproductive Immunology*, 59 (1), 2-11.

Ali Asgari, E., Mirzaie, A., Noorbazargan, H., & Bagheri Kashtali, A. (2017). Cytotoxicity effect of lactobacillus casei cell extract as indigenous probiotic bacterium on colon cancer cell line (HT29) and analysis of Bax and Bcl2 apoptosis gene expression. *Armaghane Danesh*, 21 (12), 1192-1206.

Campos-Murguía, A., Ruiz-Margáin, A., González-Regueiro, J. A., & Macías-Rodríguez, R. U. (2020). Clinical assessment and management of liver fibrosis in non-alcoholic fatty liver disease. *World Journal of Gastroenterology*, 26 (39), 5919.

Chu, H., Duan, Y., Yang, L., & Schnabl, B. (2018). Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. *Gut*, gutjnl-2018-316307.

Darfeuille-Michaud, A., Boudeau, J., Bulois, P., Neut, C., Glasser, A.-L., Barnich, N., ... & Colombel, J. F. (2004). High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology*, 127 (2), 412-421.

Duary, R. K., Batish, V. K., & Grover, S. (2014). Immunomodulatory activity of two potential probiotic strains in LPS-stimulated HT-29 cells. *Genes & Nutrition*, 9, 1-16.

Dudek-Wicher, R., Junka, A., Paleczny, J., & Bartoszewicz, M. (2020). Clinical trials of probiotic strains in selected disease entities. *International Journal of Microbiology*, 2020.

Ekpanyapong, S., & Reddy, K. R. (2019). Infections in cirrhosis. *Current Treatment Options in Gastroenterology*, 17, 254-270.

Elbaz, H., Hamed, M., Abdelhamid, F., & Abdalla, O. (2020). Effect of cefepime on hematological, immunological and oxidant/antioxidant parameters in rats experimentally infected with Escherichia coli ATCC 25922. *Mansoura Veterinary Medical Journal*, 21 (1), 36-45.

Fernández, J., Acevedo, J., Wiest, R., Gustot, T., Amoros, A., Deulofeu, C., ... & Arroyo, V. (2018). Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. *Gut*, 67 (10), 1870-1880.

Frank, D. N., & Pace, N. R. (2008). Gastrointestinal microbiology enters the metagenomics era. *Current Opinion in Gastroenterology*, 24 (1), 4-10.

Gareau, M. G., Sherman, P. M., & Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology & Hepatology*, 7 (9), 503-514.

Giannelli, V., Di Gregorio, V., Iebba, V., Giusto, M., Schippa, S., Merli, M., & Thalheimer, U. (2014). Microbiota and the gut-liver axis: bacterial translocation, inflammation and infection in cirrhosis. *World Journal of Gastroenterology*, 20 (45), 16795.

Gutiérrez, S., Martínez-Blanco, H., Rodríguez-Aparicio, L., & Ferrero, M. (2016). Effect of fermented broth from lactic acid bacteria on pathogenic bacteria proliferation. *Journal of Dairy Science*, 99 (4), 2654-2665.

Jia, Z., Chen, A., Bao, F., He, M., Gao, S., Xu, J., ... & Zhang, X. (2018). Effect of nisin on microbiome-brain-gut axis neurochemicals by Escherichia coli-induced diarrhea in mice. *Microbial Pathogenesis*, 119, 65-71.

Kai, A., Konishi, N., & Obata, H. (2010). Diarrheagenic Escherichia coli. *Nihon Rinsho*, 68, 203-207.

Kwiat, V. R., Reis, G., Valera, I. C., Parvatiyar, K., & Parvatiyar, M. S. (2022). Autoimmunity as a sequela to obesity and systemic inflammation. *Frontiers in Physiology*, 13, 887702.

Lapaquette, P., Glasser, A. L., Huett, A., Xavier, R. J., & Darfeuille-Michaud, A. (2010). Crohn's disease-associated adherent-invasive Escherichia coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cellular Microbiology*, 12 (1), 99-113.

Law, D. (2000). Virulence factors of Escherichia coli O157 and other Shiga toxin-producing Escherichia coli. *Journal of Applied Microbiology*, 88 (5), 729-745.

Liu, Y., Tran, D. Q., & Rhoads, J. M. (2018). Probiotics in disease prevention and treatment. *The Journal of Clinical Pharmacology*, 58, S164-S179.

Makvana, S., & Krilov, L. R. (2015). Escherichia coli infections. *Pediatrics in Review*, 36 (4), 167-170.

Marteau, P., Seksik, P., & Jian, R. (2002). Probiotics and intestinal health effects: a clinical perspective. *British Journal of Nutrition*, 88 (S1), s51-s57.

Martyniak, A., Medyńska-Przęczek, A., Wędrychowicz, A., Skoczeń, S., & Tomasik, P. J. (2021). Prebiotics, probiotics, synbiotics, paraprobiotics and postbiotic compounds in IBD. *Biomolecules*, 11 (12), 1903.

Mayorgas, A., Dotti, I., & Salas, A. (2021). Microbial metabolites, postbiotics, and intestinal epithelial function. *Molecular Nutrition & Food Research*, 65 (5), 2000188.

Mehaisen, G. M., Eshak, M. G., El Sabry, M., & Abass, A. O. (2016). Expression of inflammatory and cell death program genes and comet DNA damage assay induced by Escherichia coli in layer hens. *PLoS One*, 11 (6), e0158314.

Nell, S., Suerbaum, S., & Josenhans, C. (2010). The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nature Reviews Microbiology*, 8 (8), 564-577.

Pang, X., Wei, X., Wu, Y., Nan, S., Feng, J., Wang, F., ... & Li, J. (2024). Capsaicin Modulates Hepatic and Intestinal Inflammation and Oxidative Stress by Regulating the Colon Microbiota. *Antioxidants*, 13 (8), 942.

Piano, S., Singh, V., Caraceni, P., Maiwall, R., Alessandria, C., Fernandez, J., ... & Angeli, P. (2019). Epidemiology and effects of bacterial infections in patients with cirrhosis worldwide. *Gastroenterology*, 156 (5), 1368-1380.

Portolés, M. T., Ainaga, M. J., & Pagani, R. (1993). The induction of lipid peroxidation by Escherichia coli lipopolysaccharide on rat hepatocytes as an important factor in the etiology of endotoxic liver damage. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1158*(3), 287-292.

Ramos, M., Batista, S., Pires, M., Silva, A., Pereira, L., Saavedra, M., ... & Rema, P. (2017). Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. *Animal*, 11 (8), 1259-1269.

Resta-Lenert, S., & Barrett, K. (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). *Gut*, 52 (7), 988.

Scripcariu, V., Apostol, D. G. C., Dumitrescu, G. F., Turliuc, D., & Sava, A. (2020). Clinical, histopathological and immunohistochemical features of brain metastases originating in colorectal cancer: a series of 27 consecutive cases. *Romanian Journal of Morphology and Embryology*, 61 (1), 81.

Shi, C., Wang, J., Zhang, R., Ishfaq, M., Li, Y., Zhang, R., ... & Li, J. (2022). Dihydromyricetin alleviates Escherichia coli lipopolysaccharide-induced hepatic injury in chickens by inhibiting the NLRP3 inflammasome. *Veterinary Research*, 53 (1), 1-16.

Stecher, B., & Hardt, W. D. (2008). The role of microbiota in infectious disease. *Trends in Microbiology*, 16 (3), 107-114.

Strober, W., Fuss, I., & Mannon, P. (2007). The fundamental basis of inflammatory bowel disease. *The Journal of Clinical Investigation*, 117 (3), 514-521.

Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., ... & Jiang, T. (2020). Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host & Microbe*, 27 (3), 325-328.

Yamamoto, S. (2007). Molecular epidemiology of uropathogenic Escherichia coli. *Journal of Infection and Chemotherapy*, 13 (2), 68-73.

Yeşilova, Y., Çalka, Ö., Akdeniz, N., & Berktaş, M. (2012). Effect of probiotics on the treatment of children with atopic dermatitis. *Annals of Dermatology*, 24 (2), 189-193.

Zhang, Y., Tan, P., Zhao, Y., & Ma, X. (2022). Enterotoxigenic Escherichia coli: Intestinal pathogenesis mechanisms and colonization resistance by gut microbiota. *Gut Microbes*, 14 (1), 2055943.

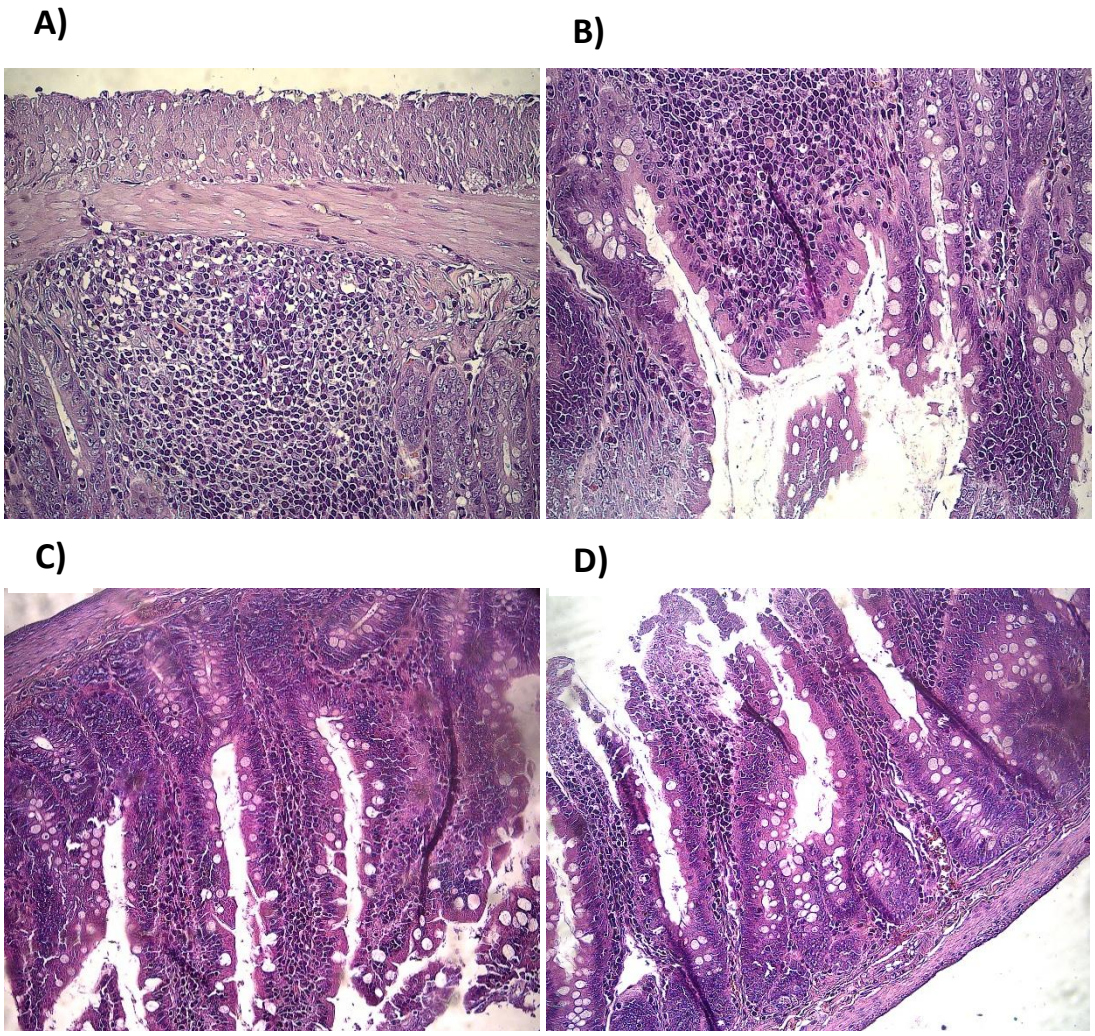


Figure 1. Comparison of microscopic alternations of small intestine tissue in different groups; A: control, B: *Escherichia coli* infected, C: *Escherichia coli* + probiotic receiver, D: *Escherichia coli* + postbiotic receiver (Hematoxylin eosin staining with 40 magnification).

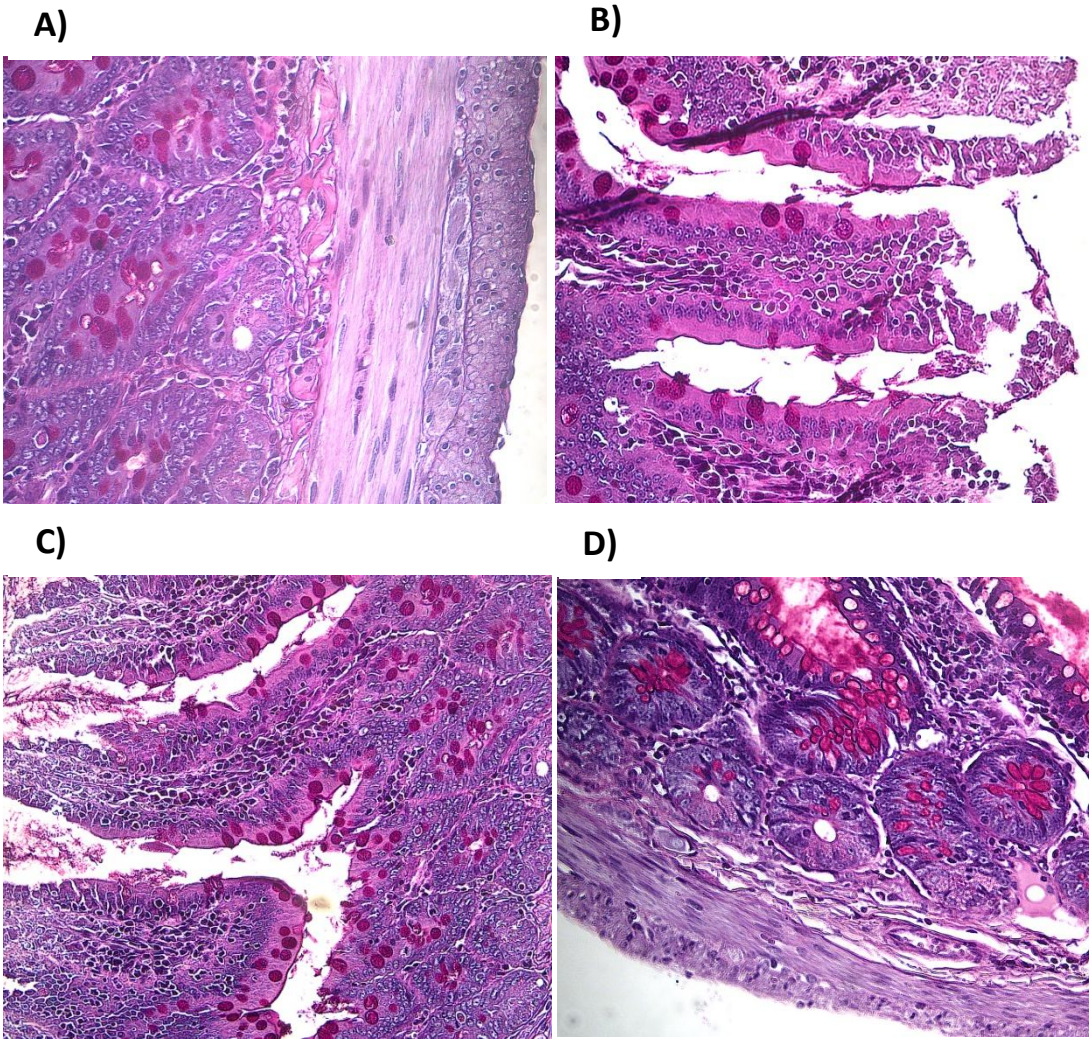
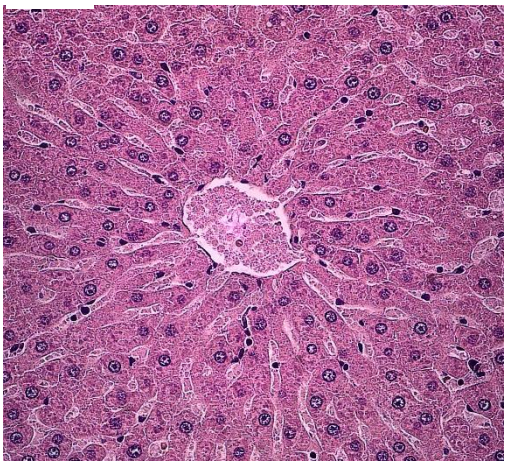
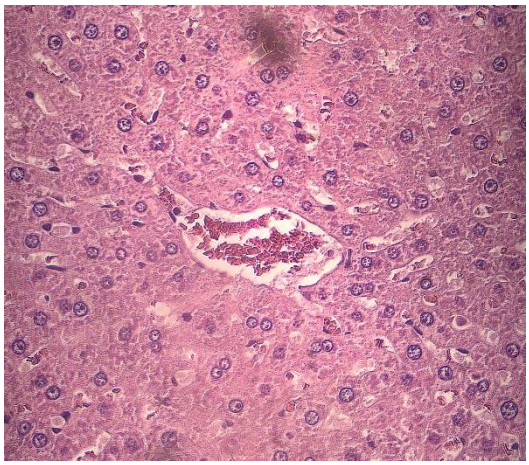


Figure 2. Comparison of microscopic alternations of small intestine tissue in different groups; A: control, B: *Escherichia coli* infected, C: *Escherichia coli* + probiotic receiver, D: *Escherichia coli* + postbiotic receiver (Pas staining with 40 magnification).

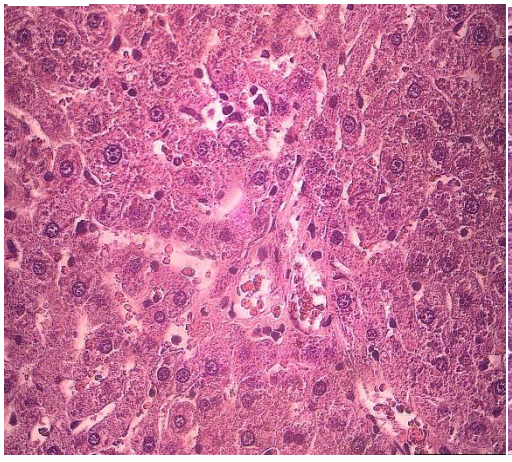
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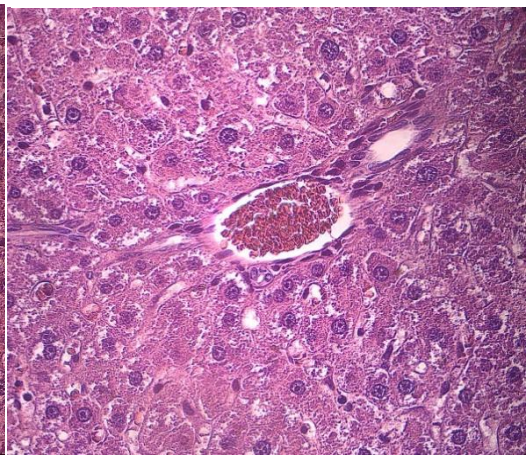


Figure 3. Comparison of microscopic alternations of liver tissue in different groups; A: control, B: *Escherichia coli* infected, C: *Escherichia coli* + probiotic receiver, D: *Escherichia coli* + postbiotic receiver (Hematoxylin eosin staining with 50 magnification).

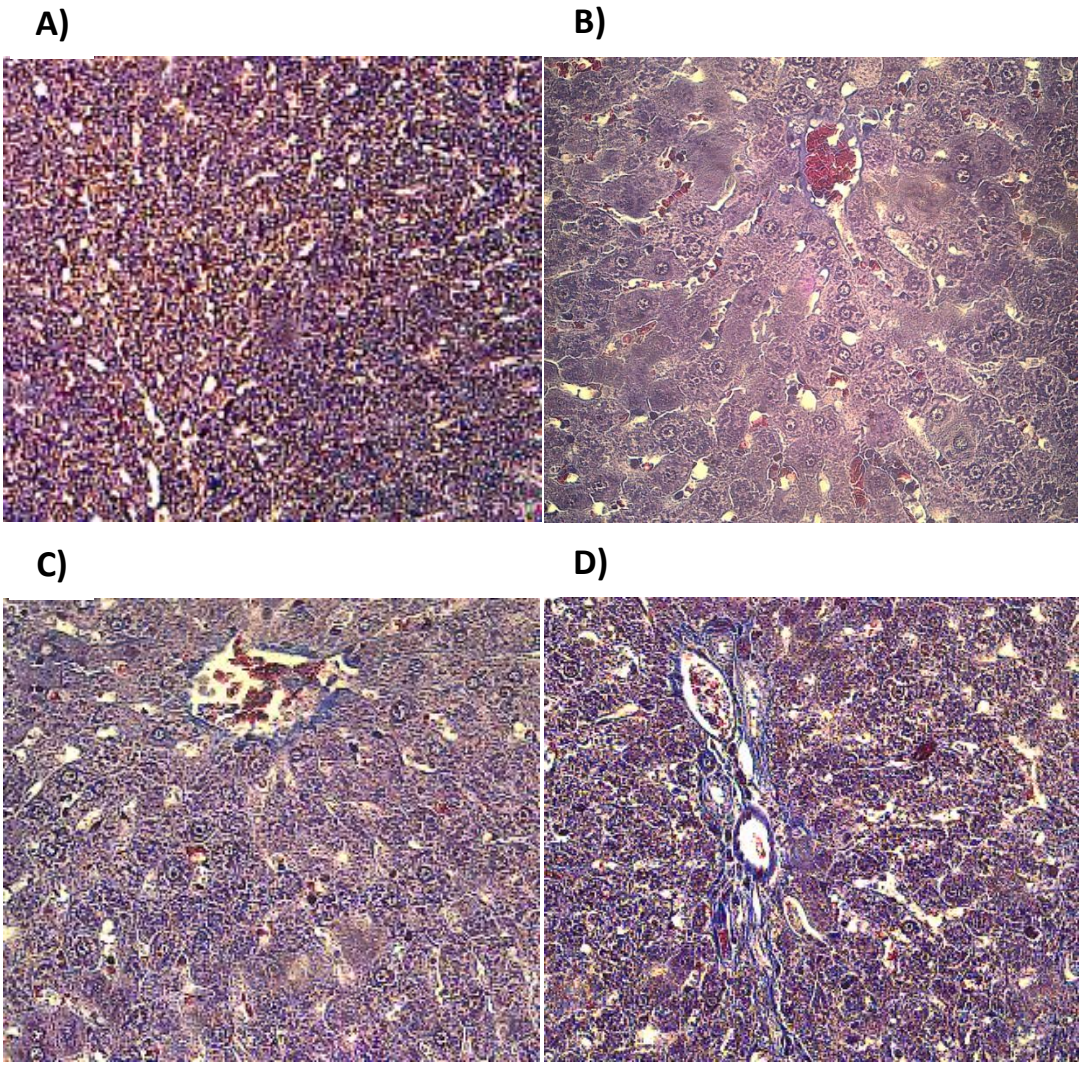


Figure 4. Comparison of microscopic alternations of liver tissue in different groups; A: control, B: *Escherichia coli* infected, C: *Escherichia coli* + probiotic receiver, D: *Escherichia coli* + postbiotic receiver (Trichomason staining with 40 magnification).

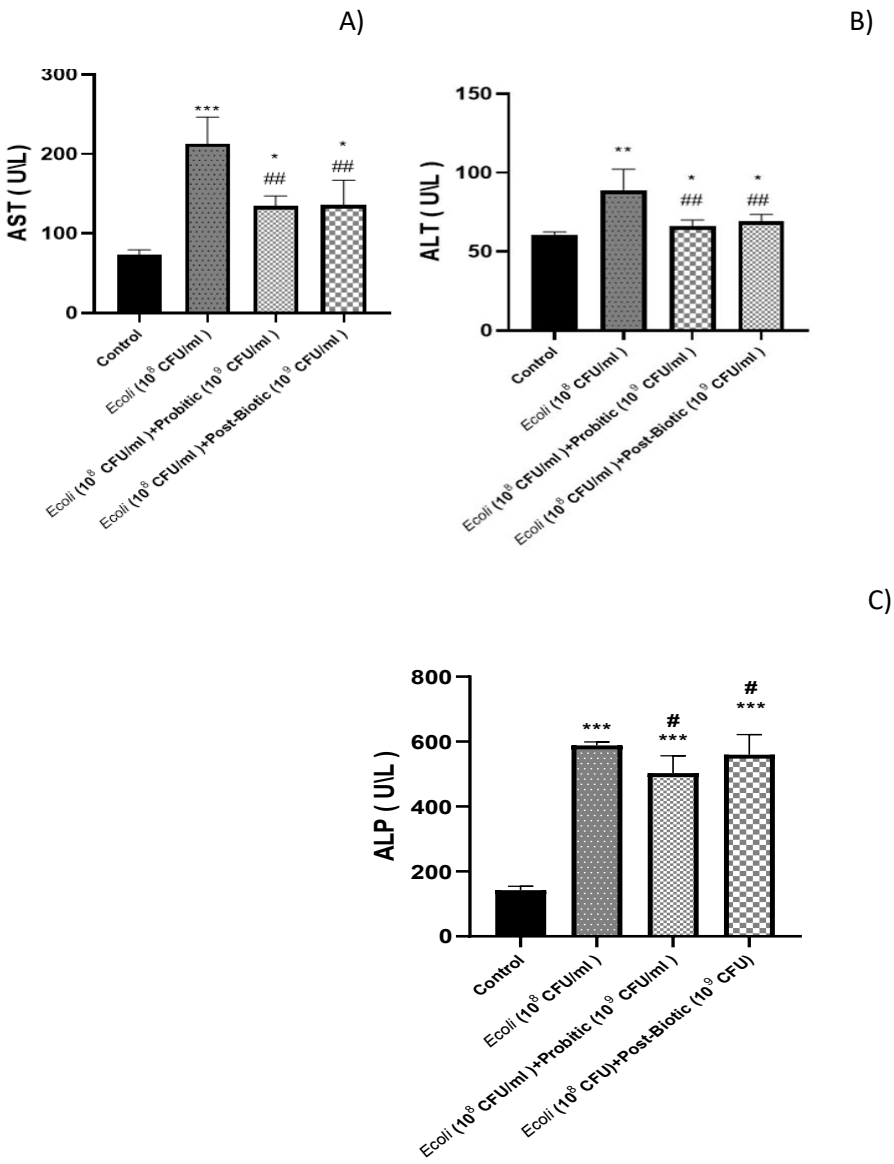


Figure 5. The levels of the liver enzymes A: the level of AST, B: the level of ALT, C: the level of ALP in *(Escherichia coli* + probiotic) and (*Escherichia coli* + postbiotic) compared to *Escherichia coli* and control group.

*: Comparison with the control group , * P<0.05, ** P<0.01, ***P<0.001.

#: Comparison with the infected group, # P<0.05, ## P<0.01

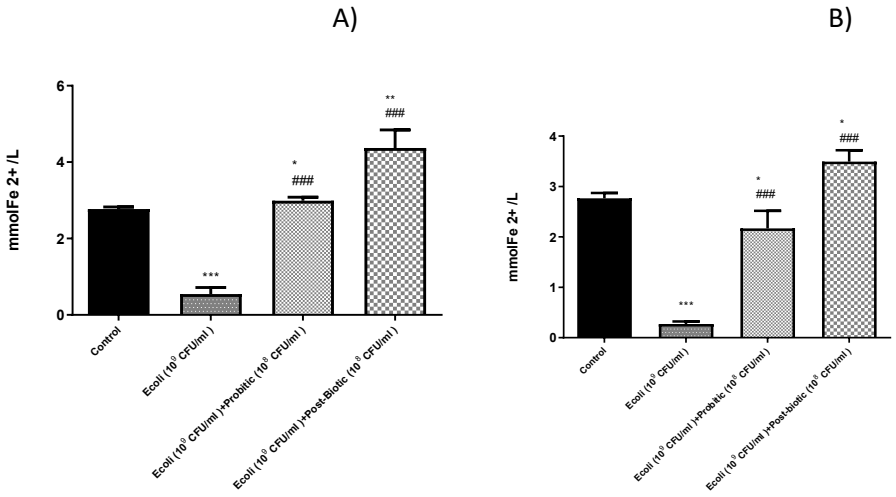
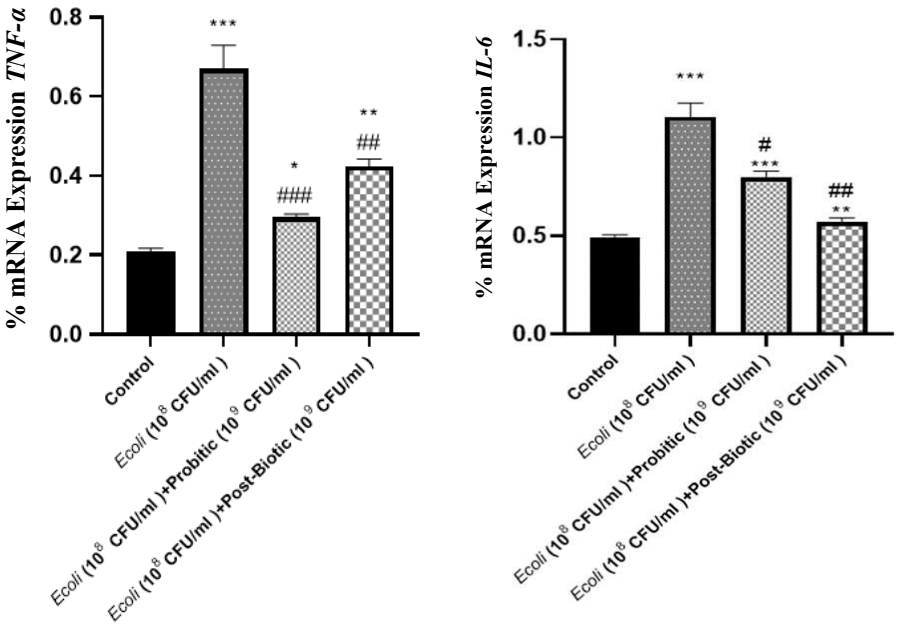


Figure 6. The levels of TAC, A: TAC results related to the liver;B: TAC results related to the small intestine in (*Escherichia coli* + probiotic) and (*Escherichia coli* + postbiotic) compared to *Escherichia coli* and control group.

*: Comparison with the control group , * P<0.05, ** P<0.01, ***P<0.001

#: Comparison with the infected group, ### P<0.001

A) B)



D)

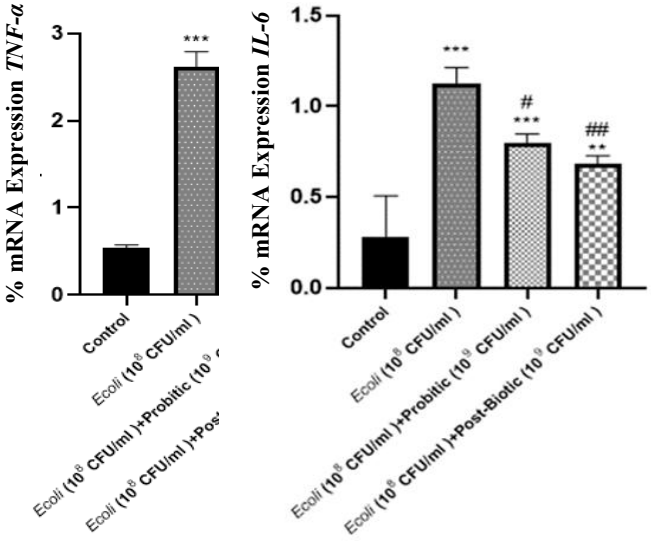


Figure 7. Real time-PCR results for inflammatory factor genes in all groups of male Wistar rats. A: *TNF-α* expression in the liver. B*: IL-6* expression in the liver. C: *TNF-α* expression in the small intestine. D: *IL-6* expression in the small intestine in (*Escherichia coli* + probiotic) and (*Escherichia coli* + postbiotic) compared to *Escherichia coli* and control group.

*: Comparison with the control group, * P<0.05, ** P<0.01, ***P<0.001.

#: Comparison with the infected group, # P<0.05, ## P<0.01

A)

B)

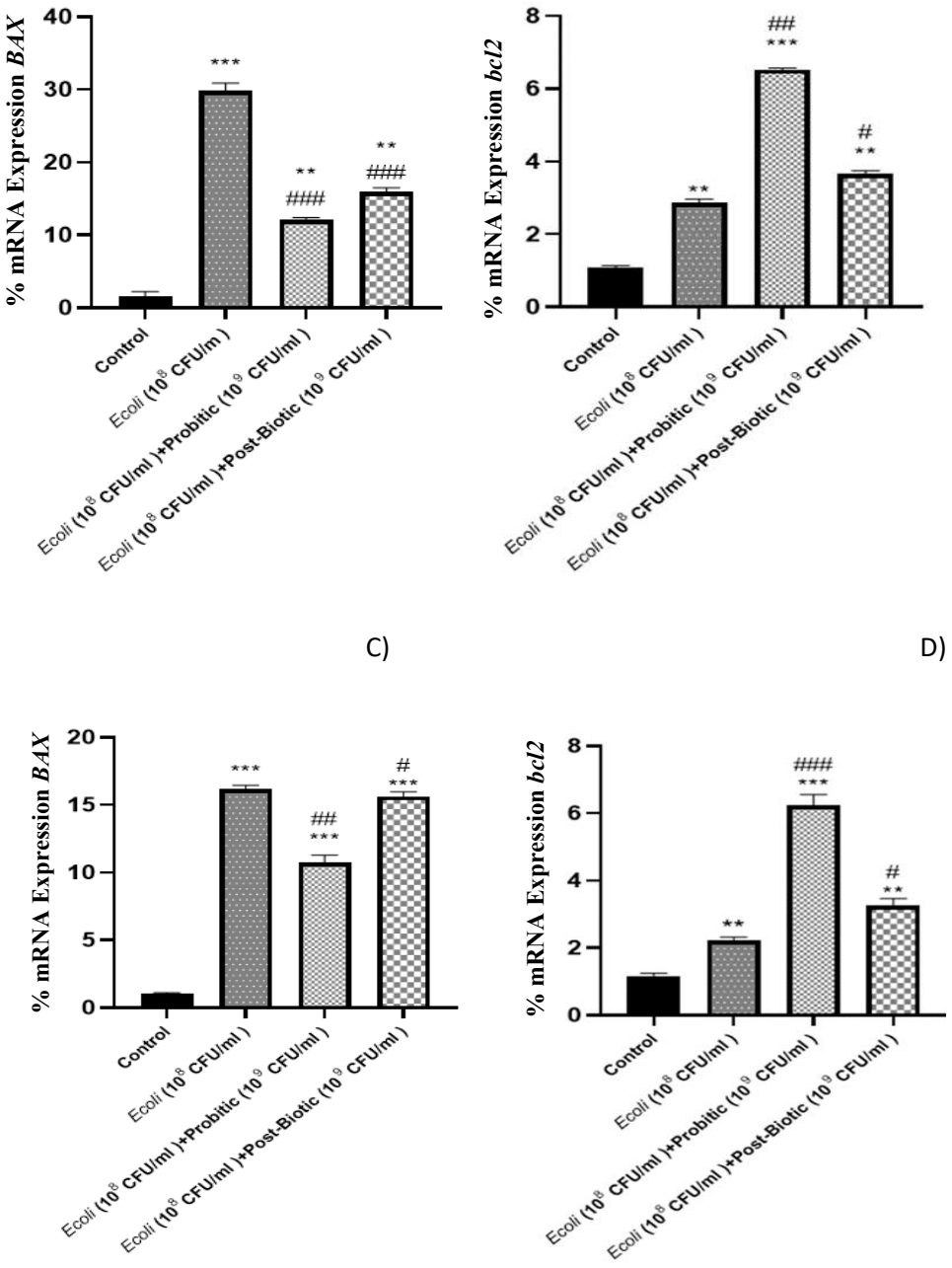


Figure 8. Real time-PCR results for apoptotic genes in all groups of male Wistar rats. A: *Bax* expression in the liver. B: *Bcl2* expression in the liver. C: *Bax* expression in the small intestine. D: *Bcl2* expression in the small intestine in (*Escherichia coli* + probiotic) and *(Escherichia coli* + postbiotic) compared to *Escherichia coli* and control group.

*: Comparison with the control group, ** P<0.01, ***P<0.001.

#: Comparison with the infected group, # P<0.05, ## P<0.01, ### P<0.001