# Effects of Probiotic Use on Bacterial Translocation in Created Rat Models with Biliary Obstructions

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## ABSTRACT

**Objective:** The aim of this study was to investigate the protective effects of the combined use of probiotic strains on the development of bacterial translocation in addition to liver and intestinal tissue damage due to biliary obstruction in rats.

**Materials and Methods:** Here, 3 groups each consisting of 10 rats were created:group 1 (sham group), group 2 (obstructive jaundice), and group 3 (obstructive jaundice+probiotic). Groups 1 and 2 were given 1 cc physiological saline solution by oral gavage twice a day; group 3 was given a probiotic solution that included Lactobacillus acidophilus, *Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium,* and *Bifidobacterium longum* microorganisms by oral gavage twice a day.

**Results:** Markers for liver damage were also found to be significantly improved ($p<0.05$) in the treatment group (group 3). Whencompared with groups 2 and 3 in terms of liver histology, damage was found to be significantly more severe in group 2 ($p<0.01$). With regard to ileal villous depth and ileal inflammation, the pathology was found to be significantly more severe in group 2 than that in group 3 ($p<0.05$). In blood, spleen, and mesenteric lymph node cultures, group 2 showed a microbiological growth rate of 33.8–58.8%, whereas group 3 showed a microbiological growth rate of 14.3–28.6%. This reduction was evaluated to be statistically significant ($p<0.05$).

**Conclusion:** Our study showed that the combined use of a probiotic in bile duct obstructions reduced bacterial translocation and alleviated pathological changes arising in the liver and terminal ileum histology.

**Keywords:** Bacterial translocation, bile duct obstructions, gram-negative microorganism, combined use of probiotic microorganisms, liver damage

## Introduction

Congestion occurring in bile ducts is a life-threatening condition that can be encountered in both children and adults [1]. The interruption of the flow of bile salts into the intestine causes bile accumulation in the liver cells and bile ducts. The absence of bile salts that can inactivate endotoxins in the intestine leads to the development of oxidative damage on the intestinal wall [2]. This condition increases the permeability of the intestinal wall by impairing the structure and functions of the intestinal mucosa [2]. Impaired barrier functions of the intestines decrease the opsonization activity of the humoral immunity and bacteriostatic capacity. Therefore, microorganisms in the intestines pass the mucosal barrier and reach the liver, spleen, and mesenteric lymph nodes in the gastrointestinal tract, thereby disrupting the functions of the immune and reticuloendothelial systems [3-6]. If timely intervention is not exercised, obstructive jaundice can lead to life-threatening clinical pictures due to bacterial translocation [7].

Probiotics, when sufficiently taken, are noninvasive, noncarcinogenic, nonpathogenic, reliable microorganisms that can temporarily colonize the gastrointestinal tract and act against the proliferation of pathogens without impairing the normal flora [8, 9]. Studies have revealed the positive effects of probiotics such as increased intestinal immunity [10], repairing of impaired intestinal mucosal barrier [11], inhibition of translocation of microorganisms [12], elimination of toxins [13], eradication of microbial pathogens [14], and regulation of intestinal functions [15].

The aim of our study is to demonstrate whether multispecies probiotics, which are easily and cheaply available in the market [15], have an improving effect on bacterial translocation caused

by liver and intestinal damage due to biliary obstruction in rats.

## Materials and Methods

### Animals

After receiving the approval of the ethics committee (decision number 40595970/168 dated August 2, 2013), 30 male Wistar albino rats between the weights of 170-230 g were accommodated in a room at a suitable temperature (22±2°C) in cages with nesting material, litter, and cardboard tubes under the standard conditions of 12-h light-dark cycles. The rats fasted according to FELASA guidelines. The rats were allowed to freely access standard feed and water whenever they wished. The animals were not given food the day before the operation, but they had free access to water.

### Experimental Design

Rats were randomly divided into 3 groups, each containing 10 rats, namely, group 1 (sham group), group 2 (obstructive jaundice group), and group 3 (obstructive jaundice+probiotic group). All the surgical procedures were carried out under intramuscular 50 mg/kg Ketalar (ketamine) and 8 mg/kg Rompun (xylazine HCl) anesthesia, which were administered in the supine position through a median incision made under sterile conditions (Figure 1 a-c). Group 1: the main bile duct was released from the surrounding tissues without being tied or incised by laparotomy. Group 2: the main bile duct was tied twice with 4/0 silk and incised from the middle of the tie. Group 3: in addition to the procedure performed in group 2, after intervention, a probiotic solution was administered to the subjects for 7 days. Animals were postoperatively fed orally for 24 h. All the rats were allowed to freely consume a standard diet and water. None of the groups had nutrition restriction. Groups 1 and 2 were given 1 cc physiological saline solution by gavage twice a dayevery 12 h; group 3 was given the prepared probiotic solution by gavage twice a day every 12 h.

A probiotic in the form of a sachet (NBL Probiotic Gold; Nobel medical, Istanbul, Turkey), containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Bifidobacterium longum* microorganisms, was mixed in water until it was homogeneous and then administered. Each probiotic dose given to the rats was adjusted to be 1cc:0.125×10$^9$CFU for 500 mg/kg of body weight [14, 15].

At the end of the 7$^{th}$ day, relaparotomy was performed through the same incision line under anesthesia and sterile conditions. Samples were taken from the blood, mesenteric lymph nodes, and spleen tissues for microbiological examinations. The blood sample was taken from the portal vein for biochemical examination. Tissue samples were taken from the terminal ileum and liver for histopathological evaluation. All the rats were sacrificed under anesthesia.

### Biochemical Analysis

The blood samples were taken into biochemistry tubes with gel. After centrifugation for 5 min at 6500 rpm, the serums were taken and the parameters of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin were studied in a biochemical autoanalyzer (Abbott, ARCHITECT c8000; Illinois, USA) using ultraviolet spectrophotometric, colorimetric, and enzymatic methods.

### Pathological Analysis

Liver and ileumtissue samples were kept in formaldehyde and embedded in paraffin blocks. Then, 3-µm-thick sections were taken and dyed with hematoxylin and eosin stains. Histopathological changes in the liver and terminal ileum were evaluated under a light microscope (Olympus BX50; Tokyo, Japan) in the presence of an experienced pathologist. The photographs of the preparations were taken with an Olympus camera, which was connected to the microscope. Hepatocyte degeneration, bile duct proliferation, microabscess foci, and capsule inflammation for the liver were evaluated. However, with regard to the ileum samples, ileumhemorrhage, ileum inflammation, reduction in villous depth, and villous atrophy were evaluated.

Liver bile duct proliferation was rated using the scoring developed by Karatepe et al., modifying the scoring system of Cheen-Shen et al. [16, 17]. Accordingly, the presence of pathological findings in less than 50% of the portal area was accepted as grade 1.The presence of pathological findings in more than 50% of the portal area was accepted as grade 2. The presence of bridging in the portal area was accepted as grade 3. Hepatocyte degeneration, microabscess focus, and capsular inflammation were graded by means of a semiquantitative evaluation [18]. Grading was labeled as follows. Grade 0: no pathology; grade 1: mild pathology; grade 2: moderate pathology; grade 3: severe pathology.

In the evaluation and classification of terminal ileum damage, scoring devised by Ay et al. was used [19]: epithelial damage overlying the villi and reduction in the villous depth, inflammation in the ileum lamina propria and serosa, and hemorrhage in the ileum were evaluated. For all these specified parameters, a semiquantitative grading system was used: 0 means normal; 1 means mild; 2 means moderate; and 3 means severe.

### Microbiological Analysis

Mesenteric lymph node and spleen samples along with the blood samples taken from a portal vein of the animals in all the groups were brought to the culture laboratory, paying attention to maintaining aseptic conditions. Mesenteric lymph nodes and splenic tissues were separated into small pieces by a sterile scalpel under aseptic conditions. Subsequently, the samples were weighed and placed in 2 mL liquid thioglycollate medium (Oxoid, UK) and then homogenized. Following homogenization, 10µL

**Figure 1. a-c.** (a) Determination of the main bile duct and making a suture through the main bile duct. (b) Bile duct after dissection. (c) After the main bile duct has been cut

of the homogenized samples was inoculated in blood agar (Oxoid, UK) and eosin methylene blue (EMB) agar (Oxoid, UK) in order to investigate the presence of both aerobic and anaerobic Gram-positive and Gram-negative bacterial growth, respectively. The culture plates were incubated for 48 h at 37°C. The samples were incubated in both aerobic and anaerobic environments. In order to create an anaerobic environment, cultured media were placed in a GasPak jar and an oxygen-free environment was provided. The blood samples were inoculated in a BACT/ALERT (bioMérieux, France) hemoculture device in aerobic and anaerobic culture bottles. After being incubated in commercially available culture bottles, the blood samples were incubated for 7 days at 37°C. At the end of the incubation, subcultures were obtained from growth-identified bottles in blood agar and EMB agar and incubated for 48 h at 37°C; subsequently, bacterial identification procedures were initiated. Conventional identification methods, as well as the automated system (VITEK2, bioMérieux), as and when needed, were used for the identification of microorganisms in the cultures detected to have growth.

### Statistical Analysis

The data were evaluated with the Statistical Package for Social Sciences for Windows (SPSS Inc., Chicago, IL, USA) version 18. Chi-squared test was used for the comparison of qualitative data. Independent two-group quantitative data were assessed by independent samples t test in the case of normal distribution and by Mann-Whitney U-test in the absence of normal distribution. The quantitative data of more than 2 independent groups were assessed by one-way ANOVA in the case of normal distribution and by the Kruskal-Wallis test in the absence of normal distribution. If there is statistical significance between the groups in the evaluation of quantitative data, post hoc analyses were performed using Least Significant Differences (LSD) test for normally distributed data.

## Results

### Biochemical Results

ALP, ALT, and AST values were significantly higher in group2 as compared to group 1 (p=0.001 for ALP and ALT; p=0.002 for AST). A statistically significant decrease was found in group 3 when compared to group 2 (p=0.04 for ALP; p=0.032 for ALT; p=0.026 for AST). The total bilirubin and direct bilirubin values were significantly higher in group 2 as compared to group 1 (p=0.013 for total bilirubin; p=0.01 for direct bilirubin), but no statistically significant difference was found between groups 2 and 3 (p=0.083 for total bilirubin; p=0.084 for direct bilirubin). There was no statistically significant difference between the groups in the comparison of GGT values (p>0.05) (Table 1).

### Histopathological Findings

Addressing all the studied parameters, no pathological finding was observed in both the small intestine and liver in group 1. Histological examination of the liver revealed no pathology in group 1 with regard to bile duct proliferation, hepatic degeneration, microabscess presence, and capsular inflammation. When compared with groups 2 and 3, the pathology was found to be significantly severe in group 2 with regard to all the parameters (p<0.01 for all the parameters). With regard to the ileum villous depth, the pathology was found to be significantly more severe in group 2 than that in group 3 (p=0.003). With regard to ileum inflammation, the pathology was found to be significantly higher in group 2 than that in group 3 (p=0.001). There was no significant difference between group 2 and group 3 (p=0.07) with regard to ileum hemorrhage (Figure 2 a–j ).

### Microbiological Findings

It was found that *Escherichia coli*was the most commonly isolated bacteria in the microbiological study, followed by *Staphylococcus spp.* and *Klebsiella* pneumonia. Percentage values were calculated for each different tissue by the ratio of the number of samples with bacteriological growth to the total number of cultures. Significant growth was found in the blood cultures of group 2 as compared to those of groups 1 and 3. There was no significant difference between group 1 and group 3(1 and 2: p<0.001;1 and 3: p>0.05;2 and 3: p<0.01).There was significant growth in the spleen cultures of group 2 as compared to those of groups 1 and 3. There was no significant difference between group 1 and group 3(1 and 2: p<0.01;1 and 3: p>0.05;2 and 3: p<0.05). In the mesenteric lymph node cultures, there was significant growth in group 2 as compared to those of groups 1 and 3. There was no significant difference between groups 1 and 3(1 and 2: p<0.001;1 and 3: p>0.05) (Table 2).

## Discussion

In patients with obstructive jaundice, the accumulation of bile salts in hepatocytes and sepsis resulting from bacterial translocation can cause damage to hepatocytes [20]. When compared with groups 2 and 3 with regard to ALT, ALP, and AST values, the biochemical markers of liver damage [21] were found to be significantly improved in the probiotic treatment group (i.e., group 3). Although GGT elevation in the blood usually indicates a liver problem, it may be indicative of other health problems, too. Its elevation along with other liver enzymes suggests that the GGT source is the liver. When compared with groups 2 and 3 with regard to GGT and bilirubin values, a marginal improvement was found, but it was not

**Table 1.** Levels of ALP, ALT, AST, DBIL, TBIL, and GGT (mean±SD)

| | | | | p *(Group Comparison)* | | |
|---|---|---|---|---|---|---|
| Parameters | Group 1 | Group 2 | Group 3 | *1-2* | *2-3* | *1-3* |
| ALP *(U/L)* | 124±28.1 | 349±61.5 | 259±101 | 0.001 | 0.004 | 0.04 |
| ALT (u/l) | 53.8±9.4 | 222±110 | 123.1±71 | 0.01 | 0.032 | 0.122 |
| AST *(U/L)* | 187±24.8 | 628±314 | 354±153 | 0.02 | 0.026 | 0.152 |
| DBIL *(µmol/L)* | 0.1±0.01 | 5.02±4.8 | 2.01±1.8 | 0.01 | 0.084 | 0.259 |
| TBIL *(µmol/L)* | 0.1±0.01 | 8.43±8.6 | 3.08±3.1 | 0.013 | 0.083 | 0.318 |
| GGT *(U/L)* | 2.17±1.1 | 6.5±7.2 | 5.29±4.6 | >0.05 | >0.05 | >0.05 |
| Blood Culture | 8.3% | 33.3% | 14.3% | <0.001 | <0.001 | >0.05 |
| MLN Culture | 1.7% | 58.3% | 21.4% | <0.001 | 0.01 | >0.05 |
| Spleen Culture | 3.3% | 50% | 28.6% | <0.001 | 0.04 | >0.05 |

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; GGT: Gamma-Glutamyl Transpeptidase; TBIL: Total Bilirubin; DBIL: Direct Bilirubin; MLN: Mesenteric Lymph Node. Continuous variablesare represented as mean±SD, while the others are represented as percentages.

**Table 2.** Bacterial growth rates in culture samples

| | Group 1 (%) | Group 2 (%) | Group 3 (%) | p (1 and 2) | p (2 and 3) | p (1 and 3) |
|---|---|---|---|---|---|---|
| Blood culture | 8.3 | 33.3 | 14.3 | <0.001 | <0.001 | >0.05 |
| Mesenteric lymph node culture | 1.67 | 58.3 | 21.4 | <0.001 | 0.01 | >0.05 |
| Spleen culture | 3.33 | 50 | 28.6 | <0.001 | 0.04 | >0.05 |

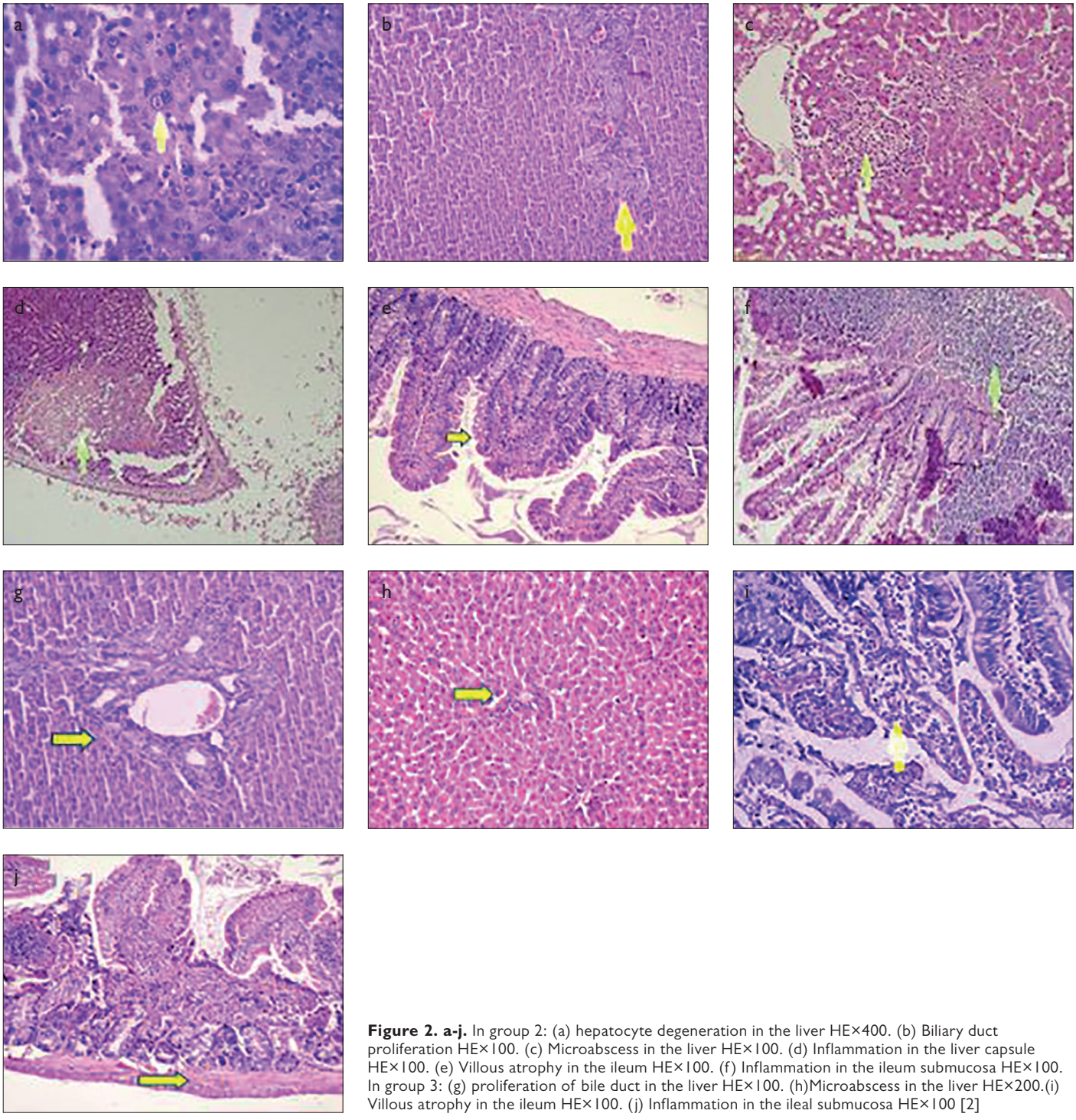


**Figure 2. a-j.** In group 2: (a) hepatocyte degeneration in the liver HE×400. (b) Biliary duct proliferation HE×100. (c) Microabscess in the liver HE×100. (d) Inflammation in the liver capsule HE×100. (e) Villous atrophy in the ileum HE×100. (f) Inflammation in the ileum submucosa HE×100. In group 3: (g) proliferation of bile duct in the liver HE×100. (h)Microabscess in the liver HE×200.(i) Villous atrophy in the ileum HE×100. (j) Inflammation in the ileal submucosa HE×100 [2]

statistically significant. These results are consistent with the results reported in the literature [22].

In the literature, several studies have shown that probiotic microorganisms have an anti-inflammatory effect and reduced inflammation [23, 24]. It was observed that the use of *Lactobacillus plantarum* decreased intestinal epithelial apoptosis and oxidative stress, while simultaneously strengthening intercellular adhesion supporting the mucosal integrity; further, the use of glutamine and probiotics reduced bacterial translocation. Improvementswere found in all the biochemical, pathological, and microbiological findings in rats that underwent choledoch ligation [25-27]. There was no pathology in group I in our study.

In accordance with the literature, a significant difference was found in group 2 as compared to group 3. The highest histopathological changes (hepatocyte degeneration, bile duct proliferation, microabscess foci, and capsule inflammation were evaluated in the liver samples, while ileal hemorrhage, ileal inflammation, reduction in villous depth, and villous atrophy were evaluated in the ileum samples)were evident in group 2, which also showed the highest bacterial translocation, suggesting that pathology was caused by endotoxemia. In biliary obstruction, endotoxemia may be due to the decreased clearance of portal endotoxins in biliary obstruction or increased endotoxin absorption from the gastrointestinal tract. Moreover, it is believed that the absence of bile salts resulting in the inactivation of endotoxin in the intestine causes the activation of endotoxin in portal circulation, thereby forming the basis for bacterial translocation [26-28].

There are experimental studies showing that probiotics reduce bacterial translocation [15, 16, 23, 24]. In some studies, probiotics have been observed to stimulate the immunity and increase IgA, providing resistance to pathogens such as viruses, *Clostridium*, and *E. coli* [27].It was observed that cecal colonization was significantly less in the group treated with *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus bulgaricus* in rats with tied bile ducts [28]; further, hepatic encephalopathy was less common due to decreased pH values in the stomach, jejunum, ileum, and colon in rats that were given probiotics [29].After giving *Lactobacillus*, rats underwent small intestine resection: the intestinal immunity of the probiotic-administered group was stable [30]. In our study in which we investigated the effects of combined probiotic (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Bifidobacterium longum*) microorganisms on rats with biliary obstructions, there was significant growth in the microbiological blood, spleen, and mesenteric lymph node cultures of group 2 as compared to those of group 1. In group 3, a reduction was observed in the growth rate and statistical analysis performed showed a significant difference. These results supported the view that the source of bacterial infection in obstructive jaundice is the gastrointestinal system [20, 30]. We assume that the cause of no growth in the cultures of group 1 is the affected intestinal flora due to surgical stress [31].

This study has certain limitations. First, we have limited cases with many stronger outcomes. This was because the ethical committee of animal experiments requested to limit the number of experimental animals to be used in the study. Second, the molecular study could not be done because of cost and lack of funding.

In conclusion, the use of probiotics in rat models with obstructive jaundice resulted in improvement in biochemical parameters, significantly reduced pathology in the liver and terminal ileum, and reduced bacterial translocation in mesenteric lymph node, spleen, and blood cultures. Therefore, cheap and easy to find probiotics [15] have been observed to have a protective effect in obstructive jaundice pathologies that are still associated with high mortality and morbidity.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Hatay Mustafa Kemal University by the decision number 40595970/168 and dated 02.08.2013.

**Informed Consent:** N/A.

**Peer-review:** Externally peer-reviewed.

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

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## References


1. Su CH, P'Eng F K, Lui WY. Factors affecting morbidity and mortality in biliary tract surgery. World J Surg 1992; 16: 536-40. [CrossRef]
2. Parks RW, Clements WD, Pope C, Halliday MI, Rowlands BJ, Diamond T. Bacterial translocation and gut microflora in obstructive jaundice. J Anat 1996; 189: 561-5.
3. Atıcı A, Karaman A, Zendroglu A, et al. Factors affecting mortality in stage 3b necrotizing enterocolitis. Turk J Pediatr 2014; 56: 133-7.
4. Sakrak O, Akpinar M, Bedirli A, Akyurek N, Aritas Y. Short and long-term effects of bacterial translocation due to obstructive jaundice on liver damage. Hepatogastroenterology 2003; 50: 1542-6
5. Alexander JW, Boyce ST, Babcock GF, et al. Theprocess of microbialtranslocation. Ann Surg 1990; 212: 496-510. [CrossRef]
6. Barber AE, Jones WG 2nd, Minei JP, et al. Bacterial over growth and in testinalatrophy in the etiology of gut barrier failure in the rat. Am J Surg 1991; 161: 300-4. [CrossRef]
7. Greig JD, Krukowski ZH, Matheson NA. Surgical morbidity and mortality in one hundred and twenty-nine patients with obstructive jaundice. Br J Surg 1988; 75: 216-9. [CrossRef]
8. El Ç, Satar M, Yıldızdaş HY, Özlü F, Asker HS. Evaluation of influence of Bifidobacterium lactis and Hindiba inulin on feeding intolerance and weight gain in premature babies. Cukurova Med J 2017; 42: 419-26. [CrossRef]
9. Verschuere L, Rombaut, G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbiol Mol Biol Rev 2000; 64: 655-671. [CrossRef]
10. Ko JS, Yang HR, Chang JY, Seo JK. Lactobacillus plantarum inhibits epithelial barrier dysfunction and interleukin-8 secretion induced by tumor necrosis factor-alpha. World J Gastroenterol 2007; 13: 1962-5. [CrossRef]
11. Zhou YK, Qin HL, Zhang M, et al. Effects of Lactobacillusplantarum on gut barrier function in experimental obstructive jaundice. World J Gastroenterol 2012; 18: 3977-91. [CrossRef]
12. Moorthy G, Murali MR, Devaraj SN. Lactobacilli facilitate maintenance of intestinal membrane integrity during Shigella dysenteriae 1 infection in rats. Nutrition 2009; 25: 350-8. [CrossRef]
13. Lindfors K, Blomqvist T, Juuti-Uusitalo K, et al. Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. Clin Exp Immunol 2008; 152: 552-8. [CrossRef]
14. Huang Y, Kotula L, Adams MC. The in vivo assessment of safety and gastrointestinal survival of an orally administered novel probiotic, Propionibacterium jensenii 702, in a male Wistar rat model. Food Chem Toxicol 2003; 41: 1781-7. [CrossRef]
15. Taylor RS. Probiotics to prevent necrotizing enterocolitis: Too cheap and easy? Paediatr Child Health 2014; 19: 351-2. [CrossRef]
16. Sheen-Chen SM, Hung KS, Ho HT, Chen WJ, Eng HL. Effect of glutamine and bile acid on hepatocyte apoptosis after bile duct ligation in the rat. World J Surg 2004; 28: 457-60. [CrossRef]
17. Karatepe O, Acet E, Battal M, et al. Effects of glutamine and curcumin on bacterial translocation in jaundiced rats. World J Gastroenterol 2010; 16: 4313-20. [CrossRef]
18. Yi ET, Liu RX, Wen Y, Yin CH. Telmisartan attenuates hepatic fibrosis in bile duct-ligated rats. Acta Pharmacol Sin 2012; 33: 1518-24. [CrossRef]
19. Tuştaş Ay B, Polat A, Tamer L, Büyükafşar K, Aksöyek S. The effect of inhibition of high mobility group box-1 and tumor necrosis factor alpha on intestinal morphology and motility in rats with peritonitis. Çocuk Cerrahisi Dergisi 2008; 22: 15-24.
20. Abdeldayem H, Ghoneim E, Refaei AA, Abou-Gabal A. Obstructive jaundice promotes intestinal-barrier dysfunction and bacterial translocation: experimental study. Hepatol Int 2007; 1: 444-8. [CrossRef]
21. Knight JA. Liver function tests: their role in the diagnosis of hepatobiliary diseases. J Infus Nurs 2005; 28: 108-17. [CrossRef]
22. White JS, Hoper M, Parks RW, Clements WD, Diamond T, Bengmark S. The probiotic bacterium Lactobacillus plantarum species 299 reduces intestinal permeability in experimental biliary obstruction. Lett Appl Microbiol 2006; 42: 19-23. [CrossRef]
23. Moore FA. The role of the gastrointestinal tract in postinjury multiple organ failure. Am J Surg 1999; 178: 449-53. [CrossRef]
24. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infantis to neonates in an intensive care unit. Int J Infect Dis 1999; 3: 197-202. [CrossRef]
25. Archer AC, Muthukumar SP, Halami PM. Anti-inflammatory potential of probiotic Lactobacillus spp. on carrageenan induced paw edema in Wistar rats. Int J Biol Macromol 2015; 81: 530-7. [CrossRef]
26. Holma R, Salmenpera P, Lohi J, Vapaatalo H, Korpela R. Effects of Lactobacillus rhamnosus GG and Lactobacillus reuteri R2LC on acetic acid-induced colitis in rats. Scand J Gastroenterol 2001; 36: 630-5. [CrossRef]

27. Geyik MF, Aldemir M, Hosoglu S, et al. The effects of Saccharomyces boulardii on bacterial translocation in rats with obstructive jaundice. Ann R Coll Surg Engl 2006; 88: 176-80. [CrossRef]
28. Yücel D, Oğuz M, Yüksel O, Yılmaz G, Paşaoğlu ÖT, Sultan N. The Effect of Glutamine and Probiotics Use on Intestinal Barrier, Bacterial Translocation, Hepatocyte Translocation, Oxidative Apoptosis, and Kupffer Cell Function in Rats with Obstructive Jaundice. Eur J Surg Sci 2014; 5: 10-9.
29. Jijon H, Backer J, Diaz H, et al. DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology 2004; 126: 1358-73. [CrossRef]
30. Sarac F, Salman T, Gun F, et al. Effect of probiotic supplementation on bacterial translocation in common bile duct obstruction. Pediatr Surg Int 2015; 31: 155-61. [CrossRef]
31. Jiang SM, Jia L, Zhang MH. Probiotic and lactulose: influence on gastrointestinal flora and pH value in minimal hepatic encephalopathy rats. Int J Clin Exp Med 2015; 8: 9996-10000.