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

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

Results: The markers of liver damage, were also found to be significantly improved (p<0.05) in the treatment group (group 3). Compared with group 2 and group 3 in terms of liver histology, the damage was found to be significantly more severe in the group 2 (p <0.01). From the point of ileal villous depth and ileal inflammation parameters, the pathology was found to be significantly more severe in the group 2 than in the group 3 (p <0.05). In blood, spleen and MLN cultures, the group 2 showed a microbiological growth rate of 33.8% to 58.8%, whereas the group 3 showed a microbiological growth rate of 14.3% to 28.6%. This reduction was evaluated to be statistically significant (p< 0.05).

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

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

INTRODUCTION

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Congestion that may occur in bile ducts is a life-threatening condition that can be encountered in both the children and adult age groups [1]. 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 inactivating 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 the bacteriostatic capacity, thereby, the microorganisms in the intestinal, pass over the mucosal barrier and reach the liver, spleen and mesenteric lymph nodes in the gastrointestinal tract, as a result, the functions of the immune system and the reticuloendothelial system disrupt [3-6]. If not timely intervened, obstructive jaundice leads to life-threatening clinical pictures due to bacterial translocation [7].

Probiotics, when taken sufficiently, are non-invasive, non-carcinogenic, non-pathogenic, reliable microorganisms that can temporarily colonize in the gastrointestinal tract and act against the proliferation of pathogens without impairing normal flora [8,9]. The conducted studies have shown positive effects of probiotics such as increased intestinal immunity [10], repairing of impaired intestinal mucosal barrier [11], and 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 the multispecies probiotic which can be easily and cheaply supplied on 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 by the decision number 40595970/168, dated 02.08.2013, 30 male Wistar albino rats between the weights of 170-230 gr were accommodated in a room with suitable room temperature (22±2°C), in cages with nesting material, litter, and cardboard tubes, under standard conditions of 12-hour light/dark cycles. The rats fasted according to FELASA guidelines. The rats were allowed to freely access standard feed and...
water whenever they wish. The animals were not given food the day before the operation but freely accessed the water.

**Experimental Design**

The rats were randomly divided into 3 groups each containing 10 rats. Group 1 (sham group), Group 2 (obstructive jaundice group), Group 3 (obstructive jaundice + probiotic group). All surgical procedures were carried out under intramuscular 50 mg/kg Ketalar (Ketamine) and 8 mg/kg Rompun (Xylazine HCl) anesthesia, in the supine position through a median incision made under sterile conditions (Figure 1). 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 the intervention, the probiotic solution was given to the subjects for 7 days. Animals fed oral-way for 24 hours postoperatively. All rats were allowed to freely reach the standard diet and water. None of the groups had nutrition restriction. The group 1 and group 2 were given 1 cc physiological saline solution by gavage twice a day, every 12 hours the group 3 was given the prepared probiotic solution by gavage twice a day every 12 hours.

A probiotic in the form of the sachet (Nbl probiotic gold), containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Bifidobacterium longum* microorganisms, was mixed into the water until homogeneous and then given. Each probiotic dose to be given to rats was adjusted to be 1cc:0.125x10⁹CFU, 500 mg/kg of body weight [14,15].

At the end of the 7th day, re-laparotomy was performed through the same incision line under anesthesia and sterile conditions. Samples were taken from the blood, mesenteric lymph nodes and spleen tissue for microbiological examinations, a 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 were sacrificed under anesthesia.
Biochemical Analysis

The blood samples were taken into biochemistry tubes with gel. After centrifugation for 5 minutes at 6500 rpm, the sera were taken and the parameters of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total bilirubin were studied in the biochemical auto-analyzer (Abbott, architect c8000, Illinois, U.S.A) using ultraviolet spectrophotometric, colorimetric and enzymatic methods.

Pathological Analysis

Liver and ileum tissue samples were kept in formaldehyde and embedded in paraffin blocks. Sections of 3-micron thickness were taken and dyed with hematoxylin and eosin stain. Histopathological changes in the liver and terminal ileum were evaluated under a light microscope (Olympus BX50) in the presence of an experienced pathologist. The photographs of the preparations were taken with an Olympus camera, which was connected to the microscope. The parameters of hepatocyte degeneration, bile duct proliferation, microabscess foci, and capsule inflammation were evaluated in the liver. Whereas, in ileum samples, the parameters of ileumhemorrhage, ileum inflammation, reduction in the villous depth and villous atrophy were evaluated.

The 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 semiquantitative evaluation [18]. Grading was labelled as following; Grade 0: no pathology, Grade 1: mild, grade 2: moderate and grade 3: severe pathology.

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

Mesenteric lymph node and spleen samples with blood samples taken from a portal vein of the animals in all groups were brought to the culture laboratory, paying attention to aseptic conditions. Mesenteric lymph nodes and splenic tissues were separated into small pieces by sterile scalpel under aseptic conditions. Following, the samples were weighed and placed in 2 ml of liquid thioglycollate medium (Oxoid, UK) and homogenized. Following homogenization process, 10μl of the homogenized samples were inoculated in blood agar (Oxoid, UK), 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. Culture plates were incubated for 48 hours 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 taken were inoculated in Back Alert (Biomerieux, 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 incubation, subculturing were performed from growth-identified bottles in blood agar and EMB agar and incubated for 48 h at 37°C, and bacterial identification procedures were started. Conventional identification methods, and when the needed automated system (Vitek-2, Biomerieux), were used for the identification of microorganisms in the cultures detected to have growth.

Statistical Analysis

The data were evaluated with Statistical Pack for Social Science for windows (SPSS Inc., Chicago, IL, USA) version 18. Chi-square 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, by Mann Whitney-U Test in the absence of normal distribution. The quantitative data of more than two independent groups were assessed by OneWay ANOVA in the case of normal distribution and by Kruskal Wallis Test in the absence of normal distribution. In the
RESULTS

Biochemical Results

ALP, ALT and AST values were significantly higher in the group 2 compared to the group 1 (p=0.001 for ALP and ALT, p=0.002 for AST). A statistically significant decrease was found in the group 3 compared to the group 2 (p=0.04 for ALP, p=0.032 for ALT, p=0.026 for AST). Total bilirubin and direct bilirubin values were significantly higher in group 2 compared to group 1 (p=0.013 for total bilirubin and p = 0.01 for direct bilirubin) but no statistically significant difference was found between the group 2 and group 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 parameters studied, no pathological finding was observed in both small intestine and liver in the group 1. Histological examination of the liver revealed no pathology in the group 1 in the parameters of bile duct proliferation, hepatic degeneration, the presence of microabscess and capsular inflammation. Compared with group 2 and group 3, the pathology was found to be significantly severe in the group 2 in all parameters (p<0.01 in all parameters). From the point of ileum villous depth, the pathology was found to be significantly more severe in the group 2 than in the group 3 (p=0.003). In terms of ileum inflammation, the pathology was found to be significantly higher in the group 2 than in the group 3 (p=0.001). There was no significant difference between group 2 and group 3 (p=0.07) in terms of ileumhemorrhage (Figure 2).

Microbiological Findings

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It was found that Escherichia coli were 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. A significant growth was found in the blood cultures of group 2 compared to that of group 1 and group 3. There was no significant difference between the group 1 and group 3. (P value of II and I: p <0.001, P value of III and I: p >0.05, P value of II and III: p <0.01). There was a significant growth in the spleen cultures of the group 2 compared to that of the group 1 and the group 3. There was no significant difference between the group 1 and group 3. (P value of I and II: p <0.01, P value of I and III: p >0.05, P value of II and III: p <0.05). In the mesenteric lymph node cultures, there was a significant growth in the group two compared to that of group 1 and group 3. There was no significant difference between the group 1 and group 3. (P value of I and II: p <0.001, P value of I and III: p >0.05) (Table 2).

DISCUSSION

In patients with obstructive jaundice, the accumulation of bile salts in hepatocytes and sepsis resulting from bacterial translocation cause damage to hepatocytes [20]. Compared with the group 2 and group 3 in terms of ALT, ALP, AST values, the biochemical markers of liver damage [21], were found to be significantly improved in the probiotic treatment group. Although GGT elevation in the blood usually indicates a liver problem, it may be indicative of other health problems. Its elevation with other liver enzymes suggests that the GGT source is liver. Compared with group 2 and group 3 in terms of GGT and bilirubin values, a little improvement was found, but it was not statistically significant. These results are consistent with the results reported in the literature [22].

In the literature, many 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, at the same time strengthened the intercellular adhesion supporting mucosal integrity, and that the use of glutamine and probiotic reduced bacterial translocation, and that an improvement was found in all of the

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In accordance with the literature; a significant difference was found in group 2 compared to group 3. Observing the highest histopathologic changes (The parameters of hepatocyte degeneration, bile duct proliferation, microabscess foci, and capsule inflammation were evaluated in the liver samples while the parameters of ileal hemorrhage, ileal inflammation, reduction in the villous depth and villous atrophy were evaluated in the ileum samples) in the group 2, which also showed the highest bacterial translocation, suggests that pathology is caused by endotoxemia. In biliary obstruction, endotoxemia may be due to the decreased clearance of portal endotoxin in biliary obstruction or increased endotoxin absorption from the gastrointestinal tract. Moreover, it is believed that the absence of bile salts inactivating endotoxin in the intestine causes the activation of endotoxin in the portal circulation, thereby forming a 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, E. coli [28]. It was observed that cecal colonization was significantly less in the group treated with Lactobacillus acidophilus, Bifidobacterium bifidum, and Lactobacillus bulgaricus in the rats with tied bile ducts [29], and that hepatic encephalopathy was less common due to decreased pH values of the stomach, jejunum, ileum, and colon in rats given probiotic [30], and that by giving Lactobacillus to the rats underwent small intestine resection, the intestinal immunity of the probiotic-given group was stable [31]. 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 obstruction; there was a significant growth in the microbiologic blood culture, spleen and mesenteric lymph node cultures of group 2 compared to that of the group 1. In group 3, a reduction was provided in the growth rate and the 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 [20,31].
think that the cause of no growth in the cultures of group 1 is the affected intestinal flora due to surgical stress [32].

We have some limitations in the study first, we have limited cases to may 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 costly, and lack of funding to support.

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

References


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Table 1. Levels of ALP, ALT, AST, D.BIL, T. BIL, GGT (mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P value (Group Comparison)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>124±28.1</td>
<td>349±61.5</td>
<td>259±101</td>
<td>0,001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>53.8±9.4</td>
<td>222±110</td>
<td>123.1±71</td>
<td>0,01</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>187±24.8</td>
<td>628±314</td>
<td>354±153</td>
<td>0,02</td>
</tr>
<tr>
<td>DBIL (μmol/L)</td>
<td>0.1±0.01</td>
<td>5.02±4.8</td>
<td>2.01±1.8</td>
<td>0,01</td>
</tr>
<tr>
<td>TBIL (μmol/L)</td>
<td>0.1±0.01</td>
<td>8.43±8.6</td>
<td>3.08±3.1</td>
<td>0,013</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>2.17±1.1</td>
<td>6.5±7.2</td>
<td>5.29±4.6</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>Blood Culture</td>
<td>8,3%</td>
<td>33,3%</td>
<td>14,3%</td>
<td>&lt;0,001, &lt;0,001, &gt;0,05</td>
</tr>
<tr>
<td>MLN Culture</td>
<td>1,7%</td>
<td>58,3%</td>
<td>21,4%</td>
<td>&lt;0,001, 0,01, &gt;0,05</td>
</tr>
<tr>
<td>Spleen Culture</td>
<td>3,3%</td>
<td>50%</td>
<td>28,6%</td>
<td>&lt;0,001, 0,04, &gt;0,05</td>
</tr>
</tbody>
</table>

Abbreviations - ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; GGT, Gamma-Glutamyl Transpeptidase; TBIL, Total Bilirubin; DBIL, Direct Bilirubin; MLN, Mesenteric Lymph Node. Continues variables are presented as the means ± SD, while the others presented as percent.

Table 1: Bacterial growth rates in the culture samples

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
<th>Group 3 (%)</th>
<th>P (1 and 2)</th>
<th>P (2 and 3)</th>
<th>P (1 and 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>8,3</td>
<td>33,3</td>
<td>14,3</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>1,67</td>
<td>58,3</td>
<td>21,4</td>
<td>&lt;0,001</td>
<td>0,01</td>
<td>&gt;0,05</td>
</tr>
<tr>
<td>Spleen culture</td>
<td>3,33</td>
<td>50</td>
<td>28,6</td>
<td>&lt;0,001</td>
<td>0,04</td>
<td>&gt;0,05</td>
</tr>
</tbody>
</table>

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Figure Legends

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

**Figure 2.** a-j. In the group 2; Hepatocyte degeneration in the liver HE X 400 (a). Biliary duct proliferation HE X 100 (b). Micro abscess in the liver HE X 100 (c). Inflammation in the liver capsule HE X 100 (d). Villous atrophy in the ileum HE X 100 (e). Inflammation in the ileum submucosa HE X 100 (f). In the group 3; Proliferation of bile duct in the liver HE x 100 (g). Micro abscess in the liver HE X200 (h). Villous atrophy in the ileum HE X 100 (i). Inflammation in the ileal submucosa HE X 100 (j).[2]