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The effects of pomegranate on bacterial translocation in rats with obstructive jaundice

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Abstract. – BACKGROUND: Obstructive jaundice may promote bacterial overgrowth and altered intestinal barrier function, with resultant increased bacterial translocation.

AIMS: This study aimed to evaluate potential effects of pomegranate on bacterial translocation after bile duct ligation in rats.

MATERIALS AND METHODS: Wistar albino rats were randomized into four groups. Group 1 underwent sham operation; Group 2 underwent sham operation and simultaneous treatment with pomegranate; Group 3 underwent common bile duct ligation, and Group 4 underwent common bile duct ligation and simultaneous treatment with pomegranate. After 8 days, the samples of systemic blood, liver, spleen and mesenteric lymph nodes (MLNs) were obtained under sterile conditions for microbiological culture. The segments of the ileum were removed for histopathological examination.

RESULTS: Bacterial translocation significantly decreased in Group 4 compared to Group 3 (p = 0.007). The bacterial counts (Colony forming unit: CFU/g) of Group 3 were significantly higher than those of Groups 1, 2 and 4 ($p < 0.05$). The mean ileal villus heights in the Groups 1, 2, 3 and 4 were 480.5±20.5 µm, 494.7±17.3 µm, 356.3±25.7 µm and 420.7±23.7 µm, respectively. The mean villus height in Group 4 was higher than that of Group 3 (p = 0.010).

CONCLUSIONS: Pomegranate has significant protective effects on intestinal mucosa barrier in obstructive jaundice and reduces bacterial translocation.

Key Words:
Pomegranate, Bacterial translocation, Ileal villus heights, Obstructive jaundice.

Introduction

Obstructive jaundice is associated with high morbidity and mortality. Despite the improvement in surgical procedures and development of potent antibiotics, septic complication after surgical operation is still a major cause of the high mortality rate in patients with obstructive jaundice[1]. Clinical and experimental works suggest that bacterial translocation is the major source of bacteria implicated in the pathogenesis of endotoxemia, sepsis, and multiorgan failure during cholestasis[2,3].

Increased intestinal permeability and bacterial translocation were demonstrated following both experimental biliary obstruction and in jaundiced patients. Bacterial translocation is defined as the passage of viable indigenous bacteria from the gastrointestinal tract to extraintestinal sites, such as the mesenteric-lymph-node complex, liver, spleen and bloodstream. Three major mechanisms promote bacterial translocation: intestinal bacterial overgrowth, deficiencies in host immune defenses, and increased permeability or damage of the intestinal mucosal barrier[4,5].

Bile does not flow into the intestines during obstructive jaundice; thus, anti-oxidant and anti-infective substances such as bile salts, bile pigments, and phospholipids cannot be used by the intestinal mucosa, and the increased number of bacteria in the intestines prepares a base for translocation of bacteria and their products. This increased intestinal permeability has been postulated to be a key factor contributing to bacterial and endotoxin translocation to mesenteric lymph nodes (MLNs), portal circulation, and the liver. Obstructive jaundice is a cause of sepsis and multiple organ failure due to translocations, cholangitis, decreased number and clearance capacity of Kupffer cells, impaired filtration function of the liver, endotoxemia, impaired host defense, bacterial overgrowth, consecutive release of proinflammatory cytokines, renal, cardiovascular and hepatic complications, coagulation defects, gastrointestinal bleeding, mucos-

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al damage and delayed wound healing, potentially leading to the development of the so-called "gut derived sepsis"[6,7]. Lipopolysaccharides, which are normally cleared by Kupffer cells (cells 80% responsible for the body's host defense mechanisms) in the healthy liver, cannot be cleared in jaundice, resulting in endotoxemia and multiorgan failure[8].

Punica granatum Linn. (Punicaceae), commonly known as pomegranate, is a shrub or a small tree, native to the Mediterranean region. *Punica granatum* flower is consumed worldwide as in Turkey and is a popular beverage. A number of biological activities such as antioxidant, anti-inflammatory, antibacterial antitumour, antidiarrhoeal, antifungal and antiulcer activities have been reported with various extracts/constituents of different parts of this plant[9-12]. Pomegranate flavonoids can exert antiinflammatory effects such as restriction of low stimuli activation of inflammatory processes[13]. In addition, *Punica granatum* is now gaining importance because of its potent antioxidant activity. *Punica granatum* L. have been highlighted in many studies as having antimicrobial activity against a range of both Gram positive and negative bacteria[14].

According to our review of the literature, this is the first *in vivo* study of the relationship between pomegranate and the development of bacterial translocation following obstructive jaundice in rats. The aim of this study was to investigate the effect of pomegranate treatment on preventing bacterial translocation in an obstructive jaundice animal model.

Materials and Methods

Animals

This experiment was approved by the Animal Ethics Committee of Dicle University and performed following standard guidelines for the Care and Use of Laboratory Animals. A total of 40, eight-week-old male Wistar albino rats weighing about 250 g were included in this study. The animals were housed under constant temperature (21° ± 2°C) and humidity, with 12-h dark/light cycles and allowed tap water and rat pellets *ad libitum* before and after the operation.

Experimental Design

The animals were randomised into 4 groups with 10 rats in each. All the surgical procedures were performed while the rats were under intraperitoneal ketamine 50 mg/kg (Ketalar, Parke-Davis, Istanbul) and xylazine HCl 10 mg/kg (Rompun®; Bayer AG, Leverkusen, Germany) anesthesia. All the operations were performed under sterile conditions. After midline abdominal incision, the common bile duct was identified and mobilized. It was then doubly ligated using 5-0 silk and divided. Sham-operated group rats had a similar incision followed by mobilization of the common bile duct, without ligation or division. Then, the abdominal wall was closed with 2-0 silk continuous sutures. Relaparotomy was performed on the 8th day.

The rats were divided in to four groups: Group 1 – sham operated group; Group 2 – drug control group (Pomella®, Verdure Sciences, Noblesville, IN, USA; 250 mg/kg/day); Group 3 – common bile duct ligation (CBDL) group; Group 4 – treatment group (Pomella®, Verdure Sciences, Noblesville, IN, USA; 250 mg/kg/day). The pomegranate was given respectively for an 8-day period as a single dose per day via temporary orogastric intubation. There was no death in any of the groups after 8 days. Eight days following the obstructive jaundice development, the animals had laparatomy under sterile conditions. The samples of systemic blood, liver, spleen and mesenteric lymph nodes (MLNs) were obtained under sterile conditions for microbiological culture. The segments of the ileum were removed for histopathological examination.

Microbiologic Evaluation

Blood samples were obtained from the portal vein and cultured aerobically and anaerobically using the BacTec™ Peds battles (Becton-Dickinson Diagnostic Inc., Sparks, MD, USA). The blood cultures were continuously monitored for 7 days. Positive cultures were plated out on blood agar, chocolate agar, eosin methylene blue (EMB) agar, or Sabouraud-dextrose agar. Identification was realized, by the Sceptor microdilution method. At the same time, MLNs, spleen and the right lobe of the liver were removed and placed in sterile glass bottles containing sterile brain-heart infusion media. The bottles were re-weighed and tissue homogenates were prepared in 2 ml brain-heart infusion using a sterile mortar and pestle. A portion (0.1 ml) of each homogenate was cultured on blood agar, chocolate agar, EMB agar and Sabouraud-dextrose agar. All the plates were examined after 24 h and 48 h of incubation at 37°C. Individual colonies were identified and quantified as

colony-forming units (CFUs) per g tissue. The results of all the CFUs were averaged and expressed as mean Log10.

Histopathologic Evaluation

One cm long portions were harvested from the terminal ileum. The intestinal lumen was carefully cannulated and gently washed with 10% formalin. The samples were fixed in 10% formalin for histopathological examination by light microscopy. Sections of 4 μm were cut and stained with hematoxylin and eosin (H&E) and were eveluated by an independent observer blinded to the experimental protocol.

Biochemical Evaluation

The sera of the blood samples were obtained by centrifugation and stored at –80ºC until analysis. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP) activities were determined by kinetic reaction, and total bilirubin (TB) and direct bilirubin (DB) levels were determined by end point reaction, which were measured in all the serum samples using a Autoanalyzer Hitachi 717 (Hitachi, Tokyo, Japan) by spectrophotometer method in the Biochemistry Laboratory of Dicle University Medical School.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 11.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as the arithmetic mean±SD. The differences among groups regarding continuous data were evaluated by Kruskal Wallis variance analysis. When the p-value of the Kruskal-Wallis test statistics was statistically significant, Mann-Whitney U test was used to determine which groups differed from each other. Nominal data were analyzed by Chi-square or Fisher's exact test, where applicable. Differences were considered as statistically significant at the level of $p < 0.05$.

Results

The predominant bacteria obtained from the blood, liver, spleen, and MLNs samples were *Escherichia coli* (40%), *Proteus mirabilis* (30%), *Klebsiella* spp. (10%), *Pseudomonas aeruginosa* (10%), *Enterococcus faecalis*(10%). The effects of the treatment on bacterial translocation are presented in Figure 1. No bacterial translocation was seen in groups 1 and 2. Bacterial translocation rates were 80% in Group 3 and 20% in Group 4. The bacterial translocation significantly decreased in Group 4 compared to Group 3 ($p = 0.007$). The bacterial counts (CFU/g) of Group 3 were significantly higher than those of Groups 1, 2 and 4 ($p < 0.05$).

The serum bilirubin levels, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were significantly higher in Groups 3 and 4 compared to Group 1 and 2 ($p < 0.05$). However, there were no statistically significant differences between Groups 3 and 4 (Table I).

The mean ileal villus height in Groups 1 was 480.5±20.5 µm, in Group 2 was 494.7±17.3 µm, in Group 3 was 356.3±25.7 µm and in Group 4 was 420.7±23.7 µm. The mean villus height in Group 4 was significantly higher than that of Group 3 ($p = 0.010$).

In the sham group and drug control group, no histopathological changes were observed (Figures 2 A and D). However, partial or subtotal villous atrophy and pronounced increases in neutrophils and lymphocytes in the lamina propria were observed in the CBDL Group (Figure 2 B). The villi were reduced in height (Figure 2 D). There were many bacteria on the surface of the epithelium and many of them were embedded in the epithelium (Figures B and C). The treatment group generally showed nearly normal histology. In some areas, partial villous atrophy, subepithelial edema, dilatation of the mucosal lymphatics, and intraepithelial lymphocyte migrations were observed, but the epithelium was generally intact

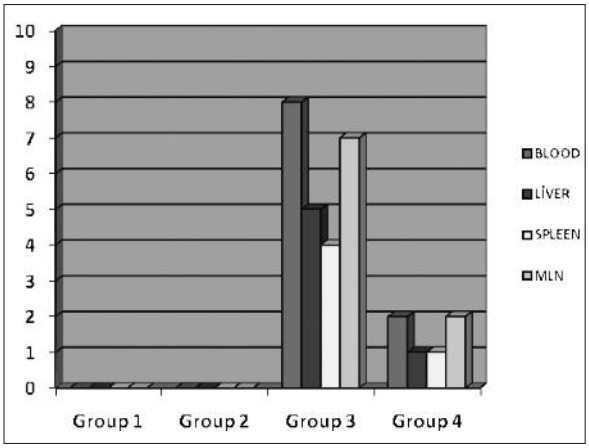

Figure 1. The number of rats with bacterial translocation for each group.

Table I. Results of biochemical parameters following the intervention (mean ± SD and n = 10).

Parameters	Groups			
	Sham (group 1)	Drug Control (group 2)	CBDL (group 3)	Treatment (group 4)
TB (mg/dL)	0.37 ± 0.13	0.32 ± 0.07	6.8 ± 1.95[a,c]	6.70 ± 1.49[a,c]
DB (mg/dL)	0.24 ± 0.12	0.19 ± 0.06	5.75 ± 1.77[a,c]	5.65 ± 1.47[a,c]
ALP (IU/L)	69.70 ± 13.49	65.80 ± 14.20	190.7 ± 42.96[a,c]	171.1 ± 36.14[a,c]
GGT (IU/L)	19.10 ± 4.48	18.10 ± 4.18	36.10 ± 10.46[b,d]	29.60 ± 8.72[f,d]
ALT (IU/L)	50.10 ± 5.41	62.90 ± 26.79	100.6 ± 28.72[a,e]	97.30 ± 19.14[a,g]
AST (IU/L)	92.00 ± 7.72	92.90 ± 12.56	233.3 ± 88.02[a,c]	229.5 ± 66.81[a,c]

[a]$p = 0.000$; [b]$p = 0.001$; [f]$p = 0.003$ versus sham group; [c]$p = 0.000$, [d]$p = 0.001$, [e]$p = 0.017$; [g]$p = 0.008$ versus drug control group; TB: Total bilirubin; DB: Direct bilirubin; ALP: Alkaline phosphatase GGT: Gamma glutamyl transferase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

with only a few areas of minimal epithelial damage (Figure 2 E). Bacteria located on the surface of the epithelium was rarely detected.

Discussion

Infectious complications such as biliary sepsis, wound infections, intra-abdominal abscess formation, and renal failure frequently occur during obstructive jaundice[5]. Bacterial translocation from the intestinal mucosal barrier implicated in the pathophysiology of complications has been associated with obstructive jaundice[15,16].

Bacterial translocation is described as living or dead microorganisms and endotoxins passing over intestinal mucosal epithelium, then through the lamina propria and mesenteric lymph nodes

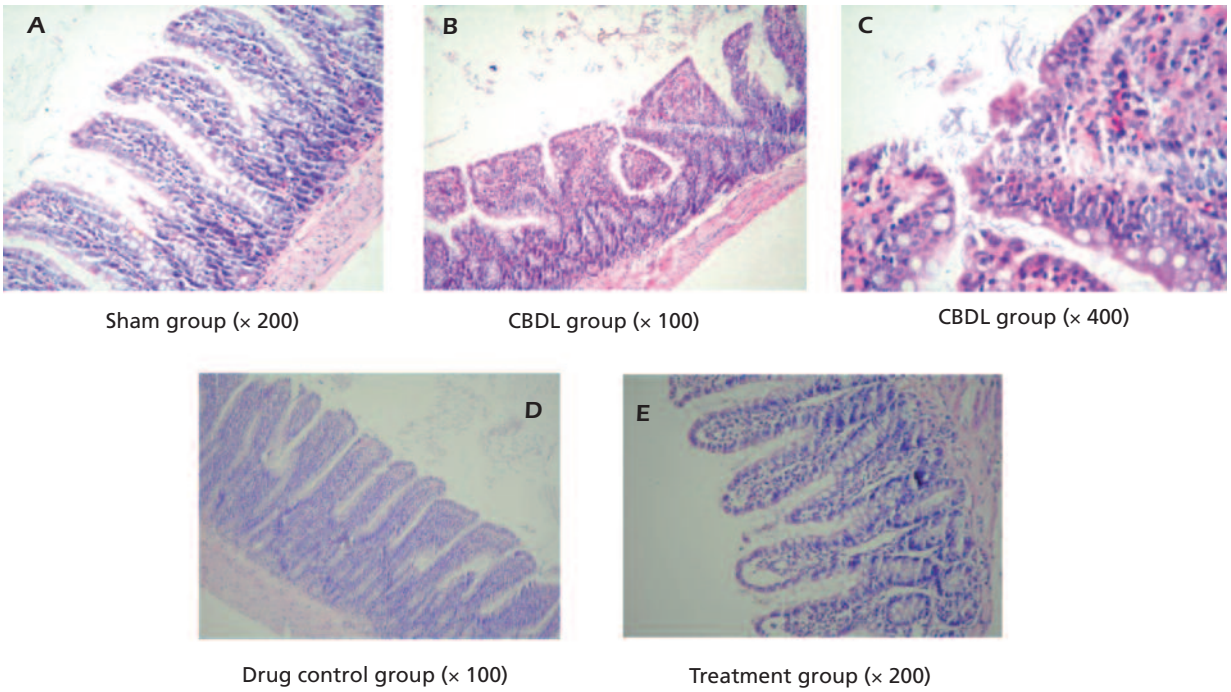

Figure 2. ***A-D,*** (Sham Group and Drug Control Group, respectively). Mucosa is normal in histological appearance. (H&E stain, × 200). ***B-C,*** (CBDL Group). Subtotal villous atrophy is evident ***(B)***. The lamina propria is infiltrated by lymphocytes and many bacteria on the surface of the epithelium, many of which embedded in the epithelium are seen ***(C)*** (H&E stain, × 400) ***E,*** (Treatment group). Nearly normal histology is observed. The epithelium is intact. There are no bacteria on the surface of the epithelium. Subepithelial edema, dilatation of the mucosal lymphatics, and lymphocyte infiltration of the lamina propria are seen (H&E stain, 200).

and spreading to other tissues[17,18]. It is believed that the disturbance of balance between intestinal microflora and the host defense mechanisms (mucosal barrier, immunologic defense, gastric acid, gastrointestinal motility) are the main factors affecting bacterial translocation. Bile duct obstruction and cholestasis cause bacterial translocation via depression in intestinal barrier function, immune system deficiency and phagocytic mononuclear function damage[19].

We showed a significant increase in the frequency of bacterial translocation in the MLN, liver, spleen and blood and significant reduction in the mean villus height in CBDL group. The increase in bacterial translocation after common bile duct ligation may be due to ileal mucosal damage presenting as a reduction in villus height. We also showed a significant decrease in the bacterial translocation and increasing mean villus height in the treatment group. The mean bacterial counts in the MLN, spleen and liver of the treatment group were significantly lower than those in CBDL group. The most commonly encountered pathogens in the MLN, liver and spleen were *E. Coli, E. cloacae*, *Klebsiella* spp. *Pseudomonas* spp. and *Proteus* spp.

Many authors have studied the effects of different drugs on preventing bacterial translocation in animal models of obstructive jaundice and demonstrated that a combination of antibiotics and immunosuppressive drugs promotes the systemic spread of bacterial translocation, resulting in lethal sepsis[20]. Bacterial colonization or infection of the intestine by bacteria such as *Escherichia, Clostridium*, *Klebsiella*, *Salmonella*, *Shigella*, *Campylobacter*, *Pseudomonas*, *Streptococcus*, *Enterococcus*, *Staphylococcus aureus*, and coagulase-negative staphylococci increases the risk of necrotising enterocolitis[21]. Bile salts are known to inhibit the growth of intestinal bacteria and may contribute to the regulation of the indigenous gut microbiota. Absence of intraluminal bile salts and their anti-endotoxic effects may result in overgrowth of bacteria[22].

Besides endotoxaemia, bacterial translocation from the gut is also considered to be an underlying mechanism of septic complications in obstructive jaundice. The luminal flow of bile salts has antibacterial effects and a direct detergent effect on endotoxins, and the increased absorption of endotoxin in obstructive jaundice has been suggested to be associated with the absence of bile salts in the small intestine. Returning bile to the gastrointestinal lumen has been assumed to be of benefit in animal models and has reduced postoperative endotoxaemia, renal impairment, and mortality[23,24]. Internal biliary drainage has also been considered to be important in the recovery of mononuclear phagocyte function[25,26]. Bacterial translocation after obstructive jaundice may be due to the inhibition of bile salts. Antibacterial and antioxidant effects of pomegranate may tolerate the absence of bile salts. Bacterial translocation after obstructive jaundice may be due to the inhibition of bile salts, reduction of villus height, or disruption of the ecological balance of the normal indigenous microbiota.

A number of biological activities such as antioxidant, antiinflammatory, antibacterial, antifungal, antidiarrheal, and antiulcer activities have been reported with various extracts/constituents of different parts of this plant[9-12,27]. Pomegranate's control of inflammation involves inhibition of both cyclooxygenase and lipoxygenase enzymes and a decline in prostaglandin release from cells[28,29]. Since pomegranate's antioxidative efficacy clinically may be impaired by poor bioavailability of active compounds. In such conditions, strengths and weaknesses of pomegranate's antioxidant activity need be considered[30,31]. In addition to these studies, other *in vivo* and clinical findings have been suggested as stemming from antioxidant effects. Examples of *in vivo* studies of beneficial effects of pomegranate's antioxidant activity include: protection of rat gastric mucosa from ethanol or aspirin toxicity, protection of neonatal rat brain from hypoxia, prevention of male rabbit erectile tissue dysfunction, and abrogation of ferric nitrilotriacetate induced hepatotoxicity evidenced by miitigated hepatic lipid peroxidation. Moreover, the actions of glutathione, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, bilirubin and albumin levels, hepatic ballooning degeneration, fatty changes, and necrosis have been reported to be decreased by pomegranate[32].

Morphometric evidence of ileal mucosal injury with reduction in villus height and total thickness in jaundiced rats has also been reported[33]. In present study, mean villus height in Groups 1, 2, and 4 was higher than that of Group 3. Obstructive jaundice may contribute to the breakdown of gastrointestinal barrier functions, thus promoting bacterial translocation. Additionally, bacterial overgrowth can promote bacterial translocation. The effect of *pomegranate* may in-

hibit overgrowth of pathogenic organisms. This study showed that pomegranate preserved mucosal integrity.

In recent years, several investigators have attempted to unravel the underlying mechanisms of beneficial effects of pomegranate. These investigations have focused mainly on the antioxidant, antiinflammatory, and antibacterial potentials of pomegranate.

Conclusions

The administration of pomegranate to rats suffering from obstructive jaundice was found as effective in the successful control of translocation and improvement of intestinal barrier function. Pomegranate is a non-toxic preparation and has been found to be experimentally effective in decreasing bacterial translocation. However, further randomized clinical trials are necessary to show the beneficial effects of pomegranate to decrease in clinical practice the infective complications in obstructive jaundiced patients.

Conflict of Interest

None to declare.

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