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Combined Effectiveness of Honey and Immunonutrition on Bacterial Translocation Secondary to Obstructive Jaundice in Rats: Experimental Study

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Background: Obstructive jaundice is a serious, life-threatening condition that can lead to death as a result of sepsis and multiorgan failure due to bacterial translocation. Treatment should be started as soon as possible after diagnosis.

Material/Methods: Forty 24-week-old male Sprague Dawley rats, with an average weight of 250 g to 300 g, were included in this study. The rats were randomly placed into five groups, each group consisted of eight rats. The sham group underwent only common bile duct (CBD) dissection and no ligation was performed. CBD ligation was applied to the other groups. After the operation, one CBD group was fed with rat chow only, the others were fed with rat chow supplemented with honey, or immunonutrients, or honey plus immunonutrients. After 10 to 12 days, all rats were sacrificed; blood and tissue samples were collected for biochemical, microbiological, and histopathological evaluation.

Results: In the groups that were fed with honey and immunonutrients, alanine aminotransferase (ALT) levels were decreased significantly compared to the other groups. Statistically significant differences were detected in terms of bacterial translocation (BT) rates among liver and spleen samples, and laboratory values of serum, except for MLNs of the BDL+HI group, when compared to other groups. We found mean mucosal thickness of ileum samples have been improved notably in the BDL+HI group compared to the other groups, especially compared to the C/BDL group.

Conclusions: Immunonutrition applied with honey had immunostimulant effects, decreased BT due to an additive effect, and had positive effects on intestinal mucosa.

MeSH Keywords: Bacterial Translocation • Honey • Intestines • Jaundice, Obstructive

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Background

Obstructive jaundice is a clinical entity that has a high morbidity and mortality rate due to septic complications, and it is associated with an impaired systemic and local immune function [1–5]. A key pathophysiological function of obstructive jaundice is bodily disruption of the intestinal barrier functionality, which consists of more than one protecting mechanism [2,6–8]. Due to the barrier characteristics of the gastrointestinal system, it prevents the dissemination of bacteria and toxins to other organs and tissues [1]. Intestinal barrier failure results in increased intestinal permeability and mononuclear dysfunction [2,3,6,9–12]; inflammatory liver injury and fibrosis obstructive cholestasis [13]; and results in elevated levels of hepatic enzymes [14].

Bacterial translocation (BT) refers to the passage of viable intestinal microorganisms through the intestinal barrier to other organs or tissues such as lymphoid tissue, spleen and liver tissue, as well as dissemination into the bloodstream [6,7,11,15]. BT is responsible for releasing inflammatory cytokines and for the activation of immunologic cells that play a crucial role in the development of sepsis and organ failure [3,4,16,17]. Bile acids can have a nourishing impact on the mucosa of the intestine by reducing the passage of enteric bacteria into the intestinal epithelium through detergent activities and protective effects against adherence and restrictions of bacterial overgrowth. Consequently, the decreased flow of bile in obstructive jaundice results in BT, and in this situation, it is important to support the immune function of the host [5,9] It has been reported that honey results in expansive range movement against gram-positive and -negative microorganisms [18]

Foods that positively regulate a patient's immune system are named immunonutrients and the administration of these nutrients to the patients is called immunonutrition. Enteral formulas of these products are supplemented with immunonutrients (arginine, omega-3 fatty acids, and ribonucleic acids), and they regulate the gut integrity and stimulate immune functions. In this way, they reduce infectious complications by preventing BT [10,15] Honey contains apalbumin 1 (Apa1), which has potential immunomodulatory effects [19,20]. It has also been reported to enhance liver function activities, decreasing serum AST and ALT levels [21].

In this study, we aimed to investigate the effects of honey and immunonutrients, such as arginine, omega-3 polyunsaturated fatty acids (PUFAs), and nucleotides) both on BT and the intestinal mucosal villi in an experimental obstructive jaundice model in rats. We compared the results separately for the use of honey and an immunonutrition, and the results obtained when honey and an immunonutrition were used together.

Material and Methods

For all the procedures used in this study, a local Animal Ethics Committee approval was provided.

Animals

Forty 24-week-old male Sprague Dawley rats, with an average weight of 250 g to 300 g were included into this study. The rats had the same organic and physiological conditions provided during the experiment with the aid of our experimental animal research center. Briefly, the rats were kept in stainless cages with standardized laboratory conditions of: warmth (22±2°C), relative humidity of 55% to 60%, and 12-hour light/dark cycles. Standard rat chow and tap water was used for feeding. All rats were handled using criteria from "Guide for the Care and Use of Laboratory Animals".

Experimental groups

The rats were divided randomly into five groups. Each group consisted of eight rats. The sham-operated group had only dissection of the bile duct performed, and were fed with standard rat chow. The control/bile-duct ligation (BDL) group (C/BDL) had a double ligation and section of the extrahepatic bile duct performed and were fed with standard rat chow. The BDL plus honey (BDL+H) group had BDL performed, followed by oral supplementation of honey in addition to standard diet during the experiment. The BDL plus immunonutrition (BDL+I) group had BDL performed, followed by oral supplementation of immunonutrition in addition to standard diet during the experiment. The BDL plus honey and immunonutrition (BDL+HI) group had BDL performed, followed by oral supplementation of honey and immunonutrition in addition to standard diet during the experiment.

Experimental procedures

All surgical procedures were performed under a warmth lamp with the rat body temperature kept at 35°C to 36°C. All operations were performed by the same surgeon using the same technique. We stopped feeding the rats twelve hours prior to anesthesia; the rats had free access to water up until two hours prior to anesthesia. The rats were anesthetized with an injection of 90 mg/kg ketamine hydrochloride (Ketalar-flk, Pfizer, Istanbul, Turkey), plus 10 mg/kg xylazine (Rompun, Bayer, Istanbul, Turkey) intramuscularly. The abdominal wall was shaved and then disinfected using 10% povidone iodine solution (Poviiodeks antiseptik 10%, Tipkimsan, Istanbul, Turkey) before the surgical intervention. First, the rats were fixed in a supine position under sterile conditions, and then the CBD was identified; followed by a 3-cm incision to the midabdominal region.

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In all groups, except the sham group, obstructive jaundice was obtained by double ligation with 4/0 polypropylene sutures (Prolene; Ethicon, USA); the transection of the CBD was performed in the supraduodenal area between the lowest part of the CBD and the upmost part of the pancreatic duct. Only an abdominal cavity dissection was performed on the rats in the sham group and no CBD ligation was done; CBD litigation was performed on the other groups. The fascia was closed by using 5/0 polyglactin (Petcryl 910, Dolphin, India) with 3/0 silk (Perma Sharp, Hu-Friedy, USA) sutures respectively, and the surgical procedure was completed. After this procedure, the rats were put into respective groups and followed in cages with controlled conditions for temperature, humidity, and lighting as previously described. The three treatment groups, as well as the sham and control groups, received standard rat fed.

Postoperatively for 10 days, the BDL+H group was fed honey (Anavarza bal, Adana, Turkey) at 10 g/kg/day [9] once daily using 1 mL via an orogastric tube (7-gauge feeding tube), in addition to standard fed,

Postoperatively for 10 days, the BDL+I group was fed immunonutrition solution (Oral Impact Nestle Nutrition, Istanbul, Turkey; 100 mL which contained 7.6 g of protein, 1.8 g of arginine, 0.6 g of omega-3 fatty acids, and 0.18 g of RNA) at 0.8–1.0 g/kg/day protein [6] twice daily using almost 1.5 mL via orogastric tube, in addition to standard fed.

Postoperatively for 10 days, the BDL+HI group was fed both the honey and the immunonutrition solution: honey (Anavarza bal, Adana, Turkey) at 10 g/kg/day [8] once daily, using 1 mL via orogastric tube and immunonutrition solution (Oral Impact, Nestle Nutrition, Istanbul, Turkey, 100 mL which contained 7.6 g of protein, 1.8 g of arginine, 0.6 g of omega-3 fatty acids, and 0.18 g of RNA), at 0.8–1.0 g/kg/day protein [6] twice daily using almost 1.5 mL via orogastric tube, in addition to standard fed.

All rats received their specific group feedings starting at 12 hours after their operation.

After 10 days post-surgery, the animals were sacrificed using an injection of high-dose thiopental sodium. Just before the injection, 7 mL of blood was taken with cardiac puncture for blood culture. After euthanasia, the stomach cavities of the rats were opened with a 3-cm midline incision and samples from the liver, mucocutaneous lymph nodes (MLNs), spleen, and tissue of the terminal ileum were collected from all rats using sterile devices and aseptic conditions standard for microbiological, biochemical, and histopathological research.

Biochemical and Microbiological analyses

Blood samples (5 mL) were put into aerobic culture flasks and incubated for a maximum of seven days in a Bact/Alert (Biomerieux, France) blood culture device. If a bacterial reproduction signal was detected, the samples were transferred to an appropriate media and incubated at 37°C in aerobic conditions. Bacteria reproduction were determined by using conventional methods and VITEK 2 (Biomerieux, France) automatized detection system.

Blood samples (2 mL) were sent to the laboratory to measure the levels of serum aspartate transaminase (AST), and alanine transaminase (ALT). Serum AST and ALT values were measured as indicators for hepatic functions using standard biochemical techniques [17]. AST and ALT results were expressed as U/L.

Approximately 1 g from each tissue sample was pulverized in a sterile mortar, and 1 mL thioglycolate was added; the samples were homogenized, and 0.01 mL homogenate was collected and placed in appropriate media and incubated at 37°C for three days. Reproduced colonies were counted, and the bacterial concentration of the tissue was calculated as CFU/gram. To determine bacterial reproduction in the media, traditional methods and VITEK 2 (Biomerieux, France) automatized identification system were used. Microbiological evaluation was performed by a microbiologist who was blinded to the study.

Histopathological evaluation

After the collection of all the terminal ileum samples, the samples were fixed with 10% neutral buffered formalin solution. Then, they were embedded in paraffin, and 5-µm thick sections were cut. Sections were stained with hematoxylin and eosin (H&E) and examined under light microscope. A pathologist who was blinded to the study performed the histopathological examination; photographs were taken with Zeiss Axioplan 2 (Jena, Germany) using the method of Gencay et al. [9]. To evaluate the structural changes in the terminal ileum, the number of villi for each centimeter (V/cm) and the mucosal thickness in micrometers (µm) were assessed in all five groups. The thickness of the mucosal wall was measured [9] in a minimum of 20 well-protected villi in each randomly selected tissue sample.

Statistical analysis

Fisher's exact test was used to evaluate categorical variables in univariate statistical analyses. The mean ± standard deviation (SD) was used to express numerical values. One-sample Kolmogorov-Simirnov test was used to determine if the calculated values correspond to the normal distribution. One-way analysis of variance (ANOVA) and post hoc analysis with Scheffe tests were used to compare parametric variables among the

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Table 1. Bacterial translocation rates of the groups.

Groups	Liver	Spleen	MLNs	Blood
Sh	0/8 (0.0%)	0/8 (0.0%)	0/8 (0.0%)	0/8 (0.0%)
C/BDL	3/6 (50.0%)	3/6 (50.0%)	5/6 (83.3%)	3/6 (50.0%)
BDL+H	2/8 (25.0%)	2/8 (25.0%)	4/8 (50.0%)	2/8 (25.0%)
BDL+I	0/8 (0.0%)	0/8 (0.0%)	6/8 (75.0%)	1/8 (12.5%)
BDL+HI	0/8 (0.0%)	0/8 (0.0%)	3/8 (37.5%)	0/8 (0.0%)
p Values				
Sh *vs.* C/BDL	0.049	0.049	0.003	0.049
Sh *vs.* BDL+H	0.467	0.467	0.077	0.467
Sh *vs.* BDL+I	–	–	0.007	1.000
Sh *vs.* BDL+HI	–	–	0.077	–
C/BDL *vs.* BDL+H	0.580	0.580	0.301	0.580
C/BDL *vs.* BDL+I	0.049	0.049	1.000	0.245
C/BDL *vs.* BDL+HI	0.049	0.049	0.301	0.049
BDL+H *vs.* BDL+I	0.467	0.467	0.608	1.000
BDL+H *vs.* BDL+HI	0.467	0.467	1.000	0.467
BDL+I *vs.* BDL+HI	–	–	0.608	1.000

Sh – sham group; C/BDL – control group; BDL+H – honey treated group; BDL+I – immunnutrients treated group; BDL+HI – honey plus immunnutrients treated group.

groups. Whether the repeated measurements were statistically significant in the same group was evaluated using the paired sample *t*-test. In the determination of statistical significance the value of $p<0.05$ was accepted. All statistical analyses were performed using the Statistical Package for Social Sciences version 15.0 (SPSS, Chicago, IL, USA).

Results

Two rats from the control group died on the first postoperative day. Autopsies detected that the reason for death was massive intraperitoneal hemorrhage. The rats that died during the experimental procedure were deducted from the study analysis and no new rats were added to replace them. All the other rats were sacrificed at the end of the experiment (on the tenth postoperative day). Dilatation of the CBD was detected in all rats at the end of the experiment.

BT was not detected in any specimens from the sham group. Significantly higher rates of BT were detected in the C/BDL group compared to the other groups. The sham and BDL+HI groups had similar levels of BT. The highest BT levels were detected in MLNs when all the groups were considered. There were statistically significant differences in the rates of BT among liver, spleen, and blood samples (except MLNs of group BDL+HI) when compared to group C/BDL (in all significant samples $p=0.049$). The BT rate for each group are shown in Table 1. Bacterial overgrowth was detected in the samples of 35 rats. Double microorganisms were proliferated together in three cultures. The most commonly isolated bacteria from the samples was *Escherichia coli* (71%). Other isolated microorganisms were *Enterococcus faecalis* (23.7%), *Proteus spp.* (2.6%), and *Enterobacter cloacae* (2.6%).

When we evaluated the terminal ileum specimens by histopathology, it was seen that the main structure of the mucosa was normal in all rats in the sham group (Figure 1). In the ileum specimens of the BDL+HI group, the mean mucosal thickness was increased significantly compared to the other groups, especially compared to the C/BDL group ($p=0.000$) (Figure 2). The number of villi per centimeter was decreased mostly in the C/BDL group. The number of villi per centimeter was higher in the supplementation groups (BDL+H, BDL+I, and BDL+HI), especially in the BDL+HI (Figure 3). There was no statistically significant difference between C/BDL and the supplementation groups ($p=0.669$). The mean number of villi per centimeter and mucosal thicknesses of the groups are presented in Table 2.

Liver function tests were performed just after blood sample collection. As expected, the results were normal in the sham group. The values of liver function tests were significantly

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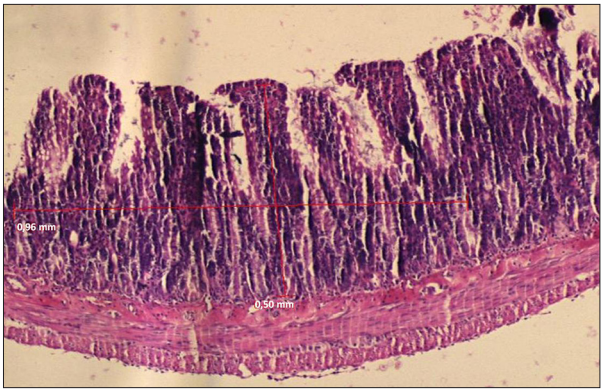

Figure 1. Mean mucosal thickness in sham group sample.

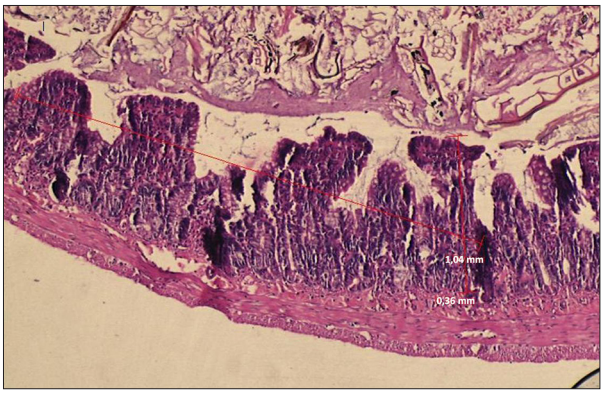

Figure 2. Mean mucosal thickness in C/BDL group sample.

decreased in the supplementation groups compared to the C/BDL group. The serum ALT levels were significantly reduced in the BDL+H and BDL+HI groups compared to the C/BDL group (p=0.002, 0.023 and p=0.000, 0.049, respectively). In terms of all groups, the distribution of differences on the liver function tests are shown in Table 3.

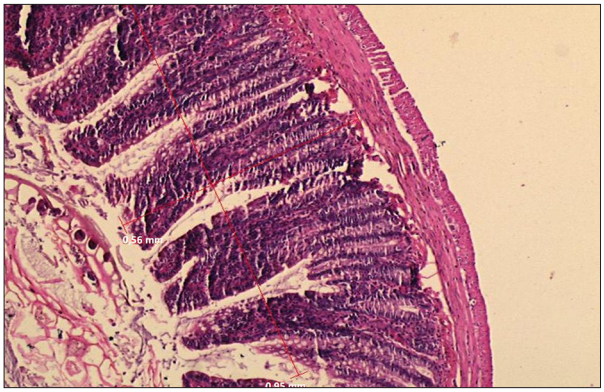

Figure 3. Mean mucosal thickness in BDL/HI group sample.

Table 2. Mean number of villi per cm and mean height of mucosa (μm).

Groups	Mean number of villi per cm	Mean mucosal thickness
Sh	71.25±9.91	500.00±50.99
C/BDL	55.00±10.49	391.70±44.01
BDL+H	61.50±18.32	431.30±56.43
BDL+I	60.00±16.90	463.80±58.54
BDL+HI	68.25±10.60	547.50±34.95
p (ANOVA)	0.669	0.000

Sh – sham group; C/BDL – control group; BDL+H – honey treated group; BDL+I – immunnutrients treated group; BDL+HI – honey plus immunnutrients treated group.

Discussion

In obstructive jaundice, the host defense is affected in different ways. The reticuloendothelial system (RES) might be damaged and the phagocytic function might be depressed. Kupffer cell function in the liver, which is the effective element of RES,

Table 3. The biochemical parameters for each group (mean ±SD).

Groups	ALT (U/L)		AST (U/L)	
	3rd day	10th day	3rd day	10th day
Sh	60.29±8.84	59.50±5.42	233.86±24.31	170.75±42.27
C/BDL	460.71±143.87	284.67±124.10	760.57±244.67	871.33±699.29
BDL+H	^{a1}392.37±139.19	^{a2}168.50±31.76	^{b1}795.00±296.20	^{b2}485.37±123.99
BDL+I	^{c1}380.37±264.44	^{c2}155.00±73.04	636.12±252.85	572.50±227.19
BDL+HI	^{d1}340.00±149.61	^{d2}132.37±56.20	^{e1}639.62±288.09	^{e2}476.00±197.69
p ANOVA	0.000	0.009	0.000	0.003

a1 *vs.* a2 p=0.002; b1 *vs.* b2 p=0.023; c1 *vs.* c2 p=0.024; d1 *vs.* d2 p=0.000; e1 *vs.* e2 p=0.049. ALT – alanine transaminase; AST – aspartate transaminase; Sh – sham group; C/BDL – control group; BDL+H – honey treated group; BDL+I – immunnutrients treated group; BDL+HI – honey plus immunnutrients treated group.

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is varied. Kupffer cell activation is very important for an efficacious immune response, thus, decreased activity of Kupffer cells results in increased incidence of BT in obstructive jaundice. The physical barrier function of the mucosa has a primary role in preventing and limiting BT in a host with a normal intestinal microbiota. However, the immune system seems to play a secondary role to the gut mucosal barrier [7]. Gut barrier failure appears with increased intestinal permeability and translocation, and concerns the activation of immunocompetent cells inside the intestinal wall and associated lymph nodes such as gut-associated lymphoid tissue (GALT) and mucosa associated lymphoid tissue (MALT), which are the largest immunological organs [12]. BT from the gut impairs systemic cell-mediated immunity [15].

Clinical studies suggest that immunonutrition prevents a decrease in phagocytosis and the number of circulating lymphocytes. At the same time, it also prevents BT from the gut by protecting the intestinal mucosal barrier integrity, stimulating the host defense system, or preventing bacterial overgrowth [10,15]. Diets that include specific substrates called immunonutrients, such as glutamine, arginine, omega-3 PUFAs, and nucleotides, have been associated with the regulation of intestinal function and a decrease in infectious complications in critically ill patients [24]. However, these diets have been comprised of multiple immunonutrients, making it difficult to identify which nutrients play the main role in modulation of the immune response. It is possible that there are different roles for each nutrient [25].

The uses of amino acid arginine in septic patients (because of its role in nitric oxide (NO) synthesis) is still controversial; and its role in the process of BT is uncertain. However, there are numerous beneficial effects on the immune system of consuming arginine, such as reducing Kupffer cell function, and enhancing proliferation of T-cells and natural killer cell activity [17,26,27].

Macrophages are organizers of lymphocyte activity. Dysfunction of macrophages in biliary obstruction could play a main role in altering the immune system. Cytotoxic effector molecules of macrophages are reactive radical NOs derived from L-arginine [15]. Glutamine is an energy source for T-lymphocytes, neutrophils, enterocytes, and other rapidly proliferating cells; and it becomes an essential amino acid under stress and sepsis. This amino acid is also necessary for normal GALT function [6,15,27,28]. Omega-3 PUFAs have been reported to defend against infection. Omega-3 PUFAs coordinate the immune response by increasing membrane fluidity, presenting free radical lipid peroxide, and by providing needed precursors for eicosanoid metabolism [5]. Nucleotides such as purine and pyrimidine are essential for cells that do not have enough nucleotide synthesis capacity like T-lymphocytes, enterocytes, and other rapidly proliferating and growing cells. Nucleotide deficiency inhibits macrophage and T-cell function and the production of T-cell dependent antibodies depends on nucleotide addition. Susceptibility to septic infections, especially gram-positive coccus and fungal infections, increases with nucleotide deprivation [5,23,25]. Administration of immunonutrients in the preoperative and early postoperative period could promote modulation of the inflammatory response, increase the cellular immune response, and improve intestinal micro-perfusion and oxygenation [30]. The results of our experimental study support the protective effects of such immunonutrients.

Several studies have looked at the immunostimulant effect of nutrients such as glutamine, arginine, omega-3 PUFAs, and nucleotides on nutritional support [1,15,16]. However, studies looking at the effects of honey and its immunostimulant effect as nutritional support are scant.

Honey is a supersaturated sugar solution produced by honeybees from the nectar of different plants. Honey has been used for the treatment of conditions such as wounds or burns, and provides some positive effects like regenerative, hepatoprotective, anti-inflammatory, antimicrobial, antioxidant, antiulcer, antitumoral, and immune-stimulant effects [1,9,23]. Alanine transaminase is a transaminase enzyme that catalyzes the interconversion of the amino acid L-alanine to L-glutamate, mutually. ALT and AST levels characteristically increase in liver diseases such as obstructive jaundice. Honey increases the level of NO in biological liquids and reduces the level of blood liver enzymes like ALT and AST [22,23]. The effectiveness of honey depends on the specific content of monosaccharides and antioxidant substances, especially phenolic acids and flavonoids that play a significant role in its effect as they inhibit prostaglandins and increase NO production [31]. Honey's antioxidant and phenolic activity has a protective effect on hepatotoxicity and hepatic injury [32]. As such, honey has a potential immunomodulatory effect in most diseases [19,20].

Gencay et al. [9] reported that the number of villi per centimeter and the intestinal mucosal thickness increased in the honey-applied group compared to the control group in an experimental rat study. Zulfikaroglu et al. [6] reported that one week after administration of immunonutrition to rats with experimental obstructive jaundice, a decrease in intestinal mucosal villous atrophy was observed. In contrast, other studies concluded that immunonutrition support was insufficient by itself for the protection of the integrity of intestinal mucosal structure [15,33,34].

In our study, among the supplementation groups, especially in the group where honey and immunonutrition solution were used together, there was a significant increase in the number of villous per centimeter in comparison to the other groups; in addition, a

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significant increase in the mean mucosal thickness level was detected especially in this combined supplement group. This suggests that immunonutrition solutions have a protective effect on the intestinal mucosal barrier. On the other hand, honey is known to have regenerative and cytoprotective effects in addition to wound healing properties. Thus, we suggest that immunonutrition solutions and honey are potentially be more useful in the protection of intestinal mucosal integrity if they are used together, taking their additive properties into consideration.

Some studies have indicated that various stress conditions can cause an increase in BT rates. Studies also suggest that arginine, which is a immunonutrient, decreases the BT rate to physiological levels and leads to recovery in intestinal mucosa disorders [25,35–37]. O'Leary et al. [38] emphasized that immunonutrients can play an important role in intestinal mucosal barriers and decrease BT rates. Likewise, Ulusoy et al. [10] found that in a head trauma model (one of the major stress conditions), BT occurred and was decreased with immunonutrition solutions. In contrast, other studies have indicated that immunonutrition support treatment prevents BT, although it is insufficient in healing the damage that occurred in the intestinal mucosal structures [33,34]. In some experimental studies [15,39] that applied immunonutrition support it was suggested that BT in the liver and MLNs after supplementation was decreased and the intestinal mucosal structure was protected. Narioka et al. [40] reported that BT was not observed in MLNs after immunonutrition support. Zulfikaroglu et al. [6] reported decreased BT rates in rats with obstructive jaundice after immunonutrition support; a finding which has been supported by other studies [41,42]; BT occurred most frequently in MLNs and *E. coli* was the most common microorganism detected.

Similar to some other studies [6,41,42], our study found that BT rates were observed most frequently in MLNs and the most frequent reproducing bacteria was *E. coli*. In our experimental study, using honey and immunonutrition solutions together resulted in a more significant decrease in BT rates in other tissue and blood samples than in MLN samples. BT is a serious complication of obstructive jaundice.

Honey and immunonutrition solutions can be applied together. The addictive effect minimizes the reproduction of bacteria by healing potential RES functions and decreasing the translocation of bacteria to tissues by reinforcing the intestinal mucosal barrier and positively contributing to the immune system.

In the literature, it was reported that in liver damage models formed experimentally, after obstructive jaundice, an increase in ALT levels noticeably regressed after the application of honey as nutritional support [22,23]. The marked release of transaminases into the circulation is thought to be indicative of severe damage to hepatic tissue membranes and a sensitive indicator of necrotic lesions within the liver [43,44].

In our study, we found that in the group that received honey and immunonutrition solution support, the ALT levels were decreased significantly compared to the other groups. The decrease in liver enzymes, especially ALT levels, was potentially related to the antioxidant effect of honey. The regression occurring in liver damage was potentially a result of possible hepatoprotective effect of honey.

Conclusions

It becomes extremely important to find new immune supportive enteral products to aid in the prevention of BT after obstructive jaundice which could result in biliary sepsis. Our study aimed to detect the effects of honey and immunonutrition solution, individually and together, on BT secondary to obstructive jaundice. We found that when immunonutrition solution was applied with honey, it had an immunostimulant effect and decreased BT, likely due to an additive effect, and had positive effects on intestinal mucosa. However, to use this method in clinical practice, more comprehensive studies are needed.

Conflict of interest

None.

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