JKKI
SCIENTIA EST BASIC VITAE

JKKI: Jurnal Kedokteran dan Kesehatan Indonesia
Indonesian Journal of Medicine and Health
Journal homepage: https://journal.uii.ac.id/JKKI
P-ISSN 2085-4145 | E-ISSN 2527-2950

Exploring the effects of ursodeoxycholic acid-probiotic combination on *Lactobacillus* and liver enzyme levels for cholestasis management: An experimental study

Muhammad Hasbi Nur*[1], Endang Sri Lestari[2], Bernadus Parish Budiono[3], Sigit Adi Prasetyo[3], Muflihatul Muniroh[4]

[1]Biomedical Science Postgraduate Program, Faculty of Medicine, University of Diponegoro, Semarang
[2]Microbiology Department, Faculty of Medicine, University of Diponegoro, Semarang
[3]Department of Surgery, Faculty of Medicine, University of Diponegoro/ Kariadi General Hospital, Semarang
[4]Physiology Department, Faculty of Medicine, University of Diponegoro, Semarang

Original Article

ARTICLE INFO

Keywords:
cholestasis,
ursodeoxycholic acid,
probiotics,
aspartate aminotransferase,
alanine aminotransferase,
Lactobacillus

***Corresponding author:**
muhammadhasbinur99@gmail.com

DOI: 10.20885/JKKI.Vol15.Iss1.art10

History:
Received: December 24, 2023
Accepted: March 25, 2024
Online: April 29, 2024

ABSTRACT

Background: Cholestasis arises from compromised bile secretion due to hepatocyte dysfunction, leading to liver impairment. Available treatments show limited efficacy, with ursodeoxycholic acid (UDCA) being a primary option. Cholestatic conditions influence gut microbiota; therefore, probiotic therapy emerges as a potential approach.
Objective: This investigation aimed to evaluate the impact of combined administration of probiotics and UDCA on *Lactobacillus* levels, as well as the levels of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats with restricted common bile ducts .
Methods: A total of 35 male Sprague Dawley rats were randomly assigned to 7 groups, each comprising 5 members: K1 (healthy control), K2 (negative control with cholestasis), K3 (positive control with cholestasis given UDCA), K4 (cholestasis given 36 mg probiotics), K5 (cholestasis given 18 mg probiotics and UDCA), K6 (cholestasis given 36 mg probiotics and UDCA), and K7 (cholestasis given 54 mg probiotics and UDCA). The treatment duration was 21 days, during which blood samples were collected for AST and ALT analysis. *Lactobacillus* count was determined by quantitative polymerase chain reaction (PCR) analysis of fecal samples.
Results: When UDCA and probiotics were given together in three different dosages, the *Lactobacillus* count significantly increased ($p<0.05$) compared to the other groups. Furthermore, compared to the other treatment groups, the UDCA-probiotic combination group exhibited noticeably lower AST and ALT values.
Conclusion: Combining UDCA and probiotics elevated *Lactobacillus* count and decreased AST and ALT levels in cases of cholestasis more effectively than single therapy.

INTRODUCTION

Cholestasis, a condition marked by impaired bile formation due to hepatocyte dysfunction or bile flow obstruction, poses a significant challenge to liver health. Its clinical implications encompass fatigue, pruritus, and jaundice.[1–3] Key biochemical markers such as serum AST and ALT rise, accompanied by hyperbilirubinemia. Bile duct obstructions cause acute cholestasis, but chronic occurrences are associated with diseases like primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC), complicating its treatment.[2,4]

Despite cholestasis frequently arising in various of liver illnesses, there are currently few and ineffective treatment options available. The only treatments available for cholestatic liver illness brought on by inherited transporter

abnormalities are liver transplantation, bile grinding, UDCA, and primary bile acid replacement. Obeticholic acid, UDCA, and several immunosuppressive medicines have shownpromise in ameliorating PBC; nonetheless, UDCA has proven to be the most efficacious treatment for PBC. Optimizing the care of cholestatic liver disease is still urgently needed, though. Research on changes in the microbiome has become more important in this context. PBC patients have been shown to have disruptions in their gut microbiota, which may indicate a relationship between cholestasis and gut health.[5–8]

Ursodeoxycholic acid is prominent in treatment, along with potential benefits from certain medications. Ursodeoxycholic acid is an example of a hydrophilic bile acid produced by the body and amounts to approximately 3% of all bile acids in the body. Ursodeoxycholic acid is formed from the modification of chenodeoxycholic acid by bacteria in the intestines and liver (tertiary bile acids). In cholestasis, there is a buildup of bile in the biliary lumen. Administration of UDCA will change the proportion of hydrophilic bile acids in stasis bile. Bile rich in UDCA is more hydrophilic and less cytotoxic, thereby reducing the degree of bile duct cell damage, portal inflammation, and ductal proliferation caused by cholestasis. Apart from accumulation in the biliary lumen, bile also accumulates in the liver. Ursodeoxycholic acid protection in hepatocytes occurs through increasing bile acid secretion so that toxic bile components do not accumulate.[9–11]

While no substantial alterations were seen in PSC, majority of published papers showed that PSC had varying impacts on the gut microbiota, suggesting that PSC is the primary cause of changes in the gut microbiome. Overall, the data point to the possibility of using alterations in the gut microbiota as a biomarker to distinguish between PSC and inflammatory bowel illness in PSC patients. Additionally, recent studies have demonstrated the potential benefits of probiotic therapy. Specifically, probiotic therapy involving *Lactobacillus rhamnosus* GG (LGG) has demonstrated promising outcomes in addressing liver injury and fibrosis in experimental settings. This exploration of probiotic effects aligns with the broader endeavour to enhance cholestasis management.[8] Moreover, recent investigations delve into the interplay between UDCA and probiotics, evaluating their combined impact on *Lactobacillus* levels and markers like AST and ALT.12 Comprehending the combined effects of these therapies on cholestasis markers could potentially enhance treatment approaches and illuminate the complex interplay of bile metabolism, gastrointestinal health, and hepatic function. This investigation aims to evaluate if the combined administration of probiotics and UDCA can impact *Lactobacillus* levels, as well as AST and ALT levels in rats with constricted common bile ducts.

METHODS

Experimental animals

Two-month-old male Sprague Dawley rats, weighing between 150-200 g were used in this study. The rats were housed in a room with a temperature of 28.0 ± 2.0 °C and a 12-hour light/dark cycle. They were provided with unlimited access to rodent chow and water. The experiment started with a seven-days acclimatization period for all the animals.

Induction of fibrosis model in rats

Cholestasis was induced by ligating the common bile duct of the rats in group K2, K3, K4, K5, K6, K7. Before surgery, the rats received a prophylaxis antibiotic injection of 18 mg cefotaxime (Indofarma®, Jakarta, Indonesia) intramuscularly. Anaesthesia was induced with a 0.5 mL intramuscular injection of ketamine hydrochloride (Dexa Medica®, Cikarang, Indonesia) . A midline laparotomy was performed under sterile conditions, and the rat's common bile duct was ligated with 5-0 silk sutures (DemeTECH®, Miami Lakes, FL, USA). To ensure the rats' comfort, oral Ibuprofen (Pharos®, Semarang, Indonesia) at a dose of 7 mg was administered every 8 hours for 3 days post-surgery.

Animal groups and study design

This study employed a randomized post-test-only study with a control group. A total of 35 male Sprague Dawley rats were randomly assigned to 7 groups, each consisting of 5 rats: K1: Healthy control group receiving standard diet and water ad libitum without ligation, K2: Negative control group receiving standard diet and water ad libitum with ligation, K3: Positive control group receiving

standard diet, water ad libitum, and 13.5mg UDCA, K4: Treatment group 1 receiving standard diet and water ad libitum, followed by oral administration of probiotic at a dosage of 36 mg, K5: Treatment group 2 undergoing the same feeding regimen as K4, along with oral administration of 13.5 mg UDCA and 18 mg probiotic daily for 21 days, K6: Treatment group 3 receiving similar diet and water schedule as K5, along with oral administration of 13.5 mg UDCA and 36 mg probiotic daily for 21 days, K7: Treatment group 4 following the same feeding pattern as K6, along with oral administration of 13.5 mg UDCA and 54 mg probiotic daily for 21 days. The dose of UDCA and probiotic were adjusted based on the pharmacokinetic of the drug for rats. On day 22 post-administration, all experimental animals were anesthetized by overdose. Blood samples were collected from mice to test AST and ALT levels. Then, the cecum was released to collect fecal samples.[13]

Biochemical analysis

On the 22nd day following the intervention, blood samples were obtained from the retroorbital plexus. The samples were centrifuged at 3,000 rpm for 10 minutes, and the serum was stored at -20 °C until biochemical analysis. Serum AST and ALT enzyme activity were determined using the calorimetric approach outlined by Reitman and Frankel with the respective kits.[14]

Bacterial Deoxyribose Nucleic Acid (DNA) extraction

This study performed the following steps to prepare and extract DNA from frozen fecal samples. Feces samples were taken from the rat's cecum by dissecting its colon. The cecum was then transferred into a 15 ml falcon tube to the laboratory and stored at -20°C as frozen cecal samples. Initially, 100 mg of fecal material was carefully weighed and placed in a 2 ml vial with glass beads. Next, 50 µl of lysozyme (10 mg/ml) and 300 µl of SDE (Sequential Detergent Extraction) 1 buffer were added. Using a cell lys homogenizer (Precellys, Bertin®, Gardais, France), the mixture was homogenized for one minute. After that, 20 µl 10 mg/mL of proteinase K was added, and the incubation was kept at 60 °C for an additional 8 hours. After incubation 100 µl of SDE 2 buffer was added after incubation, and the mixture was vortexed before being incubated on ice for five minutes. The mixture was centrifuged at 15,000 x g for 5 min, and the supernatant was combined with µl SDE 3 buffer. Incubation was performed for 2 min at room temperature, followed by centrifugation at 15,000 x g for 2 min. In another step, 250 µl of supernatant was taken 250 µl of cold isopropanol and 250 µl of SDE 4 buffer were added and was followed by vortexing and transferred to an SDE column after drying by centrifugation for 3 minutes, 50 µl of hot solution buffer was added, incubated and centrifuged at 15,000 x g for 1 min, and the resulting eluate of the products were quantified and DNA quality was analyzed using a nanodrop spectrophotometer.[15]

Quantitative polymerase chain reaction (PCR)

The methodology included the design of primer probes aimed at *Lactobacillus* detection based on the 16S rRNA (ribosome-Ribonucleic Acid) gene sequence obtained from the BLAST (Basic Local Alignment Search Tool) database followed by the development of seven tubes with 9 ml of 0.9% NaCl. The targeted bacteria were *Lactobacillus* with primer F in sequence (5'-3') AGCAGTAGGGAATCTTCCA and primer R in sequence (5'-3') CACCGCTACACATGGAG.

Bacterial DNA targets were diluted (from 10^9 CFU/ml) to yield 10^2 CFU/ml to 10^9 CFU/ml. After centrifugation to obtain the precipitate, 300 µl of SDE 1 buffer and 50 µl of lysozyme (10 mg/ml) were added. The DNA was then extracted according to the protocol for targeted DNA. Reagents were prepared, including 2x QPCR Mastermix (Smobio®, Bekasi, Indonesia), forward-reverse primers, template DNA, and nuclease-free water, were prepared. After vortexing, the mixture was transferred to qPCR tubes and sealed tightly. Subsequent qPCR was performed using a qPCR instrument (Biorad CFX96®, Bogor, Indonesia), with cycling conditions set as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 min and annealing at 60°C for 1 min. Using duplicate tenfold dilutions of the DNA and a minimum of five standard concentrations ranging from 10^4 to 10^{10} DNA copies per reaction, the standard curve analysis was created. The logarithmic concentration of the control bacterial culture was

plotted against the number of threshold cycles (Cq), which is the number of cycles needed for the fluorescence signal in each reaction tube to reach the predetermined threshold, to create the graph. Relative quantitation was used to assess the caliber of PCR amplification. Using the usual curves, the target group's copy numbers for each response were determined.[16]

Statistical analysis

Results data were analyzed using SPSS 26.0 for Windows Software. Data were expressed as a median. The Shapiro-Wilk test was used for the normality test. Then, all groups' data were compared using the Kruskall-Wallis and Mann-Whitney tests. All data were significant if $p < 0.05$.

Ethics

This study was approved by the Medical Research and Ethics Committee of Diponegoro University approved this study (protocol number: 49/EC/H/FK-UNDIP/VI/2023) and was conducted in compliance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) criteria.[17]

RESULTS

In this study, 35 male Sprague Dawley rats were procured from the Animal Research Unit laboratory at Gadjah Mada University. The rats had an average weight of 200 g, were actively mobile, had no physical defects, and were 2 months old before treatment. All samples underwent a seven-day acclimatization period in the laboratory, during which they had access to food and water ad libitum. Common bile duct ligation was performed in all samples, after which samples were divided into 7 groups by simple randomization method No mice in groups K1, K2, K3, K4, K5, K6, and K7 died during the experiment. Thus, 35 rats were used for this study.

***Lactobacillus* DNA count**

According to these findings, the K7 group which received choledochal duct ligation, 13.5 mg of UDCA, and 54 mg of probiotics exhibited the greatest level of Lactobacillus count, followed by the K6, K5, K4, K3, and K1 groups. In contrast, the K2 group as negative control group that received choledochal duct ligation without UDCA showed the lowest level of *Lactobacillus* count (Table 1). Higher levels of Lactobacillus were discovered in all other groups compared to the negative control. For *Lactobacillus* species, the following standard curve was created and discovered: $Y = -3.3789x + 47.084$ ($R2 = 0.9926$). The curve was within the dilution range and displayed a linear relationship.

The Kruskal-Wallis test revealed a significant difference ($p<0.001$) in the amount of *Lactobacillus.* The number of *Lactobacillus* was significantly different in the negative control group (without therapy) compared to the healthy controls, controls who received probiotics, UDCA, and controls who received both probiotics and UDCA together. These differences were revealed by the Mann-Whitney test results. These results suggest that therapy can increase *Lactobacillus* count. Significant differences were also found between probiotics versus UDCA alone. They mean that giving probiotics alone can increase *Lactobacillus* better than UDCA alone. However, combining UDCA and probiotics increased *Lactobacillus* better than UDCA alone or probiotics alone (Table 1).

Analysis of ALT and AST levels

According to these findings, the K2 group—the negative control group that received choledochal duct ligation without UDCA—had the highest level of AST levels, followed by the K3, K4, K5, K6, and K7 groups. Conversely, the K1 group had the lowest level of AST levels. Lower results of AST levels were found as UDCA and higher doses of probiotics were given to research subjects, indicating the effectiveness of the treatments (Table 2).

These results showed the highest level of ALT levels was found in the K2 group as the negative control group which was given choledochal duct ligation without UDCA followed by K3, K4, K5, K6 and K7 groups (Table 1). In contrast, the lowest level of ALT levels was found in the K1 group. Lower results of ALT levels were found as UDCA and higher doses of probiotics were given to research subjects, indicating the effectiveness of the treatments.

The Kruskal Wallis test demonstrated a significant difference in the levels of ALT and AST (Table 2). The Mann-Whitney test further confirmed significant differences between the negative control group and the healthy controls, as well as between the groups receiving probiotics, UDCA, and the combination of both.

These findings suggest that therapy can reduce AST and ALT levels, with the combination of UDCA and probiotics showing the most pronounced effect (Table 2, Table 3).

DISCUSSION

Table 1. *Lactobacillus* DNA Count and Mann Whitney p-value test

Groups	Mean ± SD	K1	K2	K3	K4	K5	K6	K7
K1	8.88 ± 0.45$^{\delta}$							
K2	7.00 ± 1.94$^{\delta}$	0.05*						
K3	9.57 ± 0.13$^{\delta}$	0.016*	0.008*					
K4	9.91 ± 0.12$^{\delta}$	0.008*	0.008*	0.008*				
K5	10.15 ± 0.08$^{\delta}$	0.008*	0.008*	0.008*	0.008*			
K6	10.36 ± 0.15$^{\delta}$	0.008*	0.008*	0.008*	0.008*	0.008*		
K7	11.72 ± 1.24$^{\delta}$	0.008*	0.008*	0.008*	0.008*	0.008*	0.056*	

Note: K1: healthy control, K2: negative control (choledochal duct ligation without UDCA), K3: positive control (choledochal duct ligation and given 13.5 mg UDCA), K4: choledochal duct ligation treatment and given 36 mg probiotics, K5: choledochal duct ligation treatment and given 13.5 mg UDCA and 18 mg probiotics, K6: choledochal duct ligation treatment and given 13.5 mg UDCA and 36 mg probiotics, K7: choledochal duct ligation and given 13.5 mg UDCA and 54 probiotics mg; *: significant from Mann-Whitney test of ($p<0.05$); $^{\delta}$: p significant from Kruskal Wallis test $p<0.001$; DNA: deoxyribose nucleic acid UDCA: ursodeoxycholic acid; SD: standard deviation

Table 2. Level of AST and Mann-Whitney p-value test between groups

Groups	Mean ± SD	K1	K2	K3	K4	K5	K6	K7
K1	37.09 ± 0.43							
K2	78.46 ± 1.67	0.008*						
K3	58.16 ± 2.56	0.008*	0.008*					
K4	49.13 ± 1.43	0.008*	0.008*	0.008*				
K5	43.31 ± 1.21	0.008*	0.008*	0.008*	0.008*			
K6	40.88 ± 1.26	0.008*	0.008*	0.008*	0.008*	0.032 *		
K7	39.81 ± 0.69	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	

Note: K1: healthy control, K2: negative control (choledochal duct ligation without UDCA), K3: positive control (choledochal duct ligation and given 13.5 mg UDCA), K4: choledochal duct ligation treatment and given 36 mg probiotics, K5: choledochal duct ligation treatment and given 13.5 mg UDCA and 18 mg probiotics, K6: choledochal duct ligation treatment and given 13.5 mg UDCA and 36 mg probiotics, K7: choledochal duct ligation and given 13.5 mg UDCA and 54 probiotics mg, * significant ($p<0.05$); AST: aspartate aminotransferase; UDCA: ursodeoxycholic acid; SD: standard deviation

Table 3. ALT Level and Mann-Whitney p-value test between groups

Groups	Mean ± SD	K1	K2	K3	K4	K5	K6	K7
K1	37.09 ± 0.43							
K2	78.46 ± 1.67	0.008*						
K3	58.16 ± 2.56	0.008*	0.008*					
K4	49.13 ± 1.43	0.008*	0.008*	0.008*				
K5	43.31 ± 1.21	0.008*	0.008*	0.008*	0.008*			
K6	40.88 ± 1.26	0.008*	0.008*	0.008*	0.008*	0.032 *		
K7	39.81 ± 0.69	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	

Note: K1: healthy control, K2: negative control (choledochal duct ligation without UDCA), K3: positive control (choledochal duct ligation and given 13.5 mg UDCA), K4: choledochal duct ligation treatment and given 36 mg probiotics, K5: choledochal duct ligation treatment and given 13.5 mg UDCA and 18 mg probiotics, K6: choledochal duct ligation treatment and given 13.5 mg UDCA and 36 mg probiotics, K7: choledochal duct ligation and given 13.5 mg UDCA and 54 probiotics mg, * significant ($p<0.05$), UDCA: ursodeoxycholic acid, ALT: alanine aminotransferase; SD: standard deviation

Recent research highlights the synergistic impact of combining UDCA and probiotics to address cholestasis. Ursodeoxycholic acid constitutes approximately 3% of the hydrophilic bile acid produced by the body. It is endogenously synthesized through the modification of chenodeoxycholic acid by bacteria in the intestine and liver. Furthermore, exogenous UDCA is now available as a drug of choice for various cholestasis diseases. The FDA has recommended ursodeoxycholic acid at a dose of 750 mg/day to treat cholestasis because studies have shown that it lowers bilirubin, aspartate aminotransferase, and alkaline phosphatase levels and increases the 10-year transplant-free rate. Additionally, UDCA has been shown to slow down the progression of liver disease, thereby reducing the risk of esophageal varices. For PBC, a daily dosage of 13–15 mg/bodyweight is advised.[18]

This study found that the K2 and K3 groups which were no given probiotic agent exhibited significantly lower *Lactobacillus* levels than the healthy control, indicating reduced *Lactobacillus* due to cholestasis. The condition of cholestasis causes a reduction in bile acids flowing into the intestines. As a result, harmful bacteria proliferate while normal intestinal flora are suppressed. Through Takeda G protein-coupled receptor-5 (TGR5) and Farnesoid X Receptor (FXR), the gut microbiota modulates bile acid signaling, where the microbiota reduces FXR suppression in the ileum so that FXR-FGF15 signaling increases and reduces bile acid synthesis in the liver. Disruption of the intestinal microbiota in carrying out its function disrupts this signaling so that unsuppressed bile acid synthesis has the potential to increase damage to the liver, especially in conditions of cholestasis.[19]

The analysis revealed that the positive control group's *Lactobacillus* count was considerably higher than that of the negative control group, indicating an improvement in the number of microbiotas with UDCA administration. The number of *Lactobacillus* in the group that received probiotic therapy alone showed significantly higher results than the group that received UDCA therapy alone. Analysis of the combination of UDCA and probiotics showed differences between the treatment group and the control, indicating that the combination of probiotics with UDCA could increase the number of *Lactobacillus* compared to UDCA or probiotic therapy alone. Meanwhile, analysis between the treatment groups showed that the 54 mg probiotic dose produced the highest number of *Lactobacillus* compared to smaller probiotic doses. This finding resonates with Sun et al. studies, which analyzed infant cholestatic biliary diseases and found elevated potential pathogens and reduced beneficial bacteria.[20] Surgical intervention induced significant microbiota changes, suggesting therapeutic effects on gut microbiota in cholestasis. The UDCA-probiotic combination elevated *Lactobacillus* levels significantly compared to UDCA or probiotics alone and surpassed probiotic-only therapy in *Lactobacillus* augmentation. Higher probiotic doses exhibited dose-dependent trends.[20]

Various studies showing the beneficial effects of particular probiotic strains, such as *Lactobacillus, Bifidobacterium*, and *Streptococcus*, in liver disorders are consistent with this result.[21,22] These probiotics modulate gut microbiota composition, reinforce intestinal barrier function, and mitigate liver damage. Probiotics also play a role in bile acid pathways, making them potential cholestasis ameliorators. Tanaka et al. findings demonstrated that probiotics like Bifidobacterium and *Lactobacillus* hydrolyze bile acids, influencing bile acid metabolism.[23] These probiotics could act through FXR receptor modulation and indirect gut flora regulation, as seen in significant *Lactobacillus* changes.[24]

Regarding hepatocellular damage assessment, markers AST and ALT reveal liver function, particularly during cholestasis. ALT, found in various tissues, exists in cytosolic and mitochondrial forms. AST, mainly hepatic and cytosolic, often surpasses ALT levels in liver diseases. AST levels are usually higher than ALT in the majority of types of liver disease, where the activity of these two enzymes is predominantly from the cytosol of hepatocyte cells. Hepatocellular damage and cell death will trigger the release of this enzyme into the circulation. This study showed that the AST and ALT levels in the negative control group are significantly higher than those in the healthy control group, indicating that there is damage to the liver organ that occurs due to the action of choledochal duct ligation. Additionally, AST and ALT values of positive control group were considerably lower than those of the negative control group, suggesting that UDCA has a hepatoprotective impact. Ursodeoxycholic acid

has multiple therapeutic applications, such as restoring various deficits, reducing the cholesterol content of bile, activating nuclear receptors on intestinal and liver cells, and shielding the liver from bile acid retention in cholestatic diseases.[25]

These findings align with Simental-Mendia, et al. meta-analyses, demonstrating UDCA's efficacy in lowering AST and ALT levels, and other liver function markers.[25] While probiotic-only therapy raised AST and ALT levels compared to UDCA alone, combining UDCA and probiotics further reduced these levels, particularly at higher probiotic doses. However, administering 36 mg of probiotics showed no significant ALT differences, suggesting limited dose dependence.[25]

Analysis of the group that received probiotic therapy alone showed lower AST levels than the group that received UDCA therapy alone. These findings indicate that the use of probiotics can reduce AST levels better than UDCA. A review by Musazadeh et al. showed from existing evidence, probiotics can reduce AST levels.[26] However, the effects are heterogeneous, where differences in factors such as dose, sample characteristics, and duration of intervention will influence the effects. Meanwhile, ALT levels in the same group were still significantly higher than those in the UDCA therapy group alone. This could be caused by the effect of UDCA acting on the liver, considering that ALT is more specific as a marker for liver damage because it is mainly contained in the cytosol of hepatocyte cells. The hepatoprotective effect produced by UDCA works directly on liver cells, compared to probiotics which are thought to work indirectly on the liver.[26]

Analysis of UDCA and probiotics combination showed differences between the treatment and control groups, indicating that the combination of probiotics with UDCA could reduce AST and ALT levels compared to UDCA or probiotic therapy alone. Meanwhile, analysis between treatment groups showed that larger probiotic doses produced lower AST and ALT levels than smaller probiotic doses. However, administering a 36 mg probiotic dose did not show any significant difference in SGPT levels with the other two doses, indicating there was no dose-dependent effect at this dose.

Liu et al. studies reinforced these findings, showing that the UDCA-probiotic combination positively impacted intrahepatic cholestasis patients.[25] This effect is attributed to probiotics' role in modulating gut microbiota, and controlling bacterial growth, and bile acid levels. *Lactobacillus rhamnosus* GG probiotic treatment improved liver function in bile duct-ligated rats by enhancing bile salt hydrolase activity, leading to bile acid deconjugation and reduced hydrophilicity. This aided bile acid excretion and minimized hepatic accumulation. However, a placebo-controlled study in patients with primary sclerosing cholangitis-related cholestasis revealed that probiotic usage had no positive effects on biochemistry or liver function in addition to not improving patient symptoms. The use of probiotics is strain- and dose-specific, so it can produce different effects on cholestasis which is triggered by various factors.

In conclusion, the study underscores the potential benefits of combining UDCA and probiotics in managing cholestasis, improving gut microbiota balance, and enhancing liver function.[27] However, it's essential to acknowledge the limitations of our study, such as the lack of histopathological examination of liver biopsies to visualize direct therapeutic effects and the limited duration of the intervention. Future research should address these limitations to provide a more comprehensive understanding of the therapeutic potential of UDCA-probiotic combination therapy in cholestasis management.

CONCLUSION

Administering probiotics alone has been shown to increase *Lactobacillus* count, while the combination of UDCA and probiotics has demonstrated an even greater ability to elevate *Lactobacillus* count in Sprague Dawley rats. Furthermore, the UDCA-probiotic combination effectively reduced both AST and ALT levels in Sprague Dawley rats. These findings suggest potential implications for further clinical research into new combination therapies for treating cholestasis with novel drug combinations.

CONFLICT OF INTEREST

There are no conflicts of interest in the process of making this article.

ACKNOWLEDGEMENT

There are no other contributors who are not qualified on the author's list. Research is funded by the corresponding author individually.

AUTHOR CONTRIBUTION

All authors contributed to this work. MHN, ESL, BPB were involved in the conception and planning of the research, MHN performed the data acquisition/collection, calculated the experimental data, and performed the analysis, and drafted the manuscript. ESL, BPB, SAP, and MM supervised all research processes. The final manuscript was read and approved by all authors.

LIST OF ABBREVIATIONS

UDCA: ursodeoxycholic acid; AST: alanine aminotransferase; ALT: aspartate aminotransferase; CFU: Colony Forming Unit; DNA: Deoxyribo Nucleic Acid; FXR: Farnesoid X Receptor; LGG: Lactobacillus rhamnosus GG; PBC: primary biliary cholangitis; PSC: primary sclerosing cholangitis; qPCR: Quantitative PCR; rRNA: ribosome-Ribonucleic Acid; SDE: Sequential Detergent Extraction; TGR5: Takeda G protein-coupled receptor-5.

REFERENCES

1. Onofrio FQ, Hirschfield GM. The pathophysiology of cholestasis and its relevance to clinical practice. Clin Liver Dis (Hoboken). 2020;15(3):110–4. https://doi.org/10.1002/cld.894.
2. Hilscher MB, Kamath PS, Eaton JE. Cholestatic liver diseases. Mayo Clin Proc. 2020;95(10):2263–79. https://doi.org/10.1016/j.mayocp.2020.01.015.
3. Shah R, John S. Cholestatic jaundice - PubMed [Internet]. [cited 2023 Aug 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/29489239/
4. Shipovskaya AA, & Dudanova OP Intrahepatic cholestasis in nonalcoholic fatty liver disease. Terapevticheskii Arkhiv. 2018;90(2):69-74. doi: 10.26442/terarkh201890269-74
5. Rini SS, Wibawa IDN. Evaluation and management of chronic cholestatic liver diseases. Middle East J. Dig. Dis. 2023;15(3):148–55. https://doi.org/10.34172/mejdd.2023.336.
6. Hasegawa S, Yoneda M, Kurita Y, Nogami A, Honda Y, Hosono K, et al. Cholestatic liver disease: Current treatment strategies and new therapeutic agents. Drugs. 2021;81(10):1181–92. https://doi.org/10.1007/s40265-021-01545-7.
7. Fickert P, Wagner M. Biliary bile acids in hepatobiliary injury – What is the link? J Hepatol. 2017;67(3):619–31. https://doi.org/10.1016/j.jhep.2017.04.026.
8. Lv LX, Fang DQ, Shi D, Chen DY, Yan R, Zhu YX, et al. Alterations and correlations of the gut microbiome, metabolism and immunity in patients with primary biliary cirrhosis. Environ Microbiol. 2016;18(7):2272–86. https://doi.org/10.1111/1462-2920.13401.
9. Abe K, Takahashi A, Fujita M, Imaizumi H, Hayashi M, Okai K, et al. Dysbiosis of oral microbiota and its association with salivary immunological biomarkers in autoimmune liver disease. PLoS One. 2018;13(7):e0198757. https://doi.org/10.1371/journal.pone.0198757.
10. Tang R, Wei Y, Li Y, Chen W, Chen H, Wang Q, et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. Gut. 2018;67(3):534–41.https://doi.org/10.1136/gutjnl-2016-313332.
11. Furukawa M, Moriya K, Nakayama J, Inoue T, Momoda R, Kawaratani H, et al. Gut dysbiosis associated with clinical prognosis of patients with primary biliary cholangitis. Hepatol. 2020;50(7):840–52. https://doi.org/10.1111/hepr.13509.
12. Chen W, Wei Y, Xiong A, Li Y, Guan H, Wang Q, et al. Comprehensive analysis of serum and fecal bile acid profiles and interaction with gut microbiota in primary biliary cholangitis. Clin Rev Allergy Immunol. 2020;58(1):25–38. https://doi.org/10.1007/s12016-019-08731-2.
13. Mori H, Svegliati Baroni G, Marzioni M, Di Nicola F, Santori P, Maroni L, et al. Farnesoid x receptor, bile acid metabolism, and gut microbiota. Metabolites. 2022;12(7). https://doi.org/10.3390/metabo12070647.
14. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56. https://doi.org/10.1093/ajcp/28.1.56. 14
15. Halim JAN, Lestari ES, Prasetyo SA, Muniroh M, Prasetyo AA. Combination of ursodeoxycholic acid and glutathione improves intestinal morphology in cholestasis by downregulating TNF-α expression. Indones Biomed J. 2022;14(4):429. https://doi.org/10.18585/inabj.v14i4.2044
16. Bestari SA, Fulyani F, Kusuma RJ, Lestari ES. A diet high in protein and fiber changes the gut microbiota of colorectal cancer rat mod-

el. Food Res. 2023;7(3):221–6. https://doi.org/10.26656/fr.2017.7(3).575.
17. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. BMC Vet. Res. 2020;16(1):242. https://doi.org/10.1186/s12917-020-02451-y.
18. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: Mechanisms of action and therapeutic use revisited. Hepatol. 2002; 36:525-31. https://doi.org/10.1053/jhep.2002.36088.
19. Sun X, Cai Y, Dai W, Jiang W, Tang W. The difference of gut microbiome in different biliary diseases in infants before operation and the changes after operation. BMC Pediatr. 2022;22(1):502. https://doi.org/10.1186/s12887-022-03570-1.
20. Koutnikova H, Genser B, Monteiro-Sepulveda M, Faurie JM, Rizkalla S, Schrezenmeir J, et al. Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: A systematic review and meta-analysis of randomised controlled trials. BMJ Open. 2019;9(3):e017995. https://doi.org/10.1136/bmjopen-2017-017995.
21. Oh RC, Hustead TR, Ali SM, Pantsari MW. Mildly elevated liver transaminase levels: Causes and evaluation. Am Fam Physician. 2017; 96(11):709–15. Available from: https://pubmed.ncbi.nlm.nih.gov/29431403/.
22. Shehata MG, El Sohaimy SA, El-Sahn MA, Youssef MM. Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity', Ann Agric Sci. 2016;61(1):65–75. https://doi.org/10.1016/j.aoas.2016.03.001.
23. Hernández-Gómez JG, López-Bonilla A, Trejo-Tapia G, Ávila-Reyes S V., Jiménez-Aparicio AR, Hernández-Sánchez H. In vitro bile salt hydrolase (Bsh) activity screening of different probiotic microorganisms. Foods. 2021;10(3):1–10. https://doi.org/10.3390/foods10030674.
24. Simental-Mendía M, Sánchez-García A, Simental-Mendía LE. Effect of ursodeoxycholic acid on liver markers: A systematic review and meta-analysis of randomized placebo-controlled clinical trials. Br J Clin Pharmacol. 2020;86(8):1476–88. https://doi.org/10.1111/bcp.14311.
25. Liu Y, Chen K, Li F, Gu Z, Liu Q, He L, et al. Probiotic Lactobacillus rhamnosus GG prevents liver fibrosis through inhibiting hepatic bile acid synthesis and enhancing bile acid excretion in mice. Hepatol. 2020;71(6):2050–66. https://doi.org/10.1002/hep.30975.
26. Musazadeh V, Roshanravan N, Dehghan P, Ahrabi SS. Effect of probiotics on liver enzymes in patients with non-alcoholic fatty liver disease: an umbrella of systematic review and meta-analysis. Front Nutr. 2022;9:1. https://doi.org/0.3389/fnut.2022.844242.
27. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27. https://doi.org/10.4103/0976-0105.177703.