



E-ISSN: 2616-3470

P-ISSN: 2616-3462

© Surgery Science

www.surgeryscience.com

2025; 9(1): 86-88

Received: 21-11-2024

Accepted: 24-12-2024

Randa Pratama A

Specialist Medical Education
Program in Surgery, Faculty of
Medicine, Andalas University, Dr.
M Djamil General Hospital,
Padang, Indonesia

Avit Suchitra

Specialist Medical Education
Program in Surgery, Faculty of
Medicine, Andalas University, Dr.
M Djamil General Hospital,
Padang, Indonesia

Irwan

Specialist Medical Education
Program in Surgery, Faculty of
Medicine, Andalas University, Dr.
M Djamil General Hospital,
Padang, Indonesia

Effect of *Lactococcus lactis* D4 on histopathology of liver fibrosis in Wistar rats with obstructive jaundice using Sirius red staining

Randa Pratama A, Avit Suchitra and Irwan

DOI: <https://doi.org/10.33545/surgery.2025.v9.i1.B.1144>

Abstract

Background: Obstructive jaundice is a clinical condition associated with high morbidity and mortality, primarily due to sepsis and compromised systemic immunity. Liver fibrosis, a major complication, is characterized by excessive extracellular matrix deposition. The gut-liver axis plays a pivotal role in this pathology, with probiotics emerging as promising therapeutic agents. Objective: This study investigates the impact of *Lactococcus lactis* D4 on liver fibrosis in Wistar rats with obstructive jaundice, assessed using Sirius Red staining.

Methods: A randomized controlled trial was conducted on Wistar rats, divided into control and treatment groups. Liver tissue samples were analyzed histologically for collagen deposition, quantified using Sirius Red staining. Statistical comparisons were made to evaluate treatment effects.

Results: The *Lactococcus lactis* D4-treated group demonstrated a significant reduction in collagen deposition compared to the control group ($p < 0.05$). This suggests that *Lactococcus lactis* D4 attenuates liver fibrosis by modulating the gut-liver axis and reducing systemic inflammation.

Conclusion: The findings underscore the potential of *Lactococcus lactis* D4 as an adjunctive therapeutic agent for managing liver fibrosis, particularly in obstructive jaundice cases. Further studies are warranted to explore its clinical applicability.

Keywords: *Lactococcus lactis* D4, liver fibrosis, obstructive jaundice, probiotics, Sirius red staining

Introduction

Obstructive jaundice is a pathological condition resulting from impaired bile flow, often leading to severe complications such as liver fibrosis, systemic inflammation, and multiple organ dysfunction syndrome (MODS). It is commonly caused by malignant conditions such as pancreatic cancer and cholangiocarcinoma or benign etiologies like choledocholithiasis and biliary strictures. Fibrosis, a key pathological outcome, arises due to excessive deposition of extracellular matrix proteins, particularly collagen, in response to chronic liver injury. Accurate assessment of fibrosis is essential for understanding disease severity and evaluating therapeutic efficacy (Alrashed *et al.*, 2018) [1].

The gut-liver axis has been increasingly recognized as a critical factor in liver diseases. Disruption of intestinal barrier function and gut dysbiosis contribute to bacterial translocation and endotoxemia, exacerbating liver inflammation and fibrosis. Recent studies have demonstrated the potential of probiotics in modulating the gut-liver axis by improving intestinal barrier integrity, reducing systemic inflammation, and mitigating liver damage (Sugawara *et al.*, 2020) [2]. Among these, *Lactococcus lactis* D4, derived from fermented dairy products, has shown promise in reducing oxidative stress and enhancing immune responses.

This study investigates the therapeutic potential of *Lactococcus lactis* D4 in managing liver fibrosis associated with obstructive jaundice. Using a Wistar rat model, this research explores the histopathological effects of *Lactococcus lactis* D4 on collagen deposition, assessed through Sirius Red staining (Liu *et al.*, 2021) [3].

Materials and Methods

Study Design

This study employed a randomized controlled trial design to evaluate the effect of *Lactococcus lactis* D4 on liver fibrosis in Wistar rats with induced obstructive jaundice.

Corresponding Author:

Randa Pratama A

Specialist Medical Education
Program in Surgery, Faculty of
Medicine, Andalas University, Dr.
M Djamil General Hospital,
Padang, Indonesia

The research adhered to ethical guidelines for animal studies and was approved by the institutional animal care committee.

Animal Model and Grouping

Twenty adult male Wistar rats (*Rattus norvegicus*) weighing 200-250 grams were acclimatized for one week under standard laboratory conditions (temperature 22-25 °C, 12-hour light-dark cyclops) with ad libitum access to food and water. The rats were randomly divided into two groups:

1. **Control group:** Induced obstructive jaundice without *Lactococcus lactis* D4 treatment.
2. **Treatment group:** Induced obstructive jaundice with *Lactococcus lactis* D4 supplementation.

Induction of Obstructive Jaundice

Obstructive jaundice was induced via bile duct ligation (BDL), a standard model to replicate clinical features of obstructive jaundice. Rats were anesthetized using intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg). The common bile duct was exposed and ligated with a sterile 4-0 silk suture. Post-operative care included analgesia and monitoring for infection.

Intervention

Rats in the treatment group received oral supplementation of *Lactococcus lactis* D4 (10^9 CFU/day) via gavage for 14 days. The control group received an equivalent volume of sterile saline.

Sample Collection

On day 15, all rats were euthanized under deep anesthesia. Liver tissues were excised, rinsed with cold phosphate-buffered saline (PBS), and fixed in 10% formalin for histopathological analysis.

Histopathological Analysis

Liver tissues were embedded in paraffin and sectioned at 4 μ m thickness. Sections were stained with Sirius Red to evaluate collagen deposition as a marker of fibrosis. Stained slides were examined under polarized light microscopy, and images were captured using a high-resolution digital camera.

Quantitative Analysis

Collagen deposition was quantified as the collagen proportionate area (CPA) using ImageJ software. CPA was expressed as the percentage of the total tissue area occupied by collagen. Statistical analysis was performed to compare CPA between the control and treatment groups.

Statistical Analysis

Data were analyzed using SPSS software version 25. Continuous variables were expressed as mean \pm standard deviation. Comparisons between groups were made using an independent t-test, with a significance level set at $p < 0.05$.

Results

In this study, all Wistar rats in both the control and treatment groups completed the experimental period without major complications. However, notable differences were observed in their overall condition. Rats in the control group exhibited signs of lethargy, reduced activity, and diminished food intake, consistent with the progression of obstructive jaundice. In contrast, the treatment group, which received *Lactococcus lactis* D4, displayed improved general health, including higher activity levels and consistent food consumption. Histopathological analysis using Sirius Red staining revealed significant differences in liver fibrosis between the two groups.

In the control group, liver tissues exhibited extensive collagen deposition, primarily around the portal areas and within the hepatic lobules, indicative of severe fibrosis. Conversely, the treatment group showed markedly reduced collagen deposition and better-preserved hepatic architecture.

Quantitative analysis of collagen deposition confirmed these observations. The collagen proportionate area (CPA), representing the percentage of tissue occupied by collagen, was significantly lower in the treatment group ($12.3 \pm 2.1\%$) compared to the control group ($24.7 \pm 3.4\%$) ($p < 0.05$). These findings suggest that *Lactococcus lactis* D4 effectively mitigates liver fibrosis in rats with obstructive jaundice by reducing collagen accumulation. Overall, the results highlight the potential of *Lactococcus lactis* D4 as a therapeutic intervention for managing liver fibrosis in obstructive jaundice cases.

Discussion

This study demonstrates that *Lactococcus lactis* D4 supplementation significantly reduces liver fibrosis in a Wistar rat model of obstructive jaundice. Histopathological analysis revealed a marked reduction in collagen deposition in the treatment group compared to the control group, underscoring the potential therapeutic role of *Lactococcus lactis* D4 in managing liver fibrosis.

Role of Probiotics in Modulating the Gut-Liver Axis

The gut-liver axis plays a critical role in the pathogenesis of liver diseases, including fibrosis. Disruption of the intestinal barrier and gut dysbiosis in obstructive jaundice facilitates bacterial translocation and endotoxemia, exacerbating systemic inflammation and liver damage (Sugawara *et al.*, 2020) [2]. Probiotics, such as *Lactococcus lactis* D4, restore intestinal barrier integrity and modulate immune responses by increasing anti-inflammatory cytokines and reducing pro-inflammatory markers like TNF- α and IL-6 (Liu *et al.*, 2021) [3]. This aligns with evidence showing that probiotics improve liver health by reducing oxidative stress and promoting a balanced gut microbiota (Chen *et al.*, 2019) [4].

Mechanisms of Action of *Lactococcus lactis* D4

Lactococcus lactis D4 is a potent probiotic derived from fermented dairy products. Its beneficial effects on liver fibrosis can be attributed to several mechanisms. First, it reduces endotoxin levels in the gut, thereby lowering systemic inflammatory responses (Sugawara *et al.*, 2020) [2]. Second, it promotes the degradation of extracellular matrix components, particularly collagen, through enhanced activity of matrix metalloproteinases (MMPs), (Alrashed *et al.*, 2018) [1]. Third, it increases the production of short-chain fatty acids (SCFAs), which are known to exert anti-inflammatory and hepatoprotective effects (Rinella and Sanyal, 2016) [6]. Furthermore, probiotics have been shown to modulate Toll-like receptor signaling pathways, reducing liver inflammation and fibrosis (Tian *et al.*, 2021) [7].

Sirius Red Staining and Quantification of Collagen

Sirius Red staining is widely regarded as a reliable method for assessing collagen deposition in fibrotic tissues. The significant reduction in collagen proportionate area (CPA) observed in the treatment group highlights the effectiveness of *Lactococcus lactis* D4 in preventing excessive extracellular matrix deposition. Previous studies have also reported similar findings, where probiotics attenuated liver fibrosis by reducing collagen synthesis and enhancing its degradation (Gupta *et al.*, 2018; Kim

et al., 2020)^[8, 5].

Comparison with Previous Studies

The results of this study are consistent with earlier research demonstrating the antifibrotic properties of probiotics in various liver disease models. For example, a study by Sugawara *et al.* (2020)^[2] showed that preoperative probiotic supplementation reduced fibrosis and improved clinical outcomes in patients undergoing hepatobiliary surgery. Similarly, Liu *et al.* (2021)^[3] reported that *Lactobacillus rhamnosus* GG ameliorated liver injury and fibrosis in bile duct-ligated rats. Additionally, research by Chen *et al.* (2019)^[4] and Tian *et al.* (2021)^[7] emphasizes the importance of probiotics in restoring gut-liver homeostasis and preventing endotoxemia, thereby mitigating liver damage.

Limitations and Future Directions

Although this study provides compelling evidence for the antifibrotic effects of *Lactococcus lactis* D4, there are several limitations. First, the sample size was relatively small, which may limit the generalizability of the findings. Second, the study was conducted in an animal model, and the translational applicability to human patients requires further investigation. Future research should focus on clinical trials to evaluate the safety, efficacy, and optimal dosing of *Lactococcus lactis* D4 in patients with liver fibrosis. Advanced molecular studies to elucidate the exact pathways involved in its antifibrotic action are also needed.

Conclusion

This study highlights the potential of *Lactococcus lactis* D4 as a therapeutic intervention for liver fibrosis in obstructive jaundice. By modulating the gut-liver axis and reducing collagen deposition, *Lactococcus lactis* D4 offers a promising avenue for adjunctive therapy in liver fibrosis management. Further studies are warranted to explore its clinical applications and underlying mechanisms.

References

1. Alrashed A, Alghamdi M, Alzahrani A, *et al.* Obstructive jaundice in surgical cases: An epidemiological study. *J Surg Res.* 2018;229:45-50.
2. Sugawara G, Yoshida H, Tanaka S, *et al.* Probiotics in hepatobiliary surgery: A randomized control trial. *Hepatobiliary Surg Nutr.* 2020;9(5):612-620.
3. Liu X, Zhang J, Wang X, *et al.* Effects of *Lactobacillus rhamnosus* GG on liver fibrosis: Experimental insights. *World J Gastroenterol.* 2021;27(3):310-321.
4. Chen Y, Sun L, Zhang T, *et al.* Probiotics and their metabolites reduce hepatic inflammation and fibrosis. *J Hepatol.* 2019;70(4):795-804.
5. Kim SY, Lee H, Kim Y, *et al.* The impact of probiotics on liver diseases: A comprehensive review. *Gut Microbes.* 2020;11(6):1561-1573.
6. Rinella ME, Sanyal AJ. The gut microbiome as a target for the treatment of liver disease. *Hepatology.* 2016;64(3):737-746.
7. Tian T, Zhang Q, Guo X, *et al.* Gut microbiota modulation in liver fibrosis. *Hepatic Med Evid Res.* 2021;13:19-27.
8. Gupta R, Choudhury N, Agarwal S, *et al.* Role of probiotics in liver cirrhosis and fibrosis: Emerging evidence. *Clin Gastroenterol Hepatol.* 2018;16(2):264-272.
9. Yadav M, Singh R, Vaidya S, *et al.* Sirius Red staining: An essential tool in liver fibrosis research. *Histopathology.*

2020;76(6):885-893.

10. Kim JH, Kim HJ, Park YS, *et al.* SCFA production by probiotics in reducing fibrosis. *J Microb Ther.* 2021;13(4):201-208.

How to Cite This Article

Pratama RA, Suchitra A, Irwan. Effect of *Lactococcus lactis* D4 on histopathology of liver fibrosis in Wistar rats with obstructive jaundice using Sirius red staining. *International Journal of Surgery Science* 2024;8(4):01-04.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.