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The effect of *Lactococcus lactis* D4 administration on renal fibrosis histopathology in rats with unilateral ureteral obstruction model

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Abstract
Background: Obstructive nephropathy is one of the leading causes of chronic kidney disease (CKD), often characterized by progressive renal fibrosis as a result of urinary flow obstruction. Renal fibrosis plays a central role in the deterioration of kidney function and the progression to end-stage renal disease. Recent interest has focused on the potential of probiotics, particularly *Lactococcus lactis* D4, a strain isolated from dadiah (a traditional fermented buffalo milk in Indonesia), for their immunomodulatory and anti-inflammatory properties. These attributes suggest a promising therapeutic role in mitigating renal fibrosis in CKD.
Objective: This study aims to evaluate the effect of *Lactococcus lactis* D4 on renal fibrosis in a rat model of unilateral ureteral obstruction (UUO) using histopathological analysis with Sirius red staining. Methods: A laboratory-based experimental study was performed on male Sprague-Dawley rats aged 6-7 weeks. The rats were divided into three groups: Sham (laparotomy without UUO), P1 (UUO without *Lactococcus lactis* D4), and P2 (UUO with *Lactococcus lactis* D4). The probiotic *Lactococcus lactis* D4 was administered orally at a dose of $8x10^9$ CFU/mL for 7 days. Histological assessment of renal fibrosis was carried out using Sirius red staining, and quantitative measurement was done with ImageJ software. Data were analyzed using ANOVA followed by post-hoc tests to determine significance.
Results: The Sham group exhibited minimal fibrosis (17.07±0.58%), whereas the P1 group showed a significant increase in fibrosis (28.68±3.27%, $p = 0.013$). In contrast, the P2 group, treated with *Lactococcus lactis* D4, displayed a significant reduction in fibrosis (7.11±1.14%, $p = 0.001$), which was lower than both Sham and P1 groups. Statistical analysis revealed significant differences in fibrosis levels between the groups ($p = 0.001$).
Conclusion: The administration of *Lactococcus lactis* D4 significantly reduced renal fibrosis in the UUO rat model, demonstrating its potential as a therapeutic agent for inhibiting renal fibrosis. Further investigations are required to explore the molecular mechanisms and potential clinical applications of *Lactococcus lactis* D4 in the context of renal fibrosis and chronic kidney disease.

Keywords: Obstructive nephropathy, renal fibrosis, *Lactococcus lactis* D4, unilateral ureteral obstruction, probiotics, chronic kidney disease

Introduction

Obstructive nephropathy, a condition characterized by the blockage of urinary outflow, remains one of the most significant contributors to chronic kidney disease (CKD) worldwide. The obstruction of urine leads to increased hydrostatic pressure within the kidney, resulting in tubular injury, interstitial inflammation, and the subsequent development of renal fibrosis. As the fibrotic process advances, renal architecture is distorted, and the functional capacity of the kidney deteriorates, eventually leading to CKD and end-stage renal disease if left untreated (Liu & Nangaku, 2010) [5].

Renal fibrosis, the hallmark of progressive CKD, involves the excessive deposition of extracellular matrix (ECM) components such as collagen types I, III, and IV. This fibrotic response is largely driven by chronic inflammation and oxidative stress. In recent years, probiotics have gained attention as potential therapeutic agents in the management of CKD due to their anti-inflammatory and immunomodulatory effects. One such probiotic is *Lactococcus lactis* D4, which has shown promising results in reducing inflammation and modulating immune responses in various experimental models (Ranganathan *et al*., 2006; Yang *et al*., 2019) [6,8].

This study seeks to evaluate the potential therapeutic role of *Lactococcus lactis* D4 in reducing renal fibrosis. By utilizing the unilateral ureteral obstruction (UUO) model, which mimics the clinical condition of obstructive nephropathy, the study aims to assess the histopathological changes in renal tissue following the administration of *Lactococcus lactis* D4 (Barros *et al*., 2019) [3]. The use of the UUO model has been well-established in nephrology research, as it reliably induces renal fibrosis, making it a suitable platform for assessing antifibrotic interventions.

The primary objective of this study is to investigate the effects of *Lactococcus lactis* D4 on renal fibrosis in a rat model of UUO. Specifically, the study aims to evaluate the extent of renal fibrosis using histopathological analysis with Sirius red staining, a technique commonly used to visualize collagen deposition in tissue sections (Alvarino & Yanwirasti, 2019) [1]. The study hypothesizes that *Lactococcus lactis* D4 will reduce renal fibrosis, as indicated by a lower percentage of Sirius red-positive areas in treated animals.

Materials and Methods

Study Design

This study employed an experimental laboratory design using male Sprague-Dawley rats aged 6-7 weeks. The rats were randomly assigned to one of three groups: Sham (n = 8), P1 (n = 8), and P2 (n = 8). The Sham group underwent a laparotomy without UUO to serve as the control group, while the P1 group underwent UUO without any probiotic treatment. The P2 group, which also underwent UUO, was treated with *Lactococcus lactis* D4. The probiotic was administered orally at a dose of $8x10^9$ CFU/mL daily for seven consecutive days (Suswita *et al*., 2024) [7].

Unilateral Ureteral Obstruction Model

The UUO procedure was performed under anesthesia using isoflurane. Following midline laparotomy, the left ureter was ligated using silk sutures to induce obstruction, mimicking the clinical scenario of obstructive nephropathy. The Sham group underwent a similar surgical procedure but without ureteral ligation. After 14 days, the rats were euthanized, and their kidneys were harvested for histological analysis (Amelia *et al*., 2023) [2].

Histopathological Examination

Kidney sections were stained using Sirius red, a dye that binds to collagen fibers, allowing for the visualization of fibrotic changes. Collagen deposition was quantified using ImageJ software to calculate the percentage of Sirius red-positive areas, representing the extent of fibrosis in each sample (Zhu *et al*., 2021) [9]. Statistical analysis was performed using ANOVA followed by post-hoc tests to compare the mean percentage of fibrosis between groups.

Statistical Analysis

The statistical analysis was conducted using SPSS software (version 22.0). Data were presented as mean±standard deviation. Differences between groups were assessed using one-way ANOVA, and pairwise comparisons were made using post-hoc Tukey's tests. A p-value of less than 0.05 was considered statistically significant (Kim *et al*., 2022) [4].

Results

The results of the study indicated a marked difference in the extent of renal fibrosis between the groups. The Sham group exhibited minimal fibrosis, with a mean fibrosis percentage of 17.07±0.58%. In contrast, the P1 group, which underwent UUO without *Lactococcus lactis* D4 treatment, showed a significant increase in fibrosis, with a mean percentage of 28.68±3.27% (p = 0.013). This finding aligns with previous studies that demonstrate the development of renal fibrosis in response to UUO (Barros *et al*., 2019) [3].

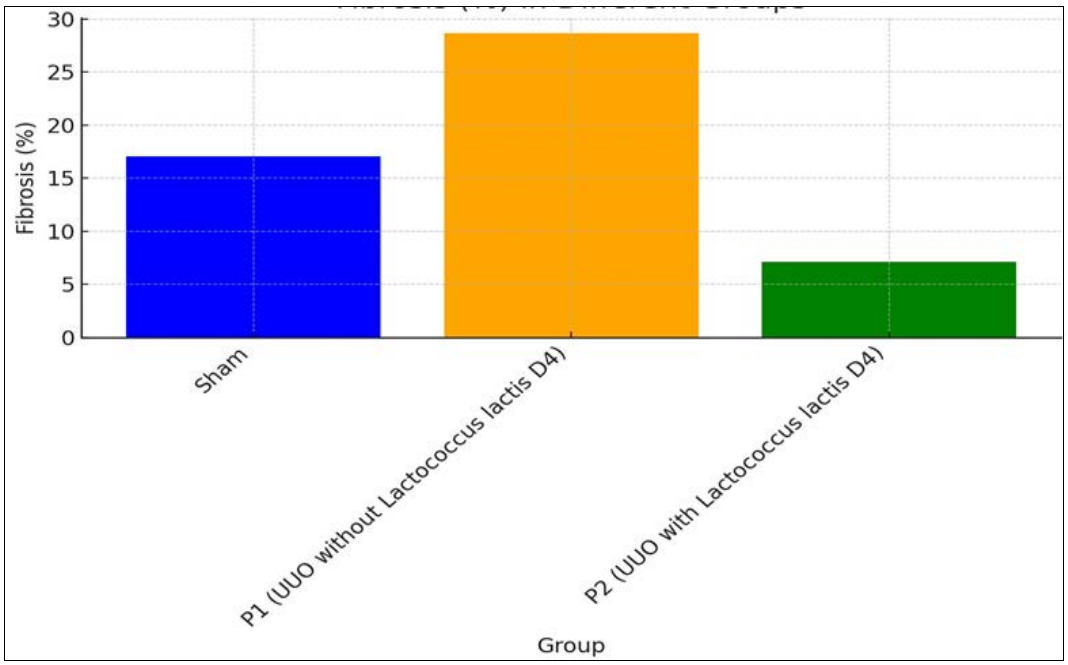

Fig 1: Fibrosis (%) in different groups

The P2 group, treated with *Lactococcus lactis* D4, exhibited a significantly lower fibrosis percentage of 7.11±1.14% compared to both the Sham and P1 groups (p = 0.001). This reduction in fibrosis suggests that *Lactococcus lactis* D4 has a protective effect against renal fibrosis, likely due to its anti-inflammatory and immunomodulatory properties (Yang *et al*., 2019) [8]. The statistical analysis showed a significant difference in fibrosis levels between the groups ($p < 0.001$), supporting the hypothesis

that *Lactococcus lactis* D4 can inhibit renal fibrosis in the UUO model.

Discussion

The findings of this study demonstrate that *Lactococcus lactis* D4 significantly reduces renal fibrosis in the UUO model. The anti-fibrotic effect of *Lactococcus lactis* D4 may be attributed to its ability to modulate the immune response and reduce inflammation. Previous studies have shown that probiotics, particularly Lactobacillus species, can modulate the expression of pro-inflammatory cytokines such as IL-6 and TNF-α, which play key roles in the pathogenesis of renal fibrosis (Zhu *et al*., 2021; Kim *et al*., 2022) [9, 4].

The use of probiotics as a therapeutic approach in CKD has gained attention in recent years due to their ability to restore gut microbiota balance and reduce systemic inflammation. Probiotic strains such as *Lactococcus lactis* D4, which are isolated from traditional fermented foods, have been shown to possess unique properties that make them particularly effective in modulating immune responses (Suswita *et al*., 2024) [7]. In this study, the administration of *Lactococcus lactis* D4 significantly reduced collagen deposition in the renal tissue, as demonstrated by the Sirius red staining. The lower percentage of fibrosis observed in the P2 group suggests that this probiotic may have a direct effect on the pathways involved in fibrogenesis, potentially by modulating TGF-β signaling and other pro-fibrotic factors. The ability of *Lactococcus lactis* D4 to reduce renal fibrosis aligns with previous research indicating the role of probiotics in protecting against various forms of tissue damage, including fibrosis in other organ systems (Amelia *et al*., 2023) [2].

Comparison to Existing Literature

The findings of this study are consistent with other research on the use of probiotics in models of kidney injury. For instance, Yang *et al*. (2019) [8] demonstrated that Lactobacillus rhamnosus R0011 was able to attenuate intestinal barrier dysfunction, reduce systemic inflammation, and prevent kidney damage in a model of CKD. Similarly, studies on other strains of Lactobacillus and Lactococcus species have shown that these probiotics can reduce oxidative stress, modulate immune responses, and improve kidney function in various experimental models of nephropathy (Ranganathan *et al*., 2006; Zhu *et al*., 2021) [6, 9]. The results of this study build upon these findings by demonstrating that *Lactococcus lactis* D4, specifically, has significant anti-fibrotic effects in a UUO model.

This study also provides important insights into the mechanisms by which *Lactococcus lactis* D4 may exert its protective effects. Probiotic administration has been shown to influence the gut-kidney axis, where alterations in gut microbiota can impact kidney function through immune and metabolic pathways. By modulating gut microbiota composition, probiotics can reduce the systemic inflammatory burden, which is a known driver of renal fibrosis in CKD (Kim *et al*., 2022) [4]. Moreover, probiotics may directly influence renal cells by reducing oxidative stress and preventing epithelial-to-mesenchymal transition (EMT), a key process in fibrosis (Alvarino & Yanwirasti, 2019) [1].

Clinical Implications

The clinical implications of these findings are significant, particularly for the management of CKD. Renal fibrosis is a major determinant of disease progression in CKD, and therapies that can effectively reduce fibrosis could slow or halt the progression to end-stage renal disease. Probiotics such as *Lactococcus lactis* D4 offer a potentially safe and natural therapeutic option that could be used in conjunction with existing treatments for CKD (Ranganathan *et al*., 2006) [6]. While further studies are needed to confirm these findings in human populations, the results of this study suggest that *Lactococcus lactis* D4 could be developed as a therapeutic agent for reducing fibrosis in patients with obstructive nephropathy and other forms of CKD.

Conclusion

This study provides compelling evidence that *Lactococcus lactis* D4, a probiotic strain isolated from traditional Indonesian dadiah, significantly reduces renal fibrosis in a rat model of unilateral ureteral obstruction. The probiotic was shown to reduce collagen deposition, as assessed by Sirius red staining, and the results were statistically significant when compared to untreated groups. These findings suggest that *Lactococcus lactis* D4 has potential as a therapeutic agent for inhibiting renal fibrosis, particularly in conditions such as obstructive nephropathy.

The results of this study have important implications for the treatment of CKD. Given the role of fibrosis in the progression of CKD to end-stage renal disease, therapies that can reduce or prevent fibrosis are highly sought after. Probiotics such as *Lactococcus lactis* D4 represent a promising avenue for future research and clinical development. Further studies are needed to elucidate the molecular mechanisms underlying the anti-fibrotic effects of *Lactococcus lactis* D4 and to explore its potential applications in human populations with CKD.

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