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Ihsan
Department of Surgery, Faculty of Medicine, Andalas University, Dr. M Djamil General Hospital, Padang, Indonesia

Alvarino
Department of Surgery, Faculty of Medicine, Andalas University / Dr. M Djamil General Hospital, Padang, Indonesia

Etriyel MYH
Division of Urology Surgery, Department of Surgery, Faculty of Medicine, Andalas University, Dr. M Djamil General Hospital, Padang, Indonesia

Yevri Zulfiqar
Department of Surgery, Faculty of Medicine, Andalas University, Dr. M Djamil General Hospital, Padang, Indonesia

Rahmat Taufik
Department of Surgery, Faculty of Medicine, Andalas University, Dr. M Djamil General Hospital, Padang, Indonesia

Ade Ricky Harahap
Department of Surgery, Faculty of Medicine, Andalas University, Dr. M Djamil General Hospital, Padang, Indonesia

Corresponding Author:
Ihsan
Department of Surgery, Faculty of Medicine, Andalas University, Dr. M Djamil General Hospital, Padang, Indonesia

Effect of probiotic *Lactococcus lactis* D4 administration on transforming growth factor-β1 (TGF-β1) expression in a rat model of unilateral ureteral obstruction-induced renal fibrosis

Ihsan, Alvarino, Etriyel MYH, Yevri Zulfiqar, Rahmat Taufik and Ade Ricky Harahap

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Abstract
Introduction: Unilateral ureteral obstruction (UUO) is a well-established animal model for studying renal fibrosis and obstructive nephropathy. Transforming Growth Factor-β1 (TGF-β1) plays a key role in renal fibro genesis by promoting fibroblast activation and extracellular matrix accumulation. *Lactococcus lactis* D4 is known for its immunomodulatory and anti-inflammatory properties; however, its effect on TGF-β1 expression in obstructive nephropathy remains underexplored.
Objective: To investigate the effect of probiotic *Lactococcus lactis* D4 on TGF-β1 expression in renal tissue of rats subjected to UUO.
Methods: A post-test-only randomized controlled design was used in 15 male Sprague-Dawley rats (170-220 g), divided into three groups (N=5 each): Control (laparotomy only), P1 (UUO without treatment), and P2 (UUO with *Lactococcus lactis* D4). The probiotic was administered orally at a dose of 8×10^9 CFU/mL, 0.5 mL/day for 7 days. After 7 days, kidney tissues were collected for histopathological analysis. TGF-β1 expression was assessed using immunohistochemistry and quantified as the percentage of positively stained cells.
Results: TGF-β1 expression was significantly different among groups (Control: 33.06±1.14%, P1: 42.67±1.67%, P2: 29.11±1.42%; P=0.000, ANOVA). Post-hoc analysis showed significant increases in TGF-β1 in P1 vs. Control (P=0.001) and significant reductions in P2 vs. P1 (P=0.000).
Conclusion: Administration of *Lactococcus lactis* D4 significantly decreased TGF-β1 expression in the kidneys of rats with UUO-induced renal fibrosis, suggesting its potential as an antifibrotic agent in obstructive nephropathy.

Keywords: Ureteral obstruction, *Lactococcus lactis* D4, TGF-β1, renal fibrosis, probiotic

Introduction
Unilateral ureteral obstruction (UUO) serves as a robust animal model for studying progressive renal fibrosis and the molecular mechanisms of obstructive nephropathy. This condition mimics clinical situations like urolithiasis and congenital abnormalities, leading to functional and structural deterioration of the kidney. Transforming Growth Factor-β1 (TGF-β1) is a pivotal cytokine that contributes to renal fibrosis by enhancing fibroblast proliferation and extracellular matrix production (Meng *et al*., 2016) [2].
Recent research has focused on targeting fibrotic pathways using natural compounds and probiotics. *Lactococcus lactis*, a Gram-positive lactic acid bacterium commonly found in fermented dairy products, has shown promise due to its immunomodulatory and anti-inflammatory actions. Certain strains, including L. lactis D4, may exert antifibrotic effects by suppressing inflammatory mediators and modulating key signaling pathways, although their effects on TGF-β1 expression in UUO models have not been well elucidated (Yamaguchi *et al*., 2014) [9]. This study aims to examine the influence of *Lactococcus lactis* D4 on TGF-β1 expression in rat kidneys following UUO induction.

Materials and Methods
Study Design
This is a laboratory-based experimental study with a "post-test-only randomized control group design."

Animals
Fifteen male Sprague-Dawley rats (6-7 weeks old, weight 170-220 g) were obtained and housed under standard conditions with ad libitum access to food and water.

Group Allocation
Control group: Laparotomy only (N=5)
P_1: UUO without treatment (N=5)
P_2: UUO with *Lactococcus lactis* D4 (N=5)

UUO Induction
UUO was induced via left ureteral ligation under anesthesia. Control rats underwent laparotomy without ureteral ligation.

Probiotic Administration
Lactococcus lactis D4 was prepared in a suspension at 8×10^9 CFU/mL. Rats in P2 received 0.5 mL orally for 7 consecutive days post-UUO.

Tissue Processing and Immunohistochemistry
On day 7, rats were sacrificed, and kidneys were harvested. TGF-β1 expression was assessed by immunohistochemistry. Staining intensity was quantified as the percentage of positively stained cells in cortical regions.

Statistical Analysis
Data were analyzed using one-way ANOVA followed by Bonferroni post-hoc tests. A p-value < 0.05 was considered statistically significant.

Results
This study investigated the impact of *Lactococcus lactis* D4 on TGF-β1 expression in rat kidney tissues under different experimental conditions. The findings are summarized as follows:

1. Control Group (No UUO, No *Lactococcus lactis* D4)
- Average TGF-β1 expression: 33.06±1.14%.
- Range: 29.60%-35.88%.

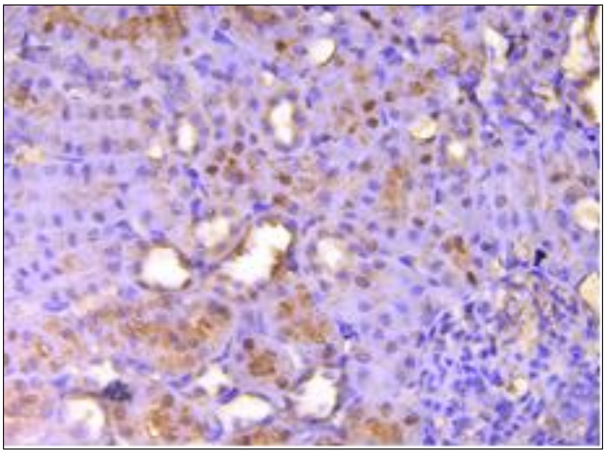

Fig 1: Showed 32% staining in a representative sample (Control-2).

2. UUO Group (Without *Lactococcus lactis* D4)
- Average TGF-β1 expression: 42.67±1.67%.
- Range: 38.85%-47.16%.

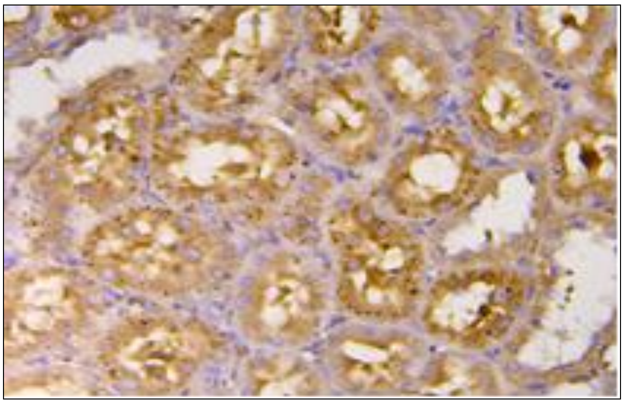

Fig 2: Showed 43.22% staining in a representative sample (P1-2).

3. UUO Group (With *Lactococcus lactis* D4)
- Average TGF-β1 expression: 29.11±1.42%.
- Range: 25.88%-33.04%.

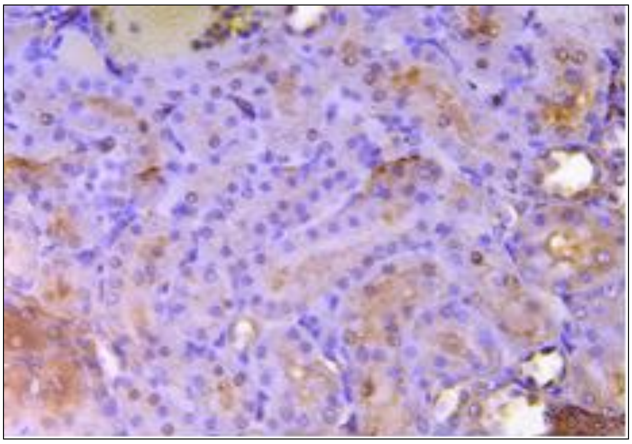

Fig 3: Showed 32% staining in a representative sample (P2-2).

4. Statistical Analysis (One-way ANOVA)
- A significant relationship was found between *Lactococcus lactis* D4 administration and TGF-β1 expression (P=0.000).
- Post hoc tests revealed:
- Significant difference between the Control and UUO (P1) groups (P=0.001).
- Significant difference between the UUO (P1) and UUO with *Lactococcus lactis* D4 (P2) groups (P=0.000).
- No significant difference between the Control and P2 groups (P=0.225).

Lactococcus lactis D4 significantly reduced TGF-β1 expression in rats with kidney fibrosis induced by UUO, bringing levels close to those of the control group. This suggests a potential therapeutic role for *Lactococcus lactis* D4 in mitigating kidney fibrosis.

Discussion
The study investigated the effects of *Lactococcus lactis* D4 (LLD4) on TGF-β1 expression in a rat model of renal fibrosis induced by unilateral ureteral obstruction (UUO). Key findings and their implications are summarized below:

1. **TGF-β1 Expression in Control Group (No UUO or LLD4):** The control group showed a baseline TGF-β1 expression of 33.06±1.14%, attributed to minor inflammation from the laparotomy procedure. This aligns

with studies highlighting surgical stress as a trigger for inflammatory responses, including TGF-β1 upregulation (Rodriguez *et al*., 2023; Gameiro *et al*., 2018) [17, 11].

2. **TGF-β1 Expression in UUO Group (No LLD4):** The UUO group exhibited significantly higher TGF-β1 expression (42.67±1.67%), consistent with the role of TGF-β1 as a central mediator of renal fibrosis. This supports prior research demonstrating TGF-β1 upregulation in obstructive nephropathy due to mechanical stress, oxidative stress, and inflammation (Martínez-Klimova *et al*., 2019; Kong *et al*., 2019) [15, 13].
3. **TGF-β1 Expression in UUO + LLD4 Group:** LLD4 administration reduced TGF-β1 expression to 29.11±1.42%, significantly lower than the UUO group. This suggests LLD4's antifibrotic potential, likely via.

- **SCFAs (e.g., butyrate):** Inhibition of histone deacetylase (HDAC) and suppression of MAPK/ERK pathways, reducing TGF-β1 transcription (Li *et al*., 2019) [14].
- **Anti-inflammatory effects:** Modulation of IL-10 and suppression of pro-inflammatory cytokines (IL-1β, TNF-α) (Del Carmen *et al*., 2011) [19].
- **Oxidative stress reduction:** Activation of Nrf2 pathway to mitigate ROS (He *et al*., 2024) [12].

4. Statistical Significance

ANOVA confirmed LLD4's significant impact on TGF-β1 levels (P=0.000). Post-hoc tests revealed:

- UUO increased TGF-β1 vs. controls (P=0.001).
- LLD4 reduced TGF-β1 vs. UUO (P=0.000), with levels comparable to controls (P=0.225), indicating restoration of near-normal TGF-β1 expression.

5. Limitations and Future Directions

- The study focused solely on TGF-β1, broader mechanistic insights (e.g., other fibrotic markers, immune cell infiltration) are needed.
- Comparative studies with other antifibrotic agents (e.g., TGF-β inhibitors) would strengthen LLD4's therapeutic rationale (Park & Yoo, 2022) [16].
- Translation to clinical models is warranted to validate efficacy in humans.

TGF-β1 is widely recognized as a central mediator of renal fibrosis. The present study confirmed that UUO significantly elevates TGF-β1 expression, consistent with previous findings (Klahr and Morrissey, 2002) [1]. However, administration of *Lactococcus lactis* D4 significantly attenuated TGF-β1 expression, supporting its potential as an antifibrotic agent.

The mechanisms by which L. lactis D4 modulates TGF-β1 may include the inhibition of pro-inflammatory cytokines and regulatory effects on gut-renal axis interactions (Lee *et al*., 2021; Nishida *et al*., 2018) [6, 7]. These findings align with studies demonstrating that probiotics can influence systemic immunity and fibrosis-related pathways. While promising, this study is limited by its small sample size and short observation period. Further research is warranted to explore the long-term benefits and molecular pathways involved.

Conclusion

This study demonstrates that probiotic *Lactococcus lactis* D4 effectively reduces TGF-β1 expression in a UUO-induced renal fibrosis rat model. These results suggest its potential utility as an adjunct therapy in preventing renal fibrosis associated with obstructive nephropathy.

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