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Relationship Between TGF Beta and Liver Regeneration Rate in Wistar Rats After Partial Hepatectomy Treated with Lactococcus lactis D4

Nugra Daary Razsky Gunawan* , Irwan, M. Iqbal Rivai
Universitas Andalas, Indonesia
Email : nugra@gmail.com*

Keywords:
Partial Hepatectomy; Lactococcus lactis D4; Transforming Growth Factor-Beta; Liver Regeneration Rate; Wistar Rat.

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ABSTRACT

Liver regeneration following partial hepatectomy is regulated by coordinated proliferative and growth-termination signals. Transforming growth factor-beta (TGF-β) plays an important role in regulating hepatocyte proliferation and tissue remodeling, while Lactococcus lactis D4 has been reported to support hepatic repair in experimental models. This study aimed to investigate the relationship between hepatic TGF-β expression and the liver regeneration rate (LRR) in male Wistar rats following partial hepatectomy and treatment with L. lactis D4. A randomized, posttest-only experimental design was employed, comprising five groups: sham-operated, partial hepatectomy control, L. lactis D4 administration before hepatectomy (P1), administration after hepatectomy (P2), and administration both before and after hepatectomy (P3). Hepatic TGF-β expression was assessed using immunohistochemistry, while LRR was calculated based on liver and body weights. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by post hoc testing, while the relationship between TGF-β expression and LRR was examined using Pearson's correlation analysis. Mean TGF-β values were 59.89 ± 6.38, 52.77 ± 5.03, 51.31 ± 1.27, 38.69 ± 0.52, and 47.53 ± 2.47 in the sham, control, P1, P2, and P3 groups, respectively ($p < 0.001$). The corresponding LRR values were 200.63 ± 66.03%, 207.50 ± 92.06%, 150.86 ± 71.79%, and 225.85 ± 95.42% in the control, P1, P2, and P3 groups, respectively, with no statistically significant differences among groups ($p = 0.670$). Pearson's correlation analysis revealed a moderate positive correlation between hepatic TGF-β expression and LRR ($r = 0.470$; $p = 0.037$). These findings indicated that L. lactis D4 administration was associated with variations in hepatic TGF-β expression, while TGF-β expression demonstrated a moderate positive correlation with liver regeneration at seven days after partial hepatectomy. However, L. lactis D4 treatment did not produce a statistically significant difference in LRR among the experimental groups.

INTRODUCTION

Hepatectomy is a major surgical procedure performed to treat both neoplastic and non-neoplastic liver diseases. Contemporary multicenter data indicate that liver resection is performed predominantly for malignant conditions, including colorectal liver metastases and hepatocellular carcinoma, although selected benign conditions may also require surgical resection. Despite advances in surgical techniques and perioperative management, postoperative morbidity remains substantial. Posthepatectomy liver failure (PHLF) continues to be one of the most serious complications because of its strong association with

postoperative mortality (Chan et al., 2020; Collaborative, 2023; Orcutt & Anaya, 2018). Consequently, the safety of liver resection depends not only on surgical technique but also on the volume and functional capacity of the future liver remnant (FLR) and its ability to restore adequate liver mass and function following tissue loss (De Gasperi et al., 2024).

The liver possesses a remarkable capacity for regeneration. Following partial hepatectomy, restoration of liver mass occurs through a highly coordinated sequence of cellular and molecular processes involving hepatocyte hypertrophy and proliferation, hemodynamic adaptations, cytokine and growth factor signaling, and interactions between parenchymal and nonparenchymal cells. Increased portal venous flow and shear stress following resection contribute to the activation of several signaling pathways, including interleukin-6/signal transducer and activator of transcription 3 (IL-6/STAT3), tumor necrosis factor-alpha/nuclear factor-kappa B (TNF-α/NF-κB), Wnt/β-catenin, Hippo/Yes-associated protein (YAP), transforming growth factor-beta (TGF-β), and Notch signaling (Korchilava, Khachidze, Megrelishvili, Svanadze, Kakabadze, Tsomaia, et al., 2025; Liu et al., 2024a). In rodent models, substantial restoration of liver mass generally occurs within approximately 7–10 days, with peak hepatocyte proliferative activity occurring during the early stages following resection (Michalopoulos & Bhushan, 2021a).

TGF-β plays a particularly important role in liver regeneration because its biological functions vary across the regenerative process. It acts as a potent inhibitor of hepatocyte proliferation and contributes to the termination of regenerative growth once sufficient liver mass has been restored. Experimental disruption of TGF-β signaling has been shown to enhance hepatocyte proliferation following partial hepatectomy, supporting its role as a negative regulator of regenerative growth (Romero-Gallo et al., 2005). Furthermore, TGF-β regulates cell-cycle progression, cellular differentiation, apoptosis, extracellular matrix deposition, and fibrogenic responses. Therefore, the biological significance of hepatic TGF-β expression depends on the timing of measurement and the corresponding phase of regeneration rather than on the assumption that increased or decreased expression consistently indicates a favorable outcome (de Haan et al., 2024a; Deng et al., 2024a).

Liver regeneration involves several overlapping phases rather than a single proliferative event. During the priming phase, quiescent hepatocytes are stimulated to reenter the cell cycle. The proliferative phase involves the expansion of hepatocyte and nonparenchymal cell populations, whereas the termination phase limits further proliferation and restores tissue homeostasis. TNF-α and IL-6, released in part by Kupffer cells, contribute to early regenerative signaling, while hepatocyte growth factor (HGF) and other mitogenic pathways promote cell-cycle progression. Subsequently, counterregulatory mechanisms prevent excessive proliferation and support the restoration of normal liver architecture. This temporal coordination is essential when interpreting TGF-β expression because its biological implications may differ during the first 12–24 hours, at peak proliferative activity, and during later stages of tissue remodeling (Hoffmann, Nagel, & Tanabe, 2020; Yagi, Hirata, & Miyachi, 2020).

A major challenge following extensive liver resection is maintaining a balance between rapid tissue regeneration and the prevention of uncontrolled proliferation or excessive fibrosis. This balance becomes particularly important when the remaining liver tissue is compromised by underlying conditions such as cirrhosis, malignancy,

chemotherapy-associated liver injury, or cholestasis. Consequently, postoperative outcomes depend not only on the volume of the remaining liver but also on the functional integrity of the hepatic parenchyma and the effectiveness of regenerative signaling. These considerations provide a rationale for investigating adjunctive biological interventions in standardized preclinical models before evaluating their potential clinical applications.

The liver regeneration rate (LRR) provides a quantitative measure of the recovery of liver mass following partial hepatectomy. The regenerative response is influenced by the extent of hepatic resection, with experimental evidence indicating that more extensive hepatectomy may induce a greater relative regenerative response while simultaneously increasing the risk of postoperative liver failure. Meier et al. (2016) reported a substantially greater relative regenerative response following 90% hepatectomy than after less extensive resections in a rat model. These findings highlight the importance of identifying adjunctive interventions that may support effective liver regeneration without exacerbating the physiological stress associated with extensive hepatic tissue loss (Cao, Zhang, & Zhang, 2015; Chari et al., 1995a; Kahn et al., 2020a; Sowa, Best, & Benko, 2008; Yuan, Dong, Zhang, Xu, Wang, Yan, et al., 2021).

One potential adjunctive strategy involves probiotic modulation of the gut–liver axis. Probiotics may influence host immune responses, intestinal barrier integrity, microbial competition, and hepatic exposure to gut-derived microbial products. Dadih, a traditional fermented buffalo milk product from West Sumatra, Indonesia, contains various lactic acid bacteria, including species belonging to the genera Lactococcus and Lactobacillus. Previous studies have identified Lactococcus lactis among the microorganisms present in dadih and have demonstrated the potential probiotic properties of bacterial isolates obtained from this fermented product (Amelia et al., 2021; Meier, Andersen, & Knudsen, 2016; Sukma et al., 2018). Furthermore, a recent investigation of L. lactis D4 isolated from dadih identified antimicrobial activity and probiotic characteristics that support further investigation of its potential biological applications (Xu, Jiang, & Xiao, 2022).

The gut–liver axis provides an important biological framework for understanding the potential effects of probiotic interventions on hepatic regeneration. Through the portal circulation, the liver is continuously exposed to microbial metabolites and products originating from the gastrointestinal tract. Surgical stress may disrupt intestinal barrier integrity, increasing the translocation of lipopolysaccharides and other microbial components into the portal circulation. These substances can activate Kupffer cells and other innate immune cells, potentially amplifying hepatic inflammatory responses. Although controlled inflammatory signaling is necessary for regenerative priming, excessive inflammation may impair tissue recovery and promote fibrogenic processes. Previous reviews and experimental investigations have identified the gut microbiota as a potential modifier of liver regeneration following hepatic injury and resection (Xu, Jiang, & Xiao, 2022). Within this context, probiotic interventions may influence liver regeneration indirectly by modulating intestinal barrier function, microbial composition, and inflammatory signaling rather than acting as direct mitogenic stimuli for hepatocytes (Dooley & ten Dijke, 2012a; Karkampouna, ten Dijke, et al., 2012; Kisseleva & Brenner, 2021a; Xie, Zhang, & Yang, 2022; Zhang et al., 2024).

Previous preclinical findings have suggested that L. lactis D4 may influence hepatic repair and regeneration. One study reported increased hepatocyte proliferation, assessed using the Ki-67 proliferation marker, in Wistar rats treated with L. lactis D4 following partial hepatectomy (Abshar et al., 2025). Another investigation identified changes in Ki-67 expression and hepatic fibrosis in a rat model of obstructive jaundice following L. lactis D4 administration (Utami, Suchitra, Irwan, Rivai, Suswita, et al., 2025). Additionally, a separate experimental study demonstrated that orally administered genetically engineered L. lactis expressing the extracellular domain of transforming growth factor-beta receptor type II (TGF-βR2) reduced TGF-β1 signaling and hepatic fibrosis [19]. Although the genetically engineered strain differed from L. lactis D4, these findings collectively provided a biological rationale for investigating the potential relationship between L. lactis D4 administration, hepatic TGF-β expression, and liver regeneration following partial hepatectomy.

Despite these findings, limited evidence was available regarding the direct relationship between hepatic TGF-β expression and LRR in experimental models of partial hepatectomy treated with L. lactis D4. This research gap was biologically relevant because TGF-β participates in regulating hepatocyte proliferation, regenerative termination, and tissue remodeling, whereas LRR reflects the macroscopic restoration of hepatic mass. Simultaneous evaluation of these parameters could provide insights into whether probiotic-associated changes in the hepatic regenerative environment were accompanied by measurable differences in liver mass recovery. Therefore, this study aimed to investigate the relationship between hepatic TGF-β expression and LRR in male Wistar rats following 70% partial hepatectomy, with L. lactis D4 administered at different perioperative time points.

METHOD

This study employed a randomized, posttest-only controlled experimental design involving 30 male Wistar rats (Rattus norvegicus), aged 7–8 weeks and weighing 150–250 g, maintained at INA Lab. The animals were randomly allocated into five groups (n = 6 per group): sham, which underwent laparotomy without partial hepatectomy or Lactococcus lactis D4 administration; control, which underwent partial hepatectomy without L. lactis D4 administration; P1, which received L. lactis D4 for seven days before partial hepatectomy; P2, which received L. lactis D4 for seven days after partial hepatectomy; and P3, which received L. lactis D4 for seven days before and seven days after partial hepatectomy. The probiotic was administered orally at a dose of 360 mg/200 g body weight/day. All animals were maintained under standardized feeding conditions with unrestricted access to food and water. The sham and control groups received physiological saline, while the treatment groups received the probiotic suspension by oral gavage.

Sample size was determined using an experimental animal sample-size approach with an allowance for potential attrition. Eligible animals met the predefined age, weight, and health criteria. Animals exhibiting signs of illness, reduced activity, or poor physical condition were excluded according to the study protocol. Randomization was performed before group allocation.

General anesthesia was induced using ketamine. Following abdominal preparation and disinfection, an upper midline laparotomy was performed. Approximately 70% of the

liver was resected using the Higgins–Anderson technique by ligating and removing the left lateral and median lobes while preserving the remaining lobes. Hemostasis was confirmed, and the abdominal wall was closed in layers. Postoperatively, the animals were allowed unrestricted access to standard feed and water. On postoperative day 7, relaparotomy was performed under anesthesia, and liver specimens were collected for histopathological and immunohistochemical examinations. The collected tissues were fixed in 10% neutral buffered formalin.

Liver specimens were processed using standard paraffin-embedding procedures, and 4-μm-thick tissue sections were prepared for immunohistochemical assessment of transforming growth factor-beta (TGF-β) expression. The liver regeneration rate (LRR) was calculated using remnant liver weight, estimated preoperative liver weight, and corresponding body weights according to the following formula:

Statistical analyses were performed using IBM SPSS Statistics, version 26.0. TGF-β expression and LRR were summarized as means ± standard deviations. Data normality was assessed using the Shapiro–Wilk test. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by post hoc comparisons for normally distributed data. The Kruskal–Wallis and Mann–Whitney U tests were specified for data that did not meet normality assumptions. Pearson's correlation analysis was performed to assess the relationship between hepatic TGF-β expression and LRR in animals with complete measurements. Statistical significance was established at $p < 0.05$, with a 95% confidence level. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas (No. 385/UN.16.2/KEP-FK/2026; July 22, 2026).

Photographic documentation of animal housing, probiotic administration, and the partial hepatectomy procedure was provided in Figures 1–3.



(A)



(B)

Figure 1. Representative experimental-animal housing during the study: (A) study cages containing the experimental animals; (B) representative cage setup with feed and drinking water



(A)



(B)



(C)

Figure 2. Representative documentation of treatment administration and study equipment: (A) oral administration using a feeding/gavage needle; (B) microtubes and syringe/feeding-needle equipment used during the experiment; (C) oral administration during the experimental period



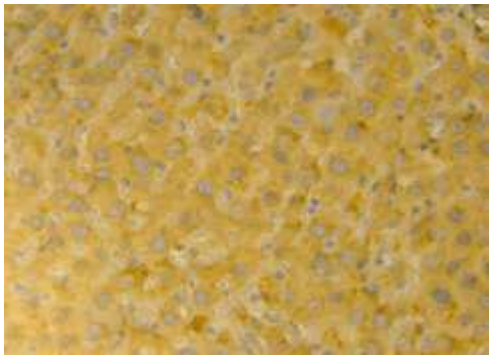


(A) **(B)**

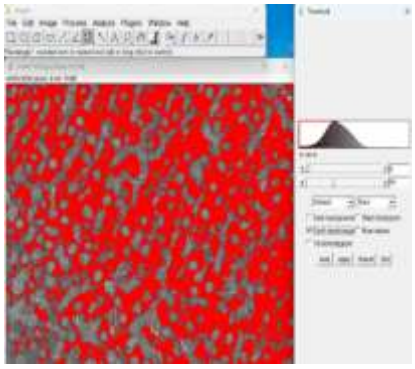
Figure 3. Representative documentation of the partial-hepatectomy procedure and postoperative condition: (A) intraoperative exposure of the liver during partial hepatectomy; (B) postoperative abdominal incision after wound closure.

RESULTS AND DISCUSSION

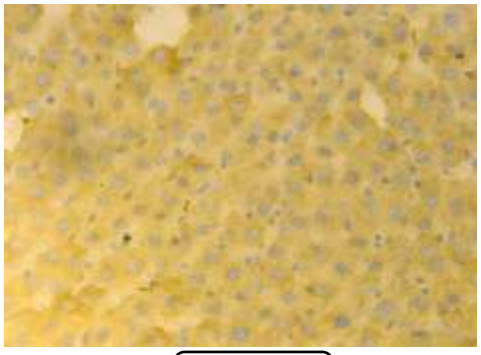
The findings are presented in three linked parts: TGF-β levels across the experimental groups, liver regeneration rate after partial hepatectomy, and the correlation between TGF-β and LRR. The sham group is included in the TGF-β comparison but not in the LRR comparison because it did not undergo partial hepatectomy.



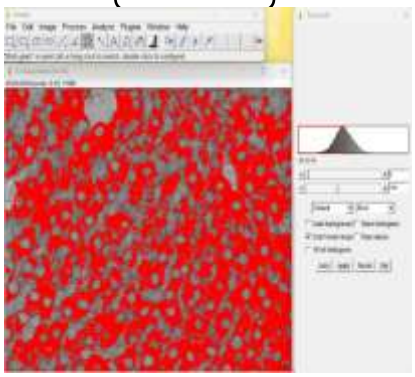
Sham



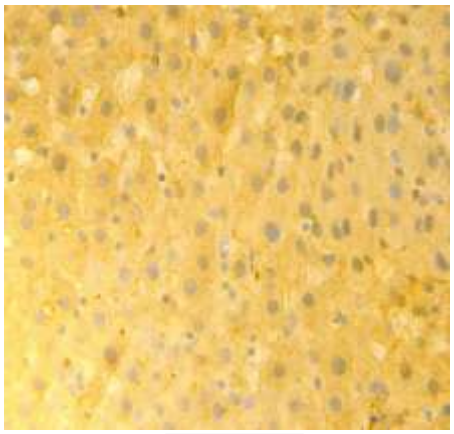
Sham



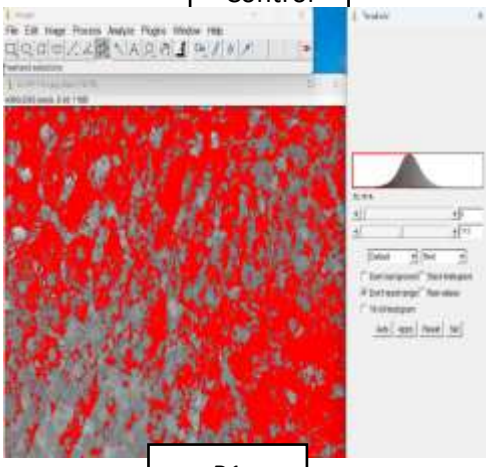
Control



Control



P1



P1

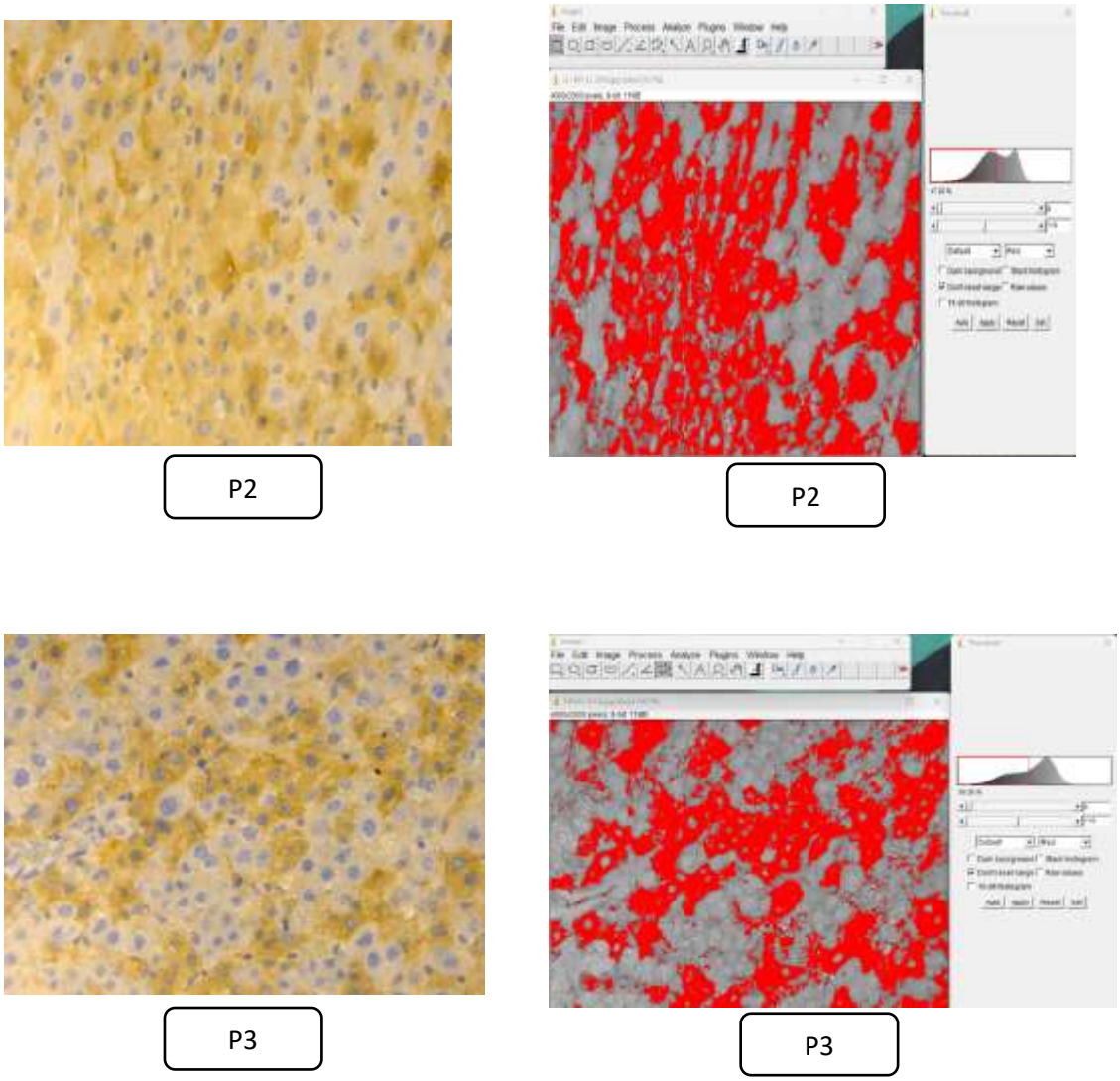


Figure 4. TGF-beta staining

TGF-β Levels Across Experimental Groups

TGF-β levels were highest in the sham group (59.89 ± 6.38), followed by the control group (52.77 ± 5.03), P1 (51.31 ± 1.27), P3 (47.53 ± 2.47), and P2 (38.69 ± 0.52). One-way ANOVA showed a statistically significant overall difference among the five groups ($p = 0.000$). Post-hoc analysis identified significant differences between sham and P2 ($p = 0.008$), sham and P3 ($p = 0.047$), control and P2 ($p = 0.014$), P2 and P1 ($p = 0.000$), and P2 and P3 ($p = 0.001$). These results indicate that the timing of L. lactis D4 administration was associated with different TGF-β profiles at the seventh postoperative day.

Table 1. TGF-β levels across sham, control, and Lactococcus lactis D4 treatment groups

Group	Intervention	TGF-β (Mean ± SD)
Sham	Laparotomy only	59.89 ± 6.38
Control	70% PH without L. lactis D4	52.77 ± 5.03
P1	L. lactis D4 before PH	51.31 ± 1.27
P2	L. lactis D4 after PH	38.69 ± 0.52
P3	L. lactis D4 before and after PH	47.53 ± 2.47

Note. Overall one-way ANOVA p = 0.000. Significant post-hoc comparisons: sham–P2 (p = 0.008), sham–P3 (p = 0.047), control–P2 (p = 0.014), P2–P1 (p = 0.000), and P2–P3 (p = 0.001). PH = partial hepatectomy.

Liver Regeneration Rate

Liver regeneration rate varied widely across the partial-hepatectomy groups. The control group had an LRR of 200.63 ± 66.03%, P1 207.50 ± 92.06%, P2 150.86 ± 71.79%, and P3 225.85 ± 95.42%. P3 therefore showed the highest mean LRR, whereas P2 showed the lowest. Despite these descriptive differences, one-way ANOVA did not demonstrate a statistically significant difference in LRR among the four groups (p = 0.670). Thus, in this experiment, L. lactis D4 administration did not produce a statistically significant between-group effect on LRR.

Table 2. Liver regeneration rate after partial hepatectomy

Group	Intervention	LRR (%) (Mean ± SD)
Control	70% PH without L. lactis D4	200.63 ± 66.03
P1	L. lactis D4 before PH	207.50 ± 92.06
P2	L. lactis D4 after PH	150.86 ± 71.79
P3	L. lactis D4 before and after PH	225.85 ± 95.42

Note. One-way ANOVA p = 0.670. LRR = liver regeneration rate; PH = partial hepatectomy.

Relationship Between TGF-β and Liver Regeneration Rate

Pearson correlation analysis showed a moderate positive relationship between TGF-β and LRR (r = 0.470; p = 0.037). The direction of the association indicates that higher TGF-β values at the study time point tended to accompany higher LRR values. This association was statistically significant, although its moderate magnitude indicates that TGF-β accounts for only part of the variation in regenerative recovery.

Table 3. *Pearson correlation between TGF-β and liver regeneration rate*

Variable pair	Pearson r	p-value
TGF-β and LRR	0.470	0.037

Note. The correlation was positive and of moderate strength. LRR = liver regeneration rate.

The first notable finding was that TGF-β was higher in sham animals than in the partial-hepatectomy control group. This pattern is compatible with the time-dependent biology of TGF-β during liver regeneration. (Cao et al., 2015) also reported differences between sham and hepatectomy groups that varied according to the postoperative interval and the assay used. TGF-β can increase during early regenerative signaling but hepatocyte sensitivity to its antiproliferative effects may be attenuated by down-regulation of TGF-β

receptors, allowing proliferation to continue despite the presence of the cytokine (Chari et al., 1995; Sowa et al., 2008). By day 7 after a 70% hepatectomy, the liver is transitioning from active proliferation toward remodeling and termination. In contrast, the sham liver has not lost tissue mass and remains closer to a homeostatic state in which TGF-β contributes to control of hepatocyte growth and tissue architecture.

All three L. lactis D4 treatment groups had lower mean TGF-β values than the sham group, and P2 also had a markedly lower value than the untreated hepatectomy control. The lowest TGF-β level occurred when L. lactis D4 was administered only after surgery, while pretreatment alone produced the highest value among the intervention groups and perioperative treatment produced an intermediate value. Direct comparison with prior studies is limited because no previous partial-hepatectomy experiment was identified that measured TGF-β after treatment with this specific L. lactis D4 isolate. Nevertheless, the direction of the findings is biologically plausible in light of studies linking Lactococcus-based interventions to modulation of TGF-β signaling and liver fibrosis (Utami et al., 2025; Yuan et al., 2021).

The timing pattern may reflect different phases of the interaction between probiotic exposure, the gut-liver axis, inflammation, and regenerative termination. One possible interpretation is that administration before and after surgery may provide a preconditioning effect before hepatic injury while maintaining immunomodulatory effects during the postoperative period. Probiotic modulation of intestinal barrier function may reduce bacterial translocation and excessive exposure to lipopolysaccharide, thereby altering Kupffer-cell activation and downstream inflammatory signaling. Evidence from liver-surgery literature supports the importance of the gut-liver axis in perioperative outcomes and the potential for pro- and synbiotics to modify this environment (Kahn et al., 2020; Xu et al., 2022). Because these mechanisms were not directly measured in the present experiment, they should be interpreted as plausible mechanistic explanations rather than as demonstrated causal pathways.

The fall in TGF-β observed in P2 should not be interpreted in isolation as either beneficial or detrimental. Lower TGF-β may indicate reduced inflammatory and fibrogenic signaling, which could create a less hostile environment for hepatocyte proliferation. Conversely, if the reduction persists into the late regenerative phase, it may also indicate that termination and remodeling signals have not yet become fully established. These two interpretations indicate that TGF-β should ideally be assessed together with other regenerative markers. This caution is supported by the broader literature showing that TGF-β signaling is context-dependent and can affect cell-cycle arrest, extracellular-matrix formation, and fibrogenesis through interconnected pathways (Deng et al., 2024; Dooley & ten Dijke, 2012).

The LRR findings were descriptively different but not statistically significant. P3 had the highest mean LRR (225.85 ± 95.42%), P1 and the control group had intermediate values, and P2 had the lowest mean LRR (150.86 ± 71.79%). The magnitude of the standard deviations indicates substantial variability within groups, which may have reduced the ability to detect differences. The precision of body-weight measurement can influence LRR because body weight is included directly in the regeneration formula. The day-7 measurement is nevertheless biologically meaningful because rat liver mass is expected to

recover rapidly after partial hepatectomy, with major regenerative changes occurring during the first postoperative week (Meier et al., 2016; Michalopoulos & Bhushan, 2021).

The high mean LRR in P3 is consistent with the interpretation that perioperative probiotic exposure may create a more favorable regenerative environment than exposure limited to the postoperative period. In earlier work, reported increased Ki-67 expression after L. lactis D4 in a partial-hepatectomy model, supporting an effect on hepatocyte proliferative activity. Other experimental work using a different probiotic species, Lactiplantibacillus plantarum, showed accelerated liver regeneration associated with changes in gut microbiota and plasma metabolites (Xie et al., 2022). These studies do not prove that the same mechanism explains the present LRR values, but they support the broader concept that microbiota-directed interventions can influence liver recovery.

The absence of a significant LRR difference also deserves emphasis because descriptive ranking alone can be misleading. P3 had the highest mean value, but the broad standard deviations show substantial overlap among groups. A biological pattern may therefore be present without sufficient statistical precision to establish a treatment effect in this dataset. This distinction is important when translating preclinical results: the data support an association between intervention timing and TGF-β, and they support a correlation between TGF-β and LRR, but they do not establish that L. lactis D4 significantly increases LRR. These three statistical findings should be kept separate rather than combined into a single efficacy claim.

The key analytical result of this study was the statistically significant moderate positive correlation between TGF-β and LRR. At first glance, a positive relationship may seem counterintuitive because TGF-β is commonly described as an inhibitor of hepatocyte proliferation. However, this apparent paradox can be resolved by considering the study time point. TGF-β has a dynamic role throughout liver regeneration: it contributes to growth control, extracellular-matrix organization, and termination of proliferation after the required liver mass has been restored (de Haan et al., 2024; Hoffmann et al., 2020). (Karkampouna et al., 2012). Therefore, a higher TGF-β level on day 7 may reflect a more advanced stage of organized regeneration rather than simple suppression of proliferative activity.

The descriptive group pattern supports this interpretation. P2 had both the lowest TGF-β level and the lowest LRR, whereas P3 had a higher TGF-β level and the highest LRR. In late regeneration, TGF-β can participate in restoring tissue organization and limiting excessive growth after a substantial portion of liver mass has already been recovered. Experimental and review literature emphasizes that successful regeneration requires not only hepatocyte division but also coordinated remodeling of extracellular matrix, vascular structures, and signaling between hepatocytes and non-parenchymal cells (Hoffmann et al., 2020; Yagi et al., 2020). Consequently, the positive correlation found here is compatible with a model in which TGF-β at day 7 is partly a marker of progression toward regenerative termination and maturation.

TGF-β also intersects with extracellular-matrix biology. Successful regeneration requires new hepatocytes to be integrated into a functional three-dimensional architecture, with restoration of sinusoidal relationships and matrix organization. Excessive TGF-β can promote stellate-cell activation and fibrosis, yet insufficient late-phase signaling could theoretically alter normal remodeling. Studies of liver fibrosis and regeneration emphasize

this dual role and the need for balanced signaling rather than maximal or minimal TGF-β activity (Karkampouna et al., 2012; Kisseleva & Brenner, 2021). The intermediate TGF-β value observed in P3, together with the highest mean LRR, is therefore an interesting pattern, but the present study did not directly measure matrix deposition or fibrotic remodeling and cannot determine whether such a balance was actually achieved.

The correlation coefficient of 0.470 also shows that TGF-β is not the sole determinant of regeneration. Recovery after partial hepatectomy is governed by multiple cytokines and growth factors, hemodynamic changes, cell-cell interactions, metabolic adaptation, and the gut microbiota. TGF-β must therefore be interpreted within a broader network of signaling rather than as an isolated predicto (Korchilava et al., 2025; Liu et al., 2024; Zhang et al., 2023). This point is especially important because the present study measured TGF-β at a single postoperative time point. Serial measurements could better demonstrate whether early suppression, later rebound, or sustained modulation is associated with the observed macroscopic recovery.

The day-7 endpoint is especially important for interpreting the observed correlation. Early after hepatectomy, regenerative signaling is dominated by priming and cell-cycle entry, whereas by the end of the first postoperative week in rats, a greater proportion of the response has shifted toward growth control, architectural restoration, and maturation. A single TGF-β measurement at this later point may therefore capture not only anti-proliferative activity but also the extent to which the liver has progressed into the termination phase. This offers a plausible explanation for why animals with greater macroscopic recovery could also display higher TGF-β values. It does not imply that increasing TGF-β would necessarily improve regeneration; rather, the measured concentration may function as a time-dependent correlate of the regenerative stage. Serial measurements at earlier and later time points would be required to distinguish these possibilities.

From a practical perspective, the findings suggest that the timing of L. lactis D4 exposure deserves further study. Although the intervention did not significantly change LRR among groups, it was associated with significant differences in TGF-β levels, and the TGF-β values were significantly correlated with LRR. The perioperative P3 regimen showed the highest mean LRR while maintaining a TGF-β level between the P1 and P2 values. This pattern supports further investigation of perioperative rather than strictly postoperative administration, but it should not be interpreted as proof of superiority because the between-group LRR comparison was not statistically significant.

This study has important limitations. The experiment used animals without pre-existing liver disease, so the findings cannot be assumed to apply to cirrhosis, hepatoma, or other conditions in which regenerative capacity is already impaired. The study also relied on a single assessment time point, limiting direct observation of the temporal pattern of TGF-β during priming, proliferation, and termination. Finally, the relatively large dispersion of LRR values and the dependence of the LRR formula on accurate body- and liver-weight measurements may have influenced the precision of the regeneration estimate. These limitations should be addressed in future studies using serial biomarker measurements, carefully standardized weight measurements, and disease-specific liver models.

CONCLUSION

Administration of Lactococcus lactis D4 was associated with statistically significant differences in hepatic transforming growth factor-beta (TGF-β) expression among male Wistar rats following 70% partial hepatectomy. The highest TGF-β expression was observed in the sham group, whereas among the L. lactis D4 treatment groups, expression was highest in P1, followed by P3 and P2. The mean liver regeneration rate (LRR) was highest in P3 and lowest in P2; however, differences in LRR among groups were not statistically significant ($p = 0.670$). Pearson's correlation analysis revealed a statistically significant moderate positive correlation between hepatic TGF-β expression and LRR ($r = 0.470$; $p = 0.037$). This positive association on postoperative day 7 may reflect the involvement of TGF-β in late-stage regenerative regulation, tissue remodeling, and termination of hepatocyte proliferation, rather than indicating a uniformly inhibitory role in liver regeneration. Nevertheless, the observed correlation did not establish a causal relationship between TGF-β expression and liver regeneration. Further studies incorporating serial measurements of TGF-β expression, multiple postoperative observation periods, and experimental models with preexisting liver disease are recommended to clarify the temporal and biological effects of L. lactis D4 on hepatic regeneration.

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CONFLICT OF INTEREST

The author declares no conflict of interest in either the conduct of this research or the preparation of this manuscript. There are no commercial, financial, institutional, or personal relationships that could potentially influence the objectivity, the research process, or the interpretation of the results.

AUTHOR CONTRIBUTIONS

The first author contributed to the conception and design of the study, data collection, experimental procedures, analysis and interpretation of data, drafting of the manuscript, and preparation of the final manuscript. The second author contributed to the supervision of the research, provision of methodological guidance, interpretation of results, and critical revision of the manuscript for important intellectual content. The third author contributed to the conception and design of the study, supervision, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content.

GENERATIVE AI STATEMENT

In preparing this manuscript, the author made limited use of artificial intelligence (AI) assistance, solely for grammar improvement and language checking. AI was not involved in generating data, conducting analysis, determining research findings, or formulating scientific

interpretations. All substantive content, data, analytical results, interpretations, and conclusions remain entirely the author's responsibility.

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