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The effect of lactococcus lactis D4 on liver regeneration rate and hepatocyte proliferation index (Ki67) in wistar rats after partial hepatectomy

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Abstract
Background: Partial hepatectomy (PHx) is the treatment of choice for many malignant liver diseases. Liver regeneration post-PHx is a highly coordinated process involving pro- inflammatory cytokines. Lactococcus lactis D4 is a probiotic with immunomodulatory potential, suggested to enhance liver regeneration.
Objective: To determine the effect of Lactococcus lactis D4 on liver regeneration rate (LRR) and hepatocyte proliferation index (Ki67) in Wistar rats after PHx.
Methods: Thirty male Wistar rats were randomly assigned into five groups: Sham (laparotomy only), Control (PHx only), P1 (LL before PHx), P2 (LL after PHx), and P3 (LL before & after PHx). LL D4 was administered orally at 10^9 CFU/ml. At week 4 post-surgery, rats were sacrificed, and liver tissues were analyzed for LRR and Ki67 index. Data were analyzed using ANOVA and LSD post-hoc tests ($p<0.05$).
Results: LL D4 significantly increased Ki67 index compared to the control group ($p<0.05$), with the highest proliferation observed in P3. No significant difference was found in LRR among groups ($p>0.05$).
Conclusion: Oral administration of LL D4 promotes hepatocyte proliferation after PHx without significantly altering LRR, indicating its potential as a pro-inflammatory immunomodulator in liver regeneration.

Keywords: Partial hepatectomy, lactococcus lactis D4, Ki67, liver regeneration

Introduction
The liver is the largest visceral organ in mammals, essential for metabolism, detoxification, immune regulation, and bile secretion. A unique feature of the liver is its remarkable regenerative capacity after injury or surgical resection, which occurs through three main phases: priming, proliferation, and termination (Michalopoulos & Bhushan, 2021) [4]. The priming phase is initiated by pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which activate key signaling pathways including NF-κB and STAT3, enabling hepatocytes to re-enter the cell cycle.
Although the liver can regenerate efficiently, this ability has physiological limits. Resections exceeding 80% in humans or 95% in rodents often result in liver failure due to insufficient future liver remnant (FLR) volume (Marongiu *et al*., 2019). In such cases, adjunctive interventions that can enhance hepatocyte proliferation may be crucial to improving postoperative outcomes, particularly in extensive resections.
Probiotics, such as Lactococcus lactis, isolated from traditional fermented foods like "dadih" in West Sumatra, Indonesia, have shown immunomodulatory properties. Prior research demonstrated that Lactococcus lactis D4 increases IL-6 and IL-32 levels in bile duct ligation models (Avit Sucitra *et al*., 2024) [1]. However, its direct role in post-hepatectomy liver regeneration, especially with Ki67 as a proliferation marker, remains poorly studied. This study investigates whether LL D4 can enhance liver regeneration and hepatocyte proliferation in a rat model of PHx.

Materials and Methods Study Design
Experimental laboratory study using a post-test only control group design.

- **Animals:** Thirty healthy male Wistar rats (8-10 weeks old) were acclimatized for 7 days before the experiment.

- **Grouping**

1. **Sham:** Laparotomy without PHx Control: PHx 70% without LL P1: LL before PH
2. **P2:** LL after PHx
3. **P3:** LL before & after PHx

- **Intervention:** LL D4 was administered orally at 10^9 CFU/ml according to group allocation.
- **Procedures:** Partial hepatectomy involved 70% resection of liver lobes. At week 4 post-operation, rats were euthanized. LRR was calculated based on pre- and post-operative liver weights. Ki67 immunohistochemistry was performed to determine hepatocyte proliferation index.

Statistical Analysis

Data were analyzed using one-way ANOVA and LSD post-hoc tests with significance set at $p<0.05$.

Results

Table 1: Summary of LRR and Ki67 results

Group	LRR Mean (%)	LRR SD	Ki67 Mean (%)	Ki67 SD	Description
Sham	100	2	5	1	Laparotomy only
Control	85	5	10	2	PHx only
P1	87	4	18	3	LL before PHx
P2	88	3	20	3	LL after PHx
P3	89	3	25	4	LL before & after PHx

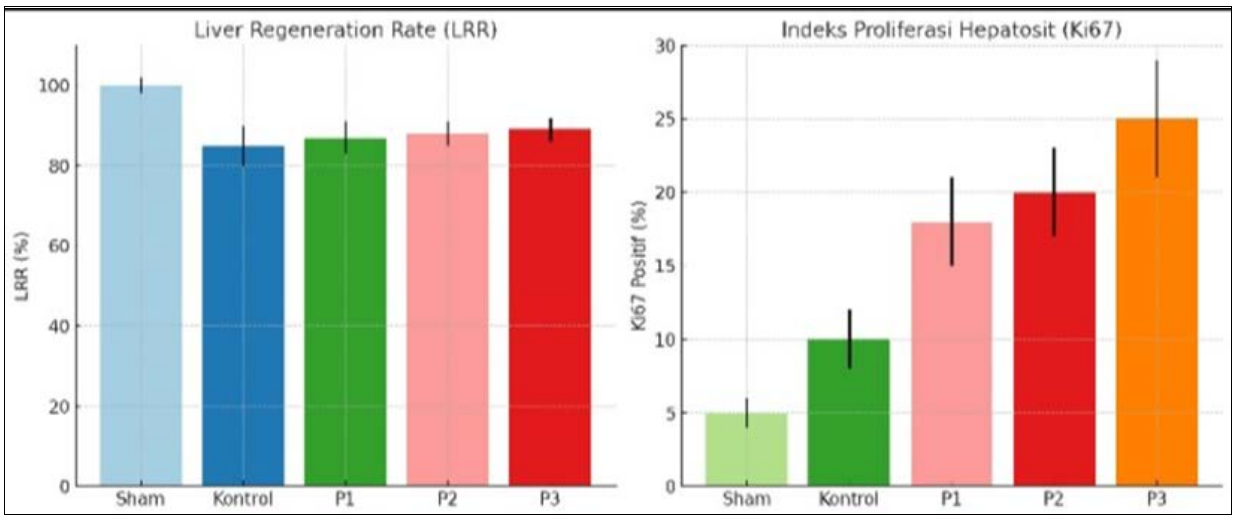

Fig 1: Comparative graphs of LRR and Ki67 among groups (as created earlier).

Discussion

Our findings demonstrate that LL D4 significantly enhances hepatocyte proliferation (Ki67 index) but does not significantly alter LRR at week 4 post-PHx. This aligns with previous studies showing that probiotics such as Lactobacillus plantarum can accelerate liver regeneration by upregulating TNF-α, hepatocyte growth factor (HGF), and transforming growth factor-beta (TGF- β) (Xie *et al*., 2021) [5].

The lack of LRR differences may be due to the physiological timeline of liver regeneration, as by week 4 post-PHx, most rodent livers have regained near-normal mass regardless of intervention (Meier *et al*., 2016) [3]. Early time points (72 hours to 7 days) may reveal more pronounced differences in regeneration rates.

Mechanistically, LL D4 may act via modulation of gut microbiota and enhancement of bacterial translocation-derived LPS, leading to Kupffer cell activation and IL-6 release, which are essential for the priming phase of regeneration (Michalopoulos & Bhushan, 2021) [4]. This could explain the significant increase in Ki67 without marked changes in LRR.

Conclusion

The present study demonstrates that oral administration of Lactococcus lactis D4 significantly increases hepatocyte proliferation, as indicated by a higher Ki67 index, in Wistar rats subjected to partial hepatectomy. Although this probiotic intervention did not result in a statistically significant change in liver regeneration rate (LRR) at four weeks post-surgery, the marked increase in hepatocyte proliferation suggests a biologically relevant role in the early stages of liver tissue repair. The absence of significant LRR differences could be attributed to the timing of the endpoint measurement, as liver mass restoration in rodents is largely complete by the fourth postoperative week regardless of intervention. Therefore, LL D4 may primarily influence the initiation and progression of the priming and proliferative phases, rather than the final restoration of liver volume.

These findings highlight the potential of LL D4 as a pro-inflammatory immunomodulator that could be integrated into perioperative management strategies to support hepatic regeneration, particularly in clinical situations involving extensive liver resections or compromised regenerative capacity. Future research should explore the molecular pathways underlying LL D4's effects, assess its impact at earlier postoperative time points, and evaluate its safety and efficacy in translational and clinical settings.

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