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ARTIKEL PENELITIAN

Exploring the Link Between Oxidative Stress Reduction and Liver Function Improvement in Jaundiced Rats with *Lactococcus lactis* D4

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Abstrak

Tujuan: Untuk mengeksplorasi korelasi antara pengurangan stres oksidatif dan fungsi enzim hati pada tikus dengan penyakit kuning obstruktif, menggunakan kadar malondialdehid (MDA) sebagai penanda biokimia, dan dampaknya terhadap kadar serum glutamat oksaloasetat transaminase (SGOT); **Metode:** Penelitian eksperimental ini menggunakan rancangan kelompok kontrol acak post-test only pada tiga kelompok tikus Rattus norvegicus strain Wistar dengan model penyakit kuning obstruktif. Tikus menjalani ligasi duktus biliaris dan menerima susu fermentasi starter Lactococcus lactis D4 selama 7 hari. Stres oksidatif dinilai dengan mengukur kadar MDA dan fungsi hati dinilai dengan mengukur kadar SGOT. **Hasil:** Hasil penelitian ini menunjukkan bahwa rata-rata kadar MDA pada Kontrol Negatif, Kontrol Positif dan Perlakuan adalah 1,68 ± 0,12, 2,56 ± 0,43, 1,82 ± 0,19. Pemberian susu fermentasi starter *Lactococcus lactis* D4 secara signifikan menurunkan kadar SGOT (Perlakuan vs Kontrol Positif: 51,11 ± 1,76 U/L vs 64,63 ± 2,39 U/L, p<0,05). Analisis korelasi menunjukkan nilai r sebesar 0,886 yang menunjukkan adanya korelasi positif yang sangat kuat antara kadar MDA dan SGOT setelah pemberian LLD4; **Kesimpulan:** Susu fermentasi starter Lactococcus lactis D4 dapat memperbaiki fungsi hati, ditunjukkan dengan penurunan kadar MDA dan kadar SGOT pada hewan percobaan.

Kata kunci: Penyakit kuning obstruktif, Susu fermentasi starter *Lactococcus lactis* D4, Malondialdehid, SGOT

Abstract

Objective: *To explore the correlation between the reduction of oxidative stress and liver enzyme function in rats with obstructive jaundice, using malondialdehyde (MDA) levels as a biochemical marker, and its impact on serum glutamic oxaloacetic transaminase (SGOT) levels;* ***Method:*** *This experimental study used a post-test only randomized control group design in three groups of Rattus norvegicus strain Wistar rats with an obstructive jaundice model. The rats underwent biliary duct ligation and received Lactococcus lactis D4 starter fermented milk for 7 days. Oxidative stress was assessed by measuring MDA levels and Liver function was assessed by measuring levels of SGOT ;* ***Result:*** *The results of this study showed that the average of MDA levels in Negative Control, Positive Control and Treatment were 1.68 ± 0.12, 2.56 ± 0.43, 1.82 ± 0.19. Administration of Lactococcus lactis D4 starter fermented milk significantly decreased SGOT levels (Treatment vs Positive Control: 51.11 ± 1.76 U/L vs 64.63 ± 2.39 U/L, p<0.05). The correlation analysis show an r-value of 0.886 revealed that*

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there a very strong positive correlation between MDA and SGOT levels after LLD4 administration; ***Conclusion:*** *Lactococcus lactis D4 starter fermented milk can improve liver function, indicated by decreased MDA levels and SGOT levels in experimental animals.*
Keywords: *Obstructive jaundice, Lactococcus lactis D4 starter fermented milk, Malondialdehyde, SGOT*

INTRODUCTION

Obstructive jaundice is a serious clinical condition with high morbidity and mortality rates due to complications such as sepsis and impaired immune function. One crucial pathophysiological aspect of obstructive jaundice is the disruption of the intestinal barrier function, which is responsible for preventing the dissemination of bacteria and toxins to other organs and tissues. Failure of the intestinal barrier leads to increased intestinal permeability, mononuclear dysfunction, liver injury due to inflammation, and obstructive cholestasis fibrosis, contributing to elevated liver enzyme levels.[1]

Cases of obstructive jaundice are common worldwide. In the United States, around 20% of the population over 65 years old is diagnosed with gallstones, and about 1 million new cases of choledocholithiasis are reported annually. Patients with obstructive jaundice often present with symptoms of yellowing skin and eyes, pale stools, dark urine, and pruritus. Additionally, they tend to experience nutritional deficits, infection complications, acute kidney failure, and cardiovascular dysfunction.Serious complications such as coagulopathy, hypovolemia, and endotoxemia increase the risk of morbidity and mortality in these patients.[2]

In the early phase of obstructive jaundice, there is an increase in hepatocyte membrane permeability and the release of indicator enzymes, causing pressure on the biliary duct system.[2,3] This pressure leads to hepatocyte dysfunction with increased production of unconjugated bilirubin in the blood and impaired bilirubin metabolism. Moreover, the increased biliary components that disrupt their metabolism can cause toxic effects on hepatocyte mitochondria, inhibit the respiratory circulation and oxidation of fatty acids, and increase free radicals in the body, contributing to oxidative stress.[4,5]

Oxidative stress is characterized by an increased production of reactive oxygen species (ROS), triggering lipid peroxidation. This process damages membranes, DNA, and proteins, contributing to pathological conditions. Malondialdehyde (MDA), a secondary product of lipid peroxidation, serves as a stable and permeable marker indicating tissue damage due to free radicals.[5,6,7]Additionally, endotoxemia in obstructive jaundice disrupts the function of Kupffer cells and triggers the release of proinflammatory cytokines such as TNF-α, IL-1, and IL-6, further aggravating hepatocyte damage chatacterized by an increase in liver enzymes (SGOT).[8]

Perioperative preparation is necessary to reduce morbidity and mortality in patients with obstructive jaundice, especially those in remote areas with limited access to healthcare facilities. Complementary therapy modalities, such as inchinkoto supplements, ursodeoxycholic acid (UDCA), and synbiotics (prebiotics and probiotics), can help improve liver function and accelerate recovery.[9] Probiotic lactic acid bacteria, as found in dadih, a traditional fermented milk product from West Sumatra, have shown potential to enhance the phagocytic capacity of leukocytes, the tumoricidal activity of NK cells, and reduce IL-6 and CRP levels as well as leukocyte count in biliary cancer patients.[10]

Previous research indicates that probiotics can help reduce hepatocyte damage and lower free radical activity.[11] Therefore, this study aims to explore the correlation between the reduction of oxidative stress in rats with obstructive jaundice, through malondialdehyde (MDA) levels as a biochemical marker, and its function against the liver enzyme SGOT.

METHODS

This study is a pure laboratory experimental study with a randomized control group posttest-only design using Wistar strain Rattus norvegicus white rats as the research subjects. The research was conducted at Ina Lab and the Biochemistry Laboratory, Faculty of Medicine, Andalas University, from September 2023 to February 2024. The protocol of this study was approved by the Ethics Committee of Faculty of Medicine, Universitas Andalas (No. 1078/UN.16.2/KEP-FK/2022).

The sample consisted of male Wistar rats aged 6-7 weeks with a body weight of 150-250 grams. The sample size was determined based on the criteria set by the World Health Organization (WHO) in 2000, which specifies a minimum of five rats per group. To account for potential dropouts due to rat mortality or illness, the sample size was increased to a minimum of six rats per group.

The study included three groups: a negative control group (rats only undergoing laparotomy), a positive control group (rats with common bile duct ligation), and a treatment group (rats with common bile duct ligation receiving Lactococcus lactis D4 starter culture from dadih at a dose of 360 mg/kg body weight). Samples were selected randomly (simple random sampling) according to inclusion and exclusion criteria.

The study began with the preparation of 18 Wistar rats weighing 150-250 grams that met the inclusion and exclusion criteria. These rats underwent an acclimatization period to adjust to the new environment, during which they were fed and watered, and their cages were maintained, with cleaning every two days. The cages had wire mesh covers and were placed in well-lit and ventilated rooms.

After acclimatization, the rats underwent surgical procedures according to their groupings. The surgeries were performed under intramuscular ketamine anesthesia at a dose of 50 mg/kg. Post-surgery, the rats were given oral feeding for 24 hours, with normal saline administered twice daily for groups 1 and 2. The treatment group received a probiotic solution homogenized with water twice daily every 12 hours. The fermented milk used was provided by the Faculty of Animal Husbandry, Andalas University, courtesy of Ade Sukma, Ph.D.

According to the American Academy of Family Physicians (AAFP) in 2008, the general dosage for probiotic consumption is 5 to 10 x 10^9 colony-forming units (CFU) per day for children and 10 to 20 x 10^9 CFU per day for adults. Dadih contains approximately 10^9 CFU/ml of lactic acid bacteria, meeting the Codex Standard for Fermented Milks established in 2003, which requires at least 10^7 CFU/ml. Based on these dosages and the lactic acid bacteria content in dadih, the dose for rats was calculated using the human-to-animal conversion factor: (20 x

10^9 CFU/ml) x 0.018 = 360 mg/200 g body weight = 0.36 ml/200 g body weight.[12]

Measurement of Malondialdehyde (MDA) Levels

MDA levels were measured by collecting 4 ml of venous blood from the test subjects, which was then placed in a tube and centrifuged at 10,000 RPM for 15 minutes. If immediate analysis was not possible, the serum was stored at -20°C to prevent changes in MDA levels. The Hunter method, utilizing spectrophotometry at a wavelength of 530 nm, was used to measure MDA levels by observing the color change to purple, indicating the formation of a thiobarbituric acid-MDA complex.

Measurement of SGOT Levels

On day seven, relaparotomy was performed through the same incision line under anesthesia and sterile conditions. The heart was exposed through the diaphragm, and blood samples were taken from the right atrium and placed in biochemistry tubes. The blood was centrifuged for 5 minutes at 5000 rpm, and total bilirubin, SGOT, SGPT, alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT) levels were measured using a semi-automated biochemistry analyzer (Elitech, Microlab 300, Italy) through ultraviolet spectrophotometry, colorimetry, and enzymatic methods.

Analysis Statistic

Data normality was tested using the Shapiro-Wilk test (for samples <30). If data were normally distributed, parametric tests were conducted using One-way ANOVA, followed by Post Hoc tests with Least Significant Differences (LSD). The relationship between malondialdehyde (MDA) levels and serum glutamic-oxaloacetic transaminase (SGOT) levels after administering Lactococcus lactis D4 fermented milk starter was assessed using Pearson correlation analysis.

RESULT AND DISCUSSION

The Effect of Lactococcus lactis D4 Administration on MDA Expression in a Rat Model of Obstructive Jaundice

The study results showed that MDA levels, as a marker of oxidative stress, increased in the group experiencing obstructive jaundice and decreased in the obstructive jaundice group that received Lactococcus lactis D4 (LLD4). The differences between these groups were statistically significant (p-value < 0.05) (Table 1).

Table 1. Analysis Results of the Effect of Lactococcus lactis D4 Administration on MDA Expression in a Rat Model of Obstructive Jaundice

Study Groups	Mean±SD	*P Value*
Negative control	1.68 ± 0.12	0.001*
Positive control	2.56 ± 0.43	
Treatment	1.82 ± 0.19	

*One Way Anova test (p value < 0.05)

Table 2. Post-hoc LSD Test Results for the Effect of Lactococcus lactis D4 on MDA Expression in Obstructive Jaundice Rat Model

Study Groups	Negative control	Positive control	Treatment
Negative control		0.001*	0.436
Positive control	0.001*		0.001*
Treatment	0.436	0.001*	

*Significant Results (p value < 0.05)

Consequently, a post-hoc analysis was conducted to assess the relationships between groups, revealing significant differences between the negative control and positive control, as well as between the positive control and treatment groups. However, there was no significant difference between the negative control and treatment groups (Table 2). This data indicates that Lactococcus lactis plays a crucial role in reducing MDA levels.[13] MDA reduction can occur with the administration of high antioxidants to scavenge free radicals produced. Probiotics like LLD4 function as such antioxidants, similar to catalase enzymes.[14]

The antioxidant metabolic activity of LLD may arise from its production of antioxidant enzymes such as superoxide dismutase and catalase, as well as metabolites like folate and glutathione. These components can reduce oxidative stress. Additionally, this probiotic is capable of synthesizing selenium nanoparticles (SeNPs). These nanoparticles can capture free radicals that contribute to cellular and molecular damage, thus inhibiting MDA formation. SeNPs can also stimulate the activity of endogenous antioxidant enzymes like glutathione peroxidase (GPx) and superoxide dismutase (SOD), which help detoxify free radicals and prevent MDA formation.[15]

The Effect of Lactococcus lactis D4 Administration on SGOT Levels in a Rat Model of Obstructive Jaundice

The study results showed that SGOT levels, as a marker of liver function, increased in the group experiencing obstructive jaundice and decreased in the obstructive jaundice group that received Lactococcus lactis D4 (LLD4). The differences between these groups were statistically significant (p-value < 0.05) (Table 3).

Table 3. Analysis Results of the Effect of Lactococcus lactis D4 on SGOT Levels in Obstructive Jaundice Rat Model

Study Groups	Mean±SD	*P Value*
Negative control	18.65 ± 1.72	0.001*
Positive control	64.63 ± 2.39	
Treatment	51.11 ± 1.76	

*One Way Anova test (p value < 0.05)

Consequently, a post-hoc analysis was conducted to assess the relationships between groups, revealing significant differences between the negative control and positive control, as well as between the positive control and treatment groups, and also between the negative control and treatment groups (Table 4).

Table 4. LSD Post-hoc Test Results of the Effect of Lactococcus lactis D4 on SGOT Expression in Obstructive Jaundice Rat Model

Kelompok	Negative control	Positive control	Treatment
Negative control		0.001*	0.001*
Positive control	0.001*		0.001*
Treatment	0.001*	0.001*	

*Significant Results (p value < 0.05)

Liver function improvement by probiotics is not only due to the high antioxidant content fighting oxidative stress, but also through the reduction of liver inflammation and the improvement of intestinal mucosa, preventing bacterial and endotoxin translocation to the portal vein.[14,16] Lactococcus lactis D4 can also reduce the population of pathogenic gut bacteria and increase the population of beneficial gut bacteria. Additionally, it enhances the production of short-chain fatty acids (SCFAs) like acetic acid, propionic acid, and butyric acid, which play a crucial role in maintaining intestinal homeostasis by preserving an acidic environment.[17]

Correlation between MDA Levels and SGOT Levels after Administration of Lactococcus lactis D4

The results of the Pearson correlation analysis in Table 5 show an r-value of 0.886, indicating a very strong positive correlation between MDA and SGOT levels after LLD4 administration.

Table 5. Correlation Results between MDA Levels and SGOT Levels after Administration of Lactococcus lactis D4

Variabel	r	*P Value*
MDA	0.866	0.026*
SGOT		

*Pearson Correlation Test (p value < 0.05)

This positive correlation means that decrease MDA levels are associated with decrease SGOT levels, and this relationship is statistically significant with a p-value < 0.05. This analysis demonstrates that liver function improvement is indeed related to the reduction of oxidative stress, particularly MDA. There have been no further studies directly linking MDA levels with SGOT after probiotic administration. However, this study found that both are closely related and interconnected. The relationship between these two biomarkers can be analyzed after LLD4 treatment through the antioxidant effects it produces.

CONCLUSION

Administering *Lactococcus lactis* D4 (LLD4) in rats with obstructive jaundice significantly reduces levels of malondialdehyde (MDA), indicating a decrease in oxidative stress, and lowers serum glutamate oxaloacetate transaminase (SGOT), reflecting improved liver function. The strong positive correlation between MDA and SGOT levels underscores the relationship between oxidative stress and liver health. LLD4 shows potential as a therapeutic option for managing oxidative stress and liver dysfunction in obstructive jaundice.

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CONFLICT OF INTEREST

No conflict of interest.

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