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The Effect of Synbiotic Intervention on Oral Microbiota and Inflammatory Markers in a Rat Model of Chemotherapy-Induced Mucositis

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ABSTRACT
This study evaluated the effects of synbiotic yogurt containing Lactobacillus plantarum and Roselle extract on oral microbiota, inflammation (IL-1β levels), and body weight in a rat model of chemotherapy-induced oral mucositis. IL-1β levels in serum showed a statistically significant difference between the oral mucositis (PC) and synbiotic-treated (T) groups ($p = 0.025$). The bacterial count revealed a decrease in beneficial lactic acid bacteria in the PC group (2 log CFU/g), while pathogenic Escherichia coli and Staphylococcus aureus increased. In the T group, synbiotics partially restored lactic acid bacteria (4 log CFU/g), though pathogenic levels remained elevated. Body weight analysis showed significant weight loss in both groups, with synbiotic treatment resulting in slightly less weight loss compared to the PC group. While synbiotics improve microbial balance, prolonged treatment may be needed for more substantial reductions in pathogenic bacteria and better maintenance of body weight.

Keywords: Synbiotics, Oral microbiota, IL-1β, Body weight changes, Mucositis

INTRODUCTION

The role of oral microbiota in systemic health has gained increasing attention in recent years. The oral microbiota plays a crucial role in maintaining the balance of the microbial ecosystem in the oral cavity, preventing pathogen colonization through competitive exclusion for space and resources (1). Disruptions to this balance, such as those seen in mucositis caused by chemotherapy, can result in microbial dysbiosis, which exacerbates inflammation and heightens the risk of secondary infections (2).

Oral mucositis, a common inflammatory condition affecting the oral mucosa, frequently occurs as a side effect of chemotherapy and radiotherapy in cancer patients. One of the hallmark features of mucositis is tissue damage to the mucosal lining, accompanied by the release of various inflammatory mediators, including pro-inflammatory cytokines like interleukin-1 beta (IL-1β) (3). IL-1β serves as a key mediator in both acute and chronic inflammatory responses and plays a pivotal role in activating the inflammasome, a multiprotein complex responsible for triggering the release of pro-inflammatory cytokines and inducing a form of programmed cell death known as pyroptosis(4).

Pyroptosis is a form of inflammasome-dependent cell death that occurs in response to microbial infection or cellular stress (5). Unlike apoptosis, which is a non-inflammatory, physiologically programmed cell death, pyroptosis is highly inflammatory, leading to the release of cytokines such as IL-1β and IL-18, which further intensify the local inflammatory environment. In cases of mucositis, excessive IL-1β production can exacerbate tissue damage, prolonging the healing process and increasing the risk of complications (6).

The use of probiotics as therapeutic agents in managing mucositis has garnered significant interest. Probiotics, as live microorganisms, confer health benefits by modulating gut and oral microbiota, making them a promising therapeutic option for mucositis management. Numerous studies have demonstrated that probiotics can balance microbial composition, reduce pathogenic colonization, and attenuate levels of inflammation (7). Furthermore, probiotics are known to enhance the host's immune response, potentially reducing inflammasome activity and IL-1β production, thereby minimizing tissue damage and accelerating mucosal recovery (8).

In the context of mucositis, one proposed mechanism is that probiotics may downregulate inflammation by suppressing the expression of the NLRP3 inflammasome, a key driver of IL-1β activation and pyroptosis. Additionally, through probiotic modulation of microbial composition, microbial balance can be restored, reinforcing the mucosal barrier and mitigating the risk of dysbiosis associated with chronic inflammation (9,10).
This study also evaluates body weight changes as an essential parameter to assess the health status of rats during the experiment. In severe cases of mucositis, rats often experience significant weight loss due to reduced appetite and oral pain. Therefore, the use of probiotics that can help modulate inflammation and improve mucosal conditions is expected to positively impact weight retention and facilitate recovery from mucositis (11). Previous research has shown that probiotic supplementation can enhance weight gain in animal models of inflammatory diseases by improving digestion and nutrient absorption. These positive effects may be mediated through the probiotic's modulation of gut microbiota and its interaction with the gut-immune axis, influencing systemic inflammation (12). Ultimately, probiotics may exert their benefits through multiple pathways, including microbiota modulation, inflammatory regulation, and improvement in nutritional status (13,14).
By understanding the complex effects of probiotics on microbiota, inflammation, and body weight, this study aims to provide deeper insights into the potential of probiotics in managing mucositis and chemotherapy-induced inflammation, paving the way for the development of more targeted and effective probiotic therapies.
Inflammation of the oral mucosa, such as mucositis, often leads to dysbiosis, worsening overall health. A healthy microbiota, particularly lactic acid bacteria, plays a crucial role in maintaining the balance of the mucosal environment (15). However, during inflammatory conditions, pathogenic bacteria such as Escherichia coli and Staphylococcus aureus tend to proliferate, exacerbating disease symptoms. Probiotics have been studied as a potential approach to modulate the microbiota and restore the balance disrupted by inflammation (16). Nevertheless, the impact of probiotics on the microbiota and overall health, particularly in animal models with oral inflammation, still requires further investigation. This study aims to evaluate the effects of probiotics on oral microbiota, serum IL-1β levels as an inflammatory marker, which also plays a critical role in pyroptosis a form of programmed cell death triggered by infection or cellular stress and closely associated with inflammasome activation as well as body weight changes in a rat model of mucositis.

MATERIALS AND METHODS

Preparation of Synbiotic Roselle Yogurt

The preparation of the synbiotic yogurt involved the pasteurization of fresh milk at a temperature of 115 °C for 3 minutes. After pasteurization, the milk was inoculated with Lactobacillus plantarum IIA-IA5 at a concentration of 5% of the milk's total volume, followed by incubation at 37 °C for a duration of 18 to 20 hours (17). For the roselle extract, dried roselle petals were boiled at 100 °C and subsequently extracted at 60 °C for 120 minutes. The yogurt was then combined with the roselle extract, which constituted 10% of the yogurt's volume. The resulting mixture was homogenized and stored in a refrigerator.

Protocol for Animal Model, Bacterial Analysis, Body Weight Measurement, and ELISA in Rats

Male Wistar albino rats (Rattus norvegicus) were sourced from the Integrated Medical Laboratory, Faculty of Medicine, Sebelas Maret University (UNS), Solo, Central Java. This study employed a true experimental design with a randomized post-test-only control group format. The rats, approximately 3 months old and weighing around 250g, were randomly assigned to two groups: the oral mucositis group (PC), induced by intraperitoneal injection of 5-Fluorouracil (5-FU) at a dosage of 200 mg/kg; and the treatment group (T), which received 5-FU along with synbiotic yogurt containing roselle extract, administered twice daily at a dosage of 1 mL for 4 consecutive days.
The rats were fed standard rodent chow (Broiler-2 feed) HI - PRO - VITE 594 K, sourced from Semarang, Indonesia, and were provided with unrestricted access to water for 4 days. The study protocol received approval from the Health Research Ethics Committee of Dr. Moewardi General Hospital Surakarta (Number: 016/I/HREC/2024).
Serum samples from each group were used in this assay. Reagent preparation began with the creation of standards in six tubes, labeled with concentrations of 40, 20, 10, 5, 2.5, and 0 pg/mL. Each tube was filled with 150 µL of standard diluent, with serial dilutions made from the stock standard. The wash buffer was prepared by diluting 20 mL of buffer into 580 mL of distilled water. For the assay, 50 µL of standards and 10 µL of rat serum samples were added to the wells, followed by 40 µL of sample diluent. The plate was incubated at 37°C for 30 minutes, washed five times with wash buffer, and then 50 µL of HRP-conjugate was added and incubated for another 30 minutes. After a second wash, 100 µL of substrate A and B mixture was added, followed by incubation at 37°C for 10 minutes. Finally, 50 µL of stop solution was added, and the absorbance was measured at 450 nm (18).
Tissue samples from each group were plated on selective media: Mannitol Salt Agar for Staphylococcus, MacConkey or Eosin Methylene Blue Agar for Escherichia coli, and Cetrimide Agar for Pseudomonas

aeruginosa.Samples were inoculated using either streaking or spread plate techniques and incubated at 37 °C for 24 to 48 hours.

Colony counts were expressed as Colony Forming Units (CFU) per gram of tissue sample. In cases where colonies exceeded countable numbers (TNTC), serial dilutions were performed to reduce the colony count to a manageable range of 30 to 300 colonies per plate (19). For total microbial counts, samples underwent serial dilution and were plated on non-selective media such as Nutrient Agar or Tryptic Soy Agar before incubation. The CFU counts were adjusted according to the dilution factor; for instance, 50 colonies from a 10^{-4} dilution would be calculated as 5 x 10^{6} CFU per gram. This method ensured precise quantification of both specific and total microbial populations.

The differentiation of colonies on Xylose Lysine Deoxycholate (XLD) Agar relies on the fermentation of xylose, lactose, or sucrose, which causes the phenol red pH indicator to turn yellow, along with the production of hydrogen sulfide (H_2S) that results in a black center in the colonies. Bacteria capable of decarboxylating lysine cause the medium to revert to red (20). This medium selectively promotes Gram-negative bacteria while inhibiting Gram-positive species, enabling identification based on colorimetric changes and colony morphology (21). The agar used for these experiments was sourced from ZA de Gesvrine - 4 rue Kepler - B.P. 4125, 44241 La Chapelle-sur-Erdre Cedex, France.

Body weight measurement in rats was conducted to evaluate changes across different treatment groups. Each rat was placed in an individual cage and allowed to calm down before the measurements were taken. Body weight was measured using a digital scale with an accuracy of 0.01 grams to ensure precision. The body weight was recorded twice: before treatment (pre-treatment) and after treatment (post-treatment). Each rat was measured individually at the same time each day to minimize diurnal variation in body weight (22).

RESULTS AND DISCUSSION

IL-1β Measurement

This study also analyzed the concentration of IL-1β protein, measured in pg/mL, in serum samples of rats in two groups: PC, and T. The independent t-test on the latest dataset yielded a t-statistic of approximately 3.49 and a p-value of 0.025. Since the p-value is less than the threshold of 0.05, this indicates a statistically significant difference in the mean concentrations (pg/mL) between the PC and T groups.

Additionally, Levene's test for homogeneity of variances produced a statistic of 0.91 and a p-value of 0.394. Since the p-value is greater than 0.05, it confirms that the assumption of homogeneity of variances is met between the PC and T groups.

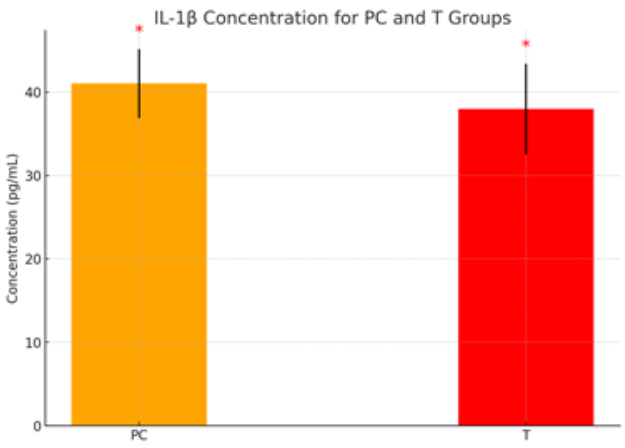

Figure 1. Concentration of IL-1β protein in serum samples of rats. The data show mean concentrations in pg/mL for oral mucositis (PC), and synbiotic-treated (T) groups.

Total Bacterial Count Using CFU

Data on the total bacterial count in the oral mucosa of rats, measured in log CFU/g, were collected at a single termination point for three bacterial groups: lactic acid bacteria, Escherichia coli, and Staphylococcus aureus.

In NC group mice Lactic acid bacteria showed a stable population of approximately 3.5 log CFU/g, suggesting a healthy balance of beneficial bacteria in the oral mucosa. Escherichia coli reached approximately 4.5 log CFU/g, while Staphylococcus aureus exhibited a similar count of around 4 log CFU/g, indicating the presence of pathogenic bacteria at manageable levels within the healthy microbial community.

In PC group mice a notable decline in lactic acid bacteria was observed, dropping to approximately 2 log CFU/g, reflecting the detrimental impact of mucositis on beneficial bacterial populations. Escherichia coli exhibited a marked increase, reaching around 5 log CFU/g, indicating a significant proliferation of pathogenic bacteria under diseased conditions. Staphylococcus aureus levels also rose slightly to about 5 log CFU/g, suggesting an increase in pathogenic presence due to the disease.

In T group mice Lactic acid bacteria levels increased to around 4 log CFU/g, demonstrating that the synbiotic treatment helped to restore the population of beneficial bacteria. However, Escherichia coli remained relatively high at approximately 4.5 log CFU/g, indicating that synbiotics had not yet fully suppressed the pathogenic bacteria. Staphylococcus aureus levels remained elevated at around 5 log CFU/g, showing a persistent pathogenic presence despite the synbiotic intervention.

Overall, the administration of synbiotics to diseased rats (T) led to an improvement in the population of beneficial bacteria, but the effects on reducing pathogenic bacteria such as Escherichia coli and Staphylococcus aureus were not immediately apparent at this termination point. A longer treatment period may be required to observe more significant reductions in pathogenic bacteria and a further increase in beneficial bacteria. The data suggest that mucositis significantly alters the bacterial composition, favoring the proliferation of pathogens, while synbiotics show potential for restoring microbial balance over time.

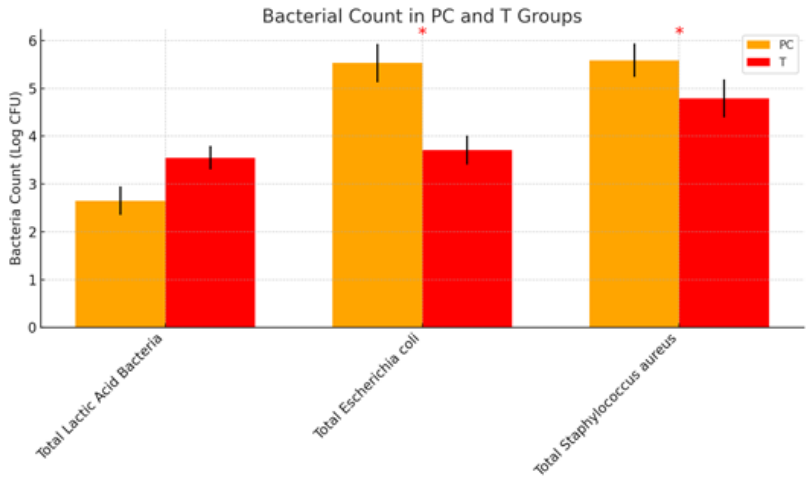

Figure 2. Total bacterial count in the oral mucosa of rats across different groups. The chart shows the log CFU/g for lactic acid bacteria, Escherichia coli, and Staphylococcus aureus in the following groups: PC (diseased control with oral mucositis), and T (synbiotic-treated group with oral mucositis). The data indicate that pathogenic bacteria, particularly Escherichia coli and Staphylococcus aureus, are more prevalent in the diseased group, while the synbiotic treatment increases the count of lactic acid bacteria, suggesting a potential benefit in restoring microbial balance.

Measurement of Body Weight

Based on the statistical analysis of the feed consumption data, we measured the delta (difference) in feed intake across four different days for two groups of rats: Synbiotic rats (T) and Sick rats (PC). In the Synbiotic rats (T) group, the mean delta in feed intake on the first day was 25 grams, with no measurable variability (standard deviation could not be calculated). On the second day, the mean delta was 19 grams, following the same pattern, with no detectable spread in the data (no standard deviation). The third day showed an increase in the mean delta to 27 grams, followed by a decrease to 19 grams on the fourth day.

In the Sick rats (PC) group, the mean delta in feed intake on the first day was 11 grams, also without measurable variability. On the second day, the mean delta increased to 21 grams. This value slightly decreased on the third day to 20 grams and then increased again to 24 grams on the fourth day. For both groups, the standard deviation could not be calculated due to the uniformity of the data or an insufficient number of data points for further variability analysis.

This analysis indicates that the Synbiotic rats (T) group consistently had a higher delta in feed consumption compared to the Sick rats (PC) group across most days of observation.

Overall, the analysis reveals that both the sick condition and synbiotic treatment significantly influenced changes in body weight, while the healthy group did not show significant weight changes. These findings underscore the importance of treatment and health status in affecting body weight in this study (23).

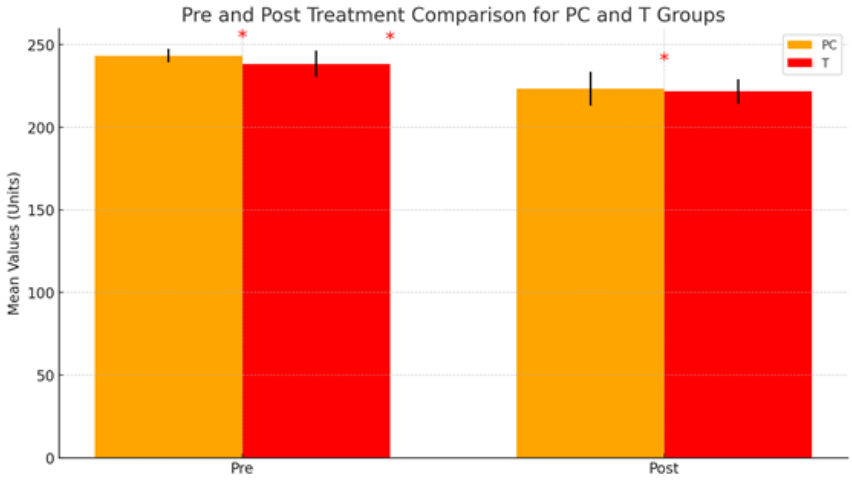

Figure 3. Changes in body weight across treatment groups before and after intervention. The data are presented as mean body weight (grams) with standard deviations for healthy, oral mucositis, and synbiotic-treated groups.

This study aimed to assess the efficacy of synbiotic yogurt containing Lactobacillus plantarum and Roselle extract in modulating oral microbiota, reducing inflammation (IL-1β levels), and maintaining body weight in a rat model of chemotherapy-induced oral mucositis. While the results suggest some beneficial effects, limitations are evident, as discussed below.

Modulation of IL-1β Levels and Inflammation

Alterations in the microbiota, as seen with the increase in pathogenic bacteria such as Escherichia coli and Staphylococcus aureus, can trigger inflammatory responses that exacerbate mucosal damage (24). Conversely, synbiotics, which enhance the population of beneficial bacteria like Lactobacillus, may help reduce IL-1β levels and mitigate inflammation by modulating the immune response, primarily through the suppression of inflammasome activation and the production of pro-inflammatory cytokines (8,25). Long-term management of post-chemotherapy patients with synbiotics could potentially lower the risk of recurrent mucositis and improve overall health (26).

In this study, a statistically significant difference in IL-1β levels was found between the T and PC groups ($t = 3.49$, $p = 0.025$), suggesting that synbiotic treatment contributed to reducing this pro-inflammatory cytokine. Levene's test confirmed the homogeneity of variances ($p = 0.394$), strengthening the reliability of these results. These findings support the idea that synbiotics can modulate inflammatory markers by influencing gut-immune interactions. However, further investigation is needed to determine if prolonged synbiotic treatment could lead to even greater reductions in IL-1β and other inflammatory markers, such as IL-18 (14). Future studies should consider extending the treatment period to fully evaluate the long-term potential of synbiotics in reducing systemic inflammation and improving overall patient outcomes.

Effects of Synbiotic Yogurt on Oral Microbiota

Synbiotic yogurt has the potential to improve oral microbiota, particularly in reducing the impact of mucositis on beneficial bacteria. In NC groups, lactic acid bacteria remained at approximately 3.5 log CFU/g, with manageable levels of E. coli (4.5 log CFU/g) and S. aureus (4 log CFU/g). However, mucositis significantly decreased the population of beneficial lactic acid bacteria (2 log CFU/g), while pathogenic E. coli and S. aureus increased (5 log CFU/g each), indicating a shift towards a disease-prone microbiota (27). In T groups, lactic acid bacteria levels improved to 4 log CFU/g, suggesting partial restoration of beneficial bacteria. However, pathogenic bacteria remained relatively high, with E. coli and S. aureus at 4.5 and 5 log CFU/g, respectively. This indicates that while synbiotics promote beneficial bacteria, they may need prolonged administration to significantly reduce pathogenic populations (28). These findings highlight synbiotics as a promising, yet not fully sufficient, treatment to modulate the microbiota in oral mucositis recovery.

Body Weight and Health Status

Both the oral mucositis (PC) and synbiotic-treated (T) groups experienced significant weight loss, consistent with prior research that links mucositis with reduced feed intake and metabolic disturbances (23). Although synbiotics have shown benefits in supporting body weight in other studies, the short duration of treatment in this experiment may have limited their effect on weight retention (11). A longer treatment period or earlier intervention might be required to achieve better outcomes in maintaining body weight and overall health status in mucositis models.

Clinical Implications and Future Directions

The findings of this study indicate that synbiotic yogurt containing Lactobacillus plantarum and Roselle extract offers promising clinical benefits in managing chemotherapy-induced oral mucositis. The reduction in IL-1β levels and the partial restoration of beneficial bacteria such as lactic acid bacteria suggest that synbiotics can modulate inflammatory responses and help restore microbial balance in the oral cavity. This could potentially reduce inflammation and support mucosal healing in patients undergoing chemotherapy, minimizing the risk of complications like secondary infections. However, the minimal effect on pathogenic bacteria, such as Escherichia coli and Staphylococcus aureus, indicates that synbiotics may require longer administration periods or additional treatments to significantly suppress harmful bacterial populations.

Further research is necessary to determine the optimal dosage, duration, and combination of synbiotic strains to maximize their therapeutic effects. Additionally, the observed impact on body weight highlights a potential link between gut microbiota modulation and metabolic health, suggesting that synbiotics may help mitigate weight loss in patients with mucositis. Future studies should focus on extending the treatment duration, exploring additional inflammatory markers such as IL-18, and assessing the safety and efficacy of synbiotics in clinical trials. These efforts could lead to the incorporation of synbiotic therapy into standard supportive care for cancer patients, improving both clinical outcomes and quality of life.

CONCLUSION

In conclusion, this study adds to the understanding of how synbiotic yogurt, combining Lactobacillus plantarum and Roselle extract, may influence oral microbiota, inflammation, and health in a chemotherapy-induced oral mucositis model. Although immediate improvements were not observed in terms of microbial balance, IL-1β levels, or body weight, the findings suggest that prolonged treatment may yield better outcomes. Future research should explore extended treatment periods, and alternative inflammatory markers, and further optimize synbiotic formulations and intervention timing to fully harness the therapeutic potential in managing mucositis.

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Declaration of competing interest

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