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Therapeutic Potential of Synbiotic Roselle Extract Yogurt in Modulating Inflammatory Markers and Oral Microbiota in a Rat Model of 5-Fluorouracil-Induced Oral Mucositis

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ABSTRACT

Mucositis is a common complication in cancer patients undergoing 5-fluorouracil (5-FU) chemotherapy. Roselle (*Hibiscus sabdariffa*) extract in synbiotic yogurt could be a beneficial alternative because it might change the inflammatory response and oral microbiota. The study aimed to investigate the effects of synbiotic roselle extract yogurt on inflammatory responses and oral microbiota in oral mucositis caused by 5-FU. An experimental study with twenty-four Sprague-Dawley rats divided into four groups (n=6): healthy control (NC), disease control (PC), synbiotic roselle extract yogurt therapy (P1), and standard therapy (P2) groups. This study evaluated the number of bacterial colonies, expression of COX-2 and caspase-1, and levels of IL-1β and VEGF (days 4 and 7). The P1 group had a significantly increased beneficial lactic acid bacteria (6.91±0.87) and a decrease in pathogenic bacteria, including S*taphylococcus aureus* (3.89±0.05), *Escherichia coli* (0), and *Enterobacter aerogenes* (1.78±0.29), compared to the PC group. Additionally, there was a statistically significant increase in VEGF levels in the tissue (0.07±0.03 ng/mL), a decrease in serum IL-1β levels (48.02±10.29 pg/mL), and an expression of caspase-1 and COX-2 compared to the PC group ($p<0.05$). Synbiotic roselle extract yogurt shows promise as a therapeutic strategy for managing mucositis by restoring microbial balance and mitigating inflammation.

1. Introduction

Mucositis is a significant complication affecting 40-80% of cancer patients undergoing chemotherapy, radiotherapy, or bone marrow transplants, with even higher incidence rates observed in bone marrow transplant recipients (Alsulami and Shaheed 2022). Mucositis is characterized by painful inflammation of the mucous membranes in the mouth or gastrointestinal tract, resulting in symptoms such as pain, ulcers, and difficulty swallowing (Chaudhry and Ehtesham 2023). Dysbiosis, an imbalance in the microbial environment, often exacerbates mucositis, complicating treatment further (Batista *et al*. 2020). Dexamethasone as standard therapy for oral mucositis has several drawbacks, namely suppression of the immune system, which can lead to infection and sepsis and increase patient mortality (Kodde *et al*. 2023; Lazar 2024). Administration of high-dose dexamethasone injection will promote the growth of

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Escherichia coli and other gram-negative bacteria (Huff *et al*. 1998). This treatment can be prevented with a new alternative treatment, which in turn lowers the death rate of patients suffering from oral mucositis (Baydar *et al*. 2005).

The 5-Fluorouracil (5-FU) is one of the most frequently used anti-cancer agents for treating head and neck malignancies (Ghafouri-Fard *et al*. 2021). Several studies have found that the most common side effect of 5-FU chemotherapy is mucositis with ulceration in the oral cavity (Atiq *et al*. 2019). There are five stages to the 5-FU-induced change in the mucosa: the production and up-regulation of messenger signals by chemotherapy, the signaling through inflammatory mediators, the amplification of mucosal damage, ulceration, and the start of the healing process (Menezes-Garcia *et al*. 2018). Chemotherapy 5-FU will cause an increase in the activity of Cyclooxygenase-2 (COX-2), Caspase-1, inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokines such as Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), and Tumor necrosis factor alpha (TNF-α) (Chang *et al*. 2012; Miao *et al*. 2011). The inflammatory response will worsen the mucosal damage in oral mucositis by inducing increased levels of COX-2, IL-1β, and caspase-1, which contribute to pain, tissue damage, and pyroptosis, a form of inflammatory cell death (Molina *et al*. 2020). COX-2 promotes the production of prostaglandins, leading to pain and inflammation, while IL-1β plays a key role in pyroptosis, exacerbating tissue injury (Opdenbosch and Lamkanfi 2019). Caspase-1 is essential for activating IL-1β and IL-18, amplifying the inflammatory cascade (Barbosa *et al*. 2018). These biomarkers are crucial for diagnosing and managing mucositis, as they assist in predicting the condition's severity (Shibuya 2011). Additionally, vascular endothelial growth factor (VEGF) expression, which promotes angiogenesis and tissue regeneration, is upregulated during mucositis (Hong *et al*. 2019). Inhibiting the inflammatory response and regulating oral bacterial dysbiosis is the most recent preventative strategy for improving oral mucositis and the patient's quality of life (Davarmanesh *et al*. 2009).

Current management strategies for mucositis primarily focus on preserving mucosal health (Chaudhry and Ehtesham 2023); however, synbiotic-based therapies, such as synbiotic yogurt containing roselle (*Hibiscus sabdariffa*) extract, offer a promising alternative by potentially modulating the inflammatory response and oral microbiota (Liu *et al*. 2022; Hakim *et al*. 2023). Previous studies have only used probiotic supplements in treating intestinal mucositis (Lopez-Gomez *et al*. 2023). The co-administration of prebiotics can enhance the effectiveness of probiotics, and this combination is known as synbiotics (Singh *et al*. 2023). To date, no studies have examined the impact of synbiotic supplements on oral mucositis. The purpose of this study was to examine the therapeutic potential of synbiotic yogurt derived from roselle extract on inflammatory responses and oral microbiota in a Sprague-Dawley rat model of oral mucositis caused by 5-FU.

2. Materials and Methods

This study is research on experimental animals, male Sprague Dawley rats (*Rattus norvegicus*), with a posttest-only control group design. It was conducted over six months, from December 2023 until June 2024, at the Laboratory of Animal Research, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. The samples were divided into four groups, namely, the healthy control group (NC), the disease control group (PC), the synbiotic rosella extract yogurt therapy group (P1), and the dexamethasone therapy group (P2). This study evaluated the number of bacterial colonies from agar media through histopathological examination, expression of COX-2 and Caspase-1 using immunohistochemistry (IHC), and IL-1β and VEGF levels using enzyme-linked immunosorbent assay (ELISA) on days 4 and 7.

The Dr. Moewardi Hospital's Ethical Committee for Health Research in Surakarta, Indonesia, authorized the study's protocol (approval number: 016/I/HREC/2024). The Animals in Research: Reporting in Vivo Experiments (ARRIVE) guidelines were followed for conducting this study (Percie *et al*. 2020).

2.1. Sample Size, Sampling Method, and Sample Allocation

The sample size for the study was determined using the Federer formula. Using this formula, the minimum sample size for each group was found to be six male white rats, resulting in a total sample of 24 rats. The samples were taken and grouped using simple random sampling. This study used an allocation method and simple randomization.

2.2. Sample Criteria

Sample criteria include those who meet the inclusion and exclusion criteria. *Rattus norvegicus* strain Sprague Dawley rats, male, healthy, weighing 350-400 g, and aged 10-12 weeks, are the inclusion criteria for the study. Sick and disabled experimental animals before the study were excluded from the research, while those that died before the treatment was completed were considered to drop out. All treatments and examinations were carried out by analysts (double blinding). All study samples were evaluated daily, and if any rat died before the completion of treatment, they were removed and considered a dropout. Meanwhile, treatment of the other rats was continued.

2.3. Preparation of Synbiotic Roselle Extract Yogurt

Fresh cow's milk from a Friesteln Holland (FH) dairy farm at Bogor Agricultural University (IPB) was pasteurized at 115°C for 3 minutes, then milk inoculated with *Lactobacillus plantarum* IIA-IA5 as much as 5% of the milk volume in the Laminar Flow Cabinet at 38-44°C for 4-6 hours to produce fermented milk. *Lactobacillus plantarum* IIA-IA5 was isolated from local Ongole crossbred beef, stored in lyophilized and slant agar at the Faculty of Animal Husbandry at IPB (registered in GenBank with access number OR47328-1). This fermented milk was incubated at 37°C for 18-20 hours to become prebiotic yogurt.

Roselle extract petals weighing 2 kg were washed and cleaned with running water, then dried at room temperature without exposure to sunlight. After drying, the dried rosella flowers were ground using a blender and sieved using a 60-mesh sieve until 150 g of rosella was obtained in the form of flour. Rosella flour was dissolved using sterile distilled water with a ratio of 20 g: 100 mL and pasteurized at a temperature of 100°C for 30 minutes. After pasteurization, the rosella solution was filtered using filter paper and evaporated using a rotary evaporator at a speed of 90 rpm at a temperature of 60°C for 120 minutes to purify the solution. Then, freeze-drying was carried out to obtain 39.750 g of rosella extract in powder form. The extract was stored in the freezer until used.

IPB produces the yogurt used, that satisfies the Indonesian National Standard (SNI), is free from heavy metals (Pb, Cd, Hg, etc.), toxins (aflatoxin M1), and pathogenic bacteria (*Salmonella* sp. and *Listeria monocytogenes*), and is safe for consumption. Yogurt was combined into a glass beaker with the roselle extract as much as 10% of the yogurt volume, homogenized using stirring for 15 minutes, and kept in its original sealed container in the refrigerator at temperatures below 40°F for up to 7 days. When stored properly, the shelf life of yogurt is seven to 14 days (Arief *et al*. 2016).

2.4. Experimental Group

The present study involved 24 male *Rattus norvegicus* Sprague-Dawley strain rats obtained from the Animal Laboratory Medical Faculty, Sebelas Maret University, aged three months and weighing approximately 250 g. The rats were randomly assigned to four groups: 1) Healthy control (NC), a single intraperitoneal injection of 0.5 mL normal saline was administered as a placebo.; 2) Disease control (PC); 3) Synbiotic roselle extract yogurt therapy (P1); and 4) Standard therapy with dexamethasone (P2).

Animal models administering 5-FU at a single dose of 200 mg/kgBW intraperitoneally will result in oral mucositis with weakened immune systems, failure to gain weight, and slowed epithelial regeneration on histopathological examination (Kim *et al*. 2023).

2.5. Animal Preparation

All groups were acclimated for a period of 7 days under controlled conditions: 12 hours of light and 12 hours of darkness, with temperatures ranging from 22°C to 24°C, 60% to 70% humidity, and unlimited access to food and water.

2.6. Synbiotic Yogurt and Standard Therapy Intervention

The NC group received a placebo with no additional treatment. The PC group had mucositis induced through an intraperitoneal injection of 5-fluorouracil (Kalbe, Jakarta, Indonesia) at a dose of 200 mg/kg body weight. The P1 group underwent mucositis induction through an intraperitoneal injection of 5-FU 200 mg/kg body weight, followed by synbiotic roselle extract yogurt therapy at a dose of 1 mL per oral using an intragastric (i.g.) sonde, twice daily (morning and evening) for 6 days, starting 2 days before undergoing mucositis induction. The P2 group had mucositis induced through an intraperitoneal injection of 5-FU 200 mg/kg body weight. It was then treated with an intraperitoneal injection of dexamethasone (Sanbe, Bandung, Indonesia) at a dose of 2.25 mg/kg body weight.

Rats from all groups were euthanized to collect buccal mucosa samples on days 4 (D4) and 7 (D7), with three samples collected per group.

2.7. Bacterial Analysis and Histopathology

The experimental animals were anesthetized using ketamine 100 mg/kg (Bernofarm, Sidoarjo, Indonesia) and xylazine 7.5 mg/kg (Interchemie-Holland, Waalre, Netherlands) via intraperitoneal injection, then euthanized by neck dislocation, and their tongue mucosal tissue was collected. Tissue samples were taken by making an incision on the ventral tongue mucosa using a number 15 scalpel, with a size of 5×3×2 mm in the lesion area. The microbiology analyst performed a circular swab on the tissue; the swab was inserted into NB media, collected, and transported to the laboratory with transport media using an ice box. The sample will be plated on selective agar media (Laboratoires Humeu, La Chapelle-sur-Erdre Cedex, France) for the isolation of Lactic acid bacteria. Mannitol salt agar (MSA) was used for the isolation of *Staphylococcus aureus* and *Staphylococcus epidermidis*, MacConkey/eosin methylene blue (EMB) agar for *Escherichia coli*, and cetrimide agar for *Enterobacter aeruginosa*. The samples were incubated with the agar medium and then inverted for bacteria at 37°C for 24-48 hours. Total Plate Count (TPC) is one method that can be used to count the number of microbes on agar media. Plates showing 30-300 colonies of bacteria will be colony counts counted using a light microscope (Olympus, Tokyo, Japan) as colony-forming units (CFU) per gram. Histopathological analysis was conducted using hematoxylin and eosin staining, with hematoxylin (catalog number ab220365, Abcam, Cambridge, UK) and eosin Y (catalog number ab246823, Abcam, Cambridge, UK). The IHC was performed to detect COX-2 and caspase-1.

2.8. Histopathological Staining

Samples taken from the tongue mucosal tissue were placed in pots containing 10% buffered formalin and labeled with the sample code. The samples were sent to the anatomical pathology laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia, and left for 24 hours. Then, the tissues to be tested were sectioned. Next, the tested tissues were melted paraffin and shaped into paraffin blocks. Paraffin-embedded oral mucosal tissues were sectioned to a thickness of 4 µm and stained with hematoxylin-eosin solution. Pathological processes in the oral mucosal tissues, including COX-2 expression, as well as the extent of inflammation (IL-1β levels), degeneration (VEGF levels), and necrosis (caspase-1 expression), were evaluated using light microscopy (Olympus, Tokyo, Japan) at 400x and 200x magnifications. Histological assessment was performed independently by a pathologist. The findings were classified according to the following scale: 0: none (normal mucosa), 1: mild (focal or diffuse alteration of basal cell layer with nuclear atypia), 2: moderate (epithelial thinning in 2-4 cell layers), 3: substantial (loss of epithelium without a break in keratinization or presence of atrophied eosinophilic epithelium and bullous formation), and 4: severe (complete loss of epithelial and keratinized cell layers; ulceration) (Kiyonaga *et al*. 2020). All samples from each group were assessed based on these criteria, and the final score was given as the mean and standard deviation (SD), as well as a description of the histopathology of each group.

2.9. Measurement of IL-1β Levels in Serum and VEGF in Tissue

The levels of IL-1β in serum and VEGF in tissue samples were measured using specific ELISA kits, with IL-1β levels measured using the Rat IL-1β Elisa Kit with ID-ER3959 (Eiyue, Wuhan, China). In contrast, VEGF was measured using the Rat VEGF Elisa Kit with ID-ER4396 (Eiyue, Wuhan, China). The measurement protocols adhered to the manufacturer's instructions provided with the kits. Biotin-conjugated antibodies as detection antibodies in 96-well plates. The wells were filled with a standard solution, samples, and biotin-conjugated detection antibody. Horseradish peroxidase (HRP)-streptavidin was then added to the wells. To see the enzymatic reaction of HRP, the substrate 3,3′,5,5′-tetramethylbenzidine is used. The optical density in a microtiter reader (xMark, Bio-Rad Laboratories, Inc., California, USA), proportional to the intended amount of sample, was recorded on the plate at 450 nm absorbance.

2.10. Immunohistochemistry (IHC) Staining

The specimens were fixed in a closed container labeled with the sample number and containing 10% formalin buffer solution for 24 hours. The specimen is processed for cutting the tissue to be studied, embedded in melted paraffin, and formed into a paraffin block. The paraffin blocks were sectioned using a microtome knife into four µm thick slices, which were subsequently stained with hematoxylin and eosin Y. The IHC staining for COX-2 (catalog number a1253, Abclonal, Massachusetts, USA) and caspase-1 (catalog number a21296, Abclonal, Massachusetts, USA) was performed. The paraffin-embedded slides were deparaffinized before staining in an oven at 55°C for 10 minutes, changing xylene twice

for 5 minutes each. Dip the slides in graded alcohol (100%, 95%, and 70%) for 5 minutes, wash with pure water (H_2O) for 5 minutes, then dry the slides. Incubate the slides in prediluted blocking serum at 25°C for 10 minutes, and add 100 µL of primary antibodies (COX-2 or caspase-1), left for 24 hours at 4°C. The smears were read using an Olympus light microscope with a wide field of view (magnification 400x), and COX-2 and caspase-1 expression scales were assessed. Counterstaining was conducted using 3,3'-diaminobenzidine (DAB) (catalog number K3468, Dako, Carpinteria, California, USA).

2.11. Study Variables

Data were collected at two time points, days 4 and 7, including oral microbiota, inflammatory markers (COX-2, IL-1β, and caspase-1), and VEGF.

Oral microbiota is the identification and counting of the number of bacterial colonies present in a sample, calculated using the Total Plate Count method, with data presented in the form of a table as mean ± SD. COX-2 is a transcription factor that produces pro-inflammatory cytokines, as determined by IHC examination, with data presented in the form of a table and images as mean ± SD. IL-1β is a multifunctional cytokine that plays an important role in regulating several cellular processes, including self-renewal and cell differentiation, as calculated using ELISA examination, with data presented in the form of a table and graphs as mean ± SD. Caspase-1 is a protease enzyme involved in the processes of pyroptosis and inflammation, as determined by IHC examination, with data presented in the form of a table and images as mean ± SD. VEGF plays a major role in the formation of new blood vessels (angiogenesis) and endothelial cells to stimulate cell growth and proliferation, as calculated using ELISA examination, with data presented in the form of a table and graphs as mean ± SD.

2.12. Statistical Analysis

The results were presented as mean and standard deviation (SD). Data normality was assessed using the Shapiro-Wilk analysis. The analysis of variance (ANOVA) test was used for normally distributed data, while the Kruskal-Wallis test was employed for non-normally distributed data, with statistical significance defined as $p < 0.05$. The two-way ANOVA test was used to determine the effect of the study group factors on the examination time (days 4 and 7). In comparison, the one-way ANOVA test was used to assess the impact of the group at one examination time. If there was statistical significance, a Post Hoc test was performed. Analyze the results using SPSS 25.0 statistical software (IBM, New York, USA) and show utilizing GraphPad Prism version 10.2.2 (GraphPad Software, Inc., San Diego, USA).

3. Results

The results reveal that synbiotic roselle extract yogurt promotes the growth of beneficial microbiota, namely lactic acid bacteria (6.91±0.87), and decreases pathogenic bacteria, such as *Staphylococcus aureus* (3.89±0.05), *Escherichia coli* (0), and *Enterobacter aerogenes* (1.78±0.29). In addition, synbiotic rose extract yogurt therapy will reduce inflammatory markers such as COX-2 and caspase-1 expression ($p<0.05$), IL-1β levels (48.02±10.29) with $p=0.007$, and increase cell growth and proliferation, namely VEGF (0.07±0.03) with $p=0.011$.

3.1. Effect of Synbiotic Roselle Extract Yogurt on Microbiological Profile

Lactic acid bacteria were detected only in the synbiotic roselle extract yogurt therapy group (6.91±0.87 on day 4 and 7.15±0.37 on day 7) and the standard therapy group (6.19±0.16 on day 4 and 6.79±0.55 on day 7), indicating enhanced growth of beneficial microbiota. No colonies were observed in the healthy control or disease control groups. Total microbial counts exceeded 10^7 CFU/g across all groups, with the highest levels in the synbiotic roselle extract yogurt therapy group, suggesting no reduction in the overall microbial population.

The count of *Staphylococcus aureus* was elevated in the disease control group and reduced in the synbiotic roselle extract yogurt therapy and standard therapy groups. *Staphylococcus epidermidis* was detected across all groups, including negative controls, with a slightly reduced population in the synbiotic roselle extract yogurt therapy group. *Escherichia coli* was detected only in the disease control group and was absent in the healthy control, synbiotic roselle extract yogurt, and standard therapy with dexamethasone groups, suggesting that synbiotic roselle extract yogurt may inhibit *Escherichia coli* growth. *Enterobacter aerogenes* was also present in the disease control group, with lower counts observed in the synbiotic roselle extract yogurt therapy group. Detailed microbiological results are presented in Table 1.

3.2. Effect of Synbiotic Roselle Extract Yogurt on IL-1β Levels

In this study, a two-way ANOVA test revealed no significant interaction between the time factor (day 4 and day 7) and the treatment groups ($p=0.174$), nor was

there a significant difference in IL-1β levels between the two time points (p=0.937). These findings indicate that IL-1β levels remained stable over time (Figure 1).

Different treatments exhibited varied effects over time, with a marked increase in IL-1β levels observed in the disease control group over the healthy control group on day 7 (p=0.011).

The synbiotic roselle extract yogurt therapy group demonstrated a reduction in IL-1β levels over the disease control group on day 7 (p=0.007), indicating its potential to mitigate inflammation. The standard therapy with the dexamethasone group exhibited a moderate increase in IL-1β levels over the same period compared to the disease control group, highlighting the progression of untreated inflammation (p=0.044). The synbiotic roselle extract yogurt therapy group showed no significant difference from the standard therapy group (p=0.292).

3.3. Effect of Synbiotic Roselle Extract Yogurt on VEGF Levels

In this study, a two-way ANOVA test revealed no significant interaction between the time factor (day 4

Table 1. Microbiological profile across groups

Type of microbiota	Population (log CFU g^{-1})						
	NC	PCa	P1a	P2a	PCb	P1b	P2b
Lactic acid bacteria	0	0	6.91±0.87	6.19±0.16	0	7.15±0.37	6.79±0.55
Total plate count	7.46±0.02	7.08±0.02	7.70±0.37	7.73±0.43	7.46±0.01	7.78±0.32	7.29±0.27
Staphylococcus aureus	2.73±0.04	4.06±0.01	3.89±0.05	3.47±0.40	3.89±0.01	3.90±0.13	4.02±0.05
Staphylococcus epidermidis	4.11±0.07	4.25±0.01	4.14±0.04	4.19±0.05	4.21±0.03	4.14±0.06	4.28±0.01
Escherichia coli	0	1.85±0.01	0	0	1.87±0.03	0	0
Enterobacter aerogenes	0	2.39±0.01	1.78±0.29	2.43±0.08	2.30±0.12	1.17±0.17	1.91±0.16

Abbreviation: NC (healthy control rats); PCa (disease control rats, day 4 termination); P1a (synbiotic roselle extract yogurt therapy rats, day 4 termination); P2a (standard therapy with dexamethasone rats, day 4 termination); PCb (disease control rats, day 7 termination); P1b (synbiotic roselle extract yogurt therapy rats, day 7 termination); P2b (standard therapy with dexamethasone rats, day 7 termination)

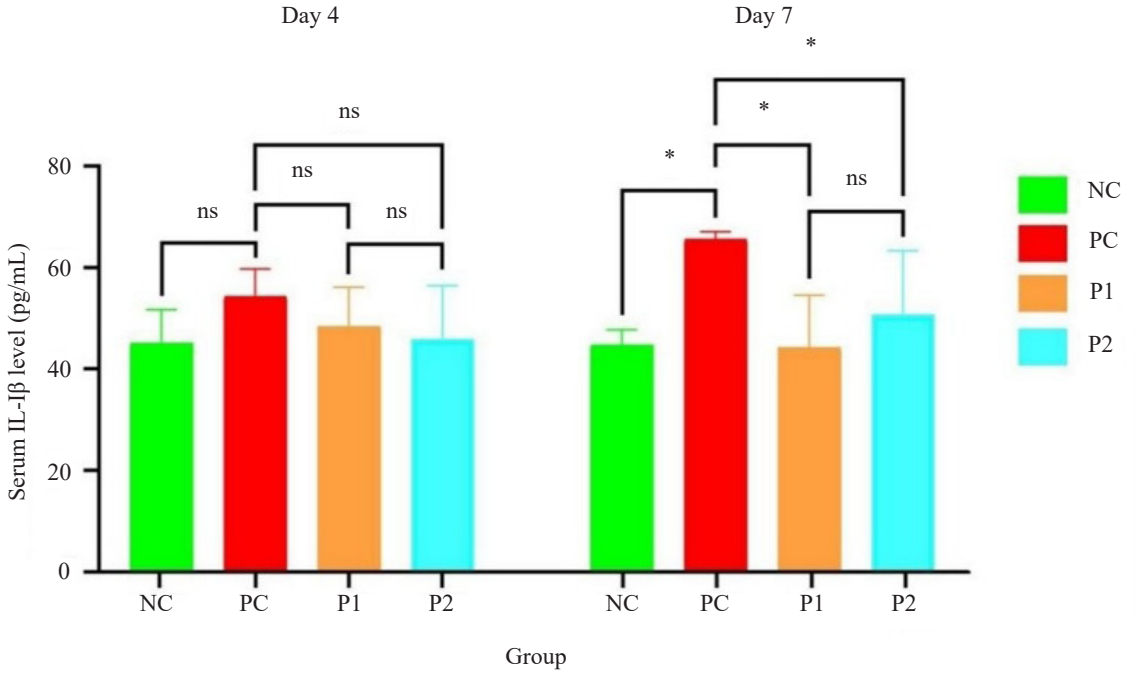

Figure 1. A bar graph depicts serum IL-1β levels in four rat groups on days 4 and 7

and day 7) and the treatment groups (p=0.251), and no significant difference in VEGF levels between the two time points (p=0.759). These findings indicate that VEGF levels remained stable over time, as illustrated in Figure 2.

A significant difference among the treatment groups on day 7 (p=0.008) indicates that treatment variations had a substantial impact on VEGF levels. The data demonstrate that VEGF levels remained relatively stable from day 4 to day 7 in the disease control (PC) and standard therapy with the dexamethasone group (P2). In contrast, the healthy control (NC) group showed a significant increase in VEGF levels on day 7 compared to the disease control group (p=0.004). The synbiotic roselle extract yogurt therapy group (P1) exhibited generally increased VEGF levels at both time points, compared to the disease control group (p=0.026; p=0.011). There was a significant difference between the synbiotic roselle extract yogurt therapy group compared to the standard therapy group (p=0.017; p=0.016).

3.4. Effect of Synbiotic Roselle Extract Yogurt on COX-2 and Caspase-1 Expression

The study findings indicate that the timing of observations (day 4 and day 7) does not significantly affect COX-2 expression, the degree of inflammation, or caspase-1 expression in the rat tissues analyzed. One-way ANOVA analysis confirmed no significant differences in COX-2 expression, inflammatory markers (lymphocyte and macrophage counts), or caspase-1 expression between the two time points, suggesting that temporal factors did not influence the measured variables.

Significant differences were observed among the treatment groups for all parameters, including COX-2 expression, the degree of inflammation, and caspase-1 expression (Figures 3 and 4). These findings underscore the critical influence of treatment type on the outcomes assessed (Tables 2 and 3).

4. Discussion

This study explores the impact of different treatments on inflammation and microbial balance in a rat model of oral mucositis, comparing synbiotic roselle extract yogurt therapy and standard therapy to disease control groups.

Lactic acid bacteria were not detected in the healthy control or disease control groups. In contrast, in the group treated with synbiotic roselle extract yogurt, there was an increase in lactic acid bacteria, indicating that this treatment supports the proliferation of beneficial bacteria. Although the total microbial count remained elevated across all groups, the highest counts were observed in the synbiotic roselle extract yogurt therapy group, suggesting selective enhancement of beneficial microbes without significantly reducing the overall microbial load.

Several studies have explained that the administration of 5-FU will cause oral mucositis in as much as 50-79%. The current strategy for mucositis therapy is to maintain

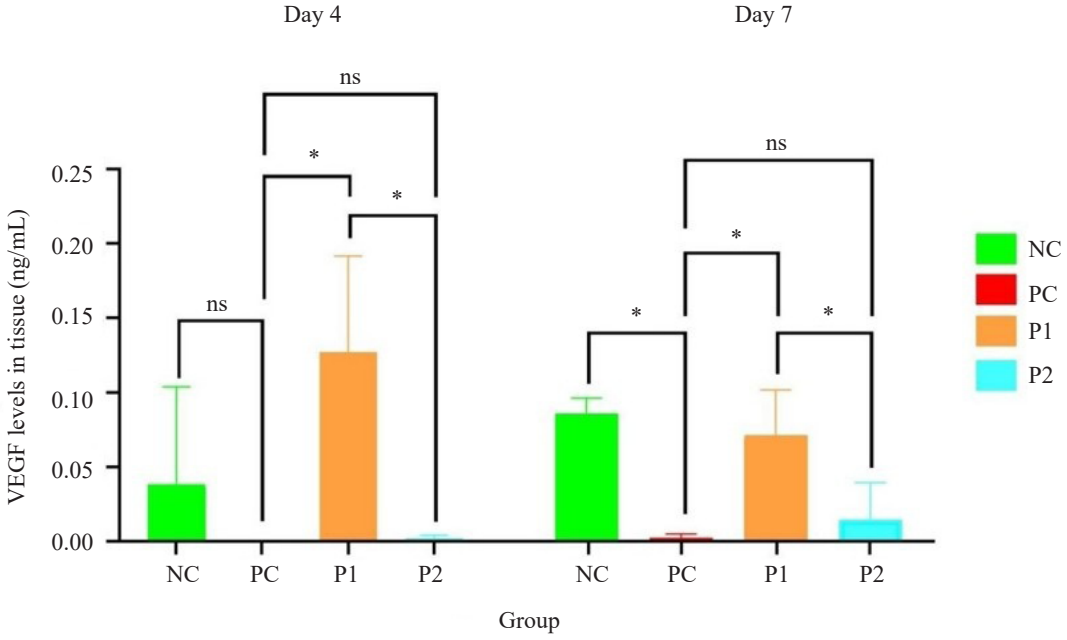

Figure 2. The graph illustrates the serum VEGF levels in different groups of rats measured on day 4 and day 7

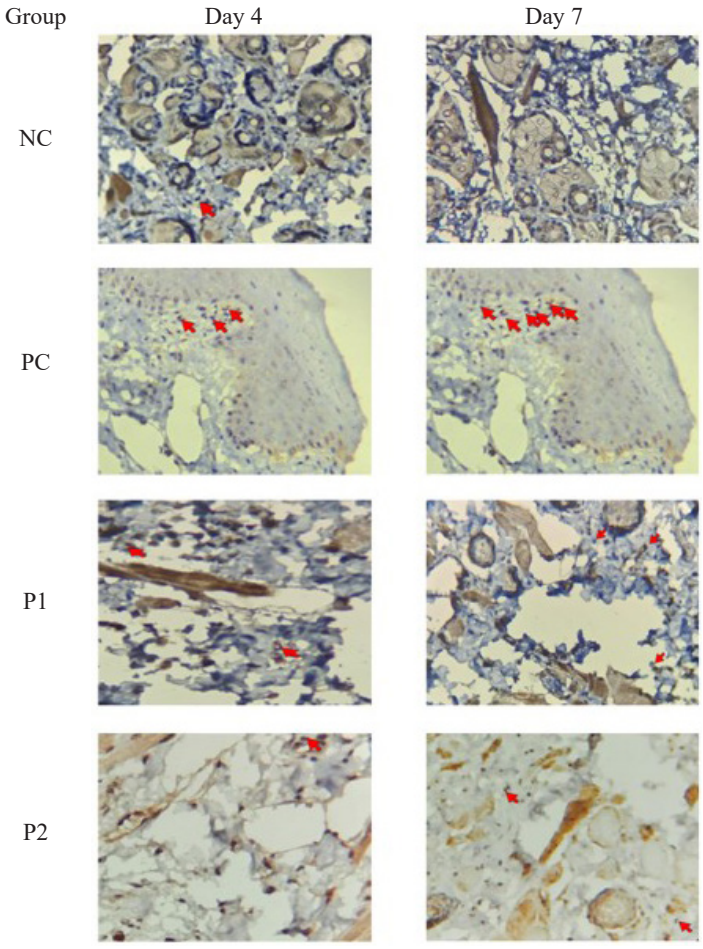

Figure 3. The results of COX-2 expression were obtained from immunohistochemistry examination in all experimental groups. Bar: 100 µm, magnification 400x. In the NC group, red arrows indicate neutrophils lacking COX-2 expression in their cytoplasm on both Day 4 and Day 7. In contrast, the PC group displayed neutrophils expressing COX-2 with a positive 2+ intensity in their cytoplasm on both days, as indicated by the red arrows. The P1 and P2 groups showed no COX-2 expression in the cytoplasm of neutrophils on either Day 4 or Day 7

Table 2. Immunohistochemistry results of COX-2 expression between experimental groups

Group	COX-2 expression					Mean± SD
	Non (0)	Mild (1)	Moderate (2)	Strong (3)	Severe (4)	
NC	6	-	-	-	-	0
PC	-	-	-	6	-	3
P1	6	-	-	-	-	0
P2	6	-	-	-	-	0

NC, healthy control; PC, disease control; P1, synbiotic roselle extract yogurt therapy; P2, standard therapy; COX-2, cyclooxygenase-2; SD, standard deviation. 0: none (normal mucosa), 1: mild (focal or diffuse alteration of basal cell layer with nuclear atypia), 2: moderate (epithelial thinning in 2–4 cell layers), 3: strong (loss of epithelium without a break in keratinization or presence of atrophied eosinophilic epithelium and bullous formation), and 4: severe (complete loss of epithelial and keratinized cell layers; ulceration)

Table 3. Immunohistochemistry results of Caspase-1 expression between experimental groups

Group	Caspase-1 expression					Mean± SD
	Non (0)	Mild (1)	Moderate (2)	Strong (3)	Severe (4)	
NC	6	-	-	-	-	0
PC	-	-	6	-	-	2
P1	6	-	-	-	-	0
P2	6	-	-	-	-	0

NC, healthy control; PC, disease control; P1, synbiotic roselle extract yogurt therapy; P2, standard therapy; SD, standard deviation. 0: none (normal mucosa), 1: mild (focal or diffuse alteration of basal cell layer with nuclear atypia), 2: moderate (epithelial thinning in 2–4 cell layers), 3: strong (loss of epithelium without a break in keratinization or presence of atrophied eosinophilic epithelium and bullous formation), and 4: severe (complete loss of epithelial and keratinized cell layers; ulceration)

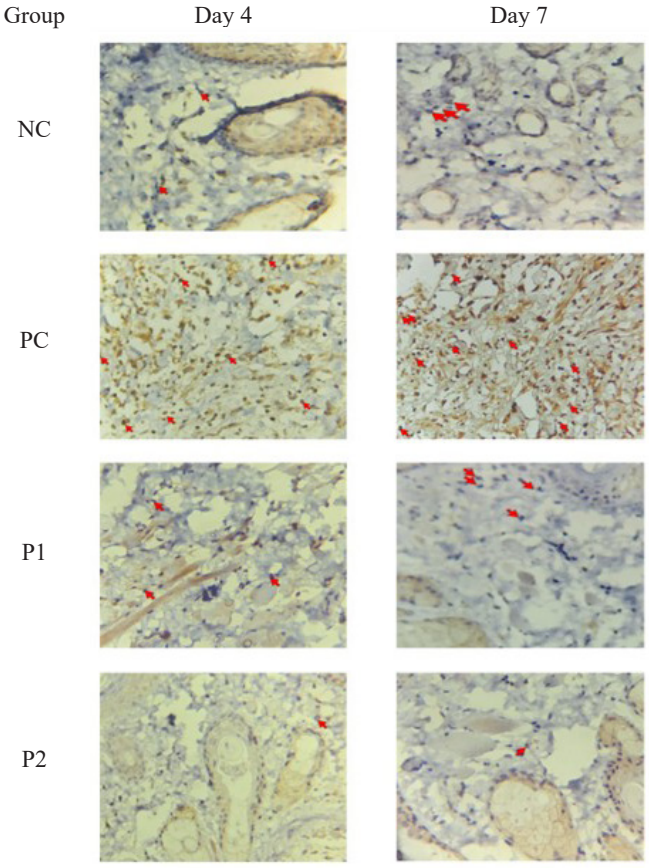

Figure 4. The results of caspase-1 expression were obtained from immunohistochemistry examination in all experimental groups. Bar: 100 µm, magnification 400x. In the NC group, neutrophils within blood vessels, indicated by red arrows, do not express caspase-1 on days 4 and 7, as evidenced by blue cytoplasm rather than brown. The PC group shows weak caspase-1 expression in the cytoplasm, marked by a brownish hue, on both days. In contrast, neutrophils in the P1 and P2 groups exhibit no caspase-1 expression in their cytoplasm on either day 4 or day 7

mucosal health and prevent the growth of harmful pathogenic bacteria (*S. aureus* and *E. coli*) (Hong *et al*. 2019). The reduction in *Staphylococcus aureus* and *Enterobacter aerogenes* in the synbiotic roselle extract yogurt and standard therapy groups, compared to the disease control group, suggests that these treatments suppress harmful pathogens. The absence of *Escherichia coli* in synbiotic roselle extract yogurt therapy groups further supports the pathogen-suppressing effect of synbiotic roselle extract yogurt, thereby preventing the onset of infections by these pathogenic bacteria. Research by Papadeas *et al*. (2007) found that the 5-FU chemotherapy directly damaged the basal epithelial cell layer and reduced the microbiota, especially lactic acid bacteria, causing clonogenic cell death, mucosal atrophy, ulceration, and inflammation due to harmful pathogenic germs. Lactic acid bacteria (LAB) have provided promising results, both in vitro and in vivo, in inflammatory models of mucositis (Papadeas *et al*. 2007). Thus, the increase in lactic acid bacteria can prevent the growth of harmful pathogenic bacteria (*S. aureus* and *E. coli*) in the oral mucosa, thereby preventing the occurrence of oral mucositis.

Total microbial counts in this study exceeded 10^7 CFU/g across all groups, with the highest levels in the synbiotic roselle extract yogurt therapy group (7.78±0.32), suggesting no reduction in the overall microbial population. The synbiotic rosella extract yogurt had higher viscosity and higher ash content, indicating changes in physical characteristics due to metabolism during fermentation (Jaman *et al*. 2022). The combination of probiotic *Lactobacillus plantarum* IIA-1A5 and rosella extract showed synergistic effects, increasing antioxidant, antihypertensive, and antimicrobial (total plate count/TPC) activities compared to yogurt without added rosella extract (Arief *et al*. 2016).

Inflammatory analysis indicated that IL-1β levels were significantly influenced by the type of treatment

(Ren and Torres 2009; Hendriyanto *et al*. 2023). The synbiotic roselle extract yogurt therapy group showed a reduction in IL-1β levels from day 4 (48.45±7.68) to day 7 (44.30±10.29) with p=0.007, suggesting that synbiotic roselle extract yogurt may mitigate inflammation through anti-inflammatory pathways and enhancement of beneficial microbiota (Mantri *et al*. 2024). In contrast, the disease control group exhibited a significant increase in IL-1β levels (65.61±1.42) compared to the healthy group (44.85±2.91), with p=0.011, reflecting the progression of inflammation in the absence of intervention (Al-Qahtani *et al*. 2024). The standard therapy group showed a decrease, indicating a protective effect with p=0.044, though less pronounced than that of synbiotics roselle extract yogurt therapy.

IL-1β, a key inflammatory cytokine, regulates immune and inflammatory responses (Lopez-Castejon and Brough 2011). Elevated serum IL-1β levels reflect inflammation severity, particularly in conditions such as mucositis, where inflammation is central (Diesch *et al*. 2021). Chemotherapy or radiation-induced damage initiates an inflammatory response, promoting immune cell migration and tissue repair (Hendriyanto *et al*. 2025). Studies suggest that targeting pyroptosis pathways can mitigate inflammation and cellular injury (Lieberman *et al*. 2019; Ashkavandi *et al*. 2024). The findings highlight the significant impact of synbiotic yogurt in modulating serum IL-1β levels in Sprague-Dawley rats.

Synbiotics roselle extract yogurt appears to exert anti-inflammatory effects, potentially through modulation of microbiota and anti-inflammatory pathways (Mahfudh *et al*. 2020). Stable IL-1β levels in the healthy control group indicate an unaffected system, whereas the substantial increase in the disease control group underscores the impact of untreated inflammation (Curra *et al*. 2022). The moderate increase in the standard therapy group suggests partial protective effects, though less effective than synbiotic roselle extract yogurt therapy.

The analysis of VEGF levels further supports these findings. While no significant differences were observed between days 4 and day 7 with p=0.251, the significant variation among synbiotic roselle extract yogurt therapy and standard therapy groups highlights the influence of the treatments. The synbiotic roselle extract yogurt therapy group showed a significant increase in VEGF levels (p=0.011), indicating a protective effect, which may lead to the formation of new endothelial cells to stimulate growth and cell proliferation. In contrast, the disease control group exhibited a significant decrease compared to the healthy group (p=0.004), reflecting an uncontrolled inflammatory response. The standard therapy group showed consistently lower VEGF levels overall (p>0.005), with a slight increase, indicating a moderate capacity to regulate VEGF expression and associated angiogenic responses (Ferrara 2004).

The observed differences in VEGF levels among the treatment groups suggest that the synbiotic treatment (P1) may exert a protective effect by maintaining stable VEGF levels. In contrast, the disease control (PC) group exhibited an increased VEGF response, indicative of a heightened inflammatory state in the absence of treatment. The standard therapy with dexamethasone group (P2) demonstrated consistently lower VEGF levels overall, with a slight increase from day 4 to day 7, potentially reflecting a moderate regulatory effect of this therapy on VEGF levels.

The expression of COX-2 and caspase-1 demonstrated that the time factor did not significantly affect these markers (p>0.05). However, significant differences between the synbiotic roselle extract yogurt therapy and standard therapy groups highlighted the importance of the treatment type. The synbiotic roselle extract yogurt therapy group showed significant reductions in COX-2 expression, caspase-1 expression, and overall inflammatory markers compared to the disease control group (p<0.05), indicating a robust anti-inflammatory effect. The standard therapy group also reduced these parameters, though to a lesser extent, suggesting some efficacy, but not as potent as synbiotic roselle extract yogurt therapy. These findings align with previous research suggesting that cytokines, such as tumor necrosis factor-alpha (TNF-α), IL-1β, and interleukin-6 (IL-6), play a crucial role in upregulating VEGF expression, thereby promoting angiogenesis in various inflammatory conditions (Geindreau *et al*. 2022; Abdullah *et al*. 2023). Emphasizing inflammatory cytokines will encourage the process of angiogenesis and the growth of mucosal cells, thus preventing the occurrence of oral mucositis (Nascimento-Junior *et al*. 2017). Furthermore, COX-2 is a key mediator that enhances VEGF production, supporting the notion that COX-2 expression contributes to increased VEGF levels observed in inflammatory diseases (Frejborg *et al*. 2020). This interplay between COX-2 and VEGF regulation is evident in the synbiotic roselle extract yogurt therapy group, where significant reductions in COX-2 expression correlate with stable VEGF levels, suggesting a potential mechanism for the observed anti-inflammatory effects (Angelo and Kurzrock 2007).

The synbiotic roselle extract yogurt therapy group demonstrated a significant reduction in COX-2 expression, the degree of inflammation, and caspase-1 expression,

compared to the disease control group. These results suggest that synbiotic roselle extract yogurt possesses a stronger anti-inflammatory effect than other treatments. The standard therapy group also showed reductions in all three parameters, albeit to a more moderate extent compared to the synbiotic roselle extract yogurt therapy group. This result indicates that standard therapy is effective in reducing inflammation and the expression of inflammatory markers such as COX-2 and caspase-1. However, it is less potent than synbiotic roselle extract yogurt therapy. In contrast, the disease control group exhibited higher levels of COX-2 expression, caspase-1 expression, inflammation, and macrophage counts. This result is consistent with ongoing inflammation in the absence of intervention. These findings highlight the critical role of treatment type in managing inflammation and modulating the expression of inflammatory markers. Synbiotic roselle extract yogurt therapy appears to be more effective in reducing inflammation than either standard therapy or no treatment, suggesting its potential as a superior intervention for inflammatory conditions such as oral mucositis.

Collectively, these findings underscore the critical role of treatment type in managing inflammation and maintaining microbial balance, so that the incidence of oral mucositis can be prevented. Synbiotics roselle extract yogurt demonstrates superior efficacy compared to standard therapy or no treatment in reducing inflammation and inhibiting pathogenic bacteria, positioning them as a potentially more effective intervention for inflammatory conditions such as oral mucositis. This study has limitations, including the use of only one dose of synbiotic roselle extract yogurt (1 mL), which prevents the determination of the optimal dose for oral mucositis therapy due to 5-FU. Additionally, this study did not examine the effects of synbiotic roselle extract yogurt on the anti-cancer effects of 5-FU. Further research is recommended using three doses of synbiotic roselle extract yogurt to obtain the optimal dose for oral mucositis therapy without interfering with the effectiveness of 5-FU chemotherapy as an anti-cancer agent.

In conclusion, the administration of synbiotics, roselle extracts, yogurt, and standard therapy with dexamethasone both influence inflammation (anti-inflammatory) and maintain microbial balance (antibacterial) in a rat model of oral mucositis. The synbiotic roselle extract yogurt therapy group showed increased beneficial lactic acid bacteria and tissue regeneration (VEGF), reduced pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter aerogenes*), and lower inflammatory markers (COX-2, IL-1β, and caspase-1), indicating a strong anti-inflammatory effect. Standard therapy of dexamethasone also regulated inflammation (COX-2, IL-1β, and caspase-1) and microbial composition (*Staphylococcus aureus* and *Escherichia coli*), though less effectively. These findings suggest synbiotic roselle extract yogurt has superior potential value over standard therapy (dexamethasone) in managing inflammation, tissue regeneration, and maintaining microbial balance.

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References

Abdullah, D., Heryadi, D.Y., Falah, M., Hendriyanto, D., 2023. The application of UV-VIS spectrophotometry method to identify Phenylbutazone in herbal packages circulating in big cities in Indonesia. *International Journal of Chemical and Biochemical Sciences*. 24, 961–967. https://www.iscientific.org/wp-content/uploads/2024/01/110-ijcbs-23-24-6-110.pdf

Al-Qahtani, A., Alhamlan, F., Al-Qahtani, A., 2024. Pro-inflammatory and anti-inflammatory interleukins in infectious diseases: a comprehensive review. *Tropical Medicine and Infectious Disease*. 9, 13. doi: 10.3390/tropicalmed9010013

Alsulami, F.J., Shaheed, S.U., 2022. Oral cryotherapy for management of chemotherapy-induced oral mucositis in hematopoietic cell transplantation: a systematic review. *BMC Cancer*. 22, 442. doi: 10.1186/s12885-022-09539-8

Angelo, L.S., Kurzrock, R., 2007. Vascular endothelial growth factor and its relationship to inflammatory mediators. *Clinical Cancer Research*. 13, 2825–2830. doi: 10.1158/1078-0432.CCR-06-2416

Arief, I., Budiman, C., Hanifah, R., 2016. Antihypertensive potency of goat milk yoghurt supplemented by probiotic and roselle extract. I*nternational Journal of Basic and Applied Science*. 30, 207–214. https://gssrr.org/index.php/JournalOfBasicAndApplied/article/view/6576/3172

Ashkavandi, Z., Tadbir, A., Rahmatabadi, A., Owji, S.H., 2024. Cell cannibalism in oral precancerous lesions and squamous cell carcinoma. *Iranian Journal of Otorhinolaryngology.* 36, 639-646. doi: 10.22038/ijorl.2024.77568.3600

Atiq, A., Shal, B., Naveed, M., Khan, A., Ali, J., Zeeshan, S., Al-Sharari, S.D., Kim, Y.S., Khan, S., 2019. Diadzein ameliorates 5-fluorouracil-induced intestinal mucositis by suppressing oxidative stress and inflammatory mediators in rodents. *European Journal of Pharmacology*. 843, 292-306. doi: 10.1016/j.ejphar.2018.12.014

Barbosa, M.M., de Araújo, A.A., Júnior, R.F., Guerra, G.C., Brito, G.A., Leitão, R.C., Ribeiro, S.B., Tavares, E.A., Vasconcelos, R.C., Garcia, V.B., Medeiros, C.A., 2018. Telmisartan modulates the oral mucositis induced by 5-fluorouracil in hamsters. *Frontiers in Physiology*. 29, 1204. doi: 10.3389/fphys.2018.01204

Batista, V.L., da Silva, T.F., de Jesus, L.C., Rocha, N.D., Barroso, F.A., Tavares, L.M., Azevedo, V., Agresti, P.M., Drumond, M.M., 2020. Probiotics, prebiotics, synbiotics, and paraprobiotics as a therapeutic alternative for intestinal mucositis. *Frontiers in Microbiology.* 17, 544490. doi: 10.3389/fmicb.2020.544490

Baydar, M., Dikilitas, M., Sevinc, A., Aydogdu, I., 2005. Prevention of oral mucositis due to 5-fluorouracil treatment with oral cryotherapy. *Journal of the National Medical Association*. 97, 1161-1164. https://pmc.ncbi.nlm.nih.gov/articles/PMC2575965/pdf/jnma00189-0115.pdf

Chang, C.T., Ho, T.Y., Lin, H., Liang, J., Huang, H., Li, C., Lo, H.Y., Wu, S.L., Huang, Y.F., Hsiang, C.Y., 2012. 5-Fluorouracil induced intestinal mucositis via nuclear factor-κB activation by transcriptomic analysis and in vivo bioluminescence imaging. *PLoS One*. 7, e31808. doi: 10.1371/journal.pone.0031808

Chaudhry, S., Ehtesham, Z., 2023. Treatment options for cancer patients suffering from oral mucositis. *Asian Pacific Journal of Cancer Care*. 8, 181-184. doi: 10.31557/apjcc.2023.8.1.181-184

Curra, M., Baldin, J., Martins, M.A., Schuch, L.F., Carvalho, A.L., Gaio, E.J., Rösing, C.K., Bittencourt, R.I., Gregianin, L.J., Paz, A.A., Daudt, L.E., Martins, M.D., 2022. Investigation of oral and general health status and IL-1β gene polymorphism as risk factors for oral mucositis in hematopoietic stem cell transplantation patients. *Brazilian Oral Research*. 36, e016. doi: 10.1590/1807-3107bor-2022.vol36.0016

Davarmanesh, M., Mardani, M., Mirkhani, H., Monabbati, S., Tanideh, N., Mehrabani, D., 2009. Effect of dexamethasone premedication on oral mucositis in hamster. *Journal of Applied Animal Research*. 36, 267-270. doi: 10.1080/09712119.2009.9707073

Diesch, T., Filippi, C., Fritschi, N., Filippi, A., Ritz, N., 2021. Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: A systematic review. *Cytokine*. 143, 155506. doi: 10.1016/j.cyto.2021.155506.

Ferrara, N., 2004. Vascular endothelial growth factor: basic science and clinical progress. *Endocrine Reviews*. 25, 581–611. doi: 10.1210/er.2003-0027

Frejborg, E., Salo, T., Salem, A., 2020. Role of cyclooxygenase-2 in head and neck tumorigenesis. *International Journal of Molecular Sciences*. 21, 9246. doi: 10.3390/ijms21239246

Geindreau, M., Bruchard, M., Vegran, F.,, 2022. Role of cytokines and chemokines in angiogenesis in a tumor context. C*ancers (Basel)*. 14, 2446. doi: 10.3390/cancers14102446

Ghafouri-Fard, S., Abak, A., Anamag, F.T., Shoorei, H., Fattahi, F., Javadinia, S.A., Basiri, A., Taheri, M., 2021. 5-Fluorouracil: a narrative review on the role of regulatory mechanisms in driving resistance to this chemotherapeutic agent. *Frontiers in Oncology*. 11, 658636. doi: 10.3389/fonc.2021.658636

Hakim, R.F., Idroes, R., Hanafiah, O.A., Ginting, B., Kemala, P., Fakhrurrazi, F., Putra, N.I., Shafira, G.A., Romadhoni, Y., Destiana, K., Muslem, M., 2023. Characterization of red algae (*Gracilaria verrucosa*) on potential application for topical treatment of oral mucosa wounds in *Rattus norvegicus*. *Narra J*. 3, e422. doi: 10.52225/narra.v3i3.422

Hendriyanto, D., Rahman, S., Tofrizal, Elliyanti, A., 2023. Molecular targets of thymoquinone protection against cisplatin-induced organ toxicity: A literature review. *International Journal of Chemical and Biochemical Sciences*. 24, 79–86. https://www.iscientific.org/wp-content/uploads/2023/10/10-ijcbs-23-24-4-10-done.pdf

Hendriyanto, D., Rahman, S., Alimuddin, T., Elliyanti, A., 2025. Thymoquinone protection against oxidative stress caused by cisplatin through increased superoxide dismutase expression in the cochlea of wistar rats. *Advances in Life Sciences*. 12, 231-236. doi: 10.62940/als.v12i1.3151

Hong, B.Y., Sobue, T., Choquette, L., Dupuy, A.K., Thompson, A., Burleson, J.A., Salner, A.L., Schauer, P.K., Joshi, P., Fox, E., Shin, D.G., Weinstock, G.M., Strausbaugh, L.D., Bagtzoglou, A.D., Peterson, D.E., Diaz, P.I., 2019. Chemotherapy-induced oral mucositis is associated with detrimental bacterial dysbiosis. *Microbiome*. 7, 66. doi: 10.1186/s40168-019-0679-5

Huff, G.R., Huff, W.E., Balog, J.M., Rath, N.C., 1998. The effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental *Escherichia coli* respiratory infection. *Poultry Science*. 77, 654-661. doi: 10.1093/ps/77.5.654

Jaman, S., Islam, M.Z., Sojib, M.S.I, Hasan, M.S., Khandakar, M.M., Bari, M.S., Sarker, A.H., Habib, R., Siddiki, M.S.R., Islam, M.A., Rashid, M.H., 2022. Physicochemical characteristics, sensory profile, probiotic, and starter culture viability of synbiotic yogurt. *Journal of Advanced Veterinary and Animal Research*. 9, 694-701. doi: 10.5455/javar.2022.i638.

Kim, D.H., Choi, J., Lim, Y.S., Huh, H.J., Cho, C.G., Kim, B.H., 2023. A rat model for oral mucositis induced by a single administration of 5-Fluorouracil. *In Vivo*. 37, 218-224. doi: 10.21873/invivo.13070

Kiyonaga, N., Moriyama, T., Kanmura, Y., 2020. Effects of dexmedetomidine on lipopolysaccharide-induced acute kidney injury in rats and mitochondrial function in cell culture. *Biomedicine and Pharmacotherapy*. 125, 109912. doi: 10.1016/j.biopha.2020.109912

Kodde, C., Timmen, F., Hohenstein, S., Bollmann, A., Bonsignore, M., Kuhlen, R., Nachtigall, I., Tasci, S., 2023. Impact of Dexamethasone on the Pathogen Profile of Critically Ill COVID-19 Patients. *Viruses*. 15, 1076. doi: 10.3390/v15051076

Lazar, A., 2024. Recent data about the use of corticosteroids in sepsis-review of recent literature. *Biomedicines*. 12, 984. doi: 10.3390/biomedicines12050984

Lieberman, J., Wu, H., Kagan, J.C., 2019. Gasdermin D activity in inflammation and host defense. *Science Immunology*. 4, eaav1447. doi: 10.1126/sciimmunol.aav1447

Liu, Y.C., Wu, C.R., Huang, T.W., 2022. Preventive effect of probiotics on oral mucositis induced by cancer treatment: a systematic review and meta-analysis. *International Journal of Molecular Sciences*. 23, 13268. doi: 10.3390/ijms232113268

Lopez-Castejon, G., Brough, D., 2011. Understanding the mechanism of IL-1β secretion. *Cytokine and Growth Factor Reviews*. 22, 189-195. doi: 10.1016/j.cytogfr.2011.10.001

Lopez-Gomez, L., Alcorta, A., Abalo, R., 2023. Probiotics and probiotic-like agents against chemotherapy-induced intestinal mucositis: a narrative review. *Journal of Personalized Medicine*. 13, 1487. doi: 10.3390/jpm13101487

Mahfudh, N., Hadi, A., Solechan, R.A., 2020. Immunomodulatory activity of yogurt fortified with roselle (*Hibiscus sabdariffa* L.) extract. *International Food Research Journal*. 28, 255-261. doi: 10.47836/ifrj.28.2.04

Mantri, A., Klumpen, L., Seel, W., Krawitz, P., Stehle, P., Weber, B., Koban, L., Plassmann, H., Simon, M.C., 2024. Beneficial effects of synbiotics on the gut microbiome in individuals with low fiber intake: secondary analysis of a double-blind, randomized controlled trial. *Nutrients*. 16, 2082. doi: 10.3390/nu16132082

Menezes-Garcia, Z., Arifa, R.D., Fagundes, C.T., Souza, D.G., 2018. Mechanisms underlying chemotherapy-associated mucositis: the role of inflammatory mediators and potential therapeutic targets. *European Medical Journal Gastroenterology*. 7, 82-91.https://doi.org/10.33590/emjgastroenterol/10310983

Miao, E.A., Rajan, J.V., Aderem, A., 2011. Caspase-1-induced pyroptotic cell death. *Immunological Reviews*. 243, 206–214. doi: 10.1111/j.1600-065X.2011.01044.x

Molina, E., Ortiz, S., Molina, F., Guaza, C., 2000. Induction of COX-2 and PGE(2) biosynthesis by IL-1beta is mediated by PKC and mitogen-activated protein kinases in murine astrocytes. *British Journal of Pharmacology*. 131, 152-159. doi: 10.1038/sj.bjp.0703557

Nascimento-Junior, L.S., Brito, W.N., Barros, D.M., Gonçalves, D.M., Matos, L.S., Nascimento, C.R., Ribeiro, L., Lima, R., Júnior, R.S., Reis, S.A.G.M., Goncalves, T.M., Amorim, E., Júnior, B.J.M., Brito, L.S., Barros, W.N., Goncalves, D.M., Matos, L.S., Nascimento, G.R.B., Ribeiro, L.A., Lima, R.S., Júnior, R.G.C., Reis, S.A.G.B, Gonçalves, T.M., Amorim, E.L.C., 2017. Anti-inflammatory and healing action of oral gel containing borneol monoterpene in chemotherapy-induced mucositis in rats (*Rattus norvegicus*). *Brazilian Journal of Pharmaceutical Sciences.* 53, e00081. doi: 10.1590/s2175-97902017000300081

Opdenbosch, N., Lamkanfi, M., 2019. Caspases in cell death, inflammation, and disease. *Immunity*. 50, 1352-1364. doi: 10.1016/j.immuni.2019.05.020

Papadeas, E., Naxakis, S., Riga, M., Kalofonos, C.H., 2007. Prevention of 5-fluorouracil-related stomatitis by oral cryotherapy: a randomized controlled study. *European Journal of Oncology Nursing*. 11, 60-65. doi: 10.1016/j.ejon.2006.05.002

Percie, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *BMC Veterinary Research*. 16, 1-7. doi: 10.1371/journal.pbio.3000410

Ren, K., Torres, R., 2009. Role of interleukin-1beta during pain and inflammation. *Brain Research Reviews*. 60, 57-64. doi: 10.1016/j.brainresrev.2008.12.020

Shibuya, M., 2011. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer*. 2, 1097–1105. doi: 10.1177/1947601911423031

Singh, N.K., Beckett, J.M., Kalpurath, K., Ishaq, M., Ahmad, T., Eri, R., 2023. Synbiotics as supplemental therapy for the alleviation of chemotherapy-associated symptoms in patients with solid tumours. *Nutrients*. 15, 1759. doi: 10.3390/nu15071759