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Original Article

The Effect of *Lactobacillus plantarum* and Selenium-enriched *Lactobacillus plantarum* on *Staphylococcus aureus* -Induced Osteomyelitis

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Abstract

Background: Osteomyelitis is a bone infection. The most common treatment is use of antibiotics, which might have many side effects and may induce antibiotic resistance in bacteria. Strengthening the body›s antioxidant system may be effective on preventing the disease progression. The aim of this study was to investigate the effects of *Lactobacillus plantarum* and *L. plantarum* enriched with selenium (Se) on osteomyelitis caused by *Staphylococcus aureus*.

Methods: Thirty-six male rats were randomly divided into the 6 groups. A group was control group. Groups B and C were respectively treated with *L. plantarum*, and *L. plantarum* enriched with Se. Osteomyelitis was induced in group D. Groups E and F were induced with osteomyelitis and treated like groups B and C. At the end of the treatment period, in order to evaluate hematological parameters, the acute phase protein, interleukin 6 and 1- levels were assessed in the rat's blood samples. Bone was stained with hematoxylin-eosin to evaluate histopathological changes.

Results: The number of white blood cells (WBCs) in groups D and E and interleukin 6 levels in groups D, E and F were significantly increased compared to the control group. There was no significant change in other factors in the experimental groups compared to the control group. Histopathological changes were less severe in groups E and F than in group D.

Conclusion: The treatment of animals with osteomyelitis by probiotics, especially Se-enriched probiotics, to some extent can prevent the distribution of bone infection to the surrounding tissues.

Keywords: Osteomyelitis, *L. plantarum*, Selenium, Rat

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Introduction

Osteomyelitis is an inflammatory process in the whole bone due to infection that leads to bone destruction (1). A wide range of bacteria including *Streptococcus*, *Pneumococcus*, *Escherichia coli*, *Salmonella* and *Brucella* can cause the disease, but the most common of them is *Staphylococcus aureus* which in 80% of cases is the causative agent of the infection (2). The bacterium can penetrate into bone tissue through bloodstream, open bone fractures, and purulent foci in a tissue close to the bone. Invasion of immune cells at the site of infection and secretion of inflammatory cytokines cause the dilation of blood vessels in the bone and fluid leakage into the intercellular space, and consequently pressure on the blood vessels in the bone and the gradual destruction of bone cells. Following this process, the accumulation of fibrous tissue around the damaged tissue blocks blood flow to this area resulting in protection of the pathogen from antibiotics access and thus treatment failure (3). The basis of treatment for acute osteomyelitis is the use of injected antibiotics, but often due to delays in diagnosis and initiation of treatment, even by the use of antibiotics, the treatment is often unsuccessful. Considering the statistics of osteomyelitis in the country, the complete failure in the treatment of this disease, and the high costs of treatment, it seems that performing studies on the use of safe supplements in conjunction with antibiotic treatment is useful in accelerating the treatment of the disease (4). In addition to the current research strategies that being progressively conducted and optimized, new and emerging technologies, including the use of non-antibiotic compounds have been considered (5). The health benefits of probiotics and trace elements including selenium have been proven for centuries. Probiotics are groups of beneficial bacteria and yeasts that their balanced consumption is of a particular importance for

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human and animals health. Probiotics are able to inhibit pathogens growth by secreting a variety of antimicrobial peptides, including bacteriocins and also by producing phytase enzyme, leading to bone repair (6).

Selenium (Se) is another effective factor in strengthening the immune system, which can reduce inflammation because of its powerful antioxidant activity (7,8). Selenocysteine, selenomethionine, sodium selenite and seleno-methyl cysteine are effective in inducing selenoenzymes such as glutathione peroxidase and thioredoxin reductase and thus preventing oxidative damage caused by infection in the body (9). It has been shown that the use of probiotic supplements enriched with Se can be effective in improving the function of a group of hormones and hemato-biochemical parameters of the body by strengthening the body's antioxidant system (10,11). The inhibitory effect of this element on the growth of pathogens including *S. aureus* has also been reported (12). Therefore, the aim of the present study was to investigate the effect of *Lactobacillus plantarum* enriched with Se on the osteomyelitis induced by *S. aureus* in rats.

Materials and Methods

Enrichment of Lactobacillus plantarum with selenium

Lactobacillus plantarum strain with ID [ATCC: 13643] was bought from microbial bank of Pasteur Institute of Iran. For enrichment of *L. plantarum* with Se, 10 mL of *L. plantarum* suspension was added to 0.04 g of Se and incubated for 47 hours at 37°C. At the end of incubation time, centrifugation was performed at 3000 rpm for 15 minutes. To remove excess Se, the precipitates were washed 3 times with sterile normal saline. Finally, Se-enriched suspension (1.5×10^8 CFU/mL) was prepared using the cell precipitates (13).

Induction of osteomyelitis

Animals were anesthetized by intramuscular injection of ketamine (60 mg/kg) and xylazine (6 mg/kg). Then the inner surface of the leg was cut with a surgical razor and a 0.5 mm diameter hole was drilled in the bone with a surgical drill, so that the drill bit reached the bone marrow. Then, 100 µL of *S. aureus* suspension (1.5×10^8 CFU/mL) was injected into the incision and finally the site was sutured to the end of the muscle and the incision site skin. To control pain, animals subcutaneously received 60 mg/kg buprenorphine and 2 mg/kg meloxicam (14). The animals were transferred to Persian Veterinary Clinic, 4 weeks after the surgery, and to ensure osteomyelitis occurrence, lateral photographs were taken from their right tibia.

Animal grouping

Thirty-six adult male Wistar rats (120 ± 20 g) were prepared from the animal nest of Islamic Azad University, Falavarjan Branch, and kept under standard healthy laboratory conditions including 12 hours light/dark period at 23 ± 2°C and 53 ± 2% humidity. Food and water were provided *ad libitum*. After one week, the animals were divided into the 6 groups:

- A: Control group. A hole (0.5 mm in diameter) was made in the proximal part of the tibia. The wound then closed and animals were intragastrically treated with 1 ml physiological serum daily for 7 weeks.
- B: *L. plantarum* treated group. Animals were prepared similar to the group A, except that instead of physiological serum, they were daily treated with 1 ml of *L. plantarum* solution (1.5×10^7 CFU/mL) for 7 weeks (13).
- C: Treatment group with Se enriched *L. plantarum*. All steps were similar to the group B except that instead of probiotics alone, the animals were treated daily with Se-enriched probiotics for 7 weeks.
- D: Osteomyelitis group. Osteomyelitis was induced by injection of *S. aureus* on the proximal part of the leg and animals were treated intra-gastrointestinally with 1 mL of physiological serum daily for 7 weeks.
- E: Osteomyelitis group treated with *L. plantarum*. Osteomyelitis was induced and animals were injected intragastrically by 1 ml of *L. plantarum* daily for 7 weeks.
- F: Osteomyelitis group treated with *L. plantarum* enriched with Se. Animals were prepared similar to the group E, except that instead of *L. plantarum*, the animals were treated with 1 ml of Se-enriched *L. plantarum*.

Measurement of blood factors

At the end of the treatment period, the rats were weighed and blood samples were taken directly from the heart. The number and index of blood cells were measured using a CBC MINDARY cell counter model CBC MINDARY. The amount of interleukin 6 (IL-6) and IL-1β were determined using the ELISA assay in accordance with the ELISA reader and according to the kit instructions. Briefly, samples and standards were prepared first. Then, 40 µL of the sample solution was added to the sample wells and 50 µL of the prepared standard was added to the standard wells. Then, 10 µL of IL-6 or IL-1β antibodies were added to the wells to measure each interleukin. The plates were incubated at 37°C for 60 min and then washed 5 times. Finally, 50 µL of chromogen solutions A and B were added to each well and incubated for 60 minutes at 37°C. After adding the solution, the optical density of each well was read at a wavelength of 450-360 nm for 15 minutes. For C-reactive protein (CRP) test, 50 µL was removed from rat serum and 50 µL was removed from the latex antigen reagent solution with a sampler and placed on a slide, then the slide placed on a shaker at 60 rpm for 5 minutes. Agglutination bleaching was a CRP-positive

indicator (15).

Evaluation of histopathological changes

A part of rat legs with its surrounding muscle was isolated and fixed in 10% formaldehyde for 24 hours after washing. Then, in order to decalcifying the samples, it was transferred to EDTA for 12.5% for two weeks. After dehydration and then paraffin molding, 5 micron sections were prepared from the tissues and the slides were stained with hematoxylin and eosin (16).

Statistical analysis

Normality of data distribution and homogeneity of variances were assessed using Kolmogorov-Smirnov. Data were expressed in mean ± SD. ANOVA was used to compare among different groups. Statistical significant level was considered at $P \leq 0.05$. The statistical analysis was performed through SPSS software version 20.

Results

As it is seen in Table 1, the number of blood cells and its indices in most groups do not show significant differences compared to the control group, except for significant increase of white blood cells (WBCs) in rats with osteomyelitis caused by *S. aureus* and in the group of osteomyelitis treated with *L. plantarum* ($P \leq 0.05$). Also, the level of red blood cell distribution width (RDW) in the group with osteomyelitis increased significantly ($P \leq 0.05$) compared to the control group.

CRP levels in the osteomyelitis group, osteomyelitis group treated with *L. plantarum* ($P \leq 0.001$) and osteomyelitis group treated with Se-enriched *L. plantarum* ($P \leq 0.05$) significantly increased compared to the control group.

Concentration of interleukin 6 in the groups with osteomyelitis ($P \leq 0.01$) and osteomyelitis group treated with *L. plantarum* ($P \leq 0.01$) and osteomyelitis group treated with Se-enriched *L. plantarum* ($P \leq 0.05$) Showed significant increases compared to the control group.

IL-1β concentration in none of the experimental groups showed a significant change compared to the control group.

Histopathological results of bone tissue

Figure 1A shows a part of the tibia with the bone marrow, the bony blades and surrounding muscles. The bony blades and the surrounding muscle have a normal appearance. Figure 1B shows a part of the bony blade at higher magnification. In this form, most lacunae have bone cells. Figure 1C shows the muscle fibers around the bone without any inflammation or damage. Due to the similarity of tissue images taken from the group of animals treated with *L. plantarum* and *L. plantarum* enriched with Se and the control group, their inclusion was refused.

Figure 2A shows inflammation of the periosteum around the bone and accumulation of inflammatory cells in part of the tibia of the osteomyelitis group. Figure 2B illustrates the proliferation of large numbers of inflammatory cells in the lining of muscle fibers. Figure 2C demonstrates the extensive destruction of bony blades and the presence of active osteoclasts around these blades. Accumulation of osteoclasts around the bony blade is seen at higher magnifications in Figure 2D.

Figure 3 shows part of the tibia in a group of animals with osteomyelitis treated with *L. plantarum*. Figures 3A and 3B show the parts of the bony blades and the muscles around these blades, respectively. In these pictures, the destruction of bone tissue and inflammation of the periosteum (star) is the same as the

Table 1. Blood factors levels of experimental groups and the control group

Parameter	A	B	C	D	E	F
WBC (10^3/μL)	5.98 ± 1.38	6.39 ± 1.85	7.19 ± 2.03	10.19 ± 1.22[c]	8.91 ± 1.75[a]	8.62 ± 1.24
RBC (10^6/μL)	7.92 ± 0.36	8.01 ± 0.79	8.19 ± 1.17	8.19 ± 0.44	8.00 ± 0.21	8.42 ± 0.58
HCT (%)	43.66 ± 1.58	42.16 ± 1.40	46.26 ± 3.46	43.81 ± 4.48	43.05 ± 0.63	46.43 ± 3.36
Hg (g/dL)	13.78 ± 6.21	13.70 ± 0.94	12.86 ± 4.00	13.58 ± 1.43	13.81 ± 0.44	14.46 ± 1.11
RDW (%)	14.13 ± 0.49	14.43 ± 0.95	14.80 ± 0.51	15.51 ± 0.65[a]	15.36 ± 0.63	15.20 ± 1.08
MCH (pg)	17.16 ± 0.51	17.30 ± 0.70	17.88 ± 1.38	17.11 ± 0.21	17.20 ± 0.48	17.18 ± 0.49
MCHC (g/dL)	31.32 ± 0.41	30.78 ± 0.55	31.40 ± 0.85	30.96 ± 0.54	31.61 ± 0.77	30.86 ± 0.83
MCV (fl)	54.81 ± 2.13	53.86 ± 2.16	57.11 ± 6.07	55.30 ± 0.79	53.70 ± 1.92	55.10 ± 1.95
PLT (10^3/)	726.67 ± 56.96	736.83 ± 40.78	768.67 ± 93.43	832.67 ± 59.78	803.67 ± 90.88	801.67 ± 83.06
CRP (μg/ mL)	302.50 ± 12.18	296.17 ± 30.70	295.17 ± 32.37	607.17 ± 104.39[c]	536.00 ± 147.65[c]	465.17 ± 47.15[a]
IL-1β (pg/ml)	1636.3 ± 256.71	1478.8 ± 258.37	1734.8 ± 174.20	1814.50 ± 225.92	1799.50 ± 205.34	1619.70 ± 251.21
IL-6 (pg/ml)	295.44 ± 58.17	290.53 ± 50.67	267.75 ± 49.88	411.60 ± 61.04[b]	408.48 ± 62.94[b]	389.85 ± 25.61[a]

Abbreviations: WBC, white blood cell; RBC, red blood cell; Hg, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; CRP, C-reactive protein; IL-6, Interleukin 6.
Data is represented as mean ± SD. Significance level is defined as [a] $P \leq 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.001$.
A: Control group, B: *L. plantarum* treated group, C: Se enriched *L. plantarum* treated group, D: Osteomyelitis group, E: Osteomyelitis group treated with *L. plantarum*, F: Osteomyelitis group treated with Se-enriched *Lactobacillus plantarum*.

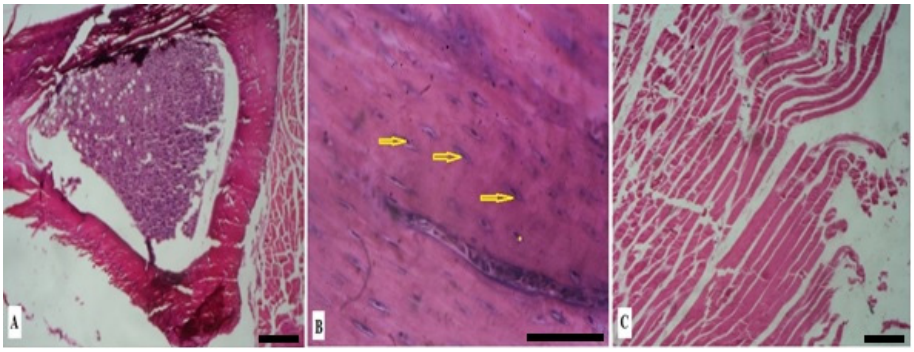


Figure 1. Part of the tibia in the control group. A: portion of leg bone tissue along with surrounding muscle (bar = 200 µm). B: part of a bony blade with lacunae containing osteoblasts (arrows) (bar = 25 µm). C: Muscle fibers around bone with normal appearance (bar = 200 µm)

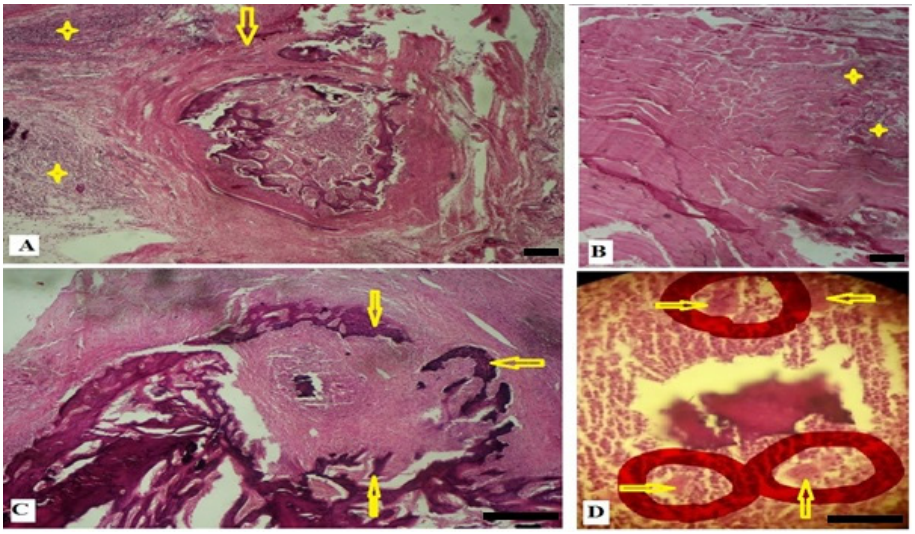


Figure 2. Part of the tibia in the osteomyelitis group. A: The star indicates the accumulation of mononuclear cells. The arrow indicates a periosteum around the bone that is inflamed (bar = 200 µm). B: Part of the muscle around the bone with the invasion of inflammatory cells between the fibers (stars) (× 40 magnification), C: Part of the tibia with extensive destruction of the bony blades (arrows) and accumulation of osteoclast cells in the center of the image (bar = 100 µm), D: Center the previous image (bar = 25 µm), Circles indicate the range of osteoclasts and arrows indicate osteoclasts

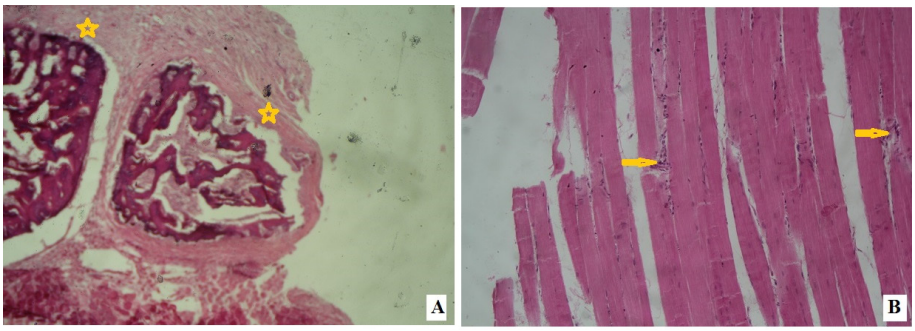


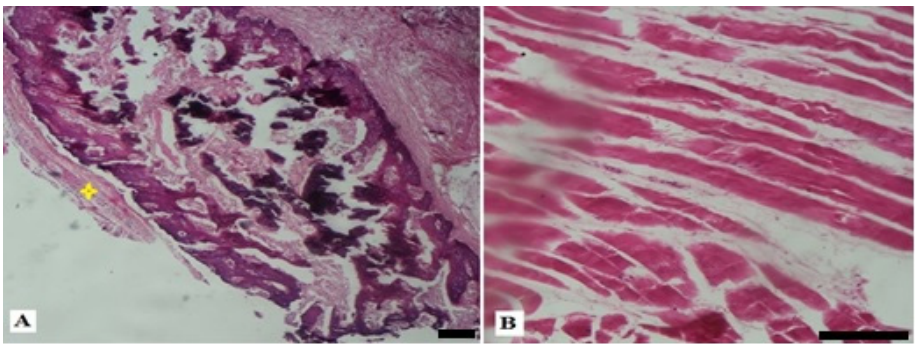
Figure 3. Part of the tibia in the group treated with osteomyelitis treated with *L. plantarum*
A: Elevation and inflammation of the peritoneum (star) (bar = 200 µm), B: Part of the muscle tissue around the bone with a mild invasion of inflammatory cells between the muscle fibers (flash) (bar = 100 µm)

group with osteomyelitis. Inflammation of inflammatory cells between muscle fibers (arrows) is also evident, but compared to the osteomyelitis group, the invasion of these cells was much less among muscle fibers.

Figure 4 shows images of animals in the osteomyelitis group treated with *L. plantarum* enriched with Se. The destruction of the bony septa is not severe in the osteomyelitis group, and although inflammation of the periosteum is still seen, the appearance of muscle fibers is normal and the invasion of inflammatory cells around these fibers is not observed.

Results of bone radiographic images in experimental groups

Figure 5A shows the images of the surgical control group



Figures 4. Part of the bone and muscle tissue of the leg in the group with osteomyelitis treated with *L. plantarum* enriched with Se. A: Bone blades with little or no degradation of inflammation in the peristomy (star) (bar = 200 µm), B: Muscle fibers with normal appearance and without invasion of inflammatory cells (bar = 100 µm)

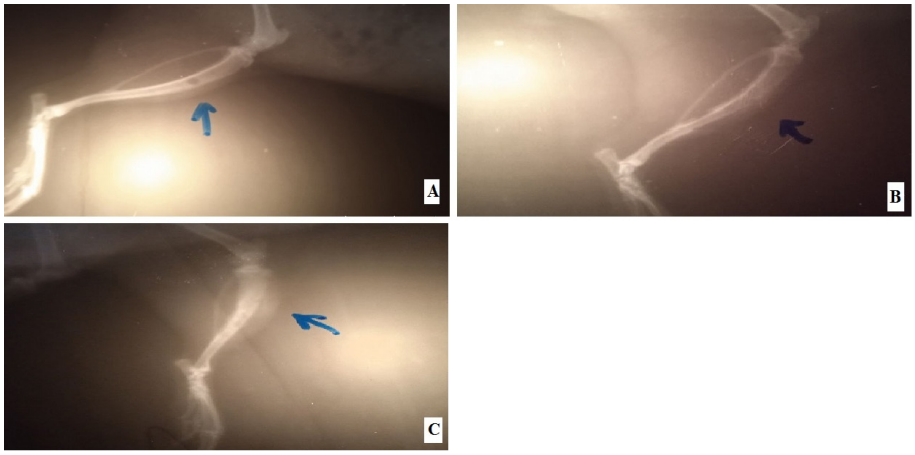


Figure 5. Bone radiographic images. A: Control group, the arrow marks the surgical place. B: Part of the tibia in the group with osteomyelitis. The arrow indicates osteomyelitis and periosteal reaction in the tibia. C: Osteolysis was seen in the tibia

showing no complication in the tibia area except the injection site. No osteomyelitis developed at the injection site.

Figure 5B shows radiographs of a group with osteomyelitis periosteum infection in the bone cortex. Figure 5C demonstrates swelling of the soft tissue around the bone, along with the formation of new tissue and bone in the bone marrow area and bone destruction in the tibia, indicating the induction of osteomyelitis.

Figure 6A illustrates leg bone tissue in the group of osteomyelitis treated with *L. plantarum*. Peristome, new bone formation, bone destruction, and no other bone complications are seen. The tibia is completely healthy. Figure 6B shows part of the tibia in the Se-treated group of osteomyelitis treated with *L. plantarum*. There are no bone complications that indicate a bone infection. Radiographic images of the osteomyelitis group treated with *L. plantarum* and Se - enriched *L. plantarum* were similar to that of the control group. Therefore, their inclusion was avoided.

Discussion

According to clinical studies, *S. aureus* is usually the main cause of implant-induced osteomyelitis. Since the most appropriate animal models for identifying new treatments for osteomyelitis are rats (17), in the present study, the effect of treatment with *L. plantarum* and Se-enriched *L. plantarum* was evaluated on *S. aureus* osteomyelitis induced rats. Histopathological images of periosteum inflammation around the bone, accumulation of inflammatory cells, extensive destruction of bone blades, the presence of active osteoclasts around the blades and the death of osteoblasts in this group of rats were well demonstrated. Swelling of the soft tissue around the bone along with the formation of new tissue and bone in the bone marrow area and bone destruction in the tibia in the taken radiographs confirmed osteomyelitis in the rats as well.

Although no complications were seen in the radiological images of the tibia of the osteomyelitis groups after the treatment with *L. plantarum* and *L. plantarum* enriched with Se, and the tibia had a similar appearance to the control group, histopathological images showed some changes. Bone tissue examination of rats treated with *L. plantarum* showed destruction and inflammation in the periosteum and inflammatory cells between muscle fibers was seen. In the experimental group with osteomyelitis treated with *L. plantarum* enriched with Se, inflammation

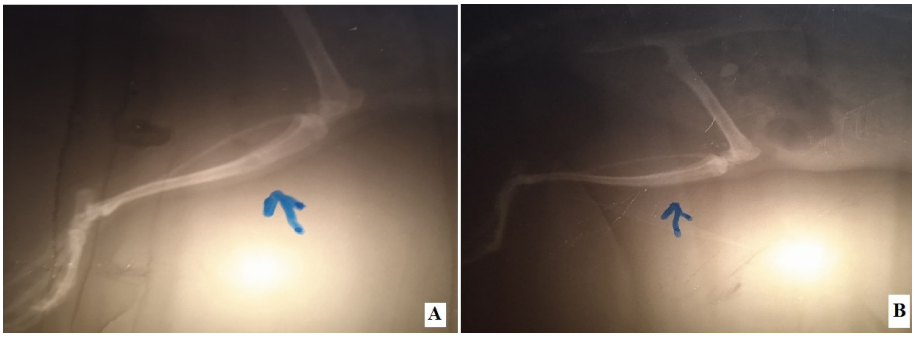


Figure 6. Bone radiographic images. A: Part of the tibia in the osteomyelitis group treated with L. plantarum. The arrow indicates normal tibial bone. Osteolysis was not seen except for the effects of injection into the tibia. B: Part of the tibia in a group of osteomyelitis treated with *L. plantarum* enriched with Se. The arrow indicates that the bone is normal

of the periosteum was evident, but the muscle fibers had a normal appearance and no invasion of inflammatory cells around these cells was observed. Se-enriched probiotics were shown to prevent inflammation spreading from the bone marrow to the muscles around the bone. Similar to the results of our study, Chundakkattumalayil et al did not see any signs of infection and inflammation after treatment with *L. plantarum* in histological images of rat bone tissue and reported that this bacterium has no harmful effect on the immune system (18). Modification of intestinal microbiota with probiotics can be a good therapeutic strategy to regulate bone regeneration (19). Alp and KuleaŞan reported that *Lactobacillus reuteri* reduces intestinal and bone inflammation by reducing receptor activator of nuclear factor kappa-B ligand and Tumor necrosis factor alpha (TNF-α) expression in rats (20). In arthritis induced rats treated with Se, a reduction in inflammation in claws with no signs of inflammation and bone erosion was observed compared to the untreated patient group (21). In a study, the improvement of damaged bone tissues in patients treated with sodium selenite has been confirmed in radiological images (22). Hosnedlova et al reported that *S. aureus* and *Staphylococcus epidermidis* are the most common causes of implant failure. They observed higher osteoblast density in the group of patients treated with Se than in the control patient group and suggested that the presence of Se increased the activity of osteoblasts and by increasing the activity of osteoblasts, damaged tissues were repaired (23). In the present study, *L. plantarum* and Se have probably prevented excessive bone destruction and the spread of infection to surrounding tissues due to their high antioxidant properties and their effect on the process of mineral uptake and stimulation of bone cell activity. In the continuation of this study, the level of hematological parameters of experimental groups were compared with the control group. The results showed a significant increase in WBC in the osteomyelitis group compared to the control group. This result was not unexpected, as other studies have shown an increase in the level of immune cells following staphylococcal infection (24,25). Following staphylococcal infection, macrophages, monocytes and neutrophils migrate to the site of infection and fight the infection by producing reactive oxygen species. This process increases the level of oxidative stress in the body and causes inflammation. Inflammation stimulates the immune system and thus increases the proliferation of immune cells (26).

In the present study, WBC in the osteomyelitis group treated with *L. plantarum* also showed a significant increase, but the rate of increase was lower than the group with osteomyelitis ($P \leq 0.05$). In the osteomyelitis group treated with Se-enriched *L. plantarum*, the increase in WBC was not significant compared to the control group. In fact, it seems that this probiotic, especially when combined with Se, has been able to prevent over-stimulation of the immune system. In line with the results of the present study, Neveling et al showed that poultry farms are susceptible to bacterial infections caused by *Escherichia coli* and *Salmonella enterica*. Probiotic supplements increase the growth of chickens and prevent the development of pathogenic infection (27).

Wang et al used Se as an antimicrobial agent for the treatment of infections in orthopedic implants and reported that Se compounds have the ability to inhibit bacterial growth and bacterial biofilm formation. Considering the above mentioned effects of Se on *S. aureus* including inhibition of growth and reduction of oxidative stress caused by this pathogen and also the ability of probiotics in the prognosis of the immune system after exposure to *S. aureus* infection, in the present study, *L. plantarum* enriched with Se was used to reduce inflammation caused by *S. aureus*. This antioxidant combination had more effectiveness than *L. plantarum* alone (28).

The number and index of other blood cells in the present study did not show significant changes after using probiotics and probiotics enriched with Se compared to the control group. Failure to change these parameters can reflect the lack of adverse effects on the body. In some studies, it has been proven that there is no change in blood parameters after taking various probiotics (18,29).

Due to the increase in WBC and the possibility of inflammatory stimulation, in the continuation of the study, CRP levels were compared among the experimental groups. The level of this factor in experimental groups with osteomyelitis showed a significant change compared to the control group. CRP is a protein from the pentraxin family that is rapidly secreted from the liver in inflammatory responses, trauma, and bacterial infections in response to factors (such as IL-6) released by lymphocytes and monocytes. CRP level increases rapidly in patients with osteomyelitis (30). Elevated CRP levels in patients with septic shock due to methicillin-resistant *S. aureus* infection (31) and infectious endocarditis due to *S. aureus* (32) have been also seen. In the two experimental osteomyelitis groups treated with *L. plantarum* and Se enriched *L. plantarum*, CRP levels increased compared to the osteomyelitis control group, but in the group treated with probiotics enriched with Se, this increase was significantly higher than the group that only treated with the probiotic. Therefore, it is possible that probiotics with Se have been somewhat effective in partially inhibiting staphylococcal infection in rats. In line with our research, Mousavi et al reported that consumption of probiotic yogurt leads to a significant reduction in CRP (33). In another study, CRP and TNFα levels significantly reduced in diabetic patients treated with probiotics (34).

Probiotics and Se supplements reduce inflammatory markers by reducing oxidative stress. Se supplements have been shown to reduce nuclear factor kappa-light-chain-enhancer of activated B cells pathway activity, which plays an important role in increasing transcription of pro-inflammatory cytokines. Cytokines in turn decrease CRP levels (35). In addition, probiotics can inhibit the enzymatic synthesis of CRP in the liver by producing short-chain fatty acids or indirectly reduce CRP by reducing the production of IL-6 (36). Since CRP levels are directly regulated by IL-1β and IL-6 (37), in this study the concentrations of these two factors were measured and compared among the experimental groups. IL-6 concentration in the osteomyelitis group showed a significant increase compared to the control group. Increased IL-6 concentration was also observed in the two groups with osteomyelitis treated with *L. plantarum* and *L. plantarum* enriched with Se, but this increase was statistically lower in the Se-enriched group treated with *L. plantarum* than in the osteomyelitis group. Also, despite the slight changes in IL-1β concentration in the experimental groups, these changes were not statistically significant compared to the control group.

In patients with osteomyelitis, IL-6 levels have increased during the first hours after the onset of infection (30). Considering that after the entry of *S. aureus* into the osteoblast cells, inflammatory factors such as cytokines, chemokines, especially IL-6 are secreted and innate immune system is activated (38), in this study, the cause of increase in IL-6 in the experimental group with osteomyelitis can attributed to the inflammation due to *S. aureus* infection. In acute pneumonia, which is often caused by *S. aureus* and is a complication of the flu virus, no change in IL-1β level was observed compared to the healthy group (39), which was in line with the results of our study. IL-6 may have been more active than IL-1β in acute infections.

In the present study, IL-6 level was significantly lower in rats with osteomyelitis treated with Se-enriched *L. plantarum* than in control rats with osteomyelitis. In addition, changes in IL-6 levels in the body have been shown to be directly related to Se deficiency. Se deficiency increases IL-6 and decreases IL-8 and IL-10 significantly (15). In a study, the increase in TNFα and IL-1β levels in mastitis caused by *S. aureus* in mice was controlled by Se treatment (40). According to the mentioned documents, it seems that Se enriched *L. plantarum*, by strengthening the body's antioxidant system, prevents the over-secretion of inflammatory factors by immune cells and the spread of inflammation to the tissues around the bone.

Conclusion

According to the results, *S. aureus* infection caused osteomyelitis, which was confirmed by radiological and histological observations as well as increased cytokines IL-6, CRP, and WBC and it seems that the decrease in the level of these factors and tissue observations in the treatment groups compared to the osteomyelitis group can indicate the positive effect of Se-enriched probiotics on the partial inhibition of adverse effects of osteomyelitis on bone and surrounding tissues. Probiotics are able to inhibit pathogens growth by secreting a variety of antimicrobial peptides, including bacteriocins and also by producing phytase enzyme, leading to bone repair. Therefore, it may be a suitable candidate along with selenium as a therapeutic supplement to strengthen the body's antioxidant system. Of course, many animal and clinical tests are needed to confirm this claim.

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Validation: Mahnoosh Fatemi.
Formal Analysis: Mahnoosh Fatemi, Fereshte Ghandehari.
Investigation: Sajedeh Mahpishanian, Mahnoosh Fatemi, Fereshte Ghandehari.
Resources: Sajedeh Mahpishanian.
Data Curation: Sajedeh Mahpishanian, Mahnoosh Fatemi.
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None declared.

Ethical Approval
The ethical guidelines on the use of laboratory animals for research were observed as approved by the Institutional Review Board of the Islamic Azad University at Flavarjan Branch, Flavarjan, Isfahan (Certificate No: IR.IAU.FALA.REC.1397.002).

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