Original Article:

Safety Assessment of a New Strain of *Lactobacillus pentosus* (IBRC=11143) As a Candidate Probiotic

Saba Bahrehvar[1], Shiva Khezri[1*], Amir Abbas Barzegari[2], Vahid Nejati[1]

1. Department of Biology, Faculty of Basic Sciences, Urmia University, Urmia, Iran.
2. Department of Biology, Faculty of Basic Sciences, University of Maragheh, Maragheh, Iran.

*** Corresponding Author:**
Shiva Khezri, PhD.
Address: Department of Biology, Faculty of Basic Science, Urmia University, Urmia, Iran.
Phone: +98 (44) 31942122
E-mail: sh.khezri@urmia.ac.ir

Article info:
Received: 28 Apr 2020
Accepted: 23 Aug 2020

Keywords:
Probiotics, *Lactobacillus pentosus*, Toxicity tests

ABSTRACT

Background: It is believed that the consumption of an adequate amount of live lactic acid bacteria (as probiotic) may improve the health of the host. Many strains of lactic acid bacteria are generally considered to be safe. However, some strains have shown some adverse effects.

Objectives: This study aimed to investigate the safety of a new strain of *Lactobacillus pentosus* (IBRC=11143) in Wistar rats.

Methods: In this experimental study, male and female Wistar rats (n=6) were used. A subacute toxicity study (for 28 days) was conducted by oral administration of *Lactobacillus pentosus* to the animals. In each sex, one group received saline, and the other two groups received the bacteria at doses of 1×108 and 1×109 Colony-Forming Units (CFU)/rat.

Results: No significant alteration in the liver and kidney tissues was seen. However, in both sexes, there were significant differences in urea and creatinine levels between the control and the experimental groups. Some blood parameters (Lymphocyte, red blood cell, hematocrit, and hemoglobin) also showed significant changes in the groups that received the bacteria. Moreover, a significant increase in alkaline phosphatase level was observed in male rats.

Conclusion: The results indicate that *Lactobacillus pentosus* (11143) is not entirely safe like other *Lactobacillus* strains. Therefore, the *Lactobacillus pentosus* (11143) strain may not be the right choice as a probiotic for human consumption.

Introduction

According to the world health organization, probiotics are microorganisms, including bacteria and yeasts, that may benefit their consumers [1-3]. Most probiotics belong to major bacteria groups in the human gut microbial flora and have harmless coexistence. The benefits of probiotics are based on the fact that the intestinal microbial flora plays an essential role against various diseases. The use of probiotics in the prevention of diseases and the improvement of the health status of humans and animals has a history of several thousand years. Nowadays, *Lactobacillus* are the most common organisms used to produce probiotic products [4-6]. Many strains of *Lactobacillus* are Generally Regarded As Safe (GRAS) be-

Citation Bahrehvar S, Khezri S, Barzegari AA, Nejati V. Safety Assessment of a New Strain of Lactobacillus pentosus (IBRC=11143) as a Candidate Probiotic. Pharmaceutical and Biomedical Research. 2021; 7(1):37-46. http://dx.doi.org/10.18502/pbr.v7i1.7355

doi: http://dx.doi.org/10.18502/pbr.v7i1.7355

cause they have been used for several years without undesirable effects [5]. However, some reports indicate clinical conditions such as liver abscess, endocarditis [7], and urinary tract infection [8] by using *Lactobacillus*-containing products. The safety assessment for any probiotic is critical [9]. Probiotics safety approval is required because most of them are marketed as food and medical supplements [5].

On the other hand, one of the characteristics that should be considered is its lack of toxicity [9, 10]. Acute and subacute oral toxicity test is proposed as a preliminary test for probiotic safety assessment [11]. Thus, in the present study, a safety assessment of a new strain of native Iranian *Lactobacillus pentosus* (IBRC [Iranian Biological Resource Center]=11143) was conducted using subacute oral toxicity tests in rats. The rats were orally administered with different doses of the strain, and then the oral toxicity, blood hematology, biochemistry parameters, and histopathological examinations were analyzed.

Materials and Methods

Lactobacillus cultures

Lactobacillus pentosus (IBRC=11143) was obtained from the Iranian Genetic Resource Center. For the preparation of *Lactobacillus pentosus* bacterial suspensions, the bacterium was cultured in an de Man, Rogosa and Sharpe (MRS) agar broth medium (Qlab Canada) and incubated for 24 h at 37°C in anaerobic jars. After growth, the samples were centrifuged at 5000×g for 10 minutes at 4°C, and the precipitate was washed three times with physiological serum. Suspensions were used afresh every day before being fed to the animals through gavage. For suspension preparation, normal saline was used as diluent.

Animals

In this research, 36 male and female Wistar rats with an average weight of 200-280 g and a mean age of 4 weeks were used. The animals were housed 6 per cage under a 12-h light/dark cycle in a room with controlled temperature (23±2°C). Food and water were available ad libitum. All the experiments were carried out according to the protocol approved by the Animal Ethics Committee of Urmia University, Urmia, Iran (No: IR-UU-AEC- 3 ﻯ/ /317).

Subacute oral toxicity study

The subacute oral toxicity study lasted for 28 days. A total of 36 male and female rats were used. After being adapted to laboratory conditions, they were randomly assigned into three groups: 1) the control group consisted of 6 male and 6 female rats. During 28 days, they received 1 mL normal saline daily by gavage; 2) the first experimental group consisted of 6 male rats and 6 female rats. During the same period, they received 1×10^8 Colony-Forming Units (CFU) of the bacteria by gavage daily; 3) the second experimental group consisted of 6 male and 6 female rats, which received 1×10^9 CFU of the bacteria by gavage for 28 days daily. The reason for choosing these doses is based on some studies that suggested 107 to 109 CFU dosage as the most effective dose in dairy and non-dairy products [12].

Hematological and serum biochemistry analyses

At the end of the experiment, the animals were anesthetized with ketamine and xylazine. Blood sampling was performed by sterile syringe from the heart, which was collected for hematology and serum biochemistry analyses and was stored in tubes containing K2EDTA and analyzed using an automated hematological analyzer (cell counter kx21-n, China). Hematological tests included Red Blood Cell count (RBC), White Blood Cell count (WBC), along with tests of Hemoglobin (Hb), Hematocrit (HCT), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet (PLT), Mean Platelet Volume (MPV) and lymphocyte. For serum biochemistry analysis, rats' blood was collected in non-heparinized tubes and centrifuged at 5000×g for 10 min at 4°C, after which serum was obtained. Serum biochemistry was analyzed using an automatic clinical chemistry analyzer (BT1500, China). Biochemistry tests included Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), urea, and creatinine.

Histopathological examination

Histopathological examination was also performed on the liver and kidney of both experimental and control groups. At the end of the experiment, liver and kidney tissues of all rats were extracted and fixed in 10% formalin solution. After alcohol dehydration and clearing, a paraffin block of liver and kidney tissues was ready and prepared for the incision. The cuts (5 µm on average) were made using a microtome (Leica RM2235, Japan). The obtained sections were stained by the Hematoxylin-Eosin (H&E) technique. The preparations were then observed under an optical microscope (Leica DM1000, Japan) and photographed [9].

Table 1. Hematological findings in rats treated with *Lactobacillus pentosus* (IBRC-M 11143), ANOVA (n=6)

Group	Gender	Mean±SD									
		WBC ×103/µL	Lymphocyte (%)	RBC ×106/µL	Hb (g/dL)	HCT (%)	MC (fL)	MC (pg)	MCHC (g/dL)	PLT×103/µL	MPV (fL)
Control	M	4.78±0.82	75.3±5.67	7.72±0.15	13.6± 0.19	41.5±0.6	53.7±0.63	17.6±0.28	32.85±0.22	958.3±74.4	6.85±0.16
	F	4.58±0.96	70.0±3.79	7.4±0.13	13.2±0.22	40.1±1.0	54.2±1.0	17.8±0.21	32.9±0.41	862.0±42.4	7.06±0.13
1×108 CFU	M	4.9±0.6	53.2±3.39*	8.53±0.16*	14.2±0.17*	45.2±1.0*	53.0±0.78	16.7±0.19	31.56±0.41*	1012±52.4	7.4±0.25
	F	3.7±1.13	65.3±7.79	6.18±0.56*	11.4±0.63*	36.9±1.37	60.6±5.42	18.36±0.95	30.53±1.11*	860±101.5	7.26±0.06
1×109 CFU	M	7.16±2.0	66.4±4.16	8.28±0.24	13.5±0.2	43.8±0.58	53.0±1.36	16.3±0.6	30.9±0.41*	932.8±33.3	6.96±0.20
	F	4.0± 0.9	67.2±4.13	7.6±0.3	13.3±0.46	42.37±1.6	55.4±0.9	17.5±0.37	31.5±0.22	770.0±60.5	6.97±0.09

PBR

RBC: Red Blood Cell count; WBC: White Blood Cell count; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean Cell Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelet; MPV: Mean Platelet Volume; M: Male; F: Female.

*P<0.05

Statistical analysis

Experimental data were analyzed by one-way Analysis of Variance (ANOVA) and followed by multiple comparisons among the means using Duncan's new multiple range test. The differences were considered significant if P<0.05.

Results

Subacute toxicity test

There were no recorded deaths during the trial, nor any significant changes observed in the appearance and behavior of rats and their daily activity during these 28 days.

Hematological parameters

The effects of feeding different doses of *Lactobacillus pentosus* strain (IBRC-M 11143) on hematological parameters are shown in Table 1. There was no significant difference in WBC, MCH, MPV, MCV, and PLT, in both males and females at any of the dosages compared to the control group. Although WBC did not show significant differences in males and females, *Lactobacillus pentosus* (IBRC-M 11143) caused a significant decrease (P<0.05) in lymphocyte at the dose 1×10^8 CFU compared to the control group. However, significant increases were observed (P<0.05) in RBC, Hb, and HCT in males at the dose 1×10^8 CFU compared to the control. There was a significant decrease (P<0.05) in Hb and RBC in the female at the doses 1×10^8 CFU compared to the control group. Also, HCT increased in the female at the dose 1×10^9 CFU and decreased in the dose 1×10^8 CFU compared to the control group, but no significant difference was observed. There was also a significant decrease (P<0.05) in MCHC in the male at the doses 1×10^8 CFU and 1×10^9 CFU compared to the control group. Also, in the female rats, there was a significant decrease (P<0.05) between 1×10^8 CFU and the control group.

Biochemical parameters of serum

The effects of feeding different doses of strain *Lactobacillus pentosus* (IBRC-M 11143) on biochemical parameters are shown in Table 2. Results of serum biochemical parameters in the males and females showed no significant difference in liver enzymes between different doses and the control group, except for ALP in the males, where significant increases (P<0.05) were observed between the control group and 1×10^8 CFU. Also, significant decreases (P<0.05) in creatinine and urea were observed in the females between the control group and 1×10^8 CFU, even between the control group and 1×10^9 CFU. But in males, creatinine and urea showed no significant difference and were stable.

Histopathological examination

Histopathological examination of the liver and kidneys of male and female rats receiving *Lactobacillus pentosus* for 28 days by gavage in two doses of 1×10^8 CFU and 1×10^9 CFU showed no changes in all groups. Microscopic observations on the liver of male and female rats in all groups showed that the portal space was completely normal and the hepatocyte nucleus was normal, and no inflammatory foci were detected. Liver sinusoids were also normal (Figures 1 and 2). Additionally, micro-

Table 2. Biochemical parameters of serum in rats treated with *Lactobacillus pentosus* (IBRC-M 11143), Mean±SD, ANOVA (n=6)

Groups	Gender	ALP (U/L)	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	M	706±229.2	56.8±12.3	131.8±29.8	40±7.3	0.73±0.7
	F	620.6±138.5	81.6±36.7	152± 35.2	47.8±4.3	0.77±0.4
1×108 CFU	M	1327±307*	70.6±23.1	123.2±21.3	46.8±6.9*	0.69±0.3*
	F	658.3±409.8	129±70.4	261±16.7	35±8.1	0.6±0.9
1×109 CFU	M	637.6±411	80.6±10.7	143±83.3	32.2±5	0.78±0.6
	F	623±171. 7	84.7±39	189± 50.3	32±2.1*	0.64±0.8*

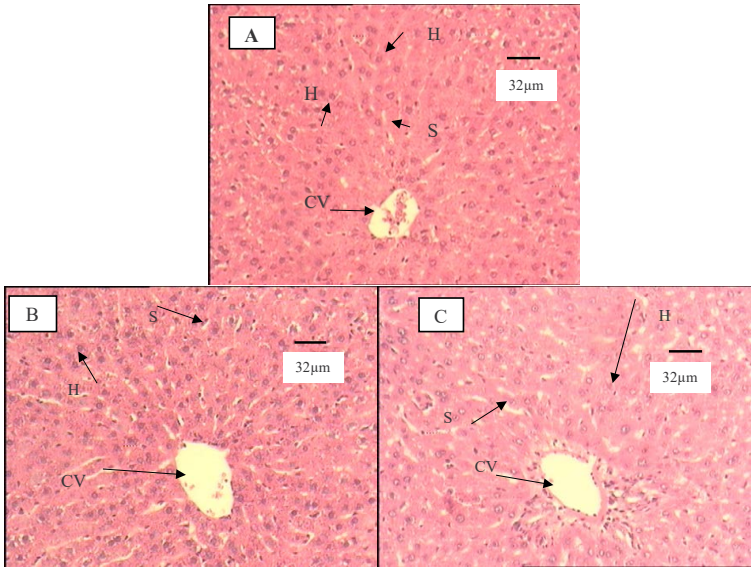
PBR

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline Phosphatase; M: Male; F: Female.
*>0.05

scopic observations of kidneys in male and female rats in all groups showed that the glomerular network was in normal size, and no dilation and stricture was observed in the urinary tract. and their blood vessels were also perfectly normal (Figures 3 and 4).

Discussion

Safety issues of *Lactobacillus pentosus* (11143), including possible side effects, should be considered before use because of their uncertainty [13]. Oral safety assessment is commonly used in the safety evaluation of probiotics [14]. We conducted a 28-day study using the 1×10^8 and 1×10^9 doses, and no significant activity or behavioral changes, diseases, or deaths were observed in the rat during the experimental protocol. Also, the results showed that *Lactobacillus pentosus* (11143) had no microscopic changes on liver and kidney tissues, but it could affect several biochemical and blood parameters separately, similar to previous reports [15, 16]. To determine the clinical side effects of *Lactobacillus pentosus* (11143), blood and serum biochemical parameters and microscopic observations of liver and kidney tissues were evaluated. Tissue damage and changes in tissue function are a sign of the



PBR

Figure 1. Histopathological examinations of the liver of male rats treated orally with *Lactobacillus pentosus* (11143)
The sections of liver from control and treated rats (×400); A. A control rat; and B. A rat given of *Lactobacillus pentosus* (11143) with the dose of 1×108 CFU; and C. A rat given of *Lactobacillus pentosus* (11143) with the dose of 1×109 CFU; H. Showing normal histological structure of Hepatocytes; S. Sinusoids; CV: Central Vein. The port space is perfectly normal, and the nuclei of the hepatocytes are normal. Also, the liver sinusoids were seen as normal, and there was no change in the size of the sinusoids. No inflammatory or cellular changes were observed in the liver of the treated rat.

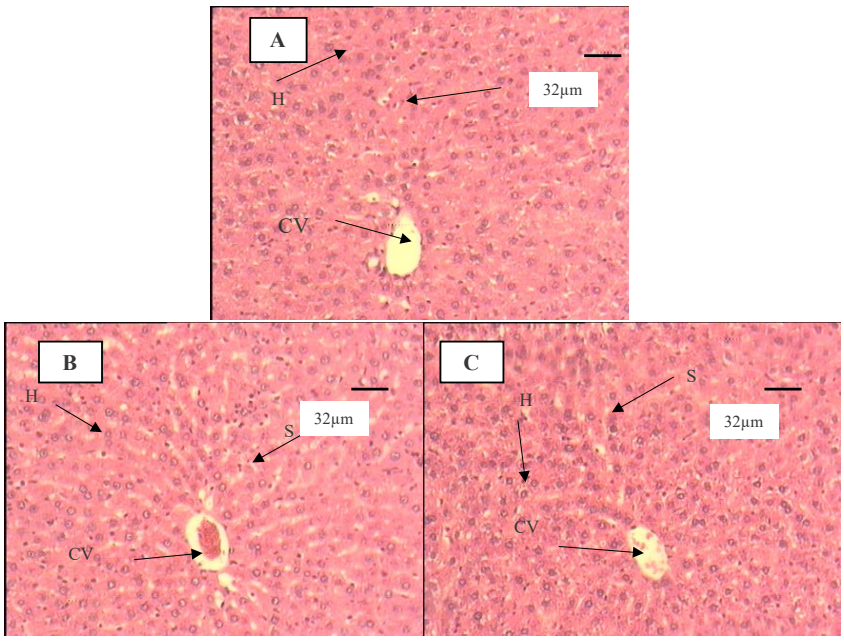


Figure 2. Histopathological examinations of the liver of female rats treated orally with *Lactobacillus pentosus* (11143)

The sections of liver from control and treated rats (×400); A. A control rat; and B. A rat given of *Lactobacillus pentosus* (11143) with the dose of 1×108 CFU; and C. A rat given of *Lactobacillus pentosus* (11143) with the dose of 1×109 CFU; H. Showing a normal histological structure of Hepatocytes; S. Sinusoids; CV: Central Vein. The results are similar to the results of males. No inflammatory or cellular changes were observed in the liver of the treated rat.

substance toxicity during the experiment. Hematological parameters are commonly used to indicate the harmful effects of test substances on blood [5].

Biochemical assays are used to detect nutrient deficiencies or imbalances in nutrient metabolism that are usually evident before any clinical symptoms. Changes in serum biochemical parameters may indicate that responsive organs are affected or even damaged [17]. The present study showed that oral consumption of *Lactobacillus pentosus* (11143) for 28 days could alter hematological and biochemical parameters in its normal range. The results of this study showed that *Lactobacillus pentosus* (11143) strain had no adverse effects on some parameters in both males and females. Most probiotics belong to a large group of the main bacteria of the intestinal microbial flora which live there harmlessly. Studies have shown that their alienation is insignificant to the body, which does not attract the immune system [18], so non-significant changes in this study are justified.

This study results in male rats are consistent with those of Aboderin, which showed that the total number of erythrocytes and hemoglobin in rats receiving *Lactobacillus plantarum* increased at different doses [19]. Hosseini did not find any significant differences in hematocrit, MCHC hematocrit, and blood parameters of probiotic Pedicoccus on Caspian salmon blood and serum parameters. Also, the number of red blood cells decreased significantly compared to the control group. The results of this study were in agreement with the findings of Hosseini's research in the case of erythrocytes in female rats [20]. Red blood cells are among essential blood factors used to diagnose anemia or polycythemia [21].

On the other hand, an increase or decrease in hematocrit and hemoglobin can also be associated with an increase and decrease in the red blood cells. Besides, MCHC changes may also be affected by changes in hematocrit and hemoglobin. Although red blood cells, hemoglobin, hematocrit, and other blood factors were significantly different from the control group, all changes were within the normal range for adults. White blood cells in males increased, but in females, they decreased in both dosages than the control group; the results were in their normal range. White blood cells play an essential role in protecting the body against infections. The number of WBC cannot provide specific information, which necessitates accounting for the number of differential leukocytes [19]. There was a decrease in the number of lymphocytes in both males and females compared to the control group. Lymphocytes play a major role in the formation of humoral antibodies and cellular immunity [19]. It has been previously reported that oral consumption of lactic

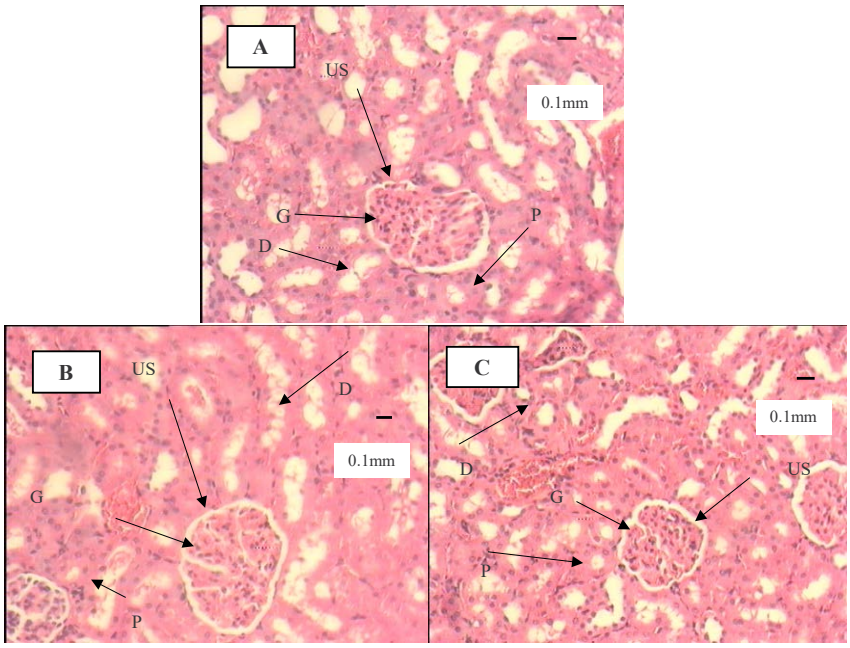


Figure 3. Histopathological examinations of the kidney of male rats treated orally with *Lactobacillus pentosus* (11143)

The sections of the kidney from control and treated rats (×400); A. A control rat; B. A rat given of *Lactobacillus pentosus* (11143) with the dose of×1 108 CFU; and C. A rat given of *Lactobacillus pentosus* (11143) with the dose of×1 109 CFU; P. Showing the normal histological structure of kidney comprising of Proximal; and G. Glomerulus; D. Distal; Urinary Space (US), the glomerular network is normal in size and there is no dilatation in the urinary tract. Convoluted tubes are entirely healthy and in their normal state. Also, the walls of blood arteries are completely normal.

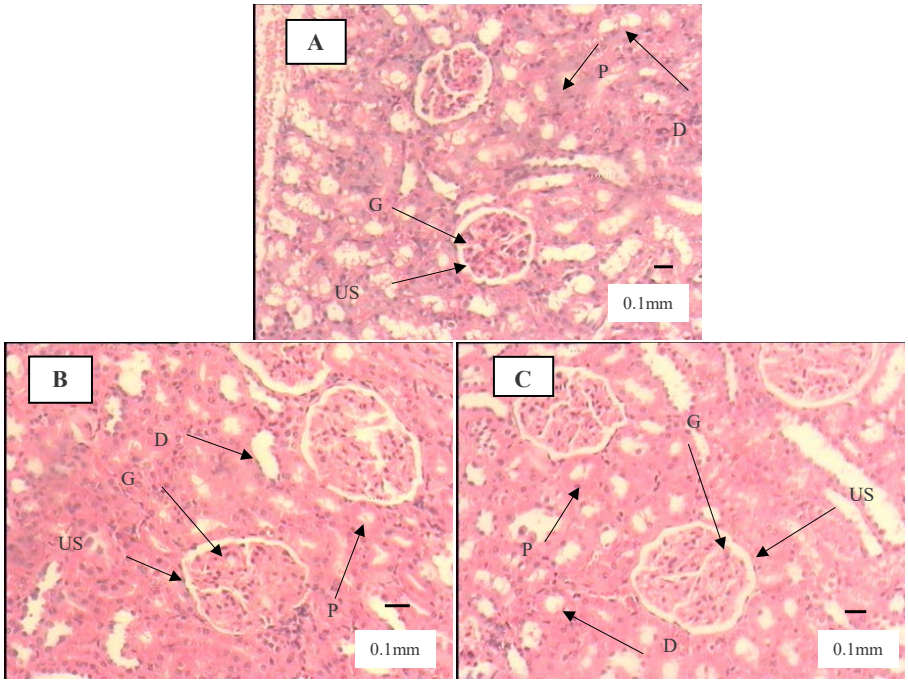


Figure 4. Histopathological examinations of the kidney of female rats treated orally with *Lactobacillus pentosus* (11143)

The sections of the kidney from control and treated rats showed glomeruli of normal size with normal tubules (×400); A. A control rat; and B. A rat given of *Lactobacillus pentosus* (11143) with the dose of×1 108 CFU; and C. A rat given of *Lactobacillus pentosus* (11143) with the dose of×1 109 CFU; P: Showing the normal histological structure of kidney comprising of Proximal ; and G. Glomerulus; D. Distal; Urinary Space (US). Like male rats, no inflammatory or cellular changes were observed in the kidney of the female rat.

acid bacteria in rats increases lymphocyte proliferation and interferon production.

The outcome of this study is different from that of Aattouri. This difference could be because of the weakening of the immune system in rats by *Lactobacillus pentosus* (11143) [22].

In Nayak's study in 2010, adding probiotics to the fish diet stimulated the immune system, increased the number of lymphocytes, thereby enhancing pathogen resistance, and ultimately, improving growth and survival [23]. The results of this study in the field of lymphocytes were not consistent with the findings of the present study, which were probably due to differences in species and diets. Liver enzymes (ALT, AST, and ALP) are used to evaluate liver function, and an increase in activity is due to hepatocyte destruction or hepatic injury [24]. This study showed that consumption of *Lactobacillus Pentosus* (11143) strain in the male and female rats increased liver enzymes. However, there was no significant difference ($P<0.05$) in liver enzyme levels between different dosages and control groups, except for ALP in males, which indicates the possibility of liver toxicity.

On the other hand, microscopic examination of liver tissue showed no damage, and the liver cells were in their normal state. It seems that consumption of the *Lactobacillus pentosus* (11143) strain did not cause liver disease. The use of LAB bacteria in food causes various levels of liver damage that can be detected by the secretion of certain enzymes in the blood. This can be confirmed by previous studies using different species of *Lactobacillus* (*L. rhamnosus* and *L. rhamnosus* + *L. plantarum*). Previous research has shown that ALT levels have decreased compared to control groups [25]. In a similar study by Son in 2015, serum enzyme activities (ALT, AST, and ALP) were not affected by oral HD1 strain administration, and no changes were reported in ALT, AST, and ALP activities that altered liver function or metabolism [26]. According to Ramli [15], the activity of liver enzymes (ALP, AST, ALT) was significantly changed by oral administration of *L. plantarum* strain, which increased the levels of ALP, AST enzymes when there were in the normal range for adults. Elevated urea levels can be a symptom of a problematic kidney, and changes in creatinine levels may reflect kidney or muscle mass problems. Kidney damage or swelling and muscle damage can increase the creatinine [11]. Diseases affecting kidney function generally elevate or lower creatinine and urea levels [27]. This study showed a significant decrease ($P<0.05$) in female urea and creatinine, which may have caused kidney disease in females. In a follow-up study by Ramli in 2012 [15], the use of *Lactobacillus plantarum* strain did not cause any significant changes in urea and creatinine levels, which was consistent with the present study results in male rats. Although the consumption of *Lactobacillus pentosus* (11143) strain caused changes in urea and creatinine levels, all changes were within their normal range and did not cause renal damage. Liver and kidney histology examinations of the male and female rats receiving *Lactobacillus pentosus* for 28 days by gavage in two doses of 1×10^8 and 1×10^9 CFU showed no changes in all groups.

In the present study, for the first time, a safety assessment of the probiotic *Lactobacillus pentosus* (11143) was performed by oral administration. In summary, feeding rats with *Lactobacillus pentosus* (11143) strain at doses of 1×10^8 and 1×10^9 CFU for 4 weeks did not affect the daily appearance, behavior, and activity as well as histological parameters. Still, they could alter some biochemical and hematological parameters like ALP, lymphocyte, RBC, HCT, and Hb. In general, it may not be completely safe like other *Lactobacillus* strains. Therefore, the *Lactobacillus pentosus* (11143) strain may not be the right choice as a probiotic for human consumption. However, further studies are needed.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by Urmia University and was carried out according to standard animal experimentation protocol of the Veterinary Ethics Committee of Urmia University (IR-UU-AEC-3/AD/317, 11/07/2020).

Funding

The Urmia University supported this research.

Authors' contributions

All authors contributed to designing, running, and writing all parts of the research.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors express their gratitude and appreciation for the support of the Urmia University and Maregheh University.

References

[1] Barzegari AA, Hashemzaei M, Majdani R, Alihemmati AR. Effects of topical treatment of second-degree burn wounds with Lactobacillus acidophilus on the wound healing process in male rats. Pharm Biomed Res. 2017; 3(3):23-30. [DOI:10.29252/pbr.3.3.23]

[2] Food and Agriculture Organization of the United Nations, World Health Organization. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria [Internet]. 2001 [Updated 2001 October 1-4]. Available from: http://pc.ilele.hk/public/pdf/20190225/bd3689dfc2fd663bb36def1b672ce0a4.pdf

[3] Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, et al. Safety assessment of probiotics for human use. Gut Microbes. 2010; 1(3):164-85. [DOI:10.4161/gmic.1.3.12127] [PMID] [PMCID]

[4] Zamani H, Rahbar S, Garakoui SR, Afsah Sahebi A, Jafari H. Antibiofilm potential of Lactobacillus plantarum spp. Cell Free Supernatant (CFS) against multidrug resistant bacterial pathogens. Pharm Biomed Res. 2017; 3(2):39-44. [DOI:10.29252/pbr.3.2.39]

[5] Zhang H, Wang Y, Sun J, Guo Z, Guo H, Ren F. Safety evaluation of Lactobacillus paracasei subsp. Paracasei LC-01, a probiotic bacterium. J Microbiol. 2013; 51(5):633-8. [DOI:10.1007/s12275-013-3336-x] [PMID]

[6] De Vuyst L, Vandamme EJ. Nisin, a lantibiotic produced by Lactococcus lactis subsp. Lactis: Properties, biosynthesis, fermentation and applications. In: De Vuyst L, Vandamme EJ, editors. Bacteriocins of Lactic Acid Bacteria. Boston, MA: Springer; 1994. [DOI:10.1007/s12275-013-3336-x]

[7] Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. Lactobacillus sepsis associated with probiotic therapy. Pediatrics. 2005; 115(1):178-81. [DOI:10.1542/peds.2004-2137] [PMID]

[8] Aznar E, Buendía B, García-Peñuela E, Escudero E, Alarcón T, López-Brea M. Community-acquired urinary tract infection caused by vancomycin-resistant Enterococcus faecalis clinical isolate. Rev Esp Quimioter. 2004; 17(3):263-5. [PMID]

[9] Doron Sh, Snydman DR. Risk and safety of probiotics. Clin Infect Dis. 2015; 60(suppl 2):S129-34. [DOI:10.1093/cid/civ085] [PMID] [PMCID]

[10] Yakabe T, Moore EL, Yokota S, Sui H, Nobuta Y, Fukao M, et al. Safety assessment of Lactobacillus brevis KB290 as a probiotic strain. Food Chem Toxicol. 2009; 47(10):2450-3. [DOI:10.1016/j.fct.2009.07.001] [PMID]

[11] Shokryazdan P, Faseleh Jahromi M, Liang JB, Kalavathy R, Sieo CC, Ho YW. Safety assessment of two new Lactobacillus strains as probiotic for human using a rat model. PLoS One. 2016; 11(7):e0159851. [DOI:10.1371/journal.pone.0159851] [PMID] [PMCID]

[12] Coman MM, Cecchini C, Verdenelli MC, Silvi S, Orpianesi C, Cresci A. Functional foods as carriers for SYNBIO®, a probiotic bacteria combination. Int J Food Microbiol. 2012; 157(3):346-52. [DOI:10.1016/j.ijfoodmicro.2012.06.003] [PMID]

[13] Abe F, Muto M, Yaeshima T, Iwatsuki K, Aihara H, Ohashi Y, et al. Safety evaluation of probiotic bifidobacteria by analysis of mucin degradation activity and translocation ability. Anaerobe. 2010; 16(2):131-6. [DOI:10.1016/j.anaerobe.2009.07.006] [PMID]

[14] Perelmuter K, Fraga M, Delucchi L, Zunino P. Safety assessment and enteric colonization ability of a native canine Lactobacillus murinus strain. World J Microbiol Biotechnol. 2011; 27(7):1725-30. [DOI:10.1007/s11274-010-0625-y]

[15] Ramli N, Omar SR, Ramakrishnan SS. Toxicological assessment of Lactobacillus plantarum as a probiotic strain in dark chocolate. Ann Food Sci Technol. 2012; 13(2):250-7. http://www.afst.valahia.ro/images/documente/2012/issue2/full/section3/s03_w05_full.pdf

[16] Zhang H, Sun J, Wang QY, He YT, Gu HY, Guo HY, et al. Safety assessment of Lactobacillus salivarius REN: A probiotic strain isolated from centenarian feces. Food Sci Technol Res. 2013; 19(6):1037-43. [DOI:10.3136/fstr.19.1037]

[17] Zhou JS, Shu Q, Rutherfurd KJ, Prasad J, Birtles MJ, Gopal PK, et al. Safety assessment of potential probiotic lactic acid bacterial strains *Lactobacillus rhamnosus* HN001, *Lb. acidophilus* HN017, and *Bifidobacterium lactis* HN019 in BALB/c mice. Int J Food Microbiol. 2000; 56(1):87-96. [DOI:10.1016/S0168-1605(00)00219-1]

[18] Panigrahi A, Kiron V, Puangkaew J, Kobayashi T, Satoh S, Sugita H. The viability of probiotic bacteria as a factor influencing the immune response in rainbow trout Oncorhynchus mykiss. Aquaculture. 2005; 243(1-4):241-54. [DOI:10.1016/j.aquaculture.2004.09.032]

[19] Aboderin FI, Oyetayo VO. Haematological studies of rats fed different doses of probiotic, Lactobacillus plantarum, isolated from fermenting corn slurry. Pakistan J Nutr. 2006; 5(2):102-5. [DOI:10.3923/pjn.2006.102.105]

[20] Hosseini AR, Oraji H, Yegane S, Shahabi H. [The effect of probiotic bactocell on growth performance, blood parameters and some serum parameters in Caspian salmon (Salmo caspius) (Persian)]. Iran Sci Fish J. 2014; 23(2):35-44. http://isfj.ir/article-1-1237-en.html

[21] Grimm RH, Neaton JD, Ludwig W. Prognostic importance of the white blood cell count for coronary, cancer, and all-cause mortality. JAMA. 1985; 254(14):1932-7. [DOI:10.1001/jama.1985.03360140090031] [PMID]

[22] Aattouri N, Bouras M, Tome D, Marcos A, Lemonnier D. Oral ingestion of lactic-acid bacteria by rats increases lymphocyte proliferation and interferon-γ production. Br J Nutr. 2002; 87(4):367-73. [DOI:10.1079/BJN2001527]

[23] Nayak SK. Probiotics and immunity: A fish perspective. Fish Shellfish Immunol. 2010; 29(1):2-14. [DOI:10.1016/j.fsi.2010.02.017] [PMID]

[24] Fatemi F, Allameh AA, Dadkhah A, Forouzandeh M, Kazemnejad S, Sharifi R. Changes in hepatic cytosolic glutathione S-transferase activity and expression of its class-P during prenatal and postnatal period in rats treated with aflatoxin B1. Arch Toxicol. 2006; 80(9):572-9. [DOI:10.1007/s00204-006-0076-2] [PMID]

[25] Adawi D, Ahrné S, Molin G. Effects of different probiotic strains of Lactobacillus and Bifidobacterium on bacterial translocation and liver injury in an acute liver injury model. Int J Food Microbiol. 2001; 70(3):213-20. [DOI:10.1016/S0168-1605(01)00550-5]

[26] Son HK, Chang HC, Lee JJ. Acute and subacute oral toxicity evaluation of crude antifungal compounds produced by Lactobacillus plantarum HD1 in rats. Prev Nutr Food Sci. 2015; 20(3):190-7. [DOI:10.3746/pnf.2015.20.3.190] [PMID] [PMCID]

[27] Muetzelfeldt L, Kamboj SK, Rees H, Taylor J, Morgan CJA, Curran HV. Journey through the K-hole: Phenomenological aspects of ketamine use. Drug Alcohol Depend. 2008; 95(3):219-29. [DOI:10.1016/j.drugalcdep.2008.01.024] [PMID]

This Page Intentionally Left Blank