ORIGINAL ARTICLE
https://doi.org/10.1590/1806-9282.20210634

Effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *caspase 3* in the testes of gamma-irradiated rats

Vahid Changizi[1], Omid Azadbakht[2], Roya Ghanavati[3], Hamid Behrouj[3], Elahe Motevaseli[4], Pegah Khanzadeh[1]*

OBJECTIVE: Ionizing radiation has various applications, including uses in medicine, industry, agriculture, and research. However, ionizing radiation is accompanied by side effects in normal radiosensitive tissues. Probiotics as natural radioprotective agents can protect normal tissues from ionizing radiation. In this regard, this study aimed to investigate the effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *caspase 3* (*CASP3*) in the testes of gamma-irradiated rats.

METHODS: A total of 30 male Wistar rats were involved in this study. The animals received the whole- body radiation with the dose rate of 2 Gy gamma-ray and were orally gavaged with 0.2 mL of 1×10^{10} *Lactobacillus* species in phosphate-buffered saline (PBS) for 4 weeks. Then, the relative gene expression levels of *BCL2*, *BAX*, and *CASP3* in the testis were assessed by using the quantitative real-time polymerase chain reaction (qRT-PCR).

RESULTS: Compared with the control group, radiation significantly downregulated the *BCL2* and upregulated the *BAX* and *CASP3* genes ($p<0.0001$). However, *Lactobacillus* species significantly reversed these effects.

CONCLUSION: All in all, according to our results, employing *Lactobacilli* probiotics as a natural radioprotector may protect radiosensitive tissue from damage.

KEYWORDS: Gamma radiation. Apoptosis. Probiotics. Lactobacillus species.

INTRODUCTION

It has been estimated that about 50–70% of all oncology patients undergo radiation therapy or a combination of chemotherapy and radiation therapy[1]. Although radiotherapy has turned into one of the most common treatments for cancer, the detrimental effects on the radiosensitive normal tissues limit the radiation exposure amount that can be applied[2]. The radiation damages radiosensitive normal tissues through various mechanisms[3]. Reactive oxygen species (ROS) are the major cause of cell apoptosis and DNA damage through increasing *BAX* and *caspase 3* (*CASP 3*) levels and decreasing *BCL2* levels in radiation-exposed normal tissues[4]. Therefore, the administration of certain antioxidants as radioprotective agents is a critical procedure to attenuate the radiation-related harmful effects on normal tissues.

Recently, probiotics, as a natural radioprotector, have attracted scientific interest. Probiotics, especially lactic acid bacteria (LAB), are live nonpathogenic microorganisms that

[1]Tehran University of Medical Sciences, Allied Medical Sciences School, Technology of Radiology and Radiotherapy Department – Tehran, Iran.
[2]Behbahan Faculty of Medical Sciences, Radiology Technology Department – Behbahan, Iran.
[3]Behbahan Faculty of Medical Sciences, Medical Laboratory Sciences Department – Behbahan, Iran.
[4]Tehran University of Medical Sciences, Advanced Technologies in Medicine School, Molecular Medicine Department – Tehran, Iran.
*Corresponding author: khanzadehpegah@gmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: this study was financially supported by the Tehran University of Medical Sciences.
Received on August 23, 2021. Accepted on August 24, 2021.

have health benefits when consumed in adequate quantity[5]. They can increase antioxidant capacity through different ways such as scavenging the excess free radicals and producing various metabolites such as glutathione (GSH), butyrate, and folate[6]. Some studies have also shown that probiotics, especially *Lactobacillus* species, exert their radioprotective effect through the regulation of the nuclear factor kappa B (NF-κB) pathway[7,8]. Additionally, the anti-apoptosis and anti-inflammation effects of *Lactobacillus* species on the irradiated normal tissues were reported by other studies[9,10].

Since the testis is one of the most radiosensitive tissues, this study aimed to investigate the effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *CASP 3* in the testes of gamma-irradiated rats.

METHODS

Irradiation

The animals received the whole- body radiation with the dose rate of 2 Gy gamma-ray (^{60}Co) and 100 cGY/min and a source-to-skin distance (SSD) of 100 cm. At the same time, the field of view (FOV) was set at 36×36, and SSD was 80 cm. The single 2 Gy dose of X-ray irradiation was conducted in Rad Source Model RS2000 Irradiator with a 0.3-mm copper filter and X-ray tube settings of 160 kVp and 24 mA (Rad Source Technologies, USA).

Preparation of probiotic strains

Lactobacillus casei (LC) and *Lactobacillus acidophilus* (LA) isolates were provided from the Iranian Biological Resource Center (IBRC). The bacteria were aerobically grown in de Man, Rogosa, and Sharpe (MRS) medium (Sigma-Aldrich, UK) at 37°C for 16 h. The live bacteria were harvested by centrifugation (10 min, 4000×g, room temperature). Thus, plate bacteria were mixed with phosphate-buffered saline (PBS) at the desired concentration. During the experiment, the rats received 0.2 ml of PBS containing 1×10^{10} colony-forming unit (CFU) of the probiotics daily.

Animals and treatment

The male Wistar rats (6–8 weeks old) weighing 220±20 g were purchased from the Animal House of Tehran University of Medical Sciences. They were housed under standard laboratory conditions (12-h light/dark cycle at 22±1°C temperature and 55±10% humidity).

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Tehran University of Medical Sciences (the ethical code: 34613).

The rats were divided into six groups (five rats in each group) and treated as it follows:

- Group 1 (healthy control): the animals only received PBS.
- Group 2: the animals only received radiation using the dose rate of 2 Gy gamma- rays.
- Group 3: the animals were orally gavaged with 0.2 mL of 1×10^{10} LC in PBS.
- Group 4: the animals were orally gavaged with 0.2 mL of a suspension of 1×10^{10} CFU LC in PBS, and their whole body was exposed to radiation.
- Group 5: the animals were orally gavaged with 0.2 mL of a suspension of 1×10^{10} CFU LA in PBS.
- Group 6: the animals were orally gavaged with 0.2 mL of a suspension of 1×10^{10} CFU LA in PBS, and their whole body was exposed to radiation.

After 4 weeks, the rats were anesthetized intraperitoneally (i.p.) with 250 mg/kg 2,2,2-tribromoethanol (TBE; Avertin®, Sigma-Aldrich) and sacrificed by cervical dislocation. Then, the rats' testes were isolated and immediately frozen in liquid nitrogen and then stored at -80°C for the subsequent analyses.

Quantitative analysis of real-time PCR (qRT-PCR)

The total RNA was extracted from the rat's testis using TRIzol®LS (Invitrogen Corp., USA) reagent according to the manufacturer's directions. Then, the single-stranded complementary DNA (cDNA) was synthesized from equal amounts of RNA using the Prime Script cDNA Synthesis Kit (Takara Bio, Japan), following the manufacturer's directions. The relative expression levels of the target gene were measured by qRT-PCR using SYBR Green with the following primer sets: *BCL2* (forward, 5'-GGTGAACTGGGGGAGGATTG-3'; reverse, 5'-GCATGCTGGGGCCATATAGT-3') (product size: 197 bp), *BAX* (forward, 5'-GGCGATGAACTGGACAACAA-3'; reverse, 5'-CAAAGTAGAAAAGGGCAACC-3') (product size: 151 bp), *CASP3* (forward, 5'-AGCTGGACTGCGGTATTGAG-3'; reverse, 5'-ATGGCGCAAAGTGACTGGAT-3') (product size: 189 bp), and *Hypoxanthine-guanine phosphoribosyl transferase* (HPRT) (forward, 5'-TCAGTCAACGGGGGACATAAA-3'; reverse, 5'-GGGGCTGTACTGCTTAACCAG-3') (product size: 142 bp). The relative amounts of *BCL2*, *BAX*, and *CASP3* mRNA were normalized against the endogenous control, HPRT, and calculated with the $2^{-\Delta\Delta Ct}$ formula.

Statistical analysis

The graphs and the statistical analysis of the data were performed using SPSS 16. The results were represented as the mean±SD. One-way analysis of variance (ANOVA) was followed by the Tukey's *post hoc* test for multiple comparisons. $p \leq 0.05$ was considered statistically significant.

RESULTS

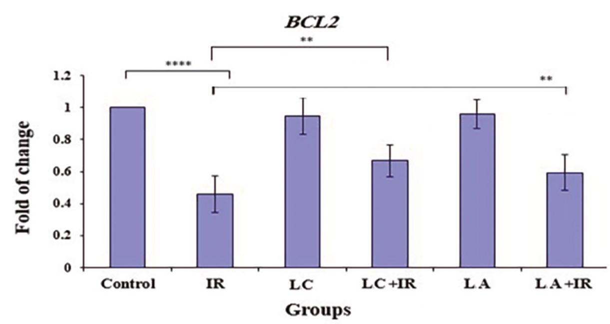
Effect of Lactobacillus species on the BCL2 and BAX gene expression in the testes of gamma-irradiated rats

As shown in Figure 1, radiation significantly downregulated the *BCL2* gene expression in the testis tissues in comparison with the control group (p<0.0001). Conversely, *Lactobacillus* spp. administration significantly reversed this effect (p<0.01). Moreover, the mRNA level of *BCL2* slightly decreased in the LC and LA groups compared with the control group.

The qRT-PCR results also showed that radiation significantly upregulated the *BAX* gene expression in the testis tissues in comparison with the control group (p<0.0001) (Figure 2). However, in the ionization radiation-treated rats and LC (IR+LC) and (ionization radiation-treated rats and LA (IR+LA) groups compared with the irradiated group, *Lactobacillus* spp. significantly decreased the expression of the *BAX* gene (p<0.05) (Figure 2). Additionally, our results showed that the mRNA level of *BAX* did not change between *Lactobacillus* spp. (LC and LA) treated groups and control groups.

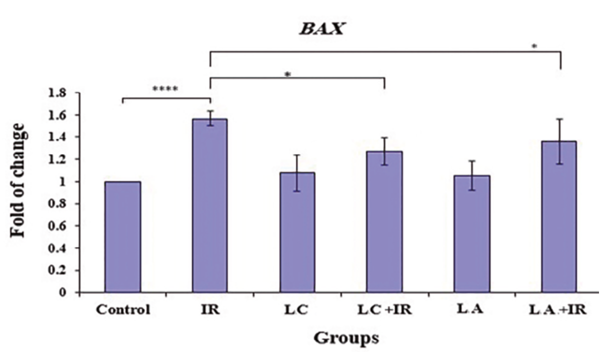
Effect of Lactobacillus species on the CASP3 gene expression in the testes of gamma-irradiated rats

Similar to the *BAX* gene, the mRNA level of *CASP3* significantly increased in the irradiated group compared with the control group (p<0.0001) (Figure 3). However, this effect was significantly reversed by treatment with *Lactobacillus* spp. in the IR+LC and IR+LA groups compared with the irradiated group (p<0.01) (Figure 3). We also found that the mRNA level of *CASP3* did not change in the LC and LA groups compared with the control group.



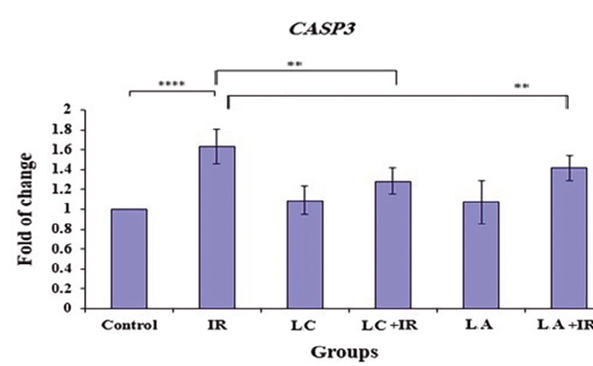
Control: PBS-treated rats as control group; IR: radiation-treated rats; LC: *Lactobacillus casei*-treated rats; LC+IR: *L.* casei and ionization radiation-treated rats; LA: *Lactobacillus acidophilus*-treated rats; LC+IR: *L. acidophilus* and ionization radiation-treated rats. Results were expressed as mean; error bars (SD); n=6. Statistical analysis was performed using one-way ANOVA test. **p<0.05; ****p<0.0001.

Figure 2. Effect of *Lactobacillus species* on the BAX gene expression in the testes of gamma-irradiated rats.



Control: PBS-treated rats as control group; IR: radiation-treated rats; LC: *Lactobacillus casei*-treated rats; LC+IR: *L. casei* and ionization radiation-treated rats; LA: *Lactobacillus acidophilus*-treated rats; LA+IR: *L. acidophilus* and ionization radiation-treated rats. Results were expressed as mean; error bars (SD); n=6. Statistical analysis was performed using one-way ANOVA test. *p<0.01; ****p<0.0001

Figure 1. Effect of *Lactobacillus species* on the BCL2 gene expression in the testes of gamma-irradiated rats.



Control: PBS-treated rats as control group; IR: radiation-treated rats; LC: *Lactobacillus* casei-treated rats; LC+IR: *L.* casei and ionization radiation-treated rats; LA: *Lactobacillus acidophilus* -treated rats; LC+IR: *L. acidophilus* and ionization radiation-treated rats. Results were expressed as mean; error bars (SD); n=6. Statistical analysis was performed using one-way ANOVA test. **p<0.01; ****p<0.0001.

Figure 3. Effect of *Lactobacillus species* on the CASP3 gene expression in the testes of gamma-irradiated rats.

DISCUSSION

Ionizing radiation plays a critical role in medical diagnosis and cancer-related therapy. It has been shown that the testis is one of the most radiosensitive organs because very low doses of radiation lead to abnormalities in spermatogenesis by mutagenesis, apoptosis, and necrosis[11]. Exposure of the testes to radiation leads to the induction of apoptosis in the radiosensitive normal cells, which may result in temporary or permanent infertility[12]. Additionally, Mingote et al. reported that changes in the brain lipid's intensities are early tissue responses to radiation exposure[13]{Mingote, 2020 #1}. The development of natural radioprotective agents with less toxicity and high effectiveness is attractive and interesting. Therefore, this study pursued the goal to determine the probiotic treatment effects on modulating apoptosis-related genes *BCL2*, *BAX*, and *CASP3* in the testes of gamma-irradiated rats. The probiotics such as LAB are the beneficial bacteria employed as an adjunct to reduce the adverse effects of ionizing radiation through several mechanisms[14]. Some studies indicated that certain probiotics modulate the activation of signaling pathways in radiation therapy[8]. The cell survival and death are regulated by the equilibrium of pro-apoptotic and the anti-apoptotic *BCL2* family proteins and *BAX*/*BCL2* ratio determines the cell susceptibility to apoptosis[15]. Our qRT-PCR results revealed that radiation downregulated the *BCL2* and increased the expression of the *BAX* gene in the normal testicular cells. The reaction of ionizing radiation with the cellular contents such as the small molecules of water in the normal testicular cells leads to the generation of ROS[16]. The high ROS level induces apoptosis *via* controlling the phosphorylation and ubiquitination of *BCL2* family proteins, which results in the upregulation of pro-apoptotic genes (e.g., *BAX*) and the downregulation of anti-apoptotic (e.g., *BCL2*)[4].

Some studies indicated that probiotics modulate the cellular signaling pathway in mammals by direct attachment to the cell surface[17]. For instance, Lutfi et al. showed that probiotic *Lactobacillus rhamnosus* negatively regulates appetite markers possibly through melatonin receptors[18]. In this study, we showed that *Lactobacillus* spp. significantly upregulates the *BCL2* gene and downregulates the *BAX* and *CASP3* genes in the testes of irradiated rats. In the human body, LAB such as LA and LC are part of the normal microbiota or microflora. The protective effects of probiotics against radiation were reported by many studies. Liu et al. demonstrated that the probiotic *Lactobacillus Plantarum* 299v reduced gastrointestinal injury and inflammation in the rats that were locally irradiated with 10 Gy[19]. In addition, it was shown that *L. rhamnosus* GG ATCC 53103 reduced intestinal epithelial apoptosis and improved crypt survival following whole-body gamma radiation at a dose of 12 Gy[8]. Intestinal bacteria also lowered the negative effect of radiation on intestinal barrier integrity by regulating the expression of tight junction-related proteins and restoring intestinal permeability[20]. A recently published study reported that probiotics improved the testes' function by neutralizing the toxins, improving sperm quality and testosterone levels, and modulating the immune system[21]. Researchers suggested that probiotics reduced the ROS activation evoked by radiation *via* the production of antioxidant enzymes such as superoxide dismutase, GSH peroxidase, GSH reductase, and catalase[22,23]. Shokri et al. demonstrated that melatonin with its antioxidant property can decrease oxidative damage induced by radiofrequency electromagnetic radiation (RF-EMR) of mobile phones on testis tissue[24]. Moreover, melatonin has been reported to have an important anti-apoptotic action by attenuating the production of ROS and pro-apoptotic proteins, such as *BAX*[25]. As above mentioned probiotics affect the melatonin pathway, therefore, it seems that melatonin might also be involved in the radioprotective effects of probiotics on testis tissue.

Certain limitations should be noted in this study. Primarily, immunohistochemical studies or tunnel analysis were not used to exactly evaluate the apoptosis induction in the testis tissue. However, in view of these findings, it is probable, therefore, that *Lactobacillus* species protect the testes of gamma-irradiated rats by modulating apoptosis-related genes *BCL2*, *BAX*, and *CASP3*. This study is the first report, to the best of our knowledge, indicating the modulatory effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *CASP3* in the testes of gamma-irradiated rats.

CONCLUSION

In summary, we concluded that *Lactobacillus* spp., particularly LC protects the testicular tissue against high- dose radiation (2 Gy) through modulating the *BAX* and *BCL2* genes expression, which play a significant role in the activation of apoptosis.

AUTHORS' CONTRIBUTIONS

VC: Conceptualization, Data curation, Formal analysis, Supervision, Validation. **OA:** Investigation, Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **RG:** Data curation, Formal analysis, Writing – original draft. **HB:** Validation, Writing – original draft, Writing – review & editing. **EM:** Conceptualization, Data curation, Validation, Methodology. **PK:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft.

REFERENCES

1. Ballas LK, Elkin EB, Schrag D, Minsky BD, Bach PB. Radiation therapy facilities in the United States. Int J Radiat Oncol Biol Phys. 2006;66(4):1204-11. https://doi.org/10.1016/j.ijrobp.2006.06.035
2. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev. 2010;4(8):118-26. https://doi.org/10.4103/0973-7847.70902
3. Meistrich ML. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. Fertil Steril. 2013;100(5):1180-6. https://doi.org/10.1016/j.fertnstert.2013.08.010
4. Li D, Ueta E, Kimura T, Yamamoto T, Osaki T. Reactive oxygen species (ROS) control the expression of Bcl-2 family proteins by regulating their phosphorylation and ubiquitination. Cancer Sci. 2004;95(8):644-50. https://doi.org/10.1111/j.1349-7006.2004.tb03323.x
5. Hijova E, Soltesova A. Effects of probiotics and prebiotics in ulcerative colitis. Bratisl Lek Listy. 2013;114(9):540-3. https://doi.org/10.4149/bll_2013_113
6. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant properties of probiotic bacteria. Nutrients. 2017;9(5):521. https://doi.org/10.3390/nu9050521
7. Dai C, Zheng CQ, Meng FJ, Zhou Z, Sang LX, Jiang M. VSL# 3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. Mol Cell Biochem. 2013;374(1-2):1-11. https://doi.org/10.1007/s11010-012-1488-3
8. Ciorba MA, Riehl TE, Rao MS, Moon C, Ee X, Nava GM, et al. Lactobacillus probiotic protects intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner. Gut. 2012;61(6):829-38. https://doi.org/10.1136/gutjnl-2011-300367
9. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology. 2007;132(2):562-75. https://doi.org/10.1053/j.gastro.2006.11.022
10. Lin PW, Myers LES, Ray L, Song SC, Nasr TR, Berardinelli AJ, et al. Lactobacillus rhamnosus blocks inflammatory signaling in vivo via reactive oxygen species generation. Free Radic Biol Med. 2009;47(8):1205-11. https://doi.org/10.1016/j.freeradbiomed.2009.07.033
11. Kim J, Lee S, Jeon B, Jang W, Moon C, Kim S. Protection of spermatogenesis against gamma ray-induced damage by granulocyte colony-stimulating factor in mice. Andrologia. 2011;43(2):87-93. https://doi.org/10.1111/j.1439-0272.2009.01023.x
12. Marci R, Mallozzi M, Di Benedetto L, Schimberni M, Mossa S, Soave I, et al. Radiations and female fertility. Reprod Biol Endocrinol. 2018;16(1):112. https://doi.org/10.1186/s12958-018-0432-0
13. Mingote MFS, Campos TPR, Augusti R, Cassali GD. Analytical methods for assessing changes induced by gamma exposure in an animal model. Rev Assoc Med Bras (1992). 2020;66(12):1651-6. https://doi.org/10.1590/1806-9282.66.12.1651
14. Riehl TE, Alvarado D, Ee X, Zuckerman A, Foster L, Kapoor V, et al. Lactobacillus rhamnosus GG protects the intestinal epithelium from radiation injury through release of lipoteichoic acid, macrophage activation and the migration of mesenchymal stem cells. Gut. 2019;68(6):1003-13. https://doi.org/10.1136/gutjnl-2018-316226
15. Raisova M, Hossini AM, Eberle J, Riebeling C, Sturm I, Daniel PT, et al. The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. J Invest Dermatol. 2001;117(2):333-40. https://doi.org/10.1046/j.0022-202x.2001.01409.x
16. Zhou R, Si J, Zhang H, Li J, Zhou X, Gan L, et al. The effects of x-ray radiation on the eye development of zebrafish. Hum Exp Toxicol. 2014;33(10):1040-50. https://doi.org/10.1177/0960327114522278
17. Liu Q, Yu Z, Tian F, Zhao J, Zhang H, Zhai Q, et al. Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. Microb Cell Fact. 2020;19(1):23. https://doi.org/10.1186/s12934-020-1289-4
18. Lutfi E, Basili D, Falcinelli S, Morillas L, Carnevali O, Capilla E, et al. The probiotic Lactobacillus rhamnosus mimics the dark-driven regulation of appetite markers and melatonin receptors' expression in zebrafish (Danio rerio) larvae: understanding the role of the gut microbiome. Comp Biochem Physiol B Biochem Mol Biol. 2021;256:110634. https://doi.org/10.1016/j.cbpb.2021.110634
19. Liu Q, Nobaek S, Adawi D, Mao Y, Wang M, Molin G, et al. Administration of Lactobacillus plantarum 299v reduces side-effects of external radiation on colon anastomotic healing in an experimental model. Colorectal Dis. 2001;3(4):245-52. https://doi.org/10.1046/j.1463-1318.2001.00244.x
20. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. J Nutr. 2011;141(5):769-76. https://doi.org/10.3945/jn.110.135657
21. Valcarce DG, Genovés S, Riesco MF, Martorell P, Herráez MP, Ramón D, et al. Probiotic administration improves sperm quality in asthenozoospermic human donors. Benef Microbes. 2017;8(2):193-206. https://doi.org/10.3920/BM2016.0122
22. Segers C, Verslegers M, Baatout S, Leys N, Lebeer S, Mastroleo F. Food supplements to mitigate detrimental effects of pelvic radiotherapy. Microorganisms. 2019;7(4):97. https://doi.org/10.3390/microorganisms7040097
23. Nath A, Haktanirlar G, Varga Á, Molnár MA, Albert K, Galambos I, et al. Biological activities of lactose-derived prebiotics and symbiotic with probiotics on gastrointestinal system. Medicina (Kaunas). 2018;54(2):18. https://doi.org/10.3390/medicina54020018
24. Shokri M, Shamsaei ME, Malekshah AK, Amiri FT. The protective effect of melatonin on radiofrequency electromagnetic fields of mobile phone-induced testicular damage in an experimental mouse model. Andrologia. 2020;52(11):e13834. https://doi.org/10.1111/and.13834
25. Ferreira CS, Maganhin CC, Simões RS, Girão MJBC, Baracat EC, Soares Jr JM. Melatonina: modulador de morte celular. Rev Assoc Med Bras. 2010;56(6):715-8. https://doi.org/10.1590/S0104-42302010000600024