Iranian Journal of Microbiology

Volume 16 Number 1 (February 2024) 90-96

ORIGINAL ARTICLE

In vitro evaluation of inhibitory effect of *Lactobacillus reuteri* supernatant on the replication of herpes simplex virus type 1 and expression of *UL54, UL52* and *UL27* genes

Faezeh Ebneali[1], Mohammad Shayestehpour[1,2*], Ahmad Piroozmand[1,2], Hossein Sedaghat[1], Shaghayegh Yazdani[3,4], Zahrasadat Fateminasab[1]**

[1]Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran

[2]Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran

[3]Department of Microbiology, Faculty of Medical Sciences, Islamic Azad University, Tehran, Iran

[4]Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran

Received: August 2023**, Accepted:** December 2023

ABSTRACT

Background and Objectives: Human herpes virus type 1 (HSV-1) is a neurotropic pathogen that is infected more than 70% of the world population. The increasing of viral resistance to antiviral drugs and the emergence of side effects has motivated researchers to study the use of probiotics as new antiviral agents. The aim of the present study was to study for the first time the potential antiviral activity of *Lactobacillus reuteri (L. reuteri)* supernatant against HSV-1.

Materials and Methods: After measuring the cytotoxicity of *L. reuteri* supernatant by MTT assay, 1:16 dilution of it was added to HeLa cells before and after HSV-1 infection, after 1.5 hours incubation with HSV-1, and simultaneously with HSV-1 infection. After 48 hours of incubation at 37°C, the viral titer and expression levels of *UL54, UL52* and *UL27* genes were measured by tissue culture infectious dose 50 ($TCID_{50}$) and Real-Time PCR methods, respectively.

Results: HSV-1 titer in the treatment conditions before infection, incubation with HSV-1, simultaneously with infection and after infection was reduced by 0.42, 3.42, 1.83, and 0.83 log 10 $TCID_{50}$/ml, respectively. When the bacterial supernatant was first incubated with the virus and then added to the cell, or when it was added simultaneously with the virus, the expression of the *UL27, UL52*, and *UL54* genes decreased significantly ($p<0.05$). When the bacterial supernatant is added to the cell before or after virus infection, the expression of *UL52* and *UL54* genes does not change significantly ($P>0.05$).

Conclusion: The study findings indicated that the supernatant of *L. reuteri* has a potent anti-HSV-1 effect especially if it is incubated with the virus before inoculation into the cell. Its possible antiviral mechanism is to inhibit the virus by binding to it or changing the surface structure of the virus. Metabolites of *L. reuteri* can be considered as a novel inhibitor of HSV-1infection.

Keywords: Antiviral; Cell culture; *Lactobacillus reuteri*; Herpes simplex virus type 1

*Corresponding author: Mohammad Shayestehpour, Ph.D, Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran; Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran. Tel: +98-9362122840 Fax: +98-3155589444 Email: shayestehpour-m@kaums.ac.ir

**Co-Corresponding author: Ahmad Piroozmand, Ph.D, Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran; Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran. Tel: +98-9132623523 Fax: +98-3155589444 Email: apiroozmand@gmail.com

INTRODUCTION

The herpes simplex virus (HSV) is a double-stranded DNA virus that belongs to the Alphaherpesvirine subfamily of the Herpesviridae family. This virus enters the host through mucosal membranes and hides in nerve ganglia after the initial infection (1). Due to internal and external stimuli, herpes simplex virus can lead to recurring infections. HSV-1 and HSV-2 are the main serotypes of the virus. HSV-1 is an important cause of eye diseases such as conjunctivitis, acute retinal necrosis, and keratitis. It can cause encephalitis and meningitis. The prevalence of HSV-1 is 42% in Iran, 52% in Finland, 57% in Netherlands, 58% in America, 84% in Bulgaria, 68% in Brazil, 67% in Belgium, and 81% in Czech Republic (2).

In order to treat herpes infections, nucleoside analogue antivirals such as acyclovir, vidarabine, famciclovir, valaciclovir, and penciclovir are typically used (3). However, in recent years, drug resistance has developed due to mutations in the DNA polymerase and thymidine kinase of the virus, which has resulted in a significant decline in their efficacy (4). Although foscarnet and cidofovir are also used to treat herpes virus infections, use of them has been constrained due to side effects such as toxicity and renal impairment (5). In recent years, researchers have tried to find a suitable alternative for nucleoside analogue drugs by using plant extracts, siRNA, probiotics and other new treatment methods (6-8).

Probiotics are defined as live microorganisms that help the host's health. Probiotic microorganisms with beneficial properties include *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces* spp., *Streptococcus* spp., and *Bacillus* spp (9). *Lactobacillus* spp. are one of the most popular probiotics that can be found in a wide range of food products throughout the world. The genus *Lactobacillus* is a group of Gram-positive, nonsporulating, facultative anaerobic bacteria which include species *L. acidophilus, L. bulgaricus, L. casei, L. reuteri,* and *L. rhamnosus* (10).

Several studies have been conducted to assess the antiviral properties of *L. reuteri.* There is evidence that the supernatant of *L. reuteri* has antiviral effects on pneumoviruses, circoviruses, rotaviruses, coxsackieviruses, and papillomaviruses (11). It has been proposed that *L. reuteri* reduces viral infection by modulating the microbiota and secreting antiviral compounds. In recent years, the effect of *L. rhamnosus* on HSV-1 replication and the effect of *L. crispatus* and *L. gasseri* on HSV-2 replication have been studied, and satisfactory results have been obtained (12-14). To date, no study has been published on the anti-herpes effect of *L. reuteri.*

Herpes virus genes are expressed in a cascade manner, with the immediate early (IE) genes being transcribed first, followed by the early (E) genes, and finally the late (L) genes. Product of *UL54* immediate early gene is involve in all steps of viral mRNA biogenesis, including transcription, RNA processing, and nuclear egress (15). *UL52* early gene produces primase protein that has a role in unwinding viral dsDNA (16). *UL27* late gene produces viral envelope glycoprotein B that is involved in the entry of HSV to host cell (17). To date, no study has been published that evaluated the effect of probiotics on the expression of the three important genes mentioned. Therefore, the present study was aimed to evaluate inhibitory effect of *L. reuteri* supernatant on the replication of herpes simplex virus type 1 and expression of *UL54*, *UL52* and *UL27* genes.

MATERIALS AND METHODS

Ethical approval. This study was approved by the Ethics Committee of Kashan University of Medical Sciences (IR.KAUMS.MEDNT.REC.1401.091).

Growth bacteria and preparation of cell free supernatant. *L. reuteri* was re-activated from freeze-dried ampoule purchased from Persian Type Culture Collection (PTCC 1655, Iran). *L. reuteri* was grown in de man, rogosa and sharpe (MRS) broth or on de man, rogosa and sharpe (MRS) agar (Ibresco, Iran) at 37°C and incubated under microaerobic condition. Ten milliliters of liquid MRS medium were inoculated with 100 ul of *L. reuteri* overnight culture (1×10^8 CFU/mL) and incubated at 37°C for 24 hours. The cell-free culture supernatant was collected from the culture suspension through centrifugation at 8,000 rpm for 15 min. Subsequently, bacterial cells were removed from the supernatant by a 0.22 µm syringe filter. The prepared cell free supernatant was neutralized (pH: 7.2) and stored at -20°C.

Preparation of cell line and culture of HSV. HeLa cells were taken from the Pasteur Institute of Iran and were grown under the following conditions: Dulbecco's Modified Eagles Medium (DMEM, Gibco,

Germany) supplemented with 5% fetal bovine serum (Bioidea, Iran) and 1% penicillin (100 U/mL)/streptomycin (100 µg/mL) (Bioidea, Iran) at 37°C with 5% CO_2. HSV-1, a gift from the Virology Department of Tarbiat Modares University (Tehran, Iran), was cultured in the HeLa cells. The virus was collected 4 days after infection by releasing from cells due to three freeze-thaw cycles.

Cytotoxicity assay of the *L. reuteri* supernatant. The dimethyl thiazolyl-diphenyl tetrazolium bromide (MTT) assay was used to measure cytotoxicity of the *L. reuteri* supernatant on cell culture. HeLa cells were seeded into a 96-well plate at a density of 2×10^4 cells/well and incubated for 24 hours at 37°C with 5% CO_2. Serial dilutions of bacterial supernatant in DMEM were prepared (up to 1/128). One hundred milliliter of each dilution was added to cell monolayer of each well. Six wells containing DMEM (without bacterial supernatant) were used as a negative control. The 96-well plate was incubated 24 hours at 37°C with 5% CO_2. Then, cells were washed with PBS. Finaly, 90 uL of fresh DMEM and 10 uL of MTT (BIO-IDEA, Iran) were added to each well, and the plate was incubated at 37°C for 4 hours. The formazan products were analyzed spectrophotometrically after dissolution in DMSO (Bioidea, Iran). Absorbance was measured at 570 nm using the enzyme-linked immunosorbent assay (ELISA) microplate reader. The percentage of cell viability was calculated using the following formula: (A treatment/A control) × 100%.

HSV-1 titration. In the present study, titer of HSV-1 was calculated by the 50 percent tissue culture infective dose ($TCID_{50}$) assay as described previously (2). Briefly, the cells were seeded at the density of 10^4 cells/well to 96-well plates and allowed to grow and reach about 80% confluency. Serial dilutions (10^{-1} to 10^{-9}) of the viral stocks in DMEM were prepared, and 100 ul of each dilution was added to each well of the 96-well plate (four replicates for each dilution). Six wells of each microplate were used as cell controls with DMEM without virus dilutions. The 96-well plates were incubated at 37°C with 5% CO_2 for 7 days, and the cytopathic effect (CPE) of HSV-1 was observed daily using an inverted microscope. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated using the Karber formula (18).

Evaluation of antiviral activity of the bacterial supernatant. Firstly, HeLa cells were seeded at the density of 0.3×10^4 cells/well to 6-well plates and incubated at 37°C with 5% CO_2 for 24 hours to reach a confluent monolayer. Medium of each well was removed and the bacterial supernatant was added to the cells in four different ways as follows:

1) before infection: HeLa cells were incubated with the bacterial supernatant for 1.5 hours at 37°C and 5% CO_2 in triplicate. cells were washed twice with PBS, infected with the virus (MOI=0.1) and incubated for 1.5 hours at 37 °C. After washing the cells with PBS, DMEM containing 2% FBS added to the cells and incubated at 37 °C for 48 hours.

2) Incubation with HSV: The virus (MOI=0.1) was mixed with the bacterial supernatant and co-incubated at 4°C for 1.5 hours, then the mixture was added to the cells and incubated at 37 °C for 1.5 hours. After washing the cells with PBS, DMEM containing 2% FBS added to the cells and incubated at 37°C for 48 hours.

3) Simultaneously with infection: The virus (MOI=0.1) and the bacterial supernatant were added into the cells simultaneously. Following 1.5 hours incubation at 37°C, the cells were washed with PBS and DMEM containing 2% FBS was added and incubated at 37°C for 48 hours.

4) After infection: The cells were infected with HSV-1 (MOI=0.1) and incubated for 1.5 hours at 37°C and 5% CO_2. The cells were washed twice with PBS and incubated with the bacterial supernatant at 37°C for 1.5 hours. After washing the cells with PBS, DMEM containing 2% FBS added to the cells and incubated at 37°C for 48 hours.

After adding the bacterial supernatant under the four conditions mentioned above, the cells were washed with PBS. For all conditions, MRS medium without supernatant was considered as control.

The cell lysate containing HSV-1 were collected after 3 Freeze-thaw cycles and stored at -70°C. Virus titer was calculated by $TCID_{50}$ method and relative expression of the *UL54*, *UL52* and *UL27* genes was measured by Real-Time PCR method.

Measuring the relative expression of *UL54, UL52* and *UL27* transcripts by Real-Time PCR. RNA was extracted from cell lysate containing HSV-1 using

the AmpliSens® RIBO-prep nucleic acid extraction kit (AmpliSens, Russia) according to the manufacturer's instructions and its quantity and quality was evaluated by the NanoDrop. One microgram of RNA was reverse transcribed using the AddScript cDNA Synthesis Kit (AddBio, South Korea). Real-time PCR analysis was performed using a RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) with ABI StepOnePlus™ instrument (ABI, USA). The primer sequences targeting human β-globin, *UL54*, *UL52* and *UL27* genes were used (Table 1) to perform Real-time PCR. Each reaction mixture contained 12.5 µL of master mix green (Ampliqon, Denmark), 0.5 µL(10 µmol) of each primer, 2 µL of cDNA, and 9.5 µL of distilled water. Amplifications were performed with the following thermal cycling: a 8-minute pre-denaturation step at 95°C, followed by 40cycles of 20-second denaturation at 95°C, 30-second annealing at 60°C, and 30-second elongation at 72°C. The $2^{-\Delta\Delta CT}$ method (19) was used to determine the relative expression of *UL54*, *UL52* and *UL27* genes after adjusting to the expression of human β-globin gene.

Statistical analysis. $TCID_{50}$ and real-time PCR data were analyzed by t student test using GraphPad Prism version 10.0. P value lessen than 0.05 was considered as statistically significant. All experiments were replicated three times, and the results were reported as mean standard deviation (SD).

RESULTS

Cytotoxicity assay and cell viability. The impact of *L. reuteri* supernatant on the viability of Hela cells is shown in Fig. 1. The results of MTT assay showed that when the cells were incubated with the 1/16, 1/32, 1/64, and 1/128 dilutions of the bacterial supernatant, more than 80% of cells were viable. dilutions 1/2, 1/4, and 1/8 of the bacterial supernatant had more than 20% cytotoxicity (Fig. 1). Therefore, the dilution of 1/16 used to evaluate the antiviral activity of the bacterial supernatant in all subsequent assays.

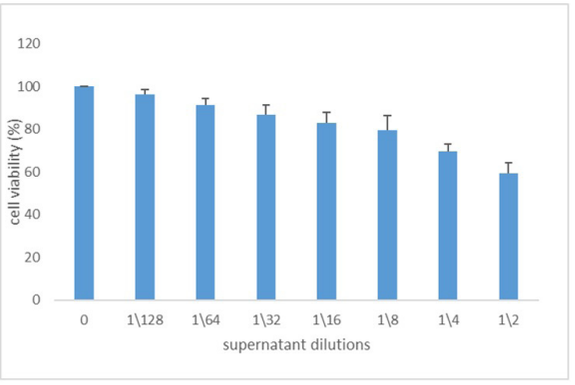

Fig. 1. Result of the cell viability determined using MTT assay. HeLa cells were treated with different concentrations of *L. reuteri* supernatant (up to 1/128) and the cell viability evaluated after 48 h.

Evaluation of virus titer reduction by $TCID_{50}$ method in the HeLa cells treated with the bacterial supernatant. After treatment of the *L. reuteri* supernatant with the cells in different conditions, the titer of HSV-1 in each condition was compared with the titer of HSV-1 in the control condition using the t-student statistical test and data were summarized in Table 2. HSV-1 titer in the treatment conditions before infection, incubation with HSV-1, simultaneously with infection and after infection was reduced by 0.42, 3.42, 1.83, and 0.83 Log_{10} $TCID_{50}$/ml, respectively, compared to the control. HSV-1 titer was significantly decreased when the bacterial supernatant was incubated with the virus and then added to the cell or when

Table 1. Primers used for measuring the relative expression of HSV-1 genes by Real-Time PCR method

Gene	Sequence (5→3)	Primer concentration (nM)	Product Size (bp)	Reference
UL54	Forward: TGGCGGACATTAAGGACATTG	300	222	(25)
	Reverse: TGGCCGTCAACTCGCAGA	300		
UL52	Forward: GACCGACGGGTGCGTTATT	300	193	(25)
	Reverse: GAAGGAGTCGCCATTTAGCC	300		
UL27	Forward: GCCTTCTTCGCCTTTCGC	300	238	(25)
	Reverse: CGCTCGTGCCCTTCTTCTT	300		
β-Actin	Forward: CAACTTCATCCACGTTCACC	300	219	(26)
	Reverse: GAAGAGCCAAGGACAGGTAC	300		

the supernatant was added to the cell simultaneously with the virus (P value < 0.0001). When the *L. reuteri* supernatant was added to the cell before infection with HSV-1, the virus titer did not decrease significantly compared to the control (P value=0.0668), but when the bacterial supernatant was added to the cell after the virus infection, the virus replication decreased (P value=0.0194, Table 2).

Relative expression of *UL54, UL52* and *UL27* genes of HSV-1 in different conditions of treatment with *L. reuteri* supernatant. The relative expression of *UL54*, *UL52* and *UL27* genes of HSV-1 in different conditions of treatment with *L. reuteri* supernatant was evaluated by Real Time PCR (Fig. 2). The results showed that when the bacterial supernatant is added to the cell before infection with HSV-1, *UL27* gene expression does not change significantly compared to the control (Fig. 2, P=0.616). When the bacterial supernatant was first incubated with the virus and then added to the cell, or when the supernatant was added to the cell at the same time as the virus, the expression of the *UL27*, *UL52*, and *UL54* genes decreased significantly. Obtaining similar results in the $TCID_{50}$ test shows that the bacterial supernatant has directly inhibited the virus, so the virus titer has decreased and the relative expression of all three viral genes has decreased significantly. When the supernatant was added to the cells after virus infection, the expression of the *UL27* gene decreased slightly (0.85 compared to the control, P=0.004). Considering that a similar result was obtained in the $TCID_{50}$ test, there is a possibility that after the virus entered the cell, the compounds in the bacterial supernatant penetrated the cell and caused a decrease in the virus replication through the effect on the expression of the *UL27* gene. When the bacterial supernatant is added to the cell before or after virus infection, the expression of *UL52* and *UL54* genes does not change significantly compared to the control (P>0.05).

Table 2. Result of HSV-1 titration using $TCID_{50}$ method in the various condition of treatment with *L. reuteri* supernatant

Treatment conditions of *L. reuteri* supernatant	Mean of HSV-1 titer (Log_{10} $TCID_{50}$/ml ± SD)		P-Value
	Sample	Control	
before infection	5.83 ± 0.14	6.25 ± 0.25	0.0668
Incubation with HSV-1	2.75 ± 0.25	6.17 ± 0.14	<0.0001
Simultaneously with infection	4.25 ± 0.25	6.08 ± 0.14	0.0001
After infection	5.50 ± 0.25	6.33 ± 0.28	0.0194

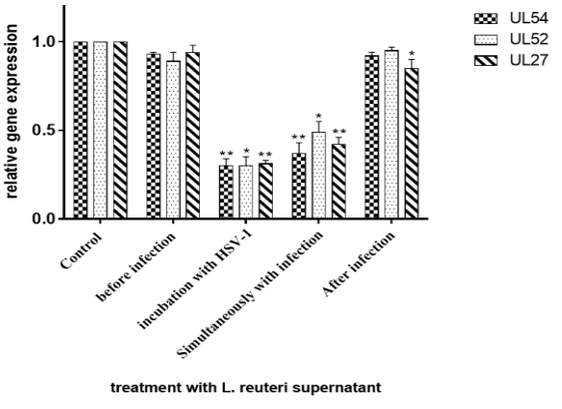

Fig. 2. Relative expression of *UL54, UL52* and *UL27* genes of HSV-1 in different experimental conditions of treatment with *L. reuteri* supernatant. *P value<0.05; **P value < 0.01.

DISCUSSION

Lactobacillus is one of the most common probiotic bacteria. In recent years, the antiviral effect of various *Lactobacillus* species has been a research interest (10). In the present study, the antiviral effect of *L. reuteri* supernatant was evaluated when it was added to the cells in the following conditions: before infection with HSV-1, incubation with HSV-1, simultaneously with HSV-1, after infection with HSV-1. Results showed that the maximum decrease in the HSV-1 replication occurred when the supernatant of *L. reuteri* and HSV-1 were incubated for 1.5 hours and then added to the cells (reduction of virus titer equal to 3.42 Log_{10} $TCID_{50}$/ml). This finding suggests that *L. reuteri* possess a potent antiviral capacity, potentially through the production of bacteriocins or other substances that can directly neutralize the virus. The pH of bacterial supernatant used in the present study was neutral. This means that the antiviral effect of *L. reuteri* was not through the production of oxygen and the release of H+ ions. It was known that the production of these products inhibits the virus replication (20).

Based on the results obtained from recent studies, *Lactobacillus* spp. has an antiviral effect through one of the following three possible mechanisms: 1) direct interaction between the bacteria and viruses, 2) production of antiviral compounds, 3) induction

of interferon production (21). The antiviral ability of *L. reuteri* was first recognized by Shornikova et al. when they introduced this probiotic as a therapeutic agent for acute rotavirus diarrhea (11). Several previous studies have shown the antiviral effect of *L. reuteri* through the direct binding of bacterial particles with viruses. In study of Ang et al., *L. reuteri* shows a significant antiviral activity against Coxsackievirus type A strain 6 and Enterovirus 71 through direct interaction between bacteria and virus (22). Several studies demonstrated the antiviral effect of bacterial supernatant. Botić et al. shown that the secreted antiviral substances during growth of *L. reuteri* decreased infectivity of the vesicular stomatitis virus (VSV) by 68% (23). Fakhri et al. investigated the antiviral activity of the *L. reuteri* supernatant against foot and mouth disease (FMD) virus serotype O. They reported that the bacterial supernatant with dilution of 1/16 had about 60% antiviral activity when it was incubated with the virus and then added to the cell, or when it was added to the cell simultaneously (24). Similar results were obtained in our study, the supernatant was able to significantly reduce virus replication when it entered the cells after incubation with the virus or at the same time. Therefore, the secondary metabolites of the *L. reuteri* may have inhibited the replication of HSV-1 by directly binding to the virus. Fakhri et al. concluded that if bacterial metabolites enter the cell after virus infection, they cannot inhibit FMD virus replication by inducing intracellular processes (24). This finding is inconsistent with the results of our study, that the addition of the *L. reuteri* supernatant after virus infection could slightly reduce virus replication. The relative expression of the *UL27* gene of HSV-1 has been significantly reduced in this treatment; therefore, the penetration of bacterial metabolites into the cell after the entry of the virus has reduced the expression of the *UL27* gene and has inhibited the viral replication.

In the present study and Faghri et al. study, when *L. reuteri* supernatant was added to the cell before virus inoculation, it did not have a significant effect in reducing virus replication, so it can be concluded that bacterial metabolites do not have a significant effect on the cell surface viral receptors. It is hypothesized that the bacteria metabolites can prevent the virus from binding to the cell receptor and entering the cell by changing the structure of the surface proteins of the virus.

Vilhelmova-Ilieva et al. showed that the cell free supernatant of *L. fermentum* had a potent antiviral effect when it was in contact with HSV-1 for one hour (25). Elebeedy et al. demonstrated a significant decrease in HSV-1 titer (1.25 $\log_{10}$ $TCID_{50}$/ml) using the *L. acidophilus* supernatant (26). Similarly, in the present study, the metabolites of *L. reuteri* reduced HSV-1 titer by 1.83 Log_{10} $TCID_{50}$/ml; therefore the supernatant of *L. reuteri*, like other mentioned *Lactobacilli*, contains anti-herpes virus compounds.

The anti-HSV-2 ability of the *L. rhamnosus* supernatant has been reported (14). In previous studies, the supernatant of some *Lactobacillus* spp. including *L. crispatus*, *L. gasseri* CMUL57, *L. acidophilus* CMUL67 and *L. plantarum* CMUL140 had not anti-HSV-2 activity (12, 14). According to the findings of the present study, which shows the presence of anti-herpes virus substances, it is suggested to evaluate the ability of *L. reuteri* supernatant against HSV-2.

CONCLUSION

The results of the study showed that the *L. reuteri* supernatant has a strong anti-HSV-1 effect, particularly when it incubates with the virus before cell inoculation. Its possible antiviral mechanism is to inhibit the virus by binding to it or changing the surface structure of the virus. Metabolites of *L. reuteri* can be considered as a novel inhibitor of HSV-1infection with potential of therapeutics, however, more studies are needed to clarify which substance in the *L. reuteri* supernatant is anti-herpes virus.

ACKNOWLEDGEMENTS

This study was part of an MSc thesis in medical microbiology, and was funded by Kashan University of Medical Sciences with grant number 401055.

REFERENCES

1. Barzoki MG, Malekshahi SS, Shayestehpour M. *In vitro* evaluation of antiviral activity of Shouchella clausii probiotic strain and bacterial supernatant against herpes simplex virus type 1. *Arch Microbiol* 2022; 204: 522.
2. Shayestehpour M, Rahimi MR, Piroozmand A, Khaledi A, Fateminasab ZS. *In vitro* evaluation of antiviral

activity effect of selenium, Bacillus clausii supernatant, and their combination on the replication of herpes simplex virus 1. *Jundishapur J Microbiol* 2022; 15(8): e129848.
3. Birkmann A, Bonsmann S, Kropeit D, Pfaff T, Rangaraju M, Sumner M, et al. Discovery, chemistry, and preclinical development of pritelivir, a novel treatment option for acyclovir-resistant Herpes simplex virus infections. *J Med Chem* 2022; 65: 13614-13628.
4. Schalkwijk HH, Snoeck R, Andrei G. Acyclovir resistance in herpes simplex viruses: Prevalence and therapeutic alternatives. *Biochem Pharmacol* 2022; 206: 115322.
5. Valladares HC, Rudd N, Leslie K. P76 Management of aciclovir-resistant mucocutaneous herpes simplex virus infection: a systematic review. *Br J Dermatol* 2023; 188(Supplement_4): ljad113.104.
6. Abd Wahab NZ, Ganasen T, Rahman NIA, Ibrahim N. *In vitro* virucidal activity of Kyllinga nemoralis aqueous extract against herpes simplex virus. *J Appl Biol Biotechnol* 2023; 11: 208-213.
7. Elebeedy D, Ghanem A, Aly SH, Ali MA, Faraag AH, El-Ashrey MK, et al. Synergistic antiviral activity of Lactobacillus acidophilus and Glycyrrhiza glabra against Herpes Simplex-1 Virus (HSV-1) and Vesicular Stomatitis Virus (VSV): experimental and In Silico insights. *BMC Microbiol* 2023; 23: 173.
8. Lasanen T, Frejborg F, Lund LM, Nyman MC, Orpana J, Habib H, et al. Single therapeutic dose of an antiviral UL29 siRNA swarm diminishes symptoms and viral load of mice infected intranasally with HSV-1. *Smart Med* 2023; 2(2): e20230009.
9. Ng QX, Lim YL, Yaow CYL, Ng WK, Thumboo J, Liew TM. Effect of probiotic supplementation on gut microbiota in patients with major depressive disorders: A systematic review. *Nutrients* 2023; 15: 1351.
10. Kullar R, Goldstein EJ, Johnson S, McFarland LV. *Lactobacillus bacteremia* and probiotics: A review. *Microorganisms* 2023; 11: 896.
11. Mu Q, Tavella VJ, Luo XM. Role of *Lactobacillus reuteri* in human health and diseases. *Front Microbiol* 2018; 9: 757.
12. Kassaa IA, Hober D, Hamze M, Caloone D, Dewilde A, Chihib N-E, et al. Vaginal *Lactobacillus gasseri* CMUL57 can inhibit herpes simplex type 2 but not Coxsackievirus B4E2. *Arch Microbiol* 2015; 197: 657-664.
13. Khani S, Motamedifar M, Golmoghaddam H, Hosseini HM, Hashemizadeh Z. *In vitro* study of the effect of a probiotic bacterium *Lactobacillus rhamnosus* against herpes simplex virus type 1. *Braz J Infect Dis* 2012; 16: 129-135.
14. Mousavi E, Makvandi M, Teimoori A, Ataei A, Ghafari S, Samarbaf-Zadeh A. Antiviral effects of *Lactobacillus crispatus* against HSV-2 in mammalian cell lines. *J Chin Med Assoc* 2018; 81: 262-267.
15. Wang Y-Y, Lyu Y-N, Xin H-Y, Cheng J-T, Liu X-Q, Wang X-W, et al. Identification of putative *UL54* (ICP27) transcription regulatory sequences binding to Oct-1, v-Myb, Pax-6 and hairy in herpes simplex viruses. *J Cancer* 2019; 10: 430-440.
16. Bermek O, Williams RS. The three-component helicase/primase complex of herpes simplex virus-1. *Open Biol* 2021; 11: 210011.
17. Li P, Gandhi D, Mutas M, Ran Y-F, Carr M, Rampini S, et al. Direct identification of the herpes simplex virus *UL27* gene through single particle manipulation and optical detection using a micromagnetic array. *Nanoscale* 2020; 12: 3482-3490.
18. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. *World J Virol* 2016; 5: 85-86.
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; 25: 402-408.
20. Ranjbar R, Goudarzi MM, Jounaidi N. *Lactobacillus acidophilus* and assessment for its antiviral effect against herpes simplex virus type I. *Biosci Biotechnol Res Asia* 2015; 12: 1351-1356.
21. Al Kassaa I, Hober D, Hamze M, Chihib NE, Drider D. Antiviral potential of lactic acid bacteria and their bacteriocins. *Probiotics Antimicrob Proteins* 2014; 6: 177-185.
22. Ang LY, Too HK, Tan EL, Chow TK, Shek LP, Tham EH, et al. Antiviral activity of *Lactobacillus reuteri* protectis against Coxsackievirus A and enterovirus 71 infection in human skeletal muscle and colon cell lines. *Virol J* 2016; 13: 111.
23. Botić T, Klingberg TD, Weingartl H, Cencič A. A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. *Int J Food Microbiol* 2007; 115: 227-234.
24. Vilhelmova-Ilieva N, Atanasov G, Simeonova L, Dobreva L, Mancheva K, Trepechova M, et al. Anti-herpes virus activity of lactobacillus' postbiotics. *Biomedicine (Taipei)* 2022; 12: 21-29.
25. Xiao J, Cai M, Wang Y, Ding P. Antiviral activities of officinaloside C against Herpes simplex Virus-1. *Molecules* 2022; 27: 3365.
26. Fakhredini K, Soleimanjahi H, Bamdad T. Prevalence of herpes simplex viruses types 1 and 2 infections among suspected children of encephalitis in Kermanshah, Iran. *Iran J Microbiol* 2023; 15: 149-154.