IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 6 Ser. III (Nov. –Dec. 2020), PP 01-07
www.Iosrjournals.Org

Antiviral effects of Myrrh against herpes virus infection compare to Acyclovir

Nancy Bigley[1], Badrieah Alamri[2]
1(Microbiology and Immunology, College of Science and Mathematics / Wright State University, United State of America)
[2](Biology, Science College/ TabukUniversity, Saudi Arabia)

Abstract:*Herpes simplex virus type 1 (HSV-1) is a highly infective human pathogen which infects a wide range of population in North America and worldwide. HSV-1 infection has two phases, lytic and latent. Recurrence of HSV-1 is a major challenge to clinicians to control the infection especially in immune depleted individuals. Acyclovir (ACV) is an antiviral drug used to treat HSV-1 infection. Low solubility of ACV in water, mutation of viral thymidine kinase, and mutation of viral DNA polymerase are major problems that cause usage limitations of ACV. Myrrh has been used as an analgesic and anti-inflammatory natural product in middle eastern countries for centuries. Recently Myrrh has shown a promising therapeutic action against fungal and parasitic infections. In the current study, low concentrations of Myrrh treatment increased cell survival of HSV-1 infected Vero cells. Prolonged exposure to Myrrh was found to be toxic to viable cells. Myrrh treatment of HSV-1 infected Vero cells was compared with ACV treatment of HSV-1 infected Vero cell. Myrrh treatment increased cell survival of infected Vero cells, similar to the effect of ACV treatment. These results provide evidence that Myrrh exerts antiviral effects against herpes virus infection.*

Key Word*: Herpes Virus Type1; Acyclovir; Myrrh; Antiviral.*

Date of Submission: 05-12-2020 Date of Acceptance: 20-12-2020

I. Introduction

Ninety percent of the human population has been infected by different types of herpes viruses[1]. HSV-1 is the most common type of herpes virus and mainly affects the epidermal cells and epithelia of the oral mucosa . life cycle of herpes simplex virus has multiple phases. The first phase is called the lytic phase, while the second phase is called the latent phase[2]. During the latency phase, there is no active virus generation and symptoms do not appear in the infected patient[3,4]. HSV-1 can reactivate spontaneously or due to stress, UV light, or immunosuppression[3,5]. One prominent aspect of HSV1 is the ability to switch between production, replication, and latency[6]. HSV-1 infection can reactivate causing infections[7, 1].

Acyclovir (ACV) is an anti-viral drug used to treat infection with herpes simplex virus (HSV1), varicella zoster (chickenpox), and herpes zoster (shingles)[8,9,10]. ACV has been used as a potent and reliable suppressor of herpes simplex virus (HSV1)for more than 25 years[9].Due to the similarity of the chemical structure of Acyclovir and the nucleoside guanosine, ACV competes with the guanosine nucleosides to inhibit viral replication[10,11,12].

Inactive ACV is phosphorylated by HSV-1 Thymidine Kinase. Two more phosphorylations activate ACV. Acyclovir triphosphate inhibits HSV-1 replication[13]. Mutation of the viral TK causes insufficiency of the ACV phosphorylation process, which prevents its therapeutic value[11]. Mutation of the DNA polymerase of the virus can be another reason for a lack of ACV activity[11]. This leads to the inability to integrate acyclovir triphosphate in the DNA molecules[13,14,15].Moreover, ACV has limitations due to its low solubility in water so it cannot be used as a muscular injection or in eye drops. Lack of absorption by transdermal application is another factor restricting ACV usage[10,9,12]. The repetitive use of antibiotics to treat bacterial disease can cause antibiotic resistance. Many studies approved that medical herbs cab be an alternative medicine to eliminate different side effects. Natural medical extracts can be more viable, cheaper, and safer compared to synthetic drugs.Myrrh oil and extract has been confirmed in the USA by the Food and Drug Administration as a safe substance for use in foods and drinks and as a fragrance in beauty agents[16].

Myrrh is a yellow resinous exudate which is harvested from the trunk of the small Myrrh tree. Many previous studies identify Myrrh as one of the oldest herbs utilized as analgesic and anti-inflammatory activity . In additional experiments, myrrh oil inhibited paw swelling in rats with a 100 mg/kg dose after 4 hours[17]. Previous finding approved the ability of myrrh to reduce parasite worms in four weeks and eliminate most of the symptoms associated with the parasite within one week parasites[16,18]. Using Myrrh as a mouthwash shows potential antiplaque and antigingivitic therapeutic effect[11].In 2019, Sarbaz, Zahra et al illustrate that the hydro-

alcoholicextract of Myrrh plant was faster andeffective on wound healing process with minimal side effects[19].Recently, Myrrh oil confirmed as a strong antibiotic against bacterial activities according to Bhattacharjee, et al experiments[20].

The aim of this study is to determine the effective concentration for Myrrh extract as inhibitor of HSV-1 replication using plaque assay. Also to evaluate the effect of Myrrh extract on cell survival of Herpes Simplex Virus Type-1 infected Vero cell then compare the result with Acyclovir treatment.

II. Material And Methods

Herpes simplex virus-1

Herpes simplex virus-1 was initially obtained from Dr. Nancy Sawtell, Children's Hospital Medical Center, Cincinnati, OH. Vero cells were infected at 90-100% confluency with 1 ml HSV-1 at 0.1 MOI and once the cells were rounded, the supernatant was harvested, aliquoted and stored at -80°C. Virus was tittered before being used for further experimentation.

Plaque Assay

The plaque method has been used to quantify infectious virus through counting of plaques (zones of killed cells) on virus-infected Vero cells.Vero cells were grown in 12 well plates until they reached 90-100% confluence. HSV-1 was added and the plate was incubated for two hours to ensure that the Vero host cell absorbed the virus. Following virus infection, 1 ml of methyl cellulose (MC) was added to each well. MC is used to prevent the diffusion and spread of virus. Following the overlaying of MC, plates were incubated for 3-5 days. Monolayers were fixed using 4% formaldehyde and kept at room temperature overnight. The fixative was then removed and the monolayers were stained using 5% Crystal Violet for about 10- 15 minutes. All wells were gently washed with distilled water (dH2O) and the plate was allowed to dry at room temperature and plaques counted. The following equation was used to calculate the viral titer.

$$\text{Average \# Plaques} = \frac{\text{PFU/ml}}{D \times V}$$

D=dilution V=Volume of diluted virus added to the plate

Vero cell

The Vero cell line is used as a host cell for the virus growth. The cell line was derived from the kidney of an African green monkey (CCL-81, ATCC). The culture medium was prepared using 90% of Dulbecco's Modified Eagle's Medium (DMEM), 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin. The cell growth was monitored every day and cells were split every 2-3 days. DMEM, FBS and antibiotics were purchased from Fisher Scientific.

Acyclovir (ACV) treatment before infection

Vero cells were cultured using 12 well tissue culture plates (Fisher Scientific) for 24 hours. The cells were treated with ACV using different doses (2, 1, and, 0.05 µg /ml of medium) and incubated for 2 and 4 hours. Then cells were infected with HSV-1 at 0.1 MOI and incubated for 2 hours at 37°C in 5% CO2. Following virus infection, 1 ml of methyl cellulose (MC) were added. Monolayers were fixed with 4% formaldehyde overnight. The fixative was removed and monolayers were stained using 5% Crystal Violet for about 10-15 minutes. Plates were washed with distilled water (dH2O) and the plaques were counted.

ACV treatment after infection

Vero cells were grown in 12 well plates with 10% DMEM overnight. The next day, cells were infected with HSV-1 at 0.1 MOI for 2 hours. After the virus in medium was aspirated, plates were incubated in culture medium containing ACV for 2 and 4 hours. The effect of virus was examined using plaque assay.

Myrrh treatment before infection

Vero cells were cultured in 12 well tissue culture plates. After 24 hours, Myrrh extracts were added at different concentrations (1%, 2%, 0.5%) for 2 and 4 hours. After that, cells were infected with HSV-1 at 0.1 MOI and incubated for 2 hours at 37°C. One ml Methylcellulose was added to each well and the dishes were incubated for 3-5 days. Monolayers were fixed with 4% formaldehyde overnight then stained with 5% Crystal Violent for 10-15 min. Plates were washed with distilled water (dH2O) and the plaques were counted.

Myrrh treatment after infection

Vero cells were plated in 12 well tissue culture plates with 10% DMEM and incubated overnight. Then, cells were infected with HSV-1 at 0.1 MOI for 2 hours. After the virus was aspirated, plates were incubated in culture medium containing different doses of myrrh extract for 2 and 4 hours. The effect on virus infectivity was

examined using plaque assay.

Cell viability

Vero cells were grown until 90- 100% confluence. They were treated with Myrrh for 2 or 4 hours either before or after virus infection. Infected, untreated cells were used as a control. Vero cells were washed using Phosphate-buffered saline (PBS). Trypsin was added to the cells. After cells were detached , 10% DMEM was added. Detached cells were centrifuged at 1500 rpm (4℃) for 5 minutes. One ml of fresh medium was added to the cell suspension. 20 µl of the suspended cells were mixed with 20 µl Trypan blue dye (Fisher Sciences) to analyze cell viability. Aemocytometer was used to count viable cells. The following equation was used to count cell viability:

Cell Viability (%) = total viable cells (unstained) ÷ total cells (stained and unstained) ×100

Statistical Significance:

All experiments were repeated three times to confirm the results. Data were analyzed by one-way ANOVA (Sigma Plot 12.0, YSTAT). Data are represented as mean ± SEM.

III. Result

The most effective concentration for Myrrh extract as inhibitor of HSV-1 replication was determined using plaque assay. Infected Vero cells were treated with 20% or 10% concentrations of Myrrh as high concentrations of Myrrh were toxic. However, infected Vero cells treated with lower concentrations of Myrrh (2%, 1%, and 0.5%) survived. Toxicity was determined visually by cell density of the infected treated cells compared with the controls infected, untreated Vero cells.

Effect of Myrrh extract on HSV-1 infection (2 hours before infection):

Cultured Vero cells at 90% confluence were treated with 2%, 1%, and 0.5% concentrations of Myrrh two hours before HSV-1 infection. Cell density reached highest value at 2% concentration (P value ≤ 0.001) while 0.5% concentration of Myrrh was the lowest (P value ≤ 0.01), compared with the untreated, virus-infected cells.

Effect of Myrrh extract on HSV-1 infection (2 hours after infection)

Cultured Vero cells (at 90- 100% confluence) were treated with different concentrations of Myrrh (2%, 1%, and 0.5%) at 2 hours post HSV-1 infection. At two hours, there was a significant reduction (P value $\leq$ 0.001) in the number of plaques formed at all Myrrh concentrations compared to the control (virus infected cells). Myrrh was most effective at 2% concentration on virus infected cells compared to other concentrations. Analyzing cell density using Image J gave the same results. Densities of the treated cells decreased with the reduction of the concentrations of Myrrh compared with the control (virus infected cell).

Effect of Myrrh extract on HSV-1 infection (4 hours before infection)

Plaque assays were used to detect the concentration of Myrrh that most effectively inhibited virus replication. Before HSV-1 infection, Myrrh treatment was added for 4 hours using different concentrations (2%, 1%, and 0.5%). A 4 hour treatment with 0.5% concentration of Myrrh before virus infection displayed a significant result (p value .031) compared to virus infected cell (control), while (1% and 2%) concentrations of Myrrh showed a slight reduction compared with the control.

Effect of Myrrh extract on HSV-1 infection (4 hours after infection)

Vero cell were grown to approximately 90-100% confluence. They were treated with Myrrh 4 hours after virus infection and the cells were stained with crystal violet to determine the cells that survived the infection. Concentrations of Myrrh 2% and 1% led to insignificant increase in cell survival density compared to the control (infected Vero cells). There was a significant difference (p value .008) between the 0.5% concentration and the control (infected untreated cells).

Cell Viability comparison between 2 and 4 hours treatment of Myrrh

There was notable decrease in the cell viability percentage at 2% and 0.5% concentrations between 2 hrs and 4 hrs of Myrrh treatment. Cell viability percentage was significantly increased higher at 2 hrs treatment compared to 4 hrs treatment (P value ≤ 0.001) as shown in Figures 1 A and C. However, 1% concentration of Myrrh treatment led to a less significant difference then 2% and 0.5% (P value 0.048) Figure 1 B.

Comparison of ACV or Myrrh treatment at 2 hours before and after infection

The scanned pictures were analyzed using Image J. Pixel densities for each of the three random wells were analyzed using Sigma Plot statistic. No significant differences in cell survival were found using a one-way analysis of variance between the ACV and Myrrh treatments at 2%, 1% and 0.5% concentrations at 2 hours before infection.

After 2 hours of the ACV or Myrrh treatments followed by HSV-1 infection, infected Vero cells treated with ACV showed greater survival rates (P value ≤ 0.001) relative to infected Vero cells treated with Myrrh in all concentrations (Figure 2).

Comparison of ACV or Myrrh treatment at 4 hours before and after infection:

At 4-hours the Myrrh treatment displayed significant results only for the 0.5% concentration. As shown in Figure 3, cell survival with ACV at a 0.5% treatment significantly increased survival compared to the Myrrh treatment before infection (P value ≤ 0.001) and after infection (P 0.01).

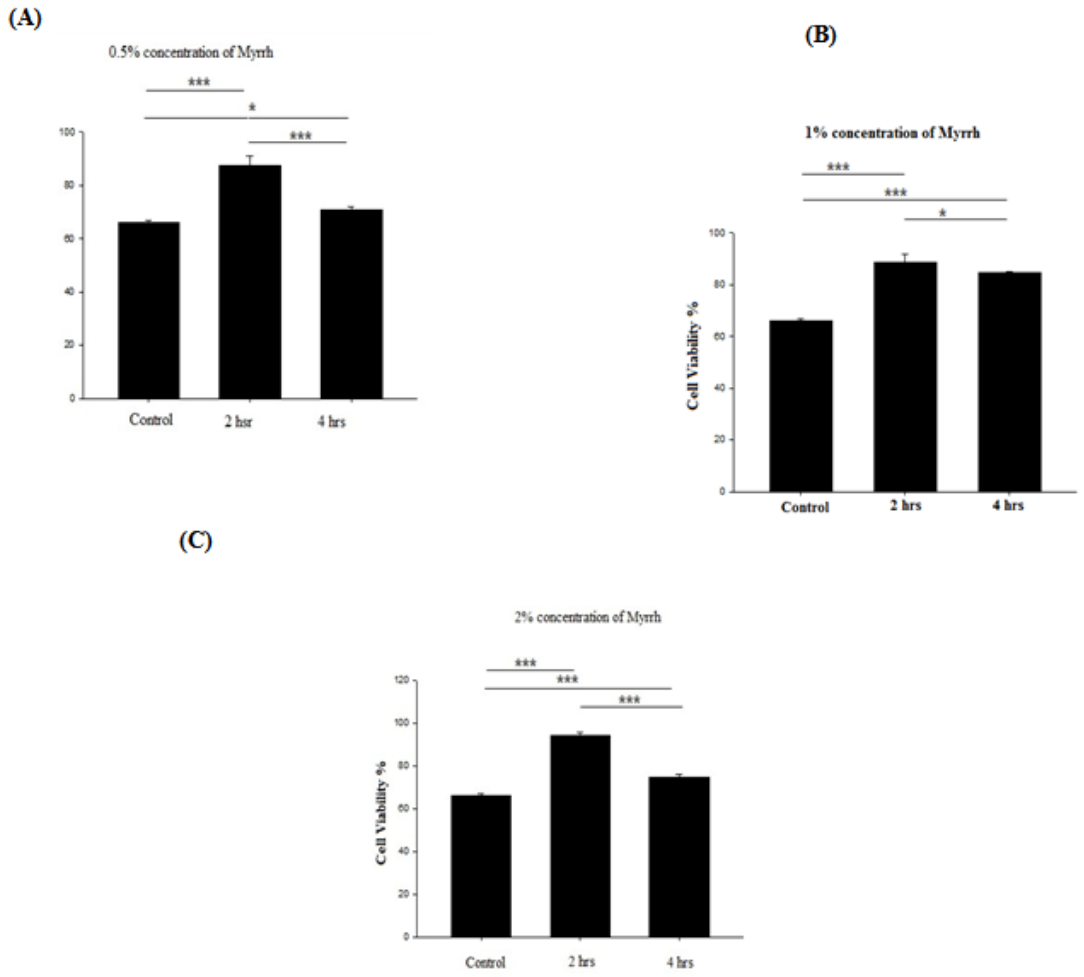


Figure 1: Comparison of present cell viability between 2 and 4 hours of Myrrh pretreatment.

(A) Cell viability percentage increased significantly at 2hrs (P value < 0.001) and at 4 hrs (P value < 0.001) of 2% myrrh treatments compared with the control (infected, untreated cells). Pretreatment with 2 hours led to a significantly higher survival than the 4 hours treatment (P value < 0.001). Figure (B) Cell viability percentage increased significantly at 2hrs (P value < 0.001) and 4 hrs (P value < 0.001) compared with the control. However, the differences between 2 hrs and 4 hrs of 1% of Myrrh were nearly insignificant (P value = 0.048). (C) At 0.5% concentration of Myrrh cell survival was greater at 2hrs compared to 4 hrs of treatment (P value < 0.001) (ME: Myrrh Extract).

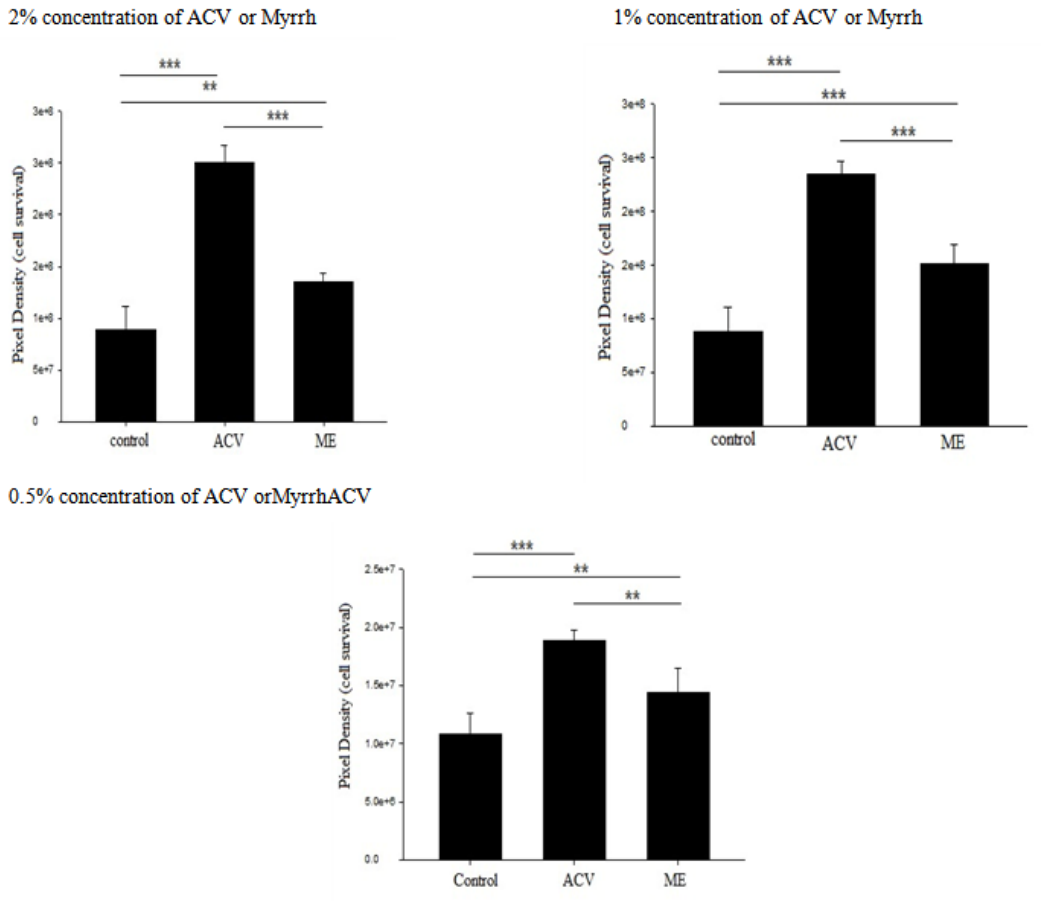
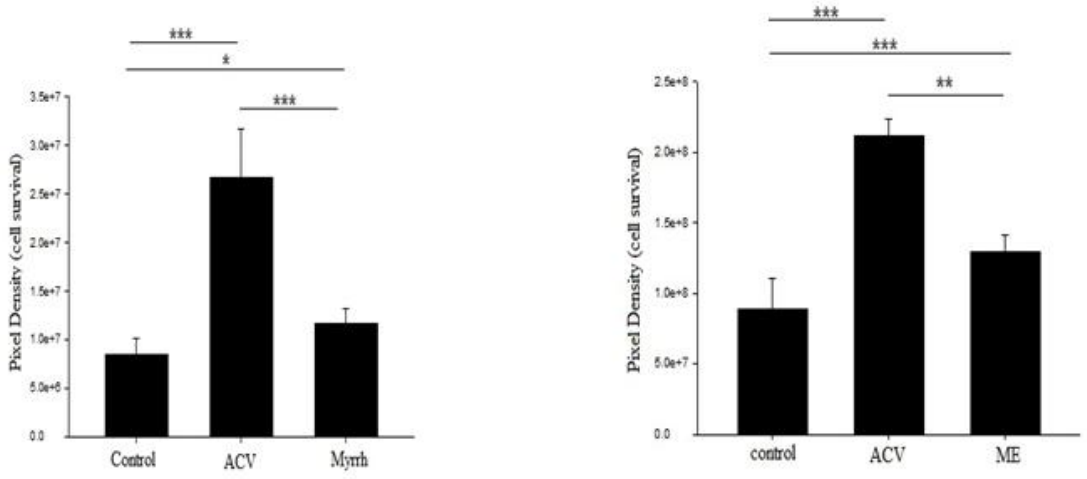


Figure 2. Comparison of cell survival between treatment with Myrrh or ACV of HSV-1 infected cells (2 Hours treatment after infection). There was a notable increase in cell survival between the ACV and Myrrh treatment compared to the control (infected untreated cell). Also infected Vero cells treated with ACV showed greater survival rate relative to infected Vero cells treated with Myrrh in all concentrations. (** $P \leq 0.01$; *** $P \leq 0.001$)

0.5% concentration of ACV orMyrrh0.5% concentration of ACV or Myrrh



(A) **(B)**

Figure 15. Comparison of cell survival between Myrrh or ACV treatments of HSV-1 infected cells (4 Hours treatment**).** (A)At 4 hours treatment with ACV before infection led to a significant increase in cell survival compared to Myrrh (P value< 0.001). (B) Treatment withACV at 4 hours after infection significantly increased cell survival compared to Myrrh ($P \leq 0.01$) (ME: Myrrh Extract).

IV. Discussion

Myrrh has been used in Middle Eastern countries for centuries, not only as a foaming material but also as a medication. Recently, researchers have demonstrated that myrrh has antibacterial effects against gram positive bacteria such as Bacillus subtilis and Staphylococcus aureus. Myrrh application has shown promising action against pathogenic Gram- negative bacteria such as Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa. Myrrh also effectively inhibits fungal pathogenic growth of Candida albicans and Apergillus niger[21]. Myrrh is used as an anti-parasitic agent to treat human trematode infections. Researchers have examined the anti-parasitic effect of myrrh with exploring its mode of action, safety, and efficacy on parasite infections[22]. In the current study, Myrrh extract's effect was examined on Vero cells infected with Herpes Virus Type-1. High concentration (10% and 5%) of Myrrh extracts were toxic to the Vero cells, while lower concentrations (2%, 1%, and 0.5%) were protective for Vero cells against the cytopathic effect of HSV-1.

Application of Myrrh Extract for 2 hours at lower concentrations of 2%, 1% and 0.5% showed significant reduction in the HSV-1 viral replication. The results were compared with infected untreated cells using plaque assay and confirmed by testing cell viability.

NF-kB is activated in HSV-1 infected Vero cells leading to increase secretion of pro-inflammatory cytokines secretion including IL6, IL8, TNF-α, and interferons[23]. Epithelial cells' ability to act as accessory immune cells is a possible explanation for the significant reduction in the virus' cytopathogenic effect on Myrrh treated Vero cells[24]. The treated Vero cells may lead to a decrease in the production of IL-1B, which in turn can reduce the production of cytokines caused by HSV-1, such as IL6 and IL8[24]. This hypothesis is worthy of further investigation in future studies.

AT 4 hours of Myrrh treatment, the 0.5% is the only concentration that showed significant reduction in cytopathogenicity before and after virus infection. 2% and 1% concentrations of Myrrh resulted in insignificant reductions. This phenomenon could be due to prolonged exposure to high toxic concentrations of Myrrh Extract that led to normal cell death. This explains why in middle eastern countries, Myrrh extract is applied to the area surrounding the wound and prohibited over the legion.

One possible mechanism of Myrrh treatment on HSV-1 infected cells could be that myrrh blocked the virus entry to the Vero cell and this could explain the effect that myrrh treatment before infection shows a significant increase in cell survival in all tested concentrations. This effect could inhibit new progeny of virus to enter new Vero cells when myrrh treatment is used after infection. The other possibility could be Myrrh treatment has effects on metabolic activity, cell membrane integrity, and IL-1B stimulated production of IL-6 and IL-8 by that lead to cytotoxic effects on infected Vero cells.

V. Conclusion

Prolonged exposure to Myrrh at high concentration could be very toxic. Myrrh treatment of HSV-1 infected Vero cells led to significant increase in cell survival in all tested concentrations (2%, 1%, and 0.5%) 2hrs treatment before and after infection. At 4 hours treatment, 0.5% concentration of Myrrh caused a notable increase in cell survival of HSV-1infected Vero cells while 2% and 1% concentrations resulted in insignificant increase in cell survival of HSV-1.2 hrs before infection, cell survival of HSV-1 infected Vero cells using Myrrh treatment matched cell survival using ACV in all tested concentrations.

Application of Myrrh extract to HSV-1 infected Vero cells showed promising results in our experiment. Future studies should investigate the possibility of similar effects on human epithelial cell lines because of the close similarities between both tissues.

Myrrh treatment inhibits the secretion of pro-inflammatory cytokines of microphages[6]. It would be beneficial to examine Myrrh effects on HSV-1 infected microphages M1 and M2 by studying the differences in cytokine and interferon levels before and after Myrrh treatment. Myrrh treatment elevated leukocyte levels even in the absence of injury; this suggest that Myrrh acts as a foreign body and initiates an immune response[25]. Future studies can investigate Myrrh effects on both T and B lymphocytes in the HSV-1 infected model animal.

References

[1]. Kukhanova MK, Korovina AN, Kochetkov SN. Human herpes simplex virus: life cycle and development of inhibitors. Biochemistry (Mosc). 2014;79(13):1635-1652.
[2]. Alfonso-Dunn R, Arbuckle JH, Vogel JL, Kristie TM. Inhibition of the Super Elongation Complex Suppresses Herpes Simplex Virus Immediate Early Gene Expression, Lytic Infection, and Reactivation from Latency. mBio. 2020;11(3):e01216-20.
[3]. Lee S, Ives AM, Bertke AS. Herpes Simplex Virus 1 Reactivates from Autonomic Ciliary Ganglia Independently from Sensory Trigeminal Ganglia To Cause Recurrent Ocular Disease. J Virol. 2015;89(16):8383-8391.
[4]. Al-Dujaili LJ, Clerkin PP, Clement C, et al. Ocular herpes simplex virus: how are latency, reactivation, recurrent disease and therapy interrelated?. Future Microbiol. 2011;6(8):877-907.
[5]. Webre JM, Hill JM, Nolan NM, et al. Rabbit and mouse models of HSV-1 latency, reactivation, and recurrent eye diseases. J Biomed Biotechnol. 2012;2012:612316.

[6]. Kim JY, Mandarino A, Chao MV, Mohr I, Wilson AC. Transient reversal of episome silencing precedes VP16-dependent transcription during reactivation of latent HSV-1 in neurons. PLoSPathog. 2012;8(2):e1002540.
[7]. Yao HW, Ling P, Tung YY, Hsu SM, Chen SH. In vivo reactivation of latent herpes simplex virus 1 in mice can occur in the brain before occurring in the trigeminal ganglion. J Virol. 2014;88(19):11264-11270.
[8]. Gopal MG, Shannoma, Kumar B C S, M R, A S N, Manjunath NC. A comparative study to evaluate the efficacy and safety of acyclovir and famciclovir in the management of herpes zoster. J Clin Diagn Res. 2013;7(12):2904-2907.
[9]. Al-Subaie MM, Hosny KM, El-Say KM, Ahmed TA, Aljaeid BM. Utilization of nanotechnology to enhance percutaneous absorption of acyclovir in the treatment of herpes simplex viral infections. Int J Nanomedicine. 2015;10:3973-3985. Published 2015 Jun 15.
[10]. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. Antimicrob Agents Chemother. 2011;55(2):459-472.
[11]. Michaelis M, Kleinschmidt MC, Bojkova D, Rabenau HF, Wass MN, Cinatl J Jr. Omeprazole Increases the Efficacy of Acyclovir Against Herpes Simplex Virus Type 1 and 2. Front Microbiol. 2019;10:2790.
[12]. Naghian E, MarziKhosrowshahi E, Sohouli E, et al. Electrochemical Oxidation and Determination of Antiviral Drug Acyclovir by Modified Carbon Paste Electrode With Magnetic CdO Nanoparticles. Front Chem. 2020;8:689.
[13]. Vashishtha AK, Kuchta RD. Effects of Acyclovir, Foscarnet, and Ribonucleotides on Herpes Simplex Virus-1 DNA Polymerase: Mechanistic Insights and a Novel Mechanism for Preventing Stable Incorporation of Ribonucleotides into DNA. Biochemistry. 2016;55(7):1168-1177.
[14]. Pan, D., Kaye, S. B., Hopkins, M., Kirwan, R., Hart, I. J., & Coen, D. M. (2014). Common and new acyclovir resistant herpes simplex virus-1 mutants causing bilateral recurrent herpetic keratitis in an immunocompetent patient. The Journal of infectious diseases, 209(3), 345–349.
[15]. Omura, N., Fujii, H., Yoshikawa, T., Yamada, S., Harada, S., Inagaki, T., Shibamura, M., Takeyama, H., &Saijo, M. (2017). Association between sensitivity of viral thymidine kinase-associated acyclovir-resistant herpes simplex virus type 1 and virulence. Virology journal, 14(1), 59.
[16]. Shalaby MA, Hammouda AA. Analgesic, anti-inflammatory and anti-hyperlipidemic activities of Commiphoramolmol extract (Myrrh). J InterculEthnopharmacol. 2014;3(2):56-62.
[17]. Su S, Wang T, Duan JA, et al. Anti-inflammatory and analgesic activity of different extracts of Commiphoramyrrha. J Ethnopharmacol. 2011;134(2):251-258.
[18]. Wanner J, Schmidt E, Bail S, et al. Chemical composition and antibacterial activity of selected essential oils and some of their main compounds. Natural Product Communications. 2010 Sep;5(9):1359-1364.
[19]. Sarbaz, Z., Yazdanpanahi, Z., Hossainkhani, A., Nazari, F., &Akbarzadeh, M. (2019). The Effect of Sitz Bath of Hydro-Alcoholic Extract of Myrrh Gum on Episiotomy Wound Healing in Nulliparous Women. Journal of Family and Reproductive Health, 13(2), 89-97.
[20]. Bhattacharjee MK, Alenezi T. Antibiotic in myrrh from Commiphoramolmol preferentially kills nongrowing bacteria. Future Sci OA. 2020;6(4):FSO458.
[21]. Mohammed S. Alhussaini, A.M. Saadabi, Mohammed I. Alghonaim and Khalid Elfakki Ibrahim, 2015. An Evaluation of the Antimicrobial Activity of Commiphoramyrrha Nees (Engl.) Oleo-gum Resins from Saudi Arabia. Journal of Medical Sciences, 15: 198-203.
[22]. Abdul-Ghani RA, Loutfy N, Hassan A. Myrrh and trematodoses in Egypt: an overview of safety, efficacy and effectiveness profiles. Parasitol Int. 2009;58(3):210-214.
[23]. Li, H., Zhang, J., Kumar, A., Zheng, M., Atherton, S. S., & Yu, F. S. X. (2006). Herpes simplex virus 1 infection induces the expression of proinflammatory cytokines, interferons and TLR7 in human corneal epithelial cells. Immunology, 117(2), 167-176.
[24]. Tipton DA, Lyle B, Babich H, DabbousMKh. In vitro cytotoxic and anti-inflammatory effects of myrrh oil on human gingival fibroblasts and epithelial cells. Toxicol In Vitro. 2003 Jun;17(3):301-10.
[25]. Haffor AS. Effect of myrrh (Commiphoramolmol) on leukocyte levels before and during healing from gastric ulcer or skin injury. J Immunotoxicol. 2010;7(1):68-75.

Nancy Bigley. "Antiviral effects of Myrrh against herpes virus infection compare to Acyclovir." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(6), (2020): pp. 01-07.