Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Inhibitory effect on acute herpes and prevention of postherpetic neuralgia in herpes simplex virus-1-infected mice using a plant extract *Ricinus communis*

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Article Info

Article history:

Received: December 14, 2023
Accepted: October 26, 2024
Published: November 30, 2024

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Abstract

The present study aimed to examine the impact of *Ricinus communis* and valacyclovir (VACV) on the progression of skin lesions and pain responses in mice infected with herpes simplex virus type 1 (HSV-1). Mice were infected with HSV-1 and treated with *R. communis* (8, 16, or 48 mg/kg) or VACV (8, 25, or 90 mg/kg) twice daily on days 2–8 post-infection. Skin lesion development and pain-associated reactions were assessed 27 days after infection. HSV-1 infection results in zosteriform skin lesions and increased pain-related scores. Both *R. communis* and VACV demonstrated a dose-dependent reduction in skin lesions and pain-related ratings. The investigation also assessed the impact of the timing of *R. communis* and VACV administration on skin lesions and pain responses and found that lesion scores were significantly reduced when *R. communis* treatment was initiated on day 2 post-infection. Additionally, the inhibitory effects of *R. communis* and VACV on HSV-1 dissemination in the dorsal root ganglia (DRG) were studied. They showed a significant reduction in HSV-1 DNA replication number after the administration of both drugs. This study aimed to investigate the impact of *R. communis* and VACV on the expressed mRNA levels of pain-associated factors in the spinal cord of HSV-1-infected mice. The findings of this study demonstrated that *R. communis* therapy exhibited an inhibitory effect on pain-related factors. Overall, these findings suggest *R. communis* may have the potential to serve as a therapeutic agent for managing skin damage and pain-related responses caused by HSV-1.

Keywords: Inhibitory, Acute herpes, Neuralgia, Herpes virus-1-infected, Mice, *Ricinus communis*

1. Introduction

Herpes Simplex Virus (HSV) can arise from either type 1 (HSV-1) or type 2 (HSV-2). HSV-1 is an exceedingly transmissible virus that exhibits a global prevalence. [1, 2]. The transmission of this condition commonly occurs through the exchange of contaminated oral secretions, and it has the potential to result in more serious sequelae, including keratitis, encephalitis, or gingivostomatitis diseases. Opportunistic infections caused by the herpes simplex virus (HSV) have the potential to give rise to a range of cancers. In recent times, a number of synthetic nucleoside analogues have emerged. In recent times, certain synthetic nucleoside analogs [3] have been utilized as medications for combating herpes infections during the early phases. Nevertheless, it has been observed that they can facilitate the emergence of drug-resistant variants, especially among individuals with impaired immune systems. [4–6]. Hence, an increasing demand exists for the exploration of novel antiviral agents. Plant-derived pure chemicals have exhibited antiviral properties against the herpes simplex virus (HSV). The disclosed mechanism of action entails the inhibition of viral replication or viral genome synthesis. Herpes simplex virus type 1 (HSV-1), like numerous other viruses, employs glycosaminoglycans (GAGs) as the primary receptors for initial attachment when infecting host cells. The targeting of HSV-1 glycoproteins that interact with GAGs by polyphenols has been demonstrated, resulting in the prevention of their connection with cell surface GAGs and subsequent binding to receptors. The inhibitory impact has been observed in various contexts: (1) in cell-free viruses, (2) in the process of viral attachment and fusion, and (3) in the propagation of HSV-1 through intercellular junctions, which is facilitated by glycoproteins. [7–9]. Antiviral activity associated phytochemicals against HSV include alkaloids [11], flavonoids [10], saponins [12], quinones [14], terpenes [13], polysaccharides [13], lignans [15], tannins [16], steroidal glycosides [18], thiosulfinates [17], and proanthocyanidins [19].

Herpes zoster, a dermatological condition caused by the reactivation of the latent varicella-zoster virus (VZV) in sensory ganglia, is a viral skin illness [20. Acute herpetic pain (AHP) is a common symptom of herpes simplex virus infection, which manifests mostly as a vesicular rash with a unilateral dermatomal pattern [21,22] The pain

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.11.4

from a vesicular rash can sometimes linger for months or even years after the rash itself has healed. Postherpetic neuralgia (PHN) is a distressing ailment that is very common and difficult to manage in the elderly population, who often have more severe cases of herpes zoster as a result of their age [23, 24]. Peripheral neuropathy (PHN) has been found to have a negative impact on the overall quality of life and functional capacities of those affected by this condition. Common symptoms associated with PHN include exhaustion, lack of appetite, weight loss, decreased mobility, physical inactivity, and disruptions in sleep patterns [25]. The development of small-animal models that accurately replicate productive infections has been hindered by the high species-specificity of VZV, which only targets humans [26, 27]. Takasaki et al [27, 28] effectively developed a murine model of acute herpetic pain (AHP) and postherpetic neuralgia (PHN) by employing herpes simplex viruses (HSV-1; Alpha-herpesviridae) alongside varicella-zoster virus (VZV). The percutaneous introduction of HSV-1 led to the development of skin lesions resembling herpes zoster within the specific area of skin where the inoculation occurred. These lesions were followed by pain-related reactions, including acute herpetic discomfort. Despite the resolution of skin lesions, the mice in question persisted in exhibiting pain-related behaviors that resembled those observed in postherpetic neuralgia (PHN) [27, 29]. The present study aimed to examine the impact of *R. communis* and valaciclovir (VACV) on the advancement of cutaneous lesions and pain-associated reactions in mice infected with herpes simplex virus type 1 (HSV-1). Our study revealed that the administration of both *R. communis* and VACV at varying doses resulted in a significant reduction in skin lesion ratings and a notable alleviation of pain-associated reactions.

2. Materials and methods

2.1. Observe the antiviral medications

The delivery of antiviral medications involved the suspension of *R. communis*, an Egyptian plant, in a 0.60% (w/v) solution of Methyl Cellulose 350 obtained from the National Institute of Health (NIH) in Pakistan. This suspension was then enriched with 1% (w/v) Tween 80 obtained from the Laboratory Scientific Supplies (Private) Limited Karachi Branch, Pakistan. VACV obtained from Fujifilm Wako Pure Chemical Corporation was dispersed in a 0.70% (w/v) Methyl Cellulose 350 solution. The prescribed dose volume was 10 mL/kg body weight, and the experimental drugs were administered orally twice daily for 6 days. Pharmaceuticals were administered 2, 4, or 6 days after HSV-1 infection. For specific details on the administration schedule for each trial, please refer to Figures 1A and 3A.

2.2. The Intersection of Animals and Ethics

Male albino mice, 6 weeks old at the beginning of the experiment and weighing between 14 and 19 g during the trial, were obtained from the National Institute of Health (NIH) in Pakistan. These mice were group-housed with six mice per cage and subjected to behavioral experiments in an environment that maintained a controlled temperature (19–25°C) and humidity (40%–60%). The lighting schedule was set from 8:00 a.m. to 9:00 p.m. and the mice had unrestricted access to food and water. This study was conducted in compliance with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The Experimental Ethics Committee approved this study, and all procedures were carried out in strict accordance with the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" established by the National Institute of Health (NIH) in Pakistan. This study was approved by the Animal Care Committee of the National Institute of Health (NIH), Pakistan. Every possible effort was made to minimize animal suffering and to reduce the number of animals used in the study.

2.3. Herpes Simplex Virus Type 1 (HSV-1) Infection

The mice were subjected to HSV-1 infection in accordance with a previously established technique [9,10]. The subjects were administered sodium pentobarbital (69 mg/kg, intraperitoneally) for anesthesia (Haisco Pharmaceutical Group Co., Ltd.China). The caudal back, flank, and hind limbs were prepared for the experiment by shaving and depilating using a chemical depilatory (Guangzhou Biying Cosmetics Co., Ltd. China). After a period of three days, the epidermis of the right shin was subjected to abrasion using a 27-gauge needle. Following this, a 10-mL suspension of HSV-1 (7401H strain, containing 1 x 106 plaque-forming units) was administered to the affected area. The rear paw on the other side of the body did not become infected, and a sham procedure was performed using HSV-1 that had been rendered inactive through exposure to heat at a temperature of 60°C for a duration of 1 hour [9]. The evaluation of the intensity of skin lesions was conducted in the following manner: The scoring system for the presence of lesions is as follows: 0 indicates the absence of any lesions, 2 indicates the presence of one or two vesicles on the back, and 4 indicates the presence of multiple vesicles either on the back, around the infected area, or both. At a severity level of 6, individuals exhibit mild herpes zoster-like lesions. At a severity level of 8, they display evident zoster-like lesions, along with paw inflammation or a combination of both. Finally, at a severity level of 10, individuals experience severe zoster-like lesions [9, 10].

2.4. AHP and PHP Evaluation

After a minimum acclimatization period of 30 min, the plantar hind paw was subjected to a gentle stroking motion using a paintbrush [Artetje Brush Camlon Pro Plata 630 #4/0 Round; AMS Artist Materials Inc., Kurashiki, Japan] through the Amazon. Prior to the experiment, the hair on the brush was cut, leaving approximately 10 pieces of hair intact [10]. The rankings of responses to stroking stimulation were as follows: the scoring system for assessing the response and movement of the stimulated paw was as follows:0 indicates no observable response or movement; 1 indicates the elevation of the stimulated paw towards the abdomen; and 2 indicates the occurrence of flinching or licking of the stimulated hind paw. The stimulation was applied six times, with each instance separated by several seconds. The pain-related scores from day 4 to day 16 post-infection (pi), or from day 18 to day 27 pi, were designated as AHP and PHP, respectively. This classification

was based on the observation that the skin lesion score reached its highest point around day 9 pi, and gradually decreased by day 18 in mice infected with HSV-1.

2.5. Histopathological analysis

On the 30th day after infection, the mice were subjected to anesthesia with a dosage of 70 mg/kg of sodium pentobarbital. Subsequently, they were permeated via the heart with a 4% paraformaldehyde phosphate buffer solution (Fujifilm Wako Pure Chemical Corporation). Following perfusion fixation, the excision of the dorsal root ganglion (DRGs) at the level of 1.5 and the placed level of spinal cord (SC) at the L5 was performed. Subsequently, both sets of samples were immersed in paraffin and divided into 2-mm slices. All these sections were subjected to staining using hematoxylin and eosin. (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) and produce the images at x100 magnification with the help of the cellSens imaging software (Olympus Corporation, Tokyo, Japan).

2.6. HSV-1 DNA Copy Levels in Dorsal Root Ganglia (DRGs)

DNA was collected from the ipsilateral dorsal root ganglia (DRGs) located at the L4-L6 spinal levels of mice infected with HSV-1 on days 4 and 8 post-infection (pi). The extraction was performed using a QIAamp DNA Mini Kit manufactured by Qiagen, a company based in Hilden, Germany. The research team used quantitative polymerase chain reaction (qPCR) experiments in order to quantify the copy number of herpes simplex virus type 1 (HSV-1) DNA. These assays were carried out according to a procedure that had been previously documented [31]. The forward primer (GbTypF: 5'-cgcatcaagaccacctcctc-3'), reverse primer (HSV1 and 2-R: 5'-agcttgcgggcctcgtt-3'), and probe (HSV1-probe: 5'-cggaacatatcgttgacatggc-3') were employed in accordance with the established methodology. [31]. The qPCR experiment was conducted using the Step-One-Plus Real-Time PCR instrument manufactured by Thermo Fisher Scientific Inc. The cycling conditions for the experiment conducted in Massachusetts, USA were as follows: an initial incubation period of 2 minutes at a temperature of 50°C, followed by denaturation at 95°C for a duration of 10 minutes. This was succeeded by a series of 45 cycles, each consisting of denaturation at 95°C for 15 seconds, and annealing/extension at a temperature of 58°C for 30 seconds. The analysis of all samples was conducted in duplicate. In order to build a standard curve, a series of dilutions were generated using the HSV-1 quantified plasmid DNA, with each dilution being ten times more diluted than the previous one. The standards were subjected to triplicate analysis and utilized as both a standard curve and positive control for every quantitative polymerase chain reaction (qPCR) assay.

2.7. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). To assess the significance of variances between the two groups, Student's or Aspin-Welch's t-test followed by an F-test was employed, except for viral load, which was evaluated using the Wilcoxon test. Dunnett's multiple comparison test was used to compare three or more groups. Statistical significance was set at $P < 0.05$. Statistical analyses were conducted using EXSUS software (CAC Croit Corporation, Tokyo, Japan).

3. Results

3.1. Ricinus communis and VACV investigation on mice

Mice were infected with HSV-1 following the procedure outlined in the Materials and Methods. They were then administered *R. communis* at doses of 8, 16, or 48 mg/kg twice daily, or VACV at 8, 25, 45, or 90 mg/kg doses twice daily, from days 2 to 8 post-infection (pi). The evaluation of skin lesion formation and pain-associated reactions was conducted 28 days following HSV-1 infection. (Fig. 1A). The inoculation of HSV-1 in the hind paw resulted in the emergence of skin lesions resembling herpes zoster inside the appropriate dermatome, beginning on the eighth-day post-inoculation. The skin lesion score reached its maximum value at approximately day 8 post-infection (pi) and thereafter declined by day 18 pi. (Fig. 1B–D). Additionally, the pain-associated score in mice infected with HSV-1 exhibited an initial increase on day 2 post-infection (pi) and reached its highest point on around day 8 pi. The pain-related score remained elevated until day 27 post-infection, even after the skin lesions of mice infected with HSV-1 had healed. The administration of both *R. communis* (at doses ranging from 8 to 48 mg/kg) and VACV (at doses ranging from 8 to 90 mg/kg) resulted in a reduction in the skin lesion score in a dose-dependent manner as compared to the corresponding vehicle groups. (Fig. 1C and D). In addition, it was observed that the administration

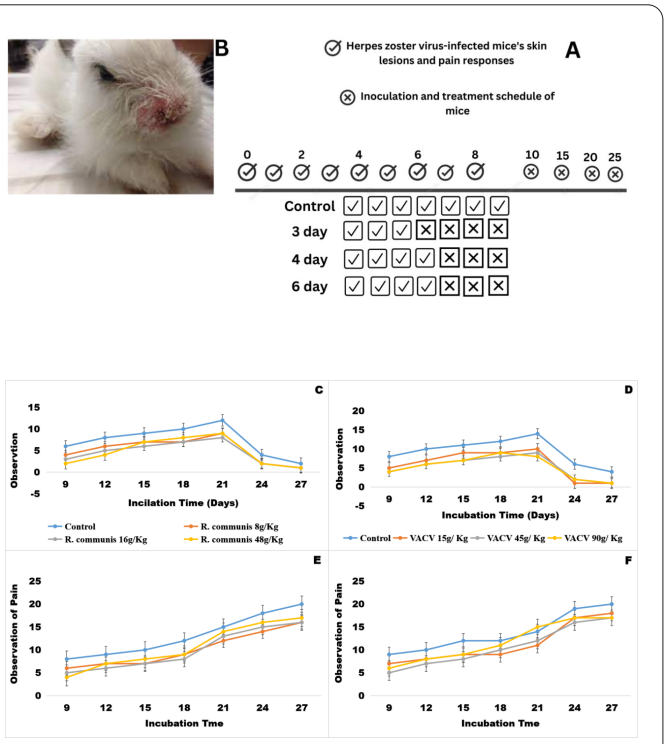

Fig. 1. Valaciclovir (VACV) and *R. communis* effects on herpes zoster virus-infected mice's skin observation and pain responses. (A) Study inoculation and treatment schedule schematic. Zoster virus was percutaneously implanted into mice, and VACV, *R. communis,* and their vehicle were given orally from days 2 to 6. Daily "bid" means twice. (B) mice, herpes zoster-like skin observation are typical. (C, E, F) The time histories of skin lesion scores (C and D) and pain-related scores (E and F) in VACV or *R. communis* treated mice are displayed. The mean pain-related score in heat-inactivated chickenpox zoster virus inoculated (-ve) control mice is shown by the dotted line. Drug oral administration start dates. Statistical significance of the comparison between $*p < 0.05$ and $**p < 0.01$ when compared to the vehicle group.

of *R. communis* at doses ranging from 8 to 48 mg/kg, as well as Vaccinia virus at doses ranging from 8 to 90 mg/kg, resulted in a significant reduction in pain-related ratings. This effect was shown to be dependent on the dosage administered. (Fig. 1E and F). In order to examine the impact of *R. communis* (48 mg/kg) and VACV (90 mg/kg) on the histological alterations in the dorsal root ganglia (DRGs) and spinal dorsal horn in mice infected with HSV-1, tissue samples were collected on day 27 post-infection (pi). Subsequently, the tissue slices were subjected to hematoxylin and eosin staining. Figures 2A and B illustrate the dorsal root ganglia (DRGs) and spinal dorsal horn in mice that were infected with heat-inactivated HSV-1. Evidence of inflammatory cell infiltration, degradation of nerve fibers, and vacuolation in the white matter was observed in the dorsal root ganglia (DRG) and spinal dorsal horn of mice infected with HSV-1. (Fig. 2C). The aforementioned pathological alterations were not detected in the dorsal root ganglia (DRGs) and spinal dorsal horn of mice infected with HSV-1 and subjected to treatment with *R. communis* or VACV, as illustrated in Figure 2D. (Fig. 2E).

3.2. Ricinus communis and VACV administration

To assess the influence of the timing of *R. communis* and VACV administration on skin lesions and pain-related responses in mice infected with HSV-1, we administered 48 mg/kg dose of *R. communis* and 90 mg/kg dose of VACV to HSV-1-infected mice for five consecutive days, starting on days 4, 5, or 6 post-infection (pi). The cumulative effects are graphically represented as the area under the curve from day 2 pi, as illustrated in Figure 3A. Notably, a significant reduction in lesion scores was observed exclusively in the group receiving *R. communis* treatment starting from day 2 pi (Figure 3A). During the assessment of pain-related responses in the AHP phase, The application of *R. communis* resulted in a notable decrease in pain levels when delivered on either day 2 or 4 post-infection (pi). In contrast, the reduction in pain scores was observed with the administration of VACV only on day 2 pi. (Figure 3B). A comparable pattern was noted during the PHP period. (Figure 3C).

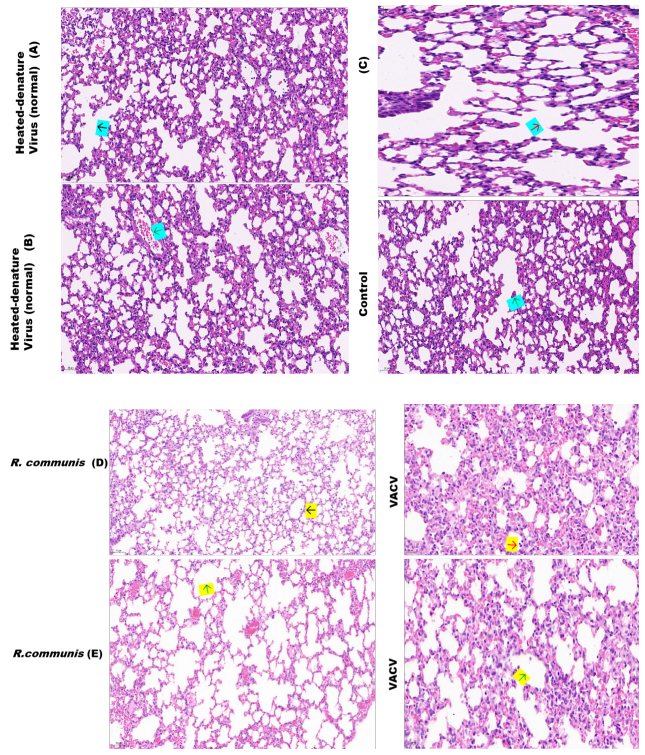

Fig. 2. (Magnification scale is 200x). Tissue samples from the dorsal root ganglion, L5 and spinal cord of mice treated with *R. communis* or valaciclovir (VACV). Dorsal root ganglia, L5 and spinal cord were obtained from mice infected with herpes simplex virus type-1 27 days post-infection. The treatment regimen involved oral administration of 48 mg/kg *R. communis*, 90 mg/kg VACV, or the vehicle *R. communis* twice a day for 4 days, starting from days 4 to 8 post-infection. Hematoxylin and eosin staining was performed on dorsal root ganglia (A) and spinal cord (B) samples from mice infected with heat-inactivated virus. The provided sections (C, F, E) display representative colour images depicting the hematoxylin and eosin (H&E) staining of the dorsal root ganglion.

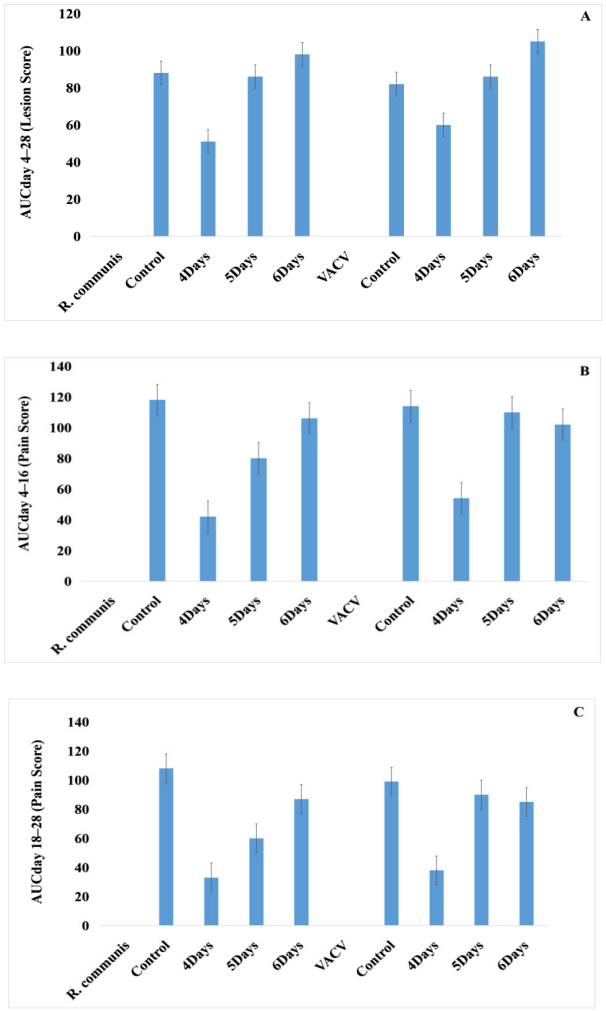

Fig. 3. In herpes simplex virus-1-infected mice, initiation timing of *R. communis* and valaciclovir (VACV) treatment affects skin blisters and pain responses. After percutaneous herpes simplex virus-1 inoculation, mice received oral 48 mg/kg *R. communis* or (90 mg/kg VACV for 4 days starting on 4, 5, or 6 post-infection days. The Control group was subjected to oral delivery twice daily from to 8 post-infection days,when neither medication was given. Bid indicates administration twice a day.

3.3. Examination of *R. communis* and VACV in mice

The findings illustrated in Figure 3 indicate that *R. communis* exhibited significant suppression of acute herpetic pain (AHP) and post-herpetic pain (PHP) in mice infected with HSV-1. This effect was observed even when RC was supplied starting from day 4 after infection. Conversely,

VACV did not show a similar inhibitory effect. In order to provide evidence for the reported differences in the ability to limit virus proliferation in dorsal root ganglia (DRGs) between *R. communis* and vaccinia virus (VACV), the quantity of viral DNA present in the DRGs of mice infected with herpes simplex virus 1 (HSV-1) was measured using real-time polymerase chain reaction (PCR). Antiviral drugs such as, 48 mg/kg *R. communis*, 90 mg/kg VACV, or a vehicle were administered orally for 6 days (starting from day 2–8 pi). DRG samples were collected on day 6 (when the highest peak of the HSV-1 was observed) and 9 pi [9]. Comparisons revealed a significant reduction in no copies of HSV-1 DNA in DRGs following administration of *R. communis* or VACV compared to the respective vehicle on both days (Fig. 4). It is worth mentioning that the DNA copy number of HSV-1 in the dorsal root ganglia (DRGs) of the *R. communis* group was considerably lower on day 6 post-infection (pi) compared to the VACV group. Nevertheless, on the ninth-day post-infection (pi), there was an absence of noticeable disparity in the copy quantity of HSV-1 DNA within the dorsal root ganglia (DRGs) among the two experimental groups.

3.4. Ricinus communis and VACV affect on mice with HSV-1

In order to provide a more comprehensive understanding of the differential inhibitory effects on afterhyperpolarization (AHP) and the transition to persistent hindlimb paralysis (PHP) between *R. communis* and vaccinia virus (VACV) when treatment was initiated on day 4 after infection, we conducted an investigation into the messenger RNA (mRNA) expression levels of activating transcription factor 3 (ATF-3), tumor necrosis factor-alpha (TNF-α), and cyclooxygenase-2 (COX-2) in the spinal cord (SC) samples obtained from the individual mice used for quantifying viral load in the dorsal root ganglia (DRGs). These expression levels exhibited marked increases in each control group of HSV-1-infected mice compared to those in the uninfected control group (Fig. 5). The group treated with *R. communis* displayed a trend toward suppression of ATF-3 and TNF-α expression in the spinal cord on day 4 pi than to the vehicle control group, whereas the VACV-treated group did not show a similar effect (Fig. 5A and B).

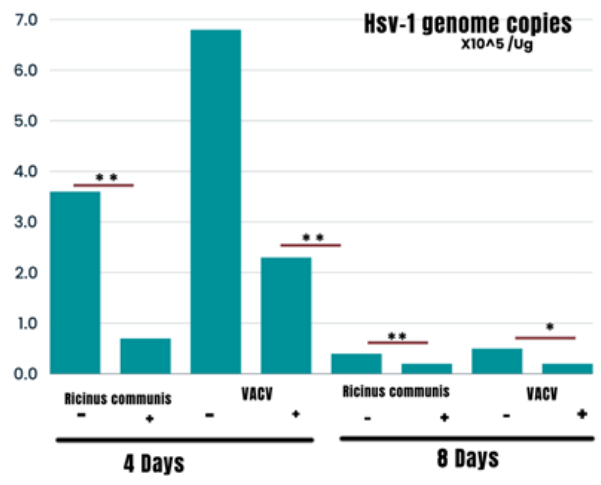

Fig. 4. Impact of valaciclovir (VACV) and *R. communis* on the levels of herpes simplex virus (HSV)-1 DNA copies within the dorsal root ganglion of mice infected with HSV-1.

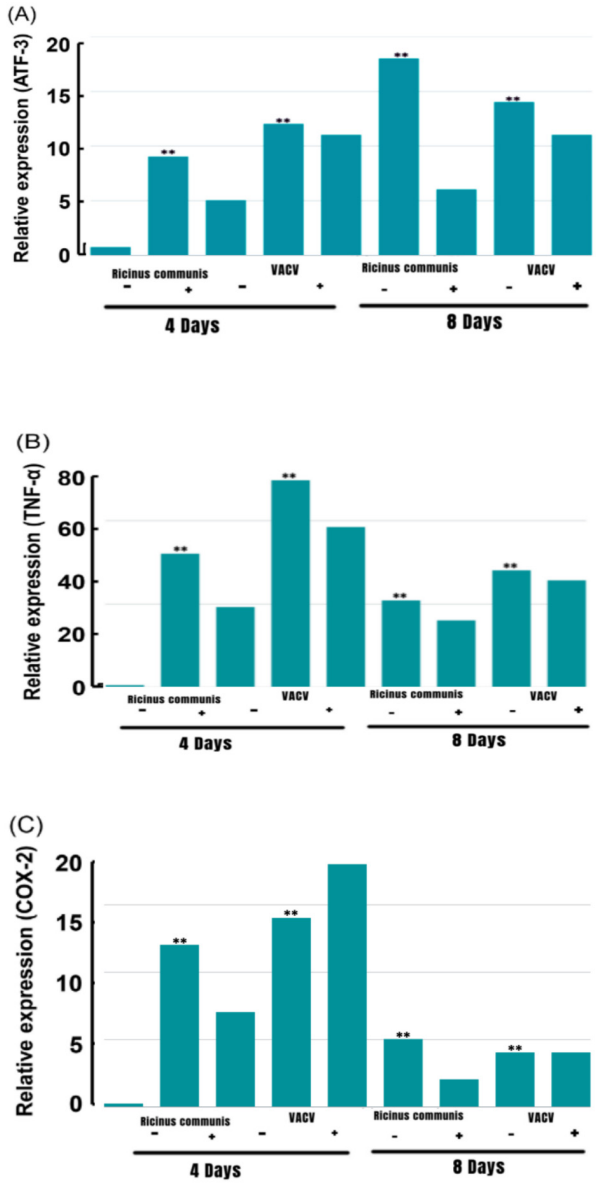

Fig. 5. Effects of valaciclovir (VACV) and *R. communis* on mRNA expression of pain-related factors in the spinal cord of mice infected with herpes simplex virus (HSV)-1 were studied. The mice were percutaneously inoculated with HSV-1 and then treated with *R. communis* (48 mg/kg), VACV (90 mg/kg), or vehicle twice daily for either 2 or 4 days, starting from day 4 post-infection. Spinal cord samples were collected on days 4 and 8 post infection. The mRNA expression of Activating transcription factor 3 (A), Tumour Necrosis Factor alpha (B), and Cyclooxygenase-2 (C) in the spinal cord of HSV-1-infected mice was quantified using. $^{**}p < 0.01$ when compared with naive. $^{*}p < 0.05$, compared to the vehicle control.

Furthermore, on days 4 and 8 pi COX-2 expression was significantly inhibited by *R. communis* but not with VACV administration (Fig. 5C).

4. Discussion

This work presents study indicate that *R. communis* demonstrates greater effectiveness in treating acute herpetic pain (AHP) and postherpetic pain (PHP) in persons with herpes zoster, as compared to *R. communis*. Oral nucleotide analogs, including aciclovir, vaccinia virus, and famciclovir, have demonstrated substantial efficacy in the treatment of immunocompetent individuals with herpes

zoster. These medications have been shown to dramatically decrease the incidence of postherpetic neuralgia (PHN) in randomized controlled clinical trials. [32]. In phase 5 clinical trial, the efficacy of *R. communis* at a dosage of 400 mg administered once daily for a duration of 6 days was found to be comparable to that of valaciclovir at a 900 mg dose administered thrice a day (with a daily dose of 2700 mg) for a duration of 7 days. This study specifically focused on the treatment of immunocompetent Japanese patients with herpes zoster, evaluating the effectiveness of these two medications for the treatment of new lesion formation and its resulting pain [37]. We found that *R. communis* and vaccinia virus prevent zosteriform-like skin lesions, AHP, PHP, and histological alterations in HSV-1-infected mice (Figs. 1 and 2). These findings imply that HSV-1-infected models can predict antiviral medication inhibitory effects on AHP and PHN in herpes zoster patients. When given within 48–72 hours of rash development, oral nucleotide analogs can speed up acute herpes zoster healing and prevent or treat AHP and PHP [33]. The above data emphasize the need for early PHP prevention. Thus, we investigated the effects of timing of *R. communis* administration at 48 mg/kg on after-hyperpolarization (AHP) and post-herpetic pain (PHP) in HSV-1-infected mice. A 90 mg/kg VACV dosage was used to compare RC's effects. When therapy began on the 4th day or 6th day post-infection (pi), *R. communis* inhibited both abortive hairpin (AHP) development and productive hairpin (PHP) formation. vaccinia virus was effective when administration was initiated from day 4 pi. (Figure 3). In addition, *R. communis* inhibited skin lesion growth when supplied alone for 3 post-infection (pi) days, similar to Vaccinia virus. The different index lengths may explain the difference in *R. communis* inhibitory effects on pain-related ratings and skin lesion scores when therapy began on day 4 post-infection (pi). This model showed that the skin lesion score increased till day 6 post-infection (pi) and resolved by day 18 pi. However, the pain-related score peaked on day 6 pi and remained high until day 27 pi. Oral *R. communis* from day 4 dramatically reduced skin lesion score in severely HSV-1-infected mice but had no effect on mice infected with VACV, as reported by Katsumata et al.[34]. In this severe condition, HSV-1 infection in a large dorsolateral area caused severe cutaneous lesions that persisted from days 2 to 18 pi. *R.* communis may be more successful in treating herpes zoster skin lesions or pain if treatment is delayed. We investigated the viral load present in the dorsal root ganglia (DRGs) at two specific time points, namely day 2 and day 8 post-infection (pi). Our objective was to understand the mechanism via which *R. communis* inhibits the development of acute herpetic pain (AHP) and post-herpetic pain (PHP) in mice infected with herpes simplex virus type 1 (HSV-1). In a study conducted by Takasaki et al. [9], it was shown that the viral load of HSV-1 in the dorsal root ganglia (DRGs) of mice reached its highest point on the fifth-day post-infection (pi) and subsequently decreased by the eighth-day pi. Thus, we examined DRG samples on 4 pi day after 1 day of therapy or 8 pi day after 5 days. As illustrated in Fig. 4, both *R. communis* and vaccinia virus significantly diminished viral replication of HSV-1 viral in DRGs than to vehicle controls, but only for AHP and PHP when RC was administered on 4 pi day. On the 5th pi day, the *R. communis* treated group had a considerably lower HSV-1 virus load than the vaccinia virus group. By day 4 pi, DRG viral load may be crucial in reducing AHP and PHP. Helicase-primase inhibitors like *R. communis* directly inhibit HSV's single-stranded DNA-dependent ATPase, primase, and helicase [35]. On the other hand, nucleotide analogs, such as aciclovir, undergo phosphorylation through the action of thymidine kinase generated from the herpes simplex virus (HSV) and cellular kinase present in the host. This process leads to the formation of aciclovir triphosphate, which then irreversibly bind to viral DNA polymerase. Consequently, the binding of aciclovir triphosphate inhibits the synthesis of herpes simplex virus genomic DNA. The replication cascade of herpesvirus genomic DNA involves the unwinding of double-stranded DNA by helicase, accompanied by the synthesis of primers by primase before DNA polymerization [36]. Our findings align with time-dependent increases in ATF-3, TNF-α, and COX-2 mRNA expression in SC. Axonal damage from VZV or HSV infection induces ATF-3, a neuronal marker of nerve injury [37]. Increased DRG ATF-3-positive neurons are positively linked with pain behavior [38]. Javed et al. found that TNF-α inhibitors may reduce the clinical incidence of PHN [39]. Therefore, ATF-3, TNF-α, and COX-2 have been proposed as valuable markers for the identification of acute hepatic porphyria (AHP) progression and the transition to porphyria hepatica (PHP). AHP development and PHP transition are aided by COX-2 and prostaglandin E2 upregulation in HSV-infected mice' DRGs on day 4 or 6 pi [40]. The administration of VACV did not provide any statistically significant impact on the upregulation of ATF-3, TNF-α, and COX-2 expression. However, it did considerably decrease the viral load on 2 and 8 post-infection days. Hence, the decrease in ATF-3, TNF-α, and COX-2 expression in the spinal cord (SC) may have implications for the alleviation of pain-associated behavior in mice infected with HSV-1. Collectively, the results of this study suggest that *R. communis* may have potential therapeutic benefits for managing acute herpetic pain (AHP) and postherpetic neuralgia (PHN) in individuals diagnosed with herpes zoster.

5. Conclusions

The impact of *Ricinus communis* and valaciclovir (VACV) on the progression of skin lesions and pain responses in mice infected with HSV-1 was investigated. Both RC and VACV administration led to a reduction in skin lesion scores and pain-related scores in a dose-dependent manner. Histological analysis showed that RC and VACV treatment prevented inflammatory cell infiltration, nerve fiber degradation, and vacuolation in the dorsal root ganglia (DRGs) and spinal dorsal horn of infected mice. Testing different initiation times for RC and VACV administration revealed that *R. communis* treatment initiated from day 2 post-infection significantly reduced lesion scores and pain levels, whereas VACV showed similar effects only when administered on day 2 post-infection. Examination of viral proliferation in DRGs indicated that RC significantly reduced HSV-1 DNA copies compared to VACV, particularly on day 6 post-infection. Analysis of mRNA expression levels in the spinal cord showed that RC treatment suppressed ATF-3, TNF-α, and COX-2 expression, indicating its potential role in inhibiting pain-related factors compared to VACV.

Funding
This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Project No. GrantA414].

Acknowledgment
The authors are grateful for the support of the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Project No. GrantA414].

Consent for publication
Not applicable.

Availability of data and materials
Not applicable.

Competing interests
The authors declare no competing interests.

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