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Antiviral activity of mangiferin from the rhizome of *Anemarrhena asphodeloides* against herpes simplex virus type 1

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ABSTRACT

Objective: To evaluate the antiviral activity of pure compounds against herpes simplex virus type 1 (HSV-1) from the rhizome of *Anemarrhena asphodeloides*.

Methods: Bioassay-guided isolation was conducted to separate the active compound and its chemical structure was elucidated by spectral analysis. *In vitro* antiviral efficacy of active compound was detected by Cell Counting Kit-8 assay, plaque reduction assay, and fluorescence observation. RT-PCR was used to determine the viral load and the cytokine-related gene expression after HSV-1 infection. *In vivo* study was also conducted to further determine antiviral efficacy of an active compound against HSV-1.

Results: An active compound was isolated and elucidated as mangiferin. Mangiferin significantly inhibited the replication of HSV-1 in Vero cells with a half maximal inhibitory concentration (IC_{50}) of 64.0 mg/L. Time-of-addition and time-of-removal assays demonstrated that mangiferin could effectively inhibit the replication of HSV-1 in the early stage (8 h). *UL12*, *UL42*, and *UL54* gene expression levels of HSV-1 in the 64 mg/L mangiferin-treated group were markedly reduced as compared with the HSV-1 group (P<0.01). Fluorescence observation showed that mangiferin attenuated the mitochondrial damage maintaining ΔΨm induced by HSV-1 in Vero cells. The expression of inflammatory factors *TNF-α*, *IL-1β*, and *IL-6* was remarkably increased in the virus-infected group as compared with that in the normal group (P<0.05), the levels of these inflammatory factors dropped after treatment with mangiferin. Mangiferin significantly decreased the viral load and attenuated the HSV-1-induced up-regulation of *TNF-α*, *IL-1β*, and *IL-6*. The relative protection rate of HSV-1-infected mice could reach up to 55.5% when the concentration of mangiferin was 4 g/kg.

Conclusions: Mangiferin exhibits promising antiviral activity against HSV-1 *in vitro* and *in vivo* and could be a potential antiviral agent for HSV-1.

KEYWORDS: *Anemarrhena asphodeloides*; Herpes simplex virus; Antiviral activity; Mangiferin

1. Introduction

Herpes simplex virus type 1 (HSV-1) is a lifelong infection in humans and could result in life-threatening consequences, especially for immunocompromised people. The WHO reported that about 66.6% of humans (under 50 years old) were infected with HSV[1,2]. Recent research also revealed that HSV was a prolegomena cause of encephalitis, dermatitis, and genitourinary infections, it was also reported that long-term HSV infection may result in cervical cancer[3]. At present, the most effective means of prevention

Significance

Herpes simplex virus type 1 (HSV-1) is widely distributed in the world and could result in life-threatening consequences, novel alternative antiherpetic drugs against HSV-1 with high activity and low toxicity are urgently needed. We isolated an active compound from the rhizome of *Anemarrhena asphodeloide*. Mangiferin exhibits promising antiviral activity against HSV-1 *in vitro* and *in vivo* and could be a potential antiviral agent for HSV-1.

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and control of herpes virus infection was drug therapy, and the most widely used drug were acyclovir, valacyclovir, famciclovir, penciclovir, and cidofovirare[4]. However, extensive use of drugs and a lack of other effective drugs may lead to the emergence of resistant viruses[5,6]. Field *et al* found that about 5% of immunocompromised patients were drug-resistant[7], as well as children[8]. Therefore, novel alternative antiherpetic drugs with high activity and low toxicity are urgently needed[6].

Recently, more and more research has been made to search for a more effective and safe drug to treat HSV. Utilization of traditional plant-based medicines has become the antiviral drug research hotspot in recent years because natural plants possess a wide range of biological and pharmacological activities with low toxicity[9,10]. Plenty of natural compounds that showed effective antiherpetic properties from medicinal plants were isolated[11,12]. Houttuynoid A exhibited antiviral activities against HSV-1 and was separated from *Houttuynia cordata* Thunb[13]. Monogalactosyl diglyceride and digalactosyl diglyceride isolated from *Clinacanthus nutans* also showed their bright application prospect as anti-HSV agents[14].

Anemarrhena asphodeloides (*A. asphodeloides*) Bunge is a kind of perennial herbaceous medicinal plant traditionally used in clinical healthcare in China. A large number of studies have shown that it contains lots of physiologically active substances such as steroid saponins[15,16], diphenylpyrones[17], flavonoids[18], organic acids, lignans[19], and polysaccharides[20]. Pharmacological research revealed that *A. asphodeloides* has biological activities such as anti-tumor, anti-senile dementia, improving learning and memory ability, anti-coagulation, anti-thrombus, anti-fungal, hypoglycemic effect, antihypertension and anti-depression[21,22]. However, little information on *A. asphodeloides* against the virus was reported.

In the present study, antiviral-guided separation was conducted to isolate antiherpetic compounds from *A. asphodeloides*. Additionally, the antiviral mechanism of the active compound was also assayed.

2. Materials and methods

2.1. Cells, viruses, and animal

Vero cells (ATCC No. CCL81, Bena Biotechnology Co., LTD) were grown at 37℃ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. HSV-1 maintained in our lab was propagated on Vero cells.

A total of 130 female BALB/c mice [(22.3±2.1) g] were obtained from the Animal Center of Zhejiang University and were kept in an IVC experimental animal breeding system. Breeding environment conditions were controlled by the Heating, Ventilating, and Air Conditioning system as follows: temperature, (22±1)℃; humidity, (60±5)%.

2.2. Plant material

The rhizomes of *A. asphodeloides* Bunge were collected from Bozhou in Anhui province and identified by professor Zhang from the College of Traditional Chinese Medicine, China Pharmaceutical University.

2.3. In vitro bioactivity–guide isolation

2.3.1. Active fraction of A. asphodeloides

Dried rhizomes of *A. asphodeloides* were extracted with petroleum ether, chloroform, ethyl acetate, and *n*-butanol, respectively 3 times and then concentrated using a rotary vacuum evaporator. The dried extracts were dissolved in dimethyl sulfoxide and then diluted into different concentrations for *in vitro* experiments. The extracts with the highest anti-HSV-1 activity were then subjected to a macroporous resin column and medium-pressure liquid chromatography.

2.3.2. Isolation of active compound

Ethyl acetate extract demonstrated the most significant anti-HSV-1 activity and was then subjected to further separation. Briefly, 580 g ethyl acetate extract was subjected to D101 macroporous resin column and eluted with a solvent mixture of ethyl alcohol and water (4∶6, *v*∶*v*), resulting in 5 fractions (Fr A-Fr E). Antiherpetic activity of the 5 fractions was determined by *in vitro* test. Fr D showed the most significant antiviral activity and was then subjected to medium-pressure liquid chromatography, successively eluted with ethyl alcohol and water (1∶9; 3∶7, 5∶5, 7∶3, 9∶1; *v*∶*v*). Fractions with antiherpetic activity were subject to further isolation, fractions or compounds with no antiherpetic activity were deleted, and continuous and repeated separation led to compounds with high antiherpetic activity.

2.3.3. Cytotoxicity assay

Vero cells were cultured in 24-well plates at 2.0×10^4 cells per well and incubated for 24 h to form monolayers. Concentrations of extracts, fractions, or compounds isolated from *A. asphodeloides* were prepared and then added to plates. The cytotoxicity of extracts, fractions, or active compounds was determined by Cell Counting Kit-8 (Beyotime, China). The 50% cytotoxic concentration (CC_{50}) of extracts, fractions, or active compounds was also assessed.

2.3.4. Antiviral activity

Vero cells at 2.0×10^4 were cultured into 24-well plates as described in cytotoxicity assay, HSV (2×10^3 $TCID_{50}$) was added and incubated for 1 h, then the culture medium containing the virus was removed, the DMEM (supplemented with 2% fetal bovine serum) containing various concentrations of crude extract, fractions, and compounds were added into each well. After being cultured for 48 h, the plaque reduction assay was conducted as described by Li *et al*[13]. Briefly, the cells were fixed with 10% formaldehyde for 10 min, then stained

with 1% crystal violet for 20 min, and finally washed with double steaming water 6 times. The 50% inhibition concentration (IC_{50}) was calculated as the concentration (crude extract, fractions, and compounds) at which HSV-1 was inhibited by 50%. The viral titers were also determined by the Karber method.

2.4. Chemical structure identification of the antiherpetic compounds

Chemical structure was determined by assaying the data of mass spectrometry, ^{1}H-NMR, and ^{13}C-NMR from the active compound as described by Han *et al*[21].

2.5. In vitro antiviral activity of mangiferin against HSV-1

2.5.1. Time-of-addition (TOA) and time-of-removal (TOR)

To determine which stage of the HSV-1 replication was affected by mangiferin, we conducted the TOA and TOR experiments. Monolayer Vero cells were cultured and then 1×10^{5} $TCID_{50}$ of HSV-1 was placed into each well. After incubation for 1 h, HSV-1 virus was removed, and DMEM was used to wash the cells. Mangiferin at 64 mg/L was added into 12-well plates at 0, 2, 4, 6, 8, 10, 14, 18, and 24 h post-infection. For TOR, HSV-1-infected cells were exposed to 64 mg/L mangiferin at 0 hour post injection (hpi). After incubation for 0, 2, 4, 6, 8, 10, 14, 18, and 24 h, the medicine medium was removed and a complete DMEM medium was added.

After 48 h post incubation, all samples were collected and the expression level of *UL12*, *UL42*, and *UL54* genes of HSV-1 was assayed. Briefly, after incubation, Vero cells were frozen in liquid nitrogen for subsequent RNA isolation. Total RNA was extracted using an E.Z.N.A. stool RNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Reverse transcription (RT) reactions were performed using the 5× All-In-One MasterMix (Applied Biological Materials, Richmond, Canada) in 20 μL reaction volume containing 2 μL of total RNA. Relative expression levels of *UL12*, *UL42*, and *UL54* were calculated (Primers are listed in Supplementary Table 1).

2.5.2. Fluorescence observation for nucleus damage

Vero cells were seeded into 24-well cell plates to form monolayers. Subsequently, 4 well cells were set as a group. Group A was incubated with HSV-1, Group B was incubated with HSV-1 and mangiferin, Group C was incubated with mangiferin, and Group D was incubated without HSV-1 and mangiferin. After incubation for 48 h, the culture fluid in the four groups were removed and then washed with phosphate buffer saline (PBS) 3 times. The cells were dyed with DAPI (Beyotime, China) for 20 min and then were observed on a fluorescence microscope.

2.5.3. Mitochondrial transmembrane potential ($\Delta\psi m$)

Vero cells were treated as mentioned in 2.5.2. After being washed with PBS, Vero cells were stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolecarbocyanide iodine (JC-1; Beyotime, China) according to the protocol of JC-1 kit and then were observed on a fluorescence microscope.

2.5.4. Cytokine production of Vero cell after treatment with mangiferin

Monolayer Vero cells were seeded. Then 1×10^{4} $TCID_{50}$ of HSV-1 were placed into each well and removed after 8 h incubation, and various concentrations (8, 16, 32, and 64 mg/L) of mangiferin were placed into 24-well plates. After incubation for 48 h, Vero cells were frozen in liquid nitrogen for subsequent RNA isolation. Real-time PCR was used for quantification using primers specific for *TNF-α*, *IL-1β*, and *IL-6*.

2.6. In vivo antiviral activity of mangiferin against HSV-1

In vivo antiviral activity of mangiferin against HSV-1 was assessed according to the method of our previous study. BALB/c mice were randomly divided into five groups. Group Ⅰ was the blank control group, mice were fed with a basal diet injected with PBS. Group Ⅱ was the virus control group which was fed a basic diet but challenged with HSV-1 (intracerebrally with 20 μL 1×10^{6} $TCID_{50}$/0.1 mL HSV-1). Group Ⅲ-group Ⅴ was the mangiferin-treated group. All the mice were challenged with 20 μL 1×10^{6} $TCID_{50}$/0.1 mL HSV-1, after 2 h infection, mice in group Ⅲ, group Ⅳ, and group Ⅴ were intraperitoneally injected with 1, 2, and 4 g/kg mangiferin for 5 d, respectively. On days 0, 1, 3, 5, and 7, the tissue samples (brain and ganglion) were collected and the expression level of *UL54* genes of HSV-1 was assayed. The gene expressions of *TNF-α*, *IL-1β*, and *IL-6* were also determined. The mortality of each group was observed daily and the protection rate was calculated.

2.7. Ethical statement

All the animal research was conducted following the internationally accepted principles and guidelines for Care and Use of Huzhou Hospital of Traditional Chinese Medicine Affiliated with Zhejiang University of Traditional Chinese Medicine (ZUTCM). All procedures and protocols were approved by the ZUTCM Animal Care and Use Committee (ZJ.ZUTCM.REC.203).

2.8. Statistical analysis

Experimental data were analyzed using SPSS for Windows, V.12.0 (IBM, Chicago, Ill, USA), and one-way ANOVA was used for analysis. All data were presented as mean ± standard error. Data were considered significant at $P<0.05$.

Table 1. Cytotoxicity, anti-HSV activity, and selectivity index of extracts and active compound from *Anemarrhena asphodeloides*.

Compound	Cytotoxicity (CC_{50}, mg/L)	Anti-HSV activity (IC_{50}, mg/L)	Selectivity index (CC_{50}/IC_{50})
PEE	1280	1280	1
EAE	1280	160	8
CFE	2560	640	4
NBE	640	320	2
RWE	640	1280	-
Active compound	1280	64	20

PEE: petroleum ether extract; EAE: ethyl acetate extract; CFE: chloroform extract; NBE: *n*-butanol extract; RWE: remaining water extract. CC_{50}: compound concentration required to reduce cell viability by 50%, as determined by the MTT method. IC_{50}: compound concentration required to reduce virus yield by 50%. -: Inhibition was lower than 50% within the range of tested concentrations.

3. Results

3.1. In vitro antiviral efficacy of extracts and compounds from A. asphodeloides

Cytotoxicity of petroleum ether extract, ethyl acetate extract, chloroform extract, *n*-butanol extract, and remaining water extract was determined as shown in Table 1. As shown in Table 1, the CC_{50} of the five extracts from *A. asphodeloides* was between 640 and 2560 mg/L. Cytotoxicity of chloroform crude extract from *A. asphodeloides* was less toxic than the other four extracts with a CC_{50} value of 2560 mg/L.

The results of antiviral efficacies for five extracts were depicted in Table 1, which indicated that ethyl acetate extract demonstrated the most active antiviral efficacy against HSV-1 with an IC_{50} value of 160 mg/L and a selectivity index (SI, CC_{50}/IC_{50}) value of 8, followed by chloroform with an IC_{50} value of 640 mg/L with a SI value of 4. The petroleum ether and water extracts exhibited the least activity with the IC_{50} value of 1280 mg/L and 1280 mg/L, respectively.

Since the ethyl acetate extract demonstrated the most significant anti-HSV-1 activity, it was then subjected to further separation and isolation. An active compound was isolated from *A. asphodeloides*. Results from the *in vitro* antiviral test showed that the active compound led to a great anti-HSV-1 activity with an IC_{50} value of 64 mg/L. The CC_{50} value of the active compound was 1280 mg/L which led to the SI on HSV-1 being 20 (Table 1).

The *UL12*, *UL42*, and *UL54* gene expressions of HSV-1 were also evaluated by RT-PCR. The results showed that the relative expression of *UL12*, *UL42*, and *UL54* was significantly inhibited by more than 85.8% when the concentration of the active compound reached 64 mg/L at 48 h after being infected with HSV-1 (Supplementary Figure 1). Following the results of RT-PCR, the viral titer in the active compound treated group was significantly lower than the control group (virus) which indicated that the release of HSV-1 was inhibited by the active compound (Supplementary Figure 2).

3.2. Chemical structure identification of the antiherpetic compound

The active compound was obtained as yellow crystal; m p:260-262℃; EI-MS (70 eV) *m/z*: calculated for $[C_{19}H_{19}O_{11}+H]^+$: 423.092; found: 423.095; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.76 (s, 1H), 7.40 (s, 1H), 6.88 (s, 1H), 6.39 (s, 1H), 4.85 (s, 1H), 4.63 (s, 1H), 4.60 (s, 1H), 4.47 (br, 1H), 4.06 (s, 1H), 3.74-3.66 (m, 1H), 3.43 (d, J=12.3 Hz, 1H), 3.35 (s, 1H), 3.25-3.12 (m, 3H); ^{13}C NMR (101 MHz, DMSO) δ 62.01, 70.77, 71.16, 73.60, 79.50, 82.08, 93.83, 101.82, 103.14, 108.14, 108.61, 112.25, 144.23, 151.28, 154.51, 156.73, 162.29, 164.34, 179.61; agreed well with the data reported[23]. Therefore, the compound was identified as mangiferin with molecular formula $C_{19}H_{18}O_{11}$.

3.3. In vitro antiviral activity of mangiferin against HSV–1

3.3.1. TOA and TOR

To determine which stage of the HSV-1 replication was affected by mangiferin, we conducted the TOA and TOR experiments. Results from the TOA study demonstrated that HSV-1 replication was significantly inhibited after adding 64 mg/L mangiferin at 0-8 h. As for TOR studies, a sharp drop was also observed when mangiferin was removed at 6-8 hpi, and an interception point between the TOA and TOR was found at around 8 hpi (Figure 1). Based on the above results we infer that mangiferin plays an important role in the early replication stage of herpes virus.

3.3.2. Mitochondrial transmembrane potential ($\Delta\psi m$)

Protective effect of mangiferin on mitochondrial damage was measured by the JC-1 assay kit. As shown in Figure 2, a decrease of $\Delta\Psi$m was found in HSV-1 infected Vero cells (green fluorescence increased significantly as compared with Vero cell), however, after treatment with 64 mg/L mangiferin, mitochondrial transmembrane potential ($\Delta\Psi$m) were significantly attenuated, which suggested that mangiferin could inhibit HSV-induced apoptosis.

Results from the nucleus damage test showed that the nucleolus Vero cell infected with HSV-1 was broken, and the number of spindle nucleus cells decreased significantly. However, in the mangiferin-treated group, the nucleolus retained a normal spindle shape, and the number of broken nucleolus decreased significantly (Figure 3).

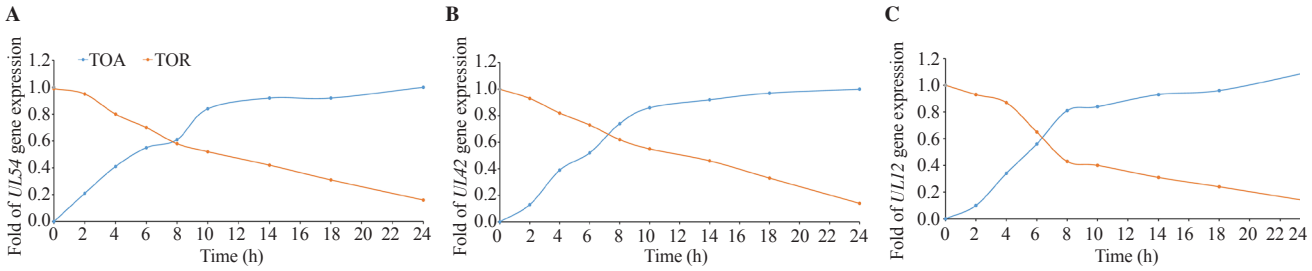

Figure 1. *UL54* (A), *UL42* (B), and *UL12* (C) gene expression in the time-of-addition (TOA) and time-of-removal (TOR).

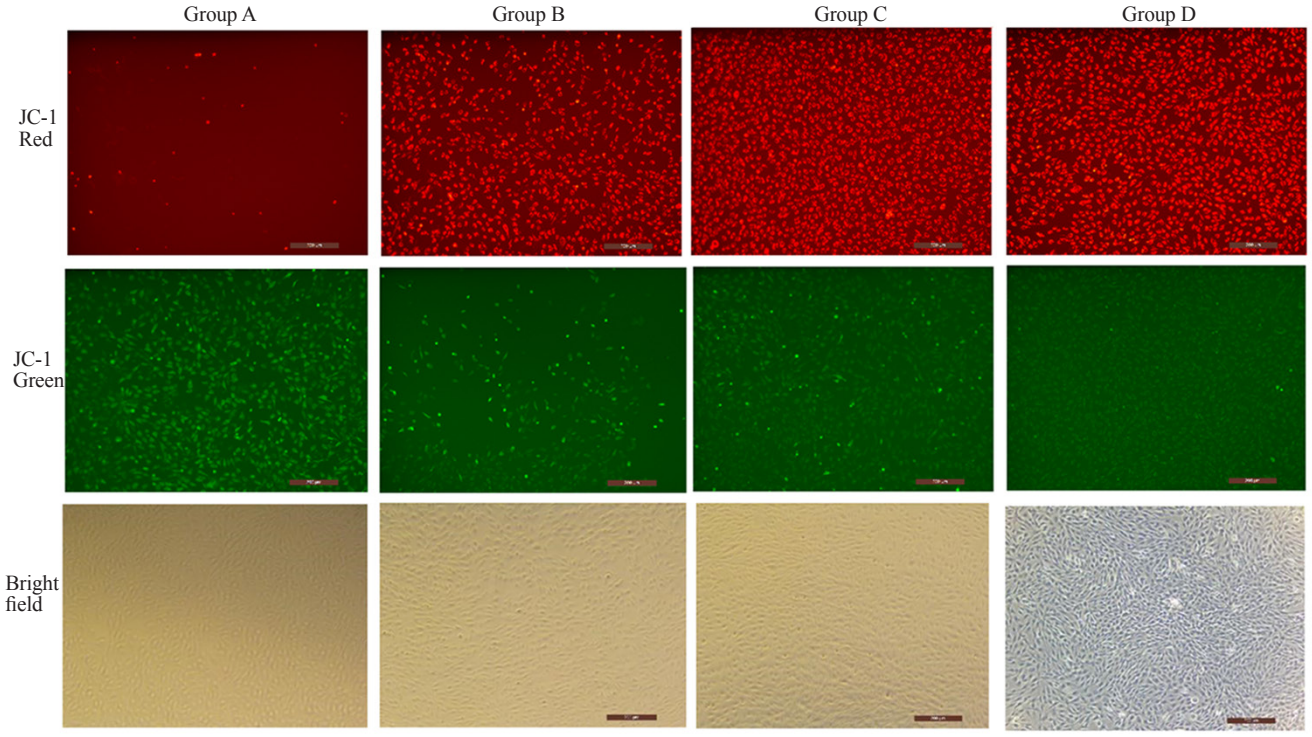

Figure 2. Fluorescence observation for mitochondrial transmembrane potential. Group A, B, and C were incubated with herpes simplex virus 1 (HSV-1), HSV-1 and mangiferin, and mangiferin, respectively, and Group D served as blank control. 100×, bar=200 μm.

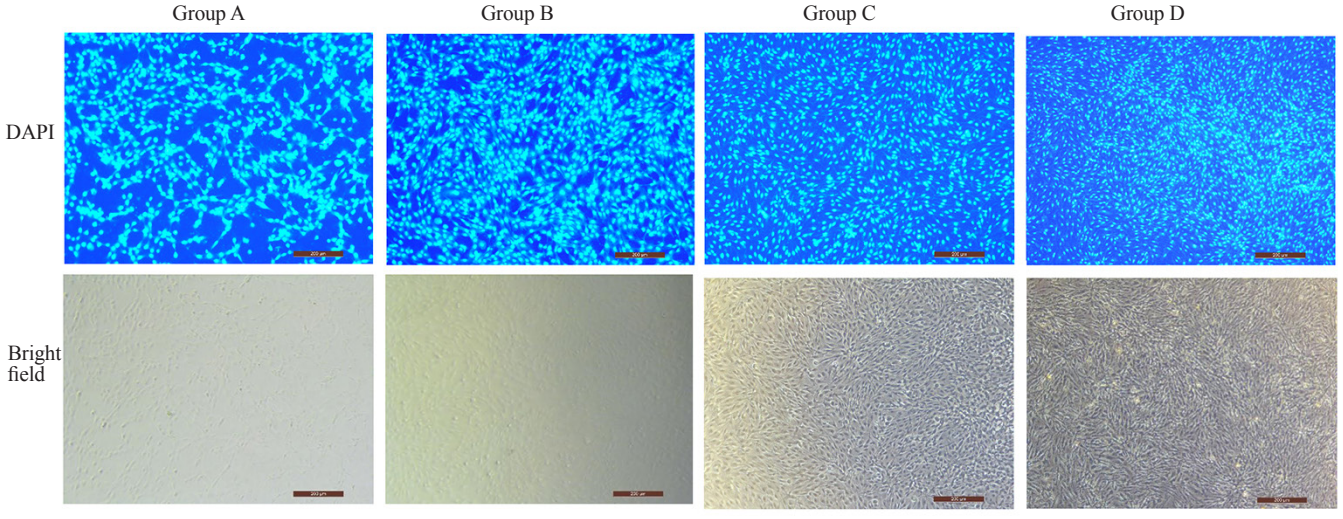

Figure 3. Fluorescence microscopy images of nucleus damage in Vero cells. 100×, bar=200 μm.

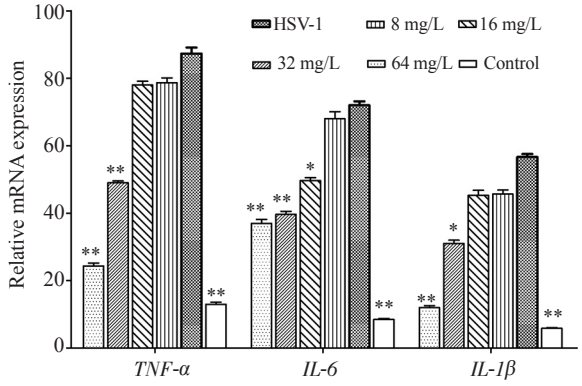

Figure 4. Relative RNA levels of *TNF-α*, *IL-1β*, and *IL-6* gene expression. All the treated group only compared with HSV-1 group, *: $P<0.05$; **: $P<0.01$.

3.3.3. Cytokine expression in Vero cell after treatment with mangiferin

Cytokine expression in Vero cells after treatment with mangiferin at non-toxic and effective antiviral concentrations was shown in Figure 4. Results from RT-PCR analysis demonstrated that all the expression levels of *TNF-α*, *IL-1β*, and *IL-6* in the mangiferin-treated group were significantly reduced in a dose-dependent manner, while these levels in HSV-1 infected Vero cells were significantly increased. Thus, the results indicated that mangiferin could reduce the cytokine induction caused by HSV-1.

3.4. In vivo antiviral activity of mangiferin against HSV–1

To further determine the antiviral activity of mangiferin against HSV-1, we conducted the *in vivo* test. After being challenged with HSV-1, the *UL54* gene expression in group Ⅱ was significantly increased which indicated that the mice were successfully infected with HSV-1. While in the mangiferin-treated group, the expression level of *UL54* was down-regulated in a dose-dependent manner. On day 7, the *UL54* gene expression level was almost one-tenth of that in the infection group when the concentration reached up to 4 g/kg (Supplementary Figure 3).

Activation of cytokine induction of *TNF-α*, *IL-1β*, and *IL-6* was observed in the HSV-1 challenged group (Figure 5). After treatment with mangiferin, all the expression levels of *TNF-α*, *IL-1β*, and *IL-6* were significantly reduced in a dose-dependent manner as compared with the HSV-1 infected group ($P<0.05$) and almost declined to a base level (group Ⅰ) when the concentration of mangiferin reached up to 4 g/kg on day 7.

After challenge with HSV-1, mice in group Ⅱ showed symptoms of drowsiness, curling, convulsion, and hair disorder and began to die on day 3. The cumulative mortality of mice in group Ⅱ was 90.0%. However, mice in the mangiferin-treated group began to die on day 4. The cumulative mortality of mice in group Ⅲ, group Ⅳ, and group Ⅴ were 76.7%, 60.0%, and 40.0%, respectively (Figure 6), and the relative protection rate were 14.7%, 33.3%, and 55.5%, respectively.

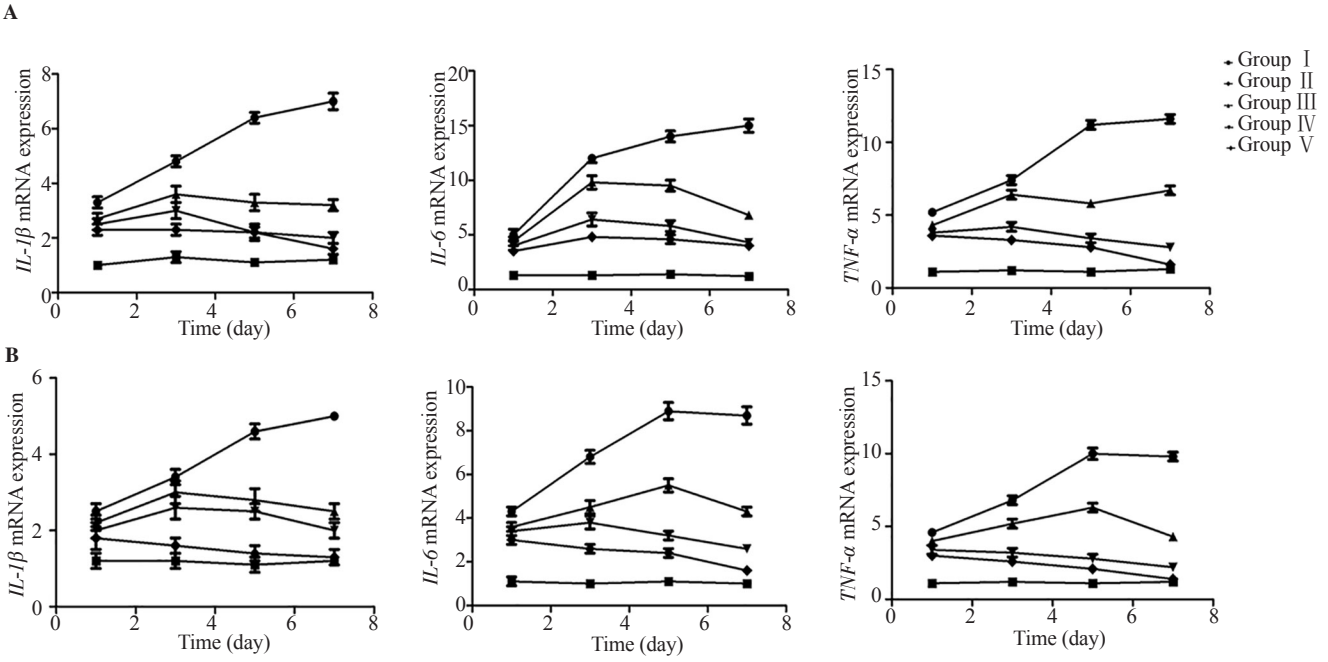

Figure 5. *In vivo* antiviral efficacy of mangiferin on the expression of *TNF-α*, *IL-1β* and *IL-6* in brain (A) and ganglion (B). Group Ⅰ served as blank control. Group Ⅱ, Ⅲ, Ⅳ, and Ⅴ were incubated with HSV-1, HSV-1 and 1, 2, 4 g/kg mangiferin, respectively.

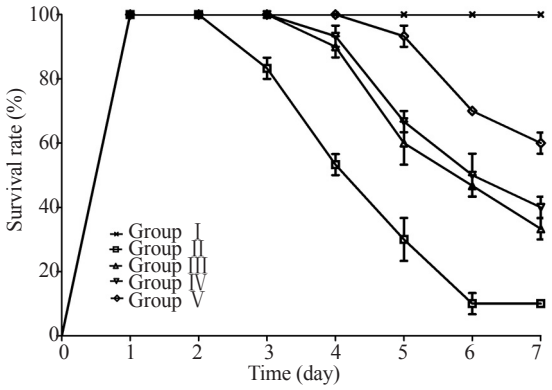

Figure 6. Survival rate of mice after challenge with HSV-1.

4. Discussion

Mangiferin is a xanthone widely distributed in *A. asphodeloides* Bunge and mango fruits[24]. The pharmacological properties of mangiferin, including antioxidant, anticancer, antiaging, antiviral, hepatoprotective, analgesic, and immunomodulatory activities, have been reported[25]. Bhatt *et al.* demonstrated that mangiferin could significantly inhibit H1N1 neuraminidase enzyme with an IC_{50} value of 0.82 μM. Moreover, medicinal plants containing mangiferin were also reported to have a protective efficiency against cardiac disease, diabetes, arthritis, certain mental health conditions, and hepatitis[26]. In the present study, mangiferin with anti-HSV activity was isolated from *A. asphodeloides* by *in vitro* bioactivity-guided isolation.

The key and core of drug screening are to establish a fast and efficient pharmacodynamic screening model. Antiviral drug screening models mainly include *in vitro* pharmacodynamic screening model and *in vivo* pharmacodynamic screening model. Compared with the *in vitro* model, although the *in vivo* model is time-consuming, it can more comprehensively reflect the actual antiviral effect of the drug[27]. The *in vitro* pharmacodynamic screening model has the advantages of convenient operation and a short experimental period[28]. Moreover, the amount of active ingredients in plants is generally low, however, very low amounts of drugs are sufficient for *in vitro* experiments as compared with *in vivo* experiments. so, *in vitro* experiments are widely used for high-throughput drug screening[29]. An active compound with antiviral activity was isolated by *in vitro* antiviral-guided separation, the results from *in vivo* challenge also proved that the *in vitro* drug screening model established in the present study was reliable and effective.

HSV-1 alkaline nuclease, encoded by the *UL12* gene, plays an important role in HSV-1 replication, Balasubramanian *et al.* found that the absence of *UL12* leads to inhibition of viral growth[30]. Viral polymerase processing factor *UL42* was identified as a potential target of antiviral and anti-tumor drug discovery because it could interact with IFN regulatory factor 3 (IRF-3) to inhibit its phosphorylation and the transcription of IFN-β-related genes[31]. Immediate-early (IE) protein ICP27 encoded by gene *UL54* is essential for HSV-1 infection[32]. Park *et al*.[33] found that leptomycin B (LMB) blocks HSV-1 replication by inhibiting the expression of ICP27. Our results demonstrated that *UL12*, *UL42*, and *UL54* gene expressions of HSV-1 were significantly inhibited after treatment with mangiferin *in vitro* and *in vivo*, which indicated that the antiviral mechanism of mangiferin may be associated with virus replication.

In general, virus infection causes multiple events in host cells. The decrease of mitochondrial membrane potential is a landmark event in the early stage of apoptosis. Hu *et al.* found that *Micropterus salmoides* rhabdovirus could significantly induce mitochondrial damage, and natural product arctigenin could significantly depress the mitochondrial damage by maintaining ΔΨm[34]. Similarly, our results showed that the membrane potential level of mitochondria of Vero cell was disrupted after infection with HSV-1, however, membrane potential levels were recovered when cells were treated with 64 mg/L mangiferin. The inhibition of any step in the viral life cycle from binding and release of virions can effectively prevent viral infection. Antivirals that target the early events of infection may be applied because virus binding or entry can induce cell injury[35,36]. Mangiferin may play an important role in the early prevention of the herpes virus. It may be a potential drug for the early-stage treatment of HSV-1.

However, the present study has some limitations. Extracts or fractions from *A. asphodeloide* with the highest activity against HSV-1 were subjected to bioassay-guided isolation while extracts or fractions with little antiherpetic activity were deleted, which may lead to the results that the compounds with low content in *A. asphodeloide* but with high efficiency may be missed. Further studies are required for the mechanism of the anti-HSV activity.

Conflict of interest statement

The authors have no conflict of interest to declare.

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Authors' contributions

All authors contributed to the conception and design. WWD contributed to data collection, article writing, and commissioning. CG contributed to the conception, analysis, and manuscript

preparation. All authors contributed to the article and approved the submitted version.

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