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The antiviral effect and potential mechanism of *Houttuynia cordata* thunb. (HC) against coxsackievirus A4

Qin Su [a,1], Hailin Wei [a,1], Yihan Xu [a,1], Yiliang Zhang [b,1], Wenlei Wang [a], Jiaxue Zhou [a], Sitong Liu [a], Xiaohui Yang [c,**], Le Zhou [d,***], Pinghu Zhang [a,e,*]

[a] *Jiangsu Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Treatment of Senile Diseases, Medical College, Yangzhou University, Yangzhou, 225009, China*
[b] *Swiss University of Traditional Chinese Medicine, Bad Zurzach, 5330, Switzerland*
[c] *Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042, China*
[d] *Yangzhou Center for Disease Control and Prevention & The Affiliated CDC of Yangzhou University, Yangzhou, Jiangsu, 225001, China*
[e] *Jiangsu Key Laboratory of Zoonosis, Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, Jiangsu, 225009, China*

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ABSTRACT

Ethnopharmacological relevance: Hand, foot, and mouth disease (HFMD) is mainly caused by various of enteroviruses such as enterovirus 71 (EVA71), coxsackievirus A16 (CVA16), CVA6, and CVA10 in infants and children under 5 years old. During the past 5 years, CVA4 has become the dominant pathogen resulting in HFMD in China. However, there are no effective vaccines and antiviral drugs available. *Houttuynia cordata* Thunb (HC). is a Chinese herbal medicine eaten as vegetables for treating viral infection diseases, but whether HC has anti-CVA4 effect remains unclear.

Aim of the study: In this study, we want to investigate the antiviral activity of HC against CVA4 *in vitro* and *in vivo* and elucidate the potential mechanism of HC against CVA4.

Materials and methods: MTT assay were used to evaluate the cytotoxicity of HC. Virus titers assay, CPE assay, violet staining and immunofluorescence were used to investigate the antiviral effect of HC against CVA4. A 13-day-old suckling mice model was established to evaluate the therapeutic efficacy of HC against CVA4 infection. Western blot, qRT-PCR and time-of-drug addition assay were performed to elucidate the potential mechanism of HC against CVA4 infection.

Results: MTT assay indicated the cytotoxicity concentration of HC on Vero cells and RD cells were more than 1 mg/ml, suggesting that the low cytotoxicity of HC. *In vitro* antiviral assay revealed that HC could dose-dependently prevent the CPE, suppress the release of newborn virus, and inhibit the replication of CVA4 by decreasing viral RNA transcription and protein expression with IC50 of 88.96 μg/mL. A time-of-addition assay showed that HC mainly exerted anti-CVA4 effect by inhibiting virus replication at the post-entry stage. *In vivo* results further demonstrated that HC could effectively prevent the lethal infection of CVA4 by promoting survival, improving clinical symptoms, prolonging the survival time, inhibiting excessive inflammatory responses, and reducing pathological injury *in vivo*. Mechanistic studies revealed inhibition of p38 MAPK and JNK pathway over-activation may be the primary mechanism of HC against CVA4 infection.

Conclusion: In summary, our results for the first time demonstrated that HC not only effectively inhibited CVA4 replication, but also partially protected the lethal infection of CVA4 *in vivo*. Furthermore, pharmacological mechanism studies revealed that the primary mechanism of HC against CVA4 infection may be associated with its effect of inhibiting over-activation of p38 MAPK and JNK signaling pathways caused by enteroviruses. Our finding indicated that HC might be a potential innovative medicine for treating HFMD.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail address: zhangpinghu@yzu.edu.cn (P. Zhang).
[1] These authors equally contributed to this work.

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Hand foot and mouth disease (HFMD), is an infectious disease caused by a variety of enteroviruses that mainly infects infants and children under 5 years of age (Yan et al., 2022). Enterovirus (EV) belongs to the genus enterovirus of the family of small RNA viruses, which causes acute clinical manifestations such as herpangina, hand, foot and mouth rash, myocarditis and pulmonary edema in young children. Thus far, 116 serotypes of EV were identified, but more than 20 kinds of EV have been confirmed to cause HFMD, including enterovirus 71 (EVA71) and coxsackievirus A16 (CVA16) (Zhang et al., 2022). Prior to 2009, EVA71 and CVA16 were the dominant epidemic strains (Upala et al., 2018). However, during the past several years, some serotypes of enteroviruses such as CVA4, CVA6, CVA8, and CVA10 have been confirmed to become epidemic strains in China (Gao et al., 2018). In 1948, CVA4 virus was firstly isolated in the United States, and then has become endemic in Kenya, Japan, India, China and other places (Melnick, 1950; Oberste et al., 2004). During the past ten years, several outbreaks have occurred in China. The earliest outbreak occurred in Taiwan of China in 2004 (Hashimoto, 1982). During the past 5 years, CVA4 has replaced EVA71 and CVA16 as the dominant pathogen resulting in HFMD in China.

Currently, only the broad-spectrum antiviral drug, ribavirin has been approved to treat HFMD in clinical. In China, it has been recommended the early use of interferon alpha (IFN-α) and ribavirin for treating symptomatic patients (Li et al., 2018), whereas they only have limited efficacy. Moreover, ribavirin exhibits serious toxic and side effects on infants. To date, there are no effective vaccine and antiviral reagents available for CVA4 infection. Therefore, it is very urgent for the development of innovative antiviral drugs against CVA4 infection.

Traditional Chinese medicine (TCM) has been widely used to treat infectious diseases such as influenza, COVID-19 in China due to its comprehensive effects of improving clinical symptoms, inhibiting viral replication, preventing cellular lesions, regulating immune functions, and controlling excessive inflammatory responses (Huang et al., 2021; Xie et al., 2023). *Houttuynia cordata* Thunb. (HC) as the “broad-spectrum antibiotic of traditional Chinese medicine" has been used for treating infectious diseases in clinical for thousands of years in China (Wei et al., 2023). Modern pharmacological studies also have demonstrated that HC has potential broad-spectrum antiviral effects. For example, Hayashi et al. found that the volatile oil of HC has direct inhibitory activity on human AIDS virus (HIV) and herpes simplex virus 1 (HSV-1) (Hayashi et al., 1995). Liu et al. confirmed that HC injection has a good preventive and therapeutic effect on influenza A (H1N1) virus infection *in vivo* by reducing lung index and preventing inflammatory injury (Liu et al., 2010). Moreover, the water extract and polysaccharide of HC has been demonstrated to have antiviral activity against coxsackievirus B3 (CV-B3), coxsackievirus B5 (CV-B5), and enterovirus 71 (EV71) *in vitro* (Fan, 2019). However, to our best knowledge, there are no reports about HC against CVA4. In the present study, our results revealed for the first time that HC could dose-dependently inhibit the replication of CVA4 and effectively prevent the lethal of CVA4 infection, suggesting that HC may be a promising pharmaceutical for the development of antiviral therapy for HFMD infection.

1. Materials and methods

1.1. Cell lines, virus and reagents

African green monkey kidney (Vero) cell line and rhabdomyosarcoma (RD) cell line were obtained from China Center for Type Culture Collection (CCTCC). CVA4 strain was isolated from the Affiliated Hospital of Yangzhou University with plaque purification. *Houttuynia cordata* Thunb was purchased from Jiangsu Lianhuan Health Pharmacy (Yangzhou, China), originated from Sichuan, China, and its main components of HC was confirmed by highperformance liquid chromatography (HPLC). Ribavirin (Lot No. 20190401) was obtained from Jiangsu Lianshui Pharmaceutical Co., Ltd (Huaian, China). DMEM was obtained from Jiangsu Kaiji Biotechnology Co., Ltd. (Jiangsu, China). Fetal bovine serum (Lot No. CP14-1260) was purchased from Capricorn Scientific (USA). Crystalline violet was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). RNA isolater (R401-01), HiScript II 1st Strand cDNA Synthesis Kit (7E710L3), and ChamQ Universal SYBR qPCR Master Mix (Q711-02) were purchased from Vazyme (Nanjing China). Polyclonal EV71 VP1 primary antibody (GTX132339) was purchased from GenTex. Phospho-p38 MAPK (AF5887), β-actin (120722230822), RIPA lysate (P0013B), 5 × loading buffer (20221224), HRP-goat anti-mouse IgG secondary antibody was purchased from Beyotime (Shanghai, China). SAPK/JNK (9252 P), p-SAPK/JNK (4668 S), p38 MAPK (8690 P) were obtained from Cell Signaling Technology (Beverly, MA, USA). HRP-goat anti-rabbit IgG secondary antibody (NMB1073155) was purchased from Biodragon (Suzhou, China).

1.2. Prepration of water extract of Houttuynia cordata thunb

Dried plant material (50 g) was immersed in 20 times of ultrapure water for 1 h, boiled for 1 h and filtered the supernatant, and then added 1 L of ultrapure water for boiling for 1 h again, filtered, and combined the filtrates twice. Concentrate the filtrate volume to 50 ml to obtain a stock concentration of 1 g/ml.

1.3. Determination of effective components by HPLC

Kromasil 100-5-C18 column was selected, with acetonitrile as mobile phase A and 0.1% phosphoric acid solution as mobile phase B. The detection wavelength was 277 nm, the column temperature was 30 °C, and the injection volume was 10 μL. Gradient elution conditions are as follows: 0–5 min, 5%–8% A; 5~12min, 8%–13% A; 12~40min, 13%–15% A; 40~55min, 15%–17% A; 55~65min, 17%–30% A; 65~75min, 30%–40% A. Accurately weigh a total of reference substances including respectively, put them in a beaker, dissolve them with 50% methanol solution by ultrasonic wave, cool them, transfer them to a 25 ml volumetric flask, and adjust the volume to the calibration line to obtain the standard substance solution.

1.4. Cell cytotoxicity assay

The cell cytotoxicity assay in Vero and RD cells was determined using MTT assay as previously described (Wei et al., 2024). Briefly, Vero cells or RD cells (1×10^4/well) were seeded into 96-well plates for overnight, and then were cultured with DMEM containing 2-fold gradient dilution of HC water extract for 72 h. After treatment, each well was added with 10 μL of MTT (5 mg/ml) for incubation 4 h and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The OD value of each well was recorded using a microplate reader (Biotech, USA) at 570 nm and cell toxicity were evaluated.

1.5. In vitro antiviral assay

The *in vitro* antiviral activity was determined via crystal violet staining assay. Vero cells infected with 100 $TCID_{50}$ of CVA4 were cultured in 96-well plate in the presence of serial concentrations of HC at 37 °C, 5% CO_2 for 72–96 h, with RBV (50 μg/ml) as a positive control. CVA4 infection results in cytopathic lesions manifested as rounded, wrinkled or even detached cells. When more than 75% of cell lesions were observed in the virus-infected group, the plates were photographed with an inverted light microscope, and then were fixed with 4% paraformaldehyde. After washing with water, the plates were stained with 0.5% crystalline violet for 1 h, and then placed in an oven for drying. 100 μl 10% glacial acetic acid was added into each well to dissolve the crystalline violet (w/v) and the absorbance of each well at 570 nm was determined by a microplate reader (Biotech, USA). The curve was plotted using Graphpad prism 5 to calculate the IC_{50} value.

Table 1
Real-time fluorescence quantitative PCR primer.

Gene name	Forward primer	Reverse primer
GAPDH	AGAGTGGGAGTTGCTGTTG	GCCTTCCGTGTTCCTACC
IL-6	AGTTGCCTTCTTGGGACTGA	TCCACGATTTCCCAGAGAAC
IP-10	TCCAGTTAAGGAGCCCTTTTAGACC	TGAAATCATCCCTGCGAGCCTAT
MIP-2	CACCAACCACCAGGCTACAG	CCTTGAGAGTGGCTATGACTTCTGT
NF-κB	CGAGAGAAGCACAGATACCA	TCAGCCTCATAGTAGCCATC

1.6. $TCID_{50}$ assay

$TCID_{50}$ assay was determined as previously described (Wei et al., 2024). Briefly, Vero cells or RD cells (4.0×10^5/well) were seeded into 12-well plates for overnight, and then infected with 100 $TCID_{50}$ of CVA4 for 1 h and treated with different concentrations of HC for 72 h. Cell supernatants were collected from 24 h to 72 h. Vero cells or RD cells (1×10^4/well) were seeded into 96-well plates for overnight. Cell supernatants were diluted with 10-fold from 10^{-1} to 10^{-8}.50 μl of dilutions were transferred to 96-well plates with 4 replicates for each dilution. The cell pathogenic effect was observed under an inverted microscope.

1.7. Western blot

Vero and RD cells were seeded into 6-well plates for overnight. After washing with PBS for twice, the plates were infected with CVA4 (100 $TCID_{50}$, about 0.01 MOI) for 1 h at 37 °C, washed with PBS for twice, and then were treated with HC (200, 50, 12.5 μg/ml) or RBV for 24 h. After incubation, the plates were washed with PBS for twice, and cells were lysed by RIPA lysis buffer containing PMSF and phosphatase inhibitor to get total protein lysates. Protein samples were separated by 12% SDS-PAGE gels and transferred onto nitrocellulose (NC) membrane (0.45 μm) using a tank transfer system as previously described (Zhang et al., 2021). After blocked in 5% FBS in Tris-buffered saline (20 mM Tris, 166 mM NaCl, and 0.05% Tween 20, pH 7.5) for 1 h, the membrane was incubated with primary antibodies (p38, JNK, p-p38, p-JNK, actin and VP1) overnight at 4 °C, and finally incubated with a horseradish peroxidase-conjugated species-specific secondary antibodies at room temperature for 1 h. Bands were visualized using an enhanced chemiluminescence kit (Millipore) with a Molecular Imager SH-523 System. Quantification relative to ACTB by densitometry analysis was performed using Image J software.

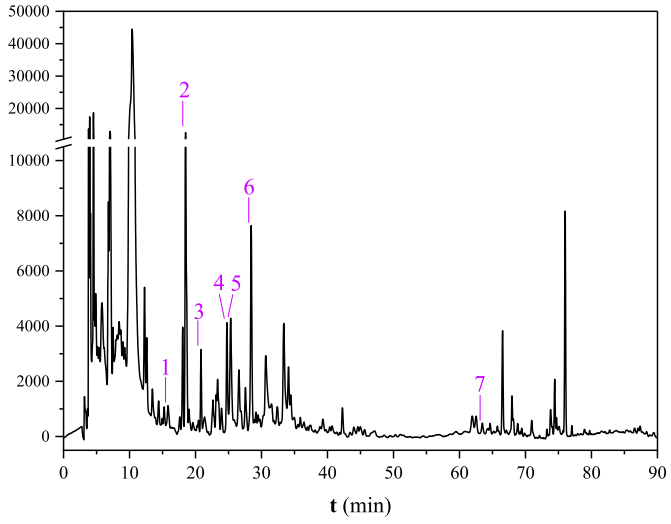

Fig. 1. The HPLC chromatographic fingerprint profile of the water extract of *Houttuynia cordata* Thunb. 1- rutin, 2- quercitrin, 3- chlorogenic acid, 4- cryptochlorogenic acid, 5- quercetin, 6- hyperoside, 7-piperolactam.

1.8. Time-of addition assay

To clarify the detailed mechanism of HC against CVA4, a time-of-drug addition assay was performed according to the administration mode diagram (Fig. 6A). After treatment, cell proteins were extracted with RIPA lysis buffer and were separated with SDS-PAGE for Western blot assay. The specific methods were as follows: (a) adsorption assay: Vero cells were pretreated with HC (200, 12.5 μg/ml) in six-well plates for 1 h and then were incubated with 0.01 MOI of CVA4 at 4 °C for 1 h. After viral solution was removed, the plates were washed with PBS for twice, and then were supplemented with 2 ml of DMEM with 2% FBS for 24 h at 37 °C; (2) Penetration assay: Vero cells were infected with 0.01 MOI of CVA4 for 1 h at 4 °C, and then were treated with HC (200, 12.5 μg/ml) for 1 h, and washed with PBS for twice, and then were cultured with 2 ml of maintenance solution for 24 h at 37 °C. (c) Proliferation assay: Vero cells were infected for 1 h, the viral solution was discarded, HC (200, 12.5 μg/ml) was added and incubated overnight at 37 °C, and cell samples were collected 24 h post infection to get total protein.

1.9. Immunofluorescence assay

Immunofluorescence assay was performed as previously described (Wei et al., 2024). Briefly, Vero cells or RD cells (1×10^4/well) infected by CVA4 were incubated with drugs for 48 h and then fixed with 10% formaldehyde for overnight. Immunofluorescence analysis was conducted as following protocol, permeabilizing with 0.5% Triton-100 for 2 h and washing with PBS. The plate was blocked with 5% FBS for 1.5 h, and then incubated with VP1 primary antibody overnight at 4 °C. After incubation with FITC-labeled rabbit secondary antibody (anti-mouse) for 1 h at 4 °C, washing with TBST for twice, the nucleus was stained with DAPI and Images were captured using a fluorescence microscope.

1.10. qRT-PCR analysis of the expression of inflammatory cytokines

qRT-PCR assay was performed as previously described (Wei et al., 2024). Total RNA was extracted by Trizol, and cDNA was synthesized by reverse transcription of Hiscript II 1st strand enzyme according to the manufacturer's instructions (Vazyme, China). qRT-PCR was performed by a Light Cycler 480 (Roche, USA). qPCR was performed as follows: 5 min at 95 °C, followed by 42 cycles of 10s at 95 °C and 60s at 59 °C, and a melting curve step. The relative expression level of CVA4 VP1, IL-6, TNF-α and IL-1β mRNA was detected by qRT-PCR using GAPDH as an internal reference, and the primer sequences are shown in Table 1.

1.11. Animal experiment

Ten-week-old ICR males and 8-week-old ICR females were purchased from Comparative Medical Center for Yangzhou University, mated at a ratio of 1:2, separated into cages after pregnancy, and 13-day-old suckling mice were infected with 5 LD_{50} of CVA4. The Ethics Committee approved all animal experiments at Yangzhou University and humane care for animals was compiled with the guidelines of Jiangsu laboratory animal welfare Laboratory. 13-day-old suckling mice were infected intramuscularly with 100 μL of viral suspension containing 5 LD_{50} of CVA4 or normal saline. After 2 h of infection, the virus-infected mice were orally administered HC (1.25 or 0.625 g/kg/day) or saline

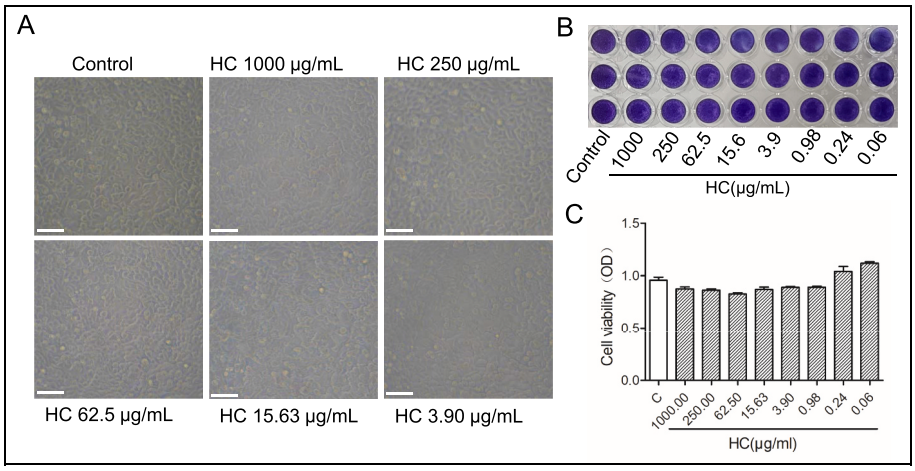

Fig. 2. The results of cell cytotoxicity assay of HC in Vero cells. (A) Cellular morphological changes treated with HC for 72 h. (B) Crystal violet staining results. (C) MTT assay results. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

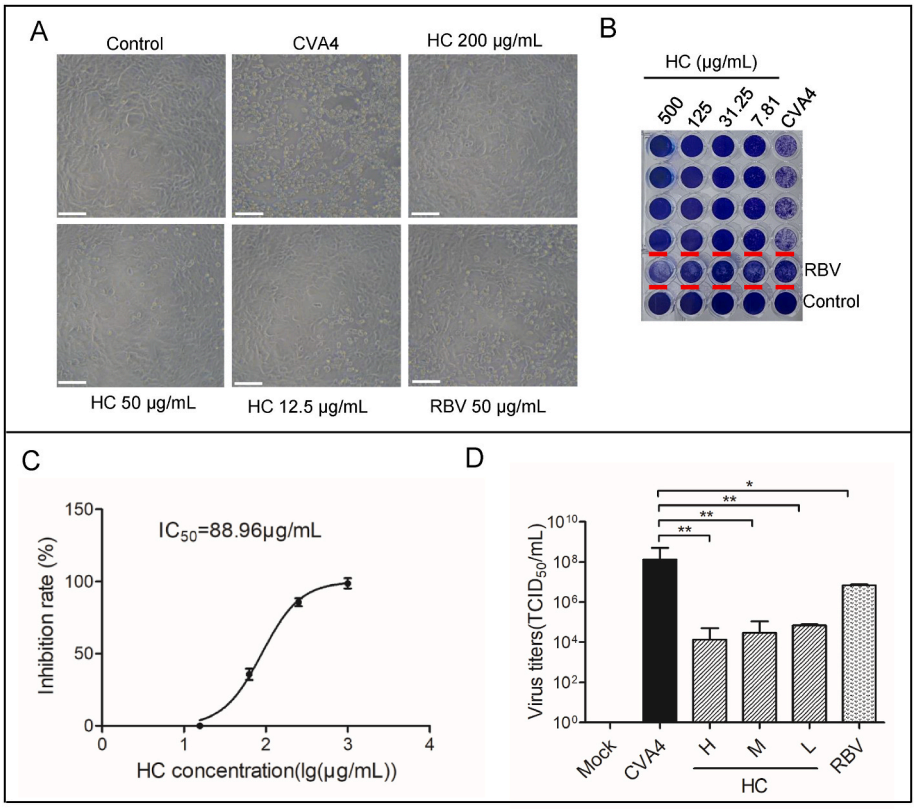

Fig. 3. Antiviral effect of HC against CVA4 infection. (A) Protective effect of HC on cytopathic effect caused by CVA4, which was further confirmed by crystal violet staining (B). (C) The IC_{50} of HC against CAV4. (D) Inhibitory effect of HC on CVA4 newborn virus. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 virus infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

once a day for 7 days. One group of suckling mice were divided into four groups (n = 10), including normal group, virus-infected group, HC high-dose group, and HC low-dose group. Body weights, deaths, and paralysis scores of all mice were recorded daily for 15 days. Other group of suckling mice (n = 9) were sacrificed at day 3 post-infection, and skeletal muscle tissues were collected for hematoxylin and eosin (HE) staining and qRT-PCR.

1.12. Statistical analysis

GraphPad Prism 5 were performed to statistical analysis. All results were expressed as mean ± standard error of triplicate determinations, and statistical differences were conducted by one-way analysis of variance (ANOVA) or Student's t-test. The $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) were considered significant, and ns was considered to be of no significant difference.

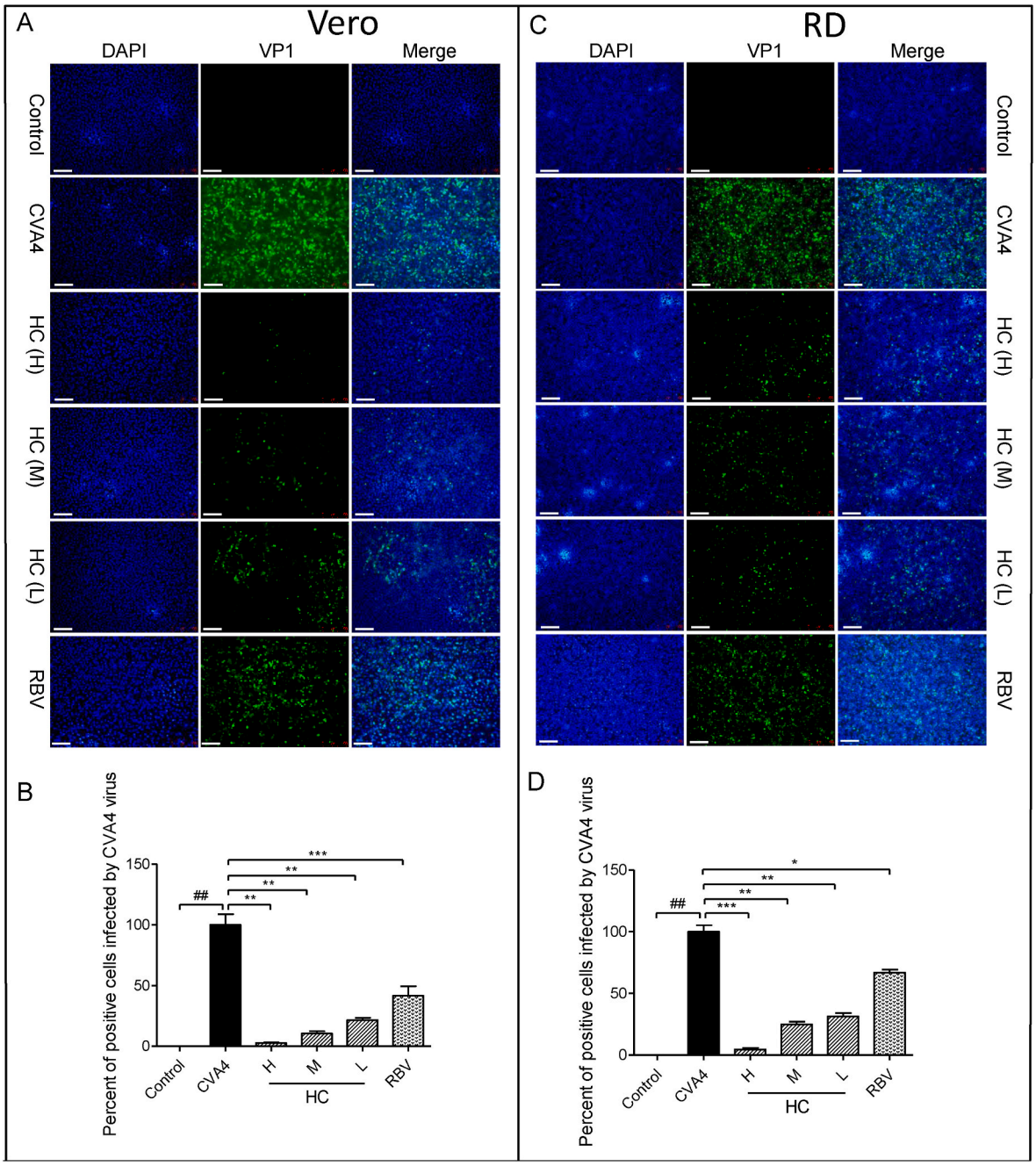

Fig. 4. Antiviral effect of HC against CAV4 confirmed by immunofluorescence assay. HC could dose-dependently inhibit CVA4 virus replication in CAV4-infected Vero cell model (A and B) or RD cell model (C and D). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 virus infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control.

2. Results

2.1. HPLC profile of HC

The chemical composition of HC was determined by HPLC (Fig. 1). Seven fingerprint peaks were identified by reference (Meng et al., 2005), and their corresponding chemical components are rutin, quercitrin, chlorogenic acid, cryptochlorogenic acid, quercetin, hyperoside and piperolactam. The contents of these compounds in HC were 0.307%, 4.87%, 0.637%, 1.008%, 1.382%, 2.222% and 0.113%, respectively.

2.2. Cell cytotoxicity assay

After treatment with HC (3.9–1000 μg/ml) for 72 h, no obvious cellular morphological changes were observed in Vero cells (Fig. 2A), which were further confirmed by crystal violet (Fig. 2B). MTT assay results also indicated that HC is safe to Vero cells even more than 1000 mg/ml (Fig. 2C). Due to the low toxicity of HC, 1000 mg/ml was selected as the highest dose of HC for further experiments.

2.3. In vitro antiviral effect of HC against CVA4

After infection with CVA4 for 72 h, Vero cells became rounded, wrinkled or even deaths, while HC treatment exhibited a good dose-

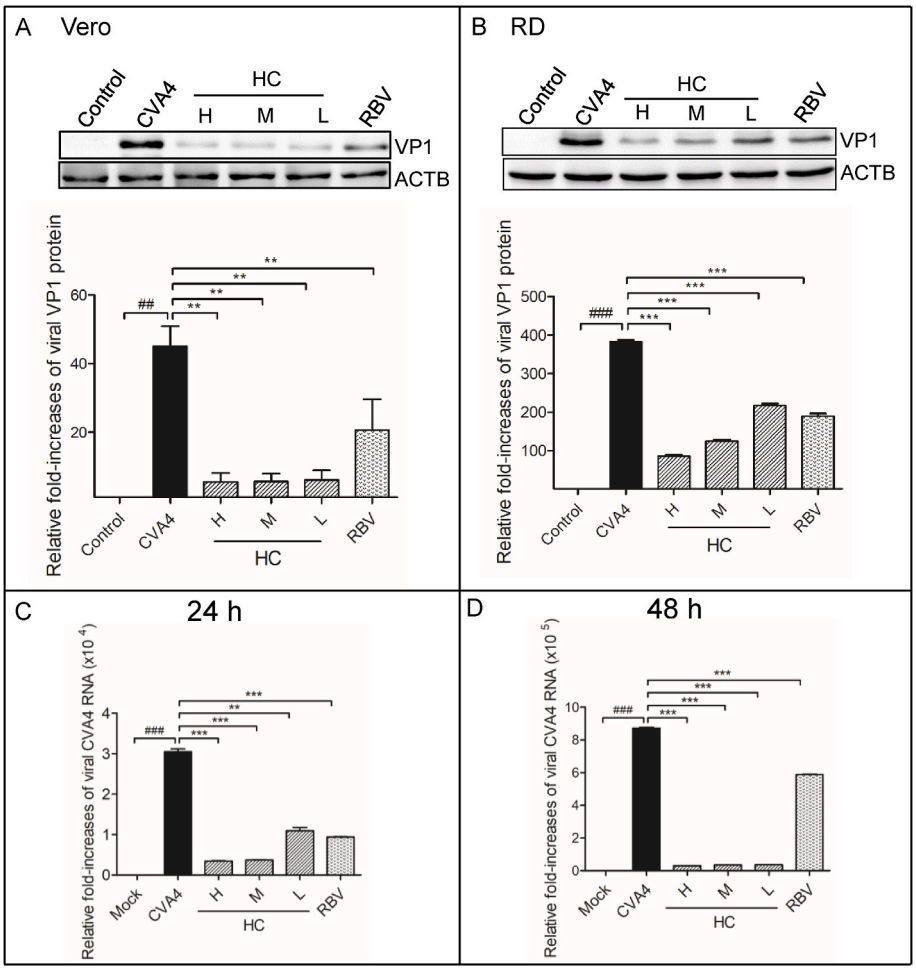

Fig. 5. Inhibitory effect of HC on CVA4 viral protein and RNA levels. Western blot assay results of viral protein expression in CAV4-infected Vero cells (A) and RD cells (B). qRT-PCR assay results of viral RNA levels in CAV4-infected Vero cells (C) and RD cells (D). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 virus infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control.

dependent protective effect on cell pathogenic effect (CPE) caused by CVA4 (Fig. 3A). Crystal violet staining further confirmed the protective effect of HC on CPE caused by CVA4 (Fig. 3B) and the IC_{50} of HC against CVA4 is about 88.96 μg/ml (Fig. 3C). To further assess the antiviral effect of HC, we examined the virus load of the HC-treated cell supernatants. $TCID_{50}$ assay results indicated that HC could dose-dependently inhibit the release of nascent virus (Fig. 3D), further confirming the inhibitory effect of HC on CAV4.

Immunofluorescence assay further demonstrated the inhibitory effect of HC on the replication of CVA4 (Fig. 4A and B). Furthermore, the antiviral effect of HC has been confirmed in the CVA4-infected RD cells (Fig. 4C and D), ruling out the cell-dependent effect of HC against CVA4. Collectively, our above results clearly indicated that HC had anti-CVA4 activity.

2.4. The inhibitory effect of HC on CVA4 viral protein and RNA levels

To elucidate the potential mechanism of HC against CVA4 virus, we firstly examined the inhibitory effect of HC on the expression of viral protein in the CAV4-infected Vero cells. Immunoblotting results indicated that HC could dose-dependently inhibit the viral VP1 protein expression (Fig. 5A), which also has been further confirmed in the CVA4-infected RD cells (Fig. 5B), ruling out the cell-dependent effect of HC against CVA4, suggesting that HC affected the expression of viral protein.

To further elucidate the potential mechanism of HC against CVA4, we investigated whether HC has influence on CVA4 RNA levels. qRT-PCR assay results revealed that HC can inhibit CVA4 viral RNA transcription in a dose-dependent manner in the CAV4-infected Vero cells (Fig. 5C). Furthermore, the inhibitory effect of HC on viral transcription has also been confirmed in the CVA4-infected RD cells (Fig. 5D). Collectively, our results indicated that HC may exert its anti-CVA4 effect by affecting viral RNA transcription and protein expression.

2.5. HC mainly exerts anti-CVA4 effect by targeting the stage of virus replication

To determine which stage of the CVA4 life cycle is primarily affected by HC, we performed a time-of-drug addition assay. Vero cells were treated with HC according to schematic illustration (Fig. 6A). Immunoblotting assay results indicated that HC exhibited the dose-dependent anti-CVA4 effect on virus binding, internalization, and replication (Fig. 6B). However, its inhibitory effect on virus binding and internalization were markedly weaker than that of HC on virus replication, which suggested that HC mainly affected the replication of CVA4 at the post-entry stage of life cycle. Therefore, it can be concluded that HC mainly inhibited the replication of CVA4 by suppressing the viral RNA transcription and viral protein synthesis. To further confirm the antiviral effect of HC against CVA4, we selected 50 μg/ml of HC to treat the CVA4 virus-infected Vero cells to investigate the time-dependent effect of HC. As shown in Fig. 6C, the expression levels of CVA4 VP1 protein gradually increased from 6 h to 24 h after post-infection. However, compared with

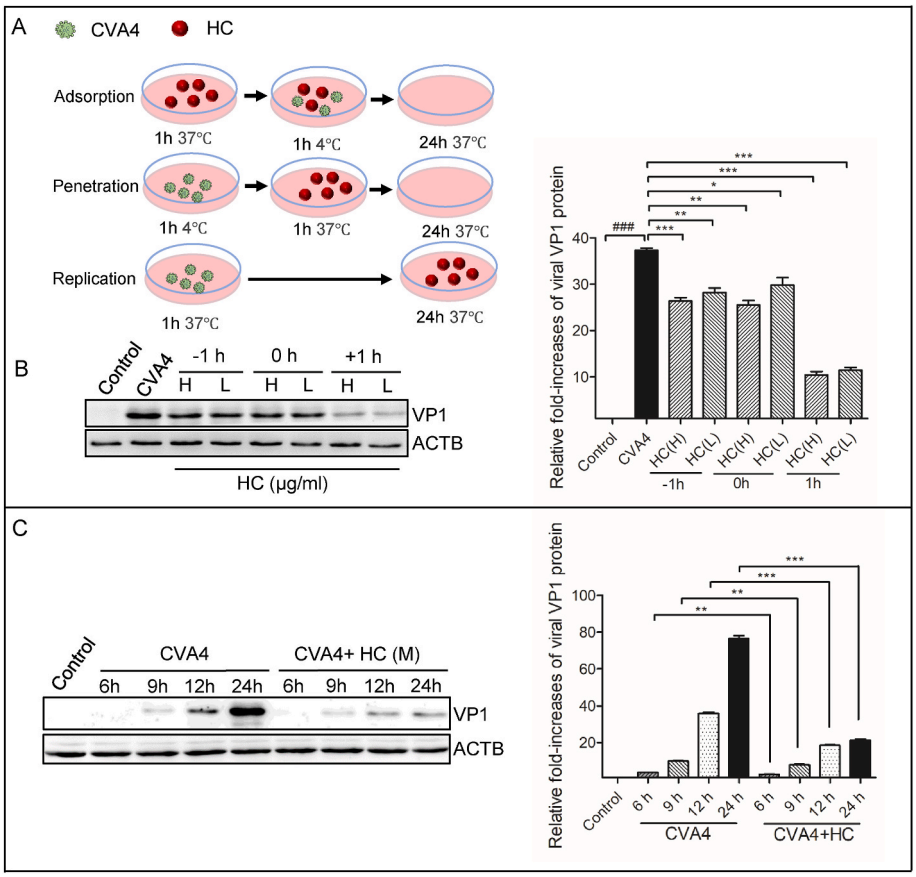

Fig. 6. Time-of-addition assay for HC. (A) Schematic diagram of time-of-addition assay. (B) Inhibitory effect of HC on CAV4 for binding, penetration and replication was confirmed by immunoblotting. (C) Time-dependent inhibitory effect of HC on CAV4 were quantified by immunoblotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control.

the CVA4-infected group, HC treatment could time-dependently inhibit the expression of viral VP1 protein expression, further confirming that HC mainly affects the replication of CAV4 virus.

2.6. Antiviral mechanism of HC against CVA4

Toll-Like Receptor (TLR) is recognized as a crucial pattern recognition molecule enabling the host to identify RNA virus infection. Upon recognition of viral RNA by TLR receptors, signal transduction ensues, thereby facilitating downstream activation of mitogen-activated protein kinase (MAPK) and JNK signaling pathways. Therefore, targeted modulation of the host's TLR-mediated innate immune signaling pathway exhibits broad-spectrum antiviral effects. Given the critical role of MAPK and JNK signaling pathway in mediating host antiviral responses, we initially investigated whether HC exerts anti-CVA4 effects through the aforementioned signaling pathway. As shown in Fig. 7, compared to the normal group, phosphorylation levels of p38 MAPK and JNK were significantly elevated in the virus-infected group, suggesting that CVA4 infection activates the above immune signaling pathway. However, HC treatment dose-dependently decreased the phosphorylation levels of p38 MAPK and JNK, suggesting that the antiviral effect of HC may be associated with inhibiting the over-activation of the above innate immune signaling pathway.

2.7. In vivo protective effect of HC on lethal CVA4 infection

Due to the good antiviral effect of HC *in vitro*, we next investigated the protective effect of HC on lethal dose of CVA4 infection in the CVA4-infected suckling mice model (Fig. 8). During the whole observation, all normal suckling mice had frequent activities, bright hair, good conditions, and high weight growth rate, while the CVA4-infected suckling mice showed obvious clinical symptoms including mental malaise, lethargy, slow movement, disinclination for activities, limb weakness or even paralysis, and slowed weight growth rate at 3 days post-infection. At 5 days after infection, the CVA4-infected mice began to die, and reached the peak of death at 6 days after infection with 100% mortality during the observation period, and the average survival time was about 6.83 ± 1.47 days. However, compared with the CVA4-infected group, the clinical symptoms of the HC treatment group were dose-dependently markedly improved, manifested as significant improvement in clinical symptoms such as delayed movement, limb disharmony, and limb paralysis. On 8th day after infection, the clinical scores of the virus-infected group reached the peak, while the clinical scores of the HC high-dose treatment group had dropped below 1. And then the HC high-dose treatment group gradually recovered. Furthermore, HC treatment could dose-dependently promote the survival of the CVA4-infected suckling mice. The survival rate of the high-dose and low-dose of HC were 50% and 33.33%, respectively. Moreover, HC treatment could dose-dependently prolong the survival time of CVA4-infected mice (HC high dose, 11.67 ± 3.78 days and HC low dose, 10.83 ± 3.25 days). Collectively, HC exhibited significant protective effect on the lethal infection of CVA4.

To further explore the antiviral effect of HC *in vivo*, we investigated the improvement effect of HC on the histopathological changes of skeletal muscle in suckling mice infected with CVA4 using HE staining. As shown in Fig. 8E, the broken fibers, a large number of myositis and myonecrosis lesions related to neutrophil infiltration appeared were observed in the CVA4-infected group. The pathological injury of skeletal muscle caused by CAV4 was dose-dependently alleviated by HC, confirming the improvement effect of HC on pathological injury induced by

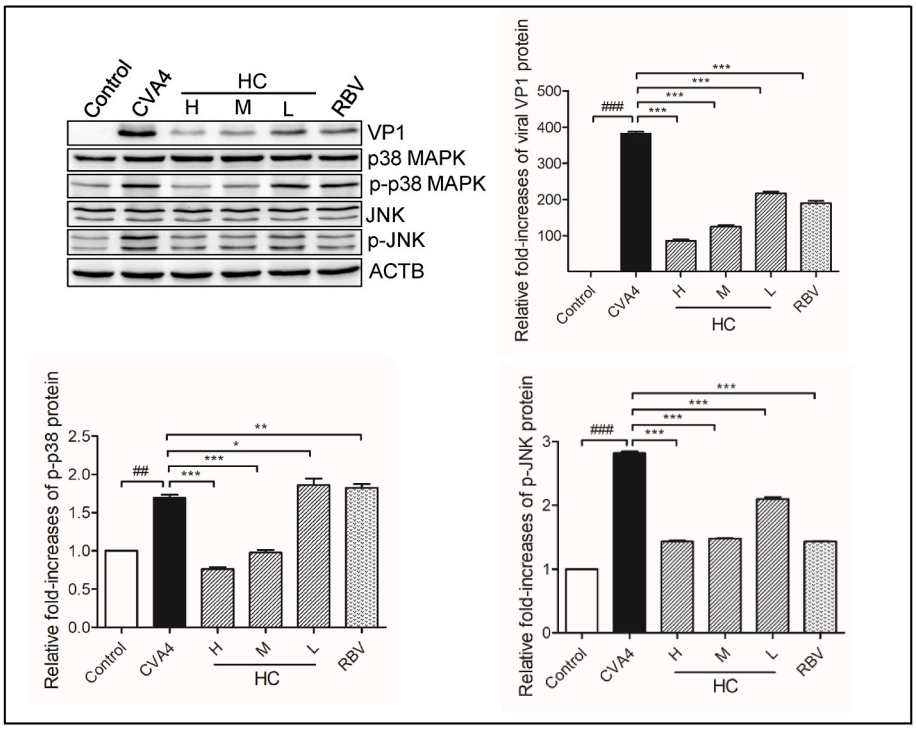

Fig. 7. Antiviral mechanism of HC against CAV4 by regulating p38 MAPK/JNK signaling pathway. RD cells infected with CAV4 were treated with HC or RBV for 12 h and the protein levels of viral VP1, p38, p-p38, JNK and p-JNK were examined with immunoblotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control.

CAV4 infection.

2.8. HC inhibited excessive cytokine response caused by CVA4 virus

To explore the antiviral effect mechanism of HC *in vivo*, we determined the inhibition effect of HC on the cytokine response. As shown in Fig. 9, compared with normal control group, the mRNA transcription level of inflammatory factors such as IL-6, IP-10, MIP-2 and NF-κB in skeletal muscle tissue of virus-infected mice increased significantly, indicating that CVA4 virus infection induced serious inflammatory reaction in skeletal muscle tissue of mice. Compared with the virus infection group, the high and low doses of HC can significantly inhibit the mRNA transcription of inflammatory factors such as IL-6, IP-10, MIP-2 and NF-κB in mice, which were also observed in the positive drug RBV treatment group, indicating that both HC and RBV administration groups can significantly inhibit the inflammatory reaction of skeletal muscle tissue in mice, but it is found that the RBV treatment group has no obvious inhibitory effect on NF-κB. The inhibitory effect of HC treatment group on inflammatory factors IL-6, MIP-2 and NF-κB was stronger than that of RBV treatment group, but its inhibitory effect on IP-10 was slightly weaker than that of RBV treatment group. The above results indicate that *Houttuynia cordata* may improve the skeletal muscle injury caused by CVA4 infection by effectively inhibiting the release of inflammatory factors in mice.

3. Discussion

In the past ten years, HFMD caused by a variety of enteroviruses outbroke sporadically worldwide, which resulted in many deaths of a few children and infants. CVA4, as one of the dominant epidemic strains after 2009, poses a significant threat and challenge to global public health. Furthermore, there are no effective antiviral drugs and vaccines currently available for the treatment of HFMD. However, traditional Chinese medicine was widely used to treat HFDM in China. The commonly used prescriptions in clinic include Qingwen Baidu decoction, Xiaoer Qingqiao Granules and Ganlu Xiaodu Dan. *Houttuynia cordata* Thunb, as a medicinal and edible herb, is widely used in treatment of a variety of diseases such as diabetes mellitus, cancer, pulmonary fibrosis, skin diseases, respiratory distress syndrome and other diseases. Pharmacological studies revealed that HC has broad-spectrum antiviral activity such as influenza A virus, Zika virus, type I herpes simplex virus (HSV-1), dengue virus, and SARS-CoV-2 (Hayashi et al., 1995; Basic et al., 2019; Gurung et al., 2021). Recently, an emerging of studies reported that HC has good anti-enterovirus effect. Lin et al. found *Houttuynia cordata* could neutralize EV71-induced cytopathic effects in Vero cells (Lin et al., 2009). Chen et al. revealed that the protective effect of HC on cytopathic effects caused by EVA71 and CVA16 (Chen et al., 2013). Moreover, the water extract and polysaccharide of HC also showed antiviral activity against coxsackievirus B3 (CV-B3), coxsackievirus B5 (CV-B5), and enterovirus 71 (EV71) *in vitro* (Fan, 2019). However, whether HC has anti-CVA4 virus effect has not been confirmed yet. In this study, our results for the first time demonstrated that HC had good anti-CVA4 effect, which were confirmed in CVA4-infected Vero and RD cell model using CPE, Western blot, immunofluorescence and qRT-PCR. The time-of-drug-addition analysis indicated that HC mainly exerts its antiviral activity at a post-entry stage. Furthermore, in 13-day-old suckling mice infected with CVA4, we also verified that HC can effectively protect the lethal infection of CVA4 by inhibiting virus replication, reducing the mortality, prolonging the survival time, controlling excessive cytokine storm, and improving the pathological damage. Collectively, our results indicated that HC has therapeutic effect on HFMD caused by CVA4.

Phytochemical studies showed that HC contains bioactive components such as volatile oil, flavonoids, polysaccharides, organic acids and alkaloids (Wei et al., 2023). Most of the active constituents of HC, such as quercetin, quercitrin, isoquercitrin, chrysin, rutin have been reported to have certain antiviral activity (Chiow et al., 2016). For example, quercetin can effectively inhibit EV71 replication by targeting viral 3Cpro activity (Dai et al., 2019; Yao et al., 2018). Here, we evaluated the anti-CVA4 activity of HC water extract. To investigate the bioactive

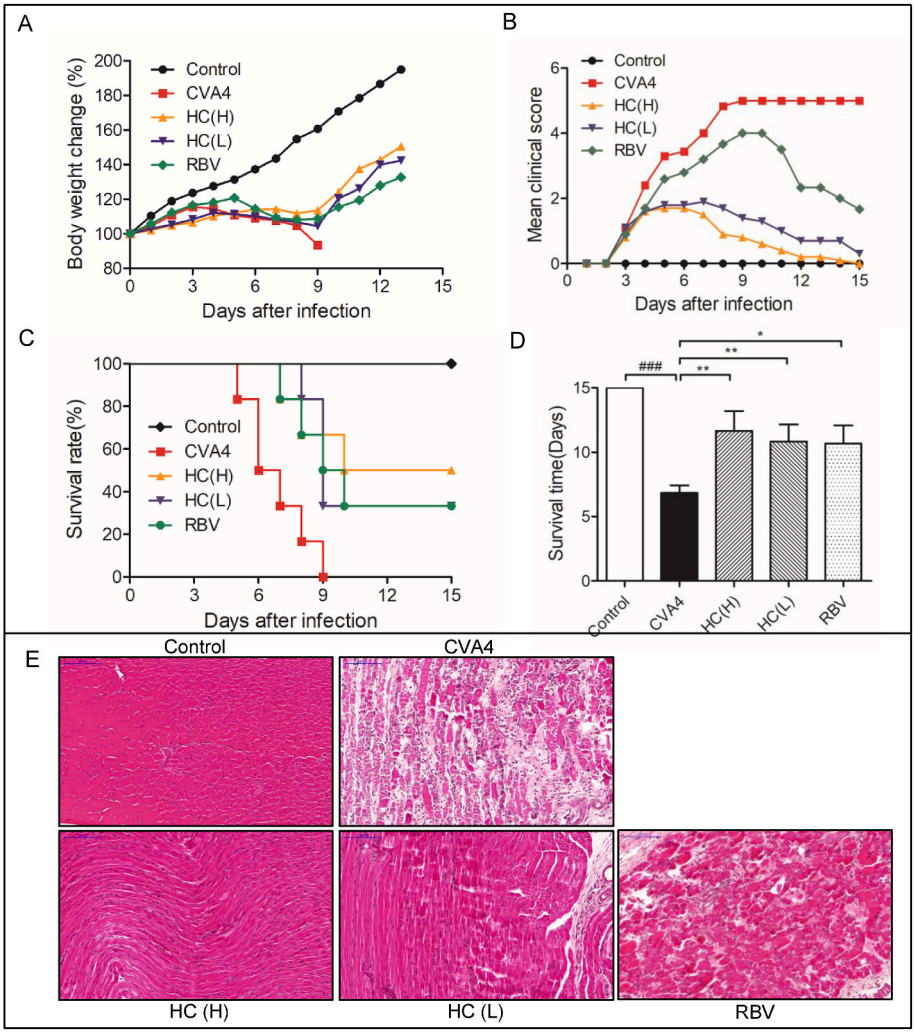

Fig. 8. *In vivo* antiviral effect of HC against CVA4 virus. The antiviral effect of HC on lethal CVA4 virus infected virus. 13-year-old ICR suckling mice were intramuscularly infected CVA4 virus and then treated with orally with HC (1.25 g/kg, 0.625 g/kg) or ribavirin (80 mg/kg) for 7 days. All mice were observed daily for 15 days. The weight, clinical symptoms and death of each group were recorded every day. (A) Body weight change, (B) Mean clinical score, (C) Survival rate, (D) Survival time, (E) Representative images of skeletal muscle injury stained by H&E staining (200 ×), Scale, 20 μm $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 virus infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control.

components of HC water extract against CVA4, we did HPLC analysis. Our results indicated that HC contains 7 active ingredients with potential antiviral effects including rutin, quercitrin, chlorogenic acid, cryptochlorogenic acid, quercetin, hyperoside and piperolactam. Lin et al. found that rutin could inhibit the activity of EV71 3Cpro in a dose-dependent manner (Lin et al., 2012). It is reported that quercitrin can regulate the oxidative stress caused by pseudorabies virus (PRV)-infected cells (Wang et al., 2022). Chlorogenic acid can inhibit virus release by directly targeting apoptosis caused by PDCoV infection (Shi et al., 2024). Hyperin can inhibit EHV-8 infection *in vitro* and reduce the lung damage caused by EHV-8 in infected mice (Wang et al., 2024). Li et al. found that chlorogenic acid has the ability to inhibit the replication and survival of EV71 *in vitro* (Li et al., 2013). Liu et al. confirmed the antiviral effect of quercetin isolated from mulberry leaves on EV71 through NF-kB signaling pathway by network pharmacology technology (Liu et al., 2023).

The innate immune response is a defensive mechanism of host defense against microbial infections. Cytokines play an important role in the body's immune response, such as type I interferon. Mitogen-activated protein kinase (MAPK) consists of C-Jun N-terminal Kinase (C-JNK), Extracellular-signal regulated protein kinase 1/2 (ERK1/2) and p38 Mitogen-activated protein kinase (p38 MAPK), which plays an important role in regulating cell proliferation, growth, differentiation and apoptosis (Chang et al., 2001). MAPK pathways are key components of intracellular signaling networks, which are often hijacked by viruses of diverse families to promote their own replication, such as infection and replication of human immunodeficiency virus type I (HIV-1), hepatitis C virus (HCV), coxsackie virus B3 (CVB3), herpes simplex virus type I (HSV) and severe acute respiratory syndrome coronavirus, these viruses all require the activation of JNK1/2 and/or p38 MAPK pathway (Du et al., 2012; Kumar et al., 2014; Kwon and Kim, 2014; Lu et al., 2006; Liu et al., 2012). Currently, direct pharmacological regulation of MAPK pathway activity resulted in a significant decrease of viral protein synthesis, viral RNA replication and progeny release, such as the anti-Influenza A Virus replication and Influenza viral pneumonia effect of emodin (Su et al., 2017). The main function of p38 MAPK and JNK signaling pathway is to promote cell apoptosis and death, which plays a vital role in the process of virus infection and pathogenesis. Porcine circovirus (PCV2) activates p38 MAPK and JNK pathways and its downstream targets c-Jun and ATF-2 with virus replication in cultured cells (Gong et al., 2011). Zhang et al. found that HC treatment decreased the level of p-p38 MAPK in a concentration dependent pattern but not the total p38-MAPK in LPS-stimulated microglia (Zhang et al., 2019). Lv et al. confirmed that apigenin significantly inhibited the increase of

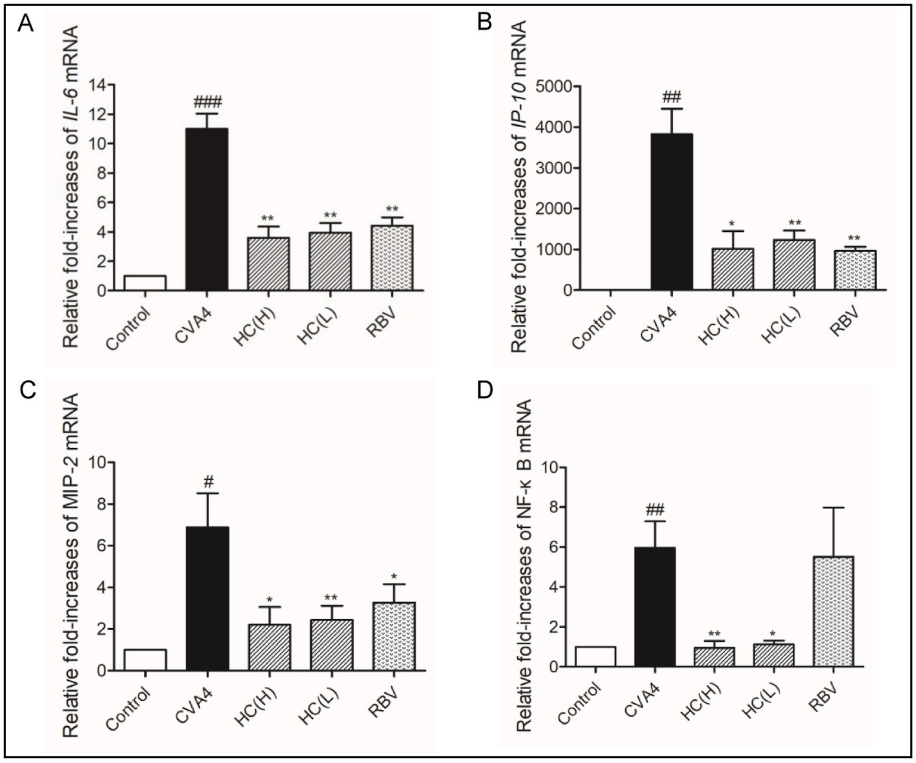

Fig. 9. *In vivo* inhibitory effect of HC on the expression of cytokines in infected mice. Furthermore, relative expression level of mRNA was also quantified by qRT-PCR. Relative transcript quantities were calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as a reference. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs CVA4 virus infected group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control.

p-JNK level induced by enterovirus 71 infection, but had no obvious effect on p-p38 level (Lv et al., 2014). In this study, our findings first revealed CVA4 infection can rapidly promote the activation of p38 MAPK and JNK signaling pathway in RD cells model, whereas these two pathways had been dose-dependently suppressed by HC, suggesting that inhibiting the over-activation of p38 MAPK and JNK signaling pathway may be the primary mechanism of HC against CVA4.

Herbal medicines or plant products were used to treat oxidative stress and inflammation-related diseases. Previous studies indicated that HC can reduce oxidative stress and inflammation through some signaling pathways (Fu et al., 2013; Lee et al., 2015; Chen et al., 2014; Hsu et al., 2016). Plant components such as astragaloside, hyperoside and quercitrin have shown anti-inflammatory effects and antioxidant properties *in vitro* and *in vivo* models. In this study, we found that HC effectively inhibited the replication of CVA4 virus *in vivo* and *in vitro*. Compared with RBV, HC exhibited better therapeutic effect on CVA4 infection, exhibiting slower weight loss (Fig. 8A), lower clinical scores (Fig. 8B), higher death protection rate (Fig. 8C), longer survival time (Fig. 8D) and lighter histopathological damage (Fig. 8E). Furthermore, HC exhibited stronger inhibitory effect on excessive cytokine storm caused by CVA4 infection than that of RBV, suggesting that HC has an advantage over RBV in improving excessive inflammatory responses.

4. Conclusion

In summary, our results for the first time demonstrated that HC not only effectively inhibits CVA4 replication *in vitro*, but also provides protection against lethal infection of CVA4 *in vivo*. Mechanistically, our findings revealed that the primary mechanism of HC against CVA4 infection may be associated with inhibiting over-activation of p38 MAPK and JNK signaling pathways. Moreover, HC exhibits better therapeutic effect than RBV in promoting survival and inhibiting excessive inflammatory responses. Our results clearly indicated that Chinese herbal medicine such as HC, is an important source of antiviral drugs for treating HFMD.

CRediT authorship contribution statement

Qin Su: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Hailin Wei:** Writing – review & editing, Supervision, Methodology. **Yihan Xu:** Writing, Data curation, Methodology, Supervision, Project administration, Formal analysis. **Yiliang Zhang:** Validation, Supervision, Conceptualization. **Wenlei Wang:** Validation, Investigation. **Jiaxue Zhou:** Project administration, Methodology. **Sitong Liu:** Validation. **Xiaohui Yang:** Validation. **Le Zhou:** Validation. **Pinghu Zhang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethics and informed consent

Animal ethics was ethically approved by Yangzhou University. All experimental protocols were performed in accordance with the Declaration of Helsinki and Ethical Standards guidelines.

Manuscript/data statement

This manuscript/data, or portions of this manuscript, has not been published in another journal or the work has not been previously published elsewhere.

Consent for publication

The authors all agreed for publication of this paper.

Declaration of competing interest

All authors disclosed no relevant relationships.

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List of Abbreviations

HC	*Houttuynia cordata* Thunb
EV-A	Enterovirus-A
EV-B	Enterovirus-B
EV-C	Enterovirus-C
EV-D	Enterovirus-D
CVA4	Coxsackievirus A4
CPE	Cell pathogenic effect
SARS	Severe acute respiratory syndrome coronavirus
EV71	Enterovirus A71
CVA16	Coxsackievirus A16
CVA6	Coxsackievirus A6
CVA8	Coxsackievirus A8
CVA10	Coxsackievirus A10
HFMD	Hand foot and mouth disease
MAPK	Mitogen-activated protein kinase
JNK	c-Jun N-terminal kinase
IC_{50}	50% inhibition concentration
TCM	Traditional Chinese Medicine
Vero	African green monkey kidney
RD	Rhabdomyosarcoma
TLR	Toll like receptor
MTT	Methyl thiazolyl tetrazolium
RBV	Ribavirin
$TCID_{50}$	Median tissue culture infective dose
PBS	Phosphate buffered saline
OD	Optical density

Data availability

Data will be made available on request.

References

Basic, M., Elgner, F., Bender, D., Sabino, C., Herrlein, M.L., Roth, Hanna, et al., 2019. A synthetic derivative of houttuynoid B prevents cell entry of Zika virus. Antivir. Res. 172, 104644. https://doi.org/10.1016/j.antiviral.2019.104644.

Chang, J.S., Chiang, L.C., Chen, C.C., Liu, L.T., Wang, K.C., Lin, C.C., 2001. Antileukemic activity of bidens pilosa L. Var. minor (blume) sherff and houttuynia cordata Thunb. Am. J. Chin. Med. 29 (2), 303–312. https://doi.org/10.1142/s0192415x01000320.

Chen, X., Wang, C., Xu, L., Chen, X.S., Wang, W., Yang, G., et al., 2013. A laboratory evaluation of medicinal herbs used in China for the treatment of hand, foot, and mouth disease. Evid Based Complement Alternat Med 2013 (3), 504563. https://doi.org/10.1155/2013/504563.

Chen, W.T., Yang, C.L., Yin, M.C., 2014. Protective effects from Houttuynia cordata aqueous extract against acetaminophen-induced liver injury. Biomedicine 4 (1), 5. https://doi.org/10.7603/s40681-014-0005-2.

Chiow, K.H., Phoon, M.C., Putti, T., Tan, B.K.H., Chow, V.T., 2016. Evaluation of antiviral activities of Houttuynia cordata Thunb. extract, quercetin, quercetrin and cinanserin on murine coronavirus and dengue virus infection. Asian Pac. J. Tropical Med. 9 (1), 1–7. https://doi.org/10.1016/j.apjtm.2015.12.002.

Dai, W.W., Bi, J.P., Li, F., Wang, S., Huang, X.Y., Meng, X.Y., et al., 2019. Antiviral efficacy of flavonoids against enterovirus 71 infection in vitro and in newborn mice. Viruses 11 (7), 625. https://doi.org/10.3390/v11070625.

Du, S.H., Li, H., Cui, Y.H., Yang, L.N., Wu, J.J., Huang, H.Y., et al., 2012. Houttuynia cordata inhibits lipopolysaccharide-induced rapid pulmonary fibrosis by up-regulating IFN-γ and inhibiting the TGF-β1/Smad pathway. Int. Immunopharm. 13 (3), 331–340. https://doi.org/10.1016/j.intimp.2012.03.011.

Fan, L., 2019. Study on Anti-infective Active Components of Houttuynia Cordata Thunb Extract. Shandong University of Traditional Chinese Medicine, Shandong. https://doi.org/10.27282/d.cnki.gsdzu.2019.000061.

Fu, J., Dai, L., Lin, Z., Lu, H., 2013. Houttuynia cordata Thunb: a review of phytochemistry and pharmacology and quality control. Chin. Med. 4 (1), 101–123. https://doi.org/10.4236/cm.2013.43015.

Gao, L., Zou, G., Liao, Q., Zhou, Y.D., Liu, F.F., Dai, B.B., et al., 2018. Spectrum of enterovirus serotypes causing uncomplicated hand, foot, and mouth disease and enteroviral diagnostic yield of different clinical samples. Clin. Infect. Dis. 67 (11), 1729–1735. https://doi.org/10.1093/cid/ciy341.

Gong, J., Shen, X., Chen, C., Qiu, H., Yang, R.G., 2011. Down-regulation of HIV-1 infection by inhibition of the MAPK signaling pathway. Virol. Sin. 26 (2), 114–122. https://doi.org/10.1007/s12250-011-3184-y.

Gurung, A.B., Ali, M.A., Lee, J., Farah, M.A., Al-Anazi, K.M., Al-Hemaid, F., 2021. Identification of SARS-CoV-2 inhibitors from extracts of Houttuynia cordata Thunb. Saudi J. Biol. Sci. 28, 7517–7527. https://doi.org/10.1016/j.sjbs.2021.08.100.

Hashimoto, I., 1982. Hagiwara A. Pathogenicity of a poliomyelitis-like disease in monkeys infected orally with enterovirus 71: a model for human infection. Neuropathol. Appl. Neurobiol. 8 (2), 149–156. https://doi.org/10.1111/j.1365-2990.1982.tb00269.x.

Hayashi, K., Kamiya, M., Hayashi, T., 1995. Virucidal effects of the steam distillate from Houttuynia cordata and its components on HSV-1, influenza virus, and HIV. Planta Med. 61 (3), 237–241. https://doi.org/10.1055/s-2006-958063.

Hsu, C.C., Yang, H.T., Ho, J.J., Yin, M.C., Hsu, J.Y., 2016. Houttuynia cordata aqueous extract attenuated glycative and oxidative stress in heart and kidney of diabetic mice. Eur. J. Nutr. 55 (2), 845–854. https://doi.org/10.1007/s00394-015-0994-y.

Huang, K., Zhang, P., Zhang, Z., Youn, J.Y., Wang, C., Zhang, H., et al., 2021. Traditional Chinese Medicine (TCM) in the treatment of COVID-19 and other viral infections: efficacies and mechanisms. Pharmacol. Ther. 225, 107843. https://doi.org/10.1016/j.pharmthera.2021.107843.

Kumar, M., Prasad, S.K., Krishnamurthy, S., Hemalatha, S., 2014. Antihyperglycemic activity of houttuynia cordata Thunb. In streptozotocin-induced diabetic rats. Adv Pharmacol Sci 2014, 809438. https://doi.org/10.1155/2014/809438.

Kwon, T.K., Kim, J.C., 2014. In vitro skin permeation and anti-atopic efficacy of lipid nanocarri-ers containing water soluble extracts of Houttuynia cordata. Drug Dev. Ind. Pharm. 40 (10), 1350–1357. https://doi.org/10.3109/03639045.2013.819883.

Lee, J.H., Ahn, J., Kim, J.W., Lee, S.G., Kim, H.P., 2015. Flavonoids from the aerial parts of Houttuynia cordata attenuate lung inflammation in mice. Arch Pharm. Res. (Seoul) 38 (7), 1304–1311. https://doi.org/10.1007/s12272-015-0585-8.

Li, X., Liu, Y., Hou, X., Peng, H., Zhang, Li, Jiang, Q., et al., 2013. Chlorogenic acid inhibits the replication and viability of enterovirus 71 in vitro. PLoS One 8 (9), e76007. https://doi.org/10.1371/journal.pone.0076007.

Li, X.W., Ni, X., Qian, S.Y., Wang, Q., Jiang, R.M., Xu, W.B., et al., 2018. Chinese guidelines for the diagnosis and treatment of hand, foot and mouth disease (2018 edition). World J Pediatr 14 (5), 437–447. https://doi.org/10.1007/s12519-018-0189-8.

Lin, T.Y., Liu, Y.C., Jheng, J.R., Tsai, H.P., Jan, J.T., Wong, W.R., et al., 2009. Anti-enterovirus 71 activity screening of Chinese herbs with anti-infection and inflammation activities. Am. J. Chin. Med. 37 (1), 143–158. https://doi.org/10.1142/s0192415x09006734.

Lin, Y.J., Chang, Y.C., Hsiao, N.W., Hsieh, J.L., Wang, C.Y., Kung, S.H., et al., 2012. Fisetin and rutin as 3C protease inhibitors of enterovirus A71. J. Virol Methods 182 (1–2), 93–98. https://doi.org/10.1016/j.jviromet.2012.03.020.

Liu, F., Shi, H., Shi, Y., Liu, Y., Jin, Y.H., Gao, Y.J., et al., 2010. Pharmacodynamics of houttuynia cordata Thunb injection against influenza virus. Acta Pharm. Sin. B 45 (3), 399–402. https://doi.org/10.16438/j.0513-4870.2010.03.006.

Liu, T., Li, Y., Wang, L., Zhang, X., Zhang, Y., Gai, X., et al., 2023. Network pharmacology-based exploration identified the antiviral efficacy of Quercetin isolated from mulberry leaves against enterovirus 71 via the NF-κB signaling pathway. Front. Pharmacol. 14, 1260288. https://doi.org/10.3389/fphar.2024.1421483.

Liu, X., Li, Q., Dowdell, K., Fischer, E.R., Cohen, J.I., 2012. Varicella-Zoster virus ORF12 protein triggers phosphorylation of ERK1/2 and inhibits apoptosis. J. Virol. 86 (6), 3143–3151. https://doi.org/10.1128/jvi.06923-11.

Lu, H.M., Liang, Y.Z., Yi, L.Z., Wu, X.J., 2006. Anti-inflammatory effect of Houttuynia cordata injection. J. Ethnopharmacol. 104 (1–2), 245–249. https://doi.org/10.1016/j.jep.2005.09.012.

Lv, X., Qiu, M., Chen, D., Zheng, N., Jin, Y., Wu, Z.W., 2014. Apigenin inhibits enterovirus 71 replication through suppressing viral IRES activity and modulating cellular JNK pathway. Antivir. Res. 109, 30–41. https://doi.org/10.1016/j.antiviral.2014.06.004.

Melnick, J.L., 1950. Studies on the Coxsackie viruses: properties, immunological aspects and distribution in nature. Bull. N. Y. Acad. Med. 26 (5), 342. https://doi.org/10.1084/jem.92.5.463.

Meng, J., Leung, K.S.Y., Jiang, Z., Dong, X.P., Zhao, Z.Z., Xu, L.J., et al., 2005. Establishment of HPLC-DAD-MS fingerprint of fresh Houttuynia cordata. Chem. Pharm. Bull. (Tokyo) 53, 1604–1609. https://doi.org/10.1248/cpb.53.1604.

Oberste, M.S., Peñaranda, S., Kaija, M., Pallansch, M.A., 2004. Complete genome sequences of all members of the species Human enterovirus A. J. Gen. Virol. 85 (6), 1597–1607. https://doi.org/10.1099/vir.0.79789-0.

Shi, C.X., Liang, W.W., Guo, M., Yuan, J., Zu, S.P., Hu, H., 2024. Chlorogenic acid inhibits porcine deltacoronavirus release by targeting apoptosis. Int. Immunopharm. 127, 111359. https://doi.org/10.1016/j.intimp.2023.111359.

Su, A.R., Qiu, M., Li, Y.L., Xu, W.T., Song, S.W., Wang, X.H., et al., 2017. BX-795 inhibits HSV-1 and HSV-2 replication by blocking the JNK/p38 pathways without interfering with PDK1 activity in host cells. Acta Pharmacol. Sin. 38 (3), 402–414. https://doi.org/10.1038/aps.2016.160.

Upala, P., Apidechkul, T., Suttana, W., Kullawong, N., Tamornpark, R., Inta, C., 2018. Molecular epidemiology and clinical features of hand, foot and mouth disease in

northern Thailand in 2016: a prospective cohort study. BMC Infect. Dis. 18 (1), 630. https://doi.org/10.1186/s12879-018-3560-4.

Wang, Q.H., Xie, X.D., Chen, Q., Yi, S., Chen, J.J., Xiao, Q., et al., 2022. Effects of quercitrin on PRV-induced secretion of reactive oxygen species and prediction of lncRNA regulatory targets in 3D4/2 cells. Antioxidants 11 (4), 631. https://doi.org/10.3390/antiox11040631.

Wang, T.T., Hu, L.Y., Li, R.B., Ren, H.Y., Li, S.W., Sun, Q., 2024. Hyperoside inhibits EHV-8 infection via alleviating oxidative stress and IFN production through activating JNK/Keap1/Nrf 2/HO-1 signaling pathways. J. Virol. 98 (4), e0015924. https://doi.org/10.1128/jvi.00159-24.

Wei, H., Wang, W., Su, Q., Zheng, Z.H., Chen, Z.H., Zhang, X.Y., et al., 2024. Network pharmacology-based study to investigate the mechanism of compound houttuynia mixture against influenza virus infection by suppressing TLR7/MyD88 signaling pathway. J. Tradit. Complement. Med. https://doi.org/10.1016/j.jtcme.2024.03.008.

Wei, P., Luo, Q., Hou, Y., Zhao, F.L., Li, F., Meng, Q.G., 2023. Houttuynia Cordata Thunb.: a comprehensive review of traditional applications, phytochemistry, pharmacology and safety. Phytomedicine 123, 155195. https://doi.org/10.1016/j.phymed.2023.155195.

Xie, Z., Jiang, N., Lin, M., He, X.S., Bo, Li, Dong, Y.J., et al., 2023. The mechanisms of polysaccharides from tonic Chinese herbal medicine on the enhancement immune function: a review. Molecules 28 (21), 7355. https://doi.org/10.3390/molecules28217355.

Yan, R., He, J., Liu, G., Zhong, J.F., Xu, J.P., Zheng, K., et al., 2022. Drug repositioning for hand, foot, and mouth disease. Viruses 15 (1), 75. https://doi.org/10.3390/v15010075.

Yao, C., Xi, C., Hu, K., Gao, W., Cai, X.F., Qin, J.L., et al., 2018. Inhibition of enterovirus 71 replication and viral 3C protease by quercetin. Virol. J. 15 (1), 1–13. https://doi.org/10.1186/s12985-018-1023-6.

Zhang, X., Zhang, Y., Li, H., Liu, L.D., 2022. Hand-Foot-and-Mouth disease-associated enterovirus and the development of multivalent HFMD vaccines. Int. J. Mol. Sci. 24 (1), 169. https://doi.org/10.3390/ijms24010169.

Zhang, Y., Wang, R., Shi, W., Zheng, Z.H., Wang, X.Q., Li, C., et al., 2021. Antiviral effect of fufang yinhua jiedu (FFYH) granules against influenza A virus through regulating the inflammatory responses by TLR7/MyD88 signaling pathway. J. Ethnopharmacol. 275, 114063. https://doi.org/10.1016/j.jep.2021.114063.

Zhang, Y.H., Ren, L.M., Wang, X.Y., 2019. Inhibitory effect of Houttuynia cordata Thunb on LPS-induced retinal microglial activation. Int. J. Ophthalmol. 12 (7), 1095–1100. https://doi.org/10.18240/ijo.2019.07.07.

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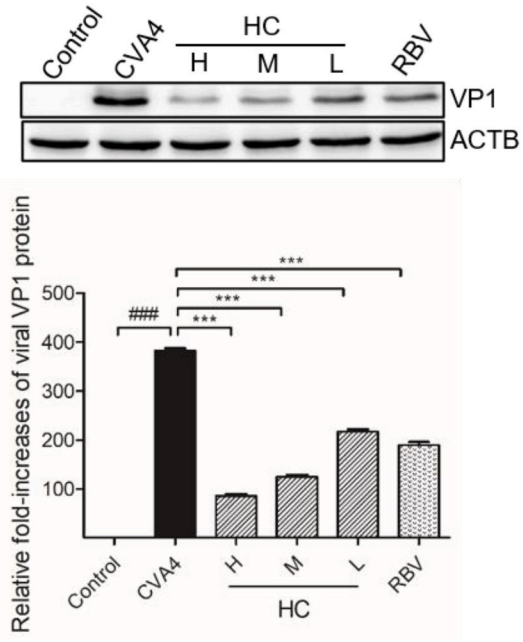
Qin Su [a,1], Hailin Wei [a,1], Yihan Xu [a,1], Yiliang Zhang [b,1], Wenlei Wang [a], Jiaxue Zhou [a], Sitong Liu [a], Xiaohui Yang [c,**], Le Zhou [d,***], Pinghu Zhang [a,e,*]

[a] *Jiangsu Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Treatment of Senile Diseases, Medical College, Yangzhou University, Yangzhou, 225009, China*
[b] *Swiss University of Traditional Chinese Medicine, Bad Zurzach, 5330, Switzerland*
[c] *Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042, China*
[d] *Yangzhou Center for Disease Control and Prevention & The Affiliated CDC of Yangzhou University, Yangzhou, Jiangsu, 225001, China*
[e] *Jiangsu Key Laboratory of Zoonosis, Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, Jiangsu, 225009, China*

The authors regret that when they carefully examined the original image, they found that there was an overlap between the WB results of RD cells in Figs. 5B and 7. They were sure that they used the same samples in Fig. 5B to investigate the antiviral mechanism analysis. But to avoid misunderstandings, they have applied to replace it with another repeat WB result in Fig. 5B.

Original Fig. 5B is as follows:



Revised Fig. 5B is as follows:

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* Corresponding author. Jiangsu Key Laboratory of Zoonosis, Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, Jiangsu, 225009, China.
** Corresponding author.
*** Corresponding author.
E-mail address: zhangpinghu@yzu.edu.cn (P. Zhang).
[1] These authors equally contributed to this work.

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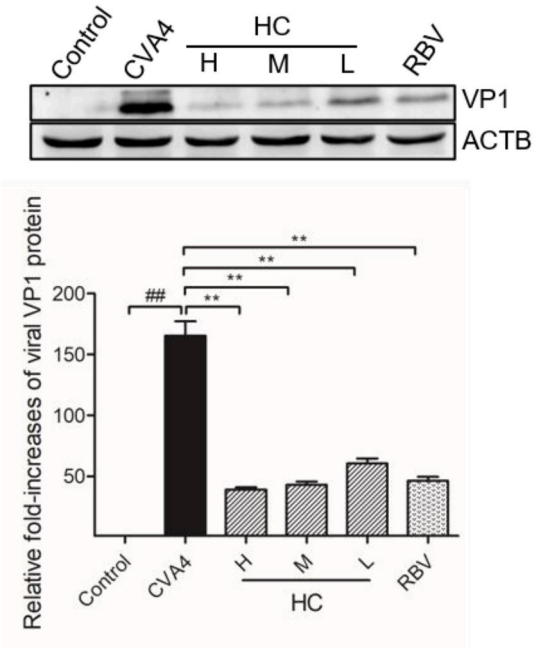

The authors would like to apologise for any inconvenience caused.