

Glycyrrhizin Modulates the Inflammatory Response Induced by Ev71 Infection by Targeting the NF- κ B pathway

Qian Li^{1,#}, Yuechuan Zhang^{2,#}, Tianjiao Wang¹, Jiamei Wu^{1,2,*}

¹Department of Basic Medical Science, Baicheng Medical College, Baicheng, Jilin, CHINA.

²Department of Basic Medical Science, Jiamusi University, Jiamusi City, Heilongjiang Province, CHINA.

[#]These authors contributed equally to this work and share first authorship.

ABSTRACT

Background: Previous research has demonstrated that glycyrrhizin exhibits antioxidant, anti-inflammatory and antiapoptotic characteristics. In this study, RD cells were infected with Enterovirus 71 (EV71) and a cell model of viral infection was established to explore the effect of glycyrrhizin on the inflammatory response triggered by EV71 infection of RD cells and its potential mechanism. **Materials and Methods:** In this study, the cells were categorized into 4 groups: the uninfected Normal control group, the EV71-infected RD group, the glycyrrhizin treatment group and the PDTC group (which received the NF- κ B pathway inhibitor PDTC). The expression levels of EV71's VP1, 3D, P65 and phosphorylated-P65 (p-P65) proteins in each group were detected by western blotting. Using ELISA, the amounts of pro-inflammatory cytokines TNF- α , IL-18 and IL-1 β in the RD cells' growth media were measured. **Results:** Compared with the uninfected regular control group, the EV71-infected RD group had increased levels of inflammatory factors IL-1 β , IL-18 and TNF- α and the expression of p-P65 protein was significantly upregulated. However, in the presence of 50 μ M glycyrrhizin or PDTC, the levels of IL-1 β , IL-18 and TNF- α were notably decreased in the p-P65 protein's expression, which was markedly down-regulated in the culture medium. **Conclusion:** Glycyrrhizin appears to mitigate the inflammatory response triggered by EV71 infection in RD cells by inhibiting the NF- κ B signalling pathway.

Keywords: Enterovirus 71, Glycyrrhizin, Inflammatory response, NF- κ B signaling pathway.

Correspondence:

Dr. Jiamei Wu

¹Department of Basic Medical Science, Baicheng Medical College, Baicheng-137000, Jilin, CHINA.

²Department of Basic Medical Science, Jiamusi University, Jiamusi City, Heilongjiang Province, CHINA.
Email: wjm838875@outlook.com

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INTRODUCTION

Enteroviruses, representing over 20 different types, are responsible for a common pediatric illness called Hand, Foot and Mouth Disease (HFMD). Although HFMD typically resolves on its own and has a favourable outcome, it can develop into serious neurological complications such as brainstem encephalitis, encephalomyelitis and aseptic meningitis. These can swiftly progress to neurogenic pulmonary oedema, potentially fatal in severe instances. Enterovirus 71 (EV71) is the leading cause of these severe manifestations.¹ Since there is currently no proven clinical treatment for HFMD, supportive care is still the mainstay of care. HFMD has triggered numerous outbreaks globally, affecting public health significantly and posing substantial risks to individuals and economic implications for affected countries.

Traditional Chinese Medicine (TCM) maintains that viruses result from the body being invaded by chilling malevolence,

which takes advantage of a weakened immune system. Like many acute viral infections, patients infected with EV71 carry high levels of cytokines and chemokines, leading to cell or tissue damage. Studies have shown that EV71 infection can induce phosphorylation of Signal Transduction and Activator of Transcription 3 (STAT3), which regulates inflammation and immunity and promotes the expression of downstream inflammatory regulators. EV71 also induces apoptosis and inflammation by promoting the production of Reactive Oxygen Species (ROS), decreasing the expression of Sirtuin 1 (SIRT1) and increasing the levels of interleukin-1 β (IL-1 β), Interleukin 6 (IL-6) and Tumour Necrosis Factor- α (TNF- α). Moreover, EV71 interacts with intracellular Toll-like Receptor 9 (TLR9). It elicits a neurotoxic glial response through Interleukin 12p40 (IL12p40)-inducible Nitric Oxide Synthase (iNOS) signalling, closely related to the development of brainstem encephalitis. TCM has been extensively applied in the treatment of viruses, with evidence indicating that various Chinese herbs can directly target viruses and boost the immune response by triggering the production of antiviral interferons. Glycyrrhizin, a compound obtained from the root of liquorice, is typically employed in treating liver conditions due to its capacity to prevent fibrosis, steatosis and necrosis in the liver while also facilitating cell regeneration.¹ Studies have



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established that glycyrrhizin possesses anti-inflammatory, anti-cancer and antiviral properties, demonstrating effectiveness against human herpesvirus type 4 (EBV), Dengue Virus (DENV) and influenza A H1N1/PDM09.²⁻⁴ However, the function and mechanism of glycyrrhizin in EV71 infection remain to be fully understood. Therefore, this research employs RD cells infected with EV71 to a cell model to examine the inflammatory response triggered by EV71 infection in these cells and investigate the potential molecular mechanisms contributing to the disease's progression.

MATERIALS AND METHODS

Experimental materials

The human malignant embryonic Rhabdomyosarcoma cells (RD) are obtained from the American Type Culture Collection. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA) supplemented with a high glucose concentration, 10% Fetal Bovine Serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) at 37°C in an atmosphere of 5% CO₂ and 95% air. The experimental materials include glycyrrhizin (5 mg/ampoule) and PDTC, sourced from Sigma (USA). The antibodies used are P65, p-P65 and β -actin from Cell Signaling Technology (USA). RIPA lysis buffer and ELISA kits for Interleukin-18 (IL-18), IL-1 β and TNF- α are used, sourced from Wuhan Boster Biological Technology.

50% Tissue Culture Infective Dose (TCID₅₀)

RD cells were evenly seeded into 96-well plates to achieve a cell density of 2×10^5 cells/mL. 100 μ L of the cell suspension was added

to each well. The EV71 virus was diluted with a maintenance solution containing 2% fetal bovine serum and used to infect RD cells at concentrations ranging from 10^{-1} to 10^{-8} . The 50% Tissue Culture Infective Dose (TCID₅₀) of the EV71 virus was calculated using the Reed-Muench method.

Creation of the EV71-infected RD model

The inoculation concentration of the EV71 virus was set to 100*TCID₅₀. EV71 was seeded with a maintenance solution containing 2% fetal bovine serum and seeded into an established monolayer of RD cells. After seeding, cells were kept in an incubator and incubated at 37°C for 24 hr in a 5% carbon dioxide atmosphere. The growth status and Cytopathic Effect (CPE) of cells were observed and the RD model of EV71 infection was established.

Grouping of the experimental subjects and associated treatments

Control Group: RD cells were grown in DMEM supplemented with 2% fetal bovine serum. EV71-Infected RD Group: Virus concentrations of 100*TCID₅₀ were used to infect RD cells. Glycyrrhizin in low concentration (25 μ M), medium concentration (50 μ M) and high concentration (75 μ M) concentration Groups: DMEM containing 2% fetal bovine serum as a solvent is used to prepare glycyrrhizin solutions at concentrations of 25 μ M, 50 μ M and 75 μ M, respectively and then applied to RD cells infected with EV71. PDTC Group: RD cells infected with EV71 were treated with a final concentration of 10 μ M of the NF- κ B pathway inhibitor PDTC.

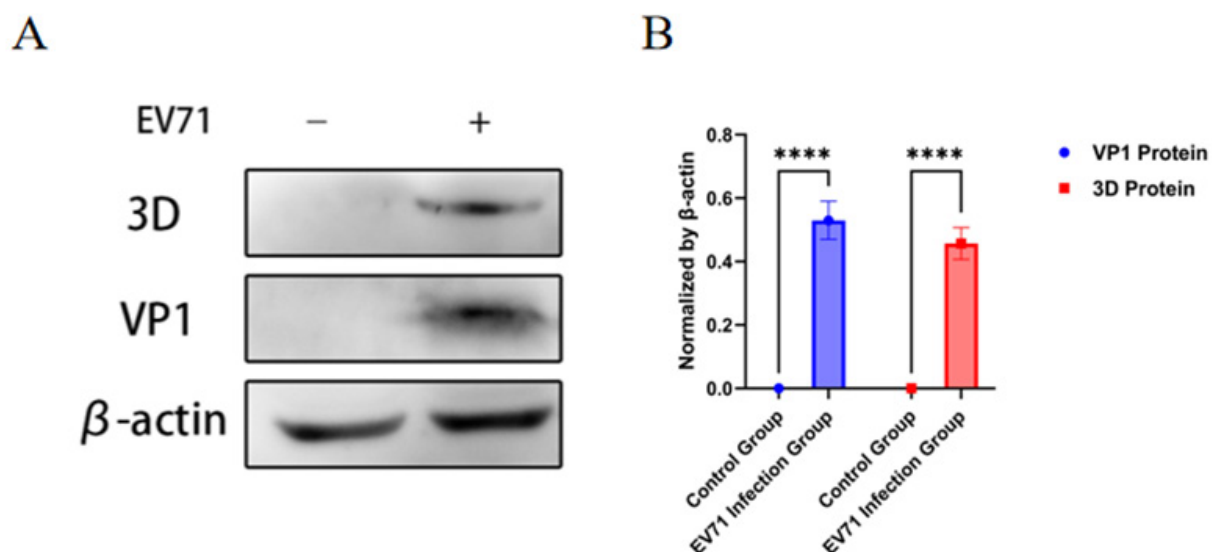


Figure 1: EV71 infection increased the expression of VP1 and 3D proteins. (A) RD cells were infected with EV71 at a concentration of 100*TCID₅₀ for 24 hr and the expression of VP1 and 3D proteins was analyzed by western blotting. (B) After normalization of the internal control β -actin, we evaluated the ratio of VP1 to 3D protein. Statistical analysis showed that the difference was statistically significant compared with the NC group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Cell viability assays

RD cells were plated in 96-well plates and cultured (2×10^5 cells/mL). Subsequently, human RD cells were infected with EV71 and treated with different concentrations of glycyrrhizin (25 μ M, 50 μ M and 75 μ M). The cell viability of RD was determined using the Cell Counting Kit-8 (CCK-8) kit (Beyotime Biotech, Shanghai, China) according to the manufacturer's instructions.

Protein extraction and western blot analysis

RD cells were treated with EV71 glycyrrhizin and PDTC for 24h, followed by harvesting and lysing in Radioimmunoprecipitation Assay (RIPA) lysis buffer (Boster Biological Technology Wuhan) containing a protease inhibitor cocktail. The lysates were centrifuged at 15,000 rpm for 15 min at 4°C. The protein concentration was determined using the bicinchoninic acid assay (Boster Biological Technology, Wuhan, China) and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The electrophoresis products were then transferred to polyvinylidene fluoride membranes (Merck, Darmstadt, Germany). Subsequently, the membranes were incubated with primary antibodies in 5% BSA in TBST (TBS with 0.05% Tween-20) overnight at 4°C. Afterward, the membranes were washed three times with TBST for 10 min each and then incubated with secondary antibodies at 37°C for 1 hr. After this, the membranes were washed thrice with TBST for 10 min each time. Finally, chemiluminescence detection is performed.

Detection of inflammatory factor secretion by ELISA

Following the manufacturer's protocol for the ELISA kits, the culture media from each RD cell group were harvested after a 24-hr incubation period to Check the levels of secreted inflammatory factors, such as TNF- α , IL-18 and IL-1 β .

Statistical Analysis

The data analysis and visualization were performed using GraphPad Prism version 8.0 software (GraphPad Software, San Diego, CA, United States). The statistical program SPSS 19.0 was used to analyse the collected data. The results were presented as mean $\pm$ standard deviation. A one-way ANOVA was used to compare different groups and LSD-t tests were used for individual comparisons. A statistically significant threshold of $p < 0.05$ was set for these tests.

RESULTS

Expression Levels of VP1 and 3D Proteins in RD cells Infected with EV71

VP1 protein, a structural component on the surface of EV71 viral particles, is crucial for the invasion of host cells. The 3D protein serves as a pivotal enzyme in replicating the EV71 virus. The EV71-infected group showed significantly higher expression of 3D proteins and VP1 in comparison to the control group, indicating the efficacy of the EV71-infected RD model (Figure 1).

EV71 infection enhances the expression of NF- κ B and other inflammatory Factors in RD cells

Referring to Figure 2, the expression of p-NF- κ B protein in the EV71-infected group was substantially higher than that in the control group. Additionally, there was a significant rise in the levels of IL-1 β , IL-18 and TNF- α in the cell culture media (Figure 3). These results suggest that EV71 infection can cause significant inflammatory pathological changes in host cells.

Glycyrrhizin's Antiviral Effect on EV71

The antiviral EV71-infected RD cells effect of glycyrrhizin showed a time-and concentration-dependent trend. The viability of RD

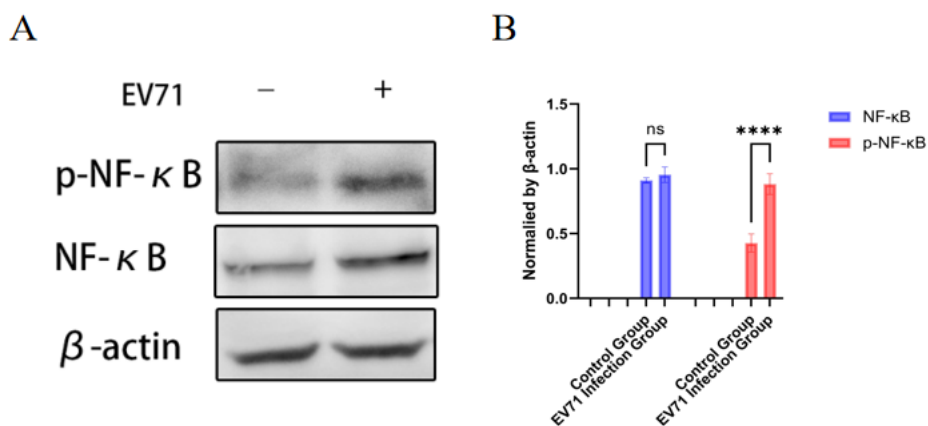


Figure 2: EV71 infection increased the expression of NF- κ B and p-NF- κ B proteins (A) RD cells were infected with EV71 at a concentration of 100*TCID₅₀ for 24 hr and the expression of NF- κ B and p-NF- κ B proteins was analyzed by western blotting. (B) After normalization of the internal control β -actin, we evaluated the ratio of NF- κ B to p-NF- κ B protein. Statistical analysis showed that the difference was statistically significant compared with the NC group ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

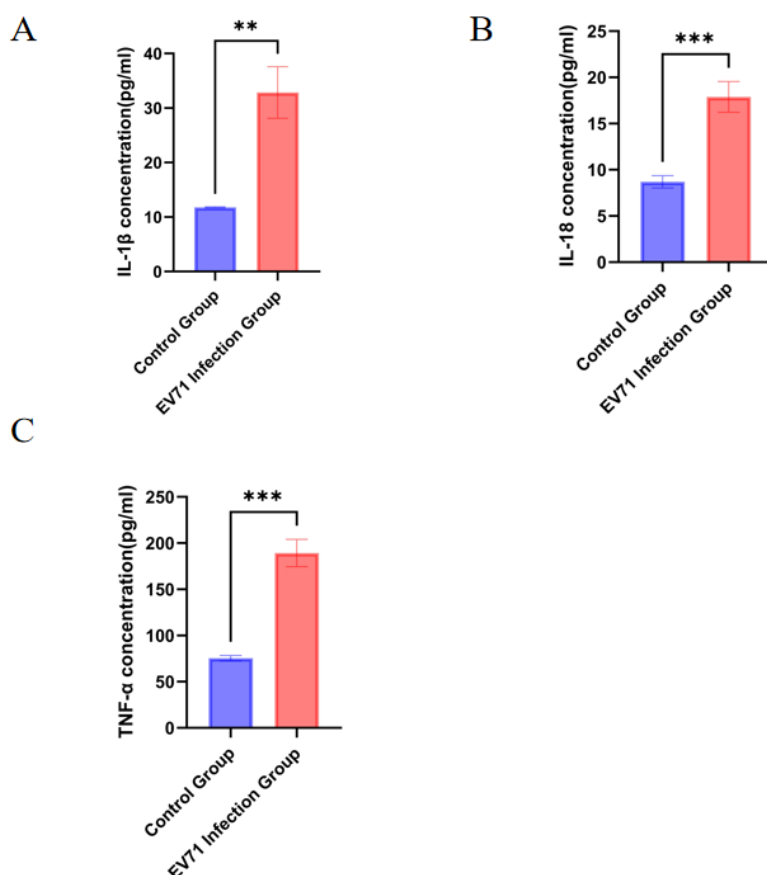


Figure 3: EV71 infection increased the expression of IL-1 β , IL-18 and TNF- α proteins (A-C) RD cells were infected with EV71 at a concentration of 100*TCID₅₀ for 24 hr and the expression of IL-1 β , IL-18 and TNF- α proteins in the cell culture supernatant was analyzed by ELISA. The results of statistical analysis showed that the difference was statistically significant compared with the NC group (* p <0.05, ** p <0.01, *** p <0.001).

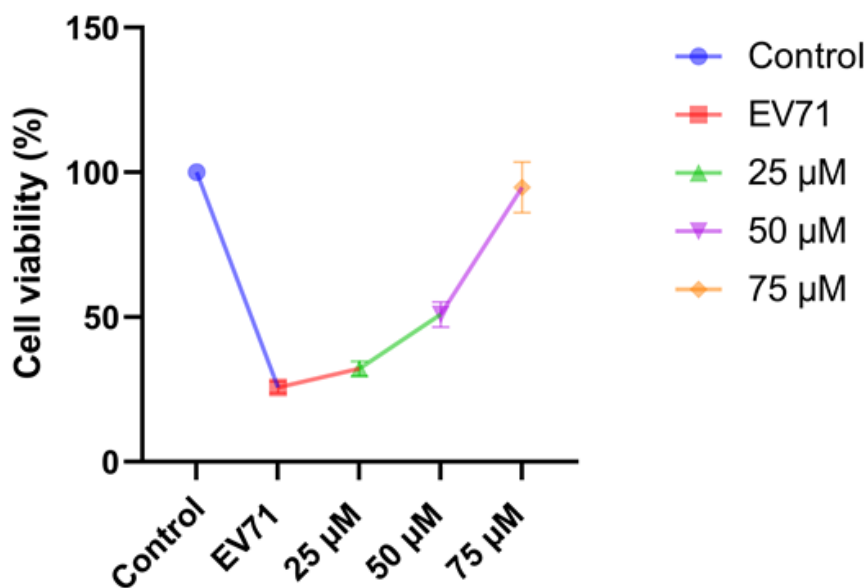


Figure 4: Glycyrrhizin improves the survival of EV71-infected RD cells. The RD cells infected with EV71 were treated with 25, 50 and 75 μ M/mL glycyrrhizin for 24 hr. The cell viability was detected by the CCK-8 method and the half-inhibitory concentration of glycyrrhizic acid was calculated.

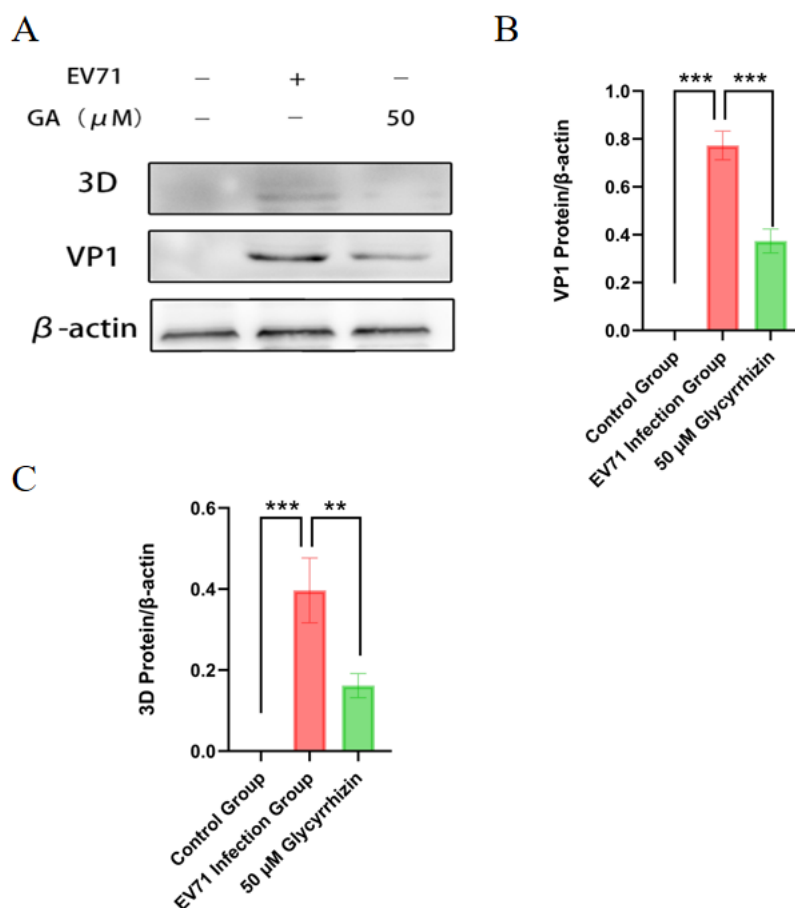


Figure 5: Glycyrrhizin reduces the expression of VP1, 3D proteins in EV71-infected RD cells. (A) EV71-infected RD cells were treated with 25, 50 and 75 μ M/mL glycyrrhizin for 24 hr, respectively. The expression of VP1 and 3D proteins was analyzed by western blotting. (B-C) After normalizing the internal control β -actin, we assessed the ratio of VP1 and 3D proteins. Statistical analysis showed that the difference was statistically significant compared to the NC group ($p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

cells infected with EV71 and subjected to treatment with 25 μ M glycyrrhizin for 24 hr was 32.19%, with 50 μ M glycyrrhizin for 24 hr was 50.88% and with 75 μ M glycyrrhizin for 24 hr was 94.83%. The IC_{50} value of glycyrrhizin for 24-hr treatment of EV71-infected RD cells was determined to be 50 μ M (Figure 4).

Subsequent tests within this investigation employed glycyrrhizin at a concentration of 50 μ M. In comparison to the EV71-infected group, the glycyrrhizin-treated group showed a significant reduction in the expression of VP1 and 3D proteins (Figure 5).

The impact of the NF- κ B Inhibitor PDTC on the inflammatory markers in EV71-infected RD cells was examined.

The PDTC group demonstrated a remarkably reduced in the relative expression of the p-NF- κ B protein Vs to the EV71-infected RD group (Figure 6). And the concentrations of IL-18, IL-1 β and TNF- α in the culture medium were substantially reduced (Figure 7).

The influence of glycyrrhizin on the inflammatory in EV71-infected RD cells was investigated.

Different dose group cells treated with glycyrrhizin showed a substantial decrease in the relative expression levels of the p-NF- κ B protein compared to the EV71-infected RD group (Figure 8). Furthermore, there was a significant decrease in the levels of IL-18, IL-1 β and TNF- α in the culture media (Figure 9).

DISCUSSION

Glycyrrhiza glabra L. is widely regarded as an important medicinal plant and has been used in traditional medicine for centuries due to its healing properties. Modern pharmacological studies have found that glycyrrhizic acid isolated from licorice has many pharmacological activities, such as anti-inflammatory, antiviral, immunomodulatory, antitumor and other activities.⁵ Glycyrrhizic acid and its metabolites have broad antiviral activity against a variety of viruses, such as hepatitis virus, herpes virus and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), among others.⁶ Although their antiviral activity has been widely reported, the specific mechanism of action involving multiple links, such as the virus itself, cells and immunity, has not yet been clarified. In this study, we found that glycyrrhizin has low

toxicity against EV71 activity in RD cells. Licorice may reduce EV71 replication and attenuate the virus-induced inflammatory response by inhibiting the NF- κ B pathway, suggesting that glycyrrhizin has the potential to develop into a novel anti-EV71 drug.

HFMD is a common childhood infectious disease caused primarily by EV71.⁷ In severe cases, HFMD can lead to serious neurological complications such as meningitis, encephalitis, acute flaccid paralysis, neurorespiratory syndrome and even death.⁸ EV71 is a single-stranded positive-sense RNA virus belonging to the genus Enteroviruses in the family Small RNA Viridae. Its genome size is approximately 7.4 kb and encodes four Viral capsid structural Proteins (VP1-VP4) as well as seven non-structural proteins (2A-2C and 3A-3D).⁹ VP1 protein is a structural protein on the surface of EV71 viral particles, which acts as the main antigenic component and is responsible for binding the virus to host cell surface receptors. In addition, VP1 protein has some degree of immunogenicity and is able to induce an immune response in the host.¹⁰ The 3D protein, also known as the virus-dependent RNA polymerase, is another key protein of EV71 and is a critical

enzyme in viral replication. It is not only involved in viral replication but may also be involved in viral transcription and translation.^{11,12} Thus, the expression levels of VP1 protein and 3D protein were indicative of enterovirus 71 replication. In this study, an EV71-infected RD cell model was established and treated with different concentrations of glycyrrhizin. The results showed that after 24 hr of incubation, the expression levels of VP1 and 3D proteins in the EV71-infected RD group were significantly higher than those in the control group, indicating that the model had been successfully established. However, the expression levels of VP1 and 3D proteins in RD cells were significantly lower in the glycyrrhizin treatment group than in the EV71-infected RD group and the effect was most significant at the glycyrrhizin concentration of 75 micromol, with an inhibition rate of 94.83%. This suggests that glycyrrhizin has a potent inhibitory effect on EV71 replication.

Cytokines are essential for cell-to-cell communication and viral clearance in the immune system, but excess cytokines can lead to severe immunopathology.¹³ In recent years, an increasing number of studies have been conducted on cytokine levels associated with

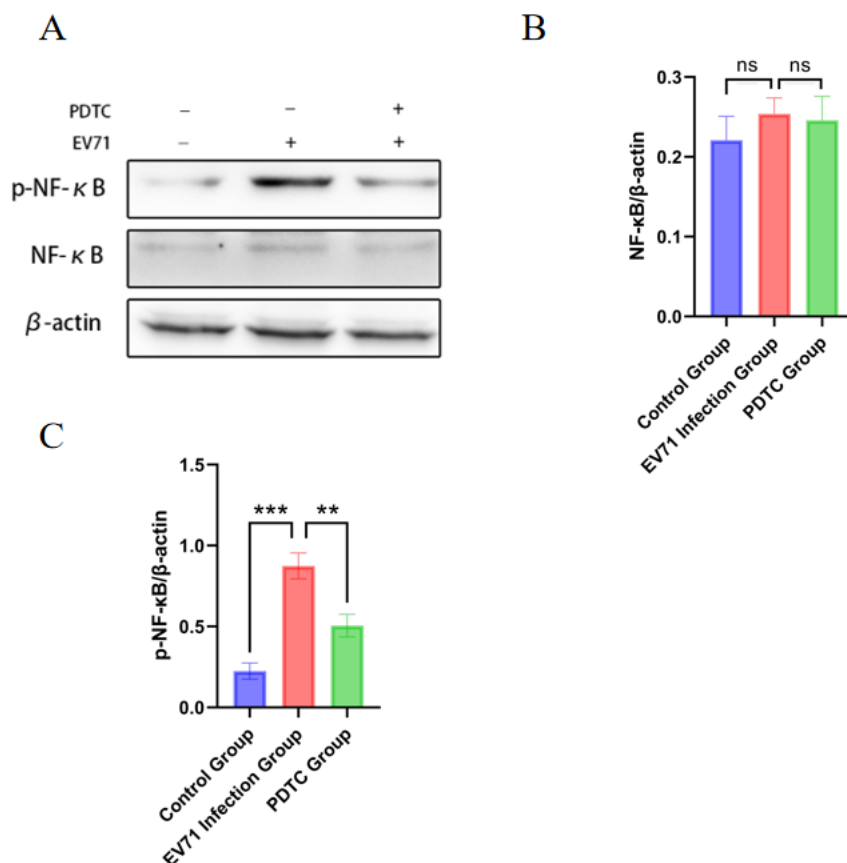


Figure 6: PDTC reduces the expression of NF- κ B and p-NF- κ B proteins in EV71-infected RD cells. (A) RD cells infected with EV71 were treated with 10 μ M PDTC for 24 hr. Western blotting was used to analyze the expression of NF- κ B and p-NF- κ B proteins. (B-C) After normalization of the internal control β -actin, we assessed the ratio of NF- κ B and p-NF- κ B proteins. Statistical analysis showed that the difference was statistically significant compared to the NC group (* p <0.05, ** p <0.01, *** p <0.001).

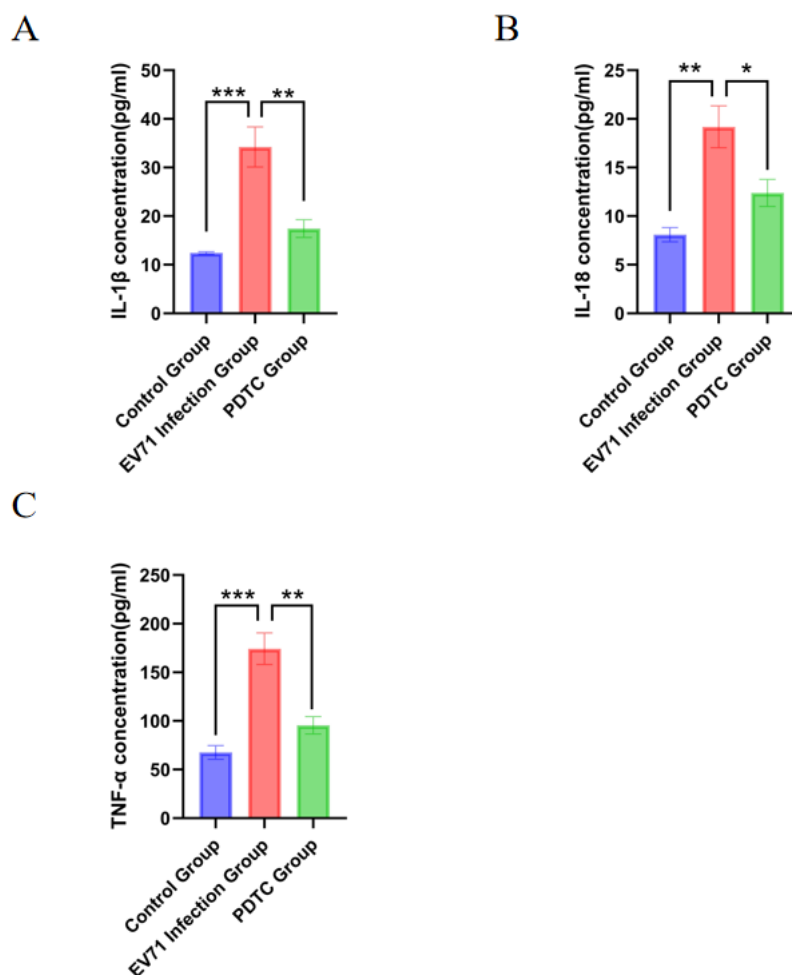


Figure 7: PDTC reduces the expression of IL-1 β , IL-18 and TNF- α proteins in EV71-infected RD cells. (A-C) EV71-infected RD cells were treated with 10 μ M/mL PDTC for 24 hr. ELISA was used to analyze the expression of IL-1 β , IL-18 and TNF- α proteins in cell culture supernatants. The results of statistical analysis showed that the difference was statistically significant compared with the NC group (* p <0.05, ** p <0.01, *** p <0.001).

serious complications caused by EV71 infection, suggesting that the important role of cytokines in the occurrence and progression of EV71 infection has been recognized. As with many acute viral infections, patients infected with EV71 carry high levels of cytokines and chemokines, leading to cellular or tissue damage.¹⁴ Following EV71 infection, susceptible cells and non-specific immune cells are first stimulated to produce cytokines such as TNF- α , IL-1 β and IL-18, which play an important role in the early control of viral replication and infection.¹⁵ Some studies have found that EV71 replication can be reduced by inhibiting the inflammatory response and alleviating severe clinical symptoms. For example, proanthocyanidins can modulate three different Mitogen-Activated Protein Kinase (MAPK) signaling pathways, attenuate virus-induced inflammatory responses and reduce EV71 replication.¹⁶ In this study, glycyrrhizin was applied to EV71-positive RD cells and the levels of corresponding inflammatory factors in the medium were evaluated. The results of the study showed that the secretion of IL-1 β , IL-18 and TNF- α

was significantly reduced after treatment with glycyrrhizin, suggesting that glycyrrhizin has a modulating effect on EV71-induced inflammation.

NF- κ B signaling has been known for nearly 40 years.¹⁷ Five structurally related proteins, namely NF- κ B1 (p50), NF- κ B2 (p52), RelA (p65), RelB and c-Rel, are involved in the NF- κ B pathway.¹⁸ In the resting state, inactive NF- κ B is bound by the Inhibitory κ B protein (I κ B) in the cytoplasm. Degradation of I κ B protein by the proteasome activates NF- κ B, mediating its translocation into the nucleus, where it functions as a nuclear transcription factor and upregulates the expression of pro-inflammatory genes.¹⁹⁻²² Previous studies have shown that the NF- κ B signaling pathway is strongly associated with the inflammatory response to EV71 infection.²³ NF- κ B inhibitors have been observed to enhance the defense mechanism of the central nervous system after EV71 infection and quercetin, extracted from mulberry leaves, significantly reduces the expression levels of VP1, TNF- α and IL-1 β in EV71-infected RD by inhibiting the NF- κ B

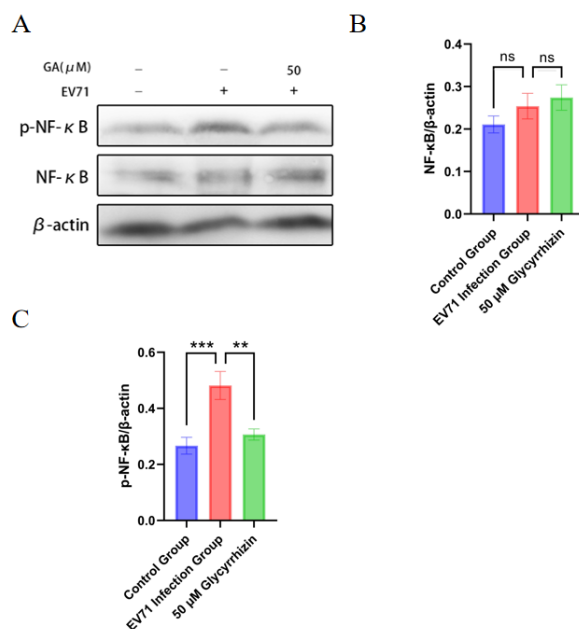


Figure 8: Glycyrrhizin reduces the expression of NF- κ B and p-NF- κ B proteins in EV71-infected RD cells. (A) EV71-infected RD cells were treated with 50 μ M glycyrrhizin for 24 hr. Western blotting was used to analyze the expression of NF- κ B and p-NF- κ B proteins. (B-C) After normalization of the internal control β -actin, we assessed the ratio of NF- κ B to p-NF- κ B protein. Statistical analysis showed that the difference was statistically significant compared to the NC group (* p <0.05, ** p <0.01, *** p <0.001).

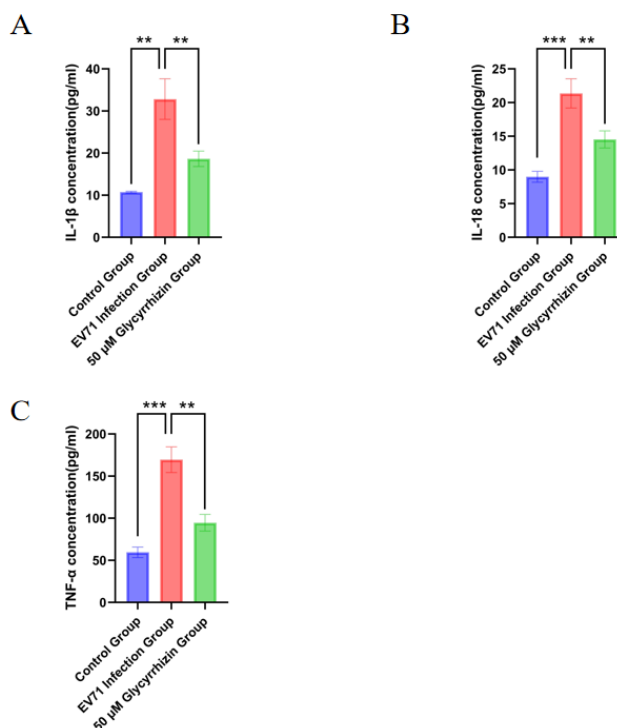


Figure 9: Glycyrrhizin reduces the expression of IL-1 β , IL-18 and TNF- α proteins in EV71-infected RD cells. (A-C) EV71-infected RD cells are treated with 50 μ M/mL glycyrrhizin for 24 hr. ELISA was used to analyze the expression of IL-1 β , IL-18 and TNF- α proteins in cell culture supernatants. The results of statistical analysis showed that the difference was statistically significant compared with the NC group (* p <0.05, ** p <0.01, *** p <0.001).

signaling pathway.^{24,25} Licorice derivatives have been found to prevent the degradation of IκB, an inhibitory protein, which in turn inhibits the activation of NF-κB. For example, in a rat model of psoriasis, glycyrrhizin regulates the NF-κB signaling pathway by inhibiting the phosphorylation of NF-κB p65, which significantly inhibits the progression of psoriasis.²⁶ In this study, it was observed that phosphorylated P65 (p-P65) expression was significantly upregulated after EV71 infection, while glycyrrhizin attenuated this effect. This suggests that glycyrrhizin may be able to counteract the activation of the NF-κB pathway induced by EV71 in RD cells.²⁷ In addition, the inhibition of nuclear NF-κB activity with Pyrrolidine Dithiocarbamate (PDTC) significantly downregulated the expression of VP1 and 3D proteins in EV71-infected RD cells.

In essence, glycyrrhizin demonstrates a significant inhibitory effect on EV71 infection in RD cells by decreasing the levels of inflammatory markers in the supernatant, which is achieved by lowering the activity of the inflammatory pathway. This effect is comparable to that of PDTC. It indicates that glycyrrhizin may modulate the inflammatory response of EV71-infected RD cells by targeting the NF-κB signaling cascade. This investigation uncovers the impact and potential mechanism of glycyrrhizin on the inflammatory response of EV71-infected RD cells, offering a theoretical foundation for the future selection of glycyrrhizin as a potential therapeutic agent against EV71 infection.

CONCLUSION

Together, we postulated glycyrrhizin affects and underlies the suppression of the inflammatory response that EV71 infection causes in RD cells. Glycyrrhizin significantly upregulated the expression of the p-P65 protein in RD cells and increased the levels of IL-1β, IL-18 and TNF-α. Additionally, glycyrrhizin or PDTC, IL-1β, IL-18 and TNF-α levels dropped and the expression of the p-P65 protein was significantly down-regulated in the culture medium. By blocking the NF-κB signaling pathway, glycyrrhizin seems to lessen the inflammatory response that EV71 infection causes in RD cells.

ABBREVIATIONS

HFMD: Hand, Foot and Mouth Disease; **EV71:** Enterovirus 71; **TCM:** Traditional Chinese Medicine; **STAT3:** Signal Transduction and Activator of Transcription 3; **ROS:** Reactive Oxygen Species; **SIRT1:** Sirtuin 1; **IL-1β:** Interleukin-1β; **IL-6:** Interleukin 6; **TNF-α:** Tumor Necrosis Factor-α; **TLR9:** Toll-like Receptor 9; **IL12p40:** Interleukin 12p40; **iNOS:** Inducible Nitric Oxide Synthase; **EBV:** Epstein-Barr Virus; **DENV:** Dengue Virus; **RD:** Rhabdomyosarcoma; **DMEM:** Dulbecco's Modified Eagle's Medium; **FBS:** Fetal Bovine Serum; **TCID50:** 50% Tissue Culture Infective Dose; **CPE:** Cytopathic Effect; **CCK-8:** Cell Counting Kit-8; **RIPA:** Radioimmunoprecipitation Assay; **BSA:** Bovine Serum Albumin; **TBST:** Tris-Buffered Saline with Tween; **ELISA:**

Enzyme-Linked Immunosorbent Assay; **PDTC:** Pyrrolidine Dithiocarbamate; **NF-κB:** Nuclear Factor Kappa B; **IκB:** Inhibitory κB; **VP1-4:** Viral Capsid Structural Proteins; **MAPK:** Mitogen-Activated Protein Kinase; **p-P65:** Phosphorylated P65; **NC:** Normal Control.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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SUMMARY

Hypotheses are being made on the basic mechanism by which glycyrrhizin suppresses the inflammatory response in RD cells brought on by EV71 infection. Western blotting was used to assess the expression levels of EV71's VP1, 3D, P65 and phosphorylated-P65 (p-P65) proteins in the cells of the glycyrrhizin treatment group, the EV71-infected RD group, the normal control group and the PDTC group (which was given the NF-κB pathway inhibitor PDTC). ELISA was used to measure the amounts of pro-inflammatory cytokines TNF-α, IL-18 and IL-1β in the RD cells' growth media. The expression of the p-P65 protein is significantly increased and levels of TNF-α, IL-18 and IL-1β increase. However, PDTC or glycyrrhizin markedly decreased TNF-α, IL-1β and IL-18 levels and p-P65 protein expression was markedly downregulated in the culture medium. In RD cells, glycyrrhizin appears to reduce the inflammatory response induced by EV71 infection by inhibiting the NF-κB signaling pathway.

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