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RESEARCH ARTICLE

Curcumol Inhibits EMCV-Induced Intestinal Injury in Mice by Modulating the DHX15-Nlrp6-IRF3-IFN-β Signaling Pathway

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ABSTRACT

Encephalomyocarditis virus (EMCV) is a potentially zoonotic virus that induces multi-systemic diseases in the host, including encephalitis, myocarditis, and reproductive disorders. The infected animals show intestinal immune imbalance and altered gut microbiota, leading to marked mucosal injury. However, currently, the host-regulated pathways and significant therapeutic strategies capable of mitigating EMCV-induced intestinal damage are not well characterized. Our previous laboratory investigations revealed that curcumol inhibited the replication of EMCV *in vitro*. In this study, an EMCV-infected mice model was established to evaluate the effect of curcumol on EMCV-induced intestinal damage and its impact on the gut microbiota. These effects were evaluated by qRT-PCR, Western blot, H&E staining, and 16S rDNA techniques. The results showed that curcumol treatment alleviated EMCV-induced intestinal injury in mice. Curcumol treatment increased the expression of intestinal tight junction mRNA (ZO-1, Occludin) and proteins. It also increased the abundance of *Corynebacterium mastitidis* and *Mucispirillum schaedleri* of the ileum microbiota. Further analysis revealed that in comparison to the virus group, curcumol administration markedly increased IFN-β protein expression in blood, ileum, and colon in mice. Western blot demonstrated that relative abundance of DHX15, NLRP6, and P-IRF3 proteins was elevated in different curcumol dose groups compared with that of the virus group. Therefore, our current study revealed that Curcumol treatment improved the gut microbiota, promoted IFN-β expression through the DHX15-NLRP6-IRF3 signaling pathway, and alleviated the intestinal pathologic injury induced by EMCV infection in mice.

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INTRODUCTION

The intestinal tract is widely regarded as the largest immune organ of the body, playing a vital role in maintaining physiological functions through the regulation of intestinal tight junctions, modulation of intestinal microbiota, and secretion of various chemicals (Di Tommaso *et al.*, 2021; Wang *et al.*, 2021). As a type I interferon produced by virus-infected cells, interferon beta (IFN-β) upregulates interferon-stimulated genes (ISGs) through the activation of the JAK-STAT signaling pathway, thereby mediating the antiviral innate immune response and regulating immune homeostasis (Schindler *et al.*, 2007; Yousaf *et al.*, 2025). Inflammatory bodies, also known as inflammasomes, are cytoplasm-associated multi-protein complexes that comprise of nucleotide binding domains and leucine rich repeat receptors (NLR) proteins. Among them, NLRP6 is mainly expressed in the intestine and can form intracellular multiprotein complexes, namely NLRP6 inflammasomes. Regulating the expression of antiviral interferons in the intestine plays a crucial function in enabling it to resist viral invasion and participate in immune responses to mitigate inflammation (Wang *et al.*, 2015; Li *et al.*, 2020).

Intestinal injury is caused by the disturbance of the intestinal barrier. Intestinal tight junctions and gut

microbiota serve as physical and microbiological barriers. Together, these barriers safeguard the intestine against viral infections and other harmful pathogens (Bertiaux-Vandaële *et al.*, 2011). Tight junction is a dynamic complex composed of transmembrane proteins such as occludin, claudin, and JAM, and cytoplasmic cytoskeletal junction proteins such as Cingulin and ZOs. They form crosslinks with the actin cytoskeleton, integrating intracellular signals to precisely regulate cell paracellular permeability, thereby maintaining barrier integrity (Günzel and Yu, 2013). Meanwhile, the gut microbiota can protect the body against viral infections by producing antiviral substances and regulating the host immune system (Bai *et al.*, 2023). Numerous enteroviruses, like rotavirus, severe acute respiratory syndrome coronavirus-2 (SARS−CoV−2) (Eleftheriotis *et al.*, 2023), can invade intestinal cells by disrupting tight junctions in the intestine (Bruno *et al.*, 2022) and disrupt the gut microbiota (Bao *et al.*, 2023).

Encephalomyocarditis virus (EMCV), which belongs to the genus Cardiovirus in the Picornaviridae family, has a broad host range and is able to cause myocarditis and encephalitis. Being zoonotic in nature, EMCV has been isolated from humans as well as different wild and domestic animal species. Its transmission is usually propagated via contaminated food and water. Importantly, EMCV has a broad range of hosts and continues to broaden (Gibson *et al.*, 2024). Some findings indicate that EMCV can be transmitted through the fecal-oral route (Wang *et al.*, 2021). EMCV infection induces diarrhea in suckling pigs and is capable of replicating in the intestinal tract of mice (Canelli *et al.*, 2010; Yang *et al.*, 2021).

Traditional Chinese medicine is a significant treasure trove of components for the advancement and discovery of highly effective and low-toxicity drugs. Curcumol is a sesquiterpenoid compound extracted from the traditional Chinese medicine Curcuma, with various pharmacological properties (Chen *et al.*, 2023; Orellana-Paucar, 2024). Studies have indicated that turmeric oil rich in curcumin exhibited significant therapeutic effects in the treatment of pediatric viral encephalitis and myocarditis in clinical practice (Orellana-Paucar *et al.*, 2022). Our previous studies revealed that curcumol elevated the expression of IFN-β in EMCV-infected HEK-293T cells, inhibited the replication of EMCV (Zheng *et al.*, 2021; Zheng *et al.*, 2022). However, it remains uncertain if curcumol can impede EMCV-induced intestinal damage, and the mechanism behind curcumol's pharmacological activity is still ambiguous.

In this study, an EMCV-infected mouse model was established. The effect of curcumol on EMCV-induced intestinal damage in vivo and its mechanism were evaluated in molecular and microbial dimensions by qRT-PCR, Western blot, histopathological observation, and 16S rDNA analysis. We quantitatively evaluated the influence of curcumol on intestinal damage caused by EMCV in-vivo and elucidated the mechanism of intestinal protection against EMCV infection across molecular and microbial dimensions.

MATERIALS AND METHODS

Virus, drugs, and Animals: EMCVNJ08 (GenBank: HM641897.1) was amplified and preserved by Professor Jiang Ping at Nanjing Agricultural University. According to the Reed-Muench method (Ming *et al.*, 2017), the virulence value was calculated to be $10^{5.25}TCID_{50}$/mL. Curcumol (99% purity) and ribavirin(100% purity) were purchased from China Food and Drug Control Institute. SPF-grade Kunming mice were purchased from Beijing Weitonglihua Experimental Animal Technology Co., Ltd. (SCXK (Beijing) 2021-0006). Mice were bred for one week at the Experimental Animal Management Center of Shanxi Agricultural University for the experimental operations. All animal tests complied with the ethical requirements of the animal committee of Shanxi Agricultural University (Ethical Review No. SXAU-EAW-2024M.HL.004011279).

Experimental Design: Model construction: Sixty-four Immune simulation predicts to a robust cellular immunological reponse Kunming mice weighing 40-45 g, with an equal ratio of males and females, were randomly distributed into two groups: a control group and a viral group. The control group was intraperitoneally injected with 0.2 mL of normal saline, whereas the viral group was intraperitoneally injected with 0.2mL of EMCV at a concentration of $100TCID_{50}$. Daily clinical assessments were performed to monitor disease progression, and at 5, 7, 9, and 11 days post-infection, eight mice from each group were randomly selected for intestinal tissue collection for further examination.

Methodological design: 48 Kunming mice, each weighing 40-45g, with an equal distribution of males and females, were randomly divided into control group, viral, positive control, and low (0.06 mg/kg), medium (0.3 mg/kg), and high (1.5 mg/kg) curcumol dose groups. All groups were intraperitoneally injected with 0.2mL of $100TCID_{50}$ EMCV. After 3 days of post-infection, 0.06 mg/kg, 0.3 mg/kg, and 1.5 mg/kg curcumol were injected intraperitoneally in three drug trial groups, and 40 mg/kg ribavirin was intraperitoneally injected into the positive control group. The control and viral groups were given intraperitoneal injections with the same amount of normal saline twice a day for five consecutive days. Clinical symptoms in mice were observed every day. At 7 days post-infection with EMCV, blood, intestinal tissue, ileum contents, and colon contents were collected for further analysis.

qRT-PCR: Blood, Intestinal, Ileum, and colon contents were collected for total RNA extraction by using RNAiso Plus reagent (TaKaRa Bio, Inc.). cDNA was synthesized using HiScript III ist Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme, China). qRT-PCR amplification was performed using a 2× M5 HiPer Realtime PCR Supermix (with Low Rox) (Mei5 Biotechnology, China). Absolute fluorescence quantification was used to detect the expression of EMCV 3D mRNA, and 2-ΔΔCT was used to detect the relative expression of β-actin, ZO-1, Occludin, IFN-β, Corynebacterium mastitidis, and Mucispirillum_schaedleri. Absolute quantification was performed on the EMCV 3D recombinant plasmid preserved in our laboratory.

Histopathology: Ileum and colon tissues were fixed in 4% paraformaldehyde solution, followed by dehydration,

paraffin embedding, and longitudinal sectioning at 5 μm. Hematoxylin and eosin (H&E) staining was used to perform the histopathological analysis.

Western Blot: Total protein was extracted from the ileum and colon tissue of each group of mice, and the protein concentration was determined using the BCA method. A Western blot was done for the detection of target-related protein expression. Antibody dilution used for Western blotting was listed as: β-actin (protein tech) (1:20,000), ZO-1 (protein tech) (1:20,000), Occludin (ab clonal) (1:500), Nlrp6 (ab clonal) (1: 700), MAVS (protein tech) (1; 5000), DHX15 (protein tech) (1; 5000) Anti-goat and anti-Rabbit IgG (boster) (1:10,000).

ELISA: Blood samples were collected from the mice of all groups to separate the serum. Ileum and colon tissues were homogenized in an appropriate volume of saline using a high-throughput grinder at 60 Hz for 5 minutes. The samples were centrifuged at a speed of 3000 rpm for 10 minutes to collect the supernatant. The levels of IFN-β in the serum and tissue homogenates were quantified using an ELISA kit.

16Sr DNA: The contents of ileum and colon of mice from each group were collected with a sterile scalpel by scraping. DNA was extracted by using the OMEGA Mag-bind soil DNA kit, followed by determination of the purity and concentration of the extracted DNA. PCR amplification was done for the selected V3-V4 variable regions by using particular primers having Barcodes and high-fidelity DNA polymerase, in accordance with the designated sequencing regions. After the electrophoresis, the PCR amplification recovery product was detected and quantified by a Microplate reader (BioTek, FLx800) fluorescence quantification system, and the library was assembled using Illumina's TruSeq Nano DNA Library Prep Kit. The 16S rDNA gene fragments were classified, species annotated, and community analyzed using the Zhongke New Life Cloud Platform.

Statistical Analysis: GraphPad Prism 8.0 (GraphPad Software, Inc., California, USA) statistical software was used for data analysis. The Bar graphs represent mean±SD. Bars sharing at least one similar letter (a–e) are not significantly different ($P>0.05$), whereas bars with different letters shows significant difference ($P<0.05$), as determined by one-way ANOVA used for multi-group variability analysis.

RESULTS

Establishment of an intestinal model for EMCV-infected mice: In order to explore the replication pattern of EMCV in the intestinal tract of mice, we detected the virus replication and pathological damage in different intestinal tracts of EMCV infected mice at different time points. qRT-PCR results indicated that the EMCV copy number in each intestinal tissue of mice peaked on day 7 post-EMCV infection and subsequently declined over time (Fig. 1A), and the highest viral copy number was detected specifically in ileum and colon tissue samples on day 7 post-EMCV infection (Fig. 1B).

H&E staining results demonstrated that ileum of control group mice had a normal structure with neatly arranged villi. In the virus-infected group, epithelial shedding in the ileum was first observed on day 5, accompanied by sparse and shortened villi. By day 7, pronounced epithelial loss (yellow arrows) and disruption with shortened villi (red arrows) were evident. By day 9, partial recovery was observed, characterized by gradual restoration of villus alignment. By day 11, the ileum morphology had largely recovered and resembled to the control group (Fig. 1C). In control group, the epithelium of the colon was intact, and the colon folds were regular. In the virus group, the epithelium of the colon was shed on day 5; the mucosa was shed on day 7 (yellow arrows), and the crypts were irregular; epithelium of the colon was gradually reduced on day 9, and the morphology appeared to be identical to control group on day 11 (Fig. 1C). Results showed that EMCV infection caused mucosal epithelial shedding of ileum and colon tissue samples, and pathological damage of ileum and colon tissue of mice gradually aggravated with rise in the duration of EMCV infection, and the effect was the most apparant at day 7.

Curcumol alleviates EMCV-induced intestinal injury in mice

Curcumol ameliorates intestinal pathologic changes caused by EMCV infection: Ileum and colon organs of EMCV-infected mice were collected, and fixation was done on day 7, and pathological changes in ileum and colon of mice were observed using H&E staining. The results showed that at day 7 of EMCV infection in mice, the villi in the mouse ileum viral group were sparse, broken and shortened (red arrows), and the mucosal epithelium was detached (yellow arrows); the ileum villi in the low-dose group of curcumol were irregularly arranged, and the arrangement of the villi in the medium- and high-dose groups was improved, but there were still broken villi in the medium-dose group of curcumol (red arrows), while in the high-dose group of curcumol, the villi were dense and the length of the villi tended to be normalized (Fig 2A). In the virus group, detachment of the colonic mucosal epithelium was observed (yellow arrows), accompanied by irregular crypt architecture. Similar epithelial detachment (yellow arrows) persisted in the curcumol low and medium-dose groups. In contrast, the colonic epithelium remained intact, and the mucosal folds appeared regular in the curcumol high-dose group.(Fig 2B). This indicates that curcumol can reduce the intestinal damage caused by EMCV.

A. Mouse ileum tissue section, B. Mouse colon tissue section. Scale Bar was 100 μm; red arrows are villi breakage, and yellow arrows are mucosal detachment

Curcumol upregulates the expression of intestinal tight junction proteins ZO-1 and Occludin in EMCV-infected mice: To analyse the tight junction proteins regulation, qRT-PCR and Western blot were used to detect the expression of tight junction proteins ZO-1 and Occludin. The results (Fig. 3) demonstrate that at day 7 post-EMCV infection, relative to the control group, the virus group exhibited a significant reduction in the expression of ZO-1 and Occludin proteins in ileum and colonic tissues ($P<0.05$). In contrast, all groups with

curcumol treatment demonstrated a significant increase in the protein levels of Occludin and ZO-1 in ileum tissues and Occludin proteins in colonic tissues compared to the virus group ($P<0.05$). Furthermore, the high-dose and medium-dose curcumol groups significantly enhanced the relative expression of ZO-1 proteins in ileum tissues ($P<0.05$). The above results indicate that curcumol repaired the intestinal tight junction barrier, resisted EMCV infection of the mouse intestine, and alleviated EMCV-induced intestinal injury in mice by up-regulating the expression levels of ZO-1 and Occludin in the ileum and colon tissues.

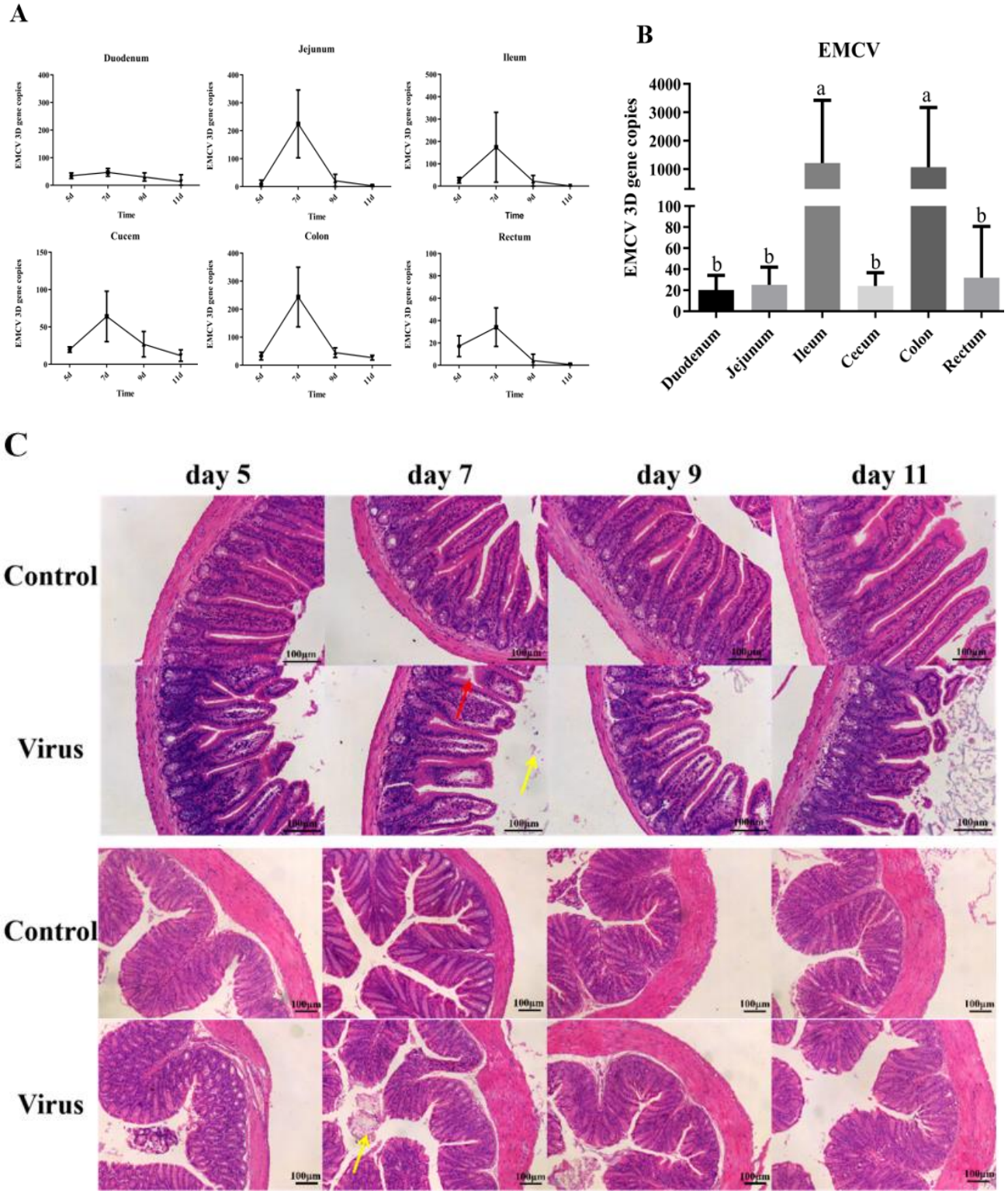

Fig. 1: Replication of EMCV and pathological damage in the mouse intestine. A. EMCV load in duodenum, jejunum, ileum, cecum, colon, and rectum tissues of mice infected with EMCV for 5 d, 7 d, 9 d, and 11 d. B. EMCV load in duodenum, jejunum, ileum, cecum, colon, and rectum tissues of mice infected with EMCV for 7d. Different letters indicate significant differences ($P<0.05$). C. H&E staining to observe the histopathological changes in the ileum and colon of mice infected with EMCV for a period of 5 d, 7 d, 9 d, and 11 d.Scale Bar was 100μm; red arrow is villi breakage, yellow arrows are the mucous membrane detachment.

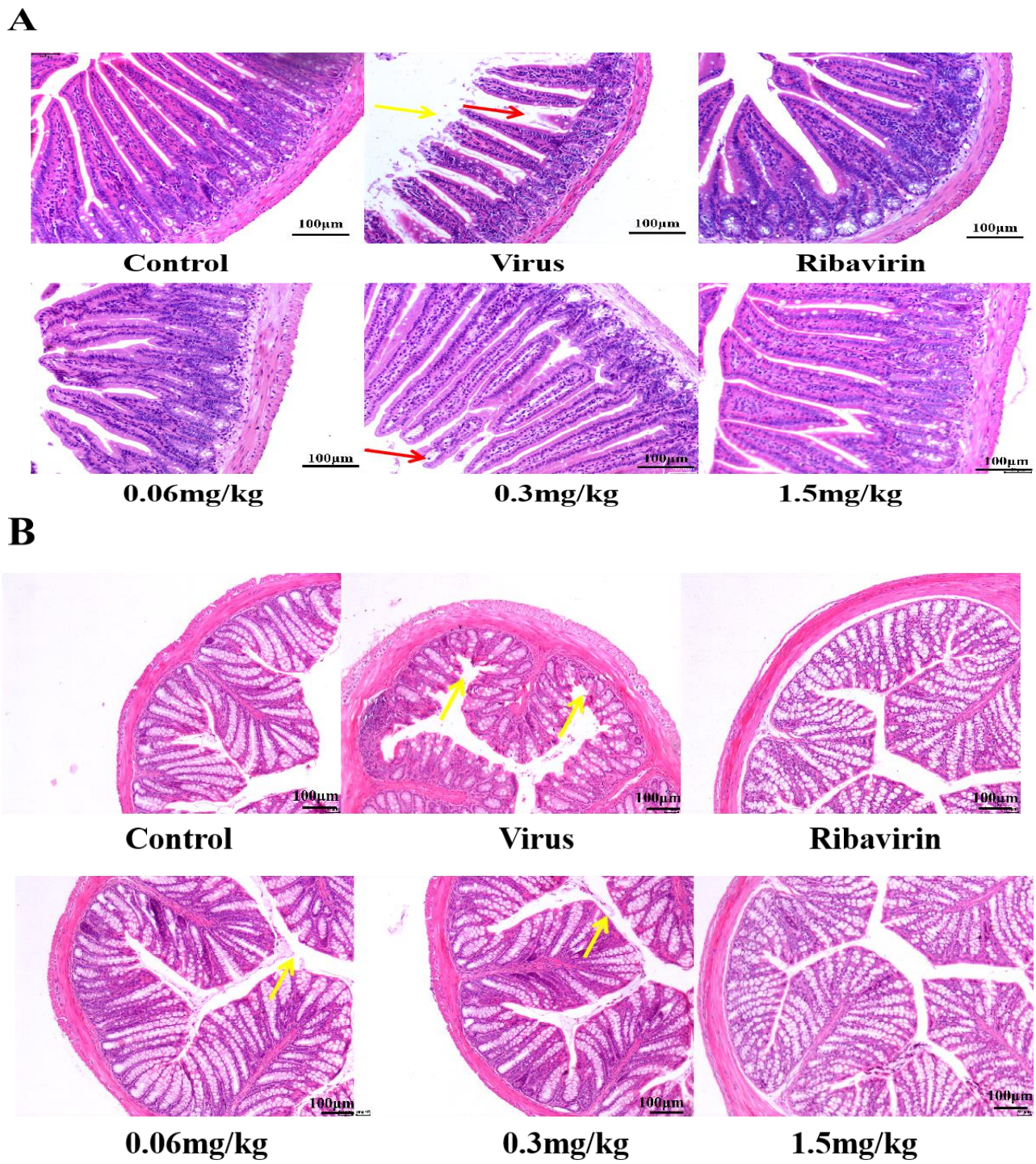

Fig. 2: H&E staining to observe the histopathological changes in the ileum and colon of mice infected with EMCV for day 7.

Curcumol enhances IFN-β inhibition of EMCV replication in mouse intestine via DHX15-NLRP6-IRF3 axis

Curcumol inhibits EMCV replication and promotes IFN-β gene and protein expression in mouse ileum and colon: qRT-PCR was performed to quantify the EMCV virus level in the ileum and colon of mice at day 7. The findings demonstrated that different concentrations of curcumol significantly reduced the EMCV load by inhibiting EMCV replication in ileum and colon of mice compared to virus group ($P<0.05$) (Fig. 4A). qRT-PCR and ELISA findings revealed that, in comparison to the control group, EMCV infection dominantly decreased the expression of the IFN-β in blood, colon, and ileum ($P<0.05$). Compared to the virus group, relative expression of IFN-β in blood, ileum, and colon was dominantly higher in different curcumol-treated groups ($P<0.05$). The results indicated that curcumol elevated IFN-β levels during EMCV infection (Fig. 4B&4C).

Curcumol enhances IFN-β expression through DHX15-NLRP6-IRF3 signaling pathway: To elucidate the mechanistic pathway, the abundance level of NLRP6, MAVS, DHX15, IRF3, and P-IRF3 proteins in ileum and colon tissue of EMCV-infected mice of each group was detected by western blot at day 7. The results, as shown in

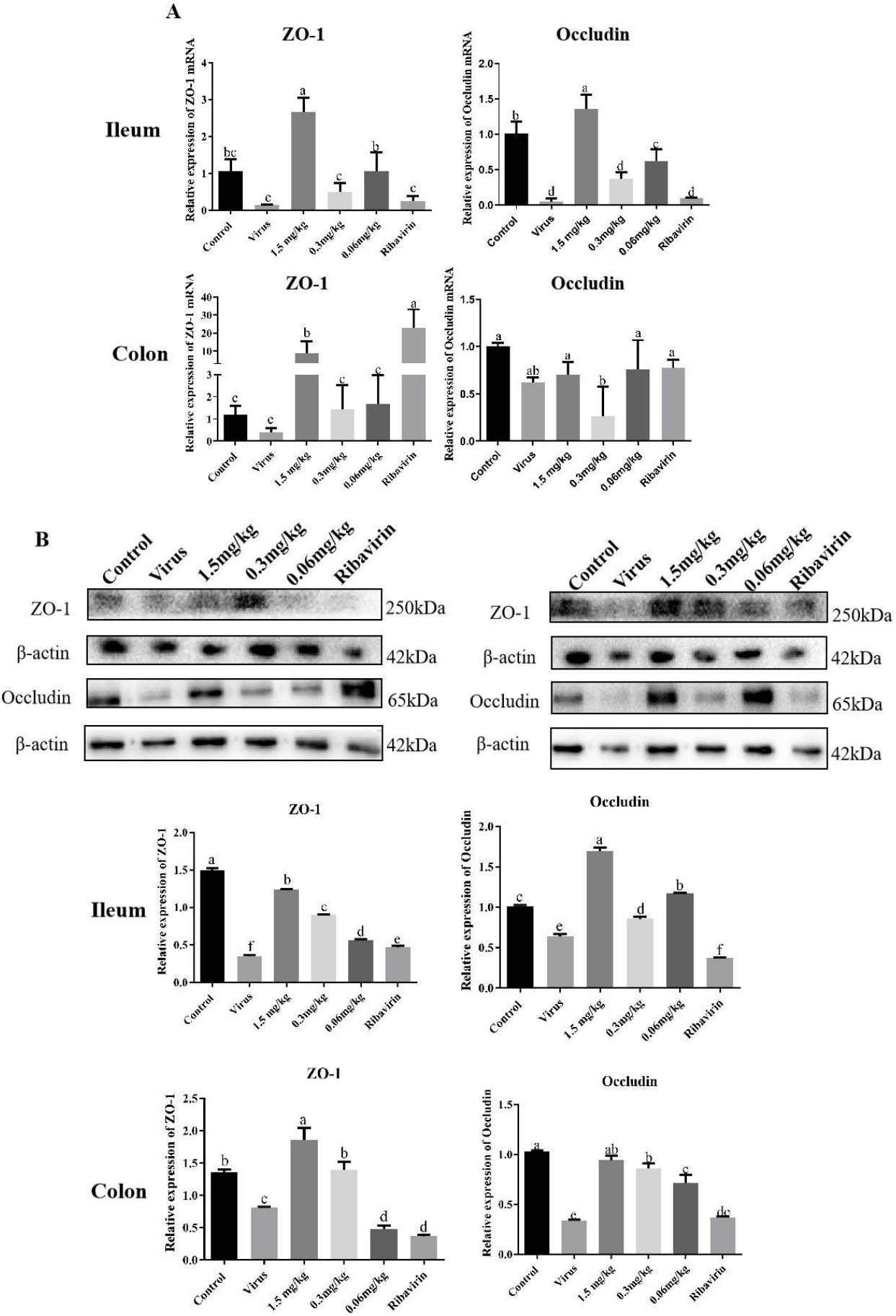

Fig. 3: qRT-PCR and Western blot detection of the expression of ileum and colon tight junctions in mice at 7d of EMCV infection. A. qRT-PCR detection of the expression of ileum and colon tight junction genes in mice, B. Western blot detection of the expression of tight junction proteins in ileum and colon tissues. Different letters indicate significant differences ($P<0.05$).

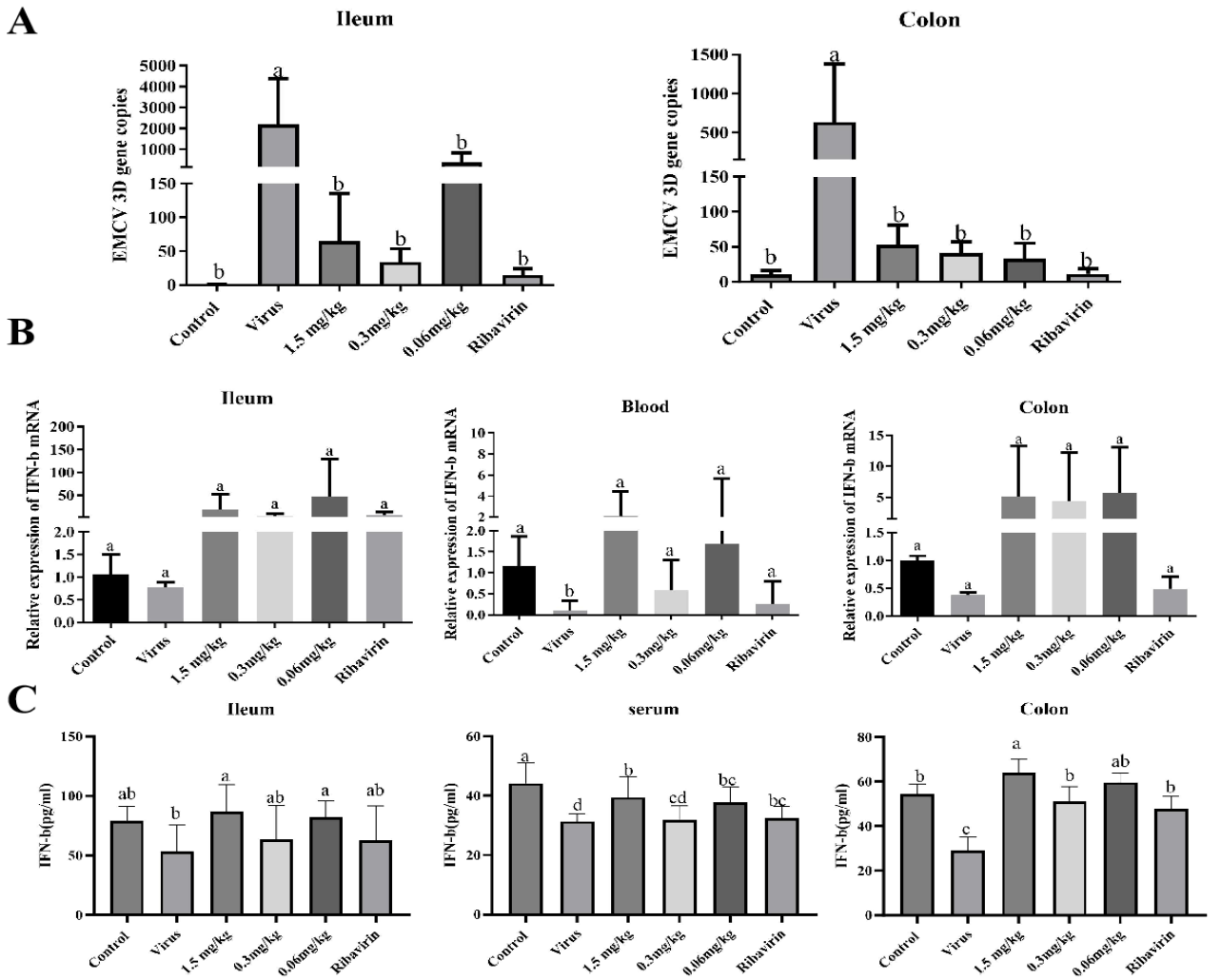

Fig. 4: Curcumol inhibits EMCV replication and promotes IFN-β gene and protein expression in mouse blood, ileum, and colon. A. The EMCV load in each group of mouse ileum and colon at day 7 of EMCV infection, B. The relative expression of IFN-β gene in mouse blood, ileum, and colon tissues at day 7 of EMCV infection, C. The serum, ileum, and colon tissue IFN-β protein content of mouse sera, ileum, and colon tissues at day 7 of EMCV infection detected by ELISA. Different letters indicate significant differences (P<0.05).

in Fig. 5, revealed that the relative expression of DHX15, NLRP6, IRF3, and P-IRF3 in tissue samples of the virus group was markedly declined in comparison with the control group (P<0.05), but the expression of MAVS didn't changed significantly (P>0.05). The relative level of DHX15, NLRP6, and P-IRF3 proteins was elevated in all curcumol-treated groups compared with that of the virus group, and the relative expression of IRF3 protein was significantly elevated in the high-dose and low-dose groups of curcumol (P<0.05). There was no significant change in the relative expression of MAVS protein in all groups treated with curcumol (P>0.05) (Fig. 5). This indicates that curcumol can promote IFN-β expression and inhibit EMCV infection of the mouse intestine through the DHX15-NLRP6-IRF3 signaling pathway.

The level of relevant proteins in the ileum tissues, B. The level of relevant proteins in the colon tissues. Different letters indicate significant differences (P<0.05).

Effect of curcumol on differential strains of gut microbiota in EMCV-infected mice: Screening of key intestinal bacteria during curcumol resistance to EMCV infection based on the results of species significant difference analysis demonstrated that in the ileum contents (Table 1), the relative abundance of *Corynebacterium mastitidis* was significantly lower in the viral group (VH) than in the control group (CH) (P<0.05, FC≤0.5). The comparative abundance of *Corynebacterium mastitidis* was not notably modulated with a significant difference by the curcumol treatment (GH) compared to the VH (P>0.05, FC≤0.5). In the contents of colon, the comparative proportion of *Mucispirillum schaedleri* was significantly lower in the viral group compared with the control group (P<0.05), and the relative abundance of *Mucispirillum schaedleri* was significantly higher in the curcumol high-dose group compared with the viral group (FC≥2) (Table 2). These results indicate that *Corynebacterium mastitidis* and *Mucispirillum schaedleri* may play important roles in the antiviral activity of curcumol.

The comparative abundance of *Corynebacterium mastitidis* was quantified using qRT-PCR. The findings demonstrated that the relative expression of *Corynebacterium mastitidis* in the ileum contents was decreased in viral group compared to the control group (P>0.05). Compared with the viral group, the high-dose curcumol group elevated the relative expression of *Corynebacterium mastitidis* (P<0.05). In the colon contents, the relative expression of *Mucispirillum*

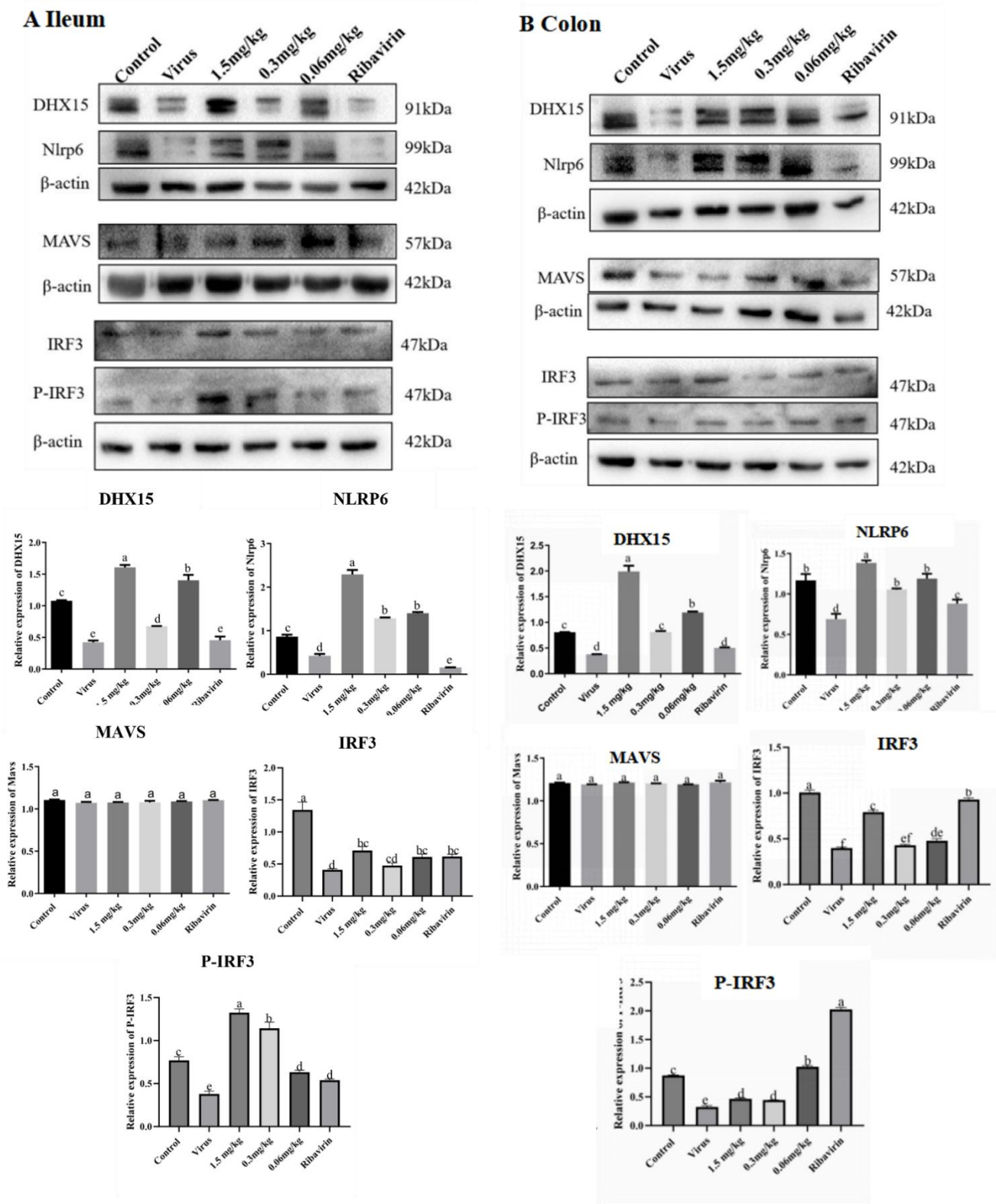

Fig. 5: Western blot detection of the expression of key proteins of the DHX15-NLRP6-IRF3 signaling pathway in the ileum and colon tissues of EMCV-infected mice at 7 d.

Table 1: Ileum contents: Species in which high-dose curcumol plays a major role in EMCV resistance

Group	Species	Mean Rel(%)	Mean Rel(%)	P value	95%CI	FC
CH vs VH	*Corynebacterium mastitidis*	0.089	0.002	0.026	2.19×10^{-5} To 0.299	0.020
VH vs GH	*Corynebacterium mastitidis*	0.002	0.022	0.182	-0.056 To 5.97×10^{-5}	12.333
CH vs GH	*Corynebacterium mastitidis*	0.089	0.022	0.192	-0.025 To 0.243	0.245

Table 2: Colon contents: Species in which high-dose curcumol plays a major role in EMCV resistance

Group	Species	Mean Rel(%)	Mean Rel(%)	P value	95%CI	FC
CJ vs VJ	*Mucispirillum schaedleri*	0.175	0.144	0.019	-0.148 To 0.271	0.826
VJ vs GJ	*Mucispirillum schaedleri*	0.144	2.125	0.149	-10.606 To 0.060	14.744
CJ vs GJ	*Mucispirillum schaedleri*	0.175	2.125	0.226	-0.186 To -0.21	1.365

schaedleri was decreased in the viral group compared with the control group (P>0.05). In contrast, the relative expression of *Mucispirillum schaedleri* was elevated in the high-dose curcumol-treated group compared with the viral group (P<0.05) (Fig. 6). These results indicate that curcumol can resist EMCV infection by upregulating the expression of *Corynebacterium mastitidis* and *Mucispirillum schaedleri,* even higher than that of the positive control group.

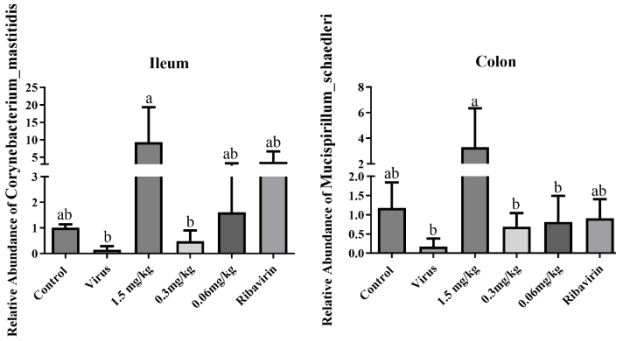

Fig. 6: The effect of curcumol on the abundance of Corynebacterium mastitidis and Mucispirillum schaedleri in ileum and colon contents of EMCV-infected mice detected by qRT-PCR. Different letters indicate significant differences ($P < 0.05$).

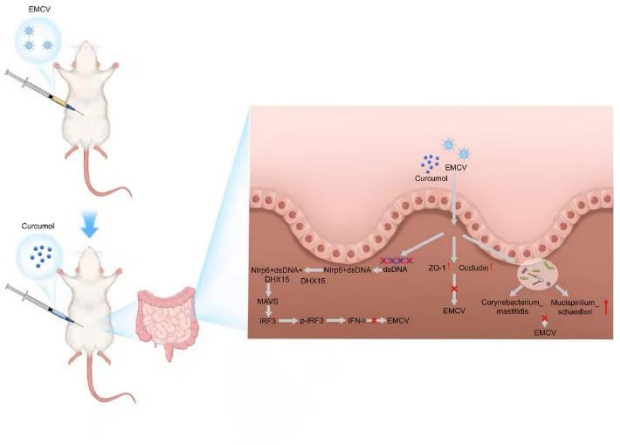

Fig. 7: Mechanistic insight of curcumol inhibiting intestinal damage in EMCV infected mice.

DISCUSSION

EMCV is potentially a zoonotic pathogen, with a significant public health importance in terms of prevention and control, specifically within underdeveloped countries with poor hygiene values. Research reported that Curcumol-rich curcumol oil can treat rotavirus-induced enterocolitis (Canelli *et al.*, 2010). Immune response plays a key role in modulating a viral infection. It has been noted that IFN-β has been majorly involved in cell mediated immune response (Sun *et al.*, 2019). Our previous study found that curcumol can increase the expression of IFN-β, thereby inhibiting the replication of EMCV. Nevertheless, the role and mechanism of curcumol in inducing intestinal injury in EMCV-infected mice are still not clear.

Type I interferons serve as the main host defense against viral infections (Orellana *et al.*, 2022; Chen *et al.*, 2023) and immune stimulator (Haseeb *et al.*, 2025), but the mechanism by which curcumol induces IFN-I to resist intestinal injury in EMCV infected mice needs to be elucidated. NLRP6, a key immunomodulator, synergistically interacts with DHX15 to recognize the cytoplasmic viral RNA, and then delivers viral RNA recognition signals through MAVS to initiate the MAVS-IRF3 cascade reaction, which induces the production of IFN-I and the expression of downstream ISGs to resist EMCV infection (Yang *et al.*, 2021). Studies have shown that traditional Chinese medicine can regulate NLRP6, and Chinese herbal medicine Qing Chang Hua Shi granule (QCHS) can increase NLRP6 expression and diminish dextran sulfate sodium(DSS) induced ulcerative colitis in mice (Cheng *et al.*, 2022). Similarly, Runchangning Shen Gao can activate NLRP6 inflammasome induced autophagy, result in colonic mucin-2 secretion, and regulate intestinal microbiota (Liu *et al.*, 2025). The expression of DHX15, NLRP6, IRF3, and P-IRF3 proteins in the ileum and colon tissue of all groups was detected by Western blot, indicating that curcumol may inhibit EMCV replication by promoting the expression of IFN-β through the DHX15-NLRP6-IRF3 axis in the intestinal tissues of mice.

Tight junctions are an important component of the intestinal barrier, characterized by proteins including ZO-1 and Occludin (Wang *et al.*, 2025). Many viruses, such as the influenza virus (Tugizov, 2021) and human cytomegalovirus (Dou *et al.*, 2024), damage the intestinal barrier by disrupting intestinal tight junction proteins ZO-1 and Occludin (Zheng *et al.*, 2025). The gut microbiota can maintain the stability of the digestive tract and sustain the intestinal barrier through multiple mechanisms (Zhou *et al.*, 2024). The herbal monomer Rg3 can resist EMCV infection by enriching acetate/propionic acid-producing commensal bacteria in the intestinal tract and enhancing type I interferon responses in macrophages via the GPR43-CGAS signaling axis (Wang *et al.*, 2023). The traditional Chinese medicine Ganlu Yin protects the intestinal mucosal barrier by hyperactivation of the tight junction proteins and microbiota (Xiong *et al.*, 2022). Moreover, it has been found that curcumol longa-derived nanovesicles can restore the intestinal epithelial barrier, by inhibiting the TLR/ MyD88 pathway to attenuate dextran sulfate sodium salt-induced colitis (Gao *et al.*, 2022; Huang *et al.*, 2023). We found that curcumol increased the levels of the tight junction ZO-1 and Occludin mRNA and protein expression in the ileum and colon tissues to resist intestinal damage in mice caused by EMCV infection.

In this study, the differential strain of *Corynebacterium mastitidis* in ileum contents and the differential strain of *Mucispirillum schaedleri* in colon contents were found by intergroup variability analysis of 16Sr DNA. EMCV infection down-regulated the ileum contents in the *Corynebacterium mastitidis* and *Mucispirillum schaedleri* abundance in colon contents, while curcumol elevated the levels of both species. Previous studies also report that microbiome datasets generated by HTS are compositional, in that they might have randomly total imposed by the instrument(Gloor *et al.*, 2017), and normalization and differential abundance strategies can have a profound impact on analysis results. *Corynebacterium mastitidis* generally defends against pathogenic Candida albicans or Pseudomonas aeruginosa

infections and can induce a commensal-specific IL-17 response in cells (Kugadas *et al.*, 2016). *Mucispirillum schaedleri* can interfere with the expression of pathogen-invasive mechanisms to resist Salmonella typhimurium-induced colitis (Herp *et al.*, 2021), and the above results suggest that gut microbiota is one of the targets of curcumol in EMCV. The dual functions of *Mucispirillum schaedleri* as an antagonist of virulence and not only to protect against colitis but also to modulate host immune responses to maintain intestinal homeostasis (Herp *et al.*, 2019). As such, *M. schaedler*i can serve as a microbial protector and an immune regulator in the gut ecosystem. However, it is needed to further investigate if the curcumol's antiviral activity was related to the metabolites produced by these two differential strains.

Conclusions: Curcumol treatment upregulates the IFN-β expression via the DHX15-NLRP-IRF3 signaling pathway and inhibits the EMCV replication in the mouse intestine. At the same time, it hyperactivated the ZO-1 and Occludin, which are tight junction proteins, in the ileum and colon tissue of mice. Simultaneously, it upregulates the levels of *Corynebacterium mastitidis* and *Mucispirillum schaedleri* in the intestine while mitigating EMCV-induced pathological damage in the ileum and colon of mice (Fig. 7)

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REFERENCES

Bai G, Tsai M, Lin S, *et al.*, 2023. Unraveling the interplay between norovirus infection, gut microbiota, and novel antiviral approaches: a comprehensive review. Frontiers in Microbiology 14: 1212582. https://doi,org/10.3389/fmicb.2023.1324539.

Bao S, Wang H, Li W, *et al.*, 2023. Viral metagenomics of the gut virome of diarrheal children with Rotavirus A infection. Gut Microbes 15(1): 2234653. https://doi,org/10.1080/ 19490976.2023.2234653

Bertiaux-Vandaële N, Youmba S, Belmonte L, *et al.*, 2011. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. The American Journal of Gastroenterology 106(12): 2165-2173. https://doi,org/10.1038/ajg.2011.257

Bruno C, Paparo L, Pisapia L, *et al.*, 2022. Protective effects of the postbiotic deriving from cow's milk fermentation with L. paracasei CBA L74 against Rotavirus infection in human enterocytes. Scientific Reports 12(1): 6268. https://doi,org/10.1038/s41598-022-10083-5

Canelli E, Luppi A, Lavazza A, *et al.*, 2010. Encephalomyocarditis virus infection in an Italian zoo. Virology Journal 7(1): 64. https://doi,org/10.1186/1743-422X-7-64

Chen L, Li X, Deng Y, *et al.*, 2023. IFITM2 presents antiviral response through enhancing type I IFN signaling pathway. Viruses 15(4): 866. https://doi,org/10.3390/v15040866

Cheng C, Hu J, Li Y, *et al.*, 2022. Qing-Chang-Hua-Shi granule ameliorates DSS-induced colitis by activating NLRP6 signaling and regulating Th17/Treg balance. Phytomedicine 107: 154452. https://doi,org/10.1016/j.phymed.2022.154452.

Di Tommaso N, Gasbarrini A, Ponziani F *et al.*, 2021. Intestinal barrier in human health and disease. International Journal of Environmental Research and Public Health 18(23): 12836. https://doi,org/10.3390/ijerph182312836

Dou B, Wu X, He Y, *et al.*, 2024. Fei-Yan-Qing-Hua decoction attenuates influenza virus infection by enhancing host antiviral response through microbiota-derived acetate. Frontiers in Pharmacology 15: 1446749. https://doi,org/10.3389/fphar.2024.1446749

Eleftheriotis G, Tsounis E, Aggeletopoulou I, *et al.*, 2023. Alterations in gut immunological barrier in SARS-CoV-2 infection and their prognostic potential. Frontiers in Immunology 14: 1129190. https://doi,org/10.3389/fimmu.2023.1129190

Gao C, Zhou Y, Chen Z, *et al.*, 2022. Turmeric-derived nanovesicles as novel nanobiologics for targeted therapy of ulcerative colitis. Theranostics 12(12): 5596. https://doi,org/10.7150/thno.73650

Gibson L, Shadbolt T, Paul P, *et al.*, 2024. Prevalence and molecular analysis of encephalomyocarditis virus-2 in the hazel dormouse. EcoHealth 21(1): 112-122. https://doi,org/10.1007/s10393-025-01709-x

Gloor G, Macklaim J, Pawlowsky-Glahn V, *et al.*, 2017. Microbiome datasets are compositional: and this is not optional. Frontiers in Microbiology 8: 2224. https://doi,org/ 10.3389/fmicb.2017.02224.

Günzel D and Yu ASL, 2013. Claudins and the modulation of tight junction permeability. Physiological Reviews 93(2): 525-569. https://doi,org/10.1152/physrev.00019.2012

Haseeb A, Yousaf W, Cao Z, *et al.*, 2025. Parvoviruses NS1 oncolytic attributes: mechanistic insights and synergistic anti-tumor therapeutic strategies. Frontiers in Microbiology 16: 1631433. https://doi,org/10.3389/fmicb.2025.1631433

Herp S, Brugiroux S, Garzetti D, *et al.*, 2019. Mucispirillum schaedleri antagonizes salmonella virulence to protect mice against colitis. Cell Host & Microbe 25(5): 681-694.e688. https://doi,org/ 10.1016/j.chom.2019.03.004.

Herp S, Durai Raj A, Salvado Silva M, *et al.*, 2021. The human symbiont Mucispirillum schaedleri: causality in health and disease. Medical Microbiology and Immunology 210(4): 173-179. https://doi,org/10.1007/s00430-021-00702-9

Huang J, Wu T, Zhong Y, *et al.*, 2023. Effect of curcumin on regulatory B cells in chronic colitis mice involving TLR/MyD88 signaling pathway. Phytotherapy Research 37(2): 731-742. https://doi,org/10.1002/ptr.7656

Kugadas A, Christiansen S, Sankaranarayanan S, *et al.*, 2016. Impact of microbiota on resistance to ocular Pseudomonas aeruginosa-induced keratitis. PLoS Pathogens 12(9): e1005855. https://doi,org/10.1371/journal.ppat.1005855

Li R, Zhu S. 2020. NLRP6 inflammasome. Molecular Aspects of Medicine 76: 100859. https://doi,org/10.1016/j.mam.2020.100859

Liu X, Jia Y, Wu C, *et al.*, 2025. Runchangningshen paste activates NLRP6 inflammasome-mediated autophagy to stimulate colonic mucin-2 secretion and modulates mucosal microbiota in functional constipation. World Journal of Gastroenterology 31(9): 102256. https://doi,org/10.3748/wjg.v31.i9.102256

Ming K, Chen Y, Shi J, *et al.*, 2017. Effects of Chrysanthemum indicum polysaccharide and its phosphate on anti-duck hepatitis a virus and alleviating hepatic injury. International Journal of Biological Macromolecules 102: 813-821. https://doi,org/10.1016/j.ijbiomac.2017.04.093.

Orellana-Paucar A, Machado-Orellana M. 2022. Pharmacological profile, bioactivities, and safety of turmeric oil. Molecules 27(16): https://doi,org/10.3390/molecules27165055.

Orellana-Paucar A. 2024. Turmeric essential oil constituents as potential drug candidates: a comprehensive overview of their individual bioactivities. Molecules 29(17): 4210. https://doi,org/10.3390/ molecules29174210

Schindler C, Levy D, Decker T. 2007. JAK-STAT signaling: from interferons to cytokines. Journal of Biological Chemistry 282(28): 20059-20063. https://doi,org/10.1074/jbc.R700016200

Sun N, Sun P, Xie N, *et al*., 2019. Antiviral and immunomodulatory effects of dipotassium glycyrrhizinate in chicks artificially infected with infectious bursal disease virus. Pakistan Veterinary Journal 39: 43-48. https://doi,org/10.29261/pakvetj/2018.109

Tugizov S. 2021. Virus-associated disruption of mucosal epithelial tight junctions and its role in viral transmission and spread. Tissue Barriers 9(4): 1943274. https://doi,org/10.1080/21688370. 2021.1943274

Wang G, Liu J, Zhang Y, *et al*., 2023. Ginsenoside Rg3 enriches SCFA-producing commensal bacteria to confer protection against enteric viral infection via the cGAS-STING-type I IFN axis. The ISME Journal 17(12): 2426-2440. https://doi,org/10.1038/s41396-023-01541-7

Wang J, Wang L, Lu W, *et al*., 2025. TRIM29 controls enteric RNA virus-induced intestinal inflammation by targeting NLRP6 and NLRP9b signaling pathways. Mucosal Immunology 18(1): 135-150. https://doi,org/10.1016/j.mucimm.2024.10.004

Wang P, Zhu S, Yang L, *et al*., 2015. Nlrp6 regulates intestinal antiviral innate immunity. Science 350(6262): 826-830. https://doi,org/10.1126/ science.aab3145

Wang Q, Wang Y, Zhang W, *et al*., 2021. Puerarin from Pueraria lobata alleviates the symptoms of irritable bowel syndrome-diarrhea. Food & Function 12(5): 2211-2224. https://doi,org/10.1039/ d0fo02848g

Xiong T, Zheng X, Zhang K, *et al*., 2022. Ganluyin ameliorates DSS-induced ulcerative colitis by inhibiting the enteric-origin LPS/TLR4/NF-κB pathway. Journal of Ethnopharmacology 289: 115001. https://doi,org/10.1016/j.jep.2022.115001

Yang XL, Wang G, Xie J, *et al*., 2021. The intestinal microbiome primes host innate immunity against enteric virus systemic infection through type I interferon. MBio 12(3): 10.1128/mbio. 00366-00321. https://doi,org/10.1128/mBio.00366-21

Yousaf W, Haseeb A, Shen Y, *et al*., 2025. Data-driven discovery of antiviral peptides against PRRSV using multiple machine learning models. Frontiers in Veterinary Science 12: 1681083. https://doi,org/10.3389/fvets.2025.1681083

Zheng J, Cao Z, Yousaf W, *et al*., 2025. Ellagic acid mitigates rotavirus-induced intestinal injury via bidirectional "immune-microbiota" regulatory effect. Frontiers in Cellular and Infection Microbiology 15: 1686918. https://doi,org/10.3389/fcimb.2025.1686918.

Zheng J, Sun P, Sun N, *et al*., 2022. Curcumol inhibits EMCV replication by activating CH25H and inhibiting the formation of ROs. BMC Veterinary Research 18(1): 453. https://doi,org/10.1186/s12917-022-03531-x.

Zheng J, Xu Y, Khan A, *et al.,* 2021. Curcumol inhibits encephalomyocarditis virus by promoting IFN-β secretion. BMC Veterinary Research 17(1): 318. https://doi,org/10.1186/s12917-021-03015-4.

Zhou L, Zhang Y, Wu S, *et al*., 2024. Type III secretion system in intestinal pathogens and metabolic diseases. Journal of Diabetes Research 2024(1): 4864639. https://doi,org/10.1155/ 2024/4864639