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# Antimicrobial peptide MPX alleviates the lethal attack of *Escherichia coli* in mice

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*Contents*



**Abstract**

*Escherichia coli* is an important zoonotic pathogen causing intestinal diseases. In recent years, due to the unreasonable use of antibiotics, the drug resistance of bacteria has been increasing, and the proportion of multi-drug resistant strains has also been rising, which directly threatens the health of animals and humans. The antimicrobial peptide MPX was isolated from wasp venom and had better antibacterial activity against Gram-positive and Gram-negative bacteria. Studies have found that MPX had better bactericidal activity against *E. coli* in vitro. However, whether MPX also has better bactericidal activity in mice is still unknown. This study found that *E. coli* infected mice lost appetite, diarrhea, and grouping together, while MPX treatment significantly alleviated these symptoms. The autopsy results found that the intestinal congestion, bleeding, thinning of the intestinal wall, yellow viscous fluid in the intestinal cavity, congestion of the lungs, necrosis in the liver, congestion, and bleeding of the spleen, and MPX treatment effectively relieved the above symptoms. The qRT-PCR results found that MPX could increase the mRNA expression of the antibacterial protein TFF3 in the jejunum and colon and reduce the expression of the antibacterial protein Remlβ and REG3γ in the jejunum and colon. H&E staining results further found that MPX could alleviate the pathological damage of mouse intestines and organs caused by *E. coli* infection. The above results show that MPX has good bactericidal activity against *E. coli* infection in mice, providing an essential reference for screening drugs for the clinical treatment of *E. coli* infection.

**Keywords**: antimicrobial peptide MPX, *Escherichia coli*, mice.

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## 1. Introduction

Escherichia coli is a gram-negative bacterium and common zoonotic pathogen, which causes many human epidemics. In the United States, more than 100,000 people are infected with EHEC 0157:H7 every year (Malavolta et al., 2019). Studies have reported that EHEC infection in pig intestinal tract contents and feces in central China is high (Bae et al., 2021). The harm of *E. coli* is not only manifested in causing animal diseases and bringing substantial economic losses to the breeding industry and animal husbandry, but also a reservoir of drug resistance genes for other pathogenic bacteria, and the drug resistance genes carried by the food chain passed to Humans (Liang et al., 2021). 80 % of *E. coli* are multi-drug resistant strains, with strong resistance to aminoglycosides, sulfonamides, tetracyclines, and chloramphenicol (Buxser, 2021). *E. coli* is exceptionally harmful and challenging to control. Therefore, there is an urgent need to find new antibacterial drugs against E. coli infection, and not easy to develop drug resistance.

Antimicrobial peptides are small-molecule peptides that can resist the invasion of pathogenic microorganisms into the body. They are an essential part of the innate immune system. Their small molecular weight, good water solubility, and resistance to resistance are considered the best alternative to antibiotics and have become a research hotspot in recent years (Santos et al., 2021). Antimicrobial peptides have various biological functions such as antibacterial, antivirus, anti-parasitic, anti-inflammatory, anti-cancer, improving animal performance and immunity (Xie et al., 2020; Al Adwani et al., 2021; Piyadasa et al., 2021; Gong et al., 2021). MPX was extracted from wasp venom consisting of 14 amino acids and had four positive charges, which had good bactericidal activity against both Gram-positive and Gram-negative bacteria (Zhao et al., 2021). Previous studies of our group found that MPX had good bactericidal activity against *E. coli* in vitro. Whether MPX also had good bacte-

ricidal activity in vivo is still unknown. This study aims to explore further the effect of MPX against *E. coli* infection in vivo.

## 2. Materials and methods

**Ethics Statement.** BALB/c mice (18–22 g, female) were purchased from Zhengzhou University (Henan Province, China). All animal experiments were approved by the Animal Ethics Committee and were performed following the guidelines of the Animal Welfare and Research Ethics Committee.

**Peptide Synthesis.** Antimicrobial peptide MPX (H-INWKGIAAMAKKLL-NH2) was synthesized and purified by Ji er sheng Hua (Shanghai, China) at a purity greater than 98 %, and antimicrobial peptide MPX was very soluble in $ddH_2O$.

**Clinical symptoms and observation of necropsy lesions.** BALB/c mice were randomly divided into 4 groups, namely control group, *E. coli*, *E. coli* + MPX, *E. coli* + enrofloxacin. The dose of *E. coli* infected BALB/c mice was 4.5x107 CFU/mice, MPX (20 mg/kg), and Enro (20 mg/kg) were treated by intraperitoneal injection after infection with *E. coli* for 2 h, and treatment was continued for 3 days. Observed the clinical manifestations and necropsy of the mice after *E. coli* infection, took out the mouse lungs, liver, spleen, and intestines with scissors and toothless forceps, observed the pathological changes of the mouse intestines and organs, and took pictures.

**qRT-PCR.** Total RNA extraction kit (Solarbio, China) extracted total RNA from mouse jejunum and colon. The jejunum and colon powder were slowly added to 1.5 mL EP, 200 μL chloroform was added to each well, and shaken on a shaker for 15 s, centrifuged at 12000 rpm, 4 ℃ for 10 min, added 500 μL isopropanol and mix well, centrifuged at 12000 rpm, 4 ℃ for 10 min, discard the supernatant, adding 1 mL to each tube 75 centrifuge in% ethanol, 12000 rpm, 4 °C for 5 min, added 20–30 μL of DEPC water and mix well, then measure the RNA concentration. A reverse transcription kit (Takala, Japan) was used to reverse RNA into cDNA. The primer sequences as shown in Table 1.

**Table 1**
The primer sequences for qRT-PCR

| Genes | Sequence |
|---|---|
| Reg3γ | F:5'-CCCGACACTGGGCTATGAAC-3'<br>R:5'-GGTACCACAGTGATTGCCTGA-3' |
| Relmβ | F:5' –CTGATAGTCCCAGGGAACGC-3'<br>R:5'-GTCTGCCAGAAGACGTGACA-3' |
| TFF3 | F:5' -CCTGGTTGCTGGGTCCTCTG-3'<br>R:5'-GCCACGGTTGTTACACTGCTC-3' |
| GAPDH | F:5' -GAGAAACCTGCCAAGTATGATGAC-3'<br>R:5'-TAGCCGTATTCATTGTCATACCAG-3' |

**H&E staining.** After wiping clean with alcohol cotton, the mouse organs and intestines were fixed with 4 % paraformaldehyde, paraffin-embedded, sectioned, and H&E stained to observe the pathology of the mouse duodenum, ileum, colon and liver, spleen, and lungs. Change, refer to the specific operation steps (He et al., 2015).

**Statistical Analysis.** GraphPad Prism 5 data processing software to carry out and difference analysis of experimental results (One-Way ANOVA), $P < 0.05$ means significant difference (marked in the text $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$).

## 3. Results and discussion

### Results

**MPX alleviates the clinical manifestations of mice.** Observation of clinical symptoms after infection of *E. coli* in mice was shown in Figure 1A and B: mice infected with *E. coli* alone showed loss of appetite, rapid heartbeat, body tremor, loose hair, bunching up, arched back, anal prolapse, feces clinical manifestations such as irregularities, while MPX treatment significantly alleviated the adverse reactions caused by *E. coli* infection. Mice increased appetite, smooth coat, and the effect was better than enrofloxacin treatment. The control group did not show any adverse reactions.

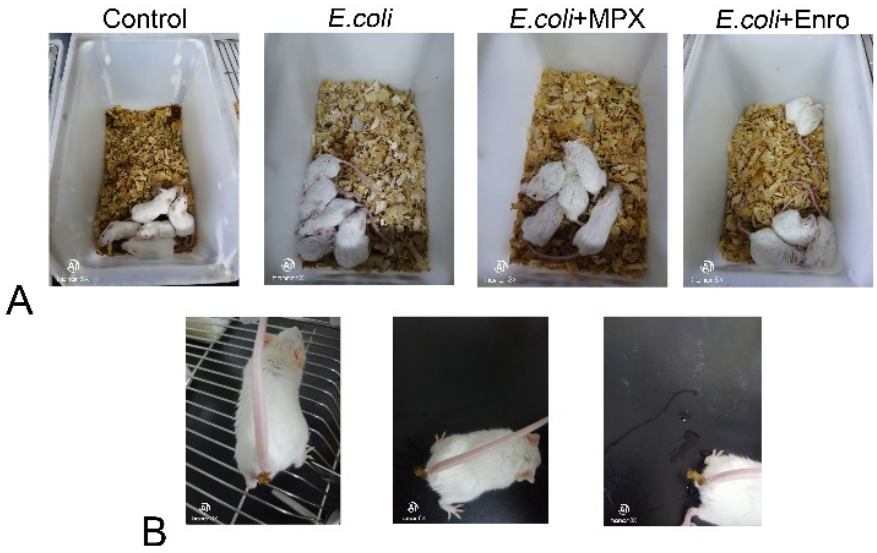


**Fig. 1**. Observation of clinical symptoms of *E. coli* infection with BALB/c mice (A, B)

**MPX alleviates the pathological changes of mice by necropsy.** The results of the necropsy were shown in Figure 2, the intestines of mice in the control group were normal, with thick and flexible intestinal walls, and no pathological changes were seen in the liver, spleen, and lungs. Mice infected with *E. coli* had intestinal congestion, hemorrhage, intestinal wall thinning, and easy to rupture, the intestinal cavity was filled with a yellow viscous liquid, the jejunum was severely congested, and the lungs, liver, and spleen were congested and bleeding. While MPX could effectively alleviate the intestinal inflammatory response and organ pathological damage caused by *E. coli* infection, its effect was equivalent to that of the antibiotic Enro.

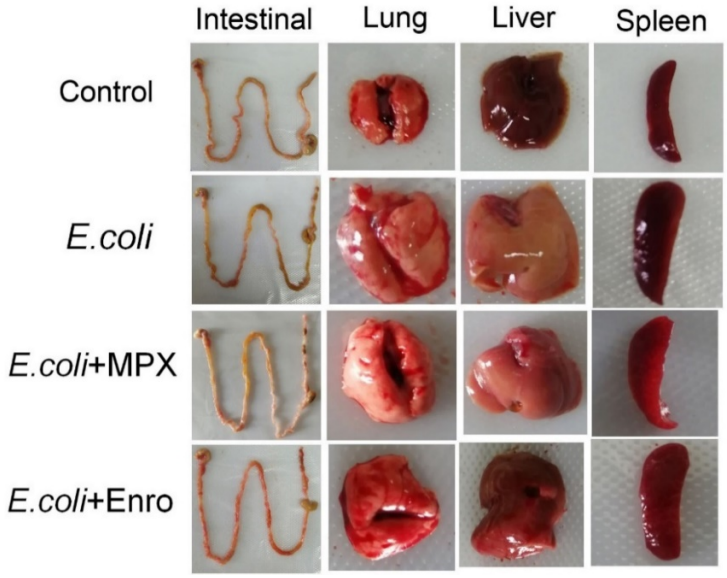


**Fig. 2.** Autopsy results of mouse intestines and organs after *E. coli* infection.

**MPX increases the expression of intestinal antimicrobial peptide protein.** The mRNA expression of intestinal antibacterial related proteins REG3γ, Remlβ, and TFF3 by qRT-PCR. In the jejunum (Figure 3A), compared with the control group, the TFF3 gene expression level in the jejunum of the *E. coli* group was increased (P< 0.05);. In contrast, the TFF3 gene expression in the jejunum of *E. coli*+MPX was significantly lower than in the *E. coli* group (P < 0.05), with no significant difference from the control group. Compared with the control group, the mRNA expression level of Remlβ in the jejunum tissue of *E. coli* infected mice was significantly increased (P < 0.001). MPX significantly reduced the mRNA expression level of Remlβ, which was equivalent to the effect of Enro. In contrast, the expression level of Remlβ in the mouse colon was not significantly different in another group. In addition, *E. coli* infection leads to increased REG3γ expression in mouse jejunum and colon, and MPX could significantly reduce REG3γ mRNA expression caused by *E. coli* infection.

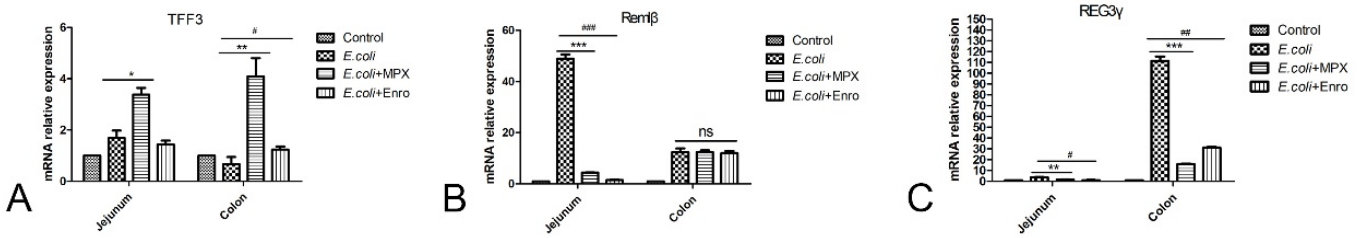


**Fig. 3.** The mRNA expression of the antibacterial protein in the mouse intestine. A: The mRNA expression of TFF3 in mouse jejunum and colon; B: The mRNA expression of Remlβ in mouse jejunum and colon; C: The mRNA expression of REG3γ in mouse jejunum and colon

**MPX relieves pathological intestine damage.** Further H&E staining was used to observe the pathological changes of the duodenum, ileum, and colon after *E. coli* infection. As shown in Figure 4, the duodenum, ileum, and colon of mice infected with *E. coli* showed intestinal villi shedding, breaking and falling into the intestinal lumen, catarrhal enteritis, degeneration, necrosis, shedding of intestinal mucosal epithelial cells, congestion of the lamina pro-pria and a large number of neutrophil infiltration, showing the pathology of necrotizing enteritis and fibrinous necrotizing enteritis Changes (Figure 4A, B, C). At the same time, the pathological changes of each bowel segment were significantly alleviated after treatment with MPX. The intestinal villi of the control were neatly arranged without the above-mentioned pathological changes.

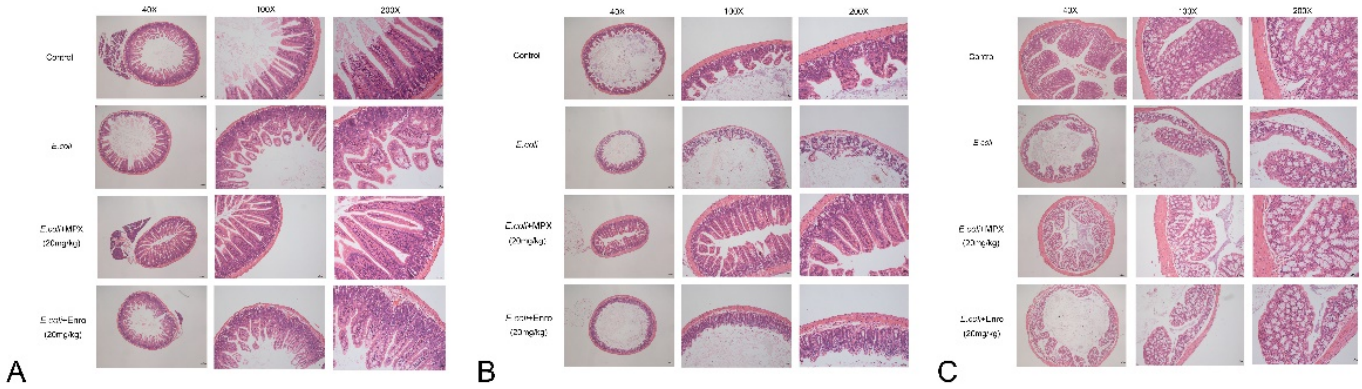

**Fig. 4**. H&E staining of intestines after *E. coli* infection in mice. A: H&E staining of duodenum after *E. coli* infection in mice; B: H&E staining of ileum after *E. coli* infection in mice; C: H&E staining of the colon after *E. coli* infection in mice

**MPX relieves pathological damage of organs in mice.** *E. coli*-infected mice developed acute interstitial pneumonia, widened alveolar septum, ruptured alveoli, neutrophil infiltration, and mild lung disease, showing local pulmonary congestion and a small amount of red blood cell and inflammatory cell infiltration (Figure 5A). Symptoms of hemorrhagic splenitis, congestion, local necrosis, small splenic corpuscles appear in the spleen, a large number of neutrophil infiltration in the splenic sinus (Figure 5B), degeneration and necrosis of hepatocytes, and acute necrosis in the liver, disintegration of liver cells, congestion, liver congestion, dilation of liver sinusoids, infiltration of red blood cells and neutrophils (Figure 5C). The above symptoms were significantly alleviated after treatment with MPX, indicating that MPX can protect mice against the damage of *E. coli* to the organs.

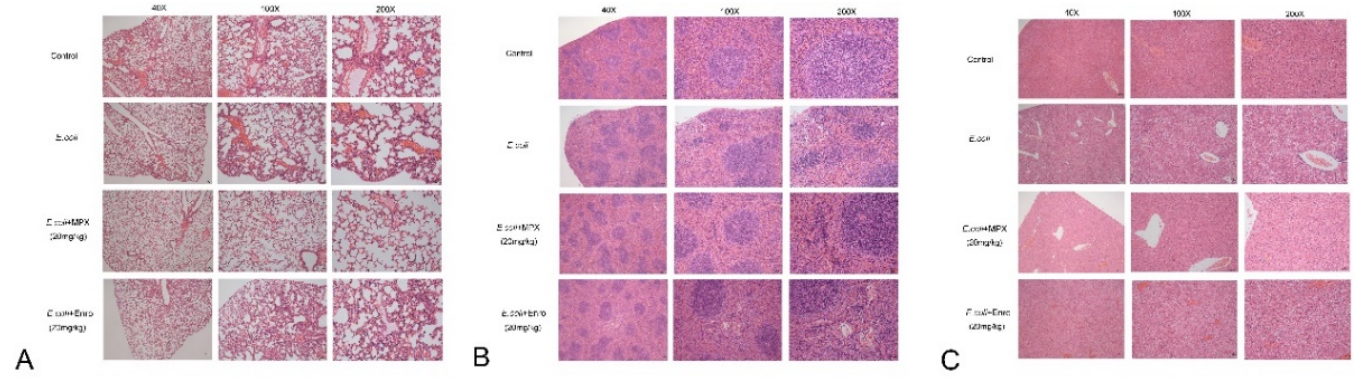

**Fig. 5.** H&E staining of the organs infected with *E. coli* in mice. A: H&E staining of lung infected with *E. coli* in mice; B: H&E staining of spleen infected with *E. coli* in mice; C: H&E staining of liver infected with *E. coli* in mice

**Discussion**

In this study, *E. coli* was used to establish the BALB/c mouse infection model and MPX treatment to evaluate the effect against *E. coli* infection in mice. The clinical symptoms, intestinal and organ necropsy, pathological changes, and the mRNA expression of the antibacterial protein in mice were evaluated. The results showed that MPX could alleviate the clinical symptoms of mice caused by *E. coli* infection, relieve the pathological changes of the intestines and organs, and increase the mRNA expression of the antimicrobial protein TFF3. This study evaluated the effect of MPX against *E. coli* in vivo, laying a foundation for the study of MPX in mice, providing a reference of drugs for treating *E. coli* infection.

MPX can alleviate the intestinal damage caused by E. coli infection in mice. The intestine is the largest digestion and absorption organ of animals and the most important immune organ of the body. Zhang et al. found that adding antimicrobial peptide plectasin to chicken diets could improve chicken performance, immune function, and intestinal health and increase intestinal villi length (Zhang et al., 2021). Roque-Borda CA et al. found that the antimicrobial peptide Ctx(Ile)-Ha could effectively alleviate pathological intestinal damage (Roque-Borda et al., 2021). Shang et al. found that the antimicrobial peptide Microcin J25 could alleviate DSS-induced intestinal Inflammation and improve intestinal morphology (Shang et al., 2021). Xiong et al. found that oral antimicrobial peptide-defensin-1 (DEFB1) could improve intestinal function and enhance intestinal barrier function (Xiong et al., 2021). The results found that MPX could effectively reduce the intestinal damage caused by *E. coli* infection in mice.

The intestine is in direct contact with the external environment and colonizes a large number of microorganisms. Antimicrobial proteins secreted by intestinal epithelial cells play an essential role in maintaining the homeostasis of the intestinal epithelium and normal microbial flora (Gallo & Hooper, 2012; Wlodarska et al., 2010). REG3γ is mainly expressed in the small intestine tissues of mice and humans. In addition, REG3γ is also conditionally expressed when pathogen infection or Inflammation occurs in the large intestine tissues (Christa et al., 1996). The study showed that REG3y was almost not expressed in the intestinal tract of sterile mice, and the expression of REG3y was significantly increased after the normal flora was colonized (Cash et al., 2006). The expression of RemLp is mainly regulated by Th2 cytokines, which play an essential role in the process of innate immunity and host defense (Hosoya et al., 2017).

TFF3 is produced by mucous secreting cells, which play an essential role in the intestinal mucus layer and mucosal repair function (Ge et al., 2015). This study found that MPX can increase the mRNA expression of the antimicrobial protein TFF3 in the jejunum and colon and reduce the expression of the antimicrobial protein Remlβ and REG3γ in the jejunum and colon.

## 4. Conclusions

MPX can resist the lethal attack of *E. coli* in mice, alleviate the pathological changes of mice intestines and organs, and increase or decrease the mRNA expression of antimicrobial proteins in the jejunum or colon to varying degrees, providing significant reference value for clinical drug screening of *E. coli* infection.

### Author's contributions

Xueqin Zhao participated in the study design, carried out data analyses, participated and performed measurements, laboratory testing's and wrote this manuscript.


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### Conflict of interest

The author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication, and claim authorship rights to this publication.

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