



Epigallocatechin-3-Gallate Ameliorates Acute Lung Damage by Inhibiting Quorum-Sensing-Related Virulence Factors of *Pseudomonas aeruginosa*

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The superbug *Pseudomonas aeruginosa* is among the most formidable antibiotic-resistant pathogens. With declining options for antibiotic-resistant infections, new medicines are of utmost importance to combat with *P. aeruginosa*. In our previous study, we demonstrated that Epigallocatechin-3-gallate (EGCG) can inhibit the production of quorum sensing (QS)-regulated virulence factors *in vitro*. Accordingly, the protective effect and molecular mechanisms of EGCG against *P. aeruginosa*-induced pneumonia were studied in a mouse model. The results indicated that EGCG significantly lessened histopathological changes and increased the survival rates of mice infected with *P. aeruginosa*. EGCG effectively alleviated lung injury by reducing the expression of virulence factors and bacterial burden. In addition, EGCG downregulated the production of pro-inflammatory cytokines, such as TNF- α , IL-1, IL-6, and IL-17, and increased the expression of anti-inflammatory cytokines IL-4 and IL-10. Thus, the experimental results supported for the first time that EGCG improved lung damage in *P. aeruginosa* infection by inhibiting the production of QS-related virulence factors *in vivo*.

Keywords: anti-virulence, quorum sensing, EGCG, *P. aeruginosa*, acute lung infection

INTRODUCTION

Globally, pneumonia is a severe public health problem with a large disease burden and a major cause of mortality and morbidity. The disease burden caused by pneumonia affects more children worldwide than other diseases (O'Brien et al., 2019). Older patients have a high risk of death afflicted by pneumonia (Quinton and Mizgerd, 2015). Some of the bacterial and viral infections causing acute pneumonia or sepsis result in serious inflammatory damage to the lungs, thereby leading to the progression of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), especially in critically ill patients (Dai et al., 2018).

Multidrug-resistant microbes (“superbugs”), such as *Streptococcus pneumoniae*, group A *Streptococcus*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, and *Pseudomonas aeruginosa* can cause severe pneumonia and were the main inducers of acute pathogenic factors (Ravi Kumar et al., 2018).

P. aeruginosa, a common opportunistic pathogen, can cause life-threatening respiratory infections and is responsible for hospital-acquired infections and ventilator-associated pneumonia (VAP). The high adaptability and an increasing number of multidrug-resistant *P. aeruginosa* constitute a threat for those who suffer from Chronic Obstructive Pulmonary Disease (COPD) or Cystic Fibrosis (CF; Aloush et al., 2006). Unfortunately, because of its intrinsic and extrinsic drug resistance and the capacity of *P. aeruginosa* to form biofilms, a *P. aeruginosa* infection is notoriously difficult to treat with antibiotics (Moore and Flaws, 2011). Multidrug-resistant pathogen-induced death is estimated to reach 10 million by 2050, and thereby exceeding deaths caused by cancer and diabetes combined worldwide. Thus, the development of alternative therapeutic methods is critical (O’Neill, 2016). The QS system is a cell-to-cell system that enables microbe populations to change their behavior based on population density. The QS system of *P. aeruginosa* plays a key role in coordinating various activities, including biofilm formation and the release of virulence factors. The QS system of *P. aeruginosa* is mainly regulated by four QS network subsystems, including *lasI/lasR*, *rhlI/rhlR*, PQS, and IQS systems (Lee and Zhang, 2015).

P. aeruginosa-induced pneumonia expresses a myriad of virulence factors, including flagella, pili, lipopolysaccharides (LPS), elastase, alkaline phosphatase, exotoxin A, as well as components of the type III secretion system (T3SS; Sadikot et al., 2005). In addition, the ability of *P. aeruginosa* to form biofilms is conducive to establishing infections in VAP and CF patients and is difficult to eradicate (Costerton et al., 1999). *P. aeruginosa*-induced pneumoniae is a complex process. Several surface-associated elements, including flagella, fimbriae, and LPS, contact host respiratory epithelia through a number of aggregated pili (Gellatly and Hancock, 2013). Once contact with host epithelia has occurred, T3SS can be activated. *P. aeruginosa* T3SS is a major determinant of virulence, and its expression is frequently associated with acute invasive infections. Moreover, *P. aeruginosa* T3SS has been linked to increased mortality in infected patients (Hauser, 2009). Elastase (*lasB lasA*) has been shown to directly injure lung tissue through disruption of epithelial tight junctions and basal membranes. Elastase may increase the recruitment of neutrophils into the airways, which can result in serious inflammation (Kipnis et al., 2006). Pyocyanin induces direct damage to the respiratory tract as epithelial necrosis, and slowing tracheal mucociliary transport results from damaged ciliary movement (Lau et al., 2004). Virulence factors, such as type III secretory proteins, QS systems, and LPS, activate the host immune response. Therefore, the activation of macrophages, neutrophilic granulocytes, and T cells induces the secretion of cytokines, chemotactic factors, and another inflammatory mediator, thus leading to lung injury and mortality (Driscoll et al., 2007).

For thousands of years, tea originating from China has gained the world’s taste. It has become the daily health drink for many individuals. In general, these health benefits result from the phenolic compounds that are present in green tea, particularly catechins. Numerous studies have demonstrated the diverse activities of Epigallocatechin-3-gallate (EGCG), including antioxidant, antibacterial, antiviral, antitumor and anti-inflammatory activities. In our previous study, we demonstrated that EGCG significantly acted against the expression of *P. aeruginosa* QS-regulated virulence *in vitro*, and significantly increased the survival rate of *Caenorhabditis elegans* infected with *P. aeruginosa* (Hao et al., 2021). QS quenching, called anti-virulence therapy, has been considered a suitable strategy to settle the multidrug resistance problem (Clatworthy et al., 2007). Taken together, our study provides additional support for drinking tea as an effective method against bacterial infections. These findings can pave the way for the use of EGCG as a therapeutic medicine to treat bacterial infections in the lungs.

MATERIALS AND METHODS

Animals, Bacterial Strains, and Chemicals

Eight-week-old male ICR mice (25–30 g) were purchased from Sibeifu Biotechnology Co. Ltd. (Beijing, China). Mice were housed at 22–25°C with a 12 h day-night cycle, were fed standard rodent chow, and sterile water *ad libitum* for 1 week of acclimation. All protocols involving animal studies were reviewed and approved by the Animal Ethical Committee of Sichuan Agricultural University (#20210020). *P. aeruginosa* (PAO1) were cultured in Luria Bertani (LB) medium (Sangon Biotech, Shanghai, China). EGCG was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China); CIP was obtained from Sichuan Chuanlong Dongke Pharmaceutical Co., Ltd.

Experimental Protocol

A total of 90 mice were randomly divided into six groups ($n=15$ mice per group): a control group, PAO 1 group, PAO 1 + EGCG (20 mg/kg) group, PAO 1 + EGCG (40 mg/kg) group, PAO 1 + EGCG (80 mg/kg) group, and a Ciprofloxacin (CIP; 20 mg/kg) group. CIP was used as positive control. Mice were administered the same volume of saline or drugs by intragastric administration for 3 days. Mice were infected by using a previously described method with modifications (Traber et al., 2019). Briefly, mice were weighed and anesthetized, and received intratracheal instillation of PAO 1 (2.5×10^8 CFU) in 20 μ l phosphate buffered saline (PBS) to model an acute infection with PAO 1. Mice were anesthetized and sacrificed at 24 h after infection. Samples were harvested rapidly for subsequent analysis. Per group, 10 mice were randomly selected to collect blood and lung tissue; another five mice were selected for the collection of bronchial-alveolar lavage fluid (BALF).

Colony Counting of the Lungs

To assess bacterial burden in the lung, fresh lung tissues were extracted aseptically, and homogenized in sterile NaCl 0.9%.

As previously described (Pylaeva et al., 2019), colony forming units (CFU) were enumerated by plating serial dilutions of lung homogenates on *Pseudomonas* Agar Medium for the detection of Pyocyanin (PDP, Haibo Biotech, Shandong, China). After incubation at 37°C for 24h, CFU were counted and recorded.

Histopathological Evaluation

Lung tissue was fixed with 4% paraformaldehyde overnight, and embedded in paraffin. Subsequently, lung tissue was cut into 5- μ m-thick sections. Sections were stained with hematoxylin and eosin (H&E) and the degree of damage to lung tissue was validated (Sen-Kilic et al., 2019).

ELISA

BALF was collected and centrifuged at 1500g for 5 min, then the supernatant was removed and transferred to a clean tube. The expression of TNF- α , IL-10, and IL-17 in the supernatant was determined by ELISA according to the manufacturer's instructions [Multisciences (Lianke) Biotech Co., Ltd., Hangzhou, China]. The absorbance was measured using a microplate reader at 450 nm.

RNA Extraction and qPCR

Total RNA was extracted from lung tissue homogenate using TRIzol reagent (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. Then, cDNA synthesis was conducted using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, K1622, Waltham, MA, United States) in a 20 μ l volume of reaction mixture. Real-time PCR was performed in a total reaction volume of 10 μ l, containing 5 μ l PerfectStartTM Green qPCR SuperMix (TransGen Biotech, Beijing, China), 2 μ l template cDNA, 1 μ l of primers (Shenzhen Huada Gene Research Institute, Shenzhen, China), and 2 μ l DNase/RNase-free water (Tiangen Biotech, Beijing, China). Finally, pvdQ of *P. aeruginosa* was chosen as the reference gene. The qRT-PCR reaction was conducted on a LightCycler[®] 480II Master Mix (Roche, Germany). Data from qRT-PCR experiments were analyzed using the 2^{- $\Delta\Delta$ CT} method (Yang et al., 2021). Primer sequences are listed in **Table 1**.

Statistical Analysis

Data are expressed as the mean \pm standard deviation (SD), and all experimental groups were compared with the control group. Data were analyzed using SPSS 20.0 by ANOVA (IBM SPSS Statistics, CA, United States), and $p < 0.05$ or $p < 0.01$ was considered significantly different.

RESULTS

Protective Efficacy of EGCG Against Acute Lung Infection

As shown in **Figure 1**, the survival of mice with a *P. aeruginosa*-induced acute lung infection was evaluated to test the protective effect of EGCG. Of the mice in the *P. aeruginosa* group, only 53.4% survived. The survival rates after EGCG early intervening

TABLE 1 | PCR primers of virulence factors and inflammatory cytokines.

Primer name	Type	Primer sequence
lasI	Fw	CGCACATCTGGGAACCTCA
	Rev	CGGCACGGATCATCATCT
lasR	Fw	CTGTGGATGCTCAAGGACTAC
	Rev	AACTGGTCTTGCCGATGG
rhlI	Fw	GTAGCGGGTTTGCGGATG
	Rev	CGGCATCAGGTCTTCATCG
rhlR	Fw	GCCAGCGTCTTGTTCCGG
	Rev	CGGTCTGCTGAGCCATC
pqsA	Fw	GACCGGTGTATTGCGATT
	Rev	GCTGAACACAGGAAAGAAC
pqsR	Fw	CTGATCTGCCGGTAATTGG
	Rev	ATCGACGAGGAAGTGAAGA
phzM	Fw	ACGGCTGTGGCGGTTTA
	Rev	CCGTGACCGTCCGATT
lasA	Fw	CTGTGGATGCTCAAGGACTAC
	Rev	AACTGGTCTTGCCGATGG
lasB	Fw	AACCGTGGTCTTACTCTGTT
	Rev	CGGTCCAGTAGTAGCGGTTG
phzA	Fw	AACGGTCAGCGGTACAGGGAAAC
	Rev	ACGAACAGGCTGTGCCGCTGTAAAC
phzH	Fw	GCTCATCGACAATGCCGAAC
	Rev	GCGGATCTCGCCGAACATCAG
phzS	Fw	CCGAAGGCAAGTCGCTGGTGA
	Rev	GGTCCCAGTCGGCGAAGAACG
RhlA	Fw	TGGCCGAACATTTCAACGT
	Rev	GATTTCCACCTCGTCTCCTT
RhlC	Fw	GCCATCCATCTCGACGGAC
	Rev	CGCAGGCTGTATTGCGTG
rpoD	Fw	GGGCGAAGAAGGAATGGTC
	Rev	CAGGTGGCGTAGGTGGAGAA
TNF- α	Fw	CTTCTGTCTACTGAACCTCGGG
	Rev	CAGGCTTGCTACTCGAATTTTG
IL-1	Fw	GAAATGCCACCTTTTGACAG
	Rev	TGGATGCTCTCATCAGGACAG
IL-6	Fw	TAGTCTTCTACCCCAATTTCC
	Rev	TTGGTCTTAGCCACTCCTTC
IL-4	Fw	GGTCTCAACCCCGAGTAGT
	Rev	GCCGATGATCTCTCAAGTGAT
IL-10	Fw	GCTGGACAACATACTGCTAACC
	Rev	ATTTCCGATAAGGCTTGCCAA
IL-17	Fw	TTAACTCCCTTGCGCAAAA
	Rev	CTTTCCTCCGCAATTGACAC
GAPDH	Fw	TCAACGGCACAGTCAAGGC
	Rev	CTCCACGACATACTCAGCAC

treatment with 80 mg/kg, 40 mg/kg, 20 mg/kg, respectively, were 71.8, 66.7, 66.7%, whereas the survival rate of the positive CIP group was 60%, thus the protective effect was less compared to that of EGCG. Next, the alleviation of *P. aeruginosa*-induced pulmonary edema by EGCG was assessed. The results showed that the wet weight of mice in the *P. aeruginosa* group increased more than 2-fold compared with mice in the control group. EGCG significantly reduced the lung edema, and no significant differences in lung weight were observed between CIP and *P. aeruginosa*. The bacterial burden in the lungs was determined by plating serial dilutions and counting viable bacteria at 24h post the challenge. As expected, the bacterial load in the lungs of EGCG-treated mice was significantly lower compared to that of mice in the *P. aeruginosa* group. These findings demonstrated the preventive effect of EGCG in decreasing acute lung infection

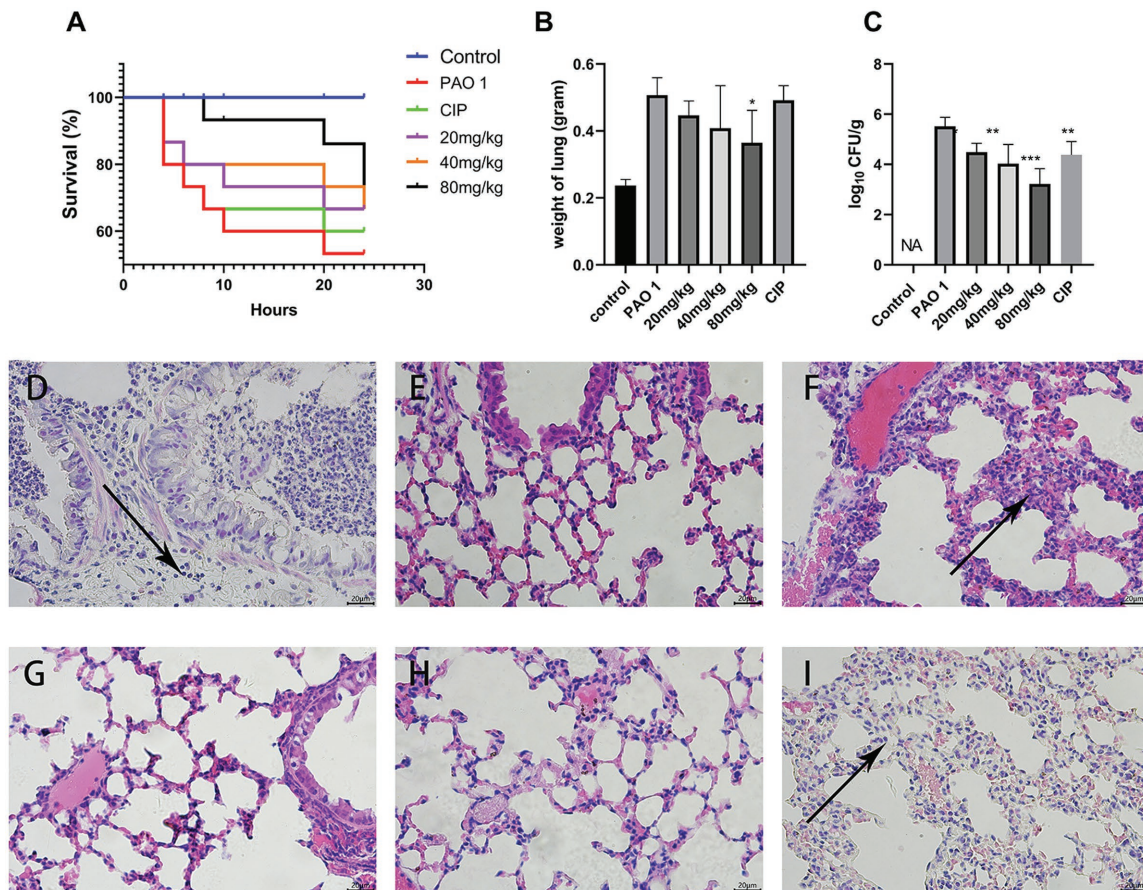


FIGURE 1 | Protective efficacy of EGCG against acute lung infection. **(A)** The survival rate was measured at different time points post *P. aeruginosa* challenged. **(B)** The weight of the lungs after *P. aeruginosa* challenged. **(C)** Bacterial load in the lungs were evaluated at 24 h post infection. **(D–I)** Pathological changes in **(D)** *P. aeruginosa* group, **(E)** control group, **(F)** EGCG (20 mg/kg) group, **(G)** EGCG (40 mg/kg) group, **(H)** EGCG (80 mg/kg) group, **(I)** CIP was used as positive control. Data are presented as mean \pm SD and analyzed with one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs. PAO 1 group.

of *P. aeruginosa*. The control group did not show any histopathological changes in the lungs under the light microscope. The results indicated that lung sections after *P. aeruginosa* infection showed significant changes, including PMN infiltration, alveolar interstitial edema, interstitial hemorrhage, and alveolar wall thickening. Importantly, our results showed that EGCG treatment reduced lung damage and maintained alveolar integrity due to structural disruptions and hemorrhage in a concentration-dependent manner compared with the control. Taken together, these results demonstrated that EGCG alleviated histopathological damage in lungs of mice challenged with *P. aeruginosa*.

EGCG Alleviated the Expression of QS System-Regulated Genes *in vivo*

In this study, the expression of QS system genes was examined in acute lung injury. As shown in **Figure 2**, EGCG and CIP significantly downregulated the expression of QS-related virulence factors *in vivo*. First, EGCG inhibited the master QS regulatory system *lasI/lasR* in a dose-dependent manner. The expression of *lasR* was significantly inhibited at 80 mg/kg. At a dose of 80 mg/kg, the expression of *lasA* and *lasB*, which are regulated *via* the

lasI/lasR pathway, was also significantly inhibited in a dose-dependent manner. Moreover, *rhlI/rhlR* and *pqsA/pqsR* systems were downregulated as well. Our data showed that the expression level of the *pqsA/pqsR* system was lower than that of *lasI/lasR* and *rhlI/rhlR* systems. Therefore, we hypothesized that *lasI/lasR* and *rhlI/rhlR* systems played a dominant role in acute lung infection (**Figure 3**). The expression of virulence factors was significantly downregulated by EGCG. The expression of *phzA*, *phzS*, *phzM*, and *phzH* was also inhibited by EGCG compared with mice in the *P. aeruginosa* group. Furthermore, the *rhlA* gene, which controls the production of rhamnolipid, was significantly inhibited.

EGCG Inhibited Biofilm Expression *in vivo*

Pseudomonas aeruginosa biofilm formation is regulated by the QS system (Sauer et al., 2002). The expression of biofilm matrix genes and subsequent development of biofilm structure are also adjusted by the QS system (Skariyachan et al., 2018). The expression of biofilm-regulated factors (*fila*, *pela*, *pila*, *pslb*) was evaluated *in vivo*. The results indicated that EGCG significantly inhibited biofilm maturation at 18 h *in vitro*, and a better inhibitory effect was found *in vivo*. The expression

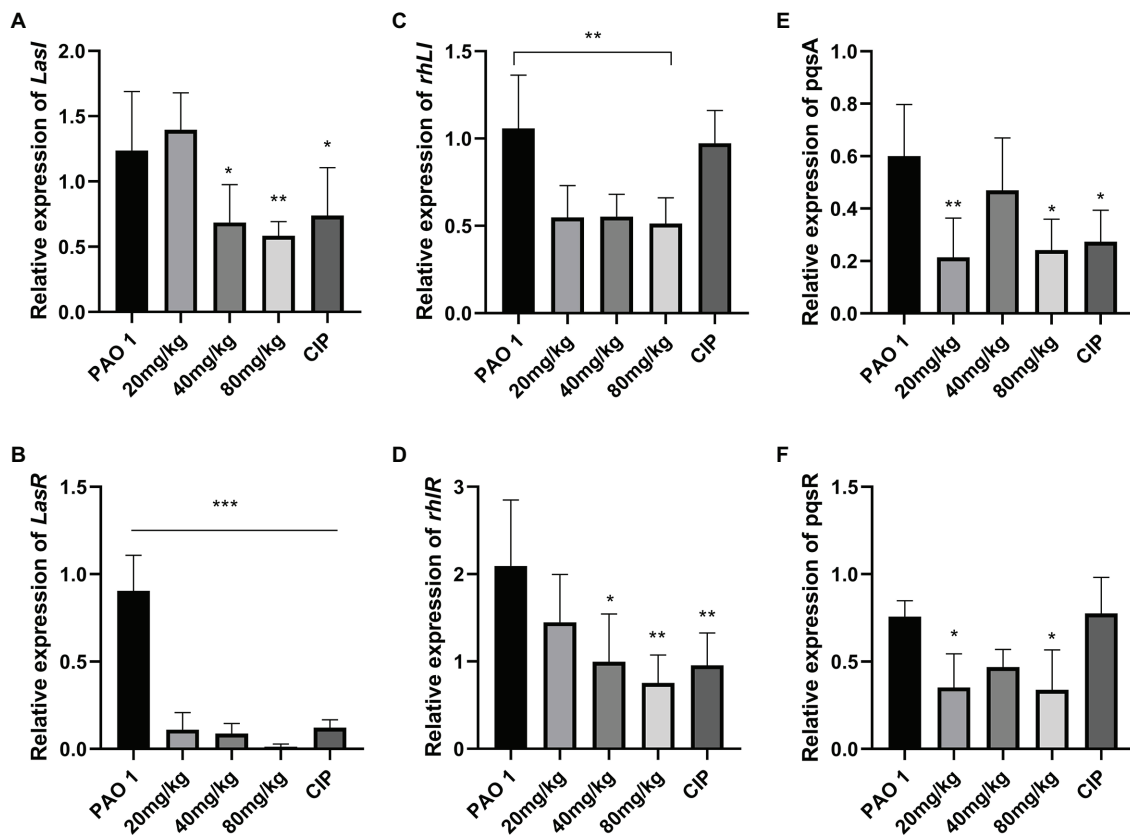


FIGURE 2 | The effect of EGCG on the expression of QS system *in vivo*. (A) *lasI*, (B) *lasR*, (C) *rhII*, (D) *rhIR*, (E) *pqsA*, and (F) *pqsR*. Data are presented as mean \pm SD and analyzed with one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs. PAO 1 group.

of *pela*, *pila*, and *pslB* was significantly inhibited by EGCG in comparison to mice in the *P. aeruginosa* infection group. Thus, these results suggested that EGCG can effectively inhibit biofilm formation and prevent the activation of virulence factors of *P. aeruginosa* *in vivo*. The data are presented in Figure 4.

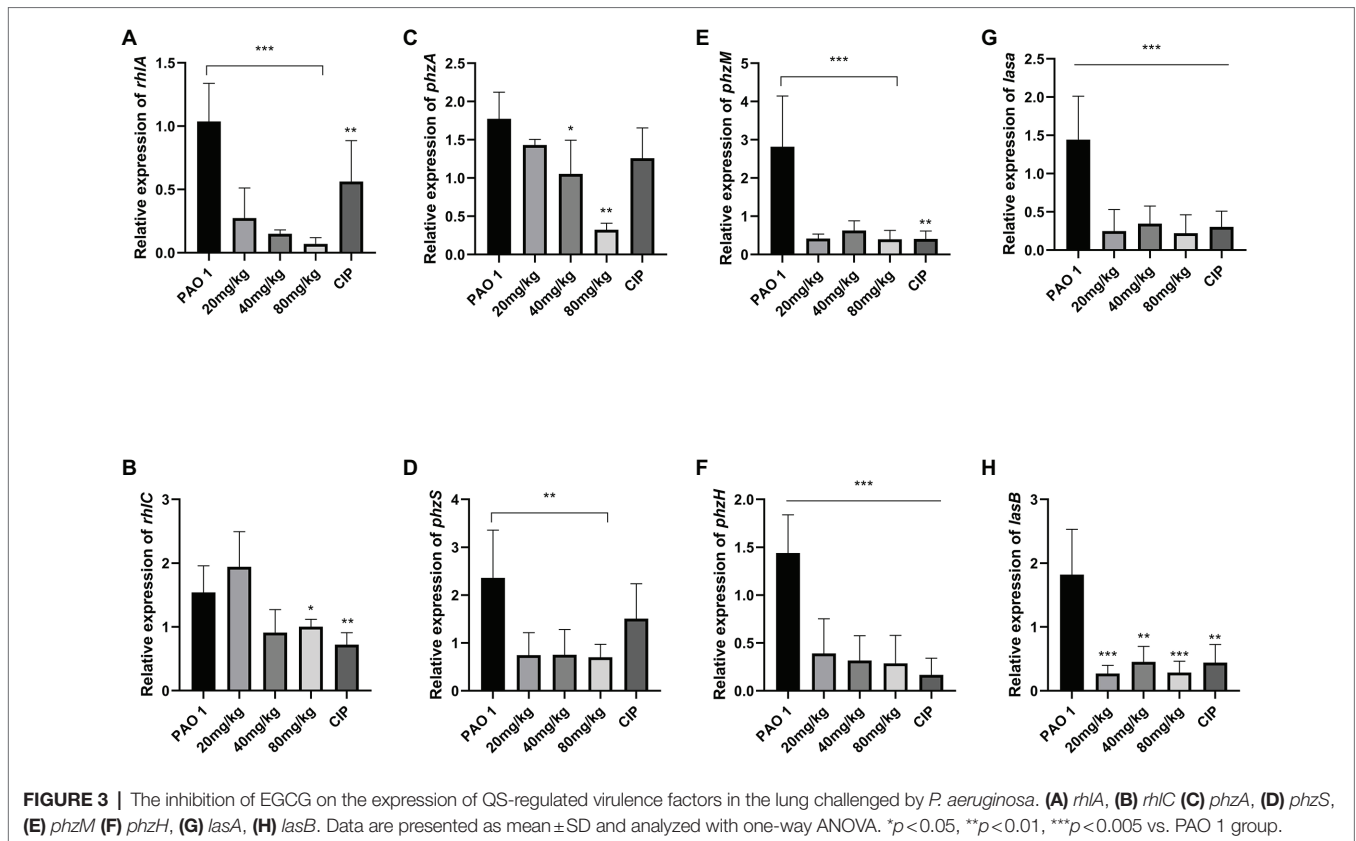
EGCG Inhibited Inflammation in *Pseudomonas aeruginosa*-Induced Lung Injury

To assess the inflammatory state of infected mice, the expression of inflammatory cytokines was determined by real-time PCR and ELISA. Compared to the control, EGCG significantly decreased the expression of proinflammatory cytokines TNF- α , IL-1, IL-6, and IL-17. Moreover, the expression of proinflammatory cytokines in the CIP group was lower than that in the EGCG group. The EGCG group had a higher expression of anti-inflammatory cytokines IL-4 and IL-10 (Figure 5).

DISCUSSION

In this study, it was demonstrated that EGCG protects mice against *P. aeruginosa*-induced lung damage by inhibiting the

virulence controlled by QS systems. *P. aeruginosa* is a major cause of acute nosocomial infections and pneumonia (Kizny Gordon et al., 2017). Nosocomial bacteremia pneumonia is associated with significant mortality, morbidity, length of hospital stays and cost. In addition, despite important medical advances, there have been no improvements in the treatment of *P. aeruginosa* infections over the past two decades (Melsen et al., 2013; Jain et al., 2015). The mechanisms by which *P. aeruginosa* resists antibiotics include intrinsic, acquired and adaptive resistance, such as efflux pumps, antibiotic-inactivating enzymes, impermeable outer membrane proteins, horizontal transfer of resistance genes or mutational changes, and adaptive resistance refers to the formation of biofilms (Livermore, 2002; Breidenstein et al., 2011). *P. aeruginosa* is resistant to diverse types of antibiotics, including quinolones, aminoglycosides, and β -lactams (Hancock and Speert, 2000). Due to the increasing frequency of antibiotic resistance of *P. aeruginosa*, novel therapeutic approaches to treat *P. aeruginosa* infections are of utmost importance. Furthermore, novel treatments may be used alone or in combination with conventional therapies, such as the inhibition of QS and bacterial lectins, the use of iron chelation, phage therapy, vaccine strategies, as well as the use of nanoparticles, antimicrobial peptides and electrochemical scaffolds (Chatterjee et al., 2016). In our previous study, the minimal inhibitory concentration (MIC) of EGCG against *P. aeruginosa* was confirmed to be 512 μ g/ml (Hao et al., 2021).



However, EGCG reduced virulence phenotypes, such as biofilm, protease, elastase activity, swimming, and swarming motility at concentrations where no growth inhibition was observed. In the present study, we aimed to explore the anti-infection ability of EGCG based on the QS mechanism in a mouse model of *P. aeruginosa* infection. Our results indicated that EGCG significantly alleviated *P. aeruginosa*-induced pulmonary edema, decreased the bacterial load and the level of proinflammatory cytokines in the lung, and enhanced the survival rates of mice. Moreover, EGCG significantly reduced the expression of QS-regulated virulence factors *in vivo*. Taken together, these findings showed that EGCG has the ability to mitigate the release of virulence and may alleviate inflammation-caused damage *in vivo*.

EGCG is one of the richest ingredients in green tea-derived polyphenols, and its biological activities have been extensively studied (Jigisha et al., 2012; Steinmann et al., 2013). EGCG can inhibit infections by reducing biofilm formation and toxin release in bacteria (Zhao et al., 2021). However, few studies have evaluated the clinical effect of EGCG in animal models. In several previous studies, the anti-inflammation effect of EGCG was demonstrated. Lee et al. demonstrated that EGCG protected against TNF- α -mediated lung inflammation by down-regulation of oxidative stress and expression of intercellular adhesion molecule (ICAM)-1 in A549 cells or human pulmonary alveolar epithelial cells (HPAEPiCs) as well as in mouse lungs (Lee et al., 2013). In a recent study, the effect of green tea (GTE) and EGCG on macrophage polarization was evaluated *in vitro* and it was determined whether the treatment could ameliorate inflammatory responses *in vivo*. Results showed

that GTE and EGCG decreased M1-macrophages and increased Treg cells in bone marrow to inhibit inflammation. EGCG and GTE prevent LPS-induced inflammatory damage contributing to restore the immune system homeostasis through increasing M2-macrophages, N2-neutrophils and Tregs in the spleen and blood (Azambuja et al., 2022). The activation of M2 macrophages, which polarized by Th2 cytokines such as IL-4 and IL-13, can enhance the effect anti-inflammatory and immunoregulatory. Consequently, increasing the release of anti-inflammatory cytokines IL-10 and TGF- β (Shapouri-Moghaddam et al., 2018). Wang et al. (2019) demonstrated that pretreatment with EGCG attenuated LPS-induced ALI as manifested by fewer pathological changes in pulmonary edema and the expression of proinflammatory cytokines TNF- α , IL-1 β , and IL-6 in the lung, serum, and BALF. The protective mechanism was associated with suppressing the TLR-4/NF-kB-p65 pathway that mediates inflammation.

These results were similar to the data obtained in our study; we demonstrated that EGCG alleviates lung damage, including pathological injury and pulmonary edema in *P. aeruginosa*-induced pulmonary infection. Moreover, EGCG decreased the *P. aeruginosa* load and infection mortality in the lung. EGCG significantly inhibited the expression of TNF- α , IL-1 β , IL-6, and IL-17 in the lung but increased the expression of anti-inflammatory cytokines IL-4 and IL-10. We speculate that EGCG may improve the immunity ability through promoting the polarization of M2 macrophages. Hence EGCG increased the level of anti-inflammatory cytokines IL-4 and IL-10. Further work needs to be performed to identify the underlying

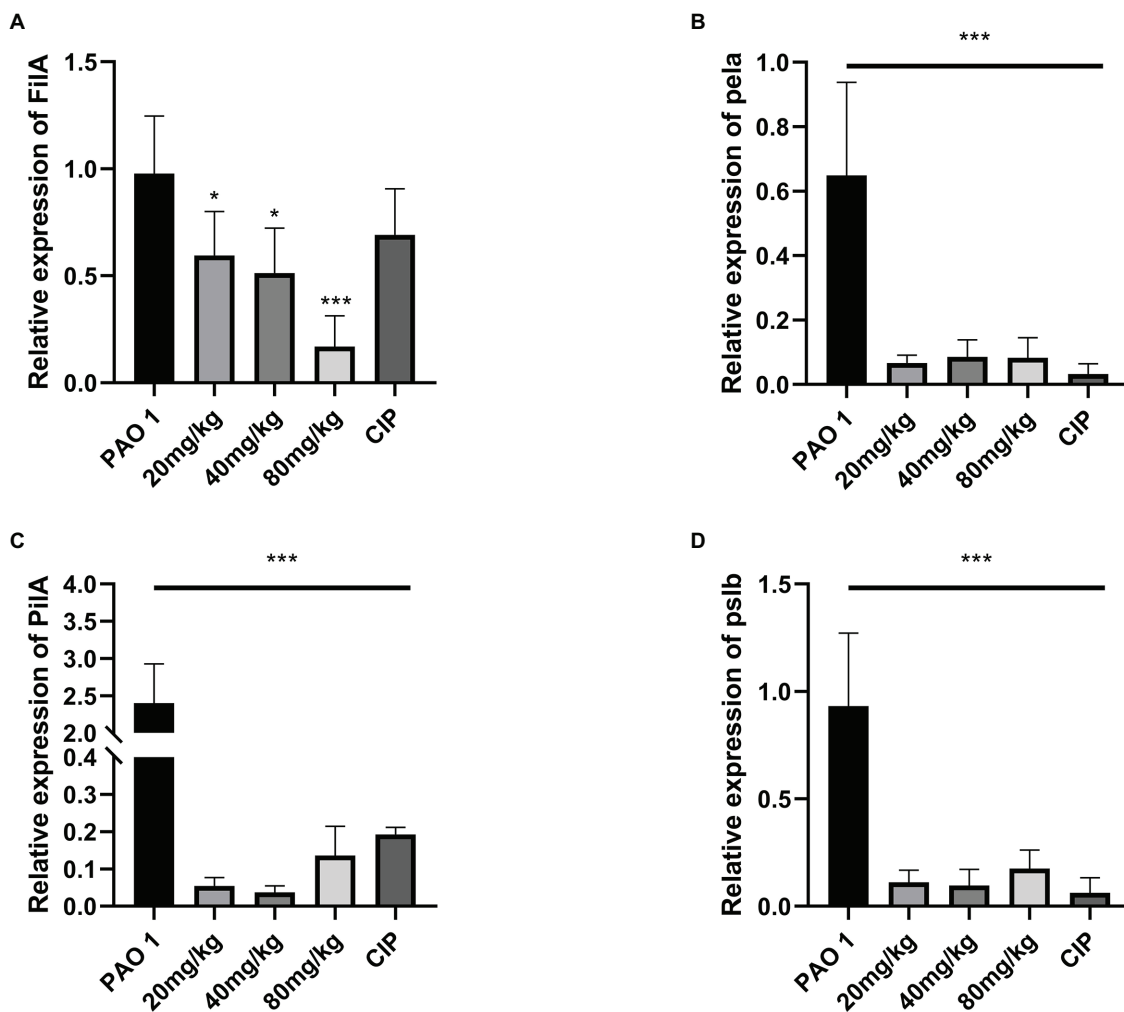


FIGURE 4 | EGCG significantly affected the expression of the biofilm formation relevant genes *in vivo*. (A) *FilA*, (B) *pelA* (C) *PiiA*, (D) *pslB*. Data are presented as mean \pm SD and analyzed with one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs. PAO 1 group.

mechanisms involved. The ELISA results showed that the protein expression levels of TNF- α and IL-17 decreased in a dose-dependent manner, compared with PAO 1 group. Therefore EGCG can reduce *P. aeruginosa*-induced inflammation in the lung. Liu et al. demonstrated that tea polyphenols increased the survival rate of *Caenorhabditis elegans* against *K. pneumoniae* infection to 73.3 and 82.2% (Liu et al., 2020). Previous results showed that microencapsulation of EGCG exhibited therapeutic outcomes by resolution of inflammation in tuberculosis bacteria-infected lungs and a significant reduction in bacterial burden (Sharma et al., 2020). Many studies have suggested that EGCG and other polyphenol compounds could be potential antivirulence agents for pulmonary infection (Sriram et al., 2009; Ling et al., 2012). The potent anti-inflammatory activities add further appeal to the medicinal use of EGCG or other green tea polyphenol-rich products.

P. aeruginosa can express a plethora of virulence factors that facilitate invasion and damage host tissues (Crousilles et al., 2015), and are controlled by complex, intersecting

regulatory circuits and multiple signaling systems (Nadal Jimenez et al., 2012; Balasubramanian et al., 2013). Among these, QS regulates virulence factors (proteases, elastase, rhamnolipid, exotoxins, and pyocyanin) and plays an important role in acute *P. aeruginosa* infections (Hauser, 2009; Lee and Zhang, 2015). QS participates in biofilm formation, which plays an important role during chronic infections and antibiotic resistance. In recent years, antimicrobial agents with great potential have been studied and are now known as antivirulence therapies (Hauser, 2009). This intervention targets virulence factors or virulence regulatory pathways that will not result in inhibition of bacterial growth or bacterial cell death but block their pathogenicity (Hauser, 2009). Consequently, the emergence of drug-resistant strains is decreasing. Interference with QS-mediated signaling and alleviation of virulence contribute to clearance of infecting bacteria by host defenses (Saeki et al., 2020). Our results confirmed the anti-QS ability of EGCG against *P. aeruginosa in vivo*. EGCG significantly inhibited the expression of QS-relevant genes, including *lasI*, *lasR*, *rhII*, *rhIR*,

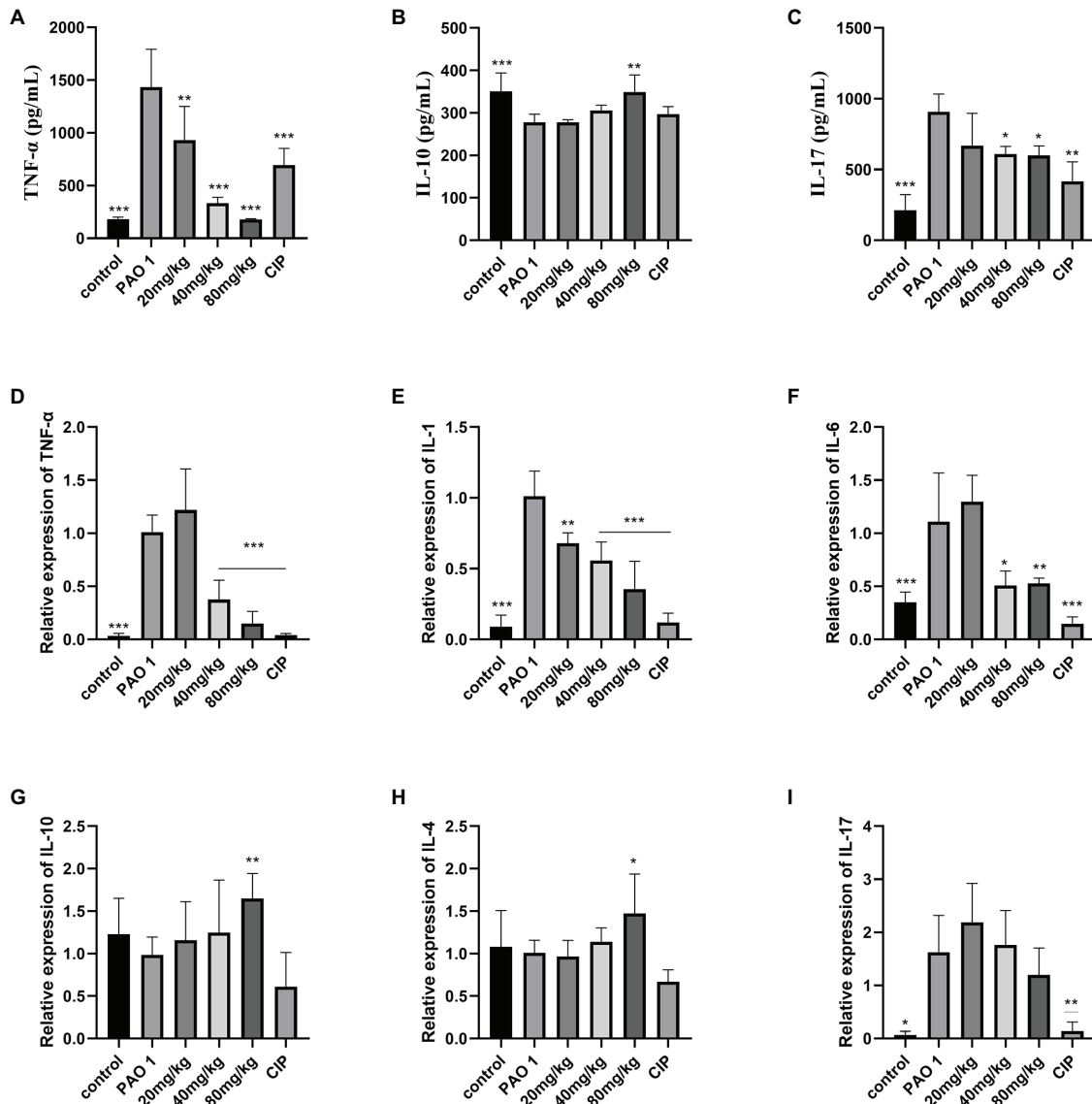


FIGURE 5 | Assay of inflammatory cytokines by ELISA and QRT-PCR. Secreted TNF- α , IL-10, and IL-17 from the bronchial-alveolar lavage fluid was assessed by ELISA assay kit (A–C). EGCG affected the expression of cytokines with a dosage dependent manner in the lung (D–I). Data are presented as mean \pm SD and analyzed with one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs. PAO 1 group.

pqsA, *pqsR*, *phzA*, *phzH*, *phzM*, *phzS*, *lasA*, *lasB*, *rhlA*, and *rhlC*. Moreover, EGCG had a better capacity to inhibit virulence than the positive CIP group. According to the gene expression results, EGCG displayed an excellent ability to inhibit proinflammatory cytokines. At 80 mg/kg, EGCG significantly increased anti-inflammatory cytokines IL-10 and IL-4, which was not observed in the CIP group. We found that the expression of *lasR/lasI* and *rhlR/rhlI* systems, the leading virulence factor during *P. aeruginosa* infection, was significantly inhibited at 80 mg/kg. Pyocyanin, a green color product of *P. aeruginosa*, can interfere with host oxidative stress responses by increasing intracellular levels of reactive oxygen species (Lau et al., 2004). Our results show that the inhibition ability of EGCG on the expression of QS-regulated virulence factors was better than

that of CIP, especially in the QS systems *lasR/lasI*, *rhlR/rhlI* and *pqsR/pqsA*. Thus, these results suggested that EGCG not only alleviates inflammation but also reduces the damage of *P. aeruginosa* infection by targeting QS system-regulated virulence, thereby resulting in a higher protective effect than CIP. EGCG is a great potential QS inhibitor against infection that not only reduces the release of virulence factors *in vitro* but also effectively inhibits the expression of virulence factors *in vivo*.

CONCLUSION

In conclusion, our results provide proof that EGCG alone can ameliorate acute *P. aeruginosa* infection in the lungs by QS and

inflammation inhibition. The *in vivo* results indicate that the QS inhibitor, EGCGs may serve as a potential approach in treating bacterial infections. EGCG, a rich ingredient of tea, is indispensable to human life and may play an important role in preventing bacterial infections.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Ethical Committee of Sichuan Agricultural University.

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AUTHOR CONTRIBUTIONS

HT and SH contributed to conceptualization, methodology, validation, and investigation. LZ and FS contributed to formal analysis and investigation. YL, QW, HG, CL, JL, and ZZ contributed to writing—original draft and resources. GY contributed to writing—review and editing, supervision, resources, and funding acquisition. All authors contributed to the article and approved the submitted version.

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