Article

Discovery Medicine 2025; 37(197): 1062–1073
https://doi.org/10.24976/Discov.Med.202537197.94

Protective Role of Quercetin in Chronic Atrophic Gastritis: Modulation of Gastric Mucosal Integrity via the Transforming Growth Factor-beta1/Smads Signaling Pathway

Peipei Cai[1], Qingqing Song[1], Fangpeng Ye[1], Rongjian Chen[1], Zhixiang Lin[1,*]

[1]Department of Gastroenterology, The Third Affiliated Hospital of Wenzhou Medical University, 325200 Wenzhou, Zhejiang, China
*Correspondence: 15958761961@163.com (Zhixiang Lin)
Published: 9 June 2025

Objective: This study aimed to elucidate the protective effects of quercetin (Que) on the gastric mucosa in a rat model of chronic atrophic gastritis (CAG), with emphasis on the regulation of the transforming growth factor-beta1 (TGF-β1)/Smads signaling pathway.

Methods: Wistar rats were randomly divided into five groups: control, model, low-dose Que (Que-L), and high-dose Que (Que-H). After 30 days of oral gavage, the rats were euthanized for further analysis. Histopathological changes, gastric function (pH, secretion volume, and pepsin activity), and serum levels of gastrin-17 (G-17), interleukin-17 (IL-17), proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and growth hormone (GH) were evaluated. The expression levels of PCNA, VEGF, TGF-β1, Smad4, and Smad7 were determined by quantitative real-time PCR (qRT-PCR) and Western blot. Gastric epithelial cells-1 (GES-1) cells were infected with *Helicobacter pylori* (*H. pylori*) and treated with Que and the TGF-β1/Smad pathway activator SRI-011381. Cell viability, morphological damage, and TGF-β1/Smads pathway expression were subsequently assessed.

Results: Rats in the model group exhibited significantly increased gastric juice pH, decreased total gastric secretion volume and pepsin activity, elevated serum levels of PCNA, VEGF, and EGF, and reduced levels of GH and G-17 ($p < 0.05$). In gastric tissues, PCNA, VEGF, Smad4, and TGF-β1 were upregulated, while Smad7 was downregulated ($p < 0.05$). Que treatment improved gastric function, normalized serum biomarkers, and decreased the expression of PCNA, VEGF, Smad4, and TGF-β1, while upregulating Smad7 ($p < 0.05$). In *H. pylori*-infected GES-1 cells, Que suppressed PCNA and VEGF expression, enhanced cell viability, and improved nuclear morphology ($p < 0.05$). It also downregulated TGF-β1 and Smad4 while restoring Smad7 expression. Pretreatment with SRI-011381 attenuated the protective effects of Que ($p < 0.05$).

Conclusion: Que alleviates gastric inflammation, improves gastric secretory function, and modulates key molecular markers (PCNA, EGF, VEGF, G-17, and GH), thereby protecting against gastric mucosal injury in CAG rats. These effects are likely mediated through inhibition of the TGF-β1/Smads signaling pathway.

Keywords: quercetin; chronic atrophic gastritis; transforming growth factor-beta1/Smads; signaling pathway

Introduction

Chronic atrophic gastritis (CAG), a type of chronic gastritis, is characterized by repeated injury to the epithelial lining of the gastric mucosa, resulting in the loss of intrinsic gastric glands. This condition may be accompanied by intestinal epithelial hyperplasia [1]. CAG is associated with impaired gastric function and persistent inflammation, posing a significant burden on gastrointestinal health [2]. In China, CAG is highly prevalent and represents a major challenge in clinical gastroenterology, with a growing need for effective therapeutic strategies [3]. Given its chronic nature and potential to progress to more severe gastric pathologies, research on the pathogenesis and treatment of CAG remains of critical importance.

Quercetin (Que) is a flavonoid compound with limited water solubility and lipophilic properties, allowing it to readily cross cell membranes and exert its biological effects. Apples and onions are among the richest dietary sources of Que glucoside [4]. Que, as a flavonoid compound widely distributed in the plant kingdom, offers several advantages, including broad availability, low cost, high safety, and minimal side effects. These features make it a promising candidate for diverse medical applications. The proliferation and invasion of gastric cancer stomach gastric cancer-7901 cells are inhibited by Que in a dose-dependent manner [5]. Que alleviates gastric inflammation and enhances gastric secretory function by inhibiting the interferon regulatory factor 8/interferon-gamma axis, thereby

improving CAG induced by *Helicobacter pylori* (*H. pylori*) infection [6]. Moreover, Que mitigates *H. pylori*-induced apoptosis in gastric epithelial cells, reduces inflammatory injury, and suppresses M1 polarization of macrophages by modulating the specificity protein 1/lipocalin 2 axis [7]. These studies collectively demonstrate that Que effectively reduces gastric inflammation, supporting its potential role in managing CAG.

The transforming growth factor-beta (TGF-β) superfamily comprises a wide range of polypeptide cytokines, including activins and bone morphogenetic proteins (BMPs), which play diverse and essential biological roles. These cytokines are pivotal in regulating cellular processes such as proliferation, adhesion, migration, differentiation, and programmed cell death. TGF-β primarily exerts its regulatory effects through the activation of two central signaling cascades: the Smad-dependent pathway, which directly influences gene transcription, and the Ras/mitogen-activated protein kinase (MAPK) pathway, which integrates multiple extracellular signals. A recent study indicates that dysregulation of the TGF-β1/Smads signaling pathway contributes to gastric mucosal damage and chronic inflammation [8]. Prior research suggests that Que, due to its antioxidant and anti-inflammatory properties, may exert protective effects by modulating the TGF-β1/Smads signaling axis. For instance, Que alleviates chronic kidney disease in mesangial cell models by regulating inflammation, oxidative stress, and the TGF-β1/Smads pathway [9]. Additionally, Que has been shown to improve atrial fibrillation by inhibiting the TGF-β1/Smads pathway via upregulation of microRNA-135b expression [10]. These findings indicate that Que can regulate the TGF-β1/Smads pathway across various disease contexts. However, the specific role of Que in regulating TGF-β1/Smads signaling in CAG remains unclear. While Que has demonstrated protective effects against CAG, its capacity to modulate the TGF-β1/Smads pathway and reduce gastric mucosal injury remains insufficiently understood. Therefore, this study aimed to investigate the potential protective effects of Que in CAG and to elucidate the underlying molecular mechanisms through which it may exert therapeutic benefits on the gastric mucosa.

Materials and Methods

Experimental Animals

Thirty-six male, 6-week-old specific pathogen free (SPF)-grade Wistar rats, weighing between 180–220 g, were purchased from Sipeifu (Beijing) Biotechnology Co., Ltd. (License No. SCXK (Beijing): 2019-0010). The rats were housed in the Experimental Animal Center of Ruian People's Hospital under standard laboratory conditions, with ad libitum access to food and water. After a one-week acclimatization period, the animals were enrolled in the study. At the end of the experiment, all animals were humanely euthanized via intraperitoneal injection of 1% (w/v) sodium pentobarbital at a dose of 180 mg/kg, ensuring deep anesthesia prior to death and minimizing animal suffering. All procedures involving animals were approved by the Institutional Animal Ethics Committee of Ruian People's Hospital and conducted following the guidelines for the care and use of laboratory animals (Approval No. SYSQ-2024-013).

Grouping, Model Preparation, and Drug Administration

A composite method was employed to establish the CAG rat model [11]. From the first day of modeling, rats were allowed free access to sodium deoxycholate (≥97%, D6750, Sigma-Aldrich, St. Louis, MO, USA), prepared as a 20 mmol/L aqueous solution (pH 7.0–7.8), along with 0.1% ammonia solution (30501, Honeywell International, Shanghai, China) provided every other day. Additionally, the starvation-refeeding method was implemented (2 days of ad libitum feeding, followed by one day of fasting). This protocol was maintained for 90 days.

Following modeling, 18 model rats were randomly allocated into three groups (n = 6 per group): model group, low-dose Que group (Que-L), and high-dose Que group (Que-H). The control group consisted of six healthy rats. All rats were treated with oral gavage once daily for 30 consecutive days. The control and model groups received physiological saline (10 mL/kg), while the Que-L and Que-H groups were administered quercetin (purity >98%, SQ8030, Solarbio, Beijing, China) at doses of 10 mg/kg and 25 mg/kg, respectively. Dosages were selected based on a previously established study [6].

Tissue Collection

Rats were anesthetized via intraperitoneal injection of 1.0% (w/v) sodium pentobarbital at 50 mg/kg. Blood samples (3 mL) were collected from the retro-orbital sinus of both eyes. Samples were left to stand at room temperature for 2 hours and then centrifuged at 3500 rpm for 15 minutes. The serum was separated and stored at –20 °C for further analyses. Stomachs were harvested, and gastric contents were collected. Each stomach was longitudinally opened along the greater curvature. A tissue block (0.3 × 0.5 cm) was excised from the lesser curvature near the pylorus and fixed in 10% neutral buffered formalin for histological analysis. Additionally, a 0.2 g sample of gastric tissue was homogenized in 1 mL saline, centrifuged at 6000 rpm for 5 minutes at 4 °C, and the supernatant was stored at –20 °C for biochemical analysis.

Hematoxylin and Eosin (HE) Staining

Fixed gastric tissues were processed using a hematoxylin and eosin (HE) staining kit (C0105S, Beyotime, Shanghai, China). Pathological alterations in the gastric mucosa were examined under a light microscope (CX23, Olympus, Tokyo, Japan).

Gastric Secretory Function Assessment

Gastric juice samples were first centrifuged at 4000 rpm for 15 minutes, followed by a second centrifugation at 13,000 rpm for 10 minutes to collect the supernatant. The total volume of gastric juice was recorded, and its pH was measured. Pepsin activity was determined using a pepsin assay kit (ml552463, Enzyme-linked Biotechnology, Shanghai, China), following the manufacturer's instructions.

Enzyme-Linked Immunosorbent Assay (ELISA)

Rat serum samples were collected and analyzed for specific biomarkers. Serum levels of gastrin-17 (G-17, ml059375), interleukin-17 (IL-17, ml028507), proliferating cell nuclear antigen (PCNA, ml003181), epidermal growth factor (EGF, ml003029), vascular endothelial growth factor (VEGF, ml064294) (all from Enzyme-linked Biotechnology, Shanghai, China), and growth hormone (GH, JL15806, JONLNBIO, Shanghai, China) were measured using ELISA kits according to the manufacturer's instructions.

Cell Culture and Stimulation

The human gastric epithelial cells-1 (GES-1) was purchased from ATCC (Manassas, VA, USA), and cultured in Dulbecco's Modified Eagle Medium (DMEM; 12491015, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; A5256701, Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin-streptomycin (15140122, Thermo Fisher Scientific, Waltham, MA, USA). The identity of the GES-1 cell line was confirmed by short tandem repeat (STR) profiling, and mycoplasma contamination was excluded using a PCR-based assay. All experiments were conducted using mycoplasma-free cells. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2. When GES-1 cells reached approximately 80% confluence, they were exposed to *H. pylori* (ATCC43504, B84182, MINGZHOUBIO, Ningbo, China) at various multiplicities of infection (MOI: 10:1, 20:1, 50:1, and 100:1) for 6 hours, or treated with different concentrations of Que (10, 20, 40, 80, 160 µM) for 24 hours. After treatment, cells were harvested for viability assessment and further analysis.

H. pylori was cultured on Brucella agar plates supplemented with 5% sheep blood and incubated at 37 °C under microaerophilic conditions for 48 hours. The bacterial suspension was prepared by resuspending colonies in sterile saline to a final concentration of 1×10^5 colony-forming units per milliliter (CFU/mL).

Subsequently, GES-1 cells were assigned into five groups: control group, model group, Que group, SRI-011381 (a TGF-β1/Smad pathway activator, HY-100347, MedChemExpress, Monmouth Junction, NJ, USA), and a combined SRI-011381 + Que group. In the Que and SRI-011381 + Que groups, cells were exposed to *H. pylori*, followed by treatment with Que (40 µM) and SRI-011381 (10 µmol/L) for 24 hours [12].

CCK-8 Assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; PG658, DOJINDO, Kumamoto, Japan). Optical density (OD) was measured at 450 nm using a Synergy H1 Hybrid Reader (Biotek, Winooski, VT, USA).

Quantitative Analysis of Cell Number and Viability

The viability and cytotoxicity of GES-1 cells were evaluated using the Calcein/PI Cell Viability and Cytotoxicity Assay Kit (C2015S, Beyotime, Shanghai, China) and Hoechst 33342 staining. Live cells were labeled with calcein acetoxymethyl ester (Calcein-AM, green fluorescence), dead cells with propidium iodide (PI; red fluorescence), and nuclei with Hoechst 33342 (blue fluorescence). Fluorescent images were captured using a fluorescence microscope (Olympus BX53, Olympus Corporation, Tokyo, Japan). Cell number and viability were quantified using ImageJ software (version 1.53k, National Institutes of Health, Bethesda, MD, USA) by counting Hoechst 33342-positive nuclei and calculating the ratio of Calcein-AM (live) and PI (dead) positive areas per field.

RNA Extraction and qRT-PCR

Total RNA was extracted from gastric tissue and GES-1 cells using TRIzol reagent, followed by DNase I treatment. One microgram (1 µg) of total RNA was reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (4368814, Thermo Fisher Scientific, Waltham, MA, USA). Quantitative real-time PCR (qRT-PCR) was performed using a qRT-PCR kit (11732088, Thermo Fisher Scientific, Waltham, MA, USA). The relative mRNA expression levels of target genes were normalized to GAPDH expression using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table 1.

Western Blot Analysis

Gastric tissue samples from rats and GES-1 cells were lysed using radioimmunoprecipitation assay lysis buffer for total protein extraction. Protein concentrations were measured using the bicinchoninic acid Protein Assay Kit (P0012, Beyotime, Shanghai, China). Equal amounts of protein (20 µg) were separated via polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes, and blocked with 5% non-fat milk for 1 hour at room temperature. The membranes were then incubated overnight at 4 °C with primary antibodies specific for VEGF (9698, Cell Signaling Technology, Danvers, MA, USA, 1:1000), PCNA (2586, Cell Signaling Technology, Danvers, MA, USA, 1:1000), TGF-β1 (3709, Cell Signaling Technology, Danvers, MA, USA, 1:1000), Smad4 (46535, Cell Signaling Technology, Danvers, MA, USA,

Table 1. Primer sequences used for qRT-PCR analysis.

Gene	Accession No	Forward primer (5′–3′)	Reverse primer (5′–3′)
VEGF (Rat)	NM_031836	CCCTGGCTTTACTGCTGTACC	GTCCATGAACTTCACCACTTCA
PCNA (Rat)	NM_022381	GGGTGAAGTTTTCTGCGAGTG	ATCTCTATGGACACAGCTTCCT
TGF-β1 (Rat)	NM_021578	TGGACCGCAACAACGCAAT	CACTGCTTCCCGAATGTCTG
Smad4 (Rat)	NM_019275	GCAGCCATAGTGAAGGACTGT	GTGGTAGTGCTGTTATGGTGG
Smad7 (Rat)	NM_030858	CCAGACGCTGTACCTTCCT	CCAGAAGAAGTTGGGAATCTGA
Gapdh (Rat)	NM_017008	GGATACTGAGAGCAAGAGAGA	TTATGGGGTCTGGGATGGAA
VEGF (Human)	NM_001025366	TTGCCTTGCTGCTCTACCTCCA	GATGGCAGTAGCTGCGCTGATA
PCNA (Human)	NM_002592	CAAGTAATGTCGATAAAGAGGAGG	GTGTCACCGTTGAAGAGAGTGG
TGF-β1 (Human)	NM_000660	TACCTGAACCCGTGTTGCTCTC	GTTGCTGAGGTATCGCCAGGAA
Smad4 (Human)	NM_005359	CTACCAGCACTGCCAACTTTCC	CCTGATGCTATCTGCAACAGTCC
Smad7 (Human)	NM_005904	TGTCCAGATGCTGTGCCTTCCT	CTCGTCTTCTCCTCCCAGTATG
Gapdh (Human)	NM_002046	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

VEGF, vascular endothelial growth factor; *PCNA*, proliferating cell nuclear antigen; *TGF-β1*, transforming growth factor-beta1; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase.

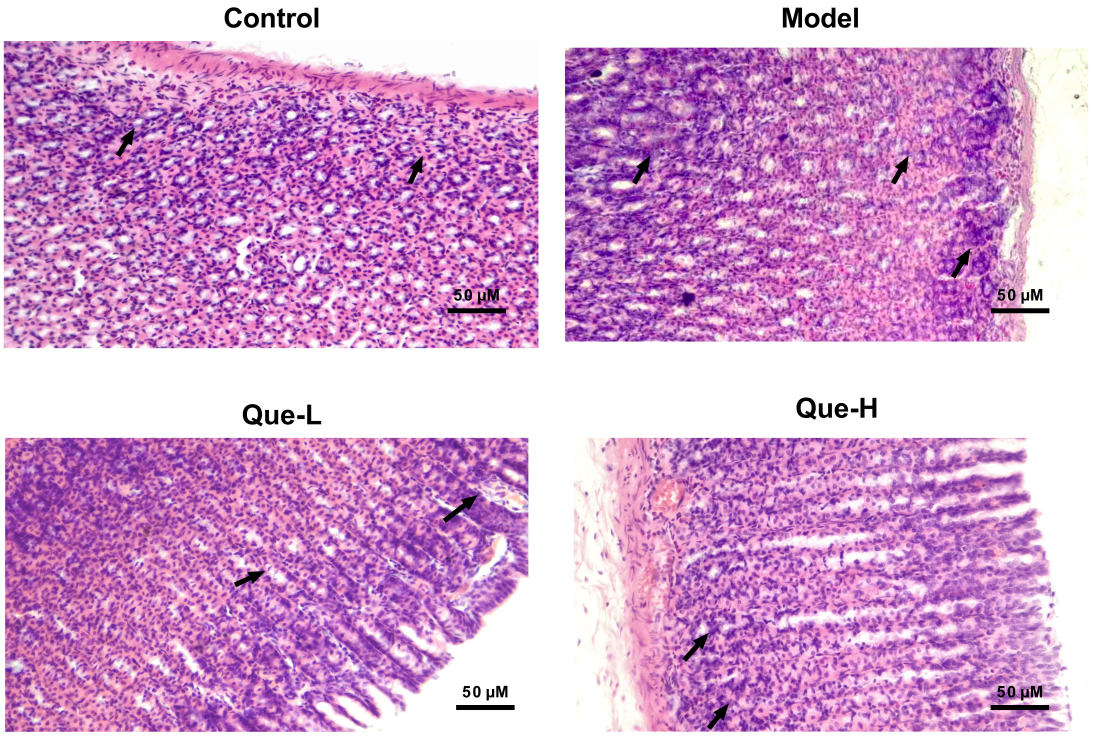

Fig. 1. Histopathological analysis of gastric tissues (Hematoxylin and eosin (HE), ×200). Representative HE-stained sections of gastric mucosa showing mucosal architecture, epithelial integrity, and inflammatory cell infiltration following different interventions. The images illustrate the extent of mucosal injury and the histopathological effects of Que treatment. Que, quercetin; Que-L, low-dose Que; Que-H, high-dose Que. The arrows indicate pathological changes in the gastric mucosal epithelial cells and glandular structures.

1:1000), Smad7 (25840-1-AP, Proteintech, Wuhan, China, 1:1000), and β-actin (4967, Cell Signaling Technology, Danvers, MA, USA, 1:3000). Membranes were subsequently incubated with secondary antibodies (7074 or 7076, Cell Signaling Technology, Danvers, MA, USA, 1:5000) for 1 hour at room temperature. After washing, bands were detected using enhanced chemiluminescence substrate (34580, Thermo Fisher Scientific, Waltham, MA, USA). Protein signals were visualized using a Bio-Rad Gel Imaging System (ChemiDoc MP, Bio-Rad Laboratories, Hercules, CA, USA) and quantified with ImageJ software (version 1.53k, National Institutes of Health, Bethesda, MD, USA) to determine relative expression levels. All experiments were performed in triplicate.

Statistical Analysis

Statistical analyses were performed using SPSS version 21.0 (version 21.0, IBM Corp., Armonk, NY, USA).

Data with a normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$). A one-way analysis of variance (ANOVA) was used to compare multiple groups, followed by Dunnett's post hoc test to compare treatment groups and the control group. Paired *t*-tests were applied for comparisons between two groups. A p-value < 0.05 was considered statistically significant.

Results

Histopathological Observation of Rat Gastric Mucosa

As shown in Fig. 1, rats in the control group exhibited intact gastric mucosal epithelium, with regularly arranged columnar epithelial cells and uniformly distributed glands within the lamina propria. The boundary between the glandular epithelium and ducts was clearly defined, and the muscularis layer appeared uniform. In the model group, gastric epithelium demonstrated atrophy, thinning, necrosis, and desquamation, with disorganized glandular structure and infiltration of lymphocytes and plasma cells. In some areas, lymphoid follicle formation and intestinal metaplasia were observed. In the treatment groups, inflammatory infiltration was reduced, and the gastric mucosa showed mild to moderate improvement, most notably in the Que-H group, which showed significant restoration of the mucosal structure.

Que Improves Gastric Secretory Function in CAG Rats

Compared to the control group, the model groups exhibited a significant elevation in gastric juice pH, along with reduced total gastric juice volume and pepsin activity ($p < 0.05$) (Fig. 2). Que treatment groups significantly reduced gastric juice pH and increased both gastric juice volume and pepsin activity relative to the model group ($p < 0.05$) (Fig. 2).

Que Regulates the Levels of Specific Serum Biomarkers in CAG Rats

Compared to the control group, rats in the model group displayed significantly elevated serum levels of IL-17, EGF, PCNA, and VEGF, along with decreased levels of G-17 and GH ($p < 0.05$) (Fig. 3). In contrast, rats in the Que treatment group demonstrated a significant reduction in IL-17, EGF, PCNA, and VEGF, accompanied by elevated levels of G-17 and GH ($p < 0.05$) (Fig. 3).

Que Reduces the Expression Levels of PCNA and VEGF in CAG Rats

The model group exhibited significantly increased mRNA and protein expression of PCNA and VEGF compared to the control group ($p < 0.05$) (Fig. 4). Conversely, rats in the Que treatment group showed a significant reduction in both mRNA and protein levels of PCNA and VEGF compared to the model group ($p < 0.05$) (Fig. 4).

Que Regulates the Expression Levels of Smad4, Smad7, and TGF-β1 in CAG Rats

Relative to the control group, the model group showed significant upregulation of TGF-β1 and Smad4 expression at both mRNA and protein levels, while Smad7 expression was markedly downregulated at both levels ($p < 0.05$). Additionally, in the Que-H treatment group, TGF-β1 and Smad4 levels were significantly decreased at both the protein and mRNA levels ($p < 0.05$). Concurrently, Smad7 expression was significantly upregulated at both mRNA and protein levels ($p < 0.05$) (Fig. 5).

Que Enhances Cell Viability in GES-1 Cells

Treatment with 160 µM Que markedly decreased cell viability compared to the control group ($p < 0.05$). However, Que at concentrations of 10 µM and 20 µM significantly enhanced cell viability, possibly due to moderate activation of protective cellular pathways or stress responses that promote cell survival without inducing toxicity. In contrast, Que at a concentration of 40 µM maintained cell viability above 90% (Fig. 6A), suggesting a beneficial effect on GES-1 cells. Based on these findings, 40 µM was chosen as the optimal concentration for subsequent experiments.

GES-1 cells were cultured with *H. pylori* for 24 hours at MOI of 10:1, 20:1, 50:1, and 100:1. At an MOI of 50:1, cell viability was approximately 50% (Fig. 6B). Therefore, this MOI was selected for subsequent experiments. GES-1 cells were co-cultured with *H. pylori* for 24 hours in the presence of Que at 40 µM and 20 µM. The results indicated that, compared to the *H. pylori* infection group, cell viability was significantly restored in Que-treated group ($p < 0.05$) (Fig. 6C), with 40 µM concentration showing the most pronounced effect. This concentration was used in subsequent experiments.

TGF-β Involvement in the Protective Effects of Que on GES-1 Cells

Compared to the control group, *H. pylori* treatment led to increased mRNA and protein levels of PCNA and VEGF in GES-1 cells. Que intervention reduced both mRNA and protein expression of PCNA and VEGF (Fig. 7A,B). However, the addition of SRI-011381 attenuated the inhibitory effect of Que on PCNA and VEGF expression compared to the Que-treated group ($p < 0.05$).

To further assess the protective effects of Que on GES-1 cells, high-content imaging was performed using Hoechst 33342, Calcein-AM, and PI dyes to stain cell nuclei (blue), live cells (green), and dead cells (red), respectively (Fig. 7C). The control group exhibited homogeneous blue and green fluorescence. *H. pylori* infection reduced cell number and viability and increased red fluorescence. Que treatment enhanced green fluorescence and reduced red fluorescence, indicating improved viability. In contrast, the Que + SRI-011381 group exhibited reduced green and increased red fluorescence relative to the Que group, sug-

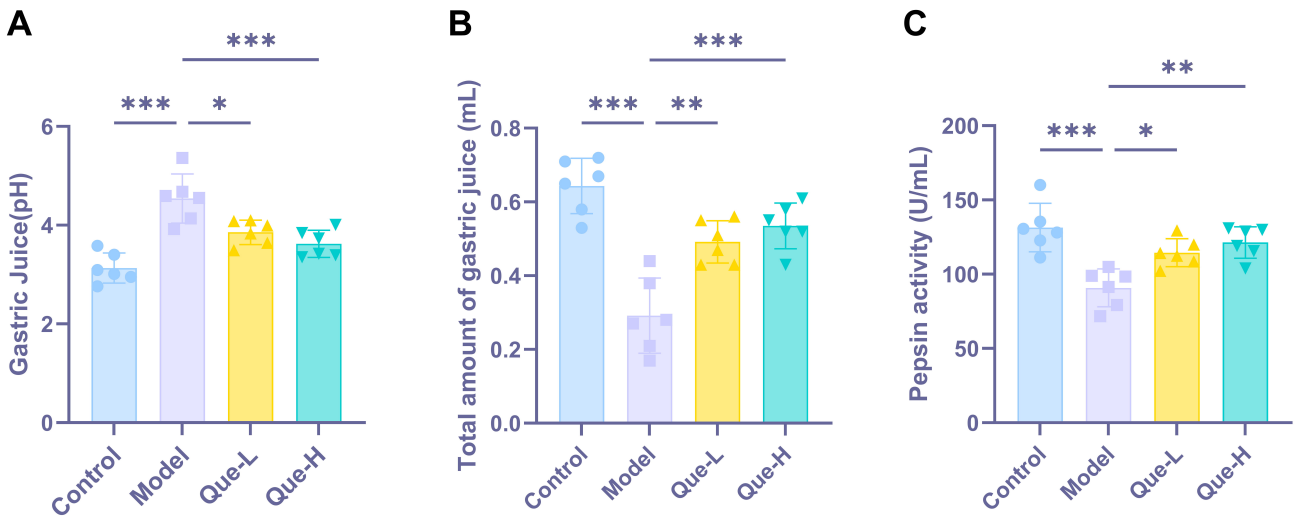

Fig. 2. Effects of Que on gastric secretory function in CAG rats. (A) Gastric juice pH, (B) total gastric juice volume, and (C) pepsin activity were measured to evaluate changes in gastric secretory function following Que treatment. Data are presented as mean ± SD, n = 6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Que, quercetin; CAG, chronic atrophic gastritis.

gesting that SRI-011381 impaired the protective effects of Que on *H. pylori*-induced damage.

Regulation of the TGF-β/Smads Signaling Pathway by Que and SRI-011381 in GES-1 Cells

Compared to the control group, *H. pylori* infection significantly upregulated TGF-β1 and Smad4 expression and downregulated Smad7 in GES-1 cells, indicating activation of the TGF-β/Smads signaling pathway. Que treatment inhibited this activation by reducing TGF-β1 and Smad4 expression and restoring Smad7 levels. However, co-treatment with SRI-011381 partially reversed these regulatory effects, increasing TGF-β1 and Smad4 and decreasing Smad7 compared to the Que group (Fig. 8, $p < 0.05$). These findings suggest that SRI-011381 counteracts the inhibitory effects of Que on *H. pylori*-induced activation of the TGF-β/Smads signaling pathway in GES-1 cells.

Discussion

Chronic atrophic gastritis (CAG), a form of chronic gastritis, is increasingly recognized as a significant public health and social concern. The underlying mechanisms driving CAG pathogenesis remain unclear, highlighting the urgent need for safer and more effective therapeutic options. Que, a bioactive flavonoid, has demonstrated wound-healing, anti-inflammatory, and antioxidant properties [13]. Notably, network pharmacology analyses have identified Que as a critical active component in numerous traditional Chinese medicine formulations used to treat CAG [14,15]. Que can modulate multiple biological processes and signaling pathways, exerting protective and therapeutic effects on the chronic inflammatory-to-cancer progression associated with atrophic gastritis. Based on these findings, Que holds promise as a novel therapeutic candidate for managing CAG effectively. However, further *in vivo* validation in large animal models and clinical trials is essential to fully evaluate the translational potential of Que. These future investigations will help to establish a more comprehensive understanding of Que's therapeutic applications in CAG and related gastric disorders.

Characteristic pathological features of CAG include atrophic changes in the gastric mucosal glands, such as glandular loss, mucosal thinning, and thickening of the muscularis mucosae. These alterations compromise the structural integrity and function of the gastric mucosa, rendering it vulnerable to injury. The present investigation demonstrated that Que intervention significantly alleviated inflammation and glandular atrophy in the gastric mucosa of CAG rats. Normally, the gastric mucosa is protected by a gel-like barrier approximately 0.5 mm thick, primarily composed of mucus and bicarbonate. This barrier serves as a critical defense mechanism, protecting against mechanical injury from food particles, microbial invasion, and chemical erosion [16]. However, when the mucosal layer undergoes atrophic changes and a reduction in secretory cells occurs, bicarbonate and pepsinogen levels are directly affected. The current findings reveal that Que treatment significantly ameliorates gastric mucosal pathology in a CAG rat model, as evidenced by restored histological architecture and enhanced mucosal integrity. These results highlight the therapeutic potential of Que in mitigating gastric mucosal atrophy and associated inflammatory damage.

IL-17 is a key cytokine that promotes the onset and progression of inflammation. It induces the production of chemokines in the body, resulting in a rapid increase in monocyte and neutrophil populations, thereby intensifying the local inflammatory response [17]. Dysregulation of

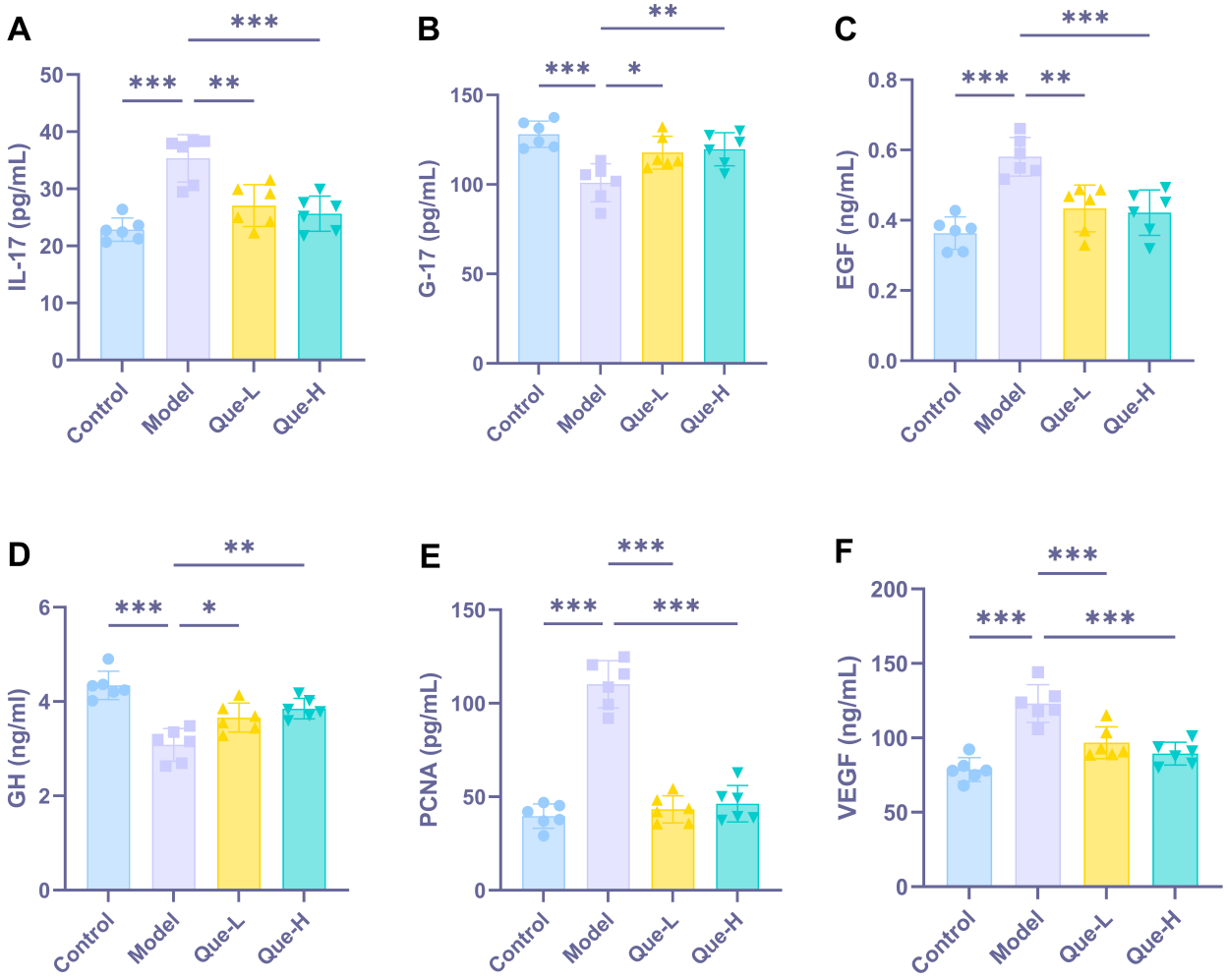

Fig. 3. Effect of Que on serum biomarkers in CAG rats. Serum levels of (A) IL-17, (B) G-17, (C) EGF, (D) GH, (E) PCNA, and (F) VEGF were measured by ELISA. Data are presented as mean ± SD, n = 6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Que, quercetin; CAG, chronic atrophic gastritis; IL-17, interleukin-17; G-17, gastrin-17; EGF, epidermal growth factor; GH, growth hormone; PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; ELISA, Enzyme-Linked Immunosorbent Assay.

IL-17 is associated with the emergence of various chronic inflammatory disorders, including autoimmune disorders, respiratory conditions, and gastrointestinal inflammation [18]. In CAG, IL-17 is closely associated with disease initiation, progression, and clinical outcomes. In the present study, Que intervention resulted in a marked reduction in IL-17 levels in CAG model rats. This decrease suggests that Que effectively mitigates the IL-17-mediated inflammatory cascade. By modulating IL-17-driven inflammation, Que demonstrates the potential to alleviate CAG-associated inflammatory responses. These findings underscore the therapeutic potential of Que in improving pathological manifestations of CAG and support its role as an anti-inflammatory agent targeting key cytokines involved in disease progression.

Proliferating cell nuclear antigen (PCNA) is crucial in several biological processes, including cell cycle regulation, DNA repair, DNA methylation, and chromatin remodeling [19]. It is widely recognized as a marker of cell proliferation and is closely associated with DNA replication. Vascular endothelial growth factor (VEGF) is a critical mediator of microvascular angiogenesis. A study has shown that VEGF levels are elevated in the serum of CAG patients compared to healthy individuals [20]. This elevation is strongly linked to the progression of precancerous lesions, underscoring its importance in gastric carcinogenesis. Epidermal growth factor (EGF) is a potent mitogen that stimulates the growth of various tissue cells. It promotes the proliferation of gastric mucosal epithelial cells and provides protective effects on the gastric mucosa through mechanisms such as suppressing gastric acid secretion, enhancing mucus secretion, and increasing gastric mucosal blood flow [21]. However, EGF overexpression may result in abnormal cell proliferation and enhanced metabolism, thereby contributing to carcinogenesis [22].

Growth hormone (GH) is a peptide hormone that regulates cell growth. It directly influences the metabolism, growth, and differentiation of gastric mucosal epithelial

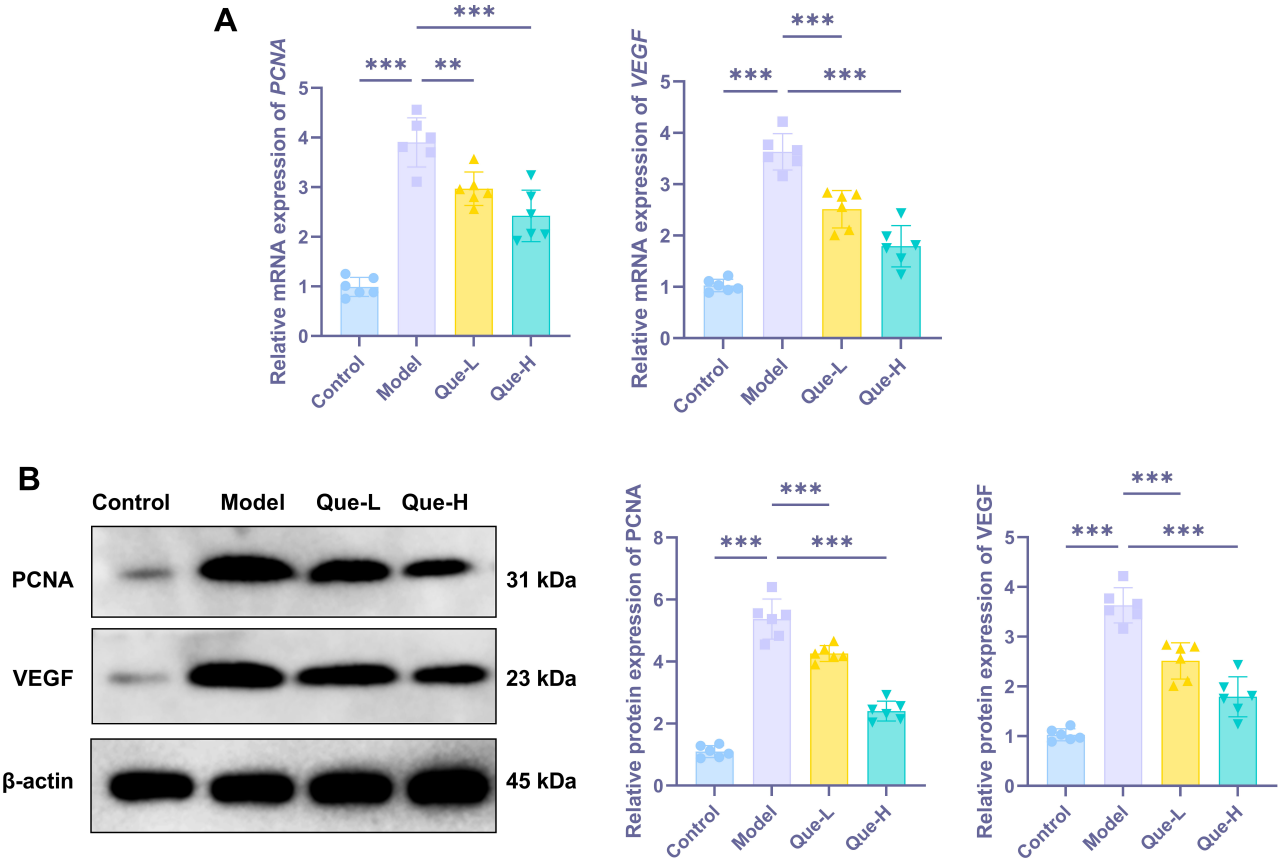

Fig. 4. Effect of Que on PCNA and VEGF expression levels in CGA rats. (A) mRNA expression levels were analyzed by qRT-PCR, and (B) protein expression levels were analyzed by Western blot for PCNA and VEGF. Data are presented as mean ± SD, n = 6. $^{**}p < 0.01$, $^{***}p < 0.001$. Que, quercetin; CAG, chronic atrophic gastritis; PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; qRT-PCR, quantitative real-time PCR.

cells [23]. Gastrin G-17 is a gastrointestinal hormone primarily secreted by G cells in the gastric antrum, and it plays a central role in regulating gastric acid secretion and gastrointestinal motility. Its expression level is closely associated with the status of gastric mucosal integrity [24]. During active CAG, gastric hypo-secretion occurs, and G-17 levels may be elevated as a compensatory response [25]. The findings of this study reveal that CAG rats exhibited significant gastric mucosal injury characterized by an imbalance between tissue injury and repair. Treatment with Que demonstrated modulatory effects on key molecular markers. Notably, Que administration downregulated the expression of PCNA, VEGF, and EGF, while upregulating GH and G-17, thereby facilitating mucosal repair and functional recovery. These results indicate that Que has the potential to regulate cell proliferation and growth metabolism, inhibit microvascular regeneration, and promote coordination and balance between gastric mucosal repair and damage in CAG rats, thereby protecting the gastric mucosa.

During the CAG stage, abnormal signaling through the TGF-β1/Smads pathway is closely associated with the progression toward gastric cancer. The classical activation pathway of Smad involves the binding of TGF-β to its receptor, which triggers Smad protein activation and facilitates the transduction of TGF-β1 signals into the cell nucleus, where they regulate gene transcription [26]. The positive expression rates of TGF-β1 in gastric cancer, gastric atypical hyperplasia, and normal gastric mucosal tissues were 83.9%, 43.3%, and 10.0%, respectively. This suggests that TGF-β1 expression progressively increases as inflammation advances toward malignancy. It is thus hypothesized that enhanced secretion of TGF-β1 may contribute to gastric cancer development [27].

Among the Smad proteins, Smad4 functions as a common mediator and serves as key messenger for conveying TGF-β1 signals to the nucleus. In contrast, Smad7 functions as an inhibitory Smad and exerts a suppressive role in this signaling pathway. Elevated levels of Smad7 can disrupt and downregulate the TGF-β1 signaling cascade, thereby attenuating its activity [28,29]. During the inflammation-to-cancer transition, the expression of Smad4 gradually decreases, particularly during the progression from CAG to gastric cancer, where this reduction is more significant [30]. This notable decrease indicates a potential association between reduced Smad4 levels and gastric cancer progression. Findings from this study reveal that

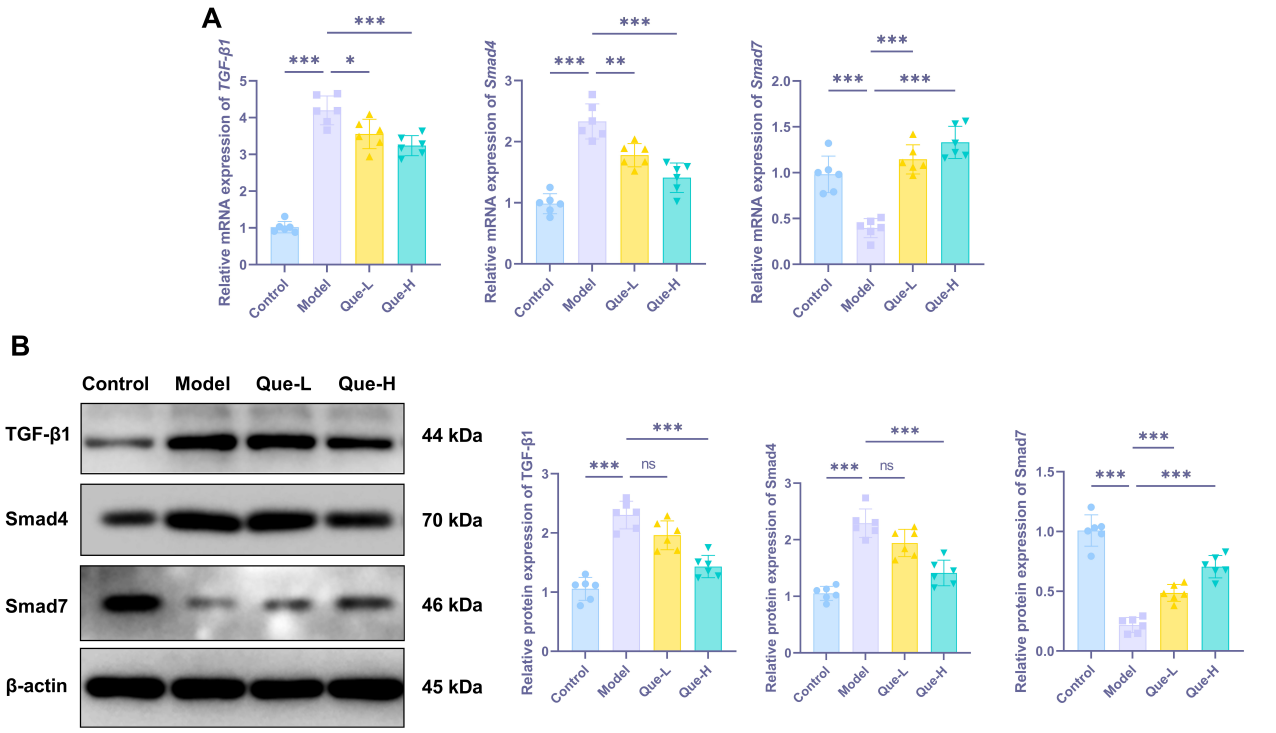

Fig. 5. Effect of Que on TGF-β1, Smad4 and Smad7 expression in CAG rats. (A) mRNA were assessed by qRT-PCR and (B) protein expression levels of TGF-β1, Smad4 and Smad7 were assessed by Western blot. Data are presented as mean ± SD, n = 6. $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Que, quercetin; CAG, chronic atrophic gastritis; qRT-PCR, quantitative real-time PCR; TGF-β1, transforming growth factor-beta1.

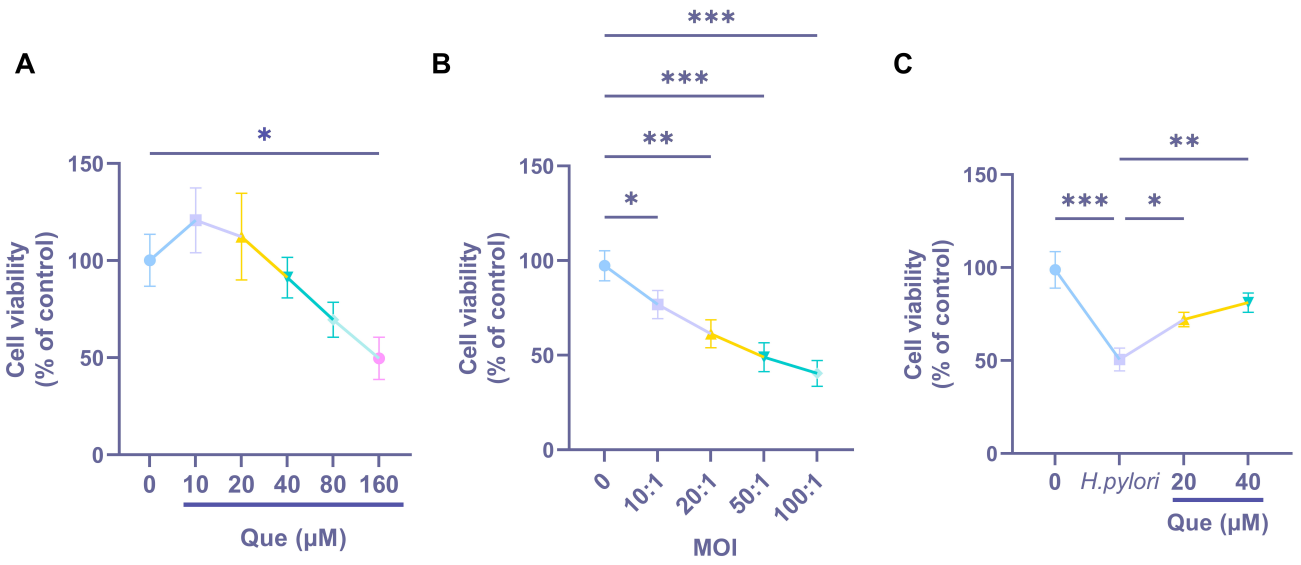

Fig. 6. Effect of Que on GES-1 cell viability. (A) GES-1 cell viability following treatment with various concentrations of Que (10, 20, 40, 80, 160 μM). (B) GES-1 cell viability after exposure to *H. pylori* at different MOIs (10:1, 20:1, 50:1, 100:1). (C) Cell viability following Que treatment in GES-1 cells pre-exposed to *H. pylori*. Data are presented as mean ± SD, n = 3. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Que, quercetin; GES-1, gastric epithelial cells-1; *H. pylori*, *Helicobacter pylori*; MOI, multiplicities of infection.

Que intervention resulted in decreased levels of TGF-β1 and Smad4 in the gastric tissues of CAG rats, while Smad7 expression was upregulated. These findings provide compelling evidence that Que may exert its protective effects by attenuating the activation of the TGF-β1/Smads signaling pathway, potentially mitigating the pathological progression associated with chronic atrophic gastritis.

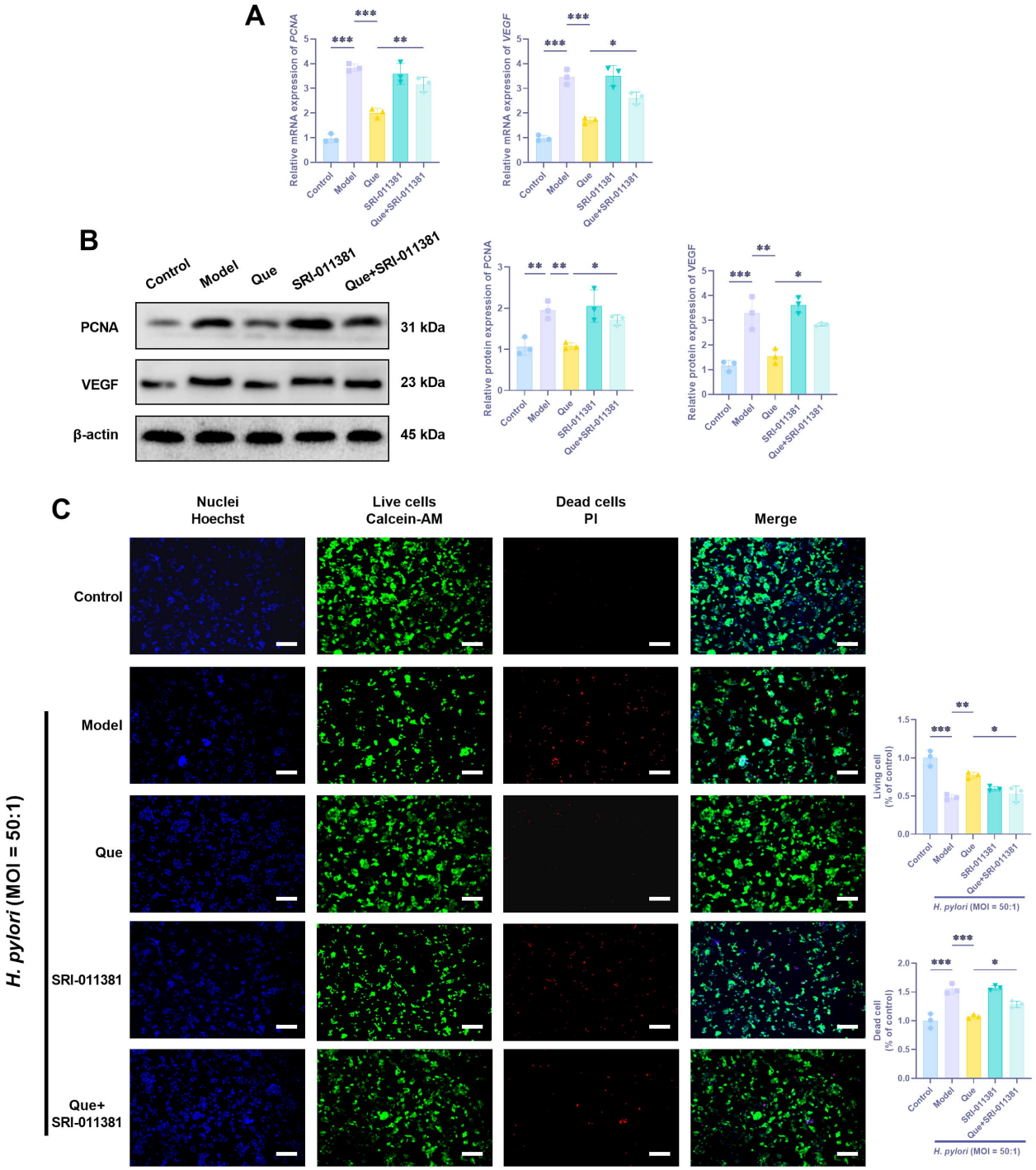

Fig. 7. Involvement of TGF-β in the protective effects of Que on GES-1 cells. (A) mRNA expression levels of *PCNA* and *VEGF* in GES-1 cells, measured by qRT-PCR. (B) Protein expression levels of PCNA and VEGF in GES-1 cells, measured by Western blot. (C) Cell viability and cell count analysis in GES-1 cells using Hoechst 33342, Calcein-AM, and PI staining. Data are presented as mean ± SD, n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The scale for all images is 50 µm. Que, quercetin; GES-1, gastric epithelial cells-1; *H. pylori*, *Helicobacter pylori*; MOI, multiplicities of infection; PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; qRT-PCR, quantitative real-time PCR; Calcein-AM, calcein acetoxymethyl ester; PI, propidium iodide.

Conclusion

Que demonstrates therapeutic effects in CAG rats by alleviating pathological changes, reducing gastric inflammation, and enhancing gastric secretory function. Additionally, it exerts protective effects on the gastric mucosa of CAG rats by regulating the expression levels of PCNA, EGF, VEGF, G-17, and GH. The underlying mechanism

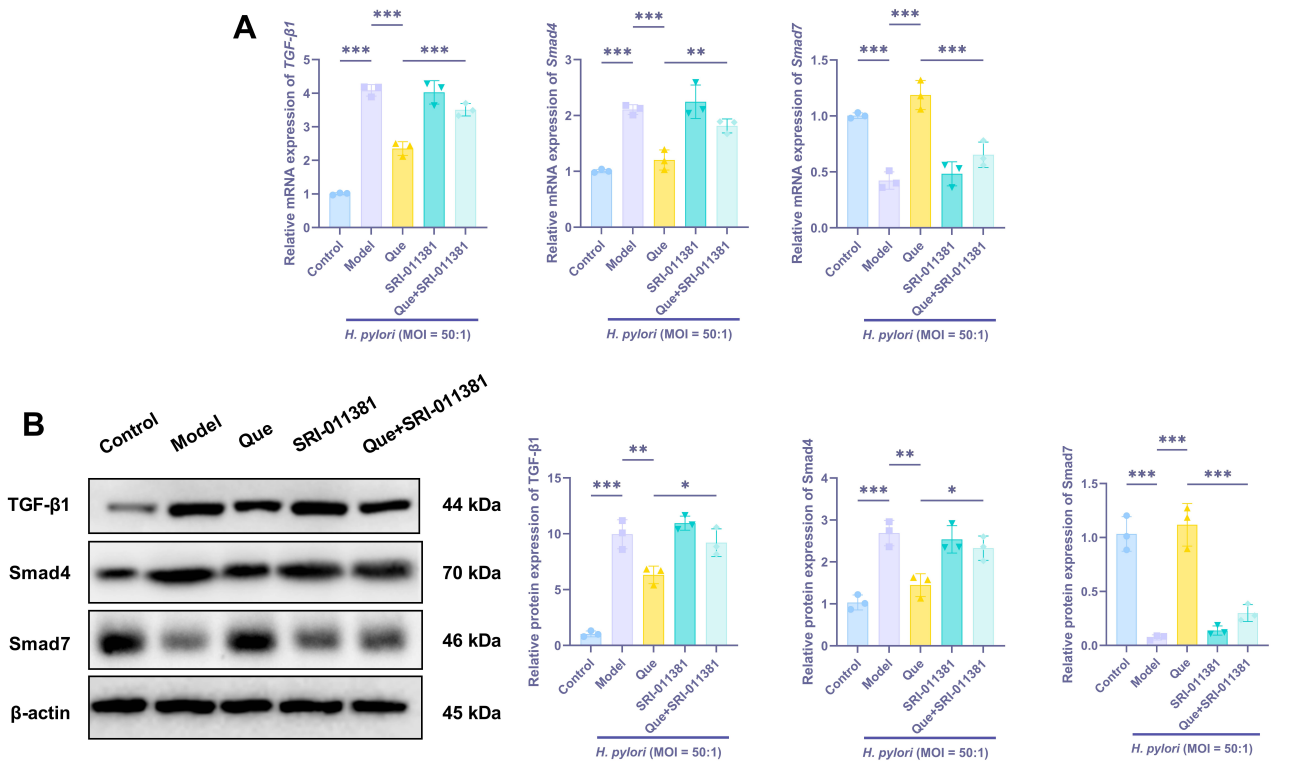

Fig. 8. SRI-011381 reverses the modulatory effects of Que on the TGF-β/Smads signaling pathway in GES-1 cells. (A) mRNA expression levels were analyzed by qRT-PCR and (B) protein expression levels of TGF-β1, Smad4 and Smad7 were analyzed Western blot. Data are presented as mean $\pm$ SD, n = 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Que, quercetin; GES-1, gastric epithelial cells-1; *H. pylori*, *Helicobacter pylori*; MOI, multiplicities of infection; TGF-β1, transforming growth factor-beta1; qRT-PCR, quantitative real-time PCR.

through which Que exerts these benefits may involve inhibition of the TGF-β1/Smads signaling pathway, although additional studies are needed to determine whether this pathway plays a central regulatory role in gastric tissue damage and inflammation. These findings offer essential experimental evidence that supports the potential therapeutic application of Que for treating CAG and underscore the need for further research into its clinical use.

Availability of Data and Materials

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

PC, QS, and FY designed and performed the experiments. RC and ZL contributed to data analysis and interpretation. PC drafted the manuscript. All authors contributed to important editorial revisions, read and approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures involving animals were approved by the Institutional Animal Ethics Committee of Ruian People's Hospital (Approval No. SYSQ-2024-013) and were conducted in accordance with the guidelines for the care and use of laboratory animals.

Acknowledgment

We are grateful to the Experimental Center for its assistance.

Funding

This study was supported by the Science and Technology Program of Wenzhou (No. Y20240222).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Wang P, Li P, Chen Y, Li L, Lu Y, Zhou W, *et al*. Chinese integrated guideline on the management of gastric precancer-

ous conditions and lesions. Chinese Medicine. 2022; 17: 138. https://doi.org/10.1186/s13020-022-00677-6.

[2] Kuang W, Xu J, Xu F, Huang W, Majid M, Shi H, *et al*. Current study of pathogenetic mechanisms and therapeutics of chronic atrophic gastritis: a comprehensive review. Frontiers in Cell and Developmental Biology. 2024; 12: 1513426. https://doi.org/10.3389/fcell.2024.1513426.

[3] Yin Y, Liang H, Wei N, Zheng Z. Prevalence of chronic atrophic gastritis worldwide from 2010 to 2020: an updated systematic review and meta-analysis. Annals of Palliative Medicine. 2022; 11: 3697–3703. https://doi.org/10.21037/apm-21-1464.

[4] Aghababaei F, Hadidi M. Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals. 2023; 16: 1020. https://doi.org/10.3390/ph16071020.

[5] Huang J, Chen J, Li J. Quercetin promotes ATG5-mediating autophagy-dependent ferroptosis in gastric cancer. Journal of Molecular Histology. 2024; 55: 211–225. https://doi.org/10.1007/s10735-024-10186-5.

[6] Yu C, Lu X, Li L, Wang A, Li X. Mechanisms of quercetin-mediated IRF8/IFN-γ in improving chronic atrophic gastritis induced by Hp infection. Chinese Journal of Nosocomiology. 2022; 32: 3073–3077.

[7] Wang Z, Zhou X, Hu X, Zheng C. Quercetin ameliorates Helicobacter pylori-induced gastric epithelial cell injury by regulating specificity protein 1/lipocalin 2 axis in gastritis. Journal of Applied Toxicology. 2024; 44: 641–650. https://doi.org/10.1002/jat.4566.

[8] Chiu HF, Venkatakrishnan K, Golovinskaia O, Wang CK. Gastroprotective Effects of Polyphenols against Various Gastro-Intestinal Disorders: A Mini-Review with Special Focus on Clinical Evidence. Molecules. 2021; 26: 2090. https://doi.org/10.3390/molecules26072090.

[9] Widowati W, Prahastuti S, Tjokropranoto R, Onggowidjaja P, Kusuma HSW, Afifah E, *et al*. Quercetin prevents chronic kidney disease on mesangial cells model by regulating inflammation, oxidative stress, and TGF-β1/SMADs pathway. PeerJ. 2022; 10: e13257. https://doi.org/10.7717/peerj.13257.

[10] Wang H, Jiang W, Hu Y, Wan Z, Bai H, Yang Q, *et al*. Quercetin improves atrial fibrillation through inhibiting TGF-β/Smads pathway via promoting MiR-135b expression. Phytomedicine. 2021; 93: 153774. https://doi.org/10.1016/j.phymed.2021.153774.

[11] Wang H, Fang F, Wang J, Liu H, Su X, Li M, *et al*. Studies on Protective Mechanism of Shaoyao Bawei Decoction on Gastric Mucosa of Rats with Chronic Atrophic Gastritis. Journal of Basic Chinese Medicine. 2021; 27: 570–572.

[12] Wang YN, Zhao SL, Su YY, Feng JX, Wang S, Liao XM, *et al*. Astragaloside IV attenuates high glucose-induced EMT by inhibiting the TGF-β/Smad pathway in renal proximal tubular epithelial cells. Bioscience Reports. 2020; 40: BSR20190987. https://doi.org/10.1042/BSR20190987.

[13] Beken B, Serttas R, Yazicioglu M, Turkekul K, Erdogan S. Quercetin Improves Inflammation, Oxidative Stress, and Impaired Wound Healing in Atopic Dermatitis Model of Human Keratinocytes. Pediatric Allergy, Immunology, and Pulmonology. 2020; 33: 69–79. https://doi.org/10.1089/ped.2019.1137.

[14] Zhou W, Zhang H, Wang X, Kang J, Guo W, Zhou L, *et al*. Network pharmacology to unveil the mechanism of Moluodan in the treatment of chronic atrophic gastritis. Phytomedicine. 2022; 95: 153837. https://doi.org/10.1016/j.phymed.2021.153837.

[15] Weng J, Wu XF, Shao P, Liu XP, Wang CX. Medicine for chronic atrophic gastritis: a systematic review, meta- and network pharmacology analysis. Annals of Medicine. 2023; 55: 2299352. https://doi.org/10.1080/07853890.2023.2299352.

[16] Nieminen AA, Kontto J, Puolakkainen P, Virtamo J, Kokkola A. Comparison of operative link for gastritis assessment, operative link on gastric intestinal metaplasia assessment, and TAIM stagings among men with atrophic gastritis. World Journal of Gastroenterology. 2020; 26: 3447–3457. https://doi.org/10.3748/wjg.v26.i24.3447.

[17] Huangfu L, Li R, Huang Y, Wang S. The IL-17 family in diseases: from bench to bedside. Signal Transduction and Targeted Therapy. 2023; 8: 402. https://doi.org/10.1038/s41392-023-01620-3.

[18] Mannion JM, McLoughlin RM, Lalor SJ. The Airway Microbiome-IL-17 Axis: a Critical Regulator of Chronic Inflammatory Disease. Clinical Reviews in Allergy & Immunology. 2023; 64: 161–178. https://doi.org/10.1007/s12016-022-08928-y.

[19] González-Magaña A, Blanco FJ. Human PCNA Structure, Function and Interactions. Biomolecules. 2020; 10: 570. https://doi.org/10.3390/biom10040570.

[20] Jia J, Zhao H, Li F, Zheng Q, Wang G, Li D, *et al*. Research on drug treatment and the novel signaling pathway of chronic atrophic gastritis. Biomedicine & Pharmacotherapy. 2024; 176: 116912. https://doi.org/10.1016/j.biopha.2024.116912.

[21] Liu F, Nong X, Qu W, Li X. Weikangling capsules combined with omeprazole ameliorates ethanol-induced chronic gastritis by regulating gut microbiota and EGF-EGFR-ERK pathway. Life Sciences. 2023; 315: 121368. https://doi.org/10.1016/j.lfs.2023.121368.

[22] Li K, Ma X, Li Z, Liu Y, Shen G, Luo Z, *et al*. A Natural Peptide from A Traditional Chinese Medicine Has the Potential to Treat Chronic Atrophic Gastritis by Activating Gastric Stem Cells. Advanced Science. 2024; 11: e2304326. https://doi.org/10.1002/advs.202304326.

[23] Kim GH, Choi MG, Kim JI, Lee ST, Chun HJ, Lee KL, *et al*. Efficacy and Safety of Fexuprazan in Patients with Acute or Chronic Gastritis. Gut and Liver. 2023; 17: 884–893. https://doi.org/10.5009/gnl220457.

[24] Tong Y, Liu L, Wang R, Yang T, Wen J, Wei S, *et al*. Berberine Attenuates Chronic Atrophic Gastritis Induced by MNNG and Its Potential Mechanism. Frontiers in Pharmacology. 2021; 12: 644638. https://doi.org/10.3389/fphar.2021.644638.

[25] Xie Y, Han Y, Zhang X, Ma H, Li L, Yu R, *et al*. Application of New Radiosensitizer Based on Nano-Biotechnology in the Treatment of Glioma. Frontiers in Oncology. 2021; 11: 633827. https://doi.org/10.3389/fonc.2021.633827.

[26] Gough NR, Xiang X, Mishra L. TGF-β Signaling in Liver, Pancreas, and Gastrointestinal Diseases and Cancer. Gastroenterology. 2021; 161: 434–452.e15. https://doi.org/10.1053/j.gastro.2021.04.064.

[27] Yuan XN, Liu Q, Shao YC, Guan XQ, Yang ZL, Chu MF, *et al*. Mettl3 synergistically regulates TGF-β/SMAD2/3 to promote proliferation and metastasis of gastric cancer. American Journal of Cancer Research. 2023; 13: 3185–3202.

[28] Liu A, Yu C, Qiu C, Wu Q, Huang C, Li X, *et al*. PRMT5 methylating SMAD4 activates TGF-β signaling and promotes colorectal cancer metastasis. Oncogene. 2023; 42: 1572–1584. https://doi.org/10.1038/s41388-023-02674-x.

[29] Humeres C, Shinde AV, Hanna A, Alex L, Hernández SC, Li R, *et al*. Smad7 effects on TGF-β and ErbB2 restrain myofibroblast activation and protect from postinfarction heart failure. The Journal of Clinical Investigation. 2022; 132: e146926. https://doi.org/10.1172/JCI146926.

[30] Ma C, Wang X, Yang F, Zang Y, Liu J, Wang X, *et al*. Circular RNA hsa_circ_0004872 inhibits gastric cancer progression via the miR-224/Smad4/ADAR1 successive regulatory circuit. Molecular Cancer. 2020; 19: 157. https://doi.org/10.1186/s12943-020-01268-5.