

# Protective effects of Baicalin injection on severe acute pancreatitis through regulating follistatin-like-1 signaling pathway by down-regulating miR-429 expression in mice

Hui Li<sup>1#</sup>, Wei Yang<sup>2#</sup>, Ming-wei Liu<sup>3#</sup>, Lin-jun Wan<sup>1</sup>, Yan-qiong Wang<sup>2\*</sup>

<sup>1</sup>Intensive Care Unit, The Second Affiliated Hospital, Kunming Medical University, Kunming, China, <sup>2</sup>Department of Anesthesiology, The First Affiliated Hospital, Kunming Medical University, Kunming, China, <sup>3</sup>Department of Emergency, The First Affiliated Hospital, Kunming Medical University, Kunming, China

In China, *Scutellaria* is used for treating inflammatory-related diseases. Baicalin is the main active component of *Scutellaria* and has protective effects on acute pancreatitis. However, the mechanism of Baicalin is still unclear. In this study, the protective effects of baicalin on acute pancreatitis induced by taurocholate and its mechanism are investigated. In this study, mice were randomly divided into three groups: sham operation, model, and treatment groups. Acute pancreatitis in mice was induced by intraperitoneal injection of taurocholate (35 mg/kg). The treatment group was given baicalin (100 mg/kg) 2 h before acute pancreatitis induction. The mRNA expression levels of miR-429, nuclear factor kappa B65 (NF-κB65), toll-like receptor 4 (TLR4), TNF receptor associated factor6 (TRAF6), NF-kappa-B inhibitor (IκB), Follistatin-like 1 (FSTL1), and interleukin-1 receptor-associated kinase (IRAK) in the liver tissues 24 h after intraperitoneal injection were detected by RT-PCR. Then, the expression levels of NF-κB65, p-NF-κB65, TLR4, TRAF6, IκB, FSTL1, IRAK, p-IRAK, and p-IκB-α proteins were detected by Western blot. IL-6, TNF-α and IL-1β in plasma were measured by ELISA, and histopathological changes in the pancreases of the mice were observed. The results showed that after baicalin treatment, miR-429 expression in the pancreatic tissues and the expression levels of NF-κB65, TLR4, TRAF6, p-IκB-α, FSTL1, and p-IRAK decreased. Similarly, pancreatic myeloperoxidase (MPO) activity and the plasma levels of IL-6, TNF-α, IL-12, IL-1β, endotoxin, serum amylase, and lipase were reduced. Thus, the pancreatic injury induced by taurocholate was alleviated. The present study indicates that pretreatment with Baicalin can alleviate acute pancreatic injury induced by taurocholate in mice. The mechanism may be associated with the decreased miR-429 expression, reduced FSTL1 signaling pathway activity, TLR4 and TLR4/MyD88 signaling pathway inhibition, and reduced pancreatic inflammation. FSTL1 is the regulatory target for miR-429.

**Keywords:** Baicalin. Acute pancreatitis. TLR4. miR-429. Follistatin-like-1. Mice.

## INTRODUCTION

Acute pancreatitis (AP) is one of the most common critical diseases encountered in clinical practice. In most cases, AP is a self-limited disease with acute onset and many

complications. Approximately 15%–20% of patients with AP may develop severe acute pancreatitis (SAP), which is accompanied by organ failure or local complications (Shi *et al.*, 2018). The occurrence of SAP is often associated with multiple organ dysfunction. The clinical manifestations of SAP are fatal, and the mortality rate is as high as 40% (Fei *et al.*, 2018). The pathogenesis of AP remains incompletely understood, although trypsin activation has been known to lead to the self-digestion of pancreatic tissues and the initiation of AP. Moreover, the pathogenesis of AP is not

\*Correspondence: W. Yan-qiong. Department of Anesthesiology, The First Affiliated Hospital, Kunming Medical University, 295, Xichang Road, Wu Hua District, Kunming, Yunnan, 650032, China. Phone: +86-20-65324888. Email: xiaoyu750529@126.com. #Contributed equally. ORCID: <https://orcid.org/0000-0002-9742-3215>

only related to the activation of digestive enzymes but also linked with the systemic inflammatory response syndrome (SIRS), which is caused by the mass release of pro-inflammatory factors. Multiple organ failure due to SIRS is one of the causes of high mortality (Nie *et al.*, 2019; Wu *et al.*, 2019). The serum levels of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) are positively correlated with the severity of AP, and these cytokines have evident cytotoxic effects on pancreatic cells; these effects directly result in pancreatic tissue injuries at the time of AP (Samanta *et al.*, 2018). Currently, SAP treatment mainly involves fasting, liquid fasting, inhibition of trypsin release, anti-infection, correction of acid–base balance, and electrolyte disturbance. The mortality rate is high without special treatment. Consequently, pathological injury in the pancreases of mice with SAP may be alleviated, and the prognosis of SAP in mice may be enhanced by inhibiting the inflammatory reactions of the pancreases.

*Scutellaria baicalensis* is a Chinese herbal medicine, was widely used in clinic for the treatment of inflammation-related diseases, such as respiratory tract infections, acute pancreatitis, hepatitis and other diseases (Wang *et al.*, 2018), with various biological activities of anti-inflammatory, anti-viral and anti-oxidation (Wang *et al.*, 2020). The aqueous extract of *Scutellaria* was also found to have a protective effect on acute lung injury induced by burning and could reduce the production of various pro-inflammatory mediators, such as IL-6, IL-1  $\beta$ , and TNF- $\alpha$ , induced by lipopolysaccharide (Bai *et al.*, 2018). The extract of *Scutellaria* directly influences the contents of prostaglandin E<sub>2</sub>, malondialdehyde, and nitric oxide in inflammatory tissues and inhibits paw swelling induced by carrageenin in rats (Xu *et al.*, 2018; Liao *et al.*, 2020). The flavonoids contained in *Scutellaria* demonstrated a strong inhibitory effect on acute inflammatory reaction, which can increase capillary permeability and inhibit the production and release of inflammatory mediators (Hung *et al.*, 2018). Baicalein (Samanta *et al.*, 2018; Adiamah *et al.*, 2018; Hung *et al.*, 2018-trihydroxyflavone) is a flavone originally isolated from the roots of *Scutellaria* (Hung *et al.*, 2018). The baicalin in *Scutellaria* can affect the metabolism of arachidonic acid by stabilizing the membrane of mast cells to inhibit the release and production of inflammatory mediators (Hang *et al.*, 2018), and effectively improve the function

of macrophages and NK cells, finally achieving the purpose of improving the immune function of the body (Qian *et al.*, 2018). Baicalin also suppressed the levels of inflammatory regulators and inhibited Akt/NF- $\kappa$ B activation, effectively alleviated chronic gastritis (Ji *et al.*, 2019), inhibited NF- $\kappa$ B and increased mTOR signaling pathways in piglets, and alleviates deoxynivalenol-induced intestinal inflammation and oxidative stress damage (Ji *et al.*, 2020). Baicalin can alleviate pancreatic injury caused by severe pancreatitis (Qian *et al.*, 2018) by inhibiting the expression of pro-inflammatory proteins, such as protein kinase D1 (PKD1) and NF- $\kappa$ B65, in the way of anti-inflammation and anti-oxidation (Liu *et al.*, 2016). However, the mechanism remains unclear

MicroRNA, also known as miRNA, is a class of single-stranded non-coding small molecule RNA with a length of approximately 22 nucleotides. Highly conserved in evolution, miRNA inhibits the expression of target gene at post-translational level (Assmann *et al.*, 2018) and participates in the regulation of many important cellular activities, such as proliferation, differentiation, inflammation, and apoptosis (Assmann *et al.*, 2018). miR-9 has been demonstrated to activate Toll-like receptor 4 and induce the upregulated expression of miRNA in neutrophils and monocytes (Bazzoni *et al.*, 2009). Additionally, many miRNAs, such as miR-21, miR-147, miR-513, let-7, and miR-98, regulate inflammatory reactions (Jiménez-Lucena *et al.*, 2018). These findings reveal the important regulatory role of miRNA in inflammatory reactions. Promoting the expression of miR-429 has been recently found to increase the production of pro-inflammatory mediators (IL-6, TNF- $\alpha$  and IL-1  $\beta$ ), which facilitates the inflammatory reaction induced by endotoxin. Inhibiting the expression of miR-429 could decrease the production of IL-6, TNF- $\alpha$ , and IL-1  $\beta$  and prevent the inflammatory reaction induced by endotoxin (Xiao *et al.*, 2015). miR-429 regulates endotoxin-induced inflammation and acute lung injury (Jin *et al.*, 2018) and the occurrence and progression of tumors by modulating multiple inflammatory pathways, such as NF- $\kappa$ B65 and TRAF6 (Wang *et al.*, 2017).

FSTL1 is a secretory glycoprotein containing 308 amino acids with a molecular weight of approximately 50-55 ku. FSTL1 was first cloned from osteoblasts induced by transforming growth factor (TGF)- $\beta$  1 (Xiao *et al.*, 2015).

FSTL1 is expressed in most organs, and the functions are varied in different tissues and cells. Moreover, FSTL1 is involved in the regulation of cell proliferation, apoptosis, differentiation, inflammation, and migration (Magadum *et al.*, 2018). FSTL1 is found to induce inflammation in 3T3-L1 adipocytes and RAW264.7 macrophages (Fan *et al.*, 2013). I $\kappa$ B kinase (I $\kappa$ B)-nuclear factor- $\kappa$ B (NF- $\kappa$ B) and (JNK) pathway of c-Jun amino-terminal kinase in adipocytes and macrophages were activated, and the expression of inflammatory factors, such as IL-6, TNF- $\beta$ , and MCP-1, were promoted (Guo *et al.*, 2016). The expression of IFN- $\gamma$  in T lymphocytes was also induced. Cytokines, such as TNF- $\alpha$  and IL-1 $\beta$ , can considerably induce the expression of FSTL1 (Zhang *et al.*, 2017).

FSTL1 and TLR4 signal pathways are two of the major pathways regulating inflammation. From well-known databases for miRNA target prediction, FSTL1 was found to be an important inflammatory regulator of acute pancreatitis and is one of the candidate targets of miR-429. Therefore, in this study, miR-429 was hypothesized to regulate the occurrence and progression of AP induced by taurocholate by regulating the FSTL1 signaling pathway. Then, whether baicalin can alleviate taurocholate-induced acute pancreatic injury by regulating FSTL1 signaling pathway via miR-429 was investigated. In this way, the protective mechanism of baicalin in the treatment of AP is revealed.

## MATERIAL AND METHODS

### Reagents

Baicalin was purchased from Sigma-Aldrich (St. Louis, MO, USA). TRIzol kit was purchased from Gibco, USA. Reverse transcription (RT) reaction kit was obtained from Takara Biotechnology Co. Ltd, (Dalian, China). PCR amplification reagent kit and the DNA ladder marker were acquired from Sangon Biological Engineering Co. Ltd. (Shanghai, China).  $\beta$ -Actin was obtained from Santa Cruz Biotechnology, Inc. (USA). Mouse TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 ELISA kits were purchased from Quantikine, R&D Systems (Minneapolis, MN, USA). The Griess reagent nitric oxide assay kit was purchased from Beyotime Biotech (Jiangsu, P. R. China). Taurocholate was purchased from Sigma.

### Cell culture

AR42J pancreatic acinar cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). AR42J cells were grown in DMEM supplemented with 5% FBS, 100  $\mu$ /ml penicillin, 100  $\mu$ /ml streptomycin, and 50  $\mu$ g/l amphotericin B. Cultures were maintained at 37 °C in a humidified atmosphere of air, and 5% CO<sub>2</sub> cells were sub-cultured into 6-well plates and maintained until subconfluence.

### MiRNA mimics, inhibitor, and gene transfection

The AR42J cells were cultured in 6-well plates to 40% confluence. miR-429 mimics, miR-429 mimic-negative control (NC), miR-429 inhibitor, and miR-429 inhibitor-NC were mixed with Lipofectamine 2000 (Invitrogen) and then added to the cell culture medium according to the manufacturer's instructions. After 24 h of transfection, total RNA and protein were prepared from the cells and subjected to qRT-PCR and Western blot analyses, respectively.

### MTT cell proliferation assay

Cell proliferation in all groups was analyzed using MTT assay kit (KeyGen Biotech Inc., Nanjing, China). Briefly, cell medium was discarded and cells were incubated with 90  $\mu$ l of FBS-free medium and 20  $\mu$ l of MTT at 37 °C for 4 h. Cells were treated with 150  $\mu$ l of dimethyl sulfoxide (DMSO) for 10 min. The optical density (OD) was detected with a microplate reader under a 490 nm wavelength. Three wells were prepared for each group. The cell proliferation inhibition rate was calculated by using the following formula: cell proliferation inhibition rate (%) = (OD value in control group – OD value in experimental group) / OD value in control group  $\times$  100%.

### miR-429 target gene prediction and dual-luciferase reporter assay

The target gene of miR-429 was predicted by Target scan (<http://www.targetscan.org>), PicTar ([www.pictar.org](http://www.pictar.org)).

org), and database (www.mirbase.org). A total of  $1 \times 10^5$  pancreatic acinar cells were cultured in 24-well plates and transfected with FSTL1-3'UTR-wt or FSTL1-3'UTR-mt and miR-429 or mi-NC using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's protocol as described. Luciferase activity was measured 24 h after transfection by using a dual-luciferase reporter assay system (Promega) and normalized to Renilla luciferase activity.

### Experimental animals

Male C57BL/6 mice weighing 20–26 g (6–8 weeks) were maintained in a climate-controlled room at 22 °C and exposed to a 12:12 h light–dark cycle. Animals were fed with standard laboratory diet and given water ad libitum, diet was from Animal Experimental Center of Kunming Medical University, its composition meet the national standards of the People's Republic of China (GB14924,3-2010). The environment was maintained at a relative humidity of 30%–70%. All the experiments were approved and performed according to the guidelines of the Animal Care Committee and Ethical Committee of Kunming Medical University. miR-429 knockdown C57BL/6 mice using locked nucleic acid-modified anti-miR-429. Briefly, Locked nucleic acid (LNA)-modified scrambled or anti-miR-429 oligonucleotides (Exiqon, Woburn, MA, USA) were diluted in saline (5 mg/ml) for administration through intraperitoneal injection (10 mg/kg) at least 30 min before taurocholate-induced severe acute pancreatitis (Wu *et al.*, 2019). In this study, 30 mice were divided into the SO group, SAP group, and SAP miR-429 knockdown groups (n = 10 for each group), and both groups received anti-miR-429 oligonucleotides.

### Taurocholate-induced severe acute pancreatitis

The mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (50 mg/kg body weight), and the operation was performed under aseptic conditions. SAP models were prepared according to the method described by Zhu *et al.* 2018, used 5% sodium taurocholate (1.5 mL/kg body weight). Sham-operated

animals underwent the same surgical procedures but did not have active infusion into the pancreas.

### Animal groupings and drug administration

The mice were randomly assigned to the sham operation group (SO group), So treatment group, severe acute pancreatitis group (SAP group), or severe acute pancreatitis treatment group (SAP treatment group), (n = 10 for each group). The mice in the SO group underwent a sham operation comprising laparotomy and puncturing of the duodenum under identical anesthesia with 1% pentobarbital sodium (50 mg/kg body weight, Wuhan Dinghui Chemical Co., Ltd., Wuhan, China) and received 300 µl of saline via injection in the tail vein once a day. In the SO treatment group, the mice underwent a sham operation and received 100 mg/kg baicalin diluted to 300 µl of saline via the tail vein once a day. In the SAP group, severe acute pancreatitis mice was induced by intraperitoneal injection of 5% sodium taurocholate and provided with 300 µl of saline via injection in the tail vein once a day. In the SAP treatment group, according to previous studies (Hung *et al.*, 2018; Bai *et al.*, 2018; Chen *et al.*, 2015), the SAP mice were induced by intraperitoneal injection of 5% sodium taurocholate, subsequently treated with either 100 mg/kg baicalin that was diluted to 300 µl of saline via the tail vein once a day. The animals were killed by cervical dislocation 24 h after duct infusion or sham operation after anesthesia with 1% pentobarbital sodium (35 mg/kg body weight, Wuhan Dinghui Chemical Co., Ltd., Wuhan, China), and samples were taken for study. Blood was collected by cardiac puncture using heparinized syringes, centrifuged at 4000 r/min for 10 min, and stored at 4 °C for further analysis. The pancreas was carefully isolated and weighed for subsequent experiments. Tissues were isolated for histological examination and fixed in 10% formalin and embedded in paraffin for sectioning.

### Real-time quantitative PCR

Total RNA was extracted from frozen pancreatic tissues by using TRIzol (Takara Bio Inc. Japan) according to the manufacturer's instructions. Real-time PCR was performed with a Lightcycler480 (Roche) using the SYBR

Green assay after the reverse transcription of 1  $\mu$ g of RNA with reverse transcriptase. All the results were normalized to the levels of  $\beta$ -actin mRNA. The primer pairs and expected lengths (in bp) are shown in Table I.

The cycling conditions were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 65 °C for 30 s, and 65 °C for 15 s. Relative gene expression was calculated as  $2^{-\Delta\Delta CT}$  (Livak *et al.*, 2011).

**TABLE I** - Primer sequences for RT-PCR analysis

miR-429	F-5'- GTCGTATCCAGTGCCTGGTGGAGTCG- 3' R-5'-GCAATTGCACTGGATACGACACGGTTT -3'	259bp
U6	F-5'GTGCTCGCTTCGGCAGCACATATAAC-3' R-5'AAAAATATGGAACGCTCACGAATTTG-3'	247 bp
FSTL1 mRNA	F-5' -TTATGATGGGCAGGCAAAGAA -3' R-5'-ACTGCCTTTAGAGAACCAGCC -3'	218 bp
IRAK mRNA	F-5'- ATGCCTATGTTTCATCGTGAAC-3' R-5'- GGCTACGACGAAGGTGGAAC-3'	194bp
I $\kappa$ B mRNA	F-5'- TGCTGAGGCACTTCTGAG-3' R-5'- CTGTATCCGGGTGCTTGG-3'	214 bp
NF- $\kappa$ B65 mRNA	F-5'- GCACGGATGACAGAGGCGTGTATAAGG-3' R-5'- GGCGGATGATCTCCTTCTCTGTCTG -3'	267 bp
TLR4 mRNA	F-5'-GAATGAGGACGTTGTGAGAAC-3' R-5'- CACAGCAAGGACTTCTCCACTT-3'	174bp
TRAF6 mRNA	F-5'- TCTGCTTGATGGCTTTACG -3' R-5'-ACCGTCAGGGAAGAATCT-3'	180 bp
$\beta$ -actin	F-5'-AGCGGTTCCGATGCCCT -3' R-5'- AGAGGTCTTTACGGATGTCAACG-3'	160 bp

### miR-429 analysis

miRNAs were purified by using the mirVana miRNA Isolation Kit (Ambion) to measure the expression of miR-429 expression. miRNAs were transcribed into cDNAs and amplified by PCR with the Taqman primers (miR-429, ID: 2169) specific for miRNAs of interest. Pri-miRNAs were purified using TRIzol (Life technologies), transcribed into cDNAs, and amplified by PCR with the primers as shown in Table I. Applied Biosystems 7900 Fast Real-Time PCR System was used for quantitative reverse transcription PCR. U6 (Taqman probe ID: 2194) was utilized as the endogenous control gene to normalize input amounts. Expressions were normalized

to endogenous controls, and then fold change in relative gene expression was calculated as  $2^{-\Delta\Delta Ct}$ .

### Western blot analysis

Pancreatic tissues were homogenized in lysis buffer containing protease inhibitors, and protein concentrations were determined with Bradford reagent (Bio-Rad Laboratories Inc., Hercules, CA, USA). Samples were loaded onto an SDS-PAGE gel. After electrophoresis at 120 V for 90 min, the separated proteins were transferred onto polyvinylidene difluoride membranes (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) by the wet transfer method (250 mA, 90 min).

Nonspecific sites were blocked with 5% non-fat dry milk in TRIS-buffered saline with Tween 20 (25 mM TRIS, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 1 h, and the blots were then incubated overnight at 4 °C with an anti-NF- $\kappa$ B65 (1:1,000; cat. no. 2307), anti-phospho -NF- $\kappa$ B65 (1:1,000; cat. no. 4283), Fstli-1 (1:1,000; cat. no. 3352), TLR4 (1:1,000; cat. no. 4557), and TRAF6 (1:1,000; cat. no. 5358) antibody (Sigma-Aldrich), anti-I $\kappa$ B (1:1,000; cat. no. 2172), phospho -I $\kappa$ B-a (1:1,000; cat. no. 4489) antibody (Cell Signaling Technology Inc.), anti-IRAK (1:1,000; cat. no. 4793), and phospho-IRAK (1:1,000; cat. no. 7831) antibody (Cell Signaling Technology Inc.). Anti-rabbit or anti-mouse horseradish peroxidase conjugated-IgG antibodies were used to detect binding of the antibodies. The membranes were stripped and reblotted using an anti-actin antibody (Sigma-Aldrich) to normalize the loading of protein in each lane. The binding of specific antibodies was visualized by exposing the membranes to photographic film after treatment with enhanced chemiluminescence system reagents (GE Healthcare).

#### **Determination of cytokine in serum and the supernatant of pancreatic acinar cells by ELISA**

Nalgene Nunc Maxisorp plates were coated with primary antibody against either IL-6, TNF- $\alpha$ , and IL-1 $\beta$  for one hour at room temperature (RT) and then washed with PBS and 0.5% TWEEN (PBS-T). After blocking with casein, the samples were added to plates for one hour at RT. Following washing, biotinylated secondary antibody was applied for one hour followed by streptavidin–HRP conjugate (Jackson ImmunoResearch, West Grove, PA) diluted at 1:20,000. The reaction was developed with 0.01% tetramethylbenzidine dissolved in DMSO and 0.5% hydrogen peroxide and measured using endpoint spectrometry.

#### **MPO activity determination**

Pancreatic tissue MPO activity was examined as previously described (Zhou *et al.*, 2018). Samples were weighed and homogenized in 1:19 (w/v) in ice-cold

homogenization buffer. MPO activity was analyzed with a commercial assay kit (Jiancheng Co., Nanjing, China) and presented as units/g of wet tissue.

#### **Measurement of serum amylase and lipase**

The serum activities of amylase and lipase were measured by Mindray Medical Automatic Biochemical Analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd. Shenzhen, China).

#### **Serum endotoxin assay**

The serum levels of endotoxin were determined with a commercially available quantitative chromogenic endpoint Limulus Amebocyte Lysate QCL-1000 kit (Lonza, Walkersville, MD) according to the manufacturer's protocols. Endotoxin levels (EU/mL) in the samples were determined from a standard curve plotted with pure endotoxin standards. All the assays were performed in duplicate.

#### **Pancreatic water content**

Changes in pancreatic weight were measured for the assessment of pancreatic interstitial edema. The entire pancreas was removed and weighed. The weight of each pancreatic sample was presented as a ratio between the weight of the pancreas and body weight and was used for the estimation of water content in the pancreas as previously described (Rongione *et al.*, 1997).

#### **Histologic examination and scoring of the pancreas**

The pancreas was removed from each mouse and fixed in 10% buffered formalin at 4 °C overnight. The pancreas was then embedded in paraffin. Full-length (4  $\mu$ m) sections were taken and stained with hematoxylin and eosin (H&E) for histologic evaluation. Edema, inflammation, hemorrhage, and necrosis of the pancreas were each graded from 0 to 3 as previously described (Rongione *et al.*, 1997).

## Statistical Analysis

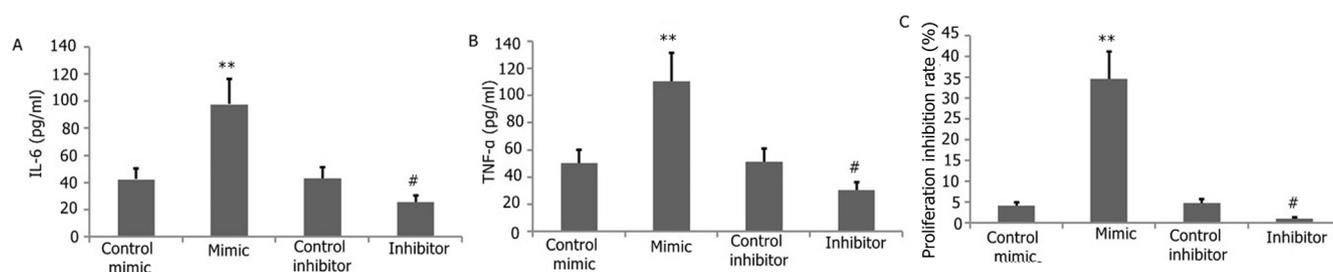
The results were statistically analyzed using SPSS 21.0 software package (Abacus Concepts Inc., Berkeley, CA, USA). Data were expressed as the mean  $\pm$  SEM unless otherwise indicated. The data was analyzed by using ANOVA and the Newman–Keuls comparison. An unpaired Student's t-test was used for two-group comparisons (GraphPad Software, San Diego, CA). A p-value  $<0.05$  was considered statistically significant.

## RESULTS

### miR-429 inhibitor attenuate pancreatic acinar cells inflammation and the cells proliferation inhibition rate of the pancreatic acinar cells

miR-429 mimics, miR-429 mimic-negative control (NC), miR-429 inhibitor, and miR-429 inhibitor-NC were

mixed with Lipofectamine 2000 (Invitrogen), and the mixture was added to the cell culture for 24 h. The cells proliferation inhibition rate of pancreatic acinar cells was compared by MTT assay. The IL-6 and TNF- $\alpha$  in the supernatant were measured by ELISA. As shown in Figure 1, the miR-429 inhibitor remarkably decreased the pancreatic acinar cells inflammation, and decreased the cells proliferation inhibition rate of the pancreatic acinar cells compared with that in all treatment group (Figure 1A–C).



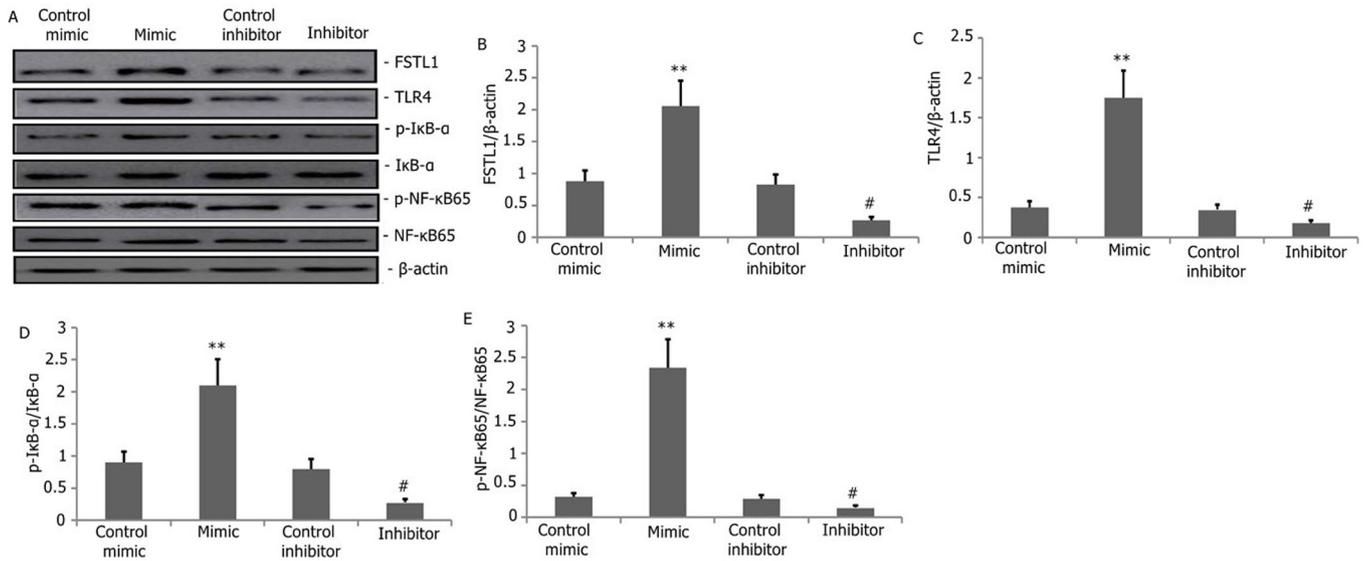
**FIGURE 1 - Effect of miR-429 on pancreatic acinar cells inflammation and proliferation.** miR-429 mimics, miR-429 mimic-negative control (NC), miR-429 inhibitor, and miR-429 inhibitor-NC were mixed with Lipofectamine 2000 (Invitrogen), then added to cell culture medium, and transfected for 48 h. (A–B) IL-6 and TNF- $\alpha$  in the supernatant of pancreatic acinar cells were measured by ELISA. (C) Cells proliferation inhibition rate was detected by MTT assay. Data were presented as means  $\pm$  SEM. \*\*P  $<0.01$ , versus control mimic. #P  $<0.05$  versus control inhibitor.

### miR-429 considerably upregulate FSTL1 and TLR4 signal pathway in the pancreatic acinar cells

FSTL1 and TLR4 signal pathway is one of the major pathways of severe acute pancreatitis. miR-429 mimics, miR-429 mimic-negative control (NC), miR-429 inhibitor, and miR-429 inhibitor-NC were mixed with Lipofectamine 2000 (Invitrogen), and the mixture was added to the cell culture for 24 h. miR-429 level was

measured by real-time quantitative PCR, and the levels of NF-kB65, p-IkB- $\alpha$ , FSTL1, and TLR4 expression were measured by Western blot. As shown in Figure 2, the decrease of miR-429 expression in miR-429 inhibitor remarkably reduced NF-kB65, p-IkB-a, FSTL1, and TLR4 expression and increased IkB expression (Figure 2A–E). The increase of miR-429 in miR-429 mimics group substantially increased NF-kB65, p-IkB-a, FSTL1, and TLR4 expression and decreased IkB expression

(Figure 2A—E). These findings suggest that miR-429 overexpression plays a role in the upregulation of FSTL1 pro-inflammatory signaling pathways.

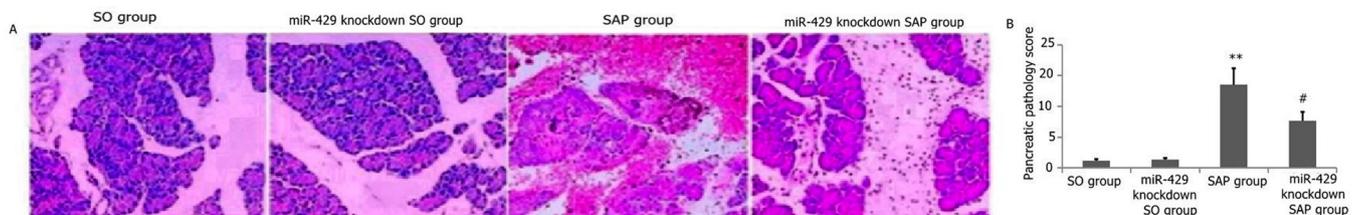


**FIGURE 2 - Effect of miR-429 on FSTL1 and TLR4 signal pathway and cell proliferation in the pancreatic acinar cells.** miR-429 mimics, miR-429 mimic-negative control (NC), miR-429 inhibitor, and miR-429 inhibitor-NC were mixed with Lipofectamine 2000 (Invitrogen) and then added to the cell culture. After 48 h of transfection, (A–E) the levels of NF-κB65, p-IkB-α, FSTL1, and TLR4 protein expression were measured by Western blot. Data obtained from quantitative densitometry and MTT assay were presented as mean ± SEM. \*\*P < 0.01, versus control mimic. #P < 0.05, versus control inhibitor.

**miR-429 knockdown ameliorated taurocholate-induced severe acute pancreatitis**

Severe acute pancreatitis was induced by intraperitoneal taurocholate challenge in miR-429 knockdown mice for 24 h to analyze the effect of miR-

429 knockdown on severe acute pancreatitis. As shown in Figure 3, histopathological examination of the pancreas of taurocholate-treated miR-429 knockdown mice revealed markedly decreased acute pancreatic injury and pancreas injury score compared with that in WT animals (Figure 3A—B).

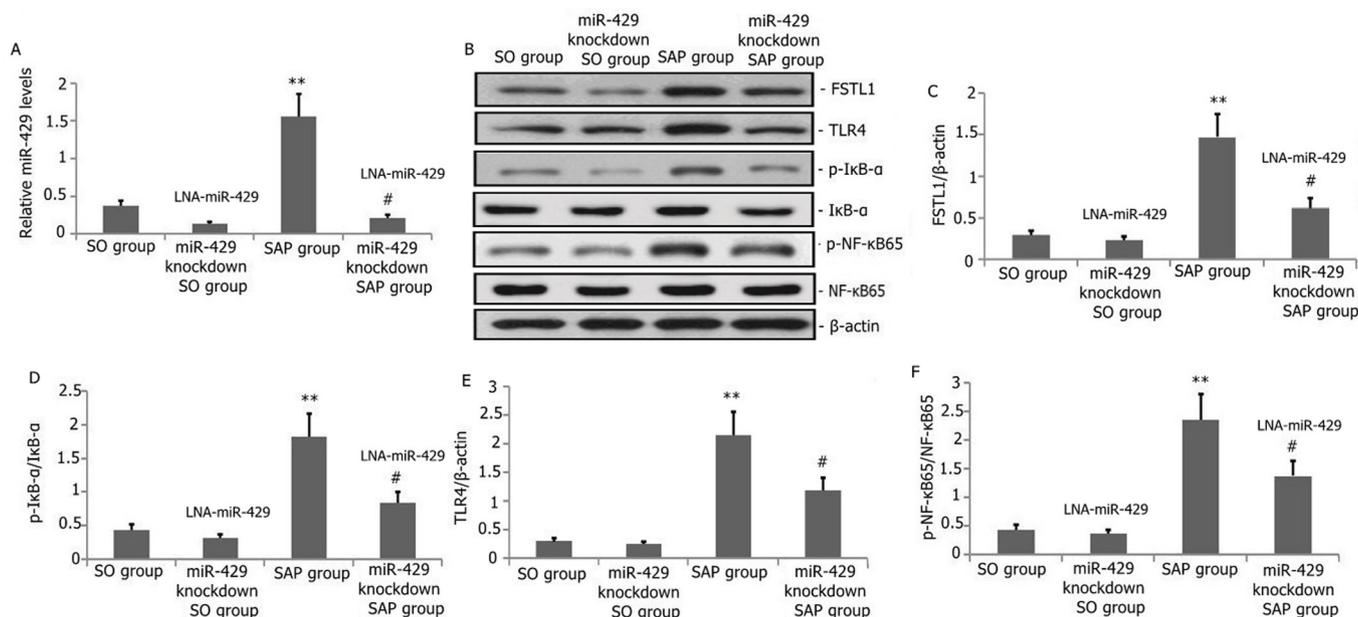


**FIGURE 3 - Effect of miR-429 knockdown on taurocholate-induced acute pancreas injury.** The anti-miR-429 oligonucleotides were intraperitoneally injected (10 mg/kg) at least 30 min before severe acute pancreatitis was induced with taurocholate. The histopathological examination of the pancreas of mice in four experimental groups was performed. (A) Representative images of hematoxylin and eosin-stained pancreas sections from three experimental groups (magnification, ×400). (B) Pancreas injury score. Each value represents as the means ± SEM. \*\*P < 0.01, versus the control and control miR-429 knockdown groups; ##P < 0.01, versus the SAP group.

### miR-429 knockdown decreased NF-kB65, p-IkB-a, FSTL1, and TLR4 expression in taurocholate-induced mice

NF-kB65, p-IkB-a, FSTL1, and TLR4 are important pro-inflammatory proteins. NF-kB65, p-IkB-a, FSTL1, and TLR4 expression were determined by Western

blot to investigate the effect of miR-429 knockdown on NF-kB65, p-IkB-a, FSTL1, and TLR4 expression in taurocholate-induced mice. As shown in Figure 4, NF-kB65, p-IkB-a, FSTL1, and TLR4 expression significantly decreased in miR-429 knockdown mice compared with that in control group (Figure 3A–F).

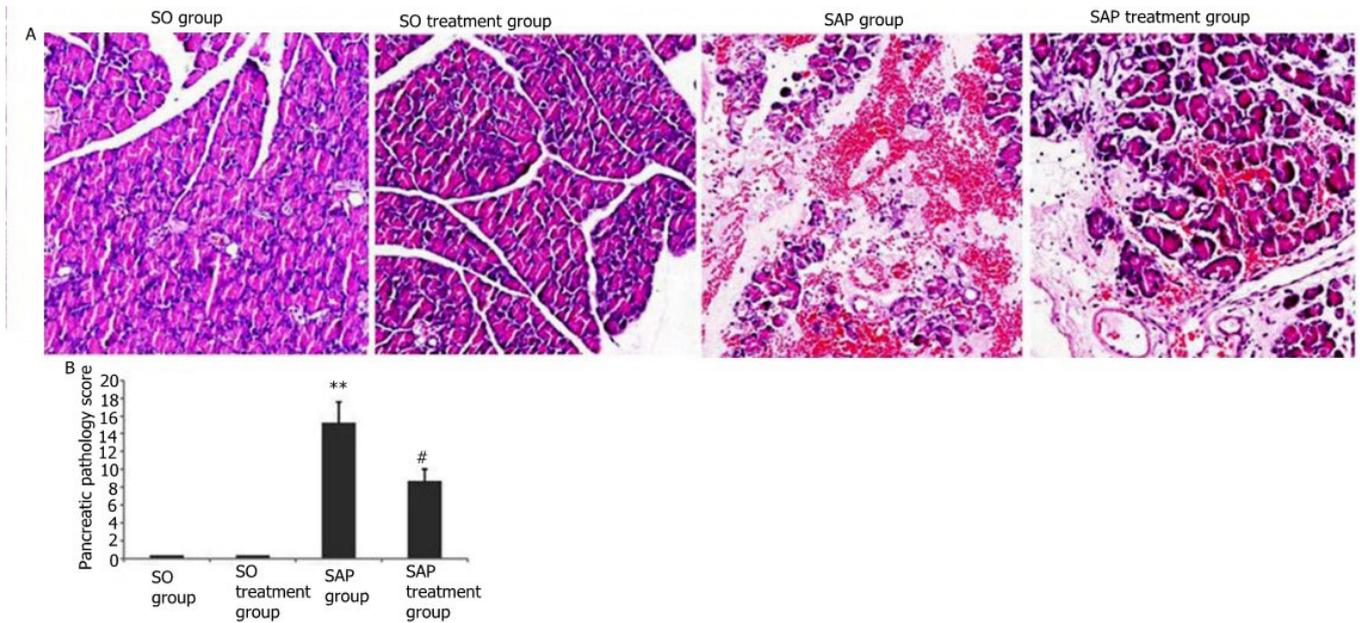


**FIGURE 4 - Effects of miR-429 knockdown on FSTL1 and TLR4 signal pathways in taurocholate-induced mice pancreatic tissue.** The anti-miR-429 oligonucleotides were intraperitoneally injected (10 mg/kg) at least 30 min before severe acute pancreatitis was induced with taurocholate. (A) Representative RT-PCR showing the miR-429 expression level in the pancreatic tissue of experimental mice 24 h after the administration of anti-miR-429 oligonucleotides. (B) Representative Western blot showing the p-NF-kB65, p-IkB-a, FSTL1, and TLR4 protein expression levels in pancreatic tissue 24 h after the administration of anti-miR-429 oligonucleotides. (C–F) Statistical summary of the densitometric analysis of p-NF-kB65, TLR4, TRAF6, FSTL1, IRAK, p-IRAK, IkB, and p-IkB-α protein expression levels in mice pancreatic tissues 24 h after the administration of anti-miR-429 oligonucleotides. Each value was represented as mean ± SEM. \*\*P < 0.01, versus the control and control miR-429 knockdown groups; ##P < 0.01, versus the SAP group.

### Baicalin treatment ameliorated taurocholate-induced acute pancreatitis in mice.

Histopathological analysis was performed on sections stained with hematoxylin and eosin-stained pancreas sections to determine the effect of baicalin on acute pancreatitis-induced histological pancreas injury. Histopathological evaluation of pancreas from the taurocholate-induced SAP group showed

massive edema, infiltration of inflammatory cells, and cytoplasmic vacuolization compared with that of the sham operation group (P < 0.05), and the pancreas injury score was obviously increased (Figure 5). Baicalin treatment after the taurocholate-induced SAP significantly reduced edema formation, inflammatory infiltrate, and cytoplasmic vacuolization (P < 0.05), and the pancreas injury score was obviously decreased (Figure 5).

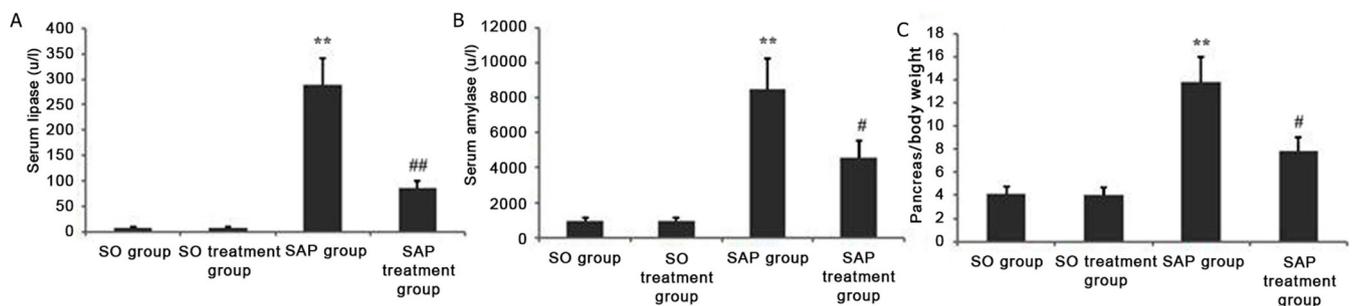


**FIGURE 5 - Changes in the pancreas pathology and pancreas injury score.** Severe acute pancreatitis mice were induced by intraperitoneal injection of 5% sodium taurocholate. Subsequently, in the SAP treatment group, the sodium taurocholate induced-mice were injected with 100 mg/kg.day baicalin for 24 h. A: Typical pathological changes in each experimental group. B: Statistical analysis of pancreas injury score in each experimental group. Data were expressed as the mean ± SEM. \*\*p < 0.01, compared with control group; #p < 0.05, compared with model group

**Baicalin treatment decreased serum amylase and lipase levels and pancreatic water content**

Serum amylase and lipase levels and pancreatic water content are important indicators for evaluating pancreatic damage. The effect of baicalin treatment was analyzed by measuring serum amylase and lipase levels and pancreatic water content. As shown in Figure 6,

serum amylase and lipase levels and pancreatic water content in the taurocholate-induced acute pancreatitis group were significantly increased (Figure 6A—C); after baicalin treatment, these levels in the taurocholate-induced acute pancreatitis group were significantly decreased (Figure 6A—C). This finding suggests that baicalin has protective effect on acute pancreatitis induced by taurocholate.

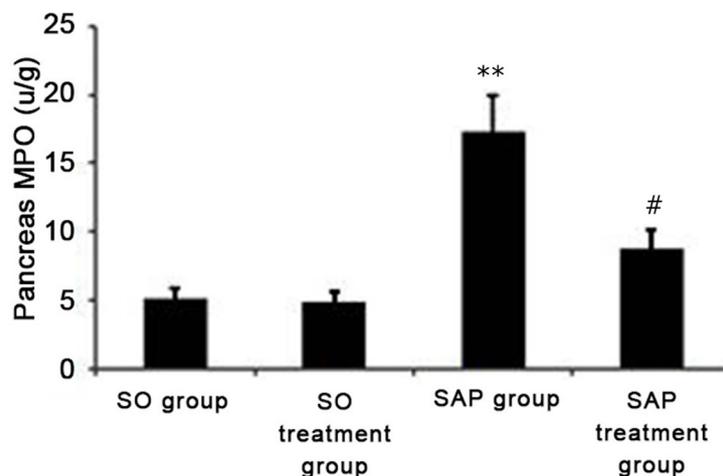


**FIGURE 6 - Changes in serum amylase and lipase and pancreas/body weight in taurocholate-induced mice.** Mice with severe acute pancreatitis were induced by the intraperitoneal injection of 5% sodium taurocholate. Subsequently, in the SAP treatment group, the sodium taurocholate induced mice were injected with 100 mg/kg.day baicalin for 24 h. (A)Serum amylase level, (B) Serum lipase level, and (C) Pancreas/body weight was measured. Data were expressed as means ± SEM. \*\*p < 0.01, compared with control group; #p < 0.05, compared with model group.

**Baicalin treatment attenuated the activity of MPO in the mice pancreas with taurocholate-induced acute pancreatitis.**

The MPO activity from mice pancreas treated with taurocholate with or without baicalin was detected to

investigate the potential mechanism underlying the protective effect of baicalin on acute pancreatitis. As shown in Figure 7, taurocholate caused a significant increase in the MPO activity in the pancreas at 24 h. These increases were reduced in the baicalin treatment group.

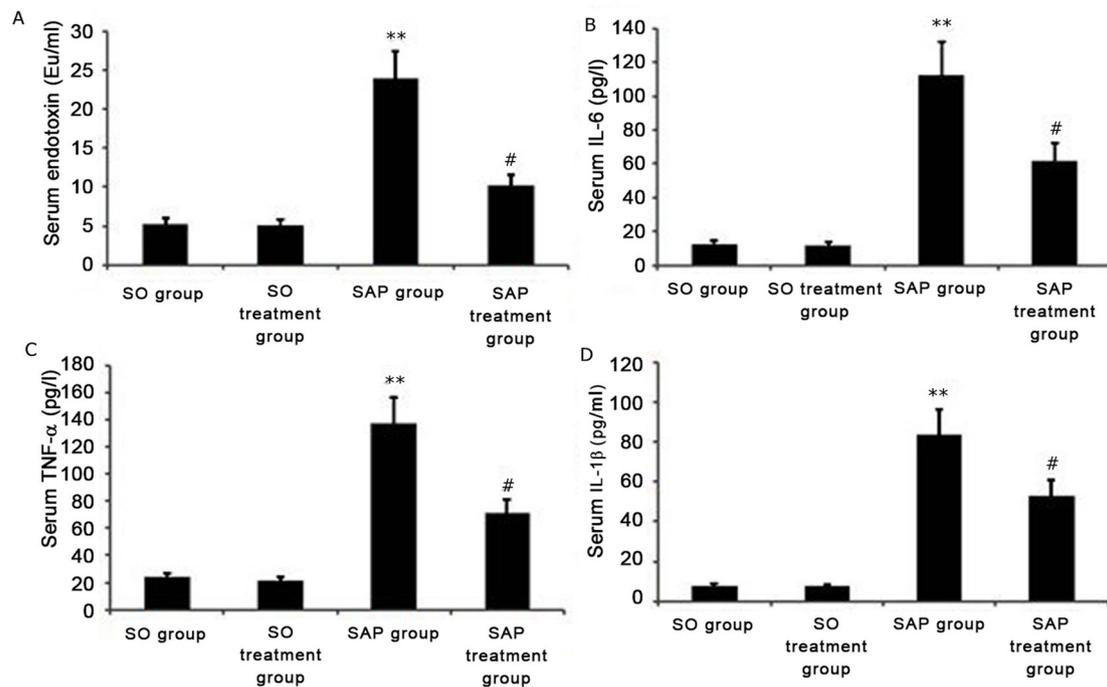


**FIGURE 7 - Baicalin treatment attenuated MPO activity in taurocholate-induced mice pancreas.** Mice with severe acute pancreatitis were induced by the intraperitoneal injection of 5% sodium taurocholate. Subsequently, in the SAP treatment group, the sodium taurocholate induced mice were injected with 100 mg/kg.day baicalin for 24 h. The activity of MPO in mice pancreas was measured. Data were expressed as means ± SEM. \*\* p < 0.01, compared with the control group; #p < 0.05, compared with the model group.

**Baicalin treatment attenuated systemic inflammation in mice with taurocholate-induced acute pancreatitis.**

The levels of TNF-a, IL-1β, IL-6, and endotoxin in serum were evaluated at 24 h to observe the effect of baicalin treatment on systemic inflammation in mice with taurocholate-induced acute pancreatitis. As shown

in Figure 8, acute pancreatitis caused a significant acute systemic inflammatory response as demonstrated by the increased serum concentrations of the pro-inflammatory mediators TNF-a, IL-1β, and IL-6 (8 A–D). The presence of baicalin reduced the increase in these pro-inflammatory cytokines and the serum concentration of the endotoxin at 24 h(8 A–D).

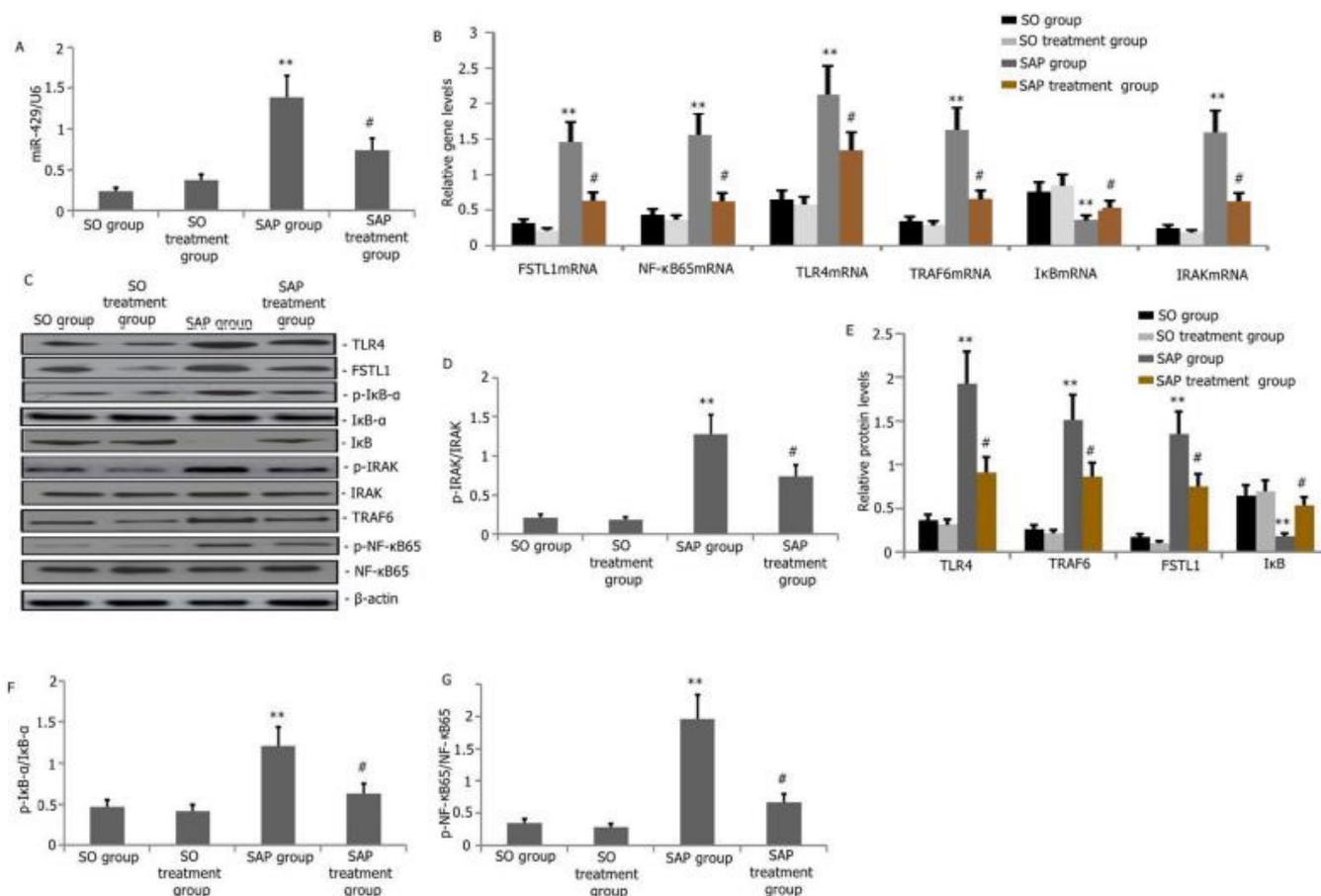


**FIGURE 8 - Baicalin treatment attenuated systemic inflammation in the taurocholate-induced mice pancreas.** Severe acute pancreatitis mice were induced by intraperitoneal injection of 5% sodium taurocholate. Subsequently, in the SAP treatment group, the sodium taurocholate induced mice were injected with 100 mg/kg.day baicalin for 24 h. (A–D) The levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in serum were determined using ELISA and endotoxin levels in serum were determined using a commercially available quantitative chromogenic endpoint Limulus Amebocyte Lysate QCL-1000 kit. Data are expressed as the mean  $\pm$  SEM. \* \*  $p < 0.01$ , compared with the control group; #  $p < 0.05$  compared with the model group.

### Effect of baicalin treatment on miR-429 expression in taurocholate-induced mice pancreas

The effects of baicalin on miR-429 expression in taurocholate-induced mice pancreas treated with

baicalin for 24 h were observed. miR-429 expression in pancreatic tissue was measured by qRT-PCR and was increased in taurocholate-induced mice pancreas (Figure 9A). Conversely, miR-429 expression was significantly increased in baicalin-treated mice (Figure 9A).



**FIGURE 9 - Effect of baicalin treatment on gene and protein expression miR-429, NF-κB65, TLR4, TRAF6, IκB, FSTL1, and IRAK from taurocholate-induced mice pancreatic tissue.** Severe acute pancreatitis mice were induced by intraperitoneal injection of 5% sodium taurocholate. Subsequently, in the SAP treatment group, the sodium taurocholate induced mice injected with 100 mg/kg.day baicalin for 24 h. (A) Relative miR-429 expression levels were determined by qRT-PCR at 24 h after administration of baicalin injection. (B) Representative RT-PCR showing the level of NF-κB65, TLR4, TRAF6, IκB, FSTL1, and IRAK gene expression in mice pancreatic tissue at 24 h after administration of baicalin injection. (C) Representative Western blot showing the level of p-NF-κB65, TLR4, TRAF6, FSTL1, IRAK, p-IRAK, IκB, and p-IκB-α protein expression in pancreatic tissue at 24 h after administration of baicalin injection. (D–G) Statistical summary of the densitometric analysis of p-NF-κB65, TLR4, TRAF6, FSTL1, IRAK, p-IRAK, IκB, and p-IκB-α protein expression in mice pancreatic tissue. Each value represents the mean ± SEM. \*P < 0.05, \*\*P < 0.01, versus the control group; #P < 0.05, ##P < 0.01, versus the model group.

**Effect of baicalin treatment on the gene and protein expression NF-κB65, TLR4, TRAF6, IκB, FSTL1, and IRAK in mice pancreas tissues with taurocholate-induced SAP**

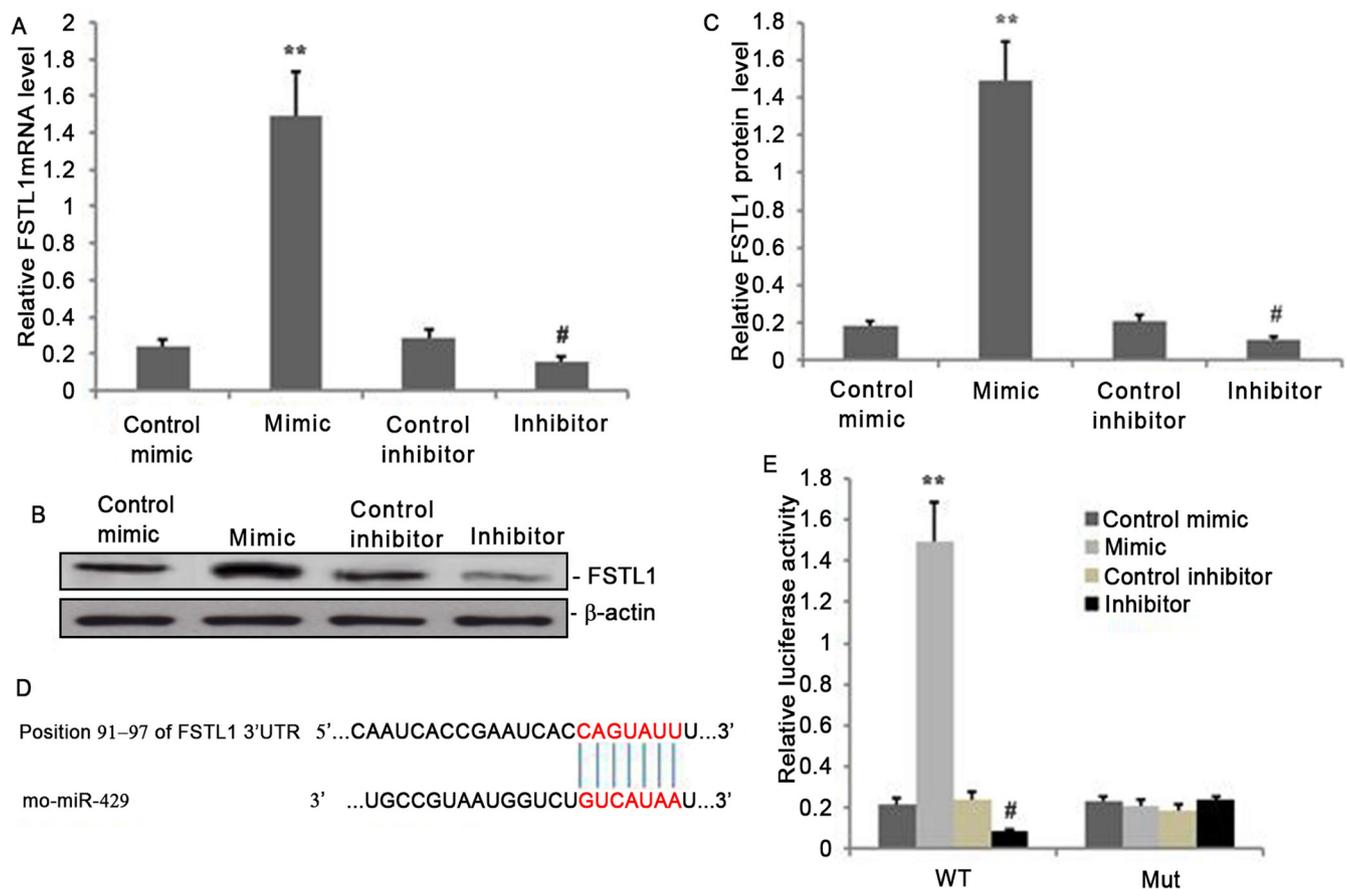
NF-κB65, TLR4, TRAF6, IκB, FSTL1, and IRAK gene and protein expression levels were measured by RT-PCR and Western blot in taurocholate-induced pancreas tissues in mice to explore the effect of baicalin treatment on the gene and protein expression

NF-κB65, TLR4, TRAF6, IκB, FSTL1, and IRAK in mice pancreas tissues with taurocholate-induced SAP. RT-PCR and Western blot analysis found that the mRNA expression of NF-κB65, TLR4, TRAF6, FSTL1, and IRAK expression and the protein expression of p-NF-κB65, p-IκB-α, TLR4, TRAF6, FSTL1, and p-IRAK expression were significantly upregulated and the protein and mRNA expression of IκB were significantly downregulated after taurocholate-induced SAP (Figure 9 B–G). However, after baicalin

treatment, the mRNA expression of NF- $\kappa$ B65, TLR4, TRAF6, FSTL1, and IRAK expression and the protein expression of p-NF- $\kappa$ B65, p-I $\kappa$ B- $\alpha$ , TLR4, TRAF6, FSTL1, and p-IRAK expression were significantly downregulated and the protein and mRNA expression of I $\kappa$ B significantly upregulated (Figure 9 B–G).

### FSTL1 are direct targets of miR-429

In the study, mRNA and protein levels of FSTL1 were dramatically increased after miR-429 overexpression (Figures 10A,C–D). The wild-type and mutant FSTL1 was constructed for dual-luciferase reporter assay to further confirm the interaction between FSTL1 and miR-429, as respectively shown in Figures 5 B and E. As expected, miR-429 bound to wild type of FSTL1 but not to the mutant type (Figure 10 E).



**FIGURE 10 - FSTL1 is a miR-429 target.** (A) FSTL1 mRNA levels were increased in miR-429 overexpressing the pancreatic acinar cells ( $p < 0.05$ ). (B–C) FSTL1 protein levels were increased in miR-429 overexpressing the pancreatic acinar cells compared with those in vector controls ( $p < 0.05$ ). (D) miR-193a bound to FSTL1-3'UTR-wt, whereas binding was blocked by FSTL1-3'UTR-mt. (E) Dual-luciferase reporter assays confirmed that the miR-429 mimic bound FSTL1-3'UTR-wt but not the mutated form ( $p < 0.05$ ).

## DISCUSSION

Inflammation is a core issue in the occurrence and progression of AP (Fei *et al.*, 2018). Especially in patients

with SAP, abnormally activated inflammatory cells can secrete a large number of cytokines and initiate the cascade reactions of inflammatory factors, resulting in multiple organ injuries in patients (Xu *et al.*, 2018; Hu *et al.*, 2011).

MPO is an indicator of leukocyte aggregation in the pancreas (Xu *et al.*, 2018). In this study, treatment with baicalin decreased the production of serum TNF- $\alpha$ , IL-1  $\beta$ , and IL-6, reduced the activity of pancreas MPO, attenuated the accumulation of inflammatory cells in pancreas, inhibited the inflammatory reaction of pancreas, thus alleviating the pancreatic injury. Consequently, baicalin can reduce the AP induced by taurocholate and has protective effects on acute pancreatic injury induced by taurocholate.

Previously, miR-429-5p was shown to regulate inflammation reactions through multiple pathways (Bazzoni *et al.*, 2009; Jiménez-Lucena *et al.*, 2018; Jin *et al.*, 2018). Upregulation of miR-429-5p significantly inhibited protein expression of LIM domain kinase 1 and reduced migration and invasion ability of malignant melanoma cells (Sheng *et al.*, 2020). miR-429 overexpression upregulated Cxcl1 expression and attenuated nuclear factor kinase B expression, and exerted protective effects against glucose deprivation/reoxygenation (OGD/R)-induced injury in vitro pathway (Leng *et al.*, 2020). However, some studies found that miR-429 inhibitors also attenuated the neurological impairment in traumatic brain injury mice by inhibiting the expression of p38 MAPK and phosphorylated NF- $\kappa$ B (Qi *et al.*, 2020). miR-429 overexpression increased apoptosis, the level of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , Bax and cleaved caspase-3, while reduced cell viability in LPS-stimulated WI-38 cells (Zhang *et al.*, 2020). Besides, miR-429 suppressor down-regulated p-NF- $\kappa$ B level in LPS-stimulated cells (Zhang *et al.*, 2020). In this study, The expression of miR-429 in AP induced by taurocholate was significantly increased, the activity of FSTL1 signaling pathway was increased, and the activity of TLR4/NF- $\kappa$ B65/I $\kappa$ B pathway was stimulated, resulting in the aggravated inflammation of the pancreas. After baicalin treatment, the expression of miR-429 in pancreatic tissues induced by taurocholate was significantly decreased, the activity of FSTL1 signaling pathway was decreased, the activity of TLR4/NF- $\kappa$ B65/I $\kappa$ B signaling pathway was inhibited, and the inflammation of the pancreas was finally alleviated.

FSTL1 was found to activate TLR4 signaling pathway to trigger inflammatory reactions, promote the release of inflammatory factors, such as IL-6 and IL-8, and further enhance inflammatory reactions (Zhang *et al.*, 2017). In addition, TLR4 can activate IRAK

through myeloid differentiation factor 88 (MyD88), thereby activating TRAF6 mediated NF- $\kappa$ B and inducing specific gene expression (Gargiulo *et al.*, 2015). Then, TNF- $\alpha$ , IL-1, IL-8, and IL-6 are activated, which promotes inflammatory reactions and aggravates injury of tissues and organs (Zhao *et al.*, 2018). In this study, the expression of FSTL1, TLR4, TRAF6, and NF- $\kappa$ B were increased in AP model induced by taurocholate, leading to the aggravated inflammation of the pancreas. After baicalin treatment, the expression of FSTL1 was significantly decreased, the expression of TLR4, TRAF6, and NF- $\kappa$ B was inhibited, and the inflammation of pancreas was reduced. Baicalin is suggested to reduce the activity of TLR4 and TLR4/MyD88 signaling pathway by inhibiting the expression of FSTL1, which finally alleviated the inflammation of the pancreas.

Severe pancreatitis often results in impaired intestinal barrier function and is prone to enterogenous endotoxemia (Kang *et al.*, 2017). Endotoxin stimulates inflammatory cells and produces a large number of inflammatory mediators, leading to the aggravation of systemic inflammatory reaction and pancreatic injury (Kang *et al.*, 2017). In this study, baicalin decrease the plasma endotoxin level, and improve pancreatitis induced by taurocholate, may help improve the intestinal barrier function in severe pancreatitis,

Both FSTL1 and miR-429 play an important role in the occurrence and development of acute pancreatitis. In this study, we further investigated the intermodulation relationship between FSTL1 and miR-429. As expected, FSTL1 was found to be the regulatory target of miR-429 by dual-luciferase reporter assay. The results suggest that miR-429 expression regulate the occurrence and development of acute pancreatitis by regulating the expression of FSTL1. Baicalin reduced the expression of FSTL1 by inhibiting the expression of miR-429, reduced pancreatic inflammation and improved acute pancreatic injury induced by taurocholate.

## CONCLUSION

No special treatment is currently available for AP. In this study, baicalin was found to reduce acute pancreatitis reaction induced by taurocholate and improve

the acute pancreatic injury, suggesting that baicalin may downregulate the expression of miR-429, inhibit the expression of FSTL1, reduce the activity of TLR4 and TLR4/MyD88 signaling pathway, and decrease the pancreatic inflammatory reaction. The pancreatic injury was eventually alleviated. The experiment also confirmed that FSTL1 is a regulatory target for miR-429, which provides a new theoretical basis for the treatment of AP with baicalin.

## ACKNOWLEDGMENTS

The authors are grateful for the excellent technical assistance provided by Prof. Mei Chen and Xu Liu.

## COMPETING INTERESTS

The authors declare that they have no competing interests

## REFERENCES

- Adiamah A, Psaltis E, Crook M, Lobo DN. A systematic review of the epidemiology, pathophysiology and current management of hyperlipidaemic pancreatitis. *Clin Nutr*. 2018;37(6 Pt A):1810-1822
- Assmann TS, Recamonde-Mendoza M, de Souza BM, Bauer AC, Crispim D. MicroRNAs and diabetic kidney disease: Systematic review and bioinformatic analysis. *Mol Cell Endocrinol*. 2018;477:90-102.
- Bai C, Li T, Sun Q, Xin Q, Xu T, Yu J, et al. Protective effect of baicalin against severe burn-induced remote acute lung injury in rats. *Mol Med Rep*. 2018;17(2):2689-2694.
- Bazzoni F, Rossato M, Fabbri M, Gaudiosi D, Mirolo M, Mori L, et al. Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. *Proc Natl Acad Sci USA*. 2009;106(13):5282-7.
- Chen HG, Xu YF, Wang JZ, Zhao W, Ruan HH. Baicalin ameliorates isoproterenol-induced acute myocardial infarction through iNOS, inflammation and oxidative stress in rat. *Int J Clin Exp Pathol*. 2015;8(9):10139-10147.
- Fan N, Sun H, Wang Y, Wang Y, Zhang L, Xia Z, et al. Follistatin-like 1: a potential mediator of inflammation in obesity. *Mediators Inflamm*. 2013; 2013:752519.
- Fei Y, Gao K, Li WQ. Artificial neural network algorithm model as powerful tool to predict acute lung injury following to severe acute pancreatitis. *Pancreatology*. 2018;18(8):892-899.
- Gargiulo S, Gamba P, Testa G, Rossin D, Biasi F, Poli G, et al. Relation between TLR4/NF- $\kappa$ B signaling pathway activation by 27-hydroxycholesterol and 4-hydroxynonenal, and atherosclerotic plaque instability. *Aging Cell*. 2015;14(4):569-81.
- Guo J, Liang W, Li JH, Long J. Knockdown of FSTL1 inhibits oxLDL-induced inflammation responses through the TLR4/MyD88/NF- $\kappa$ B and MAPK pathway. *Biochem Biophys Res Commun*. 2016;478(4):1528-1533.
- Hang YX, Qin X, Ren TL, Cao JN. Baicalin reduces blood lipids and inflammation in patients with coronary artery disease and rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Lipids Health Dis*. 2018;17(1):146.
- Hung CH, Wang CN, Cheng HH, Liao JW, Chen YT, Chao YW, et al. Baicalin ameliorates imiquimod-induced psoriasis-like inflammation in mice. *Planta Med*. 2018;84(15):1110-1117.
- Hu G, Shen J, Cheng L, Guo CY, Xu XF, Wang F, et al. Reg4 protects against acinar cell necrosis in experimental pancreatitis. *Gut*. 2011;60(6):820-828.
- Ji W, Liang K, An R, Wang X. Baicalin protects against ethanol-induced chronic gastritis in rats by inhibiting Akt/NF- $\kappa$ B pathway. *Life Sci*. 2019;239:117064.
- Jiménez-Lucena R, Rangel-Zúñiga OA, Alcalá-Díaz JF, López-Moreno J, Roncero-Ramos I, Molina-Abril H, et al. Circulating miRNAs as predictive biomarkers of type 2 Diabetes Mellitus development in coronary heart disease patients from the CORDIOPREV study. *Mol Ther Nucleic Acids*. 2018;12:146-157.
- Jin F, Hu H, Xu M, Zhan S, Wang Y, Zhang H, et al. Serum microRNA Profiles serve as novel biomarkers for autoimmune diseases. *Front Immunol*. 2018;9:2381.
- Kang X, Lu XG, Zhan LB, Liang ZK, Guo WX, Ma Q, et al. Dai-Huang-Fu-Zi-Tang alleviates pulmonary and intestinal injury with severe acute pancreatitis via regulating aquaporins in rats. *BMC Complement Altern Med*. 2017;17(1):288.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408.
- Liao P, Li Y, Li M, Chen X, Yuan D, Tang M, et al. Baicalin alleviates deoxynivalenol-induced intestinal inflammation and oxidative stress damage by inhibiting NF- $\kappa$ B and increasing mTOR signaling pathways in piglets. *Food Chem Toxicol*. 2020;140:111326.
- Magadam A, Singh N, Kurian AA, Sharkar MTK, Chepurko E, Zangi L. Ablation of a Single N-Glycosylation Site in

Human FSTL 1 Induces Cardiomyocyte Proliferation and Cardiac Regeneration. *Mol Ther Nucleic Acids*. 2018;13:133-143.

Leng J, Liu W, Li L, Wei FY, Tian M, Liu HM, et al. MicroRNA-429/Cxcl1 Axis Protective Against Oxygen Glucose Deprivation/Reoxygenation-Induced Injury in Brain Microvascular Endothelial Cells. *Dose Response*. 2020;18(2):1559325820913785.

Liu J, Wei Y, Luo Q, Xu F, Zhao Z, Zhang H, et al. Baicalin attenuates inflammation in mice with OVA-induced asthma by inhibiting NF- $\kappa$ B and suppressing CCR7/CCL19/CCL21. *Int J Mol Med*. 2016;38(5):1541-1548.

Nie S, Cui X, Guo J, Ma X, Zhi H, Li S, Li Y. Inhibiting role of rosiglitazone in the regulation of inflammatory response and protective effects for severe acute pancreatitis in mice. *J Cell Biochem*. 2019;20(1):799-808.

Qi R, Wang X. Inhibition of miR-429 improves neurological recovery of traumatic brain injury mice and attenuates microglial neuroinflammation. *Int Immunopharmacol*. 2020;79:106091.

Qian Y, Chen Y, Wang L, Tou J. Effects of baicalin on inflammatory reaction, oxidative stress and PKD1 and NF- $\kappa$ B protein expressions in rats with severe acute pancreatitis. *Acta Cir Bras*. 2018;33(7):556-564.

Rongione AJ, Kusske AM, Kwan K, Ashley SW, Reber HA, McFadden DW. Interleukin 10 reduces the severity of acute pancreatitis in rats. *Gastroenterology*. 1997;112:960-967.

Samanta J, Singh S, Arora S, Muktesh G, Aggarwal A, Dhaka N, et al. Cytokine profile in prediction of acute lung injury in patients with acute pancreatitis. *Pancreatol*. 2018;18(8): 878-884.

Shi C, Hou C, Zhu X, Huang D, Peng Y, Tu M, et al. SRT1720 ameliorates sodium taurocholate-induced severe acute pancreatitis in rats by suppressing NF- $\kappa$ B signalling. *Biomed Pharmacother*. 2018;108:50-57.

Sheng H, Guo YH, Cao DS, Li XJ, Zhao Y, Ding H, et al. MiR-429-5p attenuates the migration and invasion of malignant melanoma by targeting LIMK1. *Eur Rev Med Pharmacol Sci*. 2020;24(5):2625-2631.

Wang L, Chen W, Li M, Zhang F, Chen K, Chen W. A review of the ethnopharmacology, phytochemistry, pharmacology, and quality control of *Scutellaria barbata* D. Don. *J Ethnopharmacol*. 2020;254:112260.

Wang P, Cao J, Liu S, Pan H, Liu X, Sui A, et al. Upregulated microRNA-429 inhibits the migration of HCC cells by targeting TRAF6 through the NF- $\kappa$ B pathway. *Oncol Rep*. 2017;37(5):2883-2890.

Wang Y, Jia Y, Yang X, Liang B, Gao H, Yang T. A potential role of Baicalin to inhibit apoptosis and protect against acute liver and kidney injury in rat preeclampsia model. *Biomed Pharmacother*. 2018;108:1546-1552.

Wu XM, Ji KQ, Wang HY, Zhao Y, Jia J, Gao XP, et al. microRNA-542-5p protects against acute lung injury in mice with severe acute pancreatitis by suppressing the mitogen-activated protein kinase signaling pathway through the negative regulation of P21-activated kinase 1. *J Cell Biochem*. 2019;120(1):290-304.

Xiao J, Tang J, Chen Q, Tang D, Liu M, Luo M, et al. miR-429 regulates alveolar macrophage inflammatory cytokine production and is involved in LPS-induced acute lung injury. *Biochem J*. 2015;471(2):281-91.

Xu J, Liu J, Yue G, Sun M, Li J, Xiu X, et al. Therapeutic effect of the natural compounds baicalein and baicalin on autoimmune diseases. *Mol Med Rep*. 2018;18(1):1149-1154.

Zhang L, Yan HL, Wang H, Wang L, Bai B, Ma Y, et al. MicroRNA (miR)-429 promotes inflammatory injury by targeting kruppel-like factor 4 (KLF4) in neonatal pneumonia. *Curr Neurovasc Res*. 2020;16(2):1126-1357.

Zhang ZM, Zhang AR, Xu M, Lou J, Qiu WQ. TLR-4/miRNA-32-5p/FSTL1 signaling regulates mycobacterial survival and inflammatory responses in *Mycobacterium tuberculosis*-infected macrophages. *Exp Cell Res*. 2017;352(2):313-321.

Zhao S, Yang J, Liu T, Zeng J, Mi L, Xiang K. Dexamethasone inhibits NF- $\kappa$ Bp65 and HMGB1 expression in the pancreas of rats with severe acute pancreatitis. *Mol Med Rep*. 2018;18(6):5345-5352.

Zhou B, Weng G, Huang Z, Liu T, Dai F. Arctiin Prevents LPS-Induced Acute Lung Injury via Inhibition of PI3K/AKT Signaling Pathway in Mice. *Inflammation*. 2018;41(6):2129-2135.

Zhu Q, Lin X, Liu X, Hou T, Zhang M, Wang N, et al. Dynamic changes of proteasome and protective effect of bortezomib, a proteasome inhibitor, in mice with acute pancreatitis. *Biochem Biophys Res Commun*. 2018;505(1):126-133.

Received for publication on 27<sup>th</sup> September 2019

Accepted for publication on 16<sup>th</sup> June 2020