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ORIGINAL RESEARCH

Naringin Attenuated Acute Lung Injury in Rat Model with Acute Pancreatitis in Pregnancy through Inactivation of p38 MAPK Pathway

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Abstract

Acute pancreatitis in pregnancy can cause fetal lung injury. Naringin exhibits anti-inflammation effect against pulmonary injuries. However, whether naringin can alleviate lung injury associated with acute pancreatitis in pregnancy has not been elucidated. Establishment of acute pancreatitis in pregnancy model by sodium taurocholate showed that sodium taurocholate induced obvious pathological changes in pancreas. Histopathological changes in lungs were also aggravated post sodium taurocholate treatment with enhanced inflammation of neutrophils, thickened alveolar walls and alveolus collapse. Diagnostic blood tests for acute pancreatitis in pregnancy indicated that serum amylase and lipase were increased post sodium taurocholate treatment. Sodium taurocholate-induced rats were intragastrically administered with naringin to investigate the protective effect of naringin on acute lung injury induced by acute pancreatitis in pregnancy. Results revealed that administration of naringin could attenuate pathological changes in pancreas and lungs and decrease amylase and lipase. Moreover, naringin treatment attenuated sodium taurocholate-induced increase in inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6/1β in lungs. Sodium taurocholate-induced increase in phosphorylation of p38 was also reduced post naringin treatment. In conclusion, naringin could ameliorate acute lung injury and inflammation induced by acute pancreatitis in pregnancy by inhibiting the p38 pathway.

Keywords

Naringin, Acute lung injury, Inflammation, Acute pancreatitis in pregnancy, p38 MAPK

1. Introduction

Acute pancreatitis during pregnancy is a severe disease affecting 1/12000 to 1/1000 pregnancies [1], and is responsible for eighteen percent of perinatal deaths and five percent of maternal deaths [2]. The most fatal features of acute pancreatitis in pregnancy are the multiple organ dysfunction syndrome or systemic inflammatory response syndrome, that could result in intrauterine fetal death [3]. Among the multiple organs, lung is the most susceptible organ affected by acute pancreatitis in pregnancy that can lead to acute lung injury [4]. Acute pancreatitis in pregnancy is devoid of effective diagnosis and therapies, and anti-inflammation therapies might be a potential effective strategy for the treatment of acute lung injury associated with acute pancreatitis in pregnancy [5].

Naringin, the glycosidic form of naringenin, is a flavonoid widely found in grapefruit [6]. It demonstrates antioxidant, anti-inflammatory, lipidemic and hypoglycemic effects, and has protective effects on multiple organs [6]. For example, naringin could attenuate lipopolysaccharide-induced acute lung injury through antioxidant and anti-inflammatory activities [7]. Naringin repressed lung inflammation and maintained the oxidation/antioxidant balance to protect lungs against cigarette smoke-induced damages [8]. Acrolein-induced pulmonary injuries were also ameliorated by naringin through anti-apoptotic and anti-inflammation properties [9]. However, the role of naringin in acute lung injury associated with acute pancreatitis in pregnancy has not been reported.

P38 MAPK (mitogen-activated protein kinase) has been reported to be critical pathway for inflammation and stress responses [10]. Study has shown that MAPK was activated in rats with acute pancreatitis in pregnancy [11], and phosphorylation of p38 MAPK (p-p38) was enhanced in rats with acute pancreatitis in pregnancy [12]. MAPK could stimulate proinflammatory response during acute lung injury [13], and down-regulation of p-p38 could facilitate the amelioration of inflammatory response during acute pancreatitis in pregnancy [10]. Thus, regulation of MAPK pathway might be a strategy for the treatment of related syndromes in acute pancreatitis in pregnancy. Considering that naringin could repress p-p38 to reduce lipopolysaccharide-induced damage [14], this

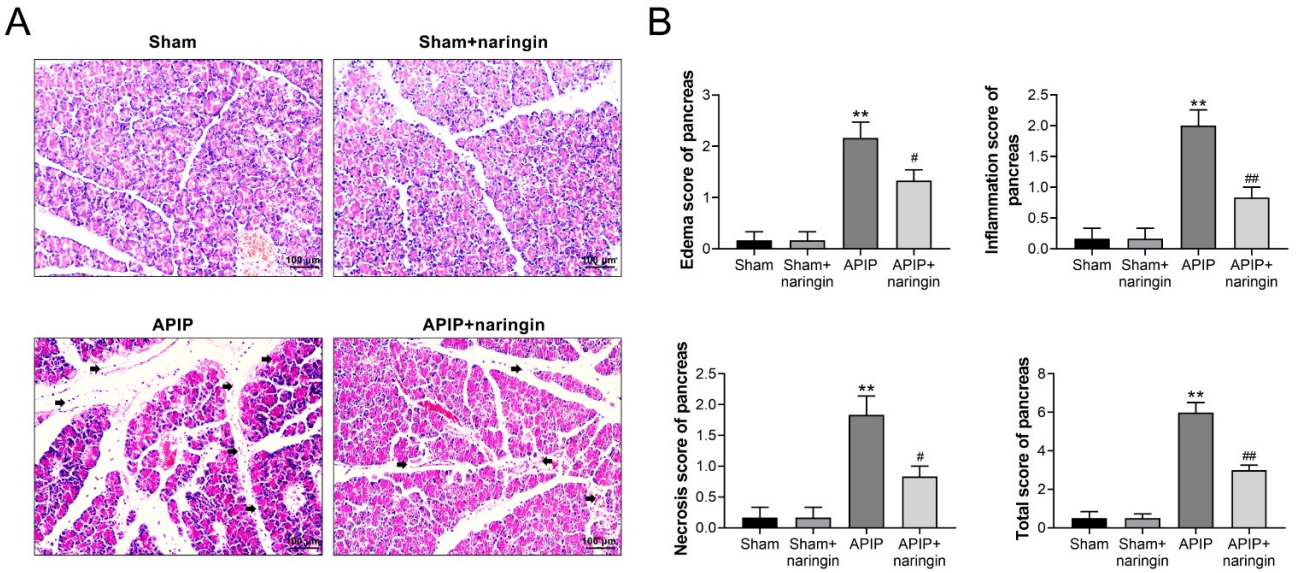

FIGURE 1. Amelioration of histopathologic injury in pancreas by naringin.
(A) Representative pathological changes in pancreas detected by H&E staining in magnification of ×200. Scale bars: 100 μm. (B) Histological scores of pancreatic tissue, including edema, necrosis, inflammation and total score of pancreas. (n = 6). ∗∗ $p < 0.01$ vs. sham group, #, ## $p < 0.05$, $p < 0.01$ vs. APIP group.

study hypothesized that MAPK might be involved in naringin-mediated acute lung injury associated with acute pancreatitis in pregnancy.

Rat model of acute pancreatitis in pregnancy was then established to investigate the protective effect and underlying mechanism of naringin against acute lung injury. These results would highlight the potential therapeutic value of naringin as an effective lung protective strategy.

2. Materials and methods

2.1 Animal experiments

Twenty-four pregnant Sprague-Dawley rats (390-450 g in weight and 17–19 days of the first gestation) were acquired from Shanghai Experimental Animal Center (Shanghai, China), and housed in facilities free to food and water. The experiments were approved by the Ethics Committee of The First Affiliated Hospital of Soochow University (Approval no.2018051) and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines. Rats were assigned into four groups: sham, sham with naringin, APIP (acute pancreatitis in pregnancy) and APIP treated with naringin. For establishment of APIP model, rats anesthetized with 3 mL/kg chloral hydrate, were retrograde infused with 1 mL/kg 5% sodium taurocholate solution (Sigma-Aldrich, St Louis, Mo, USA) into biliary-pancreatic duct. Pancreas in rats post sodium taurocholate treatment showed hemorrhaged and necrotic 5 minutes later, were appeared to be successful APIP model. For sham groups, rats were administered with 5% DMSO diluted in saline. The incision was closed, and 20 mL/kg of saline was subcutaneously injected into the back to compensate for fluid loss. For rats in the naringin groups, 40 mg/kg naringin were intragastrically administered into the rats one hour before sodium taurocholate treatment.

2.2 Histopathology analysis

Six hours after the indicated treatment, rats were euthanized and pancreatic and lung tissues were harvested. Formaldehyde-fixed and paraffin-embedded tissues were sliced into 4 μm sections. The sections were then stained with hematoxylin and eosin and visualized under microscope (Olympus, Tokyo, Japan). The pancreatic and lung histological assessment were determined according to previous study [10].

2.3 Determination of serum amylase and lipase

Six hours after the indicated treatment, rats were euthanized and blood samples were collected via BD vacutainer (Bio-Rad, Hercules, CA, USA). Samples were centrifuged at 1500 g for 10 minutes, and the supernatant was collected and conducted with automatic biochemical analyzer (Olympus, Tokyo, Japan) for determination of serum amylase and lipase.

2.4 Determination of cytokines

Six hours after the indicated treatment, rats were euthanized and lung tissues were harvested and homogenized. Homogenized tissues were centrifuged at 12000 g for 20 minutes, and the supernatant was collected and conducted with ELISA kits (Elabscience, Wuhan, China) for determination of TNF-α, IL-6 and IL-1β.

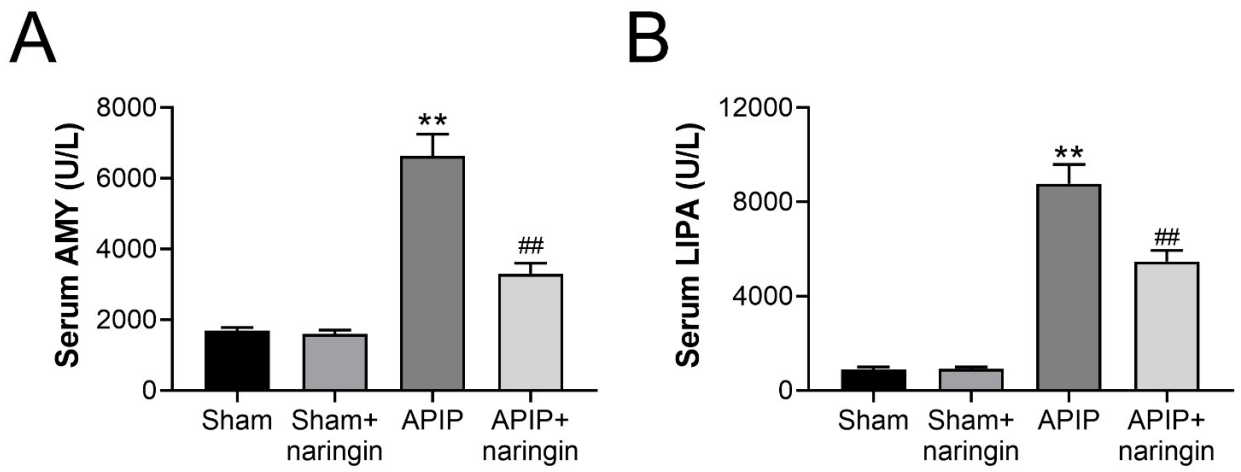

FIGURE 2. Reduce of serum amylase and lipase by naringin.
(A) Serum amylase was determined by the automatic biochemical analyzer.
(B) Serum lipase was determined by the automatic biochemical analyzer.
$** p < 0.01$ vs. sham group, ## $p < 0.01$ vs. APIP group.

2.5 Western blot

Protein concentration of homogenized lung tissues in RIPA buffer (Beyotime Institute of Biotechnology, Beijing, China) was determined by bicinchoninic acid assay kit (Pierce Biotechnology, Rockford, IL, USA). Samples (20 μg) were separated by SDS-PAGE and then transferred to polyvinylidene difluoride membranes (Merck Millipore, Billerica, MA, USA). After blocking in 5% fat-free milk, the membranes were subsequently incubated with the primary antibodies against p38 (1:2000, Abcam, Cambridge, UK), p-P38 (1:2500, Abcam) and β-actin (1:3000, Abcam). Following incubating with fluorescently labeled secondary antibody, the specific protein bands were visualized with Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA), and quantified by Quantity One 4.6.2 software (Bio-Rad).

2.6 Statistical analysis

Results of three independent experiments were presented as mean ± standard deviation. Following analyzing by Graphpad Prism 7.0, statistical analysis was performed with Mann–Whitney rank sum test or one-way analysis of variance followed by Tukey's test. $p < 0.05$ was considered as statistically significant.

3. Results

3.1 Amelioration of histopathologic injury in pancreas by naringin

Rat model with acute pancreatitis in pregnancy was established through injection with sodium taurocholate. Hematoxylin and eosin staining showed that sodium taurocholate induced severity of histopathologic injury in pancreas (Fig. 1A) with obvious pancreatic edema, necrosis and inflammatory cell infiltration (Fig. 1B). However, the histopathologic injury was attenuated by intragastric administration of naringin (Fig. 1A), as demonstrated by decreased edema, necrosis, inflammation and total score of pancreas (Fig. 1B). These results showed that naringin could protect pancreas against sodium taurocholate-induced histopathologic injury.

3.2 Reduce of serum amylase and lipase by naringin

Diagnostic blood tests of biomarkers for acute pancreatitis in pregnancy were then performed, and the results showed that serum amylase (Fig. 2A) and lipase (Fig. 2B) were increased by sodium taurocholate treatment. However, naringin attenuated sodium taurocholate-induced increase in serum amylase (Fig. 2A) and lipase (Fig. 2B), suggesting that naringin could reduce the severity of acute pancreatitis in pregnancy.

3.3 Amelioration of histopathologic injury in lung by naringin

Acute lung injury associated with acute pancreatitis in pregnancy was evaluated by hematoxylin and eosin staining. Results indicated that sodium taurocholate induced conspicuous infiltration of inflammatory cells, thickness of alveolar walls alveolus collapse and severe hemorrhage of alveolus compared with normal alveolar morphology in sham-operated rats (Fig. 3A and 3B). The histopathologic injury in lung induced by sodium taurocholate was alleviated by naringin treatment with decreased inflammatory cells, thickness of alveolar walls alveolus collapse and total score of lung (Fig. 3A and 3B). These results showed that naringin could reduce acute lung injury.

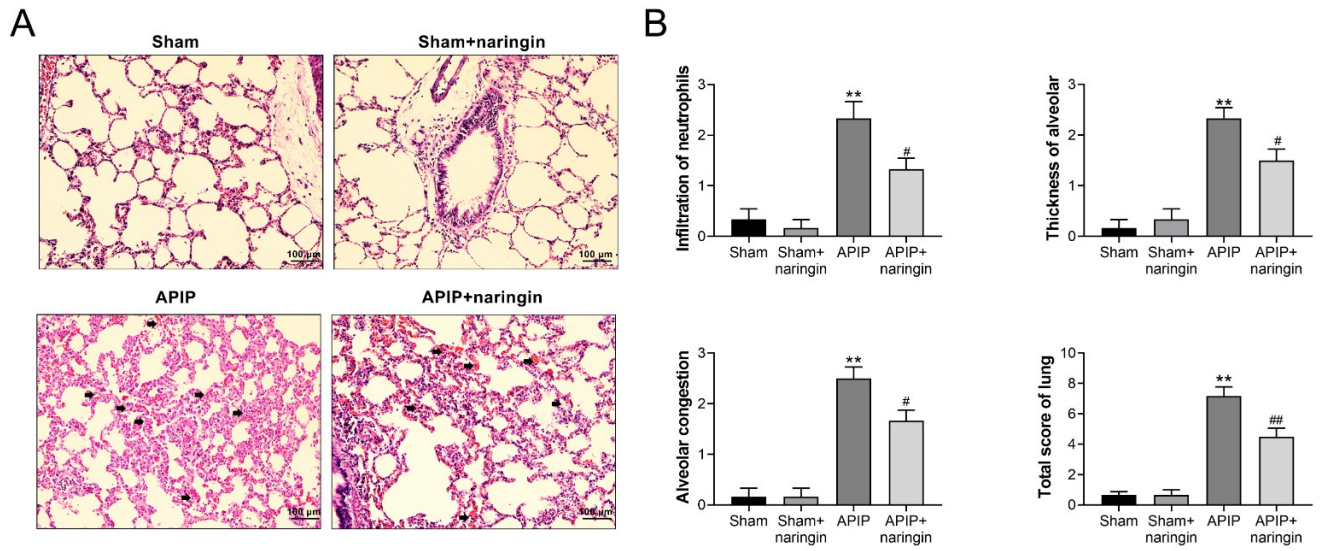

FIGURE 3. Amelioration of histopathologic injury in lung by naringin.
(A) Representative pathological changes in lungs detected by H&E staining in magnification of ×200. Scale bars: 100 μm.
(B) Histological scores of inflammatory cells, thickness of alveolar walls alveolus collapse and total score of lung of lungs. (n = 6).
∗∗ $p < 0.01$ vs. sham group, #, ## $p < 0.05$, $p < 0.01$ vs. APIP group.

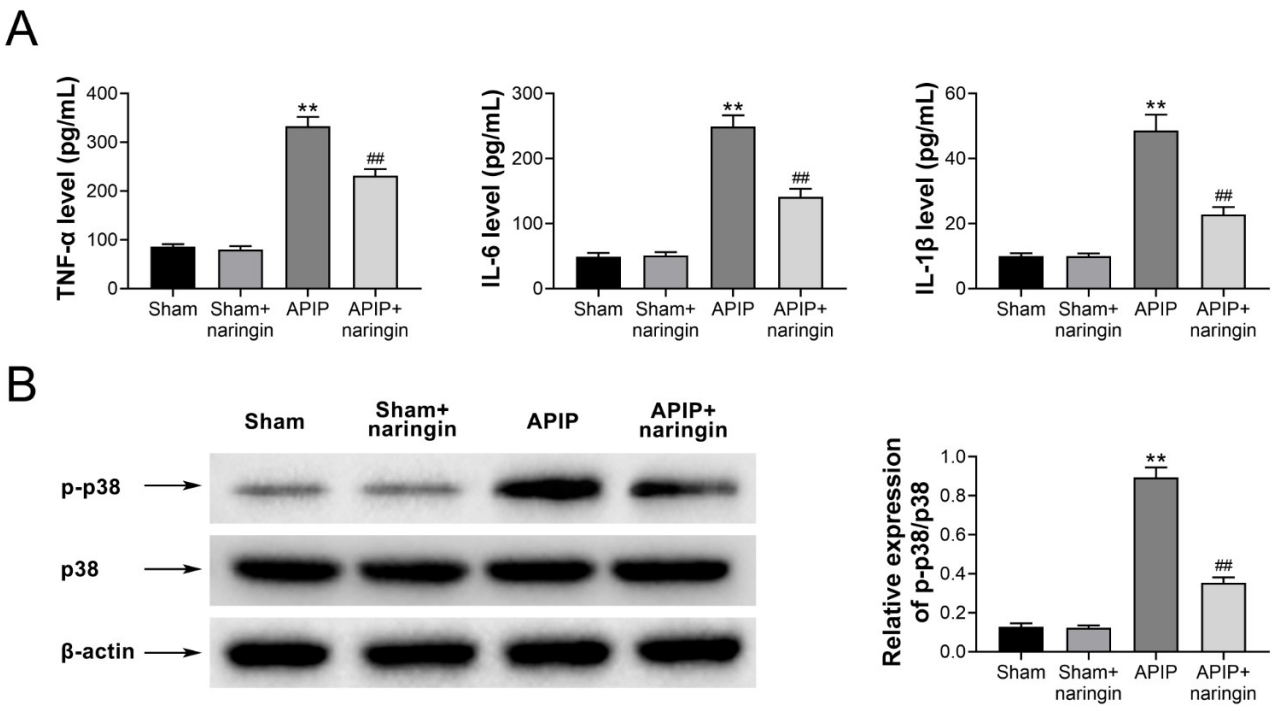

FIGURE 4. Naringin reduced cytokines in lungs of rats with acute pancreatitis in pregnancy through inactivation of MAPK.
(A) Levels of TNF-α and IL-6/1β in lungs detected by ELISA.
(B) Levels of p38 and p-p38 in lungs detected by western blot.
∗∗ $p < 0.01$ vs. sham group, ## $p < 0.01$ vs. APIP group.

3.4 Naringin reduced cytokines in lungs of rats with acute pancreatitis in pregnancy through inactivation of MAPK

Since inflammation contributes to acute lung injury, the levels of inflammatory cytokines were then evaluated. The levels of TNF-α and IL-6/1β (Fig. 4A) were increased in the lungs of rats with acute pancreatitis in pregnancy, suggesting severe pulmonary histopathology. Treatment with naringin decreased the level of TNF-α and IL-6/1β (Fig. 4A), and repressed

inflammation in acute lung injury. Sodium taurocholate significantly increase the expression of p-p38 in lungs (Fig. 4B), while treatment with naringin reversed the elevation of p-p38 (Fig. 4B), suggesting that naringin might reduce cytokines in lungs of rats with acute pancreatitis in pregnancy through inactivation of MAPK signaling pathway.

4. Discussion

Acute lung injury is a common complication of acute pancreatitis in pregnancy, showing obvious accumulation and infiltration of inflammatory cells, thereby leading to alveolar edema, leakage of alveolar capillary barrier and microvascular dysfunction [15]. Flavonoids have the antioxidative and anti-inflammatory functions and have been shown to prevent acute lung injury [16]. Flavonoid was also reported to attenuate acute pancreatitis [17]. Naringin was widely known as antioxidant and antiinflammatory flavonoid to attenuate acute lung injury [7, 9, 14]. Therefore, the involvement of naringin in acute lung injury during acute pancreatitis in pregnancy was then evaluated in this study.

Sodium taurocholate which can induce typical pathological damage in pancreas with edema and necrosis, is the most widely used agent to induce acute pancreatitis in pregnancy model [10]. Moreover, presence of abdominal pain or enhanced serum amylase and lipase have been widely used in diagnosis of acute pancreatitis in pregnancy [18]. Here, the results in this study showed that sodium taurocholate induced severity of histopathologic injury in pancreas and increased serum amylase and lipase, confirming the successful establishment of acute pancreatitis in pregnancy model in rats. However, pretreatment with naringin attenuated the severity of histopathologic injury in pancreas and decreased serum amylase and lipase, suggesting the potential protect role of naringin on acute lung injury during acute pancreatitis in pregnancy.

Previous study has shown that naringin treatment could ameliorate lipopolysaccharide-induced recruitment of neutrophils, edema and inflammation in lungs [7]. Here, the data from hematoxylin and eosin staining of lungs showed that sodium taurocholate-induced conspicuous infiltration of inflammatory cells, thickness of alveolar walls alveolus collapse and severe hemorrhage of alveolus in lungs were ameliorated by naringin, confirming that naringin could reduce acute lung injury.

Acute pancreatitis in pregnancy was reported to lead to infiltration of neutrophils, that can secret inflammatory cytokines, including TNF-α and IL-6, and IL-1β, to stimulate systemic inflammatory response and multi-organ dysfunction [10]. TNF-α, an indicator of inflammation, could activate cytokines and chemokines to aggravate the acute lung injury and is associated with the severity of acute pancreatitis in pregnancy [10]. IL-6 and IL-1β could also promote uncontrolled inflammation to induce cell apoptosis and damage, demonstrating cytotoxic effects on cell membrane permeability and extravasation of more inflammatory cytokines [10]. Here, this study indicated that sodium taurocholate induced severe inflammation in lungs with increased TNF-α and IL-6 and IL-1β. However, naringin treatment repressed infiltration of neutrophils to reduce levels of TNF-α and IL-6 and IL-1β in lungs, thus preventing inflammation in acute lung injury. Moreover, increased cell membrane permeability in lung tissues and endothelial cells apoptosis were shown to be closely associated with acute lung injury [19], and naringin demonstrated anti-apoptotic role in acrolein-induced pulmonary injuries [9]. The role of naringin on cell apoptosis involved in acute pancreatitis in pregnancy needs to be further investigated.

Naringin was reported to inhibit activation of p38 MAPK to prevent ultraviolet B-induced skin damage [20], high glucose-induced injury [21] and lipopolysaccharide-induced damage [14]. p38 MAPK was activated during acute pancreatitis in pregnancy with increase in phosphorylated p38MAPK in pancreatic and lung tissues [22]. Inactivation of p38 MAPK with decreased p-p38 MAPK could attenuate the severity of acute pancreatitis in pregnancy [23]. As anticipated, p-p38 was increased in lungs of rats post sodium taurocholate treatment, while treatment with naringin decreased p-p38 to reduce the acute lung injury associated with acute pancreatitis in pregnancy. Additionally, NF-κB is also an essential pathway involved in inflammation in the pathogenesis of acute pancreatitis in pregnancy [24], and naringin could repress NF-κB pathway to ameliorate lipopolysaccharide-induced acute lung injury [7]. Whether NF-κB is implicated in the regulatory role of naringin on acute pancreatitis in pregnancy should also be investigated in the further studies.

5. Conclusion

In summary, this study provided evidence that naringin could participate in acute lung injury associated with acute pancreatitis in pregnancy, and naringin markedly ameliorated pancreatic and lung injuries through inactivation of p38 MAPK pathway. Therefore, the findings might help for the prevention and treatment of acute pancreatitis in pregnancy in clinic.

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CONFLICTS OF INTEREST

The authors state that there are no conflicts of interest to disclose.

ETHICS APPROVAL

Ethical approval was obtained from the Ethics Committee of The First Affiliated Hospital of Soochow University (Approval no.2018051).

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

AUTHORS' CONTRIBUTIONS

Yaqin Li designed the study, supervised the data collection, analyzed the data, interpreted the data, prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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