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# Calycosin alleviates cerulein-induced acute pancreatitis by inhibiting the inflammatory response and oxidative stress via the p38 MAPK and NF-κB signal pathways in mice

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ABSTRACT

Acute pancreatitis (AP) is a common acute abdominal disease accompanied by systemic inflammatory response syndrome, and could even be complicated by multiple-organ damage. This study aimed to examine whether calycosin, an isoflavone isolated from *Radix astragali* with antioxidant and anti-inflammatory activity, could protect against AP induced by cerulein. To this end, Balb/C mice were injected with cerulein (50 μg/kg) to establish the animal model of AP. Calycosin (25 and 50 mg/kg, p.o.) was administered 1 h prior to the first cerulein injection. After the last injection of cerulein, the mice were sacrificed and blood was obtained for cytokine analysis. The pancreas was removed for morphological examination, myeloperoxidase (MPO) and malondialdehyde (MDA) analyses, immunohistochemistry, and western blot analysis. Calycosin treatment reversed the increased serum levels of amylase and lipase, alleviated the pathological damage in the pancreas, and decreased the levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β in mice with AP. Additionally, calycosin significantly reduced cerulein-induced pancreatic edema, inhibited MPO activity and increased superoxide dismutase (SOD) activity, and inhibited the expression of NF-κB/p65 and phosphorylation of the inhibitor of NF-κB (IκBα) and p38 MAPK. These results suggested that calycosin protects against AP by exerting anti-inflammatory and anti-oxidative stress effects via the p38 MAPK and NF-κB signal pathways. Calycosin's benefits for AP patients need to be explored further.

## 1. Introduction

Acute pancreatitis (AP) is a common acute abdominal disease, characterized by a pancreatic inflammatory response that can range from mild edema to severe tissue necrosis; it could be complicated by multiple-organ damage [1]. In the recent years, the incidence of pancreatitis has increased worldwide in the general population; in most cases, it is caused by factors such as biliary abnormalities, alcohol consumption, drugs, trauma, infection, anatomical abnormalities, and autoimmune conditions [2,3]. Thus far, the pathophysiology of AP has not yet been fully understood. The development of AP is considered to be a multistep process. AP manifests as locally limited inflammation and is amplified by the actions of diverse inflammatory mediators such as cytokines, reactive oxygen species (ROS), chemokines, leukocyte adhesion molecules, lipids, and gaseous mediators, resulting in the induction of a pancreatic inflammatory response [4,5]. Recent experimental studies have suggested that the cellular mechanism orchestrating these inflammatory mediators involves transcription factors such as nuclear factor kappa-B (NF-κB) in the initiation of AP. Studies have shown that the NF-κB is able to regulate a series of inflammatory mediators involved in AP, such as cytokines and adhesion molecules [6,7]. In addition, mitogen-activated protein kinases (MAPKs) such as p38, c-Jun N-terminal kinase (JNK), and extracellular regulated protein kinases1/2 (ERK1/2) have been proven to be involved in the mechanism underlying cytokine expression in AP [6,8,9].

Calycosin is the major active component in *Radix astragali* (Huangqi in Chinese), which is widely used in traditional Chinese medicines. It was reported that calycosin exhibits various biological activities such as antitumor [10,11], neuroprotective [12], proteogenesis [13], anti-Alzheimer [14], antidiabetic [15], anti-inflammatory [16], and proangiogenic activities [17]. Calycosin also exhibited very effective antioxidative activity like many flavonoids, such as decreasing

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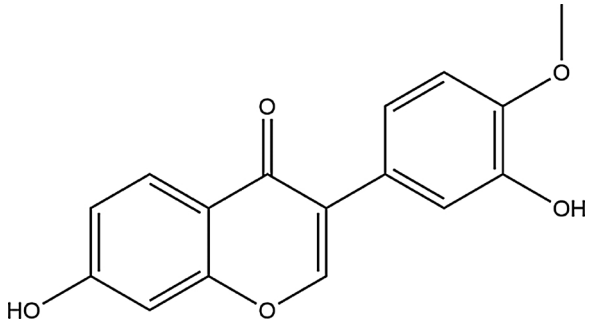


**Fig. 1.** The structure of calycosin.

**Table 1**
The criteria of pancreatic histological score.

| Pathological Factors | Histological Score | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Cell necrosis | none | < 10% necrosis | < 40% necrosis | > 40% necrosis |
| Vacuolization | none | < 20% acini with vacuoles | < 50% acini | > 50% acini |
| Inflammation | none | inflammatory cells present at interlobular areas | present at intralobular areas | present at interacini |
| Acinar edema | none | interlobular edema | intralobular edema | interacinar edema |

malondialdehyde (MDA) and ROS level and increasing superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities [18,19]. Recently, it was reported that the calycosin exhibited a significant therapeutic effect against inflammatory bowel disease in a mouse model of colitis induced by dextran sodium sulfate, which was associated with anti-inflammatory and antioxidant activities [20]. Based on the above evidence, we hypothesized that calycosin will have a good therapeutic effect on AP. Thus, the purpose of our present study was to investigate the therapeutic effect of calycosin against AP in a mouse model of cerulein-induced AP and elucidate the potential underlying mechanism.

## 2. Materials and methods

### 2.1. Reagents

Calycosin ($C_{16}H_{12}O_5$, the structure is shown in Fig. 1) and cerulein were purchased from Sigma Chemical Co (St. Louis, MO, USA). The cytokines enzyme-linked immunosorbent assay (ELISA) kits including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, and IL-10 were purchased from R&D System, Inc. (Minneapolis, MN, USA). The amylase, lipase, myeloperoxidase (MPO), and SOD assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The antibodies of NF-κB/p65, inhibitor of NF-κB (IκBα), p-IκBα, p-p38, p38, p-ERK1/2, ERK1/2, p-JNK, JNK, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Cell Signaling Technology (Boston, MA).

### 2.2. Animals

Male Balb/C mice (weight: 20 ± 2 g, age: 8–10 weeks) were obtained from Shanghai Laboratory Animal Co., Ltd. (Shanghai, China). The mice were housed under standard laboratory conditions (temperature: 22 ± 2 °C, humidity: 50–60%). All animal experiments were performed in accordance with the guidelines of the Experimental Research Institute of Jining No. 1 People's Hospital.

### 2.3. Animal model and experimental design

AP was induced by intraperitoneally (i.p.) injecting cerulein (50 μg/kg) every hour for eight consecutive hours. Calycosin was administered intraperitoneally (i.p.) (25 or 50 mg/kg) 1 h before the first cerulein injection in the calycosin treatment group. The mice were randomly allocated to the following four experimental groups (n = 20 for each group): (1) normal control (NC) group receiving equal volume of normal saline (NS) instead of cerulein; (2) cerulein group receiving cerulein for AP induction; (3) high-dose calycosin group intraperitoneally injected with 50 mg/kg calycosin 1 h before cerulein administration; and (4) low-dose calycosin group intraperitoneally injected with 25 mg/kg calycosin 1 h before cerulein administration. Blood samples and pancreas were obtained when all animals were sacrificed at 16 h after the last cerulein injection.

### 2.4. Pancreatic edema analysis

The pancreas was obtained after the mice were sacrificed and weighed using aluminum foil, after which it was dried for 12 h at 95 °C and reweighed. The pancreatic edema index was determined as a percentage of tissue wet weight [21].

### 2.5. Serum enzymes and cytokine analysis

Blood samples were obtained from the eyeballs of the mice to determine serum enzymes and cytokines. Plasma was separated after centrifugation at 12,000 rpm for 10 min. Serum levels of amylase and lipase were determined by amylase activity assay and lipase assay kits. Serum concentrations of TNF-α, IL-6, TL-1β, and IL-10 were determined using commercial ELISA kits.

### 2.6. Analysis of MPO and SOD activity in pancreas tissue

Part of the pancreas was collected for MPO and SOD activity assays. Pancreas tissues (100 mg) was homogenized in NS. The homogenate was then centrifuged at 12,000 rpm for 10 min at 4 °C to obtain the supernatant. MPO and SOD activity in the pancreas tissues was determined using the MPO assay kit and SOD assay kit, respectively. MPO activity was expressed as U/g tissue, and SOD activity was expressed as U/mg tissue.

### 2.7. Histological examination

Paraffin-embedded pancreas samples were sectioned (5 μm) and stained with hematoxylin and eosin. All the assessments were performed by an experienced pathologist blinded to the experimental design according to the criteria described previously [8]. Specimens were scored as normal to severe in terms of the extent and severity of pancreatitis, including the degree of vacuolization, edema, acinar cell necrosis, and inflammatory cell infiltration on a scale of 0–3 (Table 1).

### 2.8. Immunohistochemical staining analysis

Immunohistochemical staining was performed to analyze the expression of NF-κB/p65 with Elivision™ detection kit (Glostrup, Denmark) according to the manufacturer's instructions. Briefly, the pancreas sections (4 μm) were deparaffinized and rehydrated using xylene and graded alcohols, and then immersed in a target retrieval solution in a water bath for 30 min. Endogenous peroxidase was blocked with 3% $H_2O_2$ for 10 min, and nonspecific binding was blocked with goat serum for 45 min. Then, the slides were incubated with the primary rabbit anti-NF-κB/p65 antibody and horseradish peroxidase (HRP)-polymer secondary antibody successively. Then, the slides were stained with diaminobenzidine (DAB) and counterstained with methyl green.