

# The effects of glycyrrhizin on acute pancreatitis in mice

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**Abstract. – OBJECTIVE:** This study aimed to investigate the effects of glycyrrhizin on acute pancreatitis in mice.

**MATERIALS AND METHODS:** Sixty Balb/c mice were randomly divided into four groups as control group, model group, low dose group and high dose group (n=15). Acute pancreatitis was induced by intraperitoneal injection of Caerulein (100  $\mu$ g/kg) hourly for 6 times. Mice in low dose group and high dose group received intraperitoneal administration of 15 mg/kg and 45 mg/kg glycyrrhizin respectively 4 hours before Caerulein injection. Mice in four groups were sacrificed in three equal lots at 8, 16 and 24 hours after model construction. High Mobility Group Box-1 (HMGB1) expression and serum levels of amylase, TNF- $\alpha$  and IL-6 were determined. The pancreatic tissues were taken for histopathologic analysis.

**RESULTS:** Amylase, TNF- $\alpha$ , IL-6 and HMGB1 levels were significantly higher and pancreas lesion was severer in model group than in control group. However, Amylase, TNF- $\alpha$ , IL-6 and HMGB1 levels in low dose group and high dose group decreased significantly compared with model group. The pancreas lesion was also improved after administration of glycyrrhizin.

**CONCLUSIONS:** Glycyrrhizin could decrease the levels of pro-inflammatory cytokines and downregulate the expression of HMGB1 which finally improved the pancreas lesion in mice with acute pancreatitis.

*Key Words:*

Glycyrrhizin, Acute pancreatitis, HMGB1.

## Introduction

Acute pancreatitis (AP) is one of the most common acute abdomen diseases which might result in intestinal mucosal injury and ectopia between intestinal flora and endotoxin. It is considered as an inflammatory disease with high mortality and morbidity<sup>1,2</sup>. However, the pathogenesis of AP is not fully understood. Recent studies have showed that AP was related with autophagy closely.

Autophagy is an intracellular catabolic pathway which degrades long-lived proteins and cytoplasmic organelles through a lysosome-driven process<sup>3,4</sup>. It was considered that inhibition of autophagy in pancreas might treat acute pancreatitis.

High Mobility Group Box-1 (HMGB1) protein is a late-acting mediator which might cause a delayed reaction and prolonged the inflammatory reaction in tissues with acute pancreatitis<sup>5-7</sup>. Recent studies have showed that HMGB1 could interact with Beclin1 directly and induce autophagy since the abnormal activation of autophagy had a close relation with acute pancreatitis<sup>8,9</sup>. It is considered that downregulation of HMGB1 to inhibit autophagy might ameliorate acute pancreatitis. Glycyrrhizin extracted from rhizome of liquorices has been shown to exhibit anti-inflammatory, anti-oxidative and antiallergic effects<sup>7</sup>. We studied the effects of glycyrrhizin on acute pancreatitis in mice and explored the potential mechanism of glycyrrhizin.

## Materials and Methods

### Animals

Balb/c mice were obtained from Academy of Military Medical Sciences. Sixty Balb/c mice were randomly divided into four groups as control group, model group, low dose group and high dose group (n=15). The mice in model group, low dose group and high dose group were injected with caerulein (Sigma, St. Louis, MO, USA) (100  $\mu$ g/kg) hourly for 6 times to induce AP while the control group only received an injection of same amount of saline. Mice in low dose group received intraperitoneal administration of 15 mg/kg glycyrrhizin and the high dose group received an injection of 45 mg/kg glycyrrhizin 4 h before caerulein injection. Mice in four groups were sacrificed in three equal lots at 8, 16 and 24 h after model construction. Blood samples were collected to test the amylase, TNF-

$\alpha$  and IL-6 levels. Pancreatic tissues were isolated for histopathologic analysis and Western blot analysis. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA). Eighth Edition, 2010. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of People's Hospital of Zhengzhou.

#### **Determination of Serum Amylase, TNF $\alpha$ and IL-6 Levels**

Blood samples with anticoagulant were used to test amylase level by Automatic Biochemistry Analyzer. Fresh blood samples were centrifuged at 5,000 g for 10 min at 4°C. Plasma supernatant was collected to determine the TNF- $\alpha$  and IL-6 levels according to the related ELISA kit instruction (Dakewe Biotech Company, Shanghai, China).

#### **Histopathologic Analysis**

The pancreatic tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. The tissues were cross-sectioned in 10 mm sections and placed in slides. The sections were put into water bath of 60°C to make the paraffin melted. The sections then received dewaxing in dimethylbenzene for 5 min followed by incubation in 95% ethanol, 80% ethanol and 70% ethanol for 15 min. The sections were stained with hematoxylin and eosin (HE) and scored with a maximum score of 24 as defined by Schmidt.

#### **Western Blot Analysis**

Pancreatic tissues were lysed with lysis buffer (Beyotime, Shanghai, China) containing a protease inhibitor cocktail. After incubation in ice for 30 min, tissue homogenates were centrifuged at 15,000 g for 10 min at 4°C. Protein concentra-

tions were determined using a BCA protein assay kit. Equal amounts of lysates were fractionated by SDS-PAGE and transferred to Hybond-c nitrocellulose membrane. The membranes were blocked for half an hour at room temperature in TBST buffer containing 5% milk. The membranes were then incubated in TBST buffer (5% milk) containing HMGB1 or GAPDH antibody (Santa-Cruz, CA, USA) at 4°C overnight. After washed 3 times by TBST buffer, the membranes were incubated for an hour at room temperature in TBST buffer (5% milk) containing goat anti-rabbit IgG or anti-mouse IgG (Abcam, Cambridge, UK). The blots were visualized by incubation with related chemiluminescence kit and exposing to light-sensitive film. The bands were quantified as "intensity $\times$ area" using Quantity One software and statistically analyzed.

#### **Statistical Analysis**

All data were analyzed using SPSS13.0 software (SPSS Inc, Chicago, IL, USA) and were expressed as the mean $\pm$ standard deviation. Comparisons between groups were tested by One-Way ANOVA analysis and LSD test.  $p < 0.05$  was considered statistically significant.

## **Results**

#### **Effects of Glycyrrhizin on Amylase, TNF- $\alpha$ and IL-6 Levels**

As shown in Table I and Table II, levels of amylase, TNF- $\alpha$  and IL-6 in model group increased significantly compared with control group at different time point ( $p < 0.05$ ). However, amylase, TNF- $\alpha$  and IL-6 levels in low dose group and high dose group decreased at three time point compared with model group ( $p < 0.05$ ) and the high dose group decreased more.

**Table I.** Comparison of the AMY level in different treatment groups.

Groups	AMY		
	8 h	16 h	24 h
Control group	651.42 $\pm$ 120.19	692.77 $\pm$ 87.77	681.22 $\pm$ 99.09
Model group	4389.11 $\pm$ 450.25 <sup>a</sup>	4491.17 $\pm$ 38.91 <sup>a</sup>	5000.13 $\pm$ 389.69 <sup>a</sup>
Low dose group	3421.55 $\pm$ 310.27 <sup>b</sup>	3839.25 $\pm$ 294.10 <sup>b</sup>	4012 $\pm$ 317.54 <sup>b</sup>
High dose group	1943.12 $\pm$ 120.44 <sup>b,c</sup>	2471.99 $\pm$ 398.90 <sup>b,c</sup>	2698.78 $\pm$ 333.77 <sup>b,c</sup>

Note: <sup>a</sup>Compared with control group,  $p < 0.05$ ; <sup>b</sup>Compared with model group,  $p < 0.05$ ; <sup>c</sup>Compared with low dose group,  $p < 0.05$ .

**Table II.** Comparison of TNF- $\alpha$  and IL-6 levels in different treatment groups.

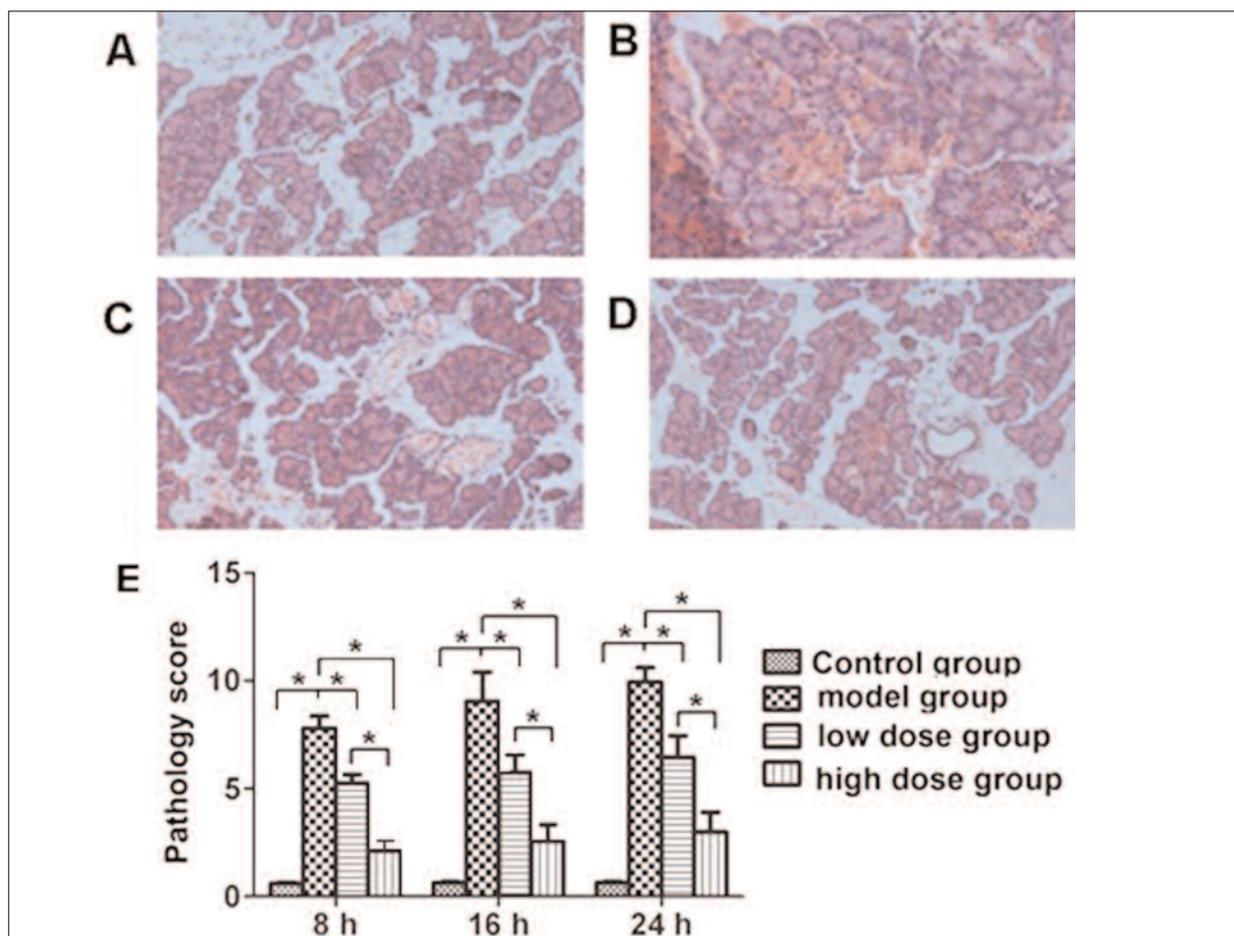
Groups	IL-6			TNF- $\alpha$		
	8 h	16 h	24 h	8 h	16 h	24 h
Control group	90.12 $\pm$ 10.33	91.34 $\pm$ 9.90	92.14 $\pm$ 11.32	21.45 $\pm$ 7.29	20.44 $\pm$ 7.82	22.14 $\pm$ 7.39
Model group	341.29 $\pm$ 19.39 <sup>a</sup>	389.01 $\pm$ 20.17 <sup>a</sup>	369.43 $\pm$ 21.10 <sup>a</sup>	57.32 $\pm$ 9.25 <sup>a</sup>	68.12 $\pm$ 9.97 <sup>a</sup>	83.12 $\pm$ 10.45 <sup>a</sup>
Low dose group	243.22 $\pm$ 17.89 <sup>b</sup>	283.12 $\pm$ 18.09 <sup>b</sup>	298.91 $\pm$ 17.90 <sup>b</sup>	45.33 $\pm$ 9.25 <sup>b</sup>	53.47 $\pm$ 8.99 <sup>b</sup>	69.91 $\pm$ 10.15 <sup>b</sup>
High dose group	151.22 $\pm$ 13.43 <sup>b,c</sup>	173.33 $\pm$ 13.90 <sup>b,c</sup>	190.25 $\pm$ 14.01 <sup>b,c</sup>	30.47 $\pm$ 8.15 <sup>b,c</sup>	39.99 $\pm$ 7.82 <sup>b,c</sup>	47.17 $\pm$ 8.54 <sup>b,c</sup>

Note: <sup>a</sup>Compared with control group,  $p < 0.05$ ; <sup>b</sup>Compared with model group,  $p < 0.05$ ; <sup>c</sup>Compared with low dose group,  $p < 0.05$ .

**Effects of Glycyrrhizin on Pancreatic Pathohistology**

As shown in Figure 1B, the pancreatic tissues in model group showed congestion, edema and disordered arrangement of pancreatic lobule at 8 h after model construction. Treatment with gly-

cyrrhizin effectively improved these symptoms (Figure 1C and D). The histological score for injury was assessed as defined by Schmidt. Compared with control group, the histological score of model group was much higher at different time point ( $p < 0.05$ ). Meanwhile, the histologi-



**Figure 1.** Effects of Glycyrrhizin on Caerulein-induced histological injury of pancreas. Representative hematoxylin-eosin stained sections of pancreatic tissues (Magnification,  $\times 400$ ) from control group (A), model group (B), low dose group (C) and high dose group (D). E, Histological scores of four groups.

cal scores of low dose group and high dose group were lower than model group at different time point ( $p < 0.05$ ) and the high dose group decreased more at three time point (Figure 1E).

### Effects of Glycyrrhizin on HMGB1 Expression

HMGB1 expression in pancreatic tissues of four groups was investigated by western blot. As shown in Figure 2A, HMGB1 expression in model group increased significantly compared with control group at different time point ( $p < 0.05$ ). Treatment with glycyrrhizin effectively decreased HMGB1 expression and the high dose group decreased more compared with low dose group.

## Discussion

AP is a kind of clinically acute abdomen with many complications. Since its high mortality, AP has become the focus of clinical research. In the progress of AP, a large number of inflammatory mediators released and caused chain reaction which might cause damage to the body<sup>10</sup>. Anti-inflammatory therapy is the common used method to treat AP. Glycyrrhizin, one of the extracts from rhizome of liquorices, has been shown to exhibit anti-inflammatory, anti-oxidative and antiallergic effects<sup>11,12</sup>.

We investigated the effects of glycyrrhizin on AP in mice. Our results showed that glycyrrhizin could effectively decrease plasma amylase, IL-6 and TNF- $\alpha$  levels and alleviate the histological injury induced by Caerulein in mice. Our work

suggested that downregulation of inflammatory mediators might contribute to the treatment of AP by glycyrrhizin.

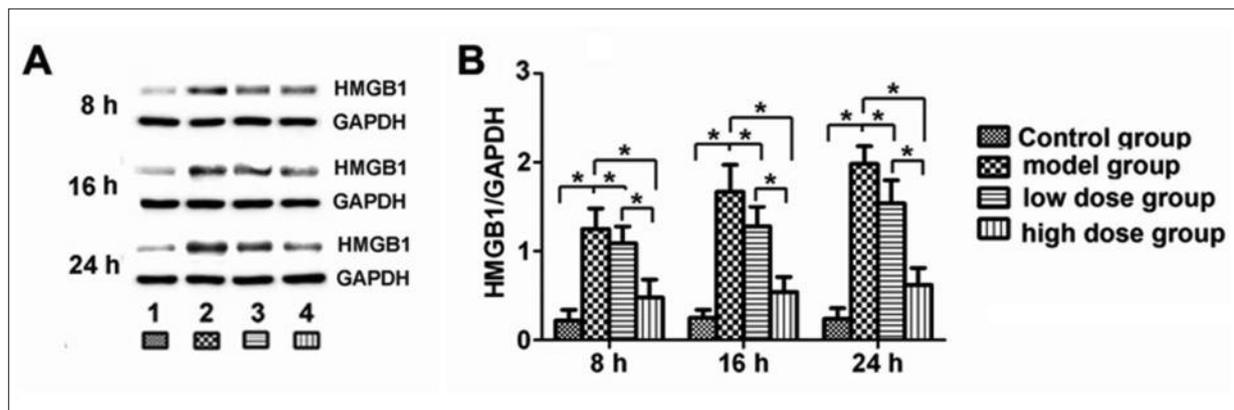
HMGB1 is a non-histone chromosomal protein of 215 amino acids and is widely expressed in brain, liver, lymph node, kidney and pancreatic tissues. It plays an important role in nucleosome stability, DNA repair, cell differentiation and other physiological processes<sup>13-16</sup>. Recent studies have showed that inflammatory cytokines promoted HMGB1 transfer from nucleus to extracellular space and the secreted HMGB1 could interact with inflammatory cytokines to maintain the inflammatory process<sup>17-19</sup>. As reported, HMGB1 expression was upregulated in AP and was positively related with pancreatic injury. Besides, HMGB1 expression was closely related with autophagy in AP<sup>20-22</sup>. We investigated the effects of glycyrrhizin on HMGB1 expression by western blot. Our results showed that glycyrrhizin could effectively decreased HMGB1 expression in AP mice induced by Caerulein.

## Conclusions

Glycyrrhizin could effectively alleviate AP in mice which might through down-regulating serum inflammatory mediators and decreasing the expression of HMGB1 in pancreatic tissues.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.



**Figure 2.** Glycyrrhizin decreased HMGB1 expression in mice with acute pancreatitis. **A**, Western blot analyzed the expression level of HMGB1 protein in different treatment mice. **B**, Quantitative analysis of the expression level of HMGB1 protein.

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