

# Gastroprotective effects of Chinese *Rana chensinensis* skin collagen against ethanol-induced gastric ulcer in mice

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**Abstract:** Gastric ulcer is a common gastrointestinal disease caused by excessive gastric acid secretion, which has been recognized as one of the most common causes of morbidity and mortality in the world. The skin of *Rana chensinensis* is rich in collagen and many previous studies have shown that it has certain bioactivity. Therefore, we extracted and purified collagen with a molecular weight less than 10000 Da from the skin of *Rana chensinensis*, and studied its gastric protective mechanism through the model of ethanol-induced gastric ulcer in Balb/c mice. The results showed that through macroscopic observation and significantly reduced ulcer index, it was proved that PCRCS could protect gastric mucosa and alleviate the damage of ethanol to gastric mucosa. PCRCS reduced ethanol-induced oxidative stress by boosting depleted SOD levels and dramatically lowering MDA levels, as well as significantly reducing lipid peroxidation. Additionally PCRCS (Protein Chinese *Rana chensinensis* Skin) additionally decreased the launch of inflammatory mediators TNF- $\alpha$  and IL-6 and more desirable the content material of protective elements NO and PGE2 in gastric mucosa. Based on these findings, we believe that PCRCS has potential stomach protective effects on ethanol-induced gastric ulcer, which may be achieved by inhibiting oxidative stress and stomach inflammation.

**Keywords:** *Rana chensinensis* skin, collagen, gastric ulcers, anti-inflammation, antioxidant.

## INTRODUCTION

With the change in people's diet and lifestyle, the incidence of gastric ulcers is increasing, which has a negative impact on people's health, quality of life and life safety. The gastric ulcer, also known as peptic ulcer, is a frequent and frequently-occurring disorder characterized by gastric mucosa injury. Its clinical incidence rate is high, affecting about 10% of the global population and the incidence rate in China is 6.1% (Sharifi-Rad *et al.*, 2018; Xie *et al.*, 2020). The disease can be caused by diet, helicobacter pylori and drug infections (such as NSAIDs) (Ciciliato *et al.*, 2022). After the onset, patients may have acid reflux, abdominal pain, bleeding and other symptoms and gastric perforation may occur in severe cases, which is not conducive to health (Mansour *et al.*, 2022). Recently, drugs for treating gastric ulcer have been effectively used, including antacids, proton pump inhibitors and histamine H<sub>2</sub> receptor antagonists. However, these drugs may still cause side effects that cannot be ignored, such as liver inflammation, nephritis and gastric acid deficiency (Devi *et al.*, 2021; Schubert 2017). Therefore, many studies have focused on new methods to prevent gastric mucosal damage.

Amphibian skin contains many bioactive substances, which have great application value and development potential (Borges *et al.*, 2022). Amphibian medicine has a long history in China and there are more than 30 species of medicinal amphibians in the "Chinese Medical

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Literature Library of Medicinal Animals". Ancient prescriptions are mainly used to treat toothache and sore poison. The existing research shows that related proteins extracted from the Chaco tree frog (*Boana raniceps*) and frog skin can be used in anti-inflammatory, anti-oxidation, bacteriostasis, inhibition of gastric acid secretion and wound healing (de Sousa *et al.*, 2022; Ye *et al.*, 2022). Up to now, most of the studies on *R.chensinensis* have focused on *in vitro* studies, showing good antibacterial and anti-inflammatory effects (Chen *et al.*, 2018; Ji *et al.*, 2022; Sang *et al.*, 2018). However, there are hardly any reports about pharmacological studies of collagen from *Rana chensinensis* skin in treating gastrointestinal diseases *in vivo*. Therefore, in this study, collagen (molecular weight less than 10000 Da) was extracted and purified from the skin of *R.chensinensis* and the protective effect on ethanol-induced gastric ulcers was discussed.

## MATERIALS AND METHODS

### Materials

L-NAME and carbenoxolone are products of Sigma Company; anhydrous ethanol and formalin are products of Tianjin Chemical Reagent Factory. Cimetidine at Shanxi Tongda Pharmaceutical Co., LTD. All reagents used in this study were of analytical grade.

### Animals

*R. chensinensis* of both sexes (weight range 15-20g) were collected in Changbai Mountain ecological park (Antu,

China). The animals were anesthetized by immersion in ice and sacrificed by decapitation and pithing. Skin was immediately removed, frozen at -80°C until the time of extraction.

Male Balb/c mice (6-7 weeks old; 20-25 g) were obtained from Yanbian University (Jilin, China). Before the experiments, acquired adaptive feeding was performed for 1 week in standard animal rearing conditions with ample water and standard chow. Keep room temperature at 25°C±1°C and the humidity was 50%±3%. Simultaneously, the period of light dark alteration was 12 h/12 h. The feeding conditions are in accordance with CPCSEA (Committee for Control and Supervision of Animal Experiments) standards. All animal experiments complied with the ARRIVE guidelines.

#### **Collagen extraction of *R. chensinensis* skin**

The skin of *R. chensinensis* (500g) was chopped and washed with distilled water thrice. After that the skins were immersed in NaCO<sub>3</sub> (200mL, 10%) for 4h and washed once again. Collagen was successively extracted from the skin with papain (100mg) in citrate buffer solution (200mL, pH=5.5). The enzymatic hydrolysate was stirred for 36 h at room temperature and filtered with gauze. Sodium chloride (40 g) was dissolved in a filtrate. After 24h, the filtrate was percolated; the precipitate was dissolved in acetic acid (10mL, 0.5mol/L) and lyophilized to obtain collagen (Neves *et al.*, 2017).

#### **Determination of the molecular weight of CRCS**

The CRCS (Chinese Rana Chesinensis Skin) molecular weight was determined by Sephadex G100 gel pillar (1.1 cm× 60 cm) and the particle size of the Sephadex G100 gel pillar was 100-200 mesh. 0.3mL samples were injected into a gel column and elution was carried out with 0.05mol/L Tris-HCl (pH 7.2 and a flow rate of 0.3mL/min) at 25°C. The absorbance at 220 Nm was detected. The correspondence relationship between the peak elution volume and molecular weight and the molecular weight of the CRCS can be estimated (Zeng *et al.*, 2018).

#### **Preparation of PCRCS and determination of protein**

Collagen (PCRCS) with molecular weight less than 10000 Da was separated and purified from CRCS by ultrafiltration membrane and freeze-dried. The content and purity of CRCS and PCRCS were determined by the hydroxyproline method (Cissell *et al.*, 2017) and the molecular weight of PCRCS was further verified by 2.4 method.

#### **Hydroxyl radicals scavenging activity (HRS) of PCRCS**

A changed technique detailed in a prior literature study was used to evaluate the hydroxyl radical scavenging effect (Dong *et al.*, 2013). 0.1 mL sample concentration, 0.2 mL PBS (0.2mM) and 0.1mL FeSO<sub>4</sub> (0.75mM) were

combined and 0.1mL H<sub>2</sub>O<sub>2</sub> was added and shocked for 1 min. After that, 0.1mL phenanthroline anhydrous ethanol (0.75mM) was added to the mixture, which was then put in a water bath at 50 °C for 1h. At 536nm, the combination was finally identified as As<sub>1</sub>. The following was interpreted as Ap, Ab and As<sub>2</sub>:

Ap: sample was replaced by distilled water

Ab: H<sub>2</sub>O<sub>2</sub> was replaced by distilled water

As<sub>2</sub>: the sample was replaced by L-ascorbic acid

The hydroxyl radicals scavenging activity is expressed as:

$$\% \text{HRS} = [ (As - Ap) / (Ab - Ap) ] \times 100\%$$

#### **Determination of the reducing activity of PCRCS**

The reducing activity of PCRCS was evaluated by the methods detailed in previous literature studies and compared with l-ascorbic acid (Vc) and the results were compared to L-ascorbic acid (Vc) (Francavilla *et al.*, 2013).

#### **Acute gastric ulcers induced by absolute ethanol**

Mice were randomly divided into 4 groups, each group had 6 mice. They are control group C (without treatment), absolute ethanol group ABE (10mL/kg), purified collagen group PCRCS (60mg/kg PCRCS dissolved in saline) and cimetidine group CIM (50mg/kg, positive control drug dissolved in saline).

Except the control group and absolute ethanol group, the mice in other groups were pretreated with PCRCS (60mg/kg) and CIM (50mg/kg) by intragastric administration 6 days before death. On the 7th day, one hour after drug treatment, mice in the ABE, PCRCS and CIM groups were given absolute alcohol (10mL/kg) orally to induce acute ulcer, while the control group had no treatment. One hour after the ulcer was induced, euthanasia was performed on all mice. Quickly remove the stomach and divide each stomach into two parts. Part of the stomach was soaked in 10% formaldehyde for histological evaluation, while the other part of the stomach tissue was kept at -80°C for biochemical analysis. The damaged area (mm<sup>2</sup>) was measured with a micrometer under a dissecting microscope (× 10) and the ulcer index (UI) was calculated (Dejban *et al.*, 2020). The rest is used to determine biochemical changes.

#### **Determination of biochemical changes**

##### **Superoxide Dismutase (SOD) assay**

Stomach tissues were homogenized using saline and the supernatant was collected by centrifugation at 10000 rpm for 5 min at 4°C. Superoxide dismutase (SOD) activity was measured separately according to Misra's protocol (Guo *et al.*, 2020; Vandi *et al.*, 2022).

##### **Malondialdehyde estimation (MDA) assay**

The color that developed during the reaction of MDA with thiobarbituric acid was detected by ultraviolet spectrophotometer. We could calculate the content of

MDA according to the absorption value of mixture (Jain *et al.*, 1989).

### Nitric oxide Assays

Based on the Grice diazotization reaction, the concentration of nitric oxide (NO) is determined by measuring the nitrite formed by NO oxidation (Li *et al.*, 2021; Roy & Wilkerson 1984).

### Matrix prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) TNF- $\alpha$ and IL-6 assay

Enzyme-linked immunosorbent assay kit (Elisa Biotech, Shanghai, China) was used to determine the levels of PGE<sub>2</sub>, IL-6 and TNF- $\alpha$  in stomach tissues. All procedures were performed according to the manufacturer's instructions.

### Gastroprotective mechanisms of PCRCs

To clarify the protective mechanism of PCRCs on the stomach, the improved method of Matsuda (1999) was used to further evaluate endogenous nitric oxide (Matsuda, Li & Yoshikawa 1999). After fasting for 24h, mice were divided into 6 groups (n=6 in each group). Three groups were intraperitoneally injected with 0.9% NaCl solution (10mL/kg), while the other three groups were intraperitoneally injected with methyl N-nitro-l-arginine (70mg/kg). After 30 min, the mice were given 1% Tween 80 (vehicle group), carbenicillin (CAR 100mg/kg) or PCRCs (60mg/kg) orally. One hour later, all mice received absolute ethanol (10mL/kg) to induce gastric injury. After 1h of ethanol intake, the animals were euthanized and the stomach was immediately dissected and treated (as mentioned earlier) to determine the ulcer index.

### STATISTICAL ANALYSIS

SPSS software (version 22.0) was used to statistically analyze all the data. A one-way ANOVA was used for finding any significant difference between treatments,  $p < 0.05$  was considered to be significant and further significance between groups was analyzed using a Duncan post hoc test. Results are presented as the mean  $\pm$  standard deviation of 3 independent experiments.

### RESULTS

#### Determination of molecular weight of CRCS

The washing volume curve of the control sample weight is shown in table.1 and the elution volume curve of the control sample is shown in fig. 1A. The molecular CRCS was calculated according to fig. 1A and B. All the molecular weight of CRCS is less than 30000 Da and the minimum is 200 Da. There were several short peptides in the structure of CRCS (about 80% less than 10000 Da).

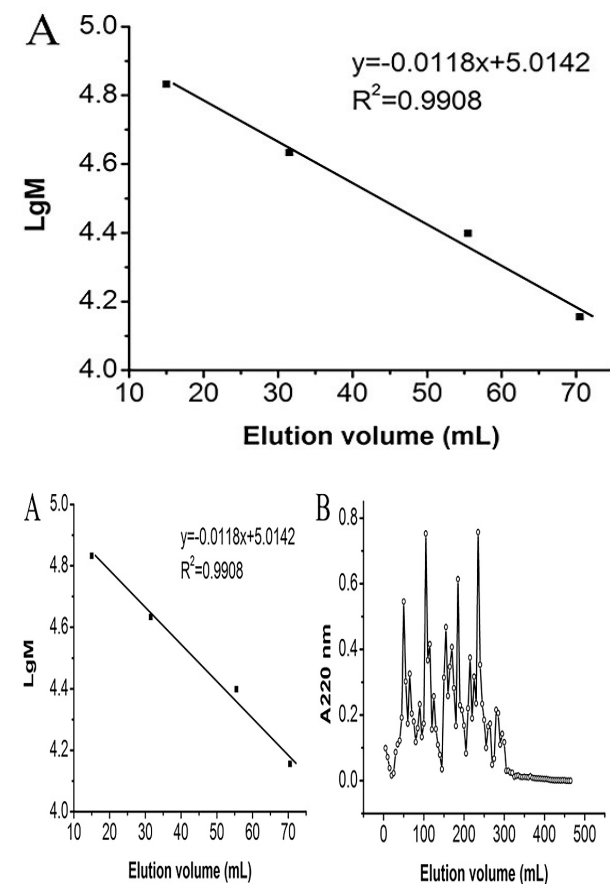
#### Protein content and purity of CRCS and PCRCs

According to the data in table 2, after separation and purification, the purity of PCRCs was significantly

improved and the purity increased to more than 80%. Sephadex G 100 gel column chromatography showed that the molecular weight of PCRCs was less than 10000 Da.

**Table 1:** Elution volume of Control sample

Control sample	Molecular weight (Da)	Washing volume (mL)
Bovine serum albumin	68 000	15
Chicken ovalbumin	43 000	31.5
Trypsin	23 300	55.5
Lysozyme	14 300	70.5



**Fig. 1:** A: Gel filtration of the control sample on Sephadex G100, eluting with 0.05 mol/L Tris-HCl (pH 7.2). The flow rate was 0.3mL/min. The elution volume curve of the Control sample was obtained. B: The elution volume curve of CRCS.

**Table 2:** Collagen content and purity

	Total protein content/%	Purity (dry weight %)
CRCS	82.47 $\pm$ 0.61	73.44 $\pm$ 0.58
PCRCs	81.27 $\pm$ 0.45	80.47 $\pm$ 0.39

Data are presented as the mean  $\pm$  SD (n = 3) per group.

#### Hydroxyl radicals scavenging activity (HRS) of PCRCs

Hydroxyl radicals are the most reactive free radicals in biological tissue proteins and readily react with DNA, proteins and lipids (Patial *et al.*, 2022). Therefore, the

hydroxyl radical scavenging ability can also estimate the antioxidant activity of proteins or peptides. Fig. 2 shows the radical scavenging activity of PCRCS at eight different concentrations ranging from 2 to 16mg/mL and compared with ascorbic acid as a standard reference. PCRCS exhibited almost similar radical scavenging activity to ascorbic acid at 16mg/mL and showed significant antioxidant activity up to 80.5% of radical scavenging activity at this concentration.

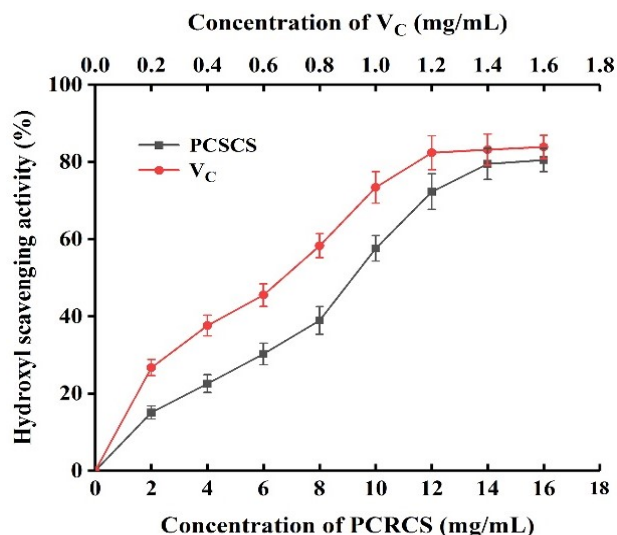


Fig. 2: Scavenging effect of collagen and Vc on hydroxyl free radical. Results are given as mean±SD of 3 independent experiments.

#### Reducing activity

The reducing activity of PCRCS and Vc is shown in fig. 3. The results show that the higher the concentration of PCRCS, the stronger the reducing activity. 16mg/mL PCRCS has comparable reducing power to 1.2mg/mL Vc.

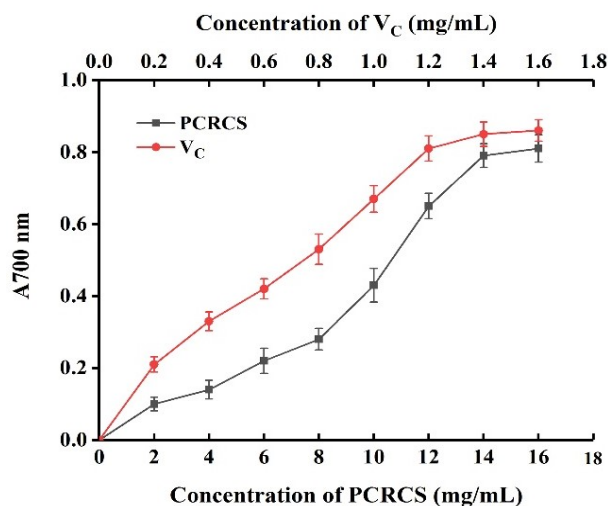


Fig. 3: Reducing the capacity of the CRCS and Vc. Results are given as mean±SD of 3 independent experiments.

#### Gastroprotective effect

Macroscopic and microscopic observations of the gastric protection effect of PCRCS are shown in fig. 4. Compared with the control group, the absolute alcohol injury group can observe obvious gastric injury (fig. 4A). Compared with the anhydrous alcohol group, the macroscopic pathological changes of gastric tissue injury after PCRCS treatment were significantly reduced (fig. 4A). This is proved by the ulcer index evaluated by microscope (fig. 4B) Compared with the control group, ABE group caused severe damage to the gastric mucosa of mice and the ulcer index was 67.3±7.10mm<sup>2</sup>. Mice treated with CIM and PCRCS showed protective effects on the ulcer and the collagen-induced protective response was close to that of cimetidine.

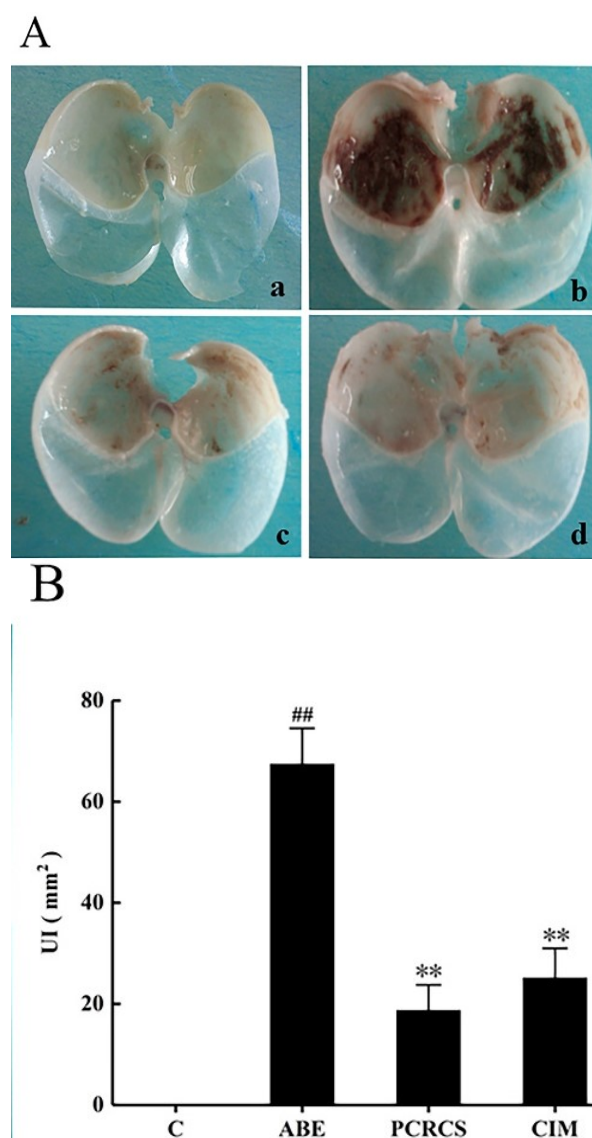


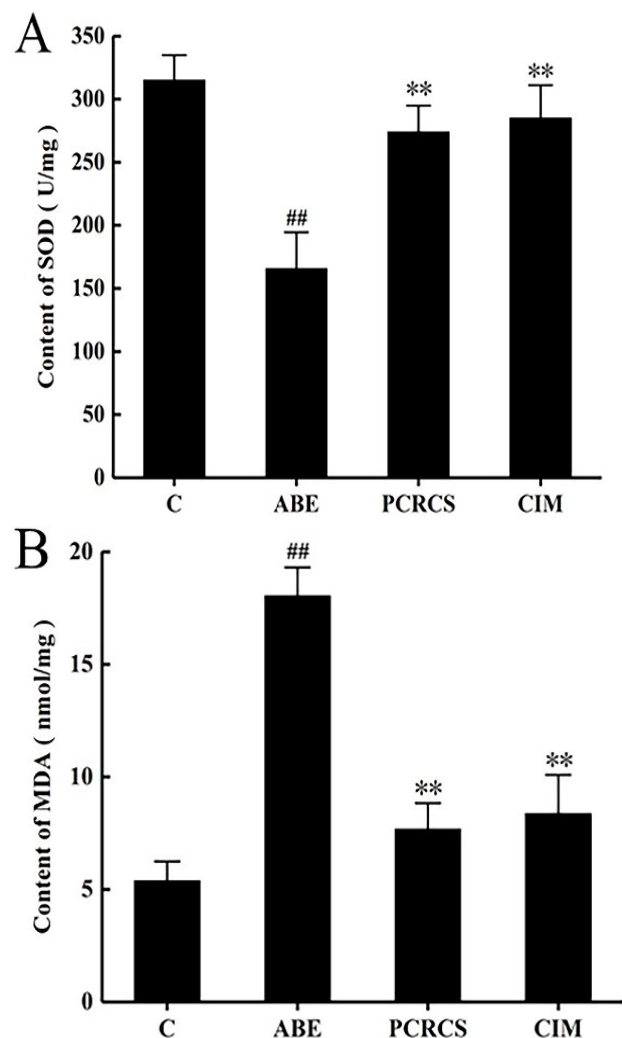
Fig. 4: Macroscopic and microscopic pathological changes of PCRCS in the protection of the stomach. (A) Macroscopic effect of PCRCS on ethanol induction. (B) Effect of PCRCS on ethanol-induced microscopic scores.

C (a): without treatment. ABE (b): with only absolute ethanol. PCRCs (c): with purified collagen (60mg/kg) and absolute ethanol. CIM (d): with cimetidine (50mg/kg) and absolute ethanol. Data are presented as the mean±SD (n=3) per group, <sup>###</sup>P<0.01 compared with the normal group; <sup>\*\*</sup>P<0.01 compared with the absolute ethanol group.

**Biochemical analysis**

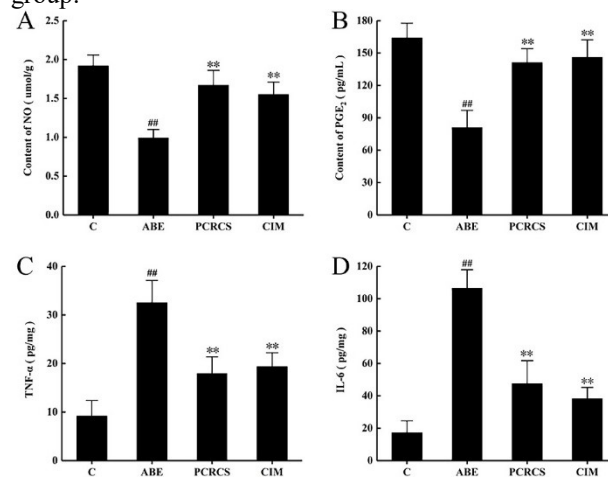
*Effects of PCRCs on SOD and MDA*

The results of SOD and MDA in the stomach tissue are shown in fig. 5. Compared with group C, the SOD level of the ABE group decreased significantly (fig. 5B, p<0.01), while that of ulcer group pretreated by PCRCs increased significantly (p<0.01). Simultaneously, the ABE group significantly increased the level of MDA in stomach (fig. 5A, p<0.01). PCRCs treatment significantly reduced the level of malondialdehyde (p<0.01).

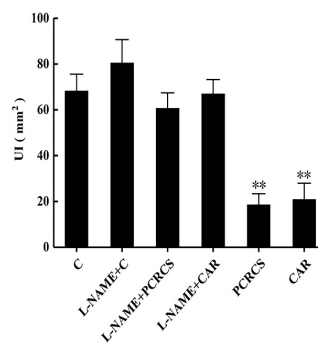


**Fig. 5:** The content of MDA(A) and SOD(B) in different group. C: without treatment. ABE: with only absolute ethanol. PCRCs: with purified collagen (60mg/kg) and absolute ethanol. CIM: with cimetidine (50mg/kg) and absolute ethanol. Data are presented as the mean±SD (n=3) per group, <sup>\*\*</sup>p<0.01 compared with the absolute ethanol group.

absolute ethanol. Data are presented as the mean±SD (n=3) per group, <sup>###</sup>P<0.01 compared with the normal group; <sup>\*\*</sup>P<0.01 compared with the absolute ethanol group.



**Fig. 6:** The content of NO(A), PGE<sub>2</sub>(B), TNF-α(C) and IL-6(D) in different group. C: without treatment. ABE: with only absolute ethanol. PCRCs: with purified collagen (60mg/kg) and absolute ethanol. CIM: with cimetidine (50mg/kg) and absolute ethanol. Data are presented as the mean±SD (n=3) per group, <sup>##</sup>P<0.01 compared with the normal group; <sup>\*\*</sup>P<0.01 compared with the absolute ethanol group.



**Fig. 7:** Effect of collagen (PCRCs) and carbenoxolone (CAR) on gastric lesions induced by absolute ethanol in mice pretreated with L-NAME (70mg/kg). C: Vehicle with 1% Tween 80 and absolute ethanol. L-NAME+C: with absolute ethanol and L-NAME. L-NAME+PCRCs: with L-NAME PCRCs (60mg/kg) and absolute ethanol. L-NAME+CAR: with L-NAME Carbenoxolone 100mg/kg and absolute ethanol. Data are presented as the mean±SD (n=3) per group, <sup>\*\*</sup>p<0.01 compared with the L-NAME processing group.

### Effects of PCRCS on NO, PGE<sub>2</sub>, TNF- $\alpha$ and IL-6

The results of determination of NO, PGE<sub>2</sub>, TNF- $\alpha$  and IL-6 in stomach tissues are shown in fig. 6. Compared with group C, the contents of NO and PGE<sub>2</sub> in stomach tissue induced by absolute ethanol were significantly decreased (figs. 6A, B,  $p < 0.01$ ). After PCRCS treatment, the levels of NO and PGE<sub>2</sub> increased significantly (figs. 6A, B,  $p < 0.01$ ) and the effect was similar to that of the CIM treatment group. It is worth mentioning that, compared with the CIM group, the activity of PCRCS on NO tends to increase. Compared with normal tissues, the content of inflammatory cytokines TNF- $\alpha$  and IL-6 in mice induced by ethanol was significantly increased (fig. 6C, D,  $p < 0.01$ ). The levels of TNF- $\alpha$  and IL-6 in the PCRCS group and CIM groups were significantly decreased (figs. 6C, D,  $p < 0.01$ ). The results showed that collagen and cimetidine had similar anti-inflammatory activities.

### Gastroprotective mechanisms of PCRCS

The ulcer indices of the PCRCS group and CAR groups with L-NAME ( $60.6 \pm 9.79 \text{mm}^2$ ,  $66.9 \pm 7.32 \text{mm}^2$ , fig. 7.) was significantly higher than that of their respective groups without L-NAME ( $18.5 \pm 5.53 \text{mm}^2$ ,  $20.8 \pm 6.48 \text{mm}^2$ ,  $p < 0.01$ ). It was previously reported that L-NAME (70mg/kg) was high enough to block the synthesis of NO (citation). L-NAME weakened the gastric protective effect of PCRCS (60mg/kg) and CAR (100mg/kg) (fig. 7).

## DISCUSSION

The digestive system's most prevalent and common ailment is the gastric ulcer. After the onset, it is easy to cause epigastric pain. Severe cases may cause ulcer perforation, gastric bleeding and death (Tanyeli *et al.*, 2017). Research shows that most peptic ulcer diseases are caused by inflammation and oxidative stress. These diseases plague 700 million people worldwide, causing a global disease burden (Lee *et al.*, 2022). Although various gastric protective drugs have been used to treat and prevent gastric ulcer diseases, there are still reports of side effects such as cardiovascular diseases, hypomagnesemia and increased risk of gastric cancer. Therefore, developing a safe and tremendous gastric protective to forestall peptic ulcers is critical.

Alcohol consumption, stress, non-steroidal anti-inflammatory drugs and *Helicobacter pylori* infection can all increase the possibility of gastric ulcer (Eraslan *et al.*, 2020). Alcohol intake is one of these risk factors that can directly affect stomach motility and metabolism, causing oxidative stress and inflammatory reaction, leading to gastric mucosa damage and ulcer (Zhang *et al.*, 2018). Therefore, we induced gastric ulcer in mice by taking absolute alcohol orally and tested the protective effect of collagen (PCRCS) extracted and purified from the skin of *R. chensinensis* on gastric ulcer. We found that compared

with the absolute ethanol group, animals pretreated by PCRCS had less macroscopic damage, the stomach damage index was lower ( $18.67 \pm 5.08$ ) and reduced oxidative stress and inflammatory response. Studies have shown that cimetidine (CIM) has an obvious inhibitory effect on gastric ulcer, so this study selected this drug as a positive control to explore the therapeutic effect of PCRCS on gastric ulcer (Yan *et al.*, 2021). The results showed that PCRCS had the same gastric protection effect as CIM and could effectively protect mice from acute gastric mucosal injury caused by ethanol.

Through *in vitro* experimental studies of hydroxyl radical scavenging activity, we found that PCRCS has good antioxidant activity *in vitro*, whereas *in vivo* antioxidant research is mainly carried out through the levels of SOD and MDA. Studies have shown that ethanol can induce oxidation reactions *in vivo* by increasing the imbalance between antioxidants and ROS and then damaging gastric mucosa (Bin Jordan *et al.*, 2020). As an important antioxidant enzyme, SOD mainly converts superoxide radicals into water molecules, providing ROS with major antioxidant defense, thus reducing oxidation reaction and protecting the stomach (Zheng *et al.*, 2021). Additionally, MDA, as the final product of polyunsaturated fatty acids, is often used as an index to evaluate lipid peroxidation in gastric mucosa (Ahmad *et al.*, 2019). Our research shows that absolute ethanol can significantly increase the amount of MDA in the stomach tissue and limit the degree of antioxidant enzyme SOD, which is consistent with previous research results (Ruiz-Hurtado *et al.*, 2021). PCRCS pretreatment significantly inhibited lipid peroxidation in the gastric tissue and enhanced the activity of SOD in gastric tissue, which indicating that PCRCS could protect the stomach by reducing the level of oxidative stress.

Both NO and PGE<sub>2</sub> are defense factors of the gastric mucosa. NO is one of the most important defense media against *Helicobacter pylori* (Lin *et al.*, 2020; Vandi *et al.*, 2022). In addition to its antibacterial effect, NO protects the integrity of mucosal barrier and gastric epithelium by reducing gastric acid secretion of parietal cells. PGE<sub>2</sub> controls gastric mucus production and enhances gastric blood flow to protect the stomach mucosa from harm (Fu *et al.*, 2021). PGE<sub>2</sub> can also improve microcirculation and promote tissue repair process (Lin *et al.*, 2020). This study found that ethanol-induced gastric ulcer can lead to severe gastric mucosal injury and this organism change can decrease NO and PGE<sub>2</sub> content in the gastric tissue. This is consistent with the results of previous studies. It was found that PCRCS pretreatment improved this phenomenon and significantly increased the contents of NO and PGE<sub>2</sub> in stomach tissue ( $p < 0.05$ ). It is worth mentioning that, compared with the CIM group, the activity of PCRCS on NO tends to increase.

To further understand the mechanism of action of PCRCS on NO, we injected L-NAME (nitric oxide synthesis inhibitor) into mice to explore the mechanism of action of NO (Dejban *et al.*, 2020). Compared with the PCRCS group, the ulcer index of the PCRCS group treated with L-NAME increased by 216.4%. Therefore, it is further proved that PCRCS can play an anti-ulcer role by increasing the NO level.

The levels of TNF- $\alpha$  and IL-6 are closely related to the severity of gastric mucosa inflammation. Inhibiting the release of inflammatory cytokines is considered as one of the effective methods to reduce the severity of gastric mucosa (Luo *et al.*, 2018; Rajamanickam *et al.*, 2020). Consistent with previous research results, alcohol intake can lead to acute inflammatory reaction and the production of pro-inflammatory cytokines TNF- $\alpha$  and IL-6 in gastric tissue induced by ulcer is significantly increased (Liu *et al.*, 2021). PCRCS pretreatment can significantly reduce the expression of TNF- $\alpha$  and IL-6 in gastric tissues of mice induced by ethanol, suggesting that PCRCS could protect the acute gastric ulcer induced by ethanol by inhibiting inflammatory reaction.

In this study, the anti-ulcer effect of purified collagen (PCRCS, molecular weight less than 10000 Da) from *R. chensinensis* skin was reported for the first time. The results showed that the physicochemical and morphological parameters of the stomach in the pretreatment group of PCRCS were improved and the lower ulcer index (18.67 $\pm$ 5.08) proved the anti-ulcer activity of PCRCS. Additionally, our research focuses on the changes of oxidative stress and inflammatory response, order to clarify the protective matrix of PCRCS against ethanol-induced gastric mucosal injury. The results showed that PCRCS could inhibit gastric mucosal injury caused by ethanol by increasing the level of antioxidant enzymes, activating gastric mucosal defense factors, inhibiting lipid peroxidation and pro-inflammatory factors in gastric tissue.

## CONCLUSION

Our research results show that PCRCS can treat gastric mucosal injury induced by ethanol and its protective mechanism is mainly through inhibiting oxidative stress and inflammatory factors. The effective intervention of PCRCS on mucosal damage caused by absolute alcohol and its non-toxic characteristics may make collagen a potential tool for preventing and treating gastric ulcer. However, further research is needed to clarify the exact chemical structure of PCRCS and its potential gastric protective mechanism.

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