

Preventive Effect of Polysaccharide of *Larimichthys crocea* Swim Bladder on Reserpine Induced Gastric Ulcer in ICR Mice

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This project's aim was to determine the reserpine-induced gastric ulcer preventive effect of polysaccharide of *Larimichthys crocea* swim bladder (PLCSB) in ICR mice. The anti-gastric ulcer effects of polysaccharide of *Larimichthys crocea* swim bladder was evaluated in mice model using morphological test, serum levels assay, cytokine levels assay, tissue contents analysis, reverse transcription-polymerase chain reaction (RT-PCR) analysis and western blot assay. High concentration (50 mg/kg dose) of PLCSB reduced IFN- γ as compared to low concentration (25 mg/kg dose) and control mice. SS and VIP serum levels of PLCSB treated mice were higher than those of control mice, and MOT and SP serum levels were lower than control mice. Gastric ulcer inhibitory index of PLCSB treatment groups mice were much lower than control mice, and the high concentration treated mice were similar to the ranitidine treated mice. The SOD and GSH-Px activities of PLCSB treated mice were higher than control mice, close to normal mice and ranitidine treated mice. PLCSB treated mice also showed the similar contents of NO and MDA to normal group. By RT-PCR and western blot assay, PLCSB significantly induced inflammation in tissues of mice by downregulating NF- κ B, iNOS, and COX-2, and upregulating I κ B- α . These results suggest that PLCSB showed a good gastric ulcer preventive effect as the gastric ulcer drug of ranitidine. Polysaccharide of *Larimichthys crocea* swim bladder may be used as a drug material from marine products.

Key Words: Cytokine, Gastric ulcer, ICR mice, *Larimichthys crocea* swim bladder, Polysaccharide

INTRODUCTION

Swim bladder is an important balancing organ and the amount of polysaccharide in it is as much as 10% of its weight. As one of the main commercial fishes in the coastal waters of China, the *Larimichthys crocea* swim bladder contains many nutrients, such as numerous proteins, microelements and vitamins. Traditional medicine advocates that it has a good curative effect on diseases including amnesia, insomnia, dizzy, anepithymia and weakness after delivery [1]. Researchers also suggested that *Larimichthys crocea* swim bladder served to remove free radicals and ward against inflammation and cancer [2]. Polysaccharide is a kind of important functional material. It's been proven that polysaccharide in swimming bladders can quicken the cure

of cuts and prevent infection as well as thrombotic events [3]. *In vivo* experiments have proved that polysaccharide in *Lentinus edodes* and *Spirulina* seaweed serves to prevent and cure injury [4,5].

Gastric ulcer is a most common disease affecting the gastro-intestinal tract and it causes inflammatory injuries in the gastric mucosa [6]. Reserpine is an indole alkaloid drug, although because of its numerous side-effects, it is rarely used as medicine [7]. Reserpine has the peripheral action in a preponderance of the effects of the cholinergic part of the autonomous nervous system on the gastro-intestinal tract and smooth muscles [8]. Reserpine can cause gastric intolerance, gastric ulcer, stomach cramps and diarrhea [9]. Base on the past researches, reserpine was used as the gastric ulcer drug revulsant in this study.

In this study, the polysaccharide was extracted from *Larimichthys crocea* swim bladder, and its pharmacological effect for stomach was studied at the first time. The preventative effect on gastric ulcer of polysaccharide of *Larimichthys crocea* swim bladder (PLCSB) was determined. The serum levels and inflammation-related cytokines levels

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ABBREVIATIONS: ICR mice, imprinting control region mice; IL-6, Interleukin 6; IL-12, Interleukin 12; TNF- α , tumor necrosis factor alpha; IFN- γ , interferon gamma; MOL, motilin; SS, somatostatin; SP, substance P; VIP, vasoactive intestinal peptide; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; NO, nitrogen oxide; MDA, maleic dialdehyde; NF- κ B, nuclear factor kappa B; I κ B- α , inhibitor kappa B alpha; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase.

were used to determine the preventative effect of PLCSB on reserpine-induced gastric ulcer mice. The gastric tissues were checked by histology, and the SOD, GSH-Px, NO and MDA contents in mice tissues were also tested. The mRNA and protein gene expressions in tissues were also determined for explaining the preventative effect. These data could help increase the useful value of PLCSB as a new medicine.

METHODS

Polysaccharide of Larimichthys crocea swim bladder (PLCSB) preparation

Wild Yellow Sea *Larimichthys crocea* was purchased from Shandong Province in China. Swim bladder of *Larimichthys crocea* (1 kg) was dried by freeze-drying, and the dried samples were crushed. 3 L petroleum ether was added into swimming bladder of large yellow croaker and then reflux extraction was done twice (1 h each time) at 60°C to remove the lipid, and the residuals were gathered after filtration. After 3 L ethyl alcohol was added and reflux extraction were performed for 3 h, the residuals without protein were filtered and gathered. At last, 3 L water was added and the residuals were extracted at 60°C for 2 h, and the filter liquor was collected. The crude polysaccharide of *Larimichthys crocea* swim bladder was collected after evaporating [10].

Animals

Seven-week-old male ICR mice (n=50) were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). They were maintained in a temperature-controlled facility (temperature 23±1°C, relative humidity 50±5%) with a 12-h light/dark cycle. The mice had unlimited access to a standard mouse chow diet and water.

Gastric ulcer experiment

The mice were divided into five groups (n=10 each). The normal group mice received no treatment during the experimental period. The control group mice received no treatment at first 4 weeks. The PLCSB group mice were received oral administration of 25 and 50 mg/kg PLCSB everyday for 4 weeks; the drug cure group mice were received 50 mg/kg dose oral administration of ranitidine for 4 weeks. Then the control, 25, 50 mg/kg dose PLCSB and ranitidine groups mice were administered single intraperitoneal injections of 10 mg/kg bw/day reserpine Sigma Co., St. Louis, MO, USA) for 3 days. After the last injection, all the mice were treated with abrosia for 24 h, the stomachs were removed, inflated by injecting 10 ml 1% formalin for 10 min to fix the tissue walls and opened along the greater curvature [11]. The gastric ulcer inhibitory index of hemorrhagic lesions developed in the stomach was measured using a digital camera (D550; Canon, Tokyo, Japan) with a square grid and the images were analyzed by ImageJ software (National Institutes of Health, Bethesda, MD, USA), gastric ulcer inhibitory index (%)=(Gastric ulcer area of control mice - Gastric ulcer area of treated mice)/Gastric ulcer area of control mice. The gastric secretion volume of each mice was measured with a 10-ml measuring cylinder.

pH of the gastric juice was measured with a pH meter (SevenEasy pH meter; Mettler Toledo, Schwerzenbach, Switzerland) after being diluted 10 times with distilled water. These experiments followed a protocol approved by the Animal Ethics Committee of Chongqing Medical University (Chongqing, China).

Analysis of inflammation-related cytokines in serum by enzyme-linked immunosorbent assay (ELISA)

For the serum cytokine assay, blood from the inferior vena cava was collected in a tube and centrifuged at 3000 r/min, 4°C for 10 min. The serum was aspirated and assayed as described below. Concentrations of inflammatory-related cytokines IL-6, IL-12, TNF- α , and IFN- γ in serum were measured by ELISA according to the manufacturer's instructions (Biolegend, San Diego, CA, USA). Briefly, biotinylated antibody reagent was added to 96-well plates, then supernatants of homogenized serum were added and the plates were incubated at 37°C for 2 h. After washing with PBS, streptavidin-horseradish peroxidase (HRP) solution was added and the plate was incubated for 30 min at room temperature. The absorbance was measured at 450 nm using a microplate reader (iMark; Bio-Rad, Hercules, CA, USA) [12].

Serum levels of MOL (motillin), SS (somatostatin), SP (substance P) and VIP (vasoactive intestinal peptide) determination

Mice blood was collected in a tube and centrifuged at 3000 r/min, 4°C for 10 min. Then the MOL, SS, SP and VIP levels of the serum were determined using commercially available kits (Beijing Pu'er Weiye Bio-Technology Co., Ltd., Beijing, China).

SOD (superoxide dismutase), GSH-Px (glutathione peroxidase), NO (nitrogen oxide) and MDA (maleic dialdehyde) contents determination

Gastric tissues homogenate were made by high-speed tissue homogenizer (T10, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 4000 r/min, 4°C for 10 min. The SOD, GSH-Px, NO and MDA contents were determined using commercially available kits (Nanjing Juli Institute of Biomedical Engineering, Nanjing, Jiangsu, China).

mRNA gene expression examination

Total RNA from gastric tissue cells was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. The RNA was digested with RNase-free DNase (Roche, Basel, Switzerland) for 15 min at 37°C and purified using an RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. cDNA was synthesized from 2 μ g of total RNA by incubation at 37°C for 1 h with avian myeloblastosis virus reverse transcriptase (GE Healthcare, Little Chalfont, United Kingdom) with random hexanucleotides according to the manufacturer's instruction. Sequences of primers used to specifically amplify the genes of interest were as follows: 5'-CAC TTA TGG ACA ACT ATG AGG TCT CTG G-3' (forward) and 5'-CTG TCT TGT GGA CAA CGC AGT GGA ATT TTA GG-3' (reverse) for NF- κ B; 5'-GCT GAA GAA GGA GCG GCT ACT-3' (forward) and 5'-TCG TAC

TCC TCG TCT TTC ATG GA-3' (reverse) for $I\kappa B-\alpha$; 5'-AGA GAG ATC GGG TTC ACA-3' (forward) and 5'-CAC AGA ACT GAG GGT ACA-3' (reverse) for iNOS; 5'-TTA AAA TGA GAT TGT CCG AA-3' (forward) and 5'-AGA TCA CCT CTG CCT GAG TA-3' (reverse) for COX-2. GAPDH was amplified as an internal control gene with the following primers: 5'-CGG AGT CAA CGG ATT TGG TC-3' (forward) and 5'-AGC CTT CTC CAT GGT CGT GA-3' (reverse). Amplification was performed in a thermal cycler (Eppendorf, Hamburg, Germany). The polymerase chain reaction (PCR) products were separated in 1.0% agarose gels and visualized with ethidium bromide staining [13].

Protein gene expression examination

Total gastric tissue protein was obtained with RIPA buffer as described [14]. Protein concentrations were determined with a Bio-Rad protein assay kit (Hercules, CA, USA). For the western blot analysis, aliquots of the lysate containing 30~50 μ g protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto a nitrocellulose membrane (Schleicher and Schuell, Keene, NH, USA). The membranes were subjected to immunoblot analysis and the proteins were visualized by an enhanced chemiluminescence (ECL) method (GE Healthcare). The cell lysates were separated by 12% SDS-PAGE, transferred onto a polyvinylidene fluoride membrane (GE Healthcare), blocked with 5% skimmed milk and hybridized with primary antibodies (diluted 1 : 1,000). The antibodies against NF- κ B, $I\kappa B-\alpha$, iNOS and COX-2 were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), then incubated with the horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology Inc.) for 1 h at room temperature. The blots were washed three times with PBS-T and then developed by enhanced chemiluminescence (Amersham Life Science, Arlington Heights, IL, USA).

Statistical analysis

Data are presented as mean \pm standard deviation (SD). Differences between the mean values for individual groups were assessed with one-way analysis of variance (ANOVA) with Duncan's multiple range test. $p < 0.05$ was considered to indicate a statistically significant difference. SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

Table 1. Prevention of reserpine-induced gastric ulcer in ICR mice by treatment with polysaccharide of *Larimichthys crocea* swim bladder (PLCSB)

Group	Rate of gastric ulcer inhibition	
	Gastric ulcer (mm ²)	Inhibitory index (%)
Normal	0.00 \pm 0.00 ^d	100.00
Control	12.87 \pm 2.45 ^a	0.0
PLCSB (25 mg/kg dose)	4.21 \pm 1.31 ^b	67.29
PLCSB (50 mg/kg dose)	2.32 \pm 0.62 ^c	81.97
Ranitidine (50 mg/kg dose)	2.41 \pm 0.58 ^c	81.27

^{a~d}Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test.

istical analyses.

RESULTS

Gastric ulcer levels

The administration of PLCSB to mice prior to the induction of gastritis led to reduced gastric ulcer. The mice of the control group demonstrated a gastric ulcer area of 12.87 \pm 2.45 mm². Treatment with 25 and 50 mg/kg PLCSB resulted in gastric ulcer inhibition index of 67.29% (gastric ulcer area, 4.21 \pm 1.31 mm²) and 81.97% (gastric ulcer area, 2.32 \pm 0.62 mm²), respectively (Table 1 and Fig. 1). Especially the greatest level of protection against gastric ulcer was achieved by high concentration PLCSB and comparable to that observed with ranitidine (81.27%) that served as the positive control.

Gastric secretion volume and pH of gastric juice

The gastric secretion volume of normal mice was the lowest (0.12 \pm 0.03 ml) among all the groups (Fig. 2A). This volume was increased in the control mice (0.67 \pm 0.08 ml), and reduced by treatment with 25 mg/kg PLCSB (0.38 \pm 0.06 ml), 50 mg/kg PLCSB (0.20 \pm 0.05 ml), or ranitidine (0.22 \pm 0.05 ml). The pH values of gastric juices were calculated as 3.52 \pm 0.38, 1.16 \pm 0.25, 2.11 \pm 0.31, 2.86 \pm 0.15 and 2.78 \pm 0.25, respectively (Fig. 2B). Compared to the other groups, 50 mg/kg PLCSB dose mice was associated with the lowest gastric secretion volume and highest pH. This could explain why the ability of PLCSB to protect against gastric ulcer was greater than that of the 25 mg/kg PLCSB.

Effect of PLCSB on serum cytokine levels of IL-6, IL-12, TNF- α and IFN- γ

The IL-6 level of normal mice was 41.3 \pm 4.1 pg/ml; however, in control mice the IL-6 level was significantly increased to 215.6 \pm 14.7 pg/ml (Fig. 3A). The levels of IL-6 in mice treated with 25 and 50 mg/kg PLCSB were 131.2 \pm 12.8 and 87.9 \pm 9.2 pg/ml, respectively. The ranitidine treated mice showed the IL-6 level in 91.8 \pm 8.8 pg/ml. The control mice showed the highest IL-12 level in 687.6 \pm 62.6 pg/ml, 25, 50 mg/kg PLCSB and ranitidine reduced the levels in 552.3 \pm 47.3, 432.6 \pm 35.7, 451.2 \pm 30.5 pg/ml, and the level of normal mice was lowest (308.7 \pm 41.2 pg/ml) (Fig. 3B). The TNF- α levels in the normal, control, 25, 50 mg/kg PLCSB

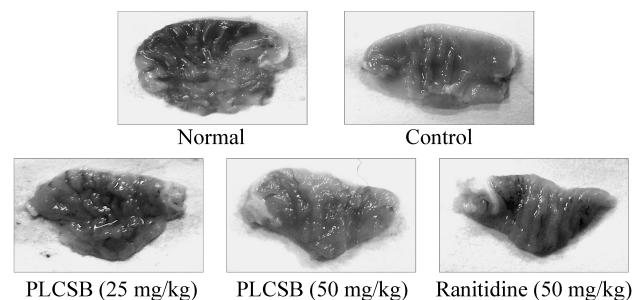


Fig. 1. Stomachs of the mice treated with polysaccharide of *Larimichthys crocea* swim bladder (PLCSB) after the induction of gastric ulcer with reserpine.

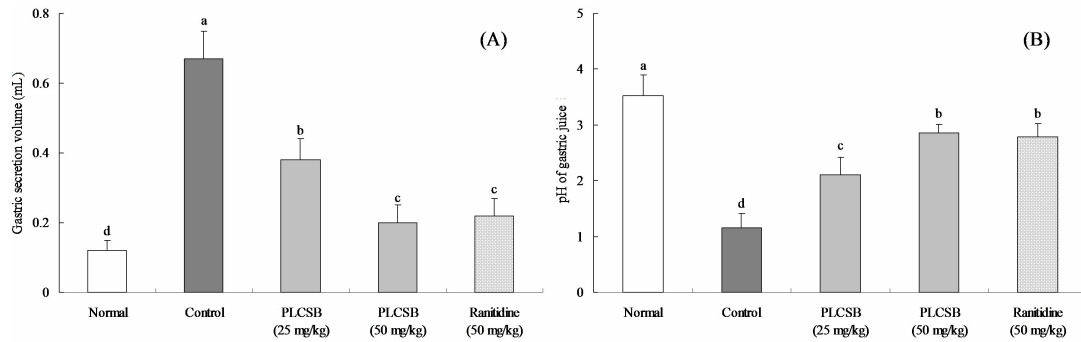


Fig. 2. Gastric secretion volume of the mice treated with polysaccharide of *Larimichthys crocea* swim bladder (PLCSB) after the induction of gastric ulcer with reserpine. ^{a-d}Mean values with different letters over the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

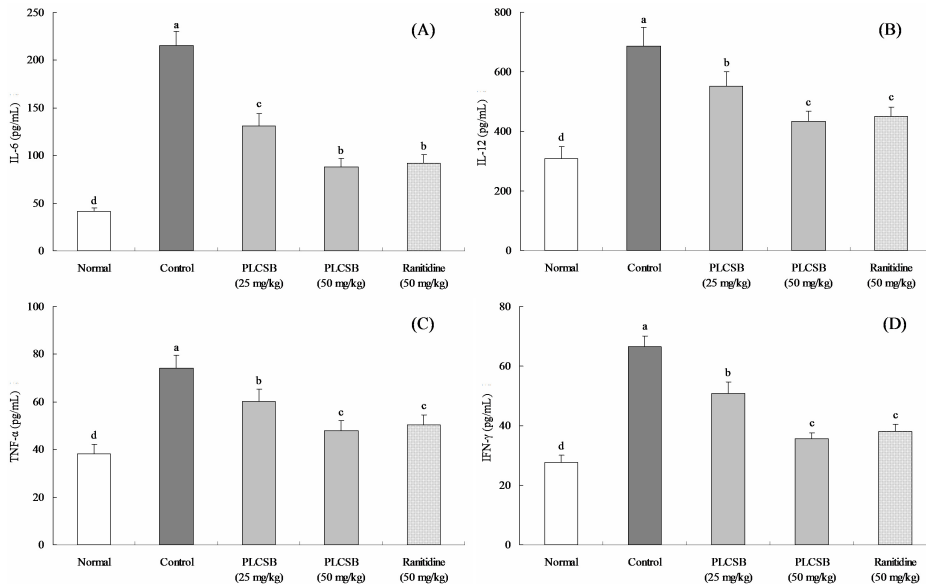


Fig. 3. Serum IL-6 (A), IL-12 (B), TNF- α (C) and IFN- γ (D) cytokine levels of reserpine-induced gastric ulcer mice treated with polysaccharide of *Larimichthys crocea* swim bladder (PLCSB). ^{a-d}Mean values with different letters over the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

and ranitidine treated mice were 38.4 ± 3.7 , 74.2 ± 5.3 , 60.3 ± 5.1 , 47.8 ± 4.2 and 50.2 ± 4.4 pg/ml, respectively (Fig. 3C). The serum IFN- γ levels in the mice in the 50 mg/kg PLCSB treated group (35.6 ± 2.1 pg/ml) and ranitidine (38.1 ± 2.3 pg/ml) were significantly lower compared with those in the control (66.4 ± 3.8 pg/ml) and 25 mg/kg PLCSB treated group (50.8 ± 4.0 pg/ml), the level of normal mice was 27.6 ± 2.4 pg/ml (Fig. 3D).

Effect of PLCSB on serum levels of MOL (motillin), SS (somatostatin), SP (substance P) and VIP (vasoactive intestinal peptide)

The MOL level of normal mice was 45.8 ± 3.1 μ g/L, however, the level in the gastric ulcer control mice was significantly increased to 96.3 ± 13.3 μ g/L (Fig. 4A). The levels of MOL in the 25 and 50 mg/kg PLCSB groups were 61.2 ± 9.9 and 55.2 ± 10.6 μ g/L, respectively. The drug control of ranitidine mice showed the level in 57.1 ± 7.8 μ g/L close to 50 mg/kg PLCSB treated group. The level of SS in mice treated with PLCSB (50 mg/kg) decreased to 86.5 ± 11.5 μ g/L,

which was higher than the level in mice treated with 25 mg/kg PLCSB (69.9 ± 7.1 μ g/L) and 50 mg/kg ranitidine (78.8 ± 8.1 μ g/L) treated mice (Fig. 4B). And the SS levels of normal and control mice were 112.3 ± 13.1 and 57.8 ± 4.6 μ g/L. The SP level in the normal group was 62.2 ± 2.5 μ g/L, whereas that of the control group was 128.7 ± 15.6 μ g/L, reflecting a marked increase. The SP levels in the 25 and 50 mg/kg PLCSB groups decreased to 102.8 ± 11.9 and 78.6 ± 7.7 μ g/L, respectively. Ranitidine (50 mg/kg) treatment also resulted in a further decreased SP level (80.3 ± 5.4 μ g/L; Fig. 4C). The levels of VIP in the 25 and 50 mg/kg PLCSB groups were 65.8 ± 3.1 and 83.2 ± 2.7 μ g/L, respectively, which were slightly higher than the level of the control group (47.6 ± 2.2 μ g/L). However, VIP level in the ranitidine group was 74.3 ± 3.0 μ g/L, whereas the normal group showed the highest level at 97.1 ± 1.9 μ g/L (Fig. 4D).

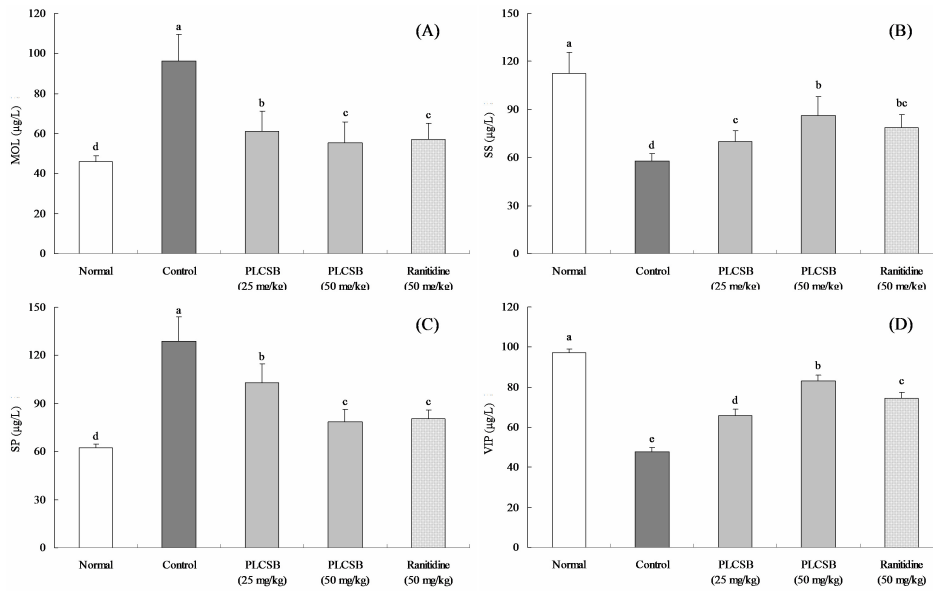


Fig. 4. Serum MOL (motilin) (A), SS (somatostatin) (B), SP (substance P) (C) and VIP (vasoactive intestinal peptide) (D) levels of reserpine-induced gastric ulcer mice treated with polysaccharide of *Larimichthys crocea* swim bladder (PLCSB). ^{a-e}Mean values with different letters over the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

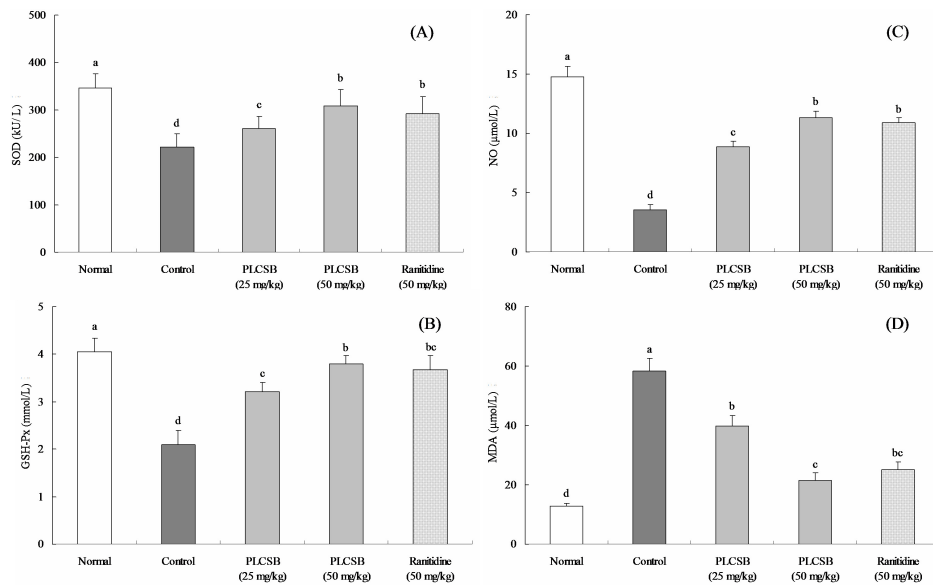


Fig. 5. Tissue SOD (superoxide dismutase), GSH-Px (glutathione peroxidase), NO (nitrogen oxide) and MDA (maleic dialdehyde) contents of reserpine-induced gastric ulcer mice treated with polysaccharide of *Larimichthys crocea* swim bladder (PLCSB). ^{a-e}Mean values with different letters over the bars are significantly different ($p < 0.05$) according to Duncan's multiple range test.

SOD (superoxide dismutase), GSH-Px (glutathione peroxidase), NO (nitrogen oxide) and MDA (maleic dialdehyde) contents in gastric tissue

The SOD and GSH-Px activities in gastric tissue of control mice were 221.3 ± 28.9 kU/L and 2.1 ± 0.3 mmol/L (Fig. 5A and B). The normal mice much increased these activities in 345.9 ± 31.3 kU/L and 4.0 ± 0.3 mmol/L. The 50 mg/kg PLCSB and ranitidine treated mice showed similar SOD activities at 308.7 ± 34.9 and 291.6 ± 36.6 kU/L, the 25 mg/kg PLCSB mice showed lower level (261.2 ± 25.6 kU/L). The GSH-Px activities of ranitidine (3.7 ± 0.3 mmol/L) and 50 mg/kg PLCSB (3.8 ± 0.2 mmol/L) treated mice were higher than 25 mg/kg PLCSB treated mice (3.2 ± 0.2 mmol/L). The NO contents of normal, control, 25 mg/kg PLCSB dose, 50 mg/kg PLCSB dose and 50 mg/kg ranitidine dose group

mice were 14.7 ± 0.8 , 3.5 ± 0.4 , 8.9 ± 0.5 , 11.3 ± 0.5 and 10.9 ± 0.4 μ mol/L, respectively (Fig. 5C). The MDA contents of these groups showed the opposite trend in 12.7 ± 1.1 , 58.4 ± 4.2 , 39.8 ± 3.6 , 21.4 ± 2.7 and 25.1 ± 2.5 μ mol/L, respectively (Fig. 5D).

Effects of PLCSB on inflammation-related gene expression of NF- κ B, I κ B- α , iNOS and COX-2

The next experiments were investigated whether the anti-inflammatory actions of PLCSB was associated with inhibited expression of the inflammation-related genes NF- κ B, I κ B- α , iNOS, and COX-2. As shown in Fig. 6, mRNA and protein expressions of NF- κ B was reduced in gastric tissues treated with PLCSB and ranitidine. PLCSB and ranitidine significantly modulated the expression of genes associated with inflammation. mRNA and protein expressions

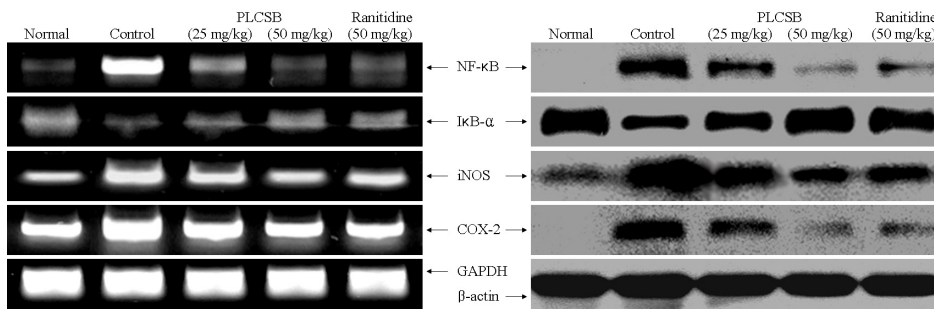


Fig. 6. Effects of polysaccharide of *Larimichthys crocea* swim bladder (PLCSB) on the mRNA and protein expression of NF- κ B, I κ B- α , iNOS and COX-2 in reserpine-induced gastric ulcer mice.

of NF- κ B was decreased while I κ B- α mRNA and protein levels were increased. Additionally, mRNA and protein expression of COX-2 and iNOS was gradually decreased in the presence of the PLCSB depending on the high concentration. These findings indicate that PLCSB may help prevent gastric ulcer by increasing anti-inflammatory activities. Overall, the results of this experiment showed that PLCSB had a strong anti-inflammatory effect on the gastric ulcer.

DISCUSSION

Swim bladder has been historically used as a Chinese folk medicine. Base on previous scientific data, swim bladder has been recently reported to ameliorate different pathological conditions of inflammation, as well as effectively strengthening the function of platelets, capillary vessels and clotting factors [15]. Polysaccharide is the main constituent of swim bladder, but there were not many studies performed regarding the polysaccharide found in the swim bladder, especially these was not any study about the stomach protective function of PLCSB. In this study, the preventive effect on gastric ulcer of polysaccharide of *Larimichthys crocea* swim bladder was researched *in vivo* at first time. *Larimichthys crocea* is used widely in Asia, PLCSB could be easy to get. The results of this study proved the medicinal value of PLCSB, the gastric ulcer inhibition index results demonstrated that the PLCSB conferred the same level of protection against gastric ulcer as ranitidine, a drug used to treat gastritis. Base on these results, PLCSB might be used as medicine for gastric ulcer therapy.

A highly acidic environment in the stomach is an important marker of gastric ulcer; gastric ulcer causes a raise of gastric secretion volume and acid output, with significantly decreased gastric pH [16]. In the present study, PLCSB was found to exhibit a preventive effect on gastric injury by increasing levels of stomach acid.

Levels of serum cytokines, including IL-6, IL-12 and TNF- α , in patients with inflammatory diseases are higher than those in healthy individuals [17]. Cytokine receptors and the inflammatory cytokines IL-6, IL-12, TNF- α and IFN- γ play pathogenic roles in gastric disease, lower levels of those levels were also indicative of improved gastric ulcer preventive effect [18,19]. IL-6 is an interleukin that functions as a proinflammatory and an anti-inflammatory cytokine, and is encoded by the IL6 gene in humans [20]. IL-6 is secreted by T cells and macrophages to stimulate an immune response, particularly during tissue damage, which leads to inflammation. IL-6 is also known to play a role

in fighting infection [21]. IL-12 through IFN- γ -dependent induction of the antiangiogenic factors interferon-inducible protein (IP) 10 and monokine induced by gamma interferon (MIG) contributes to inflammation eradication [22]. Tumor necrosis factor alpha (TNF- α) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The primary role of TNF is the regulation of immune cells. As an endogenous pyrogen, TNF induces fever, apoptotic cell death, sepsis (via IL-1 and IL-6 production), cachexia and inflammation and inhibits tumorigenesis and viral replication [23]. Inflammatory cytokines, IL-6 and TNF- α , play a pathogenic role in diseases of the stomach [24]. Although systemic IL-6 levels are elevated following traumatic hemorrhage, hepatocellular function is impaired and gastric injury occurs [25]. TNF- α has also been identified as a key mediator in a number of experimental stomach injury models [26]. In this study, we observed that the colonic levels of IL-6, IL-12, TNF- α , and IFN- γ in the reserpine induced gastric ulcer mice were markedly decreased by PLCSB treatment. Base on this study, PLCSB showed a good gastric ulcer preventive effect, and the high concentration could increase the anti-ulcer effect.

MOT and SP are excitatory gastrointestinal hormones. Their content would increase after stimulation [27]. Once MOT is stimulated, it would cause surplus secretion of gastric acid. Too much gastric acid would make the inner part of the stomach be acidic, thus compounding the gastric ulcer [28]. From these results, MOT and SP levels increased with the reserpine incitement. SS and VIP are inhibitive gastrointestinal hormones. They can inhibit the secretion of gastric acid [27]. It has been proved that gastric mucosa would result in surplus secretion of gastric juice and the pH value of it would be lower than the normal value [29]. SS and VIP increased with the high concentration PLCSB treatment compare to the control mice, the gastric secretion volume might decrease and the pH of gastric juice might increase by reason that the SS and VIP levels increased. In this study, the gastric secretion volume and pH of gastric juice also proved these relationships.

After gastric tissue suffering from gastric ulcer, partial tissues would be oxidized due to damage. As important antioxidase, SOD and GSH-Px would reduce peroxide in the gastric tissue into harmless substance or substance of little harm, which is beneficial to the recover of gastric ulcer [30]. Ulcer is caused by imbalance between the damage of gastric tissue and protective factors. NO serves to protect gastric mucosa and keeps blood flow smoothly. Its content decreases substantially in patients suffering from gastric ulcer. It has been proved to be effective component to guard against gastric ulcer [31]. MDA is the marker of oxidative

stress and it would generate in large amounts in the damaged areas of gastric tissue. Therefore, it can be regarded as an indicator of gastric ulcer to test [32]. These results demonstrate that a higher concentration of PLCSB decreased the degree of gastric ulcer.

NF- κ B, I κ B- α , COX-2 and iNOS genes in the tissue could potentially be used as biomarkers to monitor viscera damage. NF- κ B is one of the most ubiquitous transcription factors, and regulates the expression of genes required for cellular proliferation, inflammatory responses, and cell adhesion [33]. NF- κ B is present in the cytosol where it is bound to the inhibitory protein I κ B. Following its induction by a variety of agents, NF- κ B is released from I κ B and translocates to the nucleus where it binds to the κ B binding sites in the promoter regions of target genes [34]. Following inflammatory stimuli, both COX-2 and iNOS have been reported to induce deleterious effects in the stomach [35]. iNOS and COX-2 in order to boost inflammatory responses in early stages [36]. Inflammatory processes are mediated by multiple molecular mechanisms. Two of the most prominent are the production of iNOS and COX-2. Inflammatory stimuli elicit the synthesis of iNOS and COX-2 proteins with similar time courses, which suggests that the two systems may interact [37].

In the past research, PLCSB significantly induced apoptosis in colon tissues of mice, it had a potent preventive against *in vivo* colon carcinogenesis [38]. In this study, the gastric ulcer preventive effect of PLCSB was further evaluated by various *in vivo* experimental methods, including serum cytokine assay of IL-6, IL-12, TNF- α and IFN- γ ; assay for serum levels of MOL, SS, SP and VIP; tissue contents of SOD, GSH-Px, NO and MDA test, tissue RT-PCR, and western blot assays for checking the inflammatory related genes of NF- κ B, I κ B- α , iNOS and COX-2. Analysis of the stomachs of various mice treatment groups revealed that PLCSB prevented reserpine induced abdominal ulcer, indicating that PLCSB represents a potentially useful agent for the treatment or prevention of drug induced gastric ulcer *in vivo*. PLCSB could be used as the new drug or health product for gastric ulcer prevention.

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