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Ameliorative effect of low molecular weight peptides from the head of red shrimp (*Solenocera crassicornis*) against cyclophosphamide-induced hepatotoxicity in mice

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ABSTRACT

Our study was aimed at investigating the protective effect of low molecular weight peptides (SCHPs-F1) from red shrimp (*Solenocera crassicornis)* head against cyclophosphamide-induced hepatotoxicity in mice. We found that SCHPs-F1 treatment dose-dependently normalized the biochemical markers, hepatic index, and total CYP450 enzyme content in CTX-induced hepatotoxicity in mice. SCHPs-F1 also ameliorated the CTX-mediated structural disorders of the hepatic tissue. Western blot results suggested that SCHPs-F1 significantly restored the levels of endogenous antioxidants (CAT, T-AOC, GSH-Px, SOD and MDA levels) through activating the Nrf2 signal by upregulating the expression of GCLM, HO-1, and NQO-1. Moreover, SCHPs-F1 improved the CTX-induced hepatotoxicity by inhibiting of NF-κB signal responses and down-regulating the expression of the inflammatory factors (IL-1β, IL-6, IFN-γ and TNF-α). These findings suggest that SCHPs-F1 can regulate Nrf2 and NF-κB signaling pathways and reduce the oxidative stress and inflammation in CTX-induced hepatotoxicity. Overall, SCHPs-F1 are value-added food ingredients for alleviating CTX-induced hepatotoxicity.

1. Introduction

Cyclophosphamide (CTX), an oxazaphosphorine nitrogen mustard alkylating agent, is widely prescribed for treating human malignant tumors and leukemia (Moignet et al., 2014). However, CTX, an inactive prodrug, is metabolized and activated by the hepatic microsomal cytochrome P450 enzyme system and subsequently exerts its pharmacological activity (Moore, 1991). Previous studies have shown that the metabolite of phosphoramide mustard primarily exerts antitumor activity, while acrolein is associated with CTX-induced hepatic toxicity (Aladaileh et al., 2019; Boddy & Yule, 2000; Moghe et al., 2015; Sherif, 2018). Accumulation of acrolein in the hepatic tissues increases ROS production, which results in lipid peroxidation, disturbs the antioxidant enzyme defense system and eventually disrupts the hepatic structure and biochemical function (El-Naggar, Abdel-Farid, Germoush, Elgebaly, & Alm-Eldeen, 2016; Singh, Prakash, Tiwari, Mishra, & Kumar, 2018). Moreover, the uncontrolled redox reaction can disturb the pro-inflammatory and anti-inflammatory homeostasis, which is manifested by oxidative stress and inflammation in the hepatic tissues (Aladaileh et al., 2019; Germoush & Mahmoud, 2014; Mansour, Saleh, & Mostafa, 2017). Therefore, adjuvant therapies should be co-administered along with CTX to maintain human health and reduce the risk of hepatotoxic side effects associated with chemotherapy.

In recent years, functional foods and nutraceuticals from natural origins are often employed as adjuvants or alternatives for chemotherapy. Among them, bioactive peptides, produced by enzymatic

Abbreviations: CTX, Cyclophosphamide; MW, Molecular Weight; ROS, Reactive oxygen species; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; AKP, Alkaline phosphatase; CYP 450, Cytochrome P450; Nrf2, Nuclear factor erythroid-2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; CAT, Catalase; T-AOC, Total antioxidant capacity; GSH-Px, Glutathione peroxidase; SOD, Superoxide Dismutase; MDA, Malondialdehyde; GCLM, Glutamate cysteine ligase modifier subunit; HO-1, Heme oxygenase 1; NQO-1, NADPH Quinone acceptor Oxidoreductase 1; NF-κB, Nuclear factor kappa-B; IKK, Inhibitor of nuclear factor kappa-B kinase; IκB, Inhibitor of NF-κB; 6IL-1β, 6, Interleukin-1β; IFN-γ, Interferon-γ; TNF-α, Tumor necrosis factor-α

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hydrolysis of natural food proteins, exhibited high safety (Udenigwe & Aluko, 2012), good bioavailability (Sun, Acquah, Aluko, & Udenigwe, 2019), and various health benefits, including immunomodulatory, antioxidant, antihypertensive and anticancer efficacies (Harnedy & FitzGerald, 2012; E. K. Kim et al., 2013; Udenigwe & Aluko, 2012). Presently, shrimp is globally cultivated or captured in huge amounts; the shrimp by-products have no benefit-cost ratio, and their accumulation threatens the environmental safety (Benjakul, Binsan, Visessanguan, Osako, & Tanaka, 2009; James, Diego, & Darryl, 2017). Several peptides and protein hydrolysates are derived from shrimps' processing, and these by-products can be further applied in diverse fields, including antiproliferative peptide hydrolysates from the shell of *Pleuroncoides planipes* (Kannan, Hettiarachchy, Marshall, Raghavan, & Kristinsson, 2011), appetite-suppressive peptides from the head of *Penaeus aztecus* (Cudennec, Ravallec-Plé, Courois, & Fouchereau-Peron, 2008), myotropic peptides from cephalothorax of *Litopenaeus vannamei* (Leduc et al., 2018). However, no studies have reported the preparation of active peptides from the head of red shrimp (*Solenocera crassicornis*).

Solenocera crassicornis is an economic marine shrimp species, belonging to the phylum *Arthropoda*, suborder *Dendrobranchiata*, family *Solenoceridae* (WoRMS, 2019). It is widely distributed in the adjacent waters of Southeast Asia, the Yellow Sea, the East China Sea, and the South China Sea (Xu, Zhang, Dai, & Wang, 2010). Shrimp is an important source of protein for humans. During aquatic processing, the by-products of shrimp (30% to 50% of the raw materials) are discarded. However, the shrimp head is a rich protein source (Prameela et al., 2017; Zha et al., 2015). In 2018, about 1.31 million tons of marine shrimp were caught in China (Bureau of Fisheries, 2019), and the by-products created environmental and disposal issues. Currently, many food protein-derived peptides or hydrolysates have exhibited protective effects against chemical hepatotoxicity. SCSP (RVAPEEHPVEGRYLV), from *Cyclaina sinensis*, could ameliorate CTX-induced hepatotoxicity through TLR4-mediated NF-kB pathway and apoptosis-related proteins (Jiang, Yang, Zhao, Tian, & Tang, 2019). *Liza klunzingeri* protein hydrolysate (FPH) can reduce the oxidative stress in carbon tetrachloride-induced toxicity in male rats (Rabiei et al., 2019). Similarly, corn germ meal (CGMAPs) could protect mice against chronic alcoholic liver injury by normalizing the liver oxidative stress markers and antioxidant capacity in the body (Y. Yu, Wang, Wang, Lin, & Liu, 2017). In the present study, low molecular weight peptides were prepared from the head by-product of red shrimp (*Solenocera crassicornis*) *via* binary-enzymes hydrolysis, and then the molecular weight (Mw) distribution and amino acid composition of SCHPs-F1 were determined. The activity of SCHPs-F1 was determined by measuring the hepatic damage markers (ALT, AST and AKP), hepatic function parameters (hepatic index and total CYP450 enzyme content), and histopathology of the hepatic tissues in CTX-induced hepatotoxicity in mice. Furthermore, the underlying mechanism of SCHPs-F1 was elucidated from the antioxidant enzymes, inflammatory parameters, and the key regulatory pathways (Nrf2 and NF-κB). Finally, we have prepared the hepatoprotective peptide-fraction from the discarded shrimp head for the first time, which offers a high-value utilization of shrimp processing by-products and a newer method for the industrial development of red shrimp.

2. Materials and methods

2.1. Materials and reagents

Solenocera crassicornis was purchased from the Zhoushan International Aquatic Center (Zhoushan, Zhejiang, China). Pepsin and trypsin were purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). The hematoxylin-eosin staining kit and RIPA lysis buffer were purchased from Beyotime Biotechnology (Shanghai, China). BCA protein quantification assay kit and commercial kits of GSH-Px, MDA, SOD, CAT, AST, ALT, AKP were procured from Jiancheng Bioengineering Institute (Nanjing, China). ELISA assay kits for IFN-γ, TNF-α, IL-6, IL-1β, and the Enhanced Chemiluminescence Western Lightning kit were purchased from Boster Biological Technology Co., Ltd. (Wuhan, China). ELISA assay kit for CYP450 was purchased from SenBeiJia Biological Technology Co., Ltd. (Nanjing, China). Rabbit antibody GCLM, NQO-1, phospho-IκB α, phospho-NF-κB p65, NF-κB p65, IKKα were supplied by Beyotime Biotechnology (Shanghai, China); mouse antibodies for Nrf2, HO-1, Keap1, IκB α, β-actin were supplied by ProteinTech Group (Chicago, IL, USA); goat anti-rabbit IgG/HRP antibody and goat Anti-mouse IgG/HRP antibody were supplied by Solarbio Sci-technology Co. Ltd (Beijing, China). Other reagents were of analytical grade.

2.2. Preparation of SCHPs-F1

SCHPs-F1 were prepared through a stepwise enzymatic hydrolysis method, as described by Z. Xu et al., 2019. The degreased shrimp head was firstly homogenized (D-500 homogenizer, Dragon Lab, Beijing, China), and then distilled water was added into the homogenate to prepare the reaction system (m/m = 1:10). Then, pepsin (2500 U/g) was added into the reaction system, and the pH value was adjusted to 3.0; the hydrolysis reaction was continued for 4 h at 37 °C. Then, the pH of the reaction system was adjusted to 8.0 with 0.1 M NaOH to inactivate pepsin, and then trypsin (2500 U/g) was added into the reaction system, and the hydrolysis reaction was continued for another 4 h. Subsequently, the solution was heated for inactivating the enzymes, then cooled and centrifuged at 10,000 rpm for 15 min (CF16RN, Hitachi, Tokyo, Japan). The supernatant was collected for microfiltration (0.22 μm). Ultrafiltration was performed with a 1 kDa membrane. The supernatant SCHPs-F1 (lower than 1 kDa) were collected and freeze-dried for further study.

2.3. Determination of MW distribution

The Mw distribution of SCHPs-F1 was performed following our previously reported method (Zhang, Hu, Lin, Ding, & Yu, 2019). The standard molecular weight samples, containing cytochrome *c* (12,400 Da), aprotinin (6512 Da), bacitracin (1423 Da), and pentapeptide PVERK (628 Da), were filtered through 0.22 μm filters and loaded into a TSK gel G2000 SWXL analytical column (7.8 × 300 mm, 5 μm, TOSOH, Tokyo, Japan) in turns, and the chromatographic analysis was performed using an Agilent 1260 Infinity II HPLC (Agilent Technologies, Inc., CA, USA). The mobile phase, acetonitrile/H_2O/TFA (45:55:0.1), was eluted at a flow rate of 1.0 mL/min and detected at a wavelength of 220 nm.

2.4. Amino acid composition analysis

The SCHPs-F1 were hydrolyzed with 6 M HCl (containing 2% phenol, m/v) in nitrogen-filled pressure screw glass tubes at 110 °C for 22 h, and then the sample assay solution was prepared according to the method of (Zha et al., 2015). The amino acid content was analyzed using a Hitachi 835–50 amino acid analyzer (Hitachi, Tokyo, Japan).

2.5. Animals and treatment

Male ICR mice (6 weeks old) were purchased from the Zhejiang Province Laboratory Animal Public Service Platform (Hangzhou, Zhejiang, China, No. SCXK ZHE 2014-0001) and fed a commercial pellet diet with free access to sterilized water under a pathogen-free environment. Mice were acclimated for 7-days under a controlled cage temperature (22 ± 2 °C) and humidity (50–60%) with a 12 h light/dark cycle. The procedure for care and use of laboratory animals was approved by the Zhejiang Ocean University Experimental Animal Ethics Committee (Zhoushan, Zhejiang, China) and complied with all applicable institutions and government regulations regarding the ethical use of the animals. Animals were randomly divided into five groups of mice

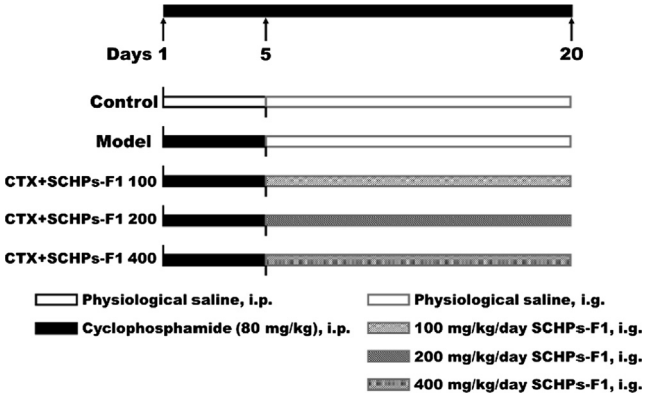

Fig. 1. The experimental protocol of mice group and treatment.

(n = 8 in each group, Fig. 1). But, the control group was administered with saline for 20 days, and the other groups were administered with CTX (80 mg/kg/day) for 5 days and saline or different doses of SCHPs-F1 (100, 200, 400 mg/kg/day) for 15 days.

2.6. Sample collection and biochemical analysis

At the end of the study, the serum was separated from the *retro*-orbital whole blood. The serum hepatic function markers, including ALT, AST and AKP, were measured using commercial assay kits. The hepatic tissue was immediately dissected and weighed. The tissue homogenate (10% hepatic tissue within normal saline, g/g) was prepared from the frozen hepatic tissues (Xu et al., 2017). After centrifuging, the supernatant of the tissue homogenate was collected for the CYP450 ELISA assay. The SOD, CAT, GSH-Px, T-AOC, MDA, IL-1β, IL-6, IFN-γ and TNF-α levels were measured in the hepatic tissue homogenate according to the manufacturer's instructions.

2.7. Histopathological examinations

The hepatic tissue of the left lobe of the liver was fixed in 4% paraformaldehyde for histopathological examination. Then, the hepatic tissue of each mouse was dehydrated stepwise in graded ethanol and embedded in paraffin. The paraffin-embedded sections were cut into 4 μm thick pieces using a microtome (Leica RM2135, Leica Instruments GmbH, Wetzlar, Germany), stained with hematoxylin and eosin (Yu et al., 2019) and then examined and photographed under an optical microscope (Biomicroscope CX31, Olympus, Japan).

2.8. Western blotting

A part of the hepatic tissue was firstly treated with pre-cooled radio immunoprecipitation assay lysis buffer, containing the protease and phosphatase inhibitors (Meabon et al., 2012), and then sonicated using a VC505 ultrasonic processor (Sonics & Materials, Inc, Newtown, USA). The protein content of each homogenate supernatant was quantified using the BCA Protein Quantification Assay Kit. The specific steps of western blot were carried out as previously described (F. Yu et al., 2019). The membranes were blocked and probed with specific primary antibodies (Nrf2, Keap1, GCLM, HO-1, NQO-1, IKKα, IB, p-IB, p65, p-p65) at 4 ˚C overnight. Finally, the membranes were incubated with appropriate secondary antibodies, and the target imprints were developed using an enhanced chemiluminescence western lightning kit and a FluorChem FC3 chemiluminescent gel imaging system (ProteinSimple, Silicon Valley, CA, USA). β-actin was used as an internal control.

2.9. Statistical analysis

All experimental data were expressed as the mean ± standard deviation ($\bar{x} \pm s$) and analyzed using SPSS software version 24.0 (SPSS Inc., Chicago, IL, USA). The statistical significance of the data was compared using a one-way analysis of variance (ANOVA). The difference between the means was considered significant at $P < 0.05$. The optical output density of the target imprints was quantified using the AlphaView image analysis software (Version 3.4.0, ProteinSimple, Silicon Valley, CA, USA).

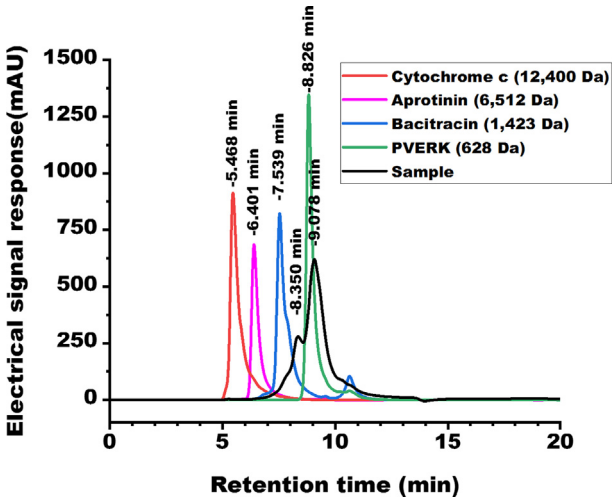

Fig. 2. The HPLC chromatograms of the standard molecular weight samples and the protein hydrolysates of shrimp head of *Solenocera crassicornis*.

3. Results

3.1. Characterization of SCHPs-F1

Previous research indicated that low molecular weight polypeptides could be readily absorbed and utilized in intact form, and their biological activities could be preserved during their gastrointestinal digestion (Chalamaiah, Yu, & Wu, 2018; Vijaykrishnaraj & Prabhasankar, 2015). The MW distributions of SCHPs-F1 were determined by HPLC under the same chromatographic conditions as standard samples (Fig. 2). Among them, SCHPs-F1 were primarily composed of molecules at the range of 180–500 Da (72.50%), while 27.50% of SCHPs-F1 were distributed in the range of 500–1000 Da. Therefore, the biological activity of SCHPs-F1 may be attributed to the short peptides.

The amino acid composition is illustrated in Table 1. From Table 1, SCHPs-F1 are rich in Glu, Asp, Gly and Leu, similarly to the protein hydrolysates of *Liza klunzingeri* (Rabiei et al., 2019), *Zea mays Linn* (G. C. Yu, Lv, He, Huang, & Han, 2012) and *Ganoderma lucidum* (Shi. Y., et al., 2008; Sun, He, & Xie, 2004); they also exhibited a protective effect against carbon tetrachloride and d-GalN -induced chemical hepatic injury. Furthermore, the essential amino acids (Thr, Val, Met, Ile, Leu, Phe and Lys) were accounted for 38.07% of the total amino acids, and the branched-chain amino acids (Val, Leu, Ile) were accounted for 13.94% in SCHPs-F1. Therefore, the low molecular weight peptides in SCHPs-F1 could be commercially developed as a dietary supplement for adjuvant CTX treatment.

3.2. Effect of SCHPs-F1 on hepatic index and function biomarkers

No significant difference was observed in the average initial body-weight of the randomly grouped mice. The treatment with CTX for 5-days significantly reduced the hepatic index in the model group mice, compared to the control group. However, the treatment with SCHPs-F1 restored the hepatic index in CTX-induced mice (Fig. 3). The serum activities of ALT, AST and AKP were significantly elevated in the CTX treated model group (Fig. 4**A, B, and C**). However, SCHPs-F1 treatment

Table 1
The amino acid composition of the low molecular weight peptides from by-product shrimp head of *Solenocera crassicornis*.

Amino Acid	Abbreviation	Ratio (g/100 g)
Valine*	Val	4.10
Leucine*	Leu	5.90
Isoleucine*	Ile	3.94
Lysine*	Lys	4.75
Phenylalanine*	Phe	2.48
Methionine*	Met	1.87
Threonine*	Thr	3.57
Histidine*	His	2.33
Aspartic acid#	Asp	7.65
Serine#	Ser	4.20
Glutamic acid#	Glu	9.56
Glycine#	Gly	6.38
Alanine#	Ala	3.79
Tyrosine#	Tyr	2.26
Arginine#	Arg	4.79
Proline#	Pro	2.30
Essential amino acids*		28.94
Non-essential amino acids#		40.93
Total amino acids		69.87

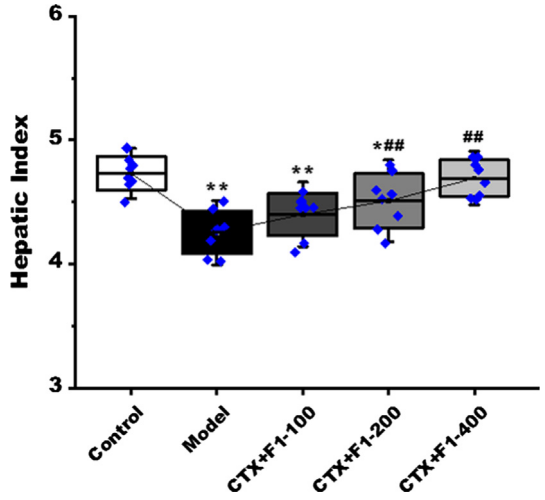

Fig. 3. Effect of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) on the hepatic index; All data were expressed as mean ± SD (n = 8 for each group), *$P < 0.05$, ** $P < 0.01$ *vs*. Control group; # $P < 0.05$, ## $P < 0.01$ *vs*. Model group.

significantly reduced the levels of AST ($P < 0.01$), ALT, and AKP following the CTX administration ($P < 0.01$). These data clearly indicated that all SCHPs-F1 doses alleviated the CTX-induced hepatotoxicity in a dose-dependent manner.

3.3. Effect of SCHPs-F1 on P450 enzyme system in hepatic microsomes

CTX treatment significantly suppressed the total P450 content in the hepatic microsomes compared to the control group ($P < 0.05$) (Fig. 4**D**). Compared to the CTX-treated group, all SCHPs-F1 administered groups significantly reduced the P450 protein content after 15 days dose-dependently ($P < 0.05$).

3.4. Effect of SCHPs-F1 on hepatic histopathology

The untreated mice displayed intact lobules in hepatic tissues, and the hepatic cords were neatly arranged, radially around the central vein (Fig. 5). In the CTX-treated mice, the hepatic lobules were discernible, and the hepatic cord and hepatic sinus were disordered and unclear. Hepatocyte enlargement was accompanied by cytoplasmic loosening. After SCHPs-F1 treatment in mice, the pathological structural disorder of hepatic tissue was ameliorated. Specifically, the alignment of the hepatic cords restored the order, the hepatic sinus gap became gradually clear, and the injuries in the hepatocytes were also repaired in a dose-dependent manner.

3.5. Effect of SCHPs-F1 on the markers of oxidative stress in liver homogenate

Compared to the control group, CTX-treatment increased the hepatic MDA level and reduced the hepatic antioxidant enzyme activities (SOD, CAT, GSH-Px, and T-AOC). Post-treatment with different doses of SCHPs-F1 reduced the MDA levels, and the activity of hepatic antioxidant enzymes was significantly increased (Table. 2). The SCHPs-F1 high-dose group exhibited significantly different results from the model group and no difference from the control group. Compared to the CTX-treatment group, the SOD level of the SCHPs-F1 high-dose group increased to 12.43 ± 0.57 U/mg protein ($P < 0.01$), the CAT level increased to 40.99 ± 5.63 U/mg protein ($P < 0.01$), the GSH-Px level increased to 229.45 ± 6.39 U/mg protein ($P < 0.01$), and the T-AOC level increased to 5.18 ± 0.69 U/mg protein ($P < 0.01$). Similarly, the MDA level decreased to 18.05 ± 2.46 nmol/mg protein ($P < 0.05$), compared to the control group.

3.6. Effect of SCHPs-F1 on the inflammatory parameters in the hepatic homogenate

The IL-1β, IL-6, IFN-γ, and TNF-α levels were significantly increased in the hepatic tissues of CTX-induced mice compared to the control group (Fig. 6), indicating that the CTX treatment caused hepatic injury and inflammation. However, co-treatment with different doses of SCHPs-F1 exhibited restored levels of proinflammatory factors. The highest dose (400 mg/kg body weight) of SCHPs-F1 effectively controlled the secretion of inflammatory factors and restored them to the normal levels. The results indicated that the concentrations of IL-1β (2235.24 ± 121.73 pg/mL, $P < 0.01$) and IL-6 (3027.14 ± 416.18 pg/mL, $P < 0.01$) in the SCHPs-F1 high-dose group were effectively reduced (Fig. 6 A, B). Similarly, the results of IFN-γ (2429.60 ± 282.08 pg/mL, $P < 0.01$) and TNF-α (552.46 ± 49.45 pg/mL, $P < 0.01$) differed significantly with the model group (Fig. 6 C, D).

3.7. Effect of SCHPs-F1 on the protein expressions of Nrf2 and NF-κB signaling pathways

To further investigate the possible hepatoprotective mechanism of SCHPs-F1 in CTX-induced mice, we examined the expression levels of proteins associated with the Keap1-Nrf2 regulatory pathway (Fig. 7). SCHPs-F1 effectively down-regulated the expression of repressor protein Keap1. Additionally, CTX treated mice substantially reduced the Nrf2 hepatic expression compared to the control group ($P < 0.01$). Mice treated with 100 mg/kg SCHPs-F1 for 15 days did not exhibit any significant changes in the hepatic Nrf2 levels, whereas the Nrf2 levels increased significantly in the 200 and 400 mg/kg SCHPs-F1 treated groups compared to the CTX-treated mice. Similarly, the expressions of Nrf2 downstream antioxidant proteins (NQO1, HO-1 and GCLM) in hepatic microsomes were up-regulated in a dose-dependent manner after SCHPs-F1 treatment.

According to Fig. 8, the level of IKKα protein in the hepatic tissue of CTX-treated mice was substantially increased, which further activated the downstream signaling cascade, resulting in an increase in the phosphorylated IκB-α level, degradation of IκB-α level, and up-regulation of NF-κB p65 level. However, the level of IKKα complex was significantly reduced in the SCHPs-F1 treated mice compared to the CTX-treated group. The protein levels of phosphorylated IκB α and

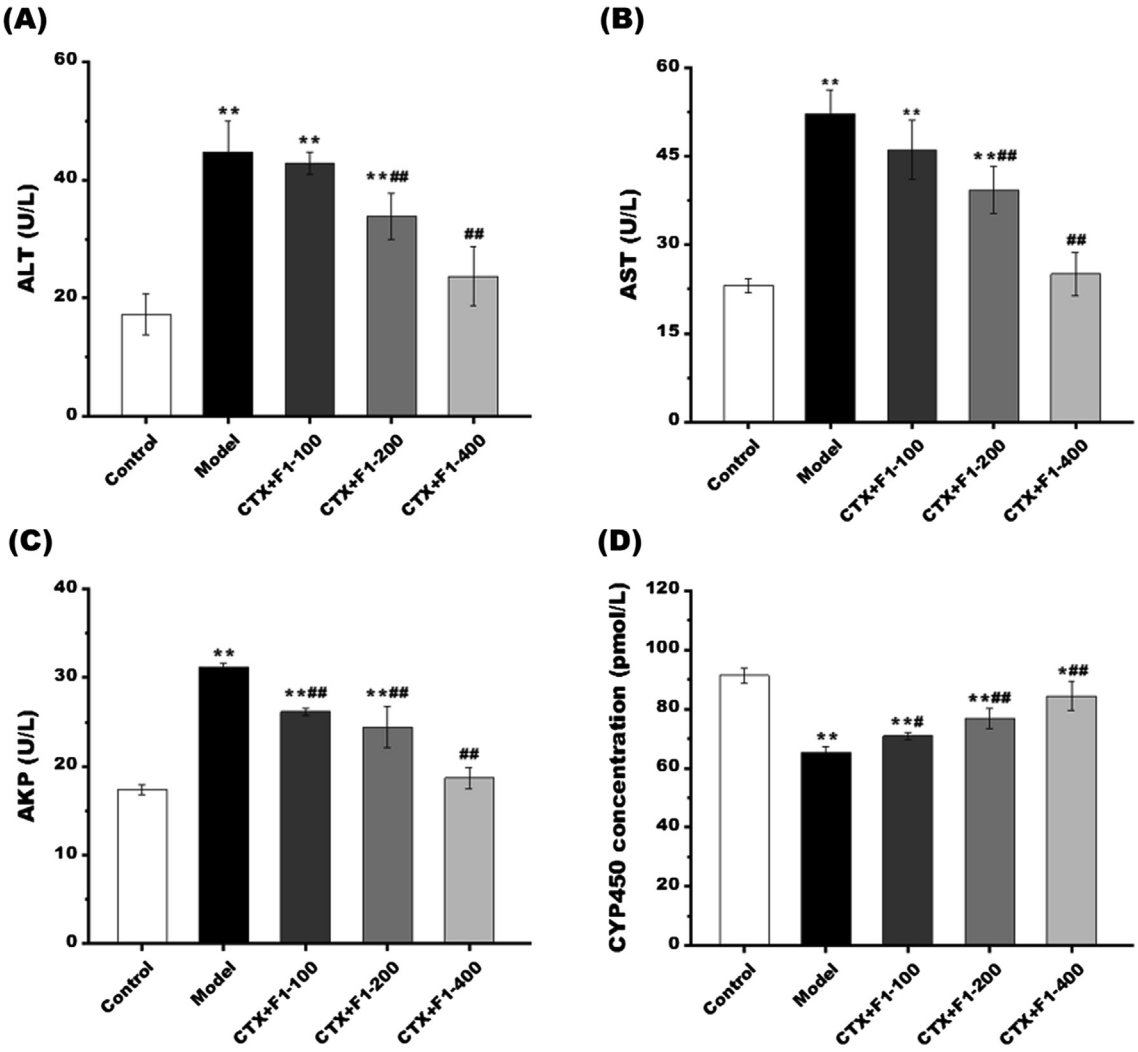

Fig. 4. Effect of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) on the serum (A) ALT (Alanine aminotransferase), (B) AST (Aspartate aminotransferase), (C) AKP (alkaline phosphatase) levels and (D) hepatic total CYP450 enzyme content (Cytochrome P450) in mice; All data were expressed as mean ± SD (n = 8 for each group), *$P < 0.05$, ** $P < 0.01$ *vs*. Control group; # $P < 0.05$, ## $P < 0.01$ *vs*. Model group.

phosphorylated NF-κB p65 were down-regulated, and IκB α, NF-κB p65 were also restored to the normal level of inactive complexes, thereby reducing the nuclear transcription and the release of inflammatory cytokines. These data suggested that SCHPs-F1 improved the CTX-induced hepatic oxidative stress by increasing the levels of SOD, CAT, GSH-Px, and T-AOC (Table. 2) and activating Nrf2-mediated antioxidant signaling pathways (Fig. 7). Moreover, SCHP also reduced the secretion of the pro-inflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α) (Fig. 6) by down-regulating proteins associated with the NF-κB signaling pathway (Fig. 8), while the hepatic inflammation was alleviated.

4. Discussion

The liver metabolizes and detoxifies exogenous drugs or compounds through biological transformation (Singh et al., 2018). CTX is an effective anti-cancer drug in the clinical chemotherapy of different lymphomas and certain types of leukemia (American Society of Health-System Pharmacists, 2019). However, its active metabolites (phosphoramide mustard and acrolein) can induce severe hepatotoxicity (Papaldo et al., 2005; Shen et al., 2019). However, adjuvants can be combined with chemotherapy to overcome CTX-induced hepatic toxicity. Herein, we prepared SCHPs-F1 from the head of red shrimp *(Solenocera crassicornis)* and investigated its potential therapeutic effect against CTX-induced hepatic toxicity. Thus, cheap marine by-products can be transformed into highly value-added functional foods or dietary supplements.

Consistent with some prior animal studies (Habibi et al., 2015; Lata, Singh, NathTiwari, & Upadhyay, 2014; Sheweita, El-Hosseiny, & Nashashibi, 2016; F. Xu et al., 2017), the results of the present study showed that the CTX treatment could increase the hepatic function marker levels (ALT, AST, AKP) and decrease the hepatic index and cytochrome P450 enzyme content in mice. ALT and AST activities are generally estimated for evaluating hepatic toxicity. When hepatic tissue is damaged, the intracellular enzymes, like AST and ALT, are released into the blood, thus their activities increase in the serum (Dzoyem, Kuete, & Eloff, 2014). Additionally, increased serum AKP activity also biochemically indicates hepatic damage (Rabie & Wong, 2012). The hepatic microsomal CYP450 system is involved in the metabolism and elimination of CTX. The toxic metabolites of CTX (phosphoramide mustard and acrolein) ultimately cause hepatic toxicity and cell damage (Ding et al., 2018). In this study, the total CYP450 isoenzyme content in the hepatic tissue was significantly reduced after CTX exposure. Furthermore, CTX causes a reduction in the hepatic index; the representative microstructural images indicated hepatocyte swelling and cytoplasmic vacuoles along with hepatocellular damage surrounded by

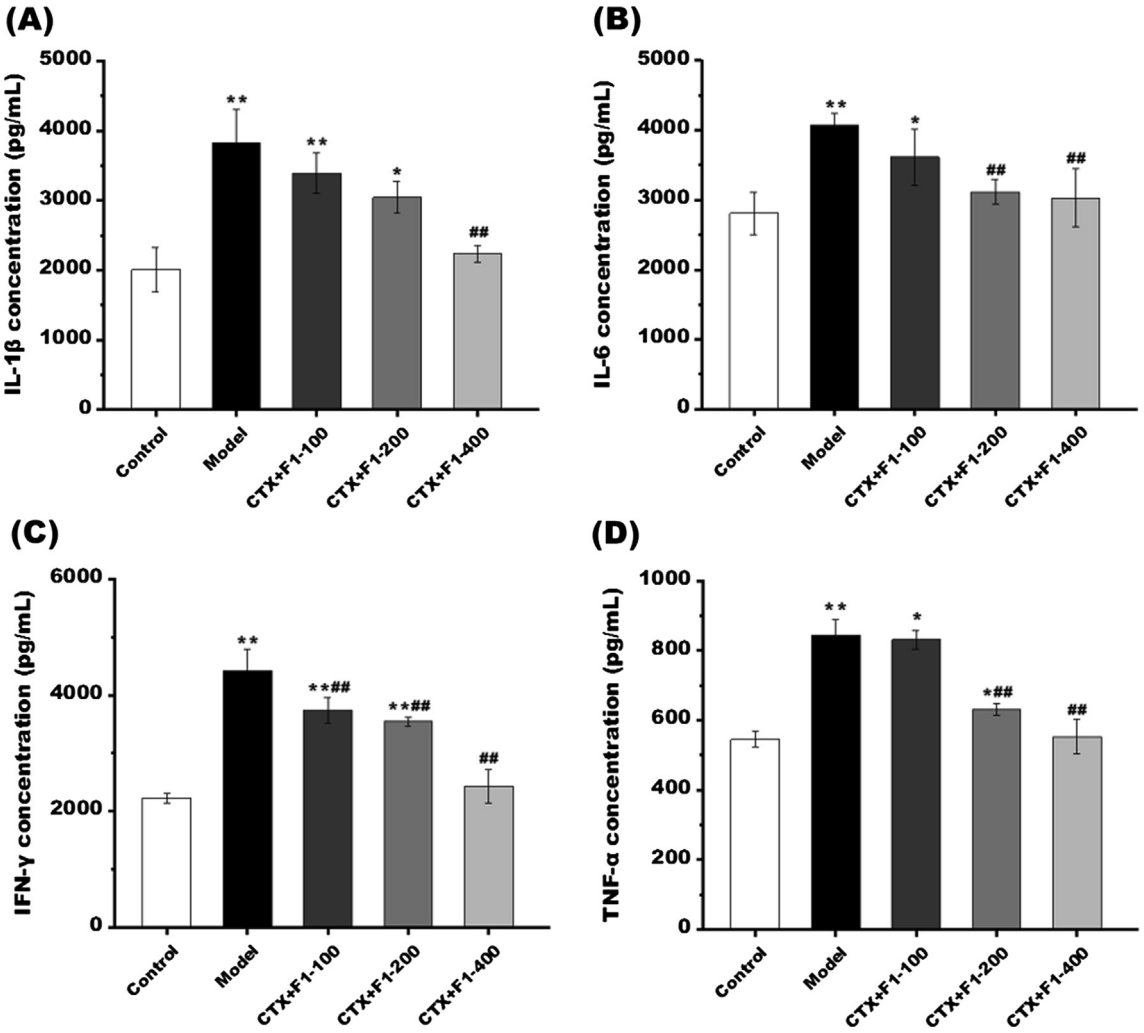

Fig. 5. Representative photomicrographs of hepatic section of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) treated mice (Hematoxylin-Eosin Staining, 200× and 400× magnification).

Table 2
Effect of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) on the hepatic CAT (Catalase), SOD (Superoxide Dismutase), GSH-Px (Glutathione peroxidase), T-AOC (Total antioxidant capacity), MDA (Malondialdehyde) levels.

Group	CAT (U/mg prot)	SOD (U/mg prot)	GSH-Px (U/mg prot)	T-AOC (U/mg prot)	MDA (nmol/mg prot)
Control	42.75 ± 2.07	13.13 ± 1.78	235.49 ± 11.79	5.47 ± 0.34	17.24 ± 2.22
Model	22.95 ± 3.90**	8.46 ± 0.21**	168.43 ± 17.93**	2.88 ± 0.40**	23.01 ± 2.52**
CP + SCHPs100	28.75 ± 1.82**#	9.51 ± 0.70**	181.67 ± 8.30**	3.53 ± 0.85**	22.27 ± 1.39*
CP + SCHPs200	35.28 ± 4.90*#	9.72 ± 1.05**	203.17 ± 11.43**##	4.72 ± 0.62##	20.22 ± 1.35
CP + SCHPs400	40.99 ± 5.63##	12.43 ± 0.57##	229.45 ± 6.39##	5.18 ± 0.69##	18.05 ± 2.46#

* $P < 0.05$.
** $P < 0.01$ *vs*. Control group.
\# $P < 0.05$.
\#\# $P < 0.01$ *vs*. Model group.

lymphocyte infiltration (Mahmoud, Germoush, Alotaibi, & Hussein, 2017). The marked changes in the liver function markers and histopathological characteristics confirmed hepatic toxicity following CTX injection.

However, compared to CTX-treated mice, the levels of the liver-function markers (AST, ALT, and AKP) were significantly decreased in the group, treated with the CTX + SCHPs-F1 combination. Additionally, SCHPs-F1 treatment markedly increased the cytochrome P450 enzyme system. Along with the microstructural images of hepatic tissue, the above findings indicated that SCHPs-F1 ameliorated CTX-mediated hepatic structural disorders and abnormal secretion of hepatic markers to reduce CTX toxicity. Similar results were observed in some previous studies with *Panax ginseng* and vitamin E (Abdelfattah-Hassan et al., 2019), fennel, cumin and clove essential oils (Sheweita et al., 2016), pentadecapeptide SCSP (RVAPEEHPVEGRYLV) (Jiang et al., 2019), and *Cichorium glandulosum* seed extract (Tong et al., 2017) against CTX-induced hepatotoxicity in mice. Specifically, the treatment interfered with the abnormally high levels of liver biomarkers, reduced the total cytochrome P450 content, and improved the pathological structural characteristics of the hepatic injury in mice.

The intracellular antioxidant enzyme defense system, including SOD, CAT, GSH-Px, and T-AOC, regulates the dynamic balance of redox

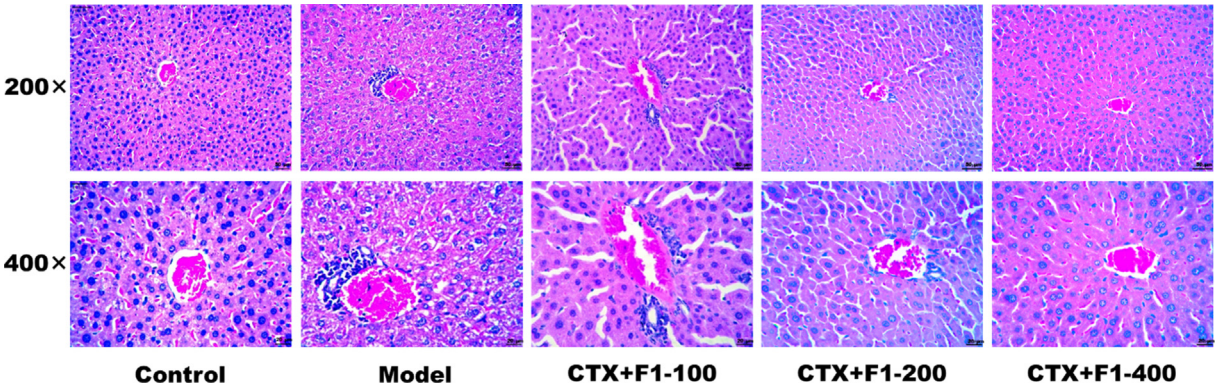

Fig. 6. Effect of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) on the hepatic (A) IL-1β (Interleukin-1β), (B) IL-6 (Interleukin-6), (C) IFN-γ (Interferon-γ) and (D) TNF-α (Tumor necrosis factor-α) levels in mice; All data were expressed as mean ± SD (n = 8 for each group), *$P < 0.05$, ** $P < 0.01$ *vs*. Control group; # $P < 0.05$, ## $P < 0.01$ *vs*. Model group.

reactions and prevents hepatic damage (Cuce et al., 2015; F. Xu et al., 2017; Yuan et al., 2020). In our study, SCHPs-F1 treatment relieved oxidative stress in hepatic tissues and prevented lipid peroxidation of the hepatic cell membranes. Also, the levels of hepatic cytoplasmic enzymes (ALT, AST, and AKP) were reduced in blood, MDA was accumulated, and the levels of endogenous antioxidants (CAT, T-AOC, GSH-Px, SOD) were restored. Furthermore, similar results were found in the protein expression of the Keap1-Nrf2 regulatory pathway. The transcription factor Nrf2 plays a key role in regulating the expression of multiple detoxifications and antioxidant defense genes in response to the hepatic oxidative stress (Kim & Ki, 2017). In the absence of any stress conditions, the specific repressors, Keap1 and Nrf2, are associated in the cytoplasm. Keap1 can mediate the ubiquitination and degradation of Nrf2 through the proteasome pathway and maintain the normal or lower intracellular levels of Nrf2. However, the conformation of Keap1 was changed in response to oxidative stress, and Nrf2 was released from ubiquitination and translocated to the nucleus to activate transcription and expression of downstream antioxidant-related genes (Covas, Marinho, Cyrne, & Antunes, 2013; Lehman-McKeeman, 2013). Compared to CTX, SCHPs-F1 treatment markedly upregulated the hepatic Keap1 and Nrf2 expressions by activating the Nrf2 functional activity in mice. Here, SCHPs-F1 activated Nrf2 signaling, as evident by the expression of GCLM (Teskey, Abrahem, Cao, Gyurjian, Islamoglu, Lucero, & Venketaraman, 2018), HO-1 (Calabrese et al., 2008; Kim et al., 2015), and NQO-1 (Vasiliou, Ross, & Nebert, 2006). Consequently, the induction of the Keap1/Nrf2/antioxidant signaling pathway could protect the liver from oxidative stress.

In response to the oxidative stress, the NF-κB regulatory pathway is activated to participate in the inflammatory response and thereby promotes the production of inflammatory cytokines and chemokines

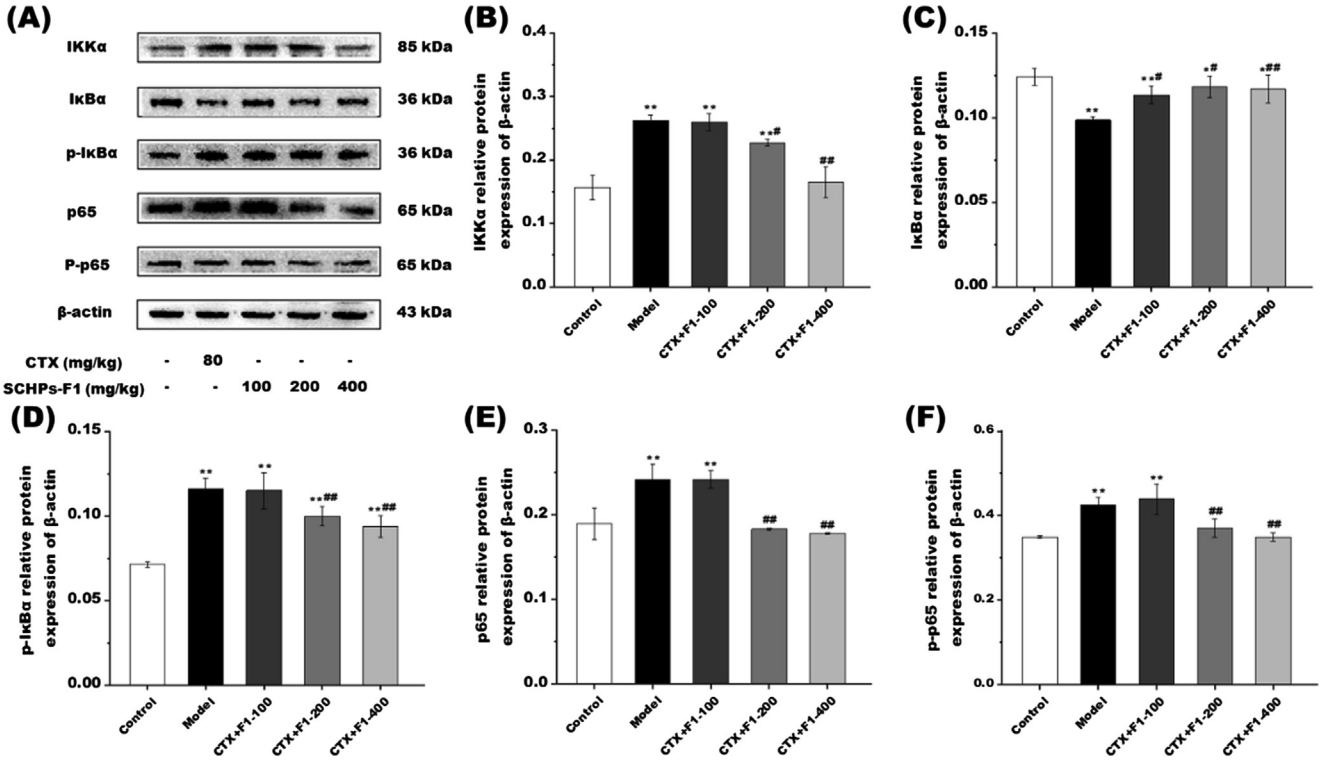

Fig. 7. Effects of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) on (A) the expression of Nrf2 signaling pathway related protein in hepatic tissues; (B) Keap1 (Kelch-like ECH-associated protein 1), (C) Nrf2 (Nuclear factor erythroid-2-related factor 2), (D) GCLM (Glutamate cysteine ligase modifier subunit), (E) HO-1 (Heme oxygenase 1), (F) NQO-1 (NADPH Quinone acceptor Oxidoreductase 1). *$P < 0.05$, ** $P < 0.01$ *vs*. Control group; # $P < 0.05$, ## $P < 0.01$ *vs*. Model group.

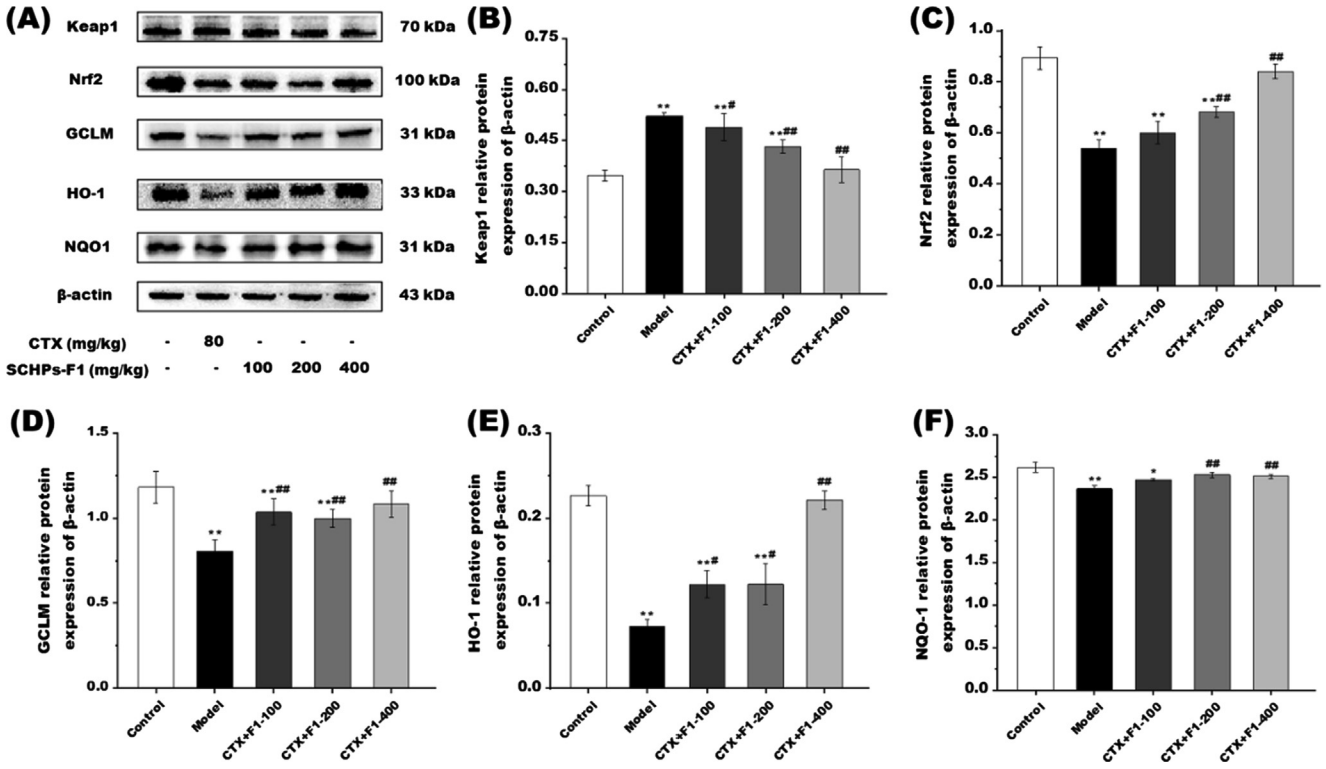

Fig. 8. Effects of cyclophosphamide (80 mg/kg b. wt., i.p., for 5 days) and its combination with either SCHPs-F1 (100, 200 and 400 mg/kg b. wt., i.g., for 15 days) on (A) the expression of NF-κB signaling pathway related protein in hepatic tissues; (B) IKKα (Inhibitor of nuclear factor kappa-B kinase β), (C) IκB α (Inhibitor of NF-κB α), (D) p- IκB α (Phosphor-IκB α), (E) p65 (Nuclear factor kappa-B p65), (F) p-p65 (Phosphor-Nuclear factor kappa-B p65). *$P < 0.05$, ** $P < 0.01$ *vs*. Control group; # $P < 0.05$, ## $P < 0.01$ *vs*. Model group.

(Reuter, Gupta, Chaturvedi, & Aggarwal, 2010; Shi et al., 2014). The results of inflammatory biomarkers confirmed the results of the previous publications (Shi et al., 2014; Shokrzadeh, Ahmadi, Naghshvar, Chabra, & Jafarinejhad, 2014; Tong et al., 2017; Tripathi & Jena, 2010) that SCHPs-F1 improved the CTX-induced hepatic toxicity by restoring the levels of the inflammatory factors, including IL-1β, IL-6, IFN-γ, and TNF-α. NF-κB is a key transcription protein involved in the inflammatory immune response. Similar to the Nrf2 transcription factor, under normal conditions, IkB-α sequesters the dimers of p50 and p65 subunits in the cytoplasm as an inactive form. In response to the oxidative stress or inflammatory signals, the IKK protein complex of the NF-κB signal pathway is rapidly activated, which thereby promotes the phosphorylation and ubiquitination of IκB-α protein. Activated NF-κB dimers are translocated to the nucleus and activate genes with NF-κB binding sites, and they initiate the transcription and expression of inflammatory mediators and pro-inflammatory cytokines (Lund, 2010). In this study, SCHPs-F1 inhibited the activation and nuclear translocation of NF-κB p65 by down-regulating the CTX-mediated IKK and phosphorylated IκB-α protein expression in mice liver. These data confirmed that SCHPs-F1 alleviated CTX-induced hepatic inflammatory damage *via* the NF-κB regulatory pathway. In-depth experiments can elucidate the ability of SCHPs-F1 to restore the CTX-induced damage to the immune system and other metabolic organs.

5. Conclusion

In summary, the low molecular weight peptides (SCHPs-F1) from the red shrimp head (*Solenocera crassicornis)* exhibited a dose-dependent ameliorative effect against CTX-induced hepatotoxicity in mice, which was reflected in the restoration of hepatic function markers and hepatic tissue damage. The hepatoprotective effect of SCHPs-F1 might be attributed to the dual effects of Nrf2/antioxidant and NF-κB/inflammatory cytokines regulatory pathways. Therefore, our research provides a possible option for the development and utilization of red shrimp head (*Solenocera crassicornis)* for preparing functionally active peptides.

Ethical statement

The procedures of animal care and use was approved by the Zhejiang Ocean University Experimental Animal Ethics Committee (Zhoushan, Zhejiang, China) and complied with all applicable institutions and government regulations regarding the ethical use of the animals.

CRediT authorship contribution statement

Shuoqi Jiang: Validation, Investigation, Writing - original draft. **Zhuangwei Zhang:** Validation, Investigation, Writing - original draft. **Fangmiao Yu:** Resources, Data curation, Software. **Zhoufeng Zhang:** Resources, Data curation, Software. **Zuisu Yang:** Resources, Data curation, Software. **Yunping Tang:** Conceptualization, Project administration, Funding acquisition, Writing - review & editing. **Guofang Ding:** Conceptualization, Project administration, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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