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# Ameliorative Effects of Peptide Phe-Leu-Ala-Pro on Acute Liver and Kidney Injury Caused by $CCl_4$ via Attenuation of Oxidative Stress and Inflammation

Shuqi He,[#] Wei Zhao,[#] Xiang Chen, Jie Li, Leifang Zhang, and Huoxi Jin*

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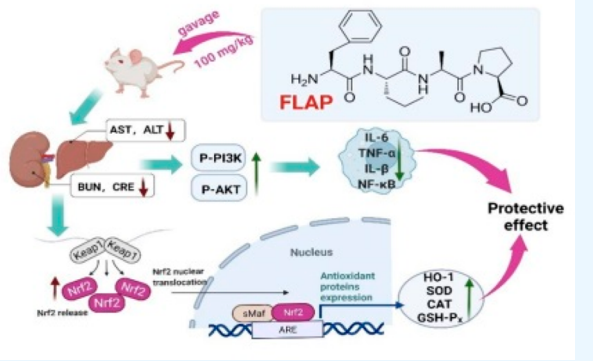
**ABSTRACT:** Acute liver injury (ALI) and acute kidney injury (AKI) are significantly affected by the antioxidant status. In the present study, the protective effect and mechanism of the collagen peptide Phe-Leu-Ala-Pro (FLAP) in mice with ALI and AKI induced by carbon tetrachloride ($CCl_4$) were examined. The results showed that FLAP effectively improved the liver mass index, the renal mass index, and the histopathological morphology. FLAP treatment significantly decreased the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen (BUN), and creatinine (CRE) but increased the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). The protein expression levels of nuclear factor E2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1), *p*-protein kinase B (*p*-AKT), and *p*-phosphatidylinositol-3-kinase (*p*-PI3K) in the liver and kidneys were significantly up-regulated after FLAP treatment. FLAP down-regulated the levels of interleukin-6 (IL-6), IL-1$\beta$, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and nuclear factor-$\kappa$ B (NF-$\kappa$B) in liver and kidney tissues. Thus, FLAP may play a protective role in ALI and AKI by attenuating oxidative stress and inflammation mediated by the Nrf2/anti-response element (ARE) and PI3K/AKT/NF-$\kappa$B pathways.




## 1. INTRODUCTION

The invertebrate sea cucumber is considered an underutilized resource and traditional nutritional food in China and Southeast Asia.[1] The body wall of a sea cucumber is the most critical edible part and is rich in many nutrients such as collagens, vitamins, proteins, amino acids, and minerals.[2] Collagen, which accounts for about 70% of the protein content of the body walls, has attracted increased interest from researchers due to its good chemical properties. Peptides prepared by the enzymatic hydrolysis of sea cucumber collagen were shown to inhibit angiotensin I-converting enzyme[3] and exert antihypertensive[4] and antioxidant effects.[5] Collagen peptides from sea cucumbers are currently used in food, pharmaceuticals, and cosmetic products.

Acute liver injury (ALI) and kidney injury (AKI) are usually caused by chemical toxins, drug overdose, and hepatitis viruses. A decrease in liver or kidney function due to ALI or AKI can lead to severe complications that can be a serious threat to human health. With increases in unhealthy lifestyles and the widespread use of drugs, the incidence of ALI and AKI is increasing annually. Animal models provide an excellent method of understanding pathophysiological mechanisms, and carbon tetrachloride ($CCl_4$) is a common chemical reagent used to induce ALI and AKI in animal models. $CCl_4$ is metabolically activated by cytochrome P450 to form trichloromethyl radicals ($CCl^+_3$) and peroxyl radicals ($OOCCl_3$), resulting in the generation of reactive oxygen species (ROS). High levels of ROS induce lipid peroxidation, thereby triggering apoptosis and the necrosis of cells.[6] Increases in the levels of tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-6 (IL-6), and IL-1$\beta$ are also contributing factors to ALI and AKI induced by $CCl_4$.[7] ALI and AKI have become global health problems with poor prognoses and high mortality. Therefore, there is an urgent need to explore new effective drugs and methods to mitigate ALI and AKI.[8] Collagen peptides have promising application prospects in preventing ALI. Peptides from *Acaudina molpadioides* collagen were shown to prevent $CCl_4$-induced ALI and AKI by reducing oxidative stress and inflammation.[9,10]

Many genes and signaling pathways have been shown to play essential roles in the pathogenesis of ALI and AKI. Nuclear

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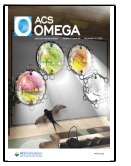





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factor E2-related factor 2 (Nrf2) is a significant regulator of antioxidant responses in the liver and kidneys, regulating the expression of antioxidant enzyme genes through the anti-response element (ARE).[11,12] Under normal conditions, Nrf2 is anchored in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1). Nrf2 dissociates from Keap1, leading to its translocation to the nucleus and accumulation.[13] Nrf2 then binds to the ARE, promoting the expression of detoxification and antioxidant defense proteins such as heme oxygenase (HO-1).[14,15] Studies have shown that the Nrf2/ARE signaling pathway is closely associated with the development of ALI and AKI.[16] Targeting the Nrf2/ARE signaling pathway is an emerging strategy for preventing and treating various liver and kidney diseases.[17−19]

Phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) is a crucial regulator of signal transduction pathways that control cell growth, metabolism, and apoptosis.[20] Numerous studies have proven that the PI3K/AKT pathway is closely associated with oxidative stress and inflammation.[10,21] Many compounds significantly ameliorated ALI or AKI by inhibiting oxidative stress and inflammation through the PI3K/AKT pathway.[17,22] In previous studies, we successfully isolated the antioxidant peptide Phe-Leu-Ala-Pro (FLAP) from sea cucumber (*Acaudina molpadioides*)[23] and confirmed that this oligopeptide significantly improved oxidative damage in cells treated with $H_2O_2$ through the Nrf2/ARE pathway.[24] However, it is unclear whether FLAP can prevent $CCl_4$-induced ALI and AKI. Therefore, this study aimed to evaluate the protective effect and explore the potential mechanism of FLAP in $CCl_4$-induced ALI and AKI, laying a theoretical foundation for the application of collagen peptides in the prevention of ALI and AKI.

## 2. MATERIALS AND METHODS

**2.1. Chemicals and Reagents.** Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), HO-1, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CRE), and urea nitrogen (BUN) kits were purchased from Nanjing Jiancheng Bioengineering Institute. TNF-$\alpha$, IL-6, IL-1$\beta$, and nuclear factor-$\kappa$ B (NF-$\kappa$B) ELISA kits were purchased from ElabScience. All other reagents can be of the highest or analytical grade on the market.

**2.2. Laboratory Animals.** Healthy male ICR mice (18−20 g, 6−8 weeks) were obtained from Hangzhou Ziyuan Experimental Animal Technology Co., Ltd. All mice were kept at room temperature (23 ± 1 °C, 55% ± 5% air humidity) with light−dark regularity (12 h−/12 h) in independent cages. The mice were fed with sterile water and conventional animal pellet feed. The formal experiment started after 1 week of acclimatization to feeding. Mice were randomly divided into four groups: the normal group, the model group, the positive group, and the FLAP group. The positive and FLAP groups were given $V_E$ (100 mg/kg) and FLAP (50 mg/kg) daily, respectively. The mice in normal and model groups were given a saline gavage daily. After 21 days, mice in all groups except for the normal group were injected intraperitoneally with 10% $CCl_4$ (1 mL), which was dissolved in soybean oil. All mice were sacrificed after fasting for 24 h, and serum was collected to detect the levels of ALT, AST, BUN, and CRE. The liver and kidneys were immediately removed, rinsed three times with saline to remove blood, then stored at −80 °C for subsequent experiments. All animal experiments were approved by the Ethics Committee of Zhejiang Ocean University (no. 2021029). All procedures were carried out in accordance with the guidelines of the Animal Protection and Use Committee of the China Animal Protection Commission.

**2.3. Calculation of Liver and Kidney Indices.** The mice were executed and weighed for their liver and kidney. Liver index (%) = liver weight/body mass × 100. Renal index (%) = kidney weight/body mass × 100.

**2.4. Determination of Serum AST, ALT, BUN, and CRE.** ALT, AST, BUN, and CRE levels were measured according to the instructions of the respective kits (Nanjing Jiancheng Institute of Biological Engineering, China).

**2.5. Histopathological Observation of the Liver and Kidneys.** Trimmed liver and kidneys tissues were fixed overnight by immersion in 4% paraformaldehyde, graded and dehydrated using anhydrous ethanol, paraffin-embedded, and cut into 3 $\mu$m thick sections. Sections were stained with standard hematoxylin-eosin (H&E), then images of morphological changes in liver and kidney tissues were captured using light microscopy.

**2.6. Determination of Antioxidant Activity in Liver and Kidney Tissues.** Liver and kidney tissue specimens were minced and placed in 9× the volume of normal saline. The mixture was ground with a tissue masher at 10000 rpm to prepare a homogenate solution. The levels of SOD, GSH-Px, CAT, and MDA were determined according to the manufacturing instructions of the respective kits. The levels of SOD at 550 nm were determined using a 1510 spectrophotometer (Thermo Fisher Scientific Oy, Vantaa, Finland). The levels of MDA, GSH-Px, and CAT were determined at 532, 405, and 412 nm using the colorimetric method, respectively.

**2.7. Detection of IL-1$\beta$, IL-6, TNF-$\alpha$, and NF-kB.** The enzyme-linked immunosorbent assay (ELISA) kits were used to detect the relevant levels of IL-1$\beta$, IL-6, TNF-$\alpha$, and NF-$\kappa$B in mouse liver and kidney samples according to the manufacturer's instructions.

**2.8. Western Blot Detection.** Liver and kidney tissues were cut into fine pieces of approximately equal size. RIPA buffer and PMSF protein inhibitor were added with a ratio of 150−250 $\mu$L/20 mg of tissue, then homogenization was performed to completely dissolve the tissue. The lysed samples were centrifuged at 4 °C and 12000 rpm for 10 min. The protein concentrations in the supernatant were measured using a BCA protein assay kit. Target proteins were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes for 2 h. They were then blocked with 5% nonfat dry milk in tris-buffered saline and Tween 20 (TBST) buffer for 1 h. The blocking buffer was discarded, the corresponding primary antibody was added to the sample, and the sample was incubated for 12 h at 4 °C. The membrane was rinsed three times with TBST buffer for 10 min each and then incubated with the corresponding secondary antibody for 1 h at room temperature. The results were detected using protein chemiluminescence imaging reagents, and the levels of protein expression were calculated using ImageJ software for gray scale value analysis.

**2.9. Statistical Analysis.** Data were analyzed using GraphPad Prism 8.0.0 software and expressed as the mean ± standard deviation. Differences between groups were analyzed using ANOVA. A *P*-value less than 0.05 indicates a significant difference. *, **, ***, and **** indicate $P < 0.05$, 0.01, 0.001, and 0.0001, respectively.



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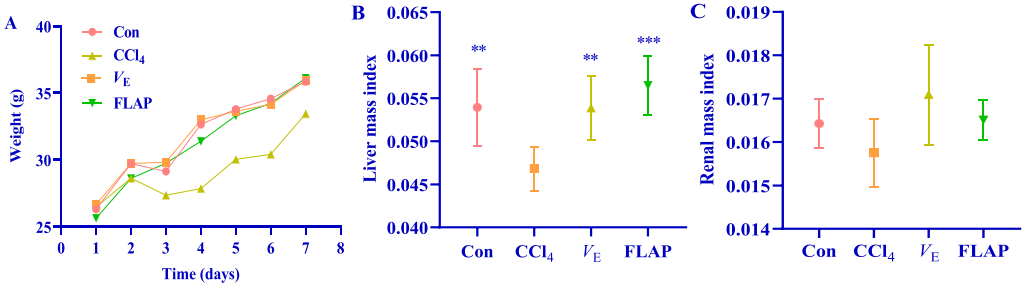


**Figure 1.** Effects of FLAP on (A) weight, (B) liver index, and (C) kidney index of mice. $P^{**} < 0.01$ and $P^{***} < 0.001$ compared with the $CCl_4$ group.

## 3. RESULTS

**3.1. Changes in Body Weight, Liver Index, and Kidney index.** Figure 1 shows the changes in body weight, liver index, and kidney index of different groups of mice. The body weights of mice in the control group, the $V_E$ group, and the FLAP group were significantly higher than those in the model group. The liver mass index in the $CCl_4$ group was significantly lower compared to that of the control group but significantly improved after pretreatment with $V_E$ or FLAP. There was no statistically significant difference in the renal mass index between the $CCl_4$ group and other groups. The results indicated that the degree of liver damage caused by $CCl_4$ treatment was significantly stronger than the kidney damage caused by $CCL_4$ treatment.

**3.2. Effect of FLAP on ALT, AST, BUN, and CRE levels.** Serum levels of ALT and AST can be used as biochemical indicators to evaluate the extent of liver injury, whereas BUN and CRE levels are indicators of renal injury. The effects of FLAP on serum ALT, AST, BUN, and CRE levels are shown in Figure 2. The ALT, AST, BUN, and CRE levels were substantially higher in the $CCl_4$-treated group compared to the control group. In contrast, serum ALT, AST, BUN, and CRE levels in the $V_E$ and FLAP groups were significantly lower than those in the $CCl_4$-treated group but still higher than those in the control group. These results suggest that FLAP had a protective effect against $CCl_4$-induced liver and kidney toxicity in mice.

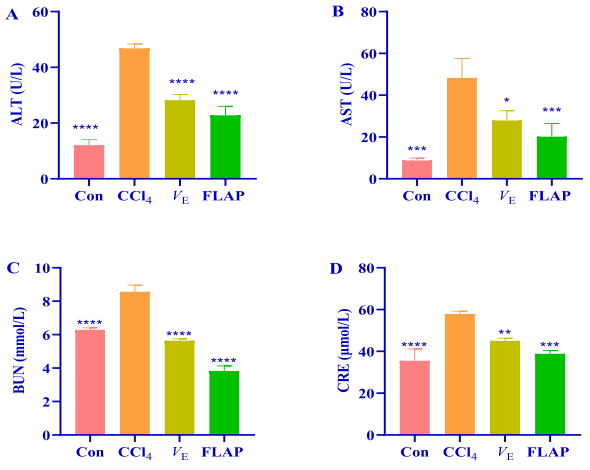


**Figure 2.** Effect of FLAP on the serum (A) ALT, (B) AST, (C) BUN, and (D) CRE levels in mice with ALI and AKI induced by $CCl_4$. $P^{**} < 0.01$, $P^{***} < 0.001$, $P^{****} < 0.0001$, compared with $CCl_4$ group.

**3.3. Histopathological Observation of the Liver and Kidneys.** The histopathological observation of the liver and kidneys was used to evaluate the protective effect of FLAP. As shown in Figure 3, the control group had a normal hepatocyte morphology, an intact hepatic lobule structure, well-aligned hepatic cords, and normal central veins and hepatic sinusoids. The $CCl_4$-treated group exhibited inflammatory cell infiltration around the hepatic lobules (indicated by red arrows) and unclear hepatic sinusoids (indicated by green arrows). FLAP treatment reduced hepatic fibrosis and hepatocyte necrosis, significantly alleviating CCl4-induced histopathological changes. In addition, $CCl_4$ treatment resulted in cytoplasmic vacuolization (indicated by blue arrows), epithelial cell degeneration (indicated by white arrows), and severe tubular vacuolation (indicated by black circles). Renal structures were recovered and regenerated after $V_E$ treatment, while glomerular swelling and vacuolation were significantly reduced and the renal cyst cavity reappeared (indicated by yellow arrows) in the FLAP group. The results showed that FLAP exerted some alleviating effects on $CCl_4$-induced liver and kidney injuries in mice.

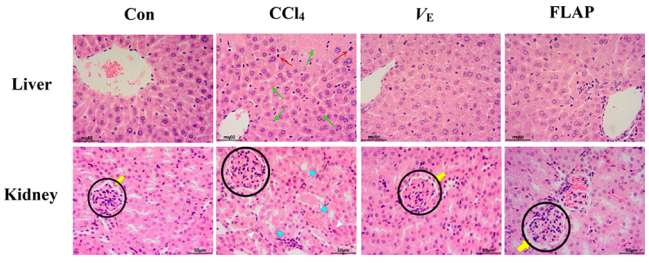


**Figure 3.** Histopathological sections of the liver and kidney (H&E, ×400).

**3.4. Effect of FLAP on MDA, SOD, GSH-Px, and CAT Levels.** Oxidative stress is one of the main mechanisms of ALI and AKI induced by $CCl_4$ in mice.[25] The SOD, GSH-Px, CAT, and MDA levels are critical indicators used to evaluate the degree of tissue oxidative stress.[26,27] As shown in Figure 4A1−A4, the activity of SOD, GSH-Px, and CAT in the livers of the $CCl_4$-treated group was significantly lower and the MDA content was higher compared to the control group. $V_E$ and FLAP treatment reversed the changes in SOD, GSH-Px, CAT, and MDA levels induced by $CCl_4$. As shown in Figure 4B1−B4, the $CCl_4$ treatment significantly reduced SOD, GSH-Px, and CAT activity and increased MDA levels in the kidneys.


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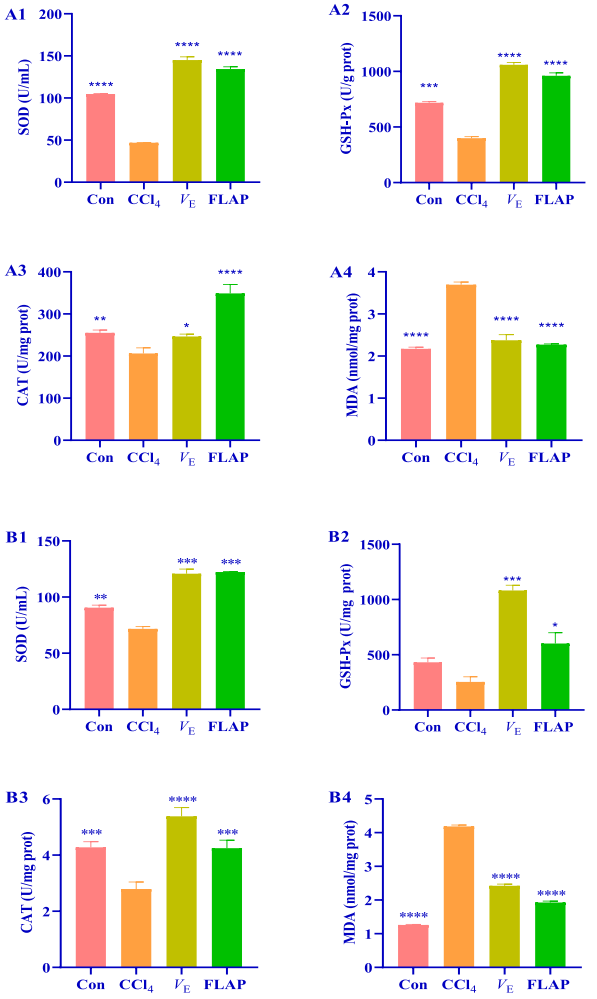


**Figure 4.** Effect of FLAP on SOD, GSH-Px, CAT, and MDA levels in (A1−A4) mouse liver and (B1−B4) mouse kidneys. $P^{*} < 0.05$, $P^{**} < 0.01$, $P^{***} < 0.001$, and $P^{****} < 0.0001$ compared to the $CCl_4$ group.

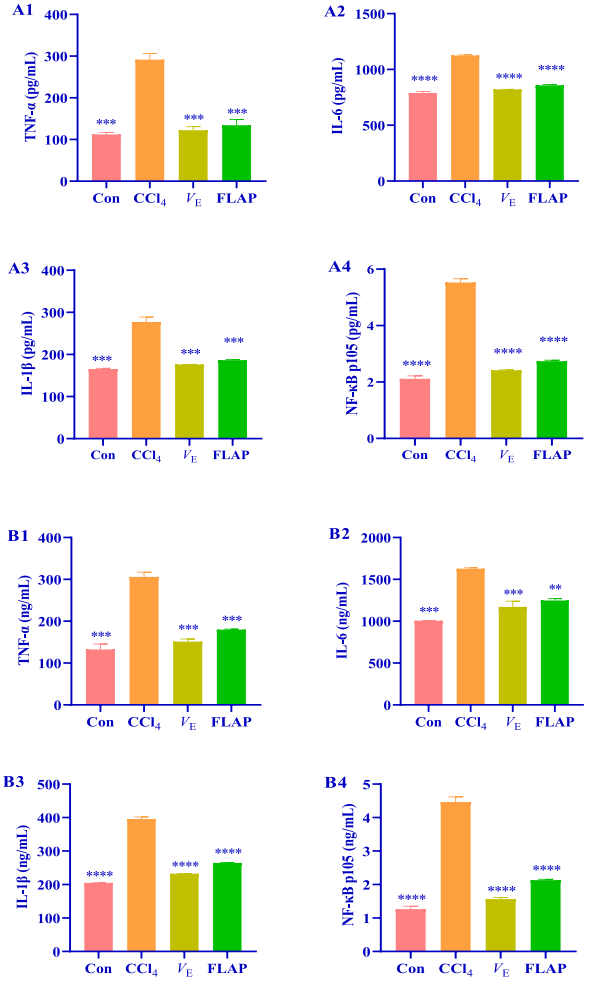


**Figure 5.** Effects of FLAP on inflammatory factors TNF-$\alpha$, IL-6, IL-1$\beta$, and NF-$\kappa$B in (A1−A4) mouse liver and (B1−B4) mouse kidney tissues. $P^{*} < 0.05$, $P^{**} < 0.01$, $P^{***} < 0.001$, and $P^{****} < 0.0001$ compared with the $CCl_4$ group.

Similarly, these metrics all improved after the FLAP treatment, and some were even better than the control group. These results suggest that FLAP attenuated $CCl_4$-induced oxidative stress in the liver and the kidneys.

**3.5. FLAP Attenuates Liver and Kidney Injury by Regulating Cytokine Expression.** The inflammatory response is one of the main expression factors and salient features of ALI and AKI.[28,29] The expression levels of IL-6 and IL-1$\beta$ as inflammatory factors directly reflect the degree of inflammation in the body. In contrast, TNF-$\alpha$, a pro-inflammatory cytokine that causes necrosis, is mainly produced by macrophages and monocytes. To determine whether FLAP treatment affected cytokine expression, enzyme-linked immunosorbent assays (ELISAs) were used to analyze the levels of TNF-$\alpha$, IL-6, and IL-1$\beta$ in the liver and kidney. In addition, the level of NF-$\kappa$B, which regulates the expression of inflammatory cytokines, was also measured.[30] As shown in Figure 5A1−A4 in liver tissues, pro-inflammatory cytokines (IL-1$\beta$, IL-6, and TNF-$\alpha$) and NF-$\kappa$B were significantly higher in the $CCl_4$-treated group compared to the control group, whereas the levels in FLAP-treated mice were comparable to those in the control group. As shown in Figure 5B1 and B2, the levels of IL-1$\beta$, IL-6, TNF-$\alpha$, and NF-$\kappa$B in kidney tissues were significantly higher after $CCl_4$ treatment, but those changes were also reversed by FLAP. The above results suggest that FLAP could attenuate the levels of IL-6, IL-1$\beta$, TNF-$\alpha$, and NF-$\kappa$B in the liver and kidneys, thus displaying a certain protective effect against $CCl_4$-induced ALI and AKI.

**3.6. Effect of FLAP on the Activation of the Nrf2 Pathway.** To elucidate the molecular mechanism of FLAP against $CCl_4$-induced oxidative stress, the expression of Nrf2 signaling pathway-related proteins was detected by Western blotting. As shown in Figure 6A1, compared to the control group, the Nrf2 and HO-1 levels in the livers of the mice in the $CCl_4$ group were reduced by 68.5% and 76.1%, respectively. FLAP pretreatment inhibited $CCl_4$-induced decreases in Nrf2, Keap1, and HO-1 levels and even increased their levels above those of the control group. In the kidneys (Figure 6B1−B4), the $CCl_4$ treatment also decreased Nrf2 expression but had no significant effect on Keap1 and HO-1 expression. These results again indicate that the oxidative damage of the liver due to $CCl_4$ was far more severe than that of the kidneys. Similarly, FLAP treatment substantially increased the levels of Nrf2, Keap1, and HO-1 in the kidneys. These results showed that



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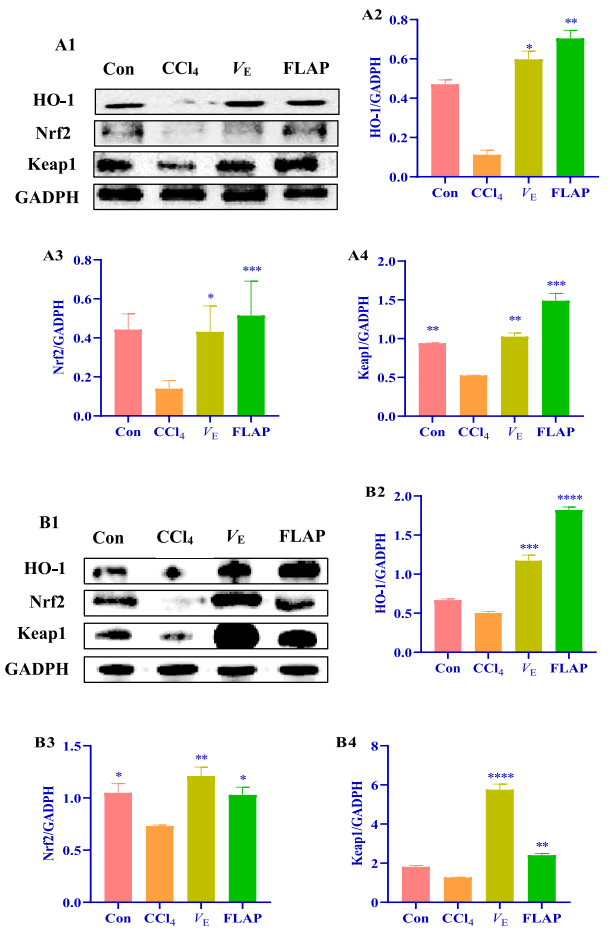


**Figure 6.** Effect of FLAP on the activation of the Nrf2/ARE pathway in (A1−A4) the liver and (B1−B4) kidneys. $P^{*} < 0.05$, $P^{**} < 0.01$, $P^{***} < 0.001$, and $P^{****} < 0.0001$ compared to the $CCl_4$ group.

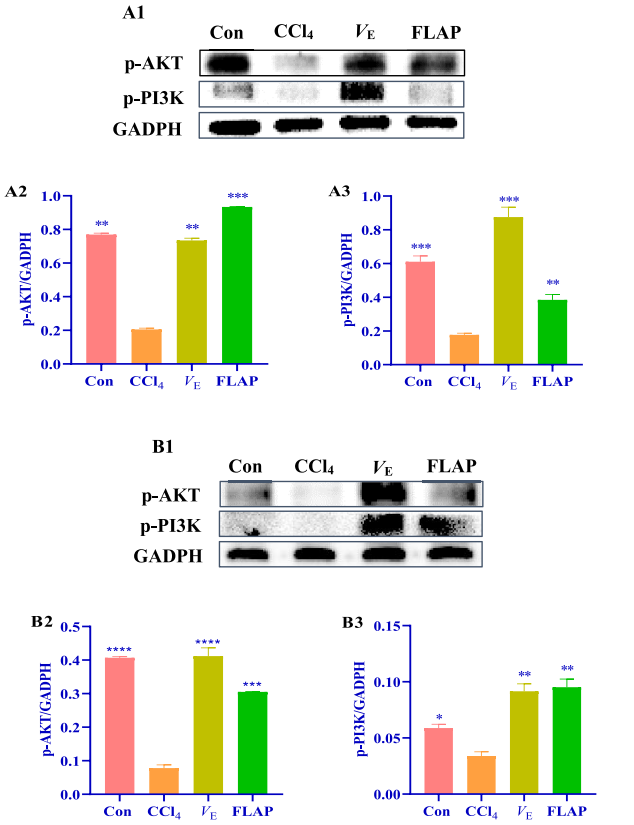


**Figure 7.** Effect of FLAP on the activation of PI3K/AKT pathway in (A1−A3) the liver and (B1−B3) kidneys. $P^{*} < 0.05$, $P^{**} < 0.01$, $P^{***} < 0.001$, and $P^{****} < 0.0001$ compared to the $CCl_4$ group.

FLAP enhanced the antioxidant capacity of the liver and kidneys in mice by activating the Nrf2/ARE signaling pathway.

**3.7. Activation of the PI3K/AKT Pathway.** The PI3K/AKT pathway is closely associated with apoptosis and autophagy. We further analyzed whether FLAP ameliorated $CCl_4$-induced liver and kidney injury by activating the PI3K/AKT pathway. As shown in Figure 7, the phosphorylation of AKT and PI3K was strongly inhibited by $CCl_4$ while it was significantly up-regulated in the FLAP and $V_E$ groups in the liver. The AKT and PI3K phosphorylation results in the kidney were consistent with those in the liver. Notably, the phosphorylation of AKT in the liver and kidneys of the mice in the FLAP group was more than three-times higher than that in the $CCl_4$ group. These results demonstrated that FLAP could activate the PI3K/AKT signaling pathway in the liver and kidneys.

## 4. DISCUSSION

ALI and AKI are common global clinical problems closely related to human health.[31] The ingestion of toxic chemicals is the leading cause of ALI and AKI. $CCl_4$ is metabolized *in vivo* to produce highly toxic trichloromethyl and trichloromethyl peroxy radicals, which cause lipid peroxidation in the renal cortex and renal microsomes.[32] $CCl_4$ treatment was also shown to lead to necrosis, steatosis, and liver fibrosis.[33] Clinical research has confirmed that oxidative stress and inflammation are the primary pathophysiological factors of ALI and AKI.[34] Antioxidants, including taurine, curcumin, and peptides, are used to prevent and treat ALI and AKI. Sea cucumber is an essential functional food source, and its collagen peptides often exhibit specific biological activities. Antioxidant peptides derived from sea cucumber collagen have great potential value in preventing ALI and AKI.[9,10] Therefore, the present study investigated whether the collagen peptide FLAP from the sea cucumber *Acaudina molpadioides* exerted protective effects against $CCl_4$-induced ALI and AKI in mice. The results showed that FLAP pretreatment could prevent decreases in the body weight and liver mass index of mice with AKI and ALI induced by $CCl_4$ treatment. FLAP also significantly prevented $CCl_4$-induced liver fibrosis, hepatocyte necrosis, glomerular swelling, and vacuolization. The results indicated that FLAP prevented liver and kidney injury caused by $CCl_4$.

Serum ALT and AST levels are important biochemical indicators for judging the degree of liver damage. Urea is the final product of protein metabolism and is taken up by the kidneys. The levels of CRE and BUN are classical indicators used to assess renal function and are usually elevated in the kidneys of patients with AKI.[35] The levels of ALT, AST, BUN, and CRE in the $CCl_4$-treated group were significantly higher than those in the control group, suggesting that $CCl_4$ caused liver and renal function impairment. However, FLAP pretreatment significantly ameliorated the increases, further reflecting the protective effect of FLAP against $CCl_4$-induced liver and kidney injury.

Oxidative stress is one of the typical pathophysiological features of ALI and AKI.[36] Antioxidative enzymes play an



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essential role in regulating oxidative stress in the body. SOD catalyzes the generation of oxygen and hydrogen peroxide from free radicals, which are then catalyzed by GSH-Px to produce nontoxic hydroxyl compounds. Thus, these two enzymes are involved in balancing free radical generation and elimination.[27] Collagen peptides have been reported to increase antioxidant enzyme levels, thus reducing intracellular oxidative stress.[37] In the present study, the collagen peptide FLAP increased the activity of SOD, GSH-Px, and CAT in the liver and kidneys of mice, indicating that FLAP reduced oxidative damage *in vivo* by scavenging more free radicals. The Nrf2/ARE pathway is an important defense response system to oxidative stress.[38,39] Nrf2 can regulate the expression levels of antioxidant genes such as SOD, GSH-Px, and HO-1. This study showed that FLAP treatment significantly up-regulated the expression of Nrf2 in both the liver and kidneys, thereby increasing HO-1 levels by more than three times relative to the $CCl_4$ group. The study suggest that FLAP may inhibit oxidative stress by activating the Nrf2/ARE pathway, which is consistent with previous studies where ALI and AKI were significantly improved by the activation of the Nrf2-mediated antioxidant pathway.[19,40]

The pro-inflammatory factors IL-1$\beta$, IL-6, and TNF-$\alpha$ are considered markers reflecting inflammatory status, which is closely associated with ALI and AKI. IL-1$\beta$ has been shown to inhibit hepatocyte proliferation, whereas the overstimulation of IL-6 is more likely to cause liver damage. TNF-$\alpha$ acts as a marker in hepatocyte apoptosis and is closely associated with $CCl_4$-induced cytotoxicity. The levels of IL-1$\beta$, IL-6, and TNF-$\alpha$ are regulated by the NF-$\kappa$B signaling pathway.[41] In the present study, FLAP treatment significantly reduced the levels of pro-inflammatory cytokines (IL-6, IL-1$\beta$, and TNF-$\alpha$) and decreased NF-$\kappa$B phosphorylation levels. These results suggest that FLAP exerted an anti-inflammatory effect by inhibiting the NF-$\kappa$B pathway. Over the years, various compounds such as dexmedetomidine, hydrogen sulfide, and melittin have been reported to ameliorate ALI or AKI by inhibiting inflammation.[19,42,43] Collagen peptides in fish scales exhibited anti-inflammatory activity by inhibiting NF-$\kappa$B activation in HaCaT cells.[44]

The PI3K/AKT signaling pathway has been shown to play a key coordinating role in protecting cells from oxidative and inflammatory responses.[21] Therefore, dysregulation of the PI3K/AKT signaling pathway may be related to liver and kidney injury. In the present study, $CCl_4$ significantly inhibited PI3K/AKT phosphorylation in mice, and FLAP effectively increased PI3K/AKT phosphorylation levels. All data suggested that the mechanism by which FLAP ameliorated $CCl_4$-induced ALI and AKI involved the activation of the PI3K/AKT and Nrf2 signaling pathways to exert anti-inflammatory and antioxidant effects, respectively. Our findings are consistent with a previous study in which collagen peptides from skate (*Raja kenojei*) skin could stimulate the phosphorylation of PI3K and AKT.[22]

In summary, this study provided strong evidence for the ameliorating effects of the oligopeptide FLAP isolated from the hydrolysates of sea cucumber collagen on $CCl_4$-induced ALI and AKI. This preventive effect of FLAP may be due to the activation of Nrf2-mediated antioxidant and PI3K/AKT-mediated anti-inflammatory pathways (Figure 8). To date, many compounds have been reported to ameliorate ALI or AKI through antioxidant and anti-inflammatory effects. However, few studies have reported the prevention of ALI or AKI by sea cucumber collagen peptides through the PI3K/AKT pathway. We previously found that the collagen hydrolysate of *Acaudina molpadioides* could protect the liver and kidneys from $CCl_4$-induced damage by reducing oxidative stress and inflammation.[9,10] However, collagen hydrolysate is a peptide mixture, and the unclear peptide sequence and content limit its application prospects in the field of medicine. This study further confirmed the function and mechanism of the oligopeptide FLAP, laying a foundation for applying sea cucumber collagen peptides in the treatment of ALI and AKI.

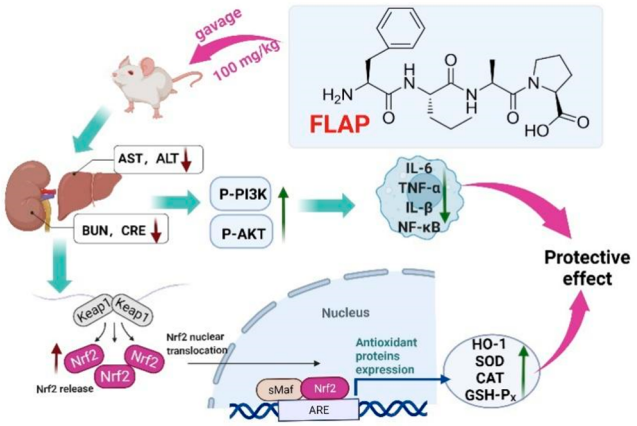


**Figure 8.** Molecular mechanism of a protective effect of FLAP on $CCl_4$-induced ALI and AKI through the PI3K/AKT and Nrf2/ARE signaling pathways. This image is free domain in BioRender.com (2022).

## 5. CONCLUSION

In this study, we observed that FLAP treatment increased both body weight and liver index values, reduced the severity of histopathological alterations in liver and kidney tissues, and decreased BUN, CRE, ALT, and AST levels in mice. In addition, FLAP treatment increased the levels of antioxidant enzymes (SOD, CAT, GSH-Px, and HO-1), Nrf2, p-AKT, and p-PI3K but decreased the levels of inflammatory factors (IL−6, IL-1$\beta$, TNF-$\alpha$, and NF-$\kappa$B). These results suggest that FLAP could protect mice from $CCl_4$-induced acute liver and kidney injury, which may be related to antioxidant effects via the Nrf2/ARE pathway and anti-inflammatory effects via the PI3K/AKT/NF-$\kappa$B pathway.

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### Notes

The authors declare no competing financial interest.

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