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As you know, selenium deficiency can lead to various diseases. Therefore, scientific selenium supplementation is urgent. Selenium-chelated peptides, a novel form of selenium supplement, offer several advantages, including high bioavailability, low toxicity, and strong stability. These peptides also demonstrate potential physiological benefits in immune regulation, lipid metabolism, and glucose control. While previous research has predominantly focused on plant-derived selenium-chelated peptides, studies on those derived from marine animals remain limited. Sturgeon processing primarily focuses on caviar and sturgeon meat products, while sturgeon heads, which account for approximately 17.1% of the total weight, are often discarded, leading to resource waste and environmental pollution. Therefore, it is urgent to develop effective methods to transform sturgeon heads into high-value products. This study not only broadens the application value of sturgeon head, but also provides a theoretical basis for the development of new organic selenium supplements of food origin.

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Selenium supplementation effect of selenium-chelating peptide from sturgeon (*Acipenseridae*) head and prevention of liver injury in selenium-deficient mice

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Abstract: Selenium deficiency leads to oxidative stress and inflammatory damage, while peptide-selenium chelation effectively alleviates this insufficiency. To develop novel selenium supplements from marine by-product resources, sturgeon head peptides (SHP) were hydrolyzed with pepsin and sturgeon head peptide-selenium (SHP-Se) chelate was also prepared. The protective effects of SHP-Se chelate against oxidative stress and liver injury were investigated in a Se-deficient mice model, which was successfully established by feeding adult Kunming mice a selenium-deficient diet (0.15 mg Se/kg diet) for 18 days. Concurrently, control mice (Se-sufficient, n=10) were fed a standard diet. Forty Se-deficient mice were randomly divided into the model group, Na_2SeO_3 group, low-dose SHP-Se chelate group (SHP-Se-L), and high-dose SHP-Se chelate group (SHP-Se-H). After 20 days of treatment, liver selenium content in the Na_2SeO_3, SHP-Se-L, and SHP-Se-H groups significantly increased compared to the model group. Compared to the Na_2SeO_3 group, the SHP-Se-H group exhibited increases in serum catalase, superoxide dismutase (SOD), reduced glutathione, and glutathione peroxidase levels by 41.42%, 26.09%, 140.54%, and 41.49%, respectively. While malondialdehyde, alanine aminotransferase, and aspartate aminotransferase levels decreased by 62.14%, 65.1%, and 28.6%, respectively. H&E histopathological staining further demonstrated that SHP-Se was more effective than inorganic selenium in restoring tissue damage. Therefore, as a novel selenium supplement, SHP-Se chelate can effectively prevent oxidative stress-induced liver injury, and shows great potential for application in the development of functional foods for dietary selenium supplementation.

Keywords: Selenium chelate; Oxidative damage; Selenium supplementation

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1. Introduction

Selenium (Se) is an essential trace element in the human body that is closely related to human health. Research indicates that Se deficiency is associated with more than 40 human diseases, such as Keshan disease and Kashin-Beck disease. Long-term Se deficiency can also lead to a variety of chronic diseases, including weakened immunity, cardiovascular diseases, hypertension, diabetes, liver diseases, and kidney diseases.[1] Approximately 1 billion people worldwide suffer from varying degrees of inadequate Se intake, with 700 million in China, making it an internationally recognized Se-deficient country.[2] Moreover, excessive intake of Se can also cause Se poisoning. Therefore, scientific Se supplementation is urgently needed.

Traditional Se supplements are mainly divided into inorganic Se and organic Se. Inorganic Se primarily includes selenides, selenates, and selenites. Organic Se mainly consists of selenocysteine, selenomethionine, dimethylselenium, and selenoproteins. Inorganic selenium not only fails to achieve the desired Se supplementation effect but also suffers from drawbacks such as low absorption rates, poor solubility, high toxicity, and gastrointestinal irritation.[3] Studies have shown that the acute neurotoxicity of inorganic tetravalent Se was 43 times higher than that of selenomethionine.[4] Hadrup *et al*.[5] found that after oral administration, the toxicity of inorganic Se was significantly higher than that of organic Se. Kim *et al*.[6] demonstrated that when dietary Se levels exceeded 15 mg/kg, inorganic Se exhibited greater toxicity than organic Se. In a recent safety assessment of Se in drinking water, Vinceti *et al*.[4] also showed that inorganic forms of Se pose greater hazards than organic Se.

To this point, chelation modification methods have been developed to convert inorganic Se into organic forms, enhancing its utilization and ensuring safer Se supplementation. For example, polysaccharides chelated selenide,[7] proteins chelated selenide,[8] and peptides chelated selenide.[9,10]

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Among them, hydrolysates and peptides obtained from proteins can bind minerals, resulting in excellent Se chelating ability.[11-12] Se-chelated peptides, as a new type of Se supplement, have the advantages of high bioavailability, low toxicity and strong stability and have potential physiological impacts on immune regulation, lipid lowering, and glucose regulation. Previous studies have explored Se-chelated peptides from various plant sources, such as soybeans,[14] wheat,[9] peas,[15] mung bean,[16] and corn,[17] while studies on Se-chelated peptides from marine animals are still very limited. Furthermore, some studies have documented the antioxidant activity of selenium-chelated peptides,[18-20] while systematic investigations into their selenium supplementation efficiency and the effects on oxidative stress-induced liver injury have not been reported.

Sturgeon, one of the largest freshwater fish in the world, is rich in various amino acids, unsaturated fatty acids, and collagen and has a protein content as high as 21%. China, as a major sturgeon farming country, ranks first in terms of farming area and production output,[21] and the annual total production of sturgeon reached 122 thousand tons in 2022.[22] Sturgeon processing has focused mainly on caviar and sturgeon meat products, whereas sturgeon (*Acipenseridae*) heads, accounting for approximately 17.1% of the total weight, are often discarded, leading to resource waste and environmental pollution. To this point, developing effective methods to convert sturgeon heads into high-value products is urgent. Fish heads are rich in collagen and myofibrillar proteins, which can be enzymatically or chemically hydrolyzed to generate low-molecular-weight peptides. The active groups (such as amino and carboxyl groups) on peptide chains enable chelation reactions with selenium ions, forming stable selenium-chelating peptides.[23,24] Compared to plant-derived proteins, animal-based peptides exhibit superior metal-binding affinity due to their distinct amino acid profiles. Notably, sturgeon heads, as processing by-products, offer economic advantages and align with the principles of

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resource recycling and sustainable utilization. Although these characteristics suggest the potential of sturgeon heads for developing selenium-chelating peptides, no studies have been reported on selenium-chelated peptides from sturgeon heads.

Therefore, in response to the current severe selenium deficiency in some regions and the significant underutilization of sturgeon head by-product resources, this study aims to utilize marine by-products to prepare SHP-Se chelate, and evaluate the improvement effects of SHP-Se chelate on the antioxidant function and liver injury using a selenium-deficient mice model. By comparing it with inorganic Se supplements, the study further elucidated its mechanism of action in regulating liver injury induced by oxidative stress. These data would provide a theoretical basis for the research and development of selenium-chelating peptides.

2. Materials and methods

2.1. Materials and reagents

The hybrid sturgeon head was provided by Quzhou Xunlong Aquatic Products Sci-tech Development Co., Ltd. (Zhejiang, China). Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px), alanine amino transferase (ALT), and aspartate aminotransferase (AST) assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other chemical reagents used in this study were of analytical grade.

2.2. Preparation of the SHP-Se chelate

Sturgeon head proteins were extracted and hydrolysed with pepsin to obtain sturgeon head peptides (SHP) using the method of Jia *et al*.[24] Subsequently, 3% (w/v) SHP powder was mixed with sodium selenite solution (0.5 M) at a 2:1 volume ratio, and the mixture was heated at 80 °C, pH 9.0, for 1 h. After the reaction, the mixture was cooled to room temperature and centrifuged at 4000 ×g for 10

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min, and the supernatant was collected. A 95% ethanol solution at five times the concentration of the supernatant was subsequently added to the supernatant, which was subsequently mixed well. The mixed solution was left to stand for 12 h and then centrifuged again. The precipitate obtained after centrifugation was washed with anhydrous ethanol and freeze-dried to obtain SHP-Se.

2.3. Animals and treatments

Adult healthy Kunming mice (male, weighing 20 ± 2.0 g) were obtained from Liaoning Changsheng Biotechnology Co. Ltd. After a 7-day acclimatization feeding period, the mice were randomly divided into 7 groups (n = 10), 2 groups of which were fed normal chow (LG, 0.013 mg Se/kg) and 5 groups were fed selenium-deficient chow (NG. 0.15 mg Se/kg) (Table 1). Fig. 1A showed the experimental framework for modelling Se-deficient mice and subsequent intervention, after 18 days of feeding, 10 mice from the normal and Se-deficient groups were randomly slaughtered. Serum and liver samples were collected for Se content measurement. The remaining 40 Se-deficient mice (n = 10) were randomly divided into 4 groups: (1) the model group, (2) the Na_2SeO_3 group (positive control), (3) the low-dose SHP-Se chelate group (SHP-Se-L), and (4) the high-dose SHP-Se chelate group (SHP-Se-H). On the basis of the Chinese Nutrition Association's recommended daily Se intake of 50-250 μg for adults,[25] a dose of 200 μg was selected for this study and converted to the Na_2SeO_3 equivalent, according to the previously determined Se content,[19] the SHP-Se-H and Na_2SeO_3 groups were administered 26 μg/kg, and the SHP-Se-L group was administered 9 μg/kg, while the control and model groups were given equal volumes of deionized water. After gavage for 20 days, the weight and behaviour of the mice were monitored every day. All procedures were approved by the Experimental Animal Ethics Committee of the National Seafood Engineering Research Centre of Dalian Polytechnic

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University, and strictly conducted the experiments in accordance with China's national standards for laboratory animal quality and the guidelines for welfare and ethical review of laboratory animals.

2.4. Determination of Se in the serum and liver

At the end of the experiment, blood was taken from the eyeballs of the mice, frozen and centrifuged (1000 ×*g*, 10 min), and the serum was separated and stored at -20 °C. After the mice were dissected, the livers were removed, washed with saline to remove excess blood and stored at -80 °C. The absorbances of different samples were determined via inductively coupled plasma-mass spectrometry (ICP-MS) (Optima 8000, PerkinElmer) to calculate the Se contents in the serum and liver.

2.5. Targeted fluorescence imaging analysis of mice *in vivo*

The effect of selenium deficiency on the digestive system of mice was determined by fluorescent labeling of SHP-Se chelate, and the biological distribution of SHP-Se chelate in major organs was also detected. Briefly, 10 mg/kg FITC-labeled SHP-Se chelate peptide was orally administered to mice in the normal feeding group and the Se-deficient feeding group, and then the mice were killed 1, 2 and 4 h later, respectively. The liver, heart, lung, kidney, and spleen were collected, and the fluorescence intensity was quantified via *in vivo* imaging system software. The distributions of the SHP-Se chelate in major organs were visualized via a multifunctional *in vivo* imager (MIIS XFP-BIX, American Molecular Devices Corporation, USA).

2.6. Measurement of liver enzyme levels in the liver

ALT and AST viability in the liver was measured according to the instructions provided in the kit instructions (Nanjing Jiancheng, China), and the absorbance of all the wells at 510 nm was measured with a microplate reader (Infinite F200 PRO, Tecan, Sweden).

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2.7. Immunofluorescence staining

After the liver tissue was cryosectioned, a circle was drawn around the tissue, and the ROS staining solution was added dropwise inside the circle and incubated at 37 °C for 30 min in a light-proof thermostat. Then, the nuclei of the cells were restained with DAPI dye, and the sections were sealed with an antifluorescence quenching sealer after being incubated at room temperature for 10 min in a light-proof incubator. Finally, a fluorescence microscope was used to observe and collect images.

2.8. Determination of the oxidative stress indicators in the serum

The SOD, CAT, GSH-Px, MDA, and GSH levels in the serum were measured according to the kit instructions (Nanjing Jiancheng, China).

2.9. Histological analysis

Some of the liver tissue was immediately fixed in formalin for 48 h, embedded in paraffin, and stained with hematoxylin and eosin, and fluorescence optical microscopy (Eclipse Ti-s, Nikon Corporation, Tokyo, Japan) was used for morphological analysis.

2.10. Statistical analysis

All the experiments were repeated at least three times, and all the data are expressed as the means ± standard deviations (SDs). SPSS software (Chicago, Illinois, USA) was used to determine the significant differences between the results through one-way ANOVA.

3. Results and discussion

3.1. Ameliorative effects of food intake and body weight on Se-deficient mice

Selenium deficiency can lead to loss of appetite and weight loss.[1] As shown in Fig. 1B, during the modelling process, the body weights of the Se-deficient group of mice gradually decreased compared

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with those of the control group. By the 9th day of modelling, the body weight of the Se-deficient group (33.39 ± 1.45 g) was significantly different from that of the normal group (39.40 ± 1.14 g). At the end of the modelling, the body weight of the Se-deficient group (42.86 ± 2.22 g) was significantly lower than that of the control group (39.79 ± 1.82 g). Fig. 1C showed that food intake began to diverge between the control and Se-deficient groups during the modelling process. By the 12th day, the food intake of the Se-deficient group (147.4 ± 4.13 g) and the control group (173.33 ± 5.45 g) gradually stabilized. After 18 days of modelling, the food intake of the control group (179.2 ± 3.30 g) was significantly greater than that of the Se-deficient group (146.13 ± 1.80 g). Bao *et al*.[26] also found that Se deficiency inhibited the appetite of mice, leading to weight loss when exploring the effect of Se deficiency on skeletal muscle cell differentiation. These results indicated that Se deficiency suppressed appetite and led to weight loss in the mice.

3.2. Effects of Se deficiency on metabolic uptake in mice

In vivo biodistribution experiments recorded and tracked by luciferase labelling have been widely used to evaluate gastrointestinal retention times and drug release sites.[27,28] To further elucidate the effects of Se deficiency on digestion and absorption in mice, the distribution of SHP-Se chelate in isolated tissues was detected using a small animal *in vivo* imaging system at different time intervals. As shown in Fig. 1D, a and b represent the effects of gastric gavage of the SHP-Se chelate in normal mice and Se-deficient mice, respectively. The results indicated that after 1 h of gavage, the fluorescent-labelled SHP-Se chelate predominantly accumulated in the stomachs of Se-deficient mice, whereas in normal mice, at the same dosage, some fluorescent-labelled SHP-Se chelates were also found in the small intestine. The fluorescence signal in Se-deficient mice significantly lagged behind that in normal mice. The distribution trend of the SHP-Se chelate at 2 h postgavage was similar to that

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at 1 h and was primarily concentrated in the small intestine. The labelled SHP-Se chelate accumulated in the small intestine of normal mice for 1-4 h and was then excreted at approximately 4 h, whereas the accumulation time in the small intestine of Se-deficient mice exceeded 4 h. Additionally, a fluorescent signal was detected in the liver at 4 h, whereas no signal was detected in other organs, suggesting that some SHP-Se chelate might accumulate in the liver.

Previous studies indicated that selenium-chelating peptides tend to form aggregates during gastrointestinal digestion, which was primarily associated with the primary sequence of hydrophobic amino acids in proteins and changes in ambient pH levels.[29] Additionally, research has shown that changes in pH can alter protein properties, thereby affecting protein-protein interactions and leading to a complex pH-dependent aggregation relationship. [30] Hou *et al*.[9] demonstrated that digestion of wheat gluten protein selenium-chelated peptides in the gastric environment significantly increased the content of hydrophobic amino acids. However, the occurrence of protein aggregation during gastrointestinal digestion has minimal impact on food applications. The results indicated that Se-deficient mice presented significantly lower metabolic rates than normal mice did, suggesting that Se deficiency influenced metabolism and absorption in these mice.

3.3. Analysis of the serum Se and liver Se contents

Table 2 presents the serum Se and liver Se contents measured in normal and Se-deficient mice after model completion. Both the liver Se concentration (0.96 ± 0.02 μg/g) and the serum Se concentration (1.52 ± 0.11 μg/mL) were significantly greater in the normal group than in the Se-deficient group (liver: 0.92 ± 0.02 μg/g; serum: 1.15 ± 0.03 μg/mL). As shown in Table 3, the serum Se and liver Se contents of the Se-deficient group (Na_2SeO_3, SHP-Se-L, and SHP-Se-H) were subsequently measured 20 days after successful modelling. Notably, significant differences in the

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serum Se and liver Se levels were detected between the Se-supplemented and Se-deficient mice. Compared with those in the model group, the liver Se contents in the Na_2SeO_3, SHP-Se-L, and SHP-Se-H groups increased by 14.74%, 7.37%, and 22.11%, respectively. The serum Se content of the SHP-Se-H group (1.09 ± 0.02 μg/mL) was the highest, which basically reached the level of the serum Se content in normal mice (1.07 ± 0.02 μg/mL). These results indicated that the Se content in the liver and serum of the SHP-Se-H group was significantly greater than that in the Na_2SeO_3 group. According to previous studies, both organic forms of Se (SeMet, SeCys, and MeSeCys) and inorganic forms of Se (selenate [Me_2SeO_4] and selenite [Me_2SeO_3]) produce SeH- after they enter the human body. SeH- reacts with selenophosphate synthetase to form selenophosphate, which is then converted into Sec-tRNA [Ser]Sec for insertion into protein sequences.[31] However, organic Se had greater biological availability, mainly because the biological activity of Se is manifested mainly through the translation of Se amino acids into selenoproteins, and SeMet and SeCys are two amino acids of Se present in proteins, especially SeCys, which can form Se-Se and S-Se bridges that are more conducive to Se absorption.[32] Therefore, compared with traditional inorganic Se supplements, the SHP-Se chelate provided better Se supplementation, which can serve as a promising source of Se supplementation.

3.4. Immunofluorescence staining analysis

Se as a key component of glutathione peroxidase (GPx), helps protect the body from stress-induced oxidative damage, and reactive oxygen species (ROS) are the main contributors to oxidative stress damage. Therefore, reducing excessive ROS release is crucial for alleviating cellular oxidative stress damage. The current theory of ROS signal transduction involves two main mechanisms: alterations in the intracellular redox state and protein oxidation. Changes in the redox state primarily depend on the thiol redox system (mainly glutathione and thioredoxin), which reduces H_2O_2 and lipid

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hydroperoxides to mitigate intracellular oxidative stress.[33] Fig. 2A showed the expression of ROS in mice liver was detected by immunofluorescence staining. Compared with the control group, the fluorescence intensity of SHP-Se-H group was significantly weaker. As shown in Fig. 2B, compared with that in the control group (0.81% ± 0.04%), the level of ROS in the livers of model mice (1.00% ± 0.08%) was significantly greater ($P < 0.05$), indicating that Se deficiency caused oxidative stress damage to the liver cells of these mice. In addition, the ROS level in the mice liver after SHP-Se-H treatment was 41.04% lower than that in the model group and 18.30% lower than that in the Na_2SeO_3 group, indicating that SHP-Se-H treatment enhanced mitochondrial autophagy and reduced ROS release. Wang *et al*.[34] also reported that ROS activity was elevated with selenium deficiency. These results suggested that Se supplementation with SHP-Se-H ensured that glutathione peroxidase functions normally, thereby reducing oxidative stress damage in the liver cells of mice.

3.5. The impact of SHP-Se on oxidative damage in Se-deficient mice

Se is an antioxidant that reduces free radical damage to cells, prevents oxidative damage, promotes endoplasmic reticulum stress, and controls inflammation. Se deficiency leads to a weakening of cellular antioxidant defences. Therefore, the reparative influence of SHP-Se on oxidative harm in Se-deficient mice was further evaluated, and the levels of CAT, MDA, SOD, GSH-Px, and GSH in mice serum were also assessed. CAT plays a pivotal role in the reactive oxygen species clearance system, acting as a primary enzyme for H_2O_2 elimination. MDA, a byproduct of polyunsaturated fatty acid oxidation, is widely used as an indicator of *in vivo* oxidative stress and lipid peroxidation.[35] SOD is considered a critical enzyme component of the antioxidant defense system and is associated with cellular damage, catalyzing the decomposition of superoxide anions. GSH is deemed an important antioxidant and signalling molecule,[36] potentially associated with signalling pathways, metabolism,

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inflammation, and apoptosis. GSH-Px, an antioxidant enzyme with Se as its active center, enhances the body's antioxidative capacity.[37] As shown in Fig. 3, compared with the control group, the Se-deficient model group presented significant decreases of 28.73%, 8.89%, 45.71%, and 134.83% ($P < 0.05$) in the CAT (Fig. 3A), SOD (Fig. 4C), GSH (Fig. 3D), and GSH-Px (Fig. 3E) levels, respectively, while the MDA (Fig. 3B) content was 1.23 times greater than that of the control group.

Studies have shown that after selenium-chelating peptides enter the body of mice, they reacted with glutathione (GSH) under the action of glutathione reductase, thioredoxin reductase (TrxR), and other enzymes to form selenodiglutathione (GSSeSG). This intermediate was subsequently reduced to glutathione selenol (GSSeH) in the presence of NADPH and glutathione reductase (GR). Subsequently, GSSeH was then converted to hydrogen selenide, which was further transformed into SectRNA[Ser]SEC under the action of selenide synthase for incorporation into protein sequences.[38] Selenate is reduced to selenide, selenoproteins, and excretable metabolites via an anion transport mechanism. After the gastrointestinal digestion phase, selenium-chelating peptides were broken down into smaller molecules and absorbed in the duodenum through amino acid absorption pathways.[39] Xu *et al*.[40] found that Se deficiency led to increased levels of oxidative stress such as ROS and MDA, which induced an inflammatory response. Abdelsalam *et al*.[41] reported that Se supplementation markedly altered the metabolic pathways of alanine, aspartate, and glutamate. Wang *et al*.[42] also found that casein phosphopeptide-selenium chelates significantly enriched amino acid metabolic pathways such as those of tryptophan, phenylalanine, cysteine, and methionine. These metabolic pathways are reported to potentially regulate oxidative stress by suppressing oxidation through free radical scavenging by selenium-chelated peptides, thereby mitigating oxidative stress-induced cellular damage.[43] The above phenomena indicated that significant oxidative stress occurred in the Se-deficient group, and compared

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with those in the Model group, the serum levels of CAT, SOD, MDA, GSH, and GSH-Px in the SHP-Se-H group were significantly different, indicating that the highest degree of oxidative damage was ameliorated in the Se-deficient group.

3.6. Influence of the SHP-Se chelate on liver enzyme levels in Se-deficient mice

Liver enzymes, specifically ALT and AST, serve as crucial indicators for evaluating liver function. Generally, increased ALT and AST levels reflect the extent of liver cell damage. ALT primarily exists in the cytoplasm of liver cells, whereas AST is distributed in both the cytoplasm and mitochondria of liver cells. Elevated levels of ALT or AST in the blood indicate liver cell membrane damage, increased membrane permeability, or cellular organelle injury.[39,44] As shown in Fig. 4, the liver enzyme levels in the model group were significantly higher than those in the control group ($P < 0.05$), and the ALT (Fig. 4A) and AST (Fig. 4B) levels were 2.41 and 2.37 times higher than those in the control group, respectively. These data indicated impaired liver function post-Se deficiency. Compared with the model group, the SHP-Se chelate group presented significantly lower liver enzyme levels ($P < 0.05$), In addition, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the SHP-Se-H group were reduced by 65.1% and 28.6%, respectively, compared with inorganic Se, which suggested that SHP-Se exhibits a stronger liver protective effect than inorganic Se. Our findings were consistent with Liang *et al.*[45] that increased dietary Se intake improved liver impairment. Overall, the SHP-Se chelate was more effective than inorganic Se supplements in reducing ALT and AST levels, indicating the effectiveness of the SHP-Se chelate in attenuating Se deficiency-induced hepatocyte injury.

3.7. Histopathological analysis

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Tissue sections of internal organs from deceased mice were prepared, and histopathological evaluations were conducted via H&E staining to study the effects of various Se supplements on the heart, liver, spleen, kidneys, and small intestines. As shown in Fig. 5, the liver tissue structure in the normal control group remained intact, exhibiting hepatic sinusoids radiating from the central vein, distinct boundaries of hepatic cell cords, and prominently discernible nuclei. The liver tissue of the model group remained undamaged but displayed disordered hepatic sinusoids surrounding the central vein, vague boundaries of hepatic cell cords, and slight hepatocyte enlargement. Instances of liver tissue disorder, cellular swelling, hepatic sinusoid loss, and minor hemorrhaging were observed in the Na_2SeO_3 group. In comparison, the hepatic lobule structure in the SHP-Se-L and SHP-Se-H groups was intact, featuring clear, orderly arranged hepatic cell cords and normal morphology (Fig. 5A, B). In the heart tissue slices from the normal control group, the myocardial cells were arranged regularly, with uniform staining and no apparent structural abnormalities or inflammatory cell infiltration (Fig. 5C). In addition, the red and white pulp of the spleen in the normal control group appeared clear, displaying normal morphology with no structural abnormalities observed.[46] In contrast, sections of the spleen tissue from the model group revealed white pulp fusion, increased lymphocyte count, and irregular morphology, whereas those from the supplemented group presented no significant structural abnormalities in the red or white pulp (Fig. 5D). Furthermore, the kidney glomerular morphology in the normal control group remained unaltered, exhibiting tightly arranged renal tubules with no apparent structural abnormalities or inflammatory cell infiltration (Fig. 5E). The intestinal mucosa of the rats in the model group was not obviously damaged, the villi were intact, and the glands were normal, suggesting that Se deficiency might not cause morphological injury to the intestinal mucosa. However, a noticeable decrease in the number of goblet cells in the model group compared with the normal group

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was observed (Fig. 5F). Strikingly, H&E staining revealed no obvious abnormalities, although slight changes were observed in the heart, kidney, and spleen tissues. The intestinal mucosa in all supplemented groups displayed normal morphology, with a notable increase in the number of goblet cells. Goblet cells primarily secrete mucus and digestive enzymes, contributing to lubrication, digestion, and immunity, and their quantity indirectly reflects a mice's digestive ability.[47] These results suggest that Se-chelated peptides can restore goblet cell quantity without causing morphological structural damage to the mice intestine, and the SHP-Se chelate was more effective at preventing liver injury than was inorganic Se, which was also consistent with the research results of Chen *et al*.[45]

4. Conclusion

In conclusion, Se deficiency results in oxidative stress and impaired liver function in mice. After Se supplementation, symptoms of Se deficiency were significantly alleviated, with SHP-Se-H being more effective than Na_2SeO_3. Compared with Na_2SeO_3, SHP-Se-H exhibited greater bioavailability and a stronger ability to alleviate oxidative stress and prevent liver injury caused by Se deficiency and prevent liver injury. These results indicate that the SHP-Se chelate is a novel organic Se supplement that could be used to alleviate Se deficiency-induced oxidative stress and liver injury. This study also provides a theoretical basis for the comprehensive utilization of sturgeon heads, paving the way for future development as an effective dietary selenium supplement in the food or pharmaceutical sectors.

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Author contributions

Jiao Jia: Methodology, Investigation, Formal analysis, Validation, Writing-original draft. **Yinan Du:** Writing-review & editing, Formal analysis. **Zhiqiang Lu:** Investigation. **Qing Liu:** Validation. **Wenfei Wu:** Supervision, Project administration, Funding acquisition.

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Conflict interest statement

The authors state no conflict of interest.

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502 **Table 1** Brief list of feed ingredients (feed content per kg diets)

Mineral substance	
Se (deficient group)	0.013mg/kg
Se (normal group)	0.15 mg/kg
Fe	100 mg/kg
Mn	75 mg/kg
Cu	10 mg/kg
Zn	30 mg/kg
Energy supply ratio (% by weight)	
Protein	15.3%
Carbohydrate	64.1%
Fat	5.9%

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504 **Table 2** Se content in serum and liver of mice

Group	Liver Se content (μg/g)	Serum Se content (μg/mL)
Normal	0.96 ± 0.02^{a}	1.52 ± 0.11^{a}
Se-deficient	0.92 ± 0.02^{b}	1.15 ± 0.03^{b}

505 Different letters in a column indicate significant differences ($P < 0.05$).

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507 **Table 3** Se content in liver and serum of mice in each group for 20 days

Group	Liver Se content (μg/g)	Serum Se content (μg/mL)
Control	0.98 ± 0.02^{d}	1.07 ± 0.02^{ab}
Model	0.95 ± 0.02^{e}	0.94 ± 0.04^{c}
Na_2SeO_3	1.09 ± 0.03^{b}	1.06 ± 0.01^{b}
SHP-Se-L	1.02 ± 0.00^{c}	1.05 ± 0.03^{b}
SHP-Se-H	1.16 ± 0.01^{a}	1.09 ± 0.02^{a}

508 Different letters in a column indicate significant differences ($P < 0.05$).

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FIGURE CAPTIONS

Fig. 1. (A) Flow chart animal experiment; (B) Body weight and (C) food intake; (D) *In vivo* imaging of main isolated tissues of mice: (a) SHP-Se-Normal, (b) SHP-Se-Deficient. Different letters indicate significant differences ($P < 0.05$).

Fig. 2. The expression of ROS in mice liver was detected by immunofluorescence staining (40×, scale bar: 50 μm), and the nuclei were detected via DAPI (blue) staining.

Fig. 3. Effects of different Se supplements on CAT (A), SOD (B), MDA (C), GSH (D) and GSH-Px (E) in mice serum. Different letters indicate a significant difference ($P < 0.05$).

Fig. 4. Effects of different Se supplements on ALT (A) and AST (B) in mice liver. Different letters indicate a significant difference ($P < 0.05$).

Fig. 5. Liver appearance (A) and H&E staining pictures of liver (B), heart (C), spleen (D), kidney (E) and intestine (F). Scale bars = 50 μm

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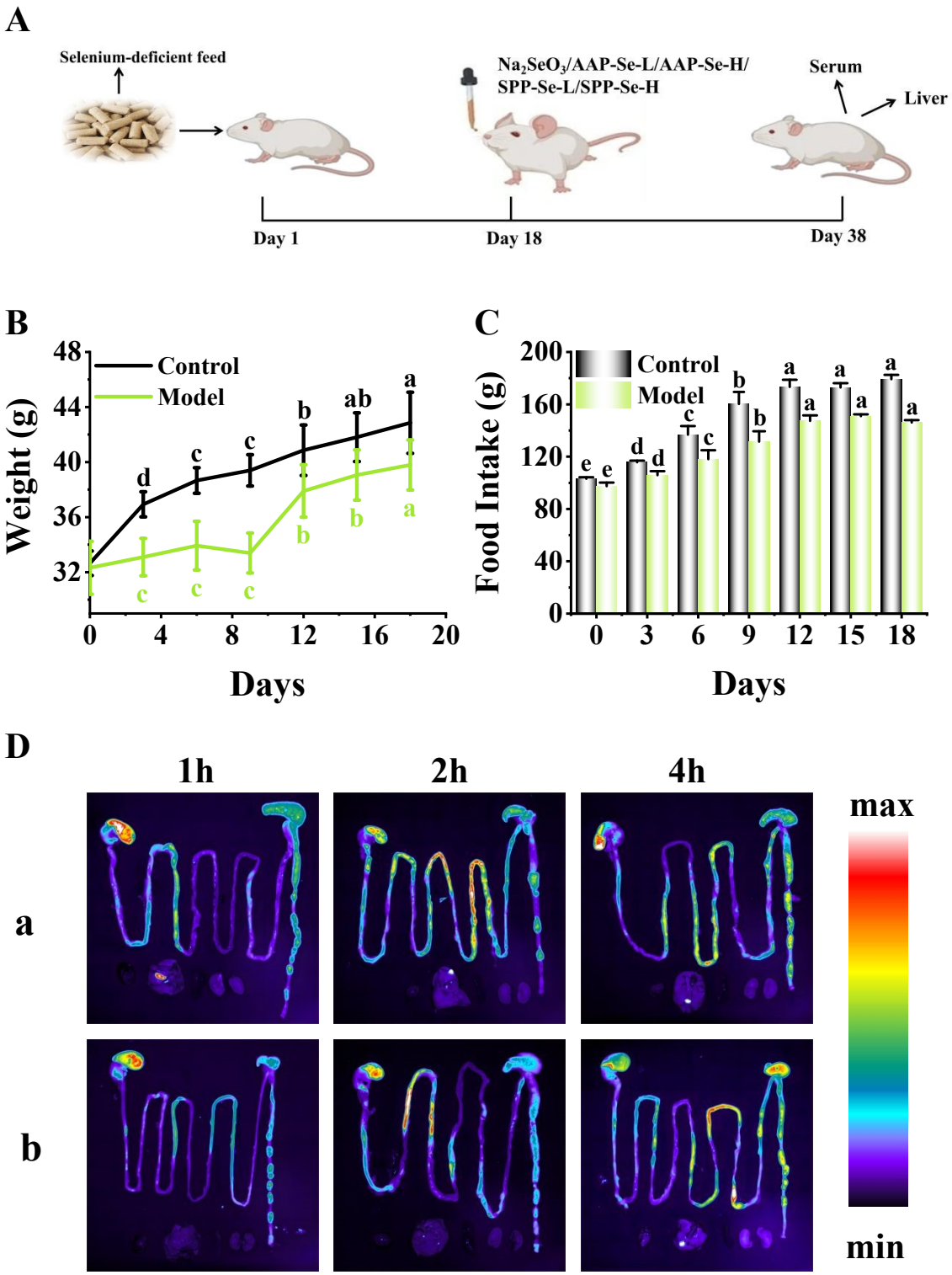

Fig. 1

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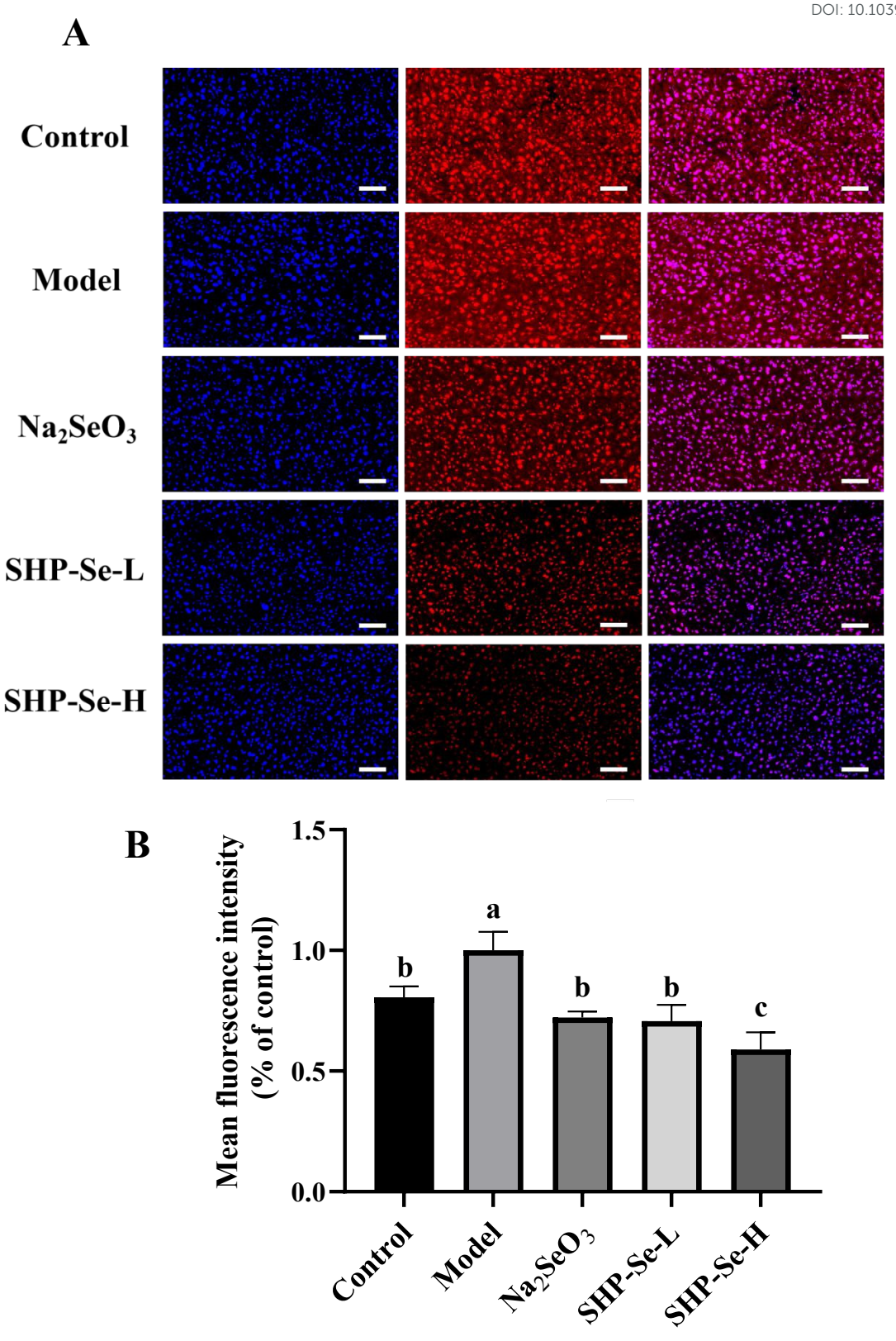

Fig. 2

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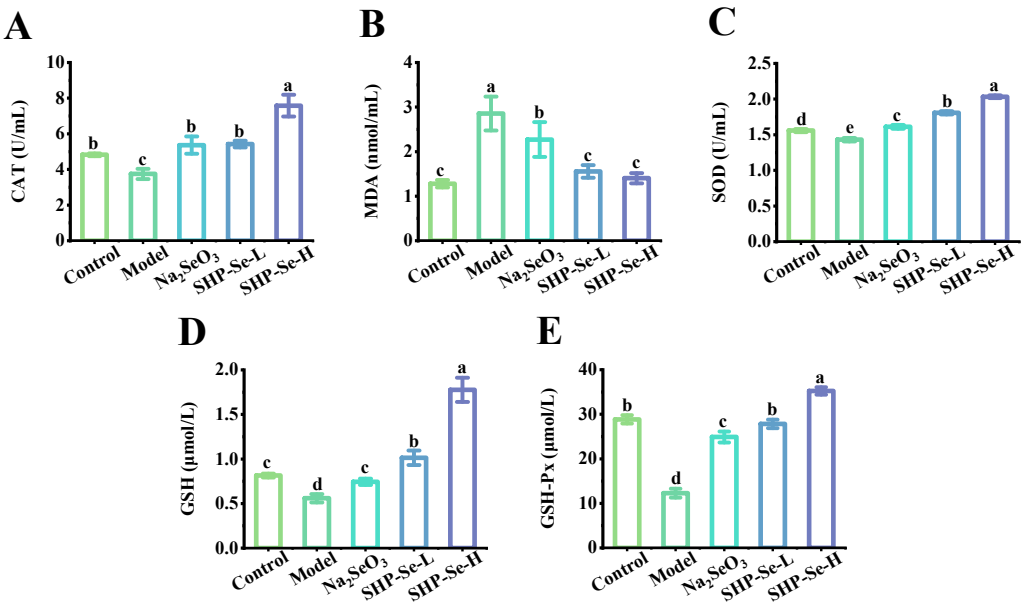

Fig. 3

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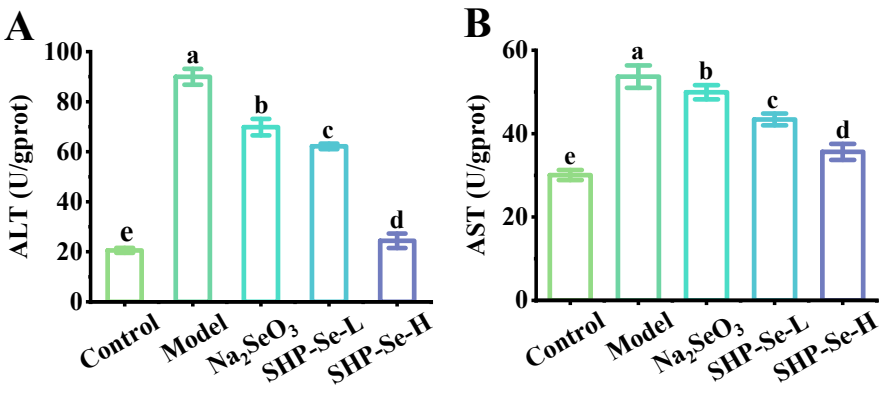

Fig. 4

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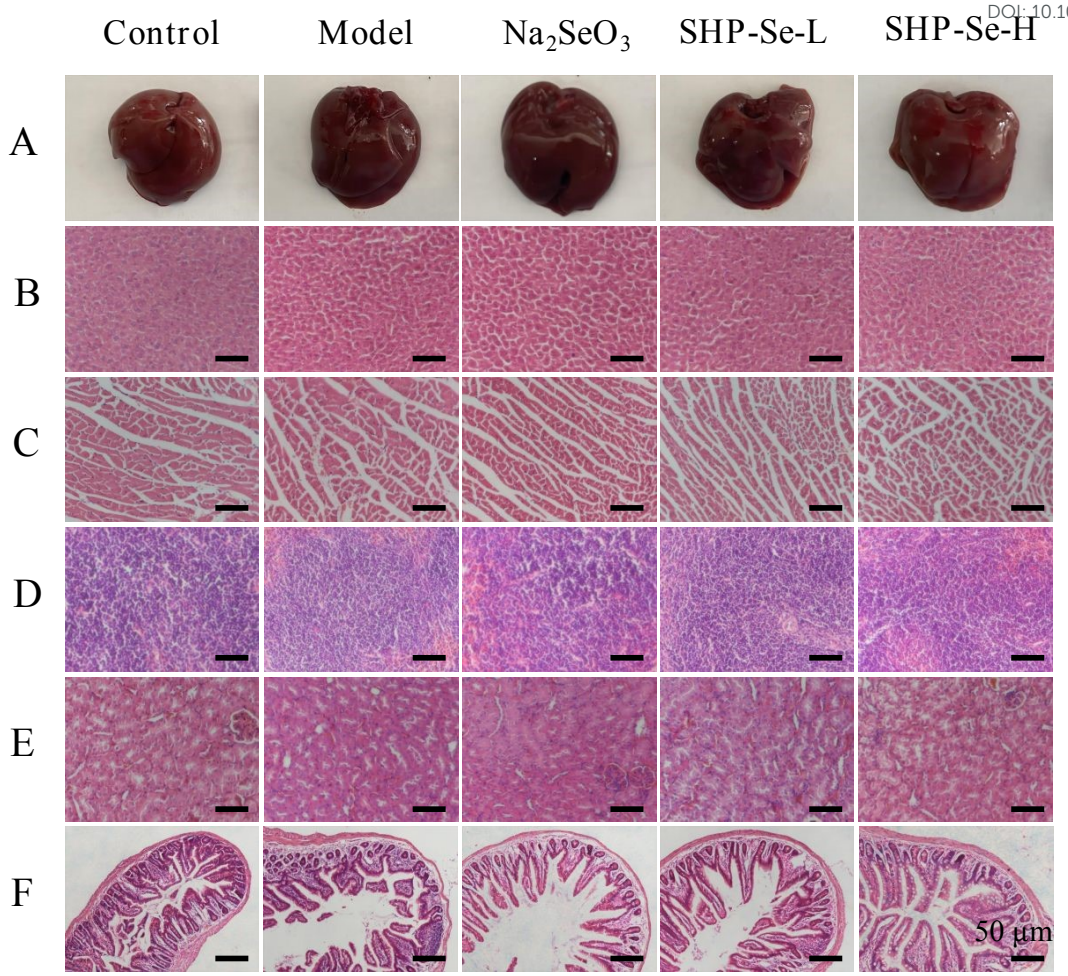

Fig. 5

The data would be available on request.

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