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# Protective effect of *Sargassum fusiforme* polysaccharides on H<sub>2</sub>O<sub>2</sub>-induced injury in LO2 cells

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**Introduction:** *Sargassum fusiforme* is considered a traditional Chinese medicinal herb to treat many diseases, *Sargassum fusiforme* polysaccharides (SFPS) were found to display many important biological activities. The purpose of this study was to investigate the in vitro antioxidant activity of crude polysaccharide from *Sargassum fusiforme* and its protective effect against H<sub>2</sub>O<sub>2</sub>-induced injury in LO2 cells.

**Methods:** SFPS was extracted from *Sargassum fusiforme* using water extraction and alcohol precipitation. High-performance liquid chromatography (HPLC) was used to measure its monosaccharide composition. The in vitro antioxidant activity of SFPS was evaluated using scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical assays. Moreover, its hepatoprotective activity was examined by establishing H<sub>2</sub>O<sub>2</sub>-induced LO2 cells injury model.

**Results and discussion:** The results showed that the SFPS was composed of Glc, Ara, Fuc, Gal, Man, Rha and Xyl. Meanwhile, SFPS exhibited strong antioxidant activity and potential hepatoprotective effect. Further studies found that SFPS could alleviate LO2 cells injury induced by H<sub>2</sub>O<sub>2</sub> via decreasing malondialdehyde (MDA) and lactate dehydrogenase (LDH) levels, increasing superoxide dismutase (SOD) and catalase (CAT) activities and inhibiting intracellular reactive oxygen species (ROS) production. These results revealed that SFPS had the ability to protect the liver cells from H<sub>2</sub>O<sub>2</sub>-induced liver injury through its antioxidant activity.

## KEYWORDS

*Sargassum fusiforme*, polysaccharides, H<sub>2</sub>O<sub>2</sub>, LO2 cell injury, protective effects

## 1 Introduction

Currently, liver disease has become a global health problem that leads to death accounting for approximately 2 million worldwide each year (Asrani et al., 2019; Neshat et al., 2021). The liver is the essential organ of people and plays a vital role in metabolism, detoxification, and protein production (Cheng et al., 2021). However, liver homeostasis can be disrupted by environmental pollutants, heavy metals, viral infections, drug, alcohol-induced liver injury and autoimmunity. Many studies have shown that liver disease is closely related to oxidative stress. ROS is usually produced as a by-product of oxidative stress, causing apoptosis and damage to lipids, proteins and DNA. Studies have shown that the antioxidant pathway can protect the body from excessive ROS damage. Therefore, inhibition of free radical-induced injury may provide a feasible way to prevent liver disease (Borrelli et al., 2018; Chen et al., 2020; Li et al., 2020).

In recent years, natural food products with elevated antioxidant activity and other health-promoting effects have gained increasing attention for the treatment of liver of diseases. Seaweed-derived polysaccharides have been found with potential in treatment or prevention of liver diseases (Bocanegra et al., 2006; Tsai et al., 2021; Zhao et al., 2021). *Sargassum fusiforme* is a kind of the seaweed and is mainly distributed in the northwest the of Pacific Ocean. Dietary intake of *Sargassum fusiforme* was found to be associated with the treatment of several diseases, including neural diseases (Bogie et al., 2019), endocrine diseases (Wu et al., 2022) and gastrointestinal diseases (Zhang et al., 2021), etc. In addition, increasing evidence showed that *sargassum fusiforme* possessed various bioactivities, such as antitumor activity (Dai et al., 2019), antioxidant activity (An et al., 2022) and immunoenhancement activity (Chen et al., 2017). Meanwhile, *sargassum fusiforme* could delay the ageing (Zhang et al., 2019). Overall, SFPS exhibits strong antioxidant capacity such as free radical scavenging activity, suggesting that SFPS may have potential therapeutic effects on oxidation-related diseases.

Exogenous H<sub>2</sub>O<sub>2</sub> can cause oxidative stress and induce apoptosis. Increasing evidence has proved that inducing human hepatocyte (LO2) injury has been widely used as a mature model for testing the biological activity of hepatocyte protective compounds *in vitro* (Fang et al., 2019; Yuan et al., 2020; Wang et al., 2021). However, there are limited reports on the antioxidant effect of SFPS based on the cultured cell model system, especially the comprehensive report on the regulatory effect of SFPS mediated antioxidant pathway on H<sub>2</sub>O<sub>2</sub>-induced liver cell injury have not been well investigated (Chen et al., 2016). The protective effect of SFPS on H<sub>2</sub>O<sub>2</sub>-induced damage in LO2 cells were demonstrated in this study. Specifically, the antioxidation activity of SFPS as well as its effect on cell viability and apoptosis in H<sub>2</sub>O<sub>2</sub>-treated LO2 cells were investigated. And the ROS, malondialdehyde (MDA), lactate

dehydrogenase (LDH), superoxide dismutase (SOD) and catalase (CAT) were evaluated. In this experiment, SFPS was used for the first time to screen the *in vitro* activity of H<sub>2</sub>O<sub>2</sub>-induced LO2 cytoprotective effect, providing a theoretical basis for the study of its hepatoprotective effect.

## 2 Materials and methods

### 2.1 Chemicals and materials

The LO2 cells used in this study were purchased from Fuheng Biological Company. DEME culture medium, fetal bovine serum (FBS), trypsin digesting solution, phosphate buffer (PBS), penicillin, streptomycin, and dimethyl sulfoxide (DMSO) were all purchased from GIBCO Company. The cell apoptosis kit by ANNEXIN-V-FITC/PI was obtained from Yeasen Biology Company. MDA, CAT, SOD, and ROS activity test kits were purchased from the Nanjing Institute of Biological Engineering.

Dried *sargassum fusiforme* was purchased from Xiapu county (Fujian Province). All the raw materials were kept in a dry and dark state.

### 2.2 SFPS preparation

SFPS was extracted by the hot water extraction method (Ji and You, 2017). The dried leaves of *sargassum fusiforme* was crushed into powder. The *sargassum fusiforme* power was pre-extracted by reflux with 95% ethanol to remove pigments. After that, the residue was extracted with boiling water (1:50, v/v) for 4 h and then cooled to room temperature. The water-extracts were filtered, concentrated, and precipitated with 4 vol. of 95% ethanol at 4°C for overnight. After centrifugation, the precipitate was deprotonated by Sevag method (Sevag et al., 1938). Ultimately, the deproteinated supernatant was lyophilized to obtain SFPS.

### 2.3 General analysis of SFPS

The total carbohydrate content in the SFPS was quantified using the phenol-sulfuric method with fucoidan as the standard (DuBois et al., 2002). The protein contents of SFPS fractions were quantified according to the Bradford method (Bradford, 1976) using bovine serum albumin as the standard. The sulfate content was quantified by Barium sulfate turbidimetry method (Dodgson and Price, 1962).

The monosaccharide composition was analyzed by PMP-HPLC analysis using Agilent 1200 system. Briefly, SFPS was hydrolyzed with 2 M TFA (1mL) at 120 °C for 6 h. After removal of TFA by rotary evaporation, the full hydrolysate was reacted

with 500  $\mu\text{L}$  2 M PMP in methanol and 400  $\mu\text{L}$  aqueous sodium hydroxide (0.3 M) at 70  $^{\circ}\text{C}$  for 1 h. The reaction products were dried and then extracted three times after addition of 0.2 mL of hydrochloric acid (0.3 M), water and chloroform, and filtered for HPLC analysis. The monosaccharide mixture solution was obtained by the same method as above. The mixtures were separated on an ZORBAX Eclipse XDB-C18 (5  $\mu\text{m}$ , 4.6 i.d. $\times$  250 mm) and eluted with PBS: Acetonitrile = 83:17 (%:%) at a flow rate of 1 mL/min.

## 2.4 UV and IR spectrum analysis

The experimental UV spectrum of polysaccharide fractions (1 mg/mL) were recorded in the region of 400–200 nm using UV-Vis spectrophotometer (TU-1901 Two-beam ultraviolet photometer, China). The extracted polysaccharides (1 mg/mL) were mixed with KBr powder and pressed into disc at the mid, and FT-IR spectrum was recorded in the range 4000–500  $\text{cm}^{-1}$  using a BRUKER TENSOR-27 Fourier infrared spectrometer (Germany).

## 2.5 Antioxidant activity assays for SFPS

### 2.5.1 Scavenging activity of DPPH radicals

The antioxidant activity was determined using DPPH as a free radical (Lim et al., 2001). Before measurement, 0.1 mol/L DPPH solution was prepared and dissolved in absolute ethanol and protected from light. Reaction mixture consisting of 0.5 mL SFPS solution and 2 mL DPPH solution was added to each test tube, and then added with 1.5 mL of ultrapure water. After shaken and left to stand for 30 min in the dark, the absorbance of the mixture was determined by spectrophotometer measurement at a wavelength of 517 nm. Vitamin C was used as a positive control. The inhibition percentage is calculated as follows:

$$\text{DPPH Scavenging rate}(\%) = [1 - (D1 - D2) / D0] \times 100\%$$

where D0 is the absorbance of the blank solution, D1 is the absorbance of the sample group by mixing test sample with DPPH solution and D2 is the absorbance of the solvent control group by mixing test sample without DPPH solution.

### 2.5.2 Scavenging activity of hydroxyl radical ( $\bullet\text{OH}$ )

The hydroxyl radical scavenging activity of SFPS was determined with a slight modification of the standard method. The absorbance of SFPS was measured at 510 nm after adding the solution of SFPS with different concentrations into the mixture of 9 mM iron<sup>2+</sup> sulfates (anhydrous) and 9 mM salicylic acid and 8 mM hydrogen peroxide at 37 $^{\circ}\text{C}$  for 1 hour.

In the control, absolute ethanol was used instead of salicylic acid ethanol solution, and in the blank, deionized water was used instead of polysaccharide sample solution.  $V_C$  was used as a positive control. The removal rate is calculated as follows:

$$\bullet\text{OH scavenging rate}(\%) = [1 - (D1 - D2) / D0] \times 100\%$$

D0 is the absorbance of the blank control, D1 is the absorbance value of the experimental group and D2 is the absorbance value of the control group.

## 2.6 Hepatoprotective activity assays for SFPS

### 2.6.1 Cell culture

LO2 cells were grown in DMEM supplemented with 100 U/mL penicillin, 10% fetal bovine serum and 100 U/mL streptomycin at 37  $^{\circ}\text{C}$  in 5%  $\text{CO}_2$ . Subculture was carried out when the degree of cell confluence reached to 80%.

### 2.6.2 Cell viability

The effect of SFPS on the  $\text{H}_2\text{O}_2$ -induced cytotoxicity was determined, and LO2 cells were plated in 96-well plates at a density of  $8 \times 10^3$  cells/mL. The cells were treated with different concentrations of SFPS (0, 0.3, 0.6, 1.25, 2.5  $\mu\text{g}/\text{mL}$ ) for 12 hours. Then, each well was added with 700  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and cultivated the cells for another 3 hours. MTT assay was utilized to examine cell viability.

### 2.6.3 Determination of LDH, CAT, SOD activity and MDA level

The assay kits were used to measure the antioxidant index. In brief, the LO2 cells were seeded in each 6-well plate with a density of  $1 \times 10^5$  cells/mL. Then cells were cultivated for 24 h. Next, the cells were treated with SFPS for 12 h before the treatment with  $\text{H}_2\text{O}_2$  for 3 h. After cells were lysed, the cells supernatant was collected. According to the manufacturer's instructions, LDH, CAT, SOD and MDA were detected with the corresponding assay kits provided by the Nanjing Jiancheng Bioengineering Institute.

### 2.6.4 Measurement of ROS production

A cell-permeable probe, DCFH-DA, was used to evaluate the effect of SFPS on ROS production by LO2 cells (Jiao and Zhao, 2002). LO2 cells were prepared into a cell suspension and incubated into a 6-well plate with  $1 \times 10^5$  cells/mL. Cells were cultured for 24 h at 37 $^{\circ}\text{C}$  in 5%  $\text{CO}_2$  medium. LO2 cells were exposed to polysaccharides (0.3, 0.6, 1.25, 2.5  $\mu\text{g}/\text{mL}$ ) for 12 h followed by  $\text{H}_2\text{O}_2$  (700  $\mu\text{M}$ ) for another 3 h. Next, cells were co-cultured with DCFH-DA for 0.5 h at 37 $^{\circ}\text{C}$  in darkness and then washed twice with PBS. The fluorescence production of DCF was

observed by a microplate reader (Varioskan Flash, Thermo Scientific, USA).

### 2.6.5 Cell apoptosis detection

To further assess the protection of SFPS against  $H_2O_2$ -induced cell apoptosis, the annexin V/PI double staining was assessed by Annexin V-FITC/PI apoptosis detection kit (Wang et al., 2017). In brief, LO2 cells were digested with trypsin, centrifuged and collected, and stained with Annexin V-FITC and PI. The cells were determined by commercially available kits according to the manufacturer's instructions and detected by the flow cytometry (Cytof, Beckman Coulter, USA).

## 2.7 Statistical analysis

SPSS 20.0 statistical software was used to examine the difference between groups using a t-test.

## 3 Results

### 3.1 The physicochemical properties and structural features of SFPS

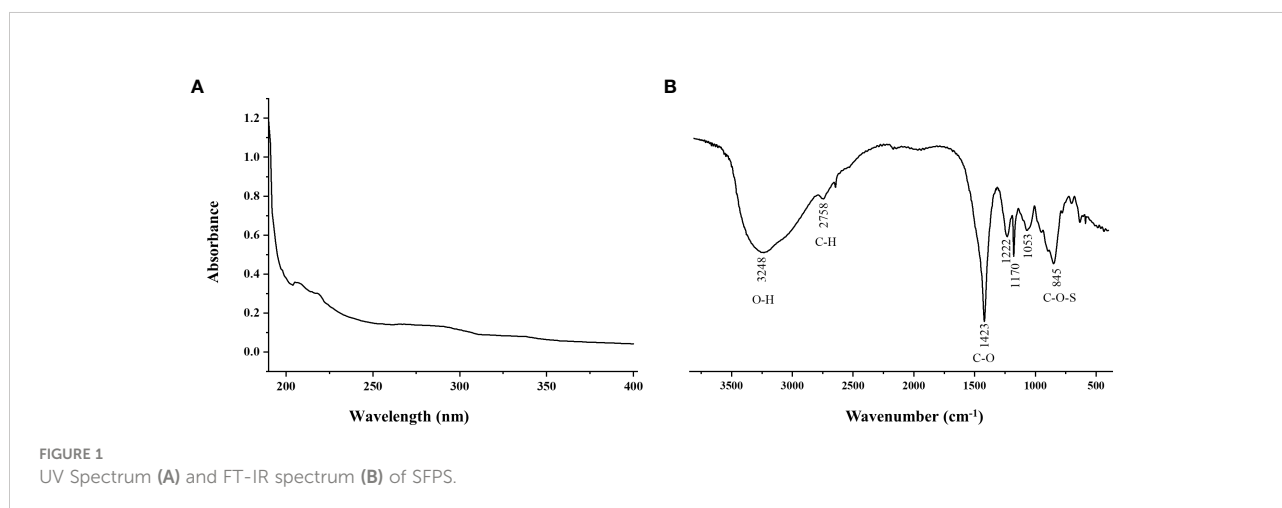
Firstly, SFPS was prepared by water extraction and alcohol precipitation with a yield of 15.47%. Then, its general composition was measured, and the results were shown in

**Table 1.** The prepared SFPS contained  $50.46 \pm 0.44\%$  total sugar,  $31.30 \pm 0.48\%$  sulfate and  $1.83 \pm 0.04\%$  protein. Further analysis of monosaccharide composition of the SFPS was performed by PMP-HPLC. SFPS were predominantly composed of glucose (Glc), arabinose(Ara), fucose(Fuc), galactose (Gal), mannose (Man), rhamnose (Rha), and xylose (Xyl) with a ratio of 21.06:15.44: 13.33:12.85:12.50:12.43:12.40, indicating that SFPS was a heteropolysaccharide. Moreover, glucose and arabinose were the major monosaccharide of SFPS.

Purity of SFPS was detected by UV spectrophotometer. There was no absorbance at 260 and 280 nm, indicating that the SFPS did not contain nucleic acid and protein (Figure 1A). The results were basically consistent with the chemical composition analysis. However, the protein concentration in SFPS was determined to be 1.88%, which might be attributed to the presence of glycoproteins. The FT-IR spectra of SFPS was shown in Figure 1B. Typical absorption peaks of SFPS was concentrated between  $500\text{ cm}^{-1}$  and  $4000\text{ cm}^{-1}$ . The absorption peaks presented at  $3248\text{ cm}^{-1}$ ,  $2758\text{ cm}^{-1}$  and  $1423\text{ cm}^{-1}$  are O-H stretching vibrations, C-H stretching vibrations, and C-O stretching vibrations within or between the polysaccharide structures. The characteristic peaks between  $950\text{--}1200\text{ cm}^{-1}$  correspond to characteristic functional groups on the glycosidic ring, overlapping with the stretching vibration of the C-OH side group and the C-O-C glycosidic band vibration (Chen et al., 2011). Besides, the absorption peak near  $845\text{ cm}^{-1}$  was attributed to the stretching vibration of symmetrical C-O-S (Lajili et al., 2019). The absorption peaks of  $1053\text{ cm}^{-1}$  and  $1170\text{ cm}^{-1}$

TABLE 1 Chemical composition of SFPS.

Sample	Total sugar(%)	Protein(%)	Sulfate (%)	Monosaccharide composition(molar ratios)(mol-%)						
				Glc	Ara	Fuc	Gal	Man	Rha	Xyl
SFPS	$50.46 \pm 0.44\%$	$1.83 \pm 0.04\%$	$31.30 \pm 0.48\%$	21.06	15.44	13.33	12.85	12.50	12.43	12.40



$\text{cm}^{-1}$  indicated that the sugar was a pyranose (Zhao et al., 2005). The characteristic peaks between  $840\text{ cm}^{-1}$  indicated that the polysaccharide was  $\alpha$ -pyranose.

### 3.2 Antioxidant activities of SFPS

The DPPH scavenging activities and hydroxyl radical scavenging activities of SFPS was determined to evaluate its antioxidant property. Different concentrations of SFPS had strong DPPH scavenging efficiency, and the DPPH scavenging rate increased by polysaccharide concentration in a certain concentration range (Figure 2A). Specifically, the DPPH scavenging rate increased smoothly within the polysaccharide concentration between 0–2.0 mg/mL, and a rapid increase of DPPH scavenging rate occurred at the polysaccharide concentration of 2.5 mg/mL, which reached to  $80.49 \pm 0.21\%$ . Meanwhile, the scavenging effect of SFPS on hydroxyl radicals was obvious at different concentrations (Figure 2B). Within a certain concentration range, the scavenging effect gradually increased by polysaccharide concentration. Specifically, the scavenging effect of SFPS on hydroxyl radical increased rapidly within the polysaccharide concentration between 0.5–1.0 mg/mL. After that, the hydroxyl radical scavenging activity grew slowly, and the highest scavenging rate was  $67.15 \pm 0.50\%$  with SFPS concentration of 2.5 mg/mL. Although the scavenging activity of SFPS was lower than VC, SFPS could dose-dependently scavenge the DPPH and hydroxyl free radical, indicating that SFPS possessed certain antioxidant activity.

### 3.3 Protective effect of SFPS on $\text{H}_2\text{O}_2$ -injured LO2

Our previous research has established a model and found that the best damage model can be obtained by treating at 700

$\mu\text{M}$   $\text{H}_2\text{O}_2$  concentration for 3 h (Chen et al., 2022). Thus, subsequent experiments were conducted based on this model. As shown in Figure 3, compared with the  $\text{H}_2\text{O}_2$  group, SFPS could markedly improve the cell viability in a dose-dependent manner. Specifically, the cell viability in SFPS treatment at 0.3, 0.6, 1.25 and 2.5  $\mu\text{g}/\text{mL}$  were  $56.84 \pm 2.92\%$ ,  $68.40 \pm 5.43\%$ ,  $71.60 \pm 1.32\%$  and  $74.57 \pm 4.31\%$ , respectively. It showed that SFPS might inhibit oxidative stress that induced by  $\text{H}_2\text{O}_2$  in LO2 cells.

### 3.4 Effect of SFPS on CAT, SOD, MDA and LDH levels

In  $\text{H}_2\text{O}_2$  treatment group, CAT activity and SOD levels were  $3.23 \pm 0.28\text{ U}/\text{mL}$  and  $1.25 \pm 0.02\text{ U}/\text{mL}$ , which were significantly decreased compared with the control group. However, CAT activity and SOD levels were significantly higher in SFPS pretreatment group than the  $\text{H}_2\text{O}_2$ -treatment group, and the two indicators were increased by SFPS concentration ( $p < 0.05$ ). Specifically, CAT activity and SOD levels were  $5.42 \pm 0.03\text{ U}/\text{mL}$ ,  $5.99 \pm 0.02\text{ U}/\text{mL}$ ,  $7.24 \pm 0.03\text{ U}/\text{mL}$ ,  $7.98 \pm 0.09\text{ U}/\text{mL}$  (Figure 4A),  $1.29 \pm 0.02\text{ U}/\text{mL}$ ,  $1.40 \pm 0.03\text{ U}/\text{mL}$ ,  $1.44 \pm 0.06\text{ U}/\text{mL}$  and  $1.62 \pm 0.06\text{ U}/\text{mL}$  at SFPS of 0.3, 0.6, 1.25 and 2.5  $\mu\text{g}/\text{mL}$  (Figure 4B).

The MDA level in  $\text{H}_2\text{O}_2$  group was the highest, indicating that  $\text{H}_2\text{O}_2$  successfully induced lipid peroxidation (Figure 4C). Meanwhile, the intracellular MDA levels in SFPS groups were significantly lower than  $\text{H}_2\text{O}_2$  group, suggesting that the addition of SFPS greatly declined oxidative injuries in LO2 cells. At the concentration of 0.3, 0.6, 1.25, 2.5  $\mu\text{g}/\text{mL}$ , the intracellular MDA levels were dramatically decreased to  $0.49 \pm 0.02$ ,  $0.42 \pm 0.02$ ,  $0.38 \pm 0.03$ ,  $0.32 \pm 0.04\text{ mmol}/\text{mgprot}$ , respectively.

The LDH levels in cultured supernatant were determined to analyze the effect of SFPS on the extent of oxidative injury in LO2 cells. As shown in Figure 4D, LDH levels were significantly

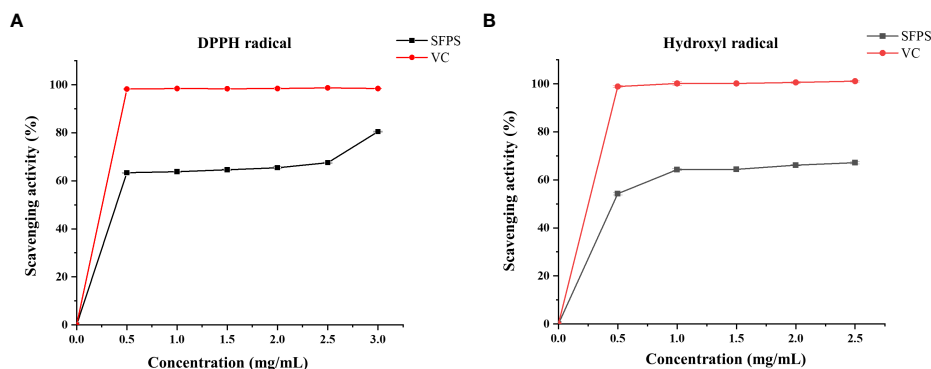
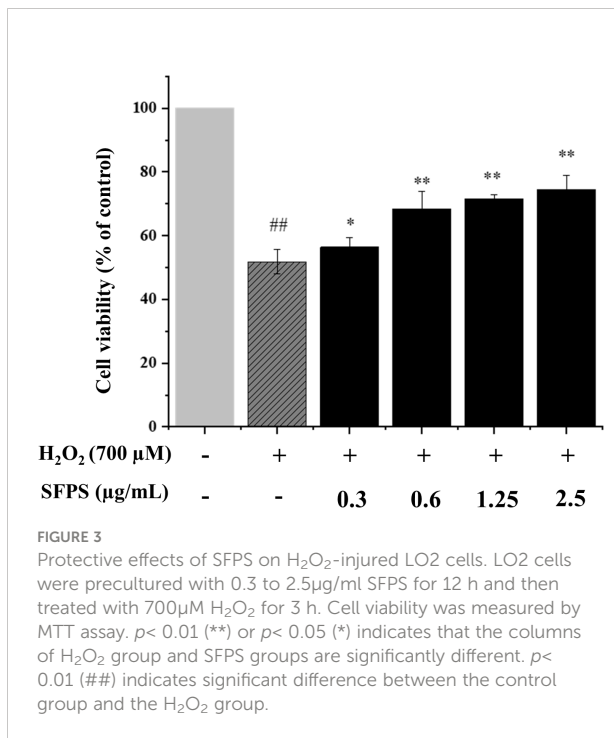


FIGURE 2

The antioxidant activities of SFPS. (A) DPPH scavenging activity (B) Hydroxyl radical scavenging activity.

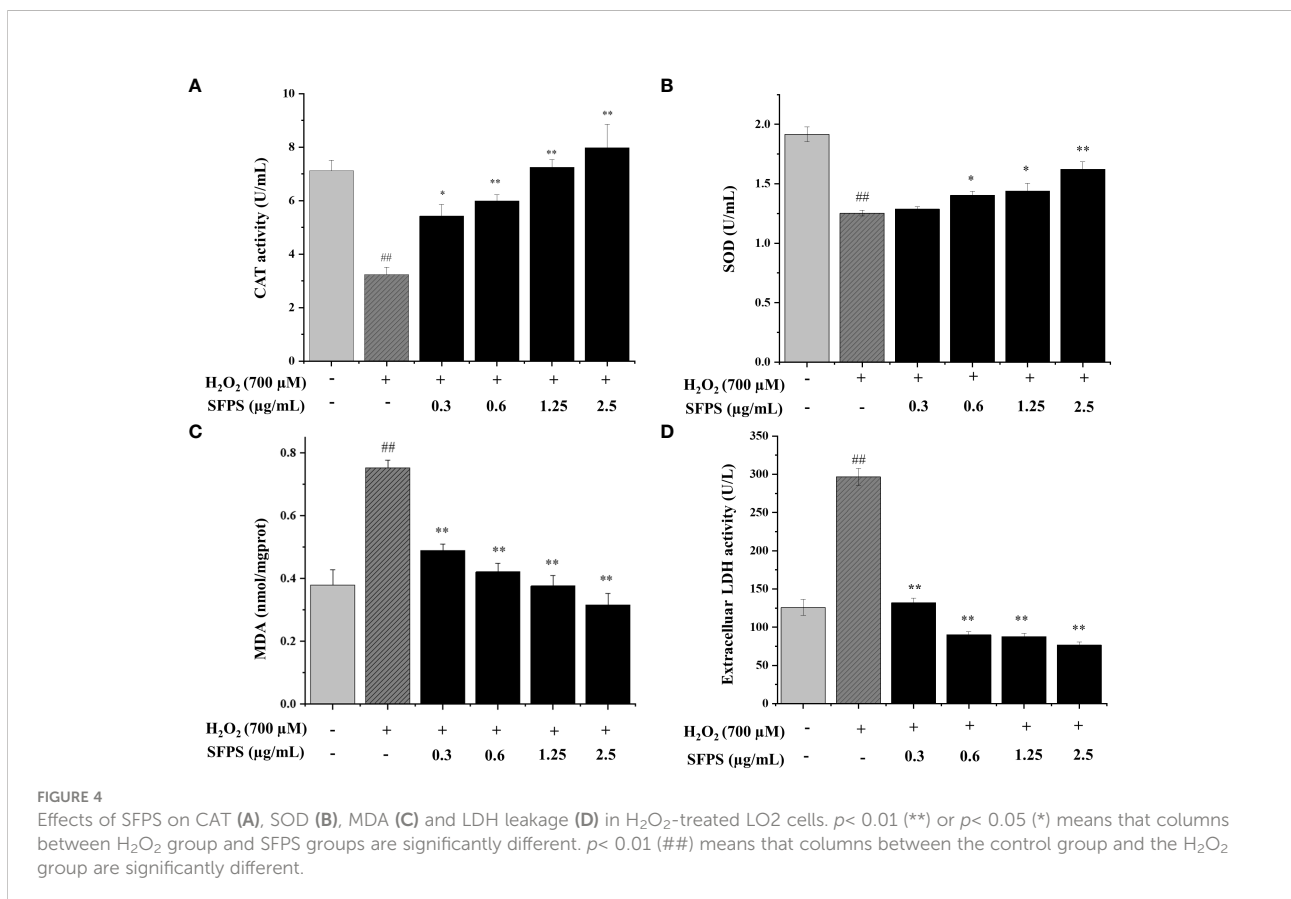




increased in H<sub>2</sub>O<sub>2</sub>-treatment group. Pretreatment with SFPS decreased LDH level in dose-dependent manner. Notably, LDH were only 76.68 ± 3.76 U/mL with the pretreatment of SFPS at 2.5 μg/mL, which was much better than the H<sub>2</sub>O<sub>2</sub> treatment group (296.48 ± 11.13 U/mL). These above results suggested that SFPS could reverse the reduction of antioxidant enzyme (SOD and CAT) levels and the increase in lipid peroxidation (MDA) and LDH levels in the H<sub>2</sub>O<sub>2</sub> treatment group.

### 3.5 Influence of SFPS on H<sub>2</sub>O<sub>2</sub>-induced LO2 cell apoptosis

The effect of SFPS on cell apoptosis in H<sub>2</sub>O<sub>2</sub>-treated LO2 was measured by detection of Annexin V/PI double staining and PI staining. The results were shown in Figure 5. H<sub>2</sub>O<sub>2</sub> caused significant apoptosis in LO2 cells. The apoptosis rate increased from 15.58 ± 1.77% to 52.22 ± 0.68% when cells were incubated with H<sub>2</sub>O<sub>2</sub> (*p* < 0.01). However, treatment with SFPS at different concentrations could attenuate the apoptosis significantly, and cell apoptosis decreased slightly with the increasing concentration of SFPS. These results indicated that SFPS inhibited apoptosis and cell death.



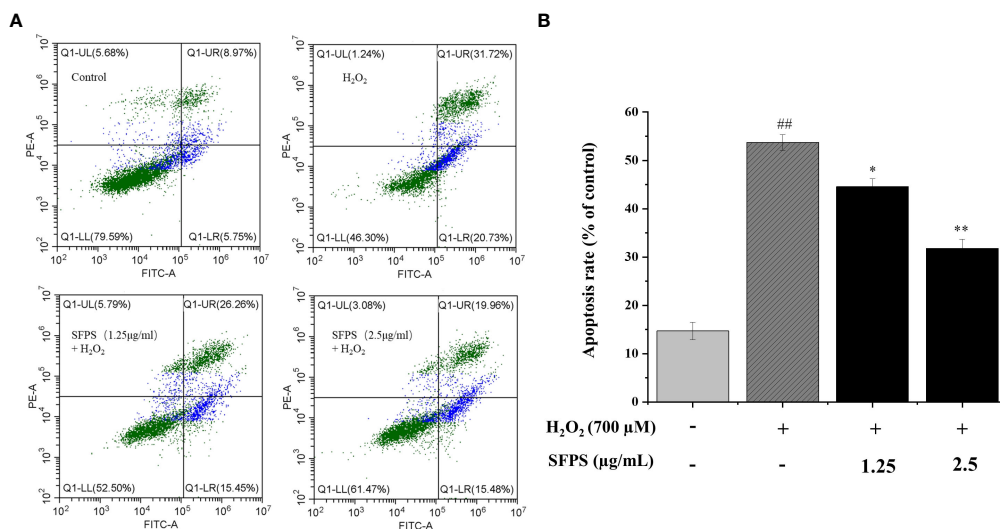


FIGURE 5

Influence of SFPS on H<sub>2</sub>O<sub>2</sub>-induced LO2 cell apoptosis. (A) LO2 cells were preincubated with SFPS (1.25 and 5 µg/mL) for 12 h before the treatment with H<sub>2</sub>O<sub>2</sub> for 3 h. Then, LO2 cells were collected and co-stained with Annexin V and PI for 10 min. The upper right quadrants and lower right quadrants represent the ratios of late apoptotic cells early apoptotic cells, respectively. (B) Effect of SFPS on the apoptosis rate of H<sub>2</sub>O<sub>2</sub>-treated LO2 cells.  $p < 0.01$  (\*\*) or  $p < 0.05$  (\*) means that columns between H<sub>2</sub>O<sub>2</sub> group and SFPS groups are significantly different.  $p < 0.01$  (##) means that columns between the control group and the H<sub>2</sub>O<sub>2</sub> group are significantly different.

### 3.6 Effect of SFPS on ROS production induced by H<sub>2</sub>O<sub>2</sub>

ROS generation was monitored by the measurement of fluorescence intensity to assess the mechanisms of SFPS for decreasing H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity and apoptosis. The results were shown in Figure 6. Compared to the blank control group, the green fluorescence in LO2 cells treated with hydrogen peroxide was significantly enhanced, which the relative amount of ROS in the damaged group was  $298.52 \pm 5.18\%$  ( $p < 0.01$ ). Treatment of cells with SFPS dramatically downregulated the levels of H<sub>2</sub>O<sub>2</sub>-induced ROS formation in LO2 cells. Specifically, the relative amount of ROS in the SFPS-treated group declined in a concentration-dependent manner, which were  $159.58 \pm 11.31\%$ ,  $138.72 \pm 6.90\%$ ,  $115.47 \pm 6.27\%$ , and  $100.38 \pm 7.62\%$ , respectively. These results indicate that SFPS exerts protective effects against oxidative stress induced by H<sub>2</sub>O<sub>2</sub> in cells.

## 4 Discussion

*Sargassum fusiform*, a kind of edible brown algae, has strong pharmacological and biological activities. *Sargassum fusiform* has been used as a therapeutic medicine 4000 years ago (Huang et al., 2006). Polysaccharides are the main constituents of *Sargassum fusiform*, and *Sargassum fusiform* polysaccharides has been shown to possess the effect of

promoting hepatocyte activity *in vitro* (Chen et al., 2016). In this study, the polysaccharide SFPS was successfully isolated from *Sargassum fusiform* by hot water extraction, ethanol precipitation and Sevage deproteinization. Then, the content of carbohydrate, sulfate and protein in SFPS, the structure of SFPS as well as its antioxidant activity were determined. The chemical composition analysis showed that SFPS was mainly composed of fucose with some sulfate heterosaccharides. Further, the analysis of infrared spectroscopy indicated that SFPS was sulfate polysaccharides. The sulfate content of SFPS was higher than that of most of the published raw polysaccharides of *Sargassum fusiform* (Meinita et al., 2021). Various studies have shown that sulfated polysaccharides exhibit different or stronger biological activities, such as antioxidant (Kim et al., 2016). Among them, the antioxidant activity of sulfate polysaccharide is positively correlated with the number of sulfate groups. Moreover, fucose and galactose have been reported to associate with antioxidant activity (Wang L. et al., 2018). In this study, we also found that the strong antioxidant activity of SFPS could be attributed to their high sulfation content and higher amount of fucose and galactose, which was consistent with the previous report (Seedevi et al., 2017; Jridi et al., 2019).

The antioxidant activity of polysaccharide is often characterized by cell damage model *in vitro*. The method of H<sub>2</sub>O<sub>2</sub> had been widely used to establish oxidative stress model *in vitro*. Oxidative stress has been linked to many diseases (Khatri et al., 2018; Daenen et al., 2019; Sadasivam et al., 2022). The liver is the most important organ in the metabolism of xenobiotic,

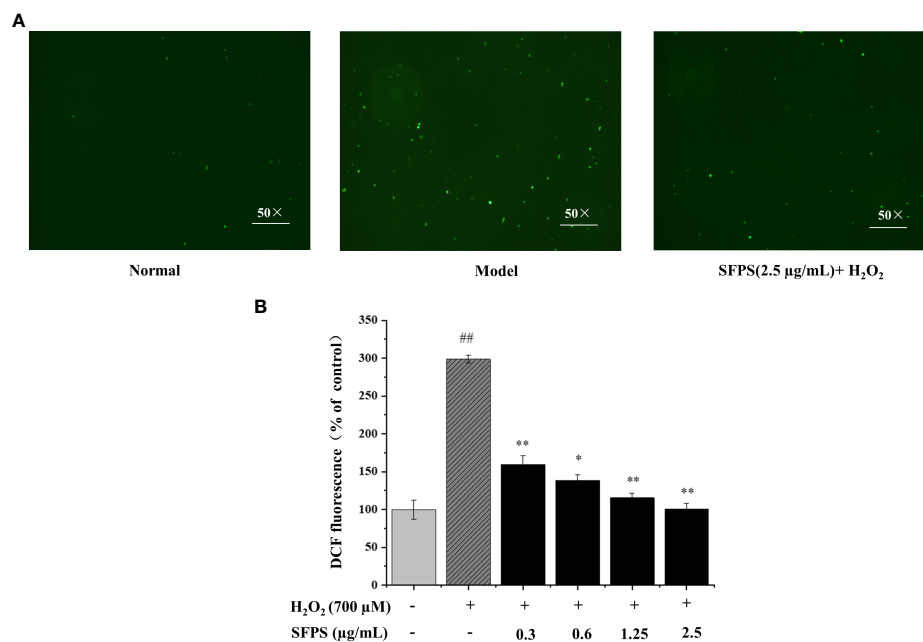


FIGURE 6

Effect of SFPS pretreatment on intracellular reactive oxygen species (ROS) in H<sub>2</sub>O<sub>2</sub>-induced LO2 cells. (A) The fluorescence intensity of DCF image of cells after treatments as examined by fluorescence microscopy (magnification 50×). LO2 cells were incubated with 2.5 μg/mL SFPS for 12 h and then treated with 700 μM H<sub>2</sub>O<sub>2</sub> for 3 h. (B) Effect of SFPS on DCF fluorescent density of H<sub>2</sub>O<sub>2</sub>-treated LO2 cells. DCF fluorescent density was observed by the ROS assay kit.  $p < 0.01$  (\*\*) or  $p < 0.05$  (\*) indicates that the columns of H<sub>2</sub>O<sub>2</sub> group and SFPS groups are significantly different.  $p < 0.01$  (##) indicates significant difference between the control group and the H<sub>2</sub>O<sub>2</sub> group.

which is particularly vulnerable to oxidative stress affects. Many studies have shown that antioxidant therapy is effective in treating these diseases. Antioxidants of natural origin have the advantage of non-toxicity and good biocompatibility to delay or reduce substrate oxidation and protect the body from oxidative damage caused by excess free radicals (Zhu et al., 2020). Maintaining an appropriate ROS level is necessary for normal physiological function. Activation of reactive oxygen species (ROS) leads to lipid peroxidation of cell membranes, and the products of peroxidation bind to biomolecules, leading to disease production (Yuan et al., 2015). CAT and SOD are the professional enzymes dedicated to preventing enzymes dedicated to preventing the build-up of intracellular H<sub>2</sub>O<sub>2</sub> (Wang Y. et al., 2018; Raza et al., 2021). Therefore, the enhancement of CAT and SOD activity indicates the enhancement of ROS scavenging activity. LDH enzyme is a typical marker of the plasma damage membrane. MDA is a biomarker of oxidative (Chen et al., 2016). Our current study showed that SFPS could attenuate H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in LO2 cells. The data showed that SFPS pretreatment significantly increased cell viability as measured by the MTT assay. SFPS pretreatment reduced MDA and LDH levels and

increased SOD and CAT enzyme activities. In addition, SFPS pretreatment also significantly inhibited the production of reactive oxygen species.

There is growing evidence that cellular stress and cell death triggered by different causes underlie most acute and chronic liver diseases. Currently, the phenotypic form of cell death in liver disease is apoptosis<sup>20</sup>. In the present study, the apoptosis of LO2 cells was significantly reduced after SFPS treatment, and the effect of the SFPS-treated group was better than that of the H<sub>2</sub>O<sub>2</sub>-treated group, indicating that SFPS could effectively protect against H<sub>2</sub>O<sub>2</sub>-induced oxidative damage of LO2. These results suggest that SFPS can reduce ROS production and attenuate oxidative stress-induced LO2 cell injury and apoptosis, thereby protecting LO2 from hydrogen peroxide oxidative stress damage.

However, the relationship between the bioactivity of SFPS and their structure remains unclear. The effectiveness and mechanism of SFPS *in vivo* need to be further verified. In addition, it is important to explore the possible synergistic mechanism between the polysaccharide and other components. To better utilize this kind of resource, further work on purification and structure analysis of SFPS is necessary in the future research.



## 5 Conclusion

In this study, the *in vitro* antioxidant activity of SFPS and its *in vitro* protective activity against LO2 injury in human hepatocytes were investigated. Our results showed that SFPS not only exhibited strong antioxidant activity *in vitro*, but also improved the viability of oxidatively damaged cells. Moreover, SFPS protected cells from H<sub>2</sub>O<sub>2</sub>-induced oxidative stress injury by attenuating the production and accumulation of ROS, reducing MDA levels and intracellular LDH leakage, increasing the activity of antioxidant enzymes such as SOD and CAT, and reducing apoptosis. In conclusion, SFPS possesses well antioxidant ability and exhibits protective effects against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in LO2 cells.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Author contributions

JL and ZW did field observations and proposed the idea for this study. JL, ZW, SZ, and JC designed the research. SZ and JC acquired the funding. JL performed the research, analyzed the data, and prepared the original manuscript. ZW, JC, BL, XC, RL, JG, XL, BS, and SZ provided logistical support, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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