American Journal of Biochemistry and Biotechnology

Original Research Paper

Protective Role of *Ganoderma lucidum* Polysaccharides Against Stress Induced by Heavy Metals in *Caenorhabditis elegans*

Huixiu Ma, Dehui Dai, Guicai Chen and Weilian Hu

School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou, China

Article history
Received: 19-01-2024
Revised: 23-02-2024
Accepted: 16-03-2024

Corresponding Author:
Weilian Hu
School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou, China
Email: weilian89@126.com

Abstract: This study aimed to investigate the protective effects and mechanisms of different *Ganoderma lucidum* Polysaccharides (GLPs) on the damage caused by heavy metal ions. The polysaccharides (GLP-B, GLP-F, and GLP-S) were obtained from *G. lucidum* fruiting body powder, fermentation powder, and spore powder. The contents of polysaccharides in these sources were 0.95, 4.69, and 1.46%, respectively. The monosaccharide composition and functional groups of polysaccharides from different sources were analyzed by PMP pre-column derivatization and Fourier transform infrared spectroscopy, respectively. Results showed that the monosaccharide composition, molar ratio, and functional groups of them were significantly different. The antioxidant activities *in vitro* were investigated based on iron ion chelating ability and scavenging hydroxyl radical, superoxide radical, and DPPH free radical assays. Results showed that GLP-F, GLP-B, and GLP-S demonstrated significant antioxidant activity. Among them, the strongest effect of GLP-F for hydroxylic group radicals; GLP-S showed the strongest antioxidant capacity in superoxide radical scavenging; GLP-B showed the strongest antioxidant capacity in iron ion chelating. The four heavy metal ions stress model (Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+}) was established using the *Caenorhabditis elegans*. Results showed that the lifespan, locomotion behavior, and antioxidant index of *C. elegans* were significantly changed by heavy metal ions ($p<0.05$). All of these three polysaccharides can significantly improve the lifespan and locomotion behavior of *C. elegans* under heavy metal stress ($p<0.05$). It was found that GLP-F, GLP-B, and GLP-S can significantly reduce the Malondialdehyde (MDA) content, as well as regulate the changes in Superoxide Dismutase (SOD) and Catalase (CAT) activity induced by heavy metal stress in *C. elegans* ($p<0.05$). This study showed that GLPs exhibited a protective effect on the toxicity of heavy metal ions, which may be related to the regulation of antioxidant index *in vivo*. Among the three polysaccharides, GLP-F and GLP-S were better than GLP-B. The mycelial fermentation powder, due to its high polysaccharide content, has promising market applications in reducing heavy metal stress.

Keywords: *Ganoderma lucidum*, Antioxidant Activity, Antioxidative Index, Heavy Metals, *Caenorhabditis elegans*

Introduction

Heavy metals cannot be disassembled, exist forever in the environment, can accumulate in living things, and pose risks to human and environmental health (Aljerf *et al.*, 2021; Elbasiouny *et al.*, 2021). It induces active oxygen production, causes damage to genomic substances such as DNA and protein and overeating lipids, and ultimately causes cell death (Ali *et al.*, 2019; Ukaogo *et al.*, 2022). Copper (Cu) is discovered in neuropathic diseases, including Alzheimer's disease and Parkinson's disease and it is suspected of copper toxicity, or copper may promote its progress (Pohanka, 2019). Manganese (Mn) accumulation is linked to abnormalities in the dopaminergic system, resulting in Parkinson 's-like motor dysfunction, ataxia, and hallucinations (Lin *et al.*, 2020). Nickel (Ni) is a known carcinogen and a common sensitizing metal (Sule *et al.*, 2020). Cadmium (Cd) is related to cardiovascular dysfunction and disease including arrhythmias, hypertension, myocardial

infarction, and heart failure (Yu *et al.*, 2020). Heavy metals move via the food chain, affecting human health in the process (Aljerf and Aljerf, 2023; Jayachandran *et al.*, 2017).

Finding new foods and dietary supplements containing natural heavy metal resistance without adverse effects on organisms is imperative. Studies have shown that *Hylocereus undatus* microencapsulated pulp extraction can improve the antioxidant system and cholinergic nervous system, thereby preventing behavioral changes before and after copper metal poisoning (Tamagno *et al.*, 2022). Additionally, it was shown that *Lactobacillus plantarum* CCFM436 can protect mice from manganese toxicity by regulating antioxidant enzymes, levels of pro-inflammatory cytokines in brain tissue, levels of beta-amyloid proteins and expression of Tight Junction (TJ) proteins (Tong *et al.*, 2020). Furthermore, Vitamin E (V_E) has indicated its potential to restore and prevent memory deficits caused by stress from heavy metal ions exposure, as well as its role in regulating memory function similar to Neuronal Calcium Sensor-1 (NCS-1) (Ye *et al.*, 2008). *Ganoderma lucidum*, a functional food and medicinal mushroom extensively used in traditional Chinese medicine, has demonstrated antioxidative properties. (Li *et al.*, 2018). Current research primarily focuses on the antioxidant, anticancer, and immune-enhancing properties of *G. lucidum* polysaccharides, but there is relatively limited research on their protective effects against heavy metal ions toxicity (Jeong and Park, 2020; Liu *et al.*, 2022a; Shahid *et al.*, 2022). Polysaccharides from *G. lucidum* fruiting bodies and spore powder are commonly used as dietary supplements. However, due to the slow growth rate and difficulty in collection, natural *G. lucidum* availability is limited. Traditional cultivation methods take time and high labor strength and it is difficult to meet the demands of large-scale production. Fermentation of *G. lucidum* mycelium offers a more efficient production method of polysaccharides, allowing for better control of their quality and purity, thereby enhancing their medicinal value. Therefore, it is necessary to investigate whether polysaccharides extracted from fermented mycelium have protective effects against heavy metal toxicity and determine differences compared to polysaccharides from natural fruiting body powder and spore powder.

This study aims to compare the content, monosaccharide composition, and antioxidant capacity of GLP-F, GLP-B, and GLP-S. Additionally, it investigates the effects of heavy metal ions (Cu^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}) on the lifespan, locomotion behavior, and antioxidant index of *C. elegans* through a heavy metal ions stress model. The protective effects of GLPs against heavy metal ions on *C. elegans* and their mechanisms were explored, as well as the differences in protective effects among GLPs from different sources.

Materials and Methods

Materials

Fruiting body powder and the spore powder of *Ganoderma lucidum* were obtained from Baishan Jiqing ginseng Trading Co., Ltd., located in Jilin province, China. The fermentation powder is obtained through liquid deep fermentation in our laboratory. The comparison results with the National Center for Biotechnology Information (NCBI) database showed that the Internally Transcribed Spacer (ITS) sequence of the strain used had a 99.67% similarity with the reported *Ganoderma lucidum* strain OM721793.1. This indicated that the strain has high homology with *Ganoderma lucidum*. Mannose (Man), Glucuronic acid (Glca), Rhamnose (Rha), Galacturonic acid (Gala), Glucose (Glc), Galactose (Gla), Arabinose (Ara), Fucose (Fuc), Trifluoroacetic Acid (TFA) and 1-Phenyl-3-Methyl-5-Pyrazolone (PMP) were purchased from Shanghai McLean biochemical technology Co., Ltd. The *C. elegans* were obtained from Shanghai Model Organisms, Shanghai, China. Under 20°C conditions, Worms were normally bred on Nematode Growth Medium (NGM) plates with *E. coli* OP50 as a food source. Cupric chloride, manganese sulfate, cobalt chloride, cadmium chloride, and other analytical-grade chemical reagents were purchased from Shanghai Lingfeng chemical reagent Co., Ltd. Malondialdehyde (MDA), Catalase (CAT) and Superoxide Dismutase (SOD) assay kits were purchased from Nanjing Jiancheng bioengineering institute.

Extraction of Polysaccharides

The extraction of polysaccharides was done according to the reported method with some modifications (Chen and Yen, 2022). Briefly, the powder using a liquid-solid ratio of 20:1 (mL/g), was incubated in the thermostat-controlled water bath at 90°C for 3 h. The extract was filtrated and concentrated. Add 95% alcohol to achieve a final alcohol concentration of 80% in the system, let it stand at 4°C, centrifuge, and obtain a precipitate. GLPs were acquired after freeze-drying. The polysaccharide content was determined by the phenol-sulfuric acid method (Liu *et al.*, 2022b). The polysaccharides obtained from fruiting body powder, fermented powder, and spore powder were labeled as GLP-B, GLP-F, and GLP-S respectively.

Monosaccharide Composition Analysis

The determination of monosaccharide composition was carried out using the modified PMP pre-column method described by Yang *et al.* (2005). In brief, the GLPs were dissolved in 2 m TFA and degraded at a temperature of 90°C for 12 h. After drying, deionized water was added to re-dissolve and obtain the samples. Samples and monosaccharide standards were mixed with 0.2 m NaOH and PMP solution (dissolved in methanol) and incubated

at 70°C for 80 min. After cooling to room temperature, the derivative reaction was halted by adding 0.5 m HCl. The obtained solution was dissolved in chloroform (1 mL). After an intense swing and centrifugal, the organic phase (under an aqueous layer) was carefully discarded and excess reagents were removed. Repeat the extraction three times; After dilution with water (150 μL), the water layer was filtered by 0.22 μm membrane and analyzed by HPLC.

FTIR Determination

Fourier transform infrared spectra of GLP-F, GLP-B, and GLP-S were determined by the KBr-pellets method on an FT-IR spectrometer (Zhao *et al.*, 2023).

Antioxidant Activity Assay in vitro

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of GLP-B, GLP-F, and GLP-S was tested using a modified procedure based on previous research methodology (Tan *et al.*, 2018). Briefly, 1 mL of sample solution (1-5 g/L) was mixed with 1 mL of $FeSO_4$ solution (9 mm), 1 mL of H_2O_2 (5 mm), and 1 mL of a salicylic acid solution (9 mm in ethanol). The mixture was sufficiently mixed and then incubated in a water bath at 37°C for 1 h. Ascorbic acid was a positive contrast. The absorbance of the obtained solution was measured at 510 nm. The percentage of hydroxylaicals was calculated in the following equation:

$$Scavenging\ effect\ (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \quad (1)$$

where, A_1, A_2, and A_0 are the absorbance values of the sample, a control reaction with 100% ethanol instead of a salicylic acid/ethanol solution, and a blank control, respectively.

DPPH Scavenging Activity

The scavenging activity of DPPH free radicals was assayed following the method described by Hu with slight modifications (Hu *et al.*, 2022). Briefly, 1 mL of sample solution (1-5 g/L) was added to 1 mL of a DPPH ethanol solution (0.2 mm). The reaction mixture was strongly shaken and uniformly and it was incubated for 30 min to avoid light. Ascorbic acid was used as a positive control. The absorbance of the resulting solution was measured at 517 nm. The ability to scavenge DPPH radicals was calculated using the following equation:

$$Scavenging\ efficiency\ (\%) = \left[1 - \left(\frac{B_1 - B_2}{B_0}\right) \times 100\right] \quad (2)$$

where, B_1, B_2, and B_0 represent the absorbance values of the DPPH solution alone, the sample/DPPH mixture, and a control reaction with water instead of the DPPH solution, respectively.

Superoxide Anion Radical-Scavenging Activities

The super anion radical-scavenging activities were assayed according to the reported method (Chen and Yen 2007). Briefly, 4.5 mL of 0.05 moL/L Tris-HCl buffer (pH 8.2) was maintained for 20 min during bathing at 25°C. Then 1 mL of polysaccharide sample solutions and 0.4 mL of 25 mmoL/L 1,2,3-phenol were added and incubated at 25°C for 5 min. 1 mL of 8 moL/L HCl was quickly added and the reaction was stopped. Ascorbic acid was a positive contrast. The absorbance was measured at 318 nm. The superoxide anion scavenging activity was calculated by using the equation:

$$Superoxide\ anion\ cavenging\ effect\ (\%) = 1 - \frac{C_2 - C_1}{C_0} \quad (3)$$

C_1 is the absorbance measured with ultrapure water instead of o-cresol solution and C_0 is the absorbance measured with ultrapure water instead of sample solution. C_2 is the result of samples.

Ferrous Ion-Chelating Activity

The ferrous ion-chelating activity was determined following the method described by Engin Celep with slight modifications (Celep *et al.*, 2012). 2 mL of sample solution (1-5 g/L) was mixed with 7.4 mL of ethanol (55%), 0.2 mL of ferrous chloride solution (2 mm) and 0.4 mL of ferrozine solution (5 mm). The mixture was thoroughly mixed and allowed to stand at room temperature for 20 min. The absorbance of the mixture was then measured at 562 nm. Ascorbic acid was used as a positive control. The ferrous ion-chelating activity was calculated using the following equation:

$$Ferrousion\_chelatingactivity = \frac{D_0 - D_1}{D_0} \quad (4)$$

where, D_1 was the absorbance of the sample, and D_0 was the result of the distilled water.

C. elegans Cultures and Treatment

The *C. elegans* were cultured on Nematode Growth Medium (NGM) plates containing 3 g/L NaCl, 2.5 g/L peptone, 17 g/L agar, 5 g/L cholesterol, 1 mmoL/L $CaCl_2$, 1 mmoL/L $MgSO_4$ and 25 mmoL/L potassium phosphate buffer at pH 6.0 (Tamagno *et al.*, 2022). The plates were seeded with *Escherichia coli* OP50 and maintained at a temperature of 20°C. To obtain a synchronized population, adult worms were placed on NGM plates and allowed to lay eggs for 6 h at 20°C. After 72 h, all *C. elegans* were in the L4 stage (Shen *et al.*, 2009), which served as the starting point for all experiments.

Table 1: Metal ion species and concentrations

Metals	Concentrations (mmol/L)			
Cu^{2+}	1	2	3	4
Cd^{2+}	10	15	20	25
Mn^{2+}	100	200	300	400
Ni^{2+}	50	60	70	80

Establishment of a C. elegans Model for Heavy Metal Stress

The four heavy metal ions (Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+}) were individually dissolved in K-medium (3.2 g/L NaCl, 2.4 g/L KCl). Based on our preliminary experiment, four concentrations were chosen for each heavy metal. Table 1 shows the four concentrations (mmoL/L) for different heavy metal ions. The appropriate heavy metal ions concentration was determined and used for subsequent experiments.

Synchronized *C. elegans* were fed on NGM agar plates containing only *E. coli* OP50 until the L4 stage. The heavy metal ions were dissolved in a k-medium solution to create the required concentrations for the experiment. The experiment consisted of a control group (k-medium solution) and different concentrations of heavy metal ions groups (heavy metal solution). A 24-well plate was used, with each well containing 20 L4-stage nematodes obtained using a picker. Subsequently, measurements were taken for lifespan, behavior, and antioxidant index *in vivo*. Each experiment was repeated independently three times.

Effects of GLPs on a C. elegans Model for Heavy Metal Stress

Synchronized *C. elegans* were fed on NGM agar plates containing only *E. coli* OP50, GLP-F, GLP-B, and GLP-S until the L4 stager, respectively. The study included a blank group (K-medium), control group (heavy metal), GLP-F group (heavy metal + GLP-F-treated group), GLP-B group (heavy metal + GLP-B-treated group), and GLP-S group (heavy metal + GLP-S-treated group). A 24-well plate was used, with each well containing 20 L4-stage nematodes obtained using a picker. Subsequently, measurements were taken for lifespan, behavior, and antioxidant index *in vivo*. Each experiment was repeated independently three times.

The Lifespan of the C. elegans

Synchronized L4 stage nematodes into their respective groups. This time point was considered as 0 h of heavy metal exposure. The plate was placed in a 20°C incubator for incubation. For the lifespan assays, the *C. elegans* were monitored every 2 h by tapping their heads with a picker. If no movement was observed after repeated probing, the worm was considered dead. Three independent experiments were conducted.

The Locomotion Behavior of C. elegans

The head thrash and body bend assay method used in this study was performed following the protocol described by Tsalik and Hobert (2003). After 4 h of exposure to heavy metal ions, the worms were transferred to a plate containing only NGM and allowed to acclimatize for 1 h. The head thrashes and body bends performed by the worms in 1 minute were recorded. A head thrash was counted when the nematode's body bend reached half of its body length. Assuming that animals move along the X-axis, the curvature of the body is considered a change in the Y-axis direction of the animal pharynx rear ball. *C. elegans* that remained still for more than five seconds were not included in the count. For each group, twenty worms were measured.

Antioxidant Index Detection

To investigate enzyme activity, the *C. elegans* were collected by centrifugation at 8000 rpm for 5 min after being exposed to heavy metals for 4 h. They were then washed and cleaned. Subsequently, the *C. elegans* were sonicated under the ice for 4 min. The obtained mixture was centrifuged at 3000 rpm at 4°C for 10 min, collected and analyzed.

The content of Malondialdehyde (MDA) as well as the activity of Catalase (CAT) and Super Oxide Dismutase (SOD) were determined by the kits' instructions.

Statistical Analyses

All the data were presented as mean ± standard error of the mean. Statistical analyses were performed using IBM SPSS statistics 26. Differences between groups were evaluated using Analysis of Variance (ANOVA) followed by Tukey tests. A probability level of 0.001, 0.01, and 0.05 was considered statistically significant.

Results and Discussion

The Polysaccharide Content of G. lucidum from Different Sources

In the study of polysaccharides, higher yield and lower production cost are crucial for meeting market demands. The contents of polysaccharides in *G. lucidum* fruiting body powder, fermentation powder, and spore powder were 0.95, 4.69 and 1.46%, respectively (Fig. 1). The polysaccharide content in fermented powder is much higher compared to that in fruiting body powder and spore powder.

Previous research reports indicated that the polysaccharide content in fruiting bodies was relatively low, with findings showing only 1.775% polysaccharide content in fruiting bodies (Song *et al.*, 2015). In comparison, the polysaccharide content in spore powder was slightly higher, Huang found that the polysaccharide content in spore powder was 2.71% (Huang *et al.*, 2023). Regarding polysaccharides obtained from mycelium fermentation, reports are indicating a total polysaccharide content of 7.6% in *G. lucidum* mycelium (Lu *et al.*, 2020). According to previous literature reports, the polysaccharide content is high in mycelium fermentation powder, followed by spore powder, and low in the fruiting body powder, consistent with our results.

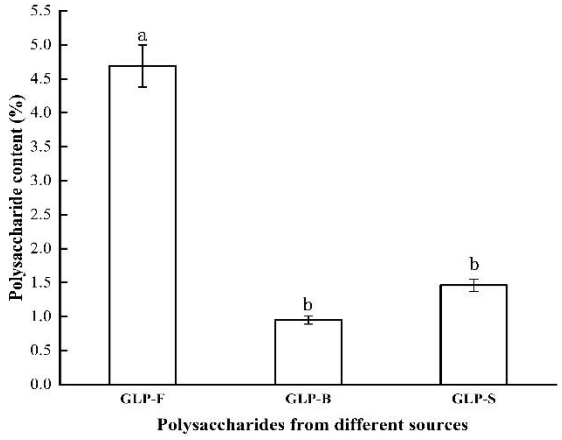

Fig. 1: The polysaccharide content of *G. lucidum* powder from different sources. Those marked with different lowercase letters indicated significant differences between groups ($p<0.05$) and those marked with the same lowercase letters indicated that the differences between groups were not significant ($p\geq0.05$)

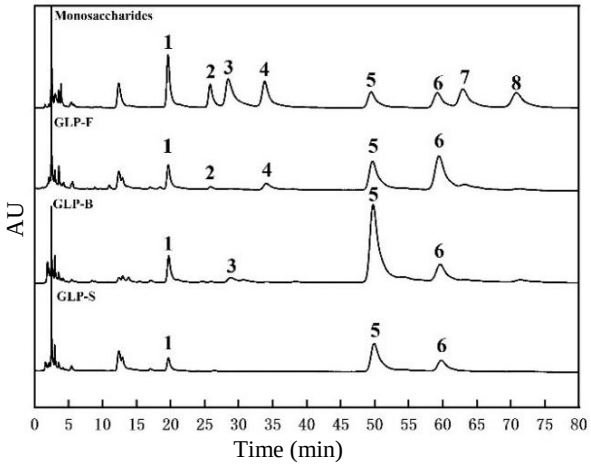

Fig. 2: Monosaccharide composition analysis of GLP-F, GLP-B and GLP-S. 1: Mannose, 2: Rhamnose 3: Glucuronic acid, 4: Galacturonic acid, 5: Glucose, 6: Galactose, 7: Arabinose, 8: Fucose

Table 2: Monosaccharide composition of GLP-F, GLP-B and GLP-S

	GLP-F		GLP-B		GLP-S	
Monosac-charides	Concentration (mol/L)	Mole ratio	Concentration (mol/L)	Mole ratio	Concentration (mol/L)	Mole ratio
Man	0.197	1.00	0.289	1.00	0.171	1.00
Rha	0.150	0.76				
Glca			0.287	0.99		
Gala	1.796	9.12				
Glc	0.538	2.73	1.913	6.62	0.574	3.36
Gal	0.766	3.89	0.550	1.90	0.322	1.88

Monosaccharide Composition Analysis of GLPs

The accurate determination of monosaccharide composition is crucial to elucidate the properties and structures of polysaccharides and is beneficial to further understanding the structure-activity relationship (Zhao *et al.*, 2023). In this study, the HPLC of polysaccharide samples was detected and the monosaccharide composition of GLP-F, GLP-B, and GLP-S was analyzed. Figure 2 and Table 2, the monosaccharide composition of GLP-F was composed of mannose, rhamnose, galacturonic acid, glucose, and galacturonic acid at a molar ratio of 1:0.76:9.12:2.73:3.89. The monosaccharide composition of GLP-B was composed of mannose, glucuronic acid, glucose, and galacturonic acid at a molar ratio of 1:0.99:6.62:1.90. The monosaccharide composition of GLP-S was composed of mannose, glucose, and galacturonic acid are at a molar ratio of 1:3.36:1.88. It indicated that the monosaccharide composition and molar ratio of these three polysaccharides were significantly different. The component with the highest concentration in GLP-F was Gala, while GLP-B and GLP-S were Glc. Compared with GLP-B and GLP-S, the content of Gal in GLP-F was increased. In contrast, the content of Glc was decreased and new monosaccharides, Rha and Gala, appeared in GLP-F.

The previous study showed that the polysaccharide in *G. lucidum* fruiting bodies were composed of mannose (5.9%), glucuronic acid (9.0%) and glucose (80.4%), along with small amounts of galactose (1.8%), xylose (1.8%) and fucose (0.9%); whereas the polysaccharide in *G. lucidum* spore powder was composed of Xylose (Xyl), Mannose (Man), Galactose (Gal), Glucose (Glc) and Glucuronic Acid (GlcA) at a molar ratio of 1.53: 15.64: 1.84: 80.6: 0.39 (Chen *et al.*, 2022; Zhao *et al.*, 2023). The polysaccharides from both the fruiting bodies and spore powders are primarily composed of glucose as the main monosaccharide constituent, this was consistent with our research findings. However, it found that monosaccharides in fruiting bodies and spore powder also contain xylose and fucose (Chen *et al.*, 2022). The diversity in source, variety, and extraction methods of polysaccharides can result in variability in monosaccharide composition. These factors may be responsible for the divergent monosaccharide composition of polysaccharides. Liu discovered that the primary monosaccharide component in polysaccharides of deeply fermented *Ganoderma lucidum* was glucose (Liu *et al.*, 2022b). This is inconsistent with our results, which might be attributed to the different carbon and nitrogen sources, as well as the variations in the cultivation conditions during the fermentation process. Different monosaccharides exhibited varying bioactivities, with a higher number of monosaccharide types linked to increased antioxidant activities of GLPs (Li *et al.*, 2020).

FT-IR Analysis of GLPs

The determination of absorption peaks of polysaccharides by FT-IR analysis can better determine the structure of polysaccharides. Figure 3, the spectra of GLP-F, GLP-B, and GLP-S were analyzed in the range of 4000-400cm^{-1}. The GLP-F, GLP-B, and GLP-S all had absorption peaks near 3432, 1630, 1250, and 950 cm^{-1}, due to the stretching vibration of hydroxyl groups in polysaccharide glycosides, the stretching vibrations of Carbonyl (C = O) groups and the stretching vibration of C-H bonds, respectively (Kang *et al*., 2019; Ye *et al*., 2011). In addition, GLP-B and GLP-S displayed absorption peaks related to the stretching vibrations of the methyl groups (C-H), the stretching vibrations of the amino groups (N-H), and the bending vibrations of C-H, compared to GLP-F (Yan *et al*., 2010). Previous studies have shown that polysaccharides in *G. lucidum* fruiting bodies also present absorption peaks attributed to O-H and C-H stretching vibrations (Liu *et al*., 2022a). Polysaccharides in *G. lucidum* spore powder showed absorption peaks attributed to the O-H stretching vibration, C-H stretching vibration, and C-H bending vibration (Zhao *et al*., 2023). In another study by Zhou, infrared spectroscopy scanning of polysaccharides from *G. lucidum* fermentation broth revealed absorption peaks linked to the stretching vibrations of O-H bonds and C = O bonds (Zhou *et al*., 2014).

Antioxidant Activity of GLPs Analysis in vitro

The scavenging effects of the GLPs on hydroxyl radicals are illustrated in Fig. 4a. In all test concentrations, GLP-F hydroxylagical removal activity showed a remarkably high level. The scavenging ability of GLP-F, GLP-B, and GLP-S increased to 91.16, 69.63, and 51.66% at the concentration of 5 g/L, respectively. These results demonstrated that GLP-F exhibited a noticeable ability to scavenge hydroxyl radicals. The outstanding hydroxyl radical scavenging ability of GLP-F may be attributed to the higher content of uronic acid than GLP-B and GLP-S.

The scavenging effects of the GLPs on superoxide radicals are shown in Fig. 4b. The superoxide radical scavenging activity of GLP-S was significantly better at all concentrations tested. The scavenging activities of GLP-F, GLP-B, and GLP-S increased with higher concentrations. At the concentration of 5 g/L, the scavenging activities of GLP-F, GLP-B, and GLP-S were 66.60, 70.57, and 81.05%, respectively. Based on the results, GLP-S appears to be the most efficient in scavenging superoxide anion radicals. The monosaccharide constituent, molecular weight, and protein content may affect the chelating properties of polysaccharides and also influence their antioxidant activities (Fan *et al*., 2012).

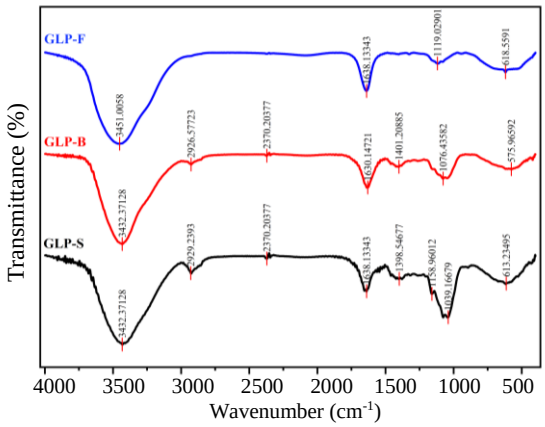

Fig. 3: FT-IR spectra of GLP-F, GLP-B and GLP-S

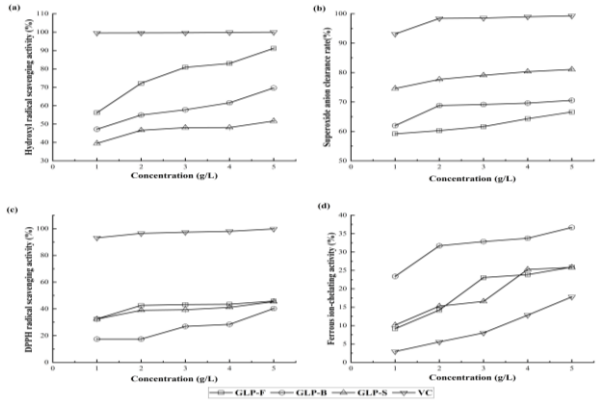

Fig. 4: Antioxidant effects of GLP-F, GLP-B, and GLP-S at different concentrations as determined by hydroxyl radicals; (a) Superoxide anion radicals; (b) and DPPH free radical; (c) scavenging activities and ferrous ion-chelating capacity; (d) Values are means ± SE (n = 3)

The scavenging effects of the GLPs on DPPH radicals are shown in Fig. 4c. The scavenging activity of polysaccharides increased in a concentration-dependent manner. The scavenging effect on the DPPH radical of GLP-F was higher than that of GLP-B and GLP-S with the increasing concentration. At 5 g/L, the scavenging activities of GLP-F, GLP-B, and GLP-S were 45.81, 40.22 and 45.38%, respectively. The results implied that three kinds of GLPs might act as an electron or hydrogen donator to scavenge DPPH. The DPPH radical scavenging activity of GLP-F was higher compared to that of GLP-B and GLP-S.

The ability to chelate iron ions is shown in Fig. 4d. The chelating capacity of metal ions from small to large was: GLP-B (36.68%), GLP-F (25.90%), and GLP-S (25.82%). GLP-B exhibited higher ability to chelate iron ions than GLP-F and GLP-S. No significant differences were found between GLP-F and GLP-S. It suggested that GLP-F, GLP-B, and GLP-S all could chelate iron ions.

Removing harmful free radicals is crucial for the antioxidant defense of cells or food systems. Based on the four *in vitro* antioxidant activity assays, GLPs had strong antioxidant activity, which was consistent with the previous studies (Chen *et al.*, 2018; Zheng *et al.*, 2020). However, there is limited literature comparing the antioxidant capacity of *Ganoderma lucidum* polysaccharides from different sources.

Establishment of A C. elegans Model for Heavy Metal Ions Stress

Determination of Heavy Metal Ions Concentration

Currently, most experiments determine heavy metal ion concentrations based on regulations from drinking water quality standards, with few studies establishing models for heavy metal ion concentrations (Zhang *et al.*, 2021a). To determine the concentration of heavy metal ions in this experiment, a model was established using *C. elegans*. Figure 5, under heavy metal stress, the lifespan of *C. elegans* significantly decreases, closely correlating with the concentration of heavy metals. Heavy metal ion concentrations that are too high or too low can result in inaccurate indicators of nematodes, potentially distorting experimental outcomes. Based on previous studies, the heavy metal ion concentrations at the 18 h time point were selected as the experiment model (Wang and Xing, 2008). Combined with our pre-experiment results, concentrations of Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+} were determined as 3, 20, 400 and 80 mm, respectively.

Analysis of Locomotion Behavior of C. elegans Under Heavy Metal Ions Stress

Heavy metal ions are not only present in different environments but also occur in trace amounts within biological organisms, playing essential roles in various biological processes. However, the accumulation of heavy metal ions in organisms can be toxic, as they can induce oxidative damage, resulting in neurological toxicity and abnormalities in locomotion behavior (Yang *et al.*, 2020; Zhao *et al.*, 2017). When worms were exposed to Cu^{2+}, Cd^{2+}, Mn^{2+} and Ni^{2+} solutions, there was a significant decrease in body bend angles from 26.95-17.65, 16.5, 14 and 10.9 and head thrash angles from 57.85-35.45, 33, 28 and 21.85, respectively ($p<.001$; Fig. 6). Heavy metal ions can enter animals through inhalation, ingestion, or skin contact, disrupting nerve conduction and normal physiological functions, consequently impairing worms motility (Wang and Xing 2009). Studies have indicated that exposure to heavy metal ions can impact the Receptor for Advanced Glycation End-products (RAGE), leading to neurodegenerative changes (Lawes *et al.*, 2020). Zhang's study found that prolonged exposure to heavy metals reduces the swimming ability of tadpoles (Zhang *et al.*, 2020). Prolonged exposure to high concentrations of Mn^{2+} may accumulate in the brain and cause neurotoxicity and nervous system symptoms such as Parkinson's disease (Lin *et al.*, 2020). Therefore, heavy metal poisoning can indeed have adverse effects on the locomotor behavior of animals (Ukaogo *et al.*, 2024).

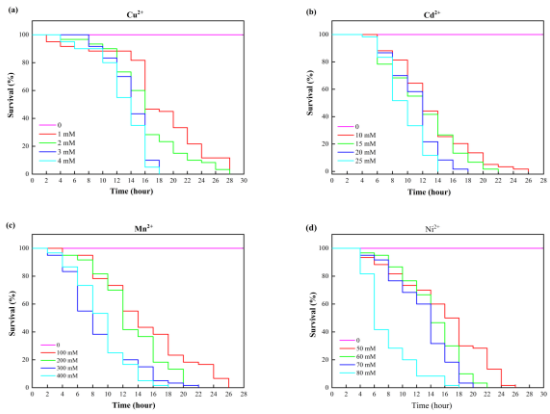

Fig. 5: Survival curves of *C. elegans* exposed to heavy metals in different concentrates; (a) Survival curves of *C. elegans* exposed to different concentrations of Cu^{2+}; (b) Survival curves of *C. elegans* exposed to different concentrations of Cd^{2+}; (c) Survival curves of *C. elegans* exposed to different concentrations of Mn^{2+}; (d) Survival curves of *C. elegans* exposed to different concentrations of Ni^{2+}

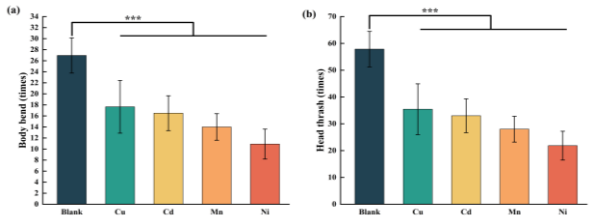

Fig. 6: (a) The body bend and; (b) The head thrash of *C. elegans* for heavy metal ions stress. Values are presented as the means ± SE (*p<0.05, ** p<0.01 and *** p<0.001)

Antioxidant Index Test Results of C. elegans Under Heavy Metal Ions Stress

To maintain biological stability following heavy metal ion-induced stress, organisms may activate conserved antioxidant enzymes and increase chelator levels. The involvement of MDA, SOD, and CAT in metal metabolism is well known (Song *et al.*, 2019).

Heavy metal ions were toxic when excessive in animals. As demonstrated in Fig. 7, exposure to Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+} significantly increased MDA levels ($p<0.05$). In the Cu^{2+} group, there was a 50% decrease in SOD and CAT activity ($p<0.01$). Conversely, the Cd^{2+} group showed a significant decrease of approximately 4.29-fold and a 0.47-fold increase in SOD and CAT activity, respectively ($p<0.01$). The Mn^{2+} group exhibited a significant increase of approximately 4.32-fold in SOD ($p<0.001$). The Ni^{2+} group showed a significant increase of approximately 3.60-fold and a 0.23-fold decrease in SOD and CAT activity, respectively ($p<0.05$).

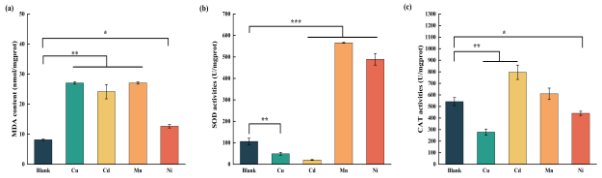

Fig. 7: (a) MDA content and; (b) SOD and; (c) CAT activities were determined to blank, Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+}.Values are presented as the means ± SE (*p<0.05, ** p<0.01 and *** p<0.001)

When cells experience oxidative stress or damage, lipid peroxidation can lead to an increase in MDA production. Elevated MDA levels are often associated with cellular oxidative damage, inflammation, neurodegenerative diseases, and other pathological conditions. Exposure of worms to Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+} caused a significant increase in MDA levels. Souid's study found significantly higher MDA levels in Cd-exposed *Sparus aurata* compared to unexposed controls (Souid *et al*., 2013). Under heavy metal stress, certain heavy metal ions may increase SOD and CAT activity by acting as cofactors or bind to the enzymes' active sites, enhancing their function (Zhang *et al*., 2021b). Conversely, other heavy metal ions may inhibit SOD and CAT activity by nonspecifically binding to the enzymes, impairing their structure and function. Furthermore, different concentrations of heavy metal ions may affect SOD and CAT activity. Low doses of heavy metal stress may activate cellular defense mechanisms, resulting in increased SOD and CAT activity. However, high concentrations of heavy metal stress may excessively inhibit or damage the enzymes, leading to reduce SOD and CAT activity. Therefore, the presence of different heavy metal ions and their concentrations may result in varying levels of SOD and CAT activity under heavy metal stress, reflecting the diverse stress responses of organisms to the toxicity of heavy metal ions. When exposed to Cu^{2+}, Cd^{2+}, Mn^{2+} and Ni^{2+}, the level of MDA in the *C. elegans* significantly increased; When exposed to Cu^{2+}, Mn^{2+} and Ni^{2+} solutions, the SOD activity of *C. elegans* increased, while when exposed to Cd^{2+} solutions, the SOD activity decreased; When exposed to Cu^{2+} and Ni^{2+} solutions, the CAT activity of *C. elegans* decreased, while it increased when exposed to Cd^{2+} and Mn^{2+} solutions. Song's research found that the exposure of *C. elegans* to zinc and copper significantly increased the MDA level and when the *C. elegans* were exposed to zinc solution, SOD activity increased, while exposed to the copper solution, SOD activity decreased (Song *et al*., 2019).

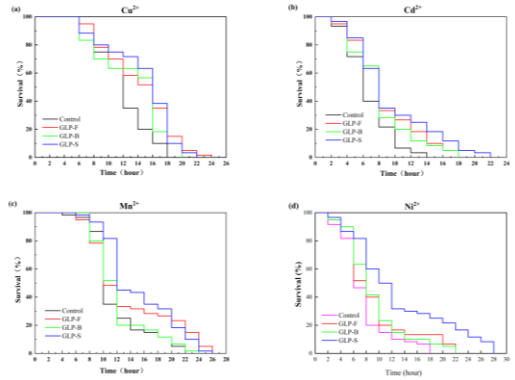

Fig. 8: Effect of GLP-F, GLP-B, and GLP-S on the lifespan of *C. elegans* poisoned with heavy metals; (a); (b); (c) and (d) worms exposed to Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+} solutions, respectively and surviving worms were measured at 2 h intervals and compared for lifespans in the absence and presence of GLPs

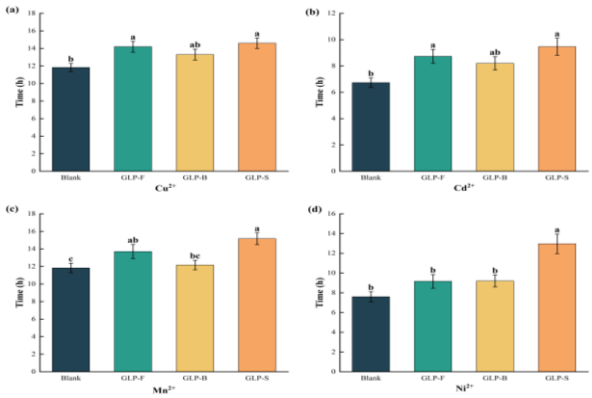

Fig. 9: The mean lifespan of *C. elegans* exposed to heavy metals; (a) The mean lifespan of *C. elegans* exposed to Cu^{2+}; (b) The mean lifespan of *C. elegans* exposed to Cd^{2+}; (c) The mean lifespan of *C. elegans* exposed to Mn^{2+}; (d) The mean lifespan of *C. elegans* exposed to Ni^{2+}. Those marked with different letters indicated significant differences between groups (p<0.05) and those marked with the same letters indicated that the differences between groups were not significant (p≥0.05)

Effect of GLPs on C. elegans for Heavy Metal Ions Stress

Effect of GLPs on the Lifespan of C. elegans for Heavy Metal Ions Stress

Figures 8-9, under normal circumstances, heavy metal toxicity led to a shorter lifespan for *C. elegans*. However, when GLPs were introduced, the survival curve shifted to the right, indicating an extended lifespan of the worms for heavy metal ions stress. Log-rank analysis showed that all three polysaccharides significantly prolonged the lifespan of nematodes after Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+} intoxication compared to the control (p<0.05). Compared to the control

group, GLP-F, and GLP-S significantly increased the lifespan of worms after Cu^{2+}, Cd^{2+}, and Mn^{2+} poisoning ($p<0.05$). GLP-S also significantly increased the lifespan of nematodes after Mn^{2+}, $Cd^{2+,}$ and Ni^{2+} poisoning ($p<0.05$). GLPs play a protective role against the toxicity of heavy metal ions in organisms. GLP-F and GLP-S demonstrated superior abilities in alleviating the toxicity of heavy metal ions compared to GLP-B. This is similar to previous research results, which indicate that GLPs can effectively prevent Cd^{2+} toxicity through various mechanisms, such as reducing the bioaccumulation of cadmium in tissues, regulating neurotransmitters, reducing lipid accumulation, and improving growth performance (Jia *et al.*, 2023). The polysaccharides from *Agaricus blazei Murill* during dietary exposure to Cd^{2+} were found to reduce histopathological damage and partially ameliorate the toxic effects of Cd^{2+} in chickens (Hu *et al.*, 2017).

Effect of GLPs on the Locomotion Behavior of C. elegans for Heavy Metal Ions Stress

Heavy metal ions toxicity may also lead to behavioral neurological disorders such as *Parkinson*'s disease and *Alzheimer*'s disease (Philippsen *et al.*, 2021). The changes were observed in all behavioral parameters assessed, such as body bending and head thrashing.

Compared to the control group, GLP-F, GLP-B, and GLP-S significantly increased the body bending frequency of Cu^{2+} stressed *C. elegans* from 17.65-21.65, 21.50, and 21.1 times per min, respectively. The head thrashing frequency also significantly increased from 35.45-43.40, 43.20, and 42.20 times per min, respectively ($p<0.05$; Fig. 10a). The GLP-F and GLP-B groups significantly increased body bending frequency of Cd^{2+} stressed increased from 16.51-19.85 and 18.95 times per min and the head thrashing frequency increased from 33-39.7 and 37.9 times per min, respectively ($p<0.05$; Fig. 10b). The GLP-F, GLP-B and GLP-S significantly increased body bending frequency of Mn^{2+} stressed increased from 14-18.85, 18.1 and 18.8 times per min and the head thrashing frequency increased from 28-37.7, 36.2 and 37.6 times per min, respectively ($p<0.05$; Fig. 10c). The GLP-F and GLP-S groups significantly increased body bending frequency of Ni^{2+} stressed increased from 10.9-20.3 and 15 times per min, as well as the head thrashing frequency increased from 22.85-40.45 and 30.1 times per min, respectively ($p<0.05$; Fig. 10d).

Our results indicated that GLP-F and GLP-S significantly improved the behavioral abilities of nematodes following heavy metal ion poisoning, showing better effects compared to GLP-B. This aligns with the research by Sun and Huang (Huang *et al.*, 2017; Xin Zhi *et al.*, 2017), which indicates that polysaccharides have a significant protective effect against neuronal apoptosis and the polysaccharides can serve as a regenerative therapeutic agent for treating cognitive decline associated with neurodegenerative diseases. This suggests that GLPs may hold potential therapeutic benefits in the treatment of heavy metal poisoning.

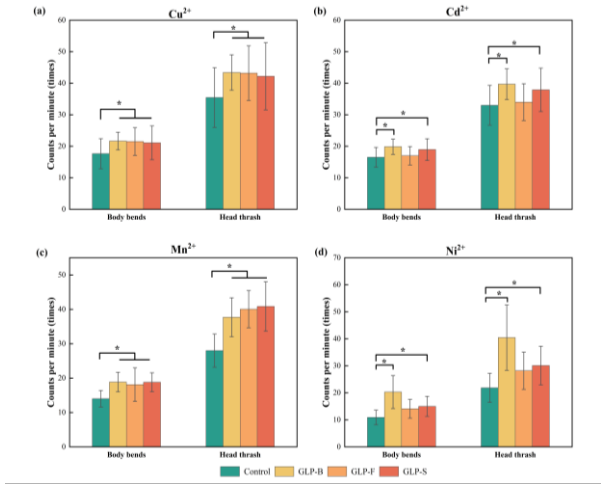

Fig. 10: Effect of GLP-F, GLP-B, and GLP-S on behavior; (a); (b); (c); and (d) denote the body bending and head thrash of worms per minute in Cu^{2+}, Cd^{2+}, Mn^{2+}, and Ni^{2+} heavy metal poisoning, respectively. Values are presented as the means ± SE (*p<0.05, ** p<0.01 and *** p<0.001)

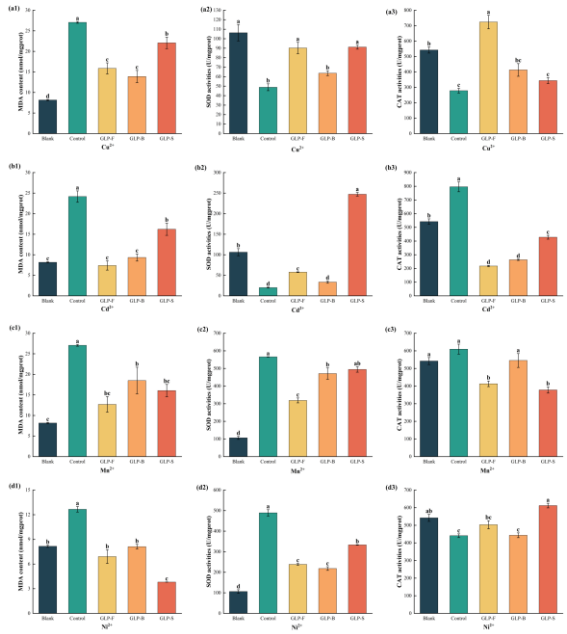

Fig. 11: MDA content and SOD and CAT activities were determined to be Blank, GLP-F, GLP-B and GLP-S. Values are presented as the means ± SE (n = 3). Those marked with different letters indicated significant differences between groups ($p<0.05$) and those marked with the same letters indicated that the differences between groups were not significant ($p\geq0.05$)

Effect of GLPs on the Antioxidant Index of C. elegans for Heavy Metal Ion Stress

Figure 11a, after exposure to Cu^{2+}, the activities of SOD and CAT in the control group were significantly lower than in the blank group ($p<0.05$), and the MDA content was significantly increased ($p<0.05$), indicating that Cu^{2+} exposure can lead to accelerated aging of nematodes. Compared with the control group, the MDA content in the GLP-F, GLP-B, and GLP-S groups were significantly lower, decreasing by 70.52 94.87 and 22.57% respectively ($p<0.05$); the SOD activity in the GLP-F and GLP-S groups were significantly increased, increase of 84.78 and 86.78%, respectively ($p<0.05$); and the CAT activity in the GLP-F group increased by approximately1.6-fold ($p<0.05$).

Figure 11b, after exposure to Cd^{2+}, the activities of CAT and the MDA content in the control group were significantly higher than in the blank group, and the activity of SOD was significantly decreased ($p<0.05$). Compared with the control group, the MDA content in the GLP-F, GLP-B, and GLP-S groups were significantly lower, decreased by 2.27-, 1.59- and 0.49-fold, respectively ($p<0.05$); the SOD activity in the GLP-F and GLP-S group was significantly increased, increase of 1.88 and 13.52-fold, respectively ($p<0.05$); and the CAT activity in the GLP-F, GLP-B and GLP-S groups decreased by approximately 2.65-2.01 and 0.86-fold, respectively ($p<0.05$).

Figure 11c, after exposure to Mn^{2+}, the activities of SOD and the MDA content in the control group were significantly higher than in the blank group ($p<0.05$). Compared with the control group, the MDA content in the GLP-F, GLP-B and GLP-S groups were significantly lower, decreased by 1.13-0.46 and 0.69-fold, respectively ($p<0.05$); the SOD activity in the GLP-F and GLP-B groups were significantly decreased, a decrease of 77.58 and 20.13%, respectively ($p<0.05$); and the CAT activity in the GLP-F and GLP-S groups decreased by approximately 0.48- and 0.61-fold, respectively ($p<0.05$).

Figure 11d, after exposure to Ni^{2+}, the activity of SOD and the MDA content in the control group were significantly higher than in the blank group, and the activity of CAT was significantly decreased ($p<0.05$). Compared with the control group, the MDA content in the GLP-F, GLP-B and GLP-S groups were significantly lower, decreased by 0.83-0.56 and 2.33-fold, respectively ($p<0.05$); the SOD activity in the GLP-F, GLP-B and GLP-S groups were significantly decreased, decrease of 1.92-0.53 and 0.63-fold, respectively ($p<0.05$); and the CAT activity in the GLP-S groups increased by approximately 0.38-fold ($p<0.05$).

The results demonstrated that GLPs can have varying effects in mitigating the impact of heavy metal ions on SOD and CAT activities, as well as MDA content. For the reduction of SOD activities caused by Cu^{2+} and Cd^{2+}, as well as CAT caused by Cu^{2+} and Ni^{2+}, GLPs can enhance their activities, effectively restoring the body's antioxidant capacity. For the increase in SOD activities caused by Mn^{2+} and Ni^{2+}, as well as CAT caused by Cd^{2+} and Mn^{2+}, GLPs can regulate their expression and activities, bringing them back to normal levels. In addition, GLPs can also reduce the elevated MDA content caused by heavy metal ions, thereby alleviating heavy metal damage in nematodes. Overall, GLPs possess the potential to regulate the effects of heavy metal ions on the antioxidant system, offering protection against heavy metal damage in organisms. Researchers have discovered the role of polysaccharides in combating the toxicity of heavy metal ions. For example, Pu observed that polysaccharides had anti-heavy metal toxicity effects in clearing free radicals to improve oxidative stress and inhibit lipid peroxidation (Pu *et al.*, 2015). Li's study showed that the protective effect of polysaccharides against central immune organ damage in cadmium-poisoned chickens was closely related to oxidative stress (Li *et al.*, 2019). Our results are consistent with these findings, indicating that GLPs can effectively alleviate the damage caused by heavy metal ion poisoning. This may be due to the ability of GLPs to scavenge heavy metal ion-induced ROS and to enhance enzymatic antioxidant activity. However, further studies are needed to understand the antioxidant mechanism of GLPs against heavy metal ion toxicity.

Conclusion

This study compared the polysaccharide content, monosaccharide composition, and antioxidant activity *in vitro* of polysaccharides from *G. lucidum* fermentation powder, fruiting bodies, and broken spore powder and investigated their potential in alleviating heavy metal ion toxicity. The results showed that polysaccharides from different sources were different in polysaccharide content, monosaccharide composition, and antioxidant activity *in vitro*. Polysaccharides may reduce the toxicity of heavy metal ions by adjusting the antioxidant index and significantly improve the life-span reduction and behavior disorder of *C. elegans* caused by heavy metal poisoning. GLP-F exhibited the most significant alleviating effect on the toxicity of Cu^{2+}, Cd^{2+}, and Mn^{2+}, while GLP-S showed the most significant alleviating effect on the toxicity of Ni^{2+}. Therefore, the addition of GLPs had the potential to protect animals from damage caused by heavy metal ion stress and had significant potential in alleviating heavy metal ion-induced injuries. Compared to spore powder and fruiting body powder, mycelial fermentation powder has a higher polysaccharide content. Moreover, its capacity for large-scale production in a shorter timeframe makes it more feasible to meet market demands.

Acknowledgment

The authors wish to acknowledge Liwei, Zhejiang University, for her help with experimental design. We

appreciate the publishers' assistance with this research work. We appreciate the editing team's efforts in publishing the content. We appreciate the publishers for providing the resources and platforms for disseminating the findings of our study.

Funding Information

This study was supported by the Key R and D program of Zhejiang province (2022C02043, 2021C02008).

Author's Contributions

Huixiu Ma: Participate in the whole process of experimental design, experimental process, result analysis, and finally write the manuscript.

Dehui Dai, Guicai Chen and Weilian Hu: Participate in the part of the process of experimental design, experimental process, and result analysis.

Ethics

This article is original and contains unpublished material. All of the authors have read and approved the manuscript and no ethical issues are involved.

References

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*, *2019*, 6730305. https://doi.org/10.1155/2019/6730305

Aljerf, L., Williams, M., Ajong, A. B., Onydinma, U. P., Dehmchi, F., Pham, V. T., Bhatnagar, S., & Belboukhari, N. (2021). Comparative Study of the Biochemical Response Behavior of Some Highly Toxic Minerals on Selenosis in Rats. *Revista De Chimie*, *72*(2), 9-18. https://doi.org/10.37358/rc.21.2.8415

Aljerf, L., & Aljerf, N. (2023). Food products quality and nutrition in relation to public. Balancing health and disease. *Progress in Nutrition*, *25*(1), e2023024. https://doi.org/10.23751/pn.v25i1.13928

Celep, E., Aydın, A., & Yesilada, E. (2012). A comparative study on the in vitro antioxidant potentials of three edible fruits: Cornelian cherry, Japanese persimmon and cherry laurel. *Food and Chemical Toxicology*, *50*(9), 3329-3335. https://doi.org/10.1016/j.fct.2012.06.010

Chen, H. Y., & Yen, G. C. (2007). Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. *Food Chemistry*, *101*(2), 686-694. https://doi.org/10.1016/j.foodchem.2006.02.047

Chen, S., Guan, X., Yong, T., Gao, X., Xiao, C., Xie, Y., Chen, D., Hu, H., & Wu, Q. (2022). Structural characterization and hepatoprotective activity of an acidic polysaccharide from *Ganoderma lucidum*. *Food Chemistry: X*, *13*, 100204. https://doi.org/10.1016/j.fochx.2022.100204

Chen, T. Q., Wu, J. G., Kan, Y. J., Yang, C., Wu, Y. B., & Wu, J. Z. (2018). Antioxidant and Hepatoprotective Activities of Crude Polysaccharide Extracts from Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Agaricomycetes), by Ultrasonic-Circulating Extraction. *International Journal of Medicinal Mushrooms*, *20*(6), 581-593. https://doi.org/10.1615/intjmedmushrooms.2018026536

Elbasiouny, H., Darwesh, M., Elbeltagy, H., Abo-alhamd, F. G., Amer, A. A., Elsegaiy, M. A., Khattab, I. A., Elsharawy, E. A., Ebehiry, F., El-Ramady, H., & Brevik, E. C. (2021). Ecofriendly remediation technologies for wastewater contaminated with heavy metals with special focus on using water hyacinth and black tea wastes: A review. *Environmental Monitoring and Assessment*, *193*(7), 449. https://doi.org/10.1007/s10661-021-09236-2

Fan, L., Li, J., Deng, K., & Ai, L. (2012). Effects of drying methods on the antioxidant activities of polysaccharides extracted from *Ganoderma lucidum*. *Carbohydrate Polymers*, *87*(2), 1849-1854. https://doi.org/10.1016/j.carbpol.2011.10.018

Hallauer, J., Geng, X., Yang, H.-C., Shen, J., Tsai, K.-J., & Liu, Z. (2016). The Effect of Chronic Arsenic Exposure in Zebrafish. *Zebrafish*, *13*(5), 405-412. https://doi.org/10.1089/zeb.2016.1252

Hu, X., Zhang, R., Xie, Y., Wang, H., & Ge, M. (2017). The Protective Effects of Polysaccharides from Agaricus blazei Murill Against Cadmium-Induced Oxidant Stress and Inflammatory Damage in Chicken Livers. *Biological Trace Element Research*, *178*, 117-126. https://doi.org/10.1007/s12011-016-0905-y

Hu, Y., Wang, S.-X., Wu, F.-Y., Wu, K.-J., Shi, R.-P., Qin, L.-H., Lu, C.-F., Wang, S.-Q., Wang, F.-F., & Zhou, S. (2022). Effects and Mechanism of *Ganoderma lucidum* Polysaccharides in the Treatment of Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats. *BioMed Research International*, *2022*, 4314415. https://doi.org/10.1155/2022/4314415

Huang, S., Mao, J., Ding, K., Zhou, Y., Zeng, X., Yang, W., Wang, P., Zhao, C., Yao, J., Xia, P., & Pei, G. (2017). Polysaccharides from *Ganoderma lucidum* Promote Cognitive Function and Neural Progenitor Proliferation in Mouse Model of Alzheimer's Disease. *Stem Cell Reports*, *8*(1), 84-94. https://doi.org/10.1016/j.stemcr.2016.12.007

Huang, L., Wang, H., & Shao, Y. (2023). Evaluation and decrease of uncertainty in determination of crude polysaccharide of broken *Ganoderma lucidum* spore powder. *Acta Agriculturae Shanghai*, *39*(2), 88-94. https://doi.org/10.15955/j.issn1000-3924.2023.02.15

Jayachandran, M., Xiao, J., & Xu, B. (2017). A Critical Review on Health Promoting Benefits of Edible Mushrooms through Gut Microbiota. *International Journal of Molecular Sciences*, *18*(9), 1934. https://doi.org/10.3390/ijms18091934

Jeong, Y.-U., & Park, Y.-J. (2020). Ergosterol Peroxide from the Medicinal Mushroom *Ganoderma lucidum* Inhibits Differentiation and Lipid Accumulation of 3T3-L1 Adipocytes. *International Journal of Molecular Sciences*, *21*(2), 460. https://doi.org/10.3390/ijms21020460

Jia, D., Tang, Y., Qin, F., Liu, B., Hu, T., & Chen, W. (2023). *Ganoderma lucidum* polysaccharide alleviates Cd toxicity in common carp (*Cyprinus carpio*): Neuropeptide, growth performance and lipid accumulation. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *271*, 109663. https://doi.org/10.1016/j.cbpc.2023.109663

Kang, Q., Chen, S., Li, S., Wang, B., Liu, X., Hao, L., & Lu, J. (2019). Comparison on characterization and antioxidant activity of polysaccharides from *Ganoderma lucidum* by ultrasound and conventional extraction. *International Journal of Biological Macromolecules*, *124*, 1137-1144. https://doi.org/10.1016/j.ijbiomac.2018.11.215

Lawes, M., Pinkas, A., Frohlich, B. A., Iroegbu, J. D., Ijomone, O. M., & Aschner, M. (2020). Metal-induced neurotoxicity in a RAGE-expressing C. elegans model. *NeuroToxicology*, *80*, 71-75. https://doi.org/10.1016/j.neuro.2020.06.013

Li, J., Gu, F., Cai, C., Hu, M., Fan, L., Hao, J., & Yu, G. (2020). Purification, structural characterization, and immunomodulatory activity of the polysaccharides from *Ganoderma lucidum*. *International Journal of Biological Macromolecules*, *143*, 806-813. https://doi.org/10.1016/j.ijbiomac.2019.09.141

Li, L. F., Liu, H. B., Zhang, Q. W., Li, Z. P., Wong, T. L., Fung, H. Y., Zhang, J. X., Bai, S. P., Lu, A. P., & Han, Q. B. (2018). Comprehensive comparison of polysaccharides from *Ganoderma lucidum* and G. sinense: Chemical, antitumor, immunomodulating and gut-microbiota modulatory properties. *Scientific Reports*, *8*, 6172. https://doi.org/10.1038/s41598-018-22885-7

Li, R., Zhang, L., Tang, Z., Li, T., Li, G., Zhang, R., & Ge, M. (2019). Effects of Fungal Polysaccharide on Oxidative Damage and TLR4 Pathway to the Central Immune Organs in Cadmium Intoxication in Chickens. *Biological Trace Element Research*, *191*, 464-473. https://doi.org/10.1007/s12011-018-1627-0

Lin, M., Colon-Perez, L. M., Sambo, D. O., Miller, D. R., Lebowitz, J. J., Jimenez-Rondan, F., Cousins, R. J., Horenstein, N., Aydemir, T. B., Febo, M., & Khoshbouei, H. (2020). Mechanism of Manganese Dysregulation of Dopamine Neuronal Activity. *The Journal of Neuroscience*, *40*(30), 5871-5891. https://doi.org/10.1523/jneurosci.2830-19.2020

Liu, G., Zhang, J., Kan, Q., Song, M., Hou, T., An, S., Lin, H., Chen, H., Hu, L., Xiao, J., Chen, Y., & Cao, Y. (2022a). Extraction, Structural Characterization, and Immunomodulatory Activity of a High Molecular Weight Polysaccharide from *Ganoderma lucidum*. *Frontiers in Nutrition*, *9*, 846080. https://doi.org/10.3389/fnut.2022.846080

Liu, L., Feng, J., Gao, K., Zhou, S., Yan, M., Tang, C., Zhou, J., Liu, Y., & Zhang, J. (2022b). Influence of carbon and nitrogen sources on structural features and immunomodulatory activity of exopolysaccharides from *Ganoderma lucidum*. *Process Biochemistry*, *119*, 96-105. https://doi.org/10.1016/j.procbio.2022.05.016

Lu, J., He, R., Sun, P., Zhang, F., Linhardt, R. J. and Zhang, A. (2020) Molecular mechanisms of bioactive polysaccharides from *Ganoderma lucidum* (Lingzhi), a review. *International Journal of Biological Macromolecules*, *150*, 765-774. https://doi.org/10.1016/j.ijbiomac.2020.02.035

Philippsen, D. F., Tamagno, W. A., Vanin, A. P., Concato, A. C., Bragagnolo, L., Prestes, E., Korf, E. P., & Kaizer, R. R. (2021). Copper uses in organic production are safe to the nervous system of Caenorhabditis elegans?. *Environmental Quality Management*, *30*(4), 61-70. https://doi.org/10.1002/tqem.21736

Pohanka, M. (2019). Copper and copper nanoparticles toxicity and their impact on basic functions in the body. *Bratislava Medical Journal*, *120*(6), 397-409. https://doi.org/10.4149/bll_2019_065

Ukaogo, P. O., Tang, J., Ahuchaogu, A. A., Igwe, O. U., Obike, A. I., Emole, P. O., Aljerf, L., Onah, O. E., Tijjani, A., & Ajong, A. B. (2022). Evaluation of concentrations of trace metal(loid)s in indigenous crab species and human health risk implications. *Emerging Contaminants*, *8*, 371-380. https://doi.org/10.1016/j.emcon.2022.07.001

Ukaogo, P. O., Aljerf, L., Nwaru, E. C., Imrana, I., Tang, J., Bethel Ajong, A., Emole, P. O., Siddhant, O., & Ukaogo, C. T. (2024). Evaluation and risk assessment of heavy metals in King tuber mushroom in the contest of COVID-19 pandemic lockdown in Sokoto state, Nigeria. *Kuwait Journal of Science*, *51*(2), 100193. https://doi.org/10.1016/j.kjs.2024.100193

Pu, X., Fan, W., Yu, S., Li, Y., Ma, X., Liu, L., Ren, J., & Zhang, W. (2015). Polysaccharides from Angelica and Astragalus exert hepatoprotective effects against carbon-tetrachloride-induced intoxication in mice. *Canadian Journal of Physiology and Pharmacology*, *93*(1), 39-43. https://doi.org/10.1139/cjpp-2014-0331

Shahid, A., Huang, M., Liu, M., Shamim, M. A., Parsa, C., Orlando, R., & Huang, Y. (2022). The medicinal mushroom *Ganoderma lucidum* attenuates UV-induced skin carcinogenesis and immunosuppression. *PLOS ONE*, *17*(3), e0265615. https://doi.org/10.1371/journal.pone.0265615

Shen, L., Xiao, J., Ye, H., & Wang, D. (2009). Toxicity evaluation in nematode Caenorhabditis elegans after chronic metal exposure. *Environmental Toxicology and Pharmacology*, *28*(1), 125-132. https://doi.org/10.1016/j.etap.2009.03.009

Song, C. F., Wang, S. G., Yang, J., Cui, Z. W., & Gu, Y. H. (2015). Optimization of Vacuum-Microwave Radiation Pretreatment on Extraction of Ganoderma Polysaccharides. *Mathematical Problems in Engineering*, *2015*, 792832. https://doi.org/10.1155/2015/792832

Song, S., Han, Y., Zhang, Y., Ma, H., Zhang, L., Huo, J., Wang, P., Liang, M., & Gao, M. (2019). Protective role of citric acid against oxidative stress induced by heavy metals in Caenorhabditis elegans. *Environmental Science and Pollution Research*, *26*, 36820-36831. https://doi.org/10.1007/s11356-019-06853-w

Souid, G., Souayed, N., Yaktiti, F., & Maaroufi, K. (2013). Effect of acute cadmium exposure on metal accumulation and oxidative stress biomarkers of Sparus aurata. *Ecotoxicology and Environmental Safety*, *89*, 1-7. https://doi.org/10.1016/j.ecoenv.2012.12.015

Sule, K., Umbsaar, J., & Prenner, E. J. (2020). Mechanisms of Co, Ni, and Mn toxicity: From exposure and homeostasis to their interactions with and impact on lipids and biomembranes. *Biochimica Et Biophysica Acta (BBA) - Biomembranes*, *1862*(8), 183250. https://doi.org/10.1016/j.bbamem.2020.183250

Xin Zhi, S., Ying, L., Wei, L., & Li mei, G. (2017). Neuroprotective effects of *ganoderma lucidum* polysaccharides against oxidative stress-induced neuronal apoptosis. *Neural Regeneration Research*, *12*(6), 953-958. https://doi.org/10.4103/1673-5374.208590

Tamagno, W. A., Santini, W., Santos, A., Alves, C., Bilibio, D., Sutorillo, N. T., Zamberlan, D. C., Kaizer, R. R., & Barcellos, L. J. G. (2022). Pitaya fruit extract ameliorates the healthspan on copper-induced toxicity of Caenorhabditis elegans. *Journal of Food Biochemistry*, *46*(3), e14050. https://doi.org/10.1111/jfbc.14050

Tan, X., Sun, J., Xu, Z., Li, H., Hu, J., Ning, H., Qin, Z., Pei, H., Sun, T., & Zhang, X. (2018). Effect of heat stress on production and in-vitro antioxidant activity of polysaccharides in *Ganoderma lucidum*. *Bioprocess and Biosystems Engineering*, *41*, 135-141. https://doi.org/10.1007/s00449-017-1850-7

Tong, Y., Hua, X., Zhao, W., Liu, D., Zhang, J., Zhang, W., Chen, W., & Yang, R. (2020). Protective effects of Lactobacillus plantarum CCFM436 against acute manganese toxicity in mice. *Food Bioscience*, *35*, 100583. https://doi.org/10.1016/j.fbio.2020.100583

Tsalik, E. L., & Hobert, O. (2003). Functional mapping of neurons that control locomotory behavior in Caenorhabditis elegans. *Journal of Neurobiology*, *56*(2), 178-197. https://doi.org/10.1002/neu.10245

Wang, D., & Xing, X. (2008). Assessment of locomotion behavioral defects induced by acute toxicity from heavy metal exposure in nematode Caenorhabditis elegans. *Journal of Environmental Sciences*, *20*(9), 1132-1137. https://doi.org/10.1016/s1001-0742(08)62160-9

Wang, D., & Xing, X. (2009). Pre-treatment with mild metal exposure suppresses the neurotoxicity on locomotion behavior induced by the subsequent severe metal exposure in Caenorhabditis elegans. *Environmental Toxicology and Pharmacology*, *28*(3), 459-464. https://doi.org/10.1016/j.etap.2009.07.008

Yan, J.-K., Li, L., Wang, Z.-M., & Wu, J.-Y. (2010). Structural elucidation of an exopolysaccharide from mycelial fermentation of a Tolypocladium sp. fungus isolated from wild Cordyceps sinensis. *Carbohydrate Polymers*, *79*(1), 125-130. https://doi.org/10.1016/j.carbpol.2009.07.047

Yang, X., Zhao, Y., Wang, Q., Wang, H., & Mei, Q. (2005). Analysis of the Monosaccharide Components in Angelica Polysaccharides by High Performance Liquid Chromatography. *Analytical Sciences*, *21*(10), 1177-1180. https://doi.org/10.2116/analsci.21.1177

Yang, X., Han, Y., Mu, Y., Yang, P., Gu, W., & Zhang, M. (2020). Multigenerational effects of cadmium on the lifespan and fertility of Drosophila melanogaster. *Chemosphere*, *245*, 125533. https://doi.org/10.1016/j.chemosphere.2019.125533

Ye, H., Ye, B., & Wang, D. (2008). Trace administration of vitamin E can retrieve and prevent UV-irradiation- and metal exposure-induced memory deficits in nematode Caenorhabditis elegans. *Neurobiology of Learning and Memory*, *90*(1), 10-18. https://doi.org/10.1016/j.nlm.2007.12.001

Ye, M., Qiu, T., Peng, W., Chen, W., Ye, Y., & Lin, Y. R. (2011). Purification, characterization and hypoglycemic activity of extracellular polysaccharides from Lachnum calyculiforme. *Carbohydrate Polymers*, *86*(1), 285-290. https://doi.org/10.1016/j.carbpol.2011.04.051

Yu, H.-T., Zhen, J., Xu, J.-X., Cai, L., Leng, J.-Y., Ji, H.-L., & Keller, B. B. (2020). Zinc protects against cadmium-induced toxicity in neonatal murine engineered cardiac tissues via metallothionein-dependent and independent mechanisms. *Acta Pharmacologica Sinica*, *41*(5), 638-649. https://doi.org/10.1038/s41401-019-0320-y

Zhang, W., Zhi, H., & Sun, H. (2020). Effects of Heavy Metal Pollution on Fitness and Swimming Performance of Bufo raddei Tadpole. *Bulletin of Environmental Contamination and Toxicology*, *105*, 387-392. https://doi.org/10.1007/s00128-020-02953-3

Zhang, Y., Cai, H., Tao, Z., Yuan, C., Jiang, Z., Liu, J., Kurihara, H., & Xu, W. (2021a). *Ganoderma lucidum* spore oil (GLSO), a novel antioxidant, extends the average life span in Drosophila melanogaster. *Food Science and Human Wellness*, *10*(1), 38-44. https://doi.org/10.1016/j.fshw.2020.05.011

Zhang, Y., Zhao, C., Zhang, H., Liu, R., Wang, S., Pu, Y., & Yin, L. (2021b). Integrating transcriptomics and behavior tests reveals how the *C. elegans* responds to copper induced aging. *Ecotoxicology and Environmental Safety*, *222*, 112494. https://doi.org/10.1016/j.ecoenv.2021.112494

Zhao, L.-L., Ru, Y.-F., Liu, M., Tang, J.-N., Zheng, J.-F., Wu, B., Gu, Y.-H., & Shi, H.-J. (2017). Reproductive effects of cadmium on sperm function and early embryonic development in vitro. *PLOS ONE*, *12*(11), e0186727. https://doi.org/10.1371/journal.pone.0186727

Zhao, Y., Li, Q., Wang, M., Wang, Y., Piao, C., Yu, H., Liu, J., & Li, Z. (2023). Structural characterization of polysaccharides after fermentation from *Ganoderma lucidum* and its antioxidant activity in HepG2 cells induced by H_2O_2. *Food Chemistry: X*, *18*, 100682. https://doi.org/10.1016/j.fochx.2023.100682

Zheng, S., Zhang, W., & Liu, S. (2020). Optimization of ultrasonic-assisted extraction of polysaccharides and triterpenoids from the medicinal mushroom *Ganoderma lucidum* and evaluation of their *in vitro* antioxidant capacities. *PLOS ONE*, *15*(12), e0244749. https://doi.org/10.1371/journal.pone.0244749

Zhou, H., Liu, G., Huang, F., Wu, X., & Yang, H. (2014). Improved roduction, purification and bioactivity of a polysaccharide from submerged cultured *Ganoderma lucidum*. *Archives of Pharmacal Research*, *37*, 1530-1537. https://doi.org/10.1007/s12272-014-0391-8