Characterization and protection on acute liver injury of a polysaccharide MP-I from *Mytilus coruscus*

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In this study, we analyzed a water-soluble polysaccharide MP-I isolated from *Mytilus coruscus*. MP-I was obtained by hot-water extraction, anion-exchange and gel-permeation chromatography. Complete hydrolysis, periodate oxidation, methylation analysis, as well as Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy were conducted to elucidate its structure. MP-I was subjected to investigate the protective effect on carbon tetrachloride (CCl4) induced liver damage in male Kunming mice. Based on the data obtained, MP-I was found to be an α-(1→4)-D-glucan, branched with a single α-D-glucose at the C-6 position every eight residue, on average, along the main chain. Based on the calibration with Dextran, the glucan had a molecular weight of about 1.35 × 106 Da. Pharmacological studies revealed that MP-I could decrease serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and hepatic malondialdehyde (MDA) levels, increase the hepatic total superoxide dismutase (T-SOD) activity, and improve hepatic damage in the CCl4 induced liver injury in mice in a dose-dependent manner. The results suggest that the possible mechanism is due to its antioxidant activity of MP-I.

Keywords: antioxidant/carbon tetrachloride/liver injury/*Mytilus coruscus* polysaccharide I/structure

**Introduction**

*Mytilus coruscus*, belongs to the family of Mytiloida, one of the main cultured species of marine shellfish in China, is used as food and medicine for thousands of years (Li and Huang 2004). As a Chinese traditional medicine, it has been frequently prescribed by practitioners of traditional Chinese medicine (TCM) to regulate the functions of liver and kidneys, strengthen the immune system, for the treatment of male impotence, female menoxenia, and so on (Li and Ding 2006).

Up to now, little attention had been paid to the carbohydrate compounds in it. Few reports have investigated the structure and bioactivity of several other shellfish polysaccharides. Mytilan, isolated from the mantle of the mussel *Crenomytilus grayanus*, possessed the high immunomodulating activity (Ovodova et al. 1992). A polysaccharide (glycogen), extracted from *Perna canaliculus*, had an anti-inflammatory activity (Miller et al. 1993). A heparin-like substance able to bind antithrombin III (ATIII), isolated from the marine clams *Anomalocardia brasiliana*, had a high anticoagulant activity (Cesaretti et al. 2004).

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis C (Roskams et al. 2003; Seki et al. 2005). A number of investigators have previously demonstrated that antioxidants could prevent CCl4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation (Teselkin et al. 2000), suppressing ALT and AST activities (Lin and Huang 2000), and increasing an antioxidant enzyme activity (Kumaravelu et al. 1995). In China, kurarinone has been used to prevent liver disease for a long time, and one important reason is its antioxidant effect (Xu et al. 2006). Polysaccharides have been used for decades to stimulate the immune system and to fight against cancer. And several researches have shown that some polysaccharide possessed an antioxidant activity (Ghiselli et al. 1998; Luo et al. 2006). But few researches have done to investigate the protective effect of polysaccharides on oxidative liver injury. Chitosan, a cationic polysaccharide made from alkaline N-deacetylation of chitin, has an antioxidative effect on chronic carbon tetrachloride induced hepatic injury in rats (Jeon et al. 2003).

Our previous researches showed that the crude polysaccharide from *M. coruscus* can enhance the immunocompetence of normal mice through improving cellular immune function, humoral immune function, monocyte-macrophase function, and natural killer (NK) cell activity (Yao et al. 2005). Besides, it showed a protective effect on mouse spleen lymphocytes from oxidative damage. However, the structure of the polysaccharide remains unclarified. In view of the above findings, the aim of the present study was to isolate and purify the polysaccharide from *M. coruscus*, elucidate its structure, and investigate its protective effect on CCl4-induced liver injury in mice through serum ALT, serum AST, hepatic MDA, hepatic SOD, and histopathological changes.

**Results**

*Isolation and structural analysis of MP-I*

The yield of the water-soluble polysaccharide from *M. coruscus* was 2.14% of the fresh material. The polysaccharide was separated and sequentially purified through DEAE-Sepharose
Table I. GC-MS of alditol acetate derivatives from the methylated product of MP-I

<table>
<thead>
<tr>
<th>Methylated sugars (as alditol acetates)a</th>
<th>Type of linkage</th>
<th>Relative retention timeb</th>
<th>Molar (%)</th>
<th>Mass fragments (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4,6-Me4-Glc</td>
<td>Terminal Glcp</td>
<td>1.00</td>
<td>1.02</td>
<td>43, 45, 71, 87, 101, 117, 129, 145, 161, 205</td>
</tr>
<tr>
<td>2,3,6-Me3-Glc</td>
<td>1,4-Linked Glcp</td>
<td>1.33</td>
<td>7.81</td>
<td>43, 45, 87, 101, 113, 117, 129, 161, 233</td>
</tr>
<tr>
<td>2,3-Me2-Glc</td>
<td>1,4,6-Linked Glcp</td>
<td>1.70</td>
<td>1.08</td>
<td>43, 45, 85, 101, 117, 127, 159, 201, 261</td>
</tr>
</tbody>
</table>

a2,3,4,6-Me4-Glc = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-glucose, etc.
bRelative retention times of the corresponding alditol acetate derivatives compared to 2,3,4,6-tetra-O-methyl-D-glucose.

and Sepharose CL-6B, each giving a single elution peak, as detected by phenol-sulfuric acid assay.

MP-I appeared as white powder had a negative response to the Bradford method (Bradford 1976), and had no absorption at 280 nm or 260 nm in the UV spectrum, indicating the absence of protein and nucleic acid. The sulfate-carbazole method showed the absence of uronic acid (Bitter and Muir 1962), while BaCl2-gelatin method showed the absence of sulfate (Dodgson and Price 1962).

The high-performance liquid chromatography (HPLC) showed a single and symmetrical sharp peak (Figure 1), indicating that MP-I was a homogeneous polysaccharide. The HPLC profiles of glucose and standard Dextrans also showed single and symmetrical peaks. The ratio of the MP-I elution volume to void volume was 1:0.972, while the ratio of the MP-I elution volume to total volumes was 1:2.070. Correlation with the calibration curve of Dextran standards showed that the molecular weight of MP-I was ~1350 kDa.

Monosaccharide analysis conducted both by thin-layer chromatography (TLC) and by gas chromatography (GC) showed that MP-I was composed of glucose monomers.

The FTIR spectrum of MP-I indicated that it had an α-configuration of the sugar units, for there was absorption at 850/cm and no absorption at 890/cm for the β-configuration.

The fully methylated product of MP-I was hydrolyzed with acid, converted into alditol acetates, and analyzed by GC-MS. MP-I furnished three types of glucose derivatives in a relative molar ratio of 1.02:7.8:1.01 according to the peak areas (Table I).

On periodate oxidation, the polysaccharide of MP-I showed abundance periodate uptake. The consumption of HIO₄ was 1.12 mol, and the production of formic acid was 0.23 mmol per sugar residue, indicating the existence of monosaccharides that were terminal-linked, 1,4-linked or 1,4,6-linked. These results were in agreement with the results of methylation analysis.

NMR spectroscopy was used to complete the structural characterization of MP-I. Chemical shifts of individual residues were assigned (Table II) according to 1D(1H, 13C) and 2D 1H-1H COSY, HSQC, and HMBC experiments. The 1H NMR spectrum of MP-I (Figure 2) showed two anomeric protons at δ 5.570 and 5.169, which were assigned as (1→4)-α-D-Glcp (Residue 1) and (1→6)-α-D-Glcp (Residue 2), respectively. It was also confirmed that the sugar residues were α-glycosidically linked, which is consistent with the presence of an IR band 850.5/cm. The chemical shifts from δ 3.5 to 4.3 ppm were assigned to protons of carbons H-2 to H-6 of the glycosidic ring (Chauveau et al. 1996). In the 13C spectrum of MP-I (Figure 3), the anomeric carbon signals for the (1→4)-D-Glcp and (1→6)-D-Glcp residues were assigned at δ 100.27 and 98.89 ppm, respectively. The typical signal of O-substituted C-4 at δ 78.31 supported the high proportion of α-D-(1→4)-linkages

Table II. 1H NMR and 13C NMR chemical shifts of the polysaccharide recorded in D₂O for MP-I

<table>
<thead>
<tr>
<th>Sugar residue</th>
<th>1 (1→4)-α-d-Glcp</th>
<th>2 (1→6)-α-d-Glcp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.27</td>
<td>98.89</td>
</tr>
<tr>
<td>2</td>
<td>71.97</td>
<td>72.19</td>
</tr>
<tr>
<td>3</td>
<td>73.12</td>
<td>73.12</td>
</tr>
<tr>
<td>4</td>
<td>73.12</td>
<td>73.12</td>
</tr>
<tr>
<td>5</td>
<td>70.85</td>
<td>69.85</td>
</tr>
<tr>
<td>6(6a)</td>
<td>61.05</td>
<td>71.69</td>
</tr>
<tr>
<td>6b</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1. Elution profile of MP-I over HPLC chromatography, eluting with H₂O at a flow rate of 0.8 mL/min.

Fig. 2. 1H NMR spectra of MP-I in D₂O as an internal chemical shift standard at 30°C.

Fig. 3. 13C NMR spectra of MP-I in D₂O as an internal chemical shift standard at 30°C.
in a linear arrangement that was previously demonstrated by GC-MS. And branchings of C-6 were shown by the signals of O-substituted C-6 at δ 71.69 and of unsubstituted C-6 at δ 61.05 (Yang et al. 2005; Wu et al. 2006; Zhao et al. 2006).

On the basis of the above-mentioned results, it can be concluded that MP-I is composed of a repeating unit having the possible structure as shown in Figure 4.

**Protective effects on CCl₄-induced liver injury of a mouse**

We initially assessed the protective effects of MP-I on CCl₄-induced liver injury of mice. Serum ALT, AST levels, hepatic MDA, SOD levels, and histological changes in livers of mice were investigated after CCl₄ treatment.

Significant elevation of serum ALT and AST levels were revealed in the CCl₄-treated mice compared to the control ones (P < 0.01). The treatment with MP-I (50 mg/kg body weight (b.w.) and 100 mg/kg b.w.) could significantly reduce the serum ALT level (P < 0.01) and serum AST level (50 mg/kg b.w., P < 0.05; 100 mg/kg b.w., P < 0.01) compared with the CCl₄-treated animals. Kurarinone (30 mg/kg b.w.) could significantly lower the serum AST level (P < 0.01), but the difference of the serum ALT level was not so obvious compared with the CCl₄-treated animals (P > 0.05) (Table III).

Evaluation of hepatic MDA and SOD was carried out to estimate free radical injury on hepatic membranes. A significant increase in hepatic MDA production and decrease in the hepatic SOD activity were found in the liver of CCl₄-treated mice compared to the control group (P < 0.01). MP-I (50 mg/kg b.w. and 100 mg/kg b.w.) could significantly decrease the hepatic MDA level (50 mg/kg b.w., P < 0.05; 100 mg/kg b.w., P < 0.01) and increase the hepatic SOD activity (P < 0.01) compared with the CCl₄ group. Kurarinone (30 mg/kg b.w.) could significantly decrease the hepatic MDA level (P < 0.05) and increase the hepatic SOD activity (P < 0.05) compared with the CCl₄ group (Table IV).

The cells of the liver sections of a control mouse appear complete, without infiltrations and haemorrhagic signs (Figure 5A). Histological changes of the CCl₄-treated mouse appear centrilobular haemorrhagic alterations, hydropic degenerations, damaged cell membranes, and fatty change with inflammatory cells. The necrosis is massive, involving whole groups of lobules in their entirety (Figure 5B). In the kurarinone (30 mg/kg b.w.) group and MP-I (50 mg/kg b.w. and 100 mg/kg b.w.) groups, liver parenchyma was well preserved with radially arranged hepatocytes around the central vein, and regular sinusoidal structures were noticed without congestion compared with the CCl₄-treated ones (Figure 5C). The necrosis of the liver cells and the immigration of inflammatory cells were reduced in a dose-dependent manner in the MP-I groups. The swelling of the liver cells was still obvious in the MP-I (50 mg/kg b.w.) group (Figure 5D), while the liver cells in the MP-I (100 mg/kg b.w.) group were well preserved (Figure 5E).

**Discussion**

Today the separation and determination of the active chemical constituents is generally recommended for the standardization and quality control of Chinese traditional medicine and related investigations. Furthermore, the identification of major compounds in it may be helpful in delineating the pharmacological activity and the underlying mechanisms. In this study, we compared the hepatic protective effect of the polysaccharide MP-I with a well-known antihepatitis medicine, kurarinone.

The structural analysis revealed that MP-I was an α-D-glucan. Other species of the polysaccharide such as hebal fuzi (Aconitum carmichaeli), marine filamentous fungus (Phoma herbarum), and mycelium (Flammulina velutipes) were also reported to be α-glucans, and the polysaccharides from Aconitum carmichaeli and Phoma herbarum have an immunostimulating activity, while the polysaccharide from Flammulina velutipes can enhance the livability of the primary culture of mouse hepatocytes and decrease the release of ALT as well as apoptosis of hepatocytes after CCl₄ toxification (Pang et al. 2007).

Over the last 20 years, considerable attention has been focused on delineating some factors involved in the pathogenesis of liver injury. It appears that several forms of hepatic damage may be caused in part by oxidative stress, a condition caused by the formation of reactive oxygen species (ROS) (Arthur 1998; Kaplowitz 2002). Experimental liver injury in mice, induced by CCl₄ administration, is a widely used model to investigate the mechanisms behind hepatotoxicity or evaluate the efficacy of some natural antioxidants or drug (Zim et al. 2002; Chen, Jiang, et al. 2005; Chen, Taguchi, et al. 2005). CCl₄ is a xenobiotic which produces hepatotoxicity in human as well as in animals (Raymond and Plaa 1997). In fact it has been shown

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**Figure 4.** Predicted structure of the polysaccharide MP-I isolated from *Mytilus coruscus*.

**Table III.** Effect of MP-I on serum ALT, serum AST of CCl₄-treated mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum ALT (U/L)</th>
<th>Serum AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.90 ± 2.63*</td>
<td>112.99 ± 22.29*</td>
</tr>
<tr>
<td>CCl₄</td>
<td>108.93 ± 7.51</td>
<td>148.94 ± 31.85</td>
</tr>
<tr>
<td>CCl₄ + kurarinone (30 mg/kg)</td>
<td>93.03 ± 15.31</td>
<td>123.26 ± 39.75*</td>
</tr>
<tr>
<td>CCl₄ + MP-I (50 mg/kg)</td>
<td>53.86 ± 9.14*</td>
<td>123.26 ± 8.89*</td>
</tr>
<tr>
<td>CCl₄ + MP-I (100 mg/kg)</td>
<td>52.06 ± 7*</td>
<td>118.12 ± 27.97*</td>
</tr>
</tbody>
</table>

*P < 0.01 versus CCl₄ control. Values are means ± SD (n = 10).

**Table IV.** Effect of MP-I on hepatic MDA and hepatic SOD of CCl₄-treated mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatic MDA (mmol/mgprot)</th>
<th>Hepatic SOD (mmol/mgprot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.93 ± 0.84*</td>
<td>51.69 ± 0.55*</td>
</tr>
<tr>
<td>CCl₄</td>
<td>5.22 ± 0.58</td>
<td>48.86 ± 0.49</td>
</tr>
<tr>
<td>CCl₄ + kurarinone (30 mg/kg)</td>
<td>4.21 ± 0.71</td>
<td>51.91 ± 1.25*</td>
</tr>
<tr>
<td>CCl₄ + MP-I (50 mg/kg)</td>
<td>4.63 ± 0.33*</td>
<td>51.93 ± 1.57*</td>
</tr>
<tr>
<td>CCl₄ + MP-I (100 mg/kg)</td>
<td>4.22 ± 0.28*</td>
<td>52.29 ± 1.13*</td>
</tr>
</tbody>
</table>

*P < 0.01, *P < 0.05 versus CCl₄ control. Values are means ± SD (n = 10).
that the trichloromethyl radical (−CCl₃), which is formed in the metabolism of CCl₄ via the liver microsomal cytochrome P₄₅₀ system, reacts rapidly with molecular oxygen to produce the trichloromethylperoxy radical (−OOCCl₃). These radicals react with unsaturated fatty acids of phospholipids present in the cell membrane, initiating the lipid peroxidation in the liver cell, and to induce hepatotoxicity eventually (Brattin et al. 1985; Nadkarni and Souza 1988; Recknagel et al. 1989).

The results showed that CCl₄ administration caused severe acute liver damage in mice, demonstrated by significant elevation of serum ALT and AST levels, and classic histopathological changes. Moreover, it is found that CCl₄ injection induced oxidative stress in the liver, as evidenced by a significant increase of hepatic MDA contents and decrease of the SOD activity. Administration of the polysaccharide MP-I and kurarinone dose-dependently reduced the ALT and AST levels in serum, elevated the SOD activity and reduced MDA contents in livers of mice. The liver histopathological study also showed milder necrotic changes in the MP-I and kurarinone pretreated mice. Table IV presents the antioxidant activities of MP-I and kurarinone in mouse livers.

In fact the first step in the liver injury induced by CCl₄ is the formation of ROS that are then capable of further interactions that lead to the process of membrane peroxidation or other mechanisms of cell injury. So, ROS is postulated to play a role in the pathogenesis of CCl₄-induced liver injury. SOD is an important enzyme that disrupts superoxide radicals and is present in all cells with high amounts in erythrocytes. It is reported that one of the possible mechanisms for lowering of SOD is that the treatment of the mouse liver with CCl₄ concurrently induces both processes in acute injury and regeneration. Injury events are dominantly expressed in the early stage but this regeneration process is latent (Taniguchi et al. 2004). Therefore, the SOD was degraded or saturated to block the CCl₄-induced massive free radical production in the early stage. The pretreatment with MP-I could significantly elevate the hepatic SOD activity in the liver injury mice. So we speculate that MP-I particularly plays an effect on the early stage in CCl₄-induced liver injury through synthesis of SOD, which can prevent the accumulation of free radicals in CCl₄-induced liver injury. Besides, many current studies have established that some polysaccharides can enhance the antioxidant potency of animals through elevating the SOD activity (Chen et al. 2007; Li, Ma et al. 2007; Li, Zhou et al. 2007).

MP-I also showed the ability to prevent a CCl₄-induced increase of the hepatic MDA level, suggesting that MP-I inhibits lipid peroxidation. Lipid peroxidation is considered a critical mechanism of tissue damage occurring during hepatic failure (Comporti 1985). Tissue MDA assay is an indicative method extensively used to evaluate lipid peroxidation. The pretreatment with MP-I (50 mg/kg b.w.) could significantly reduce the hepatic MDA, indicating a significant attenuation of liver injury.

These findings indicated that administration of MP-I decreased lipid peroxidation, improved antioxidant status, and thereby prevented the damage to the liver and leakage of enzymes ALT and AST.

In conclusion, the present study showed that MP-I can improve significantly in a dose-dependent manner in the liver of mice treated with CCl₄. Induction of the SOD activity and decreased MDA concentration might well provide an important protective effect against CCl₄-induced oxidative injury, but further studies should be carried out to clarify the detailed mechanisms in the future.
Materials and methods

Isolation and purification of the polysaccharides

Adult seawater clams, *M. coruscus*, were collected from their natural environment from Zhoushan City in Zhejiang Province, and authenticated by Professor S.L. Zhao, Marine Science and Technology Institute, Zhejiang Ocean College. Ground fresh mussel meat (700 g) was extracted with hot water (100°C) for 5 h. Insoluble material was removed by filtration, and the filtrate was deproteinized four times using the Sevag method (Staub 1965). Add threefold volume of cold ethanol to precipitate the glycans after standing at 4°C overnight. The precipitate obtained by subsequent centrifugation (3000 g × 10 min), after washed sequentially with ethanol, acetone, and ether, was dried in vacuo to give a crude product (31 g). The crude product was subjected to a DEAE-Sepharose anion-exchange column (2.0 × 40 cm), eluting at a flow rate of 32 mL/h successively with distilled water. The fraction (21 g) collected from the main peak was further fractionated on a Sephrose CL-6B column (1.0 × 100 cm) eluted with distilled water at a flow rate of 16 mL/h and lyophilized to give 14.2 g of the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was kept at 190°C.

**Determination of molecular weight**

The homogeneity and molecular weight of the polysaccharide was detected on a Bio-Rad 1330 HPLC pump and a Shodex RI-71 refractive index detector with a flame ionization detector. High purity helium was used as the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was kept at 190°C. The polysaccharide was hydrolyzed in 2 mol/L TFA (4 mL, 110°C, 2 h). Reduction of the hydrolysates with NaBH₄, followed by acetylation with Ac₂O. The alditol acetates were analyzed by GC-MS (Sweet et al. 1975; Deng and Tian 2002).

**Monosaccharide identification**

The polysaccharide (3 mg) was hydrolyzed (2 mL of 2 M TFA, 120°C, 2 h) in a sealed test tube, then rotary evaporated to completely remove TFA, the hydrolysate dissolved in water (0.5 mL), and 4 µL was analyzed by TLC on a precoated PEI-cellulose plate. The remaining hydrolysate was dissolved in H₂O (5 mL) and reduced with NaBH₄ (30 mg, 12 h), treated with AcOH (1.5 mL), dried. Methanol (3 mL) was added and the mixture was dried three times, the residue acetylated with Ac₂O (5 mL, 120°C, 1 h) (Johnes and Albersheim 1972; Blakeney et al. 1983), dried and analyzed by GC on the DB-5 capillary column with a flame ionization detector. High purity helium was used as the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was kept at 190°C for 3 min, following injection of 1 µL samples in a split ratio of 1:10 and then raised at 4°C/min to 230°C.

**NMR spectroscopy**

The sample was dissolved in 99.99% D₂O. Spectra were recorded at 323 K on a Bruker AMX-600 spectrometer operating at 600.13 MHz for ¹H and 150.90 MHz for ¹³C. Chemical shifts are given in ppm, with acetone standard (2.225 ppm for ¹H and 31.5 ppm for ¹³C). The 2D homonuclear ¹H-¹H COSY spectra were acquired using the double quantum filtered (DQF) method with a Bruker standard pulse sequence. The HMBC and HSQC data were also obtained.

**Methylation analysis**

Sample (20 mg) in DMSO (5.0 mL) was methylated by the modified Ciucanu method (Parente et al. 1985). The methylated polysaccharide was hydrolyzed in 2 mol/L TFA (4 mL, 110°C, 2 h). Reduction of the hydrolysates with NaBH₄, followed by acetylation with Ac₂O. The alditol acetates were analyzed by GC-MS (Sweet et al. 1975; Deng and Tian 2002).

**Periodate oxidation**

Sample (50 mg) was dissolved in 0.015 mol/L NaIO₄ (100 mL) and kept in the dark at 4°C (Wu et al. 2005; Mondal et al. 2006), and the consumption of NaIO₄ was measured by a spectrophotometric method. The solution of periodate product (20 mL) was sampled to calculate the yield of HCO₂H by titration with 0.01 M NaOH.

**Acute liver injury protection activity**

Male Kunming mice 4 weeks old, weighing 20 ± 2 g, were purchased from Laboratory Animal Subdivided Center of Second Military Medical University (Shanghai, China). The animals were maintained under climate-controlled conditions with a 12-h light/dark cycle, and were fed standard rodent chow and water. Mice were randomly subdivided into five groups (10 for each): (A) CTRL; (B) CCl₄; (C) CCl₄ + kurarinone (30 mg/kg b.w.); (D) CCl₄ + MP-I (50 mg/kg b.w.); (E) CCl₄ + MP-I (100 mg/kg b.w.).

Groups 1 and 2 were intraperitoneally injected with saline at a dose of 0.1 mL/kg b.w. Group 3 were intraperitoneally injected with kurarinone injection at a dose of 30 mg/kg b.w. as a positive control group. Group 4 and Group 5 were intraperitoneally injected with MP-I at the dose of 50 mg/kg b.w. and 100 mg/kg b.w., respectively. And all groups were treated once a day. One hour after the seventh administration, Group 1 was injected with olive oil at a dose of 10 mL/kg b.w. and other groups were injected with a 0.2% CCl₄ olive oil solution at the same dose, intraperitoneally. Immediately after the treatment, the chow was taken away.

Twenty-four hour after CCl₄ treatment, blood was collected from the inner canthus cava and separated into serum to evaluate the ALT and AST levels using commercial clinical test kits (ALT/GPT and AST/GOT). Then the animals were sacrificed and the livers were quickly removed. The caudal portion of the livers were homogenized with 10 volumes (w/v) of a 0.9% NaCl solution using an ultra-turrax homogenizer and centrifuged at 3000 × g for 10 min at 4°C. Then, the supernatants were collected and used to evaluate the hepatic MDA and SOD levels using commercial clinical test kits.
the protein content of the supernatants was determined by the Bradford method.

Statistical analysis
The results obtained for each group of mice tested was expressed as the mean ± SD of 10 values. Statistical analysis of the data was performed by one-way ANOVA. Differences were considered statistically significant when \( P < 0.05 \).

Acknowledgements
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Conflict of interest statement
None declared.

Abbreviations
ALT, alanine aminotransferase; AST, aspartate aminotransferase; FTIR, Fourier transform infrared spectroscopy; HPLC, high-performance liquid chromatography; MDA, malondialdehyde aldehydes; MP-I, *Mytilus coruscus* polysaccharide I; NMR, nuclear magnetic resonance; SOD, superoxide dismutase; TLC, thin-layer chromatography.

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