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# Antioxidant and hepatoprotective effects of pigment-protein complex from *Chlorella vulgaris* on carbon tetrachloride-induced liver damage *in vivo*

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## ABSTRACT

The antioxidant and hepatoprotective activities of pigments in the natural form of pigment-protein complex were investigated. Pigment-protein complex (PPC) was isolated from *Chlorella vulgaris* through thylakoid protein solubilization, anion exchange chromatography and gel filtration chromatography. PPC possessed a chlorophyll : lutein : protein ratio of 94 : 153 : 100 (w/w/w) according to HPLC and spectrophotometry analysis. Various antioxidant evaluation systems were used to evaluate the antioxidant activity of PPC *in vitro*. Results showed that PPC exhibited significant DPPH radical scavenging activity with $IC_{50}$ of 313 μg $mL^{-1}$. Distinct $Fe^{2+}$ ions chelating activity, reducing capacity and lipid peroxidation inhibition activity were observed at the concentration of 1 mg $mL^{-1}$. For the hepatoprotective effects, administration of PPC at different concentration (50, 100 mg $kg^{-1}$ BW) could significantly decrease the carbon tetrachloride-induced elevation of hepatosomatic index. The increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were attenuated with PPC pretreatment. In addition, PPC effectively restored the suppressed hepatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities. Moreover, PPC significantly reduced the formation of malondialdehyde (MDA). The results obtained from this study clearly verified the hepatoprotective effect of PPC on $CCl_4$-induced hepatotoxicity *in vivo*, suggesting the potential of PPC to be exploited as dietary supplements against free radicals oxidation and enhancing resistivity against oxidative stress in human body.


**Keywords** Pigment-protein complex; Chlorella; Isolation; Antioxidant; Hepatoprotective effect

## 1. Introduction

Free radicals such as superoxide anion radical ($\cdot O_2^-$) and hydroxyl radical ($\cdot$OH) are high reactive species with single and unbalanced electrons, which are involved in biological molecules oxidation process, and will cause many adverse effects on food and biological systems. [1] In human organ, free radicals, which are inevitably produced through oxidative metabolism, induce several diseases like arteriosclerosis and cancer. Liver damage is a widespread disease which can be caused by several compounds, such as ethanol, $CCl_4$ and bromobenzene. The mechanism of liver injury induced by chemical compounds is thought to involve excess free radicals generation and lipid peroxidation. [2] It has been a hot topic to search safe antioxidant in the field of biochemical nutrition to prevent free radical-induced damage. [3]

Natural pigments, extracted from animals, plants and microorganisms, are one of the most important antioxidant systems that can neutralize free radicals of the cells. [4, 5] It showed that high radiant energy presumably increased carotenoid concentration in the cells to prevent photooxidation [6] and some carotenoids strongly contributed to the protection of plants against photooxidative damage as direct quenchers of reactive oxygen species (ROS). [7] The antioxidant activity of carotenoids such as luteins may be due to the conjugated double bonds and the phenolic hydroxyl groups on both ends of their chemical structures. [8] Chlorophylls are also important antioxidants. They can act as lipid antioxidants in stored edible oils [9] and prevent oxidative DNA damage and lipid peroxidation both by reducing ROS and chelating metal ions. [10] However, solvent extraction is the main industrially applicable method for pigment extraction, [11] and consequently, the massive use of organic solvent goes against

environmentally friendly and destroys the natural matrix of pigments. Actually, hydrophobic pigments in cells are found in associated with the hydrophobic domains of lipid-protein complex, [12] and they play the function of photosynthesis in the form of light-harvesting complex (LHC) in most plants and algae. [13] Previous studies of LHCs mainly focused on their pigment composition, structure and energy transfer, [14, 15] by contrast, the functional activities of pigments in the natural form of pigment-protein complex are rarely reported.

Marine algae, which have traditionally formed part of the Oriental diet, especially in China, Japan and Korea, have been becoming a hot research topic because of their biological substances. [16] *Chlorella vulgaris,* which belongs to marine microalgae, contains a large number of active substances beneficial to human body, such as pigment, fat, unsaturated fatty acids, proteins, polysaccharides, squalene et al. It usually possesses 0.2-0.5 % of lutein, 2-4 % of chlorophyll and the protein content reaches up to 50 % (dry weight). [17] It would be interesting to isolate the natural matrix of pigments extract through aqueous extraction instead of organic solvent, and investigate the activities of pigments in the natural form of pigment-protein complex (PPC). Therefore, this study aims to isolate the natural pigment-protein complex from *Chlorella vulgaris* and evaluate its antioxidant activities *in vitro* and hepatoprotective effects in $CCl_4$-induced hepatotoxicity *in vivo*. The study is of significance in suggesting that natural protein-based pigment complex has the potential in making dietary supplements against oxidation and increasing resistivity against oxidative stress in human body.

## 2. Materials and Methods

### 2.1. Materials

The material of algae *Chlorella vulgaris* STIO02 was kindly provided by Third Institute of Oceanography, State Oceanic Administration, P. R. China and was stored at - 20 °C before used.

Toyopearl DEAE-650M was the product of TOSOH Co. (Japan). Sephadex G-50 Fine was purchased from GE Healthcare (USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) and lutein were obtained from Sigma Chemical Co. (USA). Bicinchoninic acid (BCA) protein kit and all the kits for biochemical indexes analysis used in the animal experiment were the products of Nanjing Jiancheng Bioengineering Institute (China). Methanol and acetonitrile used in liquid chromatography were of HPLC grade. All other chemicals and reagents used were of analytical grade and commercially available.

### 2.2. Solubilization of thylakoid proteins

Algae *Chlorella vulgaris* were broken in phosphate buffer (20 mM, pH 7.0) by ultrasonic wave in ice bath. Cell debris was removed by centrifugation at 10000 rpm for 10 min at 4 °C. Then 25 % saturation ammonium sulfate precipitation was applied to collect the membranes. Here, CHAPS, a zwitterionic detergent that can well maintain protein activity, was used to solubilize the intrinsic membrane-associated pigment-protein complex. The membrane fraction was re-suspended in Tris-HCl buffer (20 mM, pH 8.0) and 1 % of CHAPS was added. The mixture was stirred in dark for 1 h at 4 °C and then centrifuged at 12500 rpm for 30 min. The supernatant was collected for further purification.

### 2.3. Isolation and purification of pigment-protein complex

Toyopearl DEAE-650M column (Φ 1.6 × 20 cm) was previously equilibrated with Tris-HCl buffer (20 mM, pH 8.0) containing 8 mM of CHAPS. The solubilized thylakoid proteins were loaded onto the column and then washed with the same buffer followed by a linear gradient of 0-0.5 M NaCl with a flow rate of 0.5 mL $min^{-1}$ and 10 min $tube^{-1}$. The absorbance of all fractions was monitored at 430 nm and 280 nm, respectively. The pigment protein fractions were pooled and concentrated by ultrafiltration. The concentrate was then applied to a Sephadex G-50 column (Φ 1.6 × 100 cm) that was pre-equilibrated with Tris-HCl buffer (20 mM, pH 8.0) containing 8 mM of CHAPS and 50 mM of NaCl. The column was run with the same buffer and the flow rate was 0.3 mL $min^{-1}$. The absorbance of the fractions was monitored and the eluted fraction containing pigment-protein complex were collected.

## 2.4. Characterization of pigment-protein complex

### 2.4.1. Absorption spectroscopy

Absorption spectrum of pigment-protein complex was recorded with a U-2910 spectrophotometer (HITACHI Co., Japan) at room temperature.

### 2.4.2. Chlorophyll determination

Chlorophyll was determined as described by Arnon. [18] The sample was vigorously shaken with 4 volume of acetone and kept in dark for 20 min, then centrifugation at 12000 rpm for 5 min was applied. The absorbance of the supernatant at 645 and 663 nm was measured respectively. Content of chlorophyll was calculated according to the following equation:

$$\text{Chlorophyll } (\mu g\ mL^{-1}) = 20.2\ A_{645} + 8.02\ A_{663}.$$

### 2.4.3. Lutein analysis

Five volumes of methanol was added to the complex solution, followed by centrifugation to

remove precipitate. The pigment extraction was applied to a Shimadzu LC-20A system equipped with a Unimicro SP-120-5-C18-AP (4.6 mm × 250 mm, 3 μm) column. The mobile phase was 80 % of methanol and 20 % of acetonitrile and the absorbance was monitored at 443 nm.

2.4.4. Protein determination

The protein component of pigment-protein complex was separated by the method described by Maxwell. [19] 50 μL of the sample was mixed with 1 mL of 90 % (v/v) acetone followed by centrifugation at 12000 rpm for 5 min to removed pigments. The pellet was re-suspended in 50 μL of 2 % (w/v) SDS and then heated at 60 °C for 30 min. The protein content was quantified using bicinchoninic acid kit according to the protocol.

## 2.5. Antioxidant activity *in vitro*

2.5.1. DPPH radical scavenging activity

DPPH radical scavenging activity was tested as described [20] with some modifications. 1 mL of PPC was mixed with 1 mL of ethanol solution of DPPH (0.1 mM) and then kept in dark for 30 min. The absorbance was measured at 517 nm. A control sample containing 1 mL of DPPH solution and 1 mL of distilled water was prepared. The antioxidant activity of the sample was evaluated by the scavenging rate of DPPH radical with the following equation:

$$\text{DPPH radical scavenging activity } (\%) = (A_{control} - A_{sample})/A_{control} \times 100 ,$$

where $A_{sample}$ and $A_{control}$ were the absorbance of sample and control group, respectively.

2.5.3. Metal chelating activity

The metal chelating activity was determined according to the method of Dinis [21] with some modifications. 1 mL of PPC was mixed with 4.7 mL of $H_2O$ and then added to 0.1 mL of 2

mM $FeCl_2$. Deionized water in the same volume instead of PPC was used in a control experiment. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. The mixture was shaken vigorously and then kept in room temperature for 20 min and the absorbance was measured at 562 nm. The metal chelating activity of samples was calculated by the following equation:

$$\text{Metal chelating activity } (\%) = (A_{control} - A_{sample})/A_{control} \times 100,$$

where $A_{sample}$ and $A_{control}$ were the absorbance of sample and control group, respectively.

2.5.4. Reducing capacity

The reducing capacity of PPC was estimated by using the method of Oyaizu. [22] 1 mL of PPC was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % of potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 mL of 10 % trichloroacetic acid was added. The mixture was centrifuged at 1000 × *g* for 10 min and 2.5 mL of the supernatant was mixed with 2.5 mL distilled water and 0.5 mL of 1 % $FeCl_3$. The absorbance was measured at 700 nm for 10 min later. Increased absorbance of the reaction mixture indicated increased reducing capacity.

2.5.5. Lipid peroxidation inhibition activity

The lipid peroxidation inhibition activity of PPC was measured in a linoleic acid emulsion system according to the method introduced by Osawa and Namiki [23] with some modification. Briefly, PPC was dissolved in distilled water to a concentration of 1 mg/mL and then mixed with 2 mL of ethanol, 26 μL of linoleic acid and 2 mL of phosphate buffer (50 mM, pH 7.0). The mixture was incubated in a colorimetric tube with plug at 40 °C in dark. Here, BHT at 1 mg/mL was used as positive control. The degree of oxidation was measured at 24 h intervals

using the ferric thiocyanate (FTC) method of Mitsuda. [24] 100 μL of the reaction solution was added to a solution of 4.7 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride solution in 3.5 % of HCl. After 3 min, the degree of color development that represented the linoleic acid oxidation was measured spectrophotometrically at 500 nm.

### 2.6. Effect on $CCl_4$-induced hepatic injury in mice

#### 2.6.1. Animals and treatments

Fifty male Kunming mice of 29-30 g weight were purchased from Slac Laboratory Animal Center (Shanghai, China). The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Fujian Medical University (Fujian, China). Fifty mice were randomly divided into five groups with ten mice in each group. Group 1 served as blank control. Group 2 was the $CCl_4$ control group in which mice were treated with $CCl_4$ alone. Group 3 was a positive control group in which mice were administrated lutein at 20 mg/kg body weight (BW) along with $CCl_4$. Group 4 and 5 were the treatment groups in which mice were administrated PPC at 100 mg $kg^{-1}$ BW and 50 mg $kg^{-1}$ BW respectively along with $CCl_4$. PPC suspended in deionized water and lutein suspended in peanut oil were given to the mice at the setting dose once daily by gavage for 11 days. Mice in group 1 and 2 were orally given the same volume of deionized water. One hour after substances administration at the 10 th day, all the groups were treated with single dose of 0.1 % of $CCl_4$ solution in peanut oil (10 mL $kg^{-1}$ BW) by gavage except for group 1 which was treated with the same volume of peanut oil. Thirty-six hours after $CCl_4$ treatment, mice were sacrificed after being decapitated.

#### 2.6.2. Determination of hepatosomatic index

Mice were weighted and then tenderly sacrificed. The liver tissues were taken, washed and weighed. Hepatosomatic index was defined as the ratio of wet liver weight to body weight.

2.6.3. Analysis of serum biochemical indexes

Blood samples of each mouse were taken into EDTA-anticoagulant tubes from the orbit before sacrificed. After centrifugation, the serum was collected and activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed according to the protocols of relevant diagnostic kits.

2.6.4. Analysis of hepatic biochemical indexes

Liver tissues were homogenized in cold normal saline. The supernatant was collected after centrifugation at 3000 rpm for 10 min at 4 °C, and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were determined with the corresponding diagnostic kits. The total protein content of liver homogenate was measured according to the method of Bradford [25], using bovine serum albumin as the standard.

### 2.7. Statistical analysis

All data are presented as means ± standard deviation (SD). Statistical evaluation was carried out with IBM SPSS 19.0 software. Comparisons of multiple treatment conditions were analyzed by one-way analysis of variance (ANOVA) with Duncan's test for post hoc analysis and $p < 0.05$ values were considered as statistically significant.

## 3. Results and discussion

### 3.1. Isolation of pigment-protein complex form Chlorella

Crude pigment-protein complex extract of Chlorella was obtain by aqueous extraction with the addition of CHAPS and chromatography was employed to improve the purity of pigment-protein complex. Through DEAE anion-exchange chromatography, the crude extract was divided into four fractions (Fig. 1a). Fractions P2 exhibited obvious absorbance at both 280 nm and 430 nm and was dark green colored. For further purification, fraction P2 was concentrated by ultrafiltration and applied to a Sephadex G-50 column. Three fractions were obtained as shown in Fig. 1b. Pigment-protein complex was mainly contained in fraction P2-2 while the other fractions contained only proteins. The absorbance spectrum of P2-2 is shown in Fig. 2. The profile was some similar to that of crude extract, indicating the reasonable recovery obtained after isolation through chromatography. The profile included obvious chlorophyll absorbance at 430 and 672 nm. The peak at 482 nm indicated the presence of lutein when compared with the standard marker pigments (data not shown).

At the same time, increased purity ratio of $A_{430}/A_{280}$ and $A_{482}/A_{280}$ of PPC could be observed. Through two-step chromatography, the relative absorbance of $A_{430}/A_{280}$ was enhanced to 3.17 compared to 1.15 of crude extract, the purity ratio increased by 3 times (Table 1a). The content of chlorophyll was determined to be 0.94 g $g^{-1}$ protein by spectrophotometry, there was 2.6 times increased in purity ratio through isolation procedure (Table 1b). The major carotenoid existed in the fraction was confirmed to be lutein by HPLC and was quantified to be 1.53 g $g^{-1}$ protein.

### 3.2. Antioxidant activity of pigment-protein complex *in vitro*

To evaluate the antioxidant activity of pigment-protein complex from *Chlorella vulgaris*, different antioxidant parameters *in vitro* were determined respectively.

3.2.1. Free radical scavenging activity

The free radical scavenging activity of PPC was determined by the scavenging rate of DPPH radical. DPPH radical has an unpaired valence electron at one atom of nitrogen bridge and it can accept a hydrogen radical or electron to become stable with the color changing from dark purple to colorless and the maximum absorbance at 517 nm reducing. [26] As results shown in Fig. 3a, PPC could scavenge DPPH radical effectively in a dose-dependent manner. The $IC_{50}$ value of PPC against DPPH radical was determined to be 313 μg $mL^{-1}$. Previous studies showed that pigments such as lutein and chlorophyll could provide hydrogen or electron to reduce free radicals such as DPPH. [10] PPC, which possesses high content of lutein and chlorophyll, presumably trapped DPPH radical as an electron or hydrogen donor.

3.2.2. Metal chelating activity

Some transition metals could trigger the formation of free radicals and chain reaction through electron transport. $Fe^{2+}$ is one of the most important pro-oxidant since it can catalyze Fenton reaction to stimulate lipid peroxidation and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. [27] Thus, the chelation of $Fe^{2+}$ can retard metal-catalyzed oxidation. Results showed that PPC could chelate $Fe^{2+}$ and block the formation of $Fe^{2+}$–ferrozine complex dose-dependently (Fig. 3b). The percentage of $Fe^{2+}$ chelated by PPC reached up to 45 % at a concentration of 1 mg $mL^{-1}$. The chelation of PPC was considered to be attributed to the chlorophyll in the complex. Chlorophylls are $Mg^{2+}$–porphyrin derivatives and porphyrin was found to be the essential structure for antioxidant activity of chlorophyll derivatives. Chlorophylls could chelate $Fe^{2+}$ by virtue of

substitution of the $Fe^{2+}$ for the $Mg^{2+}$ in the porphyrin. [10]

3.2.3. Reducing capacity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the measurement of reducing capacity of PPC, the transformation of $Fe^{3+}$ to $Fe^{2+}$ in the presence of the complex was investigated. As shown in Fig. 3c, the reducing capacity of PPC increased with increasing concentration. At the concentration of 1 mg $mL^{-1}$, PPC exhibited reducing capacity of 0.33. Results indicated that PPC has the potential in reacting with free radicals and blocking radical chain reactions.

3.2.4. Lipid peroxidation inhibition activity

Lipid peroxidation is a complicated process, which is thought to proceed via radical mediated abstraction of hydrogen atoms from methylene carbons, resulting in various highly reactive electrophilic aldehydes, such as peroxyl and alkoxyl radicals that can form pre-existing lipid peroxide to initiate lipid peroxidation. [28] The inhibition effect of PPC towards lipid peroxidation was investigated in a linoleic acid emulsion system. Results showed that PPC could almost completely suppress lipid peroxidation at 1 mg $mL^{-1}$, which was comparable to the effect of BHT at the same concentration. The antioxidant effects of chlorophyll and lutein on the autoxidation of oils were verified previously. [8, 9] Present study showed that PPC exhibited multiple antioxidant activities, supposing the possibility that the action as lipid peroxidation protector of PPC may be related to its radical scavenging activity, iron binding capacity and reducing capacity.

### 3.3. Effect on $CCl_4$–induced hepatic injury in mice

$CCl_4$ is a widely known hepatotoxicity revulsant, the metabolism of which results in the rapid

generation of reactive radicals such as trichloromethyl peroxyl radical ($CCl_3O_3\cdot$) and then initiate lipid peroxidation, leading to cell membrane damage, intracellular enzyme leakage and even cell necrosis. It has been demonstrated that the hepatoprotective effects may be associated with antioxidant capacity. [29] The natural pigment, carotenoid lutein at 20 mg $kg^{-1}$ BW, which has been verified to have significant hepatoprotective effect on $CCl_4$-induced liver damage, was used as positive control. [30]

3.3.1. Effect of PPC on mice hepatosomatic index

As shown in Fig. 4, the hepatosomatic index of the mice treated with $CCl_4$ increased markedly when compared to the control group ($p < 0.05$), which was the symptom of fatty liver. The biotransformed metabolites of $CCl_4$, trichloromethyl radical ($CCl_3\cdot$) can result in accumulation of triglycerides (TG) in liver and the development of fatty liver through blocking the protein synthesis and lipid metabolism disorder. [31] Pretreatment with PPC at different doses (50, 100 mg $kg^{-1}$ BW) could significantly decrease the $CCl_4$-induced increase of hepatosomatic index and the effects were comparable to the positive control (Fig. 4).

3.3.2. Effect of PPC on serum ALT and AST

Fig. 5 presents the effects of PPC on serum ALT and AST. Mice treated with $CCl_4$ alone showed serious acute liver damage, as revealed by a significant increase in the serum ALT and AST ($p < 0.05$), which indicated the loss of hepatocyte membrane integrity and the release of transaminase from cytoplasm to serum. Treatment with PPC at 100 and 50 mg $kg^{-1}$ BW attenuated the increase in ALT activity by 29.3 % and 13.1 % respectively, and exhibited 35.5 % and 24.11 % suppression in AST activity, indicating the stabilization of cell membrane and mitigation of hepatic damage caused by $CCl_4$. Besides, the effects of PPC at 100 mg $kg^{-1}$

BW on the serum ALT and AST were superior to the positive control.

3.3.3. Effect of PPC on hepatic MDA, SOD, CAT and GSH-Px

Treatment with $CCl_4$ promoted lipid peroxidation in liver, the extent of which was specified by the level of MDA. MDA is the end-product of lipid peroxidation and is widely used as a marker of lipid peroxidation mediated by free radicals. [2] Enhanced hepatic MDA level could be observed after $CCl_4$ treatment (Fig. 6a). Pretreatment of PPC at 50, 100 mg $kg^{-1}$ BW could reduce the formation of MDA dose-dependently, indicating that PPC could inhibit lipid peroxidation induced by $CCl_4$ in liver.

Antioxidant enzymes such as SOD, CAT and GSH-Px act as the first line of defense against free radicals induced oxidative stress. SOD is an effective defense enzyme that catalyze reduction of super anions into $H_2O_2$ and $O_2$, and $H_2O_2$ can be further converted into $H_2O$ and $O_2$ by CAT and GSH-Px. [32] The status of these enzymes is an appropriate indirect assessment of the pro-oxidant-antioxidant status in tissues. [29] Results showed that the activities of SOD, CAT and GSH-Px were remarkable decreased after exposed to $CCl_4$ by 13.1 %, 32.1 % and 17.3 % respectively (Fig. 6b-d), indicating anabatic oxidative damage to the liver. However, the suppressed enzyme activities could be restored by PPC pretreatment and the effects were significant at the concentration of 100 mg $kg^{-1}$ BW ($p < 0.05$), implying that the hepatoprotective effects of PPC on $CCl_4$-induced liver damage result from the stabilization in intracellular antioxidant defense systems.

The antioxidant activity of an antioxidant compound has been attributed to various mechanisms, among which are radical scavenging, binding of transition metal ion catalysts, reductive capacity, prevention of chain initiation, decomposition of peroxides and prevention

of continued hydrogen abstraction. [27] The results obtained from this study are clearly indicate that PPC had powerful antioxidant activity against various antioxidant systems *in vitro*. Protein provided the pigments with good stability and solubility in aqueous solution, and the hepatoprotective effects of PPC might be attributed to the various antioxidant effects of pigments.

## 4. Conclusions

Antioxidant and hepatoprotective activities of pigments in the form of pigment-protein complex were studied in present study. Results showed that the natural pigment-protein complex isolated from *Chlorella vulgaris* exhibited significant antioxidant activity *in vitro*, including DPPH radical scavenging, metal chelation, reducing capacity and lipid peroxidation inhibition. Moreover, PPC manifested discernible protective action in $CCl_4$-induced hepatotoxicity *in vivo*, indicating that PPC can be a promising candidate for the use of dietary supplements against free radical-induced damage.

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Table 1a Chlorophyll and purity ratio of pigment-protein complex from *Chlorella vulgaris*

| | Chlorophyll ($g\ g^{-1}$ protein) | Purity ratio ($A_{430}/A_{280}$) |
|---|---|---|
| Crude extract | 0.40 | 1.15 |
| PPC | 0.94 | 3.17 |

Table 1b Lutein and purity ratio of pigment-protein complex from *Chlorella vulgaris*

| | Lutein ($g\ g^{-1}$ protein) | Purity ratio ($A_{482}/A_{280}$) |
|---|---|---|
| Crude extract | 0.69 | 0.37 |
| PPC | 1.53 | 0.95 |

## Figure captions

**Fig. 1**

Purification of pigment-protein complex from Chlorella. a) DEAE anion-exchange chromatography, b) Sephadex G-50 gel filtration chromatography.

**Fig. 2**

Absorption spectrum of pigment-protein complex.

**Fig. 3**

Antioxidant activity of PPC *in vitro*. a) DPPH radical scavenging activity, b) metal chelating activity, c) reducing capacity, d) Lipid peroxidation inhibition activity.

**Fig. 4**

Effect of PPC on mice hepatosomatic index.

* Statistical significance $p < 0.05$, compared with $CCl_4$-treated group.

$^{\triangle}$ Statistical significance $p < 0.05$, compared with control group.

**Fig. 5**

Effect of PPC on the activities of serum a) ALT and b) AST.

* Statistical significance $p < 0.05$, compared with $CCl_4$-treated group.

$^{\triangle}$ Statistical significance $p < 0.05$, compared with control group.

**Fig. 6**

Effect of PPC on the activities of hepatic a) MDA, b) SOD, c) CAT and d) GSH-Px.

* Statistical significance $p < 0.05$, compared with $CCl_4$-treated group.

$^{\triangle}$ Statistical significance $p < 0.05$, compared with control group.

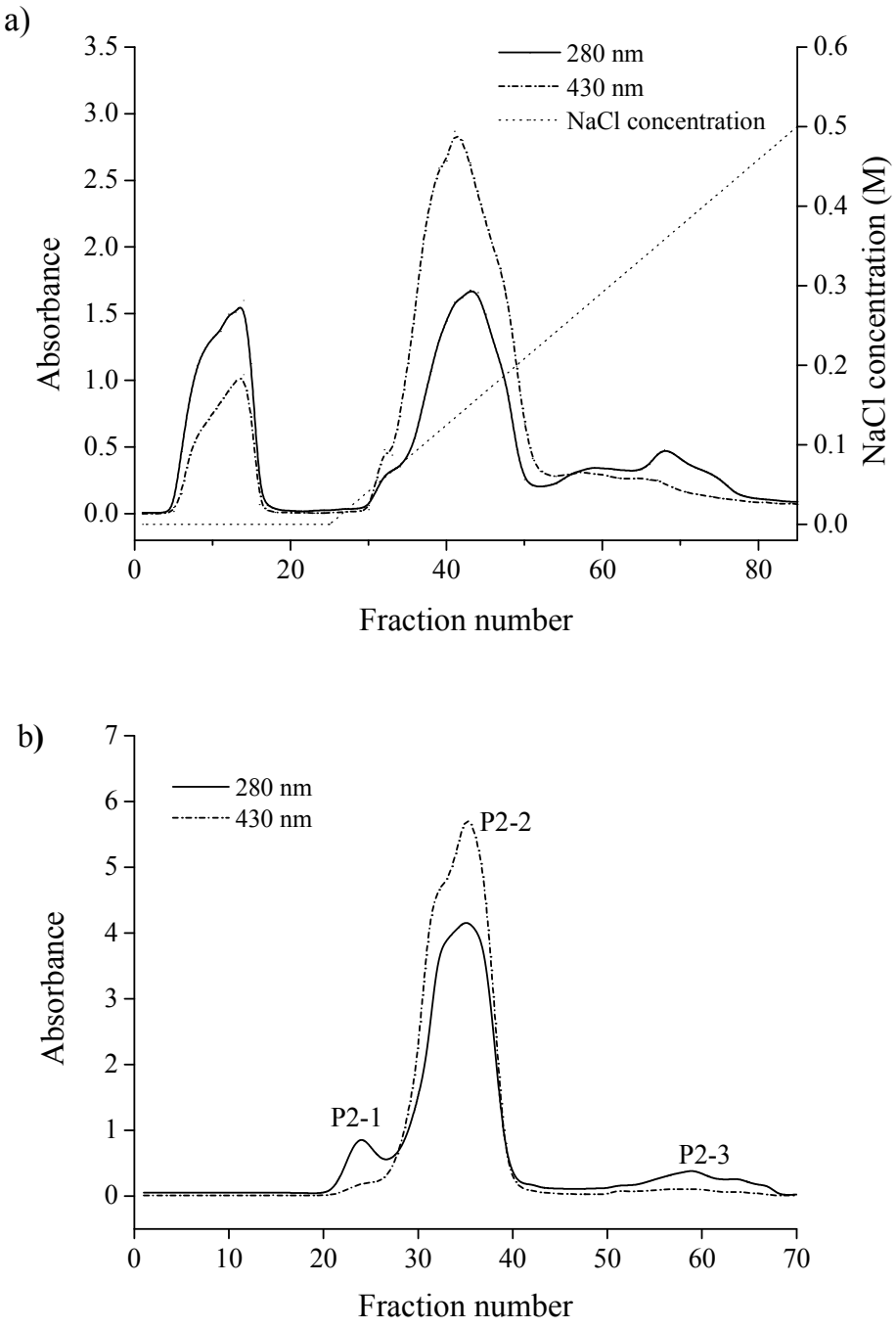



Fig. 1

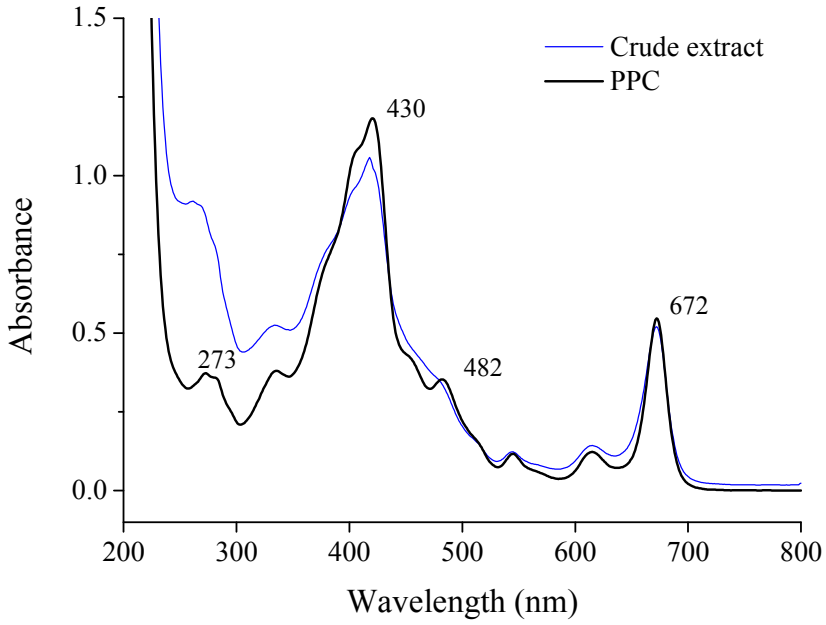
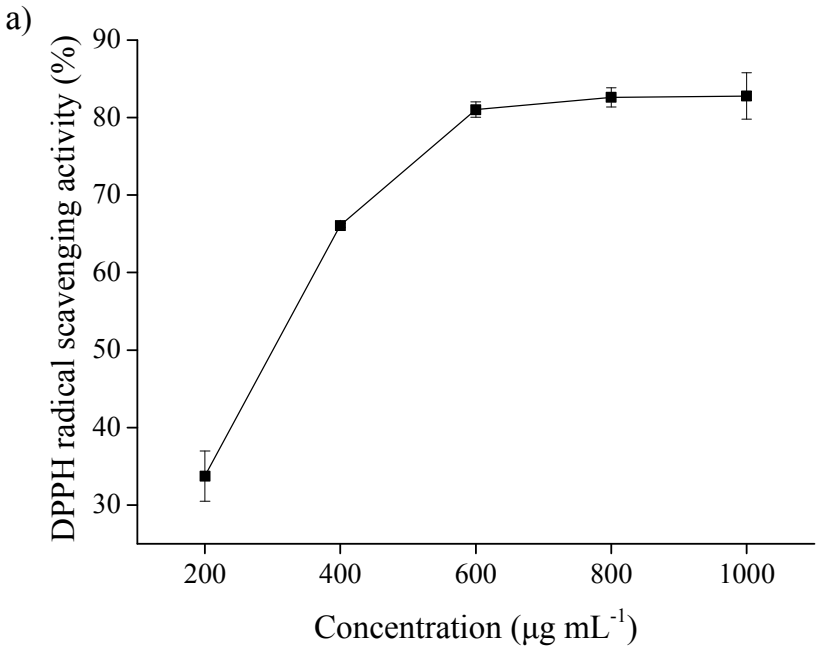
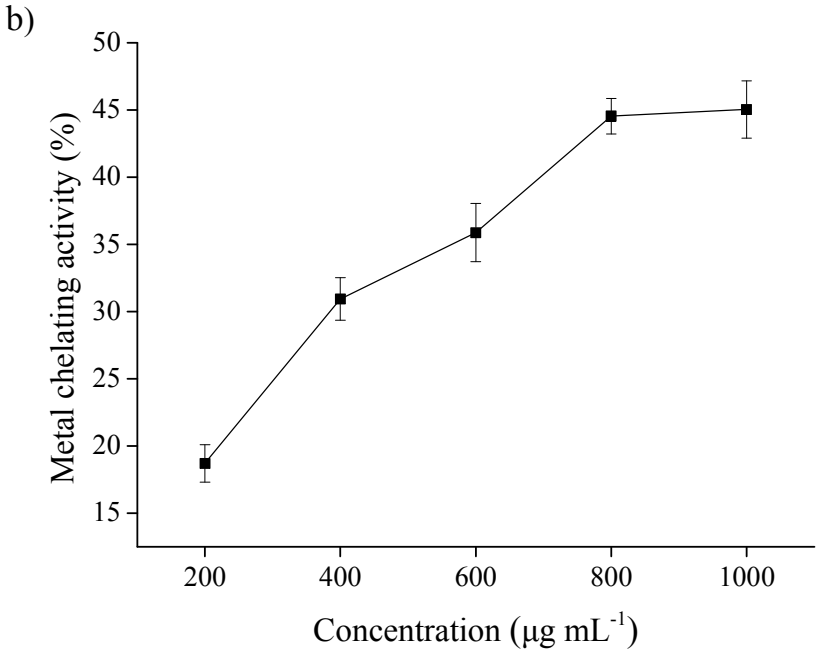



Fig. 2



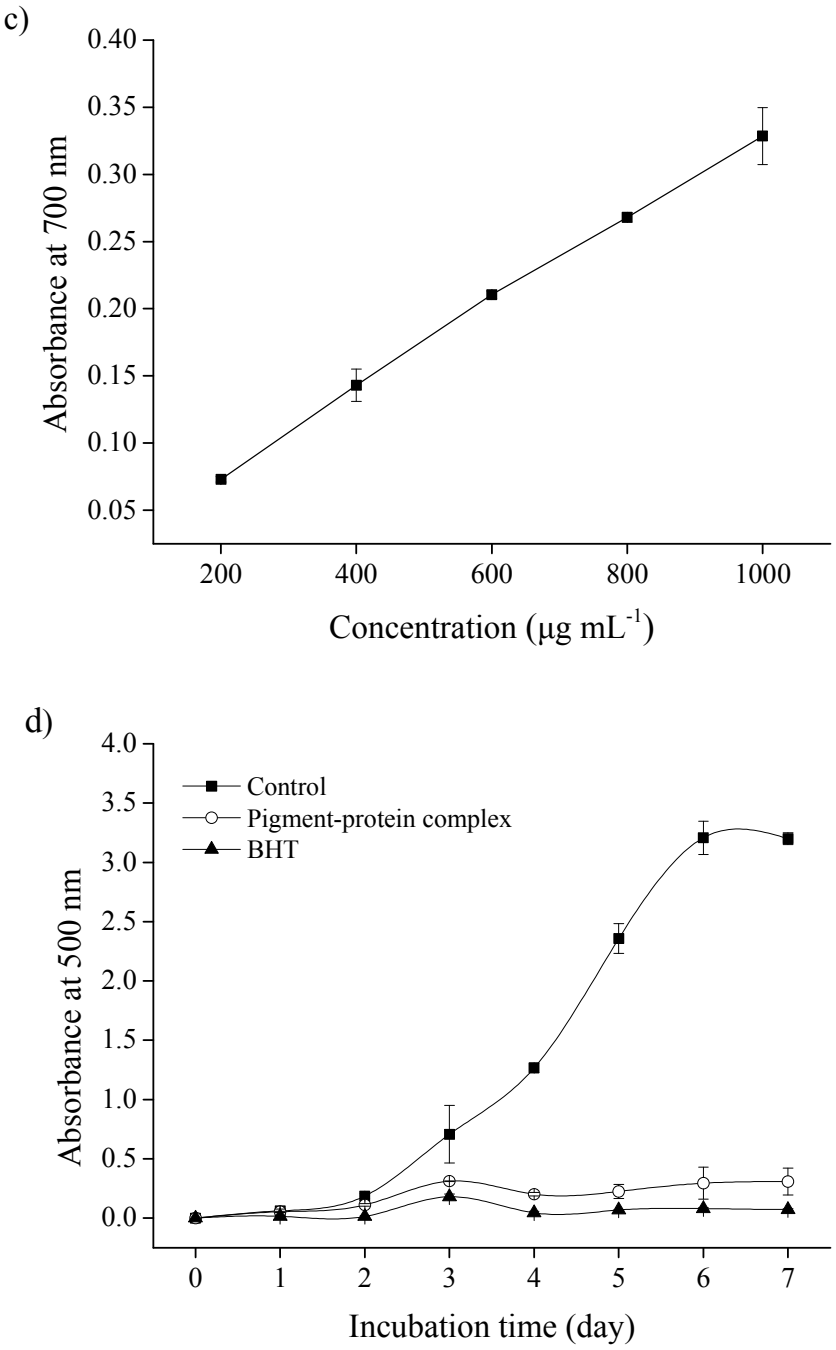



Fig. 3

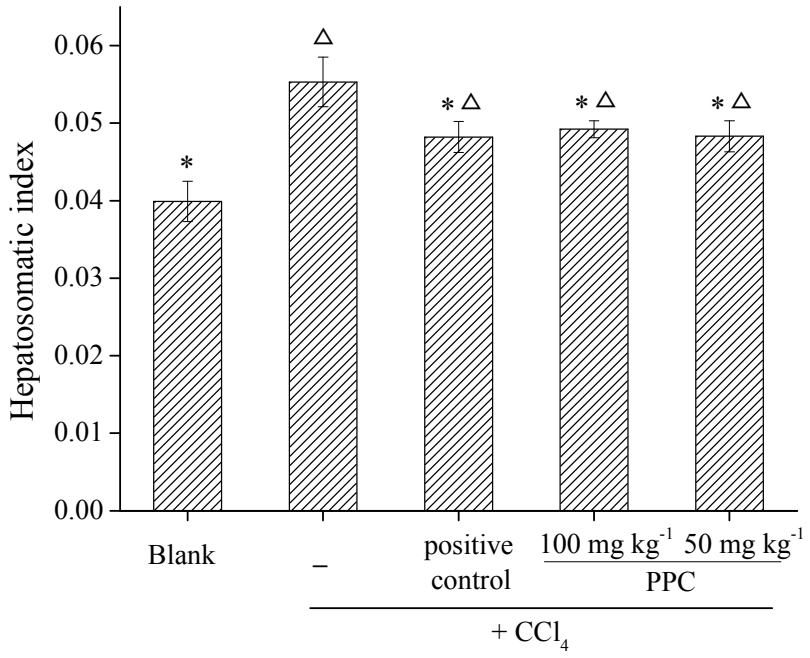



Fig. 4

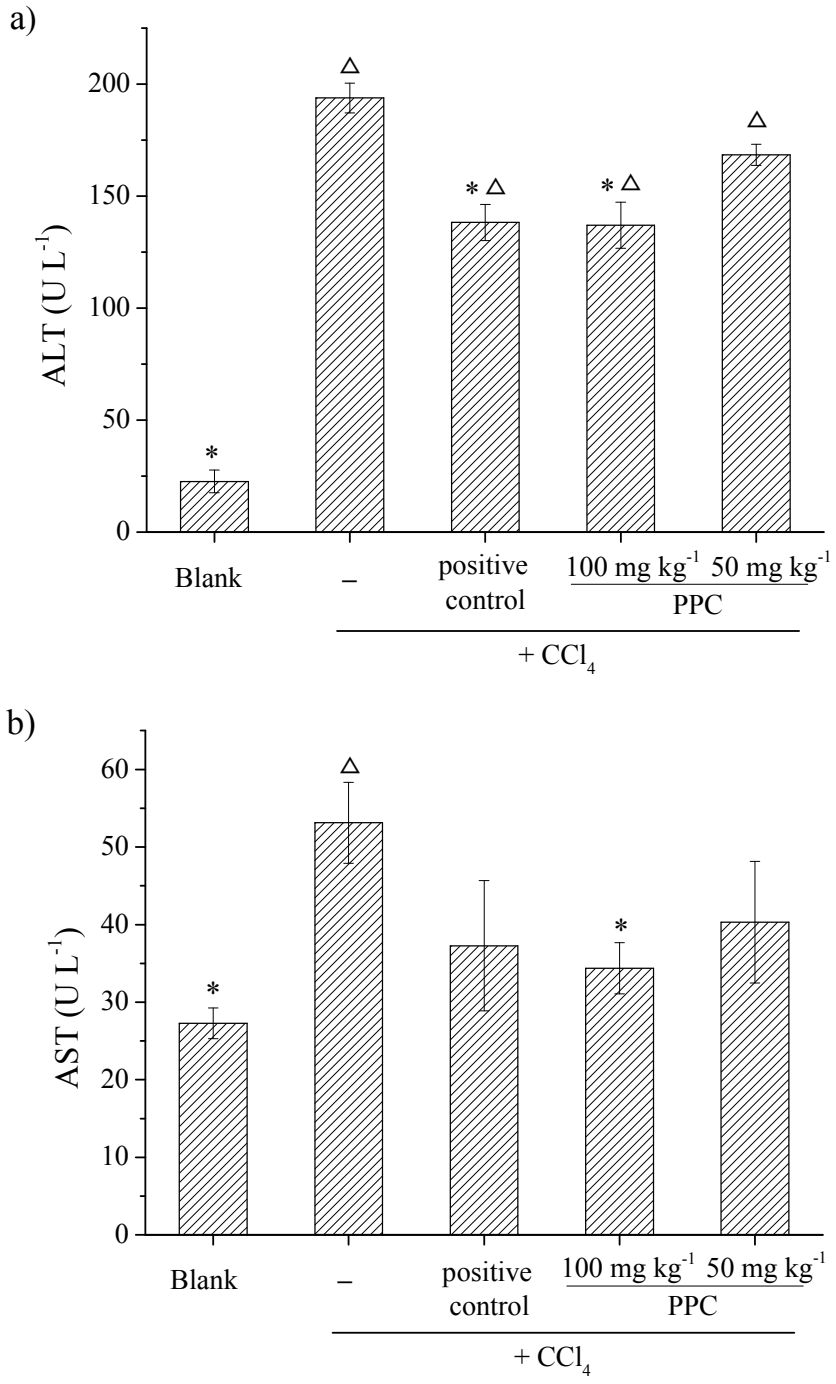
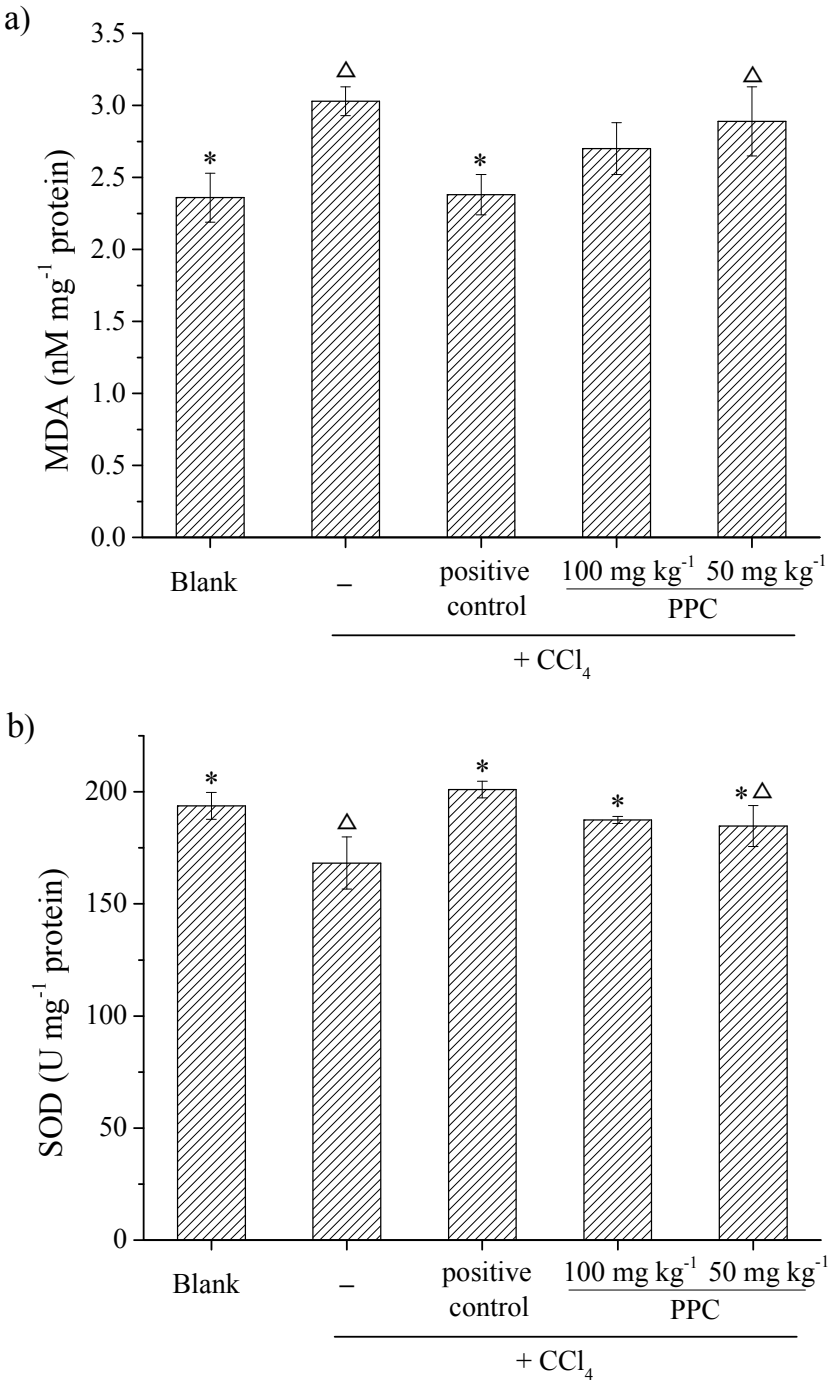



Fig. 5



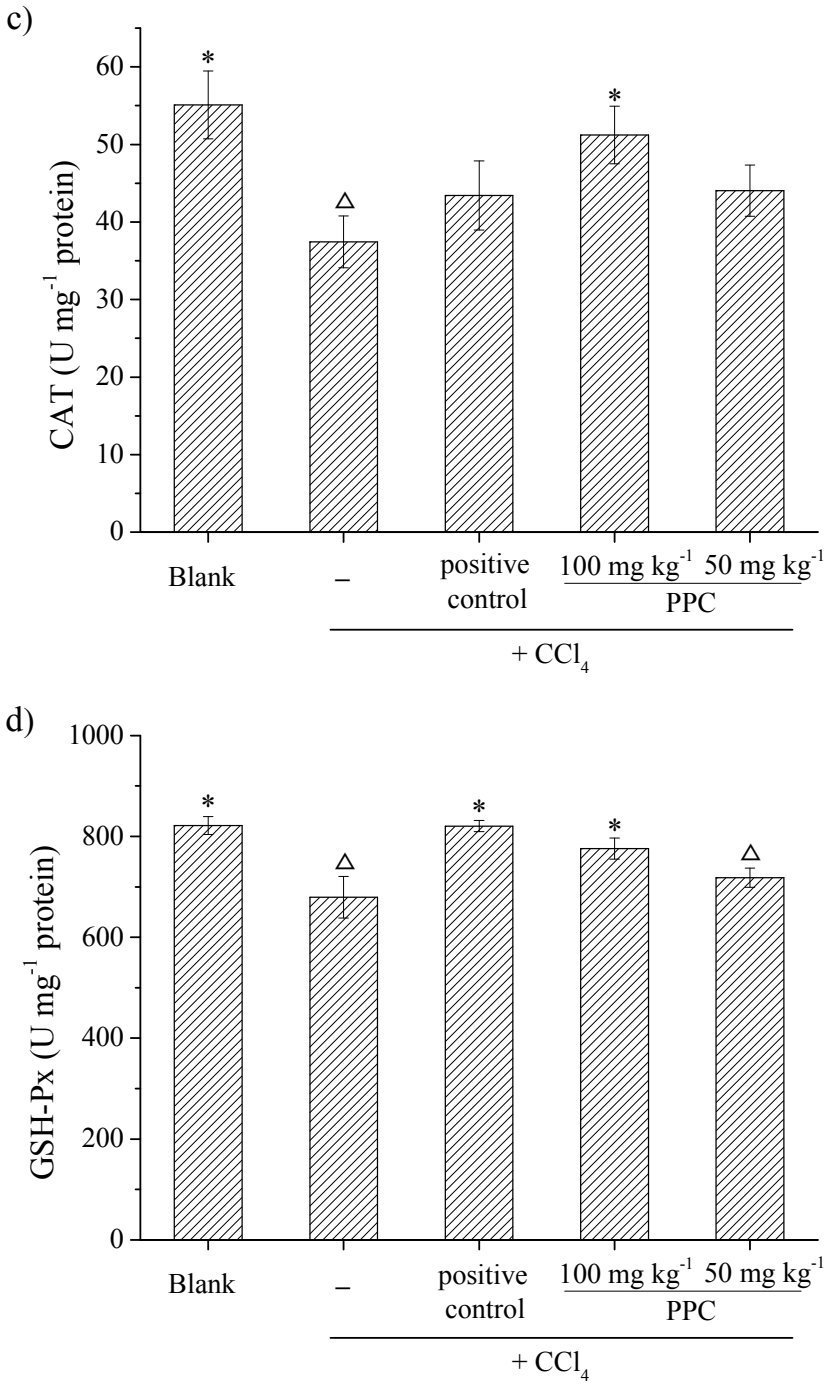



Fig. 6