Document heading　　　　doi:

Efficacy evaluation of the protein isolated from *Peganum harmala* seeds as an antioxidant in liver of rats

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ARTICLE INFO

ABSTRACT

Objective: To evaluate the curative effect of the 132 KD protein isolated from the seeds of *Peganum harmala* (*P. harmala*) L. against carbon tetrachloride (CCl_4) induced oxidative stress in rats. **Methods:** Animals were post treated intraperitoneally with 132 KD isolated protein at doses of 4 and 8 mg/kg body weight and bovine serum albumin (BSA) (8 mg/kg body weight) as well as vitamin C (250 mg/kg body weight p.o.) for 7 d after they challenged with CCl_4 orally (1 mL/kg body weight) in olive oil (50%) for 2 d. **Results:** The purified protein from seeds of *P. harmala* plant showed *in vitro* antioxidant activity with DPPH assay. Administration of CCl_4 induced induction in serum aminotransferases (AST, ALT), alkaline phosphatase (ALP), lipid profile parameters and liver malondialdehyde (MDA) and decrease in serum total protein, liver superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels. 132 KD protein treatment of rats post CCl_4 intoxication successfully alleviated the toxic effects of CCl_4. **Conclusions:** The isolated protein possessed strong antioxidant activity comparable to that of BSA (negative control) and vitamin C (positive control).

1. Introduction

Exposure to various organic compounds including a number of environmental pollutants, clinically useful drugs and xenobiotics can cause cellular damages through metabolic activation of those compounds to highly reactive oxygen species (ROS). Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations[1].

Carbon tetrachloride (CCl_4) is one of the most extensively studied of the environmental toxicants. In the principle of liver damage of CCl_4 is reductively bioactivated by CYP2E1 into a trichloromethyl radical (CCl_3^-), which is subsequently converted into a trichloromethyl peroxyl radical ($CCl_3OO−$) in the presence of oxygen. These reactive free radical metabolites may cause cellular damage by initiating lipid peroxidation and covalently binding

to macromolecules[2]. Carbon tetrachloride is a potent environmental hepatotoxin[3]. Recent studies have been reported that inhibition of CYP2E1, which is involved in CCl_4 biotransformation decrease CCl_4 hepatotoxicity. Otherwise, the induction of this cytochrome increase the drug hepatotoxicity[4]. Carbon tetrachloride is a model for studying free radical induced liver injury and screening hepato−protective drugs[5]. It is widely used in animal models to induce acute liver injury which is closely analogue of hepatotoxicity in human[6]. Several investigators reported that the principle causes of CCl_4 induction of hepatic damage is lipid peroxidation and decrease activities of antioxidant enzymes and generation of free radicals[7].

Antioxidants and anti−inflammatory agents play a critical role against CCl_4 intoxication by scavenging active oxygen and free radicals and neutralizing lipid perioxides[8]. Therefore, there is need for a natural product that protects the body against various xenobiotics, but is also cost−effective, safe and without side effects. This led to new investigations into assessing the antioxidant potential of substances or molecules from natural sources which plays an important role in body protection by scavenging active oxygen and free radicals and neutralizing lipid

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peroxidation[9]. However, studies are going on throughout the world for the search of protective molecules that would provide maximum protection of the liver and kidney as well as other organs, due to their easy availability, low cost and minimal side effects would be exerted during their function in the body[10].

The *P. harmala* L. (Syrian rue) is a wild–growing flowering plant belonging to the Zygophylaceae family and is found abundantly in middle East and North Africa[11], it grows widely in semi arid areas in Egypt especially in the coastal region from Sallum to Rafa. The *P. harmala* plant was claimed to be an important medicinal plant. Its seeds are known to possess hypothermic and hallucinogenic properties[12], and antioxidative action[13]. Moreover, several reports in the literature indicating a great variety of pharmacological activities for *P. harmala* plant such as anti–bacterial, anti–fungal, antiviral and antiprotozoal effects of *P. harmala* extract[14,15]. Therefore, the present study was designed to evaluate the curative role of the protein isolated from seeds of *P. harmala* plant as a novel antioxidant protein with a molecular mass of 132 KD[16] against CCl₄–induced of liver damage and the possible mechanism of this protective effect in male albino rats.

2. Materials and methods

2.1. Plant material

P. harmala L. is a herb belonging to the Zygophylaceae family. Dry plant seeds were obtained from the faculty of agriculture farms, Cairo University.

2.2. Experimental animals

Male albino rats (*Rattus norvegicus*) weighing 120–130 g were grouped and housed in polyacrylic cages, six animals per cage in the animal house (Faculty of science, Cairo University), at 23–25 ℃ with 12 h light/12 h dark cycle. The animals were provided with standard diet and water *ad libitum*. They were acclimatized under laboratory conditions for 7 d before commencement of the experiments.

2.3. Chemical materials

1,1–Diphenyl–2–picrylhydrazyl (DPPH), DEAE–cellulose and Phenyl methyl sulfonyl fluoride were purchased from Sigma Aldrich. Carbon tetrachloride and bovine serum albumin were purchased from International Company for scientific and medical supplies, Egypt. Kits for serum biochemical estimation and kits for determination of oxidative stress markers were purchased from Biodiagnostic Company, Egypt.

2.4. Extraction and purification of antioxidant protein

The seeds were homogenized in 20 mM Tris–HCl buffer, pH 7.4 and the homogenate was brought to 60% ammonium sulphate saturation. The pellet was reconstituted and dialyzed in Tris–HCl buffer, passed through DEAE sephadex column and eluted in linear gradient of 0.1 M NaCl in Tris buffer. The active fraction eluted with 0.2 M NaCl was concentrated and applied on a Sephadex G–50 column. The bioactive fraction obtained was subjected to a C18 hydrophobic column for reverse phase column chromatography. The homogeneity of preparation was determined by SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

2.5. Determination of in vitro antioxidant activity of the purified protein

In vitro antioxidant activity of the purified protein from seeds of *P. harmala* plant, was estimated by using the 1,1 diphenyl–2–picrylhydrazyl (DPPH) radical according to the previous method[17]. The methanolic solution of DPPH was prepared (250 mM of DPPH in 200 mL of methanol), whereas, various concentrations (300–2 000 μg/mL) of the protein were added to 2 mL of the methanolic DPPH solution. The solution was shaken vigorously and incubated at 37 ℃ for 30 min. at dark. At the end of the incubation period, the absorbance of each sample was determined at 517 nm (spectrophotometer, U–2001, model 121–0032 Hitachi, Tokyo, Japan). Methanol was used as the blank, DPPH solution was used as control and vitamin C used as positive control under same condition. DPPH radical scavenging activity was calculated by the following equation: % DPPH radical scavenging activity = (Abscontrol − Abssample) / (Abscontrol) × 100

2.6. Experimental design

Thirty six rats were divided into six groups. Rats of the first group which served as control group were administered olive oil for two days followed by distilled water for 7 consecutive days. The animals of the other 5 groups were orally given CCl₄ (1 mL/kg body weight) for 2 d followed by 7 d of oral administration of distilled water (2nd group), intraperitoneal injection of 132 KD protein at a dose of 4 mg/kg body weight (3rd group), 8 mg/kg body weight (4th group), intraperitoneal injection of bovine serum albumin (BSA) at a dose of 8 mg/kg body weight (negative control group) (5th group) and oral administration of 250 mg/kg body weight vitamin C (6th group).

2.7. Methods and technique

At the end of experiments, the rats were sacrificed. Blood samples were immediately collected into centrifuged tubes,

kept at room temperature for one hour and centrifuged at 3 000 rpm for 20 min, to obtain serum for biochemical assays, the serum were kept at −20 ℃ until used. Samples of liver were homogenized (10 % w/v) using Potter Elvehjem homogenizer in ic−cold 0.2 M phosphate buffer (pH7.4), then centrifuged at 9 000 rpm for 15 min at 4 ℃ and the resultant supernatant was used for different oxidative stress markers determination. The protein content in the tissue homogenates was measured according to the described method[18]. Other sample of liver was excised and fixed in a suitable fixative for histological studies.

2.8. Biochemical assessment

The appropriate kits (Bio−Diagnostic, Dokki , Giza, Egypt) was used for the determination of serum aminotransferase enzyme activities (AST & ALT)[19], alkaline phosphatase (ALP) activity[20], serum total protein[18], total lipids[21], triglycerides (TG) level[22], total cholesterol concentration[23], high density lipoprotein (HDL) level[24], low density lipoprotein (LDH) level[25], liver superoxide dismutase (SOD)[26], catalase (CAT)[27], glutathione reduced (GSH)[28], and malondialdehyde (MDA)[29], determinations using test kit (Bio−Diagnostic−Egypt).

2.9. Histopathological technique

For the histological preparation, small pieces from liver from all groups were immediately fixed in Bouin fluid dehydrated by 70% ethanol, cleared in xylene embedded in paraffin wax. Tissues were sectioned using sledge microtome at 4 microns thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains (H&E) for histopathological examination through the light microscope[30].

2.10. Statistical analysis

The data were analysed using IBM computer and SPSS statistical package. The student′s $t-$ test and the analysis of variance (ANOVA) were used to detect differences between control group and CCl$_4$ group and between CCl$_4$ group and other experimental groups of animal[31]. The significant differences were considered at $P<0.05$.

3. Results

3.1. In vitro antioxidant activity of the purified protein

Figure 1 showed *in vitro* antioxidant activity of different

Table 1
Effect of the purified protein from seeds of *P. harmala* plant post−treatment on serum ALT, AST and ALP activities of rats against CCl$_4$ toxicity.

Parameters	Experimental animal groups					
	Control	CCl$_4$	CCl$_4$+Protein (4 mg)	CCl$_4$+Protein (8 mg)	CCl$_4$+BSA	CCl$_4$+Vit.C
ALT(U/mL)	37.58±86.00	57.77±1.39[a] [53.73%]	49.60±1.02[b] (−14.14%)	48.30±0.35[b] (−16.39%)	56.70±0.57 (−1.85%)	38.35±0.18[b] (−33.62%)
AST(U/mL)	35.80±0.12	47.10±0.21[a] [31.56%]	38.08±0.44[b] (−19.15%)	37.72±0.50[b] (−19.92%)	46.33±0.93 (−1.63%)	36.18±0.43[b] (−23.18%)
ALP(IU/L)	80.57±0.98	130.97±0.75[a] [62.55%]	93.00±0.20[b] (−28.99%)	86.03±1.45[b] (−34.31%)	120.50±0.86[b] (−7.99%)	84.47±1.01[b] (−35.50%)

All data were mean ± SE of six male albino rats in each groups. a: significant at $P<0.05$ as compared to the control group. b: significant at $P<0.05$ as compared to the CCl$_4$ group. [ ]: % of change as compared to the control group. ( ): % of change as compared to the CCl$_4$ group.

Table 2
Effect of the purified protein from seeds of *P. harmala* plant protein post−treatment on serum total protein and lipids profile parameters of rats against CCl$_4$ toxicity.

Parameters	Experimental animal groups					
	Control	CCl$_4$	CCl$_4$+Protein (4 mg)	CCl$_4$+Protein (8 mg)	CCl$_4$+BSA	CCl$_4$+Vit.C
Total protein(g/dL)	6.22±0.19	5.85±0.14[a] [−5.95%]	6.05±0.25 (3.42%)	6.27±0.30 (7.18%)	5.85±0.19 0	6.72±0.14[b] (14.87%)
Total lipids(mg/dL)	232.72±1.4	661.43±24.7[a] [184.21%]	346.97±14.2[b] (−47.54%)	381.98±0.92[b] (−42.25%)	655.72±.83 (−0.86%)	244.45±5.43[b] (−63.04%)
Triglycerides(mg/dL)	70.03±1.36	105.53±0.81[a] [50.69%]	76.00±1.04[b] (−27.98%)	79.30±0.61[b] (−24.86%)	106.78±0.59 (−1.18%)	75.83±0.90[b] (−28.14%)
Total cholesterol(mg/dL)	72.95±0.75	125.60±1.90[a] [72.17%]	75.45±0.29[b] (−39.92%)	97.22±0.42[b] (−22.60%)	111.85±1.65[b] (−10.94%)	74.87±0.85[b] (−40.39%)
HDL−Cholesterol(mg/dL)	37.27±0.47	86.93±1.16[a] [133.24%]	43.50±0.69[b] (−49.60%)	42.57±0.46[b] (−51.03%)	86.52±0.41 (−0.47%)	38.27±0.27[b] (−55.98%)
LDL−cholesterol(mg/dL)	75.28±0.75	150.73±0.45a [50.07%]	93.65±−0.52[b] (−37.87%)	85.70±0.67[b] (−43.14%)	146.48±0.73[b] (−2.82%)	76.27±0.59[b] (−49.39%)

All data were mean ± SE of six male albino rats in each groups. a: significant at $P<0.05$ as compared to the control group. b: significant at $P<0.05$ as compared to the CCl$_4$ group. [ ]: % of change as compared to the control group. ( ): % of change as compared to the CCl$_4$ group.

concentrations of the purified protein from seeds of *P. harmala* plant with DPPH assay, which was parallel with the same concentrations of vitamin C which used as standard known DPPH radical scavenger. It can be noticed that purified protein has nearly the same antioxidant activity as vitamin C. The result obtained indicate that purified protein has *in vitro* antioxidant activity as indicated from its decrease in the concentration of DPPH radical due to scavenging ability of this protein.
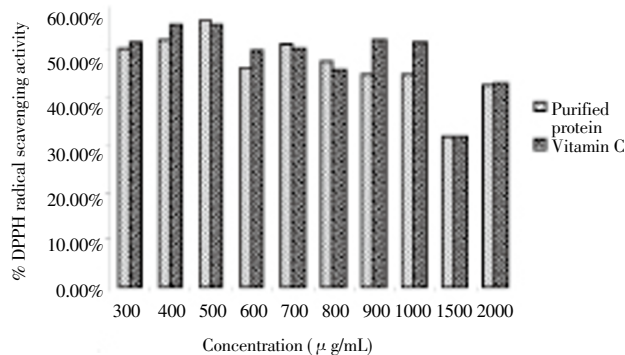

Figure 1. *In vitro* antioxidant activity of the purified protein at different concentrations (μ g/mL) on DPPH radical. Vitamin C was used as positive control.

3.2. Serum biochemical parameters

Oral administration of CCl_4 (1 mL/kg body weight) for 2 d caused a significant increase ($P<0.05$) in serum ALT, AST and ALP activities. The percentages of change were 53.73, 31.56 and 62.55, respectively as compared to that of the control group (Table 1). However, post–treated rats with the purified protein at the two doses (4 and 8 mg/kg body weight *i.p*) and vitamin C (250 mg/kg body weight p.o) daily for 7 d after CCl_4 administration caused a significant decrease ($P<0.05$) in the activities of all tested enzymes as compared to that of the CCl_4 treated rats (Table 1). It can be noticed that the administration of the purified protein at the two doses has nearly the same decreasing effect on the serum ALT, AST and ALP activities. While, post–treatment rats with BSA (8 mg/kg body weight i.p) for the same period of time after CCl_4 administration caused a non–significant change in serum ALT and AST activities and a significant decrease ($P<0.05$) in serum ALP activity with percentages of change −1.85 and −1.63 and −7.99, respectively as compared to that of CCl_4 treated rats.

Table 2 shows that the rats administered of CCl_4 (1 mL/kg body weight p.o) for 2 d caused a significant decrease ($P<0.05$) in serum total protein concentration with percentage of change −5.95, as compared to that of the control group. However, intraperitoneal injection with the purified protein at the two doses (4 and 8 mg/kg body weight) and BSA (8 mg/kg body weight *i.p*) for 7 d after CCl_4 administration caused a non–significant change in serum total protein concentration with percentage of changes were 3.42 and 7.18, respectively as compared to that of the CCl_4 treated rats (Table 2). While, post–treatment rats with vitamin C (250 m/kg body weight p.o) for 7 d subsequent to CCl_4 administration caused a significant increase ($P<0.05$) in

serum total protein concentration with percentage of change 14.87, as compared to that of CCl_4 treated rats.

From the data obtained in Table 2, it appears that administration of CCl_4 (1 mL/kg body weight p.o) for 2 d caused a significant increase ($P<0.05$) in the serum total lipids, triglycerides, total cholesterol, HDL–cholesterol and LDL–cholesterol concentrations with percentage of changes of 184.21, 50.69, 72.17, 133.24 and 50.07, respectively as compared to that of the control group. On the other hand, post–treatment of the rats with the purified protein at two doses (4 and 8 mg/kg body weight i.p) and oral administration of vitamin C (250 mg/kg body weight p.o) for 7 d after CCl_4 administration caused a significant decrease ($P<0.05$) in the concentrations of all studied lipid profile parameters, as compared to that of CCl_4 treated rats (Table 2).

3.3. Oxidative stress markers in liver tissue

Table 3 revealed that the liver activities of SOD, CAT and GSH concentration of CCl_4 treated rats were significantly decreased ($P<0.05$). The percentages of change were −31.07, −36.33 and −36.24, respectively as compared to that of the control group. On the other hand, post–treatment with purified protein at the two doses (4 and 8 mg/kg body weight *i.p*) for 7 d and vitamin C (250 mg/kg body weight *p.o*) for the same period of time after CCl_4 administration caused a significant increase ($P<0.05$) in the liver activities of SOD, CAT and GSH concentration, as compared to that of the CCl_4 treated rats. While, post–treatment with BSA (8 mg/kg body weight *i.p*) for 7 d caused a non–significant change in SOD activity and GSH level and significant decrease ($P<0.05$) in the liver CAT activity as compared to that of the CCl_4 treated rats (Table 3).

Administration of CCl_4 (1 mL/kg body weight p.o) for 2 d caused a significant increase ($P<0.05$) in MDA level. The percentage of change was 542.85 as compared to that of control rats (Table 3). Meanwhile, intraperitoneal injection with the purified protein at the two doses (4 and 8 mg/kg body weight) for 7 d after CCl_4 administration and oral administration of vitamin C (250 mg/kg body weight) for 7 d after CCl_4 administration caused a significant decrease ($P<0.05$) in MDA level. The percentage of change was −80.74, −82.96 and −82.22, respectively as compared to that of the CCl_4 treated rats. It seems that the administration of the purified protein at the two doses and vitamin C have nearly the same decreasing effect on MDA level. While, post–treatment rats with BSA (8 mg/kg body weight *i.p*) for 7 d after CCl_4 administration did not cause any significant changes in the MDA level of liver tissue with percentage of change was −1.48 as compared to that of the CCl_4 treated rats (Table 3).

3.4. Histological observation

Figure 2 shows the liver of control rats. The architecture is normal. Liver lobules consist of a central vein (c) and hepatocytes (h) arranged in anastamosing cards vertical to the central vein. Administration of CCl_4 (1 mL/kg body weight *p.o*) for 2 d induced histopathological changes in the rat liver, including fatty change in the hepatocytes (Figure 3), associated with inflammatory cells infiltration (m) in the portal area (p) (Figure 4). Liver section of rats post–treated with the purified protein at a dose of (4 mg/kg body weight

i.p) for seven days, showed fatty change in the cytoplasm of the hepatocytes (Figure 5). Liver sections of rats post–treated with the purified protein at a dose of (8 mg/kg body weight i.p), showed inflammatory cells infiltration (m) associated with diffuse kupffer cells (k) proliferation in between the degenerated hepatocytes (dh) (Figure 6), as well as dilatation of central vein (c) (Figure 7). Inflammatory cells infiltration (m) in the portal area (p) and degeneration in the hepatocytes (dh) were observed in liver section of rats intraperitoneal injected with BSA for seven days after CCl$_4$ administration for 2 d (Figure 8). Liver section of rats post–treated with vitamin C, showed inflammatory cells infiltration (m) in the portal area (p) associated with degeneration in the hepatocytes (dh) (Figure 9).
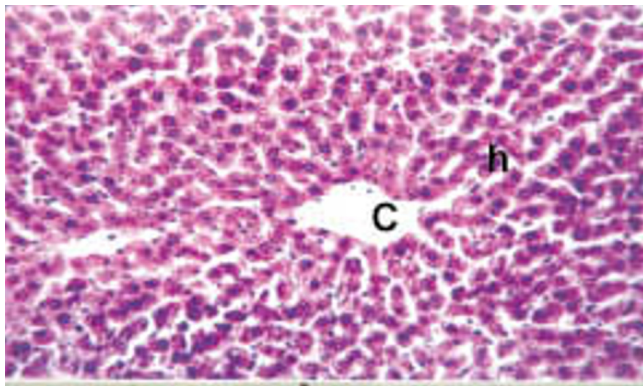

Figure 2. Liver section of rat in control group showing the normal histological structure of central vein (C) and surrounding hepatocytes (h) (H&E×64).
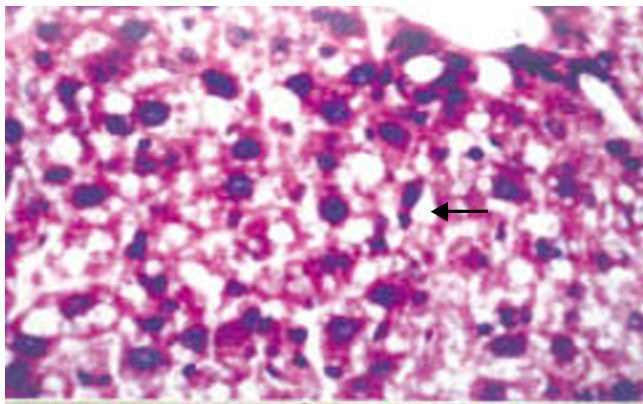

Figure 3. Section of rat liver exposed to CCl$_4$ showing the fatty change

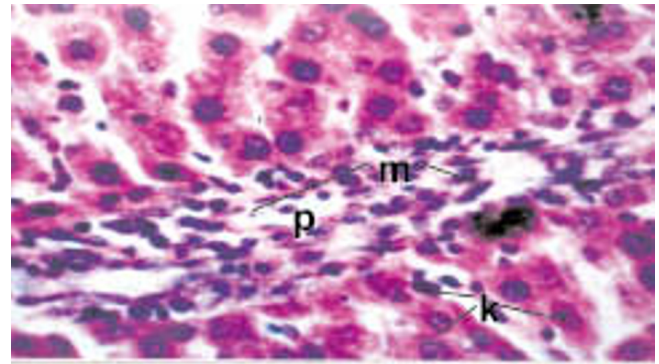in the hepatocytes (arrow) (H&E×160).

Figure 4. Section of rat liver exposed to CCl$_4$ showing inflammatory cells infiltration (m) in the portal area (p) with kupffer cells proliferation (k) between the cytomegalic degenerated hepatocytes (H&E×160) .
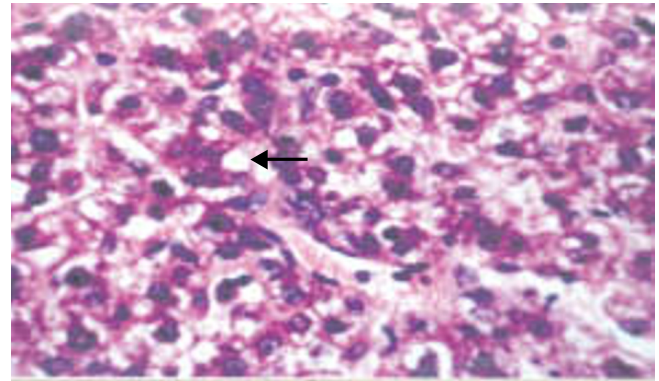

Figure 5. Section of rat liver exposed to CCl$_4$ + protein (4 mg) showing the fatty in hepatocytes (arrow) (H&E×160).
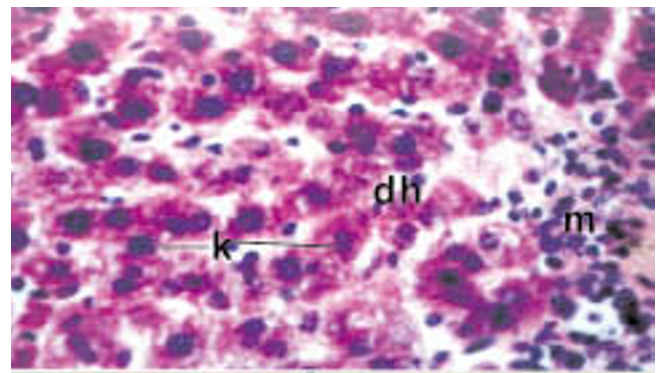

Figure 6. Section of rat liver exposed to CCl$_4$+protein (8 mg) showing the inflammatory cells infiltration (m) in the portal area with kupffer cells

Table 3
Effect of the purified protein from seede of *P. harmala* plant post–treatment on SOD, CAT, GSH and MDA in the liver of rats against CCl$_4$ toxicity.

Oxidative stress parameters	Experimental groups					
	Control	CCl$_4$	CCl$_4$+Protein (4 mg)	CCl$_4$+Protein (8 mg)	CCl$_4$+BSA	CCl$_4$+Vit.C
SOD(U/mg protein)	6.92±0.32	4.77±0.14[a] [−31.07%]	6.48±0.32[b] (35.85%)	6.65±0.40[b] (39.41%)	4.85±0.13 (1.68%)	6.86±0.23[b] (43.82%)
CAT(U/mg protein)	7.35±0.46	4.68±0.29[a] [−36.33%]	6.85±0.12[b] (46.37%)	6.58±0.20[b] (40.59%)	3.77±0.27[b] (−19.44%)	6.97±0.26[b] (48.93%)
GSH(mg/mg protein)	4.36±0.02	2.78±0.07[a] [−36.24%]	4.42±0.09[b] (58.99%)	4.05±0.23[b] (45.68%)	2.82±0.20 (1.44%)	4.28±0.10[b] (53.97%)
MDA(nmol/mg protein)	0.21±0.01	1.35±0.12[a] [543.85%]	0.26±0.02[b] (−80.74%)	0.23±0.01[b] (−82.96%)	1.33±0.04 (−1.48%)	0.24±0.005[b] (−82.22%)

 All data were mean ± SE of six male albino rats in each groups. a: significant at $P<0.05$ as compared to the control group. b: significant at $P<0.05$ as compared to the CCl$_4$ group. [ ]: % of change as compared to the control group. ( ): % of change as compared to the CCl$_4$ group.

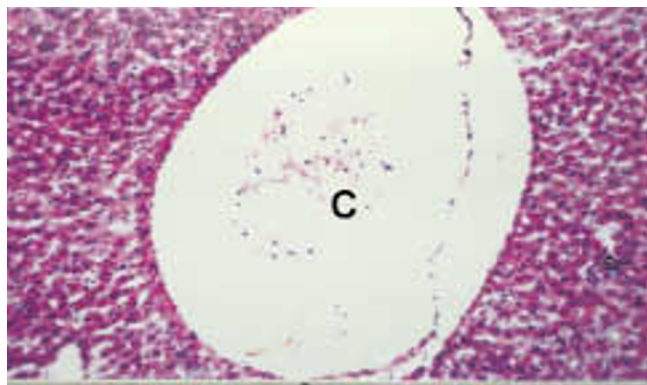proliferation (k) between degenerated hepatocytes (dh) (H&E×160).

Figure 7. Section of rat liver exposed to CCl$_4$ + protein (8 mg) showing severe dilatation of central vein (C) (H&E×64).
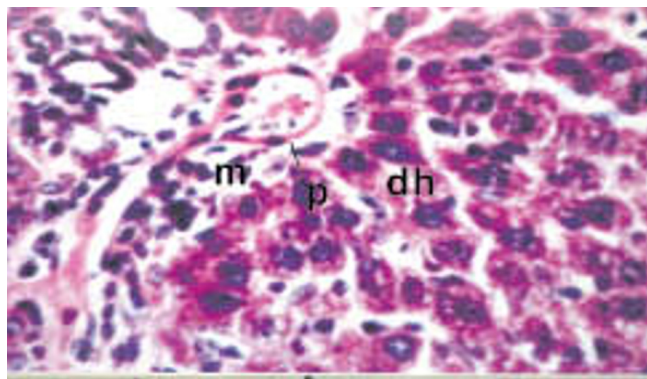

Figure 8. Section of rat liver exposed to (CCl$_4$ + BSA) showing the inflammatory cells (m) in the portal area (p) and degeneration in the hepatocytes (dh) (H&E×160).
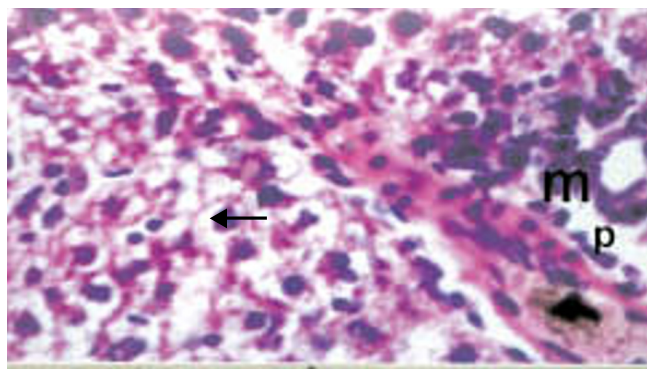

Figure 9. Section of rat liver exposed to (CCl$_4$+Vitamin C) showing inflammatory cells infiltration (m) in the portal area (p) with degeneration in the hepatocytes (arrow) (H&E×64).

4. Discussion

1,1–Diphenyl–2–picrylhydrazyl (DPPH) is a synthetic free radical which can be effectively scavenged by antioxidants[32]. It has been widely used for rapid evaluation of the antioxidant activity of plant extracts[33]. In the present study the ability of 132 KDa purified protein to scavenging DPPH radicals was determined by of the decrease in its absorbance at 517 nm and has nearly the same scavenging activity as the well known antioxidant, vitamin C. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability[34] which is one of the major antioxidant mechanisms to inhibit the chain reaction of lipid peroxidation[35].

Liver injury induced by chemicals, drugs and viruses has been well recognized as a toxicological problem[36]. The liver injury induced by CCl$_4$ is the best characterized system for xenobiotic induced hepatotoxicity and are commonly used models for screening of antihepatotoxic and/or hepatoprotective activities of drug[37].

The plasma levels of various enzymes may be increased or decreased, have diagnosed the hepatic injury. These enzymes can be divided into two groups; the first one consists of cytoplasmic enzymes that leak from the damaged hepatic cells into the circulating fluid, whose concentration in the plasma increases proportionally with the extent of the damage. The second consists of enzymes secreted only by the normal liver, therefore, decreases as a consequence of hepatic damage[38]. Members of the first group have already been used as indicators of the hepatic injury; they include AST and ALT. The two aminotransferases (AST and ALT) occur in almost all animals and human cells, but their activities in different tissues vary considerably, however, ALT exceeds that of AST. The liver damage leads to increase serum level of both enzymes (AST & ALT) but in general ALT elevation is more specific for liver damage than AST[39].

Regarding the effect of carbon tetrachloride administration on serum ALT and AST activities, the present study revealed that CCl$_4$ administration (1 mg/kg body weight) for 2 d caused a significant increases in serum ALT and AST activities of rats as compared to the control group which provide evidence of the adverse effect of CCl$_4$ on the liver functions[40]. Meanwhile, the present results indicated that administration of CCl$_4$ induced significant histopathological changes in rat's liver which may confirm the aforementioned results. It suggests that hepatotoxicity induced by CCl$_4$ depends on the cleavage of the CCl$_4$ bond to generate CCl$_3$– free radicals that react rapidly with oxygen to form CCl$_3$OO– radicals. These metabolites possibly attack membrane polyunsaturated fatty acid, thereby causing lipid peroxidation leading to impairment of membrane function and liver injury[41]. In agreement with these results, several authors[42,43] reported that CCl$_4$ caused a peroxidative degradation in adipose tissue resulting in fatty infiltration of the hepatocytes or may be due to the dehalogenation of CCl$_4$ catalyzed by CYP in liver cell endoplasmic reticulum lead to generation of an unstable high radicals attack microsomal lipids and proteins causing lipid peroxidation[44].

The present study revealed that the treatment of rats with the purified protein from *P. harmala* seeds at the two studied doses for 7 d after CCl$_4$ administration caused significant decrease in serum ALT and AST activities as compared to the CCl$_4$ group. This decrease in both enzymes was considered as an indicator for the improvement of the functional status of liver cells which may be due to

the action of the free radical scavenging activity and antioxidant property of this purified protein or may be due to the antioxidant activity of cysteine or cysteine residue which containing SH group responsible for its antioxidant property[45].

Alkaline phosphatase (ALP) is excreted normally via bile through liver and involves in active transport across the capillary wall. The increased activity of alkaline phosphatase, which occurs due to de novo synthesis by liver cells, is a reliable marker of hepatobiliar dysfunction due to damage[46]. Administration of CCl_4 (1 mL/kg body weight) for 2 d in the present study was revealed a significant increase in serum ALP activity of rats as compared to the control group. ALP is sensitive marker employed in the diagnosis of hepatic damage because it is a cytoplasmic enzyme and is released into the circulation after cellular damage[47]. So, the increase in ALP activity in the present study may be due to the fact that CCl_4 induced hepatocellular damage. Recently, several authors reported significant increases in the ALP activity of rats subsequent to different doses of CCl_4 administration[48–50].

The present study showed that the treatment of rats with the purified protein at two doses (4 and 8 mg/kg body weight) for 7 d post CCl_4 administration caused a significant decrease in the serum ALP activity. This decrease may be due to the functional improvement of hepatocytes, which may be caused by accelerated regeneration of parenchyma cells due to free radical scavenging action of protein against free radicals product by CCl_4 biotransformation by cytochrome P450 within liver or may be due to stability of biliary dysfunction of rat liver during hepatic injury with toxicants. This finding agrees with results of Ghosh and Sil[51] who observed that the administration of the protein isolated from leaves of *Cajuns indicus* plant at dose (2 mg/kg body weight) for 3 d after and before mercuric chloride, induced a hepatic damage, lowered the level of serum ALP of mice. They suggested that this protein might have inhibitory effects on CYP activity in microsomal liver enhanced by toxins through its antioxidative mechanism action.

Proteins are synthesized in liver and inhibition of protein synthesis indicates disruption and dissociation of polyribosomes from endoplasmic reticulum[52]. Liver has a great capacity to detoxicate the toxic effect of many substances and synthesis useful metabolites[53]. The decline in total protein content can be seemed as a useful index of the severity of cellular dysfunction in liver diseases[54].

The present study disclosed a significant decrease in serum total protein concentration of rats after 2 d of administration of CCl_4 at a dose of 1 mL/kg body weight, as compared to the control group. It suggests that the site specific oxidative damage of some susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis[55]. This decrease may be due to lipid peroxidation produced by exposure to CCl_4 which initiate pathological changes such as depression of protein synthesis[56] or the damage produced and localized in the endoplasmic reticulum resulted in the loss of CYP leading to

its functional failure with a decrease in protein synthesis[57].

The present study revealed that post treatment of rats with 132 KDa protein isolate at the two studied doses after CCl_4 induced liver damage caused a non significant change in serum total protein concentration as compared of CCl_4 treated rats. These findings may be due to the stabilization of endoplasmic reticulum leading to protein synthesis[58]. The stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and production of liver cells[59].

Regarding serum total lipids concentration of rats, the present study revealed that serum total lipids concentration was significantly increased after administration of CCl_4 (1 mL/kg body weight) for 2 d as compared to the control group. Similarly, some reports demonstrated that total lipid concentration was found to increased either in serum, plasma or different tissues (liver, kidney, heart and brain) due to administration of different doses of CCl_4 on different periods by different routes of administration[60–62]. This increase in total lipid content may be attributed to the hepaotoxic effect of CCl_4 intoxication or to the leakage of lipids from cells to blood.

Regarding the effect of isolated 132 KDa protein on serum total lipids concentration in the present study, a significant decrease in serum total lipids concentration of treated rats was recorded. These results suggest that the purified protein could preserve the liver against lipid accumulation and peroxidation due to the presence of hydrophobic amino acids[16] .

Alterations in hepatic triglycerides content have been used as one of series of tests to determine the relative hepatotoxic potential of various halogenated hydrocarbons in rats[63]. The present study indicated that serum triglycerides concentration showed a significant increase in rats treated with CCl_4 (1 mL/kg body weight) for 2 d as compared to the control group. This finding revealed the toxic effect of CCl_4 on serum triglycerides concentration of rats. This increase may be due to the accumulation of triglycerides due to the degradation of phospholipids as a result of the increase in lipid peroxidation or may be due to decreased activity of lipoprotein lipase, which involved in the uptake of triglycerides−rich lipoprotein by the extrahepatic tissue[64].

On the other hand, a significant decrease in serum triglycerides concentration of rats treated with the purified protein at the two studied doses (4 and 8 mg/kg body weight) daily for 7 d after CCl_4 intoxication was recorded as compared to the CCl_4 group. This finding suggested that the purified protein may be preserving the liver against lipid accumulation by preventing lipid peroxidation of phospholipids in the component of hepatocytes membrane[65].

A significant increase was noticed in serum total cholesterol concentration of rats administered CCl_4 in the present work. This finding revealed that carbon tetrachloride exerts significant toxic effect on serum total cholesterol concentration of rats, which may be due to the presence of damage in liver. This finding was confirmed by histological

sectioning of liver tissue which indicates inflammatory cells, degenerated hepatocytes and fatty changes in liver of rats administered to CCl_4. In this regard, it was suggested that the intoxication with CCl_4 resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesized in liver or derived from plasma lipids, which leading to increase in total cholesterol level[58].

Regarding the effect of the purified protein on serum total cholesterol concentration of rats, the present study revealed a significant decrease in serum total cholesterol of rats treated with protein extract at the two studied doses. This finding revealed a hepatoprotective effect of the purified protein on ameliorating the effect of CCl_4 on serum total cholesterol concentration, which may be due to the direct antioxidant activity of the purified protein or due to the inhibition of cholesterol synthesis.

Hypercholesterolemia is a risk factor for coronary artery disease (CAD), and numerous clinical studies have shown that LDL–cholesterol plays a major role in the pathogenesis of CAD[66]. The present study showed a significant increase in serum HDL and LDL–cholesterol concentrations of rats treated with CCl_4 (1 mL/kg body weight) for 2 d as compared to the control group either pre or post–treatment with CCl_4.

The present study revealed a significant decrease in serum HDL and LDL–cholesterol levels of rats treated with the purified protein at the studied doses (4 and 8 mg/kg body weight) daily for 7 d after CCl_4 administration as compared to rats treated with CCl_4. Similarly, a significant decrease was found in serum HDL and LDL–cholesterol levels of rats fed on diet containing pumpkin seed protein isolate (PSPI) for casein at 25%, 50%, 75% and 100% diet after CCl_4 intoxication[67].

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols[68]. They are intracellular antioxidant enzymes that protect tissues against oxidative process[69]. CAT protects SOD against inactivation by H_2O_2. Reciprocally, SOD protects CAT against superoxide anion. Thus the balance of this enzymatic system is essential to dispose the superoxide anion and peroxides generated in the tissues[70].

The present study recorded a significant decrease in liver SOD and CAT activities of rats exposed to carbon tetrachloride (1 mL/kg body weight) for 2 d as compared to the control group. This reduction may be due to the enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals which cause inactivation of the antioxidant enzymes. In this regard, it was suggested

that enhanced free radical concentration resulting from oxidative stress conditions can cause loss of enzymatic activities[71]. In agreement with the present results, there are significant decreases in both SOD and CAT activities of liver tissue of mice treated with CCl_4 at a dose of 1 mL/kg body weight for 2 d. They[72,67] suggested that this decrease would cause an increased accumulation of superoxide and peroxyl radicals, which could further stimulate lipid peroxidation. It was suggested that the enhancement of lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals[73].

Concerning the possible curative role of the protein isolated from seeds of *P. harmala* plant in the present study, significant increases in hepatic SOD and CAT activities were reported in rats treated with this protein at the two studied doses (4 and 8 mg/kg body weight) after CCl_4 administration as compared to the rats treated with CCl_4. This increase in serum SOD and CAT activities may be due to the antioxidant activity of this protein which recover the damage caused by CCl_4 or may be due to inhibition in the activity of CYP2E1 responsible for biotransformation of carbon tetrachloride[37]. Reduced glutathione (GSH) plays a common role in cellular resistance to oxidative damage as a free radical scavenger as protein–bound glutathione and by generation of ascorbate or tocopherol in liver[74]. It is one of the most abundant tripeptide non–enzymatic biological antioxidant present in the liver. Its functions are concerned with the removal of free radicals such as hydrogen peroxide and superoxide radicals, maintenance of membrane protein, detoxification of foreign chemicals and biotransformation of drugs[75]. The depletion of hepatic GSH has been showed to be associated with an enhanced toxicity to chemicals, including carbon tetrachloride[76]. The liver is the major site for synthesis of GSH. The detoxification of different drugs and xenobiotics, in the liver involves GSH[77].

The present study reported that rats administered of CCl_4 (1 mL/kg body weight) for 2 d caused a significant decrease in hepatic GSH contents as compared to the control group after treatment with CCl_4. This decrease in GSH contents might be due to the decrease availability of GSH resulted during the enhanced lipid peroxidation. This decrease may be due to increased accumulation of superoxide and peroxyl radicals, which could further stimulate lipid peroxidation and inactivation of antioxidant enzyme and depletion of non– enzymatic antioxidant[48–50].

Concerning the possible ameliorative role of the protein isolated from seeds of *P. harmala* plant the present study, revealed that rats treated with this protein at the two studied doses (4 and 8 mg/kg body weight) daily for 7 d both pre and post to CCl_4 administration caused a significant increase in hepatic GSH contents as compared to rats treated with CCl_4. These results may be due to de novo GSH synthesis or

GSH regeneration due to antioxidant activity of this protein, which was previously mentioned with DPPH assay. In this regard, it was suggested that the increase in glutathione contents in liver plays a primary role in the protection against trichloromethyl radicals induced liver damage[78], therefore, lipid peroxides generated after CCl₄ treatment is eliminated by glutathione peroxidase in the presence of glutathione, which lead to curbing the propagation of lipid peroxidation.

Lipid peroxidation is a free radical induced process leading to oxidative deterioration of poly unsaturated fatty acid. It arising from the reaction of free radicals with lipids is considered to be an important feature of the cellular injury brought by free radical attack[79]. MDA is the one of end products of lipid peroxidation and measure of free radical generation[80–84]. Some studies have demonstrated that acute or chronic CCl₄ administration to experimental animals increased the formation of lipid peroxidation products, such as malonodialdehyde (MDA)[85-87]. The present study indicate that rats administered of CCl₄ at a dose (1 mL/kg body weight) for 2 d caused a significant increase in hepatic MDA levels as compared to the control group. This increase suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals[75].

The present study reported that liver MDA levels of rats treated with 132 KD protein isolate at two doses (4 and 8 mg/kg body weight) daily for 7 d after CCl₄ intoxication significantly decreased. This finding may be due to the presence of hydrophobic, sulphur, aromatic and antioxidant amino acids contents[16], as the increment of the hydrophobic character will increase their solubility in lipid and therefore enhance their antioxidant activity[88].

Conflict of interest statement

We declare that we have no conflict of interest.

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