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Purification of antioxidant protein isolated from *Peganum harmala* and its protective effect against CCl_4 toxicity in rats

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Abstract: The present study was conducted to determine the protective effect of the purified protein from seeds of *Peganum harmala* against carbon tetrachloride (CCl_4)-induced toxicity in male albino rats. The purification steps included ammonium sulfate fractionation and chromatography on DEAE-cellulose, CM-Sepharose, and Superdex 75 columns. The molecular mass of the purified protein was 132 kDa by gel filtration technique; it consisted of 2 subunits with molecular masses of 30.199 kDa and 38.018 kDa by SDS-PAGE. Results of the dose-dependent experiment with purified protein prior to CCl_4 administration were higher at 4 mg/kg body weight. The antioxidant activity of the purified protein was determined in vitro by DPPH radical scavenging test. Administration of CCl_4 significantly increased the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in serum. However, a significant decrease in the level of total serum protein as well as the activities of superoxide dismutase, catalase, and reduced glutathione in liver tissues, and a significant increase in malondialdehyde level, were recorded. Pretreatment with 4 mg/kg body weight of the purified protein significantly altered the deteriorating damage induced by CCl_4 toxicity to a near normal range, which was similar to treatment with vitamin C. These results suggest that the purified protein possesses a protective effect against CCl_4-induced toxicity and probably acts as an antioxidative defense through free radical scavenging activity.

Key words: *Peganum harmala*, CCl_4, hepatic toxicities, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, superoxide dismutase, catalase, glutathione, malondialdehyde

1. Introduction

Exposure to various organic compounds including a number of environmental pollutants can cause cellular damage through metabolic activation of the compounds to highly free radical products. These free radical products induce lipid peroxidation, which is believed to be one of the major causes of cell membrane damage leading to a number of pathological events. Carbon tetrachloride (CCl_4) is a model for studying free radicals that can induce liver injury and in screening hepatoprotective drugs. The principle effects of CCl_4 are hepatic damage induction by increasing lipid peroxidation; decreasing activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and nonenzymatic reduced glutathione (GSH); generation of free radicals; and elevation of hepatic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (1–3). ALT and AST are the most sensitive biomarkers employed in the diagnosis of hepatic damage. ALP is a reliable marker of hepatobiliary dysfunction due to damage. Malondialdehyde (MDA) is one of the end products of lipid peroxidation and a measure of free radical generation. Some studies have demonstrated that acute and chronic CCl_4 administration to experimental animals increased the formation of lipid peroxidation products such as MDA (4,5).

The new approach to finding protective molecules that provide maximum protection of body organs with easy availability and minimal side effects is going on throughout the world. Many researchers report that proteins isolated from plant sources such as *Curcuma comosa* rhizomes (6), *Cicer arietium* seeds (7,8), *Cajanus indicus* leaves (9), wheat germ (10), *Murraya koenigii* (11), *Solanum torvum* seeds (12), *Curcuma longa* waste grits (13), and *Ginkgo biloba* seeds (14) show antioxidant activity in vitro by DPPH assay.

Peganum harmala L. (Syrian rue) is a wild-growing flowering plant belonging to the family Zygophyllaceae and is abundant in the Middle East and North Africa (15). In Egypt, it grows wildly in arid areas, especially in the coastal

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region from Sallum to Rafa. It is claimed to be an important medicinal plant. *P. harmala* seeds are known to possess hypothermic, hallucinogenic properties and antimicrobial (16) as well as antioxidative effects (17). It is also effective in the treatment of dermatosis, hypothermia, and cancer; in addition, it has a hepatoprotective effect (18).

Thus, the aim of this study was to extract and purify protein from the seeds of *P. harmala* and determine the in vitro antioxidant activity of the purified protein by DPPH method. Additionally, the possible protective role of the purified protein against CCl_4 toxicity in male albino rats was investigated by determining some of the biochemical parameters including activities of ALT, AST, ALP, and total protein in serum, as well as oxidative stress parameters such as SOD, CAT, GSH, and MDA in liver tissues.

2. Materials and methods

2.1. Biological materials and reagents

Dry plant seeds of *Peganum harmala* L. growing naturally in Mersa Mattrouh, Egypt, were used. Male albino rats (*Rattus norvegicus*) weighing 120–130 g were obtained from an agricultural research center in September 2009. The rats were grouped and housed in polyacrylic cages (7 animals per cage) in the animal house of Cairo University's Faculty of Science at 23–25 °C with a 12-h light/12-h dark cycle. The animals were provided with a standard diet and water ad libitum. They were acclimatized under laboratory conditions for 7 days before commencement of the experiments. CM-Sepharose (Cl-6B) and Superdex 75 were purchased from Cornell Lab Company. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), DEAE-cellulose, and phenylmethylsulfonyl fluoride (PMSF) used in the experiment were purchased from Sigma Aldrich. The CCl_4 and bovine serum albumin (BSA) were purchased from the International Company for Scientific and Medical Supplies, Egypt. Kits for serum biochemical parameters and oxidative stress marker enzymes were purchased from Biodiagnostic Company, Egypt.

2.2. Methods for extraction and purification of antioxidant protein

2.2.1. Preparation of crude extract

The dry seeds (100 g) were ground and then mixed with 3 volumes of ice-cooled extraction buffer consisting of 25 mM phosphate buffer (pH 7.0), 225 mM NaCl, 10 mM EDTA, 5 mM DTT, 1.5% polyvinylpyrrolidone (PVP) (w/v), and 1 mM PMSF, as recommended by Park et al. (19). The mixture was homogenized by blender and the homogenized sample was filtered. The filtrate was centrifuged at 10,000 rpm for 20 min at 0 °C. The protein content in the crude extract was determined according to the method of Lowry et al. (20). DPPH radical scavenging activity was assayed according to the methods of Liyana-Pathiranan and Shahidi (21).

2.2.2. Ammonium sulfate fractionation

According to the method described by Segel (22), the prepared crude extract was subjected to ammonium sulfate fractionation at 20%, 40%, 60%, and 80%. In each step, pellets were formed after incubation for 1 h at 4 °C. The pellets were collected by centrifugation at 10,000 rpm for 20 min at 0 °C, resuspended in the extraction buffer, and dialyzed against it.

2.2.3. Determination of DPPH radical scavenging activity of protein fractions

The DPPH radical scavenging activity of the ammonium sulfate fractions (20%, 40%, 60%, and 80%) (w/v) of *P. harmala* were estimated (21). The absorbance of the resulting color was determined at 517 nm. The radical scavenging capacity of the tested protein was measured as a decrease in the absorbance of the DPPH radical and was calculated by the following equation:

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

where $Abs_{control}$ is the absorbance of DPPH methanolic solution and Abs_{sample} is the absorbance of DPPH methanolic solution + sample or vitamin C (positive control).

2.2.4. Ion exchange chromatography

A DEAE-cellulose column was used as recommended by Peterson and Sober (23). The protein was precipitated with 40% (w/v) ammonium sulfate, which showed the maximum DPPH radical scavenging activity, and was loaded into a DEAE-cellulose column (18 × 2 cm) that was previously equilibrated with the same extraction buffer. The proteins were eluted from the column with a discontinuous gradient of 0.0, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 M NaCl. At a flow rate of 0.6 mL/min, 3-mL fractions were collected. The protein content was routinely determined spectrophotometrically at 280 nm as described by Warburg and Christian (24). DPPH radical scavenging activity was assayed in all fractions. Fractions containing a target protein that showed DPPH radical scavenging activity after the DEAE-cellulose column were collected, lyophilized, and loaded into a CM-Sepharose (Cl-6B) column (18 × 2 cm). Equilibration and elution were carried out as in the case of DEAE-cellulose. At a flow rate of 2.5 mL/min, 3-mL fractions were collected. Protein content and DPPH radical scavenging activity were assayed in all fractions.

2.2.5. Gel filtration chromatography

The final fractions eluted from the CM-Sepharose (Cl-6B) column that showed DPPH radical scavenging activity were collected, lyophilized, and chromatographed to a Superdex 75 column (15 × 2 cm). The protein was eluted with 500 mL of 25 mM phosphate buffer. At a flow rate of 0.5 mL/min, 3-mL fractions were collected. The protein

content and DPPH radical scavenging activity were assayed in all fractions.

2.3. Determination of molecular mass

The molecular mass of the purified protein from seeds of *P. harmala* was determined with the Sephadex G-100 (30 × 1.5 cm) column according to the method of Andrews (25). Standard proteins were used to calibrate the column and calculate the molecular mass of the purified protein from the calibration curve. At a flow rate of 0.6 mL/min, 3-mL fractions were collected. This relative molecular mass was confirmed using sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) as described by Walker (26). Samples of 5 µL were applied to 10% gel. Electrophoresis was carried out with a current of 30 mA until the bromophenol blue dye reached the bottom of the gel. The molecular mass of the provided protein subunits was calculated after calibration of the gel using protein standards.

2.4. Analysis of amino acid composition

Amino acid composition of the purified protein from seeds of *P. harmala* was determined with an amino acids analyzer (Beckman 6300) (27).

2.5. Determination of dose-dependent effect of the purified protein

To determine the dose of the purified protein providing the maximum effect, 4 different doses of the purified protein [0.5, 1, 2, and 4 mg/kg body weight (b.wt)] were intraperitoneally injected into rats for 7 days prior to CCl_4 treatment and for 2 days at a dose of 1 mL/kg b.wt (in olive oil, 1:1, v/v). All rats were euthanized 24 h after the final dose of CCl_4 was administered. The serum ALT levels were measured for all the experimental animal groups.

2.6. Groups of animals under investigation

Male albino rats were randomly divided into 5 groups (7 rats per group) as follows: 1) Rats injected daily with 1 mL/kg b.wt distilled water intraperitoneally for 7 days, followed by oral administration of 1 mL/kg b.wt olive oil by gastric gavage for 2 days; normal control group. 2) Rats injected daily with 1 mL/kg b.wt distilled water intraperitoneally for 7 days followed by oral administration of CCl_4 (1 mL/kg b.wt in olive oil, 1:1, v/v); toxicity group. 3) Rats injected daily with the purified protein at a dose of 4 mg/kg b.wt intraperitoneally for 7 days. 4) Rats injected daily with 8 mg/b.wt BSA intraperitoneally for 7 days; negative control group. 5) Rats orally administered 250 mg/kg b.wt vitamin C daily for 7 days; positive control group. Rats from the last 4 groups were orally administered CCl_4 (1 mL/kg b.wt in olive oil, 1:1, v/v) for 2 days after the end of the 7 days of treatment. At the end of the experiments, the rats were euthanized. Blood samples were immediately collected into EDTA-treated tubes, kept at room temperature for 1 h, and centrifuged at 3000 rpm for 20 min to obtain serum for biochemical assays. The serum was stored at −20 °C until use. Samples of liver tissues were homogenized (10% w/v) using a Potter-Elvehjem homogenizer in ice-cold 0.2 M phosphate buffer (pH 7.4) at 9000 rpm for 15 min at 4 °C, and the resultant supernatant was used for different oxidative stress markers.

2.7. Biochemical analysis

Using the Biodiagnostic kit, ALT and AST activities were determined according to the methods of Reitman and Frankel (28), ALP was determined according to the methods of Belfield and Goldberg (29), SOD according to Nishikimi et al. (30), CAT according to Aebi (31), GSH according to Beutler et al. (32), and MDA according to Ohkawa et al. (33). Total protein was determined in serum using the colorimetric method and the Biodiagnostic kit (34).

2.8. Statistical analysis

Statistical analysis was performed using SPSS 10.0. Differences were considered significant at $P < 0.05$ (35).

3. Results and discussion

Extraction and purification of a novel antioxidant protein isolated from the seeds of *P. harmala* were performed. The total protein content of the crude extract was 141 mg with 42.3% DPPH radical scavenging activity using 50 µg/mL protein (Table 1). In this context, it was reported that 51% DPPH scavenging activity was recorded using 1 mg of crude extract of protein isolated from curry leaves (11), and 95.94% was reported using 0.25 mg of crude extract

Table 1. Purification steps of protein purified from seeds of *Peganum harmala*.

Purification steps	Total protein (mg)	DPPH radical scavenging activity (%)	Yield (%)
Crude extract	141.0	42.30	100.0
Amm. sulfate	68.50	61.13	48.60
DEAE-cellulose	56.31	76.90	39.36
CM-Sepharose	38.64	85.50	27.40
Superdex 75	23.67	73.20	16.78

of seaweed protein (36). The steps to purification began with ammonium sulfate fractionation at 20%, 40%, 60%, and 80%. DPPH radical scavenging activity was measured in each fraction (Figure 1). It is clear that the protein fraction precipitated with 40% ammonium sulfate showed the maximum DPPH radical scavenging activity, using 50 µg/mL protein, as compared to vitamin C (as a positive control). In this context, several investigators used different concentrations of ammonium sulfate for precipitating the antioxidant proteins from different plants. For precipitation of antioxidant protein from the leaves of *Cajanus indicus* (9), 60% ammonium sulfate was used, whereas 50% was used to precipitate protein isolated from Sundakai seeds (12).

In the present study, the 40% ammonium sulfate fraction was loaded on a DEAE-cellulose column. The elution profile is illustrated in Figure 2, where it is clear that only the first protein peak, which was unbound to the DEAE-cellulose beads, showed DPPH radical scavenging activity. This peak exhibits a low density of negative charges as the column contained positively charged beads. This peak was designated as the active protein fractions and was used in the next purification steps. The total protein content of this peak was 56.31 mg with 39.36% yield and 76.9% DPPH radical scavenging activity (Table 1). In the literature, the DEAE-cellulose column was used for purification of antioxidant proteins from *Phyllanthus niruri* (37) and *Ginkgo biloba* (14).

In the present work, the second purification step was by CM-Sepharose, which is a weak cation exchange. The elution profile is illustrated and represented in Figure 3. It is clear from Figure 3 that only the first protein peak exhibited DPPH radical scavenging activity. The total protein content of this peak was 38.64 mg with 27.40% yield and 85.50% DPPH radical scavenging activity (Table 1). Concerning the use of Superdex 75 for protein purification, the separation of antioxidant proteins on this column revealed one protein peak (Figure 4). This peak was eluted with 500 mL of phosphate buffer (pH 7.0). The total protein content of this peak was 23.67 mg, which exhibited 73.2% DPPH radical scavenging activity with a 16.78% yield (Table 1). As far as we are aware, no report exists concerning the use of CM-Sepharose or Superdex 75 in the purification of antioxidant proteins from plant sources.

In the literature, the DPPH radical scavenging activity of protein isolated from Sundakai seeds was 76% by 0.8 µM protein (12), 85.82% for protein purified from chickpea using 1.0 mg/mL protein (8), and 42% and 80% using 1.8 µM and 3 µM of purified protein from curry leaves, respectively (11). In addition, 3 purified protein fractions from *Chrysaora quinquecirrha* showed 59.7%, 82.0%, and 53.8% DPPH radical scavenging activity at concentrations ranging from 20 to 120 µg/mL protein (38).

In the present work, the molecular mass of purified protein from *P. harmala* was calculated from the calibrated column of a Sephadex G-100 column using protein markers as indicated in Figure 5. The calculated molecular mass was 132 ± 2.0 kDa. Several authors reported on the molecular mass of antioxidant proteins isolated from different plant sources, such as 34 kDa for *Curcuma*

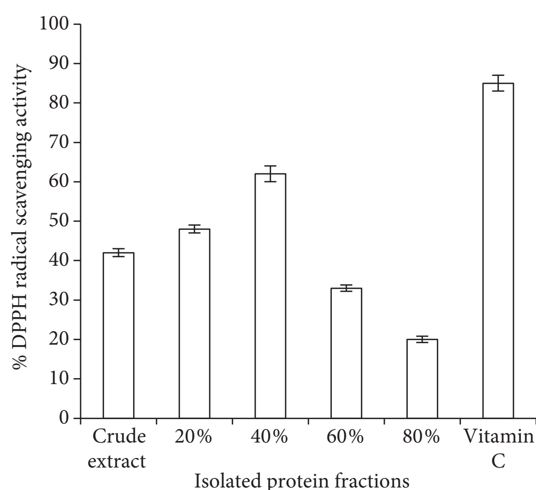

Figure 1. DPPH radical scavenging activity of protein fractions isolated from seeds of *Peganum harmala* using various concentrations of ammonium sulfate (20%, 40%, 60%, and 80% w/v). Vitamin C was used as a positive control. Bars indicate ±standard error.

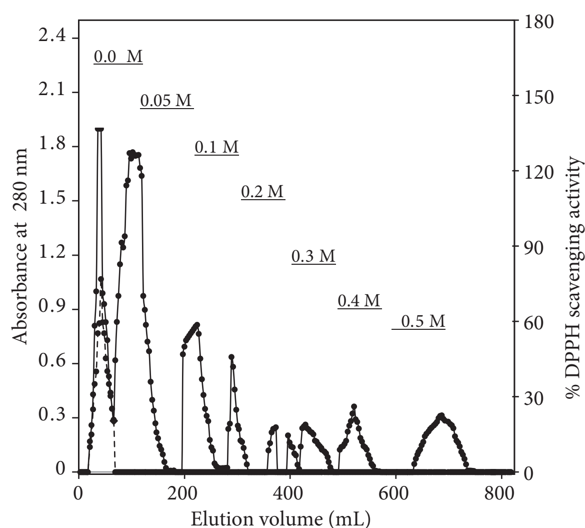

Figure 2. A typical elution profile for behavior of protein isolated from seeds of *Peganum harmala* on DEAE-cellulose column (18 × 2 cm). Proteins were eluted with a stepwise gradient of 0.0–0.5 M NaCl. The solid line represents absorbance at 280 nm and the dotted line represents activity.

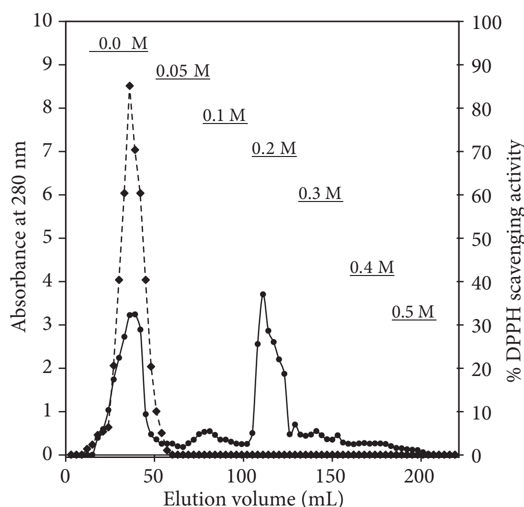

Figure 3. A typical elution profile for behavior of protein isolated from seeds of *Peganum harmala* on CM-Sepharose column (18 × 2 cm). Proteins were eluted with a stepwise gradient of 0.0–0.5 M NaCl. The solid line represents absorbance at 280 nm and the dotted line represents activity.

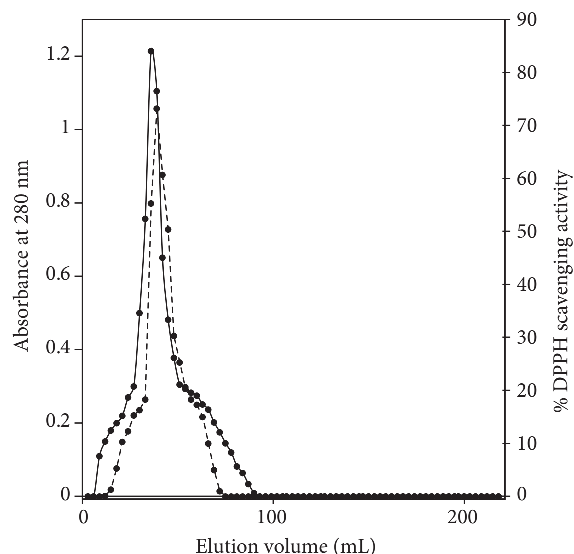

Figure 4. A typical elution profile for behavior of protein isolated from seeds of *Peganum harmala* on Superdex 75 column (15 × 2 cm). Proteins were eluted with a phosphate buffer (pH 7.0). The solid line represents absorbance at 280 nm and the dotted line represents activity.

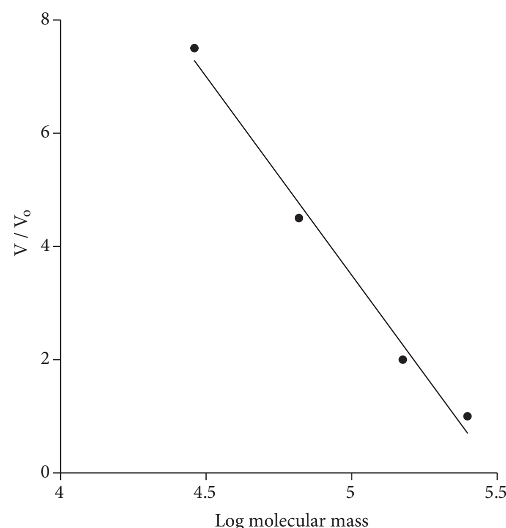

Figure 5. Calibration curve for estimation of the molecular mass by gel filtration by Sephadex G-100 (30 × 1.5 cm). The marker proteins of the known molecular masses used (expressed as kDa) are: 1) carbonic anhydrase (29), 2) bovine serum albumin (66), 3) alcohol dehydrogenase (150), and 4) catalase (250). The void volume (V_0) was determined with dextran blue 2000 kDa.

longa (13) and 28 kDa for seeds of *Solanum torvum* (7). Very small molecular masses were also reported, such as 1.5 kDa from wheat germ (10), and relatively high molecular masses were reported such as 105 kDa for *Chrysaora quinquecirrha* (38) and 92 kDa for *Gracilaria edulis* (36).

In this study, the purified protein from *P. harmala* consisted of 2 subunits, with molecular masses of 30.2 ± 1.0 kDa and 38.02 ± 2.0 kDa as estimated by SDS-PAGE (Figure 6). Several investigators recorded the molecular mass of subunits of antioxidant proteins isolated from different plants such as 18 kDa for *Curcuma comosa* (6); 43 kDa for *Cajanus indicus* (9); and 105, 65, and 9 kDa for 3 protein bands of *Chrysaora quinquecirrha* (38). For protein purified from *Gracilaria edulis*, 3 protein bands with molecular masses of 31.4, 69.5, and 92 kDa were reported (36).

The analysis of the amino acid composition of the purified protein from seeds of *P. harmala* is represented in Table 2. The highest content was recorded for tyrosine, whereas the lowest levels were recorded for arginine, alanine, and histidine. The total essential amino acids of the purified protein were 51.94 µg/mg protein. The antioxidant activity of a purified protein may be due to its hydrophobic and/or antioxidant amino acids, as increments in hydrophobicity will increase its solubility in lipids and therefore enhance antioxidant activity (8). In the present study, the protein of *P. harmala* seeds possessed antioxidant activity, and this activity was due to the presence of hydrophobic amino acids, which include Gly, Ala, Val, Pro, Met, Phe, Leu, Ile, Tyr, and Trp according to the classification reported by Zhu et al. (10), and antioxidant amino acids that include Cys, His, Trp, Lys, Arg, Leu, Val, Gly, Tyr, and Met, as according to Chen

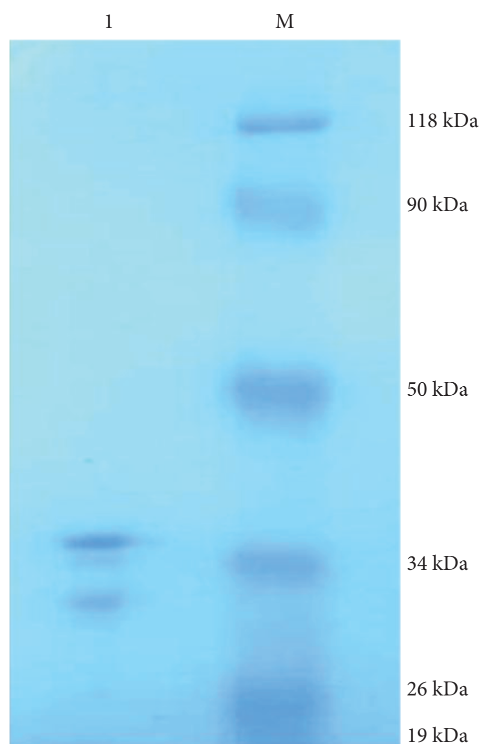

Figure 6. SDS-PAGE of purified protein from seeds of *Peganum harmala* by Superdex 75 column. Lane (M) contained 5 μg of protein standards with molecular masses as follows: lysozyme (19 kDa), myoglobin (26 kDa), carbonic anhydrase (34 kDa), ovalbumin (50 kDa), phosphorylase b (90 kDa), and β-galactosidase (118 kDa). Lane 1 contained 2 μg of the investigated protein [10% (v/v) gel was used]. Protein bands were visualized with Coomassie Blue.

et al. (39). According to these reports, the hydrophobic amino acids of *P. harmala* seeds amounted to 60.59%, and the antioxidant amino acids amounted to 58.11% of the total amino acids (Table 3).

To investigate the possible protective effect of the isolated protein from *P. harmala* seeds against CCl_4 toxicity in male albino rats, a dose-dependent test of the purified protein was carried out (Figure 7). It is clear from Figure 7 that the maximum hepatic-protective effect of the isolated protein occurred with the administration of 4 mg/kg b.wt for 7 days prior to CCl_4 administration (1 mL/kg b.wt) for 2 days. This was seen by evaluating the level of serum ALT activity, which is more specific to liver function, as recommended by several investigators (1,40).

The present results reveal that CCl_4 administration (1 mg/kg b.wt) for 2 days caused a significant increase in serum ALT and AST activities as compared to the control group (Table 3). These increases may be due to liver damage induced by CCl_4 intoxication. The same results were reported by other researchers (1,40,41). In the present study, pretreatment of rats with the purified protein from *P. harmala* resulted in significant decreases in ALT and AST activities in serum as compared to the CCl_4 group (Table 4). These decreases in the activities of both enzymes were considered as indicators of the improvement in the functional status of liver cells that may be due to the free radical scavenging activity and antioxidant properties of this purified protein that contains hydrophobic and antioxidant amino acids. These results are in agreement with those of other researchers, although the designs of the experiments were different (11,41).

Table 2. Amino acid composition of the purified protein from seeds of *Peganum harmala*.

Amino acids	μg/mg protein	Amino acids	μg/mg protein
Alanine (AlN)	1.65	Lysine (Lys)	3.81
Arginine (Arg)	1.91	Methionine (Met)	2.51
Aspartic acid (Asp)	2.56	Phenylalanine (Phe)	3.05
Cysteine (Cys)	2.63	Proline (Pro)	2.46
Glutamic acid (Glu)	2.11	Serine (Ser)	3.28
Glycine (Gly)	3.33	Threonine (Thr)	3.07
Histidine (His)	1.09	Tryptophan (Trp)	2.81
Isoleucine (Ile)	3.16	Tyrosine (Tyr)	6.18
Leucine (Leu)	3.19	Valine (Val)	3.14
Total amino acids			51.94

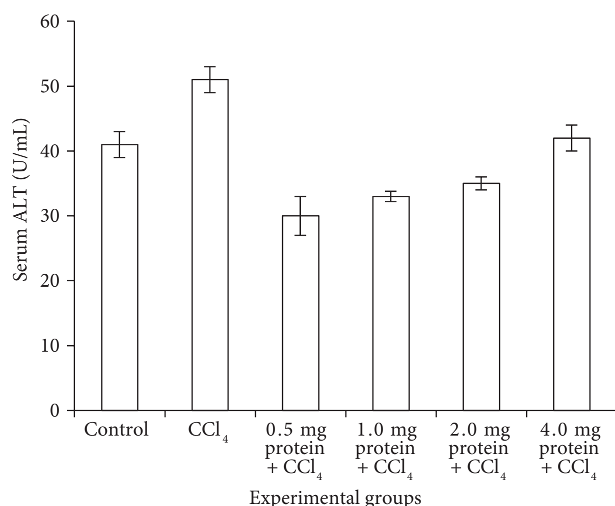

Figure 7. Dose-dependent effect of pretreatment of purified protein from *Peganum harmala* seeds on ALT activity in serum of rats against CCl_4-induced hepatic damage. All values expressed as means ± standard errors (bars) of the 7 male albino rats in each group.

It is clear from Table 4 that pretreatment with BSA produced nonsignificant effects in all studied parameters.

ALP reflects the pathological alteration in biliary flow, and its increase was considered an indicator of cellular leakage and loss of functional integrity of the cell membrane. In the present study the administration of CCl_4 for 2 days revealed a significant increase in ALP activity of the serum of rats as compared to the control group (Table 4). This increase may be due to the fact that CCl_4 induced hepatocellular damage. The same results were reported by many authors (11,42). In the present study, pretreatment of rats with 4 mg of the purified protein caused a significant decrease in serum ALP activity. This decrease may be due to the functional improvement of hepatocytes, which may be the result of the acceleration in parenchyma cell regeneration due to the free radical scavenging of the purified protein from *P. harmala* seeds.

A significant decrease in the total serum protein of the rats was recorded after 2 days of CCl_4 administration as compared to the control group (Table 4). This decrease may be due to the lipid peroxidation produced by the action of CCl_4 accompanied by hepatopathy and the inhibition of protein synthesis (43,44); however, other researchers found a significant increase (42) or a nonsignificant change (2). In the present study, pretreatment with 4 mg/kg b.wt of purified protein from *P. harmala* caused a significant increase in the total protein content in the serum of male albino rats. This increase may be due to the stabilization of the endoplasmic reticulum and improvement in the synthesis of protein. The stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism of the studied protein, which accelerates the regeneration process and production of liver cells.

Regarding the activity of antioxidant enzymes in tissues, significant decreases in liver SOD and CAT activities in rats were recorded after exposure to CCl_4 as compared to the control group (Table 4). This decrease may be due to the enhancement of lipid peroxidation, leading to tissue damage and failure of the antioxidant defense mechanism to prevent the formation of excessive free radicals, causing inactivation of the antioxidant enzymes (3,11,41).

Concerning the possible protective role of the protein

Table 3. Hydrophobic and antioxidant amino acids of the purified protein from seeds of *Peganum harmala.*

Hydrophobic amino acids	µg/mg protein	Antioxidant amino acids	µg/mg protein
Alanine (AlN)	1.65	Cysteine (Cys)	2.63
Methionine (Met)	2.51	Histidine (His)	1.09
Phenylalanine (Phe)	3.05	Tryptophan (Trp)	2.81
Proline (Pro)	2.46	Lysine (Lys)	3.81
Tryptophan (Trp)	2.81	Arginine (Arg)	1.91
Glycine (Gly)	3.33	Leucine (Leu)	3.19
Tyrosine (Tyr)	6.18	Valine (Val)	3.14
Isoleucine (Ile)	3.16	Glycine (Gly)	3.33
Leucine (Leu)	3.19	Tyrosine (Tyr)	6.18
Valine (Val)	3.14	Methionine (Met)	2.51
Total	31.48		30.6

Table 4. Effect of pretreatment with purified protein from *Peganum harmala* seeds on activities of ALT, AST, and ALP; total protein content in serum; and some oxidative stress parameters in liver of male albino rats against CCl_4-induced toxicity. All data were expressed as means ± standard error of the 7 male albino rats in each group. a: significant at P < 0.05 as compared to control group; b: significant at P < 0.05 as compared to CCl_4 group.

	Experimental animal groups				
Serum parameters	Control	CCl_4	Protein + CCl_4	BSA + CCl_4	Vit. C + CCl_4
ALT (U/mL)	40.70 ± 0.18	51.45 ± 0.49[a] [26.41%]	42.40 ± 1.25[b] (–17.59%)	48.78 ± 0.53[b] (–5.19%)	38.50 ± 0.32[b] (–25.17%)
AST (U/mL)	36.42 ± 0.82	48.90 ± 0.22[a] [34.27%]	35.48 ± 0.93[b] (–27.44%)	42.40 ± 1.61[b] (–13.29%)	37.37 ± 0.48[b] (–23.58%)
ALP (IU/L)	72.43 ± 0.41	98.83 ± 033[a] [35.82%]	76.60 ± 0.57[b] (–22.49%)	93.68 ± 0.55[b] (–5.21%)	72.88 ± 0.30[b] (–26.26%)
Total protein (g/dL)	7.70 ± 0.10	5.72 ± 0.20[a] [–25.71%]	6.32 ± 0.13[b] (10.49%)	5.67 ± 0.14 (–0.87%)	7.02 ± 0.29[b] (22.73%)
Liver oxidative stress parameters					
SOD (U/mg protein)	8.00 ± 0.32	5.42 ± 0.05[a] [–32.25%]	7.52 ± 0.20[b] (38.75%)	5.22 ± 0.16 (–3.69%)	7.43 ± 0.29[b] (37.08%)
CAT (U/mg protein)	8.60 ± 0.46	2.38 ± 0.40[a] [–72.33%]	6.93 ± 0.19[b] (191.18%)	2.00 ± 0.14 (–15.97%)	6.22 ± 0.19[b] (161.34%)
GSH (mg/mg protein)	6.03 ± 0.27	4.28 ± 0.10[a] [–29.02%]	5.58 ± 0.28[b] (30.37%)	4.22 ± 0.12 (–1.40%)	5.02 ± 0.05[b] (17.29%)
MDA (nmol/mg protein)	0.26 ± 0.01	0.60 ± 0.04[a] [130.77%]	0.32 ± 0.02[b] (–46.67%)	0.58 ± 0.03 (–3.33%)	0.27 ± 0.01[b] (–55%)

[ ]: % change as compared to control group; ( ): % change as compared to CCl_4 group.

purified from seeds of *P. harmala*, significant increases in hepatic SOD and CAT activities were observed in rats pretreated with this protein (Table 4). In agreement with the present findings, the protective effect of pumpkin seeds against liver damage was reported (41). Increases in serum SOD and CAT activities as a result of pretreatment with the protein purified from seeds of *P. harmala* may be due to the antioxidant activity of this protein, which repairs the damage caused by CCl_4. Moreover, this effect may be due to inhibition of the activity of cytochrome P2E1 (CYP2E1), which is responsible for the biotransformation of CCl_4 (45). These results suggest that plant protein might have a direct effect in inhibiting reactive oxygen species-induced membrane damage and enhancing the activity of endogenous antioxidants.

GSH plays a common role in cellular resistance to oxidative damage as a free radical scavenger and protein-bound glutathione, and through its generation of ascorbate or tocopherol in the liver. Its functions are concerned with the removal of free radicals such as hydrogen peroxide and superoxide radicals, maintenance of membrane proteins, and detoxification of foreign chemicals and biotransformation of drugs (46). The present study shows that rats administered CCl_4 alone exhibited a significant decrease in hepatic GSH content as compared to the control group (Table 4). A significant increase in hepatic GSH level was recorded in rats pretreated with 4 mg/kg b.wt of *P. harmala* protein prior to CCl_4 administration as compared to those treated only with CCl_4 (Table 4). These results may be due to de novo synthesis or regeneration of GSH as a result of pretreatment with *P. harmala* seed protein. In the literature, it was reported that mice administered 5 mg/kg b.wt protein isolated from the leaves of *Phyllanthus niruri* for 5 days prior to CCl_4 administration had a significant increase in hepatic GSH levels, suggesting that this protein may reduce oxidative stress by scavenging reactive oxygen species as an antioxidative defense mechanism (47).

The present study indicated that rats administered CCl_4 (1 mg/kg b.wt) for 2 days had a significant increase in hepatic MDA levels as compared to the control group. This increase suggests enhanced lipid peroxidation, which

leads to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (46,48,49). Similarly, several investigators demonstrated a significant increase in hepatic MDA levels in rats and mice subsequent to administration of CCl_4 by different routes and in different doses (50–52). In the present work, a significant decrease in the MDA level in liver tissues was recorded as a result of pretreatment with 4 mg/kg b.wt *P. harmala* seed protein. In the literature, a significant decrease in the hepatic MDA level of mice treated with puerarin from *Pueraria lobata* (53) or from pumpkin seeds (54) before CCl_4 administration was reported.

In conclusion, the purified protein examined in this study possesses a protective role against CCl_4 toxicity, and the selected dose has nearly the same protective effect on most biochemical and oxidative stress parameters as does vitamin C. The protective effect of the purified protein may be due to its ability to block the bioactivation of the toxicant and its potent antioxidant activity, and/or its ability to scavenge free radicals and inhibit lipid peroxidation. The analysis of amino acid composition demonstrated that the antioxidant activity of the purified protein may be due to the presence of hydrophobic amino acids, sulfur-containing amino acids, and aromatic amino acids. There is a need for further research to identify the mechanism of interaction between CCl_4 toxicity and the purified protein from *P. harmala* seed in order to understand how this protein inhibits or prevents CCl_4 toxicity.

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