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Protective and curative effects of the 15 KD isolated protein from the *Peganum harmala* L. seeds against carbon tetrachloride induced oxidative stress in brain, tests and erythrocytes of rats

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Abstract. – *Background and Objectives:* **The present study aimed to evaluate the protective and curative effects of the 15 KD protein isolated from the seeds of *Peganum harmala* L. against carbon tetrachloride (CCl_4) induced oxidative stress in rats.**

Materials and Methods: **In the protective study, animals were pretreated intraperitoneally with 15 KD isolated protein at doses of 4 and 8 mg/kg body weight as well as vitamin C (250 mg/kg body weight p.o) for 7 days and then challenged with CCl_4 orally (1 ml/kg body weight) in olive oil (50%) for 2 days. In the curative study, rats were administered CCl_4 orally for 2 days, then treated intraperitoneally with 15 KD protein (4 and 8 mg/kg body weight) and orally with vitamin C.**

Results: **Administration of CCl_4 induced induction in malondialdehyde (MDA) and decrease in reduced glutathione (GSH) levels as well as glutathione-S-transferase (GST) activity in brain, testes and erythrocytes. The activity of acetylcholinesterase (AchE) in brain was also inhibited by CCl_4 administration.**

Conclusions: **Treatment of rats either pre or post CCl_4 intoxication successfully alleviated the oxidative stress in the brain, testes and erythrocytes of the experimental animals. Data also showed that the isolated protein possessed strong antioxidant activity comparable to that of vitamin C.**

Key Words:

Carbon tetrachloride, *Peganum harmala*, Oxidative stress, 15 KD isolated protein.

Introduction

Carbon tetrachloride (CCl_4) is an extensively studied xenobiotic that induces lipid peroxidation and toxicity[1]. Carbon tetrachloride is frequently used in animals to produce an experimental model to study the mechanisms involved in the progression of hepatic disease and the impact of various drugs on this progression[2]. Metabolic activation of CCl_4 by cytochrome P_{450} to the free radicals, namely trichloromethyl and trichloromethyl peroxy radicals, is reported to enhance lipid peroxidation and protein oxidation in the liver, resulting in widespread membrane damage and liver injury[3,4]. Hepatic failure denotes a devastating clinical condition that often results in multiorgan failure and death[5,6].

Animal tissues are constantly coping with highly reactive species, such as superoxide anion, hydroxyl radicals, hydrogen peroxide, and other radicals generated during numerous metabolic reactions[7-10]. Oxidative stress resulting from increased free radical production after CCl_4 intoxication may play an important role in the degenerative processes in the tissues[11].

Antioxidants and anti-inflammatory agents play a critical role against CCl_4 intoxication by scavenging active oxygen and free radicals and neutralizing lipid peroxides[12,13]. 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. Nowadays, plant extracts are used in the treatment of the various brain diseases[14,2] and diseases related to testes[15,16].

Peganum harmala (L.) is a member of the family Zygophyllaceae[17] commonly known as "Harmal" grows spontaneously in semi arid and predesertic regions of south-east Morocco and distributed in north Africa and the middle east[18]. Protective effects of *Peganum hamala* L. extract, hasmine and harmaline against human low-density lipoprotein oxidation and also its analgesic effect were studied[19,20].

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In modern medicine, plants play a significant role since they posses various therapeutically important compounds having minimum side effects. Several studies have been made to study the antioxidative actions of isolated molecules such as proteins from medicinal plants against hepatotoxicity induced by toxins such as thioacetamide[21] and chloroform[22].

Erythrocytes are important constituents of blood and are very susceptible to free radical induced oxidative stress causing damage to their cell membranes and ultimately leading to death[23,24]. The present study was aimed to evaluate the protective and curative effects of the isolated protein from the seeds of *Peganum harmala* L. against CCl_4 induced oxidative stress in the brain, testes as well as erythrocytes of the male rats.

Materials and Methods

Plant and Animal

Peganum harmala L. is a herb belonging to the Zygophyllaceae family. Dry plant seeds were obtained from the Agriculture Faculty, Cairo University, Egypt.

Male albino rats (*Rattus norvegicus* weighing 100-120 g) were acclimatized in the laboratory for one week before starting the experiments. They were provided with standard diet and water *ad libitum*. The animals were divided into different groups, each group having six rats.

Chemicals

CCl_4 was purchased from Merk Egypt. Vitamin C was bought from SAS Chemicals Co. Mumbai, India. Kits for hemoglobin, malondialdehyde (MDA), reduced glutathione (GSH) and glutathione-S transferase estimations were purchased from Biodiagnostic Co., Egypt.

Preparation of 15 KD 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 dialysed 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 C_{18} hydrophobic column for reverse phase column chromatography (Knauer, Berlin, Germany). The homogeneity of preparation was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Treatment of Animals with 15 KD Protein

Protective Effect of 15 KD Protein

In this experiment the animals (30 rats) were divided into five groups (6 rats/ group). The rats of the first and second groups were administered distilled water orally by gastric gavage for seven days followed by olive oil (control group) and CCl_4 (50%, 1 ml/kg, dissolved in olive oil) (CCl_4) group for two days. The rats of the third and fourth groups were administered 15 KD isolated protein (at doses of 4 and 8 mg/kg b.wt, i.p., respectively) for seven consecutive days followed by CCl_4 (50%, 1 ml/kg, dissolved in olive oil) orally for two days. Animals of the fifth group were orally administered vitamin C (250 mg/kg b.wt.) for seven consecutive days followed by oral administration of CCl_4 for two days at the same dose.

Curative Effect of 15 KD Protein

Thirty rats were divided into five 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 4 groups were orally given CCl_4 (1 ml/kg b.wt.) for 2 days followed by 7 days of oral administration of distilled water (2^{nd} group), intraperitoneal injection of 15 KD protein at a dose of 4 mg/kg b.wt. (3^{rd} group), 8 mg/kg b.wt. (4^{th} group) and oral administration of 250 mg/kg b.wt. vitamin C (5^{th} group).

Rats were sacrificed and blood samples were collected in ethylene diamine tetraacetic acid (EDTA) containing tubes. Brain and testes were removed rapidly and stored at –80°C.

Samples Preparation

Hemolysate Preparation

After collecting blood samples in EDTA containing tubes, they were centrifuged at 3000 rpm for 15 min, the plasma were removed and the packed cells of the bottom were washed thrice with saline solution (0.9% NaCl). A known amount of erythrocytes was lysated with hypo-

tonic 0.015 M Tris-HCl buffer. After removing cell debris by centrifugation at 9000 rpm for 15 min. at 4°C, the hemolysate was obtained; it was used for the following biochemical assay. The hemoglobin content in the red blood cell lysate was measured according to cyanomethemoglobin method[25] using Biodiagnostic kit (Dokki, Giza, Egypt).

Tissue Preparation

Brain and testes were homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min. at 4°C and the resultant supernatant was used for different oxidative stress markers.

Determination of Antioxidant Activity in Cell-Free Systems Quenching of DPPH Radical

The free radical activity of the protein was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical[26]. The DPPH radical in ethanol (100 mM, 2 ml) was added to 2 ml of various concentrations (5-50 mg/ml) of the protein. The solution was then shaken vigorously and held for 30min at room temperature in dark. The decrease in absorbance of DPPH was measured at 517 nm. Ethanol was used as the blank solution and DPPH solution in ethanol was used as control. Percent inhibition was calculated by comparing the absorbance values of the control and the protein (spectrophotometer, U-2001, model 121-0032 Hitachi, Tokyo, Japan).

Biochemical Assay

Acetylcholinesterase (AchE) activity was determined according to Ellman et al[27]; malondialdehyde level was measured according to Ohkawa et al[28] by using 2-thiobarbituric acid (2.6-dihydroxypyrimidine-2-thiol, TBA). Reduced glutathione (GSH) was measured by the method of Aykac et al[29]. Total glutathione-S-transferase (GST) was determined according to Habig et al[30].

Statistical Analysis

Values were expressed as mean ± SEM. To evaluate differences between the groups studied, one way analysis of variance (ANOVA) with LSD post hoc test was used to compare the group means. $p<0.05$ was considered statistically significant. Statistical Package for Social Sciences (SPSS), for Windows (version 15.0, Chicago, IL, USA); was used for statistical analysis.

Results

SDS-PAGE of the Purified Protein from the Seeds of *Peganum harmala*

Figure 1 shows the SDS-PAGE pattern of the final step purified protein from *Peganum harmala*. The protein was purified to homogeneity by 5 different steps including homogenization, $(NH_4)_2SO_4$ precipitation, ion exchange chromatography, gel filtration and high performance liquid chromatography (HPLC). A single symmetrical band appeared in the region of 15 kD, indicating the homogenous preparation of the protein. Present study was carried out using this purified protein.

Scavenging Effect of Purified Protein From The Seeds of *Peganum harmala* on DPPH Radical

Figure 2 illustrates the DPPH radical scavenging ability of purified protein from the seeds of *Peganum harmala*. The isolated protein showed dose dependent scavenging of DPPH.

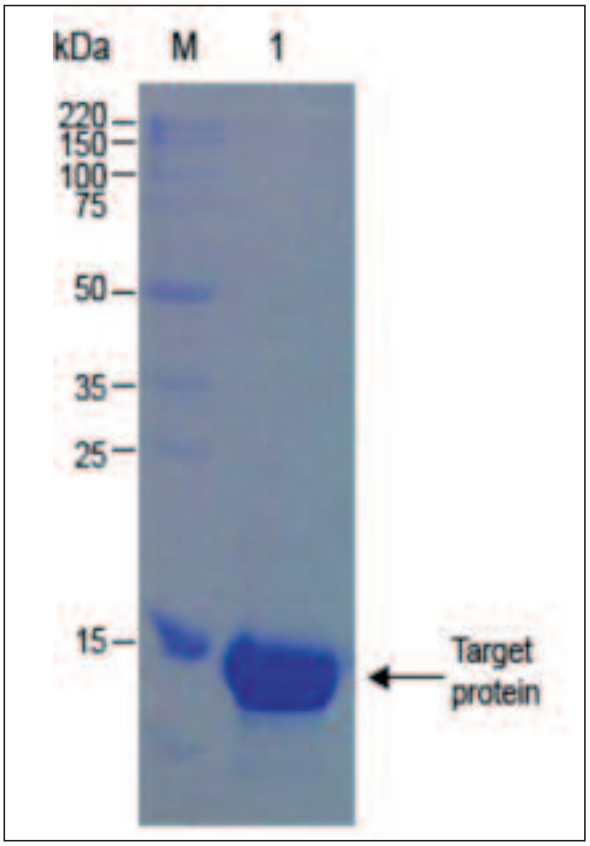

Figure 1. SDS-PAGE pattern of the purified protein from the seeds of *Peganum harmala* L. The protein appeared as a single band with an approximate molecular mass of 15 kD.

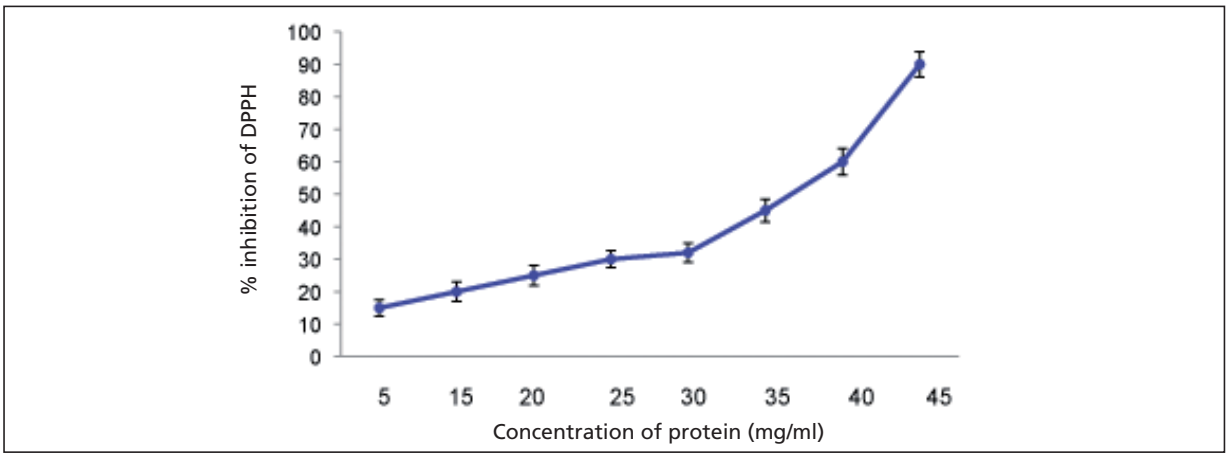

Figure 2. DPPH radical scavenging activity of the protein. The curve is obtained by plotting concentration of the protein (mg/ml) against percent inhibition of DPPH radical.

Effect of the 15 KD Protein on Brain Malondialdehyde (MDA) Level

Table I shows that, the level of brain malondialdehyde (MDA) increased significantly ($p<0.05$) subsequent to CCl_4 administration (1 ml/kg b.wt. p.o.) for two days, as compared to the control group. Protein pretreatment (4 or 8 mg/kg b.wt. i.p.) and vitamin C (250 mg/kg b.wt. p.o) administration for seven days significantly decrease ($p<0.05$) the brain MDA levels as compared to CCl_4 group (Table I). The level of MDA in the protein pretreated groups (4 or 8 mg/kg b.wt. i.p) was significantly ($p<0.05$) higher than that of the control group (Table I).

Administration of CCl_4 for 2 days significantly increased ($p<0.05$) the MDA level of brain as compared to the control group (Table I). Posttreatment of the rats with the protein at the two selected doses and vitamin C decreased the brain MDA level significantly ($p<0.05$) as compared to CCl_4 group (Table I). However, the level of brain MDA was significantly increased ($p<0.05$) subsequent to posttreatment for 7 days with isolated protein (4 or 8 mg/kg b.wt.) as compared to the control group (Table I).

Effect of the 15 KD Protein on Brain Reduced Glutathione (GSH)

Brain GSH level of rats administered CCl_4 for two days after 7 days of distilled water administration was found significantly decreased ($p<0.05$) as compared to the control rats (Table I). Pretreatment with 15 KD protein isolated from the *Peganum harmala* L. seeds (4 mg or 8

Table I. Effect of 15 KD protein on brain AChE, MDA, GSH, and GST in CCl_4- treated rats.

		Parameters			
	Group	AChE (µmol/min/g tissue)	MDA (nmol/g tissue)	GSH (mmol/g tissue)	GST (U/g tissue)
Pretreatment	Control	37.35 ± 1.13	26.74 ± 1.32	4.29 ± 0.34	0.16 ± 0.01
	CCl_4	32.09 ± 0.38[a]	44.29 ± 0.49[a]	2.14 ± 0.08[a]	1.38 ± 0.13[a]
	$P_1 + CCl_4$	38.79 ± 1.62[b]	35.94 ± 1.17[a b]	4.83 ± 0.13[b]	0.70 ± 0.05[a b]
	$P_2 + CCl_4$	41.22 ± 1.98[a b]	35.66 ± 0.50[a b]	4.61 ± 0.20[b]	0.67 ± 0.04[a b]
	Vit. C	36.25 ± 0.38[b]	28.99 ± 0.84[b]	5.44 ± 0.21[a b]	0.65 ± 0.05[a b]
Posttreatment	Control	36.54 ± 1.20	31.98 ± 3.23	5.20 ± 0.34	0.18 ± 0.01
	CCl_4	31.99 ± 0.66[a]	54.01 ± 1.28[a]	2.19 ± 0.16[a]	1.41 ± 0.08[a]
	$CCl_4 + P_1$	34.24 ± 0.69	45.51 ± 3.00[a b]	5.36 ± 0.19[b]	0.52 ± 0.05[a b]
	$CCl_4 + P_2$	34.71 ± 0.41	41.25 ± 2.17[a b]	5.25 ± 0.19[b]	0.83 ± 0.11[a b]
	Vit. C	31.97 ± 2.00[a]	36.31 ± 1.26[b]	5.14 ± 0.13[b]	0.56 ± 0.03[a b]

All data are mean of six rats ± SEM. [a]Significant as compared to control. [b]Significant as compared to CCl_4.

mg/kg b.wt. i.p) and vitamin C (250 mg/kg b.wt. p.o) caused significant increase ($p<0.05$) in the level of brain GSH as compared to CCl_4 group (Table I). Pretreatment with vitamin C was found to increase significantly (P<0.05) the brain GSH level as compared to the control group (Table I).

Table I shows that, in post treated group, CCl_4 administration caused significant decrease ($p<0.05$) in brain GSH level as compared to control group. Posttreatment with isolated 15 KD protein (4 or 8 mg/kg b.wt. i.p) and vitamin C (250 mg/kg b.wt. p.o) for 7 days caused significant increase ($p<0.05$) in the brain GSH level, as compared to CCl_4 group (Table I).

Effect of the 15 KD Protein on Brain glutathione-S-transferase (GST)

Table (1) shows that CCl_4 administration, protein pretreatment (4 or 8 mg/kg b.wt. i.p) and vitamin C (250 mg/kg b.wt. p.o)) administration prior to CCl_4 intoxication were found increased significantly ($p<0.05$) the activity of brain GST as compared to the control group. Pretreatment with 15 KD protein and vitamin C administration caused significant decrease ($p<0.05$) in the activity of brain GST, as compared to CCl_4 group (Table I).

Brain glutathione-S-transferase activity increased significantly ($p<0.05$) in all experimental post treated groups as compared to the control group (Table I). Protein posttreatments and vitamin C administration for 7 days subsequent to two days of CCl_4 administration were found to decrease significantly ($p<0.05$) the activity of brain GST, as compared to CCl_4 group (Table I).

Effect of the 15 KD Protein on Brain Acetylcholinesterase (AchE)

Carbon tetrachloride administration (1 ml/kg b.wt.) for two days caused significant decrease ($p<0.05$) in the activity of brain AchE as compared to the control group (Table I). Pretreatment with 15 KD isolated protein (4 mg and 8 mg/kg b.wt i.p) and vitamin C for 7 days prior to CCl_4 administration significantly increased ($p<0.05$) the activity of brain AchE, as compared to CCl_4 group. Meanwhile, administration of 8 mg/kg b.wt. isolated protein prior to CCl_4 treatment caused significant increase ($p<0.05$) in the activity of AchE of rats as compared to the control group (Table I). The curative effect of the 15 KD protein on brain AchE activity is shown in Table I. Brain acetyl cholinesterase activity decreased significantly ($p<0.05$) subsequent to CCl_4 administration for two days, as compared to control group (Table I). Meanwhile, vitamin C administration for 7 days after CCl_4 intoxication caused significant decrease ($p<0.05$) in the activity of brain AchE of rats, as compared to the control group (Table I). On the other hand, posttreatment with 15 KD isolated protein from *Peganum harmala* L. seeds (4 or 8 mg/kg b.wt. i.p) for 7 days insignificantly changed the activity of brain AchE, as compared to CCl_4 intoxicated group (Table I).

Effect of the 15 KD Protein on Testis MDA Level

Table II shows that CCl_4 administration was significantly increased ($p<0.05$) the value of MDA in the testis of rats, as compared to the

Table II. Effect of 15 KD protein on testes MDA, GSH, and GST in CCl_4- treated rats.

		Parameters		
	Group	MDA (nmol/g tissue)	GSH (mmol/g tissue)	GST (U/g tissue)
Pretreatment	Control	44.23 ± 0.83	3.93 ± 0.25	0.15 ± 0.01
	CCl_4	71.20 ± 4.52[a]	1.98 ± 0.21[a]	2.62 ± 0.29[a]
	P_1	51.60 ± 2.78[a b]	4.23 ± 0.18[b]	1.14 ± 0.13[a b]
	P_2	51.15 ± 1.21[b]	3.67 ± 0.38[b]	1.06 ± 0.08[a b]
	Vit. C	41.85 ± 0.73[b]	3.50 ± 0.25[b]	0.73 ± 0.07[a b]
Posttreatment	Control	59.49 ± 2.37	2.75 ± 0.25	0.15 ± 0.01
	CCl_4	99.77 ± 6..85[a]	1.14 ± 0.09[a]	2.62 ± 0.29[a]
	P_1	81.31 ± 6.51[a b]	5.60 ± 0.34[a b]	1.14 ± 0.13[a b]
	P_2	49.80 ± 2.08[b]	4.59 ± 0.35[a b]	1.05 ± 0.08[a b]
	Vit. C	48.02 ± 1.13[b]	4.27 ± 0.18[a b]	0.73 ± 0.07[a]

All data are mean of six rats ± SEM. [a]Significant as compared to control. [b]Significant as compared to CCl_4.

control group. Protein treatments (4 mg and 8 mg/kg b.wt.) and vitamin C administration significantly decreased ($p<0.05$) the level of MDA in the testis of rats, as compared to CCl_4 group (Table II). Pretreatment with isolated protein at a dose of 4 mg/kg b.wt. significantly increased the testis MDA level of rats as compared to the control group (Table II).

In post treated groups, administration of CCl_4 caused significant increase ($p<0.05$) in the level of testis MDA as compared to the control group (Table II). Posttreatment with the isolated protein (4 or 8 mg/kg b.wt. i.p) and vitamin C administration was found to ameliorate the damaged effect of CCl_4 by decreasing the level of testis MDA significantly ($p<0.05$) as compared to CCl_4 group. Posttreatment with 4 mg/kg b.wt. of isolated protein was found to increase significantly ($p<0.05$) the level of MDA in the testis of rats, as compared to that of control rats (Table II).

Effect of the Protein on Testis GSH

Significant decrease ($p<0.05$) was noticed in the level of testis GSH of rats orally administered CCl_4 as compared to the control group (Table II). Pretreatment with 4 or 8 mg/kg b.wt. (i.p) isolated 15 KD protein and vitamin C (250 mg/kg b.wt. p.o) for 7 days caused significant increase ($p<0.05$) in testis GSH level of rats, as compared to CCl_4 groups.

Table II shows that, in post treated group CCl_4 administration for two days caused significant decrease ($p<0.05$) in testis GSH level, as compared to control group. Posttreatment with isolated 15 KD protein (4 or 8 mg/kg b.wt. i.p) and vitamin C administration for 7 days, was found significantly increased ($p<0.05$) the testis GSH level, as compared both to control and CCl_4 groups (Table II).

Effect of the Protein on testis GST Activity

In the present study, testis GST activity was increased significantly ($p<0.05$) after CCl_4 administration for two days as compared to the control group (Table II). Pretreatment for 7 days with 15 KD isolated protein from the *Peganum harmala L.* seeds (4 or 8 mg/kg b.wt. i.p) and vitamin C (250 mg/kg b.wt. p.o) caused significant either, increase or decrease in the activity of testis GST, as compared to control and CCl_4 groups, respectively (Table II).

Posttreatment with the isolated protein at the two selected doses and vitamin C administration significantly ($p<0.05$) increased and decreased the activity of testis GST as compared both to the control and CCl_4 groups, respectively (Table II).

Effect of the Protein on Erythrocyte MDA Level

Figure 3 shows that carbon tetrachloride administration in pretreatment group decreased significantly the MDA level ($p<0.05$) as compared to the control group. Isolated 15 KD protein pretreatment (4 mg/kg b.wt.) and vitamin C administration for 7 days prior to CCl_4 administration caused significant increase ($p<0.05$) in the level of MDA of the erythrocytes, as compared to CCl_4 group (Figure 3). However, pretreatment with

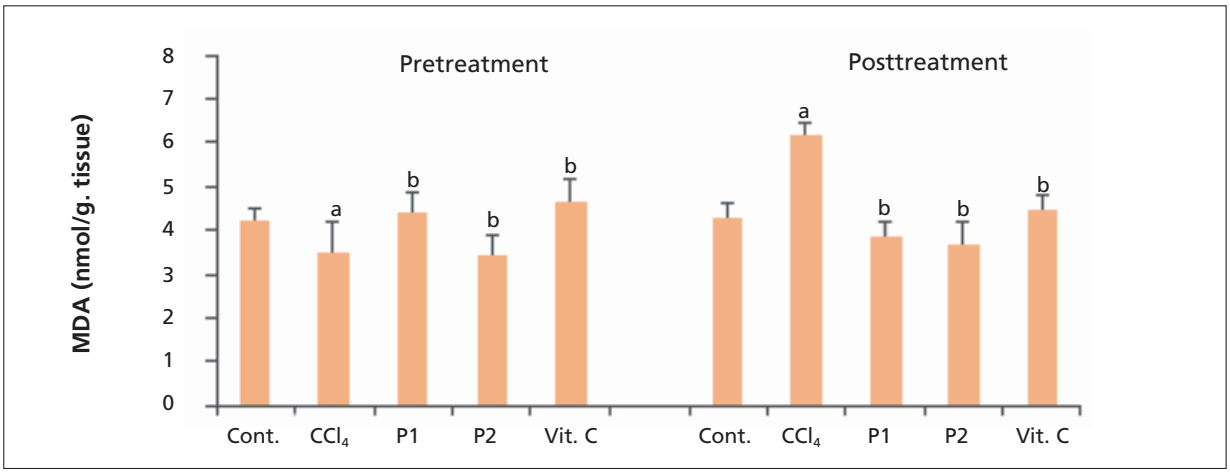

Figure 3. Effect of the protein pre and posttreatments on the erythrocyte MDA level in CCl_4 treated rats. ***A,*** Significant as compared to control. ***B,*** Significant as compared to CCl_4.

protein (8 mg/kg b.wt i.p) significantly decreased (p<0.05) the level of erythrocytes MDA, as compared to CCl_4 group (Figure 3).

Rats administered CCl_4 for two days, followed by distilled water for 7 days showed significant increase (p<0.05) in their erythrocytes MDA level as compared to the control group (Figure 3). Posttreatment for 7 days with isolated protein from the *Peganum harmala* L. seeds (4 or 8 mg/kg b.wt. i.p) and oral administration of vitamin C caused significant decrease (p<0.05) in the erythrocyte MDA level as compared to that of CCl_4 group (Figure 3).

Effect of the Protein on Erythrocytes GSH Level

In pretreatment group, CCl_4 administration caused significant decrease (p<0.05) in erythrocyte GSH level as compared to the control group (Figure 4). Isolated 15 KD protein (4 or 8 mg/kg b.wt.) and vitamin C administration prior to CCl_4 administration were significantly increase (p<0.05) the level of GSH in the erythrocytes, as compared to the CCl_4 group (Figure 4). Pretreatment with 4 mg/kg b.wt. isolated protein and vitamin C caused significant increase (p<.05) in the level of erythrocytes GSH as compared to the control group (Figure 4).

Figure 4 shows that CCl_4 administration for two days in the post treated group caused nonsignificant change in the erythrocyte GSH level as compared to the control group. Posttreatments with isolated protein and vitamin C administration for 7 days significantly increase (p<0.05) the erythrocyte GSH level, as compared both to control and CCl_4 groups (Figure 4).

Effect of the Protein on Erythrocyte GST Activity

CCl_4 administration for two days caused nonsignificant change in the activity of erythrocytes GST as compared to the control group (Figure 5). Pretreatment with isolated 15 KD protein at the two selected doses non insignificantly changed the activity of erythrocytes GST. However, pretreatment with vitamin C (250 mg/kg b.wt. p.o) significantly increased the erythrocyte GST activity as compared both to the control and CCl_4 groups (Figure 5).

Figure 5 shows that CCl_4 administration for 2 days followed by distilled water administration for 7 days caused significant increase (p<0.05) in erythrocytes GST activity as compared to the control group. On the other hand, erythrocyte GST activity was significantly decreased (p<0.5) subsequent to posttreatments with isolated 15 KD protein and vitamin C as compared to CCl_4 group (Figure 5).

Discussion

Toxic effects of carbon tetrachloride (CCl_4) on liver have been extensively studied[31-33]. Although it is primarily metabolized in liver, its determinal effects on the brain and testes are well documented[2,16,34]. The lipid solubility of CCl_4 allows it to

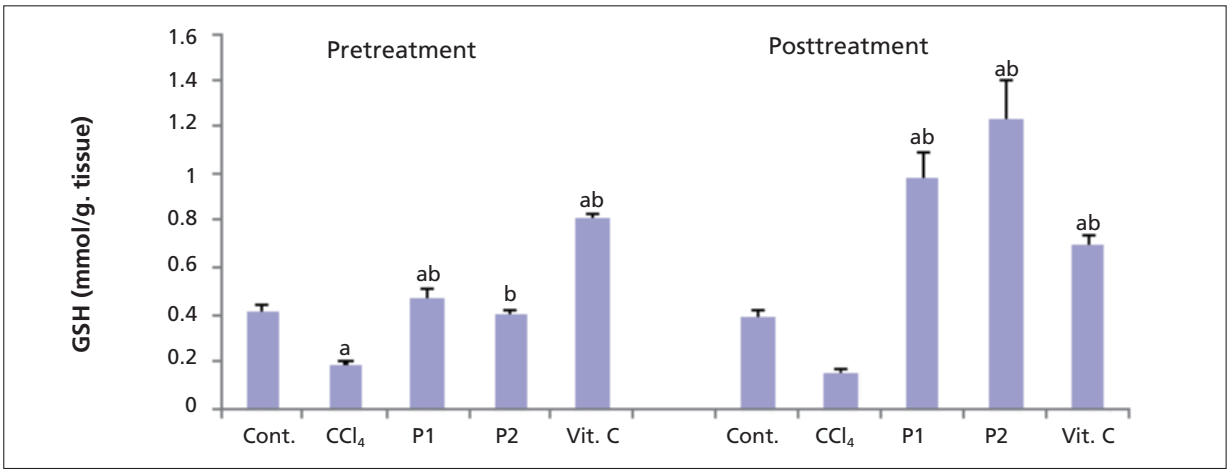

Figure 4. Effect of the protein pre and posttreatments on the erythrocyte GSH level in CCL4 treated rats. ***A,*** Significant as compared to control. ***B,*** Significant as compared to CCl_4.

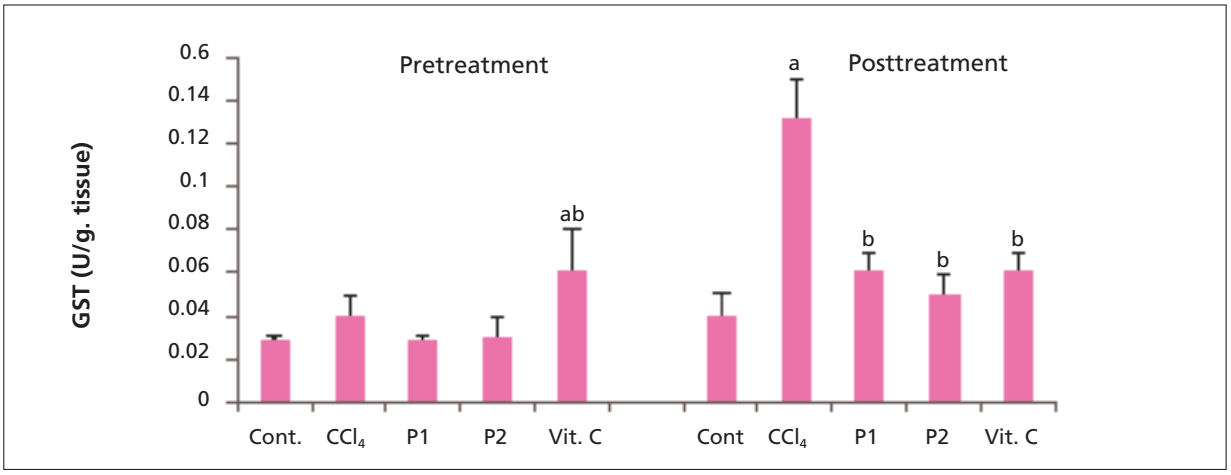

Figure 5. Effect of the protein pre and posttreatments on the erythrocyte GST activity in CCL4 treated rats. ***A,*** Significant as compared to control. ***B,*** Significant as compared to CCl_4.

cross cell membranes and deposits it to different tissues such as liver, brain and testes[11].

Free radicals and reactive oxygen species (ROS) in biology gains more attention and there is increasing awareness of the ubiquitous role of oxidative stress in neuropathology[35-36]. Free radicals have been implicated in multiple central nervous system disorders[37]. This is understandable since this tissue is highly sensitive to oxidative stress due to its high oxygen consumption, its high iron and lipid contents, especially polyunsaturated fatty acids, and the low activity of antioxidant defenses[38].

In conjunction with the reports of Pentyala et al[34]; Dani et al[14] and Boer et al[2], data from the present investigation reflects that oxidative stress in brain is a common feature of CCl_4 toxicity. The commonly used indicator of tissue oxidative damage is the peroxidation of lipids in the form of aldehydic products such as malondialdehyde (MDA), which, is widely accepted to be one of the principle causes of CCl_4-induced toxicity[39]. Administration of CCl_4 in the current study provoked significant increase in MDA level in the brain tissue. In agreement with our results Pushpakiran et al[40], Dani et al[14] and Jayakumar et al[41] recorded induction in the MDA levels following CCl_4 administration in the brain tissues of the treated rats.

Glutathione reduced (GSH) is the major endogenous antioxidant which counterbalances free radical mediated damage. It is well known that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions[40]. The present study confirmed the finding of Srivastava et al[42] who suggested that enhancement of lipid peroxidation is a consequence of depletion of reduced glutathione (GSH) to certain critical levels. Insufficiency in non-enzymatic antioxidant GSH following CCl_4 intoxication could be the consequence of increased utilization for trapping free radicals. In consonance with our study Pushpakiran et al[40] and Jayakumar et al[41] have reported depletion in GSH level in the brain of CCl_4 intoxicated rats.

Glutathione-S-transferase (GST) is an enzyme that participates in the detoxification process due to conjugation reaction between GSH and xenobiotics[43]. The present study showed significant increase in GST activity in the brain of CCl_4 treated rats. This increase could be due to the increased formation of S-conjugates between trichloromethyl radical and GSH to counter the effect of increased oxidative stress. In agreement with the present investigation, Smith and Litwack[44] reported that increased GST activity is known to serve as protective response to eliminate xenobiotics.

Acetylcholinesterase (AchE) is one of the most crucial enzymes of nerve response and function. AchE catalyzes the hydrolysis of acylcholinesters with a relative specificity for acetylcholine. Administration of CCl_4 in the present research induced decrease in AchE activity in the brain tissues of intoxicated rats. This decrease could be due to oxidation of the presynaptic protein thiol groups which subsequently reduced the AchE release[45]. Escobar et al[46] indicated that en-

hanced free radical concentration resulting from oxidative stress condition can cause loss of enzymatic activities. Furthermore, Szymonik-Lesiuk et al[11] reported that products of peroxidation, MDA is known to inhibit protein synthesis and the activities of certain enzymes. The present study extended the previously reported finding that CCl_4 exert neurotoxicity from one hand by altering signal transduction pathways in brain[34] and from the other hand by the inhibition of different complexes from mitochondrial respiratory chain[2].

Testicular tissue and seminal plasma are endowed with antioxidant enzymes and free radicals scavengers to protect spermatogenic functions from oxidative stress[47]. The present investigation showed that CCl_4 administration induced oxidative stress in the testes of the treated rats as indicated by enhanced MDA level, depletion in GSH content and increase in GST activity. Oxidative stress is known to show detrimental effects on the testis function via the induction of peroxidative damage to the plasma membrane[48]. In consonance with the present study, Fadhel and Amran[49], Manjrekar et al[15] and Khan and Ahmed[16] showed that CCl_4 intoxication induced enhancement in MDA level and depletion in GSH in the testes of the treated rats.

Oxidative stress in the erythrocytes in the present investigation can be assessed by induction in MDA level, reduction in GSH and hyperactivity of the GST. Erythrocytes are prone to oxidative stress because they are exposed to high oxygen tension, have polyunsaturated fatty acids in the membrane and hemoglobin-bound iron[50-52].

However, the naturally occurring biochemical defense system including enzymes such as GST and compounds such as GSH could not provide complete protection against the attack of ROS in conditions of severe oxidative stress[53-55]. Therefore, scientists have been trying to find bioactive substances possessing cytoprotective ability against cellular oxidative damage, as well as enhancing the ability of the antioxidant enzyme activities[56].

The present research reveals the protective and curative activities of the protein purified from *Peganum harmala* L. seeds against CCl_4 toxicity. Treatment of rats with 15 KD isolated protein prior or post to the CCl_4 administration had a profound effect on lipid peroxidation products as it significantly lowered MDA level in the brain and testes but can not normalized its level as compared to the control. This finding suggests that this protein could play a positive role in minimizing lipid peroxidation and act through an antioxidant mechanism. In agreement with our data Ghosh et al[22] demonstrated that 43 KD protein isolated from the herb *Cajanus indicus* L. attenuates oxidative stress and reverses lipid peroxidation in mice. Results from the posttreatment studies suggest that the isolated protein also possesses the same curative activity of the vitamin C in the erythrocyte against CCl_4 intoxication in rats. In consonance with our investigation, Devi et al[57] and Rai et al[52] have reported that supplementation of vitamin C to rats resulted in decreased oxidative stress in erythrocytes.

The DPPH radical, because of its odd electron gives a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in presence of a radical scavenger, its absorption vanishes and the resulting decolourisation is stoichiometric with respect to the number of electrons taken up. The 15 kD protein at various concentration was able to quench the synthetic DPPH radical. Having established a concentration based radical scavenging activity of the protein we, then, proceeded to investigate the ability of the protein to react with O_2 – and H_2O_2, both of which are potentially cytotoxic. Results showed that the protein could not react with H_2O_2 and potentiate its breakdown to water. However, it exhibited a SOD-like effect, inhibiting O_2– formation in a dose dependent manner.

However, treatment of rats either pre- or post-CCl_4 intoxication in the brain tissues with protein by intraperitoneal means bring back the levels of GSH near the normal levels as do vitamin C. The restoration of GSH level by the isolated protein could be due either to its effect on the *de novo* synthesis of glutathione, its regeneration, or both[58].

Findings from the Pretreatment studies in the present investigation showed that isolated protein also possesses protective effect in the testes and erythrocytes of CCl_4 treated rats as it restores the GSH level near to the normal level, again suggesting its antioxidant role as vitamin C. However, regarding to posttreatment effect of the isolated protein and vitamin C, it can be noticed that the level of the GSH significantly increased even over the normal range. This may be happen due to two reasons: the protein may act directly and scavenges the ROS derived by oxidation-reduction cycle with the cell or it may work in union with the existing antioxidant compounds and helps to prevent their loss during the oxidative injury caused by CCl_4[22].

In most studies on xenobiotic induced toxicity, the protective agent has been shown to be able to protect the damaged tissue when administered prior to the toxicant. Our research, however, reveals that the protein has got equal potential as a protective agent in both pretreated and post-treated rats regarding to GST activities in brain and testes. This efficient recovery in GST activity highlights the therapeutic efficacy of this protein in alleviating the CCl_4-induced oxidative stress. Results from the posttreatment studies suggest that the isolated protein at 4 mg/kg or 8 mg/kg restored the activity of the GST in the erythrocytes near to the normal level indicating that this protein could be possesses not only the capacity to scavenge the ROS, but also the capacity to block the CCl_4-induced massive ROS production.

However, treatment of rats with this protein in the present investigation, prior or post-CCl_4 application had a profound effect on the oxidative stress in brain which is the common feature of CCl_4 toxicity in brain. This can be indicated from the direct effect of this protein on the AchE activity in the brain of CCl_4 intoxicated rats as it restored the enzyme activity near the normal values during both preventive and the curative effects.

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