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Full Length Research Paper

Antioxidant effect of the Egyptian freshwater *Procambarus clarkii* extract in rat liver and erythrocytes

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The present study aims to evaluate the curative activity of freshwater crustacean extract (FCE) from freshwater crayfish *Procambarus clarkii* and silymarin on oxidative stress induced by carbon tetrachloride (CCl_4) in rat liver and erythrocytes. The degree of treatment in this activity has been measured by using biochemical parameters such as serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP) and total protein as well as malondialdehyde (MDA), glutathione reduced (GSH), glutathione-S-transferase (GST) and catalase (CAT) in liver tissue and erytheocyte hemolysate. The FCE and silymarin produced significant antihepatotoxic effect by decreasing the activity of serum enzymes and MDA level, and increasing the serum total protein, GSH levels and the activities of GST and CAT in liver and erytheocyte hemolysate. From these results, it was suggested that FCE could ameliorate the liver and erythrocytes injuries perhaps, by its antioxidative effect, hence eliminating the deleterious effect of toxic metabolites from CCl_4.

Key words: *Procambarus clarkii,* freshwater shrimp, liver, CCl_4, erythrocytes, oxidative stress.

INTRODUCTION

Oxidative stress is a redox disequilibrium in which the pro-oxidant/antioxidant balance is shifted in favour of the pro-oxidants (Sies, 1986) a phenomenon related to the aerobic nature of cellular metabolism, in which O_2 reduction is a major event. The latter proceeds through electron transfer reactions due to the electronic structure of O_2 in the ground state, with generation of reactive oxygen species (ROS), including (1) primary oxidants [superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$)]; and (2) secondary oxidants [hydroperoxides or alkoxy and peroxy radicals of biomolecules, in addition to electronically excited states derived from free-radical reactions (singlet oxygen, triplet carbonyls)] (Videla and Fernandez, 1988). The detoxication of ROS is a major prerequisite of aerobic life (Sues, 1986), which is accomplished via several enzymatic and non-enzymatic antioxidant mechanisms that are available in different cell compartments (Fernandez and Videla, 1996).

CCl_4 is a common industrial solvent which is well-known for its hepatotoxicity (Abraham et al., 1997; Guven et al., 2003). CCl_4 is commonly used for free radical induced liver injury (Junnila et al., 2000; Amin and Mahmoud - Ghoneim, 2009; Cui et al., 2009; Kim et al., 2010). Liver is not the only target organ of CCl_4 but it also affect several organs like kidneys, lungs, heart, testes, brain and blood (Ahmad et al., 1987; Ozturk et al., 2003). Through the investigation of acute CCl_4 induced liver damage in animal models, it is now generally accepted that CCl_4 toxicity results from bioactivation of CCl_4 into trichloromethyl free radical by cytochrome P_{450} system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver injury (Ohata et al., 2008; Cui et al., 2009; Kim et al., 2010). Erythrocytes are permanently in contact with potentially damaging levels of oxygen, but their metabolic activity is capable of reversing this injury under normal conditions (Kenan and Bulbuloglu, 2005; Ghandrasena et al., 2006; Avci et al., 2008). Erythrocytes are equipped by many defence systems representing their antioxidant capacity (Kurata et al., 1993). This protective system includes superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR).

However, the cellular antioxidant action is reinforced by

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the presence of dietary antioxidants (Kenan and Bulbuloglu, 2005; Ghandrasena et al., 2006; Avci et al., 2008). It was known that CCl_4 – induced changes in erythrocytes membrane composition and membrane bound Na^+/K^+ and Ca^{+2} dependant ATPase activities (Muriel et al., 1993; Vajdovich et al., 1995; Adaramoye and Akinloye, 2000). Antioxidant and anti-inflammatory agents play a critical role against CCl_4 intoxication by scavenging active oxygen and free radicals and neutralizing lipid peroxides (Murugesan et al., 2009; Gutierrez and Navarro, 2010). As a consequence of an increasing demand for the biodiversity in screening programs, seeking therapeutic drugs from natural products there is now a greater interest in the freshwater organisms (Chijimatsu et al., 2008; Chakraborty et al., 2010; Hsu et al., 2010).

Products from freshwater sources have become attractive as nutraceutical and functional foods and as a source material for the development of drugs and specific health foods (Chakraborty et al., 2010; Hsu et al., 2010). Recent attention has been focused upon supplements derived from freshwater foods and their utilization as hepatoprotective agents (Chijimatsu et al., 2008; Chakraborty et al., 2010; Hsu et al., 2010). Freshwater crayfish, *Procambarus clarkii,* has been widely spread all over most of the River Nile (Elmossalami and Emara, 1999). *P. clarkii* is heavily exploited as a fishery product and used widely in aquaculture. It is represented an important food source. The yield of its abdominal muscles ranges from 10 to 40% of the total body weight, depending on size, and maturity (Mona et al., 2000).

Recently, we have investigated the curative effect of the freshwater crustacean extract (FCE) from the freshwater shrimp *P. clarkii* on CCl_4 induced nephrotoxicity in rats (Fahmy et al., 2009). Based on the results from our experiment, we showed that FCE has antioxidant activity related to its high taurine content as well as glutamic acid, cysteine and glycine (the amino acids components of GSH). So, FCE might be effective against diseases in which ROS play a role as potent causative factors because it has a strong antioxidant activity. Silymarin is the most known hepatoprotective drug used as a reference standard also exhibited significant protective effect against CCL4 induced liver damage (Lieber et al., 2003). Therefore, the present study aimed to examined the curative effect of FCE on CCl_4 induced hepatic and erythrocytes injuries in the male rats and elucidate the possible mechanisms of this effect.

MATERIALS AND METHODS

Preparation of crude freshwater crustacean extract (FCE)

Freshwater crayfish, *P. clarkii,* specimens were collected from the River Nile at Abu- Rawash area-Giza Governorate. Freshwater crustacean extract powder was prepared as follows: fresh raw specimens (1 kg) were used. All appendages were cut and the fresh whole bodies away from the carapace and stored at -20°C until needed. After thawing, the specimens were homogenized with a mixer. The homogenate was extracted with water for 3 h. After filtration, the filtrate obtained was then concentrated and lyophilized to a brownish residue using (LABCONCO lyophilizer, shell freeze system, USA). The freshwater crustacean extract (FCE) was stored in dry place avoiding water vapor until used.

Chemicals

Carbon tetrachloride was purchased from Merk Egypt. Silymarin was purchased from Sedico (Pharmaceutical Co., 6 October City, Egypt).

Experimental animals

The experimental animals used in this study were the adult male albino rats (*Rattus norvegicus*) weighing 100 to 120 g. The animals were obtained from National Research Center (NRC), Dokki, Cairo, Egypt. Animals were caged in groups of ten and given food and water *ad libitum*. Rats were kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt.

Experimental protocol

Animals were divided into three main groups, the 1st group serves as control; animals of this group (6 rats/group) administered olive oil orally by gastric gavage for 2 days, and followed by distilled water for 7 consecutive days. Animals of the 2nd group (6 rats/group) administered FCE (250 mg/kg body weight, p.o.) for 9 days. Third group (30 rats), given CCl_4 orally (2.5 ml/kg body weight of 50%, dissolved in olive oil) for 2 days, this group then divided into 5 subgroups (6 rats/ subgroup), animals of these subgroups treated for 7 consecutive days as follows:

Subgroup I (CCl_4): Rats of this subgroup administered distilled water orally.

Subgroup II (Silymarin): Rats treated orally with standard drug silymarin (150 mg/kg body weight, dissolved in distilled water).

Subgroups III, IV, V: Animals of these subgroups treated orally with FCE (50, 100 and 250 mg/kg body weight), respectively. All animals were sacrificed on the 10th day of treatment after being fasted overnight; blood was collected in EDTA containing tubes and centrifuge tubes. Liver was removed rapidly and stored at - 80°C.

Sample preparation

Serum preparation

Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm for 20 min. Serum, stored at -20°C until used for biochemical assays.

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

Table 1. Effect of FCE and silymarin on the total protein content and liver function markers ASAT, ALAT, ALP following CCl_4 intoxication in rats.

Groups		Parameters			
		Total protein (g/100 ml)	ASAT (IU/ml)	ALAT (IU/ml)	ALP (IU/L)
Control		6.45 ± 0.39	13.92 ± 0.66	24.90 ± 0.41	79.76 ± 2.34
FCE (250 mg/kg)		7.98 ± 0.08[a]	13.94 ±0.90	26.48 ± 0.54 [a]	80.75 ± 0.62
CCl_4		5.82 ± 0.39 [a]	38.21± 0.78 [a]	77.38 ± 2.35 [a]	198.65 ± 3.87 [a]
CCl_4 + Silymarin		6.28 ± 0.22 [b]	15.24 ± 0.77 [b]	27.73 ± 0.69 [b]	82.88 ± 2.75 [b]
CCl_4 + (FCE)	50 mg/kg	6.04 ± 0.21 [b]	14.22 ± 0.35 [b]	30.45 ± 0.32 [b]	88.60 ± 1.79 [b]
	100 mg/kg	6.46± 0.42 [b]	15.28 ± 0.33 [b]	27.33 ± 0.64 [b]	97.31 ± 4.53 [b]
	250 mg/kg	6.71 ± 0.70 [b]	16.75 ± 1.01 [b]	38.42 ± 0.76 [b]	86.56 ± 5.66 [b]

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

with hypotonic 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 further biochemical assay. The hemoglobin content in the red blood cell lysate was measured according to cyanomethemoglobin method (Henry, 1964) using Biodiagnostic kit.

Liver tissue preparation

Liver was 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.

Assessment of liver functions

The appropriate kits (Biodiagnostic kits) were used for the determination of serum total protein according to Lowry et al. (1951), aminotransferase activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) according to Reitman and Frankel (1975) and alkaline phosphatase (ALP) activity (Young et al., 1975).

Assessment of oxidative stress markers

Oxidative stress markers were detected in the resultant supernatant of liver homogenate and hemolysate. The appropriate kits (Biodiagnostic kits) were used for the determination of glutathione reduced (GSH) (Aykac et al., 1985), lipid peroxidation which was measured by the formation of malondialdehyde (MDA) (Ohkawa et al., 1979), activity of glutathione- S- transferase (GST) (Habig et al., 1974) and catalase activity (CAT) (Aebi, 1984).

Statistical analysis

Values were expressed as mean ± SEM. To evaluate differences between the studied groups, one way analysis of variance (ANOVA) with LSD post hoc test was used to compare the group means and P<0.05 was considered statistically significant. SPSS, for Windows (Version 15.0) was used for statistical analysis.

RESULTS

Improvement of liver functions in FCE treated rats

The levels of total proteins, activities of aminotransferase (ASAT and ALAT) and ALP in the control, CCl_4 injured, silymarin treated and FCE administered rats are presented in Table (1). Administration of CCl_4 induced significant decrease (P< 0.05) in the total protein content as compared to control (Table 1). Treatment with silymarin and FCE at all tested doses significantly (P < 0.05) increased the total protein content which decreased after CCl_4 intoxication. Activities of aminotransferases (ASAT and ALAT) and ALP of rats administered CCl_4 were significantly increased (P<0.05) as compared to the control (Table 1). Administration of FCE at all tested doses and silymarin following CCl_4 intoxication showed significant decrease (P< 0.05) in the ASAT, ALAT and ALP activities as compared to CCl_4 intoxicated group. The results showed that administration of FCE did not show a significant change in the liver function markers of CCl_4 untreated rats except ALAT activity which showed a significant increase (P<0.05) as compared to control.

Anti-lipidperoxidation activity and antioxidant levels in the liver of FCE treated rats

Oxidative stress markers, reduced glutathione (GSH) and malondialdehyde (MDA) levels as well as glutathione- S-transferase (GST) and catalase (CAT) activities in control, CCl_4 intoxication, silymarin treated and FCE administered rats are shown in Table (2).Data presented in Table (2) showed that CCl_4 administration caused significant decrease (P<0.05) in GSH level as compared to control. Treatment of rats either with silymarin or FCE (50, 100 and 250 mg/kg body weight) significantly increased (P<0.05) the level of GSH as compared to CCl_4 treated group. MDA levels were assessed as indicator of

Table 2. Effect of FCE and silymarin on the liver oxidative stress markers (GSH, MDA, GST and CAT) following CCl_4 intoxication in rats.

Groups		Parameters			
		GSH (mg/g. tissue)	MDA (nmol/g. tissue)	GST (U / g. tissue)	CAT (U/g. tissue)
Control		44.39 ± 2.95	367.76 ± 59.43	0.16 ± 0.05	1.81 ± 0.21
FCE (250 mg/kg)		41.14 ± 3.66	335.29 ± 38.81	0.18 ± 0.02	1.75 ± 0.28
CCl_4		24.53 ± 1.54[a]	816.60 ± 47.96[a]	0.05 ± 0.01[a]	0.60 ± 0.09[a]
CCl_4 + Silymarin		40.98 ± 3.96[b]	453.36 ± 50.53[b]	0.16 ± 0.03[b]	1.34 ± 0.22[b]
CCl_4 + (FCE)	50 mg/kg	28.46 ± 3.55[b]	429.00 ± 51.95[b]	0.13 ± 0.01[b]	1.75 ± 0.18[b]
	100 mg/kg	40.74 ± 2.38[b]	450.74 ± 99.03[b]	0.16 ± 0.07[b]	1.50 ± 0.13[b]
	250 mg/kg	38.54 ± 2.04[b]	450.81 ± 88.57[b]	0.22 ± 0.03[b]	1.54 ± 0.30[b]

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

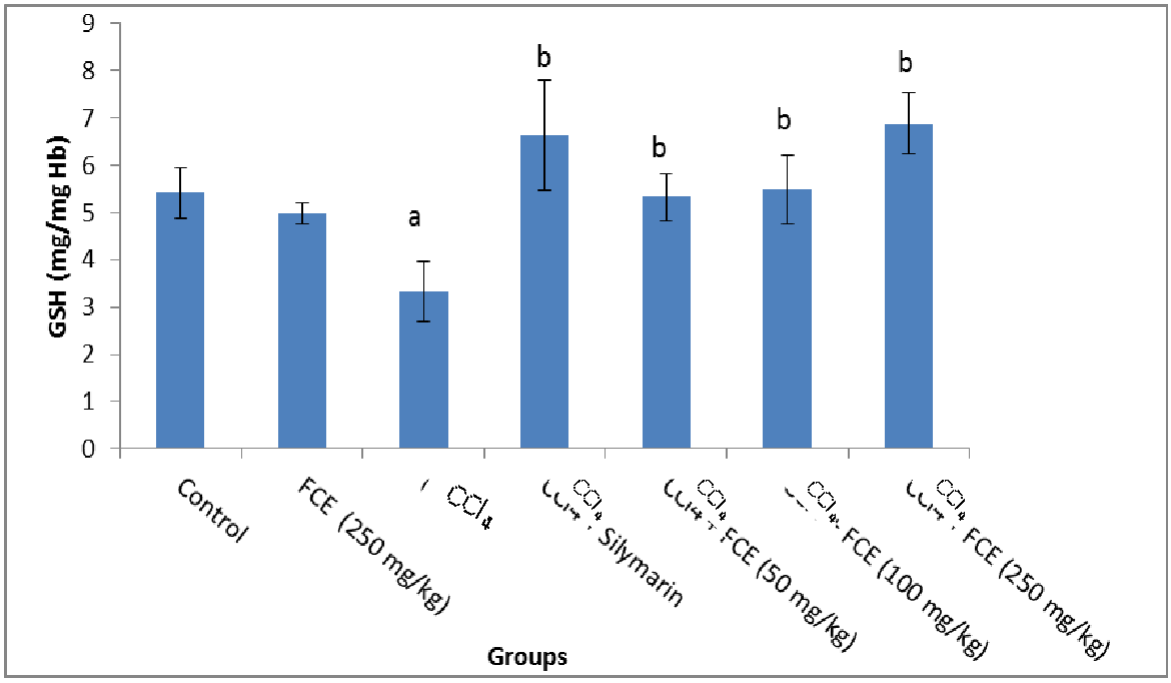

Figure 1. Effect of FCE and silymarin on the erythrocytes glutathione reduced (GSH) following CCl_4 intoxication in rats.

lipid peroxidation, CCl_4 treatment significantly ($P<0.05$) increased the level of MDA in the liver tissue as compared to control (Table 2).

However, treatment with silymarin and FCE at the three tested doses significantly ($P<0.05$) decreased the level of MDA as compared to the CCl_4 treated rats. As shown in Table (2), CCl_4 challenge significantly decreased GST activity ($P<0.05$) as compared to control. Treatment with silymarin and FCE at all the tested doses significantly increased ($P<0.05$) the GST activity as compared with CCl_4 treated group. Concerning the effect of CCl_4 on the catalase (CAT) activity, a significant decrease ($P<0.05$) in the CAT activity was recorded as compared to the control rats (Table 2). Meanwhile, CAT activity increased significantly ($P<0.05$) after all treatment either with silymarin or FCE at the three tested doses. Administration of FCE to CCl_4 untreated rats, had no effect on the studied oxidative stress markers in the liver as compared to control.

Antioxidative activity of FCE in the hemolysate of CCl_4 treated rats

Data presented in Figures 1 to 4, showed the effect of CCl_4 administration, silymarin and FCE treatment on oxidative stress markers in the erythrocyte hemolysate.

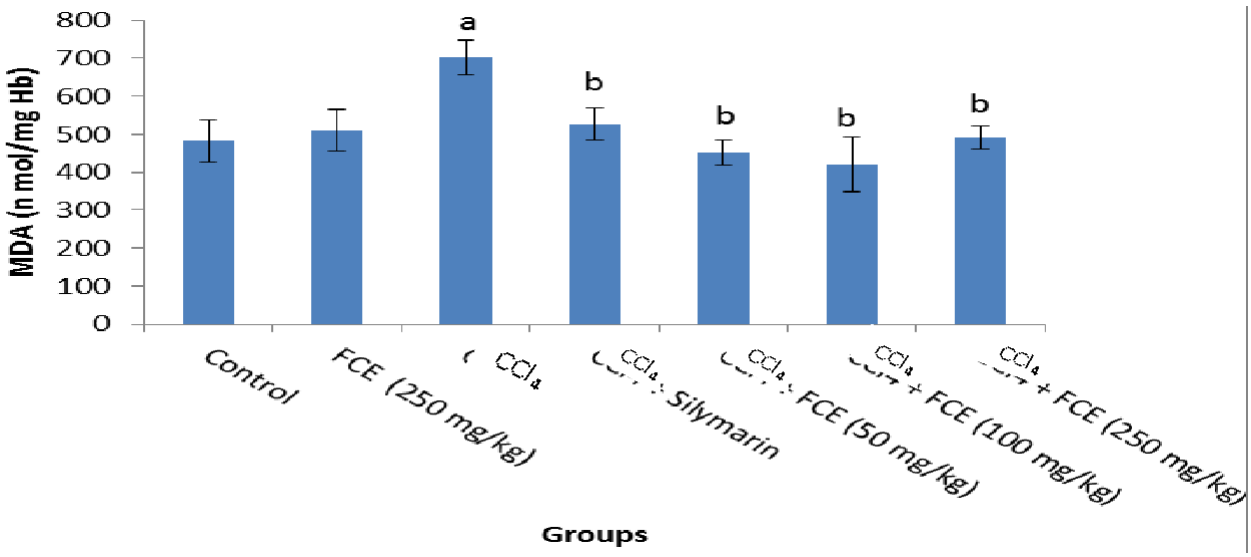

Figure 2. Effect of FCE and silymarin on the erythrocytes malondialdehyde (MDA) following CCl_4 intoxication in rats.

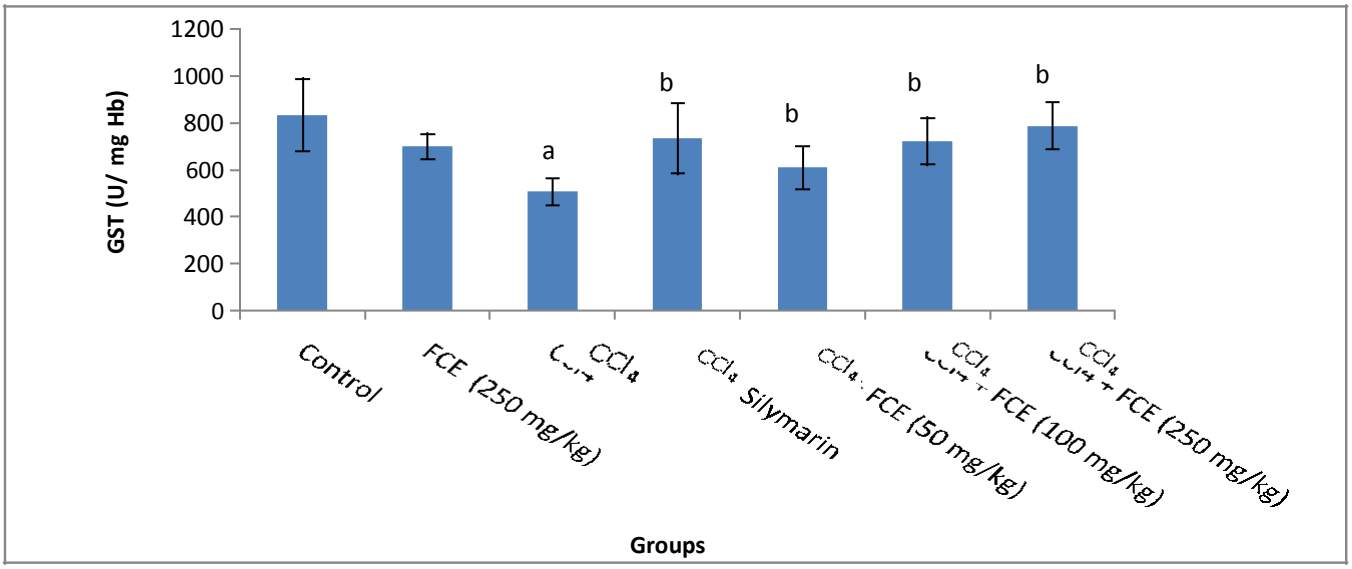

Figure 3. Effect of FCE and silymarin on the erythrocytes glutathione –S-transferase (GST) following CCl_4 intoxication in rats.

Results showed that administration of CCl_4 for 2 days caused significant decrease ($P<0.05$) in GSH level as compared to control. Treatment of rats either with silymarin or FCE (50, 100 and 250 mg/kg body weight) significantly increased ($P<0.05$) the level of GSH (Figure 1). The localization of radical formation resulting in lipid peroxidation, measured as MDA in the hemolysate asshown in Figure (2). CCl_4 treatment significantly ($P<0.05$) increased the level of MDA in the hemolysate as compared to the control.

However, treatment with silymarin or FCE at all tested doses significantly ($P<0.05$) decreased the increased level of MDA as compared to the CCl_4 - treated rats (Figure 2). Nine-days treatment with FCE (250 mg/kg) did not result in a significant alteration of MDA levels as compared to the control group (Figure 2). Concerning the effect of CCl_4 on the GST activity, a significant decrease ($P<0.05$) in the GST activity was recorded as compared to the control rats (Figure 3). Meanwhile, all the treatments either with FCE and silymarin caused significant increase in the GST activity as compared to the CCl_4-treated rats. However, the administration of FCE (250 mg/kg) to CCl_4 untreated rats did not result in any significant change in the GST activity as compared to the

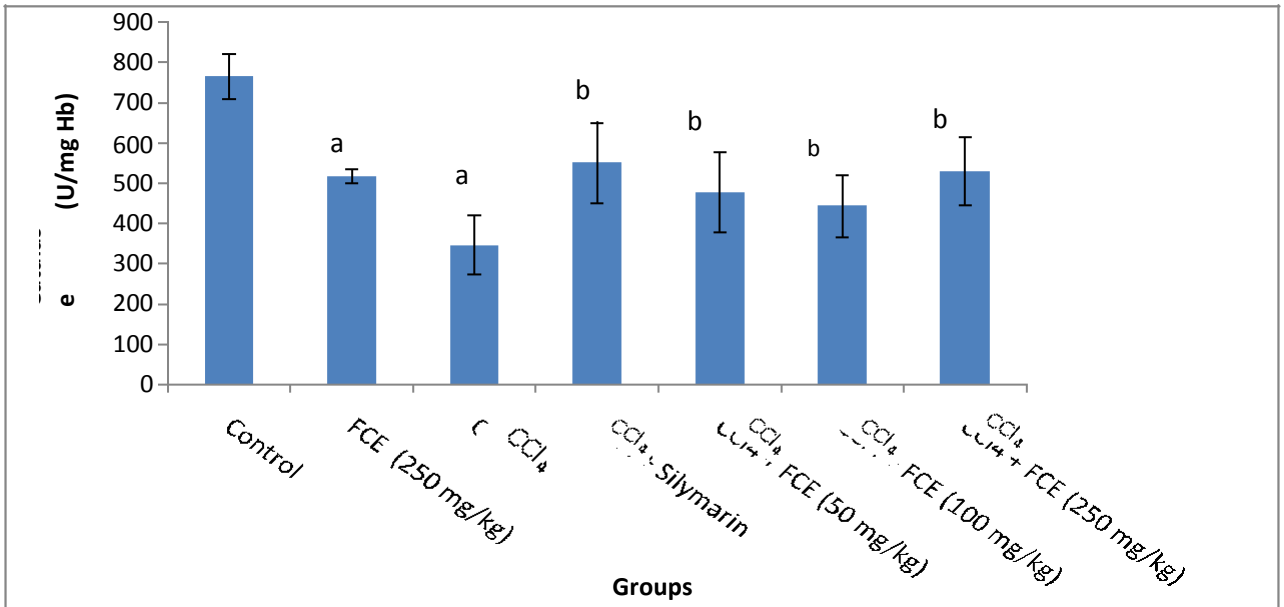

Figure 4. Effect of FCE and silymarin on the erythrocytes catalase (CAT) following CCl_4 intoxication in rats.

control group (Figure 3). As shown in Figure 4, CCl_4 challenge significantly decreased ($P<0.05$) the activity of catalase (CAT) as compared to control rats. However, treatment with silymarin and FCE at different doses significantly ($P<0.05$) increased CAT as compared to CCl_4 - treated group (Figure 4). However, the administration of FCE (250 mg/kg) to CCl_4 untreated rats did not result in any significant change in the CAT activity as compared to the control group (Figure 4).

DISCUSSION

For the therapeutic strategies of liver injury and disease, it is important to find antioxidant compound that are able to block liver injuries through free radicals generated due to toxic chemicals. Therefore, the present study speculated that the freshwater crustacean extract (FCE) which extracted from freshwater crayfish *P. clarkii* specimens protects against diseases that are caused by reactive oxygen species (ROS) because it has radical scavenging ability based on its antioxidant activity against CCl_4 in rats (Fahmy et al., 2009). Liver damage induced by carbon tetrachloride (CCl_4) involves biotransformation of free radical derivatives, increased lipid peroxidation and excessive cell death in liver tissue (Recknagel et al., 1989). Toxic effects of CCl_4 on liver have been extensively studied (Junnila et al., 2000; Amin and Mahmoud, 2009; Cui et al., 2009; Kim et al., 2010). Serum ASAT, ALAT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari and Murugan, 2004). Serum ALAT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner.

Therefore, serum ALAT is more specific to the liver, and is thus a better parameter for detecting liver injury (Williamson et al., 1996). In conjunction with the reports of (Cho et al., 2009; Hegde and Joshi, 2009; Kim et al., 2010), data from the present study showed that CCl_4 caused hepatic damage with a significant increase in serum levels of ASAT and ALAT. Serum ALP level is also related to the status and function of hepatic cells. CCl_4 administration in the present study also caused significant increase in the serum ALP which may be due to increased synthesis in presence of increasing biliary pressure (Moss and Butterworth, 1974). Treatment with FCE at the three tested doses significantly decreased the levels of serum ASAT, ALAT and ALP activities in CCl_4-treated rats indicating maintenance of functional integrity of hepatic cell membrane.

This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thebrews and Joice, 1987) In recent years, attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemicals to highly reactive compounds such as free radicals. CCl_4 has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin

(Cui et al., 2009; Kim et al., 2010). The mechanism of CCl_4 injury involves oxidative damage by metabolism of CCl_4 to $CCl_3^{\bullet}$ in hepatocytes; this causes cell death with accumulation of lipid peroxidation and intracellular calcium ions and triggers secondary damage from the inflammatory process (Medina and Moreno-Otero, 2005).

The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis (Bandyopadhyay et al., 1999). In accord with the studies of Wang et al. (2007), Koneri et al. (2008) and Hedge and Joshi (2009), the present study showed that CCl_4 induced significant decrease in the serum total protein content. It was reported that hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases (Koneri et al., 2008), hence decline in total protein can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. However, treatment of rats with FCE at the three tested doses and silymarin bring back the level of total proteins near to normal levels. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells (Awang, 1993). CCl_4 and its metabolites are capable of initiating a chain of lipid peroxidation reactions by abstracting hydrogen from polyunsaturated fatty acids (PUFA).

Peroxidation of lipids, particularly those containing PUFA, can dramatically change the properties of biological membranes, resulting in severe cell damage and play a significant role in pathogenesis of some diseases (Aleynik et al*.,* 1997). Enhanced lipid peroxidation (LPO) is a measure of membrane damage as well as alteration in structure and function of cellular membranes (Hallowell et al*.,* 1995). The increased MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (Szymonik et al., 2003; Liu et al., 2009; Kim et al., 2010). However, treatment with silymarin or FCE at all tested doses decreased the LPO levels, which may be due to the free radical scavenging activity of the extract.

Glutathione reduced (GSH) is a 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 (Pushpakiran et al., 2004). The present study confirmed the finding of Srivastava et al. (1983) who suggested that enhancement of lipid peroxidation is a consequence of depletion of 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, Hong et al. (2009), Liu et al. (2009) and Kim et al. (2010) have reported depletion in GSH level in the liver of CCl_4 intoxicated rats. Treatment with silymarin and FCE at all tested doses in the present study restored GSH content.

In accord with our results, Gate et al. (1998) have reported that dietary supplementation of the marine extract of the *Crassostrea gigas* clams increased GSH level in the liver of rats. This increase is a reflection of increased synthesis of GSH in the liver (Lieber et al., 1990; Jayaraman et al., 2008). Since, FCE is high in taurine and the precursor amino acids of GSH (glycine, glutamic acid and cysteine) (Fahmy et al., 2009), it may be an effective source of direct precursors for stimulating GSH biosynthesis. Glutathione-S-transferase is another scavenging enzyme that binds to many different lipophilic compounds. GST catalyzes the conjugation of the thiol functional groups of glutathione to electrophilic xenobiotics and increases their solubility. Then the xenobiotic-GSH conjugate is either eliminated or converted to mercapturic acid. Since GST increases solubility of hydrophobic substances, it plays an important role in storage and excretion of xenobiotics.

Compounds that increase the activity of GST are thought to have an increased hepatoprotective activity. The depression of hepatic GST activity might be an adaptive response to the increased production of oxidized glutathione in the tissue of CCl_4-hepatotoxic animals since the efflux of oxidized glutathione and GST use the same transport system (Adang et al., 1990). In accord with our result, Escobar et al. (1996) and Sanzgiri et al. (1997) have reported that the enhanced free radical concentration resulting from the oxidative stress conditions can cause loss of enzymatic activity. Moreover, Szymonik- Lesiuk et al. (2003) reported that CCl_4 intoxication would lead to damage of antioxidant enzymes or reactive intermediates formed in the course of bioactivation of CCl_4 may bind to these enzymes that are responsible for their inactivation. Administration of either silymarin or FCE at all tested doses in the present study causes significant enhancement in the GST activity. The efficient recovery in GST activity highlights the therapeutic efficacy of FCE in alleviating the CCl_4 – induced oxidative stress.

In consonance with our study, Gate et al. (1998) have reported that supplementation of the marine extract of the *C. gigas* clams serves to stimulate GST activity in the liver of treated rats. Catalase (CAT) is one of the important enzymes in the supportive team of defense against reactive oxygen species (ROS). Catalase is a haemoprotein containing four haeme groups, that catalyses the decomposition of H_2O_2 to water and O_2 and thus, protects the cell from oxidative damage by H_2O_2 and OH (Gupta et al., 2004). Viewed in conjunction with the report of Szymonik et al. (2003), the inhibition of CAT activity following CCl_4 intoxication in the present study may be due to the enhancement of the peroxidation end product MDA, which is known to inhibit protein synthesis and the activities of certain enzymes.

Administration of FCE and silymarin enhanced the activity of CAT in CCl_4 – induced liver damage. The enhancement in CAT activity may be due to prevent the accumulation of excessive free radicals and protect liver from CCl_4 – intoxication. In conjunction with the report of Balamurugan et al. (2009) who reported that the liver cells innate ability to arouse and maintain defense against oxidant by secreting more antioxidants is overpowered by the CCl_4. FCE may overpowers CCl_4 onslaught by suppressing the formation of ROS and protecting the antioxidant machinery. Since the erythrocytes oxidative stress increased in human patients with severe hepatic disease (Smith et al., 1975; Solov'eva, 2009). So, the current investigation studied the erythrocytes' oxidative stress as a consequence of hepatic injury induced by CCl_4. Oxidative stress in the erythrocytes in the present investigation can be assessed by induction in MDA and reduction in GSH levels as well as inhibition of the GST and CAT activities. In conjunction with the earlier studies of Hatherill et al. (1991), Eritsland (2000) and Rai et al. (2009), erythrocytes may be prone to oxidative stress because they exposed to high oxygen tension, have polyunsaturated fatty acids in the membrane and hemoglobin – bound iron. Jayaraman et al. (2008) reported that the decrease in the enzymatic antioxidant activities in erythrocytes following ethanol intoxication in rats could be attributed to either inhibition of enzymes synthesis or damage to the enzyme protein.

Supplementation of FCE at the three tested doses or silymarin to CCl_4- intoxicated rats in the present study, decreased the oxidative stress in erythrocytes, which can be manifested by reduction in lipid peroxidation end product MDA and an increase in GSH levels. In consonance with the report of Lieber et al. (1990) and Jayaraman et al. (2008) enhancement of GSH level in the erythrocytes may be a reflection of its increased synthesis in the liver.

In conclusion, the results of the present study indicate that oral administration of FCE at all tested doses attenuate disrupted hepatic and erythrocytes ROS metabolism associated with hepatic injury progression in rats intoxicated with CCl_4 through their antioxidant action which may be related to their contents of sulpher containing amino acids and taurine. The present investigation also throws light on the effect of dietary supplementation of freshwater crayfish *P. clarkii* on liver diseases in Egypt. However, further research must be carried out to elucidate the mechanisms of the antioxidant effect of FCE.

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