Abstract. – Objectives: The purpose of the present work was to investigate the effect of marine crustacean extract (MCE) from marine mantis shrimp *Eurigosquilla massavensis* and silymarin on oxidative stress induced by carbon tetrachloride (CCl₄) in rat liver and erythrocytes.

Materials and Methods: Male rats were randomly divided into 3 main groups, (1) control group which administered olive oil orally for 2 days, followed by distilled water for 7 consecutive days, (2) MCE group in which rats administered orally MCE, 250 mg/kg body weight for 9 consecutive days and (3) CCl₄-treated group in which rats given CCl₄ orally (2.5 ml/kg body weight) for 2 days. This group then subdivided into 5 subgroups. All subgroups treated orally for 7 consecutive days with distilled water (subgroup I), silymarin, 150 mg/kg body weight (subgroup II) and MCE at three tested doses 50, 100 and 250 mg/kg body weight (subgroups III, IV and V).

Results: The MCE and silymarin produced significant hepatoprotective effect by decreasing the activity of serum aminotransferases (ASAT and ALAT) and alkaline phosphatase (ALP) as well as malondialdehyde (MDA) level, and increasing the serum total protein, glutathione reduced (GSH) levels and the activities of glutathione-S-transferase (GST) and catalase (CAT). The MCE and silymarin also showed the same antioxidant effect on erythrocytes.

Conclusions: The results of the present study, suggested that the MCE could protect the liver and erythrocytes injuries perhaps, by its antioxidative effect, hence eliminating the deleterious effect of toxic metabolites from CCl₄.

Key Words: *Eurigosquilla massavensis*, Liver, CCl₄, Erythrocytes, Oxidative stress.

Introduction

Reactive oxygen species (ROS) including oxygen free radicals are causative factors in the etiology of degenerative diseases, including some hepatopathies. The enhanced production of free radicals and oxidative stress can be induced by a variety of factors such as ionizing radiation or exposure to drugs and xenobiotics (e.g. carbon tetrachloride). It is well known that carbon tetrachloride (CCl₄) induces hepatotoxicity in humans and experimental animals. According to the views of the initial developmental process of CCl₄-induced hepatotoxicity, the process is dominated by factors such as CCl₄ activation to trichloromethyl radical (CCl₃) and trichloromethyl peroxy radical (CCl₃O₂), the covalent binding of CCl₄ to membrane lipids and proteins, and the hydrogen abstraction from polyunsaturated fatty acids by the CCl₃O₂ and the CCl₄ to initiate lipid peroxidation. It has been shown that an increase in hepatic lipid peroxidation, which occurs via ROS, such as superoxide radical (O²⁻) and hydroxyl radical, contributes to not only the formation but also the progression of CCl₄-induced acute liver injury.

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. It was known that CCl₄-induced changes in erythrocytes membrane composition and membrane bound 

Antioxidant and anti-inflammatory agents play a critical role against CCl₄ intoxication by scavenging active oxygen and free radicals and...
neutralizing lipid peroxides\textsuperscript{17,18}. 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 marine organisms\textsuperscript{19-21}. Products from marine sources have become attractive as nutraceutical and functional foods and as a source material for the development of drugs and specific health foods\textsuperscript{22}. Supplements derived from marine foods have been used to treat and prevent a wide variety of lifestyle-related diseases such as unsaturated fatty acids\textsuperscript{23,24} and functional peptides\textsuperscript{25,26}. Marine mantis shrimp \textit{Erugosquilla massavensis} is well established in the eastern Mediterranean, displaying and dominating the local species \textit{Squilla mantis}\textsuperscript{27}. \textit{Erugosquilla massavensis} is an edible crustacean that have a small yet growing economic important in our markets\textsuperscript{28,29}. Recently, we have investigated the curative effect of the marine crustacean extract (MCE) from the marine mantis shrimp \textit{Erugosquilla massavensis} on CCl\textsubscript{4} – induced nephrotoxicity in rats\textsuperscript{30}. Based on the results from our experiment, we showed that MCE has antioxidant activity related to its high taurine content as well as glutamic acid, cysteine and glycine (the amino acids components of GSH). So, MCE might be effective against diseases in which ROS play a role as potent causative factors because it has a strong antioxidant activity. Therefore, the present study aimed to examined the curative effect of MCE on CCl\textsubscript{4} – induced hepatic and erythrocytes injuries in the male rats and elucidate the possible mechanisms of this effect.

\textbf{Materials and Methods}

\textbf{Preparation of Crude Marine Crustacean Extract (MCE)}

Marine \textit{Erugosquilla massavensis} species were collected from Mediterranean Sea at Port-Said Governorate.

Marine crustacean extract powder was prepared as follow: fresh raw specimens (1 kg) was 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 hr. After filtration, the filtrate obtained was then concentrated and lyophilized to a brownish residue using (LABCONCO lyophilizer, Shell freeze system, Kansas, MO, USA). The marine crustacean extract (MCE) was stored in dry place avoiding water vapor until used.

\textbf{Chemicals}

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

\textbf{Experimental Animals}

The experimental animals used in this study was the adult male albino rats (\textit{Rattus norvegicus}) weighing 100-120 g. The animals were obtained from a fixed local supplier. Animals were caged in groups of ten and given food and water \textit{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.

\textbf{Experimental Protocol}

Animals were divided into three main groups, the 1\textsuperscript{st} 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 2\textsuperscript{nd} group (6 rats/group) administered MCE (250 mg/kg body weight p.o.) for 9 days. Third group (30 rats), given CCl\textsubscript{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 follow:

\textbf{Subgroup I (CCl\textsubscript{4})}. Rats of this subgroup administered distilled water orally.

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

\textbf{Subgroups III, IV, V}. Animals of these subgroups treated orally with MCE (50, 100 and 250 mg/kg body weight) respectively.

All animals were sacrificed on the 10\textsuperscript{th} day of treatment after being fasted over night; 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 minutes. 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 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 following biochemical assays. The hemoglobin content in the red blood cell lysate was measured according to cyanmethemoglobin method using Biodiagnostic kits (Biodiagnostic Dokki, Giza, Egypt).

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., aminotransferase activities of aspartate aminotransferase ASAT and alanine aminotransferase ALAT according to Reitman and Frankel and alkaline phosphatase ALP activity.

Assessment of Oxidative Stress Markers

Oxidative stress markers were detected in the resultant supernatant of liver homogenate and hemolysate. The appropriate kits (Biodiagnostic kits, Biodiagnostic Dokki, Giza, Egypt) was used for the determination of glutathione reduced (GSH)35, lipid peroxidation which was measured by the formation of malondialdehyde (MDA)36, activity of glutathione-S-transferase (GST)37 and catalase activity (CAT)38.

Statistical Analysis

Values were expressed as means ± SE. 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 and $P<0.05$ was considered statistically significant. SPSS, for Windows (version 15.0) was used for the statistical analysis.

Results

Improvement of Liver Functions in MCE Treated Rats

The levels of total proteins, activities of aminotransferase (ASAT and ALAT) and ALP in the control, CCl4 injured, silymarin treated and MCE administered rats are presented in Table I. Administration of CCl4 induced significant decrease ($P<0.05$) in the total protein content as compared to control (Table I). Treatment with

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>Group</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein (g/100 ml)</td>
<td>ASAT (IU/ml)</td>
<td>ALAT (IU/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>6.45 ± 0.39</td>
<td>13.92 ± 0.66</td>
<td>24.90 ± 0.41</td>
</tr>
<tr>
<td>MCE (250 mg/kg)</td>
<td>6.95 ± 0.32</td>
<td>14.24 ± 0.90</td>
<td>25.48 ± 0.74</td>
</tr>
<tr>
<td>CCl4</td>
<td>5.92 ± 0.49a</td>
<td>38.21 ± 0.78a</td>
<td>77.38 ± 2.35a</td>
</tr>
<tr>
<td>CCl4 + Silymarin</td>
<td>7.27 ± 0.23b</td>
<td>15.24 ± 0.77b</td>
<td>27.73 ± 0.69b</td>
</tr>
<tr>
<td>CCl4 + MCE</td>
<td>50 mg/kg</td>
<td>7.04 ± 0.23b</td>
<td>15.61 ± 0.55b</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>6.69 ± 0.16</td>
<td>14.18 ± 1.33b</td>
</tr>
<tr>
<td></td>
<td>250 mg/kg</td>
<td>6.18 ± 0.23</td>
<td>17.75 ± 2.00b</td>
</tr>
</tbody>
</table>

All data are mean of six rats ± SEM. $^a$Significant as compared to control. $^b$Significant as compared to CCl4.
silymarin and MCE at a dose of 50 mg/kg body weight 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 I). Administration of MCE 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 the administration of MCE did not show a significant change in the liver functions of CCl$_4$ – untreated rats as compared to control.

**Antilipidperoxidation Activity and Antioxidant Levels in the Liver of MCE 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$_4$ intoxicated, silymarin treated and MCE administered rats are shown in Table II. Administration of CCl$_4$ caused significant decrease ($P<0.05$) in GSH level as compared to control. Treatment of rats either with silymarin or MCE (50, 100 and 250 mg/kg body weight) significantly increased the level of GSH as compared to CCl$_4$ – treated group. MDA levels were assessed as indicator of lipid peroxidation, CCl$_4$ treatment significantly ($P<0.05$) increased the level of MDA in the liver tissue as compared to control (Table II). However, treatment with silymarin and MCE at the three tested doses significantly ($P<0.05$) decreased the increased level of MDA as compared to the CCl$_4$ – treated rats. As shown in Table II, CCl$_4$ challenge significantly decreased GST activity ($P<0.05$) as compared to control. Treatment with silymarin and MCE at all tested doses caused marked increase in the GST activity as compared with CCl$_4$ treated group. However, this increase was significantly only after treatment with 250 mg/kg body weight MCE. 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 II). Meanwhile, CAT activity increased after all treatment either with silymarin or MCE at the three tested doses. However, this increase was significantly only ($P<0.05$) following treatment with 250 mg/kg body weight of MCE. Administration of MCE to CCl$_4$ – untreated rats had no effect on the studied oxidative stress markers in the liver as compared to control.

**Antioxidative Activity of MCE in the Hemolysate of CCl$_4$ Treated Rats**

Data presented in Table III showed the effect of CCl$_4$ administration, silymarin and MCE treatment on oxidative stress markers in the erythrocyte hemolysate. 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 MCE (100 and 250 mg/kg body weight) significantly increased the level of GSH. The localization of radical formation resulting in lipid peroxidation, measured as MDA in the hemolysate as shown in Table III. CCl$_4$ treatment significant-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GSH (mg/g tissue)</th>
<th>MDA (nmol/g tissue)</th>
<th>GST (U/g tissue)</th>
<th>CAT (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.39 ± 2.95</td>
<td>367.76 ± 59.43</td>
<td>0.16 ± 0.05</td>
<td>1.81 ± 0.21</td>
</tr>
<tr>
<td>MCE (250 mg/kg)</td>
<td>50.36 ± 7.58</td>
<td>302.09 ± 88.81</td>
<td>0.22 ± 0.05</td>
<td>1.97 ± 0.13</td>
</tr>
<tr>
<td>CCl$_4$</td>
<td>24.53 ± 1.54</td>
<td>816.60 ± 47.96</td>
<td>0.04 ± 0.01</td>
<td>0.60 ± 0.09</td>
</tr>
<tr>
<td>CCl$_4$ + Silymarin</td>
<td>40.98 ± 3.96</td>
<td>453.36 ± 50.53</td>
<td>0.16 ± 0.03</td>
<td>1.34 ± 0.22</td>
</tr>
<tr>
<td>CCl$_4$ + MCE</td>
<td>50 mg/kg</td>
<td>45.46 ± 6.24</td>
<td>0.16 ± 0.01</td>
<td>1.29 ± 0.08</td>
</tr>
<tr>
<td>CCl$_4$ + MCE</td>
<td>100 mg/kg</td>
<td>48.09 ± 2.89</td>
<td>0.17 ± 0.05</td>
<td>1.51 ± 0.16</td>
</tr>
<tr>
<td>CCl$_4$ + MCE</td>
<td>250 mg/kg</td>
<td>42.14 ± 2.03</td>
<td>0.16 ± 0.02</td>
<td>1.74 ± 0.16</td>
</tr>
</tbody>
</table>

All data are mean of six rats ± SEM. *Significant as compared to control. "Significant as compared to CCl$_4$. 

---

**Table II.** Effect of MCE and silymarin on the liver oxidative stress markers GSH, MDA, GST and CAT following CCl$_4$ intoxication in rats.
ly ($P < 0.05$) increased the level of MDA in the hemolysate as compared to the control. However, treatment with silymarin or MCE at all tested doses significantly ($P < 0.05$) decreased the increased level of MDA as compared to the CCl$_4$-treated rats (Table III). Nine-days treatment with MCE (250 mg/kg) did not result in a significant alteration of MDA levels as compared to the control groups (Table III). 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 (Table III). Meanwhile, all the treatments either with MCE and silymarin caused significant increase in the GST activity as compared to the control rats (Table III). Meanwhile, all the treatments either with MCE and silymarin caused significant increase in the GST activity as compared to the CCl$_4$-treated rats. As shown in Table III, CCl$_4$ challenge significantly decreased ($P < 0.05$) the activity of catalase (CAT) as compared to control rats. However, treatment with MCE and silymarin at different doses significantly increased CAT as compared to CCl$_4$-treated group (Table III). However, the administration of MCE (250 mg/kg) to rats did not result in any significant change as compared to the control group.

### Discussion

During the course of evolution, many invertebrates have established as a selective advantage by endogenous production of protective chemicals which may have a deleterious effect on other animals, such as shrimp carotenoids$^{39}$; protein-enriched fraction from the *Musca domestica*$^{40}$; fresh water clam *Corbicula fluminea* extract$^{41}$ and *Lampito mauritii* extract$^{42}$ in rats. 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 marine crustacean extract MCE which extracted from marine mantis shrimp *Eugosquilla massavensis* 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$^{30}$. Liver injury induced by carbon tetrachloride (CCl$_4$) is the best characterized system of xenobiotic-induced hepatotoxicity and is commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs$^{1}$. Toxic effects of CCl$_4$ on liver have been extensively studied$^{2-5}$. In the assessment of liver damage by CCl$_4$ hepatotoxin, the determination of enzyme levels such as serum ASAT and ALAT is largely used. Necrosis or membrane damage releases the enzymes into circulation. Therefore, they can be measured in serum. High levels of serum ASAT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. 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$^{43}$. In conjunction with the reports of$^{44-47}$, 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GSH (mg/mg Hb)</th>
<th>MDA (nmol/mg Hb)</th>
<th>GST (U/mg Hb)</th>
<th>CAT (U/mg Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.42 ± 0.53</td>
<td>483.20 ± 55.59</td>
<td>835.88 ± 153.94</td>
<td>765.09 ± 55.69</td>
</tr>
<tr>
<td>MCE (250 mg/kg)</td>
<td>5.24 ± 0.39</td>
<td>412.40 ± 31.08</td>
<td>732.69 ± 102.67</td>
<td>524.92 ± 58.01</td>
</tr>
<tr>
<td>CCl$_4$</td>
<td>3.32 ± 0.63$^a$</td>
<td>701.60 ± 45.85$^a$</td>
<td>508.27 ± 56.98$^a$</td>
<td>347.41 ± 73.75$^a$</td>
</tr>
<tr>
<td>CCl$_4$ + Silymarin</td>
<td>6.64 ± 1.16$^b$</td>
<td>526.60 ± 43.15$^b$</td>
<td>745.85 ± 172.47</td>
<td>550.94 ± 123.92</td>
</tr>
<tr>
<td>CCl$_4$ + (MCE) 50 mg/kg</td>
<td>7.33 ± 0.52$^b$</td>
<td>491.00 ± 30.95$^b$</td>
<td>610.53 ± 90.74</td>
<td>460.45 ± 54.25</td>
</tr>
<tr>
<td>CCl$_4$ + (MCE) 100 mg/kg</td>
<td>7.66 ± 0.52$^b$</td>
<td>426.80 ± 87.65$^b$</td>
<td>746.77 ± 121.98</td>
<td>482.68 ± 23.36</td>
</tr>
<tr>
<td>CCl$_4$ + (MCE) 250 mg/kg</td>
<td>6.98 ± 1.75$^b$</td>
<td>406.20 ± 42.53$^b$</td>
<td>782.85 ± 115.41$^b$</td>
<td>630.52 ± 83.62$^b$</td>
</tr>
</tbody>
</table>

All data are mean of six rats ± SEM. $^a$Significant as compared to control. $^b$Significant as compared to CCl$_4$. 

Table III. Effect of MCE and silymarin on the erythrocytes oxidative stress markers GSH, MDA, GST and CAT following CCl$_4$ intoxication in rats.
also related to the status and function of hepatic cells. CCl₄ 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. Treatment with MCE at the three tested doses significantly decreased the levels of serum ASAT, ALAT and ALP activities in CCl₄-treated rats indicating maintenance of functional integrity of hepatic cell membrane.

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. CCl₄ has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin. CCl₄ hepatotoxicity depends on the reductive dehalogenation of CCl₄ catalyzed by cytochrome P₄₅₀ in the liver cell endoplasmic reticulum leading to the generation of an unstable complex CCl₃ radical. These free radicals attack microsomal lipids leading to its peroxidation and also covalently binds to microsomal lipids and proteins ultimately initiating a site of secondary biochemical processes which is the ultimate cause for the pathological consequences of CCl₄ metabolism.

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. In accord with the studies of, the present study showed that CCl₄ 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, 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 MCE (50 mg/kg body weight) 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.

Liver cell injury induced by CCl₄ involves initially the metabolism of CCl₄ to trichloromethyl free radical by the mixed-function oxidase system of the endoplasmic reticulum and secondary, the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl₄ metabolism or from peroxidative degeneration of membrane lipids. In the present study, elevations in the levels of the end products of lipid peroxidation, malondialdehyde (MDA) in the liver of CCl₄-treated rats were observed. 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. Treatment with either silymarin or MCE at all tested doses significantly reversed these changes, suggesting that the mechanism of MCE hepatoprotection may be due to its antioxidant effect.

Glutathione reduced (GSH) is an intracellular reductant and plays major role in catalysis, metabolism and transport. It protect cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects, for example, a decrease in the rate of gluconeogenesis or an increase in glycogenolysis. The present study confirmed the finding of Srivastafa et al. who suggested that enhancement of lipid peroxidation is a consequence of depletion of GSH to certain critical levels. Insufficiency in non-enzymatic antioxidant GSH following CCl₄ intoxication could be the consequence of increased utilization for trapping free radicals. In consonance with our study, Gate et al. have reported depletion in GSH level in the liver of CCl₄ intoxicated rats. Treatment with silymarin and MCE at all tested doses in the present study restored GSH content. In accord with our results, Ger et al. 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. Since, MCE is high in taurine and the precursor amino acids of GSH, glycine, glutamic acid and cysteine, it may be an effective source of direct precursors for salvage GSH biosynthesis.

Glutathione-S-transferase (GST) is an enzyme that participates in the detoxification process due to conjugation reaction between GSH and xenobiotics. The present investigation showed significant decrease in GST in the CCl₄ treated rats as compared to the control group. In accord with our results, Escobar et al. and Sanzgir et al. have reported that the enhanced free radical concentration resulting from the oxidative stress con-
Conclusions can cause loss of enzymatic activity. Moreover, Szymonik-Lesiuk et al. reported that CCl₄ intoxication would lead to damage of antioxidant enzymes or reactive intermediates formed in the course of bioactivation of CCl₄ may bind to these enzymes that are responsible for their inactivation. Administration of either silymarin or MCE at 250 mg/kg body weight in the present study causes significant enhancement in the GST activity. The efficient recovery in GST activity highlights the therapeutic efficacy of MCE in alleviating the CCl₄-induced oxidative stress.

Catalase (CAT) is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Viewed in conjunction with the report of Szymonik-Lesiuk et al. the inhibition of CAT activity following CCl₄ 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 MCE at 250 mg/kg body weight and silymarin enhanced the activity of CAT in CCl₄-intoxicated liver damage. The enhancement in CAT activity may be due to prevent the accumulation of excessive free radicals and protect liver from CCl₄-intoxication. In view conjunction with the report of Balamurugan et al. who reported that the liver cells innate ability to arouse and maintain defense against oxidant by secreting more antioxidants is overpowered by the CCl₄. MCE may overpowers CCl₄ 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. So, the current investigation studied the erythrocytes’ oxidative stress as a consequence of hepatic injury induced by CCl₄. 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. 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. 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 MCE at the three tested doses or silymarin to CCl₄-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., Jayaraman et al. enhancement of GSH level in the erythrocytes may be a reflection of increased its synthesis in the liver.

In conclusion, the present study serve to extend the growing number of earlier investigations on therapeutic products from marine sources and confirm that MCE decreased lipid peroxidation, improved antioxidant status, and thereby prevented the damage to the liver and erythrocytes. The present research throw the light on the effect of dietary supplementation of Erugosquilla massavensis on liver diseases in Egypt. However, further studies must be carried out to elucidate the mechanisms of the hepatoprotective effect of MCE.

References


