ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF THYMOQUINONE AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN ISOLATED RAT HEPATOCYTES

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ABSTRACT

Herbs are known to play a vital role in the management of various liver diseases. Thymoquinone (TQ) is the bioactive constituent of Nigella sativa seed. The present work was planned to evaluate the potential hepatoprotective effects of TQ against the cytotoxic effects and the oxidative stress induced by carbon tetrachloride (CCl4) in isolated primary rat hepatocytes. Cytotoxicity was determined by assessing cell viability and leakage of cytosolic enzymes, such as lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST). Oxidative stress was assessed by determining reduced glutathione (GSH) level and lipid peroxidation as indicated by thiobarbituric acid reactive substances (TBARS) production. Exposure of isolated rat hepatocytes to CCl4 (5mM) caused cytotoxicity and oxidative injury, manifested by loss of cell viability and significant increase in LDH, ALT and AST leakages. As well as, CCl4 caused progressive depletion of intracellular GSH content and significant enhancement of TBARS accumulation. Preincubation of hepatocytes with either TQ (1mM) or silymarin (5 mM) which is a known hepatoprotective agent, ameliorated the hepatotoxicity and oxidative stress induced by CCl4, as indicated by significant improve in cell viability, significant decrease in LDH, ALT and AST leakages, significant prevent GSH depletion and significant decrease in TBARS formation as compared to CCl4 alone-treated cells. The present results indicate that CCl4 has a potential cytotoxic effect in rat hepatocytes; and TQ can afford a significant protection against CCl4–induced hepatotoxicity.

Keywords: Thymoquinone; Carbon Tetrachloride; Hepatotoxicity; Isolated Rat Hepatocytes.

INTRODUCTION

Liver diseases remain one of the serious health problems. The high prevalence of bilharziasis and viral hepatitis in Egypt resides behind the wide occurrence of liver disorders specially hepatic fibrosis and cirrhosis (el-Zayadi et al., 1992). Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Carbon tetrachloride (CCl4), a clear, heavy, and nonflammable liquid is most widely used for experimental induction of hepatic cirrhosis (Bahcecioglu et al., 1999). It is known to be hepatotoxic as well as nephrotoxic to humans (Abraham et al., 1999). Furthermore, it has been identified as a probable human carcinogen based on evidence of tumors in animals (Thrall et al., 2000). In years past, CCl4 was widely used as a dry cleaning solvent until it was recognized as a potent hepatotoxin and carcinogen. Recently, it is primarily used as an organic solvent (Kovacic et al., 2002).

In the absence of reliable liver protective drugs in medical practices, herbs play a role in the management of various liver disorders. A number of plants have been documented to exhibit hepatoprotective properties (Handa et al., 1986). The black seed (Nigella sativa, Ranunculaceae family), also known as Black Caraway Seed and “the Blessed Seed”, is an annual herb that grows in countries bordering the Mediterranean Sea. Although black seed is not a significant component of the human diet, it is regarded in the Middle East as part of an overall holistic approach to health and is thus incorporated into diets and everyday lifestyles. Numerous studies have shown that the seeds and oil of this plant are characterized by a very low degree of toxicity (Ali and Blunden, 2003).

TQ is the bioactive constituent of the volatile oil of black seed (54%) and was first extracted by El-Dakhakhany (1963). Previous studies suggested that TQ or Nigella sativa oil might have a variety of pharmacological actions such as
antihistaminic (Marozi et al., 1970), antibacterial (Hanafy and Hatem, 1991), antihypertensive (El-Tahir et al., 1993), hypoglycemic (Al-Hader et al., 1993), anti-inflammatory (Houghton et al., 1995), antinoceptive (Abdel-Fattah et al., 2000), and immunopotentiating (Swamy and Tan, 2000). Recent studies indicate that TQ also has antioxidant activity (Houghton et al., 1995 and Mansour, 2000); therefore, it may be protective against cisplatin-induced nephrotoxicity (Badary et al., 1997), doxorubicin-induced cardiotoxicity (Al-Shabanah et al., 1998) and experimental carcinogenesis (Badary et al., 1999). Moreover, El-Dakhakhny et al. (2000) suggested that Nigella sativa oil protects against ethanol-induced ulcers in rats.

Other studies have focused on the role of TQ on tissue lipid peroxidation. Houghton et al. (1995) found that TQ inhibited non-enzymatic lipid peroxidation in ox brain phospholipid and was found to be a potent inhibitor of eicosanoid generation, namely thromboxane B2 and leucotriene B4, by inhibiting both cyclooxygenase and lipooxygenase enzymes, respectively. Thromboxane B2 was implicated in the mechanism of hepatocyte plasma membrane bleb formation (disassociation of the membrane lipid bilayer from the underlying cytoskeleton) which is an early event in hepatocyte injury when exposed to oxidative stress (Horton and Wood, 1991). The plasma membrane bleb formation plays a critical role in liver cell injury. Moreover, Nagi et al. (1999) indicated that TQ and its metabolite, dihydrothymoquinone (DHTQ), inhibit in vitro non-enzymatic lipid peroxidation in mouse liver.

Despite this knowledge about the potent hepatoprotective, antioxidant and anti-inflammatory effects of TQ, the molecular pathways involved in its activities are not well understood. Therefore, the present study was undertaken to investigate the potential protective effect of TQ on CCl₄ induced hepatotoxicity in isolated rat hepatocytes compared with known hepatoprotective agent (silymarin). In addition, the mechanism underlying these protective potentials was also studied.

**MATERIALS AND METHODS**

**Animals and Chemicals**

Male Sprague–Dawley rats of locally bred strains (225–250 g) were supplied by the Faculty of Veterinary Medicine, Cairo university, Egypt. They were kept under good ventilation and standard hygienic conditions and allowed free access to balanced standard laboratory chow (El-Nasr Co., Abo-Zaable, Egypt) and tap water ad libitum. Bovine serum albumin, carbon tetrachloride, collagenase (type IV), dimethyl sulfoxide (DMSO), GSH, thioarbituric acid (TBA), thymoquinone (2-iso-propyl-5-methyl-1,4 benzoquinone) and tritonX-100 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Silymarin was obtained from Medical Union Pharmaceuticals, Abou-Sultan, Ismailia, Egypt. All other chemicals were of the highest analytical grade.

**Isolation of Hepatocytes:**

Hepatocytes were isolated using a collagenase two-step perfusion technique as described by Berry and Friend (1969) with slight modifications as published by Figliomeni and Abdel-Rahman (1997). Briefly, a rat was anaesthetized by intraperitoneal injection with 100 mg ketamine/kg (Ketalar, Park-Davis, Morris Plains, NJ, USA), restrained, and an incision was made in the abdominal cavity to expose the portal vein. A polyethylene cannula was inserted into the portal vein and the liver was perfused in situ for 8 min with calcium-free Hank’s bicarbonate buffer maintained at 37°C. The liver was then mechanically dislocated from the abdomen with the cannula in place and recirculated for 10 min in collagenase (0.67 mg/ml) containing 5 mM calcium chloride. The isolated liver cells were filtered through four layer of cotton gauze and centrifuged for two minutes at 50 g. The cells were washed twice and suspended in HEPES-bicarbonate buffer (pH 7.4) containing 0.5% bovine albumin. The isolated hepatocytes were counted in a hemocytometer, while the viability of the cells was assessed by 0.4% trypan blue exclusion technique (Baur et al., 1985). Freshly prepared cell suspension had 90% or greater viability prior to each experiment.

**Incubation and treatment of hepatocytes**

Freshly isolated hepatocytes (5x10⁶ cells/ml) were suspended in a HEPES-bicarbonate buffer (pH 7.4) and incubated at 37°C in a shaking water bath at 30 oscillations per minute. Hepatocytes were incubated in plastic vials equipped with covers and two main experiments were performed.

The first experiment involved determination of cytotoxicity of CCl₄, TQ and silymarin in isolated hepatocytes at different time intervals (30, 60, 120 min). CCl₄, TQ and silymarin were dissolved in 0.5% dimethyl sulfoxide (DMSO) and their concentrations in the incubation medium were adjusted to reach a final concentration of 5mM CCl₄ (Dvorak et al., 2003), 1mM TQ (Daba and Abdel-Rahman, 1998) and 5mM silymarin (Farghali et al., 2000). Twelve replicates from six rats (2 replicates per rat) were used for each chemical. Cytotoxicity was determined by assessing of cell viability using trypan blue exclusion method, cytosolic enzymes leakage percent [lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AST)], GSH content and thioarbituric acid reactive substances (TBARS) accumulation. Control replicates were carried out simultaneously under the same conditions and at the same time intervals, using DMSO at a final concentration of 0.5% (Dvorak et al., 2003).

The second experiment involved determination of cytoprotection of TQ and silymarin against CCl₄-induced hepatocytes injury at different time intervals (30, 60, 120 min). Prior to intoxication with CCl₄ (5mM), hepatocytes were pre-incubated for 30 min (Farghali et al., 2000) with TQ (1mM) and silymarin (5mM). Aliquots were taken during the time-course studies 30, 60 and 120 min after...
CCl₄ addition and subjected to cytoprotection assessment similar to the cytotoxicity study.

**Sample preparation for enzyme leakage**

Enzyme activities (LDH, ALT and AST) were monitored using Sigma kits (Sigma Chemical Co., St. Louis, MO, USA) in an aliquot of cell-free medium and compared to the total activity achieved after lysis of the cells (Moldeus et al., 1978). The cell-free medium was obtained by centrifugation of the aliquots at 1300 g for 15 min to obtain the supernatant. Lysate was obtained by addition of 1% triton X-100 and shaking for 15 min followed by centrifugation at 1300 g. The leakage was expressed as percentage of total lysate activity at each time point.

**Assay for cellular GSH**

Because GSH accounts for the majority of soluble-reduced sulfhydryls in cells (Kosower and Kosower, 1978), Reduced GSH levels in hepatocytes were determined by measuring total soluble-reduced sulfhydryl content. Aliquots were collected at specified time points and centrifuged with phosphate buffer saline (PBS) at 3000 g for 5 min. The obtained precipitate was mixed with 0.7 ml of 0.2% triton X-100 and 2.5% sulfosalicylic acid. Solutions were recentrifuged at 3000 g for 5 min. A 0.5 ml aliquot of the acid-soluble supernatant medium was then added to 1.0 ml of 0.3 M Na₂HPO₄ solution. Spectrophotometric determinations were performed at 412 nm immediately after the addition of 0.125 ml of 5,5'-dithiobis-(2-nitrobenzoic acid) (Beutler et al., 1963).

**Lipid peroxidation assay**

Lipid peroxidation was assessed by determining thiobarbituric acid reactive substances (TBARS) in hepatocyte culture media by the method of Uchiyama and Mihara (1978). Test solutions obtained from cultured cells were cooled to 4°C and centrifuged for 10 min at 1000 g to remove cells. Aliquots (750 µl) of the supernatant were cooled to 4°C and centrifuged for 10 min at 1000 g at 4°C to remove precipitated protein. One ml of supernatant was added to 1.0 ml of thiobarbituric acid reagent (0.6% thiobarbituric acid in 0.1N NaOH), and the mixture was heated at 100°C for 20 min. The mixture was allowed to cool, and TBARS were extracted with 3ml of 1-butanol. Appropriate blanks were concurrently made. A 1.0M stock solution of 1,1,3,3-tetraethoxypropane in water was diluted in various amounts of 0.01 N HCL to produce malondialdehyde (MDA); these solutions were used as TBARS standards. The 1-butanol fractions and MDA standards were concurrently made. A 1.0mM stock solution of 1,1,3,3-tetraethoxypropane in water was diluted in various amounts of 0.01 N HCL to produce malondialdehyde (MDA); these solutions were used as TBARS standards. The 1-butanol fractions and MDA standards were determined at a wavelength of 553 nm. TBARS content was always expressed as nanomoles per milligram protein. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

**Data Analysis**

The GRAPHPAD (ISI Software, Philadelphia, PA, USA) computer program was used to conduct regression analysis and to plot collected data. Data were expressed as means ± standard error of means (SEM). Assessment of the results was performed using one-way analysis of variance (ANOVA) procedure followed by Tukey-Kramer multiple comparison post-tests. Statistical analyses were performed using Software GRAPHPAD INSTAT (Version 2). The 0.05 level of probability was used as the criterion for significance.

**RESULTS**

Cell survival was assessed by trypan blue exclusion method after exposing isolated rat hepatocytes to CCl₄ (5mM), TQ (1mM), and silymarin (5mM). A significant progressive time dependent decrease in cell viability was observed as early as 30 min after exposure to CCl₄ compared to control cells. Neither TQ nor silymarin exposure produced any significant difference in the percentage of viability from the control. On the other hand, concomitant incubation of the cells with silymarin and CCl₄ inhibited the decrease in the cell viability caused by CCl₄ alone. Also, TQ showed similar protection in viability, when the cells were preincubated with TQ before exposure to CCl₄ (Fig. 1).

Plasma membrane damage was assessed by monitoring LDH, ALT and AST enzyme leakages from hepatocytes exposed to CCl₄, TQ and silymarin. Table 1 demonstrates the time course of LDH leakage in the perfusion medium of hepatocytes treated with CCl₄ alone and those pretreated with TQ or silymarin followed by CCl₄. Exposure of hepatocytes to CCl₄ resulted in a significant increase in the leakage of LDH enzyme into the culture medium as early as 30 min of incubation. Pretreatment of hepatocytes with TQ or silymarin ameliorated the effects of CCl₄ on LDH enzyme leakage.

Table 2 demonstrates the effect of CCl₄, TQ and silymarin on ALT leakage of isolated hepatocytes. CCl₄ caused time dependent significant increase in the leakage of ALT in comparison to control. On the other hand, neither TQ nor silymarin caused any change in the ALT leakage. Pre-incubation of isolated hepatocytes with TQ or silymarin decreased the ALT leakage compared to CCl₄ treated cells.

The time course of AST leakage from isolated hepatocytes is demonstrated in Table 3. CCl₄ caused significant time dependent increase in AST leakage in comparison to control. Both TQ and silymarin significantly decreased the AST leakage in hepatocyte medium induced by CCl₄. Silymarin shows a higher protective effect than TQ on AST leakage at the 60 and 120 min time point studied.

Assessment of oxidative stress-induced by CCl₄ in isolated hepatocytes was done by measuring cellular GSH level and lipid peroxidation. Fig. 2 depicts the time-course effects of CCl₄ on hepatocytes glutathione content and its possible protection by either TQ or silymarin. CCl₄ caused significant depletion of glutathione content from isolated rat hepatocytes.
El-Tawil and Moussa (2006) Antioxidant and Hepatoprotective Effects of Thymoquinone Against Carbon Hepatocytes compared to control during the 2-h incubation period. Neither TQ nor silymarin showed any effect on glutathione content. However, concomitant incubation of cells with silymarin and CCl₄ or TQ and CCl₄ prevented the depletion of glutathione induced by CCl₄ exposure alone. The protection against glutathione depletion by silymarin or TQ in the presence of CCl₄ was almost the same at the incubation time studied.

The effect of CCl₄, TQ and silymarin on lipid peroxidation, as indicated by TBARS formation, was estimated. Fig. 3 shows a significant increase of TBARS production in hepatocytes exposed to CCl₄ as early as 30 min of incubation. Both TQ and silymarin significantly decreased the TBARS formation induced by CCl₄. However, Silymarin shows a higher protection than TQ in lipid peroxidation specially at 120 min time point studied.

Table 1: Effects of CCl₄, Thymoquinone and Silymarin on LDH enzyme leakage % of isolated rat hepatocytes

<table>
<thead>
<tr>
<th>Incubation Time (min)</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.7 ± 1.11</td>
<td>24.9 ± 1.31</td>
<td>27.9 ± 1.49</td>
</tr>
<tr>
<td>CCl₄</td>
<td>36.9 ± 2.21 a</td>
<td>47.4 ± 2.87 a</td>
<td>50.2 ± 3.14 a</td>
</tr>
<tr>
<td>Thymoquinone alone</td>
<td>22.4 ± 1.87</td>
<td>26.7 ± 1.23</td>
<td>29.4 ± 2.10</td>
</tr>
<tr>
<td>Silymarin alone</td>
<td>21.1 ± 1.22</td>
<td>24.2 ± 1.54</td>
<td>24.6 ± 1.33</td>
</tr>
<tr>
<td>Thymoquinone + CCl₄</td>
<td>26.1 ± 1.48 b</td>
<td>31.3 ± 2.14 b</td>
<td>35.4 ± 2.65 b</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>24.2 ± 1.35 b</td>
<td>29.4 ± 1.29 b</td>
<td>31.5 ± 2.30 b</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M. of 12 hepatocyte replicates.

(*) Significantly different from corresponding control (DMSO) group at P<0.05.
(¹) Significantly different from CCl₄ group at P<0.05.

Table 2: Effects of CCl₄, Thymoquinone and Silymarin on ALT enzyme leakage % of isolated rat hepatocytes

<table>
<thead>
<tr>
<th>Incubation Time (min)</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.5 ± 2.33</td>
<td>37.2 ± 2.86</td>
<td>39.6 ± 2.64</td>
</tr>
<tr>
<td>CCl₄</td>
<td>59.5 ± 3.22 a</td>
<td>64.6 ± 3.17 a</td>
<td>71.2 ± 4.35 a</td>
</tr>
<tr>
<td>Thymoquinone alone</td>
<td>34.7 ± 2.55</td>
<td>41.1 ± 3.74</td>
<td>45.8 ± 3.67</td>
</tr>
<tr>
<td>Silymarin alone</td>
<td>31.3 ± 1.95</td>
<td>35.7 ± 2.34</td>
<td>38.1 ± 2.88</td>
</tr>
<tr>
<td>Thymoquinone + CCl₄</td>
<td>39.1 ± 1.48 b</td>
<td>44.8 ± 4.74 b</td>
<td>49.4 ± 4.33 b</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>36.2 ± 3.35 b</td>
<td>40.5 ± 3.92 b</td>
<td>46.6 ± 3.70 b</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M. of 12 hepatocyte replicates.

(*) Significantly different from respective control (DMSO) group at P<0.05.
(¹) Significantly different from CCl₄ group at P<0.05.

Table 3: Effects of CCl₄, Thymoquinone and Silymarin on AST enzyme leakage % of isolated rat hepatocytes

<table>
<thead>
<tr>
<th>Incubation Time (min)</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.8 ± 3.11</td>
<td>40.3 ± 2.74</td>
<td>43.2 ± 2.13</td>
</tr>
<tr>
<td>CCl₄</td>
<td>63.4 ± 4.27 a</td>
<td>70.2 ± 4.32 a</td>
<td>77.4 ± 4.64 a</td>
</tr>
<tr>
<td>Thymoquinone alone</td>
<td>39.4 ± 3.55</td>
<td>43.4 ± 3.88</td>
<td>46.7 ± 4.38</td>
</tr>
<tr>
<td>Silymarin alone</td>
<td>35.1 ± 2.34</td>
<td>41.5 ± 3.14</td>
<td>44.8 ± 4.27</td>
</tr>
<tr>
<td>Thymoquinone + CCl₄</td>
<td>48.1 ± 4.21 b</td>
<td>55.2 ± 2.78 b</td>
<td>60.3 ± 4.68 b</td>
</tr>
<tr>
<td>Silymarin + CCl₄</td>
<td>44.7 ± 2.88 b</td>
<td>49.2 ± 4.21 b</td>
<td>51.7 ± 3.11 b</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M. of 12 hepatocyte replicates.

(*) Significantly different from respective control (DMSO) group at P<0.05.
(¹) Significantly different from CCl₄ group at P<0.05.
El-Tawil and Moussa (2006) Antioxidant and Hepatoprotective Effects of Thymoquinone Against Carbon

**Fig. 1: Effects of CCl4, Thymoquinone and Silymarin on Viability % of isolated rat hepatocytes**

Data expressed as mean ± S.E.M. of 12 hepatocyte replicates.

(a) Significantly different from corresponding control (DMSO) group at P<0.05.
(b) Significantly different from CCl4 group at P<0.05.

**Fig. 2: Effects of CCl4, Thymoquinone and Silymarin on GSH level of isolated rat hepatocytes**

Data expressed as mean ± S.E.M. of 12 hepatocyte replicates.

(a) Significantly different from corresponding control (DMSO) group at P<0.05.
(b) Significantly different from CCl4 group at P<0.05.
DISCUSSION

Isolated hepatocytes have been extensively used to characterize the metabolism of xenobiotics. They offer the possibility of analyzing the pathways of metabolism in a model system under different conditions while largely maintaining the cell integrity and the intracellular interrelationship between enzyme systems and cofactors (Schlemper et al., 1993). The isolated hepatocytes as in vitro models provide the benefits of requiring fewer animals, reducing variability between samples, requiring less test material, and enabling higher throughput (Jessen et al., 2003). These advantages make isolated hepatocytes the system of choice in the studying of the hepatoprotective effect of some plant extracts which may be available in prohibitive amount (Reen et al., 2001). In general, chemically induced liver damage is more readily investigated in tissue culture than in whole animals (LeDuc et al., 1994).

Various pharmacological and chemical substances which belong to the intrinsic or idiosyncratic group of hepatotoxins may induce a level of hepatic damage varying from asymptomatic hepatic functional disturbance to widespread liver necrosis. CCl₄, which is an intrinsic hepatotoxin, was selected as a model of chemically induced liver injury in this study. CCl₄ commonly used as a model for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs (Recknagel et al., 1989). It produces a wide array of hepatic dysfunctions including centrilobular necrosis (Bernacchi, 1987), hepatic cancer, hypomethylation of cellular components as RNA which lead to inhibition of protein synthesis (Weber et al., 2003).

The data presented reflect the utilization of isolated liver cells to investigate the hepatoprotective effects of TQ against CCl₄ induced toxicity using different parameters and compared with silymarin as standard hepatoprotective. As membrane damage occurs, hepatocytes release the cytosolic enzymes into incubation media and lose the ability to exclude trypan blue. In this study, trypan blue exclusion was used to assess cell viability. Staining of the cells by trypan blue indicates severe irreversible damage and reflects the end point to evaluate the toxic effect of CCl₄ (Baur et al., 1985). Consequently, cell damage exhibits a good correlation with enzyme leakage (Berg and Aune, 1987). Hence, cellular injury caused by toxic substances is frequently accompanied by increased plasma membrane permeability (Smith and Orrenius, 1984). Cell viability and LDH and ALT enzyme leakages are indices to measure the degree of the cell membrane damage produced by toxicant, while mitochondrial damage is responsible for the major portion of AST leakage (Story et al., 1983).

In this study, CCl₄ (5mM) induced rapid loss of cell viability and increased leakage of intracellular enzymes (LDH, ALT, and AST) from isolated hepatocytes together with rapid depletion of intracellular glutathione and increased lipid peroxidation as evidenced by elevated levels of thiobarbituric acid reactive substances in isolated rat hepatocytes. These results are in harmony with those of other investigators who reported that the incubation of isolated hepatocytes with CCl₄ (5mM) resulted in marked
cell death preceded by intracellular glutathione depletion and extensive lipid peroxidation (Kim, 1995 and Dvorak et al., 2003).

The mechanisms by which CCl\textsubscript{4} produces its potent hepatotoxicity have been much studied. CCl\textsubscript{4} as a solvent, highly soluble in cell lipids that can damage cell membranes directly by physiochemical effects (Berger et al., 1986). This leads to leakage of intracellular enzymes and electrolytes within three minutes of exposure and could also contribute to mitochondrial toxicity (Farrell, 1994). Some morphological alterations were observed in the plasma membrane, endoplasmic reticulum, and mitochondria within two min of exposure of isolated rat hepatocytes to CCl\textsubscript{4} (Berger et al., 1987). Moreover, CCl\textsubscript{4} toxicity was reported to be associated with depletion of GSH where the carbon trichloromethyl free radicals (CCl\textsubscript{3}) can react with compounds containing sulfhydryl groups such as GSH and protein thiols leading to membrane lipid peroxidation and finally cell necrosis (Recknagel et al., 1989).

In the present study, both TQ and silymarin showed protective effects against CCl\textsubscript{4} induced toxicity in isolated rat hepatocytes. Silymarin was slightly more potent in preventing loss of cell viability and enzyme leakages, but both compounds have almost the same effect in preventing GSH depletion which induced by CCl\textsubscript{4}. However, GSH contents never reach the control values.

Silymarin, the standard hepatoprotective used in the present study, exerted marked protective effects against CCl\textsubscript{4} induced liver injury. Several studies in vitro and in vivo have shown that silymarin possesses hepatoprotective effects (Letteron et al., 1990). The crucial protective mechanism of silymarin is an inhibition of lipid peroxidation by silymarin’s free-radical-scavenging properties (Farghali et al., 2000). Silymarin prevented CCl\textsubscript{4} induced lipid peroxidation and hepatotoxicity in mice by decreasing the metabolic activation of CCl\textsubscript{4} and by acting as a chain-breaking antioxidant (scavenging free radicals) (Letteron et al., 1990).

The data in this study demonstrated that enzymes leakage and loss of cell viability are parallel to GSH depletion. The GSH system is an important endogenous antioxidant that is found particularly in high concentration in the liver and is known to have key functions in cellular protective mechanisms. The depletion of intracellular thiol notably GSH induced by exposure to oxidant stress (e.g. CCl\textsubscript{4}) can increase the susceptibility to irreversible injury by oxidative intoxication and by free radicals which can result in lipid peroxidation, protein oxidation, protein inactivation, disturbance in calcium homeostasis and consequent loss of cell viability (Shertzer et al., 1994).

In our study, TQ (1 mM) inhibited the decrease of cell viability and enzyme leakage from isolated hepatocytes induced by CCl\textsubscript{4}. Also, TQ caused preservation of intracellular GSH which therefore could explain the hepatoprotective effect. Both TQ and silymarin caused equal preservation of GSH with different percentages of protection as described by the indices of ALT and AST leakages. TQ has been reported to have potent superoxide anion (O\textsuperscript{2−}) scavenging abilities and to inhibit iron-dependent microsomal lipid peroxidation (Badary et al., 2003). TQ may protect against CCl\textsubscript{4}-induced hepatotoxicity by a combination of two mechanisms, TQ antioxidant potential (Houghton et al., 1995) and a previous report of the quinone to have in vitro and in vivo superoxide anion radical scavenging ability (Nagi and Mansour, 2000). TQ provided good protection against lipid peroxidation and the oxidative damage caused by several toxic agents, as in cisplatin nephrotoxicity (Badary et al., 1997), doxorubicin cardiotoxicity (Al-Shabanah et al., 1998), and benzo(a) pyrene-induced forestomach carcinogenesis (Badary et al., 1999). Burits and Bucar (2000) found that TQ had a scavenger effect against the OH\textsuperscript{−} radical in vitro. Moreover, Houghton et al. (1995) reported that TQ had potent anti-inflammatory and inhibitory effects on non-enzymatic peroxidation of ox brain phospholipid liposomes.

In conclusion, the results of the present study indicate that TQ is efficient cytoprotective agents against CCl\textsubscript{4}-induced hepatotoxicity in isolated rat hepatocytes. These hepatoprotective effects were probably due to their antioxidant activities.

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