Possible Prophylactic Effects of Vitamin E or Lycopene Treatment on Renal Toxicity Induced by CCL₄ Administration in Albino Rats

Karima M. Moawad
Zoology Department, Girls College for Arts, Science and Education, Ain Shams University, Egypt

Abstract: The main purpose of this study was to evaluate whether vitamin E (40mg/kg) or lycopene (1 mg / kg) treatments for 4 weeks are effective in preventing renal toxicity in male rats due to carbon tetrachloride (CCL₄ 1ml / kg, 3 times weekly) administration for 2 weeks. CCL₄ administration induced a significant decrease in serum total protein and albumin, a significant increase (P<0.001) in serum urea and creatinine as well as in sodium concentrations, accompanied with a significant decrease in circulating calcium level as compared to the control group. CCL₄-treatment induced a significant increase (P<0.001) in kidney total cholesterol, triglycerides, total lipids, lipid peroxidation (MDA) and total protein levels as compared to control rats. Moreover, intoxication with CCL₄ caused a significant reduction in kidney glutathione (GSH) content associated with the inhibition of gamma-glutamyl-transferase (GGT) and superoxide dismutase (SOD) activities. On the other hand, vitamin E or lycopene administration for 4 weeks (2 weeks prior to and 2 weeks during CCL₄ administration) caused improvement in serum and kidney parameters. The efficiency of vitamin E or lycopene treatment to alleviate the serum concentrations of total protein, albumin, urea and creatinine as well as sodium level was provoked. The reduction in serum calcium was modulated. Also, attenuation in the kidney cholesterol, triglycerides, total lipids and MDA levels and total protein content was recorded when compared to CCL₄ group values. Moreover, administration of vitamin E or lycopene prior to and concomitant with CCL₄ injection induced marked protection as detected by significant elevation in kidney antioxidants GSH, SOD and GGT levels. The present results indicate that treatment of rats with vitamin E or lycopene might have produced amelioration in kidney tissue through protection lipids indices and oxidative stress parameters against CCL₄-induced nephrotoxicity. Therefore, vitamin E or lycopene treatments may have therapeutic values in preventing CCL₄-induced nephrotoxicity.

Key words: Vitamin E · lycopene · rats · kidney · CCL₄

INTRODUCTION

Carbon tetrachloride (CCL₄) a clear, heavy and nonflammable liquid is widely used in the dry-cleaning industry and is a highly toxic chemical agent. Thus,CCL₄ is most widely used for experimental induction of hepatic cirrhosis [1-3]. Exposure to CCL₄ results in hepatic steatosis, centrilobular necrosis and ultimately, cirrhosis in the liver and acute tubular necrosis in the kidney [4]. CCL₄ induces oxidative stress in many settings and it also inhibits the activity of antioxidant enzymes in renal tissue [5]. Lipids peroxidation is a major mechanism by which free radical can induce tissue injury [6]. Against these types of oxidative injuries, tissues have a variety of defense mechanisms including the non-enzymatic glutathione (GSH) and the enzymatic superoxide dismutase (SOD) scavenger systems [7]. It has been suggested that certain vitamins and antioxidant compounds may act as preventive or protective factors such as vitamin E [8] and carotenoids (Alpha-carotene, beta – carotene, lycopene and lutein) [9]. Lycopene is the pigment that gives tomatoes their red color and is one of four main carotenoids normally found in human blood and tissue. There is about 5 mg of lycopene per 100 gram of ripe tomato fruit [10]. Although not considered an essential nutrient, research has shown that lycopene may have various benefits for human health. Recently, it was found that lycopene is an antioxidant known to decrease the risk of age-related chronic diseases, such as cancer [11] and cardiovascular disease in women [12]. The pathogenesis of carbon tetrachloride (CCL₄)-induced renal dysfunction is not completely known. Although
different experimental animal models have been utilized in order to explain such pathophysiological state, none of them have completely explained the mechanisms involved. It may be due to the functional state of the liver, or renal injury may develop independently of hepatic events [13].

Therefore, the aim of this study is to evaluate renal dysfunction established as a consequence of acute liver damage after CCL administration in rats and to observe the changes in the antioxidative defense enzymes. Also, to investigate the possible prophylactic effect of lycopene against oxidative damage of CCL in rats, compared with the well-known antioxidant vitamin E.

**MATERIALS AND METHODS**

Sixty male albino rats (*Rattus norvigicus*) weighing about 100-120 g each were used in this experiment. The animals were housed in the usual metal cages at room temperature and fed commercial laboratory show (solid) and had free access to water.

The drugs used:

- **CCL₄**: A colorless non-flammable liquid, of molecular weight 153.84 was obtained from El-Nasr Pharmaceutical Chemical Co., A.R.E.
- **Vitamin E**, lycopene and other chemicals used for estimation of SOD and GSH were obtained from Sigma Chemical Co., St-Lowis. Mo., USA.

**The experimental procedures**: The animals were randomly allocated to six groups each of ten rats:

- **Control group**: rats received orally saline solution (0.2 ml).
- **Vitamin.E (Vit. E)** group: rats received oral dose (40 mg/kg) dissolved in saline solution twice a week for 4 weeks.
- **Lycopene (Lycop.)** group: rats received oral dose (1 mg/kg) dissolved in saline solution twice a week for 4 weeks.
- **CCL₄ group**: rats received CCL₄ (1 ml/kg) 3 times weekly for 2 weeks through intraperitoneal injection (i.p.).
- **CCL₄ plus Vit.E group**: rats received CCL₄, 3 times weekly for 2 weeks through i.p. plus oral dose of Vit. E (40 mg/kg) for 4 weeks (2 weeks prior to and 2 weeks during CCL₄ administration).
- **CCL₄ plus Lycop group**: rats received CCL₄, 3 times weekly for 2 weeks through i.p. plus oral dose of lycopene (1 mg/kg) for 4 weeks (2 weeks before and 2 weeks during CCL₄ administration).

The doses of CCL₄, Vit E and Lycopene used in this study were based on the efficacy of these drugs in rats [5, 8, 9].

At the end of experimental period, rats were dissected under anaesthesia then blood samples were collected from heart, centrifuged and the sera were separated and stored at -20°C.

Samples, approximately 0.5 g from kidney tissue, were taken and homogenized in ice-cold distilled water, centrifuged at 3000 r.p.m. for 15 minutes and the supernatant was separated and kept frozen at -20°C until assayed.

**Biochemical measurements:**

- Total protein content was measured by the Biruet method [14], while albumin content was assessed using the method of [15].
- Serum urea level was determined according to the method of [16].
- Serum creatinine was determined using kits purchased from Stanbio laboratory Co. USA.
- Calcium and sodium contents were determined using reagent kits of Quimica Clinica Aplicada S.A (QCA), Spain, through Gamma Trade Co. Cairo.
- Total cholesterol and triglycerides (TG) were estimated by using enzymatic colorimetric kits purchased from Stanbio laboratory Co. (USA), through Gamma Trade Co. Cairo.
- Total lipid was detected according to the method of White *et al.* [17].
- Determination of malondialdehyde (MDA) a marker of lipid peroxidation was performed using thiobarbituric acid reacting substance (TBARS) according to the method of Ohkawa *et al.* [18].
- Gamma-glutamyl trasferase (GGT) was determined using kit purchased from Stanbio Chemicals (USA) through Gamma Trade Company, Cairo.
- Determination of glutathione reduced form (GSH) was performed according to the method of Beutler *et al.* [19].
- Superoxide dismutase (SOD) activity was determined by the method of Minami and Yoshikawa [20].
Statistical analysis: Data were performed using Students "t" test according to the method of Hin and Wetherill [21]. Analysis of variance (ANOVA) and Fisher's significant difference test were also used [22]. P values of 0.05 or less were considered as statistically significant.

RESULTS

The effects of vitamin E or lycopene on serum parameters of male rats treated with CCL₄ are shown in Table (1). The present data indicate that CCL₄ administration for 2 weeks induced a significant reduction (P<0.001) in serum total protein (-34.88%) and albumin (-28.47%) and a significant increase (P<0.001) in urea and creatinine levels as compared to the corresponding control values. It induced also a significant decrease (P<0.001) in circulating calcium level. These changes were accompanied with a significant increase (P<0.001) in serum sodium (27.83%) concentration compared to control group.

In comparison to the control values, CCL₄ - treated rats showed a significant increase (P<0.001) in kidney total cholesterol (6.07±0.27 mg/g), triglycerides (17.69±0.62), total lipids (37.04±0.77) and MDA (500.67±23.92) levels when compared to the control values. Also, kidney total protein level increased (97.8%) significantly in rats treated with CCL₄ (Table 2 & 3).

The intoxication of rats with CCL₄ caused a significant reduction (P<0.001) in kidney levels of GSH (-48.11%), GGT (-28.42%) and SOD (-44.61%) as compared to control group (Table 3).

On the other hand, treatment of CCL₄ intoxicated rats with vitamin E or lycopene for 4 weeks caused improvement in serum and kidney parameters. The reduction in serum total protein was abolished and recorded a significant elevation (P<0.001) after treatment with vit.E and lycopene as compared to CCL₄ group. Elevation in serum albumin level was also recorded in those animals, compared to the same group. Furthermore, the treatment with tested drugs caused detectable decrease in serum urea and creatinine levels.

Table 1: Changes in some serum parameters of rats treated with CCL₄ (1 ml/kg) or /and vitamin E (40 mg/kg) and lycopene (1 mg/kg)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T.Protein</th>
<th>Albumin</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Calcium</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co.</td>
<td>6.80±0.28</td>
<td>3.69±0.19</td>
<td>22.85±1.70</td>
<td>0.91±0.05</td>
<td>7.19±0.16</td>
<td>38.18±1.53</td>
</tr>
<tr>
<td></td>
<td>6.68±0.59</td>
<td>3.49±0.14</td>
<td>23.41±0.53</td>
<td>0.96±0.07</td>
<td>7.07±0.14</td>
<td>37.99±1.70</td>
</tr>
<tr>
<td>Vit.E</td>
<td>6.97±0.30</td>
<td>3.39±0.16</td>
<td>23.06±0.88</td>
<td>1.07±0.06</td>
<td>6.85±0.26</td>
<td>39.33±1.76</td>
</tr>
<tr>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
<tr>
<td>Lycop.</td>
<td>4.43±0.318</td>
<td>2.64±0.18</td>
<td>36.03±0.72</td>
<td>2.07±0.07</td>
<td>3.51±0.16</td>
<td>48.81±1.71</td>
</tr>
<tr>
<td>CCL₄</td>
<td>-34.88%</td>
<td>28.47%</td>
<td>57.64%</td>
<td>126.69%</td>
<td>51.12%</td>
<td>27.83%</td>
</tr>
<tr>
<td></td>
<td>a**</td>
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<td>a**</td>
<td>a**</td>
<td>a**</td>
<td>a**</td>
</tr>
<tr>
<td></td>
<td>6.70±0.31</td>
<td>3.23±0.11</td>
<td>22.84±1.54</td>
<td>1.08±0.14</td>
<td>5.15±0.27</td>
<td>39.51±0.83</td>
</tr>
<tr>
<td>CCL₄+Vit.E</td>
<td>-1.38%</td>
<td>-12.50%</td>
<td>0.05%</td>
<td>28.89%</td>
<td>-28.40%</td>
<td>3.48%</td>
</tr>
<tr>
<td></td>
<td>b**</td>
<td>a*</td>
<td>b**</td>
<td>b*</td>
<td>a** b**</td>
<td>b**</td>
</tr>
<tr>
<td></td>
<td>6.78±0.56</td>
<td>3.09±0.08</td>
<td>21.99±0.81</td>
<td>1.26±0.21</td>
<td>4.72±0.17</td>
<td>40.66±0.81</td>
</tr>
<tr>
<td>CCL₄+Lycop.</td>
<td>0.92%</td>
<td>-16.38%</td>
<td>-3.76%</td>
<td>38.03%</td>
<td>-34.29%</td>
<td>6.49%</td>
</tr>
<tr>
<td></td>
<td>b**</td>
<td>a*</td>
<td>b**</td>
<td>b*</td>
<td>a** b**</td>
<td>b**</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>F (5,30)</td>
<td>5.94</td>
<td>6.05</td>
<td>23.4</td>
<td>13.54</td>
<td>57.89</td>
<td>7.89</td>
</tr>
</tbody>
</table>

Data are given as: means± S.E for 6 rats/ group.

a: differs from control rats
b: differs from rats treated with CCL₄
% of change from control.
n.S.: Non-significant

Significant at * p<0.01 or **p<0.001
Table 2: Changes in some kidney parameters of rats treated with CCL4 (1 ml/kg) or and vitamin E (40 mg/kg) and lycopene (1 mg/kg)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cholesterol</th>
<th>T.G</th>
<th>T.lipids</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g</td>
<td>mg/g</td>
<td>mg/g</td>
<td>nmol/g</td>
</tr>
<tr>
<td>Co.</td>
<td>1.57±0.10</td>
<td>11.81±0.42</td>
<td>23.37±1.51</td>
<td>257.83±13.70</td>
</tr>
<tr>
<td>Vit.E</td>
<td>1.64±0.09</td>
<td>11.14±0.52</td>
<td>24.20±0.74</td>
<td>246.83±47.48</td>
</tr>
<tr>
<td></td>
<td>4.03%</td>
<td>-5.69%</td>
<td>3.55%</td>
<td>-4.27%</td>
</tr>
<tr>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
<tr>
<td>Lycop.</td>
<td>-1.38%</td>
<td>-10.19%</td>
<td>2.92%</td>
<td>2.20%</td>
</tr>
<tr>
<td></td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
<tr>
<td>CCL4</td>
<td>285.90%</td>
<td>49.76%</td>
<td>16.2%</td>
<td>58.47%</td>
</tr>
<tr>
<td>CCL4+Vit.E</td>
<td>81.97%</td>
<td>-8.13%</td>
<td>1.13%</td>
<td>28.31%</td>
</tr>
<tr>
<td></td>
<td>a**</td>
<td>b**</td>
<td>b**</td>
<td>b**</td>
</tr>
<tr>
<td>CCL4+Lycop.</td>
<td>40.40%</td>
<td>-10.74%</td>
<td>-5.18%</td>
<td>32.19%</td>
</tr>
<tr>
<td></td>
<td>a*</td>
<td>b**</td>
<td>a*</td>
<td>b**</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>F (5,30)</td>
<td>88.48</td>
<td>34</td>
<td>25.27</td>
<td>14.15</td>
</tr>
</tbody>
</table>

Data are given as: means± S.E for 6 rats/group.

Table 3: Changes in some kidney parameters of rats treated with CCL4 (1 ml/kg) or and vitamin E (40 mg/kg) and lycopene (1 mg/kg)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T.protein</th>
<th>GGT</th>
<th>GSH</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g</td>
<td>u/g</td>
<td>mg/g</td>
<td>u/g</td>
</tr>
<tr>
<td>Co.</td>
<td>105.02±6.09</td>
<td>22.85±1.70</td>
<td>171.56±18.73</td>
<td>69.48±1.99</td>
</tr>
<tr>
<td>Vit.E</td>
<td>96.82±2.70</td>
<td>23.41±0.53</td>
<td>174.68±8.03</td>
<td>70.50±2.16</td>
</tr>
<tr>
<td></td>
<td>98.79±1.73</td>
<td>23.03±0.88</td>
<td>162.90±6.72</td>
<td>75.73±0.97</td>
</tr>
<tr>
<td>Lycop.</td>
<td>-5.93%</td>
<td>0.88%</td>
<td>-5.05%</td>
<td>8.98%</td>
</tr>
<tr>
<td></td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
<tr>
<td>CCL4</td>
<td>97.80%</td>
<td>-28.42%</td>
<td>-48.11%</td>
<td>-44.61%</td>
</tr>
<tr>
<td>CCL4+Vit.E</td>
<td>78.24%</td>
<td>2.45%</td>
<td>1.82%</td>
<td>1.47%</td>
</tr>
<tr>
<td></td>
<td>a**</td>
<td>a**</td>
<td>a**</td>
<td>a**</td>
</tr>
<tr>
<td>CCL4+Lycop.</td>
<td>62.04%</td>
<td>-3.76%</td>
<td>-1.98%</td>
<td>11.93%</td>
</tr>
<tr>
<td></td>
<td>a*</td>
<td>b**</td>
<td>b**</td>
<td>a**</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>F (5,30)</td>
<td>88.48</td>
<td>34</td>
<td>25.27</td>
<td>14.15</td>
</tr>
</tbody>
</table>

Data are given as: means± S.E for 6 rats/group.

DISCUSSION

The health benefits of lycopene including the antioxidative effects are still under evaluation. Vitamin E is a well-known antioxidant. Thus the present study was carried out to investigate the protective effect of lycopene against oxidative damage of CCL4 in rats, compared with the well-known antioxidant vitamin E.

The current data showed that administrations of vitamin E or lycopene to normal rats for 4 weeks did not exert significant changes in all the investigated parameters in both serum and kidney tissue.

Versus to normal control rats, CCL4 injection induced significant decrease in both serum total protein and albumin levels and a significant (P<0.001) increase in serum urea and creatinine concentrations. These changes in urea and creatinine levels may indicate a reduction in the glomerular filtration rate (GFR) as a result of CCL4 intoxication, since the serum concentration of these two parameters depends largely on the glomerular filtration [23]. Confirming these results Ogeturk et al. [24] found that serum urea and creatinine and renal malondialdehyde (MDA) levels were increased significantly in rats compared to CCL4 treated rats, but creatinine values were still higher than those of the corresponding control values (Table1).

Improvement of serum sodium was observed with vit E and lycopene treatments recording (3.48 % and 6.49 %) respectively, compared to CCL4 group. Also, a marked improvement in the circulating level of calcium was provoked in response to tested drugs compared to those of CCL4 group (Table1).

In addition, treatments of vit. E and lycopene were found to reduce the degree of kidney tissue damage in biochemical findings (Tables 2 & 3). Also, these results indicate that treatment with lycopene might have produced amelioration in lipids indices and oxidative stress parameters against CCL4-induced nephrotoxicity. Vit E and lycopene therapies prevented significantly lipids elevation, induced by CCL4 intoxication, results as amelioration in kidney cholesterol (81.97%, 40.40%) and triglycerides (-8.13%, -10.74%) as well as total lipids (1.13%, -5.18%), compared to CCL4 group (Table 2).

In comparison to CCL4 intoxicated rats, Vit E or lycopene treatment induced a significant decrease (P<0.001) in both kidney MDA and total protein levels and a significant increase (P<0.001) in kidney GSH content and GGT and SOD activities compared to CCL4 group (Tables 2 & 3).
following CCl₄ exposure and also caused prominent damage in the kidney tissue as compared to the control group. Also, Rincon et al. [13] and Huizar et al. [25] observed that tubular changes, glomerular hypercellularity and capillary obliteration were significantly higher in the CCl₄-treated group than with control rats followed by a decreased in the glomerular filtration rate.

The pathogenesis of renal function alteration associated with CCl₄-induced liver disease remains to be elucidated. Rincon et al. [13] suggested that the effect of CCl₄ on kidney structure and function depended on the functional state of the liver. In a previous study, Abd El Dayem and Moawad [1] observed liver dysfunction and tissue damage of CCl₄ intoxicated rats. CCl₄ induced oxidative stress in many settings [26, 27]. It is metabolized through the mitochondrial monoxygenase system (P450 2E1). During metabolism, an unstable free radical, trichloromethyl, exits and is rapidly converted to trichloromethyl peroxide [28, 29]. Consequently, the cell membrane structure and membranes of intracellular organelles are totally damaged. In addition, CCl₄ induced injury of the basement membrane [13] which, in turn, will increase its permeability and thus increases protein loss in the urine. In this line, Ozturk et al. [30] suggested that the alterations in kidney functions and structure observed in CCl₄ treated rats, probably occurred as a result of the lipid peroxidation and breakdown of the membrane structure. Therefore, the present findings are in accordance with Lin et al. [3] and Ogeturk et al. [24] who found that CCl₄ intoxication significantly decrease serum total protein and albumin levels in rats.

On the other hand, vitamin E or lycopene administration for 4 weeks (2 weeks prior to and 2 weeks during CCl₄ administration) caused improvement in serum and kidney parameters. The efficiency of vitamin E or lycopene treatment to improve the serum concentrations of total protein, albumin, urea and creatinine was provoked. It has been well established that vitamin E attenuates the hepatotoxicity induced by carbon tetrachloride [1, 31]. The esters of vitamin E and synthetic vitamin E-like antioxidant have been also found to reduce carbon tetrachloride-induced liver injury [8, 32]. Also, Kim [9] found that carotenoids (alpha-carotene, beta–carotene, lycopene and lutein) have protective effects on oxidant-induced injury of rat hepatocytes and suppression of lipid peroxidation. The increases observed in serum total protein and albumin levels may be due to the improvement in protein synthesis in the liver as a result of antioxidant effect of both vitamin E and lycopene, which act as free radical scavengers and could protect against lipid peroxidation [1], or may be due to the improvement in kidney function. Supporting this explanation Karahan et al. [33] found that lycopene as a novel natural antioxidant might have protective effects against gentamicin-induced nephrotoxicity and oxidative stress in rats. Also, Dillioglugil et al. [34] suggested that kidney function appears to be improved by vitamin E supplementation due to its antioxidant and antithrombus action. Moreover, the present study revealed a decrease in circulating calcium level accompanied with highly significant (P<0.001) increase in serum sodium concentration. The decrease in serum calcium post CCl₄ injection was also reported by Nakano et al. [35] who found that in rats with both chronic non-cirrhotic liver injury and CCl₄-induced cirrhosis, tibial bone volume was significantly lower than in controls and the osteoid volume decreased while the urinary calcium/creatinine ratio increased. Moreover, albumin is considered as the main Ca’ carrier through blood [36], so the decrease in albumin level is postulated to be the main cause of serum Ca’ reduction.

However, the increase in serum sodium may be a result of inhibited kidney excretion by CCL₄ intoxication. This is supported by the findings of Yu et al. [37] who reported that liver cirrhosis is associated with enhanced renal tubular sodium retention and they hypothesized that the mechanism of which is associated with increased expression of renal epithelial sodium transporters. Also, Huizar et al. [25] studied bile duct ligation (BDL) of variable etiology, either surgical or induced by CCl₄ in rats and found that urinary volume and urinary sodium concentration were significantly decreased, while plasma renin activity and concentration, serum creatinine and BUN values increased after BDL in cirrhotic rats. Also, glomerular filtration rate was substantially decreased.

On the other hand, the present results indicated that the treatment of CCL₄ intoxicated rats with vitamin E or lycopene caused improvement in serum calcium and sodium levels as well as kidney total protein content, compared to those of CCL₄ treated group. These changes may be due to the protection against peroxidative injury induced by reactive oxygen species (ROS), which is associated with the risk of osteoporosis and can be reduced by certain dietary antioxidants. Rao et al. [11] suggest that the dietary antioxidant lycopene reduces oxidative stress and the levels of bone turnover markers in postmenopausal women and may be beneficial in reducing the risk of osteoporosis. In this line, Choi and Rhee [38] indicated that vitamin E supplementation in
chronic cadmium-poisoned rats normalized renal dysfunction, blood pressure regulation and the glomerular filtration rate (GFR).

It was observed in the present study that the administration of CCL\textsubscript{4} induced a significant increase (P<0.001) in kidney total cholesterol, triglycerides, total lipids and lipid peroxidation (MDA) levels. These changes recorded displayed lower levels in kidney tissue of rats treated with vitamin E or lycopene. The increments of kidney lipids recorded after CCL\textsubscript{4} administration are in agreement with the finding of Kamalakkannan et al. [39] who found that the levels of lipids, cholesterol, triglycerides and free fatty acids were increased in plasma and tissues (liver, kidney, heart and brain) of rats treated with CCL\textsubscript{4} (3 ml kg\textsuperscript{-1} wk\textsuperscript{-1}). Also, they found that phospholipid levels increased in plasma, heart and brain but decreased in liver and kidney. The mechanism of CCL\textsubscript{4} action on kidney lipids is complex and has not been elucidated completely. The formation of lipid peroxides induced by carbon tetrachloride has been observed in many studies [5, 40], results in oxidative damage to the lipids and proteins of the kidney in rats [26]. Also, a decrease in the activity of enzymes protecting from lipid peroxidation in the kidney was provoked [41]. The abnormally high kidney cholesterol level detected in the present experiment might be explained by raised levels of serum LDL-fractions [42]. These fractions are cleared chiefly through specific binding to LDL-receptors demonstrated in the glomerular tissue [43]. Therefore, the high lipids levels detected in CCL\textsubscript{4}-treated rats may be associated with the accumulation of filtered LDL-fractions in the glomeruli, leading to a reduction in the glomerular filtration surface area. On the other hand, the accumulation of triglycerides may be due to the degradation of phospholipids as a result of the increase in lipid peroxidation [39]. The blockade of hepatic triglycerides secretion by CCL\textsubscript{4} accounts for characteristic fatty liver [1,44] which caused damage of kidney structure and function. Rincon et al. [13] suggest that the effect of CCL\textsubscript{4} on kidney structure and function depended on the functional state of the liver.

In this respect, recent studies have shown that cirrhotic rats with CCL\textsubscript{4} intoxication exhibited a significant decrease in mean arterial pressure followed by a decreased glomerular filtration rate (GFR) [24, 25]. The aforementioned reduction in GFR and renal dysfunction run in agreement with the increase in kidney total lipids, cholesterol, triglycerides and total protein contents recorded in the present study.

Another mechanism for increase kidney lipids suggest a novel renal uptake pathway for Liver-type fatty acid binding protein (L-FABP), a carrier of hydrophobic molecules, some of which may exert nephrotoxic effects. These explanations agree with Oyama et al. [45] who detected high levels of L-FABP in the circulation and in the kidney of rats with CCL\textsubscript{4}-induced acute liver injury compared with those in the control rat by immunoblotting. These circulatory L-FABP was found to be filtered by glomeruli and internalized by proximal tubule cells -mediated endocytosis.

In the present study, a marked increase in MDA concentration (p<0.001) was observed in kidney of CCL\textsubscript{4} intoxicated rats, which reflect, increased oxidative stress in kidney tissue. The increase in lipid peroxidation in kidney post CCL\textsubscript{4} administration was also reported by Abraham et al. [26]; Ozturk et al. [30] and Yoshida et al. [31]. Reactive oxygen species have been implicated in the pathogenesis of renal injury by direct cellular toxicity, partly through liberation of vasoconstrictor-bioactive lipids and inactivation of nitric oxide (NO) [30]. In addition, oxidized LDL is injurious to renal tubular epithelial cells and may contribute to tubulo-interstitial disease [46].

Versus CCL\textsubscript{4} treated rats, administration of vitamin E or lycopene revealed attenuation in the levels of kidney total protein, total lipids, cholesterol, triglycerides and MDA levels.

There are several defense mechanisms that protect living organisms against free radicals. Vit-E, a fat-soluble vitamin, is believed to be the first line of defense against cell membrane damage due to peroxidation [38] and oxidative modification of LDL [47]. In support of this notion, Reckelhoff et al. [48] and Kwag et al. [49] found that kidney function appears to be improved by vitamin E supplementation due to its antithrombus action, which in turn controls the arachidonic acid cascade system and effective in the suppression of oxidative stress. Also, Ardestani et al. [50] concluded that irradiated mice that received Vit-E were able to restore the changes of lipid peroxidation and lipid profile and tolerated biomembrane damage provoked by 1.09 Gy for 3 days.

Bud et al. [51] reported that tomato lycopene, alone or in combination with other natural antioxidants, inhibits LDL oxidation, cholesterol synthesis and augments LDL receptor activity in macrophages. These results were also confirmed by clinical trials [52]. In this line, Hosomi et al. [53] reported that HgCl\textsubscript{2}-induced increase of lipid peroxidation in kidney was prevented by lycopene. Also, Fuhrman et al. [54] suggested that lycopene may act as
moderate hypocholesterolemic agents, secondary to their inhibitory effect on macrophage 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase, the rate limiting enzyme in cholesterol synthesis, also to augment the activity of macrophage LDL receptors.

Moreover, The present study demonstrated that CCl₄ intoxication induces a highly significant reduction in kidney GSH content associated with the inhibition of kidney GGT and SOD activities. These changes are in agreement with recent studies [7, 55, 56]. They observed that rats received CCl₄ recorded decrease in the levels of GSH content and enzyme activities of SOD, glutathione peroxidase (GSPx) and glutathione reductase (GR) in liver and kidney in comparison with controls as well as a significant increase in MDA level in the kidney was recorded. The decrease in the activity of SOD in the kidney of CCl₄-treated rats may be due to the increased lipid peroxidation or inactivation of the enzyme by cross-linking with MDA. This will cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. This explanation runs in agreement with the findings of Anandan et al. [57] who reported that depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH consumption and with Naik and Panda [58] who found that CCl₄ significantly altered SOD, GSH-Px and catalase (CAT) levels, indicating that oxygen radicals play an important role in CCl₄-induced renal damage. The decline observed in kidney GGT enzyme in the present study post CCl₄ administration is an indication of impaired GSH synthesis. GGT enzyme is the first enzyme in the degradation of GSH to its constituent amino acids, which were introduced in the resynthesizing of GSH to replenish the tissue stores [59]. At the molecular level CCl₄ activates tumor necrosis factor (TNF)alpha and transforming growth factors (TGF)-alpha and -beta in the cell, processes that appear to direct the cell primarily toward self-destruction or fibrosis [29]. The increase in kidney total protein observed in the present study confirm the latter suggestions.

Administration of vitamin E or lycopene prior to and concomitant with CCl₄ injection induced marked protection as detected by significant elevation in kidney antioxidants GSH content, SOD and GGT activities as well as reduction in MDA levels. In the rat kidney, vitamin E attenuates the chronic renal injury associated with scavenges free radicals [60] and attenuates the redox-sensitive mechanisms [61]. Thamilselvan and Menon [62] showed that excess vitamin E completely prevented calcium oxalate deposition, by preventing peroxidative injury and restoring renal tissue antioxidants and glutathione redox balance. Lycopene is one of the most potent singlet oxygen quenchers, which suggests that it may have comparatively stronger antioxidant properties than other major plasma carotenoids [63].

In this line, Bhuvaneswari et al. [64] suggest that lycopene-induced increase in the levels of GSH, the enzymes of the glutathione redox cycle and phase II (glutathione S-transferase -GST) in the buccal pouch mucosa treated with DMBA. Another possible explanation for lycopene alleviation of the CCl₄-induced nephrotoxicity is its antioxidant effects. According, Hosomi et al. [53] reported that glutathione peroxidase and catalase activities were enhanced, while superoxide dismutase activity was depressed in HgCl₂-treated rats when compared to control and these effects were prevented by lycopene. Moreover, Atessahin et al. [65] reported that lycopene administration produced amelioration in biochemical indices of nephrotoxicity in both plasma and kidney tissues as MDA, GSH, GSH-Px and CAT activities when compared to Cisplatin, which induced nephrotoxicity and oxidative stress in rat.

Therefore, it is possible suggested that the positive effect of lycopene on CCl₄-induced nephrotoxicity might be the result of its antioxidant effects.

CONCLUSIONS

The present study suggested that CCl₄ causes renal damage in rats through enhanced lipid peroxidation and significantly altered GSH, SOD and GGT levels, indicating that oxygen radicals play an important role in CCl₄-induced renal damage. Vitamin E or lycopene treatment normalized these changes, but treatment with lycopene lead to more protection in kidney tissue than vitamin E. This protection was indicated by the highly increase of kidney GSH content as well as SOD and GGT activities.

Therefore, vitamin E or lycopene treatments may have therapeutic values in preventing CCl₄-induced nephrotoxicity.

REFERENCES


