Kidney failure is frequently reported in fatal poisoning.\cite{4,5} Furthermore, based on results of animal studies, the US Environmental Protection Agency has classified CCl$_4$ as a Group B2, probable human carcinogen.\cite{5,6}

In spite of the fact that the harmful effects of CCl$_4$ are obvious, this compound is still used as a solvent for oils, fats, lacquers, varnishes, rubber waxes and resins and as a starting material in the production of a number of organic compounds.\cite{6,7}

It has been established that trichloromethyl (CCl$_3$) radical and Cl are formed as a result of the metabolic conversion of CCl$_4$ by cytochrome P450, which in turn, initiate lipid peroxidation process.\cite{8-10}

Among horticultural crops, fruits are sources of diverse nutrient and non-nutrient molecules, which display antioxidant properties,\cite{11} and can protect the human body against oxidant damage.

Cornus mas, known as the European and Asiatic Cornelian Cherry, has been used for the treatment of diarrhea, intestinal inflammation, fever and malaria.\cite{12} Furthermore, it has been mentioned for the treatment of kidney stones.

**Original Article**

**Protective effects of Cornus mas fruit extract on carbon tetrachloride induced nephrotoxicity in rats**

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**ABSTRACT**

Oxidative damage is implicated in the pathogenesis of kidney injury. *Cornus mas* is used for in renal ailments traditionally in Iran. The present study was aimed to investigate the antioxidant activity of *C. mas* fruit extract (CMFE) on carbon tetrachloride (CCl$_4$) treated oxidative stress in Wistar albino rats. Forty two male albino rats were divided into seven groups. Group I served as a sham; Group II served as a normal control; Group III served as a toxic control, with CCl$_4$ (1 ml/kg body weight; 80% in olive oil); Groups IV and V received CMFE at doses of 300 and 700 mg/kg before CCl$_4$ injection; Groups VI and VII received extract at same doses orally at 2, 6, 12, 24 and 48 h after CCl$_4$ intoxication. CCl$_4$ injection produced a significant rise in serum markers of oxidative stress and lipid peroxidation product malondialdehyde along with the reduction of antioxidant enzymes such as superoxide dismuta, catalase and glutathion peroxidase. Serum creatinine, urea and uric acid concentrations were increased whereas level of protein and albumin were reduced. Treatment of rats with different doses of fruit extract (300 and 700 mg/kg) significantly ($P < 0.05$) ameliorated the alterations induced with CCl$_4$ in lipid peroxidation, antioxidant defenses, biochemical and renal lesions. Based on these results, we conclude that CMFE protects kidney from oxidative stress induced by CCl$_4$.

**Key words:** Carbon tetrachloride, *Cornus mas*, lipid paroxidation, nephrotoxicity, oxidative stress

**Introduction**

Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damage through generation of reactive oxygen species (ROS). Carbon tetrachloride (CCl$_4$), a clear, colorless, volatile, heavy and nonflammable liquid, causes free radical generation and causes kidney injury in rats.\cite{1,2} Free radicals induce lipid peroxidation and can damage cell membranes.\cite{3} The average daily intake of CCl$_4$ for the general population is estimated to be 0.1 µg.

Kidney failure is frequently reported in fatal poisoning.\cite{4,5} Furthermore, based on results of animal studies, the US Environmental Protection Agency has classified CCl$_4$ as a Group B2, probable human carcinogen.\cite{5,6} In spite of the fact that the harmful effects of CCl$_4$ are obvious, this compound is still used as a solvent for oils, fats, lacquers, varnishes, rubber waxes and resins and as a starting material in the production of a number of organic compounds.\cite{6,7}

It has been established that trichloromethyl (CCl$_3$) radical and Cl are formed as a result of the metabolic conversion of CCl$_4$ by cytochrome P450, which in turn, initiate lipid peroxidation process.\cite{8-10}

Among horticultural crops, fruits are sources of diverse nutrient and non-nutrient molecules, which display antioxidant properties,\cite{11} and can protect the human body against oxidant damage.

*Cornus mas*, known as the European and Asiatic Cornelian Cherry, has been used for the treatment of diarrhea, intestinal inflammation, fever and malaria.\cite{12} Furthermore, it has been mentioned for the treatment of kidney stones.
kidney treatment and bladder infections in traditional system of medicine in Iran. Chemical characterization of C. mas fruit has shown that it is a rich source of phenolic and antioxidant, anthocyanins and flavonoids compounds.\cite{12,13} Despite the favorable ethnopharmacological properties, its protective effect against CCl\textsubscript{4} nephrotoxicity has not been explored. In the present study, we investigated the effects of C. mas fruit on oxidative stress parameters in CCl\textsubscript{4}-induced nephrotoxicity in rats.

Materials and Methods

Chemicals
Trichloroacetic acid (TCA) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma-Aldrich Chemical Co. Ltd. (USA). Thiobarbituric acid (TBA) and CCl\textsubscript{4} were obtained from Merck Co. (Germany). Assay kits for the estimation of creatinine, urea and acid uric were purchased from Pars Azma (Iran) and all other chemicals used were of analytical grade were obtained from either Sigma-Aldrich or Merck (Germany).

Plant material
C. mas plant and its fruits were authenticated by the Botany Department of Tabriz University, Iran and obtained from suburbs of Arasbaran protected jungle (East Azerbaijan, Iran) at the end of spring 2012. The fruits were air-dried, protected from direct sunlight and powdered. The powder was kept in a closed container at 8°C.

Extraction
A total of 500 g of powder was extracted with a mixture of methanol: water (7:3) at 25°C. The solvent was completely removed by rotary vacuum evaporator at 50°C. C. mas fruit extract (CMFE) was frozen at −20°C until use. The yield of the extract was 50% with reference to dry starting material.

Toxicity study
For toxicity studies, groups of 10 mice were administered (i.g.) the test compounds in the doses 100-1650 mg/kg. The LD50 (LD50 = 1270) was determined using the graphical methods of Litchfield and Wilcoxon.\cite{14} Two different doses were selected to evaluate the dose dependent effect of CMFE on CCl\textsubscript{4}-induced nephrotoxicity.

Furthermore, based on previous studies about evaluation of toxicity effects of CCl\textsubscript{4} like Zargar (2010), desired dose of CCl\textsubscript{4} was selected.

Animals and treatment
Male albino rats of Wistar strain (250-300 g) were purchased from Pasteur Institute (Tehran, Iran). The animals were housed in polypropylene cages in a temperature-controlled room (22 ± 2°C) with relative humidity (44-55%) under 12/12 h light and dark cycles for 1 week before and during the experiments. Animals were provided with a standard rodent pellet diet and clean drinking water ad libitum. Animals were divided into seven groups of six animals each:

- Group I served as a sham for both prophylactic and curative studies and received raw water and free access to food for 16 days
- Group II served as a normal control for both prophylactic and curative studies and received distilled water for 16 days orally and on the 16\textsuperscript{th} day received olive oil (1 ml/kg body weight; i.p.)
- Group III served as a toxic control for both prophylactic and curative studies and received distilled water for 16 days orally and on the 16\textsuperscript{th} day received CCl\textsubscript{4} (1 ml/kg body weight; 80% in olive oil)
- Groups IV and V served as pre-treatment groups (prophylactic). They received CMFE at doses of 300 and 700 mg/kg, orally for 16 days respectively and on the 16\textsuperscript{th} day received CCl\textsubscript{4} (1 ml/kg body weight; 80% in olive oil), 2 h after administration of the last dose of extract
- Groups VI and VII served as post-treatment groups (curative). They received distilled water orally for 16 days and on the 16\textsuperscript{th} day they received CCl\textsubscript{4} (1 ml/kg body weight; 80% in olive oil), followed by CMFE at doses of 300 mg/kg and 700 mg/kg (orally) respectively to Groups VI and VII at 2, 6, 12, 24 and 48 h after CCl\textsubscript{4} intoxication.

Assessment of renal functions
All animals were sacrificed 50 h after CCl\textsubscript{4} administration. Blood samples were collected from left ventricle. Serum was separated by centrifugation at 3000 rpm for 15 min and used for biochemical estimations and was used freshly for the assessment of kidney function tests. The urea, acid uric, creatinine and total protein levels were estimated by standard diagnostic test kits (Pars Azma, Iran).

Preparation of kidney homogenate
Renal tissues were homogenized in KCl (10 mM) phosphate buffer (1.15%) with EDTA: pH 7.4 and centrifuged at 12,000 rpm for 20 min. The supernatant was used for the measurement of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Total protein contents were determined by the method of Lowry et al. (1951),\cite{15} using bovine serum albumin as a standard.

Measurement of lipid peroxidation
Lipid peroxidation was measured by the TBA reaction method.\cite{16} In brief, samples were mixed with TBA reagent consisting of 0.375% TBA and 15% TCA in
Results are expressed as mean±SE. (n=6). *Indicate significance at P<0.05 probability from control group. *Indicate significance at P<0.05 probability from CCl₄ group. CMFE: Cornus mas fruit extract

Table 1: Effect of CMFE on serum profile in rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.66±1.11</td>
<td>0.89±0.06</td>
<td>1.13±0.13</td>
<td>7.85±0.15</td>
<td>3.65±0.06</td>
</tr>
<tr>
<td>Olive oil</td>
<td>59.66±2.23</td>
<td>1.07±0.04</td>
<td>1.35±0.07</td>
<td>7.93±0.18</td>
<td>3.38±0.07</td>
</tr>
<tr>
<td>1 ml/kg CCl₄</td>
<td>1.50±3.76</td>
<td>1.65±0.04</td>
<td>3.61±1.6</td>
<td>5.83±0.23</td>
<td>1.31±0.08</td>
</tr>
<tr>
<td>300 mg/kg CMFE+CCl₄</td>
<td>84.50±3.60</td>
<td>1.24±0.04</td>
<td>1.88±0.02</td>
<td>7.10±0.11</td>
<td>2.20±0.09</td>
</tr>
<tr>
<td>700 mg/kg CMFE+CCl₄</td>
<td>94.33±3.06</td>
<td>1.09±0.03</td>
<td>1.88±0.17</td>
<td>7.25±0.14</td>
<td>3.10±0.09</td>
</tr>
<tr>
<td>CCl₄+300 mg/kg CMFE</td>
<td>67.50±2.99</td>
<td>1.33±0.05</td>
<td>1.78±0.14</td>
<td>7.71±0.15</td>
<td>2.85±0.12</td>
</tr>
<tr>
<td>CCl₄+700 mg/kg CMFE</td>
<td>69.83±4.04</td>
<td>1.02±0.05</td>
<td>1.76±0.13</td>
<td>7.91±0.16</td>
<td>3.20±0.09</td>
</tr>
</tbody>
</table>
Discussion

The present study demonstrated the protective potential of CMFE on CCl₄ induced nephrotoxicity. CCl₄ has been used in rat experimental models to investigate the oxidative stress induced in various organs. To the best of our knowledge, this is the first study to evaluate these effects of CMFE in an attempt to prevent kidney damage from CCl₄.

The mechanism of CCl₄ hepatotoxicity is well documented in the rat model. CCl₄ intoxication generates free radicals that trigger a cascade of events resulting in organ toxicity in rats. It is well-known that the kidneys play a pivotal role in the regulation of various chemicals. CCl₄, a nephrotoxin, was used for the purpose of inducing renal damage in this study since it has previously been shown to exert its toxic effects on the kidney. According to previous reports, CCl₄-induced toxicity is due to the conversion of CCl₄ to CCl₃⁺ and CCl₃O₂⁻ by the liver cytochrome P450 enzyme. These highly reactive free radicals cause cell damage.

Elevations in the serum concentrations of urea and creatinine as seen here are indicative of renal injury.

Table 2: Effect of CMFE on renal antioxidant enzymes activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.69±0.77</td>
<td>10.91±0.49</td>
<td>62.19±1.64</td>
</tr>
<tr>
<td>Olive oil</td>
<td>29.73±0.77</td>
<td>9.91±0.34</td>
<td>58.25±1.79</td>
</tr>
<tr>
<td>1 ml/kg CCl₄</td>
<td>19.54±0.39</td>
<td>4.46±0.24</td>
<td>27.33±1.74</td>
</tr>
<tr>
<td>300 mg/kg CMFE+CCl₄</td>
<td>22.35±0.47</td>
<td>8.90±0.33</td>
<td>39.20±1.10</td>
</tr>
<tr>
<td>700 mg/kg CMFE+CCl₄</td>
<td>23.22±0.35</td>
<td>9.60±0.22</td>
<td>42.36±0.95</td>
</tr>
<tr>
<td>CCl₄+300 mg/kg CMFE</td>
<td>25.61±0.36</td>
<td>10.35±0.45</td>
<td>51.67±1.64</td>
</tr>
<tr>
<td>CCl₄+700 mg/kg CMFE</td>
<td>24.30±0.36</td>
<td>9.49±0.23</td>
<td>47.14±1.64</td>
</tr>
<tr>
<td>CCl₄+300 mg/kg CMFE+CMFE</td>
<td>25.61±0.36</td>
<td>10.35±0.45</td>
<td>51.67±1.64</td>
</tr>
<tr>
<td>CCl₄+700 mg/kg CMFE+CMFE</td>
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<td>47.14±1.64</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SE. (n=6). *Indicate significance at P<0.05 probability from control group. +Indicate significance at P<0.05 probability from CCl₄ group. CMFE: Cornus mas fruit extract

The lipid peroxidation is an autocatalytic process and common consequence of cell death. It causes tissue damage during inflammation, cancer and aging. Lipid peroxidation is reported to be a major causes of CCl₄-induced nephrotoxicity, mediated by the production of free radical derivatives of CCl₄. The renal MDA content, which is one of the end products of lipid peroxidation in the renal tissue, is used as an important indicator of CCl₄-induced oxidative stress. In the present study, which was accompanied by histological changes.

In addition, reduced level of serum albumin and protein in CCl₄-treated rats might have resulted from leakage in glomeruli and tubules. Similar results have been shown by Khan et al. The present study revealed that the treatment of CMFE to CCl₄-administrated rats ameliorated the toxic affect of CCl₄. Results obtained in this study are in agreement with earlier findings.

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administration of CCl₄ resulted in significant elevation in MDA concentration [Figure 1] indicating elevation of lipid peroxidation along with histopathological injury [Figure 2]. Interestingly, treatment by CMFE markedly the MDA concentration.

Although there are numerous studies demonstrating that CCl₄ leads to increase in MDA levels in various tissues,[27,28] a limited number of studies have investigated the effects of CMFE. In addition, no published data has ever demonstrated the influence of CMFE on CCl₄-induced elevation in renal lipid peroxide levels.

It has been suggested that a decrease in the activities of primary antioxidant; CAT, SOD and GPx may be due accumulation of ROS. An observation that strengthens this hypothesis is that SOD activity can be inhibited by hydrogen peroxide treatment.[29] The inhibition of antioxidant system may lead to accumulation of H₂O₂ or products of its decomposition may also be aided by a decrease in CAT, SOD and GPx activities.[2,30] Measurement of these antioxidant enzymes is an appropriate indirect way to assess the pro-oxidant antioxidant status in tissues.[30,31] The level of antioxidant enzymes such as SOD, CAT and GPx decreased in CCl₄-treated group, and improved by treatment with CMFE. Results obtained in this study suggest the protective effects of CMFE against the CCl₄-induced nephrotoxicity, could be attributed to its high level of phenol[32] and other antioxidants.[33-37] These compounds could scavenge the free radicals of CCl₄ generated through P450 enzyme system thereby diminished the oxidative injuries.

In this study, the kidneys of CCl₄-treated rats have shown severe morphological abnormalities in the glomerular and tubular compartments. These changes were not observed in the groups treated with CMFE, that suggesting the protective effects of CMFE in attenuating CCl₄-induced morphological changes.

**Conclusion**

The present study suggests the antioxidant potential of CMFE against the toxic effects of CCl₄ in the kidney of rats. Research is needed about each of these components against CCl₄ induced nephrotoxicity.

**References**


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