Regeneration of Coenzyme Q₉ Redox State and Inhibition of Oxidative Stress by Rooibos Tea (*Aspalathus linearis*) Administration in Carbon Tetrachloride Liver Damage

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Summary
The effect of rooibos tea (*Aspalathus linearis*) on liver antioxidant status and oxidative stress was investigated in rat model of carbon tetrachloride-induced liver damage. Synthetic antioxidant N-acetyl-L-cysteine (NAC) was used for comparison. Administration of carbon tetrachloride (CCl₄) for 10 weeks decreased liver concentrations of reduced and oxidized forms of coenzyme Q₉ (CoQ₉H₂ and CoQ₉), reduced α-tocopherol content and simultaneously increased the formation of malondialdehyde (MDA) as indicator of lipid peroxidation. Rooibos tea and NAC administered to CCl₄-damaged rats restored liver concentrations of CoQ₉H₂ and α-tocopherol and inhibited the formation of MDA, all to the values comparable with healthy animals. Rooibos tea did not counteract the decrease in CoQ₉, whereas NAC was able to do it. Improved regeneration of coenzyme Q₉ redox state and inhibition of oxidative stress in CCl₄-damaged livers may explain the beneficial effect of antioxidant therapy. Therefore, the consumption of rooibos tea as a rich source of natural antioxidants could be recommended as a market available, safe and effective hepatoprotector in patients with liver diseases.

Key words
CCl₄-liver damage • Oxidative stress • Rooibos tea • Coenzyme Q₉ • α-tocopherol

Introduction
Increasing evidence indicates the role of oxidative stress in liver injury, cirrhosis development and carcinogenesis (Yamamoto *et al.* 1998, Yamamoto and Yamashita 1999, Stal and Olsson 2000). Chronic administration of carbon tetrachloride (CCl₄) is widely used as an animal model of liver damage caused by formation of trichloromethyl and trichloromethylperoxyl radicals, initiating lipoperoxidation and resulting in
CCL4-induced liver damage in rats in which blood 2003) we described a beneficial effect of rooibos tea on experimental models of CCl4-induced cell necrosis (Murrel et al. 1997, Kadiiska et al. 2000). Of course, some other hepatotoxic agents have also been used (Ferenčíková et al. 2003). It has been shown that oxidative stress can modulate fibroblast and hepatic stellate cells proliferation (Murrel et al. 1990, Lee et al. 1995) and collagen synthesis (Parola et al. 1993). Participation of defense antioxidant systems in liver protection under the conditions of oxidative stress has been confirmed, however, the exact pathogenetic and protective mechanisms have not been fully explained. Coenzyme Q (ubiquinone) besides its bioenergetic function in mitochondrial respiratory chain is a powerful lipid-soluble antioxidant synthesized in the liver (Littarru et al. 1994, Ernster and Dallner 1995, Rauchová et al. 1995). Dominant form of coenzyme Q in rats is coenzyme Q0 and about 70 % of the total coenzyme Q in the liver is kept in reduced form (ubiquinol) by the activities of enzymatic systems (Takahashi et al. 1993, Kishi et al. 1997). The reduced form of coenzyme Q exerts its antioxidant function either directly on superoxide radicals or indirectly on lipid radicals, both alone and in cooperation with α-tocopherol (Kagan et al. 2000). Changes in endogenous coenzyme Q concentrations have been found in patients and experimental animals under conditions of oxidative stress (Kucharská et al. 1998, Gvozdjáková and Kucharská, 2000, Štefek et al. 2000). Protective effects of vitamin E and coenzyme Q administration found in experimental animals under conditions of oxidative stress (Parola et al. 1992, Naziroglu et al. 1999, Canturk et al. 1999).

Research of natural hepatoprotective compounds has become attractive in recent years. Beneficial effects of rooibos tea (Aspalathus linearis), indigenous to South Africa, based on its antioxidant activities, have been reported (Lamošová et al. 1997, Simon et al. 2000, Standley et al. 2001). In our previous paper (Uličná et al. 2003) we described a beneficial effect of rooibos tea on CCl4-induced liver damage in rats in which blood biochemical parameters and histological examination of the liver tissue confirmed its hepatoprotective effect.

In this study, we tested whether rooibos tea as a rich source of natural antioxidant compounds could affect liver antioxidant capacity, the redox state of coenzyme Q and oxidative stress in a rat model of carbon tetrachloride liver damage. The effect of rooibos tea administration has been compared with a known synthetic antioxidant N-acetyl-L-cysteine (NAC).

**Methods**

Male Wistar rats weighing 210-280 g were used in our experiment. Animals were divided into four groups, each of 10 rats.

**Control group (C):** Animals were injected intraperitoneally with olive oil (1 mg/kg) twice a week for 10 weeks and received daily 5 mg/kg of water orally by gastric tube.

**Carbon tetrachloride (CCl4) group:** Animals were injected intraperitoneally with 50 % CCl4 in olive oil (1 ml/kg) twice a week for 10 weeks and received water orally as control group.

**CCl4 + rooibos tea (CCl4 + RT) group:** Instead of tap water the animals drank rooibos tea (Aspalathus linearis) freshly prepared by boiling of 2.5 g of dry tea in 1 l of water for 10 min, starting 7 days before CCl4 administration and they also were given 5 ml/kg of rooibos tea once a day by gastric tube.

**CCl4 + N-acetyl-L-cysteine (CCl4 + NAC) group:** Animals received NAC 150 mg/kg in solution orally by gastric tube, starting 7 days before CCl4 administration.

The rats had free access to standard Larsen pellet food and tap water. All experiments were carried out according to guidelines for the care and use of experimental animals and approved by the State Veterinary Administration of the Slovak Republic.

The rats were anesthetized with thiopental 48 h after the last treatment with CCl4 and samples of the liver tissue were taken for biochemical analyses. Concentrations of oxidized and reduced forms of coenzyme Q0 (CoQ0 and CoQ0H2) and α-tocopherol were determined by high-performance liquid chromatography (HPLC - LKB, Sweden) according to Lang et al. (1986) with some modifications as follows. The liver tissue (50-100 mg) was homogenized using of Ultra-Turrax in water with an addition of t-butylhydroxytoluene and sodium dodecyl sulphate and extracted twice by mixture of hexane-ethanol (5/2, v/v, Merck). Collected organic layers were evaporated under nitrogen and the residue taken up in ethanol was injected on the column SGX C18 (7 µm, Tessek, Czech Republic). The mobile phase consisted of methanol-acetonitrile-ethanol (6/2/2, v/v/v, Merck). Concentrations of compounds were detected spectrophotometrically at 275 nm using external standards (Sigma). Reduced coenzyme Q0 standard was prepared by reduction of CoQ0 with sodium dithionite. Malondialdehyde (MDA) in liver tissue was determined...
by HPLC (Pilz et al. 2000), cholesterol in liver tissue was
determined according to Abell et al. (1952) and
triacylglycerols according to Jover (1963).
The results were evaluated using Student’s t-test
for unpaired data and linear regression analysis with
Pearson’s correlation coefficient. Values of p<0.05 were
considered statistically significant.

Table 1. Liver concentrations of α-tocopherol, oxidized and reduced coenzyme Q9 (CoQ9 and CoQ9H2), redox state of CoQ9 and malondialdehyde (MDA) concentration in CCl4-damaged rats and the effects of rooibos tea (RT) and N-acetyl-L-cysteine (NAC).

<table>
<thead>
<tr>
<th>Liver</th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+RT</th>
<th>CCl4+NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=10</td>
<td>n=10</td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>32.5±1.57</td>
<td>18.5±0.81</td>
<td>26.9±4.13</td>
<td>33.1±4.62</td>
</tr>
<tr>
<td>(nmol/g ww)</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.05</td>
<td>p&lt;0.002</td>
<td></td>
</tr>
<tr>
<td>CoQ9</td>
<td>71.0±5.16</td>
<td>58.2±4.03</td>
<td>60.7±4.85</td>
<td>78.8±4.09</td>
</tr>
<tr>
<td>(nmol/g ww)</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td></td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>CoQ9H2</td>
<td>132.8±12.5</td>
<td>40.8±6.34</td>
<td>155.0±16.1</td>
<td>131.1±19.7</td>
</tr>
<tr>
<td>(nmol/g ww)</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td></td>
<td>p=0.0002</td>
</tr>
<tr>
<td>CoQ9H2/total CoQ9 (%)</td>
<td>65.2</td>
<td>41.2</td>
<td>71.9</td>
<td>62.5</td>
</tr>
<tr>
<td>MDA</td>
<td>99.8±7.79</td>
<td>152.2±12.1</td>
<td>80.8±6.41</td>
<td>104.3±8.81</td>
</tr>
<tr>
<td>(nmol/g ww)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td></td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Data are means ± S.E.M., CCl4 is compared to controls, CCl4 + RT and CCl4 + NAC are compared to CCl4.

Table 2. Liver concentrations of total cholesterol (Chol) and triacylglycerols (TAG) in CCl4-damaged rats and treated with rooibos tea (RT) and N-acetyl-L-cysteine (NAC).

<table>
<thead>
<tr>
<th>Liver</th>
<th>Control</th>
<th>CCl4</th>
<th>CCl4+RT</th>
<th>CCl4+NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=10</td>
<td>n=10</td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>Chol</td>
<td>5.19±0.21</td>
<td>10.4±0.21</td>
<td>10.7±0.53</td>
<td>8.97±0.48</td>
</tr>
<tr>
<td>(nmol/g)</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>TAG</td>
<td>7.56±0.74</td>
<td>39.4±2.59</td>
<td>32.9±1.98</td>
<td>25.3±1.86</td>
</tr>
<tr>
<td>(nmol/g)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.05</td>
<td></td>
<td>p=0.0001</td>
</tr>
</tbody>
</table>

Data are means ± S.E.M., CCl4 is compared to controls, CCl4 + RT and CCl4 + NAC are compared to CCl4.

Results

The administration of carbon tetrachloride to
experimental rats for 10 weeks developed significant
changes in liver concentrations of lipophilic antioxidants
and malondialdehyde (MDA) formation as a marker of
lipid peroxidation (Table 1). Concentrations of reduced
coenzyme Q9 (CoQ9H2) and α-tocopherol were reduced
highly significantly (by 69 % and 43 %, respectively),
whereas oxidized coenzyme Q9 (CoQ9) decreased by
18 % (p<0.05). Treatment with rooibos tea (RT)
increased antioxidant status in CCl4-damaged livers.
Concentrations of α-tocopherol and CoQ9H2 increased to
the values comparable with healthy animals. Similar
effects on α-tocopherol and CoQ9H2 concentrations were
found when N-acetyl-L-cystein (NAC) was administered.
Rooibos tea did not counteract the decrease of oxidized
CoQ9, whereas NAC was able to do so. Malondialdehyde
formation in the liver was suppressed significantly both
with RT and NAC administration. Redox state of CoQ9,
expressed in percentage of reduced CoQ9 from total CoQ9,
was restored with RT and NAC treatment in CCl4-treated
rats. CCl4 treatment of rats caused a severe increase of
hepatic total cholesterol and triacylglycerols (Table 2).
Rooibos tea did not affect cholesterol concentration, but
decreased triacylglycerols concentration. N-acetyl-L-
cysteine lowered both cholesterol and triacylglycerols. Standardized concentration was used for expressing the ratio of the measured concentration of lipophilic antioxidant to cholesterol and triacylglycerol concentrations. In CCl₄-treated rats, these ratios were decreased (Table 3). Similarly to the absolute antioxidant concentrations, lipid-standardized concentrations of α-tocopherol and reduced CoQ₉ also increased with RT and NAC treatment. Rooibos tea did not counteract the decrease of oxidized CoQ₉, whereas NAC did. Concentrations of CoQ₉H₂ correlated inversely with MDA significantly in control (r = −0.70, p = 0.05) and non-significantly in RT-treated rats (r = −0.54, NS). Non-significant positive correlations were found in CCl₄-damaged livers (r = 0.58, p = 0.08, NS) and with NAC treatment (r = 0.55, p = 0.162) (Fig. 1).

Table 3. Cholesterol- and triacylglycerol-standardized liver concentrations of lipophilic antioxidants in CCl₄-damaged rats and the effects of rooibos tea (RT) and N-acetyl-L-cysteine (NAC).

<table>
<thead>
<tr>
<th>Liver</th>
<th>Control n=10</th>
<th>CCl₄ n=10</th>
<th>CCl₄+RT n=10</th>
<th>CCl₄+NAC n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol/Chol</td>
<td>6.38±0.44</td>
<td>1.77±0.07</td>
<td>2.61±0.39</td>
<td>3.89±0.72</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.05</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>α-tocopherol/TAG</td>
<td>5.08±0.71</td>
<td>0.48±0.03</td>
<td>0.81±0.11</td>
<td>1.28±0.15</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.02</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>CoQ₉/Chol</td>
<td>13.90±1.24</td>
<td>6.12±0.61</td>
<td>6.00±0.55</td>
<td>9.76±1.03</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>CoQ₉/TAG</td>
<td>8.20±0.65</td>
<td>1.75±0.25</td>
<td>1.90±0.17</td>
<td>3.54±0.48</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>NS</td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>CoQ₉H₂/Chol</td>
<td>25.5±2.73</td>
<td>3.96±0.65</td>
<td>15.0±1.91</td>
<td>15.3±2.51</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0002</td>
<td></td>
</tr>
<tr>
<td>CoQ₉H₂/TAG</td>
<td>20.4±3.20</td>
<td>1.08±0.19</td>
<td>4.80±0.60</td>
<td>5.26±0.83</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± S.E.M., CCl₄ is compared to controls, CCl₄ + RT and CCl₄ + NAC are compared to CCl₄.

Fig. 1. Correlations between liver concentrations of reduced coenzyme Q₉ (CoQ₉H₂) and malondialdehyde (MDA) in CCl₄-damaged rats and treated with rooibos tea (RT) and N-acetyl-L-cysteine (NAC). ♦ Control; ● CCl₄; ▲ CCl₄+RT; ■ CCl₄+NAC

Discussion

Chronic administration of carbon tetrachloride provides a suitable animal model for free radical damage in liver. CCl₄ is metabolically activated by cytochrome P450 into a trichloromethyl radical which is in the presence of oxygen converted into a peroxyl radical (Recknagel et al. 1989). As reported previously, under
our experimental conditions, CCl₄ treatment caused a severe increase of hepatic triacylglycerols, total cholesterol and plasma activities of aminotransferases as well as a decrease of albumin concentration as markers of liver function. Malondialdehyde formation, as marker of lipid peroxidation, increased significantly. In these livers, pathological changes developed, i.e. steatosis and fibrosis, which were confirmed histomorphologically and histometrically (Uličná et al. 2003).

The role of coenzyme Q in liver diseases has been investigated in few clinical and experimental studies. Bianchi et al. (1994) and Eaton et al. (2000) found a reduction in plasma coenzyme Q₁₀ levels in patients with liver cirrhosis and in chronic alcohol abusers. Yamamoto and Yamashita (1999) and Kontusch et al. (1999) observed a lower percentage of plasma ubiquinol-10 (reduced form of coenzyme Q₁₀) in patients with liver diseases which may reflect its diminished reduction by the liver.

Kadiiska et al. (2000) measured several plasma antioxidants in an animal model after a single dose of CCl₄. Concentrations of total coenzyme Q (oxidized and reduced CoQ₉ + CoQ₁₀) and α-tocopherol decreased and consumption of total coenzyme Q was more sensitive to CCl₄ exposure than that of α-tocopherol. Ratio of oxidized to reduced glutathione decreased, suggesting an early oxidative stress. Kishi et al. (1997) published decreased concentrations of reduced coenzyme Q₉ plus Q₁₀, α-tocopherol, ascorbic acid and reduced glutathione in rat livers after CCl₄ injection. Stal and Olson (2000) exposed rats to the combination of diethylnitrosamine as initiator, followed by repeated injections of carbon tetrachloride to induce liver cirrhosis. Forty-eight hours after CCl₄ administration, the content of hepatic α-tocopherol was increased and after 23 weeks of CCl₄ administration once a week, both reduced and oxidized forms of CoQ₉ were enhanced. The authors explain these results as an adaptation of the liver to toxic chemicals. In our experimental model of liver damage induced by CCl₄ administration for 10 weeks, liver concentrations of coenzyme Q₉ and α-tocopherol were significantly reduced. Redox state of coenzyme Q expressed as percentage of reduced CoQ₉ from total CoQ₉ decreased from 65 % in control rats to 41 % in CCl₄-treated rats and simultaneously increased MDA formation by 52 %. These findings support the presence of oxidative stress in CCl₄-damaged livers and its consequences on antioxidant status. We found higher utilization of CoQ₉H₂ than of α-tocopherol. This is in agreement with theories of Kishi et al. (1997) and Thomas and Stocker (2000) that CoQ₉H₂ is the first lipophilic antioxidant utilized under conditions of oxidative stress.

The administration of rooibos tea and N-acetyl-cysteine prevented the decline in liver concentrations of CoQ₉H₂ in CCl₄-treated rats (concentrations increased by 280 % with RT and 221 % with NAC, respectively). Concentration of α-tocopherol was increased with RT by 45 % and with NAC by 79 % to the values comparable with healthy animals. Both RT and NAC prevented the increased formation of malondialdehyde in CCl₄-damaged livers. In spite of little differences in liver antioxidant state of the rats treated with rooibos tea or NAC, we found a negative correlation between CoQ₉H₂ and MDA in RT-treated rats only. Although this correlation was not significant, it was similar as in control rats. It seems that sufficient antioxidant protection is important for the prevention of liver damage but treatment with natural antioxidants may be more advantageous to the liver protection. Improved regeneration of coenzyme Q redox state may explain the beneficial effect of antioxidant therapy. Therefore, the consumption of rooibos tea as a rich source of natural antioxidants could be advised as a market available, safe and effective hepatoprotector in patients with liver diseases.

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References


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