Relation between Antioxidants and Pollution on Experimental Animals

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Abstract

Objective: The aim of the present work is to study the protective effect of some antioxidants and trace elements against the hazardous effects of carbon tetrachloride (CCl₄) on hamsters for the possibility of further application on humans.

Methods: One hundred twenty hamsters weighing 104-128g were divided into 13 groups as follows: 1-Negative Control group fed standard diet, 2-positive control group given carbon tetrachloride (CCl₄) only, 3-CCl₄ + zinc, 4 - CCl₄ + β-carotene, 5- CCl₄ + α-tocopherol, 6- CCl₄ + selenium, 7-CCl₄ + vitamin C, 8- CCl₄ + zinc + β-carotene, 9- CCl₄ + zinc + vitamin C, 10- CCl₄ + selenium + α-tocopheral, 11- CCl₄ + selenium + β-carotene, 12- CCl₄ + selenium + vitamin C and 13- CCl₄ + selenium + α-tocopheral.

Results: Carbon tetrachloride (CCl₄) has a direct toxic effect on liver and kidney. Depending on biochemical results, the more antioxidants of liver protection against CCl₄ toxicity are α-tocopherol, followed by selenium, selenium + β-carotene, vitamin C, zinc + β-carotene and selenium + α-tocopherol. The more antioxidant for kidney protection against CCl₄ toxicity is β-carotene followed by selenium, zinc + β-carotene, zinc + α-tocopherol, and zinc + vitamin C. Conclusion: Trace elements should not be given individually especially zinc or selenium as deleterious effects, in spite of their protective effect.

Key words: Carbon tetrachloride-Antioxidants

Introduction

Antioxidants are organic components, they act in the body as scavengers intercepting reactive molecules called free radicals before they have a chance to damage cells (Wu, 1997). The general strategy for prevention and treatment of liver damage includes reducing the production of reactive metabolites by using antioxidants (Wu et al., 1999; Bansal et al., 2005). Free radicals are highly reactive and attack membranes or lipoproteins, start lipid peroxidation, damage proteins and cause DNA mutation. Free radicals are result of pollution, aging, malnutrition, radiation and oxidation. Oxidation is triggered by environmental pollutants as well as by individual own metabolism (Halliwell, 1997). Trace elements are micronutrients. They include: Zinc (Zn), which is essential constituent of superoxide dismutase and of glutathione peroxidase. Both enzymes protect tissues against oxidative stress requiring these trace elements for their catalysis (Hirayama et al., 1993). Pollution is one of the major problems the world faces nowadays. Pollution of indoor air, water, soil, clay, groundwater, warm ocean surface water and shallow wells is caused by dangerous gases including carbon tetrachloride (CCl₄) resulting from factories, industries, waste water, pesticides used in agriculture and radioactive waste management (Kuo and Hines, 1988). Recently the rapid and large industrial development in Egypt has lead to new and great pollution potential hence there are large amounts of air pollutants including CCl₄ and benzene. However, the evaluation of public awareness towards environmental pollution side by side with the governmental efforts to put the restricting laws for environmental protection necessitate great care with pollution control measures (Mahrus, 1997).
Carbon tetrachloride is highly toxic. Its toxicity is due to LPO and the generation of reactive oxygen species (ROS) (Pérez et al., 2003). It has been successfully used as a model of liver injury in experimental animals. Similarities have been shown between CCl₄ induced liver cirrhosis in experimental animals and human liver cirrhosis (Tamayo, 1983, Ariosto et al., 1989 and Natargian et al., 2006). Also similarities have been shown between hepatorenal syndrome in cirrhotic patients and renal affection in CCl₄ cirrhotic rats (Rincon et al., 1999).

Material and Methods

Materials
The chemicals and vitamins constituted the materials of the study were: Carbon tetrachloride (BDH), zinc chloride (Zn Cl) as a source of zinc, β-Carotene, vitamin C, vitamin E (α-tocopherol acetate as (oil)), sodium selenite (Na₂SeO₃) as a source of selenium (all were in powder form).

Biological Investigations
Diets
Standard diet was prepared to give the efficiency for normal growth and maintenance of experimental animals. The protein used in the present study was provided as casein which was evaluated to contain only 80% protein, so normal amount represented about 20% of the diet according to Reeves(1993) on the expense of starch. Fats were in the form of sunflower oil which supply as well the required quantity of vitamin E. The chemical materials were added to the experimental diet with different concentrations as shown in (Table 1).

Table (1): Experimental Diet / 100 g

<table>
<thead>
<tr>
<th>Composition of diet ingredient</th>
<th>-ve control</th>
<th>+ve control</th>
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<td>Sunflower oil</td>
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<td>Salt mixt.</td>
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<td>CCl₄ ml</td>
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<td>Vit. E mg</td>
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<td>Vit. C mg</td>
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</table>
**Animals**

Six weeks old male golden hamsters weighing about 104-128 g were obtained from Teodor Bilharz Institute, El-Waraak, Giza. The animals were kept in wire cages with wire bottoms. The cages were suspended above a plate made of galvanized metal and there is a suitable space between cages and plates to receive faeces and urine from cages. The diet was introduced to the animals in special food cups that kept food spilling to a minimum; also water was provided to the hamsters.

**Experimental Design**

One hundred and twenty growing male golden hamsters, six weeks old weighing about 104-128 g were obtained from Teodor Bilharz Institute, El-Waraak, Giza. The animals were placed in wire cages with wire bottoms. The first group fed standard diet without any chemical additives (negative control), second group fed experimental diet + CCl₄ (positive control), third group fed experimental diet + CCl₄ + Zn, fourth group fed experimental diet + CCl₄ + β-carotene, fifth group fed experimental diet + CCl₄ + α-tocopherol (vit.E), sixth group fed experimental diet + CCl₄ + Se, seventh group fed experimental diet + CCl₄ + vit.C, eighth group fed experimental diet + CCl₄ + Zn + β-carotene, ninth group fed experimental diet + CCl₄ + Zn + vit.C, tenth group fed experimental diet + CCl₄ + Zn + α-tocopherol, eleventh group fed experimental diet + CCl₄ + Se + β-carotene, twelfth group fed experimental diet + CCl₄ + Se + α-tocopherol, and thirteenth group fed experimental diet + CCl₄ + selenium + vit.C. The animals were divided into 13 groups, each containing 9 hamsters except group 2 (contains 12 hamsters). The animals were placed in individual cages for 6 weeks, food and water were provided *ad libitum* and checked daily.

The first group fed standard diet without any chemical additives (negative control), second group fed experimental diet + CCl₄ (positive control), third group fed experimental diet + CCl₄ + Zn, fourth group fed experimental diet + CCl₄ + β-carotene, fifth group fed experimental diet + CCl₄ + α-tocopherol (vit.E), sixth group fed experimental diet + CCl₄ + Se, seventh group fed experimental diet + CCl₄ + vit.C, eighth group fed experimental diet + CCl₄ + Zn + β-carotene, ninth group fed experimental diet + CCl₄ + Zn + α-tocopherol, tenth group fed experimental diet + CCl₄ + Zn + vit.C, eleventh group fed experimental diet + CCl₄ + Se + β-carotene, twelfth group fed experimental diet + CCl₄ + Se + α-tocopherol, and thirteenth group fed experimental diet + CCl₄ + selenium + vit.C. The animals were divided into 13 groups, each containing 9 hamsters except group 2 (contains 12 hamsters). The animals were placed in individual cages for 6 weeks, food and water were provided *ad libitum* and checked daily.

**Biochemical Methods**

Biochemical Colorimetric measurements were used (the Bio Kits and Spectrophotometer (SPEKOL, 11). Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) were determined by method of Bergmeyer, and Horder (1980), and serum alkaline phosphatase (ALP) was determined by kinetic procedure kits according to the method of John (1982). Serum Total protein was determined by the method of (Doumas 1975). Serum albumin was determined according to method of (Doumas et al., 1971). Serum globulin concentration was estimated by the difference of serum total protein and serum albumin, blood urea nitrogen in serum (BUN) was determined by method of (Tabacco et al., 1979), serum creatinine was determined by method of (Larsen, 1972), serum uric acid was determined according to method of (Fossati et al., 1980).

**Statistical Analysis**

The data were subjected to statistical analysis according to the method of (Sokal and Rohif, 1981). Mean value, standard error, F-test and least significant difference (L.S.D) were calculated.

**Results**

The results indicated that there is a highly significant increase (p ≤ 0.01) in serum ALT, and AST as shown in (Table 2) in positive control group when compared with negative control group. Also, there is non significant decrease in serum ALT and AST between (CCl₄ + β-carotene); (CCl₄ + α-tocopherol) and (CCl₄ + vitamin C) groups when compared to positive control group. The more non significant decrease in ALT is found in (CCl₄ + α-tocopherol) group followed by (CCl₄ + vitamin C) group then (CCl₄ + β-carotene) group (Table 2). The increase in serum ALP in positive controls is significant (p<0.005) (Table 2) when compared to negative control. While, there is slight decrease in sALP in (CCl₄ + B-carotene), (CCl₄ + α-tocopherol) and (CCl₄ + vitamin C) groups when compared to positive controls.

There is non significant increase in total serum protein and globulin (Table 2) in positive control group when compared to negative control group. But, there is non significant decrease in serum total protein and globulin of (CCl₄ + β-carotene) group when compared to positive control group.

There is a significant increase (p < 0.01) in serum blood urea nitrogen (BUN) and creatinine (Table 2) in positive control group when compared to negative control group accompanied with non significant decrease in serum BUN and serum creatinine in (CCl₄ + α-tocopherol) and (CCl₄ + vitamin C) groups when compared to positive control group. But there is non significant
decrease in serum BUN and significant decrease (P<0.05) in serum creatinine in (CCl4+β-carotene) group, compared to positive control group. In addition, there is a significant decrease (P<0.01) in serum uric acid (Table 2) in positive control group when compared to negative control group, but there is a significant increase (p<0.01) in serum uric acid in (CCl4 +β-carotene) group; (CCl4 + α-tocopherol) group and (CCl4 + vitamin C) group when compared to positive controls. The (CCl4 +B-carotene) group induced the highest increase when compared to positive controls.

Regarding the minerals groups the increase in serum sALT and sAST (Table 3) in (CCl4 + Zn) group is non significant but the increase in sALP, is significant (P<0.01) when compared to positive controls. In (CCl4 +Se) group, there is non significant decrease of sALT and sALP. Also, in (CCl4 + Se) group, there is non significant decrease in sATL and no significant change in sAST but significant decrease (P<0.01) of sALP was recorded when compared to (CCl4 + Zn) group. There is non significant increase in serum total protein and non significant change of serum albumin and significant increase (P<0.05) of serum globulin (Table 3) when compared to positive control in (CCl4 +Zn) group, but, in (CCl4 +Se) group, there is non significant increase in serum total proteins, and serum total globulin, no significant change in serum albumin when compared to positive control group. There is no significant change in serum total protein, globulin and serum albumin in (CCl4 +Se) group when compared to (CCl4 +Zn) group, suggesting beneficial effect of Selenium. The decrease in serum BUN and creatinine in (CCl4 +Zn) group is non significant (Table 3) when compared to positive controls suggesting non significant slight effect of Zn on kidney function tests.

In (CCl4 +Se) group, there is a significant decrease in serum BUN (P<0.01) and serum creatinine (P<0.05) when compared to positive controls. (CCl4 + Se) group shows non significant decrease of serum BUN and serum creatinine when compared to (CCl4 + Zn) group. While, there is a significant increase (p<0.05) in serum uric acid in (CCl4 + Zn) group when compared to (CCl4 +Se) group. There is a significant decrease in (CCl4 +β-carotene) in serum creatinine (P<0.05) and a significant increase in serum uric acid (P<0.01) (Table 4). There is non significant decrease sALT, sAST, sALP, serum total protein, albumin, globulin, and BUN (Table 4) when compared to positive control group. CCl4 + β-carotene + Zn group shows a significant decrease of serum albumin, BUN (P<0.01), in creatinine (P<0.05). There is a significant increase in serum uric acid (P<0.05) but non significant increase in sALT and serum total globulin. There is non significant decrease of sALT, serum total protein, and non significant change of sALP when compared to positive control group (Table 4).

CCl4 + β-carotene + Se shows a significant decrease (P<0.05) in serum total globulin, there is also a significant increase (P<0.01) in serum uric acid, but there is no significant decrease of sALT, sAST, sALP, serum total protein, BUN, creatinine and also non significant increase in serum albumin when compared to positive control group (Table 4). In (CCl4 +α-tocopherol) group there is a significant increase (P<0.01) in serum uric acid (Table 5) and no significant decrease in sALT, sAST, sALP, serum total protein, total globulin, BUN, creatinine and non significant change in serum albumin (Table 5) when compared to positive control group.

In (CCl4 +α-tocopherol +Zn) group there is a significant increase (P<0.01) of serum uric acid, non significant increase of sALT, total protein, albumin and total globulin, non significant decrease of, sAST, sALP, BUN, and serum creatinine, when compared to positive control group. In (CCl4 +α-tocopherol +Se) group there is a significant increase in serum uric acid (P=0.01), significant decrease of sAST (p<0.05). There is also non significant decrease of sALT, sALP, BUN, creatinine, no significant change of serum total protein, albumin, and total globulin when compared to positive control group.

There is no significant difference between (CCl4 + α tocopherol), (CCl4 +α tocopherol + Zn) and (CCl4 -α tocopherol +Se) groups in all parameters, except that there is significant decrease of sALT (p<0.05) in (CCl4 +α tocopherol), when compared to (CCl4 -α tocopherol +Zn) group. In (CCl4 + Vit.C) group there is a significant increase in serum uric acid (p <0.01), non significant decrease in sALT, sAST, sALP,
serum BUN and Creatinine (Table 6) and non significant change in serum total protein, albumin and globulin when compared to positive control group (Table 6).

In (CCl₄ + Vit.C + Zn) group, there is a significant decrease of BUN, creatinine, but significant increase in uric acid (P<0.01), non significant decrease in AST,ALP, total protein ,albumin and globuline , and non significant increase of ALT when compared to positive control group.

In (CCl₄ + Vit.C + Se ) group there is a significant increase in serum uric acid (p<0.01), non significant decrease in sALT, sAST, sALP, serum total proteins, globuline ,BUN, and creatinine and non significant change in serum albumin when compared to positive control group.

**Table (2):** Effect of β-carotene, α-tocopherol and vitamin C on some liver enzymes activity, total protein, albumun, globulin and kidney functions in serum of male hamsters fed a diet contaminated by carbon tetrachloride 1 ml/100 g diet (Mean±S.E).

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>(1) Negative control</th>
<th>(2) Positive control (CCl₄)</th>
<th>(4) CCl₄ + β-carotene</th>
<th>(5) CCl₄ + α-tocopherol</th>
<th>(7) CCl₄ + Vitamin C</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sALT(U/L)</td>
<td>67.33±3.57</td>
<td>292.54±76.98ᵃ</td>
<td>267.85±96.96ᶜ</td>
<td>142.83±25.15ᶜ</td>
<td>191.25±61.66ᶜ</td>
<td>N.s</td>
<td></td>
</tr>
<tr>
<td>sAST(U/L)</td>
<td>53.77±3.48</td>
<td>269.09±61.70ᵃ</td>
<td>164.00±47.34ᶜ</td>
<td>134.33±31.81ᶜ</td>
<td>215.50±72.46ᶜ</td>
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<tr>
<td>sALP(U/L)</td>
<td>68.22±3.88</td>
<td>109.36±18.80ᵃ</td>
<td>103.42±13.56ᶜ</td>
<td>98.00±12.77ᶜ</td>
<td>101.62±11.79ᶜ</td>
<td>N.s</td>
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</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.30±0.40</td>
<td>7.62±0.40</td>
<td>6.28±0.80</td>
<td>7.35±0.58</td>
<td>7.42±0.59</td>
<td>N.s</td>
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<td>Albumin (g/dL)</td>
<td>3.87±0.23</td>
<td>4.80±0.19ᵃ</td>
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<td>N.s</td>
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<tr>
<td>Total globulin (g/dL)</td>
<td>2.42±0.21</td>
<td>2.82±0.32</td>
<td>2.18±0.40</td>
<td>2.60±0.34</td>
<td>2.67±0.29</td>
<td>N.s</td>
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<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>9.62±0.82</td>
<td>25.46±3.11ᵃ</td>
<td>18.61±2.03ᶜ</td>
<td>19.88±1.17ᶜ</td>
<td>19.63±3.21ᶜ</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>0.66±0.03</td>
<td>1.60±0.31ᵃ</td>
<td>0.90±0.05ᵇ</td>
<td>1.07±0.17ᶜ</td>
<td>1.13±0.15ᶜ</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>4.30±0.32</td>
<td>2.64±0.20ᵃ</td>
<td>5.11±0.56ᵇ</td>
<td>4.78±0.70ᵇ</td>
<td>4.86±0.38ᵇ</td>
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*p<0.05  **p<0.01  N.s=Non significant

ᵃ = Significant ↑ when compared to negative control group.
ᵇ = Significant ↓ when compared to negative control group.
ᵇ’ = Significant ↓ when compared to positive control group.
ᶜ = Non significant ↓ when compared to positive control group.
**Table (3)**: Effect of Zinc and selenium on some liver enzymes activity, total protein, albumin, globulin and kidney functions in serum of male hamsters fed a diet contaminated by carbon tetrachloride 1 ml/100 g diet (Mean ± S.E).

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>(1) Negative control</th>
<th>(2) Positive control (CCl₄)</th>
<th>(3) CCl₄+ Zinc</th>
<th>(6) CCl₄+ Selenium</th>
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<tr>
<td>sALT (U/L)</td>
<td>67.33±3.57</td>
<td>292.54±76.98</td>
<td>297.83±57.67c</td>
<td>219.20±31.65c</td>
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<td>sAST (U/L)</td>
<td>53.77±3.48</td>
<td>269.09±61.70</td>
<td>312.83±31.25c</td>
<td>320.60±67.60c</td>
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<tr>
<td>sALP (U/L)</td>
<td>68.22±3.88</td>
<td>109.36±18.80</td>
<td>371.16±66.46b</td>
<td>69.60±11.60c</td>
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<tr>
<td>Total protein (g / dl)</td>
<td>6.30±0.40</td>
<td>7.62±0.40</td>
<td>8.68±1.14</td>
<td>8.04±0.76</td>
<td>N.S</td>
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<tr>
<td>Albumin (g / dL)</td>
<td>3.87±0.23</td>
<td>4.80±0.19</td>
<td>4.46±0.38</td>
<td>4.48±0.22</td>
<td>N.S</td>
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<tr>
<td>Total globulin (g / dL)</td>
<td>2.42±0.21</td>
<td>2.82±0.32</td>
<td>4.21±0.82b</td>
<td>3.56±0.61</td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>9.62±0.82</td>
<td>25.46±3.11</td>
<td>19.60±2.31c</td>
<td>12.24±1.39b</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.66±0.03</td>
<td>1.60±0.31</td>
<td>0.99±0.08c</td>
<td>0.86±0.05b</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.30±0.32</td>
<td>2.64±0.20</td>
<td>5.31±0.29b</td>
<td>4.28±0.24b</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05  **p<0.01  N.S = Non significant
b = Significant ↑ when compared to positive control group.
b' = Significant ↓ when compared to positive control group.
c = Non significant ↑ when compared to positive control group.
c' = Non significant ↓ when compared to positive control group.

Relation between Antioxidants and….
Table (4) : Effect of β-carotene and its combinations on some liver enzymes activity, total protein, albumin, total globulin and kidney functions in in serum of male hamsters fed a diet contaminated by carbon tetrachloride 1 ml/100 g diet (Mean±S.E).

<table>
<thead>
<tr>
<th>Group (Test)</th>
<th>(1) Negative control</th>
<th>(2) positive control (CCL₄)</th>
<th>(4) (CCL₄+) B-carotene</th>
<th>(8) (CCL₄+) β-Carotene +Zinc</th>
<th>(11) (CCL₄+) β-carotene +Selenim</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sALT(U/L)</td>
<td>67.33±3.57</td>
<td>292.54±76.98</td>
<td>267.85±96.96&lt;sup&gt;C&lt;/sup&gt;</td>
<td>221.85±46.29&lt;sup&gt;C&lt;/sup&gt;</td>
<td>131.20±29.57&lt;sup&gt;C&lt;/sup&gt;</td>
<td>N.s</td>
</tr>
<tr>
<td>sAST(U/L)</td>
<td>53.77±3.48</td>
<td>268.54±61.20</td>
<td>164.00±47.34&lt;sup&gt;C&lt;/sup&gt;</td>
<td>285.85±62.40&lt;sup&gt;C&lt;/sup&gt;</td>
<td>124.40±27.98&lt;sup&gt;C&lt;/sup&gt;</td>
<td>**</td>
</tr>
<tr>
<td>sALP(U/L)</td>
<td>68.22±3.88</td>
<td>109.36±18.80</td>
<td>103.42±13.56&lt;sup&gt;C&lt;/sup&gt;</td>
<td>110.71±25.45&lt;sup&gt;C&lt;/sup&gt;</td>
<td>74.40±9.46&lt;sup&gt;C&lt;/sup&gt;</td>
<td>N.s</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.30±0.40</td>
<td>7.62±0.40</td>
<td>6.28±0.80</td>
<td>6.61±0.36</td>
<td>6.98±0.53</td>
<td>N.s</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.87±0.23</td>
<td>4.80±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.10±0.44</td>
<td>3.55±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.36±0.33</td>
<td>**</td>
</tr>
<tr>
<td>Total globulin (g/dL)</td>
<td>2.42±0.21</td>
<td>2.82±0.32</td>
<td>2.18±0.40</td>
<td>3.05±0.34</td>
<td>1.62±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N.s</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>9.62±0.82</td>
<td>25.46±3.11</td>
<td>18.61±2.03&lt;sup&gt;C&lt;/sup&gt;</td>
<td>15.38±0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.90±4.83&lt;sup&gt;C&lt;/sup&gt;</td>
<td>**</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.66±0.03</td>
<td>1.60±0.31</td>
<td>0.90±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.93±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04±0.28&lt;sup&gt;C&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.30±0.32</td>
<td>2.64±0.20</td>
<td>5.11±0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.54±1.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>**</td>
</tr>
</tbody>
</table>

*p<0.05             **p<0.01          N.s=Non significant
b = Significant ↑ when compared to positive control group.
b'= Significant ↓ when compared to positive control group.
c = Non significant ↑ when compared to positive control group.
c'= Non significant ↓ when compared to positive control group.
Table (5): Effect of α-tocopherol and its combinations on some liver enzymes activity, total protein, albumin, total globulin and kidney functions in serum of male hamsters fed a diet contaminated by carbon tetrachloride 1 ml/100 g diet (Mean± S.E).

<table>
<thead>
<tr>
<th>Group Test</th>
<th>(1) Negative control</th>
<th>(2) positive control [CCl₄]</th>
<th>(5) CCl₄+ α-tocopherol</th>
<th>(10) CCl₄+ α-tocopherol + Zinc</th>
<th>(13) CCl₄+ α-tocopherol + Selenium</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sALT(U/L)</td>
<td>67.33±3.57</td>
<td>292.54±76.98</td>
<td>142.83±25.15 C</td>
<td>351.00±76.01 C</td>
<td>183.40±56.06 C</td>
<td>*</td>
</tr>
<tr>
<td>sAST(U/L)</td>
<td>53.77±3.48</td>
<td>269.09±61.70</td>
<td>134.33±31.81 C</td>
<td>191.25±47.77 C</td>
<td>117.40±32.94 b</td>
<td>*</td>
</tr>
<tr>
<td>sALP(U/L)</td>
<td>68.22±3.88</td>
<td>109.36±18.80</td>
<td>98.00±12.77 C</td>
<td>99.50±17.33 C</td>
<td>72.80±14.91 C</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Total protein (g/dl)
- 6.30±0.40
- 7.62±0.40
- 7.35±0.58
- 8.41±0.78
- 7.80±0.38

Albumin (g/dL)
- 3.87±0.23
- 4.80±0.19
- 4.75±0.35
- 5.31±0.25
- 4.74±0.13

Total globulin (g/dL)
- 2.42±0.21
- 2.82±0.32
- 2.60±0.34
- 3.10±0.59
- 3.06±0.41

Blood urea nitrogen (mg/dL)
- 9.62±0.82
- 25.46±3.11
- 19.88±1.17 C
- 18.77±2.50 C
- 20.32±3.40 C

Creatinine (mg/dL)
- 0.66±0.03
- 1.60±0.31
- 1.07±0.17 C
- 1.19±0.28 C
- 1.26±0.35 C

Uric acid (mg/dL)
- 4.30±0.32
- 2.64±0.20
- 4.78±0.70 b
- 4.55±0.71 b
- 4.54±0.45 b

*p<0.05       **p<0.01       N.s=Non significant
b = Significant ↑ when compared to positive control group.
b' = Significant ↓ when compared to positive control group.
c = Non significant ↑ when compared to positive control group.
c' = Non significant ↓ when compared to positive control group.
Table (6): Effect of vitamin C and its combinations on some liver enzymes activity, total protein, albumin, total globulin and kidney functions in serum of male hamsters fed a diet contaminated by carbon tetrachloride 1 ml/100 g diet (Mean+S.E).

<table>
<thead>
<tr>
<th>Group Test</th>
<th>(1) Negative control</th>
<th>(2) Positive control (CCl₄)</th>
<th>(7) CCl₄+ Vitamin C</th>
<th>(9) CCl₄+ Vitamin C+ Zinc</th>
<th>(12) CCl₄+ Vitamin C+ Selenium</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sALT (U/L)</td>
<td>67.33±3.57</td>
<td>292.54±76.98</td>
<td>191.25±61.66</td>
<td>385.20±112.83</td>
<td>159.60±20.87</td>
<td>*</td>
</tr>
<tr>
<td>sAST (U/L)</td>
<td>53.77±3.48</td>
<td>269.09±61.70</td>
<td>215.50±72.46</td>
<td>111.20±18.33</td>
<td>207.20±52.21</td>
<td>*</td>
</tr>
<tr>
<td>sALP (U/L)</td>
<td>68.22±3.88</td>
<td>109.36±18.80</td>
<td>101.62±11.79</td>
<td>94.00±10.52</td>
<td>78.80±2.80</td>
<td>N.s</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.30±0.40</td>
<td>7.62±0.40</td>
<td>7.42±0.59</td>
<td>6.24±0.44</td>
<td>6.76±0.59</td>
<td>N.s</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.87±0.23</td>
<td>4.80±0.19</td>
<td>4.75±0.36</td>
<td>4.30±0.28</td>
<td>4.60±0.44</td>
<td>N.s</td>
</tr>
<tr>
<td>Total globulin (g/dL)</td>
<td>2.42±0.21</td>
<td>2.82±0.32</td>
<td>2.67±0.29</td>
<td>1.94±0.59</td>
<td>2.16±0.26</td>
<td>N.s</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>9.62±0.82</td>
<td>25.46±3.11</td>
<td>19.63±3.21</td>
<td>14.92±1.84</td>
<td>17.04±2.71</td>
<td>**</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.66±0.03</td>
<td>1.60±0.31</td>
<td>1.13±0.14</td>
<td>0.84±0.11</td>
<td>0.95±0.11</td>
<td>*</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.30±0.32</td>
<td>2.64±0.20</td>
<td>4.86±0.38</td>
<td>5.90±0.58</td>
<td>5.10±0.97</td>
<td>**</td>
</tr>
</tbody>
</table>

*p<0.05  **p<0.01  N.s=Non significant
b = Significant ↑ when compared to positive control group.
b' = Significant ↓ when compared to positive control group.
c = Non significant ↑ when compared to positive control group.
c' = Non significant ↓ when compared to positive control group.
Discussion

The results indicated that there is a highly significant increase in serum ALT, and AST in positive control group when compared with negative control group agreeing with (Rincon et al., 1999, Aniya et al., 2000). These findings also agree with (Carini et al., 1987) who found that there is significant and progressive increase of sAST starting after 4h of giving CCl₄ by intragastric tube. This increase may be explained by the exposure of hamesters to CCl₄ by 3 ways:

Inhalation from the diet.

Ingestion from the diet as CCl₄ is volatile and has a sweet odor.

Absorption through the skin.

(Ferre et al., 1999) explained higher percentage of liver fibrosis and plasma alanine aminotransferase activity by the elevated concentration of highly toxic 2, 4 dialkenals in hepatic tissues of rats given CCl₄. The more non significant decrease in ALT is found in (CCl₄ + α-tocopherol) group followed by (CCl₄+ vitamin C) group then (CCl₄+β-carotene) group. This can be explained by the fact that vitamin C cannot suppress the oxidation of lipids when the radicals are initially generated within lipid region and oxidation proceeds in the lipophilic domain (Niki, 1991 and Kaio., 2004) and also 40-60% of β-carotene is transformed in the liver into vitamin A, this agrees with the results of (Olson, 1989).

The non significant decrease in sALT and sAST can be due to beneficial effects of vitamins on lowering these serum enzymes as they act as free radical scavengers agreeing with (Wu1997) thus preventing damage of liver cells. The increase in serum ALP in positive controls is significant when compared to negative control. This finding agrees with (Naziroglu et al., 1999 and Hsu et al., 2008) and also (Carini et al., 1987) who found that cholestatic effect on CCl₄ appeared to be mainly a consequence of the hepatocyte irreversible damage following the intoxication.

There is slight decrease in sALP in (CCl₄+B-carotene), (CCl₄+ α-tocopherol) and (CCl₄+ vitamin C) groups when compared to positive controls. This may be due to the effect of vitamin on CCl₄ cholestasis. There is non significant increase in total serum protein and globulin in positive control group when compared to negative control group. This may be due to increase of liver proteins subsequent to necrosis of liver cells, also due to toxic effect of CCl₄ which may cause fragility of membranes of hepatocytes leading to leakage of proteins to the circulation. This observation is agreeing with (Anttinen et al., 1984) and (Elewa et al., 1997). There is non significant decrease in serum total protein and globulin of (CCl₄ + β-carotene) group when compared to positive control group suggesting the beneficial effect of β carotene on serum total protein and globulin. This can be due to protective effect of β-carotene against membrane fragility decreasing leakage of proteins into the circulation agreeing with (Olson, 1989).

There is a significant increase in serum BUN and creatinine in positive control group. This result agrees with that of (Bishayee et al., 1995) and this may be due to direct toxic effect of CCl₄ on the kidney or it may secondary to CCl₄ hepatic affection. This suggestion agrees with (Stephen et al. 1980) and (Roomi et al. 2008).

There is non significant decrease in serum BUN and serum creatinine in (CCl₄ +α-tocopherol) and (CCl₄ +vitamin C) groups when compared to positive control group. But there is non significant decrease in serum BUN and significant decrease in serum creatinine in (CCl₄+β-carotene) group when compared to positive control group. β-carotene is the best effective vitamin lowering serum BUN and serum creatinine.

There is a significant decrease in serum uric acid in positive control group but there is a significant increase in serum uric acid in (CCl₄+β-carotene) group; (CCl₄+ α-tocopherol) group and (CCl₄ +vitamin C) group when compared to positive controls. This phenomenon may be explained by replacing the action of urate by vitamins which act as scavengers of free radicals. The (CCl₄ +B-carotene) group induced the highest increase when compared to positive controls.

Regarding the minerals groups the increase
in serum sALT and sAST in (CCL4 + Zn) group is non significant but the increase in sALP, is significant when compared to positive controls. These observations are in agreement with Anttinen et al., (1984) who found that a clear and significant increase in serum levels of ALP, ALT and AST in acute CCl4 injured animals and these increases were not prevent by Zn. Also (Jiang et al., 1992) found that Zn did not seem to play a role in preventing liver injury by CCl4 as serum ALT remained high. The significant increase in sALP in (CCL4 + Zn) group may be due to increase enzyme induction of ALP in the liver. This result agrees with (Yuzbasiyan et al., 1989) while, (Ademuyiwa et al. 2002) found that 10 mg Zn/kg b.wt zinc acetate reversed the hypoglycaemia, hyperbilirubinanaemia and hypercreatininaemia in rat induced by CCl4. In (CCL4+Se) group, there is non significant decrease of sALT and sALP. These decreases may be due to the protective effect of Se on rat liver cells intoxicated by CCl4. In (CCL4+Zn) group, there is non significant increase in serum total protein and non significant change of serum albumin and significant increase of serum globulin. This agrees with (Anttinen et al. 1984).

In (CCL4+Se) group, there is non significant increase in serum total proteins, and serum total globulin, no significant change in serum albumin when compared to positive control group. (Casaril et al., 1989) found that serum Se was positively correlated with two serum proteins synthesized by the liver: albumin and fibronectin. Low Se itself being an essential element for the protection of cell structures from radical action and may decrease protein synthesis by enhancing the peroxidation of microsomal membranes.

There is no significant decrease of serum total protein and globulin and no significant change in serum albumin, Alpha tocopherol) group when compared to (CCL4+Zn) group suggesting beneficial effect of selenium. The decrease in serum BUN and creatinine in (CCL4+Zn) group is non significant suggesting non significant slight effect of Zn on kidney function tests.

In (CCL4+Se) group, there is a significant decrease in serum BUN and creatinine when compared to positive controls. These results are in agreement with (Guidi et al., 1990), who studied the effect of Se supplementa-

ntation in the form of sodium selenite in humans and found that serum creatinine decreased (13%) and creatinine clearance increased significantly than the initial results. They stated that Se is very concentrated in the kidney cortex. (CCL4+ Se) group shows non significant decrease of serum BUN and creatinine when compared to (CCL4+ Zn) group. So, Se has more beneficial effect on kidney function tests than Zn.

There is a significant increase in serum uric acid in (CCL4+ Zn) group when compared to (CCL4+Se) group. This observation may be suggested to the beneficial effect of Zn in raising uric acid.

There is a significant decrease in (CCL4+β-carotene) in serum creatinine and a significant increase in serum uric acid. There is non significant decrease sALT, sAST, sALP, serum total protein, albumin, globulin, and BUN when compared to positive control group. These findings are in agreement with (Bishayee et al., 1995). CCL4+ β-carotene + Zn group shows a significant decrease of serum albumin, BUN and creatinine. There is a significant increase in serum uric acid but non significant increase in sAST and serum total globulin. There is non significant decrease of sALT, serum total protein, and non significant change of sALP when compared to positive control group.

CCL4+ β-carotene + Se shows a significant decrease in serum total globulin, there is a significant increase in serum uric acid, but there is no significant decrease of sALT, sAST, sALP, serum total protein, BUN, creatinine and also non significant increase in serum albumin when compared to positive control group. These findings may be due to the synergistic beneficial effect of β-carotene and Selenium.

In (CCL4 +α tocopherol) group there is a significant increase in serum uric acid and no significant decrease in sALT, sAST, sALP, serum total protein, total globulin, BUN, creatinine and non significant change in serum albumin when compared to positive control group. These findings are in agreement with (Maurizio et al., 1992) who observed that dietary supplementation of rats with high doses of oral vitamin E for 3 weeks before the injury as well as concurrent with the injury protected against
liver disease induced by chronic CCl₄ administration.
In (CCl₄ + α-tocopherol +Zn) group there is a significant increase of serum uric acid and non significant increase of sALT, total protein, albumin and total globulin as well as non significant decrease of sAST, sALP, BUN and serum creatinine, when compared to positive control group. In (CCl₄ +α- tocopherol +Se) group there is a significant increase in serum uric acid (P=0.01) and significant decrease of sAST. There is also non significant decrease of sALT, sALP, BUN, creatinine, no significant change of serum total protein, albumin and total globulin when compared to positive control group. These findings are in agreement with (Manna et al., 1996) who found that combination of Se and Vitamin E caused significant decrease of sALT in rats injured by CCl₄ and ethanol suggesting the possibility of their cytoprotective and/ or anti-inflammatory nature. It is suggested that the combination of α- tocopherol + Se has synergestic effect in all parameters. There is no significant difference between (CCl₄ + α tocopherol), (CCl₄ +α tocopherol + Zn) and (CCl₄ -α tocopherol +Se) groups in all parameters, except that there is significant decrease of sALT in (CCl₄ +α tocopherol), when compared to (CCl₄ -α tocopherol +Zn) group.
In (CCl₄ + Vit.C) group there is a significant increase in serum uric acid accompanied with non significant decrease in sALT, sAST, sALP, serum BUN and Creatinine. Also, non significant change in serum total protein, albumin and globulin when compared to positive control group. These findings agree with (Maellaro et al.,1994 and Kojo., 2004) who concluded that vitamin C acts as an efficient antioxidant in isolated rat liver cells and that this effect results in a protection against cell injury induced by CCl₄ . The antioxidant action of vitamin C appears to be primarily directed to oxidative damage involving membrane lipids. This effect can be independent from the cellular content of vitamin E thus suggesting that vitamin C can play a direct and independent role in the intake cell, besides its synergistic interaction with vitamin E (Haliwell, 1994 and sun etal., 2001). In (CCl₄+ Vit.C + Zn) group, there is a significant decrease of BUN, creatinine, but significant increase in uric acid and non significant decrease in AST, ALP, total protein, albumin and globuline, and non significant increase of ALT when compared to positive control group. It is suggested that the synergistic effect of vitamin C and Zn has improved all parameters except sALT.
In (CCl₄ + Vit.C + Se ) group there is a significant increase in serum uric acid while, non significant change in sALT, sAST, sALP, serum total proteins, globulin, BUN, creatinine and albumin levels was recorded when compared to positive control group. It is suggested that the synergistic effect of (vitamin C + Se) led to the improvement of all parameters. Comparing (CCl₄+Vit.C), (CCl₄+Vit.C+Zn), and (CCl₄+Vit.C+Se) groups, there is no significant difference in all parameters.

REFERENCES
induced cirrhosis. Mol Cell Biochem., 198 (1, 2): 57-60.


العلاقة بين مضادات الأكسدة والتلوث على حيوانات التجارب

مديحة هميم حسن

قسم الكيمياء الحيوية والتغذية - كلية البنات - جامعة عين شمس

**قمك كيمياء التغذية والتمثيل الغذائي - معهد التغذية - وزارة الصحة**

الفاهر

الغرض من هذا البحث هو دراسة التأثير الوقائي لمضادات الأكسدة والأملاح المعدنية في حيوانات التجارب (هامستر) من أجل امكانية تطبيقها على الإنسان.

تمت الدراسة على عدد 120 فأر (هامستر) بتوزيع وزنهم بين 104-128 جم، وتم تقسيمهم إلى 13 مجموعة. مجموعة ضابطة سلالة مجموعة ضابطة موجبة تمتعت رابع كربون كاليوم فقط. مجموعة رابع كربون كاليوم وزيت زنك- مجموعة رابع كربون كاليوم + بيتا كاروتين. مجموعة رابع كربون كاليوم + سيليسيوم - مجموعة رابع كربون كاليوم + سيليسيوم + فيتامين ج. مجموعة رابع كربون كاليوم + سيليسيوم + ألفا توكوفيرو

تحتوي كل مجموعة على تسعة هامستر واحدا المجموعة الثنائية التي تشمل اثنين عشر هامستر وكانت مدة التجربة سته أسابيع.

وقد تم إجراء الفحوصات البيوكيميائية وشملت تقدير كل من:

- الأنزيمات الخاصة بوظائف الكبد وهي الناقلة لمجموعة الأمينوسائين - أستيرتات + الفوسفات تيز القلوية 
- البروتينات الكلية، الاليبيوم والجلوبولين الكلي، بولينا، الكرياتينين - حامض الاليكي بالمصل.

وأثبتت النتائج التي تم التوصل لها أن أكثر مضادات الأكسدة في حماية الكبد هو (الفلو-توكوفيرو) يليه السيليسيوم-الفيتامين ج. (البيتا- كاروتين) - فيتامين ج-الزنك-البيتا- كاروتين) - سيليسيوم-الفيتامين ج-الزنك-البيتا- كاروتين والذين السيليسيوم-الفيتامين ج-الزنك-البيتا- كاروتين.)

كما أظهرت النتائج أن أفضل مضادات الأكسدة في حماية الكبد هو (البيتا- كاروتين) يليه السيليسيوم-الزنك-البيتا- كاروتين + الفيتامين ج.

كما يستخلص من الدراسة أن الأملاح المعدنية وخاصة الزنك والسيليسيوم يجب ألا تتناول منفردة كعوامل حماية و ذلك لفتا تأثيرها الوقائي لأثار الضار للتلوث.