Evaluation of silymarin and / or ginger effect on induced hepatotoxicity by carbon tetrachloride in male albino rats

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Abstract

Liver disease appears to be increase. Part of this increase may be due to our frequent contact with chemicals and other environmental pollutants. The amount of medicine consumed has increased greatly which could be danger to the liver.

The hepatoprotective flavonoid, silymarin (7.56 mg/Kg b. wt.) and ginger (Zingiber officinale) (1% of diet) for 4 weeks were used to ameliorate the liver injury in rats intoxicated with carbon tetrachloride (CCL₄, single acute dose 4 ml/ Kg b. wt. of 50% v/v CCL₄ in olive oil, subcutaneous).

The obtained results showed that CCL₄ decreased serum and liver total protein and albumin. Also reduced glutathione content. The antioxidant enzymes; glutathione-S-transferase, superoxide dismutase and catalase activity decreased in the liver of CCL₄ toxicity group. On the other hand, CCL₄ toxicity increased serum and liver bilirubin, total lipid and total cholesterol levels. In addition, liver function (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and liver lipid peroxidation content showed significant increase in the CCL₄ treated group.

Previous parameters turned back to normal values in carbon tetrachloride intoxicated rats after treating with silymarin and/ or ginger for one month. Meanwhile, the most interesting effect was induced by a combination of silymarin and ginger. Hence, the present findings will provide a potential scope for future use of ginger for the treatment of liver disorders.

Key words: Silymarin-ginger-carbon tetrachloride-hepatotoxicity- rats.

Introduction

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of healthy liver is vital to overall health and well being (Treadway, 1998 and Aris et al., 2001). Unfortunately, the liver is often abused by environmental toxins, poor eating habits and over the counter drug use, which can damage and weaken the liver and eventually leads to hepatitis, cirrhosis and liver disease. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis. (Treadway, 1998).

Silymarin is the active constituent of Silybum marianum cited as one of the oldest known medicinal plants, since 1970s, silymarin has been used for treatment of liver disease (Giese, 2001) and a dose of 420 mg/day was shown to improve indices of liver function (AST, ALT, GGT and bilirubin) in patients with liver diseases of various aetiology (Wellington and Jarvis, 2001). Silymarin is used for the treatment of several hepatic disorders (Hakova and Misuruva, 1993) and is mainly indicated for acute and chronic hepatitis, liver cirrhosis, fatty degeneration and toxic metabolic liver...
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Silymarin and ginger have been studied for their potential health benefits, particularly in the context of treating liver disease (Feher et al., 1987). Silymarin has antioxidant activity (Valenzuela and Garrido, 1994). It inhibited free radical induced lipid peroxidation in microsomal and mitochondrial preparations of human red blood cells, thereby stabilizing the structure of the cell membrane (Koch and Loffler, 1985). Silymarin inhibited hepatotoxicity induced by carbon tetrachloride (Letteron et al., 1990). Intraperitoneal or intragastric administration of silymarin (15-800 mg/Kg body weight) to dogs, mice and rats prevented carbon tetrachloride induced liver damage (Mourelle and Favari, 1989 and Muriel and Mourelle, 1990). This effect of silymarin was attributed to its antioxidant activity, a decrease in the metabolic activation of carbon tetrachloride and stabilization of hepatocyte membranes (Muriel and Mourelle, 1990). Silymarin also increased the redox state and the total glutathione content in the liver, intestine and stomach of rats after intraperitoneal administration (200 mg/Kg body weight) (Valenzuela and Garrido, 1994).

Ginger rhizome (Zingiber officinale) is one of the best known spices and it has also been universally used throughout history for its health benefits. It is used traditionally to promote digestion (Bone, 1997). The dried extract of ginger contains monoterpenes and sesquiterpenes. The main antioxidant active ingredients in ginger are the gingerols and shogaols and some related phenolic ketone derivatives. Ginger extract possesses antioxidative characteristics, since it can scavenge superoxide anion and hydroxyl radicals (Reddy and Lokesh, 1992; Krishnakanta and Lokesh, 1993 and Cao et al., 1993). Gingerol from ginger inhibited, at high concentrations, ascorbate/ferrous complex induced lipid peroxidation in rat liver microsomes (Reddy and Lokesh, 1992). Furthermore, ginger acts as a hypolipidemic agent in cholesterol fed rabbits (Sharma et al., 1996 and Bhandari et al., 1998). Feeding rats ginger significantly elevated the activity of hepatic cholesterol-7-hydroxylase, the rate limiting enzyme in bile acids biosynthesis, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body (Srinivasan and Sambaiah 1991). In addition, a pure constituent from ginger [E-8beta, 17 epoxylabd-12-ene-15,16-dial (ZT)], was shown to inhibit cholesterol biosynthesis in homogenated rat liver (Tanabe et al., 1993). Recently, Fuhrman et al. (2000) concluded that consumption of ginger extract may decrease atherosclerosis development, since it is associated with reduced macrophage-mediated oxidation of LDL, reduced uptake of oxidized LDL by macrophages, reduced oxidative state of LDL and reduced LDL aggregation. All these effects lead to a reduced cellular cholesterol accumulation and foam cell formation, the hallmark of early atherosclerosis.

Carbon tetrachloride is a xenobiotic, which produce hepatotoxicity in humans, as well as, in animals (Chenery et al., 1981). The hepatotoxic effect of carbon tetrachloride is known to result from its reductive dehalogenation by the cytochrome P_450 enzyme system to the highly reactive free radical, trichloromethyl radical CCL_3 (McCay et al., 1984). This radical quickly adds molecular oxygen to form trichloromethylperoxy radical CCL_3OO^-(Letteron et al., 1990). Removal of hydrogen atoms from unsaturated fatty acids by such radicals creates carbon-centered lipid radical (McCay et al., 1984). These later radicals quickly add molecular oxygen to form lipid peroxyl radicals, thereby initiating the process of lipid peroxidation (Letteron et al., 1990). Unless scavenged by radical scavengers, these lipid peroxyl radicals in turn abstract hydrogen atoms from other lipid molecules, thereby propagating the process of lipid peroxidation (Recknagel, 1983).

Therefore, the objective of the present study was to examine the possible curative effect of silymarin and/or ginger in rat-induced hepatotoxicity by carbon tetrachloride. Also, to compare the effectiveness of ginger in relation to silymarin in treating hepatotoxicity.

Materials And Methods

Thirty male Sprague-Dawley rats (120-140 g), obtained from the Egyptian
Organization for Biological products and vaccines were used in the present study. Animals were kept under standard conditions throughout the experimental period. The animals were fed on standard diet. Liberal water intakes were available.

Carbon tetrachloride (CCL₄), Silymarin and Ginger were used for treatment in the present study. Carbon tetrachloride was obtained from ADWIC Co, Egypt and used as 50% solution in olive oil. Silymarin was purchased from Sedico company (Six-October City, Egypt) in the form of sachets, each sachet contains 140 mg. Ginger (Zingiber officinale) powder was obtained from spice shop in Mansoura. Animals were divided into five groups of six rats each as follow:

**Group 1**: Control group, rats of this group were injected subcutaneously with 4 ml/Kg b. wt. of olive oil.

**Group 2**: Carbon tetrachloride intoxicated group, rats of this group were injected 4 ml/Kg b. wt. subcutaneously by a single dose of 50% v/v of carbon tetrachloride in olive oil (Jayasekhar et al., 1997).

**Group 3**: Carbon tetrachloride intoxicated rats and treated with silymarin. Rats were injected with the same dose of CCL₄ as in the second group and simultaneously given daily gavage administration of silymarin at a dose of 7.56 mg/Kg b. wt. For 4-weeks (Ghosh, 1971).

**Group 4**: Carbon tetrachloride intoxicated rats and treated with ginger. Rats were injected with the same dose of CCL₄ as in the second group and administered daily with ginger (1% w/w in diet) for 4 weeks (Newall et al., 1996).

**Group 5**: Carbon tetrachloride intoxicated rats and treated with both silymarin and ginger. Rats were injected with the same dose of CCL₄ as in the second group and simultaneously given silymarin as group (3) and ginger as group (4).

By the end of the experimental period, normal and treated rats were fasted for 12 hours, sacrificed and the blood sample were taken and centrifuged at 3000 r.p.m. for 20 minutes. The clear, non-hemolysed supernatent sera were taken and kept at 20 °C for biochemical analysis. The animals were rapidly dissected and small pieces of liver were removed out and tissue homogenates were prepared in ice cold physiological saline solution and kept at -20 °C for further analysis.

Stanbio kits (Stanbio Laboratory, INC. 2930 East Houston Street San Antonio, Texas, USA) were used for Biochemical investigation. Total protein (TP) was assayed by Biuret reaction according to Doumas (1975). Albumin (A) was estimated by the method of Doumas and Gigg (1972). Total bilirubin was measured according to Jendrassik and Grof (1938) method. Total lipid and total cholesterol were estimated according to the methods described by Knight et al. (1972) and Stein (1986) respectively. Serum and liver activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were carried out by the technique of Reitman and Frankle (1957). Alkaline phosphatase (ALP) activity was estimated according to Teitz (1976) method.

Also thiobarbituric acid reactive substances (TBARS) was determined as described by Esteribauer and Cheesman (1990). Liver content of glutathione (GSH) was estimated by the method of Prins and Loose (1969). The activity of superoxide dismutase (SOD) was assayed according to the method of Niskikimi et al. (1972). Glutathione- S- transferase (GSH-S-T) activity was determined by the method of Habig et al. (1974) and catalase activity was detected by Aebi (1983).

All results were expressed as mean ±S.E of 6 animals. The obtained data were compared using Student t-test as described by Snedecor and Cochran (1982). The level of significance was expressed as significant at P<0.05. Percent of changes in relation to control and CCL₄ intoxicated groups were calculated.

**Results**

After one month of a single acute dose of carbon tetrachloride intoxication, rats showed decreases in serum and liver total protein and albumin. On the other hand, improvement of the above mentioned parameters in the serum and liver were
Evaluation of silymarin and/or ginger 

recorded in CCL4 intoxicated rats treated with silymarin and/or ginger (table 1&2).

As shown in table (1&2), increased serum and liver total bilirubin, total lipid and total cholesterol were recorded in CCL4 intoxicated rats when compared with control group (P<0.05). Both silymarin and ginger individually improved in these parameters (bilirubin, total lipid and total cholesterol) in serum and liver. Combination of silymarin and ginger in CCL4 treated rats decreased serum total bilirubin (-13.48 & -31.86) comparing with control and CCL4 intoxicated group respectively, total lipid (+7.95 & -22.66) in comparison with control and CCL4 intoxicated groups respectively and total cholesterol (-9.88 & -38.14) in relation to control and CCL4 intoxicated groups respectively. Liver total bilirubin showed a marked decrease (-22.57 & -42.69) in CCL4 intoxicated rats treated with silymarin and ginger compared with control and CCL4 treated groups respectively. Also, decreased contents of liver total lipid in CCL4 intoxicated rats treated with silymarin and ginger (-19.33 & -23.25) was observed when compared with control and CCL4 treated group respectively. Another decreases of liver total cholesterol (-27.98 &-39.59) were recorded in CCL4 intoxicated rats treated with silymarin and ginger compared with control and CCL4 treated ones respectively.

As shown in table (3), the activities of AST, ALT and ALP in the serum increased significantly in all groups compared to control group, while decreased significantly compared with CCL4 intoxicated group, except serum ALP which decreased significantly in CCL4 intoxicated group treated with both silymarin and ginger (-5.19 & -26.56) compared with control and CCL4 intoxicated groups respectively.

Activities of AST and ALT also increased in the liver of CCL4 treated group (+16.49 & +5.75) when compared with control group respectively. Both silymarin and ginger individually decreased liver AST and ALT activities when compared with CCL4 intoxicated group. The recorded liver AST and ALT activities showed significant changes when compared with CCL4 intoxicated rats.

Combination of silymarin and ginger in CCL4 intoxicated rats showed decreased liver activities of AST and ALT which was significant when compared with control and CCL4 intoxicated groups (table 3). On the other hand, Liver ALP decreased significantly in CCL4 intoxicated rats and silymarin treated CCL4 intoxicated group but increased in ginger treated CCL4 intoxicated rats and silymarin plus ginger treated CCL4 intoxicated groups when compared with control group as shown in table (3).

There were an increase in liver lipid peroxidation (TBARS) (+81.51) in CCL4 intoxicated rats compared with control group, however, TBARS decrease significantly in other groups when compared to CCL4 intoxicated group. These decreases still above that of control group, except compination of silymarin and ginger treatment which showed insignificant changes of liver lipid peroxidation in comparison to control group (+3.93 ) (table 4). Reduced glutathione (GSH) in the liver of CCL4 intoxicated group decreased significantly (-40.39 ) in relation to control group, while high liver concentrations of GSH were reported in other treated groups when compared to control group (table 4).

In addition to changes observed in the liver GSH and lipid peroxidation, there were also changes in the activities of glutathione-S-transferase (GSH-S-T), superoxide dismutase (SOD) and catalase enzymes. In CCL4 intoxicated rats and CCL4 intoxicated treated with silymarin, activities of antioxidant enzymes decreased significantly comparing to control group. CCL4 intoxicated rats treated with ginger alone or combined with silymarin had increased activities of liver enzymes (GSH-S-T, SOD and catalase) when compared with both control and CCL4 intoxicated groups (table 4).
Table (1): Effect of silymarin and/or ginger on some serum metabolic parameters in different rat groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCL₄</th>
<th>CCL₄ + silymarin</th>
<th>CCL₄ + ginger</th>
<th>CCL₄ + silymarine+ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>7.72±0.26</td>
<td>6.66±0.19</td>
<td>7.23±0.17</td>
<td>8.54±0.18</td>
<td>9.02±0.20</td>
</tr>
<tr>
<td></td>
<td>a -13.73*</td>
<td>b -6.35&amp;+8.56</td>
<td>a+b+10.62&amp;+28.23</td>
<td>a+b+16.84&amp;+53.44</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.80±0.008</td>
<td>4.04±0.005</td>
<td>5.32±0.056</td>
<td>5.65±0.084</td>
<td>6.57±0.103</td>
</tr>
<tr>
<td></td>
<td>a -15.83*</td>
<td>a+b+10.83&amp;+31.68</td>
<td>a+b+17.71&amp;+39.85</td>
<td>a+b+36.88&amp;+62.62</td>
<td></td>
</tr>
<tr>
<td>Total lipid (mg/dl)</td>
<td>1012.44±8.75</td>
<td>1413.26±8.70</td>
<td>1249.46±8.16</td>
<td>1201.62±21.06</td>
<td>1092.96±19.01</td>
</tr>
<tr>
<td></td>
<td>a&amp;b+39.59*</td>
<td>a+b+23.41&amp;-11.59</td>
<td>a+b+18.69&amp;-14.98</td>
<td>a+b+7.95&amp;-22.66</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>262.03±4.74</td>
<td>381.71±2.02</td>
<td>285.53±5.6</td>
<td>256.60±2.36</td>
<td>236.14±4.18</td>
</tr>
<tr>
<td></td>
<td>a+b+45.67*</td>
<td>a+b+8.97&amp;-25.20</td>
<td>b-2.07&amp;-32.78</td>
<td>a+b-9.88&amp;-38.14</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.17±0.01</td>
<td>0.22±0.004</td>
<td>0.17±0.004</td>
<td>0.19±0.003</td>
<td>0.15±0.004</td>
</tr>
<tr>
<td></td>
<td>a+b+26.97*</td>
<td>a+b-2.25&amp;-23.01</td>
<td>a+b+11.24&amp;-12.39</td>
<td>a+b-13.48&amp;-31.86</td>
<td></td>
</tr>
</tbody>
</table>

All results are expressed as M± SE of six animals and % of changes.  
a = significant at P<0.05 when compared with control group.  
b=significant at P<0.05 when compared with CCL₄ intoxicated group  
* = % of change in relation to control group.  
** = % of change in relation to CCL₄ intoxicated group.

Table (2): Effect of silymarin and/or ginger on some liver metabolic parameters in different rat groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCL₄</th>
<th>CCL₄ + silymarin</th>
<th>CCL₄ + ginger</th>
<th>CCL₄ + silymarine+ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/100g)</td>
<td>30.47±0.75</td>
<td>24.89±0.44</td>
<td>29.04±0.19</td>
<td>29.59±0.61</td>
<td>34.90±0.39</td>
</tr>
<tr>
<td></td>
<td>a -18.31*</td>
<td>b -4.69&amp;+16.67</td>
<td>b -2.89&amp;+18.88</td>
<td>a+b+14.54&amp;+40.22</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/100g)</td>
<td>7.87±0.096</td>
<td>6.88±0.082</td>
<td>8.40±0.194</td>
<td>8.28±0.136</td>
<td>9.65±0.191</td>
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<tr>
<td></td>
<td>a -12.58*</td>
<td>a+b+6.73&amp;+22.09</td>
<td>a+b+5.21&amp;+20.35</td>
<td>a+b+22.62&amp;+40.26</td>
<td></td>
</tr>
<tr>
<td>Total lipid (mg/g)</td>
<td>272.73±1.17</td>
<td>286.66±3.75</td>
<td>198.92±3.02</td>
<td>236.88±2.61</td>
<td>220.0±3.50</td>
</tr>
<tr>
<td></td>
<td>a +5.11*</td>
<td>a+b-27.06&amp;-30.61</td>
<td>a+b-13.14&amp;-17.37</td>
<td>a+b-19.33&amp;-23.25</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/100g)</td>
<td>581.10±12.55</td>
<td>692.70±4.60</td>
<td>492.80±9.97</td>
<td>501.16±4.70</td>
<td>418.46±5.77</td>
</tr>
<tr>
<td></td>
<td>a+b+19.20*</td>
<td>a+b-15.20&amp;-29.86</td>
<td>a+b-13.76&amp;-27.65</td>
<td>a+b-27.98&amp;-39.59</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/100g)</td>
<td>66.14±1.70</td>
<td>89.36±1.50</td>
<td>68.12±0.69</td>
<td>60.60±0.84</td>
<td>51.21±0.71</td>
</tr>
<tr>
<td></td>
<td>a+b+35.11*</td>
<td>a+b+2.99&amp;-23.77</td>
<td>a+b-8.38&amp;-32.18</td>
<td>a+b-22.57&amp;-42.69</td>
<td></td>
</tr>
</tbody>
</table>

All results are expressed as M± SE of six animals and % of changes.  
a=significant at P<0.05 when compared with control group.  
b=significant at P<0.05 when compared with CCL₄ intoxicated group  
* = % of change in relation to control group.  
** = % of change in relation to CCL₄ intoxicated group.
Table (3): Effect of silymarine and/or ginger on some serum and liver enzyme activities in different rat groups (liver function enzymes).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCL₄</th>
<th>CCL₄ + silymarin</th>
<th>CCL₄ + ginger</th>
<th>CCL₄ + silymarin + ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AST (U/l)</strong></td>
<td>19.28±0.33</td>
<td>31.00±0.71</td>
<td>27.84±0.36 (a)</td>
<td>29.60±0.23 (a)</td>
<td>25.48±0.63 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+60.79 (a)</td>
<td>+44.40* (a)</td>
<td>+53.53* (a)</td>
<td>+32.16* (a)</td>
</tr>
<tr>
<td><strong>ALT (U/l)</strong></td>
<td>11.11±0.38</td>
<td>17.46±0.30</td>
<td>14.36±0.55 (a)</td>
<td>16.14±0.24 (a)</td>
<td>12.44±1.39 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+57.16 (a)</td>
<td>+29.25* (a)</td>
<td>+45.27* (a)</td>
<td>+11.97* (a)</td>
</tr>
<tr>
<td><strong>ALP (U/l)</strong></td>
<td>143.88±2.28</td>
<td>185.74±2.05</td>
<td>168.42±1.09 (a)</td>
<td>157.28±2.9 (a)</td>
<td>136.41±1.99 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+29.09 (a)</td>
<td>+7.32* (a)</td>
<td>+9.31* (a)</td>
<td>+11.97* (a)</td>
</tr>
</tbody>
</table>

All results are expressed as M± SE of six animals and % of changes.

a= significant at P<0.05 when compared with control group.
b=significant at P<0.05 when compared with CCL₄ intoxicated group.

* %= % of change in relation to control group.
** %= % of change in relation to CCL₄ intoxicated group.

Table (4): Effect of silymarin and/or ginger on liver lipid peroxides (TBARS) and some liver antioxidant parameters in different rat groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCL₄</th>
<th>CCL₄ + silymarin</th>
<th>CCL₄ + ginger</th>
<th>CCL₄ + silymarin + ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TBARS</strong> (n.mol/g wet tissue)</td>
<td>38.93±0.96</td>
<td>70.66±1.77</td>
<td>51.75±2.32 (a)</td>
<td>47.21±2.21 (a)</td>
<td>40.46±0.88 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+81.51* (a)</td>
<td>+32.93* &amp; +21.27* (a)</td>
<td>+19.94* &amp; -33.19* (a)</td>
<td>+3.93* &amp; -42.74* (a)</td>
</tr>
<tr>
<td><strong>GSH</strong> (mg/g wet tissue)</td>
<td>9.16±0.37</td>
<td>5.46±0.32</td>
<td>10.88±0.14 (a)</td>
<td>17.46±0.29 (a)</td>
<td>20.01±0.46 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+40.39* (a)</td>
<td>+18.78* &amp; +99.27* (a)</td>
<td>+90.61* &amp; +219.78* (a)</td>
<td>+11.45* &amp; +266.48* (a)</td>
</tr>
<tr>
<td><strong>SOD (U/g wet tissue)</strong></td>
<td>48.14±0.99</td>
<td>33.50±0.99</td>
<td>35.58±1.24 (a)</td>
<td>40.18±1.16 (a)</td>
<td>48.06±1.74 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-30.41* (a)</td>
<td>-26.09* &amp; +6.21* (a)</td>
<td>-16.54* &amp; +19.94* (a)</td>
<td>-0.17* &amp; +43.46* (a)</td>
</tr>
<tr>
<td><strong>GSH-S-T</strong> (µmol/min/g )</td>
<td>20.42±0.52</td>
<td>12.92±0.37</td>
<td>14.76±0.38 (a)</td>
<td>24.28±0.40 (a)</td>
<td>25.52±0.55 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-36.73* (a)</td>
<td>-27.62* &amp; +14.40* (a)</td>
<td>+18.90* &amp; +87.93* (a)</td>
<td>+24.98* &amp; +97.52* (a)</td>
</tr>
<tr>
<td><strong>Catalase</strong> (KU/sec/g wet tissue)</td>
<td>7.46±0.23</td>
<td>5.57±0.24</td>
<td>5.94±0.18 (a)</td>
<td>7.70±0.19 (a)</td>
<td>9.67±0.52 (a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-25.34* (a)</td>
<td>-20.38* &amp; +6.45* (a)</td>
<td>+3.22* &amp; +38.24* (a)</td>
<td>+29.62* &amp; +73.61* (a)</td>
</tr>
</tbody>
</table>

All results are expressed as M± SE of six animals and % of changes.
a= significant at P<0.05 when compared with control group.
b=significant at P<0.05 when compared with CCL₄ intoxicated group.

* %= % of change in relation to control group.
** %= % of change in relation to CCL₄ intoxicated group.
Discussion

The hepatotoxicity of halogenated hydrocarbons, particularly CCL$_4$, has been the subject of numerous investigations in experimental animals (Recknagel and Glende, 1973; Dashti et al., 1989; Klatskin and Conn, 1993; Amer and selim, 2002 and Amer, 2003).

Carbon tetrachloride is an occupational chemical agent widely used as a solvent in insecticide industry and is correlated with high incidence of certain types of cancer (Petrelli et al., 1993). Previous observations demonstrated that pretreatment with different herbal extracts provide various levels of protection against the hepatocellular damage resulting from administration of CCL$_4$ (Johnston and Kroening, 1998 and Amer, 2003).

Since liver injury is used to occur in human without pretreatment with protective medicine, this study was carried out to evaluate the possible use of ginger alone or combined with silymarin in already CCL$_4$-induced hepatotoxicity in rats.

The present study revealed that acute CCL$_4$ intoxication resulted in a decrease in serum and liver total protein and albumin contents. Similar findings were recorded in liver diseases (Tanaka et al., 1998). Sherlock and Dooley (1993) reported that albumin is the most abundant plasma protein produced by hepatocytes, its depreciation usually reflects decreased hepatic synthesis. This fall is often attributed to hepatic impairment of albumin synthesis. The decrease may also be due to leakage in kidney function leading to the release of albumin with the urine. As well as significant increases in the activities of serum and liver transaminase enzymes (AST and ALT) were noticed. These effects may reflect the hepatocellular damage. Scherlock and Dooley (1993) reported that also the activity of plasma AST and ALT are standard tests for hepatocellular integrity. These measurements assess leakage of hepatic enzymes into the blood stream. The obtained results support the findings of Naziroglu et al. (1999); Ahmed et al. (2000) and Amer (2003). They observed similar effect of CCL$_4$ on the same and other hepatic related parameters after CCL$_4$ intoxication in different animals.

Treatment of CCL$_4$ intoxicated rats with silymarin or ginger improved these parameters (total protein, albumin ,AST and ALP) in the serum and liver indicating amelioration of hepatotoxic effect of CCL$_4$. Furthermore, the combination of silymarin and ginger treatment in CCL$_4$ intoxicated rats increased serum and liver total protein and albumin contents. Hepatoprotective activity of silymarin was reported by and Bean (2002) in patients with alcohol-induced cirrhosis. Also, an improvement of liver function was noticed in patients exposed to toluene and/or xylene vapours for 5-20 years after treatment with silymarin (Szilard et al., 1988). Ginger treatment individually or combined with silymarin improved liver function by decreasing the activities of AST and ALT and increasing serum and liver total protein and albumin contents. These results reflect the effectiveness of ginger in improving hepatic injury induced by CCL$_4$.

Alkaline phosphatase (ALP) is mainly involved in metabolite transport across cell membranes found in a decreasing order of abundance in placenta, ileal, mucosa, kidney, bone and liver (Fishman, 1990). The level of serum ALP is associated mainly with cholestasis, bile salts and to a less extent with liver cell damage (Kaplan, 1972 and Moss, 1997). Carbon tetrachloride intoxicated rats showed elevated serum ALP but decreased liver ALP, which confirm the linkage of this enzyme impairment. Disorders of serum and liver ALP were improved after using silymarin and/or ginger in CCL$_4$ intoxicated rats. This improvement of ALP may reflect liver repair of these groups.

Elevated serum and liver total bilirubin was noticed in CCL$_4$ intoxicated group. Similar increase in serum total and direct bilirubin was seen in patients having cirrhosis, which may reflect abnormal conjugation of bilirubin by the liver due to generalized hepatocellular damage (El-sherbiny et al., 2003). Serum and liver total bilirubin were decreased in CCL$_4$ intoxicated rats after treatment with silymarin or ginger. Combination of both silymarin and ginger recorded more decrea-
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ase in serum and liver total bilirubin which became below the normal value. The obtained results were confirmed by Feher et al. (1987) who reported that silymarin is known to have a reduction effect on total bilirubin in different liver diseases. Similar effect of ginger may indicate the effectiveness of ginger in normal functional status of the liver.

Hyperlipidemic effect of CCL₄ was noticed in the serum and liver of CCL₄ intoxicated group, might suggest enhanced lipogenesis. Since total lipid and total cholesterol were increased significantly. The observed hyperlipidemia might reflect the impairment of liver cells to metabolize lipids and reduced transformation of cholesterol to bile acid and excretion. In the present study, silymarin and/or ginger reduced serum and liver total lipid and total cholesterol. These results are in agreement with Sharma et al. (1996) and Bhandari et al. (1998) who reported that ginger acts as a hypolipidemic agent in cholesterol fed rabbits. Also, feeding rats ginger significantly elevated the activity of hepatic cholesterol-7-hydroxylase, the rate limiting enzyme in bile acid synthesis, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body (Srinivasan and Sambarah, 1991). In addition, pure constituent from ginger [E-8 beta, 17 epoxylabol-12-ene 15,16- dial (ZT)] was shown to inhibit cholesterol biosynthesis in homogenated rat liver (Tanabe et al., 1993). Also, Fuhrman et al. (2000) observed that cholesterol synthesis was inhibited by some ginger extract derived metabolite or by secondary mediator.

The present work also showed an increase in lipid peroxidation after CCL₄ intoxication as evidenced by the increased TBARS in the liver. This observation is confirmed by other studies (Riely et al., 1974; Bechtold et al., 1982; Ohta et al., 2000 and Amer, 2003). They concluded the ability of CCL₄ to induce lipid peroxidation of liver membrane lipids in rodents. Rapid, extensive lipid peroxidation of the membrane structural lipid has been proposed as the basis of CCL₄ hepatotoxicity (Recknagel and Glende, 1973). A correlation between the metabolism of CCL₄, lipid peroxidation and hepatocyte damage provides the strongest supporting evidence. Both CCL₄-induced lipid peroxidation and CCL₄ toxicity are dependent on retudtive metabolism by microsomal mixed function oxidase with the generation of trichloromethyl radical (Castro et al., 1972), CCL₃ and CCL₃OO•, with ox-radical which arises from the reaction of CCL₃ with oxygen, are capable of initiating lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid (Castro et al., 1972 and Packer et al., 1978). Thus while CCL₄ causes free radical induced oxidative damage to cells and causes lipid peroxidation, these processes may be parallel responses to the generation of free radicals. Under some condition, lipid peroxidation may determine the extent of injury by amplifying the injury through propagation of free radical processes, generating toxic compounds and impairing detoxication systems (Tribble et al., 1987).

Impairing detoxication systems was seen in CCL₄ intoxicated group, since liver GSH content and antioxidant enzymes (GST, SOD and catalase) activities were reduced. Similar results were obtained by Lii et al. (1998) and Amer (2003), they detected a significant decrease in liver GSH content after CCL₄ intoxication. Since reduced glutathione (GSH) is an important constituent of cellular protective mechanisms against a number of toxic stimuli including oxygen derived free radicals (Cembalos-Picot et al., 1996). The observed decrease in GSH level in the hepatotoxicated animals might have been due to an increased scavenging of reactive substances that were produced as a result of the necrotic and/or steatic state of the hepatocytes and/or possible decreased hepatic production of GSH (Lii et al., 1998).

Administration of silymarin and/or ginger for one month significantly lowered the lipid peroxidation level induced by CCL₄. Furthermore, our study have shown that the supplementation of silymarin and/or ginger regulate the lipid peroxidation by altering the liver GSH and antioxidant enzymes activities. Antioxidant activity of silymarin was reported by Muriel and Mourelle (1990) and Feher and Lang.
(1988), as well as, anti-lipid peroxidation (Basaga et al., 1997) and enhanced detoxification (Baer-Dubowska et al., 1998) were recorded. The hepatoprotective effect of silymarin based on free radical scavenging activity (Abd-Allah et al., 2003). Ginger, also possesses antioxidative characteristics, since it can scavenge superoxide anion and hydroxyl radicals (Reddy and Lokash, 1992 and Cao et al., 1993). Also, antioxidative effect and free radical scavenging capacity of ginger were detected by Fuhrman et al. (2000). However, treatment with ginger alone or combined with silymarin significantly maintained the level of GSH and antioxidant enzymes activities nearly close the control value. The increased levels of GSH after ginger alone or combined with silymarin treatment may be attributed to an increased rate of GSH synthesis or due to increased hepatic tissue uptake of extracellular GSH. In this connection, GSH has been shown to be mobilized from blood and other tissues to protect target organs in conditions associated with increased oxidative stress (An-Sik et al., 1982 and Lii et al., 1998).

In conclusion, the results of the present study confirm the curative effect of ginger against CCL4 induced hepatotoxicity in rats. The potency of ginger compares well with silymarin. Moreover, combination of ginger and silymarin is more effective. These encouraging results may have future clinical importance because of the increased use of natural herbs by the general population.

References

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تقييم تأثير السيليرمرين و/أو الزنجبيل على التسمم الكبدى المستحدث برابع كلوريد الكربون في ذكور الجرذان
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تزايد أمراض الكبد يومًا بعد يوم بسبب تعرض الإنسان للكيماويات والملوثات، وسوء استخدام الأدوية.
والأمل في الاعتماد على المصادر الطبيعية (الكائنات) أصبح هدفًا للعلماء في جميع أطراف المعمورة. ويتناول هذا البحث علاج التسمم الكبدى المستحدث برابع كلوريد الكربون باستخدام دواء السيليرمرين المستخرج من شجرة سيليبيوم ماريانو متعددًا أو متحدًا مع الزنجبيل، وكذا مقارنة تأثير نتائج العلاج بالزنجبيل متفردًا بنتائج السيليرمرين، ومدى تأثير تواجدهما معًا في العلاج.

تم معاملة ذكور الجرذان بجرعة واحدة من رابع كلوريد الكربون لإحداث التسمم الكبدى وبدأ العلاج بالسيليرمرين و/أو الزنجبيل بعد 24 ساعة من استخدام رابع كلوريد الكربون.

أشارت جميع النتائج المتحصل عليها أن رابع كلوريد الكربون قد أحدث خللًا في كبد الجرذان تمثل في ما يلي:

1. نقص مستوى البروتين الكلي والألبومين في مصل الدم والكبد.

2. ارتفاع نشاط انزيمات وظائف الكبد (إنزيمات نقل مجموعة الأمين) في MCL الدم والكبد.

3. ارتفاع نشاط الفوسفات القاعدى (ALP) في مصل الدم وقصه في الكبد.

4. ارتفاع البيريلون الكلى والدهون الكلية والكولسترول الكلى في مصل وكد المجموعة المذكورة.

و هذا بالإضافة إلى حدوث خللًا واضحًا في حالة الأكسدة ومضادات الأكسدة في كبد الجرذان المصابة بالتسمم الكبدى كما يلي:

1. ارتفاع مستوى الوعي التأكسدي للدهون في الكبد . (TBARS)

2. نقص مستوى مضادات الأكسدة غير إنزيمية (الجلوتاتثيون المختزل) GSH و/أو إنزيمية (GSH-S-T) السوء أو أكسيد ديموثيديناتي (SOD) والكاثاتليز.

بينما كان العلاج بالسيليرمرين والزنجبيل متفردًا أو متحددين أيجابيًا على نتائج وظائف الكبد المقاسة في مصل الدم والكبد، وكذا في تحسين مستوى الأكسدة ومضادات الأكسدة بالكبد. وكان اتحاد السيليرمرين والزنجبيل الأكثر تأثيرًا.

ويمكن القول في ضوء النتائج السابقة أن هذه الدراسة تشجع استخدام الزنجبيل في علاج أمراض الكبد وتوصي بإجراء المزيد من التجارب عليه واكتشاف المواد الفعالة به.