Potential Effect Of Garlic Oil and Silymarin on Carbon Tetrachloride-Induced Liver Injury

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Abstract: Oxidative stress is a condition in which cellular antioxidant defenses are inadequate to completely inactivate the reactive oxygen species. It plays an important role in the pathophysiology of many diseases. Oxidative damage of any of the biomolecules; nucleic acid bases, lipids and proteins if unchecked can theoretically contribute to disease development. The present study was conducted to investigate the modulatory effects of (Silymarin and garlic oil ) on Carbon tetrachloride (ccl₄) - induced hepatotoxicity in male albino rats. Animals were orally intoxicated with ccl₄ followed by oral administration of Silymarin or garlic oil. A group of diagnostic enzymes and oxidant-antioxidant markers were assessed. Intoxication of rats with ccl₄ induced significant elevation in serum liver enzymes as well as oxidative stress through the increase in oxidation markers over the antioxidant one as compared to that of the controls. Oral administration of silymarin or garlic oil improved these adverse effects. Silymarin and garlic seen to be highly promising compound in protecting the hepatic tissue against oxidative damage and preventing hepatic dysfunction due to ccl₄ induced hepatotoxicity in rats

Key words: Advanced oxidation protein product, F2 isoprostane, DNA damage.

INTRODUCTION

Reactive oxygen species (ROS) are implicated in the pathogenesis of most liver diseases, including ischemia/reperfusion injury, endotoxemia, chronic hepatitis C, alcoholic and non-alcoholic fatty liver disease, and cholestasis (Rost et al., 2007). Carbon tetrachloride (CCl₄), a hepatotoxin, has been used extensively for decades to induce liver injury in various experimental models to elucidate the mechanisms behind hepatotoxicity. It has been known for a longtime that a part of the liver injury caused by this solvent may have originated through the free radical reactions to the metabolism of CCl₄ in the liver and subsequent initiation of lipid peroxidation (Rasha et al., 2009). In CCl₄ induced liver damage, there is an excessive lipid peroxidation leading to functional and structural disruption (Muriel et al., 2001).

The damage or death of hepatocytes usually results in the leakage of the enzymes in the affected tissue into the blood stream (Obi et al., 2001). Serum or plasma enzyme levels have been used as markers for monitoring chemically induced tissue damages. The enzymes Alanin aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma glutamate transferase (GGT) are important enzymes that are often employed in assessing liver injury (Obi et al., 1998).

Attention has been developed on the protective biochemical function of the natural antioxidants contained in the dietary plants that are candidates for prevention or protection of oxidative damage caused by free radicals species (Vincevt et al., 2004). Garlic (Allium sativum), is a member of the lily family that has been cultivated by the humans as a food for over 10,000 years.

Silymarin a flavonolignan from milk thistle (Silybum marianum) plant is used almost exclusively for hepatoprotection. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals where it alters the structure of the outer membrane of the hepatocytes in such away as to prevent penetration of the liver toxin into the interior of the cell. In addition it stimulates the action of nuclear polymerase A, resulting in ribosomal protein synthesis and thus stimulates the regenerative ability of the liver and formation of new hepatocytes (http://www.medidea.com.2011).

Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts as an antioxidantive, antilipid peroxidation(Hubert et al, 2011),antifibrotic,anti-inflammatory, membrane stabilizing and immunomodulatory (Pradhan and Girish, 2006).

This study was designed to evaluate the potentially effect of garlic oil and silymarin on the oxidative stress status in experimental rat models exposed to carbon tetrachloride induced liver injury.

MATERIALS AND METHODS

Materials:

Garlic oil was obtained from a commercial supplier, Silymarin was purchased from chemical industries.
development (CID) Giza and 10% liquid solution of CCl4, was obtained from El-Gomhurya company for chemical industries, Cairo, Egypt.

**Experimental Design:**
Sixty adult male albino rats weighing 150-170 g were randomly chosen from the animal house of the National Research Center, Cairo-Egypt. All animals received professional humane care in compliance with the guidelines of the Ethical Committee of the National Research Center, Cairo-Egypt. They were housed in standard cages and left to acclimatize for 7 days to laboratory condition before the commencement of the experiment. The animals were maintained on standard laboratory diet ad libitum.

The rats were divided into four groups; 15 rat each. Group I: was orally administered normal saline and served as control. Group II, III and IV: received feed and equal volume of (CCl4 and Paraffin oil) orally in a dose of 1 ml/kg b.wt according to Manoj and Aqued (2003). After 72 hours, liver enzymes were estimated in sera to confirm liver toxicity. Then groups III & IV were given Silymarin in a dose of 200 mg/Kg b.wt according to Tsai et al., (2008) and garlic oil in a dose of 200 mg/Kg b.wt according to Wu et al., (2001) respectively for 4 weeks.

**Urine Collection:**
The day before the termination of the experiment, rats were housed individually in metabolic cages for 24 hours to collect urine then centrifuged at 2000 g for 10 min and supernatants were withdrawn. Each sample was aliquoted into two parts; one was adjusted at pH 4.5 and stored at -20°C until assayed for the analysis of 8-hydroxydeoxyguanosine (8-OHdG) and the other was treated with 0.005% butylated hydroxy toluene (BHT) and stored at -80°C for the assessment of F2 isoprostane.

**Blood Collection:**
At the final day of the experiment, the rats were subjected to light ether anesthesia and blood was withdrawn from the optical vein. The blood was collected in polypropylene tubes, centrifuged at 3000 rpm for separation of sera. All samples were frozen at -20°C till assayed.

**Biochemical Parameter:**

**Diagnostic Enzymes:**
Serum ALT and AST measured by automated chemistry analyzer Olympous AU 400 and GGT was measured according to the method of Nielsen and Ash, (1978).

**Antioxidant Markers:**
Serum Superoxide-dismutase (SOD) and Catalase were determined according to Nishikimi et al., (1972) and Aebi, (1984) respectively using the colorimetric method.

**Oxidant Markers:**
Serum advanced oxidation protein products (AOPP) was measured according to Witko-Sarat et al., (1996). Urinary F2 isoprostane level was determined by enzyme immunoassay according to Montuschi et al., (2004). Analysis of urinary 8-OHdG was modified from the method described by Kim et al., (2001). Briefly, 8-OHdG was extracted from 1 ml urine. The eluents were dried under ultra-pure N2 stream and reconstituted in 5 ml de-ionized water for injection in HPLC.

**HPLC Condition:**
HPLC column for 8-OHdG was C18 (250 ×4.6, particle size 5µ). The mobile phase consists of acetonitrile / methanol / phosphate buffer (25/10/65) (v/v). Phosphate buffer was prepared by dissolving 8.8 g of potassium dihydrogen phosphate in 1000 ml de-ionized water and pH was adjusted to 3.5, the buffer then filtered 2 times before using. Flow rate was 1 ml/min using electrochemical detector with cell potential 600 mv.

**Statistical Analysis:**
The data analysis was carried out using the statistical package for social science (SPSS software version 16, Chicago, Illinois). All numeric variables were expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one way analysis of variance (ANOVA) test followed by Post Hoc LSD multigroup comparison. Pearson’s Correlation test was used for correlating parametric variables. For all tests a probability (p < 0.05) was considered significant.

**Results:**
Data of the present study revealed that the intoxication of rats with CCl4 caused a significant increase in ALT, AST and GGT activity in serum as compared to control group (group I). Oral administration of silymarin...
or garlic showed a significant decrease in all of these enzyme activity induced by CCl4 (Table1).

Table 1: Serum liver enzymes in the different studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl4</th>
<th>Silymarin</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>55.75 ± 3.7</td>
<td>102.5 ± 17*</td>
<td>65.07 ± 14.19*</td>
<td>61.99 ± 10.48*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>75.58 ± 4.17</td>
<td>124.3 ± 28.7*</td>
<td>92.57 ± 7.96*</td>
<td>88.72 ± 2.31*</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>3.64 ± 0.46</td>
<td>6.21 ± 1.16*</td>
<td>4.79 ± 0.71*</td>
<td>4.19 ± 0.44*</td>
</tr>
</tbody>
</table>

* = significant difference compared to control group (p<0.05).

A significant decrease in the serum levels of antiperoxidative enzymes (SOD & CAT) were observed in CCl4 – intoxicated rats as compared with levels of normal control rats. On the other hand, oral administration of silymarin or garlic significantly improved these adverse effects (Table2).

Table 2: Oxidant and antioxidant markers in the different studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl4</th>
<th>Silymarin</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum SOD (U/ml)</td>
<td>103.90 ± 1.7</td>
<td>63.68 ± 3.1*</td>
<td>85.61 ± 2.17*</td>
<td>93.92 ± 1.8*</td>
</tr>
<tr>
<td>Serum Catalase (U/ml)</td>
<td>1.307 ± 0.21</td>
<td>0.66 ± 0.15*</td>
<td>0.95 ± 0.21*</td>
<td>0.89 ± 0.16*</td>
</tr>
<tr>
<td>Urinary Isoprostane (pg/ml)</td>
<td>144.5 ± 16.18</td>
<td>783.5 ± 65.1*</td>
<td>344.85 ± 25.81*</td>
<td>351.54 ± 23.38*</td>
</tr>
<tr>
<td>Serum AOPP (µmol/L)</td>
<td>36.83 ± 5.7</td>
<td>70.66 ± 11.2*</td>
<td>48.23 ± 9.7*</td>
<td>45.65 ± 7.7*</td>
</tr>
<tr>
<td>Urinary 8-OHdG (ng/mg creatinine)</td>
<td>5.1 ± 1.1</td>
<td>28.14 ± 4.3*</td>
<td>15.73 ± 1.7*</td>
<td>11.13 ± 1.6*</td>
</tr>
</tbody>
</table>

* = significant difference compared to control group (p<0.05).

The oxidative DNA damage marker urinary 8-OHdG showed a significant increment in the rats received CCl4 alone as compared to the control group. Rats that received silymarin or garlic (groups III and IV) showed a significant decrease of the urinary 8-OHdG level as compared to the intoxicated group (group II) although its level is still higher than that of the control (Table 2).

The correlation between the investigated parameters using Pearson’s Correlation, Table (3) revealed a significant negative correlation with antioxidant markers and a significant positive correlation with the oxidant parameters in the different studied groups.

Table 3: Pearson’s Correlation coefficients between the serum liver enzymes and the Oxidant-antioxidant parameters in the different studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALT</th>
<th>AST</th>
<th>GGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>r = -0.587</td>
<td></td>
<td>r = -0.584*</td>
</tr>
<tr>
<td>SOD</td>
<td>r = -0.807*</td>
<td></td>
<td>r = -0.799*</td>
</tr>
<tr>
<td>Isoprostane</td>
<td>r = 0.815*</td>
<td></td>
<td>r = 0.795</td>
</tr>
<tr>
<td>AOPP</td>
<td>r = 0.631*</td>
<td></td>
<td>r = 0.617*</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>r = 0.789*</td>
<td></td>
<td>r = 0.729*</td>
</tr>
</tbody>
</table>

r: Correlation coefficient
** is significance at p < 0.01.

Discussion
Carbon tetrachloride (CCl4) is a common hepatotoxin used in the experimental study of liver diseases (Shenoy et al., 2001). This intoxication results in the stimulation of lipid peroxidation and the production of free radicals (Basu, 2003) which causes necrosis of hepatocytes, induces inflammation, and promotes the progression of hepatic fibrogenesis (Fu et al., 2008). The serum levels of ALT, AST, and GGT reflect the physiological state of the liver; they are changed according to the distortion of liver, resulting from cellular injury of the organ caused by toxic metabolites and diseases (Patrick-Iwuanyanwu et al., 2007).

Results of the present work indicate that CCl4 caused an increase in serum levels of the diagnostic enzymes (ALT, AST and GGT ) in rats that received CCl4 as compared to the control group .Such elevation suggests that intoxication was able to reach the liver and induce a detectable damage, as previously reported by Hukkeri and his colleagues., (2002) who proved the elevation in the plasma level of cytoplasmic and mitochondrial...
Urinary 8-OHdG levels were significantly diminished by the administration of silymarine or garlic in the present study. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage (Shaarawy et al., 2009). Administration of silymarin or garlic in the present work significantly reduced the activity of liver enzymes in CCl4 induced rats, a finding which is consistent with those shown before by Pradeep et al., (2007) and are almost definitely suggestive of protection of the structural integrity of the hepatocytes membrane or regeneration of damaged liver cells by test samples (Patrick-Twuanyanwu et al., 2007).

Free radical scavenging enzymes such as superoxide dismutase (SOD) and catalase (CAT) protect the biological systems from oxidative stress. The current study showed a significant decrease in SOD and CAT activity in rats intoxicated with CCl4 as compared to the control group. On the other hand, there was a significant increase in SOD and CAT activities in groups that received silymarin and garlic oil as compared to the intoxicated group. This improvement in the antioxidant status approached or even exceeded the control counterparts, a finding that may explain the modulatory effect of silymarin and garlic oil involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative stress (Shaarawy et al., 2009).

F2 isoprostane has been emerged as the most reliable marker of oxidative stress (lipid peroxidation) and can be used to evaluate the oxidative status in a number of human pathology (Basu, 2003). This isoprostane initially formed in situ on phospholipids, released into the circulation and could be found easily in plasma and urine because of its less reactivity than other lipid peroxidation products such as lipoperoxides and aldehydes (Comporti et al., 2008).

In the present study rats intoxicated with CCl4 showed an extremely elevated level of urinary F2 isoprostane as compared to the control group. These results are in agreement with Signorini et al., (2003). The toxicity of CCl4 to the liver is largely a result of the active metabolite, trichloromethyl radical that binds to tissue macromolecule inducing peroxidative degradation of membrane lipid of the endoplasmic reticulum rich in polyunsaturated fatty acids (Wood et al., 2003). Shenoy and his coworkers., (2001) postulated that such development would ultimately lead to the formation of lipid peroxides that in turn yield other products, among which are malondialdehyde and isoprostanes. On the other hand the third and fourth groups of animals who were administered silymarin or garlic after intoxication by CCl4 showed decline in the mean urinary F2 isoprostane level to an extent which was significantly lower than the second group intoxication with CCl4 alone and at the same time more or less similar to that of control group. Thus it may be inferred that silymarin and garlic have a potent hepatoprotective activity. Advanced oxidation protein product (AOPP) was also assessed in the present work. The data obtained showed that the increment and decrement of its level in the different studied groups were clearly parallel to those of F2 isoprostane. Telci and his colleagues (2000) explained that, damage to proteins may be more important than damage to lipids in oxidative stress in vivo. Oxidized proteins are functionally inactive and their unfoldings are associated with enhanced susceptibility to proteinases. Thus cells can generally remove oxidized proteins by proteolysis. However, certain oxidized proteins are poorly handled by cells together with possible alteration in the rate of production of oxidized proteins, this may contribute to their observed accumulation such as in diabetes and atherosclerosis (Woods et al., 2003). The present results have confirmed that AOPP has increasingly been used as a marker, instead of lipid peroxidation products, in demonstrating oxidative stress (Aller et al., 2008). Markers for DNA oxidation were few. Only in recent years 8-hydroxy-2-deoxy guanosine (8-OHdG) emerged as a sensitive marker of oxidative stress (Subash et al., 2010). Urinary 8-OHdG in particular, has been measured most frequently to indicate the extent of oxidative damage because it is non invasive, technically less involved and it reflects extremely low levels of oxidative damage (Evans et al., 2004). It is well known that the study of oxidative DNA damage is clinically important and it has been investigated in many diseases, including bladder and prostatic cancer (Chiou et al., 2003), cystic fibrosis (Brown et al., 1995), Rheumatoid arthritis (Rall et al., 2000) and essential hypertension (Subash et al., 2010).

The current study demonstrated that urinary 8-OHdG levels increased significantly in rats intoxicated with CCl4 as compared to the control rats. Azad et al., (2008) indicated that the imbalanced reactive oxygen species formation results in oxidative modification of macromolecule and subsequently genomic instability, which may explain the cause of elevation in 8-OHdG levels, occurred in the present study.

Urinary 8-OHdG levels were significantly diminished by the administration of silymarine or garlic in the third and fourth groups as compared to the second one which intoxicated with CCl4.

Restoration in the levels of 8-OHdG in the third group that received silymarine resulted from changing the tissue redox system by scavenging the free radicals and improving the antioxidant status in the liver during CCl4 hepatotoxicity (Pradeep et al., 2007). On the other hand the restoration occurred in the fourth group which received garlic may be attributable to the effectiveness of garlic in exerting anti-genotoxic, anti-clastogenic effects by modulating oxidative stress (Kumaraguruparan et al., 2005). The mechanism involved is unclear but garlic contains a number of organo- sulfur compounds which are widely believed to be the active agent (Isabella et al., 2006). Also garlic contains certain compounds such as germanium and selenium that play an important role in normalizing the oxygen utilization in the cell(Hussein et al., 2007).
The results of this study show significant statistical correlation between the level of liver functions enzymes in one side and the different oxidant-antioxidant parameters in the other one which are in line with the above mentioned mechanisms.

The present study provides a probable insight on the ability of silymarine and garlic to suppress the occurrence of CCL4 induced hepatotoxicity in rats by alleviating oxidant status through scavenging of free radicals, or by enhancing the activity of endogenous antioxidants thereby reversing the altered biochemical variables.

**REFERENCES**


