Patho-physiological studies on the Reverse Effect of Curcumin (Curcuma longa, Zingiberaceae) and Ursofalk (Ursodeoxycholic acid) against the Toxicity of Carbon Tetrachloride on Albino Rats

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Abstract

This study, was established five groups of albino rats to determine the therapeutic effect of Curcuma longa and ursofalk against the toxicity of CCl₄ in liver and kidney. Group (1) was received orally NaCl 0.9% and used as a normal group. Group (2) was injected intraperitoneal (i.p.) with CCl₄ (1 ml/kg), 3 times weekly, for 2 weeks. Group (3) was given orally Ursofalk (100 mg/kg per body weight), group (4) was given orally Curcuma longa (100 mg/kg body weight) and group (5) was given the same doses of Ursofalk plus Curcuma longa for 30 days respectively, post-injected intraperitoneal (i.p.) with CCl₄ (1 ml/kg) 3 times weekly, for 2 weeks. Two blood samples were collected, for hematological and biochemical parameters. Specimens from liver and kidney were collected for histopathological examination. Group (2) revealed a highly significant decrease in total RBCs, platelets, Hb and PCV, serum uric acid, albumin, glucose, HDL-cholesterol, besides catalase, GSH, SOD activities in liver tissue, while WBCs, serum ALT, AST, ALP, γ-GT, creatinine, urea, cholesterol, triglycerides and LDL-cholesterol levels, besides Malondialdehyde and Nitric Oxide levels in liver tissue showed a highly significant increase. Meanwhile, groups (3, 4 and 5) displayed reverse effect in all parameters and return to normal. The histological results displayed inflammation with necrosis and degenerative changes in group (2), while remain groups showed mild changes particularly in group (5). It could be concluded that CCl₄ induced destruction in liver and kidney, which showed a clear improvement by using of Ursofalk and Curcuma longa as treatment.

Keywords: Albino rats; Carbon tetrachloride; Ursodeoxycholic acid; Curcuma longa; Patho-physiological; Antioxidant enzymes

Introduction

Carbon tetrachloride (CCl₄) is considered as one of the environmental pollutants which mainly caused hepatotoxic effect; it is most widely used for experimental induction of hepatic cirrhosis [1] CCl₄ induces liver necrosis, fibrosis, cirrhosis and acute tubular necrosis in the kidney [2] In addition, CCl₄ also alters the antioxidant profile of the liver including the antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH) [3]. Thus, CCl₄ was chosen in this study as model for investigating radical-induced damage and its prevention in albino rats.

Ursodeoxycholic acid (UDCA) is normally present in human bile even in a low concentration of only about 3% of total bile acids; it has been used in Chinese traditional medicine for the treatment of liver diseases [4]. A number of clinical and experimental data have been obtained on a beneficial effect of UDCA in noncholestatic liver injury, UDCA prevents damaging liver mitochondrial function and structure in chronic alcohol intoxication [5], improves biochemical parameters and physical properties of liver plasma membranes [6].

Plants have played a significant role in maintaining human health and improving the quality of human life. Some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties [7]. Curcuma longa is one of the most common medicinal plants; it is a perennial herb and member of the Zingiberaceae (ginger) family cultivated extensively in Asia, India, China, and other countries with a tropical climate. It is widely used as a food additive and coloring agent [8]. It has been used as a traditional remedy for the treatment of inflammation and other pharmacological effects [9]. It acts as a free-radical scavenger or blocker to inhibit peroxidation of membrane lipids [8,10].

The aim of the work was, to elucidate the reverse effect of Curcumin (Curcuma longa, Zingiberaceae) and Ursofalk (Ursodeoxycholic acid) against the toxicity of carbon tetrachloride on albino rats.

Materials and Methods

Chemicals
- CCl₄ was obtained from El-Nasr Pharmaceutical Chemical Company.
- Ursofalk capsules (Ursodeoxycholic acid 250 mg per capsule) was purchased from local pharmacy in Egypt.
- Curcuma longa fresh rhizomes were purchased from local market, powdered and left in 95% (v/v) ethanol for 48 hours using Soxhlet apparatus. The extract was filtered and concentrated to dark yellow residue on a rotary evaporator [11].

Animals
Fifty adult males albino rats of age range (2.5-3 months) and weight about (230-280 g), where maintained in animal house in cages (10 rats/cage), in air conditioned room, they fed on adequate stable commercial food.
balanced diet and examined daily for two weeks, before starting the experiment.

**Experimental design**

The experimental animals were classified into five groups, each containing 10 rats.

- **Group 1**: The rats were received orally NaCl 0.9% used as (normal group).
- **Group 2**: The rats were injected i.p. with CCl₄ (1 ml/kg per b.wt.), 3 times weekly, for 2 weeks used as (control group).
- **Group 3**: The rats were injected i.p. with CCl₄ (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by oral administrated doses of Ursolfalk drug (100 mg/kg per body weight) daily for 30 days.
- **Group 4**: The rats were injected i.p. with CCl₄ (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by oral administrated of Curcuma longa (100 mg/kg b.wt.) daily for 30 days.
- **Group 5**: The rats were injected i.p. with CCl₄ (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by oral administrated of Curcuma longa and Ursolfalk drug (100 mg/kg per b.wt.) plus Curcuma longa (100 mg/kg body weight) daily for 30 days.

The doses used in this study were chosen based on previous researches. CCl₄ [12], Ursolfalk drug [13] and curcuma longa [11]. All experimental animals were sacrificed at the end of the experiment. The blood, serum, besides liver tissue were collected from the experimental groups for hematological and biochemical analysis, respectively. Specimens from liver and kidneys were collected from the sacrificed animals for histopathological examinations.

**Haematological analysis**

Complete blood picture red blood cells count (RBCs), leukocytes count (WBCs), total hemoglobin, platelets count and hematocrit assays done by Automated Hematology Analyzer (Diff3) Mek-6410/Mek-6420.

**Biochemical analysis in serum**

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [14], alkaline phosphatase (ALP) [15], albumin [16], γ-Glutamyl transferase (γ-GT), creatinine [17], urea [18], uric acid [19], glucose [20], cholesterol [21], triglycerides [22], HDL-Cholesterol [23] and LDL-Cholesterol [24], were analyzed according to the reported methods.

**Biochemical analysis of liver tissue homogenate**

Reduced glutathione (GSH) [25], Superoxidedismutase (SOD) [26], Catalase (CAT) [27], Malondialdehyde (MDA) [28] and nitric oxide [29] were analyzed as described in the reported methods. All mentioned kis were bought from bio-diagnostic co. Giza, Egypt.

**Preparation of tissue homogenate**

Liver tissues were homogenized as following:

a) Prior to dissection, perfuse tissue with phosphate buffered saline (PBS) solution pH=7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots.

b) Homogenize the tissue in 10-15 ml cold buffer per gram tissue in pastel hemogenezor.

c) Centrifuge at 4000 rpm for 15 min at 4ºC.

d) Remove the supernatant for biochemical assay

**Histopathological examination**

Specimens from liver and kidneys were collected from the sacrificed animals. The tissues were kept in 10% neutral buffered formalin and processed by paraffin embbeiding technique, sectioned at 5 µ thickness and stained with hematoxylin and eosin [30].

**Statistical analysis**

The results expressed as means ± S.E. and made by one-way analysis of variance (ANOVA) using Graph Pad Prism 03n software. Statistical significance was set at p<(0.05).

**Results**

**Haematological results**

As shown in Table 1 RBCs count, PCV value and platelets count were highly significantly decreased (p<0.01) and Hb concentration was significantly decreased (p<0.05) while WBCs count was highly significantly increased (p<0.01) in control group when compared with normal group. RBCs count, Hb concentration, PCV value and Platelets count were significantly increased in rats treated with ursofalk (group3), increased in rats treated with curcuma longa (group 4) and highly significantly increased in rats treated with ursofalk drug+curcuma longa (group 5), while WBCs count was significantly decreased in groups (3 and 4) and highly significantly decreased (group 5) when compared with control group.

**Biochemical results**

The activities of serum ALT, AST, ALP and γ-GT were highly significant increased while serum albumin was significantly decreased in control group when compared with normal group as shown in Table 2. A significant decrease of ALT, AST, ALP and γ-GT showed in (group curcuma longa 4) and highly significantly increased in rats treated with ursofalk (group3), increased in rats treated with curcuma longa (group 4) and highly significantly increased in rats treated with ursofalk drug+curcuma longa (group 5), while WBCs count was significantly decreased in groups (3 and 4) and highly significantly decreased (group 5) when compared with control group.

**Table 1**: Effect of daily oral administration doses of Ursolfalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma longa+Ursolfalk drug after 30 days of treatment on complete blood count (RBCs, WBCs, Hb, platelets and PCV) of Albino rats, injected with CCl₄ (1 ml/kg. b. w.).

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBCs count <em>(×10³/mm³)</em></th>
<th>WBCs count <em>(×10³/mm³)</em></th>
<th>Hb Conc. (g/dl)</th>
<th>Platelets count <em>(×10³/mm³)</em></th>
<th>P C V value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
</tr>
<tr>
<td>Group 1</td>
<td>7.029 ± 0.21</td>
<td>7.043 ± 0.30</td>
<td>13.5 ± 0.28</td>
<td>505.0 ± 4.83</td>
<td>37.50 ± 1.51</td>
</tr>
<tr>
<td>Group 2</td>
<td>5.35 ± ± 0.13</td>
<td>10.99 ± ± 0.25</td>
<td>11.49 ± ± 0.22</td>
<td>412 ± ± 3.395</td>
<td>29.5 ± ± 1.68</td>
</tr>
<tr>
<td>Group 3</td>
<td>6.1 ± ± 0.23</td>
<td>8.48 ± ± 0.19</td>
<td>12.63 ± ± 0.23</td>
<td>451.6 ± ± 9.54</td>
<td>34.57 ± ± 1.31</td>
</tr>
<tr>
<td>Group 4</td>
<td>5.80 ± ± 0.22</td>
<td>9.07 ± ± 0.27</td>
<td>12.29 ± ± 0.33</td>
<td>443.1 ± ± 3.47</td>
<td>33.59 ± ± 1.16</td>
</tr>
<tr>
<td>Group 5</td>
<td>6.62 ± ± 0.21</td>
<td>7.33 ± ± 0.32</td>
<td>13.20 ± ± 0.41</td>
<td>485.7 ± ± 3.14</td>
<td>36.67 ± ± 0.83</td>
</tr>
</tbody>
</table>

a= significantly decreased from normal at p<0.05
+b= significantly increased from control at p<0.05
++a= highly significantly increased from normal at p<0.01
++b= highly significantly increased from control at p<0.01
--b= highly significantly decreased from control at p<0.01

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3), the same parameters decreased in (group 4). ALT, AST, ALP showed a highly significant decrease while γ-GT showed a significant decrease in (group 5) when compared with control group. Serum albumin was significantly increased in (group 3), increased in (group 4) and highly significant increase in (group 5) when compared with control group.

As shown in Table 3 the concentration of serum creatinine and urea in control group were highly significantly increased, while serum uric acid concentration was significantly decreased when compared with normal group. Creatinine and urea concentration showed a significant decrease (group 3), decreased (group 4) and highly significant decreased in (group 5) while uric acid concentration recorded a significant increase in (group 3), increased in (group 4) and highly significant increase in (group 5) when compared with control group.

Serum glucose level as shown in Table 3 recorded a significant decrease in control group when compared with normal group. A significant increase showed in glucose level (group 3), increased (group 4) and highly significant decreased in (group 5) while uric acid concentration was highly significantly increased, while serum HDL-cholesterol was significantly decreased in control group when compared with normal group. A significant decrease in serum cholesterol, triglycerides and LDL-cholesterol and a significant increase in serum HDL-cholesterol were recorded in groups (3, 4 and 5) when compared with control group.

### Liver homogenate biochemical results

The results recorded in Table 5 revealed that the activities of liver GSH and CAT were highly significantly decreased and the activity of liver SOD was significantly decreased while activities of liver MDA and NO were highly significant increased in control group when compared with normal group. The activities of liver GSH, SOD and CAT were significantly increased (group 3), increased in (group 4) and highly significantly increased in (group 5) when compared with control group. The activities of liver MDA and NO were significant decreased in (group 3), decreased in (group 4) and highly significant decreased in (group 5) when compared with control group.

### Histopathological results

The liver in group (2) showed congestion with severe dilatation

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (Units/ml)</th>
<th>AST (Units/ml)</th>
<th>Albumin (g/dL)</th>
<th>ALP (IU/L)</th>
<th>γ -GT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
</tr>
<tr>
<td>Group 2</td>
<td>70.71 ± 1.90</td>
<td>77.86 ± 2.44</td>
<td>3.36 ± 0.173</td>
<td>64.35 ± 1.65</td>
<td>35.07 ± 1.42</td>
</tr>
<tr>
<td>Group 3</td>
<td>106.4 ± 3.50</td>
<td>119.9 ± 2.75</td>
<td>1.49 ± 0.171</td>
<td>104.1 ± 3.79</td>
<td>74.75 ± 2.01</td>
</tr>
<tr>
<td>Group 4</td>
<td>87.86 ± 1.57</td>
<td>91.43 ± 2.33</td>
<td>2.20 ± 0.141</td>
<td>87.66 ± 2.80</td>
<td>75.80 ± 2.61</td>
</tr>
<tr>
<td>Group 5</td>
<td>77.43 ± 2.20</td>
<td>81 ± 1.45</td>
<td>2.77 ± 0.169</td>
<td>73.67 ± 2.21</td>
<td>41.46 ± 1.75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dL)</th>
<th>Urea (g/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Glucose (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.425 ± 0.05</td>
<td>29.70 ± 2.44</td>
<td>3.23 ± 0.24</td>
<td>88.26 ± 2.71</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.912 ± 0.061</td>
<td>75.96 ± 1.91</td>
<td>1.43 ± 0.14</td>
<td>60.36 ± 2.14</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.628 ± 0.054</td>
<td>48.80 ± 2.73</td>
<td>2.26 ± 0.17</td>
<td>73.24 ± 2.17</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.757 ± 0.024</td>
<td>57.53 ± 1.92</td>
<td>2.08 ± 0.20</td>
<td>70.24 ± 2.19</td>
</tr>
<tr>
<td>Group 5</td>
<td>0.494 ± 0.027</td>
<td>36.77 ± 1.67</td>
<td>2.70 ± 0.168</td>
<td>82.71 ± 2.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>HDL-Cholesterol (mg/dL)</th>
<th>LDL-Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
<td>Mean ± S. E.</td>
</tr>
<tr>
<td>Group 2</td>
<td>65.26 ± 2.69</td>
<td>62.75 ± 1.98</td>
<td>42.22 ± 2.10</td>
<td>28.87 ± 1.71</td>
</tr>
<tr>
<td>Group 3</td>
<td>89.19 ± 2.28</td>
<td>83.62 ± 1.87</td>
<td>29.26 ± 2.37</td>
<td>40.07 ± 2.91</td>
</tr>
<tr>
<td>Group 4</td>
<td>76.66 ± 1.60</td>
<td>74.32 ± 1.96</td>
<td>35.07 ± 2.07</td>
<td>33.36 ± 2.10</td>
</tr>
<tr>
<td>Group 5</td>
<td>81.90 ± 2.23</td>
<td>77.90 ± 1.46</td>
<td>33.25 ± 1.93</td>
<td>35.29 ± 1.67</td>
</tr>
<tr>
<td>Group 6</td>
<td>68.86 ± 2.52</td>
<td>66.74 ± 2.36</td>
<td>40.87 ± 2.23</td>
<td>29.24 ± 2.01</td>
</tr>
</tbody>
</table>

Table 2: Effect of daily oral administration dosages of Ursolfalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursolfalk drug after 30 days of treatment on serum ALT, AST, albumin, ALP and γ-GT levels of Albino rats, injected with CCl4 (1 ml/kg b. w.).

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<tr>
<th>Groups</th>
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Table 3: Effect of daily oral administration dosages of Ursolfalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursolfalk drug after 30 days of treatment on serum creatinine, urea, uric acid and glucose levels of Albino rats, injected with CCl4 (1 ml/kg b. w.).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dL)</th>
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<th>HDL-Cholesterol (mg/dL)</th>
<th>LDL-Cholesterol (mg/dL)</th>
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Table 4: Effect of daily oral administration dosages of Ursolfalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursolfalk drug after 30 days of treatment on serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels of Albino rats, injected with CCl4 (1 ml/kg b. w.).
in the blood sinusoid causing hepatic atrophy, besides vacuolation in some hepatocytes. Focal areas of dilated blood vessels replaced the necrotic hepatic cells (Figure 1). The kidneys in the same group showed thickening in the wall of the blood vessels with perivascular edema. Perivascularitis characterized by inflammatory edema surrounded the congested blood vessels, besides thrombosisis in its components (Figure 2). Fibrous tissues proliferated among the renal tubules forming lobules in the kidneys (Figure 3).

The liver in the rats of group (3) noticed moderate degeneration and necrosis in some of hepatocytes. Congestion in the blood vessels with aggregation of rounded cells around it (Perivascularitis) was seen. Regeneration was detected in the hepatic cells (Figure 4). The hepatocytes displayed normal in the architecture of the hepatic cells adjacent to the central vein vacular degeneration in the hepatocytes with congestion in the central veins (Figure 5). The kidneys in group (3) showed perivascular edema with congestion and hyalinization in the wall of the blood vessels (Figure 6).

The Liver in group (4) showed few fibrous tissues proliferation surrounded the blood vessels and bile ducts in the portal tract (Figure 7). The kidneys in group (4) showed congestion in the glomerular capillaries. The renal tubules displayed mild necrosis and degenerative changes. Hyalinization in the wall of the blood vessels was detected. The liver in group (5) showed congestion in the blood vessels, besides necrosis in few of hepatic cells. The Kidneys in group (5) showed congestion in the glomerular capillaries and periglomerular blood vessels (Figure 8).

**Discussion**

In the present study carbon tetrachloride (1 ml/kg body weight) induced a highly significant decrease in RBCs count, plateles count and PCV value and significant decrease in Hb concentration. According to [31], depletion in RBCs count and Hb content leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture. Furthermore, the depression in RBCs count and Hb content recorded in the present work could be attributed to disturbed hemopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation [32]. CCl4 induced a highly significant increase in WBCs count; this increase may be attributed to the increased utilization of enzymes action; this could explain the reduction in serum liver marker enzymes could be attributed to the free radicals which caused structural integrity damage of the liver cell membrane and hence a leakage of the cellular enzymes in to the blood [37]. The significant reduction of all the serum liver enzymes and the significant increase in serum albumin in case of ursofalk drug has been attributed to its mechanism on membrane stabilizing [39]. Curcumin longa may enhance the molecular mechanism of enzymes action; this could explain the reduction in serum liver enzymes [40]. Also this reduction in serum ALT and AST levels due to the antioxidant activity of curcuma longa [41].

CCl4 (1 ml/kg body weight) in the present study induced alternations in creatinine urea and uric acid (control group) the increase in serum creatinine and urea levels may indicate a reduction in the glomerular filtration rate (GFR) as a result of acute renal dysfunction as mentioned by Gavin [42]. The reduction in serum uric acid level in the present study may be attributed to the increased utilization of uric acid against increased production of the free radicals since it has a capable especially of reacting with free radicals [43]. Treatment with ursofalk drug (group3) and curcuma longa (group4) ameliorates the elevation of kidney functions parameters, as mentioned by Wong [44] Ursodeoxycholic acid is an antioxidant, and its vascular effects could be mediated via a reduction in oxidative stress. The improvements in renal function markers in (group 4) may be due to the protective effect of curcumin against renal injury by suppressing oxidative stress, increasing kidney GSH content and glutathione peroxidase activity [45]. The present study declared that, administration of CCl4 induced a significant reduction in serum glucose compared with normal group this may be due to the decreased hepatic glycogen content after treatment with CCl4 which reflecting decreased gluconeoegenesis by the liver [46]. The improvements in glucose levels in (group3) could be explained by Zavodnik [47] who mentioned that ursodeoxycholic acid completely normalized the blood glucose level at alloxan-treated rats where it may prevents induction and development of diabetes and its complications by protecting the β-cell membrane. In (group 4) curcuma longa improvement mechanism explained by Kanitkar [48] who stated that curuminoid, which is component of turmeric, inhibited the formation of oxygen species associated with damage and dysfunction of langerhans islets. The present results showed that CCl4 induced a highly significant increase in serum Cholesterol, Triglycerides and LDL-Cholesterol levels while it induced a reduction in serum albumin, compared with normal animals, the hepatotoxic effects of CCl4 are largely due to its active metabolite, trichloromethyl radical [36]. These elevations in the serum liver marker enzymes could be attributed to the free radicals which caused structural integrity damage of the liver cell membrane and hence a leakage of the cellular enzymes in to the blood [37]. The reduction in serum albumin (control group) is due to the hepatic injury which caused by CCl4 [38]. The significant reduction of all the serum liver enzymes and the significant increase in serum albumin in case of ursofalk drug has been attributed to its mechanism on membrane stabilizing [39]. Curcumin longa may enhance the molecular mechanism of enzymes action; this could explain the reduction in serum liver enzymes [40]. Also this reduction in serum ALT and AST levels due to the antioxidant activity of curcuma longa [41].

<table>
<thead>
<tr>
<th>Groups</th>
<th>GHS (μL)</th>
<th>Catalase (u/g)</th>
<th>SOD (u/g)</th>
<th>MDA (n mol /g)</th>
<th>NO μmol / L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group. 1</td>
<td>302.9 ± 30.92</td>
<td>1.86 ± 0.172</td>
<td>598 ± 39.25</td>
<td>5.594 ± 0.372</td>
<td>65.12 ± 3.723</td>
</tr>
<tr>
<td>Group. 2</td>
<td>194.4 ± 18.87</td>
<td>0.847 ± 0.231</td>
<td>434 ± 37.98</td>
<td>13.56 ± 1.142</td>
<td>90.62 ± 5.329</td>
</tr>
<tr>
<td>Group. 3</td>
<td>243.9 ± 18.47</td>
<td>1.291 ± 0.225</td>
<td>545 ± 39.48</td>
<td>8.917 ± 1.174</td>
<td>75.05 ± 4.561</td>
</tr>
<tr>
<td>Group. 4</td>
<td>234.6 ± 18.66</td>
<td>1.10 ± 0.223</td>
<td>508.6 ± 43.82</td>
<td>9.930 ± 1.098</td>
<td>80 ± 4.490</td>
</tr>
<tr>
<td>Group. 5</td>
<td>279.9 ± 24.18</td>
<td>1.653 ± 0.154</td>
<td>580.6 ± 40.92</td>
<td>6.481 ± 0.912</td>
<td>68.69 ± 3.033</td>
</tr>
</tbody>
</table>

- a=significantly decreased from normal at p<0.01
- b=significantly increased from control at p<0.05
- ++=highly significantly decreased from normal at p<0.01
- ++b=highly significantly increased from normal at p<0.01
- --=highly significantly decreased from control at p<0.01

**Table 5:** Effect of daily oral administration doses of Ursfoalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursfoalk drug after 30 days of treatment on GSH, catalase, SOD, MDA and NO of Albino rats, injected with CCl4 (1 ml/kg b. w.).
may have altered membrane structure and function as suggested by the increase in cholesterol level [49]. Ursodeoxycholic acid may exert significant changes on cholesterol metabolism in patients with primary biliary cirrhosis and, ultimately, significantly reduce the risk associated with hypercholesterolemia [50,51]. The hyperlipidemic effect of CCl4 ameliorated in rats treated with curcuma longa (group 4) [52], reported that, the effect of curcumin on cholesterol could be due to an effect on cholesterol absorption, degradation or elimination.

The present study showed that administration of CCl4 produced marked oxidative impact as evidenced by the highly significant decreased in the hepatic SOD, catalase, and GSH activities. Peroxidative damage by CCl4 is the result of reductive dehalogenation, which is catalysed by P-450 and forms the highly reactive trichloromethyl-free radical CCl3. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical CCl3OO. Both radicals are capable of binding to proteins or lipids or of abstracting a hydrogen atom from an unsaturated lipid, which initiates liver damage and plays a significant role in the pathogenesis of diseases [53]. In the present study, administration of CCl4 induced a highly significant increase in MDA and nitric oxide levels in liver of treated rats, this increase explained by Fraga et al. [54], who stated that the high significant elevation of MDA level in liver homogenate of rats treated with CCl4 indicated excessive formation of free radicals and activation of lipid peroxidation of cell damage [55], proposed that a high level of nitric oxide is associated...
with CCl₄-induced acute liver injury. In rats treated with Ursokalk drug, the elevation in GSH, SOD, catalase, MDA and NO activities explained by Lukivskaya et al. [56] who stated that Ursodeoxycholic acid administration reduced the production of reactive oxygen forms in the liver, the content of lipid peroxidation carbonyl-containing products (alkenals, alkanals, ketones, oxyalkenals and MDA), and the activities of antioxidant defense enzymes (SOD). Moreover, Ursodeoxycholic acid normalized liver microsomal cytochrome P-450 level. The ability of *Curcuma longa* to increase GSH, catalase and SOD activities and decreased MDA and NO levels in liver tissue of treated rats explained by Sreejayan et al. [57], who stated that the presence of phenolic groups in the structure of *Curcuma* is fundamental to explain its ability to eliminate oxygen free radicals from the medium and that methoxy group increases this activity. Besides, the phenolic moiety of the curcumin structure can donate hydrogen atoms to deleterious oxy radicals and form the less reactive phytoxid radicals in the process [58,59]. stated that *Curcuma longa* extracts produced significant reduction in NO level which may be attributable to the bioactive substance curcumin, which scavenges free radicals and inhibits nitric oxide synthesis activity. Rats treated with Ursokalk drug plus *Curcuma longa* (group 5) showed marked improvements in all biochemical parameters this may be attributed to the strong antioxidant effects of the both treatments [60,61].

The results of biochemical alterations were insured by histopathological examination of the liver and kidney in the intoxicated rats (control group). Treatment with Ursokalk drug (group 3) and *Curcuma longa* (group 4), as antioxidants treatment, could improve these pathological changes, even more (group 5) showed a marked improvements in liver and kidney tissues. It could be concluded that [62] CCl₄-induced severe destruction in most organs, which showed a clear improvement by using combination of Ursokalk and *Curcuma longa*.

**Acknowledgment**

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**References**


