Hepatoprotective Role of Zinc Sulphate in Carbon Tetrachloride Induced Liver Toxicity

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Abstract

Objective: To evaluate the protective role of zinc sulphate in carbon tetrachloride induced hepatic changes in rabbits.

Study Design: Experimental study.

Place of Study: Department of Pharmacology, ISRA University Hyderabad.

Duration of Study: July 2011 to November 2011.

Materials and Methods: A total of 45 rabbits were divided equally into three groups including A, B and C. These groups were further subdivided into (A1, B1 and C1) consisting of 5 animals each who received treatment for one week whereas sub groups (A2, B2 and C2) consisting of 5 animals each received treatment for two weeks and sub groups (A3, B3, and C3) consisting of 5 animals each received treatment for three weeks. The 15 animal included in group A treated as (Control) received normal saline. The 15 animals in group B were treated with carbon tetrachloride (CCl4). The 15 animals in group C were treated with CCl4 along with zinc sulphate.

Result: In present study revealed the hepatotoxic effects on liver morphology showing sinusoidal congestion, periportal inflammation, kupffer cell hyperplasia, steatosis, necrosis and fibrosis produced by carbon tetrachloride. These findings were reduced in rabbits treated with zinc sulphate.

Conclusion: This study concludes that zinc sulphate has hepatoprotective effects in CCl4 induced hepatotoxicity.

Abbreviations: Carbon Tetrachloride; Zinc Sulphate; Hepatotoxicity; Hepatoprotective

Introduction

CCl4 has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin. CCl4, catalyzed by CyP450 in the liver cell and yield CCl3 radical. This trichloromethyl radical attack microsomal lipids leading to peroxidation [1]. The experimental intoxication induced by CCl4 is widely used for modeling liver Injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCl4; this is cytochrome P-450 dependant. Free radicals initiate the process of lipid peroxidation, which is generally cause of inhibition of enzyme activity [2]. Exposure to high concentrations of carbon tetrachloride (including vapor) can affect the central nervous system, degenerate the liver [3] and kidneys and may result (after prolonged exposure) in coma and even death [4]. Chronic exposure to carbon tetrachloride can cause liver [5] and kidney damage and could result in cancer [6].

Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement. Zinc is an essential mineral of "exceptional biologic and public health importance" [7]. Lobster and red meats, especially beef, lamb and liver have some of the highest concentrations of zinc in food [8]. Zinc is also found in beans, nuts, almonds, whole grains, pumpkin seeds, sunflower seeds and blackcurrant, Wheat (germ and bran) and various other seeds (sesame, poppy, alfalfa, celery, mustard) [9].

Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes [10]. It is believed to possess antioxidant properties, which may protect against accelerated aging of the skin and muscles of the body; studies differ as to its effectiveness [11]. Zinc could reduce the symptoms of Allergic Rhinitis possibly by decreasing inflammation. Zinc plays a protective role in Age-related macular degeneration (AMD). Zinc deficiency causes alterations in immune
response that probably contribute to increased susceptibility to infections, such as those that cause diarrhea, especially in children [12].

The investigation of the mode of action of zinc has also attracted considerable attention recently because of its hepatoprotective effects on hepatic injury involving the inhibition of lipid peroxidation [13], but the mechanisms underlying the antioxidant effects of zinc remain unclear. Several studies on the acute administration of zinc to rats reported a depression of hepatic cytochrome P450 activity associated with a protection against hepatotoxicity. This phenomenon was probably, secondary to a decrease in the production of free radical metabolites [14].

Thus the present study is conducted to evaluate the protective role of zinc sulphate in Carbon tetrachloride induced hepatic changes in rabbits.

Materials and Methods

The present experimental study consisted of 45 healthy adult male and female rabbits who were weighing 1.5 kg or more. The study was conducted in the Department of Pharmacology, ISRA University Hyderabad. The three groups A, B and C each consisted of 15 animals. Group A was treated as control group whereas Group B was treated with carbon tetrachloride and Group C with carbon tetrachloride along with zinc sulphate.

Each group was further divided into 03 sub groups consisting of 05 animals each into (A1, A2 and A3), (B1, B2 and B3) and (C1, C2 and C3). The sub groups (A1, B1 and C1) received treatment for one week and were sacrificed, whereas sub group (A2, B2 and C2) received treatment for two weeks and were sacrificed while. However, sub groups (A3, B3 and C3) received treatment for three weeks.

Group A: All sub groups (A1, A2 and A3) were treated as controls and were given 0.9% isotonic saline solution at a dose level 4 ml/kg on alternate day. The animals were sacrificed at the end of their respective period of time [15].

Group B: The animals in the sub groups (B1, B2 and B3) were treated with CCl4 dissolved in olive oil (1:1 ratio) at a dose level of 1.9 ml/kg orally on alternate day. The animals were sacrificed at the end of their respective period of time [15].

Group C: The animals in the sub groups (C1, C2 and C3) were treated with CCl4 dissolved in olive oil at a dose level of 1.9 ml/kg along with zinc sulphate 1 mg/kg body weight on alternate day. The animals were sacrificed at the end of their respective period of time [15].

At the end of respective period of treatment followed by and sacrifices the animals skin layers and fascia was separated away and liver was identified and removed. The liver tissue specimens were further processed for gross and microscopic examinations. The routine Haematoxyline and Eosin staining was applied and slides were prepared for microscopic examination.

The statistical analysis was carried out by using Chi-square to compare the different histological findings of animals in group A, B and C. The animals were sacrificed at week 1, 2 and 3 to determine the significance at the P value of <0.05 considered as significant.

Results

In the present study 45 liver specimens were processed with paraffin embedding and blocks were prepared for staining with Hematoxylin and Eosin. Microscopic examination of liver slides was carried out at 1st, 2nd, 3rd weeks.

a) At 1st week: 5 rabbits of sub group A1, B1 and C1 were sacrificed respectively. Normal liver histology was noted in rabbit’s liver treated with isotonic saline. Whereas microscopic examination of the 5 rabbit’s liver treated with CCl4 revealed sinusoidal congestion and periportal inflammation in two cases and sinusoidal congestion, perportal inflammation and kupffer cell hyperplasia in three animals. Histopathological findings of 5 rabbit’s liver which were given CCl4 along with Zinc sulphate, 3 of them showed no changes and 2 of them showed sinusoidal congestion and perportal inflammation (Table 1).

Table 1: Histopathological findings of liver at 1st Week.

<table>
<thead>
<tr>
<th>Rabbit Distribution</th>
<th>No</th>
<th>Findings</th>
<th>Sinusoidal congestion + periportal inflammation</th>
<th>Sinusoidal congestion + periportal inflammation + Kupffer cell</th>
<th>Steatosis</th>
<th>Piecemeal necrosis</th>
<th>Bridging necrosis</th>
<th>Fibrosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>CCl4</td>
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<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>CCl4 + ZS</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
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<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>

*Significance value (P-value) = 0.06 (Non-significant).

b) At 2nd week: 5 rabbits from sub group A2, B2 and C2 were sacrificed. Normal liver histology was noted in rabbit’s liver treated with isotonic saline. Whereas microscopic examination of the 5 rabbit’s liver treated with CCl4 revealed sinusoidal congestion, periportal inflammation and kupffer cell hyperplasia in three animals while steatosis and piecemeal necrosis each was observed in one animal. Histopathological findings of 5 rabbit’s liver given CCl4 along with Zinc sulphate showed normal histology in one animal, 3 of them showed sinusoidal congestion and periportal inflammation and 1 of them showed sinusoidal congestion, periportal inflammation and kupffer cell hyperplasia (Table 2).
The present study revealed the hepatotoxic effects of CCl₄ proved by presence of sinusoidal congestion, steatosis, necrosis and fibrosis. These effects are consistent with the findings reported in a study conducted by Weber et al. [17]. Cytochrome 2E1 and others convert the CCl₄ to CCl₃⁺, which in turn binds the various cellular and nuclear molecules thus impairing the lipid metabolism and results in fatty change within the hepatocytes [17]. Khan et al. [18] also reported that CCl₄ has damaging effects on the liver. These results correlate with the findings of present study. In present study, Zinc sulphate proved to be hepatoprotective by reducing the toxic effects of CCl₄. However, Steven et al. [19] reported that there were no histological changes seen in animal model treated with zinc which is inconsistent with our findings. Goela et al. [20] have reported that zinc improved the histomorphological features of the liver of male rats in chlorpyrifos induced liver injury. This study is consistent with our present study, in which we also observed that zinc reduces steatosis, necrosis and fibrosis in liver. The possible mechanism reducing the toxic effects by zinc treatment was by increasing the antioxidant levels of Glutathione and catalase and also by decreasing the exidant levels of superoxide dismutase [20]. Miranda et al. [21] have reported that zinc appeared to be safe and possibly protective against CCl₄ induced hepatotoxicity, the study is consistent with present study.

### Table 2: Histopathological findings of liver at 2nd Week.

<table>
<thead>
<tr>
<th>Rabbit Distribution</th>
<th>No Findings</th>
<th>Sinusoidal congestion + periportal inflammation</th>
<th>Sinusoidal congestion + periportal inflammation + Kupffer cell</th>
<th>Steatosis</th>
<th>Piecemeal necrosis</th>
<th>Bridging necrosis</th>
<th>Fibrosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>CCl₄</td>
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<td>0</td>
<td>0</td>
<td>5</td>
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<tr>
<td>CCl₄ + ZS</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
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<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
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<td>0</td>
<td>15</td>
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</table>

*Significance value (P-value) = 0.025 (Significant).

c) At 3rd week: 5 rabbits from subgroup A₃, B₃ and C₃ were sacrificed. Normal liver histology was noted in rabbit’s liver treated with isotonic saline. Whereas microscopic examination of the 5 rabbit’s liver treated with CCl₄ revealed steatosis and piece meal necrosis each in one animal, 2 of them showed bridging necrosis and 1 of them showed fibrosis. Histopathological findings of 5 rabbit’s liver treated with CCl₄ along with Zinc sulphate showed normal histology in one animal, 3 of them showed sinusoidal congestion and periportal inflammation and 1 of them showed sinusoidal congestion, periportal inflammation and kupffer cell hyperplasia (Table 3). The results revealed that prolong duration of CCl₄ exposure for three weeks time has more toxic effects which were also reduced with treatment with zinc sulphate for the longer time.

### Table 3: Histopathological Findings of Liver at 3rd Week.

<table>
<thead>
<tr>
<th>Rabbit Distribution</th>
<th>No Findings</th>
<th>Sinusoidal congestion + periportal inflammation</th>
<th>Sinusoidal congestion + periportal inflammation + Kupffer cell</th>
<th>Steatosis</th>
<th>Piecemeal necrosis</th>
<th>Bridging necrosis</th>
<th>Fibrosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>CCl₄ + ZS</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

*Significance value (P-value) = 0.049 (Significant).

### Discussion

As in this study, zinc sulphate also played a protective role in CCl₄ treated group, which correlate with the study of Rogalska et al. [22] showing that zinc also normalized the CCl₄ induced necrosis. Zinc administration resulted in improvement in the structure of hepatocytes in Acetaminophen induced hepatotoxicity in mice [23]. These results are consistent with this study that zinc administration halts the hepatocytes damage.

### References