COMPARATIVE EFFECT BETWEEN CHITOSAN AND CHITOSAN-Cu COMPLEX ON CARBON-TETRACHLORIDE (CCL₄) INDUCED LIVER DAMAGE IN RATS
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Zoology Dept., Faculty of Science, Mansoura University, Mansoura, Egypt

ABSTRACT
BACKGROUND: Carbon tetrachloride (CCL₄) is a toxic material known to induce lipid peroxidation and liver damage.

The possible protective roles that involved by chitosan or chitosan-Cu complex against CCL₄ induced liver intoxication were investigated in male rats.

RESULTS: CCL₄ administrated at dose 20 mg/kg body weight i.p., exceed malondialdehyde (MDA) and protein carbonyl (PC), depleted superoxide dismutase (SOD) and glutathione (GSH), in concomitant with marked increase in investigated liver function parameters, alanine aminotransferase and aspartate aminotransferase (ALT, AST), impaired serum and liver total protein, albumin and globulin. An elevation in serum and hepatic total lipids, total cholesterol, triglycerides and serum LDL and VLDL levels as well as a low level of HDL were recorded. In the same time, there was a significant increase in sodium and iron contents in the serum while a significant decrease in potassium and zinc contents were recorded.

Animals pretreated with chitosan (200 mg /kg body weight) orally by stomach tube for 21 consecutive days prior to CCL₄ challenge significantly attenuated most of the tested parameters, strengthen antioxidant defense system, ameliorated liver function effectively. Chitosan-Cu complex has a protective effect by a higher degree than that of chitosan only.

CONCLUSION: These findings suggest that pretreatment with chitosan-Cu complex has higher hepato-protective effects than that of only chitosan against CCL₄ induced toxicity in rat.

Key words: Carbon tetrachloride (CCL₄) – Chitosan – lipid peroxidation – liver functions.

INTRODUCTION
Liver disease is considered to be a serious health problem, as the liver is an important organ for the detoxification and deposition of endogenous and exogenous substances (Yang et al., 2008).

Single administration of carbon tetrachloride (CCL₄) can rapidly lead to both oxidative stress via the excessive production of free radicals and acute liver injuries such as centrilobular necrosis and steatosis in rats (Weber et al., 2003).

Chitosan, is a polysaccharide of marine origin which is prepared from the shells of crustaceans (Sini et al., 2005). Chitosan has attracted much attention as a biomedical material, owing to its antitumer, antiulcer, immunostimulatory, antibacterial and other unique biological activities (Xue et al., 2001). Chiang et al., (2000), showed that the scavenging effect of chitosan on hydroxyl radicals inhibits lipid peroxidation (LPO) of phosphatidylcholine and linoleate lysosomes in vitro.

Le Houx and Grondin (1993) showed that chitosan maintained adequate cholesterol homeostasis in rats, despite a greatly intake of cholesterol. Chitosan can form complexes with many metal ions because it contains multiple amino, hydroxyl and acetamide groups (Varma et al., 2004).

Various copper complexes have been tested for antibacterial and antitumer properties (Hirano, 1995). The copper complexes interact with DNA, leading to chemically
induced cleavage of DNA and thus have antitumor activity (Liang et al., 2003).

In the present study, we investigated which of either chitosan or chitosan – Cu complex has more protective effect against CCl4-induced oxidative stress and hepatotoxicity in rats.

MATERIAL AND METHODS

Adult male Albino rats (Rattus rattus) weighing 120±8 g purchased from Eye Bank, Giza, Egypt, were kept in stainless steel bottom cages within an air-conditioned animal house at 23±2 °C. Rats were fed commercial diet and allowed water ad libitum for a week, then randomly classified into six groups, each with five rats. Group I was kept normal and served as a control, each of the rats of group II was administered a single oral dose of CCl4 (1.5 ml / kg body weight) .Group III received chitosan daily 200 mg/kg for 21 days (water soluble chitosan with M.W.6x105 and degree of deacetylation more than 85%, purchased from Aldrich) . Group IV received orally chitosan-Cu complex (prepared locally by Reicha and his Co-workers, Physics Departement, Faculty of Science, Mansoura University, Egypt) at the same dose and period of chitosan . Group V received chitosan for 21 days followed by CCl4 and group VI received chitosan-Cu complex for 21 days followed by administration of CCl4.

Twenty four hours after receiving CCl4 rats were sacrificed by decapitation, blood was collected in nonheparinized tubes, allowed to centrifuge after 15 mints and sera were separated.

Animals were dissected, livers were removed, weighed, and part of each liver was excised and homogenized in cold distilled water 10% (w/v). Assay for lipid peroxidation product malondialdehyde (MDA) in liver tissue was performed spectrophotometrically at 512 nm (Ohkawa et al., 1982). The assessment of protein oxidation was performed by a spectrophotometric method (Smith et al., 1991).The level of reduced glutathione (GSH) and the activity of liver superoxide dismutase (SOD) were determined as described by Nishikimi et al. (1972) and Prins and Loose (1969) respectively. Serum and liver ALT & AST activities were estimated by the methods of Reitman and Frankle(1957). Total protein content and albumin level were determined by using Diamond diagnostic kit according to the technique described by Zollner and Kirsch (1962) and Ratliff and Hall (1973) respectively, high density lipoprotein (HDLc) was assessed using atomic absorption spectrophotometry (Zettner and Seligson 1964).

All data were expressed as mean ± S.E. The data analysis was performed by one-way ANOVA test. P value of ≤0.05 was considered significant.

RESULTS

CCl4 administration to the rats significantly elevated hepatic MDA and PC levels while GSH and SOD activities were decreased significantly (table 1) , it was noticed that chitosan and chitisan-Cu complex administration in concomitant with CCl4 significantly affect these estimated parameters positively.

Table (2) shows that serum and liver ALT & AST, were significantly increased, but the total protein content, albumin and globulin were decreased in CCl4 administered group .Pretreatment with chitosan or chitosan-cu complex markedly attenuated most of these measured liver functions parameters compared to that treated only with CCl4.

Serum and liver total lipids, triglyceride, total cholesterol and serum LDL were elevated significantly while serum HDL and VLDL were decreased in CCl4 treated rats, pretreatment with chitosan or chitosan-cu complex improved most of these parameters (table 3).
A decline in the level of serum Na and Zn but K and Fe levels were increased in CCL4 treated group, amelioration noticed when the rats treated with chitosan or chitosan-cu complex prior to CCL4 (table 4).

Table (1): Liver MDA, PC, GSH and SOD.

<table>
<thead>
<tr>
<th>Animal Estimated parameters</th>
<th>(I) Control</th>
<th>(II) CCl4</th>
<th>(III) Chitosan</th>
<th>(IV) Chitosan-Cu</th>
<th>(V) Chitosan + CCl4</th>
<th>(VI) Chitosan- Cu + CCl4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g) % of change</td>
<td>159.76±14.2</td>
<td>253.38±18.7</td>
<td>136.0±15.0</td>
<td>151.3±13.8</td>
<td>191.4±14.5</td>
<td>203.2±11.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0±58.60</td>
<td>0±14.87</td>
<td>0±5.29</td>
<td>19.80</td>
<td>-27.19</td>
</tr>
<tr>
<td>PC(μmolNPH/g) % of change</td>
<td>0.162±0.01</td>
<td>0.276±0.023</td>
<td>0.166±0.00</td>
<td>0.162±0.01</td>
<td>0.22±0.007</td>
<td>0.198±0.004</td>
</tr>
<tr>
<td></td>
<td>9</td>
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<td>2</td>
<td>+35.80</td>
<td>+22.22</td>
</tr>
<tr>
<td>GSH (mg/g) % of change</td>
<td>0.25±0.02</td>
<td>0.104±0.02</td>
<td>0.256±0.01</td>
<td>0.238±0.03</td>
<td>0.148±0.013</td>
<td>0.158±0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+58.4</td>
<td>5</td>
<td>-4.8</td>
<td>-40.8</td>
<td>-36.8</td>
</tr>
<tr>
<td>SOD (μ/g) % of change</td>
<td>34.40±1.57</td>
<td>18.60±1.36</td>
<td>36.40±1.03</td>
<td>36.20±1.66</td>
<td>25.60±1.63</td>
<td>23.80±1.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-45.93</td>
<td>5.23</td>
<td>-25.58</td>
<td></td>
<td>-30.81</td>
</tr>
</tbody>
</table>

a = Significant compared to control (I).
b = Significant compared to CCl4 (II).
c = Significant compared to Chitosan(III).
d = Significant compared to Chitosan-Cu (IV).

Table (2): Serum and liver ALT, AST, total protein and serum albumin.

<table>
<thead>
<tr>
<th>Animal Estimated parameters</th>
<th>(I) Control</th>
<th>(II) CCl4</th>
<th>(III) Chitosan</th>
<th>(IV) Chitosan-Cu</th>
<th>(V) Chitosan + CCl4</th>
<th>(VI) Chitosan- Cu + CCl4</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. ALT (U/L) % of change</td>
<td>39.48± 1.00</td>
<td>71.86±3.13</td>
<td>40.98±2.50</td>
<td>41.64±2.30</td>
<td>63.84±1.46</td>
<td>60.76±1.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+82.02</td>
<td>+3.80</td>
<td>+5.47</td>
<td>+61.70</td>
<td>+53.90</td>
</tr>
<tr>
<td>S. AST (U/L) % of change</td>
<td>33.16± 1.2</td>
<td>42.30±0.59</td>
<td>36.48±1.05</td>
<td>34.08±0.63</td>
<td>40.28±1.2</td>
<td>39.04±0.56</td>
</tr>
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<td>+10.01</td>
<td>+2.77</td>
<td>+21.47</td>
<td>+17.73</td>
</tr>
<tr>
<td>S. total protein (g/dl) % of change</td>
<td>7.74±0.52</td>
<td>5.02±0.17</td>
<td>7.60±0.68</td>
<td>7.32±0.66</td>
<td>6.34±0.10</td>
<td>6.62±0.24</td>
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<tr>
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<td></td>
<td>-35.14</td>
<td>-1.81</td>
<td>-5.43</td>
<td>-18.09</td>
<td>-14.47</td>
</tr>
<tr>
<td>S. Albumin (g/dl) % of change</td>
<td>3.78±0.09</td>
<td>2.66±0.07</td>
<td>3.56±0.4</td>
<td>3.30±0.13</td>
<td>3.22±0.08</td>
<td>2.98±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-29.63</td>
<td>-5.82</td>
<td>-12.70</td>
<td>-14.81</td>
<td>-21.16</td>
</tr>
<tr>
<td>Liver ALT (u/g) % of change</td>
<td>33.76±1.12</td>
<td>39.12±0.34</td>
<td>34.90±0.24</td>
<td>33.32±1.00</td>
<td>37.36±0.37</td>
<td>36.82±0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+15.88</td>
<td>+3.37</td>
<td>+1.3</td>
<td>+10.66</td>
<td>+9.89</td>
</tr>
<tr>
<td>Liver AST (U/G) % of change</td>
<td>34.78±0.26</td>
<td>41.26±0.89</td>
<td>35.22±0.94</td>
<td>35.60±0.97</td>
<td>37.78±0.25</td>
<td>38.06±0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+18.63</td>
<td>+1.26</td>
<td>+2.36</td>
<td>+8.62</td>
<td>+9.43</td>
</tr>
<tr>
<td>Liver total protein (mg/g wet tis.) % of change</td>
<td>29.52±1.7</td>
<td>22.36±1.5</td>
<td>26.92±1.2</td>
<td>30.52±1.4</td>
<td>22.44±1.7</td>
<td>24.94±2.14</td>
</tr>
</tbody>
</table>

a = Significant compared to control (I).
b = Significant compared to CCl4(II).
c = Significant compared to Chitosan(III).
d = Significant compared to Chitosan-Cu(IV).
**Table (3): Serum total lipids, triglycerides, total cholesterol and HDL. Liver total lipids, triglycerides, total cholesterol and HDL.**

<table>
<thead>
<tr>
<th>Animal group Estimated parameters</th>
<th>(I) Control</th>
<th>(II) CCl4</th>
<th>(III) Chitosan</th>
<th>(IV) Chitosan-Cu</th>
<th>(V) Chitosan + CCl4</th>
<th>(VI) Chitosan-Cu + CCl4</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. total lipids (mg/dl) % of change</td>
<td>966.08±22.5 +16.45</td>
<td>1125.02±9.90 +4.97</td>
<td>881.04±18.36 +9.19</td>
<td>877.22±36.4 +8.00</td>
<td>1043.4±20.6 +8.00</td>
<td>980.6±35.5 +1.50</td>
</tr>
<tr>
<td>S. triglycerides (mg/dl) % of change</td>
<td>90.66±2.30 +38.10</td>
<td>125.20±5.2 +24.37</td>
<td>68.56±4.9 +2.06</td>
<td>80.48±10.8 +1.0</td>
<td>105.54±8.8 +14.1</td>
<td>94.58±5.4 +4.32</td>
</tr>
<tr>
<td>S. total cholest. (mg/dl) % of change</td>
<td>183.58±9.5 +69.13</td>
<td>106.3±5.5 +2.06</td>
<td>118.52±7.2 +9.19</td>
<td>137.88±4.8 +27.03</td>
<td>980.6±35.5 +1.50</td>
<td>145.6±6.9 +34.14</td>
</tr>
<tr>
<td>S. HDL (mg/dl) % of change</td>
<td>36.22±1.6 +19.64</td>
<td>30.16±1.7 +16.73</td>
<td>34.34±1.9 +9.19</td>
<td>22.54±1.5 +27.77</td>
<td>26.88±1.7 -25.77</td>
<td></td>
</tr>
<tr>
<td>L. total Lipids (mg/g) % of change</td>
<td>53.44±2.3 +69.82</td>
<td>47.87±1.0 +30.65</td>
<td>54.70±1.8 +2.36</td>
<td>62.76±2.2 +17.44</td>
<td>63.30±1.6 +18.45</td>
<td></td>
</tr>
<tr>
<td>L. triglycerides (mg/g) % of change</td>
<td>59.72±7.2 +114.0±6.9</td>
<td>59.90±3.6 +11.69</td>
<td>66.70±2.8 +24.41</td>
<td>74.30±4.6 +35.64</td>
<td>94.68±0.6 +58.54</td>
<td></td>
</tr>
<tr>
<td>L. total cholesterol(mg/g) % of change</td>
<td>23.78±0.9 +40.72</td>
<td>24.24±2.9 +1.93</td>
<td>28.34±0.9 +19.17</td>
<td>34.04±1.4 +43.14</td>
<td>32.58±1.0 +37.0</td>
<td></td>
</tr>
</tbody>
</table>

a = Significant compared to control (I).
b = Significant compared to CCl4(II).
c = Significant compared to Chitosan(III).
d = Significant compared to Chitosan-Cu(IV).

**Table (4): Serum Na, K, Fe and Zn.**

<table>
<thead>
<tr>
<th>Animal Estimated parameters</th>
<th>(I) Control</th>
<th>(II) CCl4</th>
<th>(III) Chitosan</th>
<th>(IV) Chitosan-Cu</th>
<th>(V) Chitosan + CCl4</th>
<th>(VI) Chitosan-Cu + CCl4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Na (mg/dl) % of change</td>
<td>157.8±1.3 +88.2±1.7</td>
<td>156.5±2.6 +52.00±2.8</td>
<td>162.3±3.8 +50.00±2.8</td>
<td>128.6±2.7 +36.13</td>
<td>125.1±5.8 -20.72</td>
<td></td>
</tr>
<tr>
<td>Serum K (mg/dl) % of change</td>
<td>6.02±0.08 +8.82±0.40</td>
<td>5.84±0.26 +52.00±2.8</td>
<td>5.46±0.35 +52.00±2.8</td>
<td>6.98±0.23 +36.13</td>
<td>7.72±0.31 +28.24</td>
<td></td>
</tr>
<tr>
<td>Serum Fe (µg/ml) % of change</td>
<td>33.33±0.33</td>
<td>52.00±2.8 +52.00±2.8</td>
<td>31.17±0.32 +52.00±2.8</td>
<td>47.5±0.79 +36.13</td>
<td>46.23±1.8 +38.24</td>
<td></td>
</tr>
<tr>
<td>Serum Zn (µg/100 ml) % of change</td>
<td>15.5±0.16 +9.90±0.13</td>
<td>13.64±0.28 +36.13</td>
<td>13.37±0.45 +12.26</td>
<td>10.97±0.21 +12.26</td>
<td>10.35±0.31 +33.22</td>
<td></td>
</tr>
</tbody>
</table>

a = Significant compared to control (I).
b = Significant compared to CCl4(II).
c = Significant compared to Chitosan(III).
d = Significant compared to Chitosan-Cu(IV).
Discussion

Carbon tetrachloride (CCl₄) is a potent hepatotoxic agent; metabolism of CCl₄ is initiated by cytochrome P450 mediated transfer of an electron to the C-Cl bond to form an anion radical that eliminates chloride resulting in the trichloromethyl radical. Co-treatment with chitosan and CCl₄ significantly reduce these harmful effects on MDA and PC formation, probably by its antioxidant nature preventing the damage caused by free radical attack, and / or the inhibition of the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins (Eidelman et al., 2002), inhibitory malondialdehyde formation triggered by CCl₄ (Yan et al., 2006) and / or antioxidant effect (Teselkin et al., 2000). And / or the scavenging effect of chitosan on hydroxyl radicals as reported by (Chiang et al., 2000). Increasing antioxidant enzyme activities may also lead to these improvements.

The reduction in liver SOD and GSH levels in CCl₄ treated animals reveal the deleterious effect on the antioxidative status, a result which concurs with the finding of Lee et al. (2007), and may be attributed to the inhibition of GSH reductase activity as reported by Ohta et al. (1997). The resulted attenuation in lipid peroxidation products and antioxidant enzymes activities obtained in rat groups treated with CCl₄ with chitosan or chitosan-Cu complex are in agreement with Yan et al. (2006). The rapid chitosan absorption by intestinal cells and the rapid distribution to other tissues in the body, can protect the hepatic tissue from CCL₄ toxicity (Zeng et al., 2007).

In the present study, the induction of CCl₄ caused increases in estimated liver enzymatic activities, in concomitant with decline in serum and liver protein reflected liver dysfunction, indicating their undesirable effects, these results agree with El-habibi and Amer (2000) and may be attributed to oxidative and reductive biotransformation and initiate biochemical events leading to liver cell necrosis (Weber et al., 2003 and Bhadauria et al., 2007).

Obtained decline in serum and liver total protein in CCl₄ treated group agree with previous report of El-Habibi and Amer (2000), may be attributed to the inactivation and degradation of CYP2E1, by CCL₄ where protein synthesis was blocked (Dai and Cederbaum, 1995). Obtained amelioration in serum and liver enzymes activities especially in group treated with CCl₄ and chitosan-Cu complex may be due to the suppressing ALT and AST activities by their antioxidant activity as reported by Lin and huang (2000) who showed that ALT and AST activities suppressed by antioxidant.

Obtained elevation in serum and liver ALT and AST are presumptive markers of CCl₄ induced hepatic injury that are in agreement with Popovic et al. (2007).These results may be due to loss in phospholipids membrane stability leading to the release of the enzymes as reported by Ahmed et al. (2000).

Significant hyperlipidemia, triglyceridemia and cholesterolemia also recorded in CCL₄ treated rats, and the restore in lipid content after chitosan and CCL₄ administration may be attributed to its antilipidemic property as mentioned by Xing et al. (2005), and / or to inhibitory action of chitosan on fat absorption. In addition to its ability to inhibit the increased accumulation of lipids in the systemic circulation (Choi et al., 2002) . These attenuation may be also as a result of excess food intake, where Le Houx and Grondin(1993) showed that rat fed chitosan ingested more food.

Co-administration of CCL₄ and chitosan or chitosan-Cu complex reduced the elevation of total cholesterol, and the maintained HDLc near the normal level, liver cholesterol content was decreased significantly, but did not reach control values. This indicates that under such conditions, the chitosan group had nearly reached the quilibrium , but not completely.

The elevated cholesterol in CCl₄ treated
group may be attributed to impaired utilization in sterodogenesis (Lin et al., 1995).

Obtained amelioration in blood levels of total cholesterol, triglycerides, LDLc and VLDLc in chitosan treated group may be attributed to ability of chitosan in depressing of these parameters, as reported by Geremias et al. (2006), and may be attributed to catabolic derangement in lipoprotein metabolism Santhosh et al. (2006) and/or through the capability of chitosan for increasing the fecal excretion of cholesterol, a view which in line with Yao and Chiang (2002). These results may be also due to increased uptake of LDL from the blood by tissue as mentioned by Kessler et al. (2005). Also, this ameliorative effect of chitosan may be due to stop the passive exchange between plasma lipoprotein and the cell membrane (Brown and Goldstain, 1986). Obtained increase in serum and liver triglycerides in CCL4 treated group may be attributed to increased lypoysis of adipose tissue stores as reported by Kruger et al. (1967), and/or the uptake of free fatty acids (FFA) from adipose tissue by the liver, leading to the hypertiglyceridemia, a view which in accordance with Stenberg (1976).

The resulted attenuation in serum and liver triglycerides levels in chitosan and chitosan-Cu complex treated animals may be as result of reduction in cholesterol and FFA levels by the hypolipidemic effect of chitosan as mentioned by Xing et al. (2005) and/or stimulating metabolic process by antioxidants that prevent formation of free radicals as mentioned by Eidelman et al. (2002).

Yonekura et al. (2004) reported that 1%dietary chitosan could increase zinc absorption in rats by formation of stable complexes with phytic acid. Zinc is very important in insulin synthesis activating anabolic process decreasing lipid deposition and increase protein synthesis. In addition chitosan residues have amino groups indeed, nitrogen atoms hold free electrons doubles that can react with metal cations (Varma et al., 2004). However, the amino groups are easily protonated in acidic solutions, facilitating protein synthesis.

The resulted disturbances in Na and K levels in CCL4 treated animals may be attributed to the degradation of membrane phospholipids in liver and to the loss of membrane fluidity, as lipoprotein levels of long-chain PUFA, were significantly decreased by CCL4 (Moody et al., 1981).

The water solubility of chitosan was dependent on the pH of solution. The free –NH2 translated into –NH3+ which had weak chelating ability with metal cations, stimulating Na-K stability, hence nearly restored Na and K levels (Zeng et al., 2007).

Conclusion

It seems that chitosan maintained normal liver functions in rats despite acute CCL4 intoxication, attention should be paid to the possible effect of chitosan-Cu complex on the bioavailability and its pertinent clinical benefits, due to its highly hypolipidimic and strengthen antioxidant system.

Acknowledgment

The authors would like to thank Prof. Dr. F. Risha, Physics Department, Faculty of Science, Mansoura University for preparation and supplying the research group with chitosan and chitosan-Cu complex.

REFERENCES


32- Stenberg D (1976): Interconvertible enzymes in adipose tissue regulated by cyclic AMP –
COMPARATIVE EFFECT BETWEEN …...


التأثير المقارن لمركب الكيتوزان و الكيتوزان مع النحاس ضد سمية الكبد

المستحاثة برابع كلوريد الكربون

السيد الحبيبي- هناء سراج - جمال ادريس

قسم علم الحيوان- كلية العلوم - جامعة المنصورة- المنصورة- مصر.

الرابع كلوريد الكربون سميته تحدث اك세سه فوقية وتلف بالكبد وقد تم دراسه الدور الوقائي المحتمل لمركب الكيتوزان والكيتوزان مع النحاس ضد سمية الكبد في ذكور الجرذان.

تم الحقن بجرعه حاده بين الغشاء البزتيوني برابع كلوريد الكربون (20 مجم / كجم من وزن الجسم). وقد احدث ذلك زيادة في مالون ثنائي الألدهيد (MDA) وكربونيل البروتين (PC) وانخفاض في فوق انزيم الديسميتويز (SOD) والجلوتاثيون (GSH) فمنذ الكبد وصحب ذلك زيادة في انزيمات وظائف الكبد مقاسه مع انخفاض في مستوي البروتين في الكبد. كما لوحظ زيادة في معدل الدهون الكلية والكوليستيرول والجلوكوز متوسط والدهون الثلاثية والدهون منخفضة الكثافة. صحب ذلك انخفاض في مستوي الدهون عالية الكثافة، كذلك أظهرت الدراسة ارتفاع محتوى الصوديوم في المصل والكبد مع انخفاض في مستوي البوتاسيوم والزنك.

الجرذان التي تم حقنها بمركب الكيتوزان أو الكيتوزان مع النحاس بجرعه 200 مجم / كجم من وزن الجسم لمدة احدى وعشرين يوما قبل الحقن برابع كلوريد الكربون أحدث وقآية منوية لمعظم المعايير ورفع من قوه جهاز مضاد الأكسده الدفاعي وقام بتحسين وظائف الكبد. وكان لمركب الكيتوزان مع النحاس دورا اقوي من الكيتوزان بمفرده في الوقاية من اثار سمية رابع كلوريد الكربون.