Evaluation Of Toxicological Effects Of Carbon Tetrachloride On Liver Enzymes Of Wistar Rats

Ezejindu DN, Ulolene G.C, Ihentuge C.J

ABSTRACT: This work is aimed at determining the effects of carbon tetrachloride on liver enzymes of adult wistar rats following oral administration. Twenty adult wistar rats weighing between 190-220kg were used. They were allocated into four groups (A,B,C &D) of five animals each. Group A animals served as the control and received 0.5ml of distilled water. The experimental groups B,C & D received 0.1ml, 0.2ml and 0.3ml of carbon tetrachloride respectively for 21 days. Twenty four hours after the last administration, the animals were weighed, anaesthetized under chloroform vapour and dissected. The liver tissues were removed and weighed. Blood samples were collected by cardiac puncture using sterile syringes and needles. Blood for serum preparation was collected into sterile plain tubes without anti-coagulant. Serum samples were separated into sterile plain tubes and stored in the refrigerator for analysis. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using randox kit method. The final body weight of the experimental groups were significantly lower (P<0.001) than the control (A). The levels of mean aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in group D were significantly higher (P<0.001) than groups B,C and control A. The levels of mean aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in group C were significantly higher (P<0.001) than group B and control A. The result from this study showed that adverse biochemical changes are associated with animals in groups B,C and D. The present study indicated dose-dependent on the liver enzymes of rats administered with CCL4. Our findings therefore suggest that chronic CCL4 consumption may put the liver at risk of adverse biochemical alterations and histopathological lesions.

Key words: Liver enzymes, Carbon tetrachloride, Liver weight, Body weight, Wistar rats.

1. INTRODUCTION
Carbon tetrachloride is a manufactured chemical that does not occur naturally. It is a clear liquid with a sweet smell that can be detected at low levels[1]. There are two interpretations of the morphological and biochemical changes found after treatment of experimental animals with carbon tetrachloride. One school of thought, represented mainly by earlier workers such as [2] considers that the effect is a direct toxic action on the liver cells. Other workers, however stated that the effect is essentially an indirect one mediated by changes in the blood supply to the liver cells. To some extent this view appears to be supported by the well know observation that liver cell damage following oral administration of carbon tetrachloride is localized predominantly in the central zone of the liver lobule [3]. Exposure of high concentrations of carbon tetrachloride can affect the central nervous system, degenerate the liver, kidney and may result to death [4], [5],[6]. Acute inhalation and oral exposures to high levels of carbon tetrachloride have been observed primarily to damage the liver (swollen, tender liver, changes in enzymes levels, and jaundice) and kidneys (nephritis, nephrosis, proteinurea) of humans[4], [5], [6], [7],[8]. Hence, thus study aims at painstakingly investigating the effects of carbon tetrachloride on liver enzymes following administration of different doses.

2. MATERIALS AND METHODS
2.1 BREEDING OF ANIMALS
Twenty wistar rats were purchased from the animal house of Anatomy Department, University of Calabar, Cross River State, Nigeria and bred in the animal house of University of Uyo Akwa Ibom State. They were allowed for a period of seven days for acclimatization under normal temperature (27°C – 30°C) before their weights were taken. They were fed ad-libitum with water and guinea feed pallets from Agro feed mill Nigeria Ltd.

2.2 EXPERIMENTAL PROTOCOLS
The twenty animals were weighed and allocated into four groups of five animals each. The groups were designated as groups A,B,C and D. Group A animals served as the control and received 0.5ml of distilled water. The experimental groups B,C and D received 0.1ml, 0.2ml and 0.3ml of carbon tetrachloride respectively. The drug was administered using intubation method once in a day between the hours of 12 – 3pm for a period of twenty one days. After the twenty first day, the animals were weighed and their weight recorded. Twenty four hours after the last administration, the animals were anaesthetized under chloroform vapour and dissected. Blood samples were collected by cardiac puncture using sterile syringes with needles. Blood for serum preparation was collected into sterile plain tubes without anti-coagulant. Serum samples were separated from the cloth by centrifugation at 3,000rpm for 5minutes using bench top centrifuge. Serum samples were separated into sterile plain tubes and were stored in the refrigerator for analysis. Liver tissues were removed from the animals and weighed. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using randox kit method.

- Ezejindu DN, Ulolene G.C, Ihentuge C.J
- Department of Anatomy, College of health sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
- Department of Anatomy, College of health sciences, Imo State University Owerri, Imo State, Nigeria
- Email: damianezejindu@gmail.com
- Telephone: +2348032715300
3. RESULTS

3.1 MORPHOMETRIC ANALYSIS OF BODY WEIGHTS.

Table 1: Comparison of mean initial and final body weight and weight change in all the groups (A, B, C & D) (Mean ± SEM given for each measurement)

<table>
<thead>
<tr>
<th></th>
<th>GP. A</th>
<th>GP. B</th>
<th>GP. C</th>
<th>GP. D</th>
<th>F-RATIO</th>
<th>PROB. OF SIG.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt.</td>
<td>190.50 ± 4.60</td>
<td>195.2 ± 5.20</td>
<td>199.4 ± 4.70</td>
<td>220.70 ± 5.60</td>
<td>72.2 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Final body wt.</td>
<td>200.10 ± 6.20</td>
<td>180.7 ± 4.60</td>
<td>162.4 ± 5.10</td>
<td>170.30 ± 6.70</td>
<td>44.4 ± 20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wt. Chang e</td>
<td>10.0 ± 5.70</td>
<td>15.00 ± 6.10</td>
<td>37.20 ± 7.70</td>
<td>50.0 ± 4.80</td>
<td>11.5 ± 50</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The final body weight for group A (Control) treated with water was significantly higher (P<0.001) than the experimental groups B, C & D. The weight change for group D showed a statistically increase (P<0.001) compared with the experiments groups B & C and A (Control).

3.2 MORPHOMETRIC ANALYSIS OF RELATIVE LIVER WEIGHT

Table 2: Comparison of mean relative liver weight in all the groups (A, B, C & D) (Mean ± SEM given for each measurement)

<table>
<thead>
<tr>
<th></th>
<th>GP. A</th>
<th>GP. B</th>
<th>GP. C</th>
<th>GP. D</th>
<th>F-RATIO</th>
<th>PROB. OF SIG.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER WT.</td>
<td>4.90 ± 0.250</td>
<td>10.4 ± 0.12</td>
<td>15.70 ± 0.040</td>
<td>19.5 ± 0.080</td>
<td>73.8 ± 0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The relative liver weight for the experimental groups were significantly higher (P<0.001) than the control. The relative liver weight change of group D showed a statistically increase (P<0.001) compared with the experimental groups B & C and group A (Control). Group C relative liver weight were significantly higher (P<0.001) than group B and control (A).

3.3 ACTIVITIES OF SERUM LEVELS OF ASPARATE AMINOTRANSFERASE (AST), ALANINE AMINOTRANSFERASE (ALT) AND ALKALINE PHOSPHOTASE (ALP)

<table>
<thead>
<tr>
<th></th>
<th>GP. A</th>
<th>GP. B</th>
<th>GP. C</th>
<th>GP. D</th>
<th>F-RATIO</th>
<th>PROB. OF SIG.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>76.80 ± 2.50</td>
<td>90.20 ± 4.80</td>
<td>132.40 ± 6.10</td>
<td>150.20 ± 6.83</td>
<td>40.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT</td>
<td>58.10 ± 4.20</td>
<td>75.30 ± 3.70</td>
<td>110.50 ± 5.77</td>
<td>129.10 ± 5.50</td>
<td>24.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALP</td>
<td>194.20 ± 4.85</td>
<td>220.20 ± 2.40</td>
<td>243.10 ± 4.60</td>
<td>271.70 ± 2.20</td>
<td>8.25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

From the results obtained from calculations of activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase (ALP), the experimental groups B, C & D showed a significant increase (P<0.001) in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase levels compared with the control. There were statistical differences in the experimental groups B, C & D. The group D activity levels in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase were significantly higher (P<0.001) than groups B & C. Also the group C activity levels in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase (ALP) were significantly higher (P<0.001) than group B.

4. DISCUSSION

The result of this study agree with previous researchers that carbon tetrachloride has toxicological effects on the liver enzymes of water rats. Observation of the body weight difference in groups reveals decrease in weight of animals in the experimental groups compared with the control. Comparing the results of weight difference among the experimental groups reveals loss of greater weight by the animals exposed to higher dosage of carbon tetrachloride. This is probably as a result of loss of appetite by the animals in the groups. The relative weights of the organ also showed significant differences in groups. There was relative increase in liver weight for the carbon tetrachloride exposed animals compared to the control group A. This could have been pathological and one may deduce that the increase in liver weight was not growth but inflammation. There were significant differences in the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase (ALP) compared with the control. The serum levels of AST, ALT and ALP in group D were significantly higher (P<0.001) than groups B, C and control A. The AST, ALT and ALP levels of group C animals were significantly higher (P<0.001) than group B and control A. Enzyme activities in the serum and
tissue are often used as “maker” to ascertain toxic effects of administered foreign compounds to experimental animals[9]. ALP is a membrane bound enzymes [10] while ALT and AST are cystolic enzymes[11]. These enzymes are highly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane becomes leaky and even completely ruptured [12],[13]. A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to the liver cells [14].

5. CONCLUSION
The present study indicated that a long term exposure to small and high dosages of carbon tetrachloride caused biochemical alterations to the enzymes of the liver. Thus there is dose-dependent effects of carbon tetrachloride administration to enzymes of the liver.

6. REFERENCES


