Effect of vitamin A supplementation on disulfiram-copper sulphate combination induced toxicity on liver function and histopathology of the liver in female Wistar Rats

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ABSTRACT

This study evaluated the effect of vitamin A supplementation on disulfiram-copper sulphate combination induced toxicity in Wistar rats. Female wistar rats weighing between 200 to 260 g were used for this study. The rats were divided into three groups of six each. Group 1: control group received normal feed and water. Group 2 received 74.6 mg/kg disulfiram and 15 mg/kg copper sulphate combination daily while group 3 received 1 mg/kg vitamin A in addition to disulfiram-copper sulphate combination daily. The duration of treatment was 28 days. Three Wistar rats from each group were sacrificed using chloroform on day 29. Blood samples were collected through cardiac puncture and analyzed for liver function. The liver was also excised for histopathological analysis. Results showed that the liver enzymes AST, ALP and ALT were significantly increased in group 2 rats while group 3 rats showed a significant reduction in the liver enzyme levels. There was also severe distortion of the liver architecture of group 2 rats while this was mild in group 3. In conclusion, vitamin A has been shown to have hepatoprotective effects against oxidative damage caused by disulfiram /copper sulphate combination.

Key words: Vitamin A, disulfiram-copper sulphate combination, Wistar rats.

INTRODUCTION

Reports from previous studies showed that disulfiram-induced cytotoxicity can mediate oxidative stress (Chen et al., 2006; Cen et al., 2002). In addition, the presence of copper can increase the chances of the oxidative stress (Chen et al., 2005).

Antioxidant is described as a substance that helps in protecting cells from damage by free radical molecules which are mostly unstable (Hamid et al., 2010). It is a molecule that has the capability to slow or prevent the oxidation of other molecules. These reactions can produce free radicals that further start chain reactions that could damage cells. Free radical damages could lead to cancer. Antioxidants cause cessations in these chain reactions by removing the free radical intermediates molecules and inhibit other oxidation reactions by being oxidized themselves. Antioxidants make this easy because only certain polyunsaturated fatty acids generate damaging free radicals; therefore, the intake level of antioxidant vitamins that can scavenge these harmful radicals is a cofounding factor.

Forms of antioxidants may include lycopene, betacarotene and vitamins especially, A, E and C, among other substances. Vitamin A (retinol) is required by humans for the normal functioning of the visual system. The precursors of vitamin A (retinol) are the carotenoids (most commonly beta-carotene). As an antioxidant, it plays an important role in antioxidant defenses as a crucial component of selenoproteins, such as glutathione peroxidase. It has been observed that oxidative processes are involved in various stages of carcinogenesis such that excessive antioxidants interfere with the cytotoxic effects of anti-neoplastic agents on cancer cells, and certain micronutrients affect cancer prevention and treatment through their antioxidant properties. These micronutrients
include vitamin A which is available singly or in a combined form (Norman et al., 2003).

**METHODOLOGY**

Laboratory female (Wistar) rats weighing an average of 230 g were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt. They were allowed a two-week acclimatization period and received normal feed and water ad libitum. At the end of the acclimatization period the animals were divided into three groups of six (6) each.

**Group 1:** Served as control group which received normal feed and water throughout the duration of the study (Figure 1).

**Group 2:** This served as a test group treated with 74.6 mg/kg disulfiram and 15 mg/kg copper sulphate (Figure 1).

**Group 3:** Served as a test group which received 1 mg/kg vitamin A in addition to disulfiram-copper sulphate combination daily (Figure 3).

The study lasted for twenty-eight (28) days in which the animals were housed and maintained under suitable conditions. The cages were cleaned and the wood shavings which served as beddings changed on alternate days. Animals were handled according to Helsinki declaration on animal care. Drugs were administered through the oral route using a 1 ml syringe. According to the report of Georgewill et al. (2015), the high dose was chosen because this was the dose at which most of the toxicity of the Disulfiram copper combination chemotherapy was observed.

**Collection of samples**

On day 29, three rats from each group were sacrificed under chloroform anesthesia and blood samples collected for hematological analysis and kidney function tests.

**Statistical analysis**

The data were analyzed using IBM SPSS version 20. Statistical values of p<0.05 were considered significant.

**RESULTS AND DISCUSSION**

Table 1 shows the effects of vitamin A supplementation on disulfiram/copper sulphate combination induced toxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.99±0.47</td>
<td>41.00±7.00</td>
<td>8.50±1.50</td>
</tr>
<tr>
<td>DSF/CuSO4</td>
<td>21.99±3.11</td>
<td>48.00±0.00</td>
<td>48.00±4.10</td>
</tr>
<tr>
<td>DSF/CuSO4 + Vitamin A</td>
<td>17.98±6.52</td>
<td>45.50±2.50</td>
<td>37.50±21.50</td>
</tr>
</tbody>
</table>

**ALP:** Alkaline Phosphatase; **ALT:** Alanine Transaminase; **AST:** Aspartate Transaminase; **DSF/CuSO4:** Disulfiram Copper Sulphate combination; **DSF/CuSO4 + Vitamin A:** Disulfiram Copper Sulphate combination plus Vitamin A; **xx:** values significantly different from control p<0.05; **xx:** values significantly different from Disulfiram copper combination group p<0.05.
on liver enzymes. Slight improvement of the liver of the rats given drug combination with vitamin A on day 28 of the study was observed (Figure 3).

On liver function, the results of this study revealed a significant increase in ALP level of test group that received drug combination when compared to the control group. There was also, a significant increase in ALT level of rats that received disulfiram / copper sulphate combination when compared with the control. The level of AST also increased significantly in the animals that received the drug combination alone when compared with the control group that received just normal feed and water ad libitum. However, significant reductions were observed in the levels of ALP, ALT and AST in the animals that received drug combination in addition to vitamin A suggesting that vitamin A provided some ameliorating effect on the toxicity induced by this drug combination through its antioxidant activity.

The photomicrographs of the liver of rats administered drug combination showed acute severe distortion of liver architecture (Figure 2) when compared to the photomicrographs of liver of rats in the control group.
(Figure 1). Photomicrographs of the liver of the rat that received drug combination in addition to vitamin A showed minimal inflammatory (Figure 3) changes when compared to those administered drug combinations alone, this also suggest that vitamin A protected the liver from the damage caused by DSF/CuSO$_4$ combination, hence, the mild distortion in the liver architecture of the rats that received vitamin A (Figure 3).

**Conclusion**

In conclusion, from this study, Vitamin A has been shown to have protective effects on the liver against oxidative damage caused by Disulfiram/Copper Sulphate combination. It may be safe to suggest the use of vitamin A as adjuvant therapy in the use of Disulfiram/Copper Sulphate combination in cancer chemotherapy in order to reduce or mitigate possible side effects that may arise from the use of Disulfiram Copper Sulphate combination alone.

**REFERENCES**


