Effect of vitamin A supplementation on disulfiram-copper sulphate combination induced toxicity on haematological and renal function 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 on haematological and renal function in Wistar rats. Female wistar rats weighing between 200 to 260 g were used for this study. The Wistar 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 haematological and renal parameters. The results showed that there was a significant increase in all hematological parameters in group 3 rats when compared to that of group 2. Similarly, there was a significant increase in the values of electrolytes, urea and creatinine in group 3 rats as compared to that of group 2. In conclusion, vitamin A has been shown to have protective effects on haematological and renal parameters studied against oxidative damage caused by disulfiram /copper sulphate combination.

Key words: Vitamin A, disulfiram-copper sulphate, haematological and renal parameters Wistar rats.

INTRODUCTION

Presently, cancer treatment is based on conventional chemotherapy, which yields an improved prognosis, particularly in young patients. However, the general toxicity caused by non-specific interactions of chemotherapeutic drugs and the development of drug resistance limit chemotherapy’s use and could seriously damage a patient’s health (Chabner and Roberts, 2005; Ta et al., 2009). Moreover, this insufficiently effective and harmful chemotherapy is significantly expensive. The society needs new effective drugs with a safe pharmacological profile and minimal side effects at a reasonable cost. However, at present, this goal is likely impossible because current drug research consumes particularly high amounts of money and cannot afford to produce low cost products (Fojo and Grady, 2009).

Moreover, new drugs used in oncology are mostly very toxic (Vera-Badillo et al., 2013). An effort to develop a truly effective and safe anticancer drug might be accomplished through other approaches. One approach is drug repurposing or off-label use (Chong and Sullivan, 2007; Boguski et al., 2009). This is what gave birth to the disulfiram /copper combination proposed for cancer chemotherapy. Reports from previous studies showed that disulfiram-induced cytotoxicity can mediate oxidative stress (Chen et al., 2006; Cen et al., 2002) and is probably its mechanism of action. Also, the presence of copper can increase the chances of the development of oxidative stress.

Consequently, many tumors have been found to contain elevated levels of copper which render them selectively susceptible to disulfiram-induced toxicity (Daniel et al., 2007). It is reported that antioxidants mop up the free radical intermediate molecules and inhibit other oxidation reactions by being oxidized themselves and in so doing reduce the toxic effects of some cancer drugs.
Antioxidants interact and stabilize free radicals and thus help to prevent the possible damage some of these free radicals could have caused.

Disulfiram/copper sulphate combination is reported to be associated with multiple side effects on blood parameters. Several studies have reported the toxicity of DSF/Copper Sulphate combination. The mechanism of this toxicity is believed to be through induction of oxidative stress. This study therefore set out to determine the effects of vitamin A supplementation on DSF/Copper Sulphate combination induced toxicity on haematological parameters in rats.

METHODOLOGY

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.

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

**Group 3:** This served as a test group that received 1 mg/kg vitamin A in addition to disulfiram-copper sulphate combination daily.

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. The high dose was chosen because this was the dose at which most of the toxicity of the Disulfiram Copper combination chemotherapy was observed (Georgewill et al., 2015).

### 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. Results were expressed as Mean ±SEM Statistical values of p<0.05 were considered significant.

### RESULTS AND DISCUSSION

The results of the current study showed a significant reduction in RBC, platelets, packed cell volume and hemoglobin levels in animals treated with the drug combination when compared with the control group (Table 1). This is in line with the study of Georgewill et al. (2015) who reported that disulfiram copper combination is toxic to hematological parameters. However, the addition of vitamin A to the test group that received the DSF/CuSO₄ combination showed an improvement in these parameters with a significant increase in RBC, platelets, PCV and hemoglobin levels towards normal when compared to those that received the drug combination alone; this may be due to the antioxidant properties of vitamin A which prevented oxidative damage to the blood cells by mopping up free radicals produced by the DSF/CuSO₄ combination.

Similarly, disulfiram/copper sulphate combination in this study induced significant reduction in the values of the white blood cell count, neutrophils, lymphocytes and monocytes of test group of rats that received disulfiram/copper sulphate combination alone when compared with the control group. This may affect the immune system of the rats; however, the results in the rats that received vitamin A in addition to DSF/CuSO₄ combination revealed significantly higher values of WBC count, neutrophils,

### Table 1: Effects of vitamin A on disulfiram copper sulphate combination induced toxicity on RBC, PLT, PCV and Hb.

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (×10¹²/l)</th>
<th>PLT (×10⁹/l)</th>
<th>PCV (%)</th>
<th>Hgb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.10±1.10</td>
<td>36.00±1.00</td>
<td>46.00±1.00</td>
<td>15.35±0.35</td>
</tr>
<tr>
<td>DSF/CuSO₄</td>
<td>4.80±0.10x</td>
<td>28.50±3.50x</td>
<td>42.50±0.50x</td>
<td>14.15±0.15x</td>
</tr>
<tr>
<td>DSF/CuSO₄ + Vitamin A</td>
<td>5.25±0.15xx</td>
<td>30.00±1.00xx</td>
<td>52.50±3.50xx</td>
<td>17.50±1.20xx</td>
</tr>
</tbody>
</table>

RBC: Red Blood Cell; PLT: Platelets; PCV: Packed Cell Volume; DSF/CuSO₄: Disulfiram Copper Sulphate combination; DSF/CuSO₄ + Vitamin A: Disulfiram Copper Sulphate combination plus vitamin A. x Values significantly different from control P<0.05; xx: Values significantly different from Disulfiram copper combination group p<0.05.
lymphocytes and monocytes when compared to the group that received drug combination alone (Table 2). This finding may suggest an immunoprotective activity of vitamin A probably through its antioxidant properties.

On MCH, MCV and MCHC, the results of this study showed a significant reduction in MCH, MCV and MCHC levels of test group rats that received the drug combination when compared to the control group. This result is also in line with the study of Georgewill et al. (2015) that evaluated the toxicological profile of disulfiram copper combination and reported that this combination is very toxic to hematological parameters. It is worth stating here, that vitamin A, when administered to the rats receiving DSF/CuSO₄ combination resulted in a significant increase in the levels of MCH, MCV and MCHC when compared with the test group that received drug combination alone, this is still likely due to the antioxidant effect of vitamin A which stabilized free radicals released by the action of the DSF/CuSO₄ combination, thus, preventing the possible damage these free radicals could have caused.

Research also showed that a good number of nutritional factors can act as antioxidants and reduce cancer morbidity and mortality; some of them are vitamins A, C and E, etc (Croce, 2001).

On the findings of the effect of vitamin A on disulfiram/copper sulphate combination induced toxicity on creatinine, urea and electrolytes, such as chloride, Potassium and bicarbonates, it was observed from the results that there was significant increase in creatinine level, urea level, chloride, potassium and bicarbonates levels in the blood of animals that received the drug combination alone when compared with the control group (Table 3); this also is in line with the work of Georgewill et al. (2015), who did a 90 days study of the effect of this drug combination on the kidneys and revealed that this drug combination is toxic to the kidneys (Table 4). The kidneys of rats that received the drug combination plus vitamin A showed better kidney function as revealed by significant reduction in the levels of creatinine, urea,

Table 2: Effects of vitamin A on disulfiram copper sulphate combination induced toxicity on WBC Count and differentials

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC count (×10⁹)</th>
<th>Neutrophils (×10⁹)</th>
<th>Lymphocytes (×10⁹)</th>
<th>Monocytes (×10⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.25±2.95</td>
<td>0.33±0.07</td>
<td>0.65±0.02</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td>DSF/CuSO₄</td>
<td>2.40±1.20</td>
<td>0.27±0.08</td>
<td>0.49±0.05</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>DSF/CuSO₄ + Vitamin A</td>
<td>3.95±1.65</td>
<td>0.31±0.05</td>
<td>0.67±0.02</td>
<td>0.30±0.10</td>
</tr>
</tbody>
</table>

WBC: White Blood Cell; DSF/CuSO₄: Disulfiram Copper Sulphate combination; DSF/CuSO₄ + Vitamin A: Disulfiram Copper Sulphate combination plus Vitamin A; X: Values significantly different from control p<0.05; XX: Values significantly different from Disulfiram copper combination group p<0.05.

Table 3: Effects of vitamin A on disulfiram copper sulphate combination induced toxicity on blood volume.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCH (fl)</th>
<th>MCV (pg)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.95±0.05</td>
<td>95.80±0.10</td>
<td>33.35±0.05</td>
</tr>
<tr>
<td>DSF/CuSO₄</td>
<td>24.85±1.15</td>
<td>74.70±3.50</td>
<td>19.30±0.00</td>
</tr>
<tr>
<td>DSF/CuSO₄ + Vitamin A</td>
<td>29.15±0.15</td>
<td>87.30±0.40</td>
<td>33.30±0.10</td>
</tr>
</tbody>
</table>

MCH: Mean Cell Hemoglobin; MCV: Mean Cell Volume; MCHC: Mean Cell Hemoglobin Concentration; DSF/CuSO₄: Disulfiram Copper Sulphate combination; DSF/CuSO₄ + Vitamin A: Disulfiram Copper Sulphate combination plus Vitamin A; X: Values significantly different from control p<0.05; XX: Values significantly different from Disulfiram copper combination group p<0.05.

Table 4: Effects of vitamin A on disulfiram copper sulphate combination induced toxicity on renal parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (Meq/L)</th>
<th>Urea (Meq/L)</th>
<th>Cl (Meq/L)</th>
<th>K (Meq/L)</th>
<th>Bicarbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>175.50±13.50</td>
<td>11.53±2.48</td>
<td>75.65±3.89</td>
<td>3.57±0.07</td>
<td>8.51±2.18</td>
</tr>
<tr>
<td>DSF/CuSO₄</td>
<td>185.78±32.34</td>
<td>26.84±19.93</td>
<td>83.21±2.67</td>
<td>7.56±0.76</td>
<td>20.64±1.73</td>
</tr>
<tr>
<td>DSF/CuSO₄ + Vitamin A</td>
<td>166.98±10.11</td>
<td>5.42±0.30</td>
<td>79.39±4.57</td>
<td>6.96±0.45</td>
<td>14.69±10.85</td>
</tr>
</tbody>
</table>

Cl: Chloride; K: Potassium; DSF/CuSO₄ = Disulfiram Copper Sulphate combination; DSF/CuSO₄ + Vitamin A: Disulfiram Copper Sulphate combination plus Vitamin A; X: Values significantly different from control p<0.05; XX: values significantly different from Disulfiram copper combination group p<0.05.
Conclusion

Disulfiram/Copper Sulphate combination is toxic to the blood tissues and kidneys. This toxicity may be due to the oxidative stress induced by this combination. The amelioration of the toxicity by vitamin A in this research buttresses the antioxidant properties of vitamin A. This study therefore concludes that vitamin A possesses haematopoietic and renoprotective properties.

REFERENCES


