— Letter —

EFFECT OF TIRON AND ITS COMBINATION WITH NUTRITIONAL SUPPLEMENTS AGAINST VANADIUM INTOXICATION IN FEMALE ALBINO RATS

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(Received November 14, 2006; Accepted February 23, 2007)

ABSTRACT — In the present study an attempt has been made to evaluate the effect of Tiron along with Zinc, Selenium and Vitamin E against vanadium intoxication in female albino rats. Toxicant caused significant increase in the activities of serum transaminases, serum alkaline phosphatase and lactate dehydrogenase. Significant decrease was observed in blood sugar, serum albumin and triglyceride levels whereas serum proteins, cholesterol and urea levels increased significantly during toxicity (p ≤ 0.001). Hepatic lipid peroxidation increased significantly, whereas significant depletion was observed in reduced glutathione after vanadium administration. The activity of glucose-6-phosphatase in the liver was also inhibited significantly after vanadium administration. A significant rise was observed in glycogen content of liver and kidney after toxicant exposure. Activities of alkaline phosphatase, adenosine triphosphatase and succinic dehydrogenase were inhibited significantly on the contrary activity of acid phosphatase elevated in kidney. Histopathological examination of the liver and kidney using light and ultramicroscopic study also substantiated the above findings. It was found that therapy with Tiron was effective but significant recovery in all the parameters was found with Tiron + Se followed by Tiron+ VitE and Tiron +Zn.

KEY WORDS: Female albino rats, Tiron, Vanadyl sulphate, Zinc, Selenium, Vitamin E

INTRODUCTION

Vanadium, a metallic element of the first transition series, is widely distributed in the environment (Aragon et al., 2005). Its salts have been used medicinally as antiseptic, spirochete, antituberculous, anti-anemic agents and as general tonic (Sankar et al., 2004). It is also used as an alloying agent in glass production, manufacturing of paints, pigments, inks, insecticides, in electronics, ceramics, superconductors, dyeing textile and as a catalyst in the pharmaceutical industry. Occupational exposure to vanadium is common among workers at workplaces living near the industries, through medicines and developing photographs. Its acute exposure causes gastrointestinal effects, headache, diarrhea, emphysema, pneumonia, irritation in skin, eyes and nervousness. Excessive amounts of vanadium may be associated with manic-depression. Due to its structural similarity with phosphate, vanadate is reported to interfere with a large variety of phosphate-dependent enzymes and it is a potent inhibitor of membrane bound ATPase (Nechay, 1984). It causes injurious effects on central and peripheral nervous system, kidney and liver (Srivastava and Mehdi, 2005; Garcia et al., 2004). Thus the present investigation was undertaken to assess the effect of Tiron (T: 4, 5-dihydroxy-1, 3-benzene disulphonic acid disodium salt) supplemented with antioxidants (zinc: Zn, selenium: Se and vitamin E: Vit E) against vanadium induced toxicity in female albino rats.

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MATERIALS AND METHODS

Animals and treatments

Sprague Dawley female albino rats weighing 160±10 g from our animal facility were given a standard pellet diet (Pranav Agro Industries, New Delhi, India having metal contents in ppm dry weight Cu 10; Mn 33; Zn 45; Co 5). Drinking water was provided ad libitum. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Ministry of Culture, Chennai. The animals were randomized in different groups of six animals each. Group I served as normal control and received physiological saline for 7 days. Group 2 consisted of six animals and they were administered therapeutic agent, Tiron at a dose of 606 mg/Kg/day, ip for 3 days. Groups 3 to 7 were given VOSO₄ at a dose of 18.7 mg/Kg/day orally for 7 days. Groups 4 - 7 were administered Tiron, Tiron+ Zn, Tiron+ Se and Tiron + VitE for three days (5th to 7th) Thus they were treated as follows:

Group 1: Normal control (saline, 4 ml/kg)
Group 2: Tiron per se (606 mg/kg, ip for 3 days)
Group 3: Experimental control (VOSO₄, 18.7 mg/kg, po for 7 days)
Group 4: VOSO₄ as in group 3 + Tiron (5th to 7th day) as in group 2
Group 5: VOSO₄ as in group 3 + Tiron + Zn (4.0 mg/kg, sc 5th - 7th day)
Group 6: VOSO₄ as in group 3 + Tiron + Se (0.5 mg/kg, po 5th - 7th day)
Group 7: VOSO₄ as in group 3 + Tiron + Vit E (50 mg/kg, po 5th - 7th day)

Animals were sacrificed under light ether anesthesia after 24 hr of the last treatment. The blood, liver and kidney were collected for different assays.

Biochemical assays

Blood was collected directly from the heart by puncturing the retro-orbital venous sinus (Riley, 1960) for blood sugar (Asatoor and King, 1969). Serum was processed for the estimation of AST and ALT (Reitman and Franklin, 1957), proteins (Lowery et al., 1951), lactate dehydrogenase (Wroblewski and Due, 1955), albumin, urea, cholesterol and triglycerides (kit method). Standard techniques were used to determine glycogen content in fresh tissue (Seifter et al., 1950). Homogenate in hypotonic solution was processed for the determination of acid and tissue/serum alkaline phosphatase activities (Fiske and Subbarow, 1925), succinic dehydrogenase (Slatter and Bonner, 1952), adenosine triphosphatase (Seth and Tangari, 1966) and glucose-6-phosphatase (Baginski et al., 1974). Hepatic lipid peroxidation (Sharma and Krishnamurthy, 1968) and glutathione (Brehe and Burch, 1976) were also estimated.

Histopathological assays

Liver and kidney were fixed in Bouin’s fluid for light microscopic studies. Electron microscopical samples were fixed in karnovsky’s fluid and post-fixed for two hours in 1% osmium tetraoxide at 4°C. Ultrathin Sections (60-80 nm thickness) by ultracut E (Reichert Jung) were taken and stained in alcoholic uranyl acetate (10 min) and lead acetate (10 min) before examining the grides in a transmission electron microscope (Philips, CM-10) operated at 60-80 kv.

Statistical analysis

Significance of difference among various groups was evaluated by one-way analysis of variance (ANOVA) followed by Student’s t-test (Snedecor and Cochran, 1989). p value taken as significant at 5% level (p ≤ 0.05, 0.01, 0.001) (Snedecor and Cochran, 1989).

RESULTS

Vanadium administration produced severe alteration in various blood/serum and tissue biochemical parameters. One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes such as transaminases, serum alkaline phosphatase and lactate dehydrogenase in the circulation (p ≤ 0.001) whereas blood sugar level was reduced significantly. With the therapy of T + Se the values were restored significantly (p ≤ 0.001) in all these parameters, whereas co administration of Zn and Vit E also showed significant recovery (p<0.001) in LDH and ALT (Table 1). Vanadium caused significant fall in albumin and triglyceride contents whereas significant (p ≤ 0.001) increases were noticed in serum cholesterol, serum proteins and urea. Treatment with T + Se showed significant recoupment in all these parameters whereas combination with Zn and Vit E recouped albumin and urea (p ≤ 0.001)(Table 2).

Data of table 3 indicated that hepatic lipid peroxidation level was increased significantly (p ≤ 0.001), on the contrary, reduced glutathione level (p ≥ 0.01) and the activity of glucose-6-phosphatase (p ≤ 0.001) decreased in the liver after vanadium exposure. The
Combination therapy against vanadium intoxication.

Body weight of vanadium-treated animals was reduced significantly (p ≤ 0.05). Animals treated with T + Se in this study showed significant restoration in LPO (p ≤ 0.001), GSH, Glucose-6-phosphatase activity (p ≤ 0.01) and body weight (p ≤ 0.05). This was confirmed by one way ANOVA followed by Student’s ‘t’ test.

Tissue glycogen content increased significantly (p < 0.05) in the liver and kidney after vanadyl sulphate administration. Vanadium caused inhibition in the activities of adenosine triphosphatase (p ≤ 0.01), alkaline phosphatase and succinic dehydrogenase (p ≤ 0.01), whereas activity of acid phosphatase was increased insignificantly (Table 4 and 5). Therapy with Tiron showed reversal in all the parameters. Co-Therapy with Se showed maximum improvement (p ≤ 0.01) followed by Zn and Vit E.

Liver and kidney showed degenerative changes after vanadium intoxication. Fatty changes, partial cell necrosis have been observed in liver along with proliferation in canaliculi, (Fig. 1). Hexagonal hepatocytes with mitotic figures were observed after Tiron treatment (Fig. 2). Treatment with T + Se showed well formed hepatocytes with clear sinusoidal spaces (Fig. 3). After the administration of vanadyl sulphate discrete patches of cellular infiltration in the medulla of kidney, glomerular hyperemia, necrosis of convoluted tubules and fatty changes were seen (Fig. 4). Treatment with Tiron showed well-formed Bowman’s capsules with glomeruli (Fig. 5) whereas combination therapy of T + Se showed better organization in kidney (Fig. 6).

Table 1. Effect of therapeutic agents on blood biochemical parameters against vanadium intoxication.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Blood Sugar (g/100 ml)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>SALP (mg Pt/100 ml/hr)</th>
<th>LDH (µ mole/min/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>111.00 ± 8.53</td>
<td>67.90 ± 4.02</td>
<td>54.0 ± 3.95</td>
<td>197.0 ± 13.5</td>
<td>40.40 ± 3.06</td>
</tr>
<tr>
<td>T per se</td>
<td>110.20 ± 6.69</td>
<td>68.10 ± 3.54</td>
<td>57.20 ± 3.25</td>
<td>190.0 ± 13.0</td>
<td>39.80 ± 3.04</td>
</tr>
<tr>
<td>V</td>
<td>63.25 ± 4.77</td>
<td>92.17 ± 5.04</td>
<td>160.14 ± 12.1</td>
<td>299.9 ± 18.9</td>
<td>97.50 ± 6.20</td>
</tr>
<tr>
<td>V+T</td>
<td>104.60 ± 8.04</td>
<td>73.26 ± 4.05</td>
<td>97.64 ± 5.32</td>
<td>229.4 ± 19.5</td>
<td>46.10 ± 3.06</td>
</tr>
<tr>
<td>V+T+Zn</td>
<td>90.99 ± 5.32</td>
<td>84.10 ± 5.21</td>
<td>97.50 ± 6.20</td>
<td>244.8 ± 21.6</td>
<td>52.40 ± 2.94</td>
</tr>
<tr>
<td>V+T+Se</td>
<td>113.60 ± 7.66</td>
<td>67.60 ± 4.67</td>
<td>57.46 ± 3.16</td>
<td>203.0 ± 18.6</td>
<td>47.60 ± 2.76</td>
</tr>
<tr>
<td>V+T+Vit E</td>
<td>94.20 ± 7.49</td>
<td>68.11 ± 6.20</td>
<td>69.40 ± 5.95</td>
<td>247.6 ± 23.5</td>
<td>43.80 ± 2.58</td>
</tr>
</tbody>
</table>

One-way ANOVA 7.53a 5.12a 42.46a 5.01a 38.65a

Values are expressed as mean ± S.E., n = 6, p values A ≤ 0.05, B ≤ 0.01, C ≤ 0.001 when compared with control group, p values a ≤ 0.05, b ≤ 0.01, c ≤ 0.001 p ≤ 0.05 when compared with toxicant administered group.

ANOVA (F values at 5% level) a = Significant.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase, G-6-Pase; Glucose-6-phosphatase, GSH; Reduced glutathione; LDH: Lactate dehydrogenase, LP0: Lipid peroxidation, MDA: Malondialdehyde, S. Proteins; Serum Proteins, SALP: Serum alkaline phosphatase, Se; Selenium, T: Tiron (4, 5-dihydroxy-1, 3-benzene disulphonic acid disodium salt), V: Vanadyl sulphate (VOSO₄), Vit E; Vitamin E, Zn; Zinc.

Table 2. Effect of therapeutic agents on blood biochemical parameters against vanadium intoxication.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cholesterol (mg/100 ml)</th>
<th>Triglycerides (mg/dl)</th>
<th>S. Proteins (mg/100 ml)</th>
<th>Albumin (g/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.96 ± 4.68</td>
<td>8.62 ± 0.51</td>
<td>24.02 ± 1.49</td>
<td>4.16 ± 0.34</td>
<td>26.10 ± 2.06</td>
</tr>
<tr>
<td>T per se</td>
<td>60.20 ± 3.67</td>
<td>8.62 ± 0.48</td>
<td>25.60 ± 1.86</td>
<td>4.20 ± 0.30</td>
<td>27.00 ± 1.97</td>
</tr>
<tr>
<td>V</td>
<td>182.46 ± 11.3c</td>
<td>6.19 ± 0.32b</td>
<td>79.84 ± 4.99c</td>
<td>2.02 ± 0.18c</td>
<td>163.80 ± 15.2c</td>
</tr>
<tr>
<td>V+T</td>
<td>130.26 ± 9.59c</td>
<td>8.70 ± 0.65b</td>
<td>42.02 ± 3.71c</td>
<td>3.02 ± 0.28c</td>
<td>42.80 ± 3.16c</td>
</tr>
<tr>
<td>V+T+Zn</td>
<td>173.24 ± 9.37c</td>
<td>9.26 ± 0.92c</td>
<td>57.24 ± 3.74c</td>
<td>3.00 ± 0.28c</td>
<td>52.80 ± 4.71c</td>
</tr>
<tr>
<td>V+T+Se</td>
<td>129.80 ± 7.35c</td>
<td>9.52 ± 0.73b</td>
<td>33.01 ± 2.74c</td>
<td>4.10 ± 0.23c</td>
<td>31.20 ± 2.04c</td>
</tr>
<tr>
<td>V+T+Vit E</td>
<td>136.26 ± 9.78c</td>
<td>6.26 ± 0.35</td>
<td>68.10 ± 4.80</td>
<td>3.18 ± 0.16c</td>
<td>46.40 ± 3.18c</td>
</tr>
</tbody>
</table>

One-way ANOVA 40.69a 6.18a 44.55a 11.47a 69.53a

Values are expressed as mean ± S.E., n = 6, p values A ≤ 0.05, B < 0.01, C ≤ 0.001 when compared with control group, p values a ≤ 0.05, b ≤ 0.01, c ≤ 0.001 p ≤ 0.05 when compared with toxicant administered group.

ANOVA (F values at 5% level) a = Significant.
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Table 3. Effect of therapeutic agents on tissue (liver) biochemical parameters against vanadium intoxication.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>G-6-Pase (μ mole Pi/mg/liver)</th>
<th>GSH (μ mole/mg)</th>
<th>LPO (n mole MDA/mg protein)</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.87 ± 0.26</td>
<td>8.78 ± 0.42</td>
<td>0.37 ± 0.03</td>
<td>155.0 ± 9.79</td>
</tr>
<tr>
<td>T per se</td>
<td>4.80 ± 0.29</td>
<td>8.09 ± 0.53</td>
<td>0.39 ± 0.02</td>
<td>158.3 ± 9.55</td>
</tr>
<tr>
<td>V</td>
<td>2.90 ± 0.26c</td>
<td>6.48 ± 0.39b</td>
<td>0.84 ± 0.04c</td>
<td>120.5 ± 7.61a</td>
</tr>
<tr>
<td>V+T</td>
<td>3.54 ± 0.34</td>
<td>8.30 ± 0.45c</td>
<td>0.40 ± 0.03c</td>
<td>130.0 ± 6.90</td>
</tr>
<tr>
<td>V+T+Zn</td>
<td>2.84 ± 0.22</td>
<td>6.60 ± 0.40</td>
<td>0.45 ± 0.03c</td>
<td>124.0 ± 7.39</td>
</tr>
<tr>
<td>V+T+Se</td>
<td>4.03 ± 0.20b</td>
<td>8.69 ± 0.44a</td>
<td>0.38 ± 0.02c</td>
<td>148.0 ± 8.99b</td>
</tr>
<tr>
<td>V+T+Vit E</td>
<td>3.36 ± 0.22</td>
<td>7.99 ± 0.62</td>
<td>0.38 ± 0.02c</td>
<td>140.0 ± 9.81</td>
</tr>
</tbody>
</table>

One-way ANOVA 11.94a 4.69a 3.42a 3.62a

Values are expressed as mean ± S.E., n = 6, p values A ≤ 0.05, B ≤ 0.01, C ≤ 0.001 when compared with control group, p values a ≤ 0.05, b ≤ 0.01, c ≤ 0.001 p ≤ 0.05 when compared with toxicant administered group.

ANOVA (F values at 5% level)  a = Significant.

Table 4. Effect of therapeutic agents on tissue (liver) biochemical parameters against vanadium intoxication.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glycogen (mg/100 g)</th>
<th>Adenosine triphosphatase (mg Pi/100 g/min)</th>
<th>Alkaline phosphatase (mg Pi/100 g/hr)</th>
<th>Acid phosphatase (mg Pi/100 g/hr)</th>
<th>Succinic dehydrogenase (n moles of KFeCN/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3411.4 ± 213</td>
<td>1968.4 ± 125</td>
<td>78.24 ± 5.05</td>
<td>241.40 ± 13.4</td>
<td>45.66 ± 2.63</td>
</tr>
<tr>
<td>T per se</td>
<td>3733.6 ± 206</td>
<td>1950.0 ± 134</td>
<td>77.60 ± 4.36</td>
<td>251.00 ± 13.5</td>
<td>46.00 ± 2.84</td>
</tr>
<tr>
<td>V</td>
<td>4193.0 ± 262a</td>
<td>1381.2 ± 115b</td>
<td>25.84 ± 2.10c</td>
<td>288.20 ± 19.8</td>
<td>27.48 ± 2.10c</td>
</tr>
<tr>
<td>V+T</td>
<td>3735.8 ± 257</td>
<td>1963.4 ± 173a</td>
<td>68.40 ± 4.14a</td>
<td>249.90 ± 12.13</td>
<td>41.52 ± 2.68b</td>
</tr>
<tr>
<td>V+T+Zn</td>
<td>3826.4 ± 274</td>
<td>1614.3 ± 130</td>
<td>65.20 ± 3.57e</td>
<td>247.00 ± 13.2</td>
<td>34.74 ± 2.78</td>
</tr>
<tr>
<td>V+T+Se</td>
<td>3487.3 ± 175a</td>
<td>1845.7 ± 106a</td>
<td>80.37 ± 5.49a</td>
<td>265.60 ± 17.9</td>
<td>45.50 ± 2.73b</td>
</tr>
<tr>
<td>V+T+Vit E</td>
<td>3500.0 ± 216</td>
<td>1704.6 ± 160</td>
<td>63.40 ± 5.20e</td>
<td>268.40 ± 14.2</td>
<td>44.60 ± 3.19p</td>
</tr>
</tbody>
</table>

One-way ANOVA 1.60a 3.13a 21.72a 13.84a 8.04a

Values are expressed as mean ± S.E., n = 6, p values A ≤ 0.05, B ≤ 0.01, C ≤ 0.001 when compared with control group, p values a ≤ 0.05, b ≤ 0.01, c ≤ 0.001 p ≤ 0.05 when compared with toxicant administered group.

ANOVA (F values at 5% level)  a = Significant, NS- non significant.

Table 5. Effect of therapeutic agents on tissue (kidney) biochemical parameters against vanadium intoxication.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glycogen (mg/100 g)</th>
<th>Adenosine triphosphatase (mg Pi/100 g/min)</th>
<th>Alkaline phosphatase (mg Pi/100 g/hr)</th>
<th>Acid phosphatase (mg Pi/100 g/hr)</th>
<th>Succinic dehydrogenase (n moles of KFeCN/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.94 ± 4.33</td>
<td>2218.7 ± 143</td>
<td>2345.2 ± 140</td>
<td>219.47 ± 11.7</td>
<td>30.26 ± 2.67</td>
</tr>
<tr>
<td>T</td>
<td>65.80 ± 4.38</td>
<td>2301.1 ± 137</td>
<td>2405.0 ± 156</td>
<td>224.20 ± 17.9</td>
<td>29.58 ± 2.17</td>
</tr>
<tr>
<td>V</td>
<td>84.44 ± 4.68a</td>
<td>1401.8 ± 131b</td>
<td>1812.0 ± 101a</td>
<td>264.83 ± 16.2A</td>
<td>20.48 ± 1.43b</td>
</tr>
<tr>
<td>V+T</td>
<td>68.40 ± 5.72</td>
<td>1911.6 ± 152a</td>
<td>1998.9 ± 133</td>
<td>199.38 ± 17.0p</td>
<td>28.60 ± 2.34b</td>
</tr>
<tr>
<td>V+T+Zn</td>
<td>73.33 ± 6.55</td>
<td>2028.6 ± 184a</td>
<td>1824.4 ± 116</td>
<td>257.80 ± 18.9</td>
<td>24.24 ± 1.72</td>
</tr>
<tr>
<td>V+T+Se</td>
<td>75.80 ± 3.76</td>
<td>2154.6 ± 158b</td>
<td>2107.0 ± 125</td>
<td>221.00 ± 10.9p</td>
<td>28.76 ± 1.43b</td>
</tr>
<tr>
<td>V+T+Vit E</td>
<td>74.20 ± 4.33</td>
<td>1837.4 ± 151</td>
<td>1910.8 ± 160</td>
<td>238.00 ± 14.2</td>
<td>27.66 ± 1.40</td>
</tr>
</tbody>
</table>

One-way ANOVA 2.29a 4.84a 3.79a 2.48a 3.88a

Values are expressed as mean ± S.E., n = 6, p values A ≤ 0.05, B ≤ 0.01, C ≤ 0.001 when compared with control group, p values a ≤ 0.05, b ≤ 0.01, c ≤ 0.001 p ≤ 0.05 when compared with toxicant administered group.

ANOVA (F values at 5% level)  a = Significant, NS- non significant.
Combination therapy against vanadium intoxication.

Administration of vanadyl sulphate showed ultrastructural changes in liver. Marked degenerative changes were observed in mitochondria, endoplasmic reticulum and nucleus of hepatocytes and large numbers of fat droplets were seen. Deformed nuclei were also noted (Fig. 7). Treatment with Tiron along with selenium showed well-maintained endoplasmic reticulum with ribosomes. Well-formed mitochondria and evenly distributed glycogen bodies were also observed (Fig. 8). These findings are substantiated with biochemical observations.

**DISCUSSION**

A chemical which is quite stable on entering a cell leads to its metabolic activation to a highly toxic metabolite thus eliciting morphological and biological alterations. Induction of reactive oxygen species by metal and subsequent depletion of antioxidant cell defenses can result in disruption of prooxidant/antioxidant balance in mammalian tissues. In the event that oxidative stress can be partially implicated in metal toxicity, a therapeutic strategy to increase the antioxidant capacity of cells may fortify the long-term effective treatment of metal poisoning. In the present study AST and ALT were elevated after exposure to vanadium which may lead to tissue lysosomal disruption phagocytosis and acute cellular injury. Cam et al. (1993) also substantiated these findings. Lactate dehydrogenase, a bifunctional enzyme of the glycolytic

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**Fig. 1.** Vanadium caused hypertrophy of hepatocytes with hyperchromatic nuclei (× 400).

**Fig. 2.** Hexagonal hepatocytes were observed after conjoint treatment with Tiron (× 120).

**Fig. 3.** Treatment with Tiron + Se liver showed maintained chord arrangement (× 120).

**Fig. 4.** After vanadium exposure glomeruli showed hypertrophy (× 400).
pathway is involved in the inter-conversion of lactate and pyruvate. Vanadate may have some interaction with the enzyme moiety and coenzyme system (Kanthasamy et al., 1988). Enhanced values of this enzyme clearly indicated damage in plasma membrane. All these biochemical changes depicted significant improvement after Tiron therapy when supplemented with Se; however the other two combinations (Zn and Vit E) also showed recoupepment in LDH and ALT.

Vanadium caused increase in glycogen contents of liver and kidney. This may be due to activation of glycogenesis and inhibition in the activity of glycogen phosphorylase enzyme. The ability of vanadium compounds is to stimulate glucose uptake and lipid synthe-

sisis in muscle, adipose and hepatic tissues and as to inhibit the activities of gluconeogenic enzymes (Srivastava and Mehdi, 2005). Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis and is firmly bound to the endoplasmic reticulum. Vanadate was reported to be a potent inhibitor of glucose-6-phosphatase (Srivastava and Mehdi, 2005) as also seen in present investigation and may be due to its hydrolytic activity.

Increased acid phosphatase activity in liver may be due to the metallic nature of vanadium which alters the cell membrane permeability and loss of lysosomal stability. A fall in the alkaline phosphatase activity may be attributed to the displacement of Mg\(^{2+}\) by VO\(^{2+}\) because its size resembles with Mg\(^{2+}\). These findings

**Fig. 5.** Treatment with Tiron showed well-formed Bowman’s capsules with glomeruli (× 400).

**Fig. 6.** Treatment with Tiron + Se showed well-formed uriniferous tubules (× 400).

**Fig. 7.** Vanadium induced vacuolation in cytoplasm & deformed nuclei (× 3400).

**Fig. 8.** Treatment with Tiron with selenium showed well-formed mitochondria and endoplasmic reticulum (× 2650).
are substantiated by various authors (Johari et al., 2002; Haider et al., 1998; Nechay, 1984). Inhibition in adenosine triphosphatase pattern is due to the unphosphorylated enzyme which interferes with metal ion. Experimental results have also shown a link between vanadium and oxidative stress in the etiology of diabetes (Valko et al., 2005). Vanadium accumulation in the tissue with its concomitant redox cycling may lead to lipid peroxidation (Younes and Strubelt, 1991). GSH forms vanadyl / GSH complex thereby reducing the GSH contents (Saxena et al., 1993). It has been reported that mitochondrial damage is prevented when oxidative stress is suppressed. Vanadium also inhibits succinic dehydrogenase activity. This fall results in overall decrease in the energy production and may be due to the structural and functional disorganization of mitochondria assembly as also seen by electron micrographical studies. Co administration of Zn and Vit E also showed recovery but Tiron +Se was significantly effective in recouping-markers like GSH, G-6-Pase, ATPase and glycogen.

The effectiveness of Tiron could be attributed to the chelating properties and available binding sites of Tiron, which leads to the decreased concentration of vanadium. The therapeutic effectiveness (TEF) of Tiron was approximately equal to one. This chelator significantly increased urinary excretion of vanadium from the body. Thus the concentration of the metal was significantly reduced in kidney and liver. Tiron was also reported as an effective antidote for vanadyl sulphate intoxication in mice (Gomez et al., 1991). The efficacy of Tiron to mobilize vanadium and to restore the alterations in biochemical parameters may be due to the available binding sites and stability constant of the metal chelator complex. Coordination number of Tiron is four and one molecule of Tiron may replace their hydrogen atoms and bind to V with its oxygen atom, thereby forming a stable complex.

Antioxidants provide protection against deleterious metal mediated free radical attacks. Zn and Vitamin E are important elements which prevent free radical generation, protecting biological membranes from damage induced by vanadium, arsenic and other metals (iron, copper, cadmium) in vitro systems and in metal loaded animals (Stefanidou et al., 2005; Ramanathan et al., 2003). The impact of zinc on the immune system in the etiology of Alzheimer’s disease is also discussed (Valko et al., 2005). In the present investigation they play only a limited role in depleting vanadium toxicity. Zn may not compete effectively with the binding sites which might partially be responsible for its protective effect. Vit E also showed comparatively partial recovery this may be due to its ability to stabilize membrane by interacting with unsaturated fatty acid chain.

Selenium, a required dietary element for health at low dose, is an integral component of ubiquitous enzyme glutathione peroxidase, an antioxidant enzyme. This enzyme helps in neutralization of reactive oxygen species (ROS). The need for selenium substitution in artificial nutrition is suggested and for this use selenomethionine appears to be the most suitable form. Selenoproteins are generally known to concentrate in cytosol of animal tissues and have a role in immune response and liver cirrhosis in human body. This protects neuronal cells against neurotoxic effects of vanadium by maintaining the availability of antioxidant nonprotein sulphydryl groups (Haider et al., 1998). The protective mechanism could be attributed due to the competition between selenium and vanadium for binding with the functional proteins and bioligand or active tissue sites or the formation of a reversible compound, metal-selenite thus reducing the availability of “free” concentration of toxic metals ions in the body might be a possible mechanism for the observed antagonism between Vanadium and Se. Thus T+Se was most effective and prevented oxidative degradation of biological membrane by catalyzing the donation of hydrogen atom from reduced glutathione.

ACKNOWLEDGMENT

Authors are thankful to Indian Council of Medical Research and University Grant Commission, New Delhi, for financial assistance.

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