Comparative effectiveness of CaNa₃DTPA and tiron along with α-tocopherol against beryllium-induced biochemical alterations in rats

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The therapeutic efficacy of chelating agents CaNa₃DTPA (calcium trisodium diethylene triamine penta-acetic acid) and Tiron (sodium-4, 5-dihydroxy-1, 3-benzene disulphonate) with and without antioxidant, α-Tocopherol was evaluated in the treatment of beryllium-induced toxicity in female albino rats. The animals were exposed to beryllium (as beryllium nitrate) at a dose of 1mg/kg (ip) once a day for 28 consecutive days followed by chelation therapy by CaNa₃DTPA (0.1 mM/kg, ip) and Tiron (471 mg/kg, ip) with and without α-Tocopherol (25 mg/kg, orally) for 5 consecutive days after toxicant administration. Tissue biochemistry revealed severe alterations in liver and kidney. A significant fall in total protein and glycogen contents, alkaline phosphatase, adenosine tri-phosphatase and succinic dehydrogenase level was noticed. On the contrary, an elevation in acid phosphatase was recorded. The significant rise in hepatic lipid peroxidation and decreased level of hepatic reduced glutathione showed toxicity due to beryllium. CaNa₃DTPA with α-Tocopherol showed moderate therapeutic efficacy while Tiron in combination with α-Tocopherol exerted statistically more beneficial effects to reverse biochemical alterations in different variables altered due to beryllium intoxication.

Keywords: Beryllium, CaNa₃DTPA, Chelation, Tiron, α-Tocopherol

The use of chelating agents along with antioxidants for possible metal detoxification is an important aspect against metal poisoning. α-Tocopherol is one of the most important lipophilic antioxidants and resides mainly in the cell membrane, thus helping to maintain membrane stability. Supplementation of α-Tocopherol serves as an effective method of preventing membrane damage caused by oxygen radicals. Tremendous decrease in the body burden of lead in various vital organs by combined administration of α-Tocopherol and conventional chelators is also reported. CaNa₃DTPA (calcium trisodium diethylene triamine penta-acetic acid) is an octadentate chelating agent. Two nitrogen atoms of amine groups of one molecule of CaNa₃DTPA form complex by covalent binding with metal ion after replacing their hydrogen atoms. Tiron (sodium-4, 5-dihydroxy-1, 3-benzene disulphonate) is reported as a superior antidote because LD₂₅₀ of Tiron is significantly higher than that of CaNa₃DTPA through ip route. It has orthodiphenolic chelate structure, which forms water soluble complex with metal ions and toxicity of this complex is less than that of the metal ion it contains. Further the complex is easily excreted out from the body.

Due to rapid industrialization and urbanization, technological use of beryllium is increasing every day. Because of its unique properties, it has found numerous applications in aerospace, defense and electronics industry. Beryllium is one of the metals having specific properties like low density, extreme stiffness and ability to add strength when small amounts are added to copper and nickel. The general population is exposed to naturally occurring beryllium from ambient air, drinking water, diet and smoking on a daily basis and estimates of total daily exposure to beryllium from background sources (including water and food) range from 0.5-20 µg/day. Emissions from burning of fossil fuels i.e. coal and oil increase beryllium level in atmosphere. Beryllium exposure can cause acute pneumonitis, contact dermatitis, bronchitis, pulmonary granulomatosis, hepatomegaly.
and Chronic Beryllium Disease (CBD). CBD is a hypersensitive immunological response to beryllium, resulting in granuloma in the lungs of affected individuals, which can result in impaired lung function\textsuperscript{12}. Ultimately accumulation of beryllium in tissues causes cellular death. The present study has been designed with the objective of evaluating the comparison of therapeutic efficacy of chelating agents CaNa\textsubscript{3} DTPA and Tocopherol in the treatment of beryllium induced toxicity.

Materials and Methods

Beryllium nitrate [Be(NO\textsubscript{3})\textsubscript{2}], was purchased from Fluka (Switzerland). CaNa\textsubscript{3} DTPA (calcium trisodium diethylene triamine penta acetic acid) and Tiron (sodium-4,5-dihydroxy-1,3-benzene disulphonate) were obtained from Sigma-Aldrich Chemicals Pvt. Ltd. (New Delhi, India) and \alpha-Tocopherol acetate from Himedia Laboratories Ltd, Mumbai, India. 35 adult female albino rats of Sprague Dawley strain (130\pm 10 g body weight) were selected from the departmental colony and kept under uniform husbandry conditions of light (14 hr) and dark (10 hr) and temperature (24° \pm 2°C). The rats were fed on standard pelleted diet (Pranav Agro Industries Ltd, New Delhi, India) and drinking water \textit{ad libitum}.

The rats were divided into 7 groups of 5 each as follows: Group 1- received sodium nitrate (1 mg/kg, ip) once a day for 28 consecutive days and served as normal; Group 2-received beryllium nitrate (1 mg/kg, ip) once a day for 28 consecutive days followed by 5 days rest and served as control; Group 3- received toxicant as in group 2 and treated with CaNa\textsubscript{3} DTPA (0.1 mM/kg, ip) after toxicant administration for 5 consecutive days and served as Experimental group-I; Group 4- received toxicant as in group 2 and treated with Tiron (471 mg/kg, ip) after toxicant administration for 5 consecutive days and served as Experimental group-II; Group 5- received toxicant as in group 2 and treated with of \alpha-Tocopherol (25 mg/kg, po) after toxicant administration for 5 consecutive days and served as Experimental group-III; Group 6- received toxicant as in group 2 and treated with CaNa\textsubscript{3} DTPA (0.1 mM/kg, ip) and \alpha-Tocopherol (25 mg/kg, po) simultaneously for 5 consecutive days after toxicant administration and served as Experimental group-IV; and Group 7- received toxicant as in group 2 and treated with Tiron (471 mg/kg, ip) and \alpha-Tocopherol (25 mg/kg, po) simultaneously for 5 consecutive days after toxicant administration and served as Experimental group-V.

The doses of chelating agents, CaNa\textsubscript{3} DTPA and Tiron were prepared daily in 0.9% saline and the pH was adjusted to 6.4 with sodium bicarbonate before administration. \alpha-Tocopherol was dissolved in olive oil and a dose of 25 mg/kg was administered orally with the help of intragastric rubber catheter. After 24 hr of final administration, animals were sacrificed under light ether anaesthesia and liver and kidneys were excised, blotted free of adhering fluid and acid phosphatase and alkaline phosphatase\textsuperscript{13}, adenosine triphosphatase\textsuperscript{14}, succinic dehydrogenase\textsuperscript{15}, total protein content\textsuperscript{16}, glycogen content\textsuperscript{17}, hepatic lipid peroxidation\textsuperscript{18} and hepatic reduced glutathione\textsuperscript{19} were assayed.

Statistical analysis—The data were subjected to statistical analysis using Student’s \textit{t} test followed by one way analysis of variance (ANOVA). All the results were considered statistically significant at \(P \leq 0.05\).

Results and Discussion

The results are presented in Tables 1 and 2.

Beryllium is reported to have toxic effects on several biological systems with liver as one of its main target organ. Present investigation revealed severe alterations in biochemical variables after beryllium exposure. Beryllium toxicity is found to be associated with the increase in the acid phosphatase activity in liver, which may be due to altered cell membrane properties, causing mitochondrial damage and stimulation of lysosomes\textsuperscript{20} hence subsequent increase in acid phosphatase activity\textsuperscript{21}. It is also possible that the enzyme may have accumulated in the intercellular space due to destruction in the membrane due to altered membrane permeability\textsuperscript{22}.

Magnesium ion (Mg\textsuperscript{2+}) is an important factor for maintaining the activity of alkaline phosphatase. \textit{In vivo} and \textit{in vitro} studies have suggested that beryllium always competes with Mg\textsuperscript{2+} and inhibition in the activity of alkaline phosphatase during beryllium toxicity may be due to the displacement of Mg\textsuperscript{2+} by beryllium ions (Be\textsuperscript{2+})\textsuperscript{23}. Inhibition may also be due to the formation of insoluble phosphate, which further interferes with the absorption of phosphates in gastrointestinal tract. A disturbance in the activity of alkaline phosphatase, which is responsible for the movement of metabolites across membrane, indicates impaired nutrients assimilation and absorption\textsuperscript{24,25}.
ATPase is a lipid dependent membrane bound enzyme. Any alteration in membrane lipids leads to change in membrane fluidity, which in turn alters the ATPase mediated cellular functions. Beryllium induced inhibition of adenosine triphosphatase activity as seen in the present investigation could be mediated either through the attachment of beryllium to the enzymes via the phosphate group or more likely due to the combination with the unphosphorylated enzyme in a way that it interferes with the Mg$^{2+}$ required for its activity. Further, inhibition of the adenosine triphosphatase activity may be due to the generation of free radicals by beryllium. Accumulation of beryllium in the cell finally leads to cell death and onset of chronic beryllium disease. Simultaneous treatment with α-Tocopherol along with chelating agents resulted in the reversal of the level of ATPase in liver and kidney of beryllium exposed rats.

Beryllium is reported to block tricarboxylic acid cycle by inhibiting the activity of succinic dehydrogenase. The fall in SDH activity results in overall decrease in the energy production and metabolic turnover, which may be attributed to structural and functional disorganization of the mitochondrial assembly. The present observations correlate with the WHO report, which states that the maleic acid, succinic and α-ketoglutaric dehydrogenase in the liver and lungs of the rats are inhibited after intramuscular administration of beryllium.

Beryllium inhibits total protein content both in liver and kidney. Several possibilities can be put forth to explain these results. Bulk of the circulating beryllium

### Table 1—Effectiveness of chelating agents along with α-Tocopherol against Be(NO₃)₂ exposed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acid phosphatase (mg P/100 g/hr)</th>
<th>Alkaline phosphatase (mg P/100 g/hr)</th>
<th>Adenosine triphosphatase (mg P/100 g/min)</th>
<th>Succinic dehydrogenase (n moles K₅Fe(CN)₆ reduced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal</td>
<td>242±6.20</td>
<td>282±3.60</td>
<td>747±90</td>
<td>2645±90</td>
</tr>
<tr>
<td>Control</td>
<td>340±4.40</td>
<td>340±39.10</td>
<td>53±78</td>
<td>174±24</td>
</tr>
<tr>
<td>Experimental-I</td>
<td>328±47.10</td>
<td>313±33.40</td>
<td>586±146</td>
<td>1979±206</td>
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<tr>
<td>Experimental-II</td>
<td>286±42.70</td>
<td>291±33.50</td>
<td>67±378</td>
<td>2014±311</td>
</tr>
<tr>
<td>Experimental-III</td>
<td>294±39.20</td>
<td>317±33.15</td>
<td>645±10</td>
<td>2270±290</td>
</tr>
<tr>
<td>Experimental-IV</td>
<td>274±31.07</td>
<td>257±36.20</td>
<td>68±71</td>
<td>215±240</td>
</tr>
<tr>
<td>Experimental-V</td>
<td>267±32.40</td>
<td>285±32.80</td>
<td>71±83</td>
<td>2443±287</td>
</tr>
</tbody>
</table>

One way ANOVA

F Variance at 5% level

3.801<sup>c</sup> 3.208<sup>NS</sup> 4.514<sup>c</sup> 5.388<sup>c</sup> 1.941<sup>NS</sup> 5.087<sup>c</sup> 26.151<sup>c</sup> 27.884<sup>c</sup>

P values < 0.05;<sup>a</sup> compared with normal group; <sup>b</sup> compared with control group; <sup>c</sup> significant F variance at 5% level; <sup>NS</sup> = non-significant.

### Table 2—Effectiveness of chelating agents along with α-tocopherol against Be(NO₃)₂ exposed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protein (mg / 100 g)</th>
<th>Glycogen (mg / 100 g)</th>
<th>Hepatic Lipid Peroxidation (n moles MDA/mg protein)</th>
<th>Hepatic Reduced Glutathione (μ moles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal</td>
<td>15.80±1.72</td>
<td>14.20±1.46</td>
<td>2792±301</td>
<td>86.20±11.30</td>
</tr>
<tr>
<td>Control</td>
<td>12.10±1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.80±1.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1929±206</td>
<td>42.80±5.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Experimental-I</td>
<td>14.48±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.92±1.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2028±225</td>
<td>76.00±7.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Experimental-II</td>
<td>14.30±1.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.12±1.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2310±244&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.70±8.65&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Experimental-III</td>
<td>13.36±1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.70±1.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>243±249&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.80±9.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Experimental-IV</td>
<td>14.96±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.50±1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2198±247</td>
<td>82.00±8.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Experimental-V</td>
<td>14.90±1.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.30±1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2645±268</td>
<td>74.04±9.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

One way ANOVA

F Variance at 5% level

3.003<sup>NS</sup> 9.131<sup>c</sup> 7.785<sup>c</sup> 14.968<sup>c</sup> 13.933<sup>c</sup> 4.483<sup>c</sup>

P values < 0.05;<sup>a</sup> compared with normal group; <sup>b</sup> compared with control group; <sup>c</sup> significant F variance at 5% level; <sup>NS</sup> = non-significant.
binds with plasma globulin because of its apparent protein binding property and forms stable beryllium-protein complex, which is transported to various organs\textsuperscript{31,32}. Thus, a large amount of beryllium is accumulated in liver and causes damage. The impaired functioning of liver, which is a major source of protein synthesis and metabolism may also diminish the protein level. The reduction in glycogen content may be due to disturbances in the carbohydrate metabolism and impairment of key enzymes such as hexokinase, glucokinase, phosphoglucomutase and glucose-6-phosphatase\textsuperscript{33}. Toxicant induced stress at biochemical level is based on the production of free radicals. Beryllium exposure indicates oxidative damage due to impairment of natural protecting system of the cells. Lipid peroxidation, a measure of membrane damage enhances in presence of some metal ions resulting in oxidative product or MDA. Lipid peroxidation could be prevented by reducing the formation of free radicals by (1) destroying the free radicals that are already formed, (2) supplying a competitive substrate for unsaturated lipids in the membrane, and (3) accelerating the repair mechanism of damaged cell membrane. In this case, the administration of exogenous antioxidant, \( \alpha \)-Tocopherol to counteract the proportionate magnitude of the cell injury plays a pivotal role in the treatment of free radical mediated cell injury. Glutathione (GSH) plays a protective role in tissue by detoxification of xenobiotics\textsuperscript{34}. In the present investigation, it was observed that level of hepatic reduced glutathione decreased after administration of beryllium nitrate. The decrease in the glutathione level observed may be due to increased utilization by the hepatocytes\textsuperscript{35,36}, because GSH seems to act as scavenger for toxic chemical agent\textsuperscript{37} and further it also acts as a natural antioxidant. Supplementation of therapeutic agents during severe liver damage conditions elevated the GSH levels, which in turn helps in recouping the liver tissue damage. \( \alpha \)-Tocopherol as an antioxidant prevents the oxidative degeneration of biological molecules along with chelation (by chelators) thereby, enhancing the effectiveness of the chelating agents.

In the present investigation, it was found that Tiron in combination with \( \alpha \)-Tocopherol exerted more beneficial effects over a combination of CaNa\textsubscript{2}DTPA and \( \alpha \)-Tocopherol treatment against beryllium induced biochemical alterations in various variables. Thus, combined therapy of Tiron and \( \alpha \)-Tocopherol could be a better choice in treatment of beryllium induced biochemical alterations. Further work in this direction is going on before a final recommendation could be made.

Acknowledgement

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References