Beryllium induced toxic manifestations in rat brain: Dose and duration dependent study

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Exposure to beryllium causes bronchitis, bronchiolitis, chronic pulmonary granulomatosis, pneumonitis, hepatic and nephrotoxicity. In the present study, we carried out a dose and duration dependent study on beryllium induced toxic manifestations in rat brain. For dose dependent experiment, four groups of female albino rats were challenged with beryllium nitrate at 0.25, 0.50, 0.75 and 1.0 mg/kg, i.p. daily for 2 weeks, respectively. Similarly, for duration dependent study, animals were challenged with 1.0 mg/kg, i.p. dose of beryllium nitrate for 2, 4 and 6 weeks, respectively. Behavioural, biochemical and histopathological variables were performed to evaluate toxic effects of beryllium. Beryllium exposure decreased body weight, decreased motor coordination and increased anxiety level in dose and duration dependent manner. Total RBCs and hemoglobin content were decreased after beryllium intoxication. Serum bilirubin, creatinine, triglyceride, AST and ALT content were increased after beryllium intoxication, whereas ALP content was decreased in dose and duration dependent manner. Peroxidative damage was noticed in different parts of brain in dose and duration dependent manner. Beryllium administration altered histoarchitecture of brain cortex in same manner. In dose dependent study, maximum alterations in various parameters were evident at 1.0 mg/kg dose, and also it showed toxic consequences of beryllium in a duration dependent manner. Thus, exposure of 1.0 mg/kg dose of beryllium for 4 weeks duration was found to be most suitable for further studies of assessment of therapeutic agent against beryllium induced neurotoxicity.

Keywords: Behavioural studies, Blood, Brain, Lipid peroxidation, Metal toxicity, Neurotoxicity

Industrial expansion and consequent pollution has led to range of health problems in general population. A diverse number of metals have been reported to exert serious health effects on human and animals. One of these metals is beryllium, which is of light density but hard and its compounds possess exceptional properties, which make them essential in several modern industries1. People living in the vicinity of power plants get affected by beryllium through burning of coal and its metallurgical processing like melting, casting, cutting and electroplating. Other activities like handling of broken florescent tubes, diamond cutting by beryllium knife are also sources of its exposure to humans. Pulmonary disease due to toxic consequences of beryllium compounds were reported during 1930s and 1940s. Hypersensitivity reactions due to exposure to beryllium salt were reported causing chronic beryllium disease (CBD) and its occupational exposure causes a number of diseases, such as bronchitis, bronchiolitis, chronic pulmonary granulomatosis and pneumonitis2. Exposure to beryllium also causes alterations in histoarchitecture of vital organs, several enzymatic activities like lysosomal instability and release of liver lysosomal enzymes, serum biochemical alterations and histological deteriorations in liver, kidney, spleen and lungs3-5.

Though considerable studies have been conducted on deleterious effects of beryllium salts on liver, kidney, spleen and lung, investigations on beryllium induced neurotoxicity are scarce. This pilot study was designed to evaluate toxic consequences of beryllium nitrate in dose and duration dependent manner so that a highly standardized dose and duration for beryllium induced toxicity could be established for developing further therapeutic strategies against beryllium induced toxic manifestations in brain. Study included physical development, behaviour, blood and brain variables for evaluation.

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Materials and Methods

Animals and chemicals

Female rats are more susceptible to beryllium toxicity, so female albino rats of Wistar strain (180±20 g) were used in this study and purchased from Defense Research and Development Establishment (DRDE), Gwalior (MP) India. Animals were acclimatized for two weeks prior to experimentation under standard husbandry conditions and allowed pelleted rat feed and water ad libitum. Experiments were carried out with the approval from institutional animal ethics committee (994/Ere/Go/06/CPCSEA). All the chemicals used in this study were of pure and analytical grade and procured from standard chemical dealers in India.

Experimental design

The whole study was divided in two sets of experiments. First experiment dealt with the selection of optimum toxic dose of beryllium nitrate whereas second experiment was conducted to evaluate optimum toxic duration of beryllium nitrate.

Dose dependent study

Two weeks study was conducted on 30 female rats which were divided into 5 groups of 6 animals in each. Group I served as control and received distilled water as vehicle. Animals of Group II to IV received different doses of beryllium nitrate viz. 0.25, 0.50, 0.75 and 1.0 mg/kg i.p., respectively for two weeks.

Duration dependent study

Second experiment dealt with the selection of optimum toxic duration of beryllium. For this purpose, 24 female Wistar rats were divided into 4 groups of 6 animals in each and Group I received vehicle and served as control. Animals from Groups II to IV were administered with beryllium nitrate (1.0 mg/kg, i.p.; as selected from experiment 1) for 2, 4 and 6 weeks, respectively.

Further, the following variables were considered during study.

Motar coordination status

Rotarod was used to monitor motar coordination status or balance skill of animals. In this experiment, rats were placed on a rotating rod to analyze the effects of beryllium nitrate on motor coordination and balance skills6.

Behavioral studies for anxiety status

Elevated plus maze and light and dark chamber are two experimental models for evaluation of anxiety, based on the assumption that unfamiliar, non-protective and bright light environmental stress provokes inhibition of normal behaviour. The elevated plus maze consists of two open arms (50 × 10 cm L × B) and enclosed arms of the same size (Elevated height from surface 40 cm), an open roof arranged in such a manner that the two open arms are opposite to each other. Rats were individually placed on the center of the maze facing an open arm, and the number of entries and the time spent in closed and open arms were recorded during a 5 min observation period7.

The light and dark chamber consists with two distinct chambers, a dark chamber (20×30×35 cm) painted black and a bright chamber (30×30×35 cm) painted white and brightly illuminated with 100 W white light sources. By observing the time duration, an animal spends in light or dark arena in light-dark chamber, one can predict the anxiety or depression status of that animal8,9.

Blood collection, preparation of serum and isolation of tissues

After 24 h of the last administration, just before the euthanasia of animals, blood was collected in glass tubes from retro orbital venous sinus10. Blood samples were left for 30 min at 37°C were centrifuged at 3000 rpm for 10 min to obtain serum that was stored at −20°C for various serological parameters. Blood was also collected in EDTA coated vials and used for hematological studies. Rats were euthanized under mild anaesthesia and different regions of brain (forebrain, midbrain and hindbrain) were quickly dissected out and kept at −20°C for further study. Anterior left side portion of forebrain was fixed immediately in Bouin’s fixative for histological studies.

Hematological analyses

The collected blood in EDTA vials was assayed by semi automated blood analyzer (Hema 2062+) to assess number of red blood corpuscles (RBCs) count and amount of hemoglobin (HGB).

Serological analyses

Serum assay kits (Erba Mannheim Company) were used for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, creatinine, triglycerides, cholesterol and albumin levels in serum.

Determination of lipid peroxidation

Tissue biochemical parameters included total protein contents and lipid peroxidation. Total protein evaluation was done by Lowry’s protocol11 and lipid peroxidation was determined according to Sharma & Krishnamurthy12.
Histological studies

Tissues were dehydrated in graded series of alcohol, embedded in paraffin wax; sections were cut into 5 µm thickness, stained with hematoxylin and eosin (H&E) and observed under light microscope.

Statistical analysis

The data was expressed as Mean ± SEM. The data was analyzed by student’s t-test and compared with controlled group. P ≤ 0.05 was considered as significant.

Results

Dose dependent study

Physical development

Different doses of beryllium (0.25, 0.50, 0.75 and 1.0 mg/kg) administered for 2 wk decreased body weight in animals in dose dependent manner. Higher doses i.e. 0.75 and 1.0 mg/kg doses significantly decreased body weight (P ≤ 0.01) (Table 1).

Motor coordination status

Administration of beryllium at different doses i.e. 0.25, 0.50, 0.75 and 1.0 mg/kg, i.p. for 14 days in animals hampered motor balance in dose dependent manner. All the doses of beryllium caused significant alterations at different significant level. Maximum alterations in motor balance were noted at 1.0 mg/kg (P ≤ 0.001). Beryllium at 0.75 and 0.5 mg/kg caused alterations at P ≤ 0.001 and 0.25 mg/kg dose found toxic at P ≤ 0.005 level (Table 1).

Behavioral observations for anxiety status

Anxiety level was measured by elevated plus maze, observing open arm entries and % time spent in open arm. Beryllium administration at different doses enhanced anxiety level (decreased % time spent in open arm) in dose dependent manner however, significant alterations were noted at all the doses except 0.25 mg/kg. All the three higher doses of beryllium (0.5, 0.75 and 1.0 mg/kg) enhanced anxiety level in animals (P ≤ 0.001). Open arm entries showed significant alterations at all the doses. Administration of 0.25 mg/kg dose of beryllium showed less toxic effect (P ≤ 0.01) in comparison to other higher doses (P ≤ 0.001).

Light and dark chamber was used as a supporting model to assess the level of anxiety in experimental animals. Different doses of beryllium for 14 days enhanced the anxiety level (decreased % time spent in bright area and number of transition) in dose dependent manner. Higher two doses of beryllium 0.75 and 1.0 mg/kg enhanced anxiety level significantly (P ≤ 0.001), whereas 0.50 mg/kg dose of beryllium significantly enhanced anxiety level at (P ≤ 0.01) but 0.25 mg/kg dose of beryllium was not found to be significant (Table 1).

Hematological observations

Beryllium exposure at different doses for 14 days decreased hemoglobin content significantly in comparison to control animals. Higher doses 0.75 and 1.0 mg/kg decreased hemoglobin contents (P ≤ 0.01) and lower doses 0.25 and 0.50 mg/kg decreased hemoglobin contents at P ≤ 0.05 significantly (Table 2). Administration of beryllium at different doses 0.25, 0.50, 0.75 and 1.0 mg/kg, i.p for 14 days decreased RBCs count significantly in dose dependent manner. Higher two doses 0.75 and 1.0 mg/kg reduced RBCs count significantly (P ≤ 0.001). Both the lower doses i.e., 0.50 and 0.25 mg/kg were found significant at 5% level (Table 2).

Table 1 — Dose dependent effect of beryllium on physical and behavioural variables

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Body weight (% increase from initial weight)</th>
<th>Rotarod seconds</th>
<th>Elevated plus maze</th>
<th>Light and dark chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.0±0.81</td>
<td>40±2.84</td>
<td>18±0.126</td>
<td>5.00±0.35</td>
</tr>
<tr>
<td>Be (0.25 mg)</td>
<td>10.1±0.78</td>
<td>32±1.90</td>
<td>15±1.33</td>
<td>2.50±0.19</td>
</tr>
<tr>
<td>Be (0.50 mg)</td>
<td>8.20±0.51</td>
<td>28±1.94</td>
<td>12±0.63</td>
<td>1.60±0.12</td>
</tr>
<tr>
<td>Be (0.75 mg)</td>
<td>7.30±0.50</td>
<td>26±1.70</td>
<td>11±0.66</td>
<td>0.85±0.05</td>
</tr>
<tr>
<td>Be (1.00 mg)</td>
<td>6.80±0.55</td>
<td>21±1.26</td>
<td>10±0.68</td>
<td>0.50±0.03</td>
</tr>
<tr>
<td>ANOVA</td>
<td>13.4@</td>
<td>15.3@</td>
<td>13.1@</td>
<td>6.03@</td>
</tr>
</tbody>
</table>

Table 2 — Dose dependent effect of beryllium on hematological and serological variable

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Hemoglobin (g/dL)</th>
<th>RBC (10^6/mm^3)</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.4±0.84</td>
<td>4.3±0.24</td>
<td>21.3±1.81</td>
<td>30.2±1.82</td>
</tr>
<tr>
<td>Be (0.25 mg)</td>
<td>11.7±0.61</td>
<td>3.43±0.19</td>
<td>27.2±2.68</td>
<td>34.6±2.35</td>
</tr>
<tr>
<td>Be (0.50 mg)</td>
<td>11.5±0.60</td>
<td>3.18±0.23</td>
<td>30.4±2.37</td>
<td>36.1±2.25</td>
</tr>
<tr>
<td>Be (0.75 mg)</td>
<td>10.7±0.62</td>
<td>2.63±0.18</td>
<td>31.9±2.85</td>
<td>39.6±2.84</td>
</tr>
<tr>
<td>Be (1.00 mg)</td>
<td>9.90±0.68</td>
<td>2.48±0.19</td>
<td>32.8±2.42</td>
<td>41.7±2.72</td>
</tr>
<tr>
<td>ANOVA</td>
<td>7.60@</td>
<td>75.7@</td>
<td>4.34@</td>
<td>4.08@</td>
</tr>
</tbody>
</table>

Table 3 — Dose dependent effect of beryllium on histological studies

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Histological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No significant changes</td>
</tr>
<tr>
<td>Be (0.25 mg)</td>
<td>Significant changes</td>
</tr>
<tr>
<td>Be (0.50 mg)</td>
<td>Significant changes</td>
</tr>
<tr>
<td>Be (0.75 mg)</td>
<td>Significant changes</td>
</tr>
<tr>
<td>Be (1.00 mg)</td>
<td>Significant changes</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Significant changes</td>
</tr>
</tbody>
</table>

[Data are mean ± SE of n = 6; @ Significant at 5% for ANOVA. Be vs. Control at P ≤ 0.05, Be vs. Control at P ≤ 0.01, Be vs. Control at P ≤ 0.001. Be= Beryllium, and RBC= Red blood corpuscles]
to be less toxic statistically and showed decrement in RBCs count significantly at 1 and 5% level, respectively (Table 2).

**Serological observations**

Beryllium administration for 14 days caused sharp elevation in the leakage of ALT at 0.75 and 1.0 mg/kg doses ($P \leq 0.001$). The 0.50 mg/kg dose of beryllium caused significant elevation in ALT activity at 1% level. No significant elevation was noted at 0.25 mg/kg dose of beryllium (Table 3). Administration of beryllium at 0.75 and 1.0 mg/kg doses caused significant elevation in leakage of AST at $P \leq 0.001$ and $P \leq 0.01$, respectively. But no significant elevation was observed in AST activity at 0.25 and 0.50 mg/kg doses (Table 3). Beryllium intoxication decreased serum albumin, whereas increased bilirubin and creatinine contents with increasing doses of beryllium. All the doses of beryllium increased serum creatinine level significantly ($P \leq 0.001$). Beryllium intoxication at 0.75 and 1.0 mg/kg dose reduced albumin content significantly ($P \leq 0.001$) in comparison to lower doses. Bilirubin was shown to be maximum with 1.0 mg/kg dose (Table 3). Serum triglycerides and cholesterol content were significantly increased with 1.0 mg/kg dose of beryllium at $P \leq 0.001$ level of significance (Table 2).

**Oxidative stress**

Beryllium exposure enhanced LPO in forebrain, mid brain and hindbrain in dose dependent manner (Table 4). Beryllium at doses 0.75 and 1.0 mg/kg enhanced LPO in forebrain significantly at $P \leq 0.01$ whereas, 0.5 mg/kg dose enhanced LPO in forebrain significantly at $P \leq 0.05$. Highest two doses of beryllium 0.75 and 1.0 mg/kg enhanced LPO in midbrain significantly at $P \leq 0.01$, whereas 0.5 mg/kg dose enhanced LPO in midbrain significantly at $P \leq 0.01$ and 0.25 mg/kg dose of beryllium enhanced LPO significantly at $P \leq 0.05$. The 1.0 mg/kg dose of beryllium for 14 days enhanced LPO significantly ($P \leq 0.001$), whereas lowermost did not register any significant increment.

Total amount of protein content was decreased in different parts of brain in dose dependent manner after beryllium administration (Table 4). Beryllium administration in different part of brain showed maximum decreased values of protein at 1 mg/kg dose of beryllium ($P \leq 0.01$). Lower doses i.e., 0.25 and 0.50 mg/kg did not show any significant changes in total protein content in forebrain, midbrain and hindbrain.

**Histopathological observations**

Fig. 1 illustrates effects of beryllium on histology of cerebral cortex of brain. Cerebral cortex tissue histopathological examination revealed that beryllium exposure caused abnormal cellular alteration in dose dependent manner. The details have been provided in the figure legends.

**Duration dependent study**

**Physical development**

Beryllium administration at doses of 1.0 mg/kg for 4 and 6 weeks, respectively decreased whole body

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**Table 3 — Dose dependent effect of beryllium on serological variables**

<table>
<thead>
<tr>
<th></th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>Bilirubin (mg/dL)</th>
<th>Creatinine(mg/dL)</th>
<th>Albumin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.9±1.82</td>
<td>72.6±3.95</td>
<td>0.29±0.02</td>
<td>0.33±0.03</td>
<td>5.13±0.29</td>
</tr>
<tr>
<td>Be (0.25 mg)</td>
<td>36.8±2.40</td>
<td>83.8±5.39</td>
<td>0.31±0.02</td>
<td>0.57±0.03</td>
<td>4.08±0.29</td>
</tr>
<tr>
<td>Be (0.50 mg)</td>
<td>48.2±3.03</td>
<td>69.1±4.06</td>
<td>0.38±0.02</td>
<td>0.64±0.04</td>
<td>3.97±0.28</td>
</tr>
<tr>
<td>Be (0.75 mg)</td>
<td>56.3±3.39</td>
<td>103±5.90</td>
<td>0.41±0.03</td>
<td>0.67±0.04</td>
<td>3.76±0.20</td>
</tr>
<tr>
<td>Be (1.00 mg)</td>
<td>64.7±4.00</td>
<td>115±6.68</td>
<td>0.49±0.03</td>
<td>0.74±0.04</td>
<td>3.56±0.21</td>
</tr>
<tr>
<td>ANOVA</td>
<td>21.1</td>
<td>16.7</td>
<td>12.8</td>
<td>25.4</td>
<td>6.55</td>
</tr>
</tbody>
</table>

[Data are mean ± SE of n = 6; ** Significant at 5% for ANOVA. *Be vs. Control at P ≤ 0.05, **Be vs. Control at P ≤ 0.01, ***Be vs. Control at P ≤ 0.001. Be = beryllium; ALT = Alanine aminotransferase; and AST = Aspartate aminotransferase]

**Table 4 — Dose dependent effect of beryllium on lipid peroxidation and protein**

<table>
<thead>
<tr>
<th></th>
<th>Forebrain</th>
<th>Midbrain</th>
<th>Hindbrain</th>
<th>Forebrain</th>
<th>Midbrain</th>
<th>Hindbrain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (n moles TBARS formed/mg protein)</td>
<td>0.58±0.03</td>
<td>1.15±0.09</td>
<td>6.49±0.35</td>
<td>6.18±0.32</td>
<td>4.81±0.33</td>
<td></td>
</tr>
<tr>
<td>Screw</td>
<td>0.75±0.05</td>
<td>1.46±0.11</td>
<td>6.12±0.44</td>
<td>5.63±0.33</td>
<td>4.62±0.27</td>
<td></td>
</tr>
<tr>
<td>Be (0.25 mg)</td>
<td>0.74±0.06</td>
<td>1.62±0.09</td>
<td>5.78±0.35</td>
<td>5.3±0.29</td>
<td>4.34±0.25</td>
<td></td>
</tr>
<tr>
<td>Be (0.50 mg)</td>
<td>0.95±0.05</td>
<td>1.72±0.09</td>
<td>5.54±0.28</td>
<td>4.5±0.26</td>
<td>3.26±0.26</td>
<td></td>
</tr>
<tr>
<td>Be (0.75 mg)</td>
<td>1.03±0.06</td>
<td>1.91±0.10</td>
<td>4.64±0.28</td>
<td>4.18±0.27</td>
<td>3.14±0.20</td>
<td></td>
</tr>
<tr>
<td>Be (1.00 mg)</td>
<td>1.15±0.11</td>
<td>10.5</td>
<td>4.93</td>
<td>9.18</td>
<td>10.37</td>
<td></td>
</tr>
<tr>
<td>Be (0.75 mg)</td>
<td>1.03±0.06</td>
<td>1.91±0.10</td>
<td>4.64±0.28</td>
<td>4.18±0.27</td>
<td>3.14±0.20</td>
<td></td>
</tr>
<tr>
<td>Be (1.00 mg)</td>
<td>1.15±0.11</td>
<td>10.5</td>
<td>4.93</td>
<td>9.18</td>
<td>10.37</td>
<td></td>
</tr>
</tbody>
</table>

[Data are mean ± S.E of n = 6; ** Significant at 5% for ANOVA. *Be vs. Control at P ≤ 0.05, **Be vs. Control at P ≤ 0.01, ***Be vs. Control at P ≤ 0.001. Be = beryllium, and TBARS = Thio barbituric acid reactive substances]
weight in duration dependent manner (Fig. 2A). Significantly decreased body weight was observed at completion of 4 and 6 weeks ($P < 0.001$).

Motor coordination status

Perturbed motor coordination was observed in duration dependent manner with administration of 1.0 mg/kg dose of beryllium. All the duration of beryllium exposure decreased ($P \leq 0.01$) motor coordination (Fig. 2B).

Behavioral assessment for anxiety status

Beryllium administration enhanced anxiety level during all the three durations significantly ($P \leq 0.001$) as represented by open arm entries and %time spent in open arm (Fig. 2 C & D). Different duration of beryllium at 1.0 mg/kg dose enhanced %time spent in bright arena and number of transitions in duration dependent manner. Decreased number of transition at 6 wk and % time spent in bright arena at 4 and 6 wk showed enhanced anxiety level ($P \leq 0.001$). Number of transition at 2 and 4 wk and % time spent in bright arena at 2 wk showed significant anxiety level at $P \leq 0.001$ (Fig. 2 E & F).

Hematological observations

Administration of beryllium at 1.0 mg/kg dose decreased the number of RBCs with increasing duration of exposure. All the durations of beryllium exposure decreased ($P \leq 0.001$) number of RBCs (Fig. 3A). Beryllium administration at 1.0 mg/kg decreased amount of hemoglobin in duration dependent manner. Significantly decreased amount of hemoglobin was found at 4 and 6 wk ($P < 0.001$) whereas, beryllium exposure for 2 wk decreased amount of hemoglobin at $P \leq 0.01$ (Fig. 3B).

Serum Biochemistry

Elevation in cholesterol and triglycerides was observed in duration dependent manner after beryllium exposure (Fig. 3 C & D). Cholesterol was increased significantly at all the three durations whereas, concentration of triglycerides in serum was increased significantly at 4 and 6 weeks duration ($P \leq 0.001$). Administration of beryllium at different durations (2, 4 and 6 wk) at 1.0 mg/kg dose caused significant elevation in ALT and AST activities ($P \leq 0.001$). The mean values showed maximum leakage of transaminases at 4 and 6 weeks (Fig. 4). Beryllium exposure for 2, 4 and 6 weeks caused elevation in bilirubin and creatinine level (Fig. 4 C & D) ($P \leq 0.001$). Beryllium doses decreased albumin content with increasing duration and of exposure to beryllium (Fig. 4E). Beryllium administration declined albumin more significantly at 4 and 6 weeks durations ($P \leq 0.001$).

Oxidative stress

Beryllium administration enhanced lipid peroxidation in forebrain, midbrain and hindbrain at all the three durations ($P \leq 0.001$) (Fig. 5 A-C). Amount of total protein was decreased in all the parts of brain in duration dependent manner after administration of
beryllium at 1mg/kg dose (Fig. 5 D-F). Beryllium administration decreased total protein content at all the three duration in forebrain ($P < 0.001$). The 4 and 6 weeks durations ($P < 0.001$) showed more significant reduction in total protein content in comparison to 2 weeks duration ($P < 0.01$).

**Histopathological observations**

The 1.0 mg/kg dose of beryllium caused histological alterations in cerebral cortex of brain in duration dependent manner (Fig. 6). Details has been provided in legends to figures.

Various behavioural, hematomal, serological and tissue biochemical indices showed duration dependent alterations. The 2 wk duration was found less toxic in comparison to 4 and 6 wk duration.

**Discussion**

Beryllium might bind at active site of enzymes as well as induced oxidative stress by excess production of reactive oxygen species and inhibited enzymatic action resulting TBARS formation and neuronal cell death. Present study established dose and duration

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**Fig 2** — Duration dependent effect of beryllium on physical and behavioural variables changes in rat. (A) Body weight; (B) Rotarod; (C) Elevated plus maze: % open arm time; (D) Elevated plus maze: open arm entry; (E) Light and dark chamber: number of transition; and (F) Light and dark chamber: % time spend in bright arena. [Values are mean ± SE of n= 6 in each group. $^aP$ value Be vs. control at ≤0.05; $^bP$ value Be vs. Control at ≤0.01, $^cP$ value Be vs. Control at ≤0.001 for Student’s t-test. $^6$Significant ANOVA at $P ≤ 0.05$. ANOVA: Body weight (93.1$^6$), rota rod (70.6$^6$), elevated plus maze % open arm time (76.6$^6$), elevated plus maze open arm entries (93.3$^6$), light and dark chamber number of transition (21.1$^6$), light and dark chamber % time spend in bright arena (50.2$^6$). Be: Beryllium]

**Fig 3** — Duration dependent effect of beryllium on serum parameter in rat. (A) Red blood corpuscles; (B) Hemoglobin; (C) Cholesterol; (D) Triglyceride. [Values are mean ± SE of n= 6 in each group. $^aP$ value Be vs. control at ≤0.05; $^bP$ value Be vs. Control at ≤0.01, $^cP$ value Be vs. Control at ≤0.001 for Student’s t-test. $^6$Significant ANOVA at $P ≤ 0.05$. ANOVA: Haemoglobin (21.1$^6$), RBC (32.7$^6$), Bilirubin (35.4$^6$), Triglyceride (13.1$^6$). Be: Beryllium; RBC: Red blood corpuscles]

**Fig 4** — Duration dependent effect of beryllium on serum parameter in rat. (A) Alanine aminotransferase; (B) Aspartate aminotransferase; (C) Creatinine; (D) Bilirubin; and (E) Albumin. [Values are mean ± SE of n= 6 in each group. $^aP$ value Be vs. control at ≤0.05; $^bP$ value Be vs. Control at ≤0.01, $^cP$ value Be vs. Control at ≤0.001 for Student’s t-test. $^6$Significant ANOVA at $P ≤ 0.05$. ANOVA: Alanine aminotransferases (48.3$^6$), Aspartate aminotransferase (19.7$^6$), Alkaline phosphatase (28.5$^6$), Creatinine (55.4$^6$). Be: Beryllium]
based toxic potential of beryllium on brain, behavior and body weight. Results explored enhanced anxiety level with perturbed motor coordination, decreased body weight and severe alterations in blood, histological and tissue biochemical variables after intraperitoneal administration of beryllium.

Beryllium exposure resulted neurotoxicity in experimental animals as neurological deficit was indicated by inability of rat to maintain equilibrium for 3 min in each trial. Beryllium exposure significantly decreased motor coordination or balance skill in the experimental rats in dose and duration dependent manner due to neuronal damage.

Elevated plus maze has been employed as bi-directionally sensitive to both anxiolytic and anxiogenic drugs studied for human. The number of entries in open arm and close arm reflects the safety of close arm with relative fearfulness of open arm. Reduction in % time spent, entry in open arm and increased defections were indication of high level of fear or anxiety. Anxiolytic drug enhances the number of entries and time spent in open arms.

In the present study, beryllium exposure to experimental animals decreased the number of entries and % time spent in open arms showing anxiogenic effect.

Light and dark transition test was originally developed by Crawley and colleagues. In the light and dark chamber variables, bright light act as environment stressor that minimize the explorative behavior of rodents. Reduction in % time spent, rearing behavior and number of entries in the light chamber is regarded as markers of anxiety. This study revealed that beryllium exposure decreased time spent, rearing behavior and number of entries in the light chamber in dose and duration dependent manner, which
confirmed anxiogenic effects of beryllium in dose and duration dependent manner.

Present investigation revealed severe alterations in blood variables after sub chronic exposure to beryllium and found decreased number of RBCs and hemoglobin content. A significant fall in hemoglobin is a reflection of hampering in synthesis of heam and globin proteins\textsuperscript{16,17}. Decrease in RBCs count was reported due to oxidative damage of RBCs in dose and duration dependent toxicity of beryllium\textsuperscript{18}. These results were also supported by increased lipid profile in serum. Biotransformation of beryllium takes place in the liver that is why liver function test was also noted for their toxic manifestations. Elevation in serum AST, ALT, triglyceride, cholesterol, creatinine content and decreased albumin after beryllium exposure were used as biomarker of cellular damage in liver, kidney and other organs\textsuperscript{19}.

Lipid peroxidation is a molecular mechanism that causes cellular damage leading to generation of free radicals\textsuperscript{20}. Metal toxicity is attributed to generate reactive oxygen species (ROS), which causes peroxidation of membrane lipids and induces alteration in structure and function of cellular membrane\textsuperscript{21}. Increased TBARS after beryllium administration indicated enhanced LPO due to failure of antioxidant defense system. Under the oxidative stress, Reactive oxygen species interact with protein molecule at specific amino acid side chain and causes modification in protein structure, fragmentation of peptide, electrical charge alteration; peroxynitrite nitrate protein is accumulated and thus, enhance the proteolysis\textsuperscript{22}. Beryllium exposure to rats decreased total protein content in this study. Furthermore, histological examination of brain cerebral cortex revealed that beryllium administration in rats altered histological integrity with few pyknotic nuclei, hemorrhage and vacuolated neuropil of cerebral cortex in dose and duration dependent manner.

**Conclusion**

This study suggests that exposure to beryllium to experimental animals increases neurotoxicity with increasing dose of beryllium nitrate i.p. 0.25 to 1.0 mg/kg and most toxic dose was found to be 1.0 mg/kg. Similarly, neurotoxicity and behavioural alterations were found in increasing manner with increasing duration of exposure to beryllium i.e. 2, 4 and 6 weeks. Thus, 1.0 mg/kg dose and 4 weeks duration of beryllium exposure may be considered for further studies to establish therapeutic/pharmacological measures against beryllium induced toxic manifestations.

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**Conflict of interest**

All authors declare no conflict of interest.

**References**


