ISSN 2249–9709

Original Article

Toxic potentials of sodium cyanide in rats upon subchronic exposure

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Received 22 August 2014; accepted 02 September 2014

Abstract

The current study was performed to assess the adverse effect of sodium cyanide (NaCN) on haematology, serum biochemistry and histology of liver and kidney in male Wistar rats. Twenty eight rats were divided into four groups (n = 7) and treated with 0, 0.64, 1.2 and 3.2 mg kg\(^{-1}\) body weight (BW) of NaCN for 90 days. Non significant differences in all investigated parameters were observed between control and 0.64 mg kg\(^{-1}\) BW treated group. The group treated 1.2 mg kg\(^{-1}\) BW significant changes in some haematological parameters, liver function tests and creatinine but cause non significant changes in urea, uric acid and total serum protein levels compared to control. The light microscopic examination of same group indicated histological changes in the liver but more or less normal in the renal histarchitecture except tubular dilation. The rats treated with 3.2 mg kg\(^{-1}\) BW showed significant changes in all investigated parameters compared to control and which were evidenced by histopathological changes in liver and kidney. With this study we conclude, higher doses of NaCN cause haemato, hepato and renal toxicity. Therefore their toxic effects should be kept in mind during chronic usage.

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Keywords: - Sodium cyanide, Hama-toxicity, Hepato-toxicity, Renal-toxicity, Serum Biochemistry

1. Introduction

Highly toxic sodium cyanide (NaCN) is widely used in various industrial process, including, electroplating, metal mining operations, manufacture of synthetic fabrics, plastics, as pesticidal agents and intermediates in agricultural chemical production [1,2]. Brasel et al., [3] reported that, effluents from the mining industry may contain cyanide concentration ranging from 0.3 to 216 ppm of free cyanide along with metallocyanide complex which can leads to environmental pollution [4-6]. Such cyanide polluted water can be frequently accessible to aquatic ecosystems, wildlife and possibly human [7].

Cyanide intoxication mainly occurs due to its high affinity towards ferrous ions (Fe\(^{2+}\)). Consequently, cyanide combines irreversibly with ferrocytochrome a/a3 thereby, inhibiting mitochondrial oxygen uptake, cellular respiration and electron transport, resulting in cellular hypoxia and cytotoxic anoxia that is potentially fatal [4]. These conditions may occur due to impaired oxygen delivery in the tissue. Thus, oxygen-dependent tissues such as heart, brain and liver are most adversely affected by cyanide intoxication [8]. Further, subchronic exposures of cyanide may elicit a number of toxic effects including neurotoxicity [9], testicular toxicity [10,11], epidemic spastic paraparesis [12], cadotoxicity [13,14], goiter [15], pancreatic diabetes [16] and lactic acidosis [14].

However, most of the cyanide absorbed by an animals is detoxified by enzymatic combination with sulfur to produce thiocyanate (SCN\(^-\)) [17]. The SCN\(^-\) is approximately 120 times less toxic than CN\(^-\) and it has been estimated that about 80% of the absorbed cyanide is transformed to SCN\(^-\), which is almost completely eliminated through the renal excretion [18,19]. Therefore, rats can tolerate sublethal doses of cyanide without harm [20]. On the other hand, several studies have demonstrated that cyanide salts and cyanide containing plants feed induce reproductive toxicity, neural toxicity, hepatotoxicity, renal toxicity and haematomatological changes in rabbits, quails, rats and pigs [10,11,21-23]. Chronic cyanide intoxication may also induce decreased animal body weight gain and cause protein depletion [11,24]. Whereas, studies pertaining to hepatotoxicity, renal toxicity with associated enzymes and haematomatological changes induced by NaCN are very limited in rat model.
Therefore, in the present study, we made attempt to evaluate the risk assessment of NaCN by evaluating the haematology, serum biochemistry and histopathological changes in liver and kidney after 90 days of NaCN treatment in male rats.

2. Materials and methods

2.1. Chemicals

Sodium cyanide of 95% purity was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Doses were prepared freshly by dissolving NaCN in double distilled water.

2.2. Animals

This study was performed on 28 male albino rats (90 days old) of approximately 170–180 g body weight. All the animals were housed in plastic cages, at the animal care facility in the Department of Zoology, Karnataka University, Dharwad. All the rats were fed a standard laboratory ration and watered ad libitum. The animals were in controlled condition of a 12 h light/dark cycle, and temperature of 23 ± 2 °C. All the rats were handled in accordance with CPCSEA guidelines for the care and use of laboratory animals.

2.3. Experimental design

After one week of acclimation, 28 rats were divided into four groups (n = 7).

- Group I: control animals, received distilled water.
- Group II: treated with 1/10th of LD₅₀ (0.64 mg kg⁻¹ BW),
- Group III: treated with 1/5th of LD₅₀ (1.2 mg kg⁻¹ BW),
- Group IV: treated with 1/2th of LD₅₀ (3.2 mg kg⁻¹ BW)

The selected LD₅₀ (6.44 mg kg⁻¹ BW) of NaCN is based on available literature [25]. The NaCN was administered in the morning (between 09:00 and 10:00 h) to non-fasted rats with dose volume of 1 mL/100 gm BW for 90 days. Oral administration of NaCN was elected since this is the main route of cyanide exposure for both humans and animals [26].

2.4. Body and organs weight

At the end of the experiment, all animals were sacrificed under light ether anaesthesia and the final body weight was measured on the electric balance. The weight of liver and kidney of respective groups were recorded after sacrificing the animals.

2.5. Hematological parameters

Blood samples were collected by cardiac puncture technique under sodium pentobarbital anaesthesia (40 mg kg⁻¹) with anti-coagulant EDTA for hematological analysis, including red blood cell (RBC) counts, hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and white blood cell (WBC) using haematology analyzer.

2.6. Serum Biochemistry

For serum biochemistry, blood was collected in a clean centrifuge tube without anticoagulant and then it was allowed to stand for a few minutes at room temperature to clot and centrifuged at 1500 g for 5 min at 4 °C. The serum was then collected into separate vial and subsequently subjected for the assessment of urea, uric acid, creatinine and total protein using specific Erba kits with automatic analyzer (Erba Chem-5, V2). The liver function enzymes including aspartate aminotransferase, AST (EC 2.6.1.1), alanine aminotransferase, ALT (EC 2.6.1.2), alkaline phosphatase, ALP (EC 3.1.3.1) were assessed in serum using a commercial spectrophotometer-enzymatic kit.

2.7. Histopathology

For histopathological examination, the liver and kidneys were dissected and the tissue samples were fixed in Bouin’s fluid for 24 h, processed by using a graded alcohol series, and embedded in paraffin. The paraffin sections were cut into 5 μm thick by using semi-automated microtome (LeicaRM 2255) and sections were stained with hematoxylin and eosin (H&E) for light microscopic examination. The sections were viewed and photographed by using an Olympus phase contrast microscope (Olympus BX51, Tokyo, Japan) with an attached camera (ProgResC3, Jenoptic-Germany).

To evaluate degree of liver and kidney injury, six slides were prepared from each rat liver and kidney. Then each slides were examined and assigned for severity of changes using scores on a scale of none (-), mild (+), moderate (+++) and severe (++++) damages (Table 4).

2.8. Statistics

The data were analyzed by using SPSS 16.0 for Windows. The significance of differences was calculated using one-way ANOVA followed by Tukey’s or Student’s t-test for multiple comparisons. P < 0.05 was considered statistically significant and values were expressed as mean ± SE.

3. Results

3.1. Effects of NaCN on body and organ weights

No mortality was occurred during the experimental period. Body weight gain and liver and kidney weight of 0.64 mg kg⁻¹ BW treated group did not alter statistically compared to control. The group treated with 1.2 mg kg⁻¹ BW showed non significant (P > 0.05) changes in the body weight gain and kidney weight, but significant (P < 0.05) decrease by 11.01% in liver weight compared to control. While in the group treated with 3.2 mg kg⁻¹ BW showed significant (P < 0.05) decrease in body weight gain and liver by 14.05% and kidney by 20.90% weight compared to the control (Table 1).

3.2. Haematology

The results of haematological parameters are presented in Table 2. The group treated with 0.64 mg kg⁻¹ BW showed no significant (P > 0.05) difference in all haematological parameters compared to control. While the group treated with 1.2 mg kg⁻¹ BW showed non significant (P > 0.05) changes in RBC count (decreased 6.77%), MCH and WBC count, but significant (P < 0.05) changes in haemoglobin (decreased 6.25%), hematocrit, MCV and MCHC compared to control. The group treated with 3.2 mg kg⁻¹ BW showed significant changes in all haematological parameters except MCH compared to control.

3.3. Serum Biochemistry

Table 3 showed some variables which indicated the liver and kidney injury in rats. Rats treated with 0.64 mg kg⁻¹ BW showed no significant (P > 0.05) changes in all serum biochemical parameters. Whereas, the group
Table 1: Effect of subchronic NaCN on body weight gain and absolute weight of the organs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight</th>
<th>Organ Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (gm)</td>
<td>Final (gm)</td>
</tr>
<tr>
<td>Control</td>
<td>172.65±2.1</td>
<td>281.0±3.3</td>
</tr>
<tr>
<td>0.64 mg kg⁻¹ BW</td>
<td>170.67±2.0</td>
<td>268.3±2.0</td>
</tr>
<tr>
<td>1.2 mg kg⁻¹ BW</td>
<td>165.85±5.0</td>
<td>259.1±5.6</td>
</tr>
<tr>
<td>3.2 mg kg⁻¹ BW</td>
<td>171.33±8.3</td>
<td>252.0±4.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE for each experimental group (n = 7).
*Significant difference (P < 0.05) as compared to the Control.
**Significant difference (P < 0.01) as compared to the Control.

Table 2: Effect of subchronic NaCN on hematological parameters of different experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>0.64 mg kg⁻¹ BW</th>
<th>1.2 mg kg⁻¹ BW</th>
<th>3.2 mg kg⁻¹ BW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC (x10⁶/cumm)</td>
<td>7.08±0.18</td>
<td>6.71±0.13</td>
<td>6.60±0.12</td>
<td>6.48±0.18</td>
</tr>
<tr>
<td>Hemoglobin (gm%)</td>
<td>13.58±0.10</td>
<td>13.43±0.14</td>
<td>12.73±0.19</td>
<td>12.58±0.28</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.31±0.18</td>
<td>39.63±1.52</td>
<td>42.10±0.89</td>
<td>43.35±1.38</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.18±0.25</td>
<td>20.02±0.30</td>
<td>19.88±0.58</td>
<td>20.47±0.37</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>52.70±1.47</td>
<td>59.07±2.31</td>
<td>59.56±1.49</td>
<td>60.59±1.89</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>35.73±0.50</td>
<td>33.68±1.31</td>
<td>31.54±0.92</td>
<td>30.65±1.36</td>
</tr>
<tr>
<td>WBC (cells/cumm)</td>
<td>7858.60±149.77</td>
<td>7899.97±215.05</td>
<td>7487.77±245.19</td>
<td>6990.07±139.82</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE for each experimental group (n = 7).
*Significant difference (P < 0.05) as compared to the Control.
**Significant difference (P < 0.01) as compared to the Control.

Table 3: Effect of subchronic NaCN on serum biochemistry of different experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>0.64 mg kg⁻¹ BW</th>
<th>1.2 mg kg⁻¹ BW</th>
<th>3.2 mg kg⁻¹ BW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>136.88±1.05</td>
<td>139.06±2.30</td>
<td>144.29±1.99</td>
<td>149.22±2.45</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>47.28±0.43</td>
<td>48.98±0.90</td>
<td>54.58±2.27</td>
<td>56.69±2.77</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>285.83±3.69</td>
<td>292.67±5.71</td>
<td>314.08±8.03</td>
<td>317.74±4.53</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>5.16±0.19</td>
<td>5.26±0.24</td>
<td>5.78±0.19</td>
<td>5.93±0.14</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>2.35±0.09</td>
<td>2.46±0.19</td>
<td>2.78±0.14</td>
<td>2.86±0.10</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.62±0.04</td>
<td>0.65±0.03</td>
<td>0.75±0.03</td>
<td>0.77±0.01</td>
</tr>
<tr>
<td>Total Protein (mg/dL)</td>
<td>6.52±0.15</td>
<td>6.46±0.24</td>
<td>6.05±0.18</td>
<td>5.74±0.12</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE for each experimental group (n = 7).
*Significant difference (P < 0.05) as compared to the Control.
**Significant difference (P < 0.01) as compared to the Control.

Table 4: Summary of results (as % change over control) showing dose dependent differences, regarding to effects of NaCN treated animals, compared to untreated animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>0.64 mg kg⁻¹ BW</th>
<th>1.2 mg kg⁻¹ BW</th>
<th>3.2 mg kg⁻¹ BW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Weight</td>
<td>-5.16</td>
<td>-11.01</td>
<td>-14.05</td>
<td></td>
</tr>
<tr>
<td>Kidney Weight</td>
<td>-4.54</td>
<td>-8.18</td>
<td>-20.90</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>-5.22</td>
<td>-6.77</td>
<td>-8.47</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>-1.10</td>
<td>-6.25</td>
<td>-7.36</td>
<td></td>
</tr>
<tr>
<td>Leucocytes</td>
<td>0.52</td>
<td>-4.72</td>
<td>-11.04</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>1.59</td>
<td>5.41</td>
<td>9.01</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>3.59</td>
<td>15.43</td>
<td>19.90</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>2.39</td>
<td>9.88</td>
<td>11.16</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>1.93</td>
<td>12.01</td>
<td>14.93</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>4.68</td>
<td>18.29</td>
<td>21.70</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>4.83</td>
<td>20.96</td>
<td>24.19</td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>-0.92</td>
<td>-7.20</td>
<td>-11.96</td>
<td></td>
</tr>
</tbody>
</table>

% of change = [(treatment - control)/control] × 100 (referred to Tables 1–3)
Table 5: Histopathological changes in the liver and kidneys of experimental rats, based on scoring severity of injury in both organs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic Injury</th>
<th>Renal Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score average (range)</td>
<td>Severity</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>0.64 mg kg⁻¹ BW</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>1.2 mg kg⁻¹ BW</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>3.2 mg kg⁻¹ BW</td>
<td>++</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Scoring was done as follows: none (-), mild (+), moderate (++), and severe (+++) damage.

Fig. 1 Histopathological changes of Liver (A-D) and kidney (E-H) tissue stained with Hematoxylin and Eosin (H&E).  (A) Liver of control rats showing normal artery (A), vein (V) and bile duct (BD).  (B) Liver of 0.64 mg kg⁻¹ treated rats showing, apparently normal histoarchitecture as in control.  (C) Liver of 1.2 mg kg⁻¹ treated rats showing vacuolation (thick arrow) degeneration (asterisk), necrosis (arrow head).  (D) Liver of 3.2 mg kg⁻¹ treated rats showing vacuolation (thick arrow), degeneration (arrow head), necrosis (asterisk).  (E) Kidney of control rats showing normal glomeruli (G) and renal tubules (T).  (F) Kidney of 0.64 mg kg⁻¹ treated rats showing, apparently normal histoarchitecture as in control.  (G) Kidney of 1.2 mg kg⁻¹ treated rats showing dilation in renal tubules (thick arrow), degeneration (arrow head).  (H) Kidney of 3.2 mg kg⁻¹ treated rats showing dilation in renal tubules (thick arrow) degeneration (asterisk), necrosis (arrow head) 100x and 200x.

Fig. 1 showed liver and kidney histology of control and NaCN treated groups. The normal arrangement of hepatocytes with visible sinusoidal space and central vein were observed in control group (Fig. 1A). The group treated with 0.64 mg kg⁻¹ BW showed normal histoarchitecture in the liver as seen in the control (Fig 1B). The group treated with 1.2 mg kg⁻¹ BW showed dilation of sinusoids, mononuclear cell infiltration and binucleated hepatocytes observed in the liver tissues (Fig. 1C), while, in the group exhibited degeneration and necrosis (Fig. 1D). In kidney normal renal histoarchitecture were observed in the control group (Fig.1E). The groups treated with 0.64 and 1.2 mg kg⁻¹ BW showed normal histoarchitecture as in control except some marginal dilations in the tubules of 1.2 mg kg⁻¹ BW treated rats (Fig. 1F,G). While the group treated with 3.2 mg kg⁻¹ BW showed degeneration, cytoplasmic vacuolization and necrosis in the tubules of the kidney (Fig.1H).

The semi quantitative analysis revealed; normal histoarchitecture in the control and 0.64 mg kg⁻¹ BW.

Table 4 recaps the results given in Tables 1–3 in terms of percent change over control in different parameters of the treated animals. The data revealed that alteration caused by NaCN is seems to be dose dependent changes. But the percent change in the higher doses of more prominent compared to the group treated with 0.64 mg kg⁻¹ BW NaCN.

3.4. Histopathology

Table 4 recaps the results given in Tables 1–3 in terms of percent change over control in different parameters of the treated animals. The data revealed that alteration caused by NaCN is seems to be dose dependent changes. But the percent change in the higher doses of more prominent compared to the group treated with 0.64 mg kg⁻¹ BW NaCN.

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3.4. Histopathology

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The semi quantitative analysis revealed; normal histoarchitecture in the control and 0.64 mg kg⁻¹ BW.
treated groups. The group treated with 1.2 mg kg\(^{-1}\) BW showed mild injury in liver and more or less normal in kidney. While the group treated with 3.2 mg kg\(^{-1}\) BW showed moderate injury in liver and mild in the kidney (Table 5).

4. Discussion

The current study was performed to investigate the haemato, hepato and renal toxicity in rats of commonly used NaCN by electro plating and the mining community, following subchronic (90 days) exposure. The haematotoxicity was evaluated by blood parameters, hepato-toxicity and renal toxicity was evaluated by biochemically (ALT, ALP, AST, urea, uric acid, creatinine and protein) in serum and evidenced by histopathological observations.

Results obtained from the present study showed significant (\(P < 0.01\)) reduction in the body weight gain and absolute weight of liver and kidney in the highest dose (3.2 mg kg\(^{-1}\) BW) treated group (Table 1). However, throughout the experimental period, we observe non significant (\(P > 0.05\)) change in the diet consumption (data not shown) of treated groups compared to control group following cyanide intoxication. Studies have demonstrated the cyanide metabolized to thiocyanate by use sulphur-containing amino acids, which cause depletion in sulphur availability. The produced SCN\(^{-}\) inhibits the intra-thyroidal uptake of iodine, and causes a reduction in thyroxin level, which is necessary for growth [27,28]. Several other studies have demonstrated that, the cyanide cause impaired thyroid function [9,29,30]. Therefore, in the present study, reduction in body weight gain might be the result of impaired thyroid function which is an eventually cause for reduced organ weight. Vargas et al., [31] demonstrated the reduction in kidney weight closely related to hypothyroidism. Additionally, the histopathological alteration in the liver and kidney may also attribute to the reduced weight. In contrast present study indicates non significant (\(P > 0.05\)) changes in body weight gain and organ weight in the lower dose (0.64 and 1.2 mg kg\(^{-1}\) BW) treated group. This might be due the catalytic action of cyanide detoxifying enzymes, which converts cyanide to thiocyanate and thereafter complete eliminate thiocyanate through the renal excretion.

In fact body is having well established detoxification mechanism to detoxify the absorbed xenobiotics. After absorption of cyanide in the body, it can be removed by enzymatic and non-enzymatic reactions. An enzymatic reaction involves enzyme rhodanese, sulfurtransferase, mercaptopyruvate sulfurtransferase and thioupane reductase in the mitochondria, cystathionine gamma lyase in the cytoplasm. However, methemoglobin in the blood is involves in non-enzymatic removal of cyanide [32]. Cyanide is initially trapped in the erythrocytes of the blood, due to its high affinity towards the methemoglobin which have an iron atom in ferric state (F\(^{3+}\)), leads to the formation of cyanmethaemoglobin [28,33]. So that, methemoglobin is able to keep cyanide away from the cytochrome c oxidase (EC 1.9.1.3), which otherwise would become blocked [34]. Due to this, most of cyanide found in the red blood cells and it has been estimated to be 98.4% [35]. But, cyanide combination with methemoglobin is reversible [1]. This in turn, cyanide gets slowly releases, and then diffuses out of the red cells in a gradual fashion, and eventually may get detoxify by hepatic rhodanese and other sulphur detoxification enzyme systems [33]. Rhodanese enzyme detoxification mechanism can efficient to convert cyanide to thiocyanate by catalysing reaction between sulphur and cyanide. Ansell and Lewis, [19] had been estimated that about 80% of the absorbed cyanide is transformed to thiocyanate, which is almost completely eliminated through the kidneys. Consequently, detoxification mechanism cause non significant (\(P > 0.05\)) changes in serum biochemistry, haematological parameters and histology of liver and kidney after 0.64 mg kg\(^{-1}\) BW treatment. In contrast higher doses (1.2 and 3.2 mg kg\(^{-1}\) BW) induce significant (\(P < 0.05\)) changes in haematological and biochemical parameters (Table 2,3,4). This may be due to exceed cyanide intoxication leads to enhance cyanmethemoglobin percentage in red blood cells and most of the haemoglobin in the red blood cells were used for the formation of cyanmethemoglobin and reduced oxygen caring capacity [33]. Cyanide intoxication may also lead inhibition of cytochrome c oxidase which ultimately results in accumulation of oxyhemoglobin in the blood as a result of blockage of oxygen utilization in the tissue. The significant (\(P < 0.05\)) decrease in the red blood cells count in highest dose (3.2 mg kg\(^{-1}\) BW) treated group may due to oxidative damage in the erythropoietic tissue as well. Additionally, high doses of cyanide are beyond the detoxication capacity of the rhodanese system in the liver [21].

Liver is first major organ to be exposed to ingested toxins due to its portal blood supply and it helps in at least partial removal of toxins, from the circulation during the first pass, providing protection to other organs [36,37]. In liver, mitochondrial metabolism and aerobic respiration are central to the physiological functions of hepatocytes. Mitochondria of hepatocytes are plays a very important role in homeostasis, biosynthetic reactions and amino acid metabolism [38]. Cyanide intoxication leads to inhibition of important mitochondrial enzyme, cytochrome c oxidase, displayed a sharp decrease in activity and expression which leads to switch on anaerobic respiration, results in accumulates lactate a harbinger of anaerobic respiration [4]. It implies that cyanide disrupts energy homeostasis and leads to oxidative stress, result in disrupting the metabolic process in hepatocytes. These findings are consistent with earlier findings of Okolie and Osagie, [21]. Sousa et al., [39] and Soto-Blanco et al., [40] who demonstrated that chronic cyanide intoxication cause deleterious effects on the liver and kidney in rabbits, rats and goats. Similar cyanide-induced histopathological changes have been reported in pigs [41] and rainbow trouts [42].

Serum enzymes, including ALT and AST are mainly used in the evaluation of hepatic damage [43]. Any alteration in these enzymes indicates liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane. Results from the present study showed significant (\(P < 0.05\)) increase in the serum ALT, ALP and AST levels in the
groups treated with 1.2 and 3.2 mg kg\(^{-1}\) BW. The elevated enzyme levels were also evidenced by histopathological alterations in the liver, including hepatocellular damage, cytoplasmic vacuolization, necrosis and degeneration in the nuclei (Fig. 1CD). These histopathological alterations subsequently results in the damages of hepatocytes and in turn to the report of leakage of liver enzymes. Histological scoring was revealed alterations were mild in the 1.2 mg kg\(^{-1}\) BW treated group and moderate in the 3.2 mg kg\(^{-1}\) BW treated group (Table 5). However, these observations were supported by the studies of Ballantyne [4] and Yamamoto et al., [44] who have reported the highest tissue concentrations of cyanide in the liver than followed by lung, blood, spleen, and brain in rats and rabbits.

In contrast, results from the current study showed no significant (P > 0.05) alteration in the liver marker enzymes with normal histoarchitecture in the liver of the group treated with 0.64 mg kg\(^{-1}\) BW (Fig. 1B). This may be due to enzymatic detoxification mechanism in the liver. Studies demonstrated the rhodanese is distributed widely throughout the body, with the highest concentrations located in the liver [45]. This helps to complete transformation of cyanide to thiocyanate which will quickly eliminate through renal excretion.

The kidney is the critical target organ for xenobiotic compounds it produces a variety of renal toxic effects involving tubular cells and glomerulus [46]. In the present study, results showed that highest dose (1.2 and 3.2 mg kg\(^{-1}\) BW) of NaCN induce elevated urea, uric acid and creatinine level in the serum and significant decrease in the absolute weight of kidney in highest dose treated group (Table 1 and 3). However, several studies demonstrate that cyanide cause hypothyroidism which will leads to deficiency of thyroid hormone [26,28,47]. Iglesias and Díez, [47] reported that renal dysfunction is closely associated with thyroid activity in human. This might be possible reason for the decreased kidney weight and dysfunction of renal cells in the 3.2 mg kg\(^{-1}\) BW. Highest dose cause histological alterations in the kidney including dilution in renal tubules, degeneration and necrosis (Fig. 1H). These histopathological changes in the kidney cause elevated urea, uric acid and creatinine level in the high dose (3.2 mg kg\(^{-1}\) BW) treated group. However, serum creatinine level used to diagnose renal diseases such as acute renal failure, chronic kidney disease, and end-stage renal disease [49]. The increase in serum uric acid level may be related to protein degeneration and failure in renal excretion and urea level may indirect renal disorder [50]. Histopathological scoring revelled renal injuries were more or less normal in the 1.2 mg kg\(^{-1}\) BW treated rats and mild in 3.2 mg kg\(^{-1}\) BW treated rats (Table 5). In contrast, the groups treated with 0.64 and 1.2 mg kg\(^{-1}\) BW, showed non significant (P > 0.05) change in the urea, uric acid and total protein levels. Due to lower doses of cyanide may be completely eliminated through body without harm.

5. Conclusion
The results of the present study evidenced that subchronic exposure of sodium cyanide induced heamato, hepato and renal toxicity in albino rats. Consequently, if animal-to-man extrapolation is permissible, the results of this study show that chronic cyanide toxicity may have deleterious effect on human liquid connective tissue (blood), liver and kidney and humans are more sensitive to the cyanide ions. Therefore, this finding is considered crucial, especially in cyanide industry where labours are continually exposing to cyanide.

Acknowledgements
The first author thankful to Karnataka University, Dharwad for awarding University Research Fellowship (KU/SC/URS/2011-2012/20045) and University Grant commission for Research Fellowship in Science for Meritorious Student (F.4-1/2006(BRS)/7-102/2007(BRS) and authors also thankful to the Department of Zoology, Karnataka University, Dharwad, Karnataka, India to carry present work.

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Source of support: University Grants Commission [Research Fellowship No: F.4-1/2006(BRS)/7-102/2007(BRS)] and Karnatak University, Dharwad [Research Fellowship No: (KU/SC/URS/2011-2012/20045)]; Conflict of interest: None declared