CHAPTER IV

ARSENIC TRIOXIDE INDUCED TOXIC EFFECTS

1. Introduction


Owing to the notoriety as a poison, treatment with arsenic trioxide is alarming to patients and physicians alike. Therefore, a thorough understanding of the potential side effects of arsenic trioxide is necessary for minimizing its toxic complications. The present study was performed in vivo to understand the toxicological mechanism of arsenic trioxide in vital organs.

2. Experimental design

Animals were randomly divided into four groups, with six rats in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
</tr>
<tr>
<td>II</td>
<td>As$_2$O$_3$ 2mg/kg b.wt</td>
</tr>
<tr>
<td>III</td>
<td>As$_2$O$_3$ 4mg/kg b.wt</td>
</tr>
<tr>
<td>IV</td>
<td>As$_2$O$_3$ 8mg/kg b.wt</td>
</tr>
</tbody>
</table>
Arsenic trioxide administration was done by oral intubations in the morning, daily for 45 days.

3. **Results - Arsenic Trioxide Induced Toxic Effects on Blood and Heart Tissue**

3.1. **Arsenic Accumulation**

**Figure. 1 Arsenic Deposition in Heart**

Data represented as mean ±SD, n=6. *p<0.05* was considered significant. a represents statistical significance in comparison to normal control, b represents statistical significance in comparison to group II, and c represents statistical significance in comparison to group III.

Arsenic trioxide treatment caused the deposition of arsenic in heart tissue. The deposition of arsenic was found increased with the increase in the dose of arsenic trioxide. All the arsenic trioxide treated groups showed significant variations (*p<0.05*), when compared to the control group (Fig.1).
3.2. Serum Glucose

Figure 2 Effect of Arsenic Trioxide on Serum Glucose

Data represented as mean ±SD, n=6. *p*<0.05 was considered significant. *a* represents statistical significance in comparison to normal control, *b* represents statistical significance in comparison to group II, and *c* represents statistical significance in comparison to group III.

Arsenic trioxide treatment significantly (*p*<0.05) increased the serum glucose (Fig. 2) when compared to the group I control. The alterations were found increased with the increase in concentration of arsenic trioxide and showed statistical significance (*p*<0.05) between groups II, III and IV.
3.3. Creatine Kinase and Lactate Dehydrogenase

Table.1 Effect of Arsenic Trioxide on Serum Creatine Kinase and Lactate Dehydrogenase

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase (U/L)</td>
<td>487.83±9.68</td>
<td>597.5±10.71</td>
<td>640.5±9.09</td>
<td>803±8.32</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>589.33±12.83</td>
<td>803.17±6.65</td>
<td>919.17±11.32</td>
<td>1053.33±11.02</td>
</tr>
</tbody>
</table>

Data represented as mean ±SD, n=6. p<0.05 was considered significant. a represents statistical significance in comparison to normal control, b represents statistical significance in comparison to group II, and c represents statistical significance in comparison to group III.

Arsenic trioxide treatment significantly (p<0.05) increased the cardiac marker enzymes CK and LDH (Tab. 1). These alterations were increased with the increase in concentration of arsenic trioxide and showed statistical significance (p<0.05) when compared between groups II, III and IV.

3.4. Serum Sodium

Table. 2 Effect of Arsenic Trioxide on Serum Sodium

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Sodium (mEq/L)</td>
<td>145.5±3.08</td>
<td>144.67±5.09</td>
<td>141.83±2.32</td>
<td>141±3.46 a</td>
</tr>
</tbody>
</table>

Data represented as mean ±SD, n=6. p<0.05 was considered significant. a represents statistical significance in comparison to normal control, and b represents statistical significance in comparison to group II.
Serum sodium concentration was significantly \((p<0.05)\) reduced at higher concentration of arsenic trioxide (Group IV) treated rats (Tab. 2).

3.5. **Serum Potassium**

*Figure. 3 Effect of Arsenic Trioxide on Serum Potassium*

Data represented as mean ±SD, \(n=6\). \(p<0.05\) was considered significant. \(a\) represents statistical significance in comparison to normal control, and \(b\) represents statistical significance in comparison to group II

Administration of arsenic trioxide significantly decreased \((p<0.05)\) the potassium concentration when compared to the control group. When compared between the arsenic treated groups they showed significant \((p<0.05)\) variation with the increase in arsenic trioxide concentration (Fig. 3).
3.6. Serum Calcium

Figure. 4 Effect of Arsenic Trioxide on Serum Calcium

Data represented as mean ±SD, n=6. p<0.05 was considered significant. \(^a\) represents statistical significance in comparison to normal control, \(^b\) represents statistical significance in comparison to group II, and \(^c\) represents statistical significance in comparison to group III.

Arsenic trioxide treatment induced significant (p<0.05) elevation in the serum calcium concentration in groups III & IV. When compared between the arsenic treated groups they showed significant (p<0.05) variation with the increase in arsenic trioxide concentration (Fig. 4).
3.7. Detection of Blood Antioxidant Status

3.7.1. Lipid Peroxidation, GSH, SOD, CAT, GPx, and GST

Table. 3 Effect of Arsenic Trioxide on Blood MDA, GSH, SOD, CAT, GPx, and GST

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µM/L)</td>
<td>4.09±0.12</td>
<td>4.68±0.17abc</td>
<td>4.90±0.09ab</td>
<td>5.05±0.11abc</td>
</tr>
<tr>
<td>GSH (µM/gHb)</td>
<td>5.87±0.32</td>
<td>5.16±0.2abc</td>
<td>4.47±0.32ab</td>
<td>3.84±0.42abc</td>
</tr>
<tr>
<td>SOD (U/mg Hb)</td>
<td>1.59±0.13</td>
<td>1.32±0.12abc</td>
<td>0.98±0.14ab</td>
<td>0.75±0.17abc</td>
</tr>
<tr>
<td>CAT (k/ml)</td>
<td>10.31±0.45</td>
<td>9.12±0.85abc</td>
<td>8.28±0.65ab</td>
<td>7.44±0.47abc</td>
</tr>
<tr>
<td>GPx (U/gHb)</td>
<td>7.94±0.18</td>
<td>7.44±0.11ac</td>
<td>7.12±0.12ab</td>
<td>6.16±0.1abc</td>
</tr>
<tr>
<td>GST (µM/min/gHb)</td>
<td>2.09±0.11</td>
<td>1.64±0.09abc</td>
<td>1.35±0.09ab</td>
<td>0.76±0.07abc</td>
</tr>
</tbody>
</table>

Data represented as mean ±SD, n=6. p<0.05 was considered significant. a represents statistical significance in comparison to normal control, b represents statistical significance in comparison to group II, and c represents statistical significance in comparison to group III.

The lipid peroxidation product MDA was significantly increased with arsenic trioxide treatment when compared to the group I control. The tripeptide GSH was reduced significantly with respect to the control in the arsenic treated groups. Arsenic trioxide treatment also decreased the GSH dependant antioxidant enzymes GST and GPx and the antiperoxidative enzymes SOD and CAT when compared to the control and between the arsenic treated groups II, III and IV. These reductions in the antioxidant enzyme activities were in accordance with the increase concentration of arsenic trioxide (Table. 3).
3.8. Effect of Arsenic Trioxide on Heart Antioxidant Status

3.8.1. Reduced Glutathione

Figure. 5 Effect of Arsenic Trioxide on GSH

Data represented as mean ±SD, n=6. *p*<0.05 was considered significant. *a* represents statistical significance in comparison to normal control, *b* represents statistical significance in comparison to group II, and *c* represents statistical significance in comparison to group III.

Arsenic trioxide treatment significantly reduced (*p*<0.05) the GSH (Fig. 5) in heart tissue when compared with the control group. When compared between the arsenic treated groups II, III and IV the GSH was found significantly reduced (*p*<0.05) with the increase in concentration of arsenic trioxide.
3.8.2. Glutathione S-Transferase

Data represented as mean ±SD, n=6. p<0.05 was considered significant. a represents statistical significance in comparison to normal control, b represents statistical significance in comparison to group II, and c represents statistical significance in comparison to group III.

Arsenic trioxide treatment significantly reduced (p<0.05) the GST (Fig. 6) in heart tissue when compared with the control group. When compared between the arsenic treated groups II, III and IV the GST was found more reduced (p<0.05) with the increase in dose of arsenic trioxide.
3.8.3. Lipid Peroxidation, GPx, SOD and CAT

Table 4  Effect of Arsenic Trioxide on Lipid Peroxidation, GPx, SOD and CAT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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</thead>
<tbody>
<tr>
<td>MDA (nM/mg protein)</td>
<td>0.72±0.02</td>
<td>0.83±0.03&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.9±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.03±0.08&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (µg of GSH consumed/min/mg protein)</td>
<td>3.94±0.29</td>
<td>3.05±0.15&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>2.1±0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.97±0.09&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>7.28±0.26</td>
<td>6.15±0.21&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>5.33±0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.37±0.21&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (µ moles of H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; consumed/min/mg protein)</td>
<td>20.37±1.35</td>
<td>14.74±0.98&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>11.58±0.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.6±0.63&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data represented as mean±SD, n=6. <sup>p</sup><0.05 was considered significant. <sup>a</sup> represents statistical significance in comparison to normal control, <sup>b</sup> represents statistical significance in comparison to group II, and <sup>c</sup> represents statistical significance in comparison to group III.

The lipid peroxidation product MDA was found increased (<sup>p</sup><0.05) in heart tissue of arsenic trioxide treated rats when compared with the control group and was found increased with the increase in concentration of arsenic trioxide. The intergroup comparison between the arsenic treated groups showed significant variation between each groups. The GPx, SOD and CAT concentrations were found decreased with the treatment with arsenic trioxide. The reduction in the antioxidant enzymes were found reduced more with the increase in concentration of arsenic trioxide (Table 4).
3.9. Histopathology

Figure 7 Histopathology of heart tissue

Histology of heart tissue; H&E staining, X100. Normal control (7A), As$_2$O$_3$ 2mg/kg b.wt (7B), As$_2$O$_3$ 4mg/kg b.wt (7C), and As$_2$O$_3$ 8mg/kg b.wt (7D). s-swelling, cc-capillary congestion, fs-fiber separation, n-necrosis.

In light microscopic examinations, no significant pathological changes were observed in the cardiac tissue of the control rats (Fig. 7A). The percentage of tissue damage was found increased with the increase in dose of arsenic trioxide. In arsenic trioxide 2mg/kg b.wt treated rats the myocardial fibers showed mild swelling more towards pericardial zone and around vessels and there was mild interstitial edema (Fig. 7B). In arsenic trioxide
4mg/kg b.wt treated rat’s myocardial fibers showed more pronounced cellular swelling, near epicardium and also near the endocardium. And also a noticeable capillary congestion and fiber separations were observed (Fig. 7C). The myocardial fibers were markedly swollen in different zones. Focal lymphocytic infiltrations towards the pericardium, capillary congestion, necrosis and micro-hemorrhages were noticed in arsenic trioxide 8mg/kg b.wt treated rats. (Fig. 7D).

4. Result - Toxic Effects of Arsenic Trioxide on Hepatic Tissue

4.1. Arsenic Accumulation

Figure. 1 Arsenic Deposition in Liver Tissue

Data represented as mean ±SD, n=6. p<0.05 was considered significant. \( ^a \) represents statistical significance in comparison to normal control, \( ^b \) represents statistical significance in comparison to group II, and \( ^c \) represents statistical significance in comparison to group III.
Arsenic trioxide treatment leads to the deposition of arsenic in hepatic tissue. The deposition of arsenic was found increased with the increase in the concentration of arsenic trioxide. When compared to the control group and between arsenic treated groups showed significant variations ($p<0.05$). The accumulation of arsenic was found increased with the increase in concentration of arsenic trioxide (Fig.1).

**4.2. Serum Transaminases**

Figure. 2 Effect of Arsenic Trioxide on Serum Transaminases

Data represented as mean ±SD, $n=6$. $p<0.05$ was considered significant. $^a$ represents statistical significance in comparison to normal control, $^b$ represents statistical significance in comparison to group II, and $^c$ represents statistical significance in comparison to group III.
Serum transaminase enzymes (AST and ALT) (Fig. 2) were increased with the treatment with arsenic trioxide. These hepatic markers were increased with the increase in the concentration of arsenic trioxide and showed statistical significance ($p<0.05$) when compared with the control and between the arsenic treated groups.

4.3. Alkaline Phosphatase

Figure. 3 Effect of Arsenic Trioxide on Alkaline Phosphatase

Data represented as mean ±SD, n=6. $p<0.05$ was considered significant. $a$ represents statistical significance in comparison to normal control, $b$ represents statistical significance in comparison to group II, and $c$ represents statistical significance in comparison to group III.

Arsenic trioxide treatment increased the ALP concentrations when compared with the normal control. These variations were increased with the increase in concentration of arsenic trioxide and showed statistical
significance \((p<0.05)\) when compared with the arsenic treated groups (Fig.3).

4.4. Hepatic Antioxidant Analysis

4.4.1. GSH, SOD and CAT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µM/g tissue)</td>
<td>52.47±1.9</td>
<td>38.11±1.19(^{ac})</td>
<td>29.11±1.28(^{ab})</td>
<td>24.82±1.14(^{abc})</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>9.26±0.19</td>
<td>8.19±0.09(^{ac})</td>
<td>7.26±0.09(^{ab})</td>
<td>5.83±0.13(^{abc})</td>
</tr>
<tr>
<td>CAT (µmol of (H_2O_2/)min/mg protein)</td>
<td>32.95±0.3</td>
<td>28.22±0.23(^{ac})</td>
<td>26.01±0.37(^{ab})</td>
<td>24.53±0.33(^{abc})</td>
</tr>
</tbody>
</table>

Data represented as mean ±SD, \(n=6\). \(p<0.05\) was considered significant. \(^a\) represents statistical significance in comparison to normal control, \(^b\) represents statistical significance in comparison to group II, and \(^c\) represents statistical significance in comparison to group III.

The tripeptide glutathione, SOD and CAT were significantly decreased in hepatic tissue of arsenic trioxide treated rats and showed statistical significance \((p<0.05)\) when compared to the control groups. Inter group comparison between arsenic treated groups showed statistical significance. The reduction of antioxidant enzymes were found more reduced with the increase in concentration of arsenic trioxide (Tab. 1).
4.4.2. Lipid Peroxidation

Figure. 4 Effect of Arsenic Trioxide on Lipid Peroxidation

The lipid peroxidation product MDA concentration was significantly ($p<0.05$) increased in arsenic trioxide treated rats when compared to the control group. The comparison between the arsenic trioxide treated groups II, III & IV showed variation in the rate of MDA production with respect to the increase in concentration of arsenic (Fig.4).

Data represented as mean ±SD, n=6. $p<0.05$ was considered significant. $^a$ represents statistical significance in comparison to normal control, $^b$ represents statistical significance in comparison to group II, and $^c$ represents statistical significance in comparison to group III.
4.4.3. Glutathione S Transferase

Figure 5. Effect of Arsenic Trioxide on GST

Data represented as mean ±SD, n=6. *p* < 0.05 was considered significant. ^a^ represents statistical significance in comparison to normal control, ^b^ represents statistical significance in comparison to group II, and ^c^ represents statistical significance in comparison to group III.

Arsenic trioxide treatment significantly reduced (*p*<0.05) the GST (Fig. 5) in heart tissue when compared with the control group. When compared between the arsenic treated groups II, III and IV the GST was found significantly reduced (*p*<0.05) with the increase in concentration of arsenic trioxide.
4.4.4. Glutathione Peroxidase

Figure. 6 Effect of Arsenic Trioxide on GPx

Data represented as mean ±SD, n=6. *p<0.05* was considered significant. *a* represents statistical significance in comparison to normal control, *b* represents statistical significance in comparison to group II, and *c* represents statistical significance in comparison to group III.

The GSH dependent antioxidant enzymes GPx (Fig. 6) concentrations were found decreased with the increase in concentration and showed statistical significance (*p<0.05*) when compared to the control group. The comparison between the arsenic treated groups II, III and IV showed significant variation between each group with respect to the concentration of arsenic.
4.5. Histopathology

Figure. 7 Histopathology of Liver Tissue

Histology of heart tissue; H&E Staining, X100. Normal control (7A), As$_2$O$_3$ 2mg/kg b.wt (7B), As$_2$O$_3$ 4mg/kg b.wt (7C), and As$_2$O$_3$ 8mg/kg b.wt (7D). md-mild degeneration, n-necrosis, cf-cholangiofibrosis, sd-sinusoidal dilatation.

Histological examination of liver tissue showed that the arsenic trioxide treatment induced mild degeneration and coagulation necrosis of hepatic parenchyma in arsenic trioxide 2mg/kg b.wt treated rats (Fig. 7B). In arsenic trioxide 4mg/kg b.wt treated rats showed moderate sinusoidal dilatation, hemorrhage, necrosis and cholangiofibrosis were identified in liver tissue (Fig. 7C) and in arsenic trioxide 8mg/kg b.wt treated rats showed widespread hepatocellular degeneration, foci of inflammation and necrosis, moderate sinusoidal dilatation and hemorrhage and cholangiofibrosis were found with arsenic trioxide treatment (Fig. 7D). Control rats showed normal histological architecture (Fig. 7A).
5. Results - Toxic Effects of Arsenic Trioxide on Renal Tissue

5.1. Arsenic Accumulation

Data represented as mean ±SD, n=6. p<0.05 was considered significant. \( ^a \) represents statistical significance in comparison to normal control, \( ^b \) represents statistical significance in comparison to group II, and \( ^c \) represents statistical significance in comparison to group III.

Arsenic trioxide treatment caused the deposition of arsenic in kidney tissue. The deposition of arsenic was found increased with the increase in the dose of arsenic trioxide. When compared to the control group all the arsenic trioxide treated groups showed significant variations (p<0.05) (Fig.1).
5.2. Blood Urea

Figure 2 Effect of Arsenic Trioxide on Urea

Data represented as mean ±SD, n=6. \( p<0.05 \) was considered significant. \(^a\) represents statistical significance in comparison to normal control, \(^b\) represents statistical significance in comparison to group II, and \(^c\) represents statistical significance in comparison to group III.

Administration of arsenic trioxide significantly decreased \((p<0.05)\) serum urea concentration when compared to the control group. When compared between the arsenic treated groups they showed significant \((p<0.05)\) variation with the increase in arsenic trioxide concentration (Fig. 2).
5.3. Creatinine

Figure. 3 Effect of Arsenic Trioxide on Creatinine

Treatment with arsenic trioxide significantly increased the creatinine (Fig. 3) concentrations and showed statistical significance ($p<0.05$) when compared to the control group. The comparison between arsenic treated groups II, III and IV showed significant differences with respect to the increase in concentration of arsenic trioxide.
5.4. Serum Uric Acid

Table 1 Effect of Arsenic Trioxide on Serum Uric Acid

<table>
<thead>
<tr>
<th>Parameters (mg/dL)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid</td>
<td>1.32±0.12</td>
<td>2±0.09&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>3.1±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.68±0.1&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data represented as mean ±SD, n=6. *p*<0.05 was considered significant. *a* represents statistical significance in comparison to normal control, *b* represents statistical significance in comparison to group II, and *c* represents statistical significance in comparison to group III.

Administration of arsenic trioxide significantly (*p*<0.05) increased the uric acid concentration when compared to the control group. The concentration of uric acid was found significantly (*p*<0.05) increased with the increase in dose of arsenic trioxide administration (Tab. 1).

5.5. Antioxidant Analysis in Kidney

5.5.1. GSH, GPx and GST

Table 2 Effect of Arsenic Trioxide on GSH, GPx and GST

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µM/g tissue)</td>
<td>16.92±0.67</td>
<td>13.19±0.8&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>10.98±0.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.75±0.87&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (µg of GSH/min/mg protein)</td>
<td>5.76±0.12</td>
<td>4.08±0.24&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>3.06±0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.83±0.17&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>GST (µM of CDNB-GSH conjugate/min/mg protein)</td>
<td>5.1±0.34</td>
<td>4.05±0.35&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>2.63±0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.09±0.29&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data represented as mean ±SD, n=6. *p*<0.05 was considered significant. *a* represents statistical significance in comparison to normal control, *b* represents statistical
significance in comparison to group II, and \(^c\) represents statistical significance in comparison to group III.

GSH, GPx and GST concentrations were found decreased with the increase in concentration of arsenic trioxide and showed statistical significance \((p<0.05)\) when compared to the control group. The comparison between the arsenic treated groups II, III and IV showed significant variation with respect to the increase in concentration of arsenic trioxide (Tab. 2).

### 5.5.2. Lipid Peroxidation

**Figure. 4 Effect of Arsenic Trioxide on Lipid Peroxidation**

Data represented as mean ±SD, n=6. \(p<0.05\) was considered significant. \(^a\) represents statistical significance in comparison to normal control, \(^b\) represents statistical significance in comparison to group II, and \(^c\) represents statistical significance in comparison to group III.

MDA the lipid peroxidation product was found significantly increased \((p<0.05)\) with the administration of arsenic trioxide than control
group. The comparison between the arsenic administered groups II, III & IV showed significant variation with the increase in dose of arsenic trioxide (Fig. 4).

5.5.3. SOD and CAT

Table 3 Effect of Arsenic Trioxide on SOD and CAT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>5.70±0.28</td>
<td>4.57±0.26abc</td>
<td>3.46±0.12ab</td>
<td>1.75±0.05abc</td>
</tr>
<tr>
<td>CAT (µ moles of H₂O₂ consumed /min /mg protein)</td>
<td>15.61±0.9</td>
<td>11.82±0.62abc</td>
<td>6.78±0.44ab</td>
<td>5.66±0.38abc</td>
</tr>
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</table>

Data represented as mean ±SD, n=6. *p<0.05 was considered significant. a represents statistical significance in comparison to normal control, b represents statistical significance in comparison to group II, and c represents statistical significance in comparison to group III.

The antiperoxidative enzymes SOD and CAT concentrations were found decreased with the administration with arsenic trioxide. The reduction in the antiperoxidative enzymes were found reduced more with the increase in concentration of arsenic trioxide and the comparison between arsenic treated groups II, III & IV showed statistical significance (Table 3).
5.6. Histopathology

Figure. 5 Histopathology of Kidney Tissue

H&E Staining, X100. Normal control (5A), As$_2$O$_3$ 2mg/kg b.wt (5B), As$_2$O$_3$ 4mg/kg b.wt (5C), and As$_2$O$_3$ 8mg/kg b.wt (5D). ms-mild sclerosis, ih-interstitial hemorrhage, n-necrosis, ss-segmental sclerosis, n&h-necrosis and hyperemia, if-interstitial fibrosis.

The hematoxylin and eosin-stained renal tissues appeared to have normal kidney histology in the control group (Fig. 5A). Arsenic trioxide treated 2mg/kg b.wt treated rats showed occasional edema and degeneration in glomerulus and mild segmental sclerosis of glomerular capsule (Fig. 5B). Moderate degeneration of glomerulus and tubules, interstitial hemorrhage, necrosis and fibrosis were identified in the renal tissue of arsenic trioxide 4mg/kg b.wt treated rats (Fig. 5C). Arsenic trioxide 8mg/kg b.wt treated rats showed degeneration of tubular structures, hyperemia of glomerular
capillaries, necrosis, moderate segmental sclerosis of glomerular capsule and interstitial fibrosis (Fig. 5D).

6. DISCUSSION

Arsenic trioxide is an effectual cancer therapeutic drug for acute promyelocytic leukemia and has impending anticancer action against an extensive range of malignancies (Lu et al, 2007). The toxic side effects that have been noted in clinical trials of arsenic trioxide are electrocardiographic changes, hepatocellular toxicity and fluid retention (Soignet, 2001). The duration of the present study was selected based on the previous clinical study (Huan et al, 2000). They have reported that arsenic trioxide treatment resulted in a significant remission in acute promyelocytic leukemia patients. The arsenic trioxide concentrations in our study are in the assortment of clinically offered concentrations for anti-leukemia treatment (Li et al, 2002).

In humans and numerous experimental animals, inorganic arsenic is enzymatically methylated into organic arsenic, such as monomethylarsonic acid and dimethylarsinic acid. They are the major organic pentavalent arsenic metabolites in human urine after the exposure to inorganic arsenic (Kojiama et al, 2005). It has also been reported that methylated arsenic compounds accumulate during chronic arsenic poisoning in human body (Yamauchi, 2000). Inorganic arsenic deposition was found to be the most significant cause of toxicities in hepatic and neuronal cells of rats treated with arsenite (Ghosh et al, 2009). In the current investigation, the depositions of arsenic in tissues were elevated with the increase in concentration of arsenic. The deposition of arsenic was found in the following order; kidney>liver>heart. Emadi and Gore (2010) reported that kidney is the prime organ for arsenic elimination and a target for accumulation and toxicity of
arsenic. Liver is the major situate of arsenic metabolism and through the circulation it reaches heart, kidney and other parts of the body (Kojjama et al, 2005). Prolonged treatment of arsenic resulted in the depletion of effective detoxification of arsenic, which could be the cause of accumulation of arsenic in tissues.

The release of marker enzymes from cardiac tissue to the plasma is directly proportional to the number of damaged cells present in the cardiac tissue (Anandan et al, 2003). Arsenic trioxide administration caused myocardial damage and increased release of cardiac marker enzymes (Manna et al, 2008; Raghu et al, 2009). Zhang et al (2013) investigated that, in response to arsenic trioxide treatment the cardiac markers were increased in the circulation. Significant increase in CK and LDH, well known diagnostic markers of cardiac toxicity were observed in arsenic trioxide administered rats. This might be due to the exudation of enzymes from cells to the systemic circulation because of cellular damage induced by the arsenic trioxide.

In the present study, the treatment with arsenic trioxide increased the glucose concentration in serum. Miller et al (2002) reported that trivalent arsenic inhibits the uptake of glucose into cells, gluconeogenesis, fatty acid oxidation and further production of acetyl CoA. Pyruvate dehydrogenase, an enzyme of glucose metabolism, is susceptible to arsenic-induced reactive oxygen species (ROS) generation (Aposhian and Aposhian, 2006). The thiol moiety is an important target for arsenic (Flora, 2011). Arsenite can react with the sulphydryl groups of proteins and enzymes; and inhibit cellular glucose uptake, gluconeogenesis, fatty acid oxidation, and activity of pyruvate dehydrogenase, which results in decreased citric acid cycle activity.
and production of cellular ATP (Bergquist et al, 2009). The increased concentration of glucose in this study may due to the binding of arsenic to the sulfhydryl groups of glucose metabolizing enzymes, and thereby blocking the uptake of glucose. The altered blood sugar level may also be due to islet cells toxicity, because arsenic administration caused severe pancreatic damage (Mukherjee et al, 2004). In our observation, the treatment with arsenic trioxide may induce ROS production; that in turn causes pancreatic cellular damage followed by an increased glucose concentration in serum.

Mandal et al (2007) reported a direct relationship between the arsenic deposition and the lipid peroxidation in liver tissue. In the current investigation the treatment with arsenic trioxide significantly damaged the hepatic tissue, so the hepatic marker enzymes transaminases and alkaline phosphatase were increased than the normal. Arsenic exposure leads to the incidence of hepatotoxicity as manifested by increase in the levels of alanine aminotransferase, aspartate aminotransferase, and malondialdehyde (Li et al, 2007). In acute promyelocytic leukemia treatment with arsenic trioxide increased the hepatic marker enzymes, and this may due to the hepatotoxic side effect (Ghavamzadeh et al, 2011). The arsenic induced hepatocellular injury may result in the release of the hepatic marker enzymes (Mandal et al, 2007).

The treatment with arsenic trioxide significantly altered the serum creatinine, urea and uric acid, which denotes the occurrence of renal toxicity with arsenic. Arsenic concentrates in the kidney during its urinary elimination process that affects the function of proximal convoluted tubules (Burton et al, 1995, Parrish et al, 1999). Acute renal dysfunction due to
arsenic exposure is characterized by acute tubular necrosis and cast formation with increase in blood urea nitrogen and creatinine levels (Kimura et al, 2006). The kidney and liver are the primary targets for arsenic-induced toxicity (Nandi et al, 2006). As like in the current observation, arsenic increased the generation of ROS, which enhanced lipid peroxidation and cellular damage in both hepatic and renal tissue, resulted in the release of urea and creatinine (Kokilavani et al, 2005).

In our observation, the administration of higher concentration of arsenic trioxide significantly altered the serum electrolytes sodium, potassium and calcium. Hofmeister et al (2008) reported that the treatment with arsenic trioxide caused hyponatremia and hyperglycemia in patients with multiple myeloma. Arsenic trioxide treatment reduced the potassium concentration in rats. According to Barbey et al (2003), hypokalemia related with arsenic trioxide therapy was known to induce cardiac QT interval prolongation. In antineoplastic patients, the arsenic trioxide induced cardiotoxicity is associated with hypokalemia (Chanan-Khan et al, 2004). Arsenic trioxide treatment also induced hypercalcaemia in rats. Excessive levels of free calcium radicals could activate calcium channels and cause arrhythmogenic myocardial changes resulting in fatal arrhythmias (Ismail, 2005). Arsenic trioxide treatment induced ROS generation and the induction of calcium overload in cardiac myocytes, which leads to apoptosis (Raghu et al, 2009).

In the current investigation, arsenic trioxide treatment increased lipid peroxidation in blood, heart liver and kidney tissues. MDA is a marker of endogenous lipid peroxidation. Liu et al (2001) reported that the treatment with arsenic caused a significant increase in the rate of formation of ROS
such as superoxide anion radical, hydroxyl radical and hydrogen peroxide. The toxic potential exerted by these compounds is through their reactivity with sulphur containing compounds and the generation of ROS (Hughes et al, 2011). According to Wang et al (2006), arsenic induced MDA production could be due to the impairment of cells’ natural protective system and could be directly related to the GSH depletion. Major arsenic-induced ROS includes superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^1$) and peroxyl radicals (Halliwell and Whiteman, 2004). Arsenic exposure is also known to stimulate the release of free iron from ferritin (Ahmad et al, 2000) and the resulting free iron is believed to be one of the potent inducer of ROS formation via the Fenton-type reaction. Arsenic intoxication decreased the ability of intracellular antioxidant power and hence attenuated the reducing ability of Fe (III) to Fe (II) (Manna et al, 2008).

The rate of lipid peroxidation is inversely related to the GSH action. The most significant alteration in the antioxidant defense was due to the decreased GSH concentration, because GSH has direct antioxidant activity (Schulz et al,2000). GSH plays a critical role in arsenic tolerance (Kojiama et al, 2005). GSH may decrease the cytolethality of arsenic through several processes, possibly through its role as an antioxidant, as a cofactor in the enzymatic methylation reaction of arsenic or by direct binding to arsenic and thereby reducing the toxic potential, or through enhanced efflux of an arsenic-GSH conjugate (Romach et al, 2000). Depletion of GSH results in the increased production of arsenic induced ROS, which may enhance the lipid peroxidation as observed in the present study. The arsenic trioxide treated rats also showed decreased concentration of GSH and GSH
dependant antioxidant enzymes GPx and GST in blood and tissues. This reduction is suggested to be due to the consumption of glutathione while protecting against the arsenic-induced oxidative stress, for maintaining cellular redox status (Hughes, 2002). GPx and GST play an important role in arsenic detoxification and the arsenic induced oxidative stress (Thompson et al, 2009). GST catalyses the formation of arsenic-GSH conjugates, which results in biomethylation of arsenic (Chen et al, 2001, Kala et al, 2004). Depletion of GSH, GPx and GST with arsenic trioxide treatment could letdown the xenobiotic mechanism of the body.

Decreased activities of the antiperoxidative enzymes may lead to formation of ROS and bring about a number of reactions harmful to the cellular and sub cellular membranes in tissues (Dhandapani et al, 2007). Arsenic intensively reduced the ROS-metabolizing enzymes, such as SOD, CAT, GPx, and GST (Flora, 2011) and this may lead to oxidative stress. CAT catalyzes the removal of arsenic trioxide produced H₂O₂ to H₂O (Wang et al, 2006). The exposure to arsenic decreased the activities of antiperoxidative enzymes SOD and CAT in our study. SOD catalyzes the dismutation of superoxide anions and prevents the subsequent formation of hydroxyl radicals in blood cells (Wang et al, 2006). In the present study, the decreased SOD activity may suggest that the accumulation of superoxide anion radical; might be responsible for increased lipid peroxidation following arsenic treatment as observed by Maiti and Chatterjee (2000). ROS can themselves reduce the activity of the antioxidant enzymes CAT and GPx (Datta et al, 2000). Exposure to arsenic decreased the blood CAT activity. CAT catalyzes the removal of H₂O₂ formed during the reaction catalyzed by SOD (Lee and Ho, 1995). In the present study, the decreased CAT activity
indicates the impaired ability to detoxify $H_2O_2$ and may lead to the accumulation of $H_2O_2$ and thereby oxidative stress in blood, heart, liver and kidney.

Arsenic-induced toxicity is mediated through three pathways. First, oxidative stress plays a role in the process of arsenic-induced damage (Emadi and Gore 2010). Free radicals are generated during the process of arsenic metabolism in the mitochondria and cytoplasm (Yamanaka and Okada, 1994; Shi et al, 2004). Moreover, arsenicals inhibit the activity of antioxidants and detoxifying enzymes (Miller et al, 2002). Free radical generation that exceeds the body’s antioxidant defense system (including antioxidant enzymes and antioxidants) disrupts the pro-oxidant/antioxidant balance and produce oxidative stress damages. Second, the deposited arsenic could inhibit cell metabolism. Arsenate is a phosphate analog, which replaces phosphate in glycolytic and cellular respiration pathways (Brazy et al, 1980). Third, arsenite, monomethylated, and dimethylated arsenicals cause tissue damage that includes chromosomal aberrations (Schwerdtle et al, 2003). Arsenic-induced oxidative stress in cardiac, hepatic and renal cells damaged cellular lipids and proteins and caused a decrease in antioxidant and xenobiotic metabolizing enzyme activity in rats (Ramanathan et al, 2003).

In the current investigation cardiac tissue of arsenic treated rats showed clear structural abnormalities and these cytotoxic effects in cardiomyocytes may be mediated through ROS leading to apoptosis. These cytotoxic effects of arsenic also has cellular manifestations such as loss of cardiac actin, reduced size and damage to the nuclei, which coordinate well with disruption of the vascular extracellular matrix (Hays et al, 2008).
Arsenic trioxide acts at the structural level, which results in infiltration, myocardial disorganization and interstitial edema in the heart (Raghu et al, 2009). In the present study, arsenic accumulation in cardiac tissue leads to the generation of free radicals and this might be the reason for the structural abnormalities. Histopathological examination reveals that arsenic treatment caused fiber swelling, necrosis and hemorrhages in the cardiac tissue. The extent of tissue damage was found sequentially increased with the increase in arsenic concentration in experimental rats.

Current study reported hepatocellular degeneration, foci of inflammation and necrosis, moderate sinusoidal dilatation, hemorrhage and cholangiofibrosis with arsenic trioxide treatment in liver. Bashir et al (2006), reported that the molecular mechanism of arsenic-mediated oxidative stress is by activating the apoptotic markers and thereby apoptosis in hepatocytes. In the renal tissue, arsenic trioxide treatment induced degeneration of tubular structures, hyperemia of glomerular capillaries, necrosis, moderate segmental sclerosis of glomerular capsule and interstitial fibrosis. Kidneys fail to excrete the complete biomethylation products of arsenic produced in the liver, so the accumulation of arsenic may be found increased in tissues and this may lead to the toxicity. Acute renal dysfunction due to arsenic exposure is characterized by acute tubular necrosis and cast formation (Kimura et al, 2006). Structural damages which occurred in the kidney probably due to the failure in removal of methylated arsenical species resulted in accumulation and damaged the cellular architecture. The severity of toxicity was increased with the increase in concentration of arsenic in tissue. The observed structural abnormalities in the current study may be due
to the deposition of arsenic in tissues, increased lipid peroxidation and oxidative stress.

The findings of the current investigation suggest that, at different clinical concentrations, arsenic trioxide induced toxic effects by tissue deposition of arsenic, altered cellular defense mechanism and causing structural aberrations in cardiac, hepatic and renal tissues. The detailed understanding of the toxic mechanism of arsenic will help in the identification of new agent for reducing the toxic effects.