HEAVY METAL INDUCED CHANGES OF RAT SERUM NITRITE AND NITRATE LEVELS

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INTRODUCTION
Alternations in the chemical composition of natural environment by industrial effluents like heavy metals usually induce changes in the behavioural, biochemical and pathological aspects of organisms (Boominathan and Ravendran, 2009). Nitric Oxide (NO), molecule of the mellennium (Shinde et al., 2000) is well studied in recent years owing to its varied physiological functions in mammals. The very end products of NO are nitrite/nitrate (Guarner et al., 1993). Earlier, from our laboratory, it is reported that certain of the heavy metals inhibit rat tissues cNOS and iNOS activities both in vitro and in vivo in experimental animals (Neelakantam, 2007). Present study is designed to investigate the in vivo effect of selected heavy metals on rat serum NO$_2$/NO$_3$ levels in vivo.

MATERIALS AND METHODS
Albino rats of the weight range 120±5gr were selected for the present study. They were maintained at constant room temperature of 20±5ºC and were fed ad libitum with commercial rat feed. They were divided into six groups of seven each.

Stock solutions of selected heavy metals like Hg$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Mn$^{2+}$ and Al$^{3+}$ were prepared in sterile water (1gr/2mL) and required IC$_{50}$ concentration of each metal was prepared by diluting stock solutions with sterile water. The IC$_{50}$ concentration selected for each metal is shown below in (µmol).

<table>
<thead>
<tr>
<th></th>
<th>Pb</th>
<th>Cd</th>
<th>Cu</th>
<th>Mn</th>
<th>Al</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$</td>
<td>312.5</td>
<td>333.30</td>
<td>56.8</td>
<td>203.46</td>
<td>416.6</td>
<td>135.9</td>
</tr>
</tbody>
</table>

The above concentration of heavy metals were reported as IC$_{50}$ Values that inhibit rat brain cNOS activity in vitro by Neelakantam (2007). Animals were gavaged with the above shown doses of each heavy metal. After 24h, the rat blood from the control and experimental ones was collected by cardiac puncture and were centrifuged at 2000g for 15 minutes to collect the serum.

In the control and experimental samples the serum NO$_2$/NO$_3$ levels were determined following the procedure of Guarner et al. (1993). Statistical analysis was done using students 't' test.

RESULTS AND DISCUSSION
The results in Table 1 shows the levels of NO$_2$/NO$_3$ levels in IC$_{50}$ dose heavy metal administered rat serum. In the control rat serum NO$_2$ levels were found to be more compared to NO$_3$ levels. All the heavy metals Screened enhanced the rat serum NO$_2$/NO$_3$ levels of rat serum. It is reported that the heavy metals by way of enhancing rat serum NO$_2$/NO$_3$ levels may induce free radical type damage to various organs of rat.

KEYWORDS
Heavy metals
Rat serum
Nitrite
Nitrate
Free radical damage

ABSTRACT
Nitrite / Nitrate (NO$_2$/NO$_3$) are well known to be the end products of nitric oxide (NO) pathway. Keeping in view the toxic effects exerted by heavy metals, the impact of selected heavy metals on rat serum NO$_2$/NO$_3$ levels is reported. Selected heavy metals like Hg$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Mn$^{2+}$ and Al$^{3+}$ were selected for the study and albino rats were treated with an IC$_{50}$ dose of each metal. All the metals caused an elevation of rat serum NO$_2$/NO$_3$ levels of rat serum. It is reported that the heavy metals by way of enhancing rat serum NO$_2$/NO$_3$ levels may induce free radical type damage to various organs of rat.

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altered H₂O₂ toxic effects. There are evidences in the literature in support of free radical formation from NO and thereby causing cellular damage/pathological conditions is well known (Poovala et al., 1999; Ahmed et al., 2000; Ali et al., 2000). Likewise, in the current experimental results as shown in Table 1, elevated levels of rat serum NO⁻²/NO⁻³ levels in IC₅₀ dose heavy metal treated rat serum may lead to either cellular damage or may cause some other pathological changes in the albino rats.

Earlier from our laboratory, we demonstrated that the pesticide treated rat serum exert higher levels of NO⁻²/NO⁻³ levels and the reasons were explained away as due to diffusion of NO from Cells/tissues of rat under pesticidal impact (Rao et al., 1997) like reasons were also available from studies of the earlier authors (Wimc et al., 1993). Similar reasons might be responsible for the elevated rat serum NO⁻²/NO⁻³ levels in heavy metal treated (rat serum) in the present study which in part becomes responsible for impairment of overall NO pathway in rats by heavy metals causing severe pathological defects in the albino rats.

REFERENCES


### Table 1: Effect of methyl mercury on the Nitrate (NO⁻³) / Nitrite (NO⁻²) levels of rat serum (values expressed as mg of nitrate/mL of serum)

<table>
<thead>
<tr>
<th>Name of the Metabolite</th>
<th>Control</th>
<th>Name of the heavy metal and their IC₅₀ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO⁻²</td>
<td>Hg²⁺</td>
</tr>
<tr>
<td>AV</td>
<td>58.36</td>
<td>81.08*</td>
</tr>
<tr>
<td>SD</td>
<td>± 1.08</td>
<td>± 3.46</td>
</tr>
<tr>
<td>PC</td>
<td>39.83</td>
<td>32.81</td>
</tr>
<tr>
<td>NO⁻³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AV</td>
<td>40.75</td>
<td>71.22*</td>
</tr>
<tr>
<td>SD</td>
<td>± 0.54</td>
<td>± 0.96</td>
</tr>
<tr>
<td>PC</td>
<td>74.77</td>
<td>52.76</td>
</tr>
</tbody>
</table>

Each value is the Mean ± SD of 5 Samples; AV = Average, SD = Standard Deviation, PC = Percent Change over the Control; * p<0.001

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Impact of selected heavy metals on the nitric oxide pathway in rats, Ph. D., thesis, S.V. University, Tirupati. A. P.


