Role of ascorbic acid supplement in amelioration of anaemia in lead intoxication
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Abstract
Objective: To assess anaemia and oxidative stress in rats that were injected lead and to evaluate the possible effects of ascorbic acid supplementation on these parameters.
Methods: This randomised control trial study was conducted at the Army Medical College, Rawalpindi, Pakistan, from October 2007 to September 2008, and comprised Sprague Dawley rats. The rats were randomly divided into three groups. The rats in Group 1 were given weekly injections of sodium acetate, and rats of Group 2 and 3 were given weekly injections of lead acetate. Ascorbic acid was supplemented in the drinking water of rats of Group 3. At the end of six weeks, terminal sampling was done and blood obtained was used to assess the serum malondialdehyde levels and red cell parameters.
Results: Of the 105 rats, each group had 35(33.33%). The overall mean age was 105±15 days and the mean weight was 225±25gm. The mean malondialdehyde level was 3.2±0.39 µmol/L in Group 1, 7.8±0.48 in Group 2 and 3.8±0.34 in Group 3 (p<0.001). The mean haemoglobin level was 13.16±0.57 g/dL, 10.64±0.86 and 12.22±0.81, respectively (p<0.001). The red blood cells count was 7.63±0.33 106/µL in Group 1, 6.29±0.54 in Group 2 and 6.83±0.45 in Group 3 (p<0.001).
Conclusion: Administration of ascorbic acid in drinking water significantly reduced the oxidative stress and anaemia caused by lead intoxication.
Keywords: Lead poisoning, Anaemia, Malondialdehyde, Ascorbic acid. (JPMA 66:1073; 2016)

Introduction
Lead exposure results in development of heavy metal intoxication in humans. Like other heavy metals, lead causes production of reactive oxygen species (ROS). ROS cause lipid peroxidation of the cell membranes, which becomes less flexible and can be ruptured easily. Lipid peroxidation of cell membrane releases malondialdehyde (MDA) as a by-product which is used as a biochemical marker to assess the quantum of oxidative stress (OS). Although isoprostane, nitrotyrosine and other markers of OS are also available in research, MDA is the most commonly used parameter of OS because of easy methodology.1,2

Erythrocytes are the most vulnerable cells to this OS. ROS causes oxidation of polyunsaturated fatty acids (PUFAs) of erythrocyte cell membrane, the fluidity of cell membrane is decreased making erythrocytes vulnerable to membrane damage. These toxic effects of lead result in decreased survival of erythrocytes and development of anaemia.3,4

Dietary intake or supplementation with antioxidant vitamins results in reduced ROS production.5-7 Result of ascorbic acid supplementation during lead intoxication appears more encouraging than other antioxidant vitamins. Ascorbic acid supplementation minimises the lead toxicity by various postulated mechanisms, including its role as a chelating agent which decreases the lead absorption and as an antioxidant which prevents the development of OS. In 2009, Hassan AA et al. evaluated the effect of ascorbic acid on neural development and biochemical derangements in the pups exposed to lead, and their work revealed that ascorbic acid significantly reversed derangements produced by lead.8 The current study was planned to evaluate the possible role of ascorbic acid on minimising OS and anaemia caused by lead. However, it was not focused on evaluating the chelating effect of ascorbic acid supplementation.

Materials and Methods
This randomised control trial (RCT) was carried out at the Department of Physiology, Army Medical College, Rawalpindi, Pakistan, from October 2007 to September 2008. Male Sprague Dawley rats were purchased from the National Institute of Health (NIH), Islamabad. The rats were divided into three groups, and every rat was injected weekly for lead administration or a placebo. Group 1 (control group) was given sodium acetate 10mg/kg body weight in the intraperitoneal injections whereas Group 2 (lead group) and Group 3 were given...
lead acetate 10mg/kg body weight by intraperitoneal injection, for six weeks. Rats of Group 3 (ascorbic acid group) were given ascorbic acid 500 mg/l in drinking water.9

After six weeks, terminal sampling was done and 4ml of blood was collected by intracardiac sampling. One ml blood of each sample was transferred to an ethylenediaminetetraacetic acid (EDTA) tube, while 3ml of blood was transferred in a plain tube for separation of serum to be used for MDA estimation. Estimation of serum MDA was determined by Thiobarbituric Acid Reactive Substances (TBARS) kit, on spectrophotometer. Blood in the EDTA tubes was used to determine haemoglobin (Hb) concentration, erythrocyte count and red cell indices by Haematology Analyser Sysmex KX-21. Each sample was thoroughly mixed and 50µl of the sample was aspirated by the haematology analyser. Inside the chambers of the haematology analyser, cell type, cell count and haemoglobin were determined. The results of the parameters were displayed on the screen and prints of the results were obtained.

SPSS 14 was used for data analysis. Means along with the standard deviations (SD) of all the parameters were determined. One-way analysis of variance (ANOVA) was used to determine the statistical significance of the differences between the means of the groups and parameters having a p-value of less than 0.05 were further subjected to post-hoc (Tukey) test.

Results

Of the 105 rats, there were 35(33.33%) in each group. Overall mean age was 105±15 days and the mean weight was 225±25 gm. The mean MDA level was 3.2±0.39 µmol /L in Group 1, 7.8±0.48 in Group 2 and 3.8±0.34 in Group 3 (p<0.001). The mean haemoglobin level was 13.16±0.57g/dL, 10.64 ± 0.86 and 12.22±0.81, respectively (p<0.001). Similarly, the red blood cells (RBC) count was 7.63±0.33 106/µL in the control group, 6.29±0.54 in the lead group and 6.83±0.45 in the ascorbic acid group (p<0.001), but the value between the latter two groups was not significant (p=0.057). The mean corpuscular volume (MCV) level was 55.69±0.93 fl in Group 1, 56.44±2.22 in Group 2 and 56.7±1.49 in Group 3 (p=0.053). The mean corpuscular haemoglobin concentration (MCHC) level was 31.45±0.53 g/dL, 28.09±1.05 and 31.42±0.93 in the three groups, respectively (p<0.001), but the value between Group 1 and Group 3 was not significant (p=0.992) (Table).

Discussion

Intraperitoneal lead acetate administration in the Sprague Dawley rats resulted in haematological derangements, and caused a decrease in haemoglobin, haematocrit (Hct) and RBC count while the MCV remained statistically unaltered. These results were consistent with the published data on lead intoxication.10,11 The decrease in the haemoglobin and haematocrit level in the lead-toxicated rats has been attributed to the reduction in life span of erythrocytes.

Development of anaemia in lead toxicity is attributed to the decreased RBC survival because of the increased membrane fragility caused by the loss of its fluidity. Cell membrane of normal erythrocyte is very flexible and capable of rapid and frequent shape alterations of the cell, due to the presence of PUFAs. Peroxidation of these

Table: Comparison of parameters between different groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n=35)</th>
<th>Lead group (n=35)</th>
<th>Ascorbic acid group (n=35)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol / l)</td>
<td>3.2 ± .39</td>
<td>7.8 ± .48</td>
<td>3.8 ± .34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb (g / dl)</td>
<td>13.16 ± 0.57</td>
<td>10.64 ± 0.86</td>
<td>12.22 ± 0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>41.93 ± 2.06</td>
<td>38.36 ± 2.61</td>
<td>39.93 ± 3.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBC Count (10 6 / µl)</td>
<td>7.63 ± 0.33</td>
<td>6.29 ± 0.54</td>
<td>6.83 ± 0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.33 ± 0.35</td>
<td>16.85 ± 0.29</td>
<td>17.81 ± 0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.69 ± 0.93</td>
<td>56.44 ± 2.22</td>
<td>56.7 ± 1.49</td>
<td>0.053</td>
</tr>
<tr>
<td>MCHC (g / dl)</td>
<td>31.45 ± 0.53</td>
<td>28.09 ± 1.05</td>
<td>31.42 ± 0.93***</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are presented as mean ± standard deviation
MDA: Malondialdehyde
Hb: Haemoglobin
Hct: Haematocrit
RBC: Red blood cell
MCH: Mean corpuscular haemoglobin
MCV: Mean corpuscular volume
MCHC: Mean corpuscular haemoglobin concentration.

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PUFAs of the cell membrane by the free radicals, especially by the ROS, ultimately leads to the loss of fluidity of the cell membrane. Damage caused by lipid peroxidation to the cell membrane of the RBCs is further potentiated by the simultaneous depletion of antioxidant reserves in the lead intoxication. Lead causes depletion of glutathione reductase (GR) system which normally scavenges free radicals. In addition, inability of the erythrocytes to resynthesise these antioxidant enzymes because of the lack of ribosomes in them also makes these erythrocytes more vulnerable cells to damage caused by OS. Haemolysis of RBC, by the rupture of cell membrane, results in anaemia, owing to the decrease in the RBC count and fall of haematocrit.

In the present study, haemoglobin, haematocrit and RBC count were increased by ascorbic acid supplementation that suggested the role of ascorbic acid in decreasing various derangements produced by lead. Possible mechanism by which ascorbic acid caused the improvement in haematological parameters could be reduction in OS which was responsible for lipid peroxidation of erythrocytic cell membrane to cause haemolysis. Ascorbic acid because of being an antioxidant neutralised the OS and so prevented the consequent damage.

In addition to the antioxidant proprieties of ascorbic acid, it ameliorates haematological toxicity of lead by reversing the activity of certain enzymes inhibited by lead. Two enzymes, the activity of which is specifically inhibited by lead are: delta-aminolevulinic acid dehydrogenase (ALAD) and GR. Lead binds with the functional sulphydryl (-SH) groups of these enzymes, rendering them non-functional to further contributing in the impairment of oxidative balance. Ascorbic acid restores the activity of these enzymes and so the toxic effect of lead on the erythrocytes is reversed. In addition to the membrane lipid peroxidation, lead exposure causes haemoglobin oxidation, by the inhibition of ALAD. This also contributes to the haemolysis of RBCs to produce anaemia. Since the activity of ALAD is restored by ascorbic acid supplementation, generation of ROS is inhibited and RBCs live a normal life span.

In the present study, when ascorbic acid group was compared with lead-intoxicated group, RBC count increased after supplementation with ascorbic acid while the MCV remains unaffected. MCV reflects size of red blood cells which depends on the volume of cytoplasm, mainly haemoglobin present in it. MCV stays unaltered in the anaemia caused by haemolysis, because it is caused by decreased cell count and is not caused by decreased cell size. In the present study, MCV was neither significantly affected by lead intoxication nor by ascorbic acid supplementation. It reflects that the main effect of ascorbic acid supplementation is to prevent the damage to already formed RBCs which result in the improvement of haemoglobin and haematocrit. Increase in the count of RBCs is due to the increased survival of the erythrocytes because of more stability of their cell membrane in the presence of ascorbic acid.

**Conclusion**

Lead intoxication produced oxidative stress and anaemia which manifested in the increased levels of serum MDA, low haemoglobin, decreased RBC count, MCH and MCHC. MCV did not change lead administration, which is indicative of the unaltered process of maturation of RBCs during erythropoiesis. Ascorbic acid supplementation in the drinking water has significantly ameliorated the oxidative stress and anaemia caused by lead.

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**Conflict of Interest:** None.

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