Amelioration of arsenic toxicity by L-Ascorbic acid in laboratory rat

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Abstract: A study, so as to confirm the protective effects of L-ascorbic acid against inorganic arsenic (As$_5$O$_7$) toxicity was made in male Wistar rats. Multiphasic observations made on iAs concentration in target organs viz. liver and kidney, liver function, histopathological changes, ultrastructural alterations, lipid peroxidation, oxidative stress and iAs-DNA interaction strongly favoured its ameliorative effects. These effects could mainly be attributed to its antioxidative property. It offers help in regeneration of GSH and α-tocopherol. The chelation of iAs by ascorbic acid has also been hypothesized. Inhibition of DNA damage by ascorbic acid in liver and kidney appears to be the most significant part of this study. On the basis of these results, we conclude that administration of L-ascorbic acid to arsenic affected population may prevent the occurrence of fatal human diseases.

Key words: Arsenic, L-ascorbic acid, Liver, Kidney, Lipid peroxidation and Oxidative stress

Introduction

Arsenic is a naturally occurring metalloid (atomic number 33), located on group V of the periodic table. Exposure to high levels of arsenic through drinking water has been recognized for many decades in some regions of the world, i.e. China, India, and some countries in Central and South America. Millions of people are at risk of cancer and other diseases because of chronic arsenic exposure (NRC, 1999, 2001). Environmental exposure to arsenic can cause a variety of cancers, most commonly nonmelanoma skin cancers, and chronic toxicity may manifest as diffuse symptoms not easily recognizable as chronic heavy metal toxicity. General adverse health effects associated with human exposure to arsenicals include cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, fibrosis of the liver and lung, hematological disorders and blackfoot disease (Abernathy et al., 1999; Sordo et al., 2001 and Tchounwou et al., 1999). In humans, arsenic is known to cause cancer of the skin (Rossman et al., 2004) lung, bladder, liver and kidney (Abernathy et al., 1999; Kitchin, 2001 and Tchounwou et al., 1999).

Arsenic is methylated by alternating reduction of pentavalent arsenic to trivalent and addition of a methyl group from S-adenosylmethionine (Marafante et al., 1985). Glutathione and possibly other thiols, serve as reducing agents

![Fig. 1: Biomethylation is the main mechanism for the metabolism of inorganic arsenic. It involves alternate steps of two-electron reduction followed by oxidative addition of a methyl group](image-url)
(Delnomdedieu et al., 1994; Styblo et al., 1995). Liver is the most important site of arsenic methylation (Marafante et al., 1985; Geubel et al., 1988) but most organs show methylating activity. The end metabolites are methylarsonic acid (MMA) and dimethyl arsenic acid (DMAA) (Fig. 1). These compounds are readily excreted in urine. However, reactive intermediates may be formed.

Arsenite is known to bind to cellular sulfhydryl, particularly vicinal ones, accounting for its ability to interfere with energy generation (Apohian, 1989). Once in the tissues, arsenic exerts its toxic effects through several mechanisms, the most significant of which is, the reversible combination with sulfhydryl groups. Arsenic also inhibits numerous other cellular enzymes, especially those involved in cellular glucose uptake, gluconeogenesis, fatty acid oxidation and product-ion of glutathione through sulfhydryl group binding.

A second major form of toxicity is termed “arsenolysis”. Pentavalent arsenate can substitute competitively for phosphate in biochemical reactions, where ADP would normally phosphorylate into ATP. In the presence of arsenic, ADP arsenate is the end product and high energy phosphate bonds are not formed. The unstable ADP-arsenate decomposes spontaneously and irreversibly resulting in loss of energy of the cell. ROSs are capable of damaging a wide variety of cellular macromolecules including DNA, lipids and proteins. Finally, cellular signal transduction can be altered (e.g. activation of trans factors, changes of gene expression), cell growth, proliferation and differentiation can be promoted and apoptosis leading to cell death or cancer developments can be induced (Yang and Frenkel, 2002; Qian et al., 2003).

The idea that arsenical induced toxicity could be modified by nutrients was initially proposed in the early 1930's by Mayer and Sulzberger (1931), who suggested that adequate levels of ascorbic acid, in the diet prevented or reduced occurrence of arsenic induced anaphylaxis. L-ascorbic acid is a primary defensive nutrient by virtue of its function as a free radical scavenger. It can react in aqueous media against in vivo peroxidants, including quenching of singlet oxygen species. It increases the turn over rate of toxic metals and reduces damage by scavenging free radicals generated by their metabolism (Hume et al., 1991). During acute response to different stressors such as metals and heat shock etc. ascorbic acid is depleted (Parihar and Dubey, 1995 and Lackner, 1998). Therefore, a study on the plausible ameliorative effects of L-ascorbic acid against iAs toxicity in rat was proposed.

Materials and Methods

**Chemicals:** Arsenic trioxide was purchased from Loba Chemie (Mumbai). L-ascorbic acid, reduced glutathione (GSH), nicotinamide adenine dinucleotide (NAD), 5-5, dithio-bis-2-nitrobenzoic acid, 1-chloro-2, 4-dinitrobenzene, bovine serum albumin, triton X-100, sodium lauryl sulphate, proteinase-K, RNAase, were obtained from Sigma Chemical Company (St Louis, Mo., USA). Thiobarbituric acid (TBA) was purchased from Wako Chemical Company (Japan). NADPH was obtained from SRL (Mumbai). All other chemicals or reagents of highest purity were procured from S. Merck, S. D. Fine Chemicals and Qualigens (Mumbai).

**Model:** Male Wistar rats (150 ± 20 g) were procured from the animal facility of Jamia Hamdard, New Delhi. They were housed individually in polypropylene cages under standard laboratory conditions (room temperature 25 ± 5°C; RH = 50 ± 10%). Each rat was offered food pellets (Golden Feeds, New Delhi) and tap water ad libitum. All animal treatments and protocols employed in this study received prior approval of the Institutional Ethical Committee and met the standards laid down by Govt. of India.

**Treatments:** Twenty healthy male rats weighing 200 ± 30 g were selected for present study. After acclimatization to laboratory conditions, rats were divided into four groups, each containing five rats. Rats of group A, in addition to food and drinking water were administered 1.0 ml saline by gavage each alternate day and treated as controls. Rats of group B were administered predetermined sublethal dose (4 mg/100 g body weight) (LD<sub>50</sub> 10 mg/100g body weight) of arsenic trioxide dissolved in saline through gavage on each alternate day for 30 days as described earlier (Allen and Rana, 2003). Rats of group C were administered the same dose of arsenic trioxide in the same concentration and manner similar to the rats of group B. Moreover, they were administered with 25 mg/100 g body weight of L-ascorbic acid simultaneously on each alternate day for thirty days. Rats of group D were administered L-ascorbic acid only (25 mg/100 g body weight) as the rats of group C and treated as controls.

**Sample preparations:** On 31<sup>st</sup> day rats were starved overnight and sacrificed next morning by light ether anesthesia. Blood was collected through cardiac puncture. Serum was separated by centrifugation at 5000 rpm for 20 min. and processed for the estimation of serum transaminases and bilirubin. Small pieces of liver and kidney were carefully removed from each rat and processed suitably for the estimation of arsenic, glutathione, microsomal lipid peroxidation, glutathione-S-transferase, isolation and quantification of DNA, histopathological and EM studies.

**Estimation of arsenic in soft tissue, serum and urine:** 1.0 gm of wet tissue/1.0 ml serum or urine sample was digested in 10.0 ml of concentrated nitric acid at 80°C for 16 hr. A.2.0 ml aliquot of the digest was analyzed for inorganic As by hydride generation at pH 6, using sodium borohydride as the reducing agent. The analyses were done using atomic absorption spectrophotometer (Electronic Corporation, India). Absorbance was recorded at 193.7 nm, using a hollow cathode lamp for arsenic.

**Determination of serum transaminases:** Alanine amino transaminase (ALT) and aspartate amino transferase (AST)
activity in serum were determined following the method of Reitman and Frankel (1957) using a kit procured from Span Diagnostics (Surat, Gujrat).

**Determination of total bilirubin in serum:** Total bilirubin in serum was determined by a commercial kit obtained from Ozone Biomedicals Pvt. Ltd. (Hyderabad) using the method of Burtis and Ashwood (1996).

**Histopathological study:** Small pieces of liver and kidney were carefully removed from experimental animals and fixed in 10% buffered neutral formalin. 5 micron thick paraffin sections thus prepared were stained with hematoxylin and eosin and examined under research microscopes (Nikon, Japan).

**Electron microscopic studies (Transmission electron microscopy):** Very small cubes (1 mm³) of liver and kidney were immersed in 2.5% glutaraldehyde, post fixed in 1% osmium tetraoxide, dehydrated through a graded series of ethanol and embedded in Epon 812 after several changes of propylene oxide. Ultra thin sections stained with uranyl acetate and lead citrate were examined under a Phillips, CMIO Transmission electron microscope, at AIIMS, New Delhi.

**Estimation of reduced glutathione:** Glutathione was measured as acid soluble sulfhydryl levels assayed by the method described by Ellman (1959). The acid soluble sulfhydryl group generated a yellow colored complex (5-thio-2-nitrobenzene) with DTNB. Its absorbance was recorded at 412 nm.

**Oxidized glutathione:** GSSG was estimated in the liver and kidney following the method suggested by Ohmori et al. (1986). Peptides having a c-terminal glycine exhibit a color reaction similar to that of glycine with acetic anhydride, p-dimethylamino-benzaldehyde and pyridine. Absorbance was recorded at 458 nm.

**Glutathione-S-transferase (E.C.2.5.1.8):** Glutathione-S-transferase was assayed by the method of Habig et al. (1974). Its activity was determined by the rate of formation of conjugate between reduced glutathione and 1-chloro 2,4-dinitrobenzene. The conjugate absorbs strongly at 340 nm wavelength.

**Table 1:** Arsenic concentration (µg/ml) in the serum and urine of arsenic and ascorbic acid treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>0.011 ± 0.003</td>
<td>ND</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>0.599 ± 0.076*</td>
<td>0.100 ± 0.01*</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + ascorbic acid</td>
<td>0.136 ± 0.014**</td>
<td>0.071 ± 0.013**</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid</td>
<td>0.0126 ± 0.003 NS</td>
<td>0.020 ± 0.016**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n = 5)

NS denotes non significant
* denotes significantly different values from control rats (p<0.05) ‘t’ test
** denotes significantly different results from arsenic treated rats (p<0.05) ‘t’ test

**Determination of lipid peroxidation:** Peroxidized membrane lipids were estimated by method described by Jordan and Schenkmann (1982). Microsomal pellets were precipitated by calcium according to the method suggested by Schenkmann and Cinti (1978).

Malondialdehyde and other reactive oxidized products of membrane lipids, under acidic conditions, react with thiobarbituric acid and form a pink colored chromogen which is strongly absorbed at 532 nm wavelength.

Protein content was determined by the method described by Lowry and coworkers using bovine serum albumin (Sigma, USA) as the standard Lowry et al. (1951).

**As⁶-DNA interaction:** DNA from liver and kidney samples were eluted using Genelute mammalian genomic DNA miniprep kit procured from Sigma. The concentration was determined by spectrophotometric analysis and the absorbance was measured at 260 nm and 280 nm using a quartz microcuvette (Sambrook et al., 1989).

**Estimation of fragmented DNA:** Cells were lysed using tris-EDTA buffer and Triton-100. Lysate was centrifuged at 13000xg and intact and fragmented DNA were separated. The respective samples were precipitated by trichloroacetic acid. DNA in each fraction was quantified using diphenylamine reaction. Absorbance was recorded at 600 nm against a blank (Sellins and Cohen, 1987).

**Statistical analyses:** Statistical evaluation of results was simultaneously made employing SPSS software.

**Results and Discussion**

Urinary excretion of arsenic increased several fold in arsenic fed male and female rats. Arsenic concentration decreased in liver, kidney, urine and serum on simultaneous treatments with ascorbic acid (Table 1, 2). Ascorbic acid has earlier been reported as a possible chelator of lead with similar potency as that of EDTA (Bratton et al., 1981). One possibility of ascorbate protection might be the mobilization of arsenic from...
Table 3: AST (Karmen Units), ALT (Karmen Units) and bilirubin (mg/dl) in the serum of arsenic treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
<th>AST (Karmen Units)</th>
<th>ALT (Karmen Units)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>42.25 ± 1.29</td>
<td>42.0 ± 1.15</td>
<td>17.98 ± 0.53</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>120.51 ± 1.48*</td>
<td>136.96 ± 2.19*</td>
<td>40.58 ± 0.921*</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + Ascorbic acid</td>
<td>89.71 ± 1.39**</td>
<td>98.99 ± 1.057**</td>
<td>28.22 ± 1.00**</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid only</td>
<td>42.90 ± 0.814*</td>
<td>41.95 ± 0.6233*</td>
<td>15.63 ± 0.6668*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n = 5)
* denotes values significantly different from control rats.
** Denotes values significantly different from arsenic treated rats.

Table 4: Reduced glutathione (GSH) (μg/g wet weight) in the liver and kidney of arsenic treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Gender</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>Male</td>
<td>0.154 ± 0.060</td>
<td>0.169 ± 0.039</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>Male</td>
<td>0.126 ± 0.006*</td>
<td>0.105 ± 0.013*</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + ascorbic acid</td>
<td>Male</td>
<td>0.130 ± 0.010**NS</td>
<td>0.170 ± 0.004**NS</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid</td>
<td>Male</td>
<td>0.139 ± 0.002**NS</td>
<td>0.136 ± 0.006**NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n = 5)
* denotes significantly different values from control rats (p<0.05) “t” test
** denotes significantly different results from arsenic treated rats (p<0.05) “t” test
NS denotes non significant

Table 5: Oxidized glutathione (GSSG) (μ moles/gm wet weight) in the liver and kidney of arsenic treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>0.535 ± 0.030</td>
<td>0.429 ± 0.077</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>3.168 ± 0.053*</td>
<td>3.644 ± 0.018*</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + ascorbic acid</td>
<td>1.627 ± 0.114**</td>
<td>0.956 ± 0.060**</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid</td>
<td>0.560 ± 0.023**NS</td>
<td>0.392 ± 0.033**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n = 5)
* denotes significantly different values from control rats (p<0.05) “t” test
** denotes significantly different results from arsenic treated rats (p<0.05) “t” test
NS denotes non significant

Table 6: Glutathione-S-transferases (GST) (n moles/NADPH/min/mg protein) in the liver and kidney of arsenic treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>0.865 ± 0.035</td>
<td>0.727 ± 0.015</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>0.426 ± 0.021*</td>
<td>0.355 ± 0.008*</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + ascorbic acid</td>
<td>0.677 ± 0.007**</td>
<td>0.570 ± 0.013**</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid</td>
<td>0.806 ± 0.013**</td>
<td>0.689 ± 0.031**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n = 5)
* denotes significantly different values from control rats (p<0.05) “t” test
** denotes significantly different results from arsenic treated rats (p<0.05) “t” test
NS denotes non significant

soft tissues. Another reason might be the reduced absorption of arsenic from intestine. Ingested arsenic that accumulates in tissues, binds with sulfhydryl groups (Aposhian and Aposhian, 1989) and the remaining is excreted in the urine. Thus it could be envisaged that ascorbic acid might play a therapeutic role against general arsenic toxicity.

Arsenic is known to produce disturbances in liver function (Fowler et al., 1977). AST and ALT are reliable determinants of liver parenchymal injury (Moss et al., 1987). Activities of both ALT and AST significantly increased in arsenic treated rats indicating liver dysfunction. In arsenic and ascorbic acid treated rat, values of ALT and AST declined significantly. Similarly serum bilirubin increased in arsenic treated rats but declined in ascorbic acid and arsenic treated rats (Table 3). These results suggest that ascorbic acid protects against hepato toxicity of arsenic by improving liver function.

In the present study low levels of GSH were observed in the liver and kidney of arsenic treated male and female rats. A moderate increase in oxidized glutathione (GSSG) was, however, recorded (Table 4 and 5). The intracellular level of GSH has been inversely correlated with cytotoxicity of arsenic (Ochi et al., 1996). GSH status improved on ascorbic acid co-treatment. The improved levels of GSH might protected the sulfhydryl groups from binding with arsenic and promoted the detoxification of arsenic by modulating arsenic methylation reactions (Buchet and Lauwerks, 1988). When GSH levels are reduced, inorganic arsenic becomes more toxic (Shimizu et al., 1998). GSH depletion causes essentially nontoxic MMAs to become toxic (Sakurai, 2003). DMAs induces apoptosis after GSH depletion (Sakurai, 2002), which could allow the survival of damaged cells.

Glutathione-S-transferase (GST) consists of a large family of GSH utilizing enzymes that play an important role in detoxification of xenobiotics in mammalian systems. Arsenic trioxide was found to inhibit glutathione-S-transferase activity significantly in liver and kidney both (Habig et al., 1974; Lee et al., 1989 and Wendel, 1980). However, ascorbate co-treatment restored enzyme activity in male rats. This was considered as an
Table - 7: Microsomal malondialdehyde (nmol/mg protein) in the liver and kidney of arsenic treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>0.120 ± 0.004</td>
<td>0.135 ± 0.005</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>0.325 ± 0.009*</td>
<td>0.460 ± 0.059*</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + ascorbic acid</td>
<td>0.211 ± 0.003**</td>
<td>0.235 ± 0.007**</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid</td>
<td>0.113 ± 0.012*</td>
<td>0.125 ± 0.017*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n = 5)
* denotes significantly different values from control rats (p < 0.05)
** denotes significantly different results from arsenic treated rats (p < 0.05)

Table - 8: Intact and fragmented DNA (μg/ml) in the liver of arsenic and ascorbic acid treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total DNA</th>
<th>% Fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>31.59 ± 0.332</td>
<td>23.46 ± 0.478</td>
</tr>
<tr>
<td>B</td>
<td>Arsenic</td>
<td>12.56 ± 0.253 *</td>
<td>34.69 ± 0.445 *</td>
</tr>
<tr>
<td>C</td>
<td>Arsenic + ascorbic acid</td>
<td>29.58 ± 0.389**</td>
<td>25.84 ± 0.345**</td>
</tr>
<tr>
<td>D</td>
<td>Ascorbic acid</td>
<td>33.22 ± 0.482**</td>
<td>21.65 ± 0.779**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE (n = 5)
* denotes significantly different values from control rats (p < 0.05)
** denotes significantly different results from arsenic treated rats (p < 0.05)

Adaptive response facilitated by ascorbic acid against arsenic induced stress (Table 6).

Lipid peroxidation has been largely considered as a molecular mechanism involved in deleterious effects of a variety of xenobiotics including heavy metals (Sunderman et al., 1985). Biomembranes and subcellular organelles are the major site of lipid peroxidation (Halliwell and Gutteridge, 1989). Co-treatments of ascorbic acid and arsenic inhibited lipid peroxidation in liver and kidney both. This effect could be attributed to its antioxidative property. Ascorbic acid is well known to inhibit oxidative damage to membranes (Li et al., 2001; Nandi et al., 2005) (Table 7).

The induction of oxidative DNA damage by arsenic in mammalian cells has been paid considerable attention by earlier workers. (Kessel et al., 2002). There is evidence that both DNA and MMA exert DNA damaging effect through an intermediate involving ROS production (Nesnow et al., 2002). Arsenic perturbs cells in numerous ways i.e. inducing chromosomal abnormalities, altering DNA repair or DNA methylation patterns and producing oxidative stress (Hamadeh et al., 2002; Liu and Jan, 2000; Mass et al., 2001). Present observations on total amount of DNA in liver and kidney showed that arsenic trioxide significantly damaged DNA. Results also indicated higher percentage of fragmented DNA in liver and kidney both after arsenic treatment. Further, it was found that cotreatments with ascorbic acid reduced the percentage of fragmented DNA and increased the amount of total DNA in liver and kidney both (Table 8, 9 and 10). Thus a protective effect of ascorbic acid on DNA damage could be envisaged. An earlier study suggested that ascorbic acid inhibited DNA damage caused by an antitumor drug (Blasiak and Kowalik, 2001).

To gather more evidence to support the protective behavior of ascorbic acid against arsenic toxicity, histopathological studies, using both light and electron microscopy were undertaken. Light microscopical observations showed that arsenic caused hepatic parenchymal degeneration, hyperplasia and vascular lesions in male rats. Sinusoids were dilated and found to be filled with foam cells. Significant protective effects on different lesions were observed in the liver of rats treated with arsenic and ascorbic acid. There was no neoplastic formation, however, mild inflammation of hepatic cells still persisted (Fig. 1, 2). While the ultrastructural study of liver, of arsenic treated rats, showed inflamed nuclei and increased number of mitochondria in hepatic cells treated with arsenic and ascorbic acid. The mitochondria of different shapes and sizes were observed and several small vacuoles were observed in the matrix of the cell (Fig. 3 and 4).

Light microscopical observations on kidney of arsenic treated rats showed glomerulonephritis, proximal tubular necrosis, epithelial damage and loss of nuclei. In cortex focal tubular necrosis was observed along with pycosis and appearance of...
Fig. 2: T.S. of liver of an arsenic fed rat shows parenchymal degeneration, hyperplasia and vascular lesions (H/E X 400)

Fig. 3: T.S. of liver of rat treated with arsenic and ascorbic acid shows binucleated cells and focal pycnosis (H/E X400)

Fig. 4: T.E.M. study of liver of arsenic treated rat shows swollen nuclei and increased number of mitochondria (M) (880X)

Fig. 5: T.E.M. study of liver of rat treated with arsenic and ascorbic acid shows normal nucleus (N). Several vacuoles are also observed (880X)

Fig. 6: T.S. of kidney of arsenic treated rat shows inflammed glomerulus, and proximal tubular necrosis (H/E X 400)

Fig. 7: T.S. of kidney of rat treated with arsenic and ascorbic acid shows mild glomerulonephritis and tubular necrosis (H/E X 400)
Ascorbic acid has a few therapeutic characters. It is a water soluble antioxidant. It predominantly works as a radical chain terminator. One ascorbate molecule reacts with a peroxyl radical to yield a hydroperoxide and ascorbyl radical, subsequently the ascorbyl radical can react with another peroxyl radical and produce the oxidized ascorbic acid i.e., dihydroascorbic acid (Combs and Gray, 1998). Thus one molecule of ascorbate can trap two molecules of peroxyl radicals. Another hypothesis suggests that ascorbic acid may be involved in the regeneration or restoration of antioxidant properties of α-tocopherol. The tocopherol is converted to α-tocopherol quinone (Chow, 1985).

Ascorbate can both chelate and reduce transition metal ions and the reduced metal ions in turn can reduce oxygen or \( \text{H}_2\text{O}_2 \) to superoxide and hydroxyl radicals, respectively.

\[
\text{AsCH} + \text{Me}^{(n+1)+} \rightarrow \text{AsC}^{+} + \text{H}^{+} + \text{M}^{n+} \quad \text{(Eq. 1)}
\]

\[
\text{H}_2\text{O}_2 + \text{M}^{n+} \rightarrow \text{OH}^{+} + \text{OH}^{-} + \text{M}^{(n+1)+} \quad \text{(Eq. 2)}
\]

Superoxide and hydroxyl radicals are scavenged by ascorbate with second order rate constant of \( 1\times10^8 \) and \( 1.1\times10^{10} \) \( \text{M}^{-1}\text{s}^{-1} \) respectively. (Carr and Frei, 1999). Therefore, ascorbic acid forms the first line of antioxidant defense (Frei, 1999; Carr and Frei, 2000). These properties of ascorbic acid make it a suitable antidote for arsenic toxicity in rodents and possibly in human subjects.

References


Buchet, J.P. and R. Lauwerys: Role of thiols in the human subjects. T.E.M. study of kidney of rat treated with arsenic and ascorbic acid shows basolateral membrane infoldings tightly associated with mitochondria (M) (1400X)

Fig. 8: T.E.M. study of kidney of arsenic treated rat shows mitochondria (M) of different shapes and sizes (880X)


