Chronic toxicity of arsenic in goats: clinicobiochemical changes, pathomorphology and tissue residues


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Abstract

Chronic inorganic arsenic toxicity was induced in goats by oral administration of one-fifth of the acute lethal dose 50 (ALD50) of sodium arsenite (25 mg kg⁻¹ body weight) packed in gelatin capsules and given daily for 12 weeks. Clinical signs of toxicity developed from 3 week post-exposure, consisting of gastrointestinal disturbances and renal insufficiency with 100% mortality in all animals. There were significant (p<0.01) decreases in total serum protein and the albumin:globulin ratio, and increases in blood glucose and various enzymatic activities of treated animals. Toxicity also induced severe pathomorphological changes, indicative of haemorrhagic and degenerative and/or necrotic lesions in most organs. In addition, proliferative pneumonia in lungs, hyperplastic goitre in thyroid and chronic proliferative lesions in skin were observed. Liver contained the largest residues of arsenic, followed by intestine, kidneys, thyroid, abomasum, spleen, skin, lungs and lowest in brain. The intensity of pathomorphological changes was proportional to the accumulated amount of arsenic in tissues/organs. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Chronic toxicity; Arsenic pollution

1. Introduction

Arsenic pollution in the environment has gained importance owing to its widespread toxic effects on humans, animals, birds, aquatic life and plants through polluted ground water and food chains. In the recent past, accumulation of arsenic in ground water (0.20–3.70 mg per litre has posed serious threats to populations of millions of human beings in India, China, Thailand, Bangladesh and Taiwan (Chakraborty and Das, 1997). A major arsenic calamity has been reported from seven districts of eastern India, covering an area of 37,493 km² with 34 million people victimized of arsenical dermatosis and skin cancer (Mandal et al., 1996). Various livestock animals are also the likely victims of such catastrophes arising from arsenic pollution. Basic informations on arsenical poisoning in cattle and small ruminants are meagre, excepting a recent report of development of anemia, gastrointestinal signs, toxic nephropathy and mortality in goats with induced chronic arsenic toxicity (Biswas et al., 1998).

Further toxico-pathologic effects of long-term ingestion of arsenic at a low dose rate on various body systems of ruminants remain obscure. It has been reported that the intensity of pathologic changes is directly related to the quantitative accumulations of mercury in various organs (Pathak and Bhowmik, 1998), but a similar correlation remains undetermined for chronic arsenic toxicity of farm animals. In view of the importance of goat meat in the human diet, chronic inorganic arsenic toxicity was induced in this species, to determine the residual concentrations of arsenic in...
various tissues, including muscle, and to correlate them with the clinico-biochemical and pathological changes.

2. Materials and methods

2.1. Determination of acute lethal dose 50 ($ALD_{50}$) of sodium arsenite in goats

Twenty-five female adult Black Bengal goats were randomly divided into five equal groups of five each. Four ascending doses viz. 75, 100, 125 and 150 mg kg$^{-1}$ body weight of reagent grade, powdercd sodium arsenite (E. Merck, India) were dissolved in 300 ml deionized water and administered once to each goats of groups I, II, III and IV, respectively, using stomach tube. The group V animals received only the deionized water, serving as control. Clinical signs of toxicity and mortality were observed and reported in all groups. The $ALD_{50}$ value of sodium arsenite was calculated as per methods of Millev and Tainter (1944), described by Ghosh (1984).

All goats of groups I and II treated with 75 and 100 mg of sodium arsenite started to show clinical signs of toxicity, consisting of gastrointestinal and mild rephrotoxic signs during 12 h post-treatment onwards without any mortality. Goats of groups III and IV dosed with 125 and 150 mg kg$^{-1}$ of sodium arsenite also developed similar signs on 4 h post-treatment, but the intensity was severe, with the advancement accompanied with manifestation of diarrhoea, red urine and nervous signs with incoordination, bulging of eye ball and opisthonus, and followed by death of all animals between 40 and 48 h. Therefore, the dose level of 125 mg kg$^{-1}$ body weight was considered as $ALD_{50}$ of sodium arsenite.

2.2. Experimental goats

Twelve clinically healthy female Black Bengal goats, approximately 12 months of age and weighing 11−11.5 kg were used. The animals were acclimatized to the experimental condition for 15 days. They were fed on 0.5 kg goat ration; 1 kg hybrid Napier grass, supplemented with feed additives twice a day, and were given deionized drinking water ad libitum. The goats were randomly divided into two equal groups of six each, and housed in groups in indoor concrete floored pens.

2.3. Arsenic treatment

One-fifth of determined $ALD_{50}$ of sodium arsenite i.e. 25 mg kg$^{-1}$ body weight was weighed, packed in gelatin capsules, and administered orally to each goat of group II daily for 12 weeks or until death. In group I, each animal received daily gelatin capsules containing sucrose, serving as control. The deionized water, as the sole source of drinking water, was available ad libitum for both the groups. All goats were examined at least twice a day, and progressive development of clinical signs was recorded, including mortality. Necropsy was performed within 1 h after the goats died or were killed with an over dose of sodium pentobarbitone after 12 weeks of exposure and gross pathological changes were recorded.

2.4. Collection of samples

Jugular blood samples and rumen content were collected in sterilized evacuated tubes from the animals of both groups at three weekly intervals after dosing with sodium arsenite. One tube of blood contained sodium fluoride for glucose measurement. The second tube of blood was allowed to clot and the separated serum was utilized for total protein and enzymatic activity values. The collected rumen content and a part of blood were used to determine arsenic content in the samples.

During necropsy, pieces of liver, abdomasum, intestine, lungs, heart, spleen, mesentric lymph nodes, kidneys, thyroid, adrenal and parotid glands, ovary, bone marrow, skin, hair, skeletal muscles and brain were collected for estimation of arsenite residues, and evaluation of histopathological changes.

2.5. Biochemical method

Blood sugar content (Nelson and Somogyi, 1969): total serum protein and its albumin: globulin (A:G) ratio (Wooton, 1974); aspartate transaminase (AST) and alanine transaminase (ALT) (Frankel et al., 1978); alkaline phosphatase (ALP) (Kind and King, 1954); and acid phosphatase (AP) (King and Jagatheesan, 1959) were measured.
2.6. Histopathology

Pieces of various organs from animals of both groups were fixed in 10% buffered formalin. Fixed tissues were processed through conventional histological procedures for tissue sectioning of 5 μm thickness, and stained with Mayer’s haematoxylin and eosin.

2.7. Estimation of arsenic

The instruments and glassware used to handle and stores the tissues were chemically cleaned, rinsed in double-distilled deionized water and air-dried. About 5.0 g (wet weight) of each tissue was dissected out and taken directly into pre-weighed 125 ml standard taper flasks, and the weight recorded. The flasks were sealed and immediately placed at −20°C until use. The tissue samples from −20°C storage were digested in double the volume of triple acid mixture (HNO₃ — 10 parts, HClO₄ — 3 parts and H₂SO₄ — 1 part) on a hot plate. Dilution to a known concentration was finally made with double-distilled deionized water (Sandel, 1950). The residual concentrations of arsenic in the tissues were measured by the Molybdenum blue method (Stewart, 1989). Likewise, a known volume of blood sample and rumen content were digested in triple acid mixture and dilution to a known concentration was made with deionized water and arsenic content was determined using same method. The total arsenic content were expressed as μg g⁻¹ of tissue and rumen content.

2.8. Statistical test

The significance of the differences in the mean values of biochemical findings and arsenic concentrations in tissues/rumen content between the groups were analysed using Student’s t-test (Snedecor and Cochran, 1968).

3. Results

3.1. Clinical signs

All arsenic-treated goats exhibited wild signs of toxicity from 3 week post-exposure, consisting of dullness and depression, with slightly reddish coloured urine. The arsenic content in blood and rumen content of treated animals were 0.51 and 0.53 mg% at this stage. In 4–6 weeks, there were partial loss of appetite, reddish coloured urine, oliguria and weakness. Both blood and rumen content had 2.05 and 2.33 mg% of arsenic at 6 weeks. During 7 and 8 weeks, brown pellet faeces with mucous, yellowish mucous membrane, rough body coat with erected hairs, and profound muscular weakness were observed. From 9 week onwards, there were polyuria, incoordination, inability to get up (knee-based posture, Fig. 1) and salivation. A significant (p<0.01) increase of arsenic content in blood (2.88±0.99 and 3.17±0.08 mg%) and rumen content 92.82±0.11 and 93.87±0.04 mg%), and increase in respiratory rate (33.0±0.23 and 40.0±0.13/min) and heart rate (99.0±1.23 and 119.0±0.35/min) were observed in 9 and 12 weeks, respectively. Arsenic-treated animals had a significant (p<0.01) decrease in body weight gain in 9 (9.80±0.11 kg) and 12 weeks (8.60±0.20 kg). Two animals died at the beginning of 12 week, and the remaining four at the end of week 12. None of the control animals developed any signs of toxicity.

3.2. Biochemical findings

Blood biochemical values of both arsenic-treated and control animals are presented in Table 1. Arsenic-treated goats showed a significant (p<0.01) decrease in
total serum protein with simultaneous reduction in A:G ratio, and significant (*p* <0.01) increase in blood glucose from 6 week post-treatment. Significantly higher AST, ALT and ALP values from 3 weeks onwards, and AP activity from 9 week were observed in arsenic-treated goats when compared with the values of control animals (Table 1).

### 3.3. Pathomorphological findings

Arsenic-treated goats showed enlargement of the kidneys, abomasum and liver with greyish-white necrotic foci on the surfaces. The abomasum, intestinal mucosa, kidneys and spleen were haemorrhagic in varying degrees, whereas the liver was pale. The intestine contained thick, viscid, slimy material. The lungs exhibited consolidation and grey hapatization.

The histopathological lesions comprised moderate to severe hyperemia and haemorrhages in the abomasum, intestinal mucosa, kidneys and spleen. The most striking cytotoxic lesions were cellular swelling with granular cytoplasm, degeneration and/or coagulative necrosis in some of the organs, the order being the hepatocytes in the centrilobular, midzonal and periportal areas of liver (Fig. 2), epithelium of abomasum and intestinal mucosa, the tubular epithelium of kidney (Fig. 3) and lymphoid cells in the Malpighian corpuscles of spleen. The lymphoid follicles in the spleen and mesenteric lymph node were reduced both in number and size.

The other changes included accumulation of fibrin admixed with red blood corpuscles, mild proliferation of von Kupffer cells and focal infiltrations of mononuclear cells in the liver; presence of cellular casts in the kidney tubules (Fig. 3); proliferation of bronchial epithelium, with thickening of both bronchial and alveolar septa due to accumulation of sero-fibrinous exudate and infiltrating cells; and, Zenker’s degeneration of cardiac muscle. The brain showed swelling and vacuolation of nerve cells. Thyroid revealed hyperplastic proliferation of acinar lining epithelium and scant colloid materials in the lumina. Skin lesions comprised intracytoplasmic oedema, acanthosis, and

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**Table 1**

Blood biochemical profiles of arsenic-treated and control goats (*n*=6, mean±S.E.)

<table>
<thead>
<tr>
<th>Item</th>
<th>Group I (control)</th>
<th>Control II (arsenic treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 weeks: 7.93±0.21</td>
<td>3 weeks: 7.88±0.03</td>
</tr>
<tr>
<td></td>
<td>6 weeks: 7.92±0.30</td>
<td>6 weeks: 5.64**±0.08</td>
</tr>
<tr>
<td></td>
<td>9 weeks: 7.93±0.02</td>
<td>9 weeks: 5.68**±0.02</td>
</tr>
<tr>
<td></td>
<td>12 weeks: 7.92±0.04</td>
<td>12 weeks: 5.39**±0.01</td>
</tr>
<tr>
<td>Total serum protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.24±0.10</td>
<td>47.17±0.30</td>
</tr>
<tr>
<td></td>
<td>47.15±0.44</td>
<td>47.64±0.24</td>
</tr>
<tr>
<td>Blood glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/100 ml)</td>
<td>20.00±0.32</td>
<td>42.75**±0.43</td>
</tr>
<tr>
<td></td>
<td>21.20±0.14</td>
<td>51.15**±0.66</td>
</tr>
<tr>
<td></td>
<td>20.38±0.85</td>
<td>62.40**±1.46</td>
</tr>
<tr>
<td>AST (K.U.)</td>
<td></td>
<td>79.10**±0.87</td>
</tr>
<tr>
<td></td>
<td>10.88±0.18</td>
<td>21.35**±0.19</td>
</tr>
<tr>
<td></td>
<td>12.60±0.08</td>
<td>34.75**±0.19</td>
</tr>
<tr>
<td>ALT (K.U.)</td>
<td></td>
<td>38.30**±0.40</td>
</tr>
<tr>
<td></td>
<td>5.20±0.09</td>
<td>9.56**±0.13</td>
</tr>
<tr>
<td>ALP (KAU)</td>
<td></td>
<td>17.74**±0.19</td>
</tr>
<tr>
<td></td>
<td>5.10±0.11</td>
<td>17.50**±0.21</td>
</tr>
<tr>
<td></td>
<td>5.07±0.09</td>
<td>17.02**±0.19</td>
</tr>
<tr>
<td>AP (KAU)</td>
<td>1.03±0.09</td>
<td>3.14±0.07</td>
</tr>
</tbody>
</table>

*a*: no. of samples; S.E.: standard error of the mean.

* p<0.05; ** P<0.01.

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![Fig. 2. Cellular swelling with granular cytoplasm, degeneration and coagulative necrosis in the centrilobular area of liver of arsenic-treated goats. H&F×80.](image-url)
hyperkeratosis (Fig. 4). The control animals did not reveal any changes in any of their organs.

3.4. Residual arsenic in tissues

Results on residual concentrations of arsenic showed significantly \( p<0.01 \) higher values in treated animals than in controls (Table 2). However, the distribution of arsenic in the various organs/tissues was not uniform. In each treated animal, the largest residual concentration of arsenic was in the liver, the next largest in the small intestine, followed by the kidneys, thyroid glands, abomasum, spleen, skin, hair, bone marrow, heart, skeletal muscles and lowest in brain tissue. Tissues from the organs of the control animals also contained less than 1/\( \mu g \) g\(^{-1}\).

<table>
<thead>
<tr>
<th>Name of the organs/tissues</th>
<th>Control (( n=6 ))</th>
<th>Arsenic-treated (( n=6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±S.E. (( \mu g ) g(^{-1}))</td>
<td>Mean±S.E. (( \mu g ) g(^{-1}))</td>
</tr>
<tr>
<td>Liver</td>
<td>0.21±0.01</td>
<td>75.70±2.21*</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.26±0.01</td>
<td>43.99±1.25*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.19±0.01</td>
<td>38.12±0.41*</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>0.20±0.01</td>
<td>36.10±0.37*</td>
</tr>
<tr>
<td>Abomasum</td>
<td>0.14±0.01</td>
<td>29.41±0.28*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.12±0.01</td>
<td>27.09±0.13*</td>
</tr>
<tr>
<td>Skin</td>
<td>0.50±0.03</td>
<td>23.23±1.03*</td>
</tr>
<tr>
<td>Hair</td>
<td>0.66±0.02</td>
<td>18.19±0.27*</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0.21±0.03</td>
<td>16.09±0.07*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.15±0.02</td>
<td>11.95±0.15*</td>
</tr>
<tr>
<td>Skeletal muscles</td>
<td>0.17±0.02</td>
<td>12.83±0.09*</td>
</tr>
<tr>
<td>Brain tissues</td>
<td>0.04±0.02</td>
<td>5.18±0.04*</td>
</tr>
</tbody>
</table>

* \( n \) no. of samples; S.E.: standard error of the mean.
* \( p<0.01 \).

4. Discussion

Long-term administration of arsenic in the form of sodium arsenite to goats at 25 mg kg\(^{-1}\) body weight i.e. one-fifth of the ALD\(_{50}\) produced nephrotoxic and gastrointestinal signs of toxicity, hypoproteinaemia, hyperglycaemia, an increased level of enzymatic activity and cytotoxic changes on many organ systems accompanied by 100% mortality. It is well known that the toxico-biological effects of an element are related to its rate of clearance and degree of accumulation in tissues (Pathak and Bhowmik, 1998). Because of a very high degree of gastrointestinal absorption (Tam et al., 1979), as well as a very slow rate of clearance of inorganic arsenicals (Underwood, 1977; Goodman et al., 1990) compared to organic compounds, high residues of arsenic were observed in all tissues. This in turn, caused severe toxicopathological changes in various organ systems.

The onset of gastrointestinal disturbances from 4 weeks onwards could be due to absorption of arsenic into the GI tract through the mucous membrane.

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**Fig. 3.** Degeneration and/or coagulative necrosis of tubular epithelium with pressure of cellular casts of arsenic-treated goats. H&E\( \times 100 \).

**Fig. 4.** Intracytoplasmic oedema, acanthosis and hyperkeratosis in the skin of arsenic-treated goats. H&F\( \times 100 \).
Clinical signs of renal insufficiency reflected damage to nephrons, as evident by the renal cytotoxic changes. The progressive loss of body weight gain was possibly because of gradual onset of anorexia due to toxaemia. The increase in cardiac and respiratory rates might have been due to retention of toxic metabolites resulting from acidosis.

Hypoproteinaemia with simultaneous reduction of A:G in arsenic-induced animals is likely to be due to marked destruction and disintegration of parenchymatous tissues. It is also possible that severe nephrotoxic lesions caused drainage of protein through the urine, resulting to hypoproteinaemia. The augmented level of blood glucose might be attributed to stress factor that increased the release of cortisol secretion by the adrenal cortex resulting gluconeogenesis. An increased level of enzymatic activity is known to occur in a wide range of degenerative and/or necrotic and inflammatory disease conditions, particularly in hepatic, cardiac, enteric and renal disease (Stogadale, 1981).

Although long-term exposure of sodium arsenite to goats caused varying degree of cytotoxic changes in most organ systems, the massive development of hyperemia, haemorrhage, cellular swelling, degeneration and/or coagulative necrosis in the liver, intestine, abomasum, kidneys, spleen and lymphnodes are characteristic. Goodman et al. (1990) also reported degenerative and necrotic lesions in the liver, intestine and kidneys in animals exposed to arsenicals. As such, the haemorrhagic and degenerative and/or necrotic changes may be considered as specific for chronic inorganic arsenic toxicity in goats. Presumably, as also suggested by Saxena et al. (1991), it is possible that arsenic caused severe toxic injury to capillary endothelium, resulting to the development of widespread vascular lesions in various organs. It is believed that arsenic may cause denaturation of cellular protein followed by denaturation of hydrolytic enzymes as well, causing degeneration and/or necrosis in parenchymatous organs. However, necrosis and depletion of lymphoid cells in the immunobiological organs clearly suggest that arsenic has a cytopathic effect on the antibody producing cells, so that immunosuppression may be a possible outcome of chronic arsenic intoxication.

This study also revealed lung lesions depicting proliferative pneumonia and thyroid changes suggesting hyperrplastic goitre in arsenic treated goats. Evidence indicating the goitrogenic cytotoxic effect of arsenic has also been reported in rats (Sharpless and Metzger, 1941).

All induced animals had widespread distribution of arsenic in the organs/tissues. However, the residual concentrations were not uniform in each tissue, possibly due to variation in the rate of accumulation and/or clearance of residues from the individual tissues. Liver retained the largest quality of arsenic residue and showed severe toxic hepatitis. Intestine, kidneys, abomasum, thyroid, spleen, skin and lungs contained larger quantities of residue and revealed widespread haemorrhagic and necrotic changes. On the other hand, brain retained least residues and presented only mild congestion, oedema and degenerative lesions. Furthermore, the evidence of moderate to higher arsenic residues in the muscles and other edible meat tissues represents a potential public health hazard since the permissible limit of arsenic ranges from 0.1 to 0.5 \( \mu g \) g\(^{-1}\) of animal tissue (Kreuzer and Logdeser, 1981).

5. Conclusion

The results suggest that long-term exposure of inorganic arsenic at low levels to goats produces severe clinical signs of toxicity and toxico-pathological changes accompanied by mortality. The cytotoxic effect of arsenic are proportional to the quantitative accumulation of its residues in the organs. Since arsenic exerts cytotoxic changes on the antibody producing cells, efforts are needed in depth to measure both cell-mediated and humoral immune responses in animals exposed to chronic arsenic toxicity as this element is rapidly becoming an unavoidable environmental pollutant.

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References


