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Male reproductive effect of arsenic in mice

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Abstract

Arsenic, a known human carcinogen, was given to mice via drinking water as sodium arsenite at a dose 53.39, 133.47, 266.95 and 533.90 μmol l for 35 days. A decrease in the activity of 17 β HSD along with increase in LDH, γGT activity were observed at 533.90 μmol l. The observed sperm count, motility and morphological abnormalities in sperm were similar to control at lower dose levels. However at 533.90 μmol l a significant decrease in sperm count and motility along with increase in abnormal sperm were noticed. Significant accumulation of arsenic in testes and accessory sex organs may be attributed to the arsenic binding to the tissues or greater cellular uptake. No effects were observed on indices studied for reproductive effects at 53.39 μmol l arsenic close to which human being are exposed through drinking water under the present set of experimental conditions.

Abbreviations: CTNB – cetyltrimethylammonium bromide; SDH – sorbitol dehydrogenase; LDH – lactate dehydrogenase; γGT – gamma-glutamyl transpeptidase; AP – acid phosphatase; βG-beta glucuronidase; 17β HSD – 17-beta-hydroxysteroid dehydrogenase; ND – not detected

Introduction

In recent years arsenic contamination in ground water has been considered as a problem of world concern. Arsenic , a ubiquitous toxic element is used in the production of agricultural pesticide, wood preservatives, metallurgic applications, glass production, as a catalyst in several manufacturing process and in medicine. The trace element is released into the atmosphere from both natural sources as well as anthropogenic activities. Human exposure through drinking water is increasing due to contamination from industrial operations and the over withdrawal of groundwater for irrigation since 1978. A large number of people are encountered with arsenic toxicity due to drinking of contaminated water in West Bengal (India), estimated in the range of 0.20 to 3.7 mg l (Chatterjee *et al.* 1995; Mandal *et al*. 1996; Saha *et al*. 1999). Chronic consumption of arsenic-contaminated drinking water is epidemiologically linked to many toxic effects including hyperpigmentation, keratosis, peripheral neuropathy , skin and lung cancer and peripheral vascular disease (Winsky & Carter 1998). Although human exposure provides evidence of the carcinogenicity and neurotoxicity of arsenic, data pertaining to reproductive toxicity are however very limited (Saha *et al*. 1999). In studies correlating reproductive outcomes with drinking water contaminant, some association was noted between arsenic and its adverse outcome (Aschengrau *et al*. 1989). Arsenic has been reported to cause reproductive failure in male workers at a copper smelter in Sweden, however the effect may not be attributed to arsenic only as subjects were also exposed to other metals (Beckman 1978). High level of radioactive arsenic has been detected in the epididymis and seminal duct of rodents (Danielsson *et al*. 1984). Low levels of sodium arsenite in drinking water for 6 weeks caused increase in testicular mass (Savabieasfahani *et al.* 1998) while treatment at 0.4 ppm/rat/day (5.33 μmol l) for 16 and 28 days showed duration dependent reduction in plasma level of LH, FSH ,estrogen and detection of arsenic in ovary, uterus, vagina

Table 1. Effect of arsenic exposure on organ weight (g) of mice treated orally for 35 days.

Group	Testes	Epididymides	Seminal Vesicle	Ventral prostrate	Coagulating gland
Control A	0.21 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.02 ± 0.01	0.01 ± 0.002
R	0.78 ± 0.03	0.34 ± 0.01	0.27 ± 0.01	0.08 ± 0.01	0.05 ± 0.01
As μmol l					
53.39 A	0.21 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.02 ± 0.001	0.01 ± 0.01
R	0.81 ± 0.03	0.36 ± 0.02	0.32 ± 0.04	0.09 ± 0.01	0.05 ± 0.003
133.47A	0.20 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.01 ± 0.03
R	0.77 ± 0.03	0.28 ± 0.04	0.28 ± 0.02	0.10 ± 0.02	0.05 ± 0.01
266.95A	0.20 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.01 ± 0.002
R	0.78 ± 0.04	0.36 ± 0.02	0.26 ± 0.02	0.08 ± 0.01	0.05 ± 0.01
533.90 A	0.21 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.01 ± 0.002
R	0.80 ± 0.04	0.32 ± 0.03	0.26 ± 0.01	0.07 ± 0.003	0.05 ± 0.002

Mean ± S.E. of 5 mice in each group.
A: absolute organ weight R: relative organ weight.

Table 2. Effect of arsenic exposure on marker testicular enzymes of mice treated orally for 35 days.

Enzymes	Control	53.39	133.47	266.95	533.90
			As μmol l		
SDH[a]	5.25 ± 0.43	5.10 ± 0.69	5.73 ± 0.76	5.82 ± 0.29	5.58 ± 0.37
17 β HSD[a]	3.28 ± 0.15	3.15 ± 0.15	3.08 ± 0.25	2.98 ± 0.14	1.50 ± 0.15*
AP [b]	195.60 ± 12.55	181.60 ± 13.48	200.20 ± 12.24	208.40 ± 13.01	215.60 ± 35.79
LDH[a]	212.18 ± 16.4	215.83 ± 10.88	213.65 ± 7.11	230.84 ± 8.83	260.62 ± 14.67*
γGT[c]	26.09 ± 2.24	24.74 ± 3.4	30.49 ± 3. 88	35.77 ± 5.87	52.38 ± 4.18*
βG[d]	0.15 ± 0.03	0.15 ± 0.02	0.17 ± 0.03	0.18 ± 0.02	0.18 ± 0.03

Mean ± SE of 5 mice in each group.
* P <0.05
[a]n mole NADH oxidised/NAD reduced/min/mg protein.
[b]n mole p-nitrophenol formed/min/mg protein.
[c]n mole p-nitroaniline liberated/min/mg protein.
[d]n mole phenolphthalein liberated/min/mg protein.

and plasma (Chattopadhyay *et al.* 1999). Thus keeping in view the persistent exposure of general population to arsenic through drinking water in India and due to the lack of pertinent male reproductive toxicity data in the literature this study was undertaken.

Materials and methods

Animals and treatment

Male mice (20 ± 2 g) bred at the Industrial Toxicology Research Centre, Lucknow, in the animal house colony were used for the study*. They were fed *ad libitum* on pellet diet and maintained under standard laboratory conditions. Mice were divided into 5 groups. Group 1 served as control and received tap water (arsenic level <0.0133 μmol l) whereas mice in group II, III, IV or V were orally fed 53.39, 133.47, 266.95 and 533.90 μmol l arsenic in the form of sodium arsenite, respectively, in drinking water for 35 days. Mice were killed by cervical dislocation on the 36th day of treatment. Testes, epididymides, seminal vesicle, ventral prostate and coagulating glands were quickly removed and weighed.

Testicular enzyme assay

A portion of testis was homogenised (1:9) in 0.2 M Tris HCl buffer (pH 7.0), having 0.1% CTNB using Potter Elvejham homogenizer for the assay of SDH and LDH. Another portion was homogenized (1:9) in 0.05 M Tris HCl buffer (pH 7.4) for the estimation of γGT. For AP and βG the testis was homogenized in

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Table 3. Effect of arsenic exposure on sperm motility and total epididymal sperm count of mice treated orally for 35 days.

Group	Motility (%)	Total sperm count (per epididymis) $\times 10^6$
Control	72 ± 3.75	5.62 ± 0.25
As μmol l		
53.39	75 ± 3.88	6.00 ± 0.07
133.47	76 ± 2.45	5.40 ± 0.24
266.95	74 ± 2.35	5.65 ± 0.26
533.90	60 ± 4.00*	3.84 ± 0.60*

Mean $\pm$ SE of 5 mice in each group.
*$P < 0.05$

ice cold water (Gerlach 1983; Vassault 1983; Roomi & Goldberg 1981; Walter & Schutt 1974; Fishman 1967). 17 β HSD and protein contents of the samples were estimated by the method of Talalay (1962) and Lowry *et al*. (1951), respectively.

Sperm count, sperm motility and morphological abnormalities assay

Epididymal sperm were obtained by mincing the epididymis in normal saline and filtering through/nylon mesh. The sperm were counted using a hemocytometer. Motility of the epididymal sperm and morphological abnormalities in sperm were enumerated using light microscopy (Pant *et al.* 1997).

Distribution of arsenic

Tissues collected in glass vials and stored at -18 °C prior to analysis were digested thrice with a mixture of 10 ml deionized water, 2 ml conc HNO_3, 0.5 ml H_2O_2 and 0.1 ml H_2SO_4 and finally with 1.0 ml of HNO_3 and H_2O_2 (1:1) until almost dryness. The residue finally dissolved in 1% HNO_3 was analysed using hydride generation system in Atomic Absorption Spectrophotometer (Varian model 250 plus) (Das *et al*. 1995). Recovery experiments in fortified samples in triplicate were performed using Certified Reference Material of As (BND 301) from National Physical Laboratory, New Delhi, India and were found to be in the range of 95% to 98%. The detection limit of the instrument was 0.30 ppb (0.004 μmol l).

Statistical analysis

The data were statistically analysed using Student's t test and $p < 0.05$ was considered to be significant (Fischer 1950).

Results

There was no difference in the uptake of water in control and treated animals. The approximate amount of arsenic intake/rat calculated on the basis of water intake ranged from 266.95–320.34, 667.35–800.82 , 1334.75–1601.70 and 2669.50–3203.40 μmol for group II, III, IV or V, respectively.

Body weight and organ weight

The body weight, testes , epididymis and accessory sex organ weight were similar to control at all the dose levels tested (Table 1).

Biochemical parameters

The activity of SDH, 17 β HSD, AP, LDH, γGT and βG were comparable to control at lower dose levels. However at 533.90 μmol l the activity of 17β HSD decreased and LDH, γGT were significantly increased. No such effects were observed in SDH, AP and βG activity (Table 2).

Sperm count, motility and morphological abnormalities in sperm

The observed sperm count, motility and morphological abnormalities in sperm were similar to control at 53.39, 133.47, 266.95 μmol l, however at 533.90 μmol l a significant decrease in sperm count and motility along with increase in abnormal sperm were noticed (Table 3 & 4).

Distribution of arsenic in tissues (mg/kg)

Arsenic was found to be present in tissues of control as well as exposed mice. An increase in arsenic content was noticed at 133.47, 266.95 and 533.90 μmol l dose level, however such an effect was not observed at 53.39 μmol l (Table 5). The presence of arsenic in tissue of normal animals may be due to the consumption of food, water and air contaminated with the traces of this universally distributed metal in the environment (Krishnamurthy & Vishwanathan 1990).

Table 4. Effect of arsenic exposure on sperm abnormalities of mice treated orally for 35 days.

Group	Per cent abnormalities								Total% abnormalities
	Head		Tail						
	Banana	Amorphous	Folded	Coil	Curved	Bent	Loop	Signet	
Control	0.80 ± 0.20	1.00 ± 0.31	0.80 ± 0.21	1.0 ± 0.31	0.60 ± 0,40	0.40 ± 0.24	0.60 ± 0.24	0.20 ± 0.10	5.60 ± 0.68
As μmol l									
53.39	0.80 ± 0.31	0.60 ± 0.40	1.40 ± 0.68	1.0 ± 0.31	0.60 ± 0.24	0.60 ± 0.21	0.60 ± 0.20	0.40 ± 0.24	6.00 ± 0.24
133.47	1.20 ± 0.20	1.40 ± 0.24	0.80 ± 0.20	0.8 ± 0.37	0.60 ± 0.40	0.80 ± 0.20	0.64 ± 0.24	1.20 ± 0.20	7.60 ± 1.12
266.95	0.80 ± 0.10	1.60 ± 0.24	1.20 ± 0.20	0.8 ± 0.20	0.60 ± 0.24	0.40 ± 0.24	1.60 ± 0.24	0.40 ± 0.24	7.40 ± 1.25
533.90	1.50 ± 0.24	1.60 ± 0.24	1.00 ± 0.30	1.2 ± 0.20	1.30 ± 0.23	1.40 ± 0.24	0.40 ± 0.24	0.30 ± 0.21	8.80 ± 0.80*

Mean ± SE of 5 mice in each group.
* $P < 0.05$

Table 5. Distribution of arsenic in tissues (mg/kg).

Doses	Testes	Epididymis	Seminal Vesicle	Ventral Prostate
Control	0.52 ± 0.07	2.70 ± 0.05	0.30 ± 0.05	ND
As μmol l				
53.39	0.55 ± 0.04	3.18 ± 0.60	0.39 ± 0.08	ND
133.49	1.01 ± 0.09*	3.16 ± 0.84	0.27 ± 0.09	12.03 ± 1.29*
266.95	1.08 ± 0.09*	3.75 ± 0.30*	1.49 ± 0.40*	14.32 ± 0.47*
533.90	5.26 ± 0.41*	4.70 ± 0.23*	3.95 ± 0.67*	16.8 ± 0.52*

Mean ± SE of 5 mice in each group.
* $P < 0.05$

Discussion

Arsenic treated mice survived the treatment period without any viable signs of clinical toxicity and showed no significant change in the body, testes and accessory organ weights contrary to a previous study which depicted increase in testicular mass of rats exposed to 5 ppm (66.74 μmol l) arsenite via drinking water for 6 weeks (Savabieasfahani *et al.* 1998). However, morphological studies depicted decrease in sperm count, motility and increase in abnormal sperm which may be due to adverse effect on spermatogenesis (Pant *et al.* 1997). Mammalian sperm contain large amount of thiol rich protamines in their nuclear chromatin and sulfhydryl group in the sperm flagellum which are thought to be involved in its stability and in the maintenance of motility in sperm (Working *et al.* 1985). As arsenic is known to be a thiol inhibiting substance, the decrease in motility could be ascribed to the high concentration of arsenic as observed in the present studies in the epididymis where the sperm undergo the process of maturation and acquire motility. Although the exact mechanism of toxicity is not understood it may be due to the electrophilic nature of arsenite as it binds to sulfydryl group on proteins or inhibition of enzymes by binding to a thiol containing active site (John *et al.* 1998). LDH and γGT are biochemical markers associated with specific cell types and related with germ cell maturation. The induction in the activity of LDH and γGT suggests the possible depletion of germ cells in the seminiferous tubule and indicate possibility of the interference with normal physiology of Sertoli cells (Pant *et al.* 1997).The interstitial cells are the principal site of steroid biosynthesis and contain steroidogenic enzyme 17 β HSD, which catalyses the interconversion of androstendione to testosterone. A similar alteration in the activity has been reported in ovary of rats exposed to 0.4 ppm sodium arsenite (5.33 μmol/l) for 16 and 28 days (Chattopadhyay *et al.* 1999). The inhibition in the activity may result in low levels of plasma gonadotropin as both plasma FSH and LH are responsible for regulating the activity of 17 β HSD (Srivastava & Srivastava 1991). An increase in the

activity of LDH and γGT has been also observed on the exposure to styrene, sodium selenite, acrylonitrile and phthalate (Srivastava *et al*. 1990). The metalloid accumulation in tissues might be the result of arsenic binding to the tissues or greater cellular uptake. This is in accordance with the presence of radioactive arsenic in the epididymis indicating risk of decreasing sperm viability and of impaired reproduction (Danielsson *et al.* 1984). No effect were observed on indices studied for reproductive effects at 53.39 μmol l arsenic close to which human beings are exposed to drinking water. However, at 533.90 μmol l vulnerable effects on biochemical and morphological indices, considered to be related with specific events of reproduction, were observed under the present set of experimental conditions. Further studies by exposing animals for a longer duration at low doses (53.39 μmol l) of arsenic may deduce the possible reproductive effects in actually prevailing conditions.

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