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Transplacental and Lactational Exposure of Mice to Arsenic: Effect on Body and Organ Weights with Special Reference to Male Reproductive Organs

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Abstract: Sodium arsenite was orally administered to mice during pregnancy and lactation at a dose level of 0.4 ppm and body weights and organ weights with special focus to reproductive organs in next generation adult male mice were analyzed. The body weight and weight gain of control and experimental pups did not differ significantly. However, the weights of testes, prostate and seminal vesicle decreased in experimental mice when compared with controls. Histology of testes indicated decrease in primary and secondary spermatocytes and spermatids in experimental mice when compared with control. These results indicated that exposure to arsenic during early stages of development suppresses the development of male reproductive organs in adults. Thus, it was conclude that the potential of reproduction is programmed, to some extent, in the early stages of development and hence any toxic insult during embryonic development and lactation suppresses male reproductive potential in adulthood.

Key words: Sodium Arsenite % Sperm Analysis % Male Reproduction % Testes % Histology % Gestation and Lactation

INTRODUCTION

Arsenic, a nonessential trace element and a potent toxic metalloid has drawn increasing attention in recent years as a major pollutant of drinking water. Higher levels of inorganic arsenic occurs naturally in ground water of many parts of the world including India and millions of people are exposed worldwide to the drinking water containing this known carcinogen in excess amount [1-4]. Epidemiological data indicated that more than six million people residing in different areas of West Bengal, India are exposed to arsenic contaminated drinking water and more than 300,000 people were reported with signs of arsenic toxicity [5].

Arsenic exposure has been associated with an increased risk of dermatitis along with hyperkeratosis, gangrene and tumors of skin, bladder, liver, kidney, lung, prostate and other tissues [6-11]. Epidemiological reports from Ukraine, Taiwan and Bangladesh revealed that the intake of arsenic contaminated food and water caused reproductive disturbances in women [12], adverse pregnancy outcomes [13] and also spontaneous abortions [14]. Arsenic has been suspected to be the cause for reproductive failure in male workers at a copper smelter in Sweden [15]. Long term exposure of arsenic is associated with abortion, low birth weight and reduced lactation [16].

There is a lack of literature and data related to the exposure to arsenic during prenatal and neonatal period on reproduction in adults, particularly at the dose levels occurring in drinking water in wide areas of India and in other countries where this element is present in the range above the admissible limit (0.01 ppm according to the World Health Organization) [17]. The present study was conducted to assess the effect of exposure to 0.4 ppm of sodium arsenite through drinking water during embryonic development and lactation on reproduction of adult male mice (F1 generation).

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MATERIALS AND METHODS

Animals: Swiss Albino mice were bred at Department of Biotechnology, S.V. University, Tirupati. Animals were maintained in polypropylene cages lined with paddy husk under a well regulated light and dark (12h:12h) schedule at 23 ±1°C. Animals were given food and water *ad libitum.* The mice feed was purchased from Kamadhenu Agencies, Bangalore, India. Healthy mice of 90 days age were selected for present study.

Experimental Design: Pregnant mice were divided randomly into two groups consisting of ten animals in each group. The animals in group 1 were allowed *ad libitum* access to tap water without sodium arsenite while the animals in group 2 were allowed *ad libitum* access to tap water containing 0.4 ppm of sodium arsenite during gestation and lactation periods. Sodium arsenite purchased from S.D. fine chemicals (Mumbai, India) was used as a test chemical. The mice were allowed to deliver the pups and the pups, after weaning, were grown on normal diet and tap water (with out sodium arsenite) up to 60 days and used for experimentation. All animal procedures were approved by the Institutional Animal Ethics Committee at S.V. University.

Body Weight and Organ Weights: The body weights of pups were recorded immediately after the delivery (day 1 of post natal) and at the time of termination of the experiment (post natal day 60). The mice were sacrificed by cervical dislocation. The organs (liver, kidney, testis, seminal vesicle, epididymis and prostate gland) were quickly excised, weighed immediately and tissue somatic index was calculated. The testes were used for histological studies.

Histology of the Testis: The mice were autopsied and the testes were dissected out. The testicular tissue was fixed in aqueous Bouin's fluid for 24 hours and dehydrated in alcoholic series, cleared in xylol and embedded in paraffin wax. Sections of 6 **9**m thickness were cut and stained in Harries haematoxylin-eosin and examined under the microscope.

Statistical Analysis of the Data: The data were presented as mean ± SEM. Statistical analysis was performed using analysis of variance (ANOVA) followed by Dunnett's test, using SPSS 10.0 version.

RESULTS

No mortalities were observed in control or in experimental groups. No behavioral abnormalities were observed in experimental mice. The body weights of experimental pups were not significantly different when compared with the control pups. The body weight gain of experimental mice was not significantly altered when compared with the control mice (Table 1). Kidney and liver indices of mice exposed to 0.4 ppm sodium arsenite during early stages of development were not significantly altered when compared with the controls (Table 1). The weight of the testes, seminal vesicle, epididymis and ventral prostate of experimental mice were significantly decreased when compared with those of control mice (Table 1).

Histological observations of the testis in control mice, reveals that it consists of seminiferous tubules and inter tubular elements (Fig. 1). The seminiferous tubules showed normal spermatogenesis with all cell types and well developed interstitial cells. Each seminiferous tubule consists of the tubular wall with outer most basement membrane. Resting on the basement membrane are the spermatogonia and the sertoli cells. Towards, the lumen the primary spermatocytes, secondary spermatocytes and spermatids adhere to the sertoli cells. Sperms are seen with heads embedded in the sertoli cells and the tails lying in the lumen.

Table 1: Effect of gestational and lactational exposure to sodium arsenite on body and organ weight in adult mice

Parameters	Control	Arsenite	T- test
Body weight (g) on 60 PND	36.21±4.45	33.43^{ns}±3.39 (-7.68)	t=1.406 p=0.1817
Liver	4.84±0.77	4.91±0.65 (1.45)	$F_{1,10}$ =1.403 P=0.3596
Kidney	1.75±0.14	1.69±0.11(-3.43)	$F_{1,10}$ =1.620 P=0.3047
Testes	0.75±0.07	0.43±0.03 (-42.67)	$F_{1,10}$ = 5.444 P<0.05
Seminal Vesicle	0.93±0.06	0.54±0.08 (-41.94)	$F_{1,10}$ =1.778 P=2715
Epididymis	1.22±0.18	0.77±0.09 (-36.89)	$F_{1,10}$ = 4.00 P=0.0772
Ventral Prostrate	0.18±0.02	0.05±0.006 (-72.22)	F_{10} =11.111 P<0.01

Values are Mean ± SEM of 8 animals

Values in parentheses are % change from the control

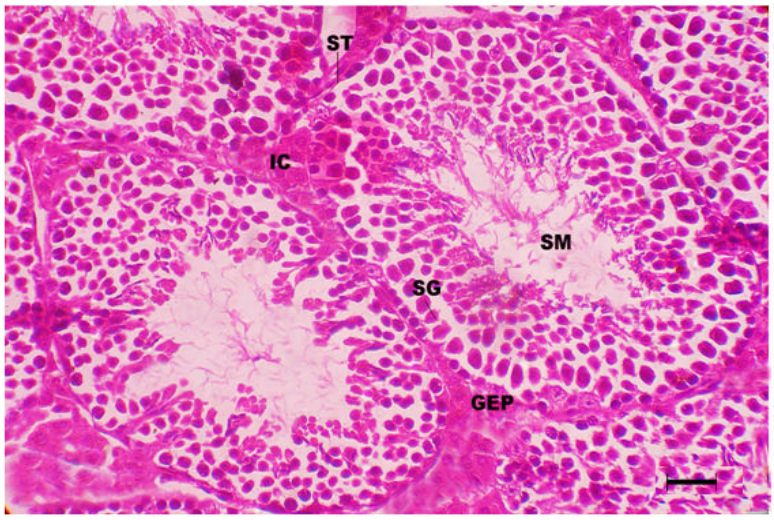

Fig. 1: Transverse section of the testis of the control mice Scale bar = 40µm

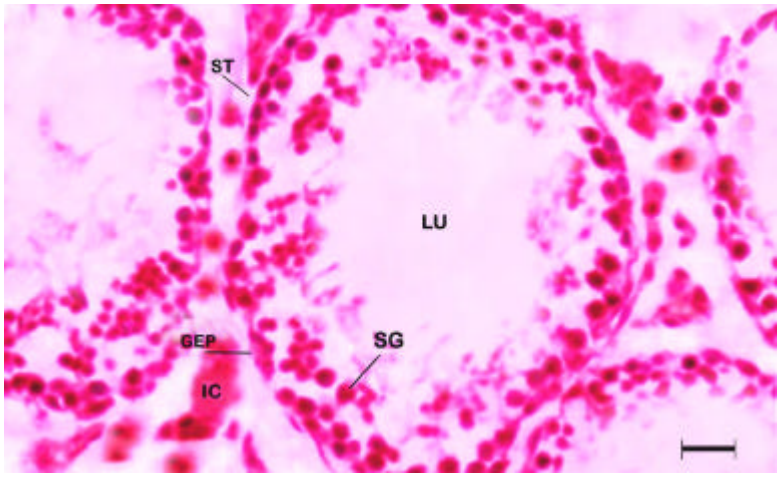

Fig. 2: Transverse section of the testis of mice exposed to 0.4 ppm sodium arsenite during embryonic development and lactation Scale bar = 30µm
SM=Sperm; LU=Lumen; ST=Seminiferous tubules; EP=Epithelium; IN=Inter tubular tissue

Transverse section of the testis of the mice exposed to sodium arsenite during embryonic development and lactation showed significant decrease in spermatogenesis. The seminiferous tubules are disorganized. The germinal epithelium, spermatogonia, spermatocytes and spermatids are severely disrupted. The lumen was empty of active sperms (Fig. 2).

DISCUSSION

The present study aimed to determine the reproductive toxic effects of mice exposed to arsenic during embryonic development and lactation. The route chosen in this study for exposure was via drinking water through mothers to mimic human exposure and to reflect the impact on fertility of next generation. In the present study reproductive potential of male mice was measured using tissue indices and changes in architecture of testes as biological parameters.

The arsenic dose selected in the present study was not resulted in any toxic symptoms in mice. No mortality and no behavioral abnormalities were recorded in experimental mice indicating the arsenic do not exhibit any toxicity at the selected dose level. Results of the present investigation demonstrated the adverse effect of sodium arsenite on reproductive organ growth of adult mice exposed to arsenic during early stages of development. The weight of testes largely depends on the mass of differentiated spermatogenic cells and it has been used as an index of spermatogenesis [18]. A strong and positive

correlation also was demonstrated between weight of the testes and number of germ cells [18]. The decreased reproductive organ weights observed in experimental mice might be responsible for decreasing male fertility. The reduction of testes weight has been shown to occur due to loss of germ cells [19]. Degenerative changes were reported in the testicular tissue of mice treated with arsenic [20]. Similar results were reported in fish [21]. Decrease in testicular mass in arsenic treated animals was consistent with elimination of germ cells [22]. The weight of the accessory sex organs depends mainly on the presence of circulatory androgens as castration causes reduction of reproductive organ weights. Since body weight gain was not altered significantly in experimental mice in comparison to the controls, this deleterious effect of arsenic on the male reproductive system may be due to the toxic effect of arsenic itself on this specific system and not to the bad health of the mice.

Significant decrease in sperm count and sperm motility was also observed in mice exposed to arsenic during embryonic development and lactation in our further studies. Histology of reproductive tissues is used as a valuable tool to measure male reproductive toxicity. Histological evaluations can be especially useful because they provide information related to architecture of testis [22]. A positive relationship exists between the seminiferous tubular diameter and the spermatogenic activity of the testes [8]. Proliferative preneoplastic lesions in testes have been reported in mice exposed to arsenic for 20 days [23] resulting in suppression of spermatogenesis.

No significant changes were observed in food and water intake levels in As-exposed animals; indicates that they were not influenced by As. There were no alterations in body weight and organ indices of liver, kidney, seminal vesicles and vasdeferens in As-exposed animals; suggests they were independent of Arsenite treatment.

The decrease in indices of testes, ventral prostrate and epididymides The seminiferous tubules are disorganized. The germinal epithelium, spermatogonia, spermatocytes and spermatids are severely disrupted. The lumen was empty in Arsenite-exposed mice indicates Arsenite-induced reproductive toxicity.

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