J. Ecophysiol. Occup. Hlth. 11 (2011) 33-38

Effects of ground water arsenic on the testes of albino rats

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Abstract : *Arsenic may cause variation in tissues which affect histopathology of reproductive organs. The aim was to investigate the morphological and anatomical changes in structure of testis. In the present study, twenty male albino rats divided into four groups of five rats each. Distilled water and arsenic water were administrated orally to control and experimental groups for 7, 14, and 21 days respectively. The body weight, organ weight, sperm count, sperm motility and sperm sluggish motility were decreased significantly compared with control. The histopathological observations of testes after 7, 14 and 21 days of ground water arsenic treatment of rat showed some predominant change in the structure of testis. There are atrophic changes in testis due to degenerative changes in spermatogenic and leydig cells. The above results confirm the toxic effect of arsenic in testis of albino rats.*

Key Words*:* Arsenic, Organ weight, Sperm count, Sperm motility and Sperm sluggish

Introduction

Arsenic a common environmental contaminant, of the various sources of arsenic in the environment drinking water causes the greatest threat to human health. Arsenic occurs in ground water in the form of arsenite, arsenate, methyl arsenic acid and dimethyl arsenic acid. Arsenite and arsenate compounds are highly toxic to human beings as well as animals. Arsenic exposure causes both acute and chronic toxicity in human. Human arsenic exposure is related to severe health problems such as skin cancer, diabetes, liver, kidney and CNS disorders (Neiger and Osweiler, 1985). It also causes many other toxic effects (Jolliffe *et al.*, 1991). Effect of male reproduction of arsenic was first studied in mice, then in fishes (WHO, 1981). The testes are composed of seminiferous tubules and the interstitial cells of leydig which are present in the angular spaces between the tubules. The interstitial cells of the testes produce the male hormones known as androgens. The testosterone is the most important androgen and is regulated by FSH. Testosterone promotes the production of functional sperms and is responsible for secondary sexual characters (Raji *et al.*, 2003).Arsenic exposure in experimental rats has shown to produce steroidogenic dysfunction leading to impairment of spermatogenesis. Few recent investigations have shown that arsenic in drinking water is associated with oxidative stress (Pershagen, 1983), genotoxicity in testicular tissue of mice (Shukla and, Pandey, 1984). On the other hand recent study suggests that arsenic causes testicular toxicity probably by affecting the pituitary testicular axis (Chaudary *et al.*, 2010; Geierhaas, 1991). The present study was designed to evaluate the effect of ground water arsenic on male reproductive organ in albino rats

Materials and Methods

Twenty male albino rats (*Rattus norvegicus*) of wistar strain weighing 120±25 gm and eight weeks old were randomly divided into four

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groups of five rats each. Each group was kept in a separate polypropylene cage, and maintained in controlled temperature (25±2^{0}C), humidity (65±10 %) and proper circadian rhythm. The animals were acclimatized for 20 days before starting the experiment. During this period animals had free access to normal diet and the water given *ad libitum*.

The arsenic water was collected from Sikandra area Agra region from water sources like hand pumps in polypropylene bottles. The concentration of arsenic in drinking water was found to be 0.102 mg/L.The concentration of arsenic in water sample was measured by the method of Aggett and Aspell (1976).

Rats of group A were treated as control group and were given distilled water while rest three groups B, C, and D were treated with ground water arsenic (10.2 mg/L)of above area daily for 7,14, and 21 days respectively.

Body weight was measured before and after the experimental period. At the end of each experimental period, the animals were scarified and testes were dissected out and weighed individually.

Epididymis sperm was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility. Epididymal sperm counts were made using the haemocytometer and were expressed as million/ml of suspension. The sperm viability was determined using Eosin stain (Raji *et al.*, 2003).

The testes were fixed in Bouin's fixative, embedded in paraffin and 5 mì thick sections were stained with routine hematoxylin-eosin. Histopathological changes in the testes were examined under optical microscope.

The data were expressed as Mean±SEM and were evaluated for statistical significance with the student "t" test.

Results and Discussion

The body weight, testicular weight, sperm count, sperm motility and sperm sluggish motile decreased significantly after ground water treatment arsenic group in the treated groups over a period of 7, 14 and 21 days in comparison to the control groups (Table 1).

The microscopic observations of testes *in placebo* control group rats show many lobules with typical circular shape in testes. Each lobule contains one or more highly convoluted seminiferous tubules, within a lobule, the space between adjacent seminiferous tubules was filled by very loose connective tissue which contained blood vessels. Lying in this connective tissue there were groups of leydig cells (Fig. 1).The histpathological observations of testes after 7, 14 and 21 days of ground water arsenic treatment of rat showed some predominant changes in seminiferous tubules. It is shrinken and had a wavy outline, apical degeneration and confluence of tubule and some time lumen was obliterated. Most of the tubule contains spermatogonia and spermatocyte which were large in size and contain nucleus. The nuclear membranes of some cells were ruptured (Fig. 2). The structure of seminiferous tubules, blood vessel, sertoli cell, spermatids, myoid cell and tunica albugenia were irregular in shape and position. Spermatids were less in number and shape. The tunica albugenia was thickened and blood vessel were sparsed and collapsed (Fig.3). The structure of seminiferous tubules, blood vessels, sertoli cells, spermatids, myoid cells and tunica albugenia were irregular in shape and position. Spermatids were less in number. The sperms also reduced in number in lumen. In some tubules, the lumen was completely obliterated. The tunica albugenia thickened and blood vessels were sparsed and collapsed and majority of seminiferous tubules were shrinken and irregular in shape. The nuclear membrane of spermatogoinia ruptured and was

Table 1. Effects of ground water arsenic on the testes of albino rats

Treatment time (in days)	Biological parameters									
	Body weight		Organ weight		Sperm count		Sperm motility		Sperm sluggish motility	
	Control Mean ± S.Em	Treated Mean S.Em	Control Mean ± S.Em	Treated Mean ± S.Em	Control Mean ± S.Em	Treated Mean S.Em	Control Mean ± S.Em	Treated Mean S.Em	Control Mean ± S.Em	Treated Mean ± S.Em
7	140.1± 1.702	132± 1.503*	1.848±0.026	1.658± 0.022*	52.4±3.826	42.0± 1.470*	43±1.789	32.0± 1.468**	22±1.414	18.0± 1.570*
14	140.0±1 702	131± 1..887**	1.848±0.026	1.476± 0.017*	52.4±3.826	30.2± 1.105**	43±1.789	24.8± 1.115***	22±1.414	11.8± 1.305**
21	140.0±1 702	130± 1.8166**	1.848±0.026	1.332± 0.0258**	52.4±3.826	22.6± 1.356***	43±1.789	18.0± 1.306***	22±1.414	5.2± 1.256***

N = 5

No. of Observations-10 [NS- non significant (p>0.05)*- Significant (p<0.05), **-Highly Significant (p<0.01),***-Very Highly Significant (p<0.001)]

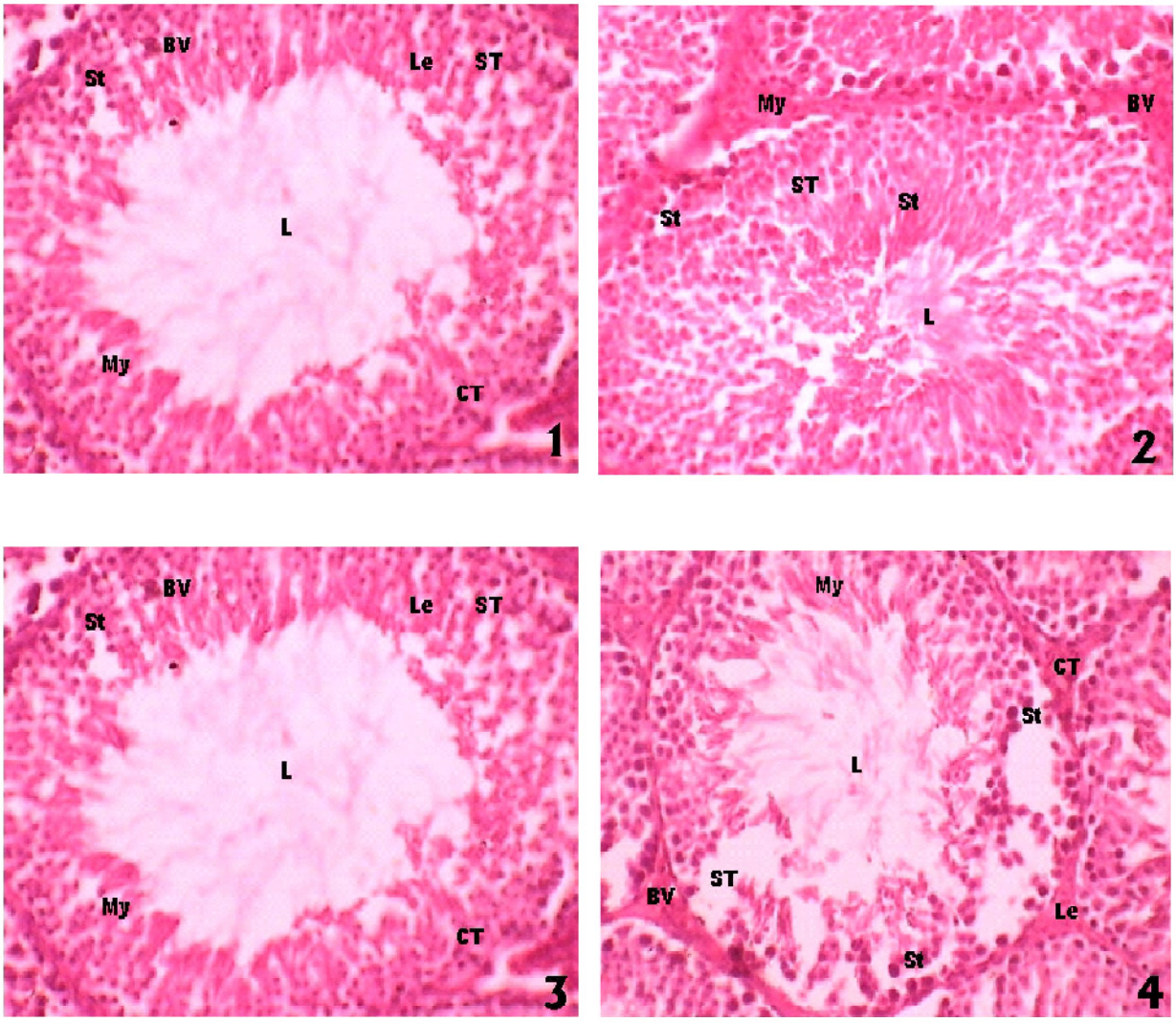

Fig. 1. T.S. of testes of rat of control group
Fig. 2. T.S. of testes of rat after 7 days of ground water arsenic treatment
Fig. 3. T.S. of testes of rat after 14 days of ground water arsenic treatment
Fig. 4. T.S. of testes of rat after 21 days of ground water arsenic treatment
Seminiferous tubule (ST), blood vessel (BV), lumen of seminiferous tubule (L), leydig cell (Le), Myoid cell (My), spermatids (St), spermatogonia and connective tissue (CT). H/E x 400

accompanied by fragmentation of nucleus. The interstitial cells of leydig also reduced in number and their nuclei were decreased in size (Fig. 4).

Arsenic is considered as a toxic metal, and this reflects on human health. Various workers have observed systemic disorders (Rossman, 2003; Majumdar *et al.,* 1998), but male reproductive study in relation to arsenic toxicity is scanty. Earlier studies indicated that heavy metals like lead, mercury and chromium causes cytotoxic effect in the male reproductive function (Jana *et al.,* 2006).

Arsenic exposure to Albino rats, in the present study, gradually decreased the testicular weight compared to control suggesting cellular regression of the testicular tissue. The

observations are in collaboration with the earlier findings of Pant *et al.* (2004).

The sperm count, sperm motility and sperm sluggish motility decreased significantly due to side effect of ground water arsenic and similar findings have been given by Mehranzani and Hemadi (2007). They have studied the effect of sodium arsenite on corpus caverlose and have reported decreased testosterone by decreasing gonadotropin releasing hormone which in turn stimulated the production of leutenizing hormones and FSH.

In the present study histopathological changes increased in the testis with an increase in time duration of arsenic. This activity may be attributed due to imbalance of sex hormones, reduction in testosterone level that suppresses spermatogenesis and also inhibits testicular enzymes. It may also be due to reactive oxygen species produced by arsenic intoxication in the cell which damages intracellular compound (Soucy, *et al.,* 2003). Testicular histology exhibited severe cellular damage in spermatogenic cell. Moreover, the appearance of eosinophilic multinucleated giant cell in the seminiferous tubule with higher treated group indicated cellular degeneration(Sarkar *et al.,* 1991; 1992). A significant gradual dose dependent regression was observed in the number of resting spermatocyte, pachytene and round spermatid after 0.102 mg/L ground water arsenic treatment for over a period of 21 days, whereas there was no significant decrease in the number of spermatogonia. These findings act as an indicator that the maturation of spermatogonia through the process of meiosis has been severely distrupted following arsenic exposure. The above observation is in agreement with the recent finding of Omura *et al.,* (2000). Degeneration of interstitial (Leydig) cells was observed in the testis of arsenic-treated mice. Moreover Leydig cell population significantly decreases in both the doses over a period of 21 days (Sarkar *et al.,* 2003). The Leydig cell nuclear diameter increased significantly in both the doses in 7 days followed by gradual diminution of the Leydig cell diameter in 14 and 21 days (Squibb and Fowler 1983). Inspite of a testosterone assay, it may be suggested that the degeneration of Leydig cell with significant decrease in the Leydig cell population probably would have resulted in decreased synthesis of testosterone, which in-turn disturbs the process of spermatogenesis. It has already been established that Leydig cell plays an important role in the structural and functional integrity of seminiferous tubules and synthesis of testosterone, which is one of the main component of regulation the post meiotic stage of spermatogenesis (Sharpe *et al.,* 1990). The exogenous arsenic exposure may cause a chemical stress on the cellular function. The initial increase in Leydig cell diameter may be a better indication to adopt the metal induced stress but due to continuous stress effect, cellular exhaust may be a result of Leydig cell atrophy (Tamaki and Frankenberger, 1992). Therefore, in conclusion, the present study revealed that arsenic induced toxicity might be responsible for testicular regression in albino rats.

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