WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

Volume 2, Issue 5, 1524-1533. Research Article ISSN 2277 – 7105

EFFECT OF ARSENIC EXPOSURE ON TESTOSTERONE LEVEL AND SPERMATOGONIA OF MICE

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Article Received on
05 June 2013,

Revised on 10 July 2013,
Accepted on 02 August 2013

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ABSTRACT

Exposure to different heavy metals causes irreversible toxic insult to male reproductive system. Heavy metals produce cellular impairments at structural and functional level in male reproductive system. Environmental toxins and radiation are suspected to be responsible in part for the deterioration of semen quality observed worldwide during the recent few decades. Arsenical exposure through drinking water is common in many areas of the world and major threat for human. Present study aims to illustrate effect of arsenic on testosterone level and spermatogonia of mice. Arsenic 3 mg/kg b.w and 4 mg/kg b.w daily administered by gavage method for eight weeks. Mice were sacrificed after completion of schedule, blood were collected for testosterone estimation and tissues were fixed for Transmission Electron Microscopic Study. It is observed that arsenic causes marked decline in testosterone level of mice. It was declined gradually with increased duration of arsenic exposure. In higher dose group it was more declined. Mitochondria, nucleus, nuclear membrane and nucleopore complex of spermatogonia were more degenerated increase duration of arsenic exposure. Thus it is concluded that arsenic exposure caused decrease in testosterone level and degeneration of spermatogonia, which finally leads to infertility in male mice.

Key words: testosterone, spermatogonia, arsenic, nucleopore complex, infertility.

1. INTRODUCTION

The rapid industrialization and overgrowing urbanization, the toxic effects of heavy metals on male reproduction system have become a major health concern in the globe for last few

decades. Reproductive hazards from metal exposure in males are one of the fastest growing areas of concern in toxicology today. Exposure to different heavy metals causes irreversible toxic insult to male reproductive system. Heavy metals produce cellular impairments at structural and functional level in male reproductive system. The effect of heavy metals, such as lead, mercury, cadmium and chromium on male reproduction has been studied in details in various experimental species.

Environmental toxins and radiation are suspected to be responsible in part for the deterioration of semen quality observed worldwide during the recent few decades [1]. Exposure to arsenicals, which is used as herbicides, fungicides and rodenticides may cause soil, air and water pollution [2] and might be a factor considering the hormonal disruption that occurs with its use. Arsenical exposure through drinking water is common in many areas of the world [3]. Metabolic disorders, hypertrophy of adrenal glands [4] and anemia [5], inhibition of the activity of testicular steroidogenic enzymes [6] and reduction in the weight of the testis and accessory sex organs [7] are associated with exposure to arsenicals.

Present study aims to illustrate effect of arsenic exposure on testosterone level and spermatogonia of mice.

2. MATERIALS AND METHODS

2.1 Animals: The mice were reared in our laboratory. The age group of mice selected for the study was 12 weeks old with 30±2 gm. b.w.

2.2 Chemicals: Arsenic trioxide, manufactured by merk India Pvt. Ltd., Mumbai was utilized for the experiment.

2.3 Study groups & sampling: The control group of 10 mice received distilled water as drinking water. The ‘treatment’ groups (n=10) received arsenic 3 mg/kg b.w and 4 mg/kg b.w daily by gavage method for eight weeks. Mice were sacrificed after completion of schedule, blood were collected for testosterone estimation. The testes from all the animals were removed and washed three times in isotonic saline (0.85 v/w%) and fixed in 2.5 % gluteraldehyde for transmission electron microscopy.

2.4 Estimation of serum Testosterone level in mice: ELISA method

Blood sample were collected after each sacrifice and there serum were isolated. Using the ELISA method Testosterone kit of LILAC Medicare (P) Ltd., Mumbai was utilized for the experiment.

2.5 Method: The normal range was calibrated and then 25 µl serum samples were taken in the well plates. 100 µl of enzyme conjugate was added in each well. After that, it was left for incubation at 37^0C in incubator for 1 hour. Then, the wells were washed with 300 µl distilled water for at least 3 times and blotted. Then, 100 µl TMB solution was added as substrate in each well plate and was again left for the incubation for 15 minutes for the colour. Finally, 100 µl stop solution was added in each well to stop the reaction. Reading was taken at 630nm through Merck ELISA reader in ng/ml value.

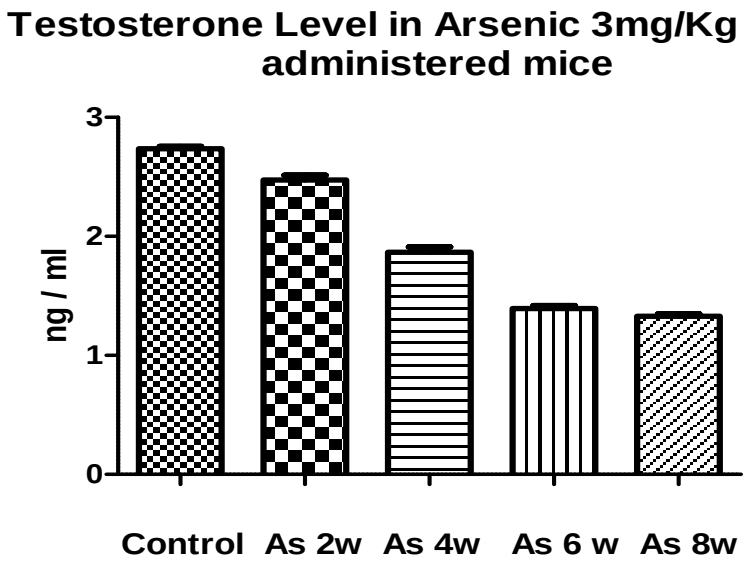
3. RESULTS AND DISCUSSION

Testosterone level in control group was 2.7 ng/ml. while in arsenic 3 mg/kg b.w administered group it was 2.5 ng/ml, 1.85 ng/ml, 1.39 ng/ml and 1.31 ng/ml after 2 weeks, 4 weeks, 6 weeks and 8 weeks (Text figure: I). It was 2.2 ng/ml, 1.6 ng/ml, 1.12 ng/ml and 1.08 ng/ml after 2 weeks, 4 weeks, 6 weeks and 8 weeks in arsenic 4 mg/kg b.w. administered group of mice(Text figure: II).

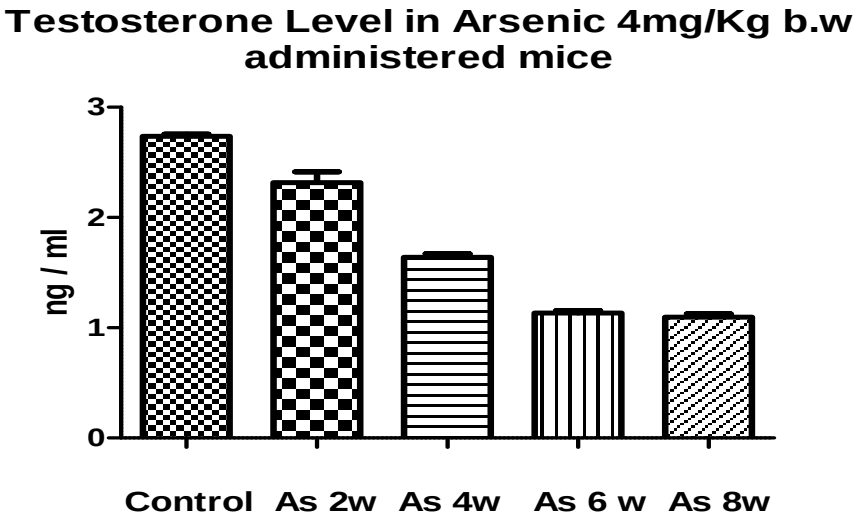
Transmission Electron Micrograph of testis showing normal nucleus and plasma membrane of spermatogonia in control mice. Chromatin material are also normal (Figure: 1). Testis of two weeks arsenic 3mg/kg b.w administered mice showing serrated nucleus of spermatogonia. Degenerated mitochondria were also observed (Figure: 2). Arsenic 3mg/kg b.w four weeks administered mice showing weavy nuclear membrane of spermatogonia. Degenerated cytoplasm and Endoplasmic reticulum were also observed (Figure: 3). Arsenic 3mg/kg b.w eight weeks administered mice showing vacuolated nuclear membrane of spermatogonia. Heterochromatin were observed. Dilated nucleopore were also observed (Figure: 4).

Testis of two weeks arsenic 4mg/kg b.w administered mice showing dilated nucleopore in nucleus of spermatogonia. Degenerated mitochondria were also observed (Figure: 5). Arsenic 4mg/kg b.w four weeks administered mice showing vacuolisation nucleus of spermatogonia. Weavy plasmamembrane were also observed (Figure: 6). Arsenic 4mg/kg b.w eight weeks

administered mice showing degenerated spermatogonia. Degenerated spermatid were also observed with many vacuolated spaces (Figure: 7).



Text Figure: I



Text Figure: II

Setchell [8] and cram [9] have observed the infertility in men and it causes due to hormonal imbalance. Ichihara [10] has observed the effect on testosterone due to chemically caused ageing. Anomalies in sperm and hormonal imbalance (Testosterone) of Mus musculus due to

pesticide exposure were studied in detailed by Ali [11]. In present study decline level of testosterone with increasing period of dose were evident but increased dose of arsenic causes more declination in testosterone due to which seminiferous tubule become rudimentary and causes infertility in mice.

Exposure to arsenic is associated with various metabolic disorders, hypertrophy of adrenal gland[12] and anemia[13]. A number of proteins and enzyme systems containing sulfhydral group have been found to be altered by arsenic[14]. Arsenic effects mitochondrial enzymes and impairs tissue respiration, which seems to be related to the cellular toxicity[15]. Gonadal effects of arsenic were fist evaluated in mice, then in fishes[16,17,18]. Most of the available data on arsenic toxicity indicates that the main concern is with the developmental toxicity on the fetus[19]. Till to date there is little study available on the effect of arsenic on the microscopic anatomy of testis[20,21].

The population of leydig cells was decreased and they were rarely seen in group or clumps. The nucleoli in majority of these cells were absent. The sertoli cells were spared because of their resistence to obnoxious agents as is documented by lesson and lesson[22]. In present study degeneration in sub cellular structure were more in higher dose and longer duration. Chromatin material, nucleus, nucleopore comples and mitochondria of spermatogonia were more degenerated in higher doses.

Photoplates

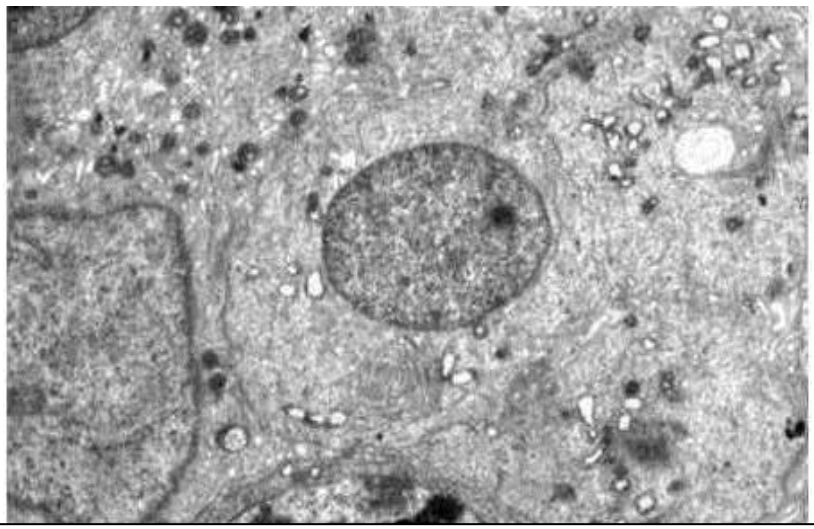


Fig: 1, Transmission Electron Micrograph of testis showing normal nucleus and plasma membrane of spermatogonia in control mice. Chromatin material are also normal.

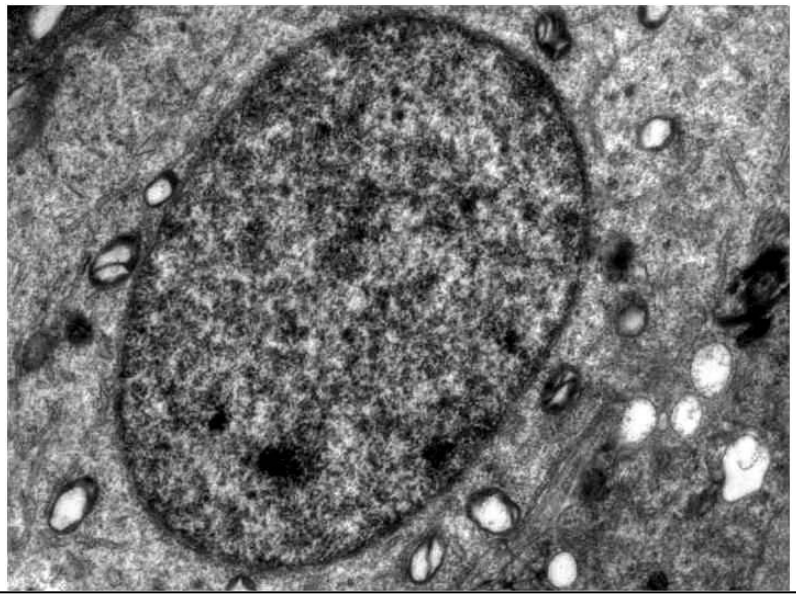


Fig: 2, Transmission Electron Micrograph of testis of two weeks arsenic 3mg/kg b.w administered mice showing serrated nucleus of spermatogonia. Degenerated mitochondria were also observed.

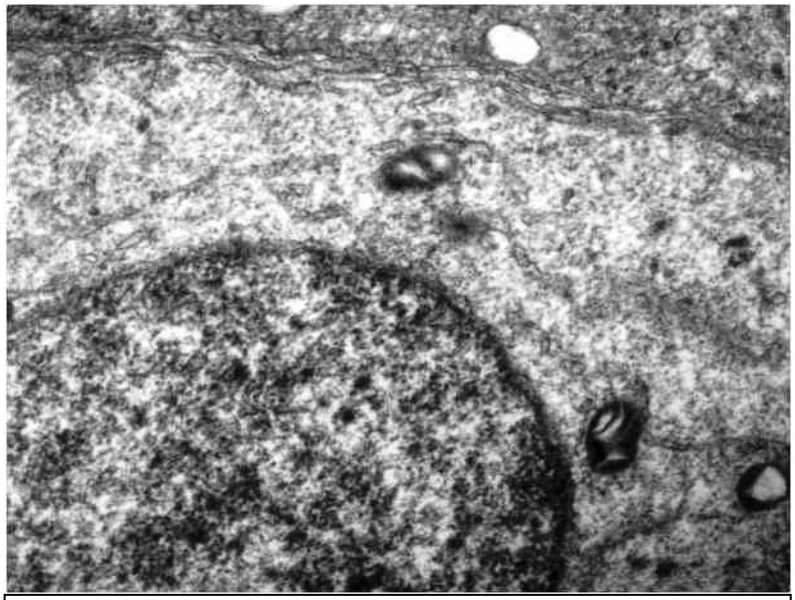


Fig: 3, Transmission Electron Micrograph of testis of four weeks arsenic 3mg/kg b.w administered mice showing weavy nuclear membrane of spermatogonia. Degenerated cytoplasm and Endoplasmic reticulum were also observed.

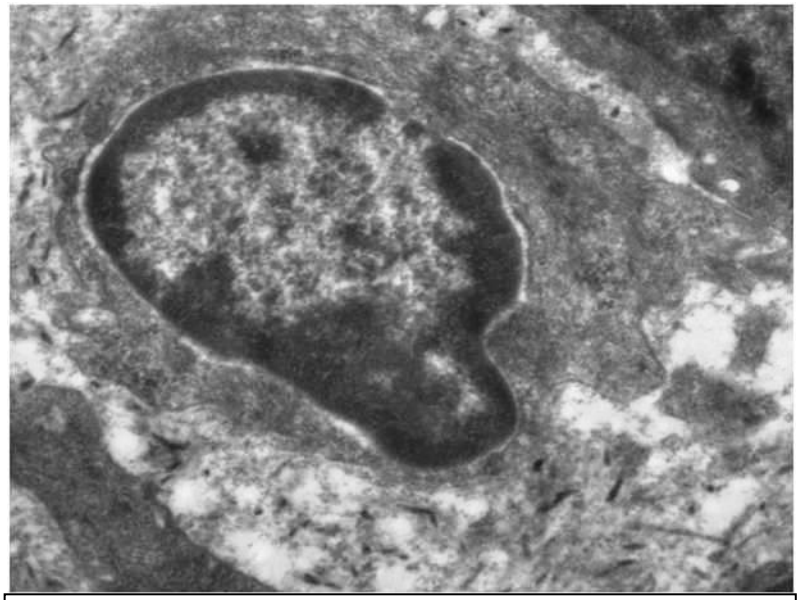


Fig: 4, Transmission Electron Micrograph of testis of eight weeks arsenic 3mg/kg b.w administered mice showing vacuolated nuclear membrane of spermatogonia. Heterochromatin were observed. Dilated nucleopore were also observed.

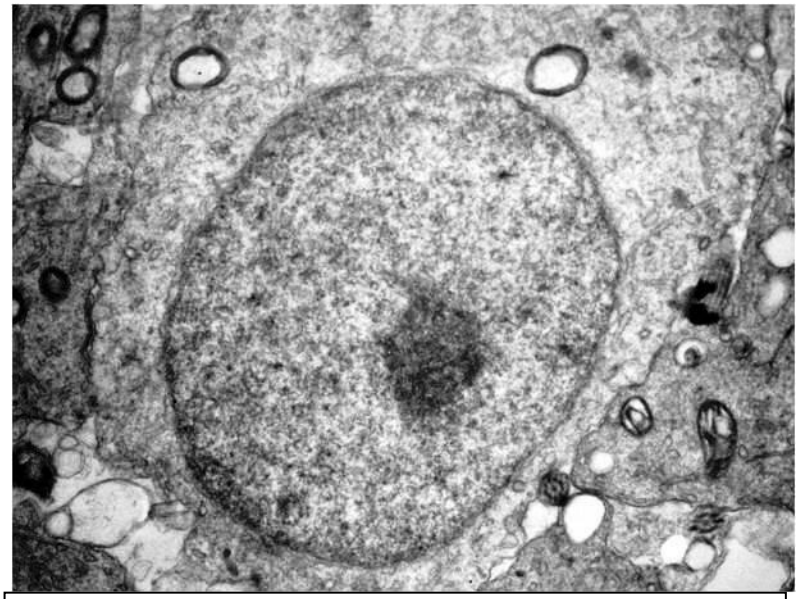


Fig: 5, Transmission Electron Micrograph of testis of two weeks arsenic 4mg/kg b.w administered mice showing dilated nucleopore in nucleus of spermatogonia. Degenerated mitochondria were also observed.

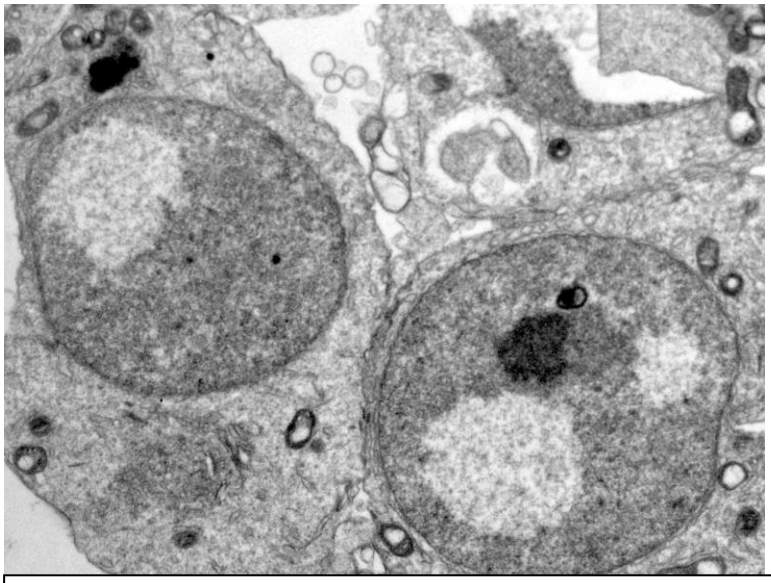


Fig: 6, Transmission Electron Micrograph of testis of four weeks arsenic 4mg/kg b.w administered mice showing vacuolisation nucleus of spermatogonia. Weavy plasma membrane were also observed.

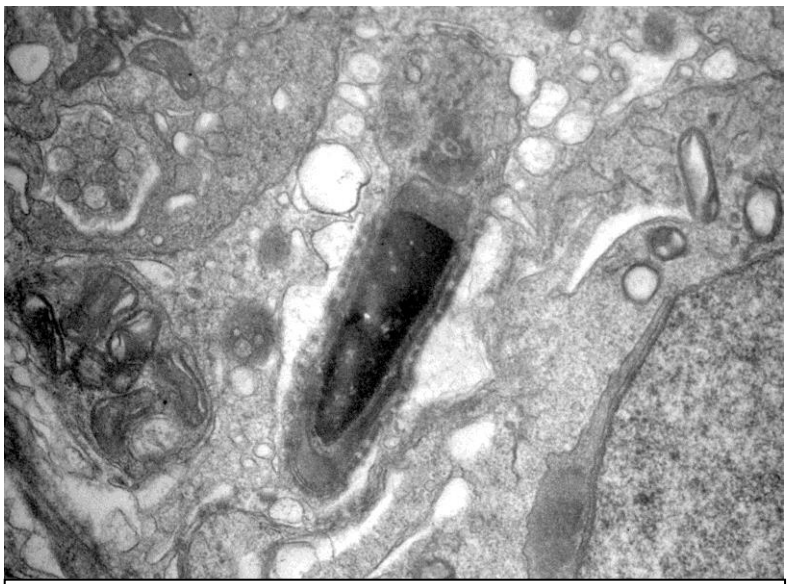


Fig: 7, Transmission Electron Micrograph of testis of eight weeks arsenic 4mg/kg b.w administered mice showing degenerated spermatogonia. Degenerated spermatid were also observed with many vacuolated spaces.

4. CONCLUSION

It is evident from entire study that arsenic causes marked decline in testosterone level of mice. It was declined gradually with increased duration of arsenic exposure. In higher dose group it was more declined. Mitochondria, nucleus, nuclear membrane and nucleopore

complex of spermatogonia were more degenerated increase duration of arsenic exposure. Thus it is concluded that arsenic exposure caused decrease in testosterone level and degeneration of spermatogonia, which finally leads to infertility in male mice.

5. ACKNOWLEDGEMENT

The authors are thankful to Mahavir Cancer Sansthan for providing infrastructural facility during this work. We are also thankful to our entire research team who provide us every support during this study.

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