

Research Article



Effect of Arsenic Toxicity in Testis of Swiss Albino Mice Leading to Low Testosterone and High MDA Level

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ABSTRACT

Arsenic exposure is a major health problem due to its toxic effect in human. Therefore, present study has been undertaken to observe the possible effects of arsenic on the testis of male mice. 2 mg/kg body weight of sodium arsenite was administered to different groups of Swiss albino mice for 2, 4, 6 and 8 weeks respectively. After each interval mice were sacrificed and histopathological study, hormonal assessment (testosterone), sperm count and MDA assessment was done. Histopathological alterations in testis of mice were observed in the present study. Sperm count and testosterone level was significantly decreased ($p < 0.0001$). Further it was observed that the level of MDA in sodium arsenite treated mice was higher than the control group. Thus, the present study reveals the toxic effect of arsenic exposure on testis of Swiss albino mice.

Keywords: Arsenic, Swiss albino mice, Testis, Testosterone.

INTRODUCTION

Arsenic is a common health concern worldwide due to its prevalence in nature and its toxic effects. It is a naturally occurring quaternary metalloid and present in more than 200 different mineral forms, which include 60% arsenate, 20% sulfides and sulfosalts and the remaining 20% are arsenites, arsenides, oxides, silicates and elemental arsenic.¹ Contamination of arsenic in the environment may be due to natural sources or by anthropogenic activities. Few studies demonstrated that coal may contain very high level (35,000 mg/kg) of arsenic.² Countries like India use coal with high arsenic contents in their power plant, polluting the environment by this toxic heavy metal.³ The International Agency for Research on Cancer (IARC) has classified arsenic and its compounds as carcinogenic to humans (Group 1), on the basis of sufficient evidence for their carcinogenicity in human.⁴ The occurrence of arsenic in drinking water has been reported from several countries which include Argentina, Bangladesh, China, India, Nepal, etc.

Exposure to arsenic is a major health problem due to its toxic effect in human and in animal model. Inorganic arsenic has been linked to develop cancer of skin, prostate, liver and lung.⁵ The non-cancer effects of arsenic include keratosis, diabetes, cardiovascular disease, pigmentation etc.⁶ Arsenic exposure to the human occurred through water, food, soil and air. Arsenic accumulation in the rice (*Oryza sativa*) has shown to have potential health risk to the high rice-consuming populations.⁷ Sodium arsenite mimics estrogen and has affinity to bind with estrogen receptor; therefore it is known as metallo-estrogen. The arsenic toxicity in animal model study is very limited.

Reactive oxygen species (ROS) and other free radicals are perpetually produced *in vivo*. Highly reactive radicals such as OH[•] tend to attack different biomolecules (including lipids), initiating free radical chain reaction.^{8,9} Lipid peroxidation (LPO) *in vivo* has severe consequences followed by some major clinical problems, cancer being one of them. LPO caused due to oxidative stress ensues eventually in cell damage or death. PUFAs (Polyunsaturated fatty acids) incorporated in lipid bilayer membrane are frequently targeted by free radicals creating lipid peroxides. PUFAs are highly susceptible to oxidation due to presence of methylene group between double bonds.¹⁰ Free radicals, primarily produced during electron transport chain in mitochondria; do activate series of chain reaction converting PUFAs into malondialdehyde (MDA). MDA is major aldehyde by-product of LPO,¹¹ which is highly mutagenic and has been denoted to form adducts with DNA bases dG, dA, and dC as m1G, m1A and m1C respectively.^{12,13}

Therefore, due to global health implications of arsenic toxicity, present study was undertaken to assess the possible effects of arsenic on the testis of male mice.

MATERIALS AND METHODS

In the present experiment, 10-12 weeks old normal male Swiss albino mice (*Mus musculus*) were selected. These mice were kept in the polypropylene cages containing paddy husk at the temperature 28 ± 1 °C, the humidity was maintained $50 \pm 5\%$ and in controlled light (12hrs light and 12hrs dark). Animals were maintained in ideal conditions as per the ethical guidelines of the CPCSEA, (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) Gov. of India and Institutional Animal Ethics Committee (IAEC).



All the mice were segregated into 5 groups, each group containing 6 mice: group I (control), group II (arsenic treatment for 2 weeks), group III (arsenic treatment for 4 weeks); group IV (arsenic treatment for 6 weeks) and group V (arsenic treatment for 8 weeks). The inorganic form of arsenic, sodium arsenite was administered to the all the mice groups at the dose of 2mg/kg body weight for 2 weeks, 4 weeks, 6 weeks and 8 weeks respectively by gavage method.

Body weight and Organ weight

Body weight was measured for each group of mice before and after the arsenic treatment. The control and treated group of mice were sacrificed as per the above mentioned treatment plan, [After each consecutive weeks gapping,] testes were dissected out and weighed.

Sperm Count

The cauda epididymis was dissected out and washed with normal saline. It was punctured at several places with 1ml distilled water to ooze out the sperm and then sperm count was performed by using Neubauer's chamber which was observed under light microscope.

Histological Study

Testis was dissected out and fixed in Bouin's fixative. 4-5 μm thick sections were cut and stained with routine Hematoxyline and Eosin. Histological changes for each group of mice testes were observed under light microscope.

Estimation of Testosterone level by ELISA assay

Blood samples were collected from mice of each group as they were sacrificed and serum was isolated. Estimation of testosterone level in the serum samples of mice were done using the testosterone kit of LILAC Medicare (P) Ltd, Mumbai.

MDA Level Assessment

Blood samples collected from each group of mice were centrifuged at 3000 rpm for 10 minutes and serum samples were collected and stored at -80°C . MDA (Malondialdehyde) levels were estimated by using standard procedure with slight modifications.¹⁴

Statistical Analysis

The values are mean \pm S.E. P-values ≥ 0.05 were considered as significant and P-values ≥ 0.0001 were considered as highly significant. Statistical analysis was performed by using one way ANOVA.

RESULTS AND DISCUSSION

Body and Organ weight

Body weight gain was not significantly altered after arsenic administered mice group in comparison to the control. Significant reduction in testicular weight was observed in 4weeks, 6 weeks and 8 weeks sodium arsenite treated mice (Figure 1).

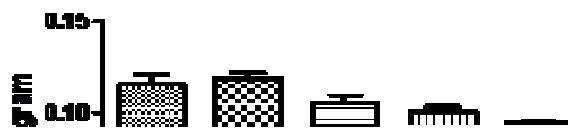


Figure 1: Testicular weight of sodium arsenite administered Swiss albino mice for the duration of 2 weeks, 4 weeks, 6 weeks and 8 weeks.

Histological Finding

The histological sections of testes of mice of control group showed the normal organization of germ cells and Leydig cell in seminiferous tubules. Normal bundles of spermatozoa (sz) are very prominent. Sperms are in normal condition and architecture of seminiferous tubules with normal functioning of spermatogenetic stages can be observed (Figure 2).

Figure 2: Photomicrograph of testis of control mice - showing normal organization of germ cells and Leydig cell inseminiferous tubule and spermatozoa (Sz) X 400.

Figure 3: Photomicrograph of testis of 2 weeks sodium arsenite administered male mice – Showing thin epithelial germ layer X 400.

2 weeks arsenic (sodium arsenite at dose of 2 mg/kg) treated testis of Swiss albino mice showing thin (Ts) and broken seminiferous epithelium (BS) and less number of Leydig cells (L). Sperm are not in normal condition. (Figure 3).

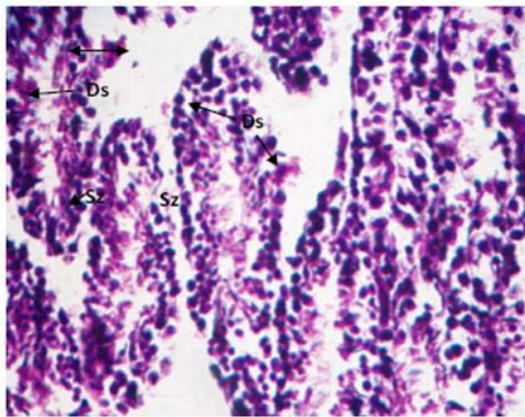


Figure 4: Photomicrograph of testis of 4 weeks sodium arsenite administered male mice – Showing thin epithelial germ layer and decreased number of spermatogonia X 400.

4 weeks arsenic (sodium arsenite at dose of 2 mg/kg b. wt.) treated testis of mice showing degeneration of seminiferous epithelium layer (Ds) and increase distance between two seminiferous tubule (). Few Leydig cells are observed and spermatozoa are in abnormal condition with decreased number of spermatogonia. (Figure 4).

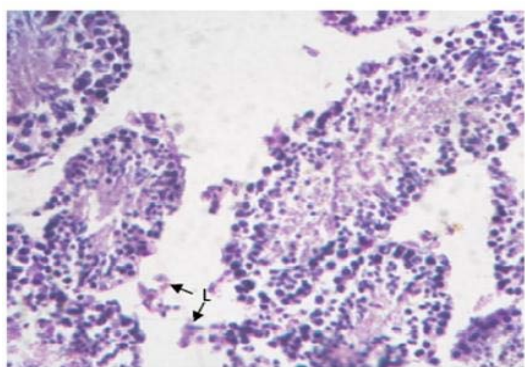


Figure 5: Photomicrograph of testis of 6 weeks sodium arsenite administered male mice – Showing damaged epithelial layer and increased interstitial spaces X 400.

6 weeks (sodium arsenite dose at 2mg/kg) treated testis shows damaged epithelial germ layer and increased distance between two seminiferous tubule. Very few number of Leydig cells are also observed. (Figure 5).

8 weeks Arsenic (sodium arsenite at dose of 2 mg/kg body weight) treated testis of mice showing damaged epithelial germ layer and few number of Leydig cells are also observed. Sperm are in abnormal condition with degeneration of tail only head are present. Degeneration of spermatogonia is observed.

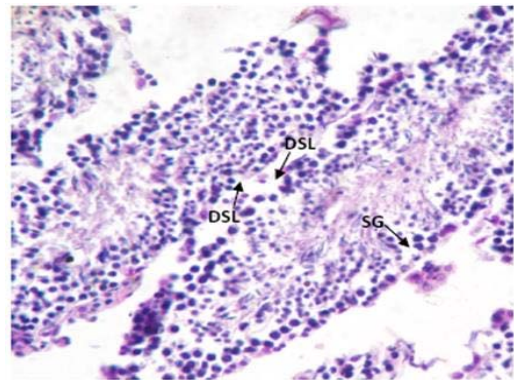


Figure 6: Photomicrograph of testis of 8 weeks sodium arsenite administered male mice – Showing damaged seminiferous epithelial layer (DSL) and degeneration of spermatogonia (SG).

Effect on Spermcount

The epididymis sperm count of mice was found decreased in sodium arsenite at dose of 2mg/kg body weight) treated experimental mice for the duration of 2 weeks, 4 weeks, 6 weeks and 8 weeks (mean value 3.24, 2.96, 2.65, 2.5 million/ml respectively) as compared to the control mice (mean value 3.49 million/ml).

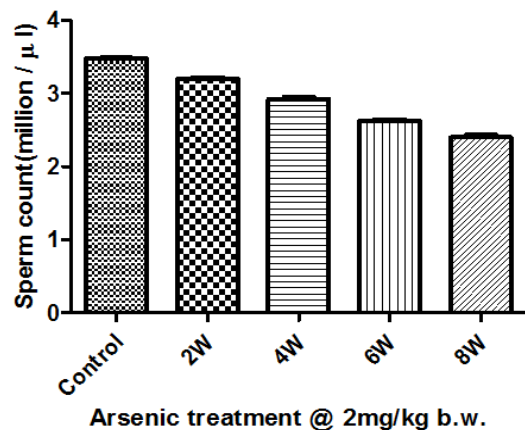


Figure 7: Sperm count of sodium arsenite treated mice for the duration of 2 weeks, 4 weeks, 6 weeks and 8 weeks. (p< 0.0001)

Effect on Testosterone

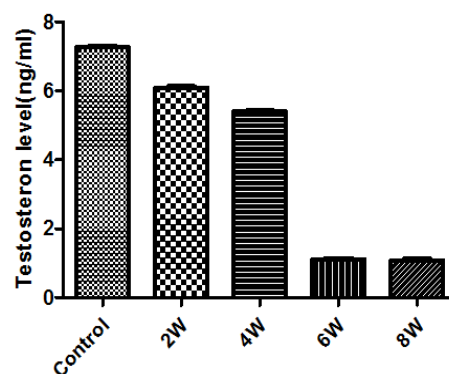


Figure 8: Testosterone level of sodium arsenite treated mice for the duration of 2 weeks, 4 weeks, 6 weeks and 8 weeks. (p< 0.0001)

Testosterone reduction was highly significant in experimental mice treated with sodium arsenite at dose of 2mg/kg of body weight (mean value 6.1 ng/ml, 5.5 ng/ml, 1.2 ng/ml and 1.2 ng/ml for 2week, 4weeks, 6 weeks and 8 weeks respectively) when compared with the results of control mice (mean value 7.4 ng/ml). The testosterone level shows a significant decline with increase in number of weeks (Figure 6).

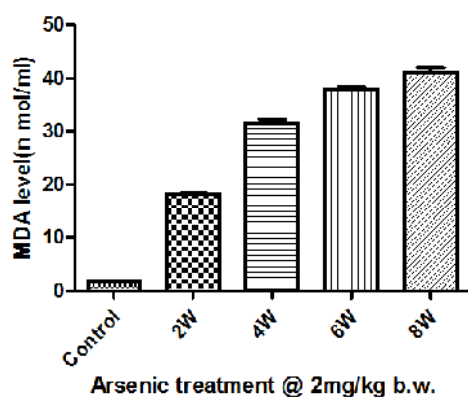


Figure 9: MDA level in sodium arsenite treated mice for the duration of 2 weeks, 4 weeks, 6 weeks and 8 weeks. ($p \leq 0.0001$)

MDA Level

Increased MDA levels in mice serum were found highly significant in sodium arsenite administered group (mean value 18.2, 31.6, 38.2 and 41.2 n mol/ml for 2 week, 4 weeks, 6 weeks and 8 weeks respectively) when compared to the control (1.7 n mol/ml).

Arsenic is a toxic heavy metal which leads to the major impairment of male reproductive functions. In the present study effect of arsenic on the male reproductive system has been observed at the definite time intervals and on particular dose. In the present investigation 2 mg/kg body weight of sodium arsenite has been associated with reduction in the weight of testis and testosterone level in mice. Further, decreased sperm count has also been observed. In the present study significant decrease in the weight of testis in mice has been found after 4, 6 and 8 weeks administration of sodium arsenite in mice. Arsenic may affect the reduction of weight of testis in mice due to the decrease in the biosynthesis of steroid in Leydig cells, spermatogenic arrest and reduced size of seminiferous tubules.¹⁵ Sodium arsenite induced tissue damage has been frequently observed in our study. Arsenic treatment has been associated with increased distance between two seminiferous tubules, degeneration of seminiferous epithelium, reduced number of Leydig cells and defective spermatozoa, leading to infertility. Fusion of two seminiferous tubules is very significant process of carcinogenesis, which is very noticeable in histopathological microphotograph in the present study. A significant, gradual dose dependent decrease of level of testosterone has been observed in the present study. Reduced number of Leydig cell was found after exposure

of arsenic in mice. Leydig cells synthesize testosterone and decrease in these cells lead to the reduced level of testosterone in the blood of male mice.¹⁶ Apart from the male reproductive disorders, the toxic effect of arsenic has also been associated with liver disorders in animal model study.^{17,21} The accumulation of these toxic arsenic has tendency to accumulate in male reproductive organs as it acts like a xenoestrogen. Some report stated that exposure to arsenic has associated with metabolic disorders, hypertrophy of adrenal glands¹⁸ and anemia¹⁹. However, a number of sulfhydryl containing proteins and enzyme systems have been found to be altered by exposure to arsenite.²⁰ Exposure of sodium arsenite to mice at 2 mg/kg b. wt. for the definite time intervals results in significant increase of MDA levels in their serum. This increased level of MDA in mice serum denotes the elevated oxidative stress induced by sodium arsenite. Oxidative stress in experimental animal due to arsenic exposure has also been reported in some studies.^{21,22}

CONCLUSION

The present study reveals the toxic effect of arsenic exposure on testis of Swiss albino mice. Thus this investigation can be very significant in studying arsenic induced testicular anomalies in human populations.

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