ejpmr, 2018,5(5), 603-607

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF ARSENIC INDUCED TOXICITY IN THE PROSTATE GLAND OF SWISS ALBINO MICE

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Article Received on 20/03/2018 **Article Revised on 11/04/2018** **Article Accepted on 02/05/2018**

ABSTRACT

Arsenic is a ubiquitous metalloid found naturally in underground water in the form of inorganic arsenic. Arsenic exposure has been associated with several diseases including cancer. Arsenic is a potent endocrine disruptor and a carcinogen. In this study, toxic effects of arsenic were examined in vivo in Swiss albino mice as animal model. The mice were selected with body weight of 28±3.0g (mean±standard deviation). Sodium arsenite was administered orally as 2.0 mg/kg body weight /ml for 04 weeks, 06 weeks, 13weeks, 26weeks. Cellular architecture was studied by histopathology. PSA (Prostate specific antigen) and accumulation of arsenic in prostate tissue of male mice was estimated by ELISA and AAS method respectively. Sodium arsenite level was observed to be significantly higher in group II than group I (P-value < 0.005). PSA levels were recorded to be significantly elevated in group II than group I (P-value< 0.028). Significant histopathological changes were also observed in prostate glands of arsenic treated mice. The present finding in mice is very significant for better understanding of endocrine disrupting potential of arsenic.

INTRODUCTION

Heavy metal is found naturally in the earth crust. Contamination of arsenic in the drinking water has adverse effect on the human health. Arsenic is a ubiquitous metalloid found naturally in water bodies like river and underground water in the form of inorganic arsenic. Among inorganic arsenic, derivatives like arenic trioxide, sodium arsenite and arsenic trichloride are the most common forms of arsenic found in environment. The possible routes of exposure to arsenic are soil, water, air, or food.[1,2]

According to a report, arsenic concentration as high as 50 ppb in drinking water of Supaul and Madhepura ditrict of Bihar was recorded, which is five times higher than the WHO limit.[3] Arsenic contamination of underground water had already been reported in 20 countries out of which major incident were from Asia.[4,5,6] Arsenic-related groundwater problems have emerged in different Asian countries, including new sites in China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan and Pakistan.[7]

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.[8] Inorganic arsenic has been suggested to develop cancer of skin, prostate, liver and lung.[9] The non-cancer effects of arsenic include keratosis, diabetes, cardiovascular disease, pigmentation etc.[10] Arsenic accumulation in the rice (*Oryza sativa*) has shown to have potential health risk to the high rice-consuming populations.[11,13] Inorganic form of arsenic are implicated to be more toxic than its organic forms (Monomethylarsenic acid and dimethyarsenic acid).[14] A very high mortality rate of prostate cancer in U.S population was reported due to exposure of arsenic.[12]

Arsenic contamination in drinking water has become serious concern and greatly impacting the human health. Hence, this study has been taken up to examine the endocrine disrupting activity of arsenic and its carcinogenic potential in prostate glands of Swiss albino mice.

MATERIALS AND METHODS

In the present study, male Swiss albino mice were chosen as an animal model to investigate the toxic effect of arsenic on prostate glands. Sodium arsenite was administered to Swiss albino mice by oral gavage method as 2.0 mg/kg body weight/day.

Animals: Swiss albino mice (Musmusculus) were reared in the animal house of Mahavir Cancer Institute and Research, Patna. 12 weeks old mice were weighed (30±2 grams) and selected for the experiment. The mice were kept in the polypropylene cages with paddy husk at room temperature 28±2ºC and humidity 50±5% in a controlledlight (12 hrs light and 12 hrs dark). Animals were maintained in ideal conditions as per the ethical guidelines of the CPCSEA, (CPCSEA

No.1840/PO/ReBi/S/5/CPCSEA) Govt. of India and Institutional Animal Ethics Committee (IAEC) of Mahavir Cancer Institute and Research Centre, Patna.

Study Groups and Sample Collection: All the mice were segregated into 5 groups, each group containing 6 mice: group A (control), group B (arsenic treatment for 4 weeks), group C (arsenic treatment for 6weeks); group D (arsenic treatment for 13 weeks) and group E (arsenic treatment for 26 weeks).

Blood was collected by orbital sinus puncture method for estimation of PSA level. Prostate tissues were collected after sacrificing of mice for estimation arsenic level and histopathological study.

Detection of Arsenic level in tissue: 0.5 gm prostate tissue sample was digested in 10 ml of HNO_3 solution at 95^0 C for 90 minutes and then allowed to cool. Atomic Absorption Spectroscopy was used to determine the concentration of arsenic in prostate tissues.

Detection of prostate specific antigen (PSA): ELISA (Enzyme Linked ImmunoSorbent Assay) was performed to analyze the level of Prostate Specific Antigen in blood serum of control and prostate cancer patients. PSA was analysed with the help of ELISA diagnostic kits from Calbioech, Austin.

Histopathological Study: Prostate organs were dissected out and fixed in 10% formalin. Tissues were dehydrated and blocks were prepared. Further, sections of 5µm thickness were cut and slides were prepared by double staining (Hematoxyline and Eosin).

Statistical Analysis: Mean, standard deviation and ANOVA were calculated and graphical representation was performed with help of GraphPad Prism 5.

RESULTS

The sodium arsenite concentrations in prostate tissue of control group and treated with sodium arsenite groups for different weeks were recorded. The sodium arsenite levels of sodium arsenite treated and control groups of mice are shown in Table - 1 and represented in the Figure- 1.

Table. 1: Mean±S.D of PSA level and sodium arsenite level in prostate tissues of control and arsenic administered Swiss albino mice for different duration.

Groups	Duration of sodium arsenite treatment	Sodium Arsenite level in prostate (ppb) Mean±SD	PSA level (ng/ml) Mean±S.D
A	Control	1.48±1.51	0.86±0.32
B	4 weeks	11.51±3.17	1.43±0.33
C	6 weeks	14.13±2.65	1.53±0.28
D	13 weeks	14.88±6.60	2.42±0.38
E	26 weeks	20.0±4.48	2.53±0.25

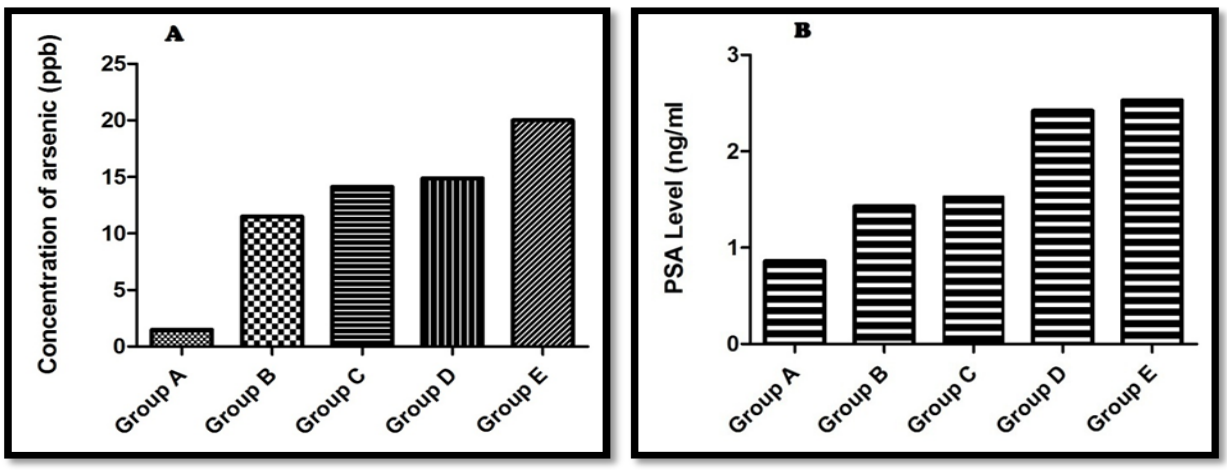
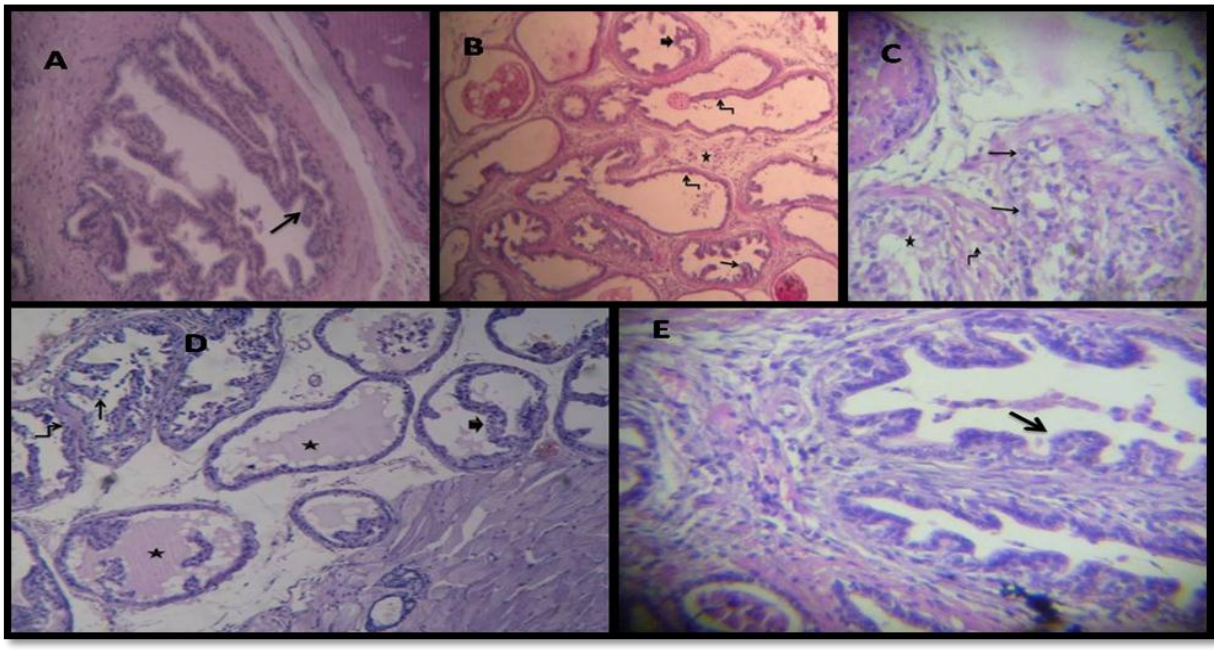


Figure. 1: Showing concentration of arsenic (ppb) in prostate tissues of control and sodium arsenite treated mice (A) and PSA level (ng/ml) in control and sodium arsenite treated mice (B).

The control group (group A) had sodium arsenite level in prostate tissue of 1.48±1.51 ppb. After treatment with sodium arsenite, the arsenic level in prostate tissue increased manifold. After treatment of 4 weeks (Group B) with sodium arsenite, arsenic concentration in prostate tissue was found to 11.51±3.17ppb. After treatment with sodium arsenite for longer duration, the arsenic level kept on increasing with duration of the treatment. After treatment of 6 weeks (Group C) with sodium arsenite, arsenic concentration in prostate tissue was found to be 14.13±2.65ppb. After treatment of 13 weeks (Group D) with sodium arsenite, arsenic concentration in prostate tissue was found to be 14.88±6.60ppb. After treatment of 26 weeks (Group E) with sodium arsenite, arsenic concentration in prostate tissue was found to be 20.0±4.48ppb. When compared to

control group the sodium arsenite levels in prostate were significantly ($p<0.001$) higher for all treated groups B, C, D and E.

After treatment with sodium arsenite, PSA levels in blood serum were found to be sequentially increasing in all the arsenic treated groups. Serum PSA level in blood serum of control (Group A) was found to be 0.86±0.32 ng/ml, after 4 weeks (Group B) 1.43±0.33 ng/ml, after 6 weeks (Group C) 1.53±0.28 ng/ml, after 13 weeks (Group D) 2.42±0.38 ng/ml, after 26 weeks (Group E) 2.53±0.25 ng/ml. PSA levels were significantly ($p<0.004$) higher in all treated groups B, C, D and E than Control (group A).



Microphotgraph: Showing histological sections of prostate tissues of control (A), 4 weeks of sodium arsenite treatment (B), 6 weeks of sodium arsenite treatment (C), 13 weeks of sodium arsenite treatment (D), and 26 weeks of sodium arsenite treatment (E).

Section of microphotograph (A) represents section of prostate of control mice (40X). Moderate to large acini are lined by cuboidal to simple columnar epithelium with flat luminal borders. Small to moderate acini is lined by simple columnar epithelial cells with sparsely located infoldings (Straight Arrow). The lining of the epithelial cells of acini is mostly surrounded by thick and dense stroma. In microphotograph B, stroma with eosinophiles can be charactized in prostate tissue (*); lining of cuboidal epithelial cells (Elbow Arrows); larger infolding (Straight Arrow) and merging of two infoldings (Arrow head) can be observed due to high mitosis (10X). Loss of glandular structure, larger cellular structure (straight arrow) and weakening of stroma (Elbow arrow) can be seen microphotograph C (X40). Prostate gland of Sodium arsenite adiministened male mice for 13 weeks shows glandular proliferation (straight arrow) and secrection in lumen (*) in microphotograph D (X10). High mitotic count (arrow head) and delineation of infolding from acini (straight arrow) can also be seen in microphotograph D (X100).

DISCUSSION

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. It was reported that low dose and long term toxicity of sodium arsenite caused induction of apoptosis, DNA broken.[15] Arsenic has been demonstrated to induce oxidative stress, apoptosis and alteration in cellular structure of prostate of male Swiss albino mice.[16] It can be reasoned that arsenic can potentially interact with several cell signal cascade leading to apoptosis or abnormal cellular structure. As the duration of dosage of sodium arsenite was increased. The level of deposition of arsenic in prostate tissue increased correspondingly (Figure 1A). It not unwise to assume that arsenic accumulated in the organ has vital role in disfiguring the structure of cells by deregulating cell signaling.[17] According to another report, it was shown that effect of exposure to sodium arsenite during male reproductive organ testosterone level increased.[18]

Arsenic is a ubiquitous metalloid and present in our environment naturally. People residing at arsenic contaminated area are more prone to developing cancer. In a report, it was documented that larger number patients belonging to Gangentic zone (arsenic contaminated area) were diagnosed with prostate cancer and had elevated level of PSA.[19] PSA is used as prognostic marker for diagnosis of prostate cancer.[20] PSA is believed to be enzymatically cleaved off from the epithelial cells of prostate organ. PSA level can be

observed to be increased as the duration of arsenic administration increases (Figure 1B). Arsenic may have role in inducing the PSA level.

As the treatment with arsenic increased, cellular architecture of prostate tissue worsened. It can be observed that clear and apparent acini around the inner surface of prostate glands in control got diminished along with the duration arsenic treatment in mice (microphotograph A to E). Glandular structures of prostate tissues were also observed to be diminished in arsenic treated mice.[21] High mitotic count can be observed in prostate tissue of mice treated with arsenic for 52 weeks (Microphotograph E).

The present study corroborates the fact that arsenic has potential role in inducing cancer suggested by data of arsenic concentration in prostate tissue, PSA level and histopathology presented. However, more data need to be acquired for landing on to a concrete conclusion.

CONCLUSION

Arsenic accumulation has been observed in many cancers. It is believed that people residing in arsenic contaminated area are more predisposed to developing cancer. Since Bihar is an arsenic contaminated region, preponderance of cancer incidences in the populace is inevitable. Arsenic is also classified as a potent carcinogen, leading to high risk of prostate cancer. The government has to take initiatives to spread awareness among people about deleterious effect of arsenic on human health.

ACKNOWLEDGEMENT

Authors would like to thank Mahavir Cancer Institute and Research Centre, Patna for providing Animal House Facilities. Authors also thank to all the scientists, doctors and technicians of Mahavir Cancer Institute and Research Centre, Patna for their co-operation and technical support. Authors extend their gratitude towards A. N. College, Patna for providing Lab infrastructure and facilities.

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