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ORIGINAL ARTICLE

Histomorphological Effects of Sodium Arsenite on Cervix of Rats: Experimental Study

AMAIDAH MIR[1], HAMMAD AHMED BUTT[2], MARIA YASMEEN[3], URFA ZARYAB MIR[4], WALEED AHMED BUTT[5], SUMAIRA ABBASI[6], TALHA LAIQUE[7*]

[1]*Department of Anatomy, CMH Kharian Medical college, Kharian-Pakistan*
[2]*Department of Pharmacology, CMH Kharian Medical College, Kharian-Pakistan*
[3]*Department of Anatomy, CPSP, Islamabad-Pakistan*
[4]*Department of Biochemistry & Molecular Biology, Army Medical College, Rawalpindi-Pakistan*
[5]*Department of Medical Education, CMH Kharian Medical college, Kharian-Pakistan*
[6]*Department of Anatomy, Federal Medical & Dental College, Islamabad-Pakistan*
[7]*Department of Pharmacology, Allama Iqbal Medical College, Lahore-Pakistan*
**All the correspondence should be made to Dr. Talha Laique, Department of Pharmacology, Allama Iqbal Medical College, Lahore-Pakistan. Email: talhalaique51@gmail.com Tel:+92-331-0346682*

ABSTRACT:

Arsenic contamination among heavy metals in ground water is a health hazard due to its toxicological concern, affecting millions of humans globally.

Purpose: To observe and analyze the histomorphological effects of sodium arsenite on cervix of rat.

Study Design: Laboratory based experimental study.

Methodology: Present study held at department of anatomy, College of Physician and Surgeon Pakistan, Regional Centre Islamabad, in two principal groups, group A (control) and group B (experimental). The animals of B were administered sodium arsenite by oral gavage daily for 14 days while group A were administered only distilled water daily for 14 days. The animals of both groups were sacrificed after day 14th of drug administration. The cervix was processed for paraffin embedding and stained with Haematoxylin and Eosin (H&E).

Statistical analysis: Data analyzed by SPSS 22.0v. Student's t-test was applied to determine the significance. *P*-value ≤ 0.05 was considered significant.

Results: The histological evaluation of experimental group animals showed decrease in cervical canal's luminal area, hyperplasia of cervical epithelium and decrease thickness of cervical muscles.

Conclusion: The nature of histomorphological effects observed in cervix showed that these changes may be due to oxidative stress produced by the formation of free radicals, decrease levels of serum estradiol, progesterone and by the denaturation of proteins.

Key Words: Arsenic, Cervix, Free Radicals, Hyperplasia and Oxidative stress.

INTRODUCTION

Arsenic contamination among heavy metals in ground water is a health hazard due to its toxicological concern, affecting millions of humans globally.[1] Arsenic is a ubiquitous naturally occurring metalloid formerly known as 'King of poisons'.[2] It was commonly used in political assassination due to its tasteless and odorless characteristic in water.[3] Recent reservoirs of water are insufficient to fulfill the demands of the growing population and of course the clean water is a big issue side by side.[4] Due to lack of awareness and unavailability of water filtration systems, these toxins and heavy metals are freely incorporating and polluting the drinking water.[5]

One of these contaminants is arsenic which has now gained a notable attention due to its increasing concentration in portable water. Word 'Arsenic' means 'bold / strong'. It is a well known naturally occurring toxic metalloid.[6] Arsenic is ubiquitously present in the earth crust and in ground water in organic and in inorganic forms.[7] Contamination of drinking water by arsenic has become a serious health issue because it has been proved that arsenic consumption even in small amounts is lethal and carcinogenic.[8] Truly, there is "no risk free or safe" limit of arsenic in food and drinking water. According to the U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO) the concentration of arsenic in drinking water should not exceed more than 10 µg/L.[9] Epidemiological data suggested that more than 65% water sources of Pakistan are contaminated by arsenic.[10,11]

Arsenic is regarded as group 1 carcinogen by International Agency for Research on Cancer (IARC) because of its toxic effects on almost all the organs and systems of the body e.g. cardiovascular, gastrointestinal, renal, neurological, hematological, hepatic, reproductive, respiratory and immunological system.[12-14]

Animal based experimental trials concluded that exposure of arsenic to the female rats caused degeneration of uterine luminal epithelial cells, stroma, endometrium and myometrium. It also affects the histology of ovaries as it decreases the number of preantral, antral, graffian follicles and corpora lutea and increases the number of regression follicles.[15-17] Animal studies showed that arsenic crosses the placental barrier even at very low doses that lead to adverse pregnancy outcomes.[18] Arsenic is also, embryotoxic, genotoxic, mutagenic andteratogenic for developing fetus as well. Similarly, a meta-analysis done in 2015 evaluated an association between exposure to arsenic and its effects on human females. This study concluded that exposure to arsenic even at low doses imposed negative effects on pregnancy and induced infant mortality.[19,20]

Over the past few decades, light has been shed on effects of arsenic on different parts of human and animal body. From the reproductive tract, uterus and ovaries of

female rat were mostly remained under concern of researches. But there was a lack of knowledge regarding the effects of arsenic on histology and morphology of cervix even at lose doses and for short duration of time. So, a comparative study was designed to fill the gap.

OBJECTIVE:

To observe and analyze the histomorphological effects of sodium arsenite on cervix of rat.

Methodology: Present study held at department of anatomy, College of Physician and Surgeon Pakistan, Regional Centre Islamabad, in two principal groups, group A (control) and group B (experimental). The animals of group B were administered sodium arsenite by oral gavage daily for 14 days while group A were administered only distilled water daily for 14 days. The animals of both groups were sacrificed after day 14th of drug administration. The cervix was processed for paraffin embedding and stained with Haematoxylin and Eosin (H&E). Area of cervical canal's lumen (µm2) at 10X, height of cervical luminal epithelium (µm) at 40X and thickness of cervical muscles (µm) at 10X were measured. They were kept under controlled temperature of 23+2 and humidity 50+5% in the experimental research lab of postgraduate medical institute Lahore. The night and day cycle was maintained for 12 hours each. The cages were labeled according to the group and number of animals. Standard diet and tap water ad libitum was given to animals. Before starting the experiment animals were acclimatized for 10 days.

Statistical Analysis: Data analyzed by SPSS 23.0v. All the histological were analyzed for detection of any significant differences between the mean in the experimental and control groups. All the data was expressed as mean ± SD. Student t-test was applied to analyze the data. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS:

Different parameters like area, height and thickness of cervical canal lumen in both study groups were shown in table-1 as mean ± SD with their p-values.

Table-1: Parameters of Cervix In Control Group And Experimental Group Rats

PARAMETERS	Group-A (n-30)	Group-B (n=30)	*p-values*
	Mean±SD	Mean±SD	
Area of cervical canal's lumen (µm^2)	1308.91± 619.73	563.79 ± 384.02	0.000*
Height of cervical luminal epithelium (µm)	31.41± 11.74	99.82± 35.82	0.002*
Thickness of cervical muscles (µm)	24.92 ± 10.80	18.19 ± 4.25	0.000*

*Statistically Significant

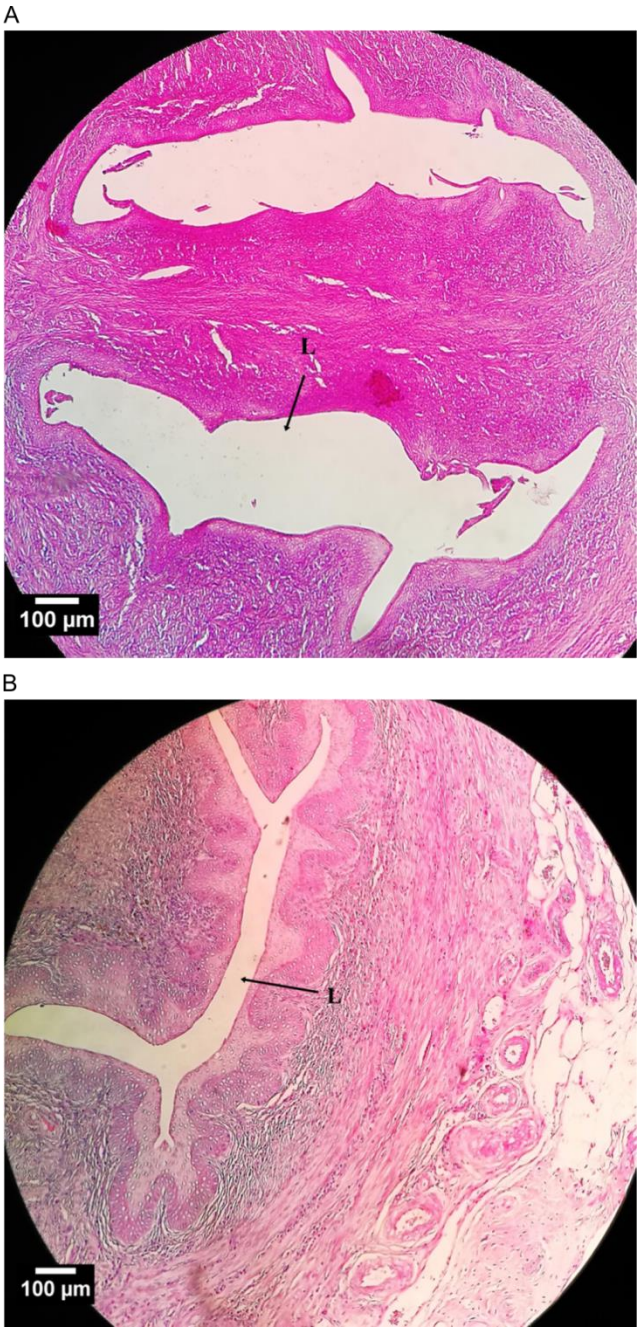
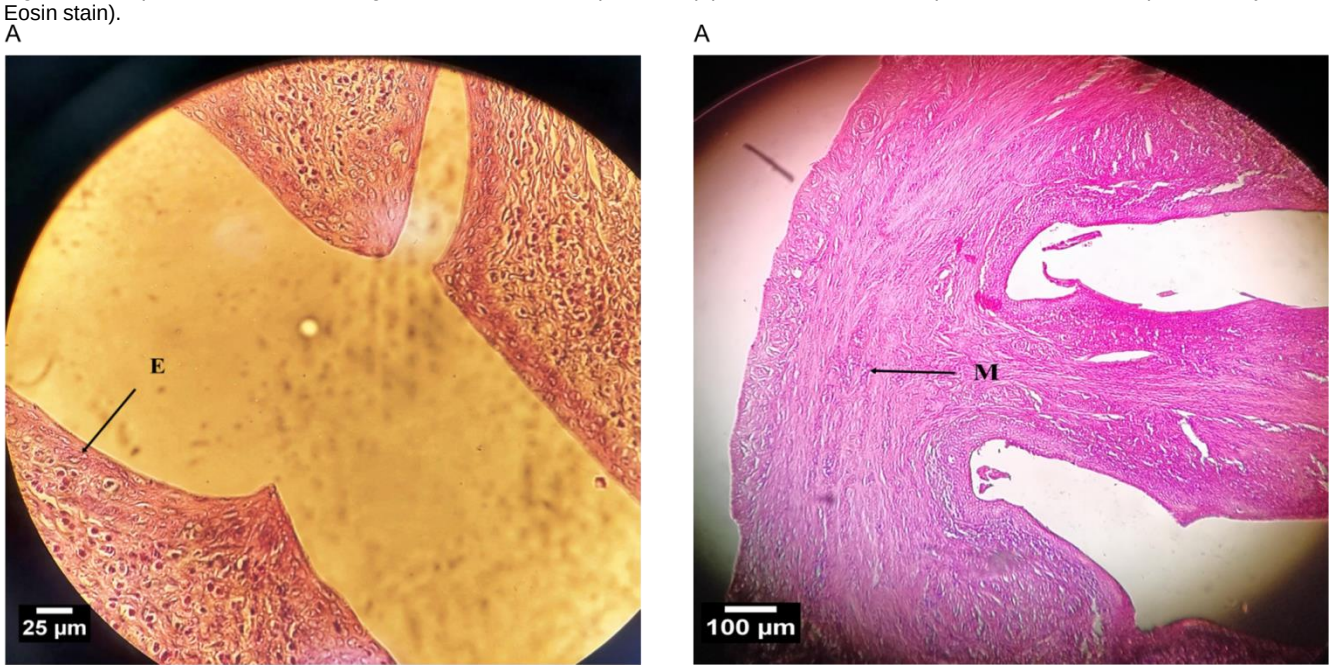


Figure-1: Comparison between the luminal area of cervical canals (L) of both control and experimental rat at 10X (Haematoxylin and Eosin stain).

Figure-2: Comparison between the height of cervical luminal epithelium (E) of both control and experimental rat at 40X (Haematoxylin and Eosin stain).



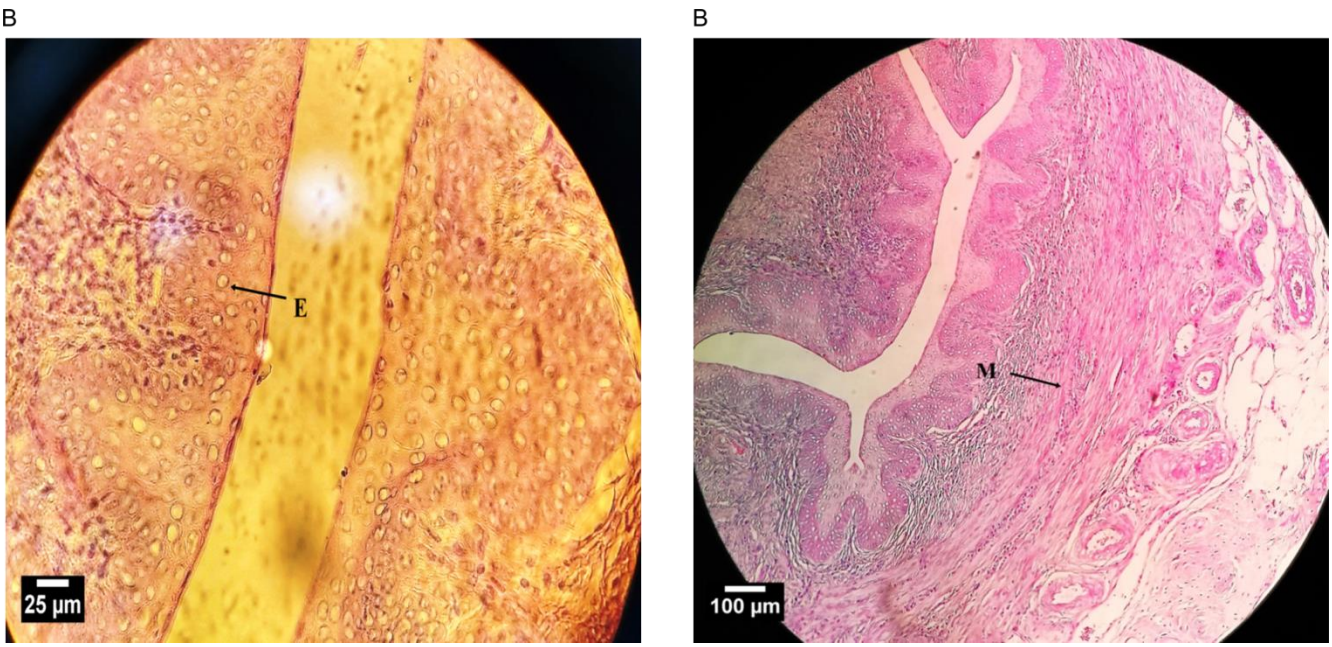


Figure-3: Comparison between the thickness of cervical muscles (M) of both control and experimental rat.

DISCUSSION

Arsenic as a toxic agent is well documented in clinical cases as well as in animal models. Arsenic is absorbed in almost all the systems of body including digestive, cardiovascular, respiratory, excretory, reproductive, biliary, integumentary and nervous system. Due to its absorption, these systems undergo many diseases like hepatotoxicity, diabetes, cancers, cardiac and neurological disorders. Arsenic is highly teratogenic and also associated with abortions and still births. In this study the effects of inorganic arsenic (4µg/ 10ml distilled water for 14 days) on female rat cervix were seen.

The mean luminal area of cervical canals were significantly reduced in experimental animals as compared to control (*p*-value= 0.000). These results are similar to a study done by Mondal S et al. in which the female rats were exposed to arsenic which diminished the uterine luminal diameter. The possible cause of decrease in cervical canal's luminal area after exposure to arsenic may be due todecrease production of E2, bonding of arsenic

with estrogen receptors and inhibition of binding of E2 with alpha ER.[21]

The experimental animals showed cervical epithelial hyperplasia (*p*-value = 0.002).These results are similar to a study done by Salnikow K who reported the effects of heavy metals including arsenic on genetic levels. Another study done by Gao Y on female reproductive organs of mice and reported the causes of endometrial hyperplasia in detail. So according to the previous studies the possible causes of cervical epithelial hyperplasia (after exposure to arsenic) may be due to epigenetic changes. Arsenic increases the expression of transforming growth factor-α (TGFα), transforming growth factor-β (TGF-β)and macrophage-colony-stimulating factor (GM-CSF). All these growth factors up regulate the production of cyclin D1 and disruption of the functions of tumour suppressor gene p53 which lead to epithelial hyperplasia.[22]

The thickness of cervical muscles in experimental animals was decreased in experimental animals (*p*-value= 0.000). These results are comparable to the study of Chatterjee A and Akram Z et al. who exposed female rats to arsenic and the thickness of myometrium in those rats were deceased. The decrease in thickness of cervical muscles may be due to production of ROS that lead to protein denaturation by exposure to arsenic. Muscles are mainly formed of proteins so the alteration in protein structure is very harmful for the normal architecture of muscles.[23]

Limitations: resent study had small sample size, financial constraints and limited resources. It was an animal based study, arsenic toxicity could be assessed in human beings who are living in those areas where the drinking water has arsenic more than the safe limit recommended by WHO.

Conclusion: This study concluded that the nature of histomorphological effects observed in cervix showed that these changes may be due to oxidative stress produced by the formation of free radicals, decrease levels of serum estradiol, progesterone and by the denaturation of proteins.

Author's Contribution: AM & HAB: Overall supervision, write up and literature review. MY & UZM: Statistics application analysis literature review, help in write up. WAB, SA & TL: Literature review help in write-up.

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