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Ameliorating Effects of Arsenic-Induced Toxicity On The Histology Of Vagina And Reproductive Hormones Of Female Sprague Dawley Rats

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Abstract

Objective: To find out the toxic effects of arsenic on the histology of the vagina and reproductive hormones of female Sprague Dawley rats.

Methods: 60 female Sprague Dawley rats were divided into control group A and experimental group B with 30 rats in each group. The gross weight of all the rats was checked before and at the end of the experiment. After one week of acclimatization, the control group was administered 10ml of distilled water by oral gavage daily for 14 days and the experimental group was administered 4µg of sodium arsenite dissolved in 10ml of distilled water by oral gavage daily for 14 days. After being euthanized by chloroform inhalation on day 15, blood from each rat was drawn by a single intra-cardiac puncture and stored for biochemical analysis. After that, rats were sacrificed and the whole reproductive tract was removed and vaginal sections were prepared and stained with hematoxylin and eosin (H&E). The histological parameters were analyzed by using Image J software. Whereas, biochemical parameters e.g. serum LH and FSH levels were analyzed by ELISA.

Results: After exposure to a low dose of arsenic administered for a short period, the gross weight of rats, weight of reproductive tract, height of vaginal epithelium and serum FSH and LH values were significantly reduced in experimental animals as compared to control animals with p-value < 0.05.

Conclusion: Low dose of arsenic given via contaminated drinking water for a short period is very toxic to the female reproductive tract which is evident by the histological changes in the vagina and deranged hormonal profile of female Sprague Dawley rats.

Keywords: Arsenic, FSH, Free radicals, LH, Oxidative stress, Vagina.

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1. Introduction

Water is indispensable for the continuance of life. Due to insufficient supply and inappropriate supervision developing countries like Pakistan are constantly facing a lack of clean and pure potable water.[1] For so long on different public forums an intense debate has been going on about the contamination of drinking water by Arsenic and its side effects.[2] Arsenic is present on the surface of the earth in various organic and inorganic forms with different oxidation states e.g., 0' 3+, 5+3-. The inorganic/trivalent form of arsenic is the most reactive and lethal to all living organisms.[3] In potable water, arsenic comes from different natural and anthropogenic sources e.g., pesticide application of arsenic in agriculture and use of arsenic in therapeutics and several industries e.g., glass manufacturing industries, wood preservative industries and in car batteries as a semi-conductor etc.[4] As reported by WHO the safe limit of arsenic in potable water is about 10 µg/L [5,6]

According to a report, millions of people across the world are badly suffering from the noxious effects of arsenic by using polluted groundwater. Excessive administration can lead to cancer of different body systems including integumentary, reproductive, cardiovascular, respiratory, nervous and gastrointestinal systems.[7] Many studies have proved that the trivalent form of arsenic is more dangerous than pentavalent and the trivalent form increases more chances of oxidative stress which is the main cause of diseases caused by arsenic.[4] In females, prolonged exposure to arsenic leads to breast and endometrial cancer, decreased lactation, menstrual problems, endometriosis, miscarriages, pre-term births and stillbirths.[8,9]

The normal growth and proliferation of the female reproductive tract and oestrous cycle are under the

control of the hypothalamic-pituitary axis that secretes luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in pulsatile manners. The biochemical function of FSH is to control the proliferation of ovarian follicles to regulate the puberty and reproductive functions of the body. Whereas, LH stimulates ovulation and luteinization of the corpus luteum. Both FSH and LH work in synergy. These two hormones, along with estrogen and progesterone, maintain homeostasis in various stages of the oestrous cycle in female Sprague Dawley rats.[10] The research data from previous studies have revealed the deleterious effects of arsenic on the levels of female reproductive hormones.[9] Arsenic not only disturbs the hormonal profile but also alters the regularity of the oestrous cycle, diminishes the ovarian reserves and stimulates dys-steroidogenesis. [11]

This study was conducted to see the hazardous effects of low doses of arsenic on the histology of the vagina and levels of reproductive hormones of female Sprague Dawley rats.

2. Materials & Methods

It was a laboratory-based experimental study that was performed at the Islamabad Regional Centre of the College of Physicians and Surgeons (CPSP). The duration of the study was 18 months (November 2017-April 2019). Ethical approval was taken by the Research Ethical Committee of CPSP Islamabad according to the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).60 female Sprague Dawley rats aged 15-16 weeks and weighing 200-300g were selected by non-probability convenience sampling method. Animals were randomly divided into two groups e.g., group A (healthy control group) and group B (experimental group). Each group comprised of 30 female rats. Before any intervention, rats were acclimatized under room temperature 25±2°C, ~60% humidity and 12 hours of day and night cycle for one week. During this period, they were given standard rat chow and distilled water *ad libitum*. After one week, group A continued with standard rat chow and distilled water *ad libitum* along with 10ml of distilled water via oral gavage daily for 14 days. Whereas, group B was given standard rat chow and distilled water *ad libitum* along with 4µg of sodium arsenite (purchased from Hamza Enterprises Sadder Rawalpindi manufactured by LAB CHEM lot # LH-1406) dissolved in 10ml of distilled water via oral gavage daily for 14 days. On day 15, the rats were taken to the animal lab of the National Institute of Health (NIH) Islamabad where with the help of a veterinarian, the rats were deeply anaesthetized by chloroform inhalation and then placed on a dissecting board with a clean sheet underneath. Blood was drawn by single intra-cardiac puncture and stored in pre-labelled clot activator vacutainers for 10-20 minutes. All the vacutainers were kept in thermocol along with ice packs to maintain the temperature at 4ºC till further processing.

After that, rats were dissected by giving a midline vertical incision with the help of a scalpel. The incision extended from the groin region to the chin. Muscles were pulled away with the help of forceps. When the muscle layers were separated the reproductive tract became visible. The whole reproductive tract was removed very carefully, trimmed and washed in 0.9% normal saline. Then it was dried and the whole vagina was separated from the rest of the reproductive tract and preserved in formalin for histological study.

For the light microscopic study, sections were first cut at 5µm thickness and then stained with hematoxylin and eosin (H&E). The height of the vaginal epithelium was taken by an ocular micrometre fitted in the eyepiece. By employing Image-J software, the height of vaginal epithelium was measured in µm from the basement membrane up to the lumen on four different fields (two on the X-axis and two on the Y-axis to remove any chance of repetition) under a 40X lens and then took a mean.

For analysis of serum FSH and LH levels, vacutainers were centrifuged at 2000-3000 rpm for 20 minutes to collect the supernatant. Serum was collected in pre-labelled aseptic Eppendorf tubes and refrigerated at 2-8 ºC. To estimate serum FSH values, BT lab FSH rat ELISA kit (cat # EA0015Ra, sensitivity of 0.022mIU/ml and detection range of 0.05-20mIU/ml) was used. While, for LH values, BT lab LH rat ELISA kit (cat # EA 0013Ra, sensitivity of 0.31 mIU/ml and detection range of 0.5-100mIU/ml) was used. Kits were purchased from Bio-diagnostic Resources Commercial Market Rawalpindi.

Mean ± SD of the values of weight of rats, weight of reproductive tract, height of vaginal epithelium, FSH and LH, were analyzed statistically with the SPSS, version 22. Comparison of means of FSH and LH levels between two groups was evaluated by the Student T-test. A *p-value* of ≤ 0.05 was taken as significant.

Graphical presentation of the weight of rats, the weight of the reproductive tract, the height of vaginal epithelium, FSH and LH values were processed on an Excel spreadsheet.

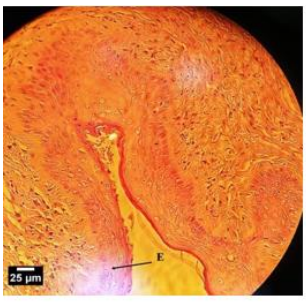
3. Results

The comparative results of the weight of rats, the weight of the reproductive tract, height of vaginal epithelium, FSH and LH are shown in Table 1. A histological view of vaginal epithelium is shown in Figure 1.

Table 1: Gross parameters of both control (A) and experimental rats (B)

PARAMETERS	A (Control Group) n=30 Mean ± SD	B (Experimental Group) n=30 Mean ± SD	Statistical Significance *p* value
Weight of rats (g)	263.51± 35.65	206.13± 45.27	< 0.01
Weight of reproductive tract (g)	1.77± 0.34	1.10± 0.36	< 0.01
Height of vaginal epithelium (µm)	84.91 ± 27.27	30.44 ± 15.61	< 0.01
Serum LH (ng/ml)	7.47 ± 1.33	1.67 ± 0.68	< 0.01
Serum FSH (ng/ml)	3.73 ± 0.82	0.64 ±0.27	< 0.01

In comparison with group A, the animals of group B showed a significant decrease in gross body weight, weight of reproductive tract, height of vaginal epithelium and serum FSH and LH levels with p-value < 0.05.



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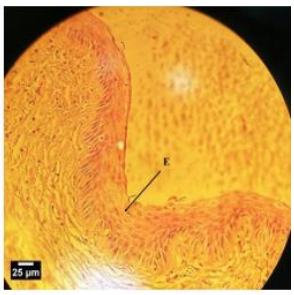

B

4. Discussion

Taking the laboratory study on the reproductive tract of female Sprague Dawley rats it is evident that the mean body weight of group B animals (experimental group) was decreased significantly as compared to the control group A (p-value<0.05). These results are by the study of Handali S, the possible causes of gross weight reduction after exposure to arsenic may be due to interference in thermogenesis with the endocrine system and hormones like leptin and adiponectin. Arsenic toxicity inhibits the utilization of glucose and production of ATP in a living cell by irreversible inhibition of glyceraldehydes 3-P dehydrogenase and pyruvate dehydrogenase complex (enzymes of glycolysis and TCA cycle respectively). To replenish the need for glucose body starts gluconeogenesis (for which muscle protein is broken down and amino acids are converted into glucose) and for the production of ATP, body fat is broken down and fatty acid oxidation is initiated. This whole phenomenon will reduce the body weight, muscle mass and activity of animals.[12-14]

The mean weight of the reproductive tract was significantly less in experimental animals in comparison with the control (p-value<0.05). The possible cause of a decrease in the weight of the reproductive tract after exposure to arsenic may be due to decreases in plasma gonadotrophins and estradiol levels which are essential for the normal morphology and histology of the reproductive organs of the female.[15]

The comparison of vaginal epithelium in both control and experimental animals has shown that the vaginal epithelium in control animals has multiple layers of stratified squamous cells. But in group B animals, the height of vaginal epithelium was significantly reduced to a few layers of stratified squamous cells (p-value< 0.005). The possible cause of the decrease in height of vaginal epithelium after exposure to arsenic may be due to decrease estrogen levels [9,16]

Biochemical parameters showed a significant reduction in serum FSH and LH levels with p-value < 0.05. Similar results were shown in a study by Hasan Huseyin Demirel where 28-day-old female rats were exposed to 100 mg/L and 600 mg/L of arsenic via drinking water for 4 weeks. Arsenic exposure caused significant deterioration of serum LFTs and RFTs along with a decline in serum FSH, LH, progesterone and estrogen levels.[17] A review of the literature implicates that arsenic produces reactive oxygen species and induces oxidative stress in the body that can disrupt the homeostasis of the endocrine system and hypothalamic-pituitary axis.[18] Altered hormonal levels damage the normal physiology of hormonally regulated cells and tissues of the body. Another mechanism of arsenic toxicity is that arsenic along with its metabolites directly or indirectly bind with the cellular proteins and disturb various cellular processes. Among other hazardous effects, it hinders the DNA

damage repair process, deranges gene expression regulation, disarrays the epigenetic regulation and damages the hormonal system of the living body thus inducing the progression of various diseases. Arsenic-protein interactions as a mechanism of arsenic toxicity.[19,20] The results of this study favour our hypothesis that arsenic is a potent toxin to the female reproductive tract and endocrine system. However, a recent study was animal-based had a small sample size and had financial hindrances.

5. Conclusion

Short-term exposure to low doses of arsenic is critical to the female reproductive tract and hormonal profile.

CONFLICTS OF INTEREST- None

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Potential competing interests: None to report

Contributions:
A.M, U.Z.M, I.K.K - Conception of study
A.M, U.Z.M - Experimentation/Study Conduction
U.Z.M, M.A, K.H - Analysis/Interpretation/Discussion
M.Y, K.H, - Manuscript Writing
M.A, I.K.K - Critical Review
M.Y - Facilitation and Material analysis

All authors approved the final version to be published & agreed to be accountable for all aspects of the work.

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