ORIGINAL ARTICLE

CYTOLOGICAL AND BIOCHEMICAL IMPACT OF ARSENIC EXPOSURE ON THE ENDOCRINE SYSTEM AND OESTROUS CYCLE OF FEMALE RATS

Urfa Zaryab Mir, Amaidah Mir*, Maria Yasmeen, Faiza Ikram***, Zahra Masood, Sana Umar†**

Department of Biochemistry, *Anatomy, CMH Medical College, Kharian, **Department of Anatomy, Watim Medical and Dental College, Islamabad, * Department of Physiology, CMH Multan Institute of Medical Sciences, Multan, †Department of Biochemistry, HITEC Institute of Medical Sciences, Taxila, Pakistan**

Background: Rising concentration of arsenic in drinking water is severely damaging the reproductive health of both humans and animals. This study was thus designed to evaluate the deleterious effects of sodium arsenite at a minimum dose on the endocrine system and oestrus cycle of female Sprague Dawley rats. **Methods:** A randomized control trial was conducted at College of Physicians & Surgeons Pakistan, Regional Centre, Islamabad. Sixty healthy female Sprague Dawley rats were randomly divided into group 1 (control) and group 2 (experimental) with 30 rats in each group. After one week of acclimatization, the control group was administered 10 ml of distilled water daily via oral gavage, and the experimental group was administered 4 µg of sodium arsenite dissolved in 10 ml of distilled water daily via oral gavage. After one week of habituation, vaginal smears were taken daily to study the oestrus cycle. Whereas, serum oestrogen and progesterone levels were assessed using ELISA after 14 days of intervention. Biochemical parameters (oestrogen and progesterone) were analyzed on SPSS-22. Comparison of means of these hormones was evaluated by the Student's *t*-test. **Results:** After exposure to sodium arsenite, the oestrus cycle of the experimental group was prolonged and halted in the diestrus phase along with significant reduction of serum estradiol and progesterone levels. **Conclusion:** Low dose of arsenic delays and disturbs regulation of oestrus cycle and disrupts the hormonal levels in female rats.

Keywords: Arsenic, Estradiol, Oestrus cycle, Progesterone

Pak J Physiol 2023;19(4):19–22

INTRODUCTION

Arsenic is a constituent of earth's crust where it exists in various organic and inorganic forms. The inorganic forms of arsenic are readily absorbable in living bodies and hence regarded as the most lethal.[1]. Arsenic is widely used in agriculture as a pesticide and in various industries like wood preservative and glass manufacturing. WHO has declared that the mean concentration of arsenic in potable water should not exceed 10 µg/L.[2] Due to the lack of water filtration systems in under developed and developing countries, arsenic in our earth crust percolates down into the underground water tables and gets entry into the living bodies.[3] Mean concentration of arsenic in various regions of Pakistan is above 40 µg/L which is an alarming situation.[4,5] Its toxic effects have been studied to various organs and systems of animal and human bodies. So far, arsenic has been proved to be neurotoxic, hepatotoxic, nephrotoxic and cardio-toxic. Arsenic is now under discussion as an endocrine disrupter also.[6]

One of the major components of the endocrine system in female rats is oestrous cycle which is controlled by the fluctuations in the serum levels of oestrogen and progesterone. This cycle enables the female rats to reproduce offspring again and again and is similar to the menstrual cycle of human females. This cycle has 4 phases, e.g., pro-oestrus, oestrus, met-oestrus and di-oestrus and it lasts for ~ 4-5 days. There is rapid development of follicles with a rise in estradiol levels in pro-oestrus phase. Ovulation occurs in the oestrus phase with a decline in estradiol levels. Whereas, the met-oestrus and di-oestrus phases are characterized by rise in progesterone levels.[7] These phases are identified by observing the types of cells in the vaginal smear of female rats.

There are certain environmental chemicals and pollutants that disrupt the endocrine system and the oestrous cycle of female rats. WHO in the year 2010, declared the endocrine disrupting chemicals as a high research priority.[8] One of these toxins is arsenic that leads to various adverse effects on the female reproductive tract, e.g., ovarian failure, low weight of ovaries and uterus and dys-steroidogenesis that lead to spontaneous abortions, teratogenesis, and still birth etc.[9,10] Hazardous effects of low dose of arsenic for a brief period of time is still largely under-discovered. This study was designed to evaluate the effects of arsenic on the oestrous cycle of female Sprague dawley rats.

MATERIAL AND METHODS

It was a laboratory based randomized controlled trial conducted at the animal house of College of Physicians and Surgeons Pakistan, Islamabad for one year (1 Jan 2019 to 4 Jan 2020). Ethical approval was obtained from 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). Sixty healthy and non-diseased female Sprague Dawley rats were included in the study. Rats with any visible physical deformity and disabilities were excluded from the study. Animals with body weight of 220–300 g and age 15–16 weeks were selected by non-probability convenient sampling method. All animals were acclimatized for one week under room temperature 25±2 °C, ~60% humidity and 12-hr day and night cycle. During this period, they were provided with distilled water and standard rat diet *ad libitum*.

Animals were randomly allocated into 2 groups, i.e., group 1 (healthy control group) and group 2 (experimental group). After one week of habituation, the intervention period started that lasted for 2 weeks. Animals of group 1 were continued with standard rat diet and distilled water *ad libitum* along with additional 10 ml of distilled water given once daily by oral gavages. The animals of group 2 were given standard rat diet and distilled water *ad libitum* along with additional 10 ml of distilled water mixed with 4 µg of sodium arsenite once daily by oral gavage.

The vaginal smear of all the animals were collected early morning daily for 14 days and visualized under microscope[9]. A blunt plastic pipette was used to collect vaginal secretions. The pipette was rotated clock-wise in the vaginal wall up to 1 Cm and removed immediately to prevent cervical stimulation in the animal.[11] Secretions were placed on the glass slides and left to dry in air. Slides were stained with Papanicolaou stain and studied under 40× of light microscope.[12]

On basis of cytological results, the phases along with duration of oestrus cycle were compared between control and experimental animals. Each cycle was identified by the types of desquamated vaginal epithelial cells in the vaginal smear, e.g., pro-oestrous phase had abundance of nucleated epithelial cells, oestrus phase had cornified cells, met-oestrus and diestrus had leukocytes. Normally the oestrous cycle lasts for 4–5 days and cycle duration beyond these days was considered as prolonged.[13]

Rats were deeply anaesthetized by chloroform inhalation and exsanguinated by single intra-cardiac puncture. Blood was stored in clot activator vials at temperature 4–8 °C. Serum was extracted by centrifugation at 3,000 g for 15 minutes and stored in disposable and sterile Eppendorf tubes at -80 °C till further hormonal analysis. Serum estradiol (E2) and progesterone (P4) were assessed by commercially prepared rat ELISA kits[14].

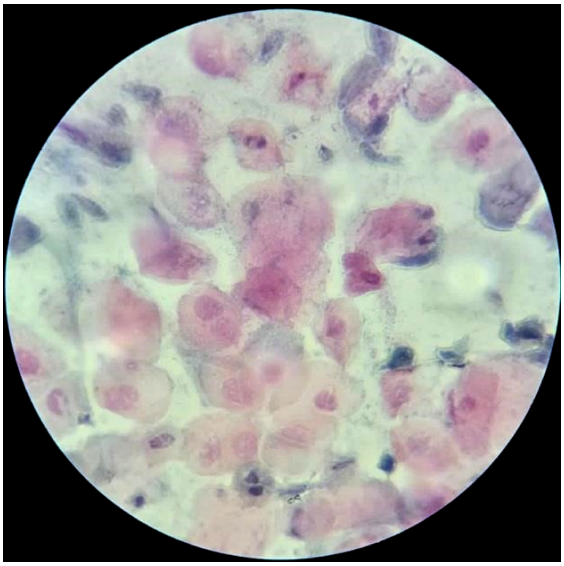
Mean±SD of serum estradiol (E2) and progesterone (P4) levels of both control and experimental groups were estimated with SPSS-22. Comparison of means of hormonal levels between two groups was done on Student's *t*-test, taking $p \leq 0.05$ as significant.

RESULTS

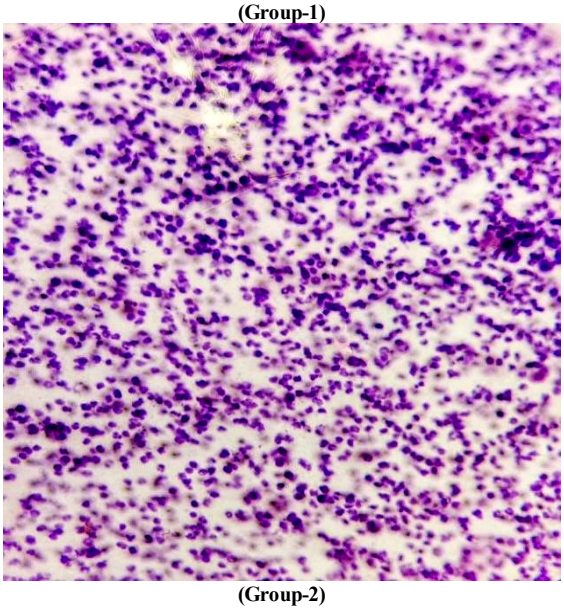
The cytology of vaginal smear of control group showed normal and regular phases of oestrus cycle. In the rats of experimental group the oestrous cycle was prolonged and remarkably hindered in diestrus phase till the end of experiment ($p<0.05$). Serum estradiol and progesterone were significantly reduced after arsenic exposure in group 2 as compared to group 1 ($p<0.05$). (Table-1 and Figure-1).

Table-1: Serum values of estradiol and progesterone of control and experimental groups

Variables	Group 1 (n=30)	Group 2 (n=30)	*p*
Estradiol (pg/ml)	92.0±14.3	35.7±8.8	<0.01
Progesterone (ng/ml)	11.5±2.4	2.1±0.4	<0.05



(Group-1)



(Group-2)

Figure-2: Comparison between the vaginal cytology of both group 1 and group 2 at 40×

DISCUSSION

Epidemiological data suggests that millions of people across the world are exposed to arsenic contaminated drinking water. Arsenic is causing numerous negative health effects, e.g., nervous system diseases, dermatological lesions, malignancies of various organs and cardiovascular problems. Arsenic has been proven as an endocrine disrupter.[15] Our study has evaluated the hazardous effects of low dose of arsenic, given for a short period of time on the female rats reproductive tract and hormonal profile. To evaluate these effects, vaginal smear visualization method by microscope was used which is cheapest, fastest and least harmful to the animals.[16] The results of our study showed that exposure of arsenic prolonged the normal length of oestrus cycle (4–5 days) and normal duration of diestrus phase (48–72 hours). The oestrus cycle of all female rats of experimental group was halted in diestrus phase till the end of experiment. These results are in accordance to the results of Esqueda *et al*[17] and Panpan Chen *et al*[18]. Their studies highlighted that exposure of various doses of arsenic in the pubertal age rats significantly disturbed the oestrus cycle. Relevant studies have also proven that disruption of oestrus cycle is related to the delayed onset of puberty and enhanced the possibility of sub-fertility and infertility in female rats.[19]

Our study has related the disruptive effects of arsenic with the levels of reproductive hormones, e.g., estradiol (E2) and progesterone (P4)[20]. These hormones are responsible for the regulation of oestrus cycle and reproductive cycle homeostasis. The results of our study showed that exposure of 4 µg of sodium arsenite dissolved in 10 ml of distilled water given for 14 days to the female rats significantly reduced the serum levels of estradiol and progesterone. Data of a recent studies by Panpan Chen *et al*[18] and F-Souza *et al*[19] showed similar results indicating arsenic as an endocrine disrupter. These reduced levels of estradiol and progesterone may be due to under stimulation by the pituitary hormones. Arsenic releases free radicals and induces oxidative stress in the body. These cytological and biochemical effects of arsenic could be due to oxidative imbalance and disturbance of reactive oxygen species (ROS) homeostasis in the reproductive tract of female rats.[21] It is, thus plausible that in our study, arsenic has been proven to be reproductive tract toxin even in lower doses.

Our study was animal-based and faced financial limitations. Managing a potent toxin like arsenic demanded a heightened level of vigilance.

CONCLUSIONS

Disruption of oestrus cycle phases and regularity along with disturbance of female hormonal profile after exposure of low dose of arsenic given for a brief period of time concludes that arsenic is a potent reproductive tract toxin and an endocrine disrupter.

IMPACT OF THE STUDY

Given the widespread presence of arsenic in our drinking water, the findings from this study undoubtedly hold substantial importance for public health.

ACKNOWLEDGMENTS

The authors duly acknowledge the intellectual guidance from (Late) Prof Muhammad Yunus Khan, and thank CPSP, Islamabad, and National University of Medical Sciences for facilitation to complete this study.

FUTURE RECOMMENDATIONS

Since the dosage utilized in this research is significantly lower than doses relevant to the environment, it is imperative to further assess the connection between exposure to arsenic and reproductive abnormalities in humans.

REFERENCES

1. Palma-Lara I, Martínez-Castillo M, Quintana-Pérez J, Arellano-Mendoza M, Tamay-Cach F, Valenzuela-Limón O, *et al*. Arsenic exposure: A public health problem leading to several cancers. Regulatory Toxicol Pharmacol 2020;110:104539. DOI: 10.1016/j.yrtph.2019.104539.
2. Ahmad A, Bhattacharya P. Arsenic in drinking water: Is 10 µg/L a safe limit? Current Pollution Reports 2019;5:1–3. DOI: 10.1007/s40726-019-0102-7.
3. Thakur BK, Gupta V, Bhattacharya P, Jakariya M, Islam MT. Arsenic in drinking water sources in the Middle Gangetic Plains in Bihar: An assessment of the depth of wells to ensure safe water supply. Groundwater for Sustainable Development 2021;12:100504. DOI: 10.1016/j.gsd.2020.100504.
4. Shah AH, Shahid M, Khalid S, Natasha, Shabbir Z, Bakhat HF, *et al*. Assessment of arsenic exposure by drinking well water and associated carcinogenic risk in peri-urban areas of Vehari, Pakistan. Envir Geochem Health 2020;42:121–33. DOI: 10.1007/s10653-019-00306-6.
5. Ur Rehman H, Ahmed S, Ur Rahman M, Mehmood MS. Arsenic contamination, induced symptoms, and health risk assessment in groundwater of Lahore, Pakistan. Envir Sci Pollution Res 2022;29(33):49796–807.
6. Fatoki JO, Badmus JA. Arsenic as an environmental and human health antagonist: A review of its toxicity and disease initiation. J Hazardous Materials Advances 2022;5:100052. DOI: 10.1016/j.hazadv.2022.100052
7. Adeniyi M, Agoreyo F. Estrous cycle ratio as a reproductive index in the rats. Am J Biomed Sci Res 2019;4(2):100-3. DOI: 10.34297/ajbsr.2019.04.000772
8. Sabir S, Akash MSH, Fiayyaz F, Saleem U, Mehmood MH, Rehman K. Role of cadmium and arsenic as endocrine disruptors in the metabolism of carbohydrates: Inserting the association into perspectives. Biomed Pharmacother 2019;114:108802. DOI: 10.1016/j.biopha.2019.108802.
9. Ommati MM, Shi X, Li H, Zamiri MJ, Farshad O, Jamshidzadeh A, *et al*. The mechanisms of arsenicinduced ovotoxicity, ultrastructural alterations, and autophagic related paths: An enduring developmental study in folliculogenesis of mice. Ecotoxicol Environ Safety 2020;204:110973. DOI: 10.1016/j.ecoenv.2020.110973.
10. Fan Y, Jiang X, Xiao Y, Li H, Chen J, Bai W. Natural antioxidants mitigate heavy metal induced reproductive toxicity: prospective mechanisms and biomarkers. Crit Rev Food Sci Nut 2023:1–13.

11. Massányi P, Massányi M, Madeddu R, Stawarz R, Lukáč N. Effects of cadmium, lead, and mercury on the structure and function of reproductive organs. Toxics 2020;8(4):94.
12. Pantier LK, Li J, Christian CA. Estrous cycle monitoring in mice with rapid data visualization and analysis. Bio-protocol 2019;9(17):e3354-e.
13. Sano K, Matsuda S, Tohyama S, Komura D, Shimizu E, Sutoh C. Deep learning-based classification of the mouse estrous cycle stages. Scientific Reports 2020;10(1):11714.
14. Abdel-Mottaleb Y, Ali HS, El-Kherbetawy MK, Elkazzaz AY, ElSayed MH, Elshormilisy A, *et al*. Saponin-rich extract of Tribulus terrestris alleviates systemic inflammation and insulin resistance in dietary obese female rats: Impact on adipokine/hormonal disturbances. Biomed Pharmacother 2022;147:112639. DOI: 10.1016/j.biopha.2022.112639.
15. Bjørklund G, Oliinyk P, Lysiuk R, Rahaman MS, Antonyak H, Lozynska I, *et al*. Arsenic intoxication: general aspects and chelating agents. Arch Toxicol 2020;94:1879–97. DOI: 10.1007/s00204020-02739-w.
16. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res Pract 2020;6(1):1–15.
17. Dávila-Esqueda ME, Jiménez-Capdeville ME, Delgado JM, De la Cruz E, Aradillas-García C, JiménezSuárez V, *et al*. Effects of arsenic exposure during the pre-and postnatal development on the puberty of female offspring. Exp Toxicol Pathol 2012;64(1–2):25–30. DOI: 10.1016/j.etp.2010.06.001.
18. Chen P, Luo Q, Lin Y, Jin J, Hu K-L, Wang F, *et al*. Arsenic exposure during juvenile and puberty significantly affected reproductive system development of female SD rats. Ecotoxicol Environ Safety 2022;242:113857. DOI: 10.1016/j.ecoenv.2022.113857.
19. Souza ACF, Ervilha LOG, Coimbra JLP, Bastos DSS, Guimarães SEF, Machado-Neves M. Reproductive disorders in female rats after prenatal exposure to sodium arsenite. J App Toxicol 2020;40(2):214–23. DOI: 10.1002/jat.3897.
20. Herbison AE. A simple model of estrous cycle negative and positive feedback regulation of GnRH secretion. Frontiers Neuroendocrinol 2020;57:100837.
21. Biswas P, Mukhopadhyay A, Kabir SN, Mukhopadhyay PK. High-protein diet ameliorates arsenicinduced oxidative stress and antagonizes uterine apoptosis in rats. Biological Trace Element Research. 2019;192:222–33. DOI: 10.1007/s12011-019-1657-2.

Address for Correspondence:

Dr Urfa Zaryab Mir, Senior Lecturer Biochemistry, CMH Medical College, Kharian, Pakistan. **Cell:** +92-335-9056574

Email: urfazaryabofficial@gmail.com

Received: 20 Nov 2023 **Reviewed:** 11 Dec 2023 **Accepted:** 11 Dec 2023

Contribution of Authors:

UZM: Running ELISA, and laboratory data collection
AM: Study design, sampling of vaginal smear, and evaluation of cytological changes
MY: Animal handling, and vaginal smear processing
FI: Data processing on SPSS
ZM: Write up, and referencing
SU: Blood sampling, and processing of serum

Funding: None
Conflict of Interest: None