IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 13, Issue 6 Ser. II (June. 2019), PP 12-16
www.iosrjournals.org

Depolarization of Plasma Membrane Components Due To Arsenic Exposure during Spermiogenesis

A.Nath[1*], Neha Mithilesh[1], Shreya Sinha[1], Priyanka[1], Amrita Srivastava[2], Priyanka Sinha[1], Mohita Sardana[1], J.K.Singh[1]
[1]*S.S. Hospital Research Institute, Kankarbagh, Patna*
[2]*Central University of South Bihar, Gaya, Patna*
Corresponding Author: A.Nath

Abstract: *Arsenic is a global threat in this century as a pollutant and it is gradually getting incorporated inside the physiological systems of plant and animals by various means. It is present in our environment in air, soil, water bodies, due to its usage in multiple fields like paints, dyes and paper industries, cotton harvesting, glass manufacturing, herbicides as well as electronics industries. Arsenic affects the animal system at the molecular level. All the complementary organ system is being exposed to environmental Arsenic. Due to these hazardous exposures cell faces a range of internal stresses that ultimately lead to disruption of its plasma membrane integrity. Our reproductive system is highly sensitive towards pollutants like arsenic. The effects of arsenic in the form of arsenite on the integrity of plasma membrane was studied under electron microscope. Plasma membrane being the outer most covering of the cell severs as penetrating barrier is very susceptible to the toxic agents like arsenic. Arsenic damages the plasma membrane of the developing spermatozoa. Finally, it causes infertility in male on a very large scale.*

Key words*: Mice, Testes, Spermatozoa, Plasma Membrane, Arsenic, Depolarization, Electron microscope.*

Date of Submission: 05-06-2019 Date of acceptance: 20-06-2019

I. Introduction

Heavy metals are found naturally in the earth. They become accumulated as a result of various, natural and anthropogenic activities and enter in plant, animal, and human via inhalation, food, and manual handling. They can bind with molecules and interfere with the functioning of vital cellular components [1].

Heavy metal toxicity can be due to membrane distortion as shown earlier by the altered lipid content [2].Similarly have indicated that saturation of fatty acid increased, whereas the level of sterols significantly decreased in the membrane of plants grown with metals. Since lipid composition and membrane fluidity are considered as significant factors regulating the plasma membrane H^+-ATPase [3], an inactivation of the proton pump could be the result of the metal-induced changes in the plasma membrane. Heavy metals may mimic the essential metals causing a disruption in cellular and enzymatic mechanism like phosphate by arsenate. Arsenic is present in environment naturally and it does not have have any significant smell or taste, thus making it difficult to mark its presence in air, water and food chain without any biochemical tests[4].

By the time the symptoms become visible, the accumulated arsenic in body reaches to significantly high levels and starts showing its adverse effect on systems like reproductive system impeding the early phases of sperm maturation. The Arsenic exposure has many adverse health effects which include various diseases related to brain, heart, liver and lungs as well as developmental abnormalities, hematologic defects and hearing disorders and cancers [5].

High levels of Arsenic exposure has been noticed since a very long period in the different regions of world, notably China, India, and some countries in Central and South America. Population living in such area are at high risk to develop cancers and other disorders due to this chronic exposure of Arsenic [6].

Arsenic family induces differentiation and apoptosis by different mechanism, such as through direct opening of the intramolecular pores between integral protein molecules and lipids. Plasma membrane is the most significant multi-molecular boundary between the cell and its external environment [7,8].

The lipids of plasma membrane are more than simple structural elements. They can have important effect on the biological properties of a membrane. It can determine the physical state of the membrane and influence the activity of particular membrane protein. Membrane lipids also provide the precursors for highly active chemical messengers that regulate cellular function [9,10].

Keeping the idea of membrane potentiality and its disruption due to toxic substances like heavy metals, the present research work has been undertaken to know the degree of disruption due to toxicity occurred by Arsenic[11].

Testicular tissues shows high degree of damage due to Arsenic toxicity especially on spermatozoa plasma membrane which shows clear depolarization under the Electron microscope. Their structure cannot be seen under Light Microscope thus Electron Microscopy study is important to note the changes at molecular level. Sodium Arsenite is very toxic to all kind of living cells and capable of inducing cell death.[12,13] Metals may vary in oxidation state by loosing one or more electrons to form cations and form organometallic compounds[14].Arsenic exhibits unique toxicology. The common mechanisms of toxicity include mimicry, oxidative damage, and adduct formation with DNA or protein[15]. In India mainly the rivers originating from Himalaya are the main source for Arsenic pollution and the area situated on the bank of such rivers like [16].

II. Materials and method

Mice grouping /sampling and dosing: Swiss Albino mice weighing between 28 to 32 gm± 4 gm were assigned to 9 experimental groups. Two sets of mice were taken as normal and control respectively. Among Arsenic compound Sodium Arsenite was the common form selected for the experiment. The dosing of sodium arsenite, dissolved in distilled water, was standardized (After LD_{50} assesment) to 2mg per kg body weight of mice. Seven models were prepared according to the duration of the sodium arsenite treatment. The experimental mice (n= 10) in each treated group were administered sodium arsenite everyday by Oral Gavage method. They were sacrificed after 21 days,1 month,2 months,3 months,6 months,8 months and10 months of Sodium Arsenite treatment respectively.

Arsenic estimation in blood and tissues: The confirmation of the presence of arsenic inside model system was done by estimating the level of arsenic in blood and tissues of normal, control and treated mice by Atomic Absorption spectrophotometer by Graphite flame. The data was compiled digitally and comparative histogram and graphs were prepared.Group of mice which shows accumulation of arsenic in tissues and blood and low testosterone only those were considered and selected for EM.

Biochemical Test: Biochemical tests were done for the assessment of methylation by spectrophotometry and the level of testosterone was detected by ELISA. (Nath et al;2014)

Light Microscopic study: The histological experiments were performed according to the standard methods.

Electron microscopic study: As the tissues were fixed for TEM in 2% Gluteraldehyde followed by osmication. After osmication the tissue turns black .Other cellular organelles also take blackish, gray stain. Tissues were processed for Transmission Electron Microscope. After dehydration blocks were prepared and ultra thin sections were cut with glass knives only silver colour sections were kept on grid. Images were captured under different magnification at SAIF,AIIMS, New Delhi.

III. Results and Discussion

The electron micrographs showing the clear damage in the lipid bilayer after 21 days,1 month,2 months,3 months,6 months,8 months and 10 months of Arsenic treated mice plasma membrane (2mg/gm of b.wt.).This depolarization of basic components of plasma membrane are clearly visible under Electron Microscope.

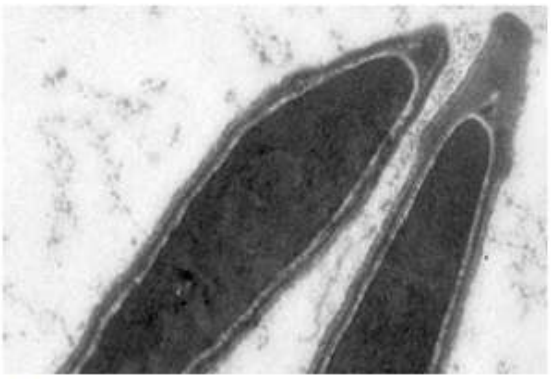

Fig.1.Microphotograph showing plama membrane of spermatozoa of normal

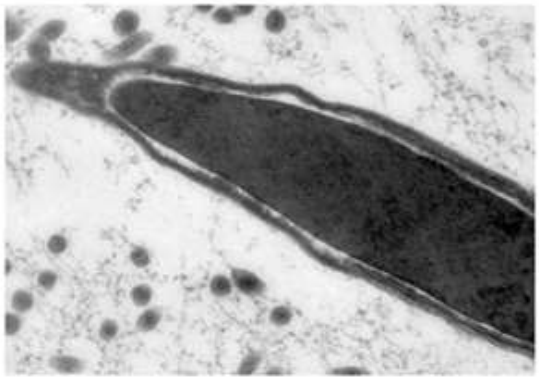

Fig. 2. Microphotograph shows intact plasma membrane consisting of as usual normal lipid & protein molecule showing normal formation of

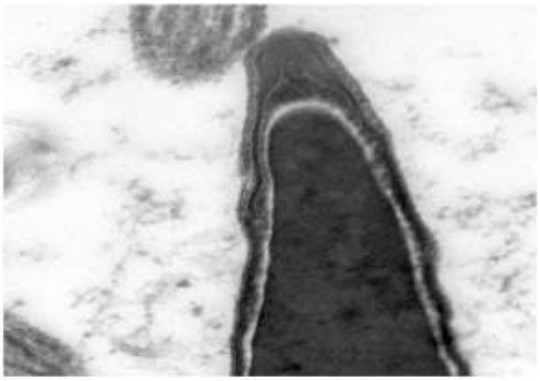

Fig.3.Microphotograph showing almost growing apermatozoa , Note-Intact the plasma membrane (1 month As @ 2mg/gm of b.wt)

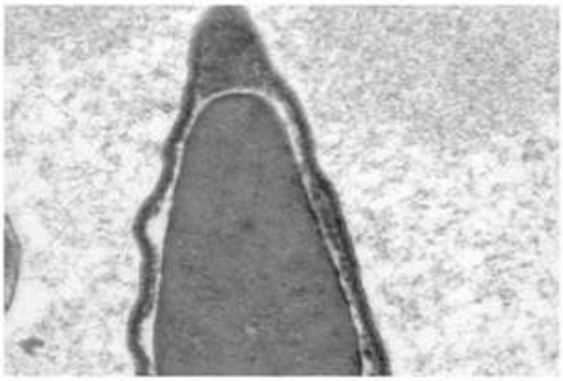

Fig.4.Microphotograph showing initiation of depolarization of plasma Membrane of growing spermatozoa.(2 months As@ 2mg/gm of b.wt)

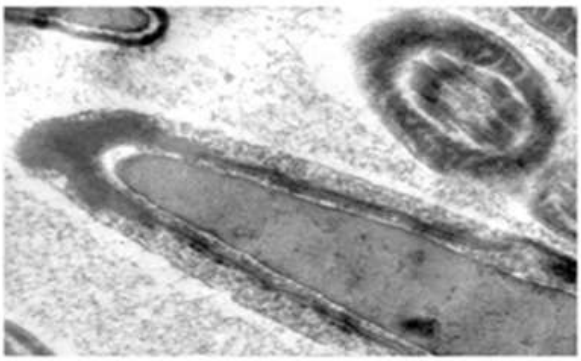

Fig. 5.Microphotograph showing effect of Arsenic very clearly visible in depolarization of plasma membrane development of Acrosome is also effected.(3 months As@ 2mg/gm of b.wt)

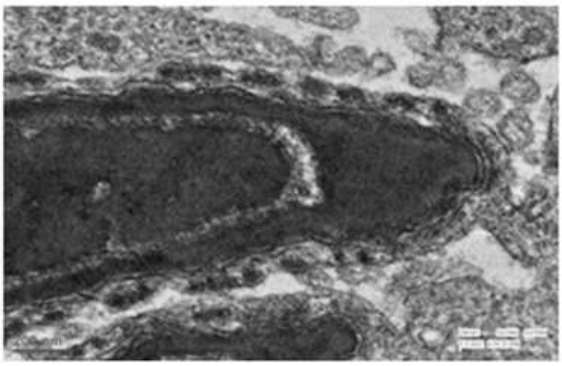

Fig.6 Note the broken plasma membrane and gap between outermembrane .(6 months As@ 2mg/gm of b.wt)

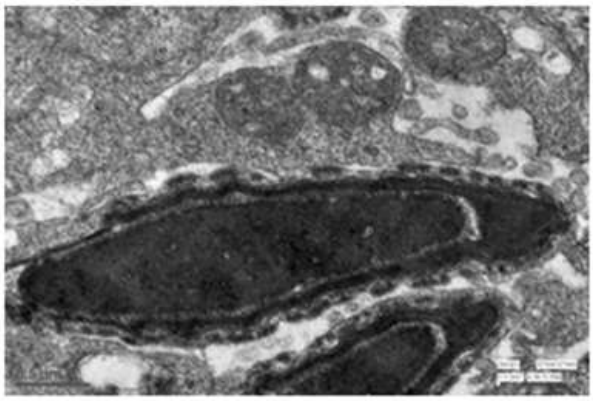

Fig.7. Effect of arsenic is very significant on pm as only black bricked shaped structure , which can be depicted as protein molecule around the head of spermatozoa.(8 months As @ 2mg/gm of b.wt)

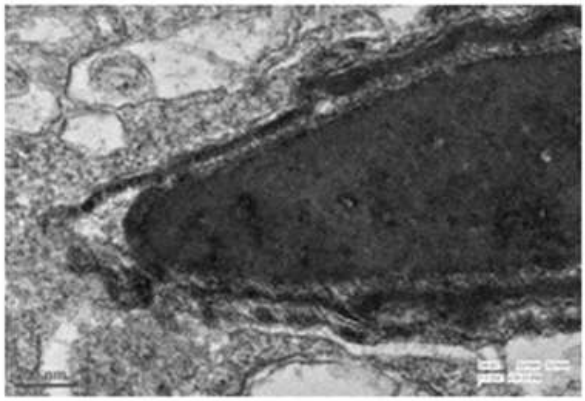

Fig.8.Deletion of lipid molecule are more in head region of spermatozoa.(10 months As@ 2mg/gm of b.wt)

The microphotographs showing gradual effect on the membrane of testescular cells as the duration increases .The 21 days treated sample is intact and usual protein and lipid arrangement is visible same like 1 Month treated sample. But as the duration increase , we can note the initiation of depolarization of molecule of phoshpholipid bilayer in the 2 months treated sample images. In microphotograph of 3 months treated sample the damage at acrosomal portion is clearly visible under EM. Microphotograph of 6 months treated sample is showing the broken plasma membrane, having large and visible gaps. Effect of Arsenic is very significant in 8 months treated microphotograph, as the black bricked shaped structure are visible as separate bodies in p m. Maximum duration 10 months treated samples sample shows highly deletion of lipid molecules in head region of acrosome.

Each cell organelle has a language and expression of its own under stressful conditions. Due to toxicological exposure to pollutants like arsenic, organelles express various forms of deformities. In the present investigation, the organelles' deformities shows significant co-relation with serum testosterone levels as well as MDA and TSH, LH levels [17, 18]. Serum FSH shows abrupt fluctuations in its levels. Even though the FSH production was increased in the test animals, there was failure of FSH to induce cells of Leydig for production of testosterone and the level of later remained significantly low. Thus there is a weak co-relation between serum FSH level and activities of cell organelles.

Toxic metals like Arsenic can bind easily with cellular molecules which leads to induction of intracellular conformational changes in the biomolecules , replacement of physiological metals from their binding sites and further induces cell cycle arrest and carcinogenesis[19].Arsenic promotes inhibition of DNA repair functions and leads to deformities in cellular activities [20].Finally they catalyze the redox reactions and produces the Reactive Oxygen Species (ROS) that can easily damages a wide variety of cellular macromolecules like DNA, lipids and proteins[21,22].

Mechano-structure of cells and particularly, the occurrence of cytoskeletal and plasma – membrane is significantly damaged when cells are chronically deformed due to toxicity of arsenic exposure, as evident from the TEM images [23]. Such chronic wounds generated due to arsenic are significant in nuclear region which is the sign of toxicity in cell mechano-biology. Mechanically subjected towards deformation which affects sperm motility causing infertility. TEM micrographs shows the clear deletion of lipid molecules from phospholipid bilayer and mechanosensitive ion channels are also effected by Arsenic and the cascade ultimately leading to disruption in plasma membrane integrity.

IV. Conclusion

The present investigation depicts that the plasma membrane, during spermiogenesis, shows clear depolarization of lipid and protein component due to arsenic toxicity. Deletion of lipid molecule is visible as the bricked blank spaces in membrane. During deletion of lipid molecule, peroxisome, black in colour, are also very predominant around the membrane of spermatozoa. This shows the significant internal cellular defense mechanism. As cells are exposed to Arsenic, the first step of defense is clearly visible under Electron microscope only. Because of deletion of lipid molecules, raised malondialdehyde level is seen in blood. Reproductive Hormone levels also get disrupted and these significant changes affect the integrity of plasma membrane during spermiogenesis. This causes infertility by degrading the plasma membrane components of

developing spermatozoa, mainly in acrosomal portion. Thus it can be concluded that Arsenic exposure causes depolarization of components of plasma membrane during spermiogenesis.

Acknowledgement

The authors are grateful to Indian Council of Medical Research (ICMR), New Delhi for the financial assistance. We are thankful to S. S. Hospital and Research Institute ,for providing a research environment and latest facilities, We are also thankful to SAIF,AIIMS, New Delhi for providing TEM facility. We extend our sinecier thanks to Prof. Raj laxmi for help in interpretation of Electron microphotograph.

References

[1]. Ng J.C., Wang J. &Shraim A. 2003. A global health problem caused by arsenic from natural sources. Chemosphere, 52: 1353-1359.
[2]. Burzyński, M. &Kolano, E. In vivo and in vitro effects of copper and cadmium on the plasma membrane H+-ATPase from cucumber (Cucumis sativus L.) and maize (Zea mays L.) roots.Acta Physiol Plant (2003) 25: 39. https://doi.org/10.1007/s11738-003-0034-z
[3]. Hernández, J A et al. "Antioxidant systems and O(2)(.-)/H(2)O(2) production in the apoplast of pea leaves. Its relation with saltinduced necrotic lesions in minor veins ." Plant physiology vol. 127,3 (2001): 817-31. doi:10.1104/pp.010188
[4]. Smedley P.L. &Kinniburgh D.G. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem, 17: 517-568.
[5]. Mandal B.K. & Suzuki K.T. 2002. Arsenic around the world: a review. Talanta, 58: 201-235.
[6]. National Research Council. 2001. Arsenic in Drinking Water: 2001 Update. Washington, DC: The National Academies Press. https://doi.org/10.17226/10194
[7]. Florea, Ana-Maria et al. "Intracellular calcium disturbances induced by arsenic and its methylated derivatives in relation to genomic damage and apoptosis induction." Environmental health perspectives vol. 113,6 (2005): 659-64. doi:10.1289/ehp.7634
[8]. Qian W1, Liu J, Jin J, Ni W, Xu W. "Arsenic trioxide induces not only apoptosis but also autophagic cell death in leukemia cell lines via up-regulation of Beclin-1."Leuk Res. 2007 Mar;31(3):329-39. Epub 2006 Aug 1; doi:10.1016/j.leukres.2006.06.021
[9]. Karp Gerald. 2003, Cell and Molecular Biology Concept and Experiments, third edition update 2003, 232-237
[10]. Devi SR, Prasad MNV. Membrane lipid alterations in heavy metal exposed plants. In: Prasad MNV, Hagemeyer J, editors. Heavy metal stress in plants. From molecules to ecosystems. Berlin: Springer; 1999. pp. 99–116.
[11]. Nath A.,Priyanka, Kumari K., Anshu A. K., Singh A., Kumar S., Anand, P.,Priyanka, Sinha P. (2015) "Effect of Arsenic Toxicity in Testis of Swiss Albino Mice Leading to Low Testosterone and High MDA Level" , Int. J. Pharm. Sci. Rev. Res., 30(2), Pages: 69-73.ISSN: 0976-044X. IF- 2.1
[12]. Yukihiro Akao, Hiroko Yamada & Yoshihito Nakagawa (2000) Arsenic-Induced Apoptosis in Malignant Cells In vitro, Leukemia & Lymphoma, 37:1-2, 53-63, doi: 10.3109/10428190009057628
[13]. Kumar, Sanjay & Yedjou, Clement & Tchounwou, Paul. (2014). Abstract 2291: Arsenic trioxide induces cell cycle arrest, apoptosis and MAPKinase signaling cascade in acute promyelocytic leukemia cells. Cancer Research. 74. 2291-2291. 10.1158/1538-7445.AM2014-2291.
[14]. Deborah E. Keil, Jennifer Berger-Ritchie, Gwendolyn A. McMillin, Testing for Toxic Elements: A Focus on Arsenic, Cadmium, Lead, and Mercury, Laboratory Medicine, Volume 42, Issue 12, December 2011, Pages 735– 742, https://doi.org/10.1309/LMYKGU05BEPE7IAW.
[15]. Tchounwou, Paul &Yedjou, Clement & K Patlolla, Anita & Sutton, Dwayne. (2012). Heavy Metal Toxicity and the Environment. EXS. 101. 133-64. 10.1007/978-3-7643-8340-4_6.
[16]. Stephane Guillot, Laurent CharletBengal arsenic, an archive of Himalaya orogeny and paleohydrology. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2007 Oct; 42(12): 1785–1794. doi: 10.1080/10934520701566702
[17]. Nath A , Anshu A.K.*, Priyanka , Singh C K, Behera S and Singh J K, Reprotoxicity and genotoxicity by arsenic ensuing to male infertility in male musmusculus, International Journal of Pharmaceutical Sciences and Research, DOI: 10.13040/IJPSR.0975-8232.8(3).1153-59, 2017
[18]. Patlolla, Anita K et al. "Arsenic-induced biochemical and genotoxic effects and distribution in tissues of Sprague-Dawley rats." Microchemical journal : devoted to the application of microtechniques in all branches of science vol. 105 (2012): 101-107. doi:10.1016/j.microc.2012.08.013
[19]. Yong Qian, Vince Castranova, Xianglin Shi, New perspectives in arsenic-induced cell signal transduction,Journal of Inorganic Biochemistry,Volume 96, Issues 2–3,2003, doi.org/10.1016/S0162-0134(03)00235-6.
[20]. Andrew, Angeline S et al. "Arsenic exposure is associated with decreased DNA repair in vitro and in individuals exposed to drinking water arsenic." Environmental health perspectives vol. 114,8 (2006): 1193-8. doi:10.1289/ehp.9008
[21]. Abernathy, C O et al. "Arsenic: health effects, mechanisms of actions, and research issues." Environmental health perspectives vol. 107,7 (1999): 593-7. doi:10.1289/ehp.99107593
[22]. De Vos CHR, Bookum WMT, Vooijs R, Schat H, de Kok LJ. Effect of copper on fatty acid composition and peroxidation of lipids in the roots of copper-tolerant and -sensitive Silenecucubalus. Plant Physiology and Biochemistry. 1993;31:151–158.
[23]. Farooq, Muhammad Ahsan & Li, Lan & Ali, Dr. Basharat & Gill, Rafaqat Ali & Wang, Jian & Ali, Shafaqat & Gill, Bilal & Zhou, Weijun. (2015). Oxidative injury and antioxidant enzymes regulation in arsenic-exposed seedlings of four Brassica napus L. cultivars. Environmental Science and Pollution Research . Doi:10.1007/s11356-015-4269-

A.Nath. "Depolarization of Plasma Membrane Components Due To Arsenic Exposure during Spermiogenesis." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 13.6 (2019): 12-16.