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# Ameliorating Effect of Selenium against Arsenic Induced Male Reproductive Toxicity in Rats

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## ABSTRACT

**Oral exposure of humans by excess amounts of arsenic may cause disturbances of the reproductive system. In the present study, such exposure was modelled in rats, with the support of sperm principal parameters and histopathological observations. Male Sprague-Dawley rats were randomly divided into three groups where the group I was served as a normal control, group II was received sodium meta-arsenite as arsenic (10 mg/kg b.w/day) and a combination of sodium meta- arsenite and sodium selenite (3 mg/kg b.w/day) in group III. After 6 weeks, there was no significant change in testis weight and in total motility of all the three experimental groups, whereas, rapid moving spermatozoa, moderately moving spermatozoa and slow moving spermatozoa were significantly decreased in arsenic treated rats as compared to control rats. The other sperm principal parameters like progressiveness, average path velocity, straightness linear velocity (VSL), curvilinear velocity (VCL), straightness, linearity sperm head elongation ratio, area, linearity amplitude of lateral head department (ALH) and beat cross frequency (BCF) were found to be reduced in arsenic intoxicated rats. These results are not correlated with the histological studies. On oral administration of selenium ameliorated the adverse effects of arsenic as compared to arsenic alone treated rats. Our findings clearly demonstrate that administration of selenium could prevent some of the deleterious effects of arsenic in the testis.**

**(Key words : Arsenic, Spermatozoa, Selenium, Reactive oxygen species, Rats)**

## INTRODUCTION

Arsenic is ubiquitous in the environment as a result of natural and anthropogenic activities (ATSDR, 1993). Groundwater contamination with arsenic is reported to be the largest arsenic calamity in the World (Nordstrom, 2002; Lubin *et al*., 2007; Halem *et al*., 2009; Kar *et al*., 2010). Other vehicles of arsenic are agricultural applications such as insecticides, herbicides, fungicides, wood preservatives and by-products of fossil fuels (Akter *et al*., 2005; Das *et al*., 2004). A large number of studies have reported the associations between arsenic exposure and multiple adverse health effects, e.g. cancer, diabetes, skin diseases, cardiovascular system, male infertility and neurological disorders (Carrillo *et al*., 2014; Hsu *et al*., 2013; Bhattacharje *et al*., 2013; Tsuji *et al*., 2014; Tanrıkut *et al*., 2014).

The acute and chronic toxicities of arsenic are largely dependent on its chemical form and physical state. The trivalent form of arsenic is considered to be the most potent toxicant than pentavalent form, because of its higher affinity with sulfhydryl group. Since, it has electrophilic nature and capable of generating reactive oxygen species, oxidative stress is the major contributor for arsenic toxicity (Michael *et al*., 2005; Nandi *et al*., 2006).

Due to the high concentration of polyunsaturated fatty acids and low antioxidant capacity, male germ cells become susceptible to oxidative stress and reproductive failure. Enhanced production of reactive oxygen species (ROS) in the testis has the propensity to cause a significant alteration in testis physiology (Sabbir *et al*., 2011; Maa *et al*., 2008).

Arsenic has been designated as an endocrine disruptor (ED), mainly due to its adverse effect on the reproductive system such as impair male fertility, degrade semen quality and cause testicular degeneration, seminiferous tubular (ST) damage and ultimately, reproductive failure (Sarkar *et al*., 2003).

However, the supplementation of antioxidants can be useful to inhibit oxidative damage. Selenium is an essential dietary trace element, which acts as an antioxidant (Penglase *et al*., 2014). Testes and epididymis con-

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tain high concentrations of selenium indicating its vital role during spermatogenesis to improve semen quality. Some studies showed that selenium deficiency may lead to various reproductive disorders, e.g. seminiferous tubule degeneration, poor spermatozoa integrity, reduced numbers of spermatozoa within the seminiferous tubules, and reduced sperm motility (Messaoudi *et al*., 2010; Ursini *et al*., 1999). Moreover, selenium is an antagonist of arsenic.

In spite of few studies on the protective effects of selenium against the toxicity of metals in the male reproductive system of experimental animals (Said *et al*., 2010). To the best of our knowledge, no comprehensive study concerning the protective effect of selenium on reproductive toxicity of arsenic. Therefore, the present study was designed to investigate the ameliorating effects of selenium on arsenic induced reproductive toxicity in rats.

## MATERIALS AND METHODS

### Chemicals and Reagents

Selenium and sodium meta-arsenite were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals utilized in the present study were obtained from local firms, South Korea and were of analytical grade.

### Animals

Adult male Sprague Dawley rats (4 weeks old) were obtained from the Central Lab, Seoul, Korea and housed in solid bottom polypropylene cages under standard environmental conditions (12 h light/dark cycle; 50±10% humidity; temperature 23±2℃). Commercial pellet diet and water were fed *ad libitum.* The care and treatment of rats were in accordance with the guidelines established by the Korean National Institute of Health at the Korean Academy of Medical Sciences and were approved by the Institutional Animal Care and Use Committee (IACUC) of Konkuk University (KU-14308).

### Preparation and Administration of Arsenic and Selenium

Arsenic and selenium dose selection in the present study was based on the previous reports (Messarah *et al*., 2012; Sarkozi *et al*., 2012). Arsenic as sodium meta-arsenite at a dose of 10 mg/kg b.w/day was dissolved in water and treated orally. Selenium as sodium selenite was dissolved in water and each rat received daily 1 mL at a dose of 3 mg/kg b.w/day administrated orally just after exposure to arsenic by intragastric intubation throughout the experimental period.

### Experimental Time Line

The rats were randomly divided into three groups of six rats in each group. Group I: Normal control rats, Group II: Normal rats received arsenic as sodium meta-arsenite and Group III: Normal rats received arsenic with co-administration of selenium.

At the end of experimental period, rats were anesthetized by intraperitoneal injection of 1 mL of 4 % avertin and epididymis and testis were dissected out, subjected for sperm quality and histology studies respectively.

### Measurement of Sperm Motility, Velocity, Movements and Morphology

After the animal sacrifice, a cauda epididymis was pricked few times with pointed forceps, and sperm were released into 2 mL of Hanks balanced salt solution (phenol red free) at 37℃. The solution containing the sperms was centrifuged at 1,000 rpm for 3 min. 20 μL aliquot spermatozoa were positioned into a pre-warmed slide glass and examined by computer assisted semen analysis (CASA, Hamilton Thorne, Beverly, MA, USA) system. Percentage of motile sperm, movement velocity such as rapid, medium, slow, and static, and movement characteristics such as progressiveness, average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), ALH, BCF, sperm head elongation, and area rate were measured for normal control and experimental rats.

### Histopathological Examination of Testis

For qualitative analysis of testis histology, the tissue samples were fixed for 48 h in bouin solution and dehydrated by passing successfully in different mixture of ethyl alcohol and water, cleaned in xylene and embedded in paraffin. Sections of the testis tissues (5~6 μm thick) were prepared by using a rotary microtome and stained with hematoxylin and eosin dye, which was mounted in a neutral deparaffined xylene medium for microscopical observations.

### Statistical Analysis

The data for various sperm functional parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p<0.05$ (Duncan 1957).

## RESULTS

### Effect of Selenium and Arsenic on Testis Weight and Sperm Motility

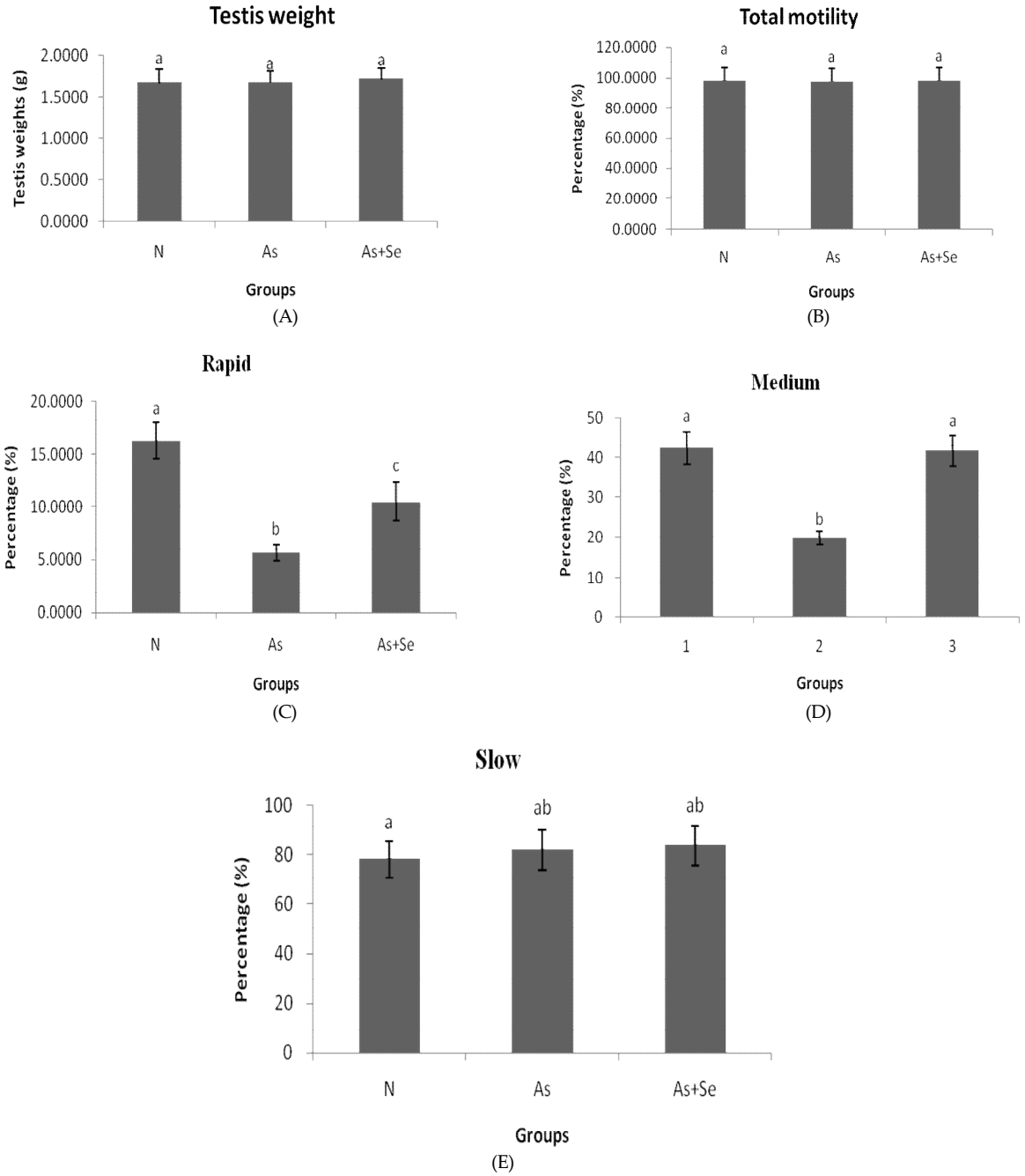



**Fig. 1. Changes in the testis weight and sperm motility in normal control and experimental rats.** (A) testis weight and (B) total motility. (C) spermatozoa with rapid, (D) medium and (E) slow motility. N: normal control, As: Arsenic, As + Se: arsenic and selenium. All the values are expressed as means±S.D. of 6 rats in each group. Values not sharing a common superscript letter $^{a\sim c}$ differ significantly at $p<0.05$.

Fig. 1 shows the testis weight and sperm motility of normal control and experimental rats. There were no significant changes in the testis weight and total sperm motility in all the experimental groups. But, the indicators of other motility parameters such as rapid moving sperm and moderately moving sperm were significantly decreased in arsenic alone treated rats as compared to the control rats. Administration of selenium at a dose of 3 mg/kg b.w to arsenic intoxicated rats significantly increased the rapid moving sperm and moderately moving sperm to near normal. In contrast to the above results, the slow moving sperm were sig-

nificantly increased in selenium along with arsenic treated groups than the other two groups.

**Effect of Selenium and Arsenic on Detailed Motion Characteristics**

Fig. 2 depicts the changes in the progressiveness, average path velocity, straightness linear velocity (VSL) and curvilinear velocity (VCL) of control and experimental rats. The detailed motion characteristics were significantly decreased in arsenic alone treated rats when compared with that control rats. Treatment of selenium alleviated the negative effects of arsenic as compared to arsenic alone treated rats.

**Changes in Motion and Morphological Index**

Fig. 3 illustrates the effect of selenium on the changes of straightness, linearity, sperm head elongation ratio and area in arsenic intoxicated rats. The motion and morphological index were significantly reduced in toxicity of sperm when compared with control rats. Selenium exerts its positive effect in arsenic intoxicated rats via the improvement of the changes in motion and morphological index.

**Effect of Selenium and Arsenic on ALH and BCF**

As shown in Fig 4, linearity amplitude of lateral head department (ALH) and beat cross frequency (BCF) were significantly decreased in arsenic alone treated rats. Upon oral administration of selenium along with arsenic significantly increased the levels of linearity amplitude of lateral head department (ALH) and beat cross frequency (BCF) when compared to arsenic alone treated rats.

**Effect of Selenium and Arsenic on Histopathological Studies**

There were no pathological changes observed in histological structure such as cell death, degeneration, or

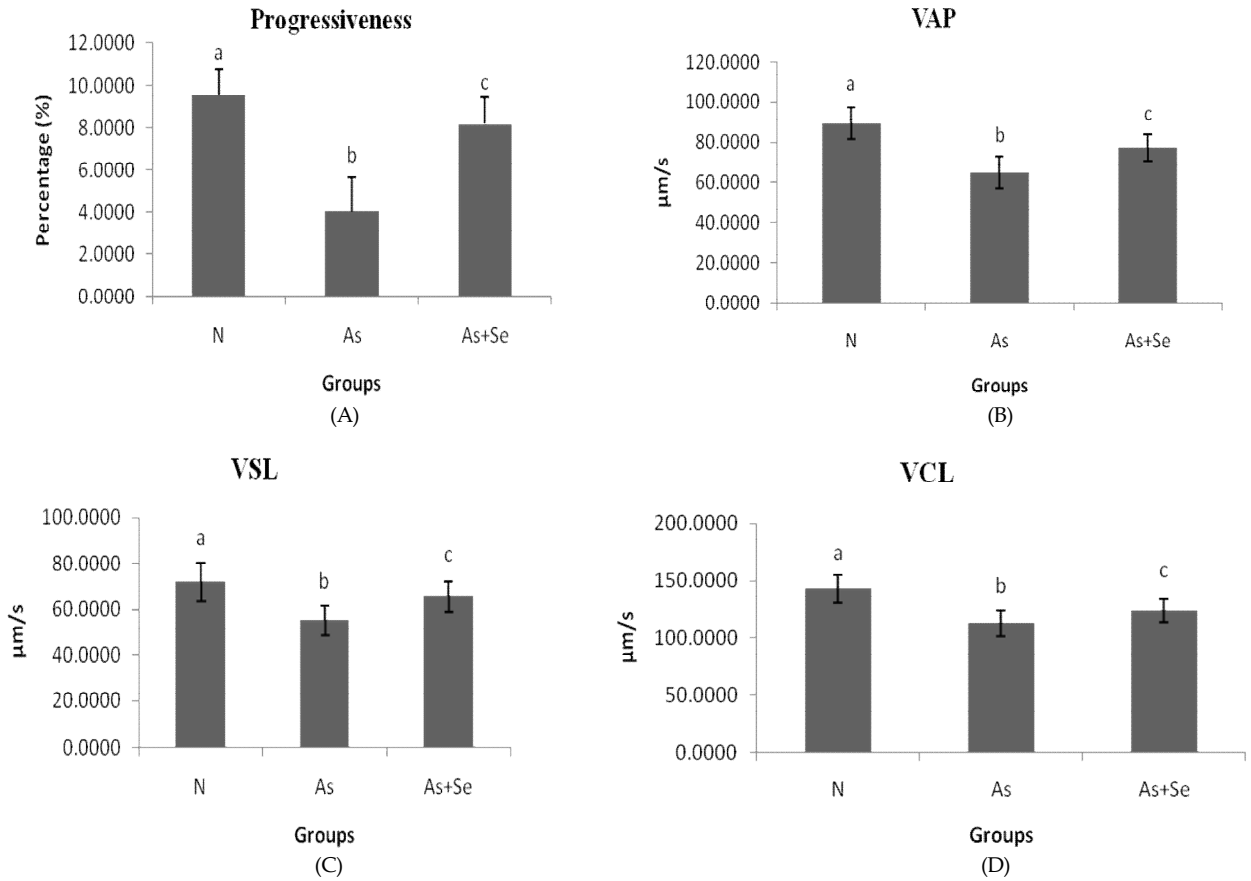



**Fig. 2. Changes in the detailed motion characteristics in normal control and experimental rats.** (A) progressiveness, (B) VAP (average path velocity), (C) VSL (straight line velocity) and (D) VCL (curve linear velocity). N: normal control, As: arsenic, As + Se: arsenic and selenium. All the values are expressed as means±S.D. of 6 rats in each group. Values not sharing a common superscript letter $^{a\sim c}$ differ significantly at $p<0.05$.

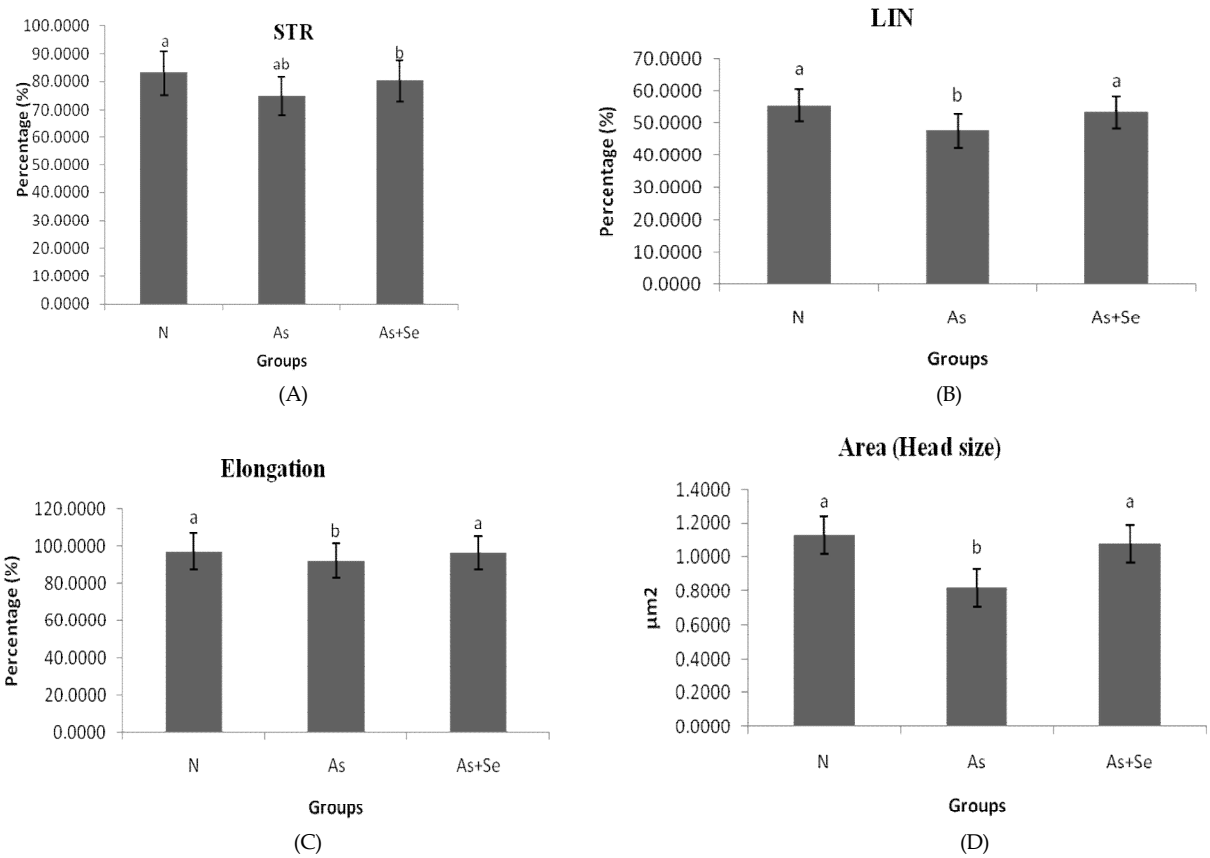



**Fig. 3. Changes in the motion and morphological index between the control and experimental rats.** (A) STR (straightness), (B) LIN (linearity), (C) elongation and (D) area (head size). N: normal control, As: arsenic, As + Se: arsenic and selenium. All the values are expressed as means±S.D. of 6 rats in each group. Values not sharing a common superscript letter. $^{a\sim c}$ differ significantly at $p<0.05$.

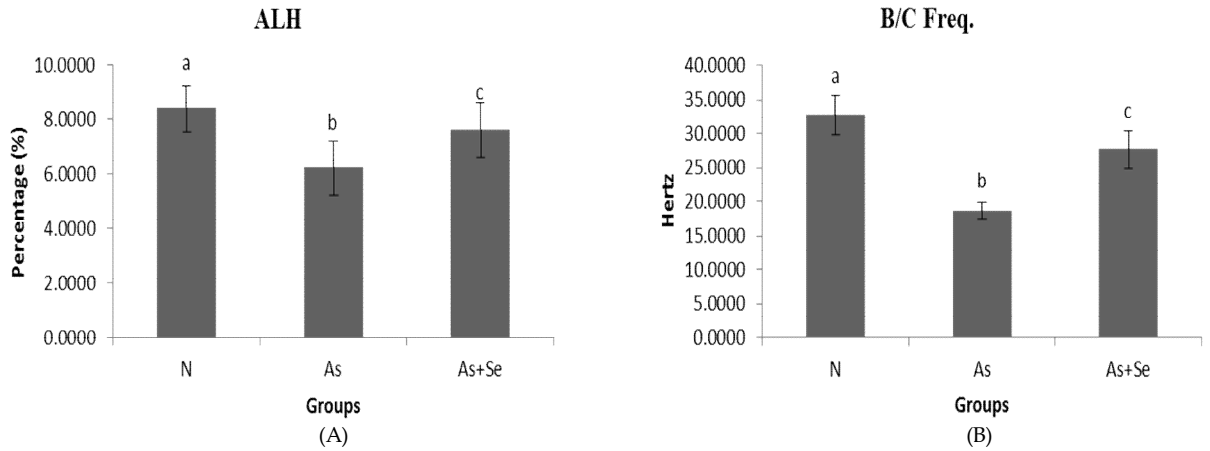
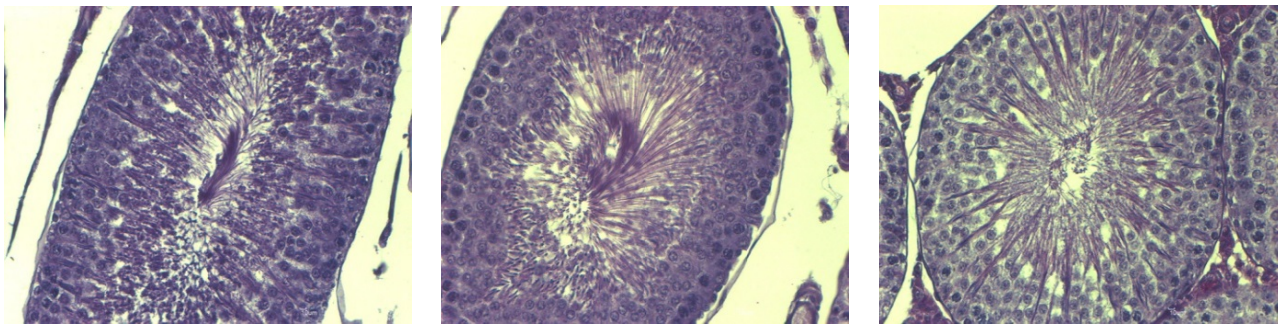



**Fig. 4. Changes in ALH and BCF in normal control and experimental rats.** (A) ALH (lateral head department) and (B) BCF (beat cross frequency). N: normal control, As: arsenic, As + Se: arsenic and selenium. All the values are expressed as means±S.D. of 6 rats in each group. Values not sharing a common superscript letter. $^{a\sim c}$ differ significantly at $p<0.05$.

hypertrophy in testis exposed to arsenic. All experimental groups showed normal histological structure of the seminiferous tubules and normal spermatogenesis as shown in Fig. 5.



(a) (b) (c)

**Fig. 5. Hematoxylin and eosin-stained sections of rat testis.** (A) normal control rat testis, (B) arsenic (10 mg/kg) treated rat testis, and (C) arsenic + selenium (3 mg/kg) treated rat testis.

## DISCUSSION

To date, there is a little information available on arsenic induced male infertility in testis. Besides, the potential ability of selenium to attenuate reproductive toxicity has not yet been investigated. Animal models provide an attractive alternate for humans to carry out the toxicological study involved in spermatogenesis (Elangovan *et al*., 2006). We observed the testis weight of rats exposed to arsenic did not show any significant change, indicating that the general condition of the rats was within normal range. Sperm motility, sperm motion characteristics parameters such as VCL, VAP, VSL, linearity (LIN), and straightness (STR) has been reliable parameters to evaluate the sperm quality (Won Young Lee *et al*., 2014). A statistically significant decrease in sperm motility was observed in arsenic intoxicated rats under the present set of experimental conditions. The decrease in sperm motility in arsenic treated rats might be due to the toxic effect of arsenic on the flagellum, the important machinery for sperm motility (Sharpe *et al*., 1992). In addition to this, the toxicity of arsenic was further justified by a decrease in detailed motion characteristics, morphological index, ALH and BCF through CASA analysis.

In our present study, detailed motion characteristics were found to be significantly decreased in arsenic groups as compared to control groups. However, sperm perturbations are not seem to be correlated with the histological studies in the testis. There were no morphological degenerative changes observed at the tissue level in histological sections would suggest that onset of tissue damage is delay, because of male germ cells are very sensitive and attractive to arsenic.

Increasing evidence has showed that mature sperms are enriched with polyunsaturated fatty acids in which the effect of oxidative stress is prominent (Kaur and Bansal, 2004). Increased sperm membrane lipid peroxidation (LPO) induced by arsenic has been shown to impede sperm progress motility and increase percent total sperm abnormalities as well as cause a dramatic loss in the fertilizing potential of sperm (Kao *et al*., 2008). The decreased sperm functional parameters observed with arsenic may be a direct outcome of increased and consistent LPO and altered membrane properties that lead to germ cell death at different stages of development (Cummins *et al*., 1994). Our results indicated that non-pathological condition coupled with abnormal in sperm quality parameters in arsenic-treated rats as compared to control rats.

Moreover, mammalian sperm contain a large amount of thiol-rich protamines in their nuclear chromatin, and sulfhydryl group in the sperm flagellum, which are thought to be involved in stability and in the maintenance of motility. As arsenic is known to be a thiol-inhibiting substance and ROS creator, the decrease in motility and its detailed features might be due to high concentration of arsenic in the epididymis where the sperm undergoes the process of maturation and acquires motility. Our results corroborated with previous findings which demonstrated that arsenic decreased sperm counts and motility in mice following oral exposure to arsenic (Pant *et al*., 2004; Garima and Madhu, 2012).

Accordingly, among the main approaches used to ameliorate arsenic induced male infertility is the use of agents with powerful antioxidant properties. The effect of selenium on spermatogenesis has been demonstrated in animal experiments (Ren *et al*., 2012; Yue *et al*., 2010; Shia *et al*., 2014). However, reports on the relationships between selenium and arsenic induced infertility were inconsistent. Recent studies have reported that selenium showed significant protective effects against liver damage induced by arsenic (Messarah *et al*., 2012).

In this study, we observed the possible protective

role of selenium against male infertility in arsenic intoxicated rats. Several mechanisms could be operating in the protective action of selenium, which could result, for example, in changed absorption of the arsenic or in a change in their action and distribution in the organism and within target organs (Sah *et al*., 2013; Pei *et al*., 2008). The protective mechanisms of selenium compound and selanoproteins are well known for their ability to scavenge ROS and enhancement antioxidant system in arsenic induced tissue damage (Rana and Verma, 1997). Our results were complementary for previous studies in line with the hepatoprotective role of selenium against arsenic toxicity.

## CONCLUSION

In conclusion, this study demonstrates that exposure to arsenic provoked male infertility and did not have any pathological changes in testis tissue as reflected by the normal histology. However, selenium treatment could protect testis against arsenic toxicity by improvement of semen quality. Further, biochemical investigations are warranted to ascertain the precise mechanisms of its action and extrapolating the data to humans. On the basis of this study, it should be taken into consideration that the nutritional supplementation of selenium may act as a protective agent against arsenic induced male infertility.

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## REFERENCES

1. Akter KF, Owens G, Davey DE, Naidu R (2005): Arsenic speciation and toxicityin biological systems. Rev Environ Contam Toxicol 184:97-149.
2. Agency for Toxic Substances and Disease Registry (ATSDR) (1993): Toxicological profile of Arsenic. US Public Health Services, Atlanta.
3. Bhattacharjee P, Dasa N, Chatterjee D, Banerjee A, Das JK, Basu S, Banerjee S, Majumder P, Goswami P, Giri AK (2013): Association of NALP2 polymorphism with arsenic induced skin lesions and other health effects. Mutat Res 755:1- 5.
4. Carrillo LL, Hernandez-Ramirez RU, Gandolfi JA, Ornelas-Aguirrec JM, Sanchez LT, Mariano E (2014): Arsenic methylation capacity is associated with breast cancer innorthern Mexico Cebrian. Toxicol Appl Pharm 280:5359.
5. Cummins JM, Jequier AM, Kan R (1994): Molecular biology of human male infertility: links with aging, mitochondrial genetics, and oxidative stress? Mol Reprod Dev 37:345-362.
6. Das HK, Mitra AK, Sengupta PK, Hossain A, Islam F, Rabbani GH (2004): Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environ Int 30:383-387.
7. Duncan BD (1957): Multiple range tests for correlated and heteroscedastic means. Biometrics 13:359-364.
8. Elangovan N, Chiou TJ, Tzeng WF, Chu ST, (2006): Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. Toxicology 222:60-70.
9. Garima S, Madhu K (2012): Antioxidant and modulatory role of Chlorophytumborivilianum against arsenic induced testicular impairment. J Environ Sci 24:2159-2165.
10. Halem VD, Bakker SA, Amy GL, van Dijk JC (2009): Arsenic in drinking water: a worldwide water quality concern for water supply companies. Drinking Water Engineering. Science 2:29-34.
11. Hsu LI, Wang YH, Chiou HY, Wud MM, Yang TY, Chen YH, Tseng CH, Chen CJ (2013): The association of diabetes mellitus with subsequent internal cancers in the arsenic-exposed area of Taiwan. J Asian Earth Sci 73:452-459.
12. Kaur P, Bansal MP (2004): Effect of experimental oxidative stress on steroidogenesis and DNA damage in mouse testis. J Biomed Sci 11:391-397.
13. Kao SH, Chao HT, Chen HW, Hwang TI, Liao TL, Wei YH (2008): Increase of oxidative stress in human sperm with lower motility. Fertil Steril 89: 1183-1190.
14. Kar S, Maity JP, Jean JS, Liu CC, Nath B, Yang H, Bundschuh J (2010): Arsenic enriched aquifers: occurrences and mobilization of arsenic in groundwater of Ganges Delta Plain, Barasat, West Bengal, India. Appl Geochem 25:1804-1814.
15. Lubin JH, Beane F LE, Cantor KP (2007): Inorganic arsenic in drinking water: an evolving public health concern. Journal of National Cancer Institute 99: 906-907.
16. Maa A, Yang X, Wang Z, Shi D, Chen Y (2008): Adult exposure to diethylstilbestrol induces spermatogenic cell apoptosis *in vivo* through increased oxidative stress in male hamster. Reprod Toxicol 25:

367-373.
17. Messarah M, Klibet F, Boumendjel A, Cherif A, Bouzerna N, Boulakoud MS, Fekic AE (2012): Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. Environ Toxicol Phar 64:167-174.
18. Messaoudi I, Banni M, Said L, Said K, Kerkeni A (2010): Involvement of selenoprotein P and GPx4 gene expression in cadmium-induced testicular pathophysiology in rat. Chem Biol Interact 188:94-101.
19. Michael F, Hughes T, Devesa V, Blakely M, Adair, Styblo M, Elaina M, Kenyon, David J, Thomas (2005): Tissue dosimetry, metabolism and excretion of pentavalent and trivalent monomethylated arsenic in mice after oral administration. Toxicol Appl Pharm 208:186-197.
20. Nandi D, Patra RC, Swarup D (2006): Oxidative stress indices and plasma biochemical parameters during oral exposure to arsenic in rats. Food Chem Toxicol 44: 1579–1584.
21. Nordstrom DK (2002): Worldwide occurrence of arsenic in groundwater. Science 296: 2143-2145.
22. Penglase S, Hamre K, Ellingsen S (2014): Selenium prevents down regulation of antioxidant selenoprotein genes by methyl mercury. Free Radical Bio Med 75:95-104.
23. Pant N, Murthy RC, Srivastava SP (2004): Male reproductive toxicity of sodium arsenite in mice. Hum Exp Toxico 23: 399-403.
24. Pei ZHQ, Sun G, Zhang S, Liang J, Gao Yi, Zhang X (2008): Low selenium status affects arsenic metabolites in an arsenic exposed population with skin lesions. Clinica. Chimica Acta 387:139-144.
25. Rana SV, Verma S (1997): Protective effects of GSH, a-tocopherol, and selenium on lipid-peroxidation in liver and kidney of copper fed rats. Bull Environ Contam Toxicol 59:152-158.
26. Ren X, Wang G, Xu D, Luo K, Liu Y, Zhong Y, Cai Y (2012): The protection of selenium on cadmium-induced inhibition of spermatogenesis via activating testosterone synthesis in mice. Food Chem Toxicol 50:3521-3529.
27. Sabbir K, Tauseef A, Parekh CV, Trivedi PP, Kushwaha S, Jena G (2011): Investigation on sodium valproate induced germ cell damage, oxidative stressand genotoxicity in male Swiss mice. Reprod Toxicol 32:385-394.
28. Said L, Banni M, Kerkeni A, Said K, Messaoudi I (2010): Influence of combined treatment with zinc and selenium on cadmium induced testicular pathophysiology in rat. Food Chem Toxicol 48:2759-2765.
29. Sarkar M, Chaudhuri GR, Chattopadhyay A, Biswas NM (2003): Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J of Androl 5:27-31.
30. Sarkozi K, Mate Z, Vezer T (2012): General and behavioral toxicological effects of subchronic inorganic arsenic and fluoride treatment in adult Wistar rats. Eur Chem Bull 1:531-534.
31. Sah S, Vandenberg A, Smits J (2013): Treating chronic arsenic toxicity with high selenium lentil diets. Toxicol Appl Pharm 272: 256-262.
32. Shia L, Zhao H, Ren Y, Yaoa X, Songa R, Yuea W (2014): Effects of different levels of dietary selenium on the proliferation of spermatogonial stem cells and antioxidant status in testis of roosters. College Anim Reprod Sci (Article in press).
33. Sharpe RM, Maddocks S, Millar M, Saunders PTK, Kerr JB, Mckinnell C, (1992): Testosterone and spermatogenesis: identification of stage dependent, androgen-regulated proteins secreted by adult rat seminiferous tubules. J Androl 13: 172-184.
34. Tanrıkut A, Karaer O, Celik E, Celik B, Otlu E, Yilmaz O (2014): Role of endometrial concentrations of heavy metals (cadmium, lead, mercury and arsenic) in the aetiology of unexplained infertility. Ozgul Eur J Obstet Gyn R B 179:187-190.
35. Tsuji JS, Perez V, Garry MR, Alexander DD (2014): Association of low-level arsenic exposure in drinking water with cardiovascular disease: A systematic review and risk assessment. Toxicology 323:78-94.
36. Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J, Flohe L (1999): Dual function of the selenoprotein PHGPx during sperm maturation. Science 285:1393-1396.
37. Won Young Lee, Ran Lee, Hee Chan Kim, Kyung Hoon Lee, Xiang Shun Cui, Nam Hyung Kim, Sang Hyun Kim, Joo Lee, Sang Jun Uhm, Min Jung Yoon, Hyuk Song (2014): Pig spermatozoa defect in acrosome formation caused poor motion parameters and fertilization failure through artificial insemination and *in vitro* fertilization. Asian-Australas J Anim Sci 10:1417-1425.
38. Yue LSW, Zhang C, Ren Y, Zhua X, Wang Q, Liguangshi, Lei F (2010): Effects of maternal and dietary selenium (Se-enriched yeast) on oxidative status in testis and apoptosis of germ cells during spermatogenesis of their offspring in goats. Anim Reprod Sci 119: 212-218.

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