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Prevention of Arsenic Induced Testicular Oxidative Stress and DNA Damage by Coenzyme Q10 and Vitamin E in Swiss Albino Mice

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Abstract

Arsenic toxicity has become one of the major public health problems in certain parts of the world. Thus, it is rational to find out a suitable compound to prevent arsenic-induced toxicity for clinical usage. Hence, the Coenzyme Q10 and Vitamin E were tested against arsenic-induced testicular oxidative stress and DNA damage. The mice were divided into five groups, animals of the four groups were exposed to 136 ppm arsenic via drinking water for 30 days. Subsequently, animals of three groups were treated with Vitamin E (50 mg/kg b.wt.), Coenzyme Q10 (10 mg/kg b.wt.), and their combination for 30 days, and animals of the 4th group were maintained without antioxidant treatment. The animals of the 5th group (without any treatment) served as control. Thereafter, blood was collected, for DNA damage study, and testis dissected out to assess oxidative stress. The body and testis weight gain was lower in the arsenic subjected group compared to the control group whereas antioxidants (Vitamin E, Coenzyme Q10, and combination) treatment checks to some extent this decline. Biochemical data indicated that lipid peroxidation level was higher while reduced glutathione, total thiol, superoxide dismutase, and total protein level was significantly lesser in the arsenic exposed group compared to the control group, and antioxidants treatment diminished arsenic-induced these alterations to some extent. Arsenic induces DNA damage in the blood cells of mice by displaying a significantly lower head DNA percentage and a higher level of tail DNA percentage, tail length, tail moment, while Vitamin E, Coenzyme Q10, and combination were able to lower these changes. The data further revealed that the combined treatment of Vitamin E, Coenzyme Q10 is more effective than the treatment of these antioxidants individually.

Keywords: Arsenic, Vitamin E, Testis, Coenzyme Q10, DNA damage

Introduction

Chronic arsenic exposure from arsenic-contaminated drinking water, foodstuffs can cause cancer, skin lesions, etc and linked with cardiovascular disease, diabetes, etc., prenatal and early childhood exposure related to adverse influences on cognitive development and elevated mortality in young adults and it is present naturally at high concentrations in the groundwater of many countries (1). Recently, Sharma and Kumar (2019) reviewed that chronic arsenic (As) exposure affects both the central and peripheral nervous system and causes depression, memory impairment, difficulty in problem-solving, impact upon body coordination, etc. They also stated that various prenatal and postnatal investigations with reverence to exposure indicated that the developing offspring and young children are vulnerable to arsenic exposure (2). A population of more than 140 million people in fifty countries is using drinking water comprising arsenic at levels of more than the prescribed WHO provisional guideline value of 10 µg/L (3). Thus, a significant proportion of the world's population is exposed to a considerable amount of arsenic through drinking water. Earlier, Wang et al.(2006) reported that arsenic is a reproductive toxicant for humans and brings malformations, especially neural tube defects in animals and murine malformations that occurred with exposure to a higher dosage of inorganic arsenic in early gestation (4). Later, Kim and Kim (2015), reported that inorganic arsenic induces discrepancies in spermatogenesis, declines of testosterone and gonadotropins levels, and interruptions of steroidogenesis (5).

Alterations in androgenic activity, reduced sperm count, motility, and disparity of hormonal level in male mice with exposure to arsenic were also reported (6, 7). In addition, arsenic has been implicated in DNA repair inhibition, cell cycle interruption, and ubiquitin (a protein) dysregulation, all these changes exaggerate the DNA damage (8).

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Several synthetic and natural compounds were tested against the toxicity of arsenic by several investigators worldwide.

Bhattacharya (2017) reviewed data on medicinal plants and natural compounds against arsenic-induced toxicity and reported that 34 medicinal plants and 14 natural compounds showed noteworthy protection against arsenic toxicity, generally in preclinical trials and a few are in clinical investigations (9). However, a suitable more protective compound is still needed for the prevention of arsenic-induced toxicity for clinical usage.

Thus, Coenzyme Q10 and Vitamin E and their combination were tested against arsenic-induced testicular oxidative stress and DNA damage in mouse blood cells. Coenzyme Q10 (CoQ10) is reported to work as a sturdy antioxidant, avoids the commencement and dissemination of lipid peroxidation in cellular bio-membranes, and supports rejuvenation of α-tocopherol (10-12). The CoQ10 was reported to effectively protect testicular injury in mice induced by magnetic field radiation (13). Vitamin E (Vit E) is a family of lipid-soluble vitamins, of which alpha-tocopherol is the most potent Vit E, has been reported to act as an antioxidant in cells, distressing the proliferation of lipid peroxidation in the plasma membrane, and preserving membrane integrity (14). Several experimental studies were conducted to assess the protective properties of Vit E against various toxicants (15, 16). Both CoQ10 and Vit E were proved to be effective against heavy metal cadmium-induced testicular toxicity in mice (17). Recently, a positive role of Vit E and CoQ10 against the toxicity of arsenic in the brain of mice was also observed from this laboratory by Sharma et al. 2018 (18). Hence, this study was conducted to evaluate the role of Vit E and CoQ10 alone or in combination against arsenic-induced testicular oxidative stress and DNA damage in mouse blood cells.

Materials and Methods

Sodium meta-arsenite, Vitamin E, and Coenzyme Q10 were procured from Sigma Aldrich St Louis, USA. Adult Swiss albino mice (6-8 weeks; 22±3 gm.) were obtained from the Zydus Research Center, Ahmedabad, and kept as per the procedures of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate change (MoEF&CC), Govt. of India. They were acclimatized for a week in an Animal house facility, National Institute of Occupational Health, Ahmedabad. The mice were kept in polypropylene cages and maintained under controlled temperature, humidity, and light and dark cycle (12hr light: dark cycle). The experimental plan was prepared by including five groups of mice having a minimum of 6 animals/group with or without arsenic treatment at the dose of 136 ppm for 30 days subsequently animals of three groups were treated with Vit E and CoQ10 and their combinations for 30 days. This paper is a part of a major study entitled "Neuroprotective role of combination of Vitamin E and Coenzyme Q10 against arsenic intoxicated mice" for which ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) of NIOH, Ahmedabad.

Dose preparation and mode of exposure

Arsenic Dose: Sodium meta-arsenite (NaAsO2) was dissolved in double-distilled water. The dose of arsenic i.e., 136 ppm was prepared every alternate day to prevent oxidation by dissolving an appropriate quantity of arsenic in double-distilled water. The mice were treated with arsenic by sipping water. The dose was based on LD 50 of sodium arsenite i.e. 41 mg/kg bwt. orally in rats (Sigma Aldrich, Safety data sheet, Revision, 30.03.2016).

Antioxidant Dose: The required quantity of both the antioxidants Vit E (50 mg/kg b. wt.) and CoQ10 (10 mg/kg b.wt.) were weighed separately and dissolved in 1% of aqueous tween-80. The antioxidants were provided to three groups of mice(Vit E and CoQ10 individually and in combination) intraperitoneally daily for 30 days of post-arsenic treatment. A separate group of arsenic-treated mice was also maintained without antioxidant treatment. A control group was also maintained on drinking water for comparison purposes.

Biochemical Estimations to Assess Oxidative Stress

During the treatment, body weight was taken daily using electronic balance to observe arsenic-induced changes in body weight. After the scheduled treatment, blood was collected through retro-orbital sinus to assess DNA damage by comet assay, and animals were euthanized using CO_2 gas in a glass chamber and were sacrificed. Testis was dissected out cleanly, washed, blotted, and weighed. Testis was homogenized in phosphate buffer (0.1 M; pH- 7) with 0.5% Triton X-100, centrifuged at 5000 rpm at 4ºC (Sigma 3-18K) and the supernatant was taken in different aliquots to measure the following biochemical parameters:

Lipid Peroxidation (LPO): The method of Buege and Aust, (1978) was used for the determination of the Malondialdehyde (MDA) level in testis homogenate (19).

Reduced glutathione (GSH): Ellman's reagent was reduced by sulphydryl group of the glutathione to 2-nitro-5 mercaptobenzoic acid which has a characteristic yellow color and it was measured at 412 nm (20).

Total thiol (TT): The free sulphydryl (-SH) groups in testis homogenate were reacted with DTNB (5',5' dithiobis-2- nitro benzoic acid) to form a colored product and the same was measured at 412 nm (21).

Superoxide dismutase (SOD): Superoxide anion radical is involved in the auto-oxidation of pyrogallol. At alkaline pH, superoxide dismutase superoxide radical and thereby inhibiting the auto-oxidation of pyrogallol which was measured at 429 nm (22, 23).

Total protein level: The sample containing protein when treated with phenol reagent of Folin-Ciocalteu, a deep blue color develops which was measured at 660nm (24).

DNA Damage

The Single Cell Gel Electrophoresis (SCGE), also known as comet assay was performed for the detection of DNA damage in mouse blood cells using a standard procedure (25). A minimum of 100 cells was scored in each animal for the determination of DNA damage.

Data analysis

The data were calculated and represented as Mean±SE in table-1 and figure 2-8. The significant level between control, arsenic-treated and antioxidant protected groups was calculated by using one-way ANOVA test and intergroup comparison was made by post-hoc analysis. The software used for data analysis was SPSS-16.0. The observation and data analysis of comet assay

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was done by using Comet assay software project (CASP) software by fluorescence microscopy (Leica, Germany).

The data were presented in table 1 and representative photographs of intact DNA, low, moderate, and high DNA damage are depicted in Figures 1-A, B, C, D, respectively.

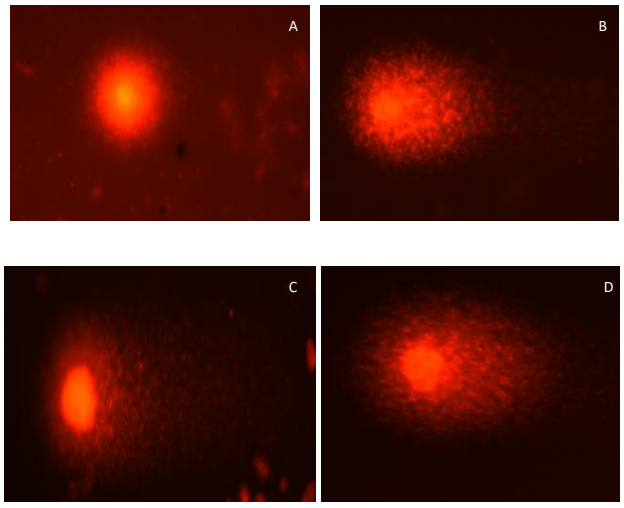

Figure 1. Representative figures of DNA damage in blood cells of mouse control and arsenic-treated groups. A: Intact cell / No DNA damage; B- Mild DNA damage; C- Moderate DNA damage; D- High DNA damage

Results

Bodyweight changes in arsenic-treated and antioxidant protected groups:

A statistically significantly lower mean body weight was observed in the arsenic-treated group compared to the control group after completion of scheduled treatment tenure. The body weight was more in all the three antioxidant (Vit E, CoQ10, and their combination) protected groups as compared to the only arsenic-treated group. However, the body weight gain was statistically significantly more in the combination of antioxidant (Vit E, CoQ10) protected group with respect to only arsenic-treated group (Figure 2).

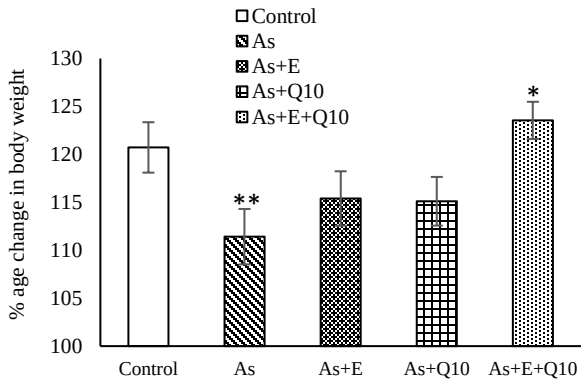

Figure 2. Arsenic induced body weight changes of mouse and its modification by vitamin E and Coenzyme Q10. p<0.05. * Compared to the arsenic-treated group; **Compared to control group

Mouse testis weight changes in arsenic-treated and antioxidant protected groups

A decline in testis weight was observed in arsenic exposed the group with respect to the control group. Whereas testis weight was higher in all the three antioxidant protected groups as compared to only the arsenic treated group. Further, the testis weight was more in the combination of the antioxidant-protected group as compared to the individual antioxidant-treated group. However, all these alterations in testis weight of antioxidant protected groups were statistically insignificant with respect to arsenic-treated and control groups (Figure 3).

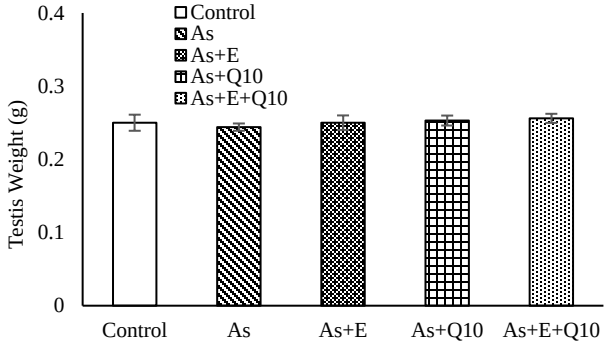

Figure 3. Arsenic induced mouse testis weight changes and its modification by Vitamin E and Coenzyme Q10.

LPO level in arsenic-treated and antioxidant protected groups

The level of MDA in testicular tissue was found to be significantly elevated in the arsenic-treated group as compared to without arsenic treated group namely control. While the MDA level was lower in all three antioxidant protected groups with respect to only the arsenic-treated group. A significantly lower level of MDA was found in CoQ10 as well as in the combination of CoQ10 and Vit E protected group with respect to arsenic-treated group (Figure 4). The level of MDA was also lower in Vitamin E protected group compared to the only arsenic exposed group but the difference between the two groups was statistically insignificant.

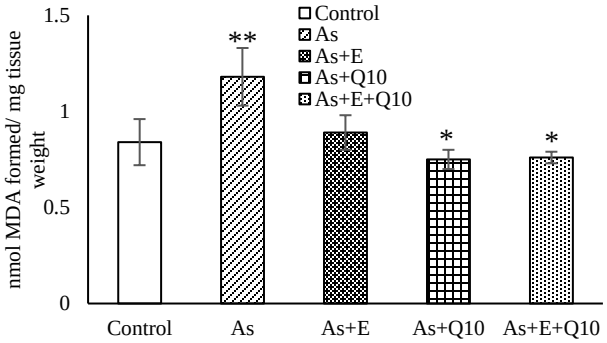

Figure 4. Arsenic induced alteration in MDA level in testicular tissue and its modification by Vitamin E and Coenzyme Q10. p<0.05.
*** Compared to the arsenic-treated group; **Compared to the control group**

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GSH level in arsenic-treated and antioxidant protected groups

The GSH level in testicular tissue was found to be significantly lower in the arsenic intoxicated group with respect to the control group. GSH level was higher in all the three antioxidant-treated groups as compared to the arsenic exposed group. The GSH level was statistically significantly higher in the combination of the antioxidant protected group while the level was non significantly higher in individual antioxidant protected groups with respect to only the arsenic exposed group (Figure 5). However, the alterations among the individual antioxidant protected groups were insignificant with respect to the arsenic-treated group.

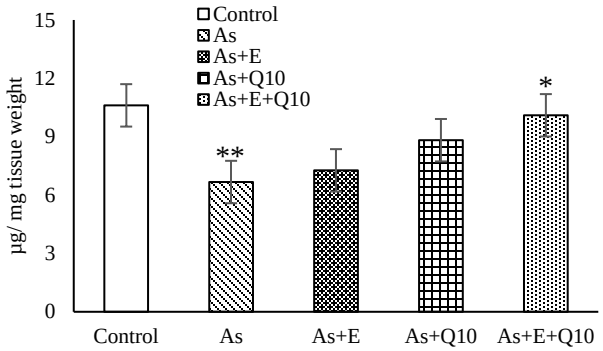

Figure 5. Arsenic induced changes in testicular GSH level and its modification by Vitamin E and Coenzyme Q10. p<0.05.*Compared to the arsenic-treated group; **Compared to the control group

Total thiol level in arsenic-treated and antioxidant protected groups

Total thiol level in testicular tissue was observed to be lower (statistically significant) in the arsenic-treated group compared to the control or without any treatment group. While thiol levels were non-significantly higher in antioxidant protected groups (Vit E and CoQ10) individually with respect to arsenic exposed group. Further, the total thiol level was significantly higher in the combination of the antioxidant protected group with respect to only arsenic exposed groups (Figure 6).

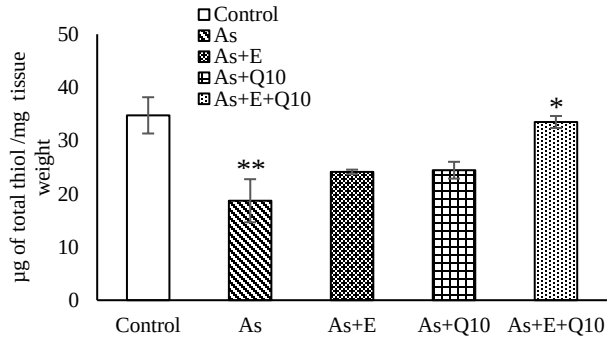

Figure 6. Arsenic induced alteration in testicular total thiol level and its modification in Vitamin E and Coenzyme Q10. p<0.05. *Compared to the arsenic-treated group; **Compared to the control group

Superoxide dismutase (SOD) activity in arsenic-treated and antioxidant protected groups

SOD activity in testicular tissue was declined significantly statistically in the arsenic exposed group with respect to the control group while the SOD activity was higher in all the three antioxidant protected groups with respect to the only arsenic exposed group which was statistically significantly higher only in the combination of the antioxidant protected group as compared to only arsenic-treated group (Figure 7).

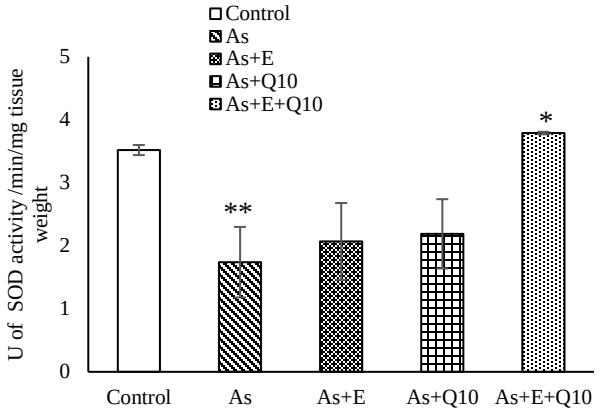

Figure 7. Arsenic induced alteration in testicular SOD activity and its modification in Vitamin E and Coenzyme Q10.p<0.05. *Compared to the arsenic-treated group; **Compared to control

The total protein level in arsenic-treated and antioxidant protected groups

A significantly lower level of total protein in testicular tissues was also recorded in the arsenic exposed group as compared to the control group. The level of total protein was found to be higher in antioxidant-treated groups with respect to the arsenic-treated group which was significantly higher in CoQ10 alone and combination (CoQ10 and Vit E) of the antioxidant-protected group as compared to only the toxicant group (arsenic-treated group) (Figure 8).

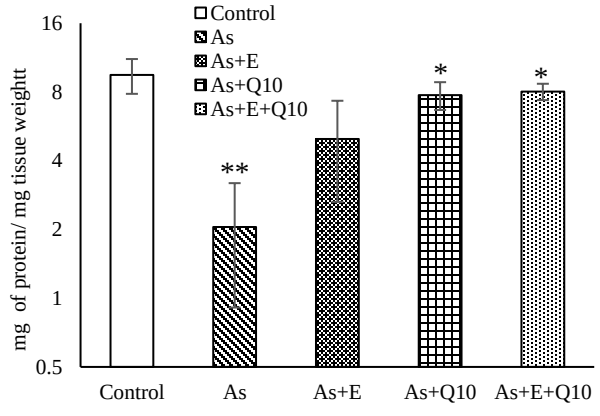

Figure 8. Arsenic induced alteration in testicular total protein level and its modification by Vitamin E and Coenzyme Q10. p<0.05. *Compared to arsenic treated group; **Compared to control

Arsenic induced DNA damage and its modification by antioxidants (using single-cell gel electrophoresis assay or Comet assay)

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A significant decline in the percentage of head DNA was recorded in 136 ppm of the arsenic-treated group with respect to the control group. The diminution in head DNA percentage was lesser in magnitude in antioxidant treated groups with respect to only the arsenic-treated group. The tail DNA percentage was statistically significantly higher in the arsenic-treated group with respect to control or without any treatment group while the elevation in tail DNA percentage in antioxidant treated groups was lower with respect to only the arsenic-treated group. The tail moment and olive tail moment were also higher in the arsenic-treated group as compared to the control group. However, tail moments were lower in the antioxidant (Vit E, CoQ10, and the combination) treated group as compared to the arsenic treated group. The DNA damage induced by arsenic was prevented by both the antioxidants and their combination to some extent but the differences were statistically non-significant between arsenic-treated and antioxidant protected groups (Table 1).

Table 1. DNA damage in blood lymphocytes of arsenic-treated (136 ppm) & antioxidant (Vit E, CoQ10 & the combination) treated groups

Groups	Head DNA (%)	Tail DNA (%)	Tail Moment	Olive Tail Moment
Control	94.43±1.62	5.56±1.62	9.47±4.08	12.51±4.32
Arsenic (136ppm)	78.9±4.22*	21.09±4.23*	38.62±2.27*	27.95±1.92
As+ Vit E	80.89±2.39	19.10±2.39	36.98±5.03	35.31±6.27
As+CoQ10	85.14±1.55	14.85±1.55	37.08±6.33	39.63±4.88
As+ Vit E +CoQ10	84.14±3.30	17.82±3.3	36.09±7.49	36.94±6.40

*p<0.05 compared to control. Vit E- Vitamin E; CoQ10- Coenzyme Q10

Discussion

Arsenic is a natural constituent of the earth's crust and is broadly distributed throughout the environment in the air, water, and soil (1). Arsenic is a lethal metalloid, ubiquitously exists in the environment and chronic arsenic exposure has toxic effects on various organs/systems. The data suggested that arsenic exposure reduces body and testis weight in mice and induces testicular oxidative stress in terms of elevation in LPO level and decline in GSH, TT, and total protein level. Earlier, Chang et al.(2007) also found that arsenic exposure exhibited a decline in mouse epididymal sperm counts and testicular weights (26). Earlier, bodyweight loss after chronic exposure to arsenic was also reported in experimental models (26, 27). Furthermore, Maharajan et al. (2007) showed that arsenic consumption was negatively associated with body mass index (BMI) and elevate the frequency of underweight individuals, with the prevalence of skin manifestations greater than normal-weight individuals (28). Recently Zeng et al. (2019) showed that arsenite destroyed the structure of the testes and reduced the sperm count. They also reported that arsenite significantly reduced the activity of total superoxide dismutase and glutathione content but elevate the levels of reactive oxygen species (ROS) and malondialdehyde levels in the mouse testes (29). Further, arsenic was reported to induce DNA damage through reactive oxygen species and elevates lipid peroxidation levels (30). The present study also advocated that arsenic exposure induces oxidative stress by enhancing the MDA level and declining the SOD, GSH, and total thiol in testicular tissues of the mouse. Earlier, Guvvala et al. (2016) reported that the mice treated with As (V) led to an elevation of the arsenic accumulation, protein carbonylation, and lipid peroxidation which is associated with alterations in the testicular SOD, GST, and CAT activities. They concluded that arsenic was found to be a testicular toxicant that diminished semen quality by inducing oxidative stress in the testicular microenvironment (31). A significant reduction in protein level was observed in the arsenic intoxicated group with respect to control. However, the protein level was more in antioxidant-protected groups compared to only the arsenic-treated group. An impaired antioxidant defense mechanism along with OS might be the reason for arsenic-associated toxicity, which might lead to a hostile impact upon reproduction. Earlier, Li et al. (2015) also stated that arsenic trioxide treated mice showed a significantly diminished sperm count, testis somatic index, activity levels of SOD, GSH, and total antioxidative capability than the control group (32).

The data on arsenic exposure and cellular DNA damage indicated that arsenic induces DNA damage in mouse blood cells as revealed by a lower percentage of head DNA and a higher level of tail DNA, tail length, the tail moment was observed in the arsenic treated group as compared to control. Earlier, Guillamet et al. (2004) also reported that some compounds of both organic (tetramethyl-arsonium iodide and tetraphenyl-arsonium chloride) and inorganic arsenic (sodium arsenite and sodium arsenate) species were able to induce an elevation in the tail moment, the parameter used for genotoxicity (33). They also reported that genotoxicity was generally observed at the higher dose of arsenic exposure (33). Further, a study on human blood samples from the arsenic-contaminated groundwater regions showed higher DNA damage with elevated levels of ROS and lipid peroxidation along with depletion in the antioxidant's levels were reported and curcumin intervention reduced the DNA damage, retarded ROS generation, lipid peroxidation, and raised the level of antioxidant indicating that curcumin may have a defensive role against arsenic-induced DNA damage (34). An association was also reported amid urinary arsenic concentrations in subjects with acute arsenic poisoning and elevated levels of urinary 8-OHdG level indicator of oxidative DNA damage) (35).

The data attained indicated that a combination of CoQ10 and Vit E can prevent oxidative stress more effectively in testicular tissues than these compounds avert oxidative stress individually indicating synergistic protective effect of these antioxidants. These results corroborate with the earlier study on CoQ10 of Ognjanović et al. (2010) with respect to toxicity of heavy metal cadmium (17) and Fouad et al. (2011) with respect to arsenic (36). Earlier, Ognjanović et al. (2010) found that the CoQ10 and Vit E protect against cadmium-induced oxidative stress in testicular

tissues of rats(17). While Fouad et al. (2011) reported that CoQ10 provides protection against arsenic-induced toxicity in testes of rats (36). Earlier, Chang et al. (2007) reported that arsenic exposure induces a reduction in testicular glutathione level and an elevation in protein carbonyl level (a marker of oxidative damage to tissue proteins) in mice and ascorbic acid treatment reversed these effects (26). Arsenic-induced reduction of glutathione levels was also observed in the present study. Momeni and Eskandari (2012) investigated the hostile impacts of sodium arsenite on the male reproductive system of rats and its alteration by Vit E and found that Vit E ameliorated toxic effects of sodium arsenite with regards to hostile impact upon sperm number and the diameters of the tubule (37). They further reported that curcumin ameliorated the lethal effect of sodium arsenite on sperm parameters in adult mice (38).

The present data coupled with our earlier findings (18) lend support to the view that antioxidants usage might be useful to reduce arsenic toxicity in an animal model. The present study also revealed that arsenic exposure induces testicular oxidative stress, reduced protein level as well as DNA damage in blood cells of the mouse which was ameliorated by the antioxidants (CoQ10 or its combination with Vitamin E) treatment to some extent. The role of antioxidant especially CoQ10 or its combination with Vitamin E may be further explored to find out their use in the clinical field as synergistic effects of CoQ10 with Vitamin E was also observed notably in the present study. These antioxidants can be tested clinically against arsenic poisoning in future studies after the accumulation of more information on these compounds with respect to arsenic toxicity.

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Conflict of interests

The authors declare that there is no conflict of interest.

Author contributions

SK was involved in concept designing, project preparation, guidance, and MS preparation. HGS and RK were involved in guidance and work supervision. AS was involved in the execution of the study, data analysis, and MS writing. CK and BP were involved in assistance during the execution of the study and data analysis.

Ethical issue

The ethical approval obtained from IAEC, ICMR-NIOH, Ahmedabad.

References

1. WHO. 2018.Arsenic, Key facts. https://www.who.int/news-room/fact-sheets/detail/ arsenic.Retrieved on 29-4-2021.
2. Sharma A, Kumar S. Arsenic exposure with reference to neurological impairment: An overview. Reviews on Environmental Health. 2019;34(4):403-414. doi: 10.1515/reveh-2019-0052.
3. Ravenscroft P, Brammer H, Richards K. Arsenic pollution: a global synthesis. John Wiley & Sons; 2008 pp 28.
4. Wang A, Holladay SD, Wolf DC. Reproductive and developmental toxicity of arsenic in rodents: a review. International Journal of Toxicology. 2006;25(5): 319-331. doi: 10.1080/10915810600840776.
5. Kim YJ, Kim JM. Arsenic toxicity in male reproduction and development. Development Reproduction. 2015;19(4):167-180. doi: 10.12717/DR.2015.19.4.167
6. Pant N, Murthy RC, Srivastava SP. Male reproductive toxicity of sodium arsenite in mice. Human and Experimental Toxicology. 2004;23(8):399-403. doi: 10.1191/0960327104ht467oa.
7. Ali M, Khan SA, Dubey P, et al. Impact of arsenic on testosterone synthesis pathway and sperm production in mice. Innovative Journal of Medical and Health Science. 2013;3(4):185-189.
8. Muenyi CS, Ljungman M. Arsenic disruption of DNA damage responses—potential role in carcinogenesis and chemotherapy. Biomolecules. 2015;5(4):2184-2193. doi: 10.3390/biom5042184
9. Bhattacharya S. Medicinal plants & natural products in amelioration of arsenic toxicity: a short review. Pharmaceutical Biology. 2017;55(1):349-354. doi: 10.1080/13880209.2016.1235207.
10. Bentinger M, Brismar K, Dallner G. The antioxidant role of coenzyme Q. Mitochondrion. 2007; 7: S41-S51. doi:10.1016/j.mito.2007.02.006.
11. Crane FL. Biochemical functions of coenzyme Q10. Journal of the American College of Nutrition.. 2001; 20(6):591-598. doi: 10.1080/07315724.2001.10719063.
12. Paunović MG, Matić MM, Ognjanović BI, Saičić ZS. Antioxidative and haematoprotective activity of coenzyme Q10 and Vitamin E against cadmium-induced oxidative stress in Wistar rats. Toxicology Industrial Health. 2017; 33(10): 746-756. doi: 10.1177/0748233717725480
13. Ramadan LA, Abd-Allah AR, Aly HA, Saad-El-Din AA. Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice. Pharmacology Research. 2002;46(4):363-370. doi: 10.1016/s1043661802001718.
14. Beyer RE. The role of ascorbate in antioxidant protection of biomembranes: interaction with Vitamin E and Coenzyme Q10. Journal of Bioenergetics and Biomembrane.1994;26(4): 349-358. doi: 10.1007/BF00762775.
15. Chen H, Tappel AL. Protection of Vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q0, coenzyme Q10, beta-carotene, canthaxanthin, & (+)-catech in against oxidative damage to rat blood and tissues in vivo. Free Radical Biology and Medicine. 1995;18(5):949-953. doi: 10.1016/0891-5849(94)00238-f.
16. Ibrahim WH, Bhagavan HN, Chopra RK, Chow CK. Dietary coenzyme Q10 & Vitamin E alter the status of these

compounds in rat tissues & mitochondria. The Journal of Nutrition. 2000; 130(9):2343-2348. doi: 10.1093/jn/130.9.2343.
17. Ognjanović BI, Marković SD, Đorđević NZ, et al. Cadmium-induced lipid peroxidation and changes in antioxidant defense system in the rat testes: Protective role of coenzyme Q10 & Vitamin E. Reproductive Toxicology. 2010;29(2):191-197. doi: 10.3390/antiox9060492.
18. Sharma A, Chaoba Ksh, Sadhu HG, Kumar S. Arsenic-induced oxidative stress, cholinesterase activity in the brain of Swiss albino mice, and its amelioration by antioxidants Vitamin E and Coenzyme Q10. Environmental Science Pollution Research. 2018;25(24):23946-23953. doi: 10.1007/s11356-018-2398-z.
19. Buege JA, Aust SD. In Methods in Enzymology (eds) Microsomal lipid peroxidation. Academic Press. 1978; pp302-310.
20. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959;82(1):70-77. doi: 10.1016/0003-9861(59)90090-6.
21. Hu ML In Methods in enzymology (eds) Measurement of protein thiol groups and glutathione in plasma. Academic Press. 1994; pp 380-385.
22. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 1974; 47(3): 469-474. doi: 10.1111/j.1432-1033.1974.tb03714.x.
23. Bakhtari A, Nazari S, Kargar-Abarghouei E, Mesbah F, Mirzaei E, Molaei MJ. Effects of dextran-coated superparamagnetic iron oxide nanoparticles on mouse embryo development, antioxidant enzymes and apoptosis genes expression, and ultrastructure of sperm, oocytes and granulosa cells. International Journal of Fertility & Sterility. 2020; 14(3): 161.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951; 193:265-275.
25. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 1988; 175 (1): 184-191. doi: 10.1016/0014-4827(88)90265-0
26. Chang SI, Jin B, Youn P, et al. Arsenic-induced toxicity and the protective role of ascorbic acid in mouse testis. Toxicology Applied Pharmacology.2007; 218(2):196-203. doi: 10.1016/j.taap.2006.11.009.
27. Neiger RD, Osweiler GD. Arsenic concentrations in tissues and body fluids of dogs on chronic low-level dietary sodium arsenite. Journal of Veterinary Diagnostic Investigation. 1992;4(3): 334-337. doi: 10.1177/104063879200400318.
28. Maharjan M, Watanabe C, Ahmad SA, et al. Mutual interaction between nutritional status and chronic arsenic toxicity due to groundwater contamination in an area of Terai, lowland Nepal. Journal of Epidemiology Community Health. 2007;61(5):389-394. doi: 10.1136/jech.2005.045062.
29. Zeng Q, Yi H, Huang L,An Q, Wang H. Long-term arsenite exposure induces testicular toxicity by redox imbalance, G2/M cell arrest and apoptosis in mice. Toxicology. 2019;411:122-132. doi: 10.1016/j.tox.2018.09.010.
30. Mukherjee S, Roy M, Dey S, Bhattacharya RK.A mechanistic approach for modulation of arsenic toxicity in human lymphocytes by curcumin, an active constituent of medicinal herb Curcuma longa Linn. Journal of Clinical Biochemistry and Nutrition.2007;41(1):32-42. doi: 10.3164/jcbn.2007005.
31. Guvvala PR, Sellappan S, Parameswaraiah RJ. Impact of arsenic (V) on testicular oxidative stress and sperm functional attributes in Swiss albino mice. Environmental Science Pollution Research. 2016;23(18):18200-18210. doi: 10.1007/s11356-016-6870-3.
32. Li SG, Ding Yu S, Niu Q, Xu S Zhi, Pang Li J, MA Ru-L, Jing M Xia, Feng GL, Liu JM, Guo SX. Grape seed Proanthocyanid in extract alleviates arsenic-induced oxidative reproductive toxicity in male mice. Biomedical and Environmental Science, 2015:28(4):272-280. doi: 10.3967/bes2015.038.
33. Guillamet E, Creus A, Ponti J, Sabbioni E, Fortaner S, Marcos R. In vitro DNA damage by arsenic compounds in a human lymphoblastoid cell line (TK6) assessed by the alkaline Comet assay. Mutagenesis. 2004;19(2):129-135. doi: 10.1093/mutage/geh005.
34. Biswas J, Sinha D, Mukherjee S, Roy S, Siddiqi M, Roy M. Curcumin protects DNA damage in a chronically arsenic-exposed population of West Bengal. Human Experimental Toxicology. 2010;29(6):513-524. doi: 10.1177/0960327109359020.
35. Yamauchi H, Aminaka Y, Yoshida K, Sun G, Pi J, P Waalkes MP. Evaluation of DNA damage in patients with arsenic poisoning: urinary 8-hydroxy deoxy guanine.Toxicology Applied Pharmacology. 2004;198(3):291-296. doi: 10.1016/j.taap.2003.10.021.
36. Fouad AA, Al-Sultan, AI, Yacoubi MT. Coenzyme Q10 counteracts testicular injury induced by sodium arsenite in rats. European Journal of Pharmacology. 2011;655(1):91-98. doi: 10.1016/j.ejphar.2010.12.045.
37. Momeni HR, Eskandari N. Effect of Vitamin E on sperm parameters & DNA integrity in sodium arsenite-treated rats. Iranian Journal of Reproductive Medicine. 2012;10(3): 249-256.
38. Momeni HR, Eskandari N. Curcumin inhibits the adverse effects of sodium arsenite in mouse epididymal sperm. International Journal of Fertility and Sterility. 2016;10(2):245-252.

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