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DNA damage by sodium arsenite in experimental rats: ameliorative effects of antioxidant vitamins C and E

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Abstract

Serious health problems in humans are caused by arsenic-exposure, which is wide spread in the environment. Sodium arsenite, capable of inducing macromolecular damage is evaluated for its DNA damaging effect in the blood, liver, kidney and bone marrow cells of Wistar rats. Also the ameliorative potential of α-tocopherol (400 mg/kg body weight) and ascorbic acid (200 mg/kg body weight) supplemented orally to arsenic-intoxicated rats (100 ppm in drinking water for thirty days) in neutralizing the genotoxic effect of arsenite was explored. Detection of DNA damage at cellular level is evaluated by single cell gel electrophoresis or comet assay, under alkaline conditions. This study demonstrated that dietary supplementation of antioxidants such as vitamins C and E could ameliorate arsenite induced toxicity in experimental rats.

Keywords: Arsenic, antioxidants, ascorbic acid, comet assay, DNA damage, genotoxicity, *in vivo*, α- tocopherol.

Introduction

Arsenic is widely distributed in nature and principally occurs in the form of inorganic or organic compounds. An inorganic arsenical compound consists of arsenite, considered to be the most toxic form, arsenate the less toxic form, and organic forms the least toxic ones (Pradosh Roy *et al*., 2002; WHO, 1993). As a result of wide occurrence of arsenic in the environment, human exposure to the metalloid becomes almost universal. The most common pathway for an elevated environmental exposure to inorganic arsenic worldwide is through drinking water. Chronic exposure to arsenic causes a wide range of toxic effects and thus this metalloid is classified as Group I carcinogen in humans (IARC, 1987). Based on substantial evidence on cancer risk associated with chronic exposure to relatively low concentrations of arsenic in drinking water, the international agencies like National Research Council, (NRC, 1999) had suggested 50 µg / litre as the maximum contaminant level (MCL) with an increase in risk for cancer. The United States Environment Protection Agency had revised the MCL for arsenic in drinking water of 10 µg /litre (USEPA, 2001) and the WHO, has suggested a provisional guideline value of 10 µg/litre of arsenic in drinking water and the European union has set 50 µg/ litre and 10 µg/ litre as limit and guidevalues of arsenic in drinking water respectively (WHO, 1981). Chronic arsenicism has been observed in workers and in groups of the general population living in areas of The United States (Welch *et al*., 1998 and Shaw *et al.,* 2005), United Kingdom (Goyer *et al*., 1995), China (Luo *et al*., 1997), Taiwan (Chen *et al*., 2005 and Tseng *et al*., 2006) Mexico (Cebrian *et al*., 1993), Chile (Smith *et al*., 1998) Argentina (Hopenhayn Rich *et al*., 1998), India (Guha Mazumdar *et al*., 1988; Rahman *et al*., 2005) and Bangladesh (Gamble *et al*., 2005). Noteworthy among them are India and Bangladesh where the arsenic concentration in drinking water is hundred times more than the recommended safety level prescribed by the international agencies.

Following ingestion, inorganic arsenic appears rapidly in the circulation, where it binds primarily to hemoglobin (Axelson, 1980). Skin, bone and muscle represent the major storage organs (Osborne, 1925). Inorganic arsenic does not appear to cross the blood brain barrier, however transplacental transfer of arsenic in human (Gibson & Gage, 1982) and mice (Hood, 1987) occurs. The metabolism of arsenic like other toxic metals is associated with the conversion of the most potent toxic form of this element to the less toxic form, followed by cellular accumulation or excretion. Biomethylation of arsenic is considered the primary detoxification mechanism, since the inorganic arsenics are more toxic to the living organisms (Yamauchi & Fowler, 1994). The conversion of arsenic between oxidation states and organo-metalloid forms alter the binding affinities of arsenic for different proteins, thus altering the relative toxicities of the various arsenic species (Fowler & Woods, 1979). These conversions of arsenic species are important in the methylation of arsenate to dimethylarsinic acid (DMA), which is believed to be principal detoxification mechanism (Aposhian, 1989). Arsenic toxicity, differs in a fundamental fashion from that of other "Protoplasmic Poison", which act by denaturing and precipitating the cellular proteins. It has been assumed that the effect of arsenic must depend on a functional activity rather than on structural integrity (Stocken & Thompson, 1946). Arsenicals can cause cellular damages through the generation of free radicals (Barchowsky, 1996; Schinella *et al*., 1996). Several studies suggest that arsenic compounds may also exert

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their toxicity through the generation of reactive oxygen species such as superoxide, hydroxyl radicals, hydrogen peroxide and nitric oxide during their metabolism in the cells (Hei & Filipic, 2004; Liu *et al.,* 2005). Though Arsenicals are unable to induce gene mutation in cultured cells, arsenite has been shown to enhance the cytotoxicity, mutagenicity and clastogenicity of UV-radiation, alkylating and DNA crosslinking agents in rodents and human cells (Lee *et al*., 1986; Okui & Fujiwara,1986). Lee *et al*. (1996) have found that Genotoxic studies of arsenic have largely yielded negative findings for gene mutations but positive results for chromosomal aberrations.

Material and methods

Male albino rats of Wistar stain (120-150 g) were used in this study. The animals were obtained from The King Institute of Preventive Medicine, Chennai in the year 2002. The animals were housed in large spacious cages and were given food and water *ad libitum*. The animal room was well ventilated with a 12 hour light/dark cycle, throughout the period of the experiment. The animals were maintained on a commercial rat-feed manufactured by Hindustan Lever Ltd., Mumbai under the trade name "GOLD MOHUR RAT FEED". The feed contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fibre with adequate mineral and vitamin contents (Anusuyadevi *et al*., 2008).

Grouping of animals

The animals were divided into five groups, namely:

Group I Rats that received vehicles alone (served as control),

Group II Rats that received arsenic as sodium arsenite in drinking water at a concentration of 100 ppm.

Group III Rats that were treated with arsenic along with ascorbic acid (200 mg/kg body wt. dissolved in water) given by oral gavage once a day.

Group IV Rats that were given arsenic along with α-tocopherol (400 mg/kgbody wt. dissolved in mineral oil) by oral gavage once a day.

Group V Rats that were administered arsenic along with ascorbic acid (200 mg/kg body wt. dissolved in water) and α-tocopherol (400 mg/kg body wt. dissolved in mineral oil) by oral gavage once a day.

Food and water intake and body weight of the animals were monitored throughout the 30 days of the period of the experiment.

Estimation of arsenic

Tissue/blood/urine samples were digested according to the method of Ballentine and Burford (1957). To 100 mg of tissues/1 ml of blood or urine, 1 ml of concentrated nitric acid was added, followed by 1ml of perchloric acid. The sample was then digested over a sand bath until the solution turned yellow in colour. If the colour of the digest was brown, more nitric acid and perchloric acid were added and the oxidation was repeated. The digest was made up to known volume with deionized water. Aliquots of this were used to estimate arsenic by using the atomic absorption spectrophotometer. The concentration of arsenic was expressed as µg/ dl blood or µg/ g tissue.

Assessment of DNA damage

DNA damage was assessed by using alkaline single cell electrophoresis (comet assay) according to the method of Singh and Mc Coy, (1988). Single cell suspensions from tissues like liver and kidney were prepared following the procedure of Singh *et al*., (1998).

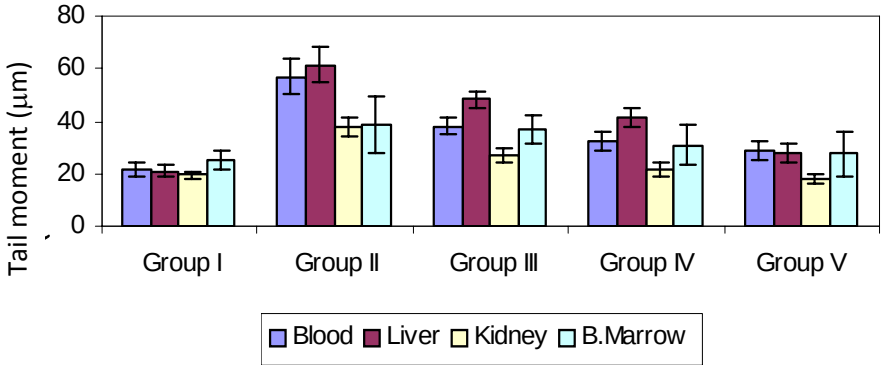
Reagents: 100mM Phosphate buffered saline (PBS); 1% Low melting point agarose (LMPA); 1% Normal Melting Point Agarose (NMPA); Hank's balanced salt solution (HBSS) in 0.02 M EDTA; Lysing solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris-HCl, pH adjusted to 10.0 with NaOH; 1% Triton X-100 and 10%DMSO were added freshly; Electrophoresis buffer (300mMNaOH and 1mM EDTA, pH 13.0); 0.4 M Tris-HCl, pH 7.5; Ethidium bromide (2 µg/ ml).

Procedure

One hundred µl of 1% NMPA in phosphate buffered saline was dropped on to frosted slide immediately covered with cover slip and kept for 10 min in refrigerator for it to solidify. Then, cover slips were removed and 100 µl of LMPA coated cells (100 µl of cell suspension in HBSS and 100 µl of LMPA) were added to the slides. The cover slips were replaced and the slides were kept in the refrigerator for another 10 min to solidify the LMPA. After this, the cover slips were removed and a top layer of 100 µl of LMPA was added the slides were again cooled for 10 min.

After removal of cover slips, the slides were immersed in cold lysing solution. The slides were kept in dark at 4 ºC for at least 1 hr. To prevent the occurrence of additional DNA damage, the following steps were performed under dim light. The slides were removed from the lysing solution and placed on a horizontal electrophoresis tank. The unit was filled with a freshly made electrophoresis buffer to a level of 0.25 cm above

Fig.1. Arsenite-induced DNA damage in rat as assessed by comet assay



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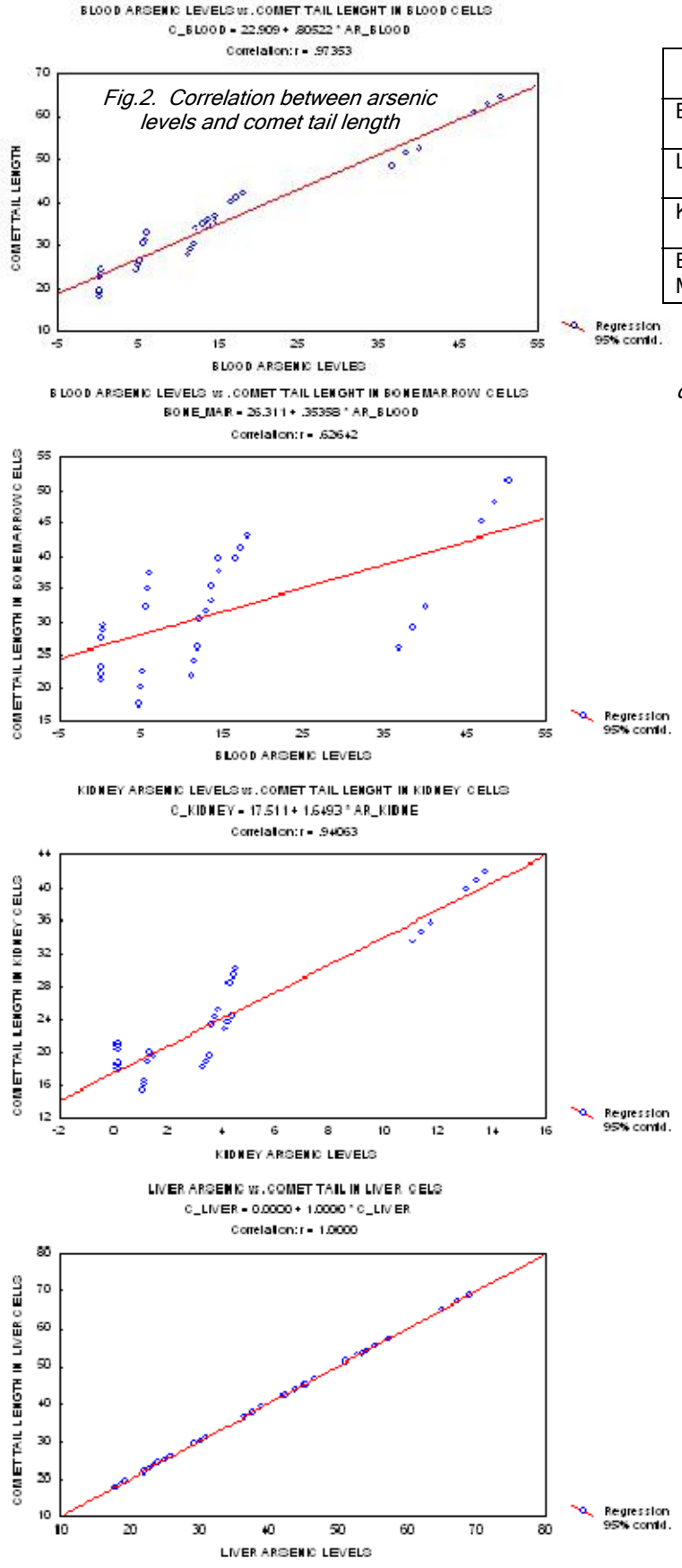


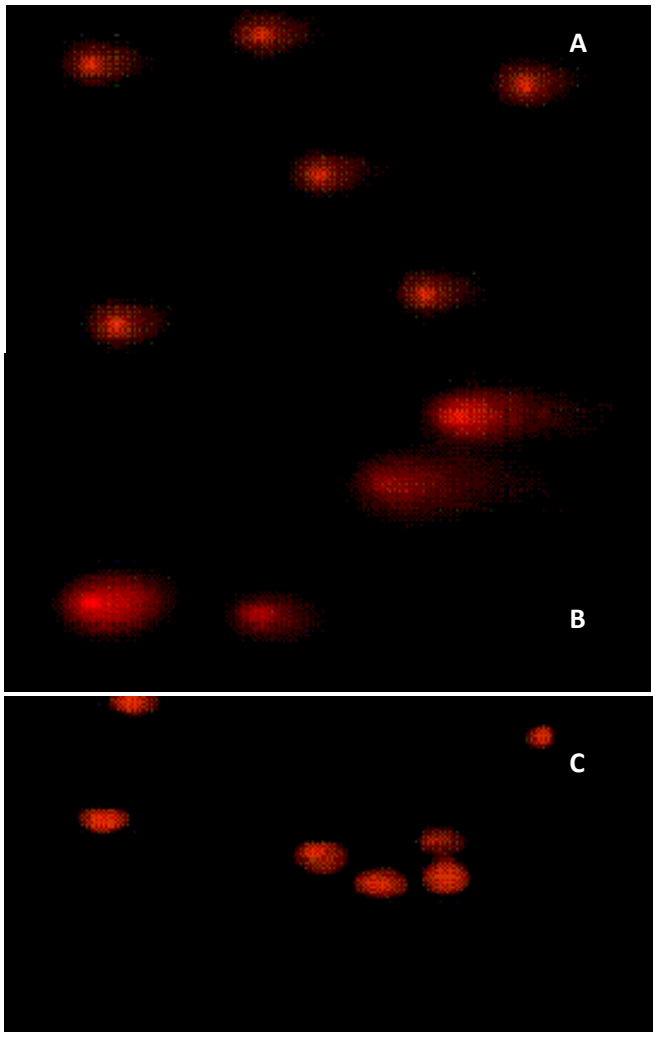
Fig.2. Correlation between arsenic levels and comet tail length

Table 1. Assessment of arsenite induced genotoxicity in rat using comet assay

	Group I	Group II	Group III	Group IV	Group V
Blood	216.0 ± 2.6	567.6 ± 6.69 [a]	381.9 ± 3.4 [b]	323.9 ± 3.7 [bc]	284.6 ± 3.5 [bcd]
Liver	207.9 ± 2.3	612.0 ± 6.6 [a]	481.9 ± 5.0 [b]	415.9 ± 4.3 [bc]	278.0 ± 2.8 [bcd]
Kidney	196.0 ± 1.3	378.0 ± 3.5 [a]	268.0 ± 2.8 [b]	213.0 ± 2.6 [bc]	177.0 ±1.9 [bcd]
Bone Marrow	253.9 ± 3.6	387.0 ± 10.8 [a]	365.0 ± 5.4 [b]	307.0 ± 7.6 [bc]	275.0 ± 8.4 [bcd]

Each value represents comet tail length in microns expressed as mean ± SD for six rats in each group. [a] As compared with group I; [b] As compared with group II; [c] As compared with group III; [d] As compared with group IV; [a, b, c, d] represent p<0.05

Fig.3. DNA fragmentation analysis by single cell gel electrophoresis (comet assay) in control and experimental rats (25X)



A. Control; B. arsenic treated; C. Arsenic + Vit.C + Vit.E

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the slides. The cells were exposed to alkali for 20 min to allow for DNA unwinding.

An electric current of 25 V and 300mA was applied for 20 min to electrophoresis. After electrophoresis, the slides were placed horizontally, and neutralized with Tris-HCl. Finally, 50 µl of ethidium bromide was added to each slide and covered with a cover slip and analyzed using a fluorescence microscope (Nikon, Japan) with a calibrated scale in ocular. Images of 50 randomly selected cells were analyzed from each sample. For each cell, the length of the image was measured and was expressed as micrometers (µm) with the help of software Komet V- single cell gel electrophoresis, Version V, 2001, Marketed by Kinetic Imaging Limited, UK. www.kineticimaging.com.

Statistical analysis

Values are expressed as mean ± SD for six rats in each group, and significance of the differences between mean values were determined by one way analysis of variance (ANOVA) followed by Duncan test for multiple comparison using Statistical Package for Social Sciences (SPSSS) version 11.0 package/ Values of $p<0.05$ were considered to be significant. Correlation analysis were done using statistical package Statistica /w 5.0 for windows.

Results

DNA strand breaks caused by free radicals analyzed through comet assay in blood liver, kidney and bone marrow cells revealed an increase by 163%, 194%, 93% and 52% in blood, liver, kidney and bone marrow respectively. Co-administration of antioxidant vitamin C and vitamin E decreased the level of DNA damage as expressed by the mean length of comets observed to 49.85%, 54.50%, 53.17% and 28.90% in blood, liver, kidney and bone marrow respectively as compared to arsenic intoxicated rats. The values are given in the Table 1 and graphically represented in Fig.1. The combination of both these antioxidants had a greater effect rather than giving individually as the results are significant at 95% confidence level ($p<0.05$).

A positive correlation was observed between the comet tail length in blood, liver, kidney and bone marrow against the concentration of arsenic in their respective organs. The correlation coefficient being r = 0.97 for blood, 1.0 for liver, 0.94 for kidney and 0.62 for bone marrow (Fig.2).

The photographs of comets as visualized under Fluorescence microscope are given in Fig.3.

Discussion

Our observation demonstrated that free radicals might be involved in arsenic induced DNA strand breakage. This is corroborated by a reduction in the formation of comet tail length in group-V supplemented with antioxidants such as vitamin C and vitamin E (Table 1). Recent studies show that ROS are involved in arsenite induced cell signaling and activation of transcription factor (Barchowsky *et al*., 1999) leading to chromosomall aberrations (Hei *et al*., 1998), DNA strand breakage (Lynn *et al*., 2000), gene mutation (Hei *et al*., 1998) generation of micronuclei (Wang *et al*., 1994; Gurr *et al*., 1998) and apoptosis (Gurr *et al*., 1999). Similarly, nitric oxide is also involved in arsenic induced DNA strand breakage (Liu and Jan, 2000) and poly (ADP-ribosylation) (Lynn *et al*., 1997). Moreover, metabolism of arsenic generates oxygen radicals (Yamanaka and Okada, 1994; Hei and Filipic, 2004; Kessel *et al*., 2002 and Valko *et al*., 2006) which may damage the cellular macromolecules and decrease cytP450 biotrasformation enzymes involved in xenobiotic metabolism (Albores *et al*., 1989). Antioxidant Vitamin E is an important lipid soluble antioxidant present in cells, as it is the major chain terminating antioxidant in biological membranes (Burton *et al*., 1983) and it scavenges a wide array of ROS including $^1O_2$, $HO^{\bullet}$, $^{\bullet}O_2^-$, Peroxyl, and alkoxyl radicals. Vitamin E is composed of a number of derivatives of tocopherols and tocotrienols. The major isomer in humans is α –TOH, which also possesses the greatest antioxidant activity of any vitamin E derivative. In homogenous solutions, α-TOH is a strong inhibitor of polyunsaturated lipid peroxidation (Samokyszyn *et al*., 1990) and *in vivo*, most of the cellular vitamin E is concentrated in the lipid membranes (Bjorneboe *et al*., 1990). The primary antioxidant activity of tocopherols is to stop chain propagation of peroxyl radicals ($k = 6 \times 10^3$- 3×10^6 M^{-1} sec^{-1}) (Niki and Matsuo, 1993). Tocopherols can typically scavenge two ROS per tocopherol molecule (Horswel *et al*., 1966).

Ascorbic acid (Vitamin C), a water soluble antioxidant, exists primarily as ascorbate at physiological pH. Ascorbate is a powerful reducing agent capable of rapidly scavenging a number of ROS including $^{\bullet}O_2^-$ ($k = 2.7 \times 10^5$ M^{-1} sec^{-1}) (Nishikimi, 1975). In addition, ascorbate reacts with other cellular prooxidants such as $^1O_2$, hypochlorous acid (HOCL) and thiol radicals ($RS^{\bullet}$) (Halliwell, 1990); Ascorbate is also able to reduce the vitamin E derived tocopheroxyl radical α-$TO^{\bullet}$. Through this mechanism, ascorbate in the aqueous phase is able to regenerate membrane-bound α-TOH, prolonging the life time of this important antioxidant in the lipid phase and effecting removal of the radical from the lipid to the aqueous phase. In its action as an antioxidant, ascorbate is rapidly oxidized to dehydroascorbate, which shows little antioxidant activity. Dehydroascorbate may be reduced back to ascorbate via GSH-dependent reductase (Sharma and Buettner, 1993). Therefore group-V animals supplemented with antioxidant vitamins E and C, show reduced DNA damage as visualized as comet tail length due to the free radical scavenging effects of vitamins.

The level of DNA strand breaks were found to be elevated in arsenic treated group-I, whereas the groups supplemented with antioxidant vitamins showed a minimal level of alteration in the above said parameters suggesting primarily, a free radicals mediated toxicity caused by arsenic.

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Our study corroborated with the similar findings of Ramanathan et al., that the supplementation of ascorbic acid and alpha-tocopherol modulated arsenic induced apoptosis in rats by improving the cellular antioxidant status and scavenging of free radicals. It also supports the perspective of co-administration of antioxidant vitamins to arsenic-induced toxicity (Peraza *et al*., 1998; Wei *et al*., 2005; Karasavvas *et al*., 2005).

Exposure to arsenic at low doses chronically causes oxidative stress and depletion of vital antioxidants in the cells. The toxicity of arsenic comprises altered membrane properties, loss of cellular functions, macromolecular damage and finally induction of apoptosis. Co-treatment of antioxidant vitamins like vitamin C and vitamin E to arsenic exposed rats has restored the antioxidant status of the cells and protected them from arsenic induced clastogenicity thereby reducing the tail lengths of comet observed in group-V supplemented with antioxidant vitamins. Therefore, it is strongly believed that the perspective of dietary supplementation of antioxidants such as vitamin C and vitamin E may in part alleviate arsenic-induced toxicity.

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References

1. Albores A, Cebrian ME, Bach PH, Connelly JC, Hinton RH and Bridges JW (1989) Sodium arsenite induced alterations in bilirubin excretion and heme metabolism. *J. Biochem. Toxicol.* 4, 73-78.
2. Anusuyadevi M, Jayachandran KS, Paneerselvam C (2008) Role of L-Carnitine and DL-a-Lipoic acid on EEG pattern in aged rat brain. *Curr. Trends in Biotech. & Pharmacy*. 2(2), 277-285.
3. Aposhian HV (1989) Biochemical toxicology of arsenic. *Rev. Biochem. Toxicol.* 10, 265-299.
4. Axelson O (1980) Arsenic compounds and cancer. *J. Toxicol. Environ. Health*. 6,1229.
5. Barchowsky A, Dudek EJ, Treadwell MD and Wetterhann KE (1996) Arsenic induced oxidant stress and NF Kappa G activation in cultured aortic endothelial cells. *Free Radical Biol. Med.* 21,783-790.
6. Barchowsky A, Roussel RR, Klei LR, James PE, Ganju N, Smity KR and Dudek EJ (1999) Low levels of arsenic trioxide stimulate proliferatiove signals in primary vascular cells without activating stress effector pathways. *Toxicol. Appl. pharmacol*. 159, 65-75.
7. Bjorneboe A, Bjornenoe GE and Drevon CA (1990) Absorption, transport and distribution of vitamin E. *J. Nutr.* 120, 233-242.
8. Burton GW, Joycen A and Ingold KU (1983) Is vitamin E the only lipid soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys.* 221, 281-290.
9. Cebrian M (1993) Heavy Metals. In: Environmental Epidemiology. Finkelman J, Correy G & Calderon R (eds.). A project for Latin America and the Caribbean metepec, Mexico: Pan American Centre for Human Ecology and Health, WHO. pp: 95-145.
10. Chen CJ, Hsu LI, Wang CH, Shih WL, Hsu YH, Tseng MP, Lin YC, Chou WL, Chen CY, Lee CY, Wang LH, Cheng YC, Chen CL, Chen SY, Wang YH, Hsueh YM, Chiou HY and Wu MM (2005) Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. *Toxicol. Appl. Pharmacol.* 206(2), 198-206.
11. Fowler BA and Woods JS (1979) The effects of prolonged oral arsenate exposure on liver and mitochondria of mice: morphometric and biochemical studies. *Toxicol. Appl. Pharmacol.* 50,117-187.
12. Gamble MV, Liu X, Ahsan H, Pilsner R, Ilievski V, Slavkovich V, Parvez F, Levy D, Factor-Litvak P and Graziano JH (2005) Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. *Environ. Health Perspect*. 113(12),1683-1688.
13. Gibson RS and Gage LA (1982) Changes in hair arsenic levels in breast and bottle fed infants during the first year of infancy. *Sci. Total Environ.* 26, 33.
14. Goyer RA, Cherian MG, Jones MM and Reigart JR (1995) Role of Chelating agents for prevention, intervention and treatment of experimental toxic agents. *Environ Health Perspect.* 105, 1048-1052.
15. Guha Mazumdar DN, Chakraborty AK, Ghose A, Gupta JD, Chakraborty DP and Dey SB (1988) Chronic arsenic toxicity from drinking water in rural West Bengal. *Bull. World Health Org.* 66, 499-506.
16. Gurr JR, Bau DT, Liu F, Lynn S and Jan KY (1999) Dithiothreitol enhances arsenic trioxide induced apoptosis in NB4 cells. *Mol. Pharmacol.* 56,102-109.
17. Gurr JR, Liu F, Lynn S and Jan KY (1998) Calcium-dependent nitric oxide production is involved in arsenite-induced micronulei. *Mutat. Res*. 416,137-148.
18. Halliwell B (1990) How to characterize a biological antioxidant. *Free Radical Res. Commun.* 9,1-32.
19. Hei TK and Filipic M (2004) Role of oxidative damage in the genotoxicityof arsenic. *Free Radic. Biol. Med.* 37(5), 574-581.
20. Hei TK, Liu SX and Waldren C (1998) Mutagenecity of arsenic in mammalian cells: Role of reactive oxygen species. *Proc. Natl. Acad. Sci.* 95, 8103-8107.
21. Hood RD, Vedel-Macrander GC, Zaworotko MJ, Tatum FM and Meck RG (1987) Distribution, metabolism and fetal uptake of pentavalent arsenic in pregnant mice following oral or intra peritoneal administration. *Teratology* 35, 19.
22. Hopenhayn Rich C, Biggs ML and Smith AH (1998) Lung and kindey cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. *Int. J. Epidemiol.* 27, 561-569.
23. Horswel EC, Howard JA and Ingold KU (1966) Alphatocopherol. *Can. J. Chem.* 44, 985-991.
24. International Agency for Research on Cancer (1987) In: IARC monograph on the evaluation of carcinogenic risk to humans- Overall evaluation of carcinogenicity. An update of IARC Monographs 1 to 42 (supp 7), p:100, International Agency for Research on Cancer Lyon.
25. Karasavvas N, Carcamo JM, Stratis G, Golde DW (2005) Vitamin C protects HL60 and U266 cell from arsenic toxicity. *Blood.* 105(10), 4004-4012.

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26. Kessel M, Liu SX, Xu A, Santella R and Hei TK (2002) Arsenic induces oxidative DNA damage in mammalian cells. *Mol. Cell Biochem*. 234-235(1-2), 301-308.
27. Lee TC, Lee KC, Tzeng YJ, Hung RY and Jan KY (1986) Sodium arsenite potenitates the clastogenicity and mutagenicity of DNA crosslinking agents. *Environ. mutagenesis*. 8,19-128.
28. Lee TC, Tseng SF, Chang YC, Lin KY and Jan MR (1996) Post treatment with sodium arsenite during G2 enhance the frequency of chromosomal aberrations induced by S-dependent clastogens. *Mutat. Res*. 163, 263-269.
29. Liu F and Jan KY (2000) DNA damage in arsenite and cadmium- treated bovine aortic endothelial cells. *Free Radic. Biol. Med*. 28, 55-63.
30. Liu SX, Davidson MM, Tang X, Walker WF, Athar M, Ivanov V and Hei TK (2005) Mitochondrial damage mediates genotoxicity of arsenic in mammalian cells. *Cancer Res.* 65(8), 3236-3242.
31. Luo ZD, Zhang YM, Ma L, Zhang ZY and He L (1997) Chronic arsenicism and cancer in inner Mangolia consequences of well- water arsenic level greater than 50 mu/l. In: Arsenic exposure and health effects. Abernathy Co, Claderon RC & Chappell WR (eds.), London: Chapman & Hall. pp:55-68.
32. Lynn S, Gurr JR, Lai HT and Jan KY (2000) NADH oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. *Cir. Res.* 86, 514-519.
33. Lynn S, Lai HT, Gurr GR and Jan KY (1997) Arsenite retards DNA breaks rejoining by inhibiting DNA ligation. *Mutagenesis.* 12, 353-358.
34. Marjorie A. Peraza, Felix Ayala-Fierro, David S. Barber, Elizabeth Casrez, and Leonard T. Rael (1998) Effects of Micronutrients on Metal Toxicity. *Environ Health Perspect.* 106, 203-216.
35. Niki E and Matsuo M (1993) Rates and products of reactions of vitamin E with oxygen radicalsIn: Vitamin E. In: Health and disease. Packer L & Fuchs J (eds). NY: Dekker. pp:121-130.
36. Nishikimi M (1975) Oxidation of ascorbic acid with superoxide anion generated by the xanthine-xanthine oxidase system. *Biochem. Biophys. Res. Commun.* 63, 463-468.
37. NRC (1999) Chemistry and analysis of arsenic species in water, food, urine, blood, hair and nails. In: Arsenic in drinking water (National Research Council, Eds.). National Acad. Press, Washington, DC. pp:27-82.
38. Okui T and Fujiwara Y (1986) Inhibition of human excision DNA repair by inorganic arsenic and the co-mutagenic effect of C79 Chinese hamster cells. *Mutat. Res*.172, 69-76.
39. Osborne ED (1925) Microchemical studies of arsenic in arsenical pigmentation and keratoses. *Arch. Dermal Syph.* 12, 773.
40. Pradosh Roy and Anupama Saha (2002) Metabolism and toxicity of arsenic: A human carcinogen. *Curr. Sci.* 82(1), 38-45.
41. Rahman MM, Sengupta MK, Ahamed S, Lodh D, Das B, Hossain MA, Nayak B, Mukherjee A, Chakraborti D, Mukherjee SC, Pati S, Saha KC, Palit SK, Kaies I, Barua AK and Asad KA (2005) Murshidabad-one of the nine groundwater arsenic-affected districts of West Bengal, India. Part I: magnitude of contamination and population at risk. *Clin Toxicol (Phila)*. 43(7), 823-834.
42. Ramanathan K, Anusuyadevi M, Shila S and Paneerselvam C (2005) Ascorbic acid and alpha-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. *Toxicol. Lett*. 156 (2), 297-306.
43. Samokyszyn VM, Miller DM, Reif DW and Aust SD (1990) Iron-catalyzed lipid peroxidation. In: Membrane lipid oxidation. Vol. 1. Vigo-Pelfrey C (ed), Boca Raton, FL: CRC Press. pp:101-127.
44. Schinella GR, Tournier, HA, Buschiazzo HO, and de Buschiazzo PM (1996) Effect of arsenic (V) on the antioxidant defense systems: *In vitro* oxidation of rat plasma lipoprotein. *Pharmacol. Toxicol.* 79, 293-296.
45. Sharma MK and Buettner GR (1993) Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. *Free Radical Biol. Med.* 14, 649-653.
46. Shaw WD, Walker M and Benson M (2005) Treating and drinking well water in the presence of health risks from arsenic contamination: results from a U.S. hot spot. Risk *Anal.* 25(6),1531-1543.
47. Singh NP (1998) A rapid method for the preparation of single-cell suspensions from solid tissues. *Cytometry.* 31, 229-232.
48. Singh NP and Mc Coy (1988) Metallothioneins. In: A simple technique for quantitation of low levels of DNA damage in individual cells. Tice RR & Schneider EL (eds.). *Exp. Cell Res.* 175,184-191.
49. Smith AH, Goycolea M, Haque R and Biggs ML (1998) Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. *Am. J. Epidemiol*.147, 660-669.
50. Stocken LA, and Thompson RHS (1946). Arsenic derivaties of thiol proteins. *Biochem. J.* 40, 548.
51. Tseng CH, Huang YK, Huang YL, Chung CJ, Yang MH, Chen CJ and Hsuch YM (2006) Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease inblackfoot disease-hyperendemic villages in Taiwan. *Toxicol. Appl. Pharmacol.* 211(2), 175.
52. US. Environmental Protection Agency (2001) National primary drinking water regulations: Arsenic and clarifications to compliance and new source contaminants monitoring: Final rule (40 CFR Part 9, 142 and 142) fed. Reg66, 6975-7066.
53. Valko M, Rhodes CJ, Moncol J, Izkovic M and Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Cancer Res.* 160 (1),1-40.
54. Wang TS and Huang H (1994) Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. *Mutgenesis.* 9, 253-257.
55. Wei M, Arnold L, Cano M and Cohen SM (2005) Effects of co-administration of antioxidants and arsenicals on the rat urinary badder epithelium. *Toxicol. Sci*. 83(2), 237-245.
56. Welch AH, Lico MS and Huges JL (1998) Arsenic in ground water of the western United States. *Ground Water* 26, 33-337.
57. WHO (1993) Guidelines for drinking water quality. Vol. 1: Recommendations. 2d eds., Geneva.
58. WHO (1981) Environmental health criteria - arsenic, Geneva.
59. Yamanaka K and Okada S (1994) Induction of lung specific DNA damage by metabolically methylated arsenics via the production of free radicals. *Environ.Health Perspect*. 3, 37-40.
60. Yamauchi H and Fowler BA (1994) Toxicity and metabolism of inorganic and methylated arsenicals. In: Arsenic in the environment, Part II: Human health and ecosystem effects. Nriagu JO (eds.), Wiley, NY. pp:35-43.