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Positive effects of Vitamin C in arsenic trioxide and sodium fluoride induced genotoxicity and oxidative stress in mice *in vivo*

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ABSTRACT: The aim of the present study was to evaluate Vitamin C (VC) as a potent natural antioxidant to mitigate the genotoxic effects of Arsenic trioxide and sodium fluoride in Swiss albino mice *in vivo*. The study was divided into eight groups consisting of control treated with normal saline (Group I), Group II, III, IV, V, VI, VII and the VC group with only Vitamin C (500 mg/kgbw). Arsenic trioxide (4 mg/kgbw) and Sodium fluoride (8 mg/kgbw) were administered singly, as well as in combination to swiss albino mice, with and without VC. In this study, the genotoxic effect of arsenic (As) and fluoride (F) in mice using comet, chromosomal aberration and lipid peroxidation assay was investigated. The results revealed that VC efficiently ameliorates the genotoxic effect of As and F by increasing the frequency of chromosomal aberrations and primary DNA damage along with increased malondialdehyde (MDA) level. In conclusion, VC mitigates the genotoxic effects of the two well-known water contaminants (As and F) effectively and efficiently at the given concentration *in vivo*.

Keywords: arsenic trioxide, comet, malondialdehyde, sodium fluoride, Vitamin C.

INTRODUCTION

Human exposure to metals is frequent due to their ubiquity, contamination, wide use in industry, and environmental persistence. Both arsenic and fluoride are considered as water contaminants raising serious health issues in India and in the world at large (ATSDR, 2003, 2005). Arsenic in drinking water poses the greatest threat to public health (Hindmarsh and McCurdy, 1986; Cui and Liu, 1988). However, it is a ubiquitous element detected in all environmental media. It occurs commonly in pentavalent [As (V)] and trivalent [As (III)] forms. [As (III)] is found to be relatively more toxic when compared to [As (V)] (Aposhian, 1989). As a result of wide occurrence of arsenic in the environment, human exposure to the metalloid becomes almost universal. Humans are potentially exposed to arsenic by reason of smoke from cigarette, arsenic contaminated water, air and food. 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 a Group I carcinogen in humans (IARC, 1987). Chronic ingestion of inorganic arsenic has been related to increased incidence of skin, bladder, liver and kidney cancer, while

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inhalation of arsenic causes lung cancer (IARC, 1987; Abernathy et al., 1999; NRC, 1999; Goering et al., 1999). Arsenic has been considered as a potent toxicant and carcinogen (Luchtrath, 1972). Arsenic compounds during their metabolism in cells, generate reactive oxygen species. These ROS contribute to the parthogenesis of various acute and chronic liver diseases (Sollott, 2006) and damage cellular macromolecules.

However, fluoride is an important environmental contaminant and the major sources of exposure to this element are through drinking water, food, dental products and pesticides. Excessive consumption of fluoride causes fluorosis, a slow progressive degenerative disorder which is known to affect predominantly the skeletal systems, teeth and also the structure and function of skeletal muscles (Kaul and Shusheela, 1974) with characteristic muscle weakness, pains in joints and fatigue with non-ulcer dyspepsia and polyurea (Susheela and Bhatnagar, 2002). The increasing exposure to fluoride has led to questions regarding its safety. There are reports both in favour (Pati and Bhunya, 1987; Podder et al., 2011) and against (Hayashi et al., 1988; Liet al., 1987; Martin et al., 1979) the controversial genotoxic potential of fluoride. In addition reports have suggested fluoride as a genotoxic agent that causes havoc through water contamination worldwide (Li et al., 1995). Fluoride leads to increased percentage DNA in comet tail and olive tail moment in rat (Zhang et al., 2008). The administration of sodium fluoride (15 mg/l) to mice for 30 days significantly increased the percentage of aberrant cells and structural chromosomal aberrations (Podder et al., 2008).

Conversely, antioxidants are thought to play a crucial role in counteracting free radical induced damage to macromolecules and has been found to repair free radical mediated cell damage. There are many toxic, mutagenic and carcinogenic metals such as arsenic, nickel and chromium with proven genotoxic potential. Vitamin E is an important lipid soluble antioxidant present in cells (Burton et al., 1983). Further, reports from *in vivo* and *in vitro* studies indicate that curcumin possesses antioxidant and anticarcinogenic properties (Naik et al., 2004; Smerak et al., 2006). Vitamin C is known to be an antioxidant that is able to scavenge free radicals and prevent oxidative damage to the cell membrane, induced by radicals in the aqueous environment (Monteiro and Stern, 1996). As observed in previous studies, Vitamin C and E have proved evident as efficient drugs against arsenic mediated toxicity (Panneerselvam et al., 2005). Vitamin C decreased the frequency of Sister-chromatid exchange induced by MMC and cyclophosphamide (Krishna et al., 1986). Also, Vitamin C has shown protection against the genotoxicity of the drug 'Cisplatin' *in vivo* (Giri et al., 1998), as well as *in vitro* (Nefic, 2001).

Taking all these evidences into consideration, this study was designed to determine the genotoxic potential of arsenic trioxide and sodium fluoride *in vivo*, and to further evaluate the protective role of VC upon As and F induced genotoxicity.

MATERIALS AND METHODS

Arsenic trioxide (CAS No. 1327-53-3) was purchased from Sigma-Aldrich Co., USA and sodium fluoride (CAS No.7681-49-4) was obtained from Merck Co., Mumbai, India. Vitamin C (CAS No.50-81-7), Normal melting point (NMP) agarose, low melting point (LMP) agarose, di-sodium salt of ethylene diamine tetra acetic acid (EDTA), Tris buffer, ethidium bromide (EtBr), thiobarbituric acid (TBA), trichloroacetic acid (TCA), Triton X-100, DMSO, sodium hydroxide, sodium chloride, acetic acid and methanol were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Mitomycin C

(CAS No. 50-07-7) was purchased from Cadila Pharmaceuticals, India, while phosphate buffered saline (Ca^{2+}, Mg^{2+} free; PBS) was obtained from Hi-Media Ltd., Mumbai, India.

This study was conducted using Swiss albino mice of both sexes, within the ages 6-8 weeks old, weighing 22-28g. The animals were obtained from the departmental animal house colony and acclimatized for two weeks prior to exposure. They were maintained at room temperature (25±2°C) and normal photoperiod conditions (12 h light and 12 h dark). Standard food pellets and water *ad libitum* was provided to the animals. This experiment was approved by the ethical Committee of the University Animal Care Unit, University of Calcutta (India).

Vitamin C (500 mg/kgbw) was administered orally (p.o.) by gavage for 7 consecutive days prior to treatment with arsenic trioxide, Sodium fluoride and in combination of both. The dose were selected and standardized on the basis of available literature. The animals in the control group were treated with normal saline. Arsenic trioxide (4 mg/kgbw) was administered intraperitoneally (i.p.) over a period of 7 days. Sodium fluoride (8 mg/kgbw) was also administered orally, which is similar to the intraperitoneal treatment of arsenic trioxide (Table 1).

The doses of arsenic trioxide and Sodium fluoride were based on the LD_{50} of Arsenic trioxide (39.4 mg/kg body weight) in swiss albino mice (Harrison et al., 1958) and sodium fluoride (54.4 mg/kg body weight) in male mice (Pillai et al., 1988). The doses of vitamin C were chosen based on earlier study on mice (Giri et al., 2008).

Table 1. Description of the experimental design

Experimental groups	Treatment	Doses
Group I	Control	
Group II	Arsenic Trioxide(AT)	4mg/kg bw
Group III	Sodium Fluoride(NF)	8mg/kg bw
Group IV	Arsenic Trioxide+Sodium Fluoride (AT+NF)	4mg/kg bw+8mg/kg bw
Group V	Arsenic Trioxide+Vitamin C (AT+VC)	4mg/kg bw+500mg/kg bw
Group VI	Sodium Fluoride+Vitamin C (NF+VC)	8mg/kg bw+500mg/kg bw
Group VII	Arsenic Trioxide+Sodium Fluoride+Vitamin C(AT+NF+VC)	4mg/kg bw+8mg/kg bw+500mg/kg bw
Group VIII	Vitamin C(VC)	500mg/kg bw

Comet Assay is recognized as one of the most sensitive technique for measuring the DNA strand breaks. The method is rapid, simple, sensitive and visual. The alkaline comet assay was carried out as described by Tice et al. (2000) with slight modifications. In this study, three layered agarose procedure was used. For the first layer, 1% normal melting point (NMP) agarose was prepared with Milli Q water. For the second layer, 1% low melting point (LMP) agarose and for the third layer, 0.5% LMP agarose were prepared in calcium and magnesium free phosphate-buffered saline (PBS). The slides were dipped in methanol and were burnt over a blue flame in order to remove the dust and machine oil. The conventional microscopic slides were dipped in molten hot NMP agarose (1%) and marked properly. The bone marrow cells were flushed with 5 ml of PBS and centrifuged @ 3000 rpm for proper mixing. For the second layer, 250 µl of 1% LMP agarose (37°C) and 250 µl cell sample were mixed and spread on the pre-coated slides by means of cover slips and the gel was solidified by placing the slides on an ice plate for 5-10 min. The cover

slips were removed and the slides were processed for the third layer, 100 µl of 0.5% LMP agarose in the same manner as the second layer. The slides were then placed in freshly prepared chilled lysis buffer (2.5 M Nacl, 100 mM EDTA and 10 mM Tris, p^H 10), with freshly added 1% Triton X-100 and 10% DMSO for 1 h at 4°C. The slides were then incubated in freshly prepared alkaline buffer (10 N NaOH and 200 mM EDTA, pH 10) for 20 min at 4°C for unwinding of the DNA. An electric current of 302 mA and 26 V was applied for 30 min to perform DNA electrophoresis. Thereafter, the slides were neutralized by washing three times for 5 min with neutralization buffer (0.4M Tris-Hcl, pH 7.5) and finally rinsed in distilled water. DNA specific fluorescent dye Ethidium Bromide (20 µg/ml) was used to stain the slides and rinsed in distilled water to wash off excess stain. Images of 150 randomly selected cells (50 cells from each of two replicate slides) were scored from each animal by using fluorescent microscope (Leica, Wetzlar, Germany). Comets were analyzed by using Komet 5.5 software (Andor Technology, Belfast, Northern Ireland).

The Chromosomal aberration (CA) assay was carried out as described by Krishna and Hayashi (2000), with slight modifications. The experimental animals were injected with 2 mg/kgbw of colchicine 1.5 h prior to sacrifice following treatment with test chemicals. Bone marrow cells were collected by flushing with 0.56% KCl (pre-warmed at 37ºC) from the femur bone and incubated for 20 min at 37ºC. The material was centrifuged at 1000 rpm for 5 min, fixed in freshly prepared aceto-methanol (acetic acid and methanol in the ratio 1:3, v/v) followed by refrigeration for 30 min. The material was centrifuged and re-suspended in aceto-methanol. The slides were prepared by dropping the sample on chilled slides and run over the flame. Staining was done using 5% buffered Giemsa. Stain (P^H 7.0) after 24 h, air dried and covered with coverslips. At least 100 well spread metaphase plates were studied per animal for structural chromosomal aberrations. The mitotic index was calculated from a scan of 2000 cells per animal. Mitotic index for cytotoxicity evaluation was calculated as percentage of dividing cells out of total bone marrow cells counted. The following formula was applied for the calculation of suppression percentage of CA and the results are presented in Table 3.

$$100 - \left[\frac{\text{\% incidence of aberrant cells in vitamin C treated and arsenic trioxide treated groups}}{\text{\% incidence of aberrant cells in only arsenic trioxide treated group}} \right] \text{X } 100$$

Lipid peroxidation (LPO) level in the liver was measured using the method of Shafiq-ur-Rehman (2003). On completion of doses, the animals were sacrificed by cervical dislocation. Liver was dissected out from each animal, blotted and weighed. MDA, the final product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a coloured complex. The tissue homogenate (1 ml) was used for the estimation of LPO and was incubated at 37°C for 2 h. To each sample, 1 ml of 10% w/v tricholroacetic acid (TCA) was added and mixed properly. The mixture was then centrifuged at 2000 rpm for 10 min. In another test tube, 1 ml of supernatant was taken and allowed to react with an equal volume of 0.67% w/v TBA for 10 min in a boiling water bath. The mixture was cooled with tap water and diluted with 1 ml double distilled water. The absorbance was read at 535 nm using a Thermo Scientific

GENESYS 20 visible Spectrophotometer. LPO was measured in the form of n mol MDA/mg wet weight of tissue.

Statistical comparisons were done with the Graph pad prism program using one-way analyses of variance (ANOVA) test. Statistically Significant was accepted at P< 0.05.

RESULTS AND DISCUSSION

In the arsenic trioxide treatment, % tail DNA increased in Group II (P<0.05). Tail extent moment and olive tail moment increased in sodium fluoride treated Group III (P<0.05). Statistically, significant increase was observed in all comet parameters in the combined treatment of arsenic trioxide with sodium fluoride (Group IV; P<0.001) when compared to control and visibly indicated arsenic and fluoride induced DNA damage at the given dose. Noteworthy decrease was observed in the percent tail DNA in the group V receiving VC along with arsenic trioxide (P<0.01). Percent tail DNA was also decreased in the treatment of sodium fluoride with vitamin C (Group VI; P<0.001). There was a significant decrease in % tail DNA (P<0.001), tail extent moment (P<0.001) and olive tail moment (0.05) with administration of vitamin C along with arsenic trioxide and sodium fluoride treatment (Group VII) when compared to group IV (Table 2). These findings visibly indicated mitigating action of VC against arsenic trioxide and sodium fluoride induced DNA damage.

Table 2. Comet parameter: % tail DNA, tail extent moment, olive tail moment in bone marrow cells of control, arsenic trioxide, sodium fluoride, vitamin c alone, and in combined treatment as measured by comet assay in mice*in vivo*

Treatment	Route	%Tail DNA [mean ± SD]	Tail Extent Moment [mean±SD]	Olive Tail Moment [mean±SD]
Control	p.o.	3.45±0.36	0.12±0.03	0.13±0.01
AT	i.p.	6.71±0.45^{*d}	0.19±0.06	0.22±0.04
NF	p.o.	6.23±0.39^{e}	0.23±0.07*	0.25±0.03*
AT+NF	i.p.+p.o.	9.69±0.48^{***c}	0.53±0.16^{***c}	0.39±0.06^{***f}
AT+VC	i.p.+p.o.	1.82±0.68^{d}	0.07±0.06	0.08±0.04
NF+VC	p.o.+p.o.	2.42±0.36^{e}	0.11±0.04	0.11±0.02
AT+NF+VC	i.p.+p.o.+p.o.	4.07±0.37^{c}	0.14±0.02^{c}	0.16±0.04^{f}
VC	p.o.	2.08±0.45	0.02±0.01	0.08±0.03

Values are significantly different from control: ***P<0.001; *P<0.05.Values having similarsuperscripts in the respective columns are significantly different from each other P<0.01 (d, e); P <0.05 (f)

Table 3 shows that the percent of aberrant cells increased with arsenic trioxide (P<0.001), sodium fluoride (P<0.01) and both arsenic with fluoride (P<0.001) treatment and found to be statistically significant when compared with untreated control. VC pretreatment significantly reduced the percent of aberrant cells in arsenic trioxide, sodium fluoride and in combined treatment of both arsenic and fluoride (P<0.05). The status of mitotic index (MI) evaluated as percentage of dividing cells was found to be 1.87±0.10 in control. In arsenic trioxide (Group II; P<0.05), sodium fluoride (Group III; P<0.01) and also in combination with arsenic and fluoride (Group IV; P<0.001) treatment, there were a decrease in MI, indicating increase in cytotoxicity. But on supplementation with VC, sodium fluoride treated Group VI and both arsenic and fluoride treated Group VII significantly increases the percentage of MI (P<0.05).

The frequency of structural aberrations increased in arsenic trioxide, sodium fluoride and combined treatment of both arsenic and fluoride and were found to be statistically significant (P<0.001). In the

present study, the efficacy of VC against arsenic trioxide and sodium fluoride induced genotoxicity is shown. In the combination, VC pretreatment apparently reduced the frequency of structural aberrations in the arsenic trioxide, sodium fluoride and both arsenic and fluoride treatment and the decrease was found to be significant (P<0.001).

Treatment of arsenic, fluoride and combination of both induced clastogenic damage and the suppression percentage in arsenic trioxide, sodium fluoride and combined treatment were 65.73, 65.56 and 46.91, respectively when VC supplementation (500mg/kgbw) was administered (Table 3).

Table 3. Percent of aberrant cells, mitotic index and frequency of structural aberrations in the bone marrow cells of mice after 7 days treatment in all groups

Treatment	Route	Total cells studied/n	% Aberrant Cells [mean±SD]	Mitotic Index (%) [mean±SD]	Structural Abberations [mean±SD]	Suppression (%)
Control	p.o.	300/3	2.33±0.30	1.87±0.10	2.33±0.52	
AT	i.p.	300/3	$9.00\pm0.89^{***f}$	$1.43\pm0.10^{*}$	$11.67\pm0.52^{***a}$	
NF	p.o.	305/3	$6.33\pm1.03^{**g}$	$1.33\pm0.10^{**f}$	$9.67\pm1.37^{***b}$	
AT+NF	i.p.+p.o.	302/3	$13.00\pm2.37^{***h}$	$1.20\pm0.09^{***g}$	$16.33\pm2.88^{***c}$	
AT+VC	i.p.+p.o.	300/3	3.66 ± 0.30^{f}	1.56±0.06	4.00 ± 0.51^{a}	65.73
NF+VC	p.o.+p.o.	301/3	3.00 ± 0.51^{g}	1.60 ± 0.08^{f}	3.33 ± 0.30^{b}	65.56
AT+NF+VC	i.p.+p.o.+p.o.	300/3	7.66 ± 0.79^{h}	1.50 ± 0.09^{g}	8.67 ± 0.30^{c}	46.91
VC	p.o.	300/3	2.67±0.52	1.67±0.10	4.67±0.52	

Values are significantly different from control: $^{***}P<0.001$; $^{*}P<0.05$. Valus having similar superscripts in the respective columns are significantly different from each other P<0.001 (a, b, c); P<0.05 (f, g, h)

In addition, MDA was measured for oxidative stress. The results thus obtained from LPO assay in arsenic trioxide, sodium fluoride and VC treated Swiss albino mice could be correlated with the results obtained from comet assay, micronucleus assay, as well as chromosomal aberration assay. Arsenic trioxide intoxication significantly increased in group II (P<0.001) in comparison to control. In group III, treatment with sodium fluoride significantly increased the level of MDA (P<0.01). In the combination studies, that is, in group IV, arsenic trioxide and sodium fluoride treatment significantly increased the MDA level (P<0.001). Treatment with VC appeared to reverse this parameter significantly towards normal in Group V (P<0.01), Group VI (P<0.05) and Group VII (P<0.001) as presented in Table 4.

Table 4. Extent of lipid peroxidation induced by Arsenic trioxide, Vitamin C, Sodium Fluoride alone, and in combination in mice liver in *vivo*

Treatment	Route	Lipid Peroxidation (nM MDA/g wet tissue) (mean±SD)
Control	p.o.	4.00±0.18
AT	i.p.	$5.57\pm0.14^{***d}$
NF	p.o.	$5.32\pm0.16^{**f}$
AT+NF	i.p.+p.o.	$7.65\pm0.66^{***a}$
AT+VC	i.p.+p.o.	3.49 ± 0.28^{d}
NF+VC	p.o.+p.o.	3.96 ± 0.07^{f}
AT+NF+VC	i.p.+p.o.+p.o.	4.08 ± 0.14^{a}
VC	p.o.	3.54±0.14

Values are significantly different from control. ***P<0.001; **P<0.01; *P<0.05.Values having similar superscripts in the respective columns are significantly different from each other P<0.001 (a); P<0.01 (d); P<0.05 (f)

Arsenic compounds have also been shown to produce clastogenic and aneugenic effects (Ostrosky-Wegman et al., 1995; Menzel et al., 1997; Pekkanen, 1998) DNA-protein cross-links and DNA strand lesions (Dunkelberg, 1998). The metabolism of arsenic generates a variety of genotoxic and cytotoxic species, damaging the DNA directly and indirectly, through the generation of reactive oxygen species and induction of DNA adducts, strand breaks and cross links and inhibition of the DNA repair process itself. Inorganic arsenic, the form found in soil, water and crops, is classified by the US Environmental Protection Agency as a Group A human carcinogen, meaning that sufficient knowledge exists to substantiate a causal relationship between human exposure and cancer occurance (NAS, 1999). Arsenic is found to alter the mitochondrial function by altering the cytochrome c oxidase and citrate synthase activity (Partridge et al., 2007). Further, immunohistochemical study reported the appearance of 8-oxodG, not only in tumor tissues but also in keratosis and normal tissues of arsenic related skin tumors (An et al., 2004). Arsenic exposure has been shown to depress the functions of antioxidant defense system leading to oxidative damage to cellular macromolecules such as DNA leading to apoptosis (Liu et al., 2001; Huang et al., 1999; Ochi et al., 1996; Wang et al., 1996).

However, the mechanism of fluoride induced genotoxicity is still not clear. Previous study indicates that oxidative stress induced by ROS is one of the major factors in the manifestation of fluoride toxicity (Jeji et al., 1985; Saralakumari and Rao, 1991; Shanthakumari et al., 2004). Sodium fluoride could induce DNA damage and apoptosis in rat's brain, oral mucosal cells and hepatocytes through the induction of oxidative stress (Chen et al., 2002; He and Chen, 2006). Earlier report indicates melatonin *in vitro* protection of lymphocytes from toxic effects of arsenic and fluoride (Pant and Rao, 2010). Sodium fluoride has been shown to be genotoxic in mammalial cells *in vitro*, inducing CA and SCE (Tsutsui et al., 1984). Fluoride is mutagenic and causes damage to DNA and chromosomes (Aardema et al., 1989; Crespi et al., 1990). Moreover, fluoride has synergistic or antagonistic effects with certain mutagens (Mukherjee and Sobels, 1968; Slacik-Erben and Obe, 1976).

Fundamentally, the present study has shown that arsenic trioxide and sodium fluoride induces DNA strand breaks in blood leukocytes of mice. Arsenic trioxide induces DNA strand breaks in blood leukocytes of mice *in vivo* (Ishaq et al., 2001). A recent study revealed that curcumin supplementation protects against the *in vitro* genotoxic effects of arsenic and fluoride in human lymphocytes (Rao and Tiwari, 2010). In the present study, VC showed decrease in arsenic trioxide and sodium fluoride induced chromosomal abnormalities, DNA damage and MDA level, indicating its protective role. The antiapoptotic activity of vitamin C and E has been reported by several researchers (Straface et al., 1995; Barroso et al., 1997; Mobio et al., 2000). An *in vivo* study has shown metallothionein (MT) induction in As (III) treated swiss albino mice to reduce its toxicity (Kreppel et al., 1993). Vitamin C protects against toxic effects of arsenic and fluoride *in vitro*, by reducing oxidative stress (Jhala et al., 2008).

The results of this study showed that arsenic trioxide and sodium fluoride are both genotoxic to mice. Thus the present study demonstrates positive effects of VC when administered to Swiss albino mice by reversing the toxicity of arsenic trioxide and sodium fluoride.

CONCLUSION

This study has supported the role of arsenic trioxide and sodium fluoride as a potent genotoxic agents, since *in vivo* exposure

caused DNA damage of the cell. Several studies confirmed the antioxidant properties of VC against arsenic induced toxicity. VC has antioxidant and free radical scavenging activities, which suggests that this vitamin may modulate oxidative DNA damage in mammalial cells (Odin, 1997).

In the present study, VC supplementation caused radical amelioration in the genotoxic effects brought about by arsenic trioxide, sodium fluoride and also in combined treatment. Conclusively, VC successfully ameliorates the *in vivo* genotoxic effects of arsenic trioxide and sodium fluoride in swiss albino mice and its potency is attributed to the antioxidant property present.

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REFERENCES

Aardema, M.J., Gibson, D.P. and LeBoeuf, R.A. (1989). Sodium fluoride-induced chromosome aberrations in different stages of the cell cycle: a proposed mechanism. Mutat. Res., 223, 191-203.

Abernathy, C., Liu, Y., Longfellow, D., Aposhian, H., Beck, H., Fowler, B., Goyer, R., Menzer, R., Rossman, T., Thompson, C. and Waalkes, M. (1999). Arsenic: health effects, mechanisms of action and research issues. Environ. Health Perspect., 107, 593–597.

An, Y., Gao, Z., Wang, Z., Yang, S., Liang, F., Feng, Y., Kato, K., Nakano, M., Okada, S. and Yamanaka, K. (2004). Immunohistochemical analysis of oxidative DNA damage in arsenic-related human skin samples from arsenic-contaminated area of China. Cancer Lett., 214 (1), 11–18.

Aposhian, H. V. (1989). Biochemical toxicology of arsenic. Rev. Biochem. Toxicol., 10, 265–299.

ATSDR. (2003). Toxicological Profile for Fluorides, Hydrogen Fluoride and Fluorine.US Departmental of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Research. Toxicological Profile for Arsenic.

ATSDR. (2005). Toxicological Profile for Arsenic. US Departmental of Health and Human Services, Public Health Service, Atlanta, GA.

Barroso, M.P., Diaz, C.G., Lluch, G.L., Malagon, M.M., Crane, F.L. and Navas, P. (1997). Ascorbate andα-tocopherol prevent apoptosis induced by serum removal independent of Bcl-2. Archiv. Biochem. Biophys., 343, 243–248.

Burton, G.W., Joyce, A. and Ingold, K.U. (1983). Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch. Biochem. Biophys., 221, 281-90.

Chen, J., Chen, X., Yang, K., Xia, T. and Xie, H. (2002). Studies on DNA damage and apoptosis in rat brain induced by fluoride. Chinese Journal of Preventive Medicine, 36 (4), 222–224.

Crespi, C.L., Seixas, G.M., Turner, T. and Penman, B.W. (1990). Sodium fluoride is a less efficient human cell mutagen at low concentrations. Environ. Mol. Mutagen., 15, 71-77.

Cui, C.G. and Liu, Z.H. (1988). Chemical speciation and distribution of arsenic in water, suspended solids and sediment of Xiangjiang river. China Sci. Total Environ., 77, 69–72.

Dunkelberg, H., Luthin, S., Birkenkamp, P. and Gebel, T. (1998). Arsenic (III), but not antimony, induces DNA–protein cross-links. Anticancer Res., 18, 4253–4258.

Giri, A., Khynriam, D. and Prasad, S.B. (1998). Vitamin C mediated protection on cisplatin induced mutagenicity in mice. Mutat. Res., 421, 139–148.

Giri, S., Mazumdar, M. and Roy, D.L. (2008). Effects of low dose radiation and vitamin C treatment on chloroquine-induced genotoxicity in mice. Environmental and Molecular Mutagenesis, 49, 488-495.

Goering, P., Aposhian, H., Mass, M., Cebrián, M., Beck, B. and Waalkes, M. (1999).The enigma of arsenic carcinogenesis: role of metabolism. Toxicol. Sci., 49, 5–14.

Harrisson, J.W.E., Packman, E.W. and Abbott, D.D. (1958). Acute oral toxicity and chemical and physical properties of arsenic trioxides. Arch. Ind. Health, 17, 118–123.

Hayashi, M., Kishi, M., Sofuni, T. and Ishidate, M. Jr. (1988). Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Food Chem.Toxicol., 26, 487–500.

He, L.F. and Chen, J.G. (2006). DNA damage, apoptosis and cell cycle changes induced by fluoride in rat oral mucosal cells and hepatocytes.World J.Gastroenterol., 12, 1144–1148.

Hindmarsh, J.T. and Mc Curdy, R.F. (1986). Clinical and environmental aspects of arsenic toxicity. CRC Crit. Rev. Clin. Lab. Sci., 23, 315–319.

Huang, C., Ma, W.Y., Li, J. and Dong, Z. (1999). Arsenic induces apoptosis through a c-junNH2-terminal kinase-dependent, p53-independent pathway. Cancer Res., 59, 3053–3058.

IARC. (1987). IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans: Overall Evaluations of Carcinogenicity, An Updating of IARC Monographs, 7 (1–42), World Health Organization, IARC, Lyon.

Ishaq, M., Rao Visweswara, K., Ahuja, R.Y., Jamil, Kaiser, Danadevi, K. and Banu, S.B. (2001). In vivo genotoxic effect of arsenic trioxide in mice using comet assay. Toxicology, 162,171-177.

Jeji, J., Sharma, R., Jolly, S.S. and Pamnani, S. (1985). Implication of glutathione in endemic fluorosis. Fluoride, 18, 117–119.

Jhala, D. D., Chinoy, N. J. and Rao, M.V. (2008). Mitigating effects of some antidotes on fluoride and arsenic induced free radical toxicity in mice ovary. Food Chem. Toxicol., 46 (3), 1138–1142.

Kaul, R.D. and Susheela, A.K. (1974). Evidence of muscle fibre degeneration in rabbits treated with sodium fluoride. Fluoride, 7, 177–181.

Kreppel, H., Bauman, J.W., Liu, J., Mc Kim, J.M. and Klaassen, C.D. (1993). Induction of metallothionein by arsenicals in mice. Fundam. Appl. Toxicol., 20, 184–189.

Krishna, G. and Hayashi, M. (2000). In vivo rodent micronucleus assay: protocol, conduct and data interpretation. Mutat. Res., 455, 155–166.

Krishna, G., Nath, J. and Ong, T. (1986). Inhibition of cyclophosphamide and Mitomycin C-induced sister chromatid exchanges in mice by vitamin C. Cancer Res., 46, 2670-2674.

Li, Y., Dunipace, A.J. and Stookey, G.K. (1987). Lack of genotoxic effects of fluoride in the mouse bone-marrow micronucleus test. J. Dent. Res.,66, 1687–1960.

Li, Y., Liang, C.K., Katz, B.P., Brizendine, E.J. and Stookey, G.K. (1995). Long-term exposure to fluoride in drinking water and sister chromatid exchange frequency in human blood lymphocytes. J. Dent. Res., 74, 1468–1474.

Liu, S.X., Athar, M., Lippai, I. and Waldren, T.K. (2001). Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. Proc. Natl. Acad. Sci., 98, 1643–1648.

Luchtrath, H. (1972). Cirrhosis of the liver in chronic arsenical poisoning. German Med. Mon., 2, 127.

Martin, G.R., Brown, K.S., Matheson, D.W., Lebowitz, H., Singer, L. and Ophaug, R. (1979). Lack of cytogenetic effects in mice or mutations in Salmonella receiving sodium fluoride. Mutat. Res., 66, 159–167.

Menzel, D. and Rasmussen, R. (1997). Variation in arsenic-induced sister chromatid exchange in human lymphocytes and lymphoblastoid cell lines. Mutat. Res., 386, 299–306.

Mobio, T., Baudrimont, I., Sanni, A., Shier, T.W., Saboureau, D., Dano, S.D., Udeno, Y., Steyn, P.S. and Creppy, E.E. (2000). Prevention by Vitamin E of DNA fragmentation and apoptosis induced by fumonisin B1 in C6 glioma cells. Archiv. Toxicol., 74, 112–119.

Monteiro, H.P. and Stern, A. (1996). Redox regulation of tyrosine phosphorylation-dependent signal transduction pathways. Free. Radic. Biol. Med., 21, 323–333.

Mukherjee, R.N. and Sobels, F.A. (1968). The effects of sodium fluoride and iodoacetamide on mutation induction by x-irradiation in mature spermatozoa of Drosophila. Mutat. Res.,6, 217-225.

Naik, R.S., Mujumdar, A.M. and Ghaskadbi, S. (2004). Protection of liver cells from ethanol cytotoxicity by curcumin in liver slice culture in vitro. J. Ethnopharmacol., 95, 31-37.

NAS. (1999). Arsenic in Drinking water. Natl. Acad. Press, Washington, DC.

Nefic, H. (2001). Anticlastogenic effect of Vitamin C on cisplatin induced chromosome aberrations in human Iymphocyte cultures. Mutat. Res., 498, 89-98.

NRC. (1999). Arsenics in the Drinking Water, National Academy Press, Washington, DC.

Ochi, T., Nakajima, F., Sakurai, T., Kaise, T. and Oya-Ohta, Y. (1996). Dimethylarsinic acid causes apoptosis in HL-60 cells viainter-action with glutathione. Archiv. Toxicol., 70, 815–821.

Odin, A. P. (1997). Vitamins as antimutagens: advantages and some possible mechanisms of antimutagenic action. Mutat. Res., 386, 39-67.

Ostrosky-Wegman, P., Gonsebatt, M. and Vega, L. (1995). Aneugenic effect of sodium arsenite on

human lymphocytes in vitro: an individual susceptibility effect detected. Mutat. Res., 334, 365–373.

Panneerselvam, C., Shila, S., Anusuyadevi, M. and Ramanathan, K. (2005). Ascorbic acid and α-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. Toxicology Letters, 156, 297-306.

Pant, H, H. and Rao, V, M. (2010). Evaluation of *in vitro* anti-genotoxic potential of melatonin against arsenic and fluoride in human blood cultures. Ecotoxicology and Environmental Safety, 73, 1333-1337.

Partridge, A., Huang, S.X.L., Hernandez-Rosa, E., Davidson, M.M. and Hei, T.K. (2007). Arsenic induced mitochondrial DNA damage and altered mitochondrial oxidative function: implications for genotoxic mechanisms in mammalian cells. Cancer Res., 67, 5239–5247.

Pati, P.C. and Bhunya, S.P. (1987).Genotoxic effect of an environmental pollutant, sodium fluoride, in mammalian in vivo test system.Caryologia,40, 79–87.

Pekkanen, J., Paldy, A., Kurttio, P. andMäki-Paakkanen, J. (1998). Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water. Environ. Mol. Mutagen., 32, 301–313.

Pillai, K.S., Mathai, A.T. and Deshmukh, P.B. (1988). Effect of subacute dosage of fluoride on male mice.Toxicol. Lett., 44, 21-29.

Podder, S., Chattopadhyay, A. andBhattacharya, S. (2008). In vivo suppression by fluoride of chromosome aberrations induced by mitomycin-cinmouse bone marrow cells. Fluoride, 41, 40–43.

Podder, S., Chattopadhyaya, A. and Bhattacharya, S. (2011). Reduction in fluoride-induced genotoxicity in mouse bone marrow cells after substituting high fluoride-containing water with safe drinking water. J. Appl. Toxicol., 31, 703–705.

Rao, V.M. and Tiwari, H. (2010). Curcumin supplementation protects from genotoxic effects of arsenic and fluoride. Food and Chemical Toxicology, 48, 1234-1238.

Saralakumari, D. and Rao, R.P. (1991). Red cell membrane alterations in human chronic fluoride toxicity. Biochem. Int., 23, 639–648.

Shafiq-ur-Rehman (2003). Lead-exposed increase in movement behaviour and brain lipid peroxidation in fish. Journal of Environmental Science and Health, 38 (4), 631–643.

Shanthakumari, D., Srinivasalu S. and Subramanian, S. (2004). Effects of fluoride intoxication on lipid peroxidation and antioxidant status in experimental rats. Toxicology, 204, 219–228.

Slacik-Erben, R. and Obe, G. (1976).The effect of sodium fluoride on DNA synthesis, mitotic indices and chromosomal aberrations in human leukocytes treated with Trenimon in vitro. Mutat. Res., 37, 253-266.

Sollott, S.J., Juhaszova, M. and Zorov, D.B. (2006). Mitochondrial ROS-induced ROS release: an update and review. Biochim. Biophy. Acta, 1757, 509–517.

Smerak, P., Polivkova, Z., Sestakova, H., Stetina, R., Barta, I., Langova, M., Turek, B. and Bartova, J. (2006). Antimutagenic effect of curcumin and its effect on the immune response in mice. Czech J. Food Sci., 24, 72–83.

Straface, E., Santini, M.T., Donelli, G., Giacomoni, P.U. and Malorni, W. (1995). Vitamin E prevents UV B-induced cell blebbing and cell death in A431 epidermoid cells. Int. J. Radiat. Biol., 68, 579–587.

Susheela, A.K. and Bhatnagar, M. (2002). Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Mol. Cell. Biochem., 234, 335–340.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C. and Sasaki, Y.F. (2000). Single cell gel / Comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen., 35, 206–221.

Tsutsui, T., Suzuki, N. and Ohmori, M. (1984). Sodium fluoride-induced morphological and neoplastic transformation, chromosome aberrations, sister chromatid exchanges, and unscheduled DNA synthesis in cultured Syrian hamster embryo cells. Cancer Res., 44, 938-941.

Wang, T.S., Kuo, C.H., Jan, K.Y. and Huang, H. (1996). Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. J. Cell Physiol., 169, 256–267.

Zhang, M., Wang, A., Xia, T. and He, P. (2008). Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF-kappaB in primary cultured rat hippocampal neurons. Toxicol. Lett., 179, 1–5.