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# Effect of the Antioxidant Butylated Hydroxytoluene on the Genotoxicity and Cytotoxicity Induced in Mice by Sodium Arsenite

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**Abstract:** In this study, we evaluated the effect of the antioxidant butylated hydroxytoluene (BHT) on the genotoxicity and cytotoxicity induced by sodium arsenite ($NaAsO_2$) in normal adult male SWR/J mouse bone marrow cells. Animals were subjected to intraperitoneal (i.p.) injection of $NaAsO_2$ at various dose levels (1, 0.5 and 0.25 $LD_5$, which corresponds to 9, 4.50 and 2.25 mg $kg^{-1}$ b. wt.) and killed 24 h later. Another group of male mice were treated with 30 mg $kg^{-1}$ b. wt. of the synthetic antioxidant and hypermethylizing agent butylated hydroxytoluene 1 h prior to $NaAsO_2$ administration. The three single doses of sodium arsenite significantly ($p<0.05$) increased the rate of total structural Chromosomal Aberrations (CAs), Sister Chromatid Exchanges (SCEs), micronucleus (MNs) formation, Poly (ADP-ribose) polymerase (PARP) and Lamina-A degradation and apoptosis compared with the negative control. In the combined treatment with BHT, no significant effect was observed in the rate of CAs or SCEs, whereas a significant decrease was observed in the rate of micronucleated polychromatic erythrocytes (MNPCEs) at medium and high doses. The present study has shown that administration of an antioxidant had a negative effect as represented in the rate of CAs, PARP and Lamina-A degradation and apoptosis. On the other hand, the antioxidant had a positive effect as represented in the decreased rate of pulverized chromosomes and MN formation.

**Key words:** Methylation, genotoxicity, cytotoxicity, sodium arsenite, CAs, SCEs, MNs, PARP, Lamina-A, apoptosis, mice

## INTRODUCTION

It has become evident that increased human activity has modified the natural cycle of metals and metalloids, including toxic elements such as arsenic (Chowdhury *et al*., 2008). The contamination of air, water, food and soil with arsenic for long periods has led to different degrees of arsenic toxicity and has become a threat to plant and animal communities, including humans (Toribio and Romanya, 2005; Florea and Büsselberg, 2008). A large number of human-made arsenic compounds were used in agriculture as effective agents against pests, parasites or weeds. These compounds have gradually accumulated in the soil and several organic arsenic compounds are still being employed in the area of human medicine (Loredo *et al*., 2006; Vardanyan and Ingole, 2006; Florea and Büsselberg, 2008).

The biological effects of one metal can be modified considerably by interaction with other metals (Biswas *et al*., 1999a). The present study employed sodium arsenite, which is classified by the International Agency for Research on Cancer (IARC) as a human carcinogen and notwithstanding the fact that arsenic has been the subject of extensive research investigations, its mechanism of action remains to be delineated (Brink *et al*., 2006; Florea and Büsselberg, 2008).

DNA methylation plays an important role in organizing the genome into transcriptionally active and inactive zones and DNA methylation levels have been observed to change following metal treatment (Lee *et al*., 1998; Klein *et al*., 2007; Reichard *et al*., 2007). Despite the large number of studies conducted concerning arsenic toxicity, the effects remain poorly understood (Dopp *et al*., 2004). Several assays performed *in vivo* and *in vitro* on mammalian cells have shown that exposure to arsenic induces chromosomal damage, as indicated by monitoring chromosomal aberrations and the formation of micronuclei (Biswas *et al*., 1999a; Bhattacharya *et al*., 2005; Klein *et al*., 2007).

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The present investigation was undertaken in an effort to determine the effect of antioxidant and hypermethylation state on the genotoxic and cytotoxic effects of sodium arsenite.

## MATERIALS AND METHODS

All of the experimental procedures were conducted in the Genetic Lab and Molecular Biology Laboratory of the King Saud University, Saudi Arabia between 2006 and 2008.

**Experimental animals:** Normal SWR/J male mice, 8-10 weeks old and weighing 25-30 g were used throughout the study. Animals were maintained and bred under standard laboratory conditions.

**Treatments:** A total of 45 males were used and divided into 9 groups, with each group containing 5 males. Group 1 was subjected to (i.p.) injection (0.2 mL/10 g b. wt.) of sterile normal saline as a negative control. Groups 2-4 were subjected to (i.p.) injection of $NaAsO_2$ in single various dose levels 2.25, 4.50 or 9 mg $kg^{-1}$ b. wt. (0.25, 0.50 or 1 $LD_{50}$ respectively). Groups 5-7 were treated with the same doses as in Groups plus 30 mg $kg^{-1}$ of the synthetic antioxidant and hypermethylizing agent butylated hydroxytoluene (BHT) 1 h prior to $NaAsO_2$ treatment. Group 8 was treated with only 30 mg $kg^{-1}$ BHT. Group 9 was treated with the organic solvent Tween-80 (0.2 mL/10 g b. wt.), which was used to dissolve the BHT.

**Test chemicals:** Sodium arsenite was obtained from Hannover, Germany. Butylated hydroxytoluene (BHT), Tween-80, 50 mg 5-Bromo-2-deoxyuridine (BrdU) tablets, hoechest and Acridine Orange (AO) were obtained from (Sigma, UAS).

BrdU was transplanted subcutaneously (Allen *et al*., 1978). The methods of Preston *et al*. (1987) were used for the chromosomal preparations. The method of Latt *et al*. (1981) was used for the staining.

**Scoring:** The slides were used to simultaneously detect Chromosomal Aberrations (CAs) and Sister Chromatid Exchanges (SCEs).

**Chromosomal Aberrations (CAs):** One hundred well-spread and clear metaphases from each slide (giving 100×5 = 500 group) were examined for the monitoring of CAs. Each selected metaphase was examined for CAs using a light microscope (Nikon, Eclipse E600W, Japan) equipped with 10x and 100x oil lenses (Scappaticci *et al*., 2000).

**Sister Chromatid Exchanges (SCEs):** Fifty well-spread and clear metaphases from each slide (giving 50×5 = 250 group) were examined for SCEs (Allen *et al*., 1978).

**Micronucleus test**

**Slide preparation:** Femoral bone marrow cells were flushed from the femur using a syringe containing Fetal Calf Serum (FCS), smeared onto clean glass slides and then fixed with absolute methyl alcohol for 15 min.

**Staining:** Slides were stained by immersion in phosphate buffer solution and followed by treatment with Acridine Orange (AO) for 1 min. Slides were then treated with phosphate buffer solution for 10 min followed by an additional treatment with fresh phosphate buffer solution for 15 min. Slides were embedded with DPX, covered and then immediately examined using an FL EPI-Fluorescence microscope (Nikon, Eclipse E600W, Japan) at 530 nm wavelength.

**Scoring:** One thousand polychromatic erythrocytes (PCEs) oil reddish from each slide, (giving 1000×5 = 5000 group) were examined in this study to evaluate the number of micronucleated polychromatic erythrocytes (MNPCEs) and micronucleated normochromatic erythrocytes (MNNCEs) in normochromatic erythrocytes (NCEs) bright reddish. The ratio of MNPCEs to MNNCEs was used as an indicator of chromosomal changes, while percent of PCEs was used as an indicator of apoptogenicity (Garcia *et al*., 2001).

**Primary antibodies (anti-PARP and anti-Lamina-A):** Both primary antibodies were obtained from Cell Signaling, USA. Primary anti-PARP was used to detect the intact PARP (116 kDa) enzyme, as well as the large (89 kDa) and small (24 kDa) fragments produced following hydrolysis of intact PARP with caspase-3. Primary anti-Lamina-A was used to detect intact Lamina-A (70 kDa) protein, as well as the small (28 kDa), but not the large (45 kDa), fragment following hydrolysis of intact Lamina-A with caspase-6. Both polyclonal antibodies were produced by immunizing rabbits and diluted with skimmed milk (1:1000).

**Secondary antibodies (anti-rabbit IgG) HRP-linked antibodies:** Secondary antibodies were obtained from Cell Signaling, USA. Antibodies were labeled with peroxidase and assayed using enhanced chemiluminescence (ECL) Western blotting detection reagents obtained from Amersham, RPN2106PC, USA.

**Protein extraction:** Protein extraction from mice liver was as follows: 10 g of mice liver was homogenized in a cold homogenizer tube containing 2 mL of homogenization buffer. The concentration of total protein in each sample was estimated spectrophotometrically (GeneQuant pro, Amersham, USA) at 595 nm. Equal volumes of 2X sample buffer and protein (30 μg $\mu L^{-1}$) were mixed in an Eppendorf tube and heated to 95°C for 5 min before loading (Hossain *et al*., 2000; Mathas *et al*., 2003).

**SDS-PAGE and immunoblotting:** The mix of protein and 2X sample buffer was electrophoresed through a 30% polyacrylamide gel using a PowerPac Basic system (S.N 37S/7159, Italy) at 50 V for 1 h and then at 100 V near the end of the electrophoresis. Protein was then transferred onto nitrocellulose membrane. The nitrocellulose membrane was washed several times with Phosphate Buffered Saline (PBS), incubated in 5% skimmed milk, followed by primary antibodies (anti- PARP or anti-Lamina-A) overnight at 4°C and then with secondary antibodies for 3 h (Moronvalle-Halley *et al*., 2005) and then protein bands were visualized using ECL according to the manufacturer's instructions. The molecular size of the visualized protein bands was determined by comparison with markers.

**Statistical analysis:** The data obtained in this study were statistically analyzed with SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) using the Mann-Whitney U-test.

## RESULTS AND DISCUSSION

### Genotoxicity

**Chromosomal Aberrations (CAs):** A number of structural and numerical chromosomal aberrations were scored in bone marrow cells of treated mice, in additional to some aberrations referred to as chromosomal instability (Table 1).

The results obtained from the recorded data in Table 1 show that single treatment with medium or high doses (4.50 and 9 mg $kg^{-1}$) of sodium arsenite induced a significant ($p<0.05$) increase in chromatid breakage compared with the negative control, whereas treatment with BHT induced a significant ($p<0.05$) decrease in chromatid breakage. Single treatment with low or high doses (2.25 and 9 mg $kg^{-1}$) of sodium arsenite induced a significant ($p<0.05$) increase in end to end association compared with the negative control. Single treatment with sodium arsenite had no significant effect on centric fusion compared with the negative control.

**Sister Chromatid Exchanges (SCEs):** The data in Table 2 show the SCEs following single treatment with three doses of sodium arsenite alone or in combination with BHT.

Single treatment with each of the three doses of sodium arsenite induced a significant ($p<0.05$) increase in the rate of SCEs compared with the negative control (Table 2). No significant effect was observed of the hypermethylation state on the rate of SCEs when sodium arsenite was combined with BHT compared with sodium arsenite treatment alone. The hypermethylation state correlated significantly ($p<0.05$) with the decrease in the rate of SCEs induced by the low dose of sodium arsenite.

**Micronucleus:** The data in Table 3 show that single treatment with medium or high doses of sodium arsenite induced a significant ($p<0.05$) decrease in the percent of PCEs compared with the negative control. No significant effect was observed of the hypermethylation state on the percent of PCEs for all combined doses of sodium arsenite with BHT compared with sodium arsenite treatment alone. Single treatment with medium or high doses of sodium arsenite induced a significant ($p<0.05$) increase in the number of MNPCEs compared with the negative control. The hypermethylation state correlated significantly ($p<0.05$) with the increase in MNPCEs induced by sodium arsenite alone at medium or high doses. Single treatment with a high dose of sodium arsenite only induced a significant ($p<0.05$) increase in the number of MNNCEs compared with the negative control. Generally, there was no correlation between the hypermethylation state and the observed decrease in the number of MNNCEs compared with the effect of sodium arsenite treatment alone.

### Cytotoxicity

**Poly (ADP-ribose) polymerase (PARP):** As shown in Fig. 1, single treatment with three doses of sodium arsenite induced apoptosis and yielded positive results (B, C and D) in terms of the degradation of intact PARP molecules (116 kDa) to generate the large (89 kDa) fragment. Figure 1 also shows that single treatment produced different bands which increased with low dose treatment, while no degradation is observed in the negative control panel (A). Combined treatment using BHT (F, G and H) at three doses yielded positive results compared to the negative control. Furthermore, treatment with BHT alone induced PARP fragmentation and led to apoptosis.

Table 1: Frequency of chromosomal aberrations induced in bone marrow cells of mice treated with sodium arsenite ($NaAsO_2$) alone and in combination with butylated hydroxytoluene (BHT)

| | | | | No. of structural chromosomal aberrations | | | | |
|---|---|---|---|---|---|---|---|---|
| Groups | Treatment (dose mg $kg^{-1}$) | No. of examined mice | No. of examined cells | Chromatid breakage | End to end association | Centric fusion | Ring chromosome | Total |
| 1 | -ve control | 5 | 500 | 1 (0.20±0.45) | 0.00 | 0.00 | 0.00 | 1 (0.20±0.45) |
| 2 | $NaAsO_2$ (2.25) | 5 | 500 | 7 (1.40±1.34) | 7[a] (1.40±1.14) | 1 (0.20±0.45) | 2 (0.40±0.55) | 17[a] (3.40±1.83) |
| 3 | $NaAsO_2$ (4.5) | 5 | 500 | 9[a] (1.80±1.48) | 4 (0.80±0.84) | 1 (0.20±0.45) | 5 (1.00±0.70) | 19[a] (3.80±2.28) |
| 4 | $NaAsO_2$ (9) | 5 | 500 | 9[a] (1.80±0.83) | 4[a] (0.80±0.45) | 4 (0.80±0.84) | 1 (0.20±0.45) | 18[a] (3.60±1.52) |
| 5 | $NaAsO_2$ + BHT (2.25+30) | 5 | 500 | 5 (1.00±1.00) | 1 (0.20±0.45) | 1 (0.20±0.45) | 2 (0.40±0.55) | 9[a] (1.80±1.48) |
| 6 | $NaAsO_2$ + BHT (45+30) | 5 | 500 | 9 (1.80±1.8) | 3 (0.60±0.79) | 6 (1.20±1.30) | 1 (0.20±0.45) | 19[a] (3.80±2.28) |
| 7 | $NaAsO_2$ + BHT (9+30) | 5 | 500 | 4[d] (0.80±0.84) | 5 (1.00±1.41) | 8[a] (1.60±0.89) | 0.00 | 17[a] (3.40±1.14) |
| 8 | BHT (30) | 5 | 500 | 9[a] (1.80±1.30) | 3 (0.60±0.55) | 2 (0.40±0.55) | 2 (0.40±0.55) | 16[a] (3.20±1.30) |
| 9 | Tween-80 | 5 | 500 | 4 (0.80±0.84) | 1 (0.20±0.45) | 0.00 | 0.00 | 5[a] (1.00±0.70) |

| | | No. of structural chromosomal aberrations | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | Treatment (dose mg $kg^{-1}$) | Total No. of cells with structural aberrations (%) | No. of cells with one aberration (%) | No. of cells with more than one aberration (%) | No. of cells with chromosome pulverization | No. of centeromerically attenuated cells | Numerical aberration No. of polyploid |
| 1 | -ve control | 1 (0.20) | 1 (0.20) | 0.00 | 2 (0.40±0.55) | 13 (2.60±1.34) | 0.00 |
| 2 | $NaAsO_2$ (2.25) | 16[a] (3.20) | 15[a] (3.00) | 1 (0.20) | 6 (1.20±0.45) | 30[a] (6.00±1.73) | 2 (0.40±0.89) |
| 3 | $NaAsO_2$ (4.5) | 16[a] (3.20) | 13[a] (2.60) | 3[a] (0.60) | 10[a] (2.00±1.00) | 42[a] (8.40±1.95) | 3 (0.60±0.55) |
| 4 | $NaAsO_2$ + (9) | 16[a] (3.20) | 14[a] (2.80) | 2 (0.40) | 6 (1.20±0.84) | 46[a] (9.20±2.39) | 2 (0.40±0.55) |
| 5 | $NaAsO_2$ + BHT (2.25+30) | 7[a] (1.40) | 5[b,e] (1.00) | 2 (0.40) | 3 (0.60±0.55) | 20[b] (4.00±0.70) | 2 (0.40±0.55) |
| 6 | $NaAsO_2$ + BHT (45+30) | 17[a] (3.40) | 15[a] (3.00) | 2 (0.40) | 4 (0.80±1.30) | 31[a,e] (6.20±2.38) | 0.00 |
| 7 | $NaAsO_2$ + BHT (9+30) | 13[a] (2.60) | 9[a] (1.80) | 4 (0.80) | 5 (1.00±1.41) | 52[a,e] (10.40±3.36) | 3 (0.60±0.55) |
| 8 | BHT (30) | 15[a] (3.00) | 14[a] (2.80) | 1 (0.20) | 5 (1.00±0.70) | 12 (2.40±1.52) | 1 (0.2±0.45) |
| 9 | Tween-80 | 5[a] (1.00) | 5 (1.00) | 0.00 | 5 (1.00±0.70) | 15 (3.00±0.70) | 1 (0.20±0.45) |

a: Significant different from group 1 at $p<0.05$, b: Significant different from group 2 at $p<0.05$, d: Significant different from group 4 at $p<0.05$, e: Significant different from group 8 at $p<0.05$

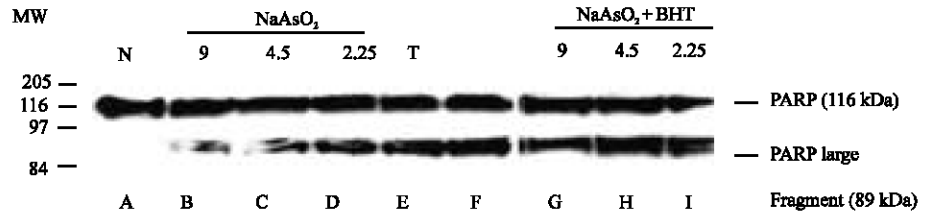


Fig. 1: N: Untreated (A), Western blot analysis of PARP from mice livers treated with sodium arsenite alone (B-D), or in T: Tween-80 (E), MW: Marker: BHT (F), combination with BHT (G-I)

**Lamina-A:** The results shown in Fig. 2 indicate that single treatment with three doses of sodium arsenite induced apoptosis and had a positive effect (B, C and D) in terms of the degradation of intact Lamina-A molecules (70 kDa) to generate small (28 kDa) fragments, however no degradation was observed in the negative control panel

Table 2: Sister chromatid exchange frequency in bone marrow cells of mice treated with sodium arsenite ($NaAsO_2$) alone and in combination with butylated hydroxytoluene (BHT)

| Groups | Treatments (dose mg $kg^{-1}$) | No. of examined mice | No. of examined cells | Sister Chromatid Exchanges ($SCE_S$) I | II | III | IV | V | Mean/group±SD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | -ve control | 5 | 250 | 5.96±2.70 | 5.14±2.40 | 4.72±1.52 | 4.60±1.80 | 4.86±1.64 | 5.05±0.54 |
| 2 | $NaAsO_2$ (2.25) | 5 | 250 | 8.14±3.42 | 8.34±2.92 | 8.60±4.22 | 8.50±4.34 | 9.36±3.00 | 8.58±0.46[a] |
| 3 | $NaAsO_2$ (4.5) | 5 | 250 | 5.16±2.00 | 6.12±2.32 | 10.86±3.80 | 9.90±3.70 | 11.60±4.76 | 8.73±2.90[a] |
| 4 | $NaAsO_2$ (9) | 5 | 250 | 12.48±3.88 | 11.00±4.07 | 8.40±3.25 | 10.84±3.55 | 9.90±3.42 | 10.53±1.50[a] |
| 5 | $NaAsO_2$ + BHT (2.25+30) | 5 | 250 | 5.54±2.90 | 5.92±2.99 | 6.80±3.10 | 8.68±3.47 | 7.18±3.32 | 6.82±1.23[a] |
| 6 | $NaAsO_2$ + BHT (4.5+30) | 5 | 250 | 11.82±3.97 | 10.54±4.28 | 8.44±3.19 | 9.70±3.50 | 10.14±3.00 | 10.12±1.23[a,e] |
| 7 | $NaAsO_2$ + BHT (9+30) | 5 | 250 | 9.42±3.35 | 15.16±5.87 | 13.81±6.23 | 8.94±3.35 | 11.44±4.34 | 11.75±2.70[a,e] |
| 8 | BHT (30) | 5 | 250 | 7.98±3.49 | 8.10±3.14 | 7.26±2.99 | 6.70±2.43 | 7.80±3.47 | 7.57±0.58[a] |
| 9 | Tween-80 | 5 | 250 | 6.84±3.16 | 6.24±2.88 | 6.58±2.13 | 6.16±2.10 | 6.14±2.42 | 6.39±0.30[a] |

a: Significant difference from group 1 at $p<0.05$; e: Significant difference from group 8 at $p<0.05$

Table 3: Effect of sodium arsenite ($NaAsO_2$) alone and in combination with butylated hydroxytoluene (BHT) on micronucleus induction in bone marrow cells of SWR/J mice

| Groups | Test substances | Dose (mg $kg^{-1}$) | Sampling time (h) | No. of examined PCEs | Percentage of $PCE_s$/Ratio of PCE/NCE | Micronucleated cells per 1000/% $PCE_s$ | $NCE_s$ |
|---|---|---|---|---|---|---|---|
| 1 | -ve control | 0.00 | 24 | 5000 | 67.87/2.5 | 0.60 | 0.24 |
| 2 | $NaAsO_2$ | 2.25 | 24 | 5000 | 53.85/5.53 | 2.60 | 0.47 |
| 3 | $NaAsO_2$ | 4.50 | 24 | 5000 | 47.40[a]/3.65 | 4.60[a] | 1.26 |
| 4 | $NaAsO_2$ | 9.00 | 24 | 5000 | 44.12[a]/2.66 | 8.00[a] | 3.00[a] |
| 5 | NaAsO2 + BHT | 2.25+30 | 24 | 5000 | 61.97/- | 1.20 | 0.00 |
| 6 | $NaAsO_2$ + BHT | 4.50+30 | 24 | 5000 | 48.83[a]/10.52 | 0.80[c] | 0.76 |
| 7 | $NaAsO_2$ + BHT | 9+30 | 24 | 5000 | 40.00[a,e]/2.33 | 2.80[d] | 1.20 |
| 8 | BHT | 30 | 25 | 5000 | 57.77/1.03 | 1.40 | 1.36 |
| 9 | Tween-80 | - | 25 | 5000 | 60.70/- | - | - |

PCEs: Polychromatic erythrocytes, NCEs: Normochromatic erythrocytes, BHT: Butylated hydroxytoluene, a: Significant difference from group 1 at $p<0.05$ c: Significant difference from group 3 at $p<0.05$, d: Significant difference from group 4 at $p<0.05$, e: Significant difference from group 8 at $p<0.05$

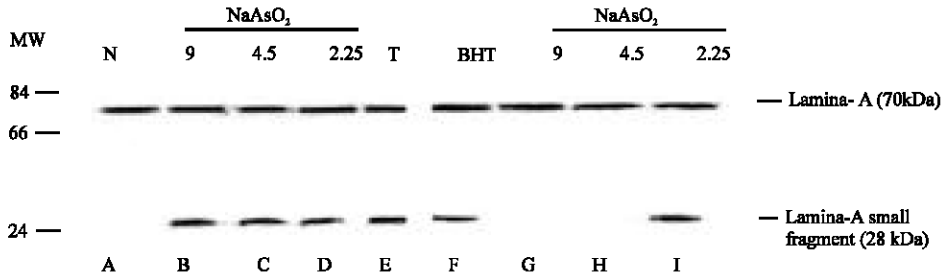


Fig. 2: N: Untreated (A), Western blot analysis of Lamina-A from mice livers treated with sodium arsenite alone (B-D), T: Tween-80 (E), MW: Marker; BHT (F), or in combination with BHT (G-I)

(A). BHT alone and combined treatment with low doses of BHT (F, G and H) induced cytotoxicity and apoptosis.

In this study, the effect of hypermethylation and the antioxidant BHT on the genotoxicity and cytotoxicity induced by sodium arsenite in mice was investigated. CAs, SCEs and MN formation were examined in an effort to evaluate the genotoxicity, while increases in apoptosis were used as indicators of cytotoxicity by monitoring PARP and Lamina-A. The *in vitro* and *in vivo* genotoxicity and cytotoxicity of arsenic has been discussed by Martínez *et al.* (2005), Hagiwara *et al.* (2006) and Florea and Büsselberg (2008). Although, much progress has recently been made in the area of arsenic carcinogenesis, no overall consensus has been reached regarding the precise mechanism of action (Chowdhury *et al.*, 2008). Here, we observed that single sodium arsenite doses correlated significantly with the frequency of chromosomal aberrations in mice *in vivo* compared to the negative control and that sodium arsenite is strongly clastogenic in bone marrow cells of exposed mice, a result consistent with earlier reports (Florea and Büsselberg, 2008). Although, several mechanisms have

been proposed to account for the toxic effects of arsenic and the ability of antioxidants to attenuate these effects, the precise nature of the mechanism or mechanisms involved remains to be delineated, a situation perhaps compounded by the paucity of dose-response relationship studies (Biswas *et al*., 1999b; Banu *et al*., 2001; Seok *et al*., 2007). Some studies reported increased chromosomal aberrations in lymphocytes in humans exposed to arsenic in drinking water (Florea and Büsselberg, 2008). Although, arsenite dose not react directly with DNA, cells treated with arsenite show evidence of oxidative DNA damage (Jhala *et al*., 2008). Structural chromosomal damage is thought to be linked mainly to exposure to direct DNA-damaging agents and/or intracellular defects in DNA replication, recombination or repair mechanisms. In contrast, numerical chromosomal aberrations are thought to be linked mainly to exposure to compounds that induce intracellular defects of the mitotic spindle, the kinetochore apparatus and/or the centrosome. Thus, an eugenic compounds act according to an indirect mechanism of genotoxicity (Patlolla and Tchounwou, 2005; Steiblen *et al*., 2005). Spindle fibers have been posited as potential cellular targets for arsenic given their major constituent tubulin, which has a relatively high sulfhydryl content and plays an important role in microtubule polymerization. The disruption of microtubule assembly and spindle formation during mitosis by arsenic can promote polyploidy (Miller *et al*., 2002; Chowdhury *et al*., 2008). Furthermore, the strong antioxidant butylated hydroxyanisole failed to rescue cells from the toxic effects of arsenic (Miller *et al*., 2002).

Both arsenite and its metabolites can have a variety of genotoxic effects, which may be mediated by oxidants or free radical species (Jhala *et al*., 2008). Arsenic is a prooxidant and thus may cause lipid peroxidation, protein and enzyme oxidation, GSH depletion and DNA adherence. Furthermore, arsenic generates Reactive Oxygen Species (ROS) which are known to induce poly ADP-ribosylation, which is implicated in DNA repair, signal transduction and apoptosis. As a result, sodium arsenite may induce DNA str and-breaks (Bhattacharya and Bhattacharya, 2007).

The DNA damage caused by sodium arsenite can be accounted for by the experimental evidence of its genotoxic effect. Its mode of action may include: (1) inhibition of various enzymes involved in DNA repair and expression, (2) induction of ROS capable of inducing DNA damage. Arsenite also induces considerable accumulation of ROS in a variety of animal cells (Wang *et al*., 2004; Patlolla and Tchounwou, 2005; Bishayi and Sengupta, 2006). Exposure to sodium arsenite in combination with BHT did not induce any significant changes in the frequency of chromosomal aberrations compared with exposure to sodium arsenite alone. Several mechanisms have been proposed to account for the observed attenuation of arsenic-induced damage by BHT. The protective action of antioxidants operates in a dose-dependent manner (Hocman, 1988). BHT itself was not generally considered genotoxic, although few studies revealed its potential to induce chromosomal aberrations (Grillo and Dulout, 1995).

The presence of pulverized chromosomes increased significantly following treatment with only single medium doses compared with the negative control. Various mechanisms have been proposed to account for the formation of pulverized chromosomes including the involvement of cell fusion, failure of cytokinesis following normal nuclear division, cell division with lagging chromosomes or chromosome fragments (Tsutsui *et al*., 2000). It is known that $NaAsO_2$ has the potential to generate genetically unstable (multi or micronucleated) cells which can result in pulverized chromosome formation in cultured Chinese hamster cells (Sci andrello *et al*., 2002; Manna *et al*., 2007). Furthermore, genomic instability can result from telomerase inhibition, which was observed in the NB4 cell line following treatment with arsenic trioxide ($As_2O_3$), given the low transcriptional activity attributed to the direct affect of arsenic on transcription factors (Chou *et al*., 2001; Miller *et al*., 2002).

The present study also reported on increased centromeric attenuation following treatment with $NaAsO_2$. A disorder in spindle fibers has been suggested to account for centromeric disruption and followed by chromatid attenuation. Pati and Bhunya (1989) showed that the presence of chromatid attenuation may be related to aneuploidy, while DeHondt *et al*. (1984) considered that an early stage of endomitosis may lead to polyploidy (Bishayi and Sengupta, 2006; Chowdhury *et al*., 2008).

Investigation of SCEs showed significant increases in the rate of SCEs following treatment with single doses of $NaAsO_2$ compared with the negative control. This result confirms the few earlier studies which monitored SCEs to evaluate the genotoxic effects of arsenic in tissue culture (Lerda, 1994; Mahata *et al*., 2003; Martínez *et al*., 2005).

The monitoring of MN formation is one of the most important assays used to determine the damaging effects induced by agents on chromosomes or spindle fibers. The present study showed that single treatment with medium or high doses of sodium arsenite induced a significant increase in the number of MNPCEs and caused genotoxic

effects in mice bone marrow cells (Seok *et al*., 2007). The data obtained is consistent with earlier studies which showed increased micronuclei in bladder epithelial cells derived from people exposed to arsenic in drinking water and in cultured Chinese ovary hamster cells (Martínez *et al*., 2005). Micronuclei are formed by unrepaired double-str and breaks. Thus, it is the only biomarker that allows for the simultaneous evaluation of clastogenic and an eugenic effects in a wide range of cell types. In this way, analysis of MN formation in cells has been shown to be a sensitive method for monitoring genetic damage (Martínez *et al*., 2005; Steiblen *et al*., 2005). Twenty four hours following treatment we expected scored PCEs to have been exposed to sodium arsenite during S-phase of last cell cycle, whereas NCEs on the same slide passed S-phase. Thus, the observed increase in MNNCEs could be accounted for if sodium arsenite acts on cells during $G_2$-phase or M-phase of last cell cycle (Adler, 1984). The significant ($p<0.05$) increase in the number of MNPCEs is a principal endpoint of the assay (Hayashi *et al*., 1994). We suggest that all doses of sodium arsenite induced genotoxic effects in the mice bone marrow cells. Furthermore, the decrease in percent of PCEs is considered to be another indicator of the cytotoxicity of sodium arsenite (Adler, 1984; Jagetia and Reddy, 2002).

The data showed PARP degradation following treatment with three single doses of $NaAsO_2$, reflecting its potential to induce cytotoxicity. Many reports have appeared detailing PARP sensitivity and its response to apoptosis (Manna *et al*., 2007). Exposure of T-cells to arsenic *in vitro* results in activation of caspase-3 and -8, together with PARP degradation and the inhibition of DNA repair following reduction of the activation signals of DNA repair enzymes (Jin *et al*., 2008; Han *et al*., 2008). Furthermore, several intranucleolar changes are generated following the activation of caspase enzymes that include active DNase, PARP and Lamina-A degradation as apoptosis markers (Reynaud and Driancourt, 2000; Kang *et al*., 2006; Mclaren *et al*., 2006). As a result of PARP activation resulting from an early DNA damage response, $NAD^+$ levels may decline rapidly which in turn may affect the activity of the enzymes involved in glycolysis and the Krebs cycle. In an attempt to restore $NAD^+$ pools cells regenerate $NAD^+$ and as a consequence cellular ATP levels become depleted and a cellular energy crisis may arise which leads to cell death. Cells that are replicating and growing and almost exclusively utilize glucose die from $NAD^+$ and ATP depletion as a consequence of PARP activation (Brock *et al*., 2004; Shi *et al*., 2004; Wijk and Hageman, 2005).

Studies have shown that $NaAsO_2$ induces apoptosis signals from the cell surface to the nucleus of lymphocytes through fragmentation of DNA, activation of caspase and PARP degradation. Recently, arsenic compounds have been shown to be a potent inducer of apoptotic death for both normal and malignant cells. Arsenic has also been shown to induce activation of Mitogen-Activated Protein Kinase (MAPK), which plays a key role in the induction of apoptosis in leukemia cells (Hossain *et al*., 2000).

Present results suggest that Lamina-A degradation is significantly correlated with the three single doses of $NaAsO_2$ examined. Earlier studies demonstrated that caspase activity was correlated with lamina cleavage and the disintegration of nuclei in the late stages of apoptosis. Caspase-6 cleavage of lamina is sufficient to result in the cessation of nuclear processes followed by apoptotic execution because lamina proteins bind specifically to most nuclear envelope proteins, histones, transcriptional regulators and gene expression regulators. Furthermore, lamina filaments interfere with chromosome segregation during mitosis. Lamina-A cleavage is linked to the apoptotic pathway and precedes DNA fragmentation (Takahashi *et al*., 1997; Chen *et al*., 2000; Cohen *et al*., 2001; Bjerke and Roller, 2006).

## CONCLUSION

The effect of hypermethylation and the antioxidant BHT on the genotoxicity and cytotoxicity induced by sodium arsenite in mice was clear but with unclear dose-response relationship. Sodium arsenite induce genotocxicity according to direct or indirect mechanism and had different potential cellular targets. When DNA is moderately damaged, PARP participates in the DNA repair process. Cells exposed to DNA-damaging agents may undergo three pathways depending on the degree of DNA damage. Mild DNA damage activates PARP, which subsequently interacts with several proteins involved in DNA repair such as polymerase II and DNA ligase III. DNA repair proceeds successfully and the cell survives. Low concentrations induce apoptosis, while higher concentrations result in necrosis. There are several biochemical and morphological differences between apoptosis and necrosis. Finally, the protective role of BHT as an antioxidant was unclear in this study perhaps due to the low BHT concentration employed, which is equivalent to 60 fold of the acceptable daily intake, the acceptable daily intake being in the range of 0-0.5 mg $kg^{-1}$ b.wt. and this area need more investigation.

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