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Antioxidation and Hypomethylation Effects on Genotoxicity and Programmed Cell Death Induced in Mice Somatic Cells by Arsenic Trioxide

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Abstract: The present study aims to evaluate the effect of antioxidation and hypomethylation on the genotoxicity and apoptosis induced in mice by arsenic trioxide (As_2O_3) in normal adult male SWR/J mouse. Animals were treated with intraperitoneally (ip) injected with (2.65, 5.35 or 10.70 mg kg^{-1} b.wt. of As_2O_3 which represent 0.25, 0.50 or 1 of LD_{50}, respectively) and killed 24 h later. Another groups were treated with 30 mg kg^{-1} b.wt. of antioxidant and hypermethylizing agent butylated hydroxy toluene (BHT) 1 h prior to As_2O_3 administration. Another different groups were treated with three doses of 5-azacitidine (5-AzaC) 5 mg kg^{-1} b.wt. As_2O_3 administered after 6 days of the last dose. The three single doses of As_2O_3 significantly ($p<0.05$) increased the rate of total structural chromosomal aberrations (CAs) compared with the negative control. No significant effect was observed in the combined treatment with BHT or 5-AzaC compared with single treatments. The histopathological analyses of mice liver cells showed significantly ($p<0.05$) increased in apoptosis markers in all three single doses of As_2O_3 compared with the negative control and also significantly ($p<0.05$) increased with combined treatment with BHT at low and high doses compared with single doses. This study showed that administration of As_2O_3 had a negative effects as represented in CAs test, antioxidant as represented in apoptosis markers and 5-AzaC as represented in rate of pulverized chromosomes, centromeric attenuations, number of polyploidy cells.

Key words: Methylation, genotoxicity, cytotoxicity, arsenic trioxide, CAs, apoptosis, mice

INTRODUCTION

With respect to human health hazard, arsenic consider one of the most important environmental toxins. And It has become evident that increasing human activities have modified the natural cycle of metals and metalloids (Chowdhury *et al*., 2008; Alarifi *et al*., 2009; Raghu and Cherian, 2009). The risk of arsenic compounds increased after exposure to deferent sources and of course that was because of increasing human activities such mining, melting and pesticides production, so for long period arsenic has led to gradually accumulated in the soil (Toribio and Romanya, 2005). Arsenic is released into the atmosphere from both natural and anthropogenic sources (Reichard *et al*., 2007) to contaminate air, water, food and soil, with different degrees of arsenic toxicity (Toribio and Romanya, 2005; Chowdhury *et al*., 2008) and has become a threat to the all livening organism including the human race (Manna *et al*., 2007). In spite of various risk arsenic trioxide is still use against some cancer diseases (Chowdhury *et al*., 2008; Florea and Büsselberg, 2008). The biological effects of one metal can be modified considerably by interaction with other metals (Biswas *et al*., 1999). Studies showed that trivalent arsenic (As^{3+}) was found to be greater toxic than pentavalent arsenic (As^{5+}) (Chowdhury *et al*., 2008). We used in this study arsenic trioxide which is classified by the International Agency for Research on Cancer (IARC) as a human carcinogen and its mechanism have been subject of extensive research but could still not be elucidated (Brink *et al*., 2006; Florea and Büsselberg, 2008).

Methylation status of DNA plays an important role in organizing the genome into transcriptionally active and inactive zones and also DNA methylation levels somewhat changed following metal treatment (Lee *et al*., 1998; Reichard *et al*., 2007; Klein *et al*., 2007). Apoptosis, which is also known as programmed cell death, is an active form of cell death which is plays a crucial role in the development and maintenance of cell homeostasis and may in fact be a key mechanism in the development of toxicity (Lee *et al*., 2008). Apoptosis is known to be activated by a cascade of factors and to be placed under tight genetic regulation. It has now been recognized as an important determinant of cell degeneration in many toxic events (Yasin *et al*., 2003). Apoptosis may occur via a death receptor-dependent (extrinsic) or independent (intrinsic or mitochondrial) pathway. Over the past few years, there has been increasing recognition of (1) the important role of cell death in determining appropriate cell number and (2) how a lack of cell death under physiologic

conditions can contribute to cellular transformation and malignant cell growth. Cell death permits the selective elimination of excess cells and permits the maintenance of tissue homeostasis in morphogenesis and in the immune system. The majority of such cell deaths share common characteristics, such as cell shrinkage, blebbing of the plasma membrane, chromatin condensation, apoptotic bodies and finally fragmentation of DNA as a biochemical hallmarks of apoptosis (Ho *et al*., 1999; Qurtam *et al*., 2009). Despite the large number of studies conducted concerning arsenic toxicity, the effects remain poorly understood (Doop *et al*., 2004; Florea and Büsselberg, 2008). But several assays performed *in vivo* and *in vitro* on mammalian cells have shown that exposure to arsenic induces chromosomal aberrations and formation of micronuclei (Biswas *et al*., 1999; Bhattacharya *et al*., 2005; Klein *et al*., 2007).

The present investigation was undertaken in an effort to determine the effects of antioxidation and hypomethylation on the genotoxicity and cytotoxicity induced by arsenic trioxide in mice somatic cells to find out the relationship between DNA methylation status and the effect of arsenic trioxide on mice cells.

MATERIALS AND METHODS

All of the experimental procedures were conducted in the Central Lab of Teachers College, King Saud University, Saudi Arabia between 2008 and 2009.

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 at a temperature of 22"11°C, a relative humidity of 45"5% and photoperiod cycle of 10/14 h. Mouse food (commercially available in Saudi Arabia) and water were offered *ad libitum*.

Treatments: A total of 65 males were used and divided into 13 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, 3 and 4 were subjected to i.p. injection of As_2O_3 in single various dose levels (2.65, 5.35 or 10.70 mg kg^{-1} b.wt. which corresponds to 0.25, 0.50 or 1 LD_{50}, respectively). Groups 5, 6 and 7 were treated with the same doses as in Groups 2, 3 and 4 plus 30 mg kg^{-1} b.wt. of the synthetic antioxidant and hypermethylizing agent butylated hydroxy toluene (BHT) one hour prior to As_2O_3 treatment. Group 8 was treated with only 30 mg kg^{-1} b.wt. of BHT. Groups 9, 10 and 11 were treated with the same doses in groups 2, 3, 4 plus three doses of 5 mg kg^{-1} b.wt. Group 12 treated with only three doses of 5-AzaC with 5 mg kg^{-1} b.wt. each and three hours intervals between them. As_2O_3 was then administered after 6 days of the last dose (Plumb *et al*., 2000).

Group 13 was treated with the organic solvent Tween-80 (0.2 mL/10 g b.wt.), which was used to dissolve the BHT.

Test chemicals: Arsenic trioxide, Butylated hydroxy toluene (BHT), 5-Azacytidine (5-AzaC), Tween-80 were obtained from (Sigma, UAS). 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

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).

Apoptosis markers: One hundred fifty liver cells from each group of five fields were examined for the monitoring of cellular and nuclear changes associated with apoptosis (Shi *et al*., 1998; Johnson *et al*., 2000; Kawasaki *et al*., 2000). Semi-thin sections (0.5 μm) from each samples were prepared by Ultramicrotome (Leica, UCT, Austria) and examined by using a light microscope (Nikon, Eclipse E600W, Japan) equipped with 10X and 100X oil lenses (Glauert, 1974).

The data obtained in this study from CAs test were statistically analyzed with SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) using the Mann-Whitney U-test. And Chi-square (χ^2) 2×2 Contingency tables was used for apoptosis markers.

RESULTS

Genotoxicity

Chromosomal Aberrations (CAs): The screened types of structural aberrations were included chromatid breakage contained (Breaks, deletions, fragments, end to end association, centric fusion and ring chromosome). Whereas the numerical changes were only polyploidy cells. The pulverized chromosome and Centromeric attenuation were scored as indicator to chromosomal instability. The data in Table 1 showed that all three single doses of As_2O_3 induced a significant ($p<0.05$) increase in chromatid breakage, total number of cells with structural

Table 1: Frequency of chromosomal aberrations induced in bone marrow cells of mice treated with arsenic trioxide (As2O3) alone and in combination with butylated hydroxy toluene (BHT) or 5-azacytidine (5-AzaC)

				No. of structural chromosomal aberrations				
				Average/Animal±SD				
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)	1 (0.20±0.45)	0	0	2 (0.40±0.55)
2	As_2O_3 (2.65)	5	500	6^{a} (1.20±0.45)	1 (0.20±0.45)	2 (0.40±0.55)	2 (0.40±0.55)	11^{a} (2.20±1.30)
3	As_2O_3 (5.35)	5	500	9^{a} (1.80±0.84)	2 (0.40±0.55)	3^{a} (0.60±0.55)	2 (0.40±0.55)	16^{a} (3.20±1.10)
4	As_2O_3 (10.70)	5	500	24^{a} (4.80±3.77)	2 (0.40±0.55)	4^{a} (0.80±0.45)	2 (0.40±0.55)	32^{a} (6.40±3.85)
5	As_2O_3+ BHT (2.65+30)	5	500	10^{a} (2.00±1.87)	0	0	1 (0.20±0.45)	11^{a} (2.20±1.79)
6	As_2O_3+ BHT (5.35+30)	5	500	27^{c,e} (5.40±3.71)	1 (0.20±0.45)	1 (0.20±0.45)	2 (0.40±0.55)	31^{a,e} (6.20±4.44)
7	As_2O_3+ BHT (10.70+30)	5	500	21^{a} (4.20±4.15)	2 (0.40±0.89)	2 (0.40±0.55)	2 (0.40±0.55)	27^{a,e} (5.40±4.16)
8	BHT (30)	5	500	6 (1.20±1.10)	1 (0.20±0.45)	1 (0.20±0.45)	1 (0.20±0.45)	9^{a} (1.80±0.84)
9	As_2O_3+5-AzaC (2.65+3×5)	5	500	24^{a,b} (4.80±3.70)	2 (0.40±0.55)	1 (0.20±0.45)	1 (0.20±0.45)	28^{a} (5.60±4.51)
10	As_2O_3+5-AzaC (5.35+3×5)	5	500	17^{a,c} (3.40±1.14)	2 (0.40±0.55)	3^{a} (0.60±0.55)	2^{a,f} (0.40±0.55)	25^{a,c} (5.00±1.22)
11	As_2O_3+5-AzaC (10.70+3×5)	5	500	29^{a} (5.80±5.36)	2 (0.40±0.55)	3^{a,f} (0.60±0.55)	3^{a,f} (0.60±0.55)	37^{a} (7.40±5.68)
12	5-AzaC (3×5)	5	500	19^{a} (3.80±3.35)	1 (0.20±0.45)	0	0	20^{a} (4.00±3.08)
13	Tween-80	5	500	3 (0.60±0.89)	0	0	0	3 (0.60±0.89)

		Number of structural chromosomal aberrations					
		Percentage					Numerical 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 (Average±SD)	No. of centromerically attenuated cells (Average±SD)	No. of polyploid cells (Average±SD)
1	-ve control	2 (0.40)	2 (0.40)	0	2 (0.40±0.55)	8 (1.60±0.55)	0
2	As_2O_3 (2.65)	11^{a} (2.20)	9^{a} (1.80)	2 (0.40)	5 (1.00±0.71)	19^{a} (3.80±0.84)	0
3	As_2O_3 (5.35)	15^{a} (3.00)	9^{a} (1.80)	6^{a} (1.20)	8 (1.60±1.14)	22^{a} (4.40±1.53)	0
4	As_2O_3 (10.70)	25^{a} (5.00)	14^{a} (2.80)	11^{a} (2.20)	11^{a} (2.20±1.10)	11 (2.20±1.10)	1 (0.20±0.45)
5	As_2O_3+BHT (2.65+30)	11^{a} (2.20)	4 (0.80)	7^{a} (1.40)	19^{a,b,e} (3.80±0.84)	29 (5.80±3.77)	1 (0.20±0.45)
6	As_2O_3+BHT (5.35+30)	31^{a,e} (6.20)	7 (1.40)	24^{a,c,e} (4.80)	17^{a,c,e} (3.40±1.14)	63^{a,c,e} (12.60±7.37)	1 (0.20±0.45)
7	As_2O_3+BHT (10.70+30)	27^{a,e} (5.40)	5 (1.00)	22^{a} (4.40)	16^{a} (3.20±2.77)	104^{a,d,e} (20.80±6.83)	1 (0.20±0.45)
8	BHT (30)	9^{a} (1.80)	4 (0.80)	5 (1.00)	6 (1.20±0.84)	11 (2.20±1.30)	0
9	As_2O_3+5-AzaC (2.65+3×5)	24^{a} (4.80)	7 (1.40)	17^{a,b} (3.40)	12^{a,b,f} (2.40±0.55)	127^{a,b} (25.40±9.53)	3 (0.60±0.89)
10	As_2O_3+5-AzaC (5.35+3×5)	21^{a} (4.20)	7^{f} (1.40)	14^{a,c} (2.80)	18^{a,c,f} (3.60±1.14)	152^{a,c} (30.40±11.67)	3 (0.60±0.89)
11	As_2O_3+5-AzaC (10.70+3×5)	30^{a} (6.00)	6 (1.20)	24^{a} (4.80)	21^{a,f} (4.20±1.92)	117^{a,d} (23.40±5.59)	17^{a,d,f} (3.40±1.34)
12	5-AzaC (3×5)	13^{a} (2.60)	5 (1.00)	8 (1.60)	3 (0.60±0.55)	103^{a} (20.60±2.61)	4 (0.80±1.10)
13	Tween-80	3 (0.60)	1 (0.20)	2 (0.40)	1 (0.20±0.45)	10 (2.00±1.00)	1 (0.20±0.45)

Chromatid breakage : includes breaks, deletion and fragments, CP: Cyclophosphamide, [a]Significant difference from group 1 at $p<0.05$; [b]Significant difference from group 2 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 $p<0.05$; [f]Significant difference from group 12 at $p<0.05$

aberrations compared with the negative control. Single treatment with medium and high doses of As_2O_3 induced a significant ($p<0.05$) increase in centric fusion. Single treatment with low and medium doses of As_2O_3 induced a significant ($p<0.05$) increase in the number of centromerically attenuated cells compared with the negative control. Whereas the combined treatment with BHT at low and medium doses induced a significant ($p<0.05$) increase in the number of cells with pulverized chromosomes compared with single treatment. And also the combined treatment with BHT at medium and high doses induced a significant ($p<0.05$) increase in the number of centromerically attenuated cells compared with single treatment.

The combined treatment with Hypomethylation reagent (5-AzaC) at low and medium doses induced a significant ($p<0.05$) increase in the chromatid breakage, number of cells with pulverized chromosomes compared with single treatment. Also, the combined treatment with (5-AzaC) at all three doses induced a significant ($p<0.05$) increase in the number of centromerically attenuated cells compared with single treatment. The combined treatment with (5-AzaC) at high dose induced a significant ($p<0.05$) increase in the number of polyploidy cells compared with single doses. However, the treatment with (5-AzaC) alone was not induced a significant increase on this phenomenon.

Histopathological analysis: The scored types of cellular and nuclear changes associated with programmed cell death (apoptosis markers) in semi thin sections of liver of different groups were included apoptosis bodies, dark cells, fragmented nuclei, condensed nuclei and irregular nuclei (Fig. 1a-f).

In histopathological observations the appearance of cells in control group was normal containing homogenous nuclei and intact cytoplasmic organelles. The data in Table 2 showed that the exposed mice groups to As_2O_3

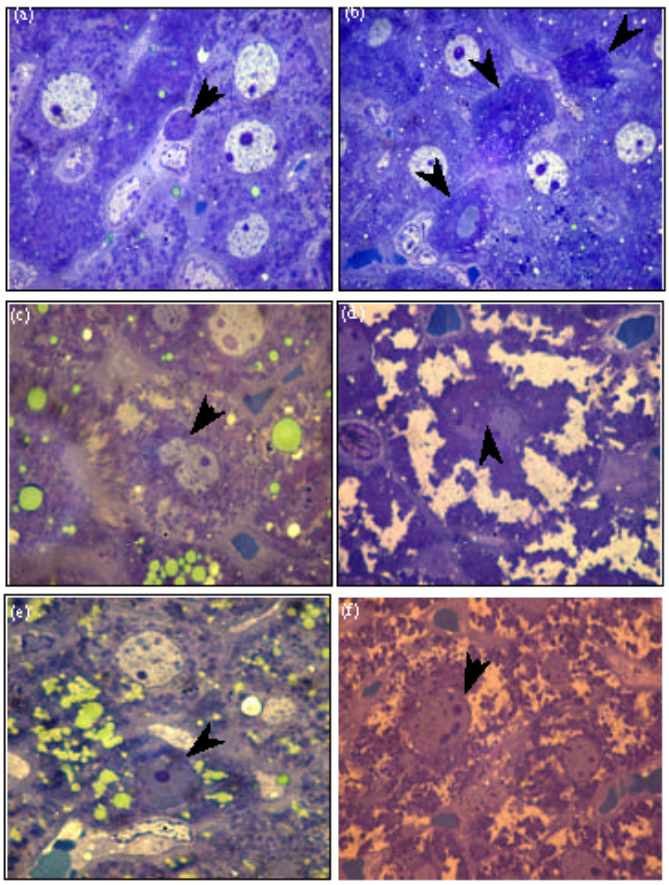

Fig. 1: Semi-thin sections from liver tissue of treated mouse showing (a): Arrows shows apoptotic body engulfed by a kupffer cell in a hepatic sinusoid (X 1500); (b): Arrows shows dark cells with markedly condensed nuclei, the dark cells are obviously shrunken with irregular outline (X 1250); (c): Arrows shows fragmentation of hepatocytes nuclei, hepatocyte nucleus revealing a deep constriction in its envelope as a initial step preceding the complete fragmentation (X 1500); (d): Arrows shows Fragmented hepatocyte nucleus. Note that the nucleus is fragmented into two unequal irregular parts (X 1400); (e): Arrows shows condensation of a hepatocyte nucleus (X 1500) and (f): Arrows shows irregular hepatocyte nucleus the nuclear envelope reveals protrusions. All sections were stained with toluidine blue

Table 2: Cellular and nuclear changes associated with programmed cell death (apoptotic changes) in semi thin sections of liver of different groups of mice

			Apoptotic changes						
Groups	Treatment dose (mg kg^{-1})	No. of examined cells	Apoptotic bodies	Dark cells	Fragmented nuclei	Condensed nuclei	Irregular nuclei	Total	Percentage
1	-ve control	150	0	1	0	0	1	1	0.66[a]
2	As_2O_3 (2.65)	150	0	1	0	3	7	11	8.21[a]
3	As_2O_3 (5.35)	150	4	0	2	6	17	29	21.01[a]
4	As_2O_3 (10.70)	150	0	16	0	2	0	18	10.65[a]
5	As_2O_3+ BHT (2.65+30)	150	2	1	1	10	15	29	18.95[a,b]
6	As_2O_3+ BHT (5.35+30)	150	4	8	0	2	8	23	17.29[a]
7	As_2O_3+ BHT (10.70+30)	150	5	3	0	0	23	31	19.87[a,d]
8	BHT (30)	150	6	3	0	3	6	18	15.25[a]
9	As_2O_3+5-AzaC (2.65+3×5)	150	6	1	1	0	8	16	11.59[a]
10	As_2O_3+5-AzaC (5.35+3×5)	150	6	3	0	9	5	23	19.32[a]
11	As_2O_3+5-AzaC (10.70+3×5)	150	1	6	0	2	4	13	9.92[b]
12	5-AzaC (3×5)	150	4	1	2	1	8	16	11.94[a]
13	Tween-80	150	-	-	-	-	-	-	nd

nd: Not detected, [a]Significant difference from group 1 at $p<0.05$; [b]Significant difference from group 2 at $p<0.05$, [d]Significant difference from group 4 at $p<0.05$

alone or with combined treatments revealed presence of cells with typical alternations of apoptosis. All three single doses of As_2O_3 induced a significant ($p<0.05$) increase in the percentage of apoptotic changes compared with the negative control. whereas the combined treatment with BHT at low and high doses induced a significant ($p<0.05$) increase in the percentage of apoptotic changes compared with single treatment. No significant effects with combined treatment with (5-AzaC).

DISCUSSION

The genotoxic effect of arsenic compounds on CAs has been reported *in vitro* and *in vivo* in several publications (Martínes *et al*., 2005; Patlolla and Tchounwou, 2005; Florea and Büsselberg, 2008; Alarifi *et al*., 2009). Many studies have been pointed to genotoxic effects of investigated arsenic trioxide (As_2O_3) (Brink *et al*., 2006; Hagiwara *et al*., 2006; Florea and Büsselberg, 2008; Qurtam *et al*., 2009). The results of present study showed that the single treatment with As_2O_3 at all used doses significantly increased the structural CAs. This structural CAs were included chromatid breakage contained (Breaks, deletions, fragments and few of chromosome-types structural aberrations such as centric fusion and ring chromosome). The results of present study were corresponded with previous studies used the live mice to detect genotoxicity of arsenic compounds (Ochi *et al*., 2008; Touriguine *et al*., 2008). As well as corresponded with Rahman *et al*. (2001) results on people have been exposed to high levels of arsenic in drinking water.

Here we noted that pulverized chromosomes were significantly increased after treatment with only single high dose compared with the negative control. Various mechanisms has been suggested to explain pulverized chromosomes formation, from these: cell fusion, failure of cytokinesis following normal nuclear division (Tsutsui *et al*., 2000; Ochi *et al*., 2008). Its known that As_2O_3 has potent to form genetically instability cells B multi or micronucleus cells-led to pulverized chromosomes formation in Chinese hamster (Seok *et al*., 2007). Furthermore the genomic instability phenomenon can result from telomerase inhibition which observed in treated NB4 cell line with arsenic trioxide (As_2O_3) because of low transcription which attributed to direct affect of arsenic on transcription factors (Chou *et al*., 2001; Miller *et al*., 2002; Shen *et al*., 2008). Also in present study, increasing in centromeric attenuation after treatment with As_2O_3 was observed at low and medium doses. And spindle fibers disorder has been suggested as a reason for centromeric disruption, followed with chromatid attenuation. Pati and Bhunya (1989) study was pointed to that present of chromatid attenuation maybe represent important noticed related to aneuploidy, while DeHondt *et al*. (1984) considered that as early stage of endomitosis which maybe led to polyploidy. Cytoskeleton has been mentioned as a potential cellular target for arsenic because it's major constituent, tubulin, which has a relatively high sulfhydryl (-SH) content (Bishayi and Sengupta, 2006; Seok *et al*., 2007; Chowdhury *et al*., 2008). Both arsenic 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, Arsenic trioxide may induce DNA strand-breaks (Bhattacharya and Bhattacharya, 2007). The DNA damage caused by arsenic trioxide can be accounted for by the experimental evidence of its genotoxic effect. Its mode of action may include: (1) Here 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). Furthermore, several intra nucleolus changes produced from activation of caspases enzymes such as active Dnase and Lamina-A degradation as apoptosis markers (Kang *et al*., 2006; Mclaren *et al*., 2006; Yu *et al*., 2008). Studies showed As_2O_3 induced apoptosis signals from the cell surface to the nucleus of lymphocytes through fragmentation of DNA, activation of caspase. Arsenic play a dual roles as anti-cancer and inducing of gentotoxicity and cytotoxicity, its these two apparently opposite effects on human life may share a common molecular mechanism. Extensive DNA damage lead to cell dysfunction and apoptosis, this kind of cell death takes place in a tissue or organ with distinct biochemical and morphological markers of apoptosis (Nguewa *et al*., 2003; Raghu and Cherian, 2009). Previously studies demonstrated that the activity of caspase that cleavage Lamina is required for the disintegration of nuclei in the late stages of apoptosis. The Lamina-cleavage caspase-6 is sufficient to drive nuclear events to shutting down nuclear processes followed by apoptotic execution because of lamina proteins bind specifically to most nuclear envelope proteins, histones, transcriptional regulators, gene expression regulators. Furthermore,

lamina filaments interfere with chromosome segregation during mitosis. Mostly the lamina cleavage links in the apoptotic pathway and precedes DNA fragmentation (Takahashi *et al*., 1997; Chen *et al*., 2000; Cohen *et al*., 2001; Bjerke and Roller, 2006). Anyway, the relationship between arsenic dose-response and its toxic effects still unclear because of different cell types, various biological endpoints studied, experimental scatter (Geble, 2001). But most studies showed that the dose-response depend on exposure protocol, time exposure, dose (Yih and Lee, 1999). Exposure to arsenic trioxide in combination with BHT did not induce any significant changes in the frequency of chromosomal aberrations compared with exposure to arsenic trioxide alone, but we observed that pulverized chromosomes were significantly increased after treatment with low and medium doses and also, the centromeric attenuated cells significantly increased after treatment with low and medium doses compared with single treatments. 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), so, the protective role of BHT as an antioxidant was unclear in this study perhaps due to the low BHT concentration employed compared with previous studies which have been used 200 and 500 mg kg^{-1} b.wt. BHT itself was not generally considered genotoxic, although few studies revealed its potential to induce chromosomal aberrations (Grillo and Dulout, 1995). Hypermethylation of DNA could cause aberrant expression of genes such as oncogenes, which in turn can cause abnormalities in cell proliferation leading to carcinogenesis, a finding consistent with the usual DNA methylation changes observed in cancers. 5-Azacytidine was used in present study to decrease DNA methylation status compared with normal level methylation (Laird *et al*., 1995). The all combined treatment with 5-AzaC led to significant increase in the total number of cells with structural aberrations at medium dose compared with single treatments of As_2O_3. Also, we observed that combined treatment with 5-AzaC led to significant increase in the cells with pulverized chromosome at low and medium doses compared with single treatments of As_2O_3 and significant increase in the cell with centromeric attenuated at all combined doses compared with single treatments of As_2O_3. A high rate in the number of cells with centromeric attenuation can be explain depend on that 5-AzaC could be interact with kinetochore protein synthesis (VanHummelen *et al*., 1992) which may led to centromer disruption, followed by chromatid attenuation (Dolara *et al*., 1994). In addition, these high rates may be indicating to that hypomethylation led to increase DNA sensitivity to toxic effect by arsenic. As mentioned in few studies that hypomethylation of DNA could cause changes in specific regions of chromatin led to genome instability throughout increase sensitivity of some DNA sequences for DNA damage agents (Keshet *et al*., 1986; Lewis and Brid, 1991; Klose and Bird, 2006). These increasing in the numbers of cells with chromosomal changes after combined treatments or 5-AzaC alone reflect genome instability (pulverized chromosome and centromeric attenuation). This is support what suggested before that DNA hypomethylation which occurs away from CpG islands led to chromosomal instability which appear in different chromosomal changes (Schulz *et al*., 2002). The results of present study refer to a clear association between genome hypomethylation and chromosomal instability. Several studies have been showed in different cancer diseases that DNA methylation affect on genes throughout different cellular pathways involve apoptosis pathways, the defect of apoptosis pathways in cancer cells arrest cells death. Anyway there are a currently examination on 5-AzaC alone or combined with another compounds such as Phenylbutyrate or Amifostine as a clinical attempting to treat some diseases like β-thalassemia, lymphoma, lung and prostate cancer (ClinicalTrials. Gov., 2001). The obtained data from histopathological analysis showed that arsenic trioxide induced apoptosis as evidenced by morphological changes of liver cells, this elucidate its potential to induce cytotoxicity. And as mentioned before that arsenic trioxide generate (ROS) in cells-free as well as in cellular systems and induce the opening of the mitochondrial permeability pore and, in addition, might exert direct effects at the plasma membrane, potentially activating the Jun kinase pathway (Dehne *et al*., 2002). Generally, ROS and cytotoxins can cause cell death, often in a dose-dependent manner high dosages of the toxicant usually result in necrosis characterized by progressive cell and organelle membrane dysfunction, leading to loss of ion homeostasis and secondly to the inability to maintain mitochondrial respiration and ATP levels essential for cellular survival. On the other hand, moderate doses of cytotoxins or ROS can activate the apoptotic pathway (Dehne *et al*., 2002). Oxidative stress is the inappropriate exposure to ROS and results from the imbalance between prooxidants and antioxidants leading to cell damage and tissue injury. The ROS generation is increased in many pathological situations. In liver diseases, excess of ROS can induce cell death by either apoptosis or necrosis. Apoptosis, or programmed cell death is an active process characterized by cell shrinkage, chromatin condensation, formation of apoptotic bodies and activation of caspases (Conde de la Rosa *et al*., 2006). In the liver, the apoptosis

could result from a combination of both pathways: the intrinsic apoptosis pathway by generation of oxidative stress and the extrinsic apoptosis pathway by activation of Kupffer cells which can secrete TNF (Moronvalle-Halley *et al*., 2005).

CONCLUSIONS

Effect of DNA methylation status on genotoxicity and cytotoxicity of arsenic trioxide in laboratory Mice was clear but with unclear dose-response relationship. Arsenic trioxide induce genotoxicity and cytotoxicity according to direct or indirect mechanism and had different potential cellular targets. The hypomethylation led to increase DNA sensitivity to toxic effect by arsenic and could cause changes in specific regions of chromatin led to genome instability throughout increase sensitivity of some DNA sequences for DNA damage agents. Finally, the study agreed with previous study by Alarifi *et al*. (2009) which has been reported that 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. Also, present study demonstrated that the potential of arsenic trioxide to induce apoptosis in mice liver cells and this positive results are an agreement with (Mikami *et al*. (2004) who are reported that the liver has a potential to regeneration and rapid clearance of apoptotic cells *in vivo* and the apoptosis was induced more rapidly in the liver than in other tissues observed *in vivo*. This may be one of the manifestations of the toxicity of arsenic trioxide.

REFERENCES

Alarifi, S.A., S. Alkahtani, F.M. Abou-Tarboush and A. Al-Qahtani, 2009. Effect of DNA hypomethylation on genotoxicity and apoptogenicity of sodium arsenite in laboratory mice. Pak. J. Biol. Sci., 12: 554-564.

Bhattacharya, K., E. Dopp, P. Kakkar, F. Jaffery and D. Schhiffmann *et al*., 2005. Biomarkers in risk assessment of asbestos exposure. Mutat. Res., 579: 6-21.

Bhattacharya, A. and S. Bhattacharya, 2007. Induction of oxidative stress by arsenic in *Clarias batrachus*: Involvement of peroxisomes. Ecotoxicol. Environ. Saf., 66: 178-187.

Bishayi, B. and M. Sengupta, 2006. Synergism in immunotoxicological effects due to repeated combined administration of arsenic and lead in mice. Int. Immunopharmacol., 6: 454-464.

Biswas, S., G. Talukeder and A. Sharma, 1999. Protection against cytotoxic effects of arsenic by dietary supplementation with crude extract of Emblica officinalis fruit. Phytother. Res., 13: 513-516.

Bjerke, S. and R. Roller, 2006. Roles for herpes simplex virus type 1 U_L34 and U_S3 proteins in disrupting the nuclear lamina during herps simplex virus type 1 egress. Virology, 347: 261-267.

Brink, A., B. Schulz, K. Kobras, W. Lutz and H. Stopper, 2006. Time-dependent effects of sodium arsenite on DNA breakage and apoptosis observed in the comet assay. Mutat. Res., 603: 121-128.

Chen, H., J. Zhou and Y. Dai, 2000. Cleavage of lamin-like proteins *in vivo* and *in vitro* apoptosis of tobacco protoplasts induced by heat shock. FEBS Lett., 280: 165-168.

Chou, W.C., A.L. Hawkins, J.F. Barrett, C.A. Griffin and C.V Dang, 2001. Arsenic inhibition of telomerase transcription leads to genetic instability. J. Clin. Invest., 108: 1541-1547.

Chowdhury, R., A. Dutta, S. Chaudhuri, N. Sharma, A. Giri and K. Chaudhuri, 2008. *In vitro* and *in vivo* reduction of sodium arsenite induced toxicity by aqueous garlic extract. Food Chemical Toxicology., 46: 740-751.

ClinicalTrials. Gov., 2001. A service of the national institutes of health. Developed by the National Library of Medicine. http://www.clinicaltrials.gov/.

Cohen, M., Y. Gruenbaum, K. Lee and K. Wilson, 2001. Transcriptional repression, apoptosis human disease and the functional evolution of the nuclear lamina. Trends Biochem. Sci., 26: 41-47.

Conde de la Rosa, L., M. Schoemaker, T. Vrenken, M. Buist-Homan, R. Havinga, P. Jansen, H. Moshage, 2006. Superoxide anions and hydrogen peroxide induce hepatocyte death by different mechanisms: Involvement of JNK and ERK MAP kinases. J. Hepatol., 44: 918-929.

DeHondt, H., A. Fahmy and S. Abdelbaset, 1984. Chromosomal and biochemical studies on the effect of Kat extract on laboratory rats. Environ. Mutagen, 6: 851-856.

Dehne, N., U. Rauen, H. Groot and J. Lautermann, 2002. Involvement of the mitochondrial permeability transition in gentamicin ototoxicity. Hear. Res., 169: 47-55.

Dolara, P., F. Torricelli and N. Antonelli, 1994. Cytogenetic effects on human lymphocytes of a mixture of fifteen pesticides commonly used in Italy. Mutat. Res., 325: 47-51.

Dopp, E., L. Hartmann, A. Florea, U. Recklinghausen and R. Pieper *et al*., 2004. Uptake of inorganic and derivatives of arsenic associated with induced cytotoxic and genotoxic effects in Chinese hamster ovary (CHO) cells. Toxicol. Applied Pharmacol., 201: 156-165.

Florea, A.M. and D. Büsselberg, 2008. Arsenic trioxide in environmenta and clinically relevant concentrations interacts with calcium homeostasis and induces cell type specific cell death in tumor and non-tumor cells. Toxicol. Lett., 179: 34-42.

Gebel, T., 2001. Genotoxicity of arsenic compounds. Int. J. Hygiene Environ. Health, 203: 249-262.

Glauert, A.M., 1974. Fixation, Dehydration and Embedding of Biological Specimens. In: Practical Methods in Electron Microscopy, Glauert, A.M. (Ed.). North-Holland, Amsterdam.

Grillo, C. and F. Dulout, 1995. Cytogenetic evaluation of butylated hydroxy toluene. Mutat. Res., 345: 73-78.

Hagiwara, M., E. Watanabe, J. Barrett and T. Tsutsui, 2006. Assessment of genotoxicity of 14 chemical agents used in dental practice: Ability to induce chromosome aberrations in Syrian hamster embryo cells. Mutat. Res., 603: 111-120.

Ho, Y., H. Lee, C. Chang and J. Lin, 1999. Induction of bax protein and degradation of lamin A during p53-dependent apoptosis induced by chemotherapeutic agents in human cancer cell lines. Biochemical Pharmacol., 57: 143-154.

Hocman, G., 1988. Chemoprevention of cancer: Phenolic antioxidants (BHT, BHA). Int. J. Biochem., 20: 639-651.

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

Johnson, V.L., S.C. Ko, T.M. Holmstrom, J.E. Eriksson and S.C. Chow, 2000. Effector Caspases are dispensable for the early nuclear morphological changes during chemical-induced apoptosis. J. Cell Sci., 113: 2941-2953.

Kang, H.M., S.K. Lee, D.S. Shin, M.Y. Lee and D.C. Han *et al*., 2006. Dehydrotrametenolic acid selectively inhibits the growth of H-ras transformed rat2 cells and induces apoptosis through caspase-3 pathway. Life Sci., 78: 607-613.

Kawasaki, M., K. Kuwano, N. Hagimoto, T. Matsuba and R. Kunitake *et al*., 2000. Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspase inhibitor. Am. J. Pathol., 157: 597-603.

Keshet, I., H. Lieman and H. Cedar, 1986. DNA methylation affects the formation of active chromatin. Cell, 44: 535-543.

Klein, C.B., J. Leszczynska, T.C. Hickey and T. Rossman, 2007. Further evidence against a direct genotoxic mode of action for arsenic induced cancer. Toxicol. Applied Pharmacol., 222: 289-297.

Klose, R.J. and A.P. Bird, 2006. Genomic DNA methylation: The mark and its mediators. Trends Biochem. Sci., 31: 89-97.

Laird, P., L. Jackson-Grusby, A. Fazeli, S. Dickinson, W. Jung, R. Weinberg and R. Jaenisch, 1995. Suppression of intestinal neoplasia by DNA hypomethylation. Cell, 81: 197-205.

Latt, S., J. Allen, S. Bloom, A. Carrano and E. Falke *et al*., 1981. Sister chromatid exchange: A report of the Gene-Tox program. Mutat. Res., 87: 17-62.

Lee, Y., L. Broday and M. Costa, 1998. Effects of nickel on DNA methyltransferase activity and genomic DNA methylation levels. Mutat. Res., 415: 213-218.

Lee, D.H, M. Szczepanski and Y. Lee, 2008. Role of Bax in quercetin-induced apoptosis in human prostate cancer cells. Biochemical Pharmacol., 75: 2345-2355.

Lewis, J. and A. Bird, 1991. DNA methylation and chromatin structure. FEBS Lett., 285: 155-159.

Manna, P., M. Sinha, P. Pal and P.C. Sil, 2007. Arjunolic acid, a triterpenoid saponin, ameliorates arsenic-induced cyto-toxicity in hepatocytes. Chemico Biol. Interact., 170: 187-200.

Marti-nez, V., A. Creus, W. Venegas, A. Arroyo and J.P. Beck *et al*., 2005. Micronuclei assessment in buccal cells of people environmentally exposed to arsenic in Northern Chile. Toxicol. Lett., 155: 319-327.

Mclaren, S.H., D. Gao, L. Chen, R. Lin and J.R. Eshleman *et al*., 2006. Oxidative stress and DNA damage-DNA repair system in vascular smooth muscle cells in artery and vein grafts. J. Cardiothroracic Renal Res., 1: 59-72.

Mikami, O., S. Yamamoto, N. Yamanaka and Y. Nakajima, 2004. Porcine hepatocyte apoptosis and reduction of albumin secretion induced by deoxynivalenol. Toxicology, 204: 241-249.

Miller, W.H., H.M. Schipper, J.S. Lee, J. Singer and S. Waxman, 2002. Mechanisms of action of arsenic trioxide. Cancer Res., 62: 3893-3903.

Moronvalle-Halley, V., B. Sacré-Salem, V. Sallez, G. Labbe and J.C. Gautier, 2005. Evaluation of cultured, precision-cut rat liver slices as a model to study drug-induced liver apoptosis. Toxicology, 207: 203-214.

Nguewa, P., M. Fuertes, C. Alonso and J. Perez, 2003. Pharmacological modulation of Poly (ADP-ribose) polymerase-mediated cell death: Exploitation in cancer chemotherapy. Mol. Pharmacol., 64: 1007-1014.

Ochi, T., K. Kita, T. Suzuki, A. Rumpler, W. Goessler and K. Francesconi, 2008. Cytotoxic, Genotoxic and cell-cycle disruptive effects of thio-dimethylarseinate in cultured human cells and the role of glutathione. Toxicol. Applied Pharmacol., 228: 59-67.

Pati, P. and S. Bhunya, 1989. Cytogenetic effects of fenvalerate in mammalian *in vivo* test system. Mutat. Res., 222: 149-154.

Patlolla, A.K. and P.B. Tchounwou, 2005. Cytogenetic evaluation of arsenic trioxide toxicity in Sprague-Dawley rats. Mutat. Res., 587: 126-133.

Plumb, J.A., G. Strathdee, J. Sluden, S.B. Kaye and R. Brown, 2000. Reversal of drug resistance in human tumor xenografts by 2-deoxy-5-azacytidine-induced demethylation of the h MLH1 Gene promoter. Cancer Res., 60: 6039-6044.

Preston, R.J., B.J. Dean, S. Galloway, H. Holden, A.F. McFee and M. Shelby, 1987. Mammalian *in vivo* cytogenetic assay. Mutat. Res., 189: 157-165.

Qurtam, A., S. Alkahtani, F. Abou Tarboush, A. Al-Qahtani and M. AL-Eissa, 2009. Effect of antioxidation butylated hydroxy toluene on genotoxicity and cytotoxicity induced in mice by sodium arsenite. J. Biol. Sci., 9: 413-422.

Raghu, K.G. and O.L. Cherian, 2009. Characterization of cytotoxicity induced by arsenic trioxide (a potent anti-APL drug) in rat cardiac myocytes. J. Trace Elements Med. Biol., 23: 61-68.

Rahman, M.M., U.K. Chowdhury, S.C. Mukherjee, B.K. Mondal and P. Kunal *et al*., 2001. Chronic arsenic toxicity in Bangladesh and West Bengal, India: A review and commentary. J. Toxicol. Clin. Toxicol., 39: 683-700.

Reichard, J.F., M. Schnekenburger and A. Puga, 2007. Long term low-dose arsenic exposure induces loss of DNA methylation. Biochemical Biophys. Res. Commun., 352: 188-192.

Scappaticci, S., C. Danesino, E. Rossi, C. Kelersy and G. Fiori *et al*., 2000. Cytogenetic abnormalities in PHA-stimulated lymphocytes from patients with Langerhans cell histiocytosis. Br. J. Haematol., 111: 258-262.

Schulz, W.A., J.P. Elo, A.R. Florl, S. Pennanen and S. Santurlidis *et al*., 2002. Genomewide DNA hypomethylation is associated with alteration on chromosome 8 in prostate carcinoma. Genes Chromosomes Cancer, 1: 58-65.

Seok, S.H., M.W. Baek, H.Y Lee, D.J. Kim and Y.R. Na *et al*., 2007. Arsenite-induced apoptosis is prevented by antioxidant in zebrafish liver cell line. Toxicol. *In Vitro*, 21: 870-877.

Shen, S., l. Yang, H. Lin, C. Wu, T. Su and Y. Shen, 2008. Reactive oxygen species-dependent HSP90 protein cleavage participates in arsenical As+ *3* and MMA+ *3* induced apoptosis through inhibition of telomerase activity via JNK activation. Toxicol. Applied Pharmacol., 229: 239-251.

Shi, J., K. Aisaki, Y. Ikawa and K. Wake, 1998. Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. Am. J. Pathol., 153: 515-525.

Takahashi, A., P. Clermont, E. Alnemri, T. Fernandes and K. Yoshizawa *et al*., 1997. Inhibition of ICE-related proteases (caspases) and nuclear apoptosis by phenylarsin oxide. Exp. Cell Res., 231: 123-131.

Toribio, M. and J. Romanya, 2005. Leaching of heavy metals (Cu, Ni and Zn) and organic matter after sewage sludge application to Mediterranean forest soils. Sci. Total Environ., 363: 11-21.

Touriguine, O.N., J. Hamelin and J. Breard, 2008. Cytoskeleton and apoptosis. Biochem. Pharmacol., 76: 11-18.

Tsutsui, T., Y. Tamura, M. Hagiwara, T. Miyachi, H. Hikiba, C. Kubo and J. Barrett, 2000. Induction of mammalian cell transformation and genotoxicity by 2-methoxyestradiol, an endogenous metabolite of estrogen. Carcinogenesis, 21: 735-740.

VanHummelen, P., A. Deleener, P. Vanparys and M. Kirsch-Volders, 1992. Discrimination of aneuploidogens from clastogens by C-banding, DNA and area measurements of micronuclei from mouse bone marrow. Mutat. Res., 271: 13-28.

Wang, Y.C., R.H. Chaung and L.C. Tung, 2004. Comparison of the cytotoxicity induced by different exposure to sodium arsenite in two fish cell lines. Aquat. Toxicol., 69: 67-79.

Yasin, K.F., M. Sabir, M.I.K. Sherwani, M. Zafar, S. Yasmin and M.I. Alam, 2003. Amelioration of Gentamicin Nephrotoxicity by vitamin B6 (a general and histochemical profile). Pak. J. Med. Res., 42: 69-73.

Yih, L. and T. Lee, 1999. Effect of exposure protocols on induction of kinetochore–plus and–minus micronuclei by arsenic in diploid human fibroblasts. Mutat. Res., 440: 75-82.

Yu, Q.T., M. Saruta and K.A. Papadakis, 2008. Visilizumab induces apoptosis of mucosal T lymphocytes in ulcerative colitis through activation of caspase 3 and 8 dependent pathways. Clin. Immunol., 127: 322-329.