RESEARCH ARTICLE

Arsenic induces oxidative stress and apoptosis in human lung cancer cells

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ABSTRACT

Arsenic is a toxic heavy metal and a well-known environmental contaminant. Often water, food, soil are found to be contaminated with arsenic and occupational exposure leads to harmful health issues. Hence, in the present study human lung epithelial A549 cells were used as *in vitro* model system. Treatment of A549 cells with arsenic for 48 h showed reduced viability with increased leakage of lactate dehydrogenase with loss of membrane damage. Arsenic significantly induces reactive oxygen species with depletion of free radical neutralizing antioxidant glutathione peroxidase and catalase enzymes. IC_{50} value of 2 µM arsenic concentration was used for mRNA expression studies. Further, pro-apoptotic Bax gene was induced and anti-apoptotic Bcl-2 was decreased. Arsenic stimulates dysfunction of cell cycle progression by downregulation of Cyclin-E1and Cyclin-D1 mRNA expressions. Thus confirms the toxic nature of arsenic and clues to oxidative redox potential alteration with cell cycle inhibition and apoptosis in lung epithelial cells.

Key words: Arsenic, A549 cells, Oxidative stress, Apoptosis, Cyclins.

INTRODUCTION

Arsenic, a naturally occurring metalloid and is being the twentieth most abundant element in earth's crust. As early as 1250 Albertus Magnus was first to document Arsenic metalloid. Arsenic being a potent environmental contaminant present in food, soil and water (Hughes 2002). The concentration of arsenite in drinking water varies in the range 0.01–3.7 mg/l (WHO 2005). Drinking water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals. However, elevated arsenic level in drinking water is the major cause of arsenic toxicity in the world (Chakraborti *et al*. 2002). The toxicity and mobility of arsenic depend on its oxidation state, where arsenite (arsenic III) is 50-100 times more toxic than arsenate (arsenic V).

In vivo studies showed that methylated arsenic monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were found to be carcinogenic or tumour promoting molecules (Hughes *et al*. 2000). Clinical case studies and reports relating to intake of inorganic arsenic in drinking water, medications or occupational or environmental exposure affects multi-organ systems of human body. The epidemiological evidence on humans exposed to arsenic is based on studies on cancer in relation to arsenic content in drinking water. International Agency for Research on Cancer observed that arsenic was potentially carcinogenic for skin cancer (IARC. 2004). Epidemiological studies indicated that people exposed to high levels of arsenic are prone to develop skin, bladder, liver, and lung cancers (Chen *et al*. 1992; Hong *et al*. 2014; Mayer and Goldman 2016).

Arsenate reacts with phosphate and sulfhydryl group and inhibits many biochemical pathways. Biologically, the arsenite III is more active than the arsenate V in toxicity as well as in the ability to induce gene induction in mammalian cells (Hughes *et al*. 2011; Yamanaka *et al*. 2001). In addition arsenic exposure appears to play a role in the development of black foot disease, type II diabetes mellitus (Tseng 2005) and cardiovascular disease (Moon *et al*. 2013). Studies showed that the arsenic generated excessive free radicals during cellular metabolism. Reactive oxygen and nitrogen species such as superoxide anion (O_2·–), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and nitric oxide (NO) are known to be important molecules involved in mutagenesis and carcinogenesis (Flora *et al*. 2007). H_2O_2 mediates apoptosis by affecting mitochondria. Arsenite in oesophageal carcinoma showed morphological alteration with mitochondrial aggregation (Shen *et al*. 2000). The mechanism of arsenic as a carcinogen and a toxin has been under intense investigation in recent years.

Altered mitochondrial membrane permeability with distorted Bcl-2/Bax ratio leads to apoptosis. Arsenic acts directly on mitochondria to destroy the mitochondrial inner transmembrane potential to promote apoptosis (Miller *et al*. 2002). Arsenite oxidise free radical scavengers such as glutathione by diminishing thiols and facilitates permeability (Flora *et al*. 2007). Enhanced oxidative stress might be associated with the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic leading to cell death. Disclosure of inorganic arsenic (100µg/m^3) is likely to cause sore throat and irritated lungs. Longer exposure at lower concentration of arsenic leads to the risk of lung cancer (Hong *et al*. 2014; IARC. 2004).

Although large body of evidence exists on the mechanism of arsenic toxicity but action on lung /lung cells are limited. Taken together with the studies on arsenic toxicity, it reveals to be an environmental health hazard and requires further insights into the mechanism of its action. In the present study human lung epithelial A549 cells were used as an *in vitro* model system to evaluate the effect of arsenic on cytotoxicity and on induction of oxidative stress. Further, the role of redox sate in stimulation of apoptosis.

MATERIALS AND METHODS

Human lung epithelial cells (A549) were purchased from National Centre for Cell Science-NCCS (Pune, India), oligo's forward and reverse primers for different cell cycle regulators and apoptotic genes (Table 1) and Lithium Lactate were purchased from Sigma-Aldrich (St Louis, USA). Fetal bovine serum (FBS), penicillin, streptomycin, glutamine, RPMI 1640, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), trypan blue, Reduced Glutathione, Nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt (NADPH), Sodium Azide, Glutathione reductase were procured from Himedia (Mumbai, India). Oligo dT's and superscript reverse transcriptase were obtained from Invitrogen BioServices, India Pvt. Ltd. (Bengalu-ru, India), Taq DNA polymerase (1 U/lµl) was purchas-ed from Merck (Mumbai, India). Iodonitro tetrazolium chloride (INT), Arsenic was used as arsenic trioxide from Rolex (Mumbai, India), Phenazine Methosulfate (PMS) and Phenyl Methane sulponyl fluoride (PMSF) was procured from Sisco Research Laboratory Pvt. Ltd. (Mumbai, India).

Culturing of A549 cells

A549 cells were grown in 25cm^2 culture flask using RPMI-1640 medium supplemented with 10 % FBS, 100 U/ml penicillin and 100 µg/ml streptomycin were cultured in a humidified atmosphere at 37 °C by supplying 5 % CO_2 in an incubator. The 80-90 % confluent flask containing cells were trypsinzed and sub cultured into 96 or 6 wells plate for further treatments (Babu *et al*. 2013).

Cell viability (MTT) assay

MTT assay measures the reduction of yellow tetrazolium bromide by mitochondrial NADH+ H+ and succinate dehydrogenase (Mosmann 1983). Reduction of MTT occurs only with metabolically active cells and absorbance is directly proportional to the number of viable cells. A549 cells treated with or without different concentrations of arsenic (1-10 µM) for 48 h, further cells were washed with PBS and 50 µl of MTT (2 mg/ml) was added to each well, incubated for 4 h at 37 °C. The insoluble dark purple formazan product dissolved in 100 µl of DMSO and measured spectrophotometrically at 540 nm.

Reactive Oxygen Species (ROS) assay

Overnight cultured A549 cells (3 × 10^3cells/well) were washed with PBS and treated with 10 µM DCF-DA in 0.1 N NaOH for 3 h (Periyakaruppan *et al*. 2007). The cells were then washed with PBS and further incubated with or without different concentrations of arsenic (1-20 µM) in complete media for different time intervals (0, 15, 30, 45, 60, 90 and 120). Fluorescence intensity was read at excitation wavelength at 485 nm and emission at 527 nm in multimode plate reader (Perkin Elmer, USA).

Lactate Dehydrogenase (LDH) assay

LDH catalyzes the conversion of lactate to pyruvate via NAD+ reduction to NADH. Diaphorase then uses NADH to reduce a tetrazolium salt (INT) to a red formazan product (Ramirez *et al*. 2014). Overnight cultured A549 cells (5 x 10^5cells /wells) were treated with or without different concentrations of arsenic (1-20 µM) for 24 h in 5 % CO_2 incubator. 50 µl of each cell-free supernatant were transferred in triplicate in a 96-well plate, and then 150 µl of LDH-assay reaction mixture was added to each well. The optical density was measured at 490 nm using a multimode microplate reader.

Catalase assay

Catalase is an enzyme found in nearly all living organisms and when exposed to oxygen, it catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is an important enzyme in protecting the cell from oxidative damage (Thomas 1988). A549 cells treated with different concentration of arsenic (1-20 µM) for 24 h. Cell lysate were prepared by using 300 µl of lysis buffer; lysate was centrifuged at 12000 rpm for 10 min at 4 °C. Equal volume of supernatant (100 µl) was mixed with equal volume of absolute alcohol (100 µl) and incubated on ice for 30 min. The reaction mixture was kept at room temperature for 15 min and 10 µl of TritonX-100 was added to each tube. Reaction mixture (10 µl) was mixed with 240 µl phosphate buffer (50 mM pH 7.0) and 250 µl of 0.066 M H_2O_2 was added and OD (Four readings in time scan of 1 min) was measured at 240 nm in Shimadzu spectrophotometer.

Glutathione peroxidase (GPx) assay

GPx assay was carried out as per the protocol described earlier (Kankofer 2002). A549 cells (5×10^5 cells/well) were cultured in a 6-wells plate and incubated overnight. Cells were treated with or without different concentrations of arsenic (1-20 µM)

Table 1 Sequence of primers used for the PCR amplification

Gene	Primer Sequence (5'-3')	Annealing temp. (°C)	Product size (bp)
Cell cycle regulator genes			
Cyclin D1	F: AGACCTGCGCGCCCTCGGTG	58	574
	R: GTAGTAGGACAGGAAGTTGTTG		
Cyclin E1	F: GTCCTGGCTGAATGTATACATGC	60	415
	R: CCCTATTTTGTTCAGACAACAT		
Apoptotic genes			
Bcl-2	F: AGATGTCCAGCCAGCTGCACCTGAC	62	365
	R: AGATAGGCACCCAGGGTGATGCAAGCT		
Bax	F: AAGCTGAGCGAGTGTCTCAAGCGC	61	366
	R: TCCCGCCACAAAGATGGTCACG		
β-actin	F: TACCACTGGCATCGTGATGGACT	62	516
	R: TCCTTCTGCATCCTGTCGGCAAT		

Columns 3 and 4 *show different annealing temperatures used and the size of the amplified products.*
F *forward,* ***R*** *reverse*

and further incubated for 24 h. Cell lysate was prepared using 200 µl of lysis buffer. To 100 µl of cell lysate 2.8 ml of glutathione peroxidase assay mixture containing 100 µl of 8.4 mM NADPH, 10 µl glutathione reductase (GSSG-R, 100 U/mg protein/ml), 10 µl of 1.125 M sodium azide, 100 µl of 0.02 M reduced glutathione in 0.05 M phosphate buffer of pH 7.0 was added. The enzymatic reaction was initiated by the addition of 100 µl of 0.02 mM H_2O_2 to the GPx reaction mixture. Rate of GSSG formation was measured by decrease in the absorbance at 340 nm, as NADPH oxidized to NADP+, using spectrophotometer.

Semi-quantitative RT-PCR analysis

Reverse transcription of RNA and PCR analysis of apoptotic genes (Bax and Bcl2) and cell cycle regulators (Cyclin D1 and Cyclin E1) were carried out as per the protocol described earlier (Sharma and Richards 2000). In brief, total RNA (2 µg) isolated from control and arsenic (1.5 and 2 µM) cells was reverse transcribed using Superscript III First-Strand Synthesis Invitrogen kit. The cDNA was subjected to 30 cycles of PCR using specific forward and reverse primers of genes with appropriate annealing temperatures as indicated (Table 1) in a gradient Eppendorf thermo cycler. Amplified PCR products were analysed on 1 % agarose gel using 1X TAE buffer. The mRNA expressions were quantified using image analysis software (ImageJ) and expression of β-actin mRNA was used as a positive control and for normalization.

Statistical analysis

Experimental data shown as mean ± standard deviation from three independent experiments. Statistical analysis was done by Student's t-test and one-way ANOVA followed by post hoc Tukey test. Difference between control and arsenic treated cell samples were considered significant if the level was *P < 0.05, **P < 0.005.

RESULTS AND DISCUSSION

Arsenic decreased the cell viability of A549 cells

A549 cells were treated with or without different concentrations of arsenic (0.5-10 µM) for 48 h. Cell viability was determined by MTT assay. Results showed that there was a dose dependent decrease in cell viability by 48 h. Maximum of 80 % decrease in cell viability was observed at 10µM concentration of arsenic (Fig. 1).

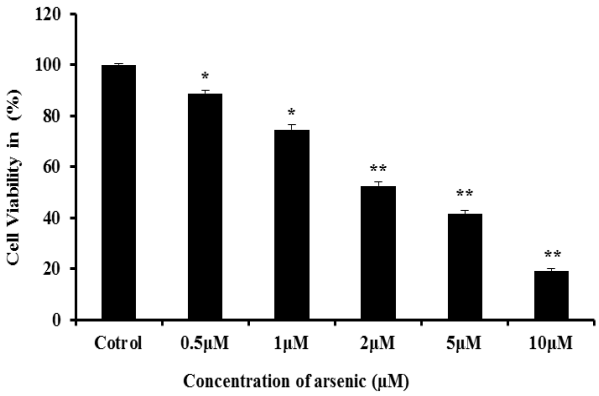


Fig. 1: Effect of Arsenic on A549 cell viability

A549 cells (5x10^{3}cells/well) cultured overnight were treated with or without different concentrations of Arsenic (0.5-10µM) and incubated for 48 h. MTT assay was carried out and absorbance was measured at 540 nm. Figure shown was a representative of three independent experiments. Results were expressed as % viability of cells compared by taking control as 100% (mean ± SD, n=4). Values are significantly different from control if * p <.05, **p <.005 by using one-way ANOVA followed by post hoc Tukey's test.

Induced production of reactive oxygen species on arsenic exposure

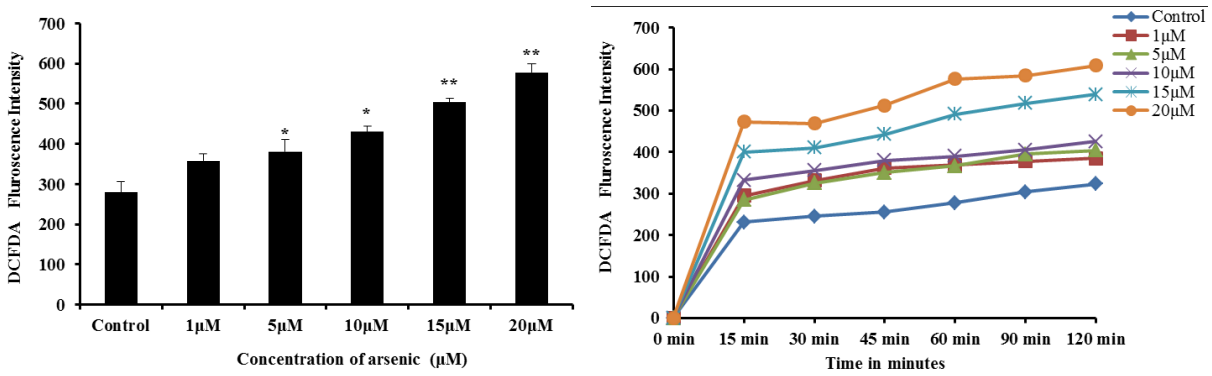
Cells treated with different concentration of arsenic induce ROS in a dose dependent manner and significant increase was seen at 15 and 20 µM arsenic treated sample (Fig. 2a). The amount of ROS increased by more than two fold with increase in time and maximum increase was seen at 20 µM for 120 min (Fig. 2b).

Leakage of lactate dehydrogenase in arsenic treated A549 cells

Lactate dehydrogenase (LDH) was measured by changes in optical density due to NAD+ reduction monitored at 490 nm. Cells treated with different concentration of arsenic show significant increase in the release of LDH in a dose dependent manner and maximum of 3.5 fold increase was recorded at 20 µM arsenic compared to control (Fig. 3).

Arsenic dimished catalase enzyme activity in A549 cells

Catalase enzyme activity on the decomposition of H_2O_2 by arsenic treatment was measured at 240 nm in a spectrophotometer. The results show that there was decrease in catalase enzyme activity in dose dependent manner and maximum of 36 % decrease was observed at 20 µM concentration of arsenic (Fig. 4).



(a) **(b)**

Fig. 2: Effect of arsenic on ROS production (a) at different concentration (b) for different time intervals

A549 cells (5x10^3 cells / well) were treated with 10 µM DCF-DA for 3 h and washed with PBS, further treated with different concentrations of arsenic (1-20 µM) for different time interval (0-120 min). Fluorescence was read at different time intervals. Results were expressed as the change in DCF-DA fluorescence which were measured as shown in the figure (mean ± SD, n=4). Values are significantly different from control if *p <.05, **p <.005 by using one-way ANOVA followed by post hoc Tukey's test. The results were shown as representatives of three independent experiments.

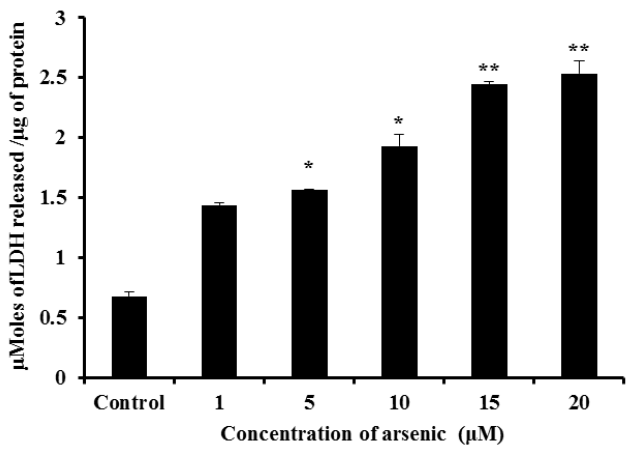


Fig. 3: Effect of arsenic on LDH leakage from A549 cells

A549 cells (5 x 10^5 cells/ well) in a 6-well plate were treated with different concentrations of arsenic incubited for 24 h and LDH in the medium was measured at 490 nm. Values are significantly different from control if *p <0.05, **p <.005 by using one-way ANOVA followed by post hoc Tukey's test. The results were shown as representative of three independent experiments.

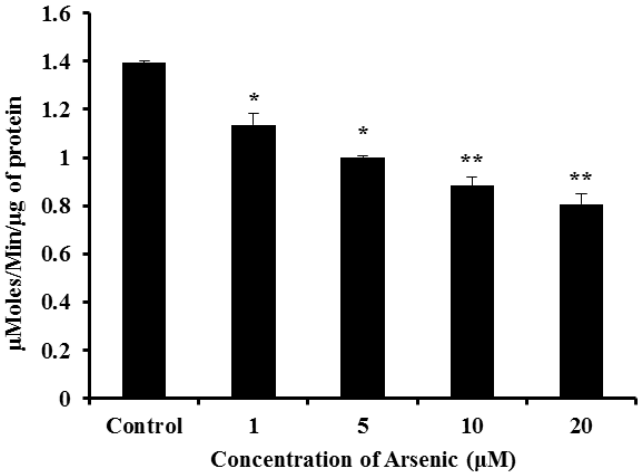


Fig. 4: Effect of arsenic on catalase enzyme activity

A549 cells (5 x 10^5 cells/ well) in a 6-well plate were treated with different concentrations of arsenic incubated for 24 h and catalase enzyme activity was measured at 240 nm. Values are significantly different from control if *p <0.05, **p <.005 by using one-way ANOVA followed by post hoc Tukey's test. The results were shown as representative of three independent experiments.

Reduced glutathione peroxidase activity on arsenic exposure

Glutathione peroxidase enzyme activity was measured by addition of H_2O_2 and the rate of GSSG formation was measured by decrease in the absorbance at 340 nm. The result shows that there was dose dependent decrease in the GPx activity and maximum of 20 and 31 % decreased activity was observed at 10 and 20 µM concentration of arsenic treated A549 cells (Fig. 5).

Arsenic induces apoptosis by increased Bax and decreased Bcl-2 mRNA expression

Cells treated with or without arsenic were analyzed for apoptotic mRNA expressions. Results show that the

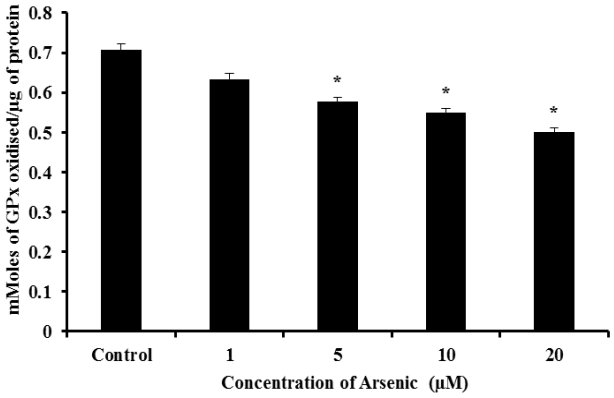


Fig. 5: Effect of arsenic on Glutathione peroxidase enzyme activity

Cells were treated with arsenic for 24 h and cell lysates were analyzed for the GPx activity. The amounts of GPx enzyme activity was carried out by measuring total 2-nitro-5-thiobenzoic acid (TNB) formed using spectrophotometer at 340 nm. Values are significantly different from control if *P<0.05, **P<0.005 by using one way ANOVA followed by post hoc Tukey test. The results were shown as representative of three independent experiments.

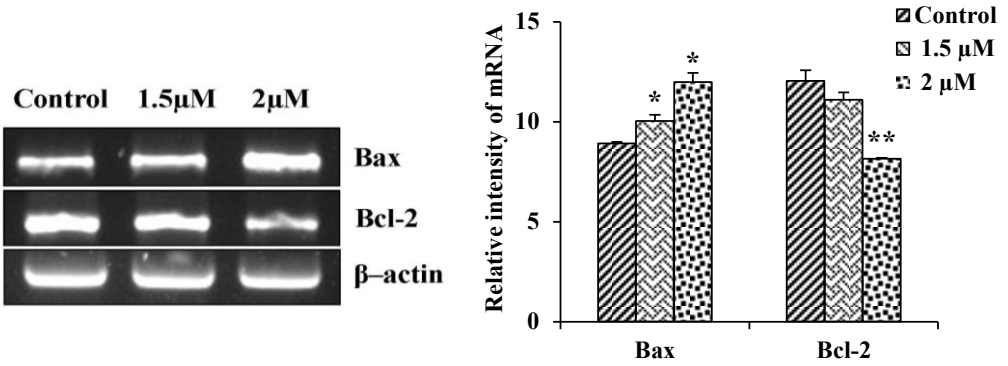


Fig. 6: Effect of arsenic on apoptotic genes in A549 cells

Cells were treated with arsenic (1.5 and 2.0 µM) for 48 h. The mRNA expression of apoptotic genes were carried out by semi quantitative RT-PCR and were analyzed on 1 % agarose gel, and the band intensity of the experimental samples was compared with the control. β-actin was used as a positive control and for normalization. Differences in the expression of mRNA levels of apoptotic genes are statistically significant: if *P < 0.05 or **P < 0.005 compared with control value using one-way ANOVA followed by post hoc Tukey test. The results were shown as representative of three independent experiments. The bar graph represents the densitometry analysis of the mRNA levels of apoptotic genes.

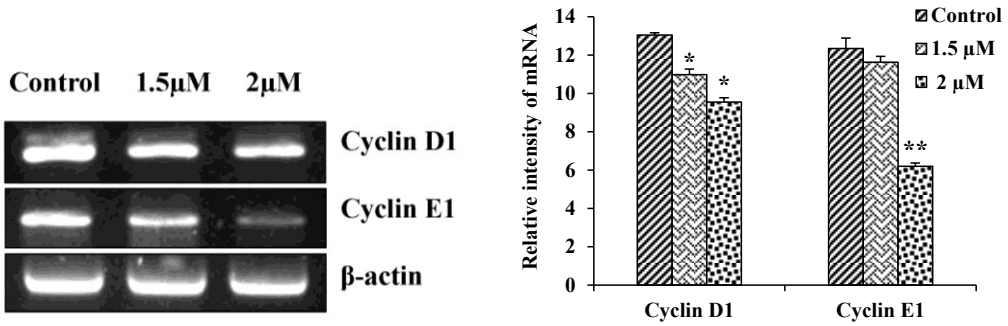


Fig. 7: Effect of arsenic on cell cycle regulator in A549 cells

Cells were treated with arsenic (1.5 and 2.0 µM) for 48 h. The mRNA expression of cell cycle regulators were carried out by semi quantitative RT-PCR and were analyzed on 1% agarose gel, and the band intensity of the experimental samples was compared with the control. β-actin was used as a positive control and for normalization. Differences in the expression of cell cycle regulators mRNA levels are statistically significant: if *P < 0.05 or **P < 0.005 compared with control value using one-way ANOVA followed by post hoc Tukey test. The results were shown as representative of three independent experiments. The bar graph represents the densitometry analysis of the mRNA levels of cell cycle regulator.

arsenic at 1.5 μM marginally increases the pro-apoptotic gene Bax by 1.2 fold and significant increase of 1.7 fold was observed at 2 μM concentration of arsenic. However with respect to anti-apoptotic gene Bcl-2 significantly decreased by 10 and 43 % at 1.5 and 2 μM concentration of arsenic respectively when compared to control (Fig. 6). Results suggested the entry of arsenic treated A549 cells into apoptosis pathway.

Downregulation of Cyclin D1 and Cyclin E1 in arsenic treated A549 cells

Expression of cell cycle regulator genes in control and arsenic treated cells shows 30 % reduction in Cyclin D1 expression at both concentrations. However, marginal decrease in the expression of Cyclin E1 at 1.5 μM and more than 50 % decreased expression at 2 μM concentration of arsenic was observed compared to control. The result confirms the decreased cell proliferation and induction in apoptotic status in arsenic treated A549 cells (Fig. 7).

DISCUSSION

Environmental pollution particularly water pollution raise concerns over their potential effects on human health and environment. Heavy metal-induced toxicity and carcinogenicity involves many mechanistic aspects, which are not clearly elucidated (Frumkin and Thun 2001). Heavy metal toxicity depends on several factors that include the dose, route of exposure and chemical species. However, arsenic is known to have unique features and physicochemical properties that confer to its specific toxicological mechanisms of action.

Arsenic is the twentieth most abundant element on earth and its inorganic forms such as arsenite and arsenate compounds are lethal to the environment and living creatures. Arsenic is introduced into soil and ground water from anthropogenic sources. Inorganic arsenic has been recognized as a human poison since ancient times in food or water would result in cell death (Jaishankar *et al*. 2014). Smaller doses of inorganic arsenic would result in irritation of cardio vascular disease and cell death (Moon *et al*. 2013; Schmidt 2014). Long-term oral exposure to inorganic arsenic results in change of skin patterns, darkening of skin with changes in the blood vessels of the skin leading to skin cancer (Kapaj *et al*. 2006; Mayer and Goldman 2016). Inhalation of arsenic would increase the risk of cancer in the liver, bladder, kidneys, prostate, and lungs (Bouaziz *et al*. 2015). People who live near waste sites with arsenic may have an increased risk of lung cancer (Smedley and Kinniburgh 2002). Thus, the route of exposure to arsenic is also largely through inhalation and therefore it makes the lung tissue as one of its target organs. Arsenic particles with small concentration can lodge deep into the lung alveoli, thus producing toxicological impact at the site of contact (Chen *et al*. 1992).

Arsenic exposure above 5 μM exerts a potent cytotoxic effect on human keratinocytes (HaCaT) by inhibiting cell proliferation and inducing cell death (Yedjou and Tchounwou 2007). There exist numerous evidences about the mechanism of arsenic toxic action on particularly skin cells, while the studies on lung /lung cells are limited. Hence, the present study was carried out to understand the cytotoxic effect of arsenic in lung A549 cells as an *in vitro* model system. The cells treated with different concentration of arsenic show dose dependent decrease in the cell viability and maximum of 80 % decreased was observed at 10 μM concentration of arsenic at 48 h, suggesting the possibility of significant damage induced by the oxidative stress in the presence of heavy metal. Arsenic treatments also show increased ROS and maximum two fold was seen at 20 μM concentration at 2 h. Increase in oxidative stress could result in failure of the antioxidant system that constantly counter act the formation of oxidative species in the cells. In addition to arsenic, heavy metals like Pb, Cd, Hg and Cr are also capable of inducing oxidative stress both *in vivo* and *in vitro* models (Tchounwou *et al*. 2012).

The cause of the oxidative stress due to metals pertains to failure in antioxidant status in the system (Kiran Kumar *et al*. 2016). Arsenic results in the production of ROS by inducing NADPH oxidase (Chou *et al*. 2004). Excessive production of free radical ROS causes damage in lipids and proteins as well as facilitating damage to mitochondria and its functions (Kim *et al*. 2002; Shen *et al*. 2001). ROS-induced oxidative stress is caused by a mitochondria dependent apoptotic pathway. The enzyme GPx is one of the major antioxidant present in cells which counteract oxidative assaults (Waalkes *et al*. 2004). Our study show that there was a decrease in the level of GPx along with the catalase enzymes to overcome the oxidative stress in arsenic treated cells, which clearly indicates a correlation between loss of

antioxidant enzymes and increase in the oxidative stress.. Therefore, the decrease in peroxidase and catalase system is effective in scavenging free radicals (Ahlemeyer *et al*. 2001). The above results suggest that the increase in the ROS by arsenic in A549 cells, possibly due to the failure of the antioxidant mechanisms to counteract the rising oxidative species. Increased release of LDH into the medium in a dose dependent manner suggested that the arsenic molecules are toxic to lung cells.

Metals are known to induce apoptosis through number of mechanisms by decreased cell proliferation/ viability (Bertin and Averbeck 2006) and regulating apoptotic players (Oh *et al*. 2004; Pettersson *et al*. 2007). Uranium acetate follows similar toxic response and induces apoptosis in normal rat kidney cells (Periyakaruppan *et al*. 2009). In the present study, we made an attempt to study arsenic-induced apoptosis in human lung epithelial cells and report the involvement of Bax and Bcl-2 apoptotic genes. Results of RT-PCR analysis of apoptotic genes show that arsenic at 2 μM significantly increase the pro-apoptotic gene Bax expression by more than 30 % and decrease the anti-apoptotic gene Bcl-2 expression significantly by more than 43 % suggested apoptosis induction by arsenic in A549 cells.

Cyclins are regulatory proteins activate cyclin dependent kinases. Each cyclin has a CDK partner and each CDK/cyclin pair has different function in cell cycle (Nagesh *et al*. 2017). Cyclin D and E families with corresponding CDKs induce transcription of growth factor signals in G1 phase and initiate the cell to start S phase (Neganova and Lako 2008). Analysis of expression of cell cycle regulator genes in arsenic treated cells show that there was 30 % reduced expression of Cyclin D and more than 50 % decreased expression of Cyclin E was seen at 2 μM concentration of arsenic by 48 h and confirms the regulation of cell cycle by arsenic metal in A549 cells.

Arsenic found to induce pro-apoptotic Bax mRNA and decreased the expression of anti-apoptotic Bcl-2 gene in A549 cells suggested that the arsenic induces apoptosis through intrinsic mechanism. However, arsenic action on caspases and cytochrome-C oxidase molecules needs to be investigated. Further, the arsenic significantly decreases the expression of cell cycle regulators cyclin-D and E suggested that the heavy metal inhibits the cell cycle. Taken together with all these findings, it is reasonable to state that arsenic metal induces oxidative stress in A549 cells by inducing ROS. The increase in the oxidative stress by heavy metal can be attributed to loss of effectiveness of the antioxidant system(s) GPx and catalase in A549 cells. Our study suggests that the Arsenic metal induces oxidative stress by inducing ROS in turn induces apoptosis by increased expression of apoptotic genes with decreased expression of cell cycle regulators.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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