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Original Article

Oxidative/ Nitrosative Stress, 8-OHdG and MMP-9: The Possible Co-Links and Early Sign of Arsenic Induced Urinary Bladder Carcinogenesis in Experimental Rats

Kshirod Bihari Sathua, Jayant Kumar Patwa, Swaran Jeet Singh Flora*

ABSTRACT

Background: Presently arsenicosis is considered as dangerous health issues including cancer. Urinary bladder is one of the common target organs for developing carcinogenesis by arsenic exposure due to accumulation of arsenic toxic metabolites in bladder. The mechanism involving urinary bladder carcinogenesis is still mysterious. Although there are multiple studies revealed about oxidative/nitosative stress, which plays main role for carcinogenesis, but no study evaluated the associated factor that linked with it as early sign to identify bladder carcinogenesis using a rat model. This study thus will be useful in predicting early sign for arsenic exposed urinary bladder carcinogenesis. **Objective:** We evaluated 8-Hydroxy-2-deoxyguanosine (8-OHdG) and Matrix Metalloproteinases-9 (MMP-9), which may be the possible associated factors along with oxidative/nitrosative stress as early sign for developing urinary bladder carcinogenesis upon arsenic and its metabolites exposure. **Methods:** Male Sprague Dawley rats were exposed to 25 ppm of sodium arsenite and its metabolite Dimethylarsinic Acid (DMA) via drinking water for a period of 16 weeks. The carcinogenic potentials were evaluated using various biochemical parameters indicative of oxidative/ nitrosative stress, ELISA of 8-OHdG as biomarker of oxidative/nitrosative stress induced DNA damage, which acts as a bridge between DNA damage and carcinogenesis. We also determined MMP-9 as potential pro-oncogenic biomarker associated with DNA damage. **Results:** High accumulation of arsenic was noted in tissues accompanied by a significant alteration of oxidative/nitrosative variables in liver and urinary bladder like significant changes in positive predictor of 8-OHdG and MMP-9 both in serum as well as urinary bladder tissue in animals exposed to arsenic and its metabolites. **Conclusion:** Early sign of urinary bladder carcinogenesis evaluated possibly by co-linking between oxidative/ nitrosative stress, 8-OHdG, MMP-9 and metal accumulation.

Key words: Sodium arsenite and dimethylarsinic acid, Urinary bladder carcinogenesis, Oxidative/Nitrosative stress, DNA damage, MMP-9.

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INTRODUCTION

Now-a-days water is the challenging resources for meeting agricultural and industrial needs, which are the major factor for the contamination of water resources. Arsenic is one such prime contaminants arises due to agricultural and industrial outcome which has assumed as the serious health concern including cancer.[1] It is considered as most widely studied toxic metalloid.[2] Although as per WHO guidelines the maximum permissible value of arsenic toxicity is 10 ppb but it has been reported that, some places in the world like Indo-Bangladesh region it exceeded by 80 times than its normal limit. Even epidemiological data revealed that, more than 200 million people worldwide getting affected by arsenic intoxication, out of more than 38 million were from the Indo-Bangladesh region only.[3-5]

Arsenic induced carcinogenesis is common. International Agency for Research on Cancer (IARC) has classified it as group 1 carcinogen. Many epidemiological studies signified that arsenic and its metabolites are carcinogenic to various organs such as liver, skin, lungs and urinary bladder exposed via drinking water.[6-8] Urinary bladder carcinogenesis via exposure to arsenic is not new. During detoxification process arsenic get metabolised and converted from pentavalent arsenicals to the trivalent arsenicals and vice versa in the liver by reduction and oxidative methylation process, respectively.[9] Finally the generated oxidative/nitrosative stress is scattering to various organs and tissue and associated with various adverse health effects.[10,11] The toxic metabolites generated from the liver will accumulate in urinary bladder via elimination process and may be responsible for the urinary bladder carcinogenesis. Various investigations have suggested that there is a relationship between arsenic and its metabolites exposure with urinary bladder carcinogenesis.[12,13]

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Toxic metabolites are generally produced during metabolism of accumulated arsenic. It has been assumed that, toxic metabolites that are generated as outcome of arsenic detoxification process are the major responsible factor for the generation of oxidative/nitrosative stress which eventually produce imbalance between generation and elimination of ROS/RNS.[10] Although, low or moderate amount of ROS/RNS have favourable effects, yet excessive generation may damage lipids, proteins, DNA and finally turn into oxidative tissue injury and cancer.[14] It is currently considered that "Arsenic-induced oxidative stress is the more accepted hypothesis for the carcinogenesis".[1,15]

To compensate the action of excessively generated oxidative stress, body has its own defence mechanism by stimulating some antioxidant enzymes like Superoxide Dismutase (SOD) and catalase. SOD accelerates the dismutation of superoxide to H_2O_2, whereas catalase catalyzes the removal of H_2O_2 in the form of H_2O and O_2.[16] If the expression of endogenous antioxidant enzymes gets continuously altered then antioxidant machinery of the system will disturbed eventually altering multiple cellular pathways and participate in carcinogenesis process.[17] Thus, there is a associative links between arsenic induced oxidative/nitrosative stress and carcinogenesis.

Endogenous damage of DNA by oxidative/nitrosative stress results into the modification of guanine and causing transversions of Guanine (G) to Thymine (T). Such modification and transversions are commonly found in mutated oncogenes and tumour suppressor genes which may participate in the altering of cell proliferation and apoptosis. Many reports considered 8-Hydroxy-2-deoxyguanosine (8-OHdG) as the outcome of DNA damaging lesions produced by oxidative/nitrosative stress during the development of cancer.[18] Hence 8-Hydroxy-2-deoxyguanosine (8-OHdG) may acts as the bridge between oxidative DNA damage and carcinogenesis.

Matrix Metalloproteinases-9 (MMP-9) is the class of endopeptidase enzymes. It is involved in pathological tissue remodelling by degrading collagen IV from basement membrane, extracellular matrix and triggering inflammatory or immune responses proteins such as cytokines, chemokines and growth factors, which may facilitates tumour progression, including invasion, metastasis, growth and angiogenesis.[19,20]

Various studies have concluded that there has been the involvement of generation of Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) mediates DNA damage, leading to the tumour development on arsenic exposure.[21] In the present study we investigated if i) chronic arsenic exposure induce oxidative stress and ii) the possible linkage between magnitude of oxidative/nitosative stress, DNA damage, Matrix Metalloproteinase-9 (MMP-9) expressions and arsenic accumulation as early sign for development of urinary bladder cancer.

MATERIALS AND METHODS

Chemicals

Sodium (Meta) arsenite was obtained from Sigma-Aldrich (St. Louis, MO, USA), Cacodylic acid from Sigma-Aldrich (St. Louis, MO, USA), while all other chemicals were of "AnalaR" or "Extra pure" grade and obtained from Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA).

Animals

All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). Male Sprague-Dawley rats (Weighing between 80-100 g, ~5-6 weeks) were procured from the animal facility of CSIR-Central Drug Research Institute, Lucknow, India. Experiments were performed in the animal house and laboratory facilities in the Department of Pharmacology and Toxicology of National Institute of Pharmaceutical Education and Research, Raebareli, India. All animal husbandry procedures were maintained as per the Standard Operating Procedures (SOPs) followed in the test facility. All experimental animals were kept in standard polypropylene cages (3 rats/cage) and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5 %) with automatically controlled cycle of 12 h light and 12 h dark. Standard laboratory animal feed (Purchased from Lipton's India Ltd) and water (aqua pure) were provided *ad libitum*. The metal contents of the animal feed (in ppm dry wt.) were Cu 10, Mn 55, Co 5, Zn 45 and Fe 70. The animals were weighed once in a week. All animals received human care incompliance with the guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)". Animals were acclimatized to the experimental conditions for a period of 1 week prior to the beginning of the experiment and were allowed a standard diet, water throughout the study.

Experimental design

Eighteen male Sprague Dawley rats (80-100 g, ~5-6 weeks) were randomized into 3 groups of 6 rats each and were treated as below for 16 weeks.

Group 1: Normal Control (received normal water).

Group 2: Inorganic arsenic as sodium (Meta) arsenite (25 ppm in drinking water).

Group 3: Arsenic metabolites as dimethylarsenic Acid (DMA) (25 ppm in drinking water).

Human gets exposed to arsenate through ground water contamination but following ingestion it gets converted into arsenite form. After methylation arsenate gets converted into inorganic arsenic (arsenite) an organic form (DMA and MMA). DMA is known to be the main causative factor for carcinogenesis. Since rats are devoid of SAM, we have chosen arsenite form to get the same effects as seen in human. In various reports from our group we have noted that 16 weeks of arsenic exposure produces almost similar biochemical responses as seen in human. Thus continuous exposures were given to all treated animals for the period of 16 weeks. After 16th weeks, animals were anesthetized with 50 mg/kg Pentobarbital and sacrificed. Blood samples were collected by via intra-cardiac puncture. Finally animals were quickly dissected under anaesthesia. Liver, Kidney and urinary bladder were removed, washed thoroughly with chilled normal saline and stored at −80°C until use for biochemical estimation.

Element analysis

Arsenic estimation in liver and kidney was done by using of Atomic Absorption spectrophotometer. A total of 0.2 g tissues were digested in 5 ml glass stopper test tubes with 1 ml of 16 N HNO_3, heated for 3 h at 95°C to near dryness (0.1 ml). Arsenic was estimated using a hydride vapour generation system (Perkin Elmer model MHS-10) fitted with an atomic absorption spectrophotometer (AAS, Perkin Elmer model AAnalyst 100).

Biochemical assays

Estimation of reduced glutathione level

Reduced glutathione (rGSH) was estimated according to the protocols described by Hissin *et al.* (1976) with some modification. In tissue homogenate equal volumes of 5 % sulfosalicylic acid were added and vortexed. The mixture was kept for 30 min in ice bath. After centrifugation, the supernatant was collected. rGSH contents were measured using Ellmann's reagent 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) solution. rGSH levels were calculated using a standard reference curve using

reduced glutathione as a standard. Results were expressed in µM rGSH/mg protein.[22]

Measurement of catalase activity

Ammonium molybdate forms a yellow complex with H_2O_2 and is suitable for measuring serum and tissue catalase activity. To analyze the catalase activity, we incubated 0.2 ml of tissue homogenate with 1 ml 65 µM H_2O_2 in 6.0 mM sodium potassium phosphate buffer, pH 7.4 for 60 sec (Sample 1). Control reactions were prepared using 1 ml H_2O_2 plus 0.2 ml buffer (No enzyme control; blank 2) and 1.2 ml buffer (No enzyme/no substrate, blank 3). The reaction was stopped by adding 1.0 ml of 32.4 mM ammonium molybdate to the samples and controls. The absorbance was read at 405.[23]

Measurement of superoxide dismutase activity

Superoxide Dismutase (SOD) activity was estimated according to the protocols described by Kakkar *et al.* (1984). Reaction mixture was prepared which contained 1.2 ml of sodium pyrophosphate, 0.3 ml of PMS, 0.3 ml of NBT, 0.2 ml of supernatant, 0.8 ml of distilled water and 0.2 ml of NADH. The control reaction mixture was prepared and contained 1.2 ml of sodium pyrophosphate, 0.3 ml of PMS, 0.3 ml of NBT, 1ml of distilled water and 0.2 ml of NADH. Both mixtures were incubated at 37°C for 90 sec and then reaction was stopped by adding 1 ml of acetic acid and the mixture was allowed to stand for 10 min. The absorbance was measured at 560nm.[24]

Measurement of malondialdehyde levels

Malondialdehyde (MDA) considered as the end product of lipid peroxidation, it was measured in liver tissues by using thiobarbituric acid reactive substance method, according to method described by Ohkawa *et al.*[25] with some modifications. In brief, the liver and bladder tissue were collected and rinsed with chilled PBS, tissue was minced and homogenate was prepared in phosphate buffer (pH 7.4) containing EDTA (1 mM). The samples were centrifuged and the supernatant was used for the determination of MDA levels. The absorbance was measured at 532 nm. MDA levels was calculated from the standard curve using the 1, 1, 3, 3-tetramethoxy propane (97 %) and expressed as µM MDA/mg protein.[25]

Measurement of nitrite content

Measurement of Nitrite content in the tissue homogenate was done according to the method previously described by Giustarini *et al.*[26] with some modifications. For the determination of nitrite content, equal volumes of Griess reagent and supernatant were added in a 96- well plate and incubated for 10 min in dark with shaking and then absorbance was measured at 540 nm. Nitrite levels were calculated using a standard curve using sodium nitrite as a standard and expressed as µM/mg protein.[26]

Measurement of serum 8-OHdG level

Serum 8-OHdG level was estimated by using ELISA kit (MyBioSource San DiegoUS.) according to the manufacturer's instruction.

Measurement of MMP-9 level

MMP-9 level was estimated in serum as well as urinary bladder by using ELISA kit (My BioSource San Diego US.) according to the manufacturer's instruction.

Statistical analysis

All results were expressed as the mean± standard error of the mean (SEM). Graph Pad (Prism 6) software were used for statistical analysis. Statistical differences in the variables between the groups were analyzed by one-way ANOVA followed by multiple comparisons with Tukey's test as well as regression analysis were performed along with correlation. The level of statistical significance was set at $p<0.05$.

RESULTS

Effect of arsenic exposure on tissue arsenic concentration

Figure 1 and Table 1 demonstrates that the concentration of arsenic in liver and kidney tissues on 16 weeks of continuous exposure to sodium arsenite and DMA led to the significant accumulation of arsenic in animals and was in the order of liver > kidney. On the other hand arsenic concentration in sodium arsenite and DMA exposed groups was in the order of sodium arsenite > DMA.

Effect of arsenic exposure on oxidative and nitrosative stress variables in liver and urinary bladder tissue

Figure 2-6 (Table 2-4) demonstrate the effects of arsenic and its metabolites on various biochemical variables on 16 weeks of exposure suggestive of oxidative and nitrosative stress. We selected reduced GSH as a free radical scavenger, SOD and catalase to evaluate tissue antioxidant status, TBARS as the end-point for lipid peroxidation due to oxidative stress and nitrite as the end-point of nitrosative stress. Exposure to sodium arsenite and DMA via drinking water for 16 weeks resulted in a significant decrease in reduced GSH level in the liver and a marginal decrease in urinary bladder compared with untreated group. However, the stimulation of both the antioxidant enzymes i.e. SOD and catalase increases significantly compared to controls both in liver as well as urinary bladder tissue. Arsenic and DMA exposure also increased the nitrite and MDA levels in the liver and urinary bladder tissues significantly. Oxidative and

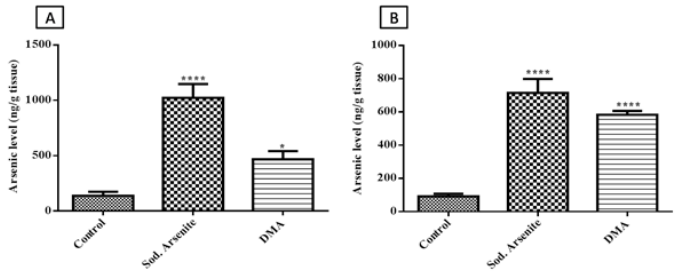

Figure 1: A) Effect of Arsenic Exposure on Liver Arsenic Level B) Effect of Arsenic Exposure on Kidney Arsenic Level. All the values are expressed as mean± SEM (n=6), *P<0.05, ****P< 0.0001 vs. Control.

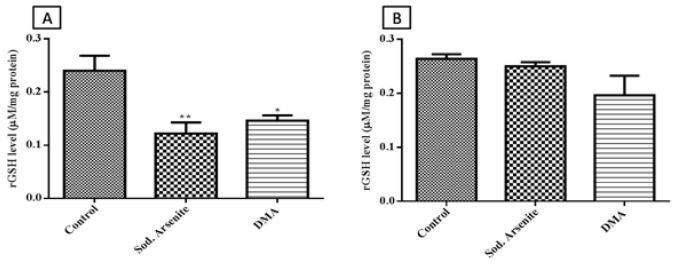

Figure 2: A) Effect of arsenic exposure on liver reduced GSH level B) Effect of arsenic exposure on urinary bladder reduced GSH level. All the values are expressed as mean± SEM (n=4-5), *P<0.05, **P<0.01 vs. Control.

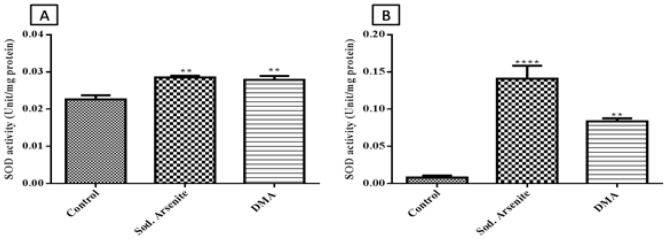

Figure 3: A) Effect of Arsenic Exposure on Liver SOD activity B) Effect of Arsenic Exposure on Urinary Bladder SOD Activity. All the values are expressed as mean± SEM (n=4-5), ***P*<0.01, *****P*<0.0001 vs. Control.

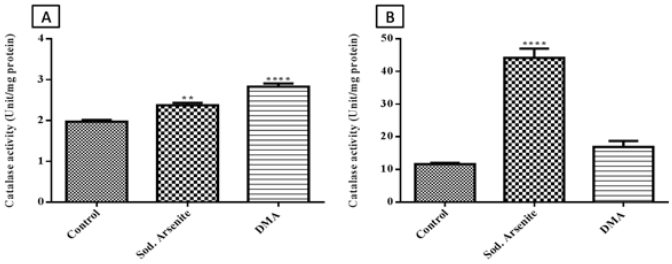

Figure 4: A) Effect of Arsenic Exposure on Liver Catalase Activity B) Effect of Arsenic Exposure on Urinary Bladder Catalase Activity. All the values are expressed as mean± SEM (n=5), ***P*<0.01, *****P*<0.0001 vs. Control.

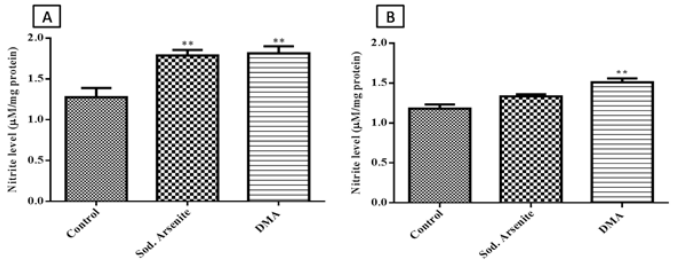

Figure 6: A) Effect of Arsenic Exposure on Liver Nitrite Level. B) Effect of Arsenic Exposure on Urinary Bladder Nitrite Level. All the values are expressed as mean± SEM (n=4-5), ***P*<0.01 vs. Control.

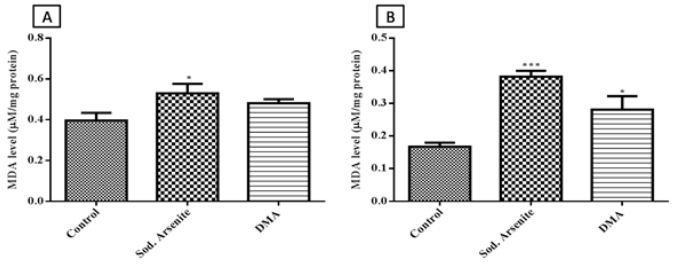

Figure 5: A) Effect of arsenic exposure on liver MDA level B) Effect of arsenic exposure on urinary bladder MDA level. All the values are expressed as mean± SEM (n=4-5), **P*<0.05, ****P*<0.001 vs. Control.

Table 1: Effect of arsenic exposure on liver and kidney arsenic level.

Parameters	Control	Sod. Arsenite	DMA
Liver arsenic level (ng/g tissue)	137.5±37.08	1023±125.1****	467.5±74.90*
Kidney arsenic level (ng/g tissue)	91.67±15.47	714.2±84.73****	582.5±23.41****

All the values are expressed as mean± SEM (n = 6), ***P** < 0.05, ******P** < 0.0001 vs. Control.

Table 2: Effect of arsenic exposure on liver oxidative stress variables.

Parameters	Control	Sod. Arsenite	DMA
rGSH (µM/mg protein)	0.24±0.028	0.12±0.021**	0.15±0.010*
SOD activity (Unit/mg protein)	0.023±0.0011	0.029±0.00047**	0.028±0.0010**
Catalase activity (U/mg protein)	12±0.37	44±2.8****	17±1.9
Nitrite (µM/mg protein)	1.3±0.11	1.8±0.068**	1.8±0.087**
MDA (µM/mg protein)	0.40±0.037	0.53±0.047*	0.48±0.020

All the values are expressed as mean± SEM (n = 5), ***P** < 0.05, ****P** < 0.01, ******P** < 0.0001 vs. Control.

nitrosative stress variables both the form of arsenic treated group was in the order of sodium arsenite > DMA.

Effects of Arsenic Exposure on Serum 8-Hydroxy-2-deoxyguanosine (8-OHdG): Bridge between oxidative DNA Damage and Carcinogenesis

Sixteen weeks of sodium arsenite and DMA exposure produced a significant increase in serum 8-Hydroxy-2-deoxyguanosine (8-OHdG) level compared to untreated group as shown in Figure 7 Table 4. Whereas the level of 8-Hydroxy-2-deoxyguanosine (8-OHdG) in sodium arsenite treated group was higher compared to the level observed in DMA treated group.

Effect of arsenic exposure on matrix Metalloproteinase-9 (MMP-9) expression in serum and urinary bladder tissue

There was a significant increase in serum matrix metalloproteinase-9 (MMP-9) expression in sodium arsenite treated group where as in DMA treated animals the expression was marginally increased compared with naive animals (Figure 8 Table 4). However, there was significant increase of Matrix Metalloproteinase-9 (MMP-9) levels in urinary bladder tissues both in sodium arsenite and DMA treated groups. The Matrix Metal-

Table 3: Effect of arsenic exposure on oxidative stress variables and MMP-9 level in on urinary bladder.

Parameters	Control	Sod. Arsenite	DMA
rGSH (μM/mg protein)	0.264±0.008	0.250±0.007	0.196±0.036
SOD activity (Unit/mg protein)	0.0079±0.0026	0.14± 0.018****	0.084±0.0041**
Catalase activity (Unit/mg protein)	2.0±0.044	2.4±0.061**	2.8±0.078****
Nitrite (μM/mg protein)	1.2±0.052	1.3±0.027	1.5±0.049**
MDA (μM/mg protein)	0.17±0.012	0.38±0.018***	0.28±0.041*
MMP-9 (pg/mg protein)	48±3.8	86±4.5***	69±4.3*

All the values are expressed as mean± SEM (n = 5), *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 vs. Control.

Table 4: Effect of arsenic exposure on serum 8 OHdG and MMP-9 level.

Parameters	Control	Sod. Arsenite	DMA
8 OHdG(ng/ml)	331±44	784±21**	685±109*
MMP-9 level (ng/ml)	5.1±0.14	6.7±0.27	5.6±0.26

All the values are expressed as mean± SEM (n = 5), *P < 0.05, **P < 0.01 vs. Control.

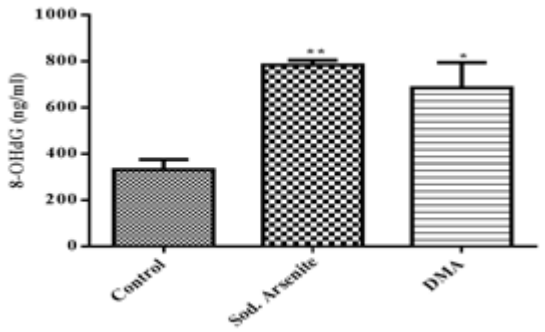

Figure 7: Effect of arsenic exposure on serum 8-OHdG level. All the values are expressed as mean± SEM (n=3), *P<0.05, **P<0.01 vs. Control.

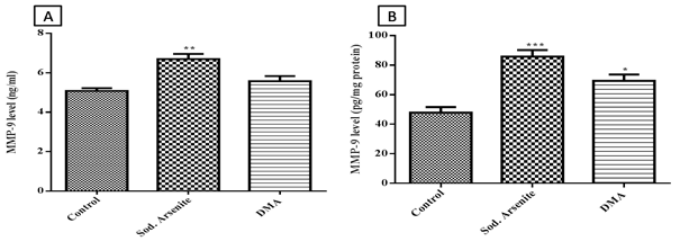

Figure 8: A) Effect of Arsenic Exposure on Serum MMP-9 Level. B) Effect of Arsenic Exposure on Urinary Bladder MMP-9 Level. All the values are expressed as mean± SEM (n = 3-4), *P < 0.05, **P < 0.01, ***P < 0.001 vs. Control.

loproteinase-9 (MMP-9) expression levels of both the form of arsenic treated group was in the order of sodium arsenite > DMA.

DISCUSSION

Arsenic is considered globally as the most serious health hazard including urinary bladder cancer.[27] Although the mechanisms of cancer initiation and progression is still a mystery yet some research evidences supports that there has been the involvement of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which eventually leads to DNA damage and carcinogenesis on arsenic exposure.[21] In the present study, we noted that the carcinogenic potential of arsenic and its metabolites in urinary bladder is possibly due to the interlinking between high levels of metal accumulations, oxidative/nitrosative stress, DNA damage and expression of Matrix Metallopeptidase-9 (MMP-9).

From our data on arsenic concentration it has been shown that there was a significant accumulation of arsenic in liver and kidney tissue in sodium arsenite and DMA exposed animals compared to control animals. These results are in agreement with earlier study which signified that extensive accumulation of arsenic in liver and kidney tissue leads to arsenic intoxication.[28,29]

Plenty of evidences from published literature indicate that reactive oxygen species and reactive nitrogen species are the main factor responsible for arsenic induced carcinogenesis.[30] Oxidative/nitrosative stress generated from the liver during detoxification process and finally scattered to various organs and tissue.[10] As liver is the prime source for generating and scattering of oxidative stress, thus we have chosen liver as the organ of consideration along with urinary bladder for carcinogenesis evaluation.

Free radical scavenger plays vital role during oxidative stress. GSH is one such scavenger which maintains the GSH pool. Our results demonstrated that chronic arsenic exposure caused a significant depletion in the reduced GSH level in the liver and marginally decreased in the urinary bladder, suggesting an imbalance in the maintenance of the GSH pool. Galaris *et al.*[31] suggested that, decrease in the intracellular GSH levels on arsenic exposure affects the transcription of a variety of stress response genes which may plays as main key factor for carcinogenesis.[1,31] Our data further gets credibility from an earlier study which suggested significant decrease in the GSH level in rats following exposure to arsenic.[32]

Interestingly, our results also showed that there is significant stimulation of SOD and catalase enzyme activities both in liver as well as urinary bladder. To compensate the over-action of continuously generated excess oxidative stress in response to external stimuli, living systems have its own defence mechanism by altering the expression of some endogenous antioxidant enzymes.[33,17] Increased SOD and catalase activities observed in our study can be attributed to compensate the over-action of H_2O_2.[34,35,16] Altering the expression of endogenous antioxidant enzymes may ultimately imbalance the antioxidant machinery of the body system resulting in the modification of multiple cellular pathways and alteration in the expression of growth factors, cell cycle checkpoint proteins etc. which ultimately might be participating in the carcinogenesis process.[17]

Determination of TBARS levels is an important indicator of lipid peroxidation occurr following various pathological conditions including cancer.[36] There is an extensive experimental evidence from oxidative stress studies on various types of cancer which suggest an increased TBARS/MDA levels compared to normal individuals.[6] These findings correlate with the levels of TBARS measured in our study where a significant increase was observed in chronic exposure of arsenic and DMA treated group compared to controls both in liver as well as urinary bladder. It has been suggested that MDA/TBARS will damage DNA by forming MDA-DNA adducts during intracellular oxidative stress.[37] Leuratti *et al.*[38] in their study suggested that MDA-DNA adducts may be involved in the initiation and progression of cancer with its mutagenic and carcinogenic effects.[38]

Excessive production of reactive nitrogen species results in the derangement of cell metabolism, breakage of DNA/RNA chains and tissue damage which is solely associated with cancer initiation and progression.[39]

Our results demonstrated that chronic arsenic exposure caused a significant increase of nitrite levels both in liver as well as urinary bladder suggesting probability of carcinogenesis via nitosative damage to tissues. These results are in agreement with earlier study.[40,41]

8-Hydroxy-2-deoxyguanosine (8-OHdG) is widely used as a biomarker for oxidative stress and carcinogenesis as it is an indicator of DNA damage.[18] Our results from oxidative and nitrosative stress parameters confirm that there is a possibilities of DNA damage, as evident from the data of 8-OHdG in our study as the prime indicators which may acts as a bridge between DNA damage and carcinogenesis. Presence of 8-OHdG residues in cells may lead to the modification of guanine and transversion of GC to TA. Such modification and transversions are commonly found in mutated oncogenes and tumour suppressor genes thus suggest that the level of 8-OHdG in cells critically participate in the carcinogenesis.[42,43] Previous studies also report elevated levels of 8-OHdG at precancerous and cancerous tissues compared to adjacent normal tissues.[44,45] For instance, our result showed that the levels of serum 8-OHdG in arsenic and DMA exposed animals were significantly higher than that of untreated animals. These results are in agreement with an earlier study which suggested that significant increase the 8-hydroxy-2′-deoxyguanosine content in Japanese patients with urinary bladder and renal cancers[46] and duration of exposure to arsenic in drinking water was significantly associated with increases in 8-OHdG.[47] All of these findings prompt us to propose that 8-OHdG may act a potential biomarker during detection and treatment of arsenic induced carcinogenesis.

It has been reported that MMP-9 has potential pro-oncogenic roles i.e. increase the level of MMP-9 precedes to oncogenesis.[19] Our data revealed that chronic arsenic and DMA exposure causes significantly raised the matrix metalloproteinase-9 (MMP-9) expression in urinary bladder whereas in serum there was significant and marginal increase in sodium arsenite and DMA exposed animals, respectively. We confirm from our 8-OHdG levels that there was an extensive DNA damage imparted by arsenic and DMA. It has been reported that alteration in the level of MMP-9 is linked with the levels of DNA fragmentation and hindrance of DNA repair machinery.[48,49] MMP-9 also binds with damaged DNA and associated with nuclear translocation.[50] Thus there is a possible link between 8-OHdG, MMP-9 and carcinogenesis. Our results signify that there is a critical involvement of both arsenic and DMA in the carcinogenesis processes. Our data of matrix metalloproteinase-9 expression level further gets credibility from the fact that "Relation of dietary inorganic arsenic to serum Matrix Metalloproteinase-9 (MMP-9) at different threshold concentrations of tap water arsenic".[51]

From the data of our present study we observed that though, both sodium arsenite and DMA are positively associated with the development of urinary bladder carcinogenesis yet the potency of DMA is less compared to sodium arsenite, suggesting inorganic to be more potent carcinogenic compared to DMA following drinking water exposure. However some *in-vitro* and *in-vivo* studies suggested that carcinogenic potency of DMA is more than that of sodium arsenite.[52,53] Our results although in some disagreement with available literature and observed arsenic (III) to be more carcinogenic than DMA possibly due to deteriorative effects of DMA via oral route.

CONCLUSION

In conclusion, our present finding signified that both sodium arsenite and DMA actively participate in the development of urinary bladder carcinogenesis in male rats which critically linked with high arsenic accumulations, oxidative/nitrosative stress, DNA damage and expression of matrix metallopeptidase-9 (MMP-9) which may be used as early predictor of arsenic exposed urinary bladder carcinogenesis. In spite of the fact that it gives solid experimental support yet additional studies are required in terms of genetic expression for advancement of arsenic exposed bladder carcinogenesis.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

ABBREVIATIONS

Sod. Arsenite: Sodium (meta) arsenite, **DMA:** Dimethylarsenic Acid, **ROS:** Reactive Oxygen Species, **RNS:** Reactive Nitrogen Species, **GSH:** Glutathione, **SOD:** Superoxide dismutase, **MDA:** Malondialdehyde, **8-OHdG:** 8-hydroxydeoxyguanosine, **MMP-9:** Matrix Metalloproteinase-9.

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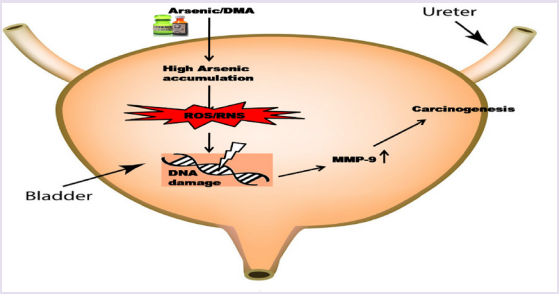
GRAPHICAL ABSTRACT



SUMMARY

- High accumulations of arsenic, oxidative/nitrosative stress, DNA damage and increase MMP-9 level may early sign of arsenic induced bladder carcinogenesis

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