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RESEARCH ARTICLE

ARSENIC AS AN ELICITOR IN CONTROLLING TOTAL ANTIOXIDANT CAPAPCITY IN HUMAN

Dalal B[1*], Mukherjee S[1], Roy S K[2] and Mukherjee A K[2]

[1]Department of Biochemistry and Toxicology, Regional Occupational Health Centre (Eastern), National Institute of Occupational Health, Indian Council of Medical Research, Block DP-1, Sector V, Salt Lake City, Kolkata-700091, India

[2]Department of Industrial Hygiene, Regional Occupational Health Centre (Eastern), National Institute of Occupational Health, Indian Council of Medical Research, Block DP-1, Sector V, Salt Lake City, Kolkata-700091, India

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ABSTRACT

It is well documented that arsenic exposure through drinking water causes different types of manifestations covering almost all human body system. The human exposure to arsenic is being evaluated by clinical signs and symptoms and by laboratory investigation like metabolic products of arsenic in urine. Objective of the present study was to determine total antioxidant capacity in human exposed to arsenic. The study was conducted at West Bengal, India and n=108 subjects were recruited for conducting study. All subjects were investigated for health status and antioxidant profile. Water samples were analyzed for arsenic analysis. Significant rise ($p<0.001$) in serum uric acid (UA) level, as preliminary oxidative stress marker, was observed in 58% cases of group of tested population consuming >50µg/lit arsenic in drinking water. Declining trend of glutathione peroxidase (GPX) and glutathione reductase (GR) were also demonstrated in our experiments. Similarly, decline in Total Antioxidant Status (57% cases) of the >50µg/lit group was in agreement with the elevation of serum UA. Arsenic induced dermal manifestations were observed in 6.4% of tested population. Our study also indicated reduced oxidative stress parameters in a significant number of cases without signs and symptoms of arsenic exposure. Therefore, it may be concluded that the high arsenic consuming group (>50µg/lit) suffer from irreversible oxidative stress, which could not be compensated leading to symptomatic disease.

INTRODUCTION

Arsenic is silent killer by exerting its toxic effect to millions of people who are exposed over the world mainly through drinking contaminated water. It is well documented that arsenic exposure causes different types of symptoms covering almost all human body system. The human health effect of arsenic toxicity ultimately leads to arsenicosis. The major features of arsenicosis are dermatological manifestations with diffused or spotted melanosis, leukomelanosis, and keratosis.

Moreover, arsenic can cause alterations of the other organs as well, such as liver, kidney, bladder or lung, which could be related with cancer development on these organs (Dalal B *et al*, 2013). Cyto-oxidative phenomenon takes place irrespective of arsenic exposure. Studies have demonstrated that toxicity of arsenic is associated with oxidative stress *in vivo* in which arsenic induces generation of reactive oxygen species (ROS) (Zhang Z *et al*, 2011; Lingzhi L *et al*, 2014). Animal studies have also demonstrated methyl arsenics and dimethyl arsenics may play a role in arsenic carcinogenesis through the induction of oxidative damage (Yamanaka K *et al*, 2001). Glutathione (GSH), master antioxidant, is normally under tight homeostatic control both intra-cellularly and extra-cellularly (Baudouin-Cornu P *et al*, 2012). Different reports have further revealed that toxic effects of arsenic are regulated by diverse mechanism [Dalal B *et al*, 2013; Biswas R *et al*, 2008; Soto-Pena GA *et al*, 2006]. Alteration of GSH and its controlling enzymes has also been demonstrated in animal system [Santra A *et al*, 2000; Han Y *et al*.2010]. Difficulties have been focused on oxidative stress in arsenic exposed human as potential source of damage in contrast to what has been well established in arsenic exposed animal system [Singh MK *et al*, 2014]. The problem resides in drawing a relation between two independent events like oxidative stress and arsenic exposure in human. We have earlier reported existence of elevated serum uric acid in arsenic exposed (above 50 µ gm /lit) human [Dalal B *et al*, 2009]. Our preliminary work indicated the possibility of establishing relationship between oxidative stress and arsenic exposure. However, the causative role of impaired oxido-reduction

Corresponding author:* **Dalal B
Department of Biochemistry and Toxicology, Regional Occupational Health Centre (Eastern), National Institute of Occupational Health, Indian Council of Medical Research, Block DP-1, Sector V, Salt Lake City, Kolkata-700091, India

process with arsenic exposure has seldom be discussed. The present paper deals with involvement of total antioxidant capacity in human exposed to arsenic.

MATERIALS AND METHODS

Categorization of subjects on arsenic concentration in drinking water

One hundred and eight subjects (n=108) were recruited from North 24 pargana,West Bengal, India where subjects are drinking water contaminated with arsenic. Subjects were divided into two groups depending upon level of exposure of arsenic in drinking water (a) >50µg/lit (b) <50µg/lit. The arsenic level in drinking water was evaluated by Atomic Absorption Spectrophotometer (AAS). Correlation between manifestation of clinical symptoms of arsenic exposure and antioxidant status, if any, among >50µg/lit and <50µg/lit subjects was evaluated.

Measurement of water arsenic

Estimation of arsenic in water was done by atomic absorption spectrophotometer (AAS-FIAS) (Model AA800, Perkin Elmer, USA) as previously described (Chatterjee A *et al*,1995). Stock solutions of arsenic (1000mg/l) prepared from 99% AS_2O_3 (Sigma, USA) in de-ionized water and working standards were prepared daily from the stock solution. Before measurement all As present was reduced to As III by acidifying the samples with two molar hydrochloric acid (2M HCl) and 0.2% potassium iodide (KI). Time period for complete reduction at room temperature was one hour. Arsenic concentration in the water samples was estimated against the working standards prepared daily from the stock solution at an absorbance at 193.7nm.

Medical study

The study comprised of recording of history with special emphasis on collection of exposure to arsenic through drinking water including duration of water consumption in a pre-designed, tested and validated proforma followed by clinical examination of the subjects. Collection of blood specimen was undertaken after administration of signed informed consent procedure approved by the Institutional Ethics Committee of Regional Occupational Health Centre (Eastern), Indian Council of Medical Research, Department of Health Research, Government of India.

Assay of Uric Acid

Serum Uric acid was estimated by Automated Biochemistry Analyser (Chemwell Awareness Technologies, USA) using commercial diagnostic kit containing internal standard according to the method of Fossati *et al*,1980.

Estimation of Total Antioxidant Status (TAS)

Freshly prepared serum was used for the assay of TAS by commercial kit of Ms. Randox as per the protocol of Miller NJ *et al*, 1993. Briefly, at 37 °C, 20 µl serum was mixed with 1000 µl chromogen 2,2-Azino-di-[3-ethylbenzenzthiazoline sulphonate (ABTS) (610 µmol/lit) and initial reading was taken. Subsequently, Xanthine Oxidase was added to it and reading of blue green color was measured after 3 minutes using Double Beam Spectrophotometer, Perkin Elmer Lambda 45 at 600nm. The assay was conducted using a standard 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid solution (2.04mmol/l) and a blank using water. The unit was expressed in mmol/lit.

Measurement of Glutathione Peroxidase (GPX)

This method of Randox assay kit is based on the method of Paglia DE *et al*,1967. 0.05 ml heparinized whole blood was diluted with 1 ml PBS and incubated for 5 minutes followed by addition of 1 ml double strength Drabkin's reagent.

It was mixed well and the resultant hemolysate was used for assay of GPX. 1000 µl reagents [Glutathione (4mmol/lit), Glutathione reductase (>=0.5 U/lit), NADPH (0.34mmole/lit) in Phosphate Buffer (PBS) (0.05 mol/lit. pH 7.2), EDTA (4.3m mol/lit)] and sample (20 µl) were pipetted into cuvette. Subsequently 40 µl Cumene Hydroperoxide (0.18 mmol/lit)] was added to it.

The assay mixture was maintained at 37 °C. Double distilled water (20 µl) acts as blank. The contents were mixed and initial absorbance of sample and reagent blank was taken after one minute and timer was started simultaneously. Reading was taken again after 1 and 2 minutes. Reagent blank value was subtracted from that of the sample. U/lit of Hemolysate = 8412 x –A 340 nm / minute.

Assay of Glutathione Reductase (GR)

This estimation was carried out using assay kit of Randox. Briefly, 0.05 ml of whole blood was centrifuged at 2000 rpm for 15 minutes. The erythrocytes obtained was washed with 3 ml of 0.09% NaCl and centrifuged at 2000 rpm for 5 minutes again. The process of washing was continued for three times as per protocol of k it. Finally the erythrocytes was ruptured by 2 ml cold distilled water and left for five minutes at +4°C for 10 minutes to get hemolysate.

The lysate was centrifuged for 5 minute at 200 minutes to remove stroma. Finally,100 µl of lysate was mixed with 1.9 ml of 0.9% NaCl solution to make it ready for assay. The assay procedure was based on method of Gold berg DM, 1983. 40 µl of lysate sample was incubated with 1 ml of substrate GSSG (oxidized glutathione) in potassium phosphate buffer containing EDTA at 37 °C. Finally, 200 µl NADPH was mixed to it and initial absorbance was measured at 340 nm using Double Beam Spectrophotometer, Perkin Elmer Lambda 45. Absorbance was again measured up to 5 minute in 1 minute interval. GR is expressed as U /gm Hb.

Statistical analysis

Data are shown as Means ±S.E. Student's T test was used to compare means of two categories of samples. Statistical significance was considered to be reached for $p< 0.001$.

RESULTS AND DISCUSSIONS

Average age group of tested males and females were as follows: Males= 42.38±16.86 years (n=61); Females = 40.55±13.81 years (n=47). Minimum duration of consuming arsenic contaminated water was 5 years and maximum was 20 years. Arsenic induced dermal manifestations were found in 6.4% (n=7) of the tested population. Table 1 showed mean arsenic concentration in drinking water. Arsenic concentration was detected and categorized in two ranges (i) Range 1.48-49.18 with an average 14.54±1.74 µg/lit and (ii) Range 51.0-900.03 µg/lit with an average 276.73±40.48 µg/lit. Based on the arsenic concentration of drinking water two categories have been made: a) subjects consuming water from the source with arsenic concentration <50µg/lit, b) subjects consuming water from the source with arsenic concentration >50µg/lit.

Table 1 Arsenic level in tube well water collected from North 24 Parganas, West Bengal, India

Parameter		Water-As level in the ranges	
		0-50 µg/lit	>50 µg/lit
Water-As Total (n=92)	Mean ± SE	14.54±1.74	276.73±40.48
	Range	1.48-49.18	51.0-900.03
	N	(n=57)	(n=35)

A series of experiments on the quantification of UA, GPX, GR and TAS as indicated in Figure 1 showed that elevation of uric acid, with respect to normal range, occurred in 58% of total tested population of >50µg/lit category. Enhancement of 8-oxo-2'-deoxyguanosine, a product of base oxidation, has also been shown as consequence of arsenic induced oxidative damage in animal system (Yamanaka K *et al* , 2001). Moreover, >50µg/lit category tested population (58% of total tested population) showed lower values of GPX than normal range.

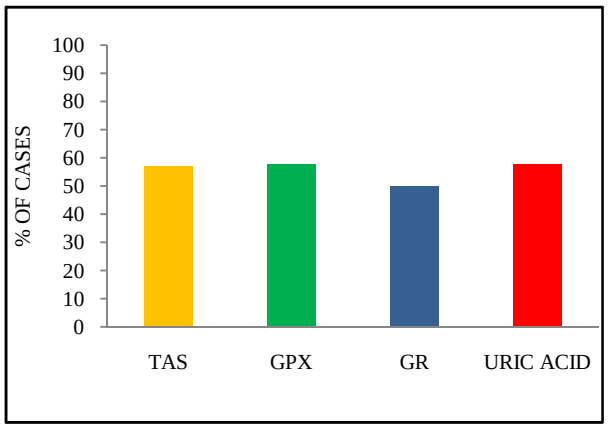

Figure 1 Percentage of cases of deviation from normal range in >50µg/lit category

Our observation is supported by the study conducted in Southern Thailand (Warangkana C *et al*, 2008). Arsenic induced decline of GPX has also been demonstrated in rats and aquatic animals (Sudha K *et al*, 2012; Wang Q *et al*,2014). Low GPX status may heighten risk of adverse health effects (Caver S *et al*, 2010). GR showed similar declining pattern (50.0%) in tested population. Arsenic is found to inhibit GR in vitro (Nemati B *et al*, 2004). Animal experimentation demonstrated arsenic induced decrease in GR (Santra A *et al*, 2000, Rodriguez VM *et al*, 2005). We further observed that TAS level decreases below normal in case of >50µg/lit category exposed subjects (57% cases of total tested population). It has been demonstrated that reactive oxygen species mediate arsenic induced alteration of physiological events in human cells (Zhang Z *et al*, 2011; Lingzhi L *et al*, 2014). Free radical mediated oxidative damage is a common denominator of arsenic pathogenesis [Jomova K *et al*, 2011].

Table 2 indicates the effect of arsenic exposure on biochemical parameters. Serum UA, as preliminary indicator of oxidative stress in >50µg/lit exposure group increased significantly ($p<0.001$) in comparison to <50µg/lit group.

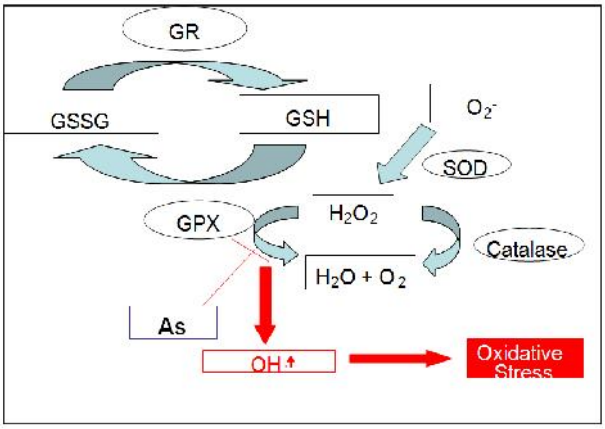

Figure 2 Schematic diagram for arsenic induced oxidative stress

Elevation of serum uric acid takes place with concomitant increase of reactive oxygen species in different patho-physiological conditions [Strazzulo P *et al*,2007; Zhang Y *et al*,2013]. Epidemiological studies also indicate that increased serum levels of uric acid are a risk factor for cardiovascular disease where oxidative stress plays an important role [Glantzounis GK *et al*, 2005]. GPX, a glutathione dependent enzyme, decreased significantly ($p<0.001$) in >50µg/lit group. Other study also demonstrated that decreased levels of GPX activities, total glutathione, and GSH among high exposure subjects when compared to low exposure subjects [Warangkana C *et al*, 2008].

Values are Mean ± SE, values in parenthesis are number of subjects GR, an enzyme responsible for conversion of GSSG to GSH, also showed significant decline ($p<0.001$) among >50µg/lit subjects compared to <50µg/lit group. Arsenic induced GS depletion with concomitant GR reduction by erythrocytes has also been demonstrated *in vitro* (Nemati B *et*

Table 2 Effect of arsenic exposure on biochemical parameters

Biochemical Parameters (Unit)	Normal Range	Arsenic< 50µg/lit	Arsenic >50µg/lit	Change p value
Hb(gm/dL)	M-13-18; F-12.1-15.1	12.28±0.20 (n=45)	11.90±0.19 (n=63)	NS
Uric acid (mg/dL)	2.4-6.0	4.62±0.12 (n=45)	6.67±0.0.07 (n=63)	P<0.001
TAS (mmol/lit)	1.3-1.7	1.44±0.01 (n=30)	1.29±0.031 (n=40)	P<0.001
GPX (U/lit)	4171-10881	6371.36±151.74(n=45)	2558.77±191.51 (n=63)	P<0.001
GR (U/gm Hb)	4.7-13.2	7.75.95±0.12 (n=12)	3.17±0.30 (n=12)	P<0.001

al,2004). Decline of TAS, as indicator of the status of oxidative stress, was significant ((p<0.001) in >50µg/lit exposure group compared to <50µg/lit group. Decreased GPX and GR activities with concomitant increased oxidized glutathione levels in human supported our observation (Warangkana C *et al*, 2008). Our experiments suggest a schematic diagram for arsenic induced oxidative stress (Figure 2).

It was also observed from our study that 6.4% of the exposed population showed dermal manifestations. This implies that these subjects already have signs of arsenic exposure. Their serum uric acid were significantly higher (than normal range), which was further corroborated by lowering of TAS, GR, GPX than normal range. Our study is in agreement with experiments carried out in human and animal system (Warangkana C *et al*, 2008; Sudha K *et al*,2012]. Moreover, our study also indicated decreased level of anti-oxidative parameters in a significant number of cases without signs and symptoms of arsenic exposure.

CONCLUSION

Therefore, it may be concluded that the high arsenic consuming group has cyto-oxidative stress which is not compensated leading to symptomatic disease. The study needs further investigation for establishment of the parameters as early biomarker of arsenic induced oxidative stress.

Author's Contribution and Competing interest

BD conceptualized, planned and executed the work. AKM and SKR are responsible for analysis of arsenic and SM for biochemical analysis respectively and provided intellectual inputs. Draft is prepared by BD in consultation with AKM and SM. The authors declare no competing interests.

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References

Baudouin –Cornu P, Lagniel G, Kumar C, Huang M E, Labarre J. Glutahione degradation is a key determinant of glutathione homeostasis. *The J. Biol. Chem*. 2012; 287 : 4552-4561.

Biswas R, Ghosh P, Banerjee N, Das J K, et.al. Analysis of T-Cell proliferation and cytokine secretion in the individuals exposed to arsenic. Human & Exp. Toxicol. 2008; 27 : 381-386.

Caver S, Bosnjak Z, Klapec T., Barisic K, Cepelak I, Jurasovic J, Milic M. Blood selenium, glutathione peroxidase activity and antioxidant supplementation of subjects exposed to arsenic via drinking water. Environ. Toxicol. Pharmacol. 2010; 29 : 138-143.

Chatterjee A, Das D,. Mandal D K, Chowdhuri T R, Samanta G and Chakraborty D. Arsenic in ground water in six district of West Bengal: the biggest arsenic calamity in the world, Part I- Arsenic species in drinking water and urine of the affected people. Analyst. 1995;120 : 643-650.

Dalal B, Lantz R C. Studies of arsenic induced action of T cell subsets in mice. *The Journal of Toxicol and Health*. 2013; 103 : 252-256.

Dalal B, Mukherjee A K, Mukherjee S. Concentration dependent expression of hepatic biomarkers of arsenic exposure in human. *The J. Public Health*. 2013; 115 : 205-210.

Dalal B, Nayak K, Gangopadhyay P K, Mukherjee A K. Identification of indicators of arsenic induced hepatic damage in human. *The Internet J. Toxicol*. 2009;7.

Fossati P, Precipe L, Berti G. Use of 3,5- dichloro 2 hydroxy benzene sulphonic acid/ aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine . Clin Chem. 1980; 26 :227-231.

Glantzounis G K, Tsimoyiannis E C, Kappas A M, Galaris D A. Uric and Oxidative Stress. Curr Pharm Des. 2005; 11 : 4145-4551.

Goldberg D M and Spooner R J . Methods of Enzymatic Analysis. Bergmeyen, H.V. 1983; Ed 3 : 258-265.

Han Y, Moon H J, You B R, Kim S Z, Kim S H, Park W H. Effects of arsenic trioxide on cell death, reactive oxygen species and glutathione levels in different cell types. *Int. J Mol. Med*. 2010; 25 : 121-8.

Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes C J, Valko M. Arsenic toxicity, oxidative stress and human disease. *J. Appl.Toxicol*. 2011; 31 : 95-107.

Lingzhi L, Qiu P, Chen B., Lu Y, Thakur C, Chang Q , Sun J, Chen F. Reactive oxygen species contribute to arsenic induced EZH2 phosphorylation in human bronchial epithelial cells and lung cancer cells. *Toxicol .Appl. Pharmacol.* 2014; 276 : 165-170.

Miller N J, Rice-Evans C, Davies M J, Gopinathan V, and Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 1993; 84 : 407-412.

Nemati B, Gregus Z. Gtutathione-Dependent Reduction of Arsenate in Human Erythrocytes- a Process Independent of Purine Nucleoside Phosphorylase. Toxicol. Sci. 2004; 82 : 419-428.

Pagilia D E, Valentine W N. Studies on the Quantitative and Qualitative Characterization of erythrocytes glutathione peroxidase. *J. Lab. Clin. Med.* 1967; 70 :158-169.

Rodriguez V M, Del Razo L M, Limon-Pacheco J H, Giordano M, Sanchez-Pena LC, Uribe –Quirol E, Gutierrez-Ospina G, Gonsebatt M E. Glutathione Reductase Inhibition and Methylated Arsenic Distribution in Cd1 Mice Brain and Liver. Toxicol. Sci. 2005; 84 : 157-166.

Santra A, Maiti A, Das S, Lahiri S, Chakraborty S K, Mazumder D N. Hepatic damage caused by chronic

arsenic toxicity in experimental animals. *J. Toxicol. Clin.Toxicol*. 2000; 38: 395-405.

Singh M K, Yadav S S, Yadav R S, Singh U S, Shukla Y, Pant K K, Khattri S. Efficacy of crude extract of Emblica officinalis (amla) in arsenic‐induced oxidative damage and apoptosis in splenocytes of mice. Tox.Int. 2014; 21: 8-17.

Soto-Pena G A, Luna A L , Acosta- L. Saavedra and Conde P et. al.. Assessment of lymphocyte subpopulations and cytokine secretion in children exposed to arsenic. *FASEB Journal. FJ Express*. 2006; 20: 779-781.

Strazzullo P, Garia Puig J. Uric acid and oxidative stress: Relative impact on cardiovascular risk. Nutr. Metab. Cardiovas. 2007; 17 : 409-414.

Sudha K. Effect of arsenic induced toxicity in testis of male rats. *Ind. J. Fund. Appl. L. Sci*. 2012; 2: 126-130.

Wang Q, Ning X, Zhang Q, Liu F, Wu H , Zhang Y, Zhao J. Molecular characterization of two glutathione peroxidase genes in Mytilus galloprovincialis and their transcriptional responses to sub-chronic arsenate and cadmium exposure. *Invert Surv.J*. 2014:11: 149-162.

Warangkana C. Decreased glutathione peroxidase activities with concomitant increased oxidized glutathione levels among residents in an arsenic contaminated community of Southern Thailand. *Walailak J Sci & Tech*. 2008; 5 : 57-65.

Yamanaka K, Takabayashi F, Mizoi M, An Y, Hasegawa A, Okada S. Oral exposure of dimethylarsenic acid, a main metabolite of inorganic arsenic, in mice leads to an increase in 8-Oxo-2'-deoxyguanosine level, specifically in target organs for arsenic carcinogenesis. *Biochem Biophys Res Commun.* 2001; 287 :66-70.

Zhang Y, Yamamoto T, Hisatome I, Li Y, Cheng W, Sun N, Cai B, Huang T, Zhu Y, Li Z, Jing X,. Zhou R, Cheng J. Uric acid induces oxidative stress and growth inhibition by activating adenosine monophosphate-activated protein kinase and extracellular signal –regulated kinase signal pathways in pancreatic β cells. Mol. Cell. Endocrinol. 2013; 375: 89-96.

Zhang Z, Wang X, Cheng S. L, Sun, Y.O. Son et. al. Reactive oxygen species mediate arsenic induced cell transformation and tumourogenesis through Wnt / β – catenin pathway in human colorectal adenocarcinoma DLD1 Cells. *Toxicol. Appl. Pharmacol*. 2011; 256 : 114-121.

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