Study of the effect of inorganic and organic complexes of arsenic metal on the status of GSH in T. cells and B. cells of blood

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Abstract: Arsenic is a major threat to large part of the population due to its carcinogenic nature. The toxicity of Arsenic varies with its chemical form and oxidation states. Glutathione (GSH), a major intra-cellular tripeptide plays a major role in arsenic detoxification. The present study was designed to provide insight into the extent of changes in GSH level by inorganic arsenic in the form of Arsenic trioxide (ATO) and organic arsenic in the form of nitro benzene arsenic acid (NBA). Lymphocytes (T.cells and B.cells) were investigated for determination of change in GSH metabolic status caused by arsenic. The depletion of GSH level positively correlated with increasing arsenic concentration and time of incubation. The decline in GSH level was consistent with increasing pH and physiological temperature. Our findings show that changes in GSH status produced by Arsenic could be due to adduct (As-$(SG)_3$) formation. This change in GSH metabolic status provides information regarding mechanism of toxicity of inorganic and organic arsenicals. These findings are important for the rational design of antidote for the prevention of arsenic induced toxicity.

Keywords: Glutathione (GSH), Arsenic Tri Oxide (ATO), Nitro Benzene Arsonic Acid (NBA), T. Cells, B. Cells

INTRODUCTION

Since the dawn of civilization, metal pollutants have been a part of human history. However, toxic metal pollution of the biosphere has intensified rapidly since the onset of the industrial revolution, posing major environmental and health problems. Among all the metal pollutants, arsenic, the 33rd element discovered by Albertus Magnus in 1250 (Rosen, 1999) is of major concern. Chemically, arsenic is a metalloid, an intermediate element possessing properties of both metals and non-metals. Pure arsenic is a gray metal-like material which is usually found in the environment combined with other elements such as oxygen, chlorine, and sulfur. Arsenic combined with these elements is called inorganic arsenic.

Arsenic combined with carbon and hydrogen is called organic arsenic (Roy, 1997). The most common oxidation numbers of arsenic are +V, +III, and -III. Arsenic can form both inorganic and organic compounds in the environment and human body. Inorganic arsenic includes arsenite (As^{III}) and arsenate (As^{V}). Arsenic is a well documented carcinogen in a number of studies (Waalkes *et al*., 2004) Humans are exposed to many different forms of inorganic and organic arsenic species (arsenicals) in food, water and other environmental media. Each of the forms of arsenic has different physicochemical properties and bioavailability and therefore the study of the kinetics and metabolism of arsenicals in animals and humans is a complex matter. Large interspecies differences compared with other metals and metalloids also characterize arsenic metabolism. The chemistry of arsenic is rather complex, and the compounds it forms are numerous. This is largely because arsenic possesses several different valence or oxidation states, which result in the markedly different biologic behavior of its compounds (ATSDR, 2006). As_2O_3 has been used for the treatment of acute promyelocytic leukaemia (Shen *et al.,* 1997; Marasca *et al.*, 1999). Historically, various compounds of arsenic have been used in medicine to treat various disorders of the skin and respiratory system. Interestingly, arsenic trioxide has been re-introduced as a potential drug to treat acute promyelocytic leukemia (ATSDR, 2007).

Both inorganic and organic forms of arsenic may cause adverse effects in laboratory animals. The effects induced by arsenic range from acute lethality to chronic effects such as cancer. The degree of toxicity of arsenic is basically dependent on the form (e.g. inorganic or organic) and the oxidation state of the arsenical. It is generally considered that inorganic arsenicals are more toxic than organic arsenicals, and within these two classes, the trivalent forms are more toxic than the pentavalent forms, at least at high doses (Gomez *et al.,* 2001). Trivalent arsenic reacts readily *in vitro* with thiol-containing molecules such as GSH and cysteine (Scott *et al*., 1993; Delnomdedieu *et al.,* 1994b). Among inorganic arsenicals, arsenic trioxide (As_2O_3) is most common in air (ATSDR, 2007), the binding of trivalent arsenic to critical thiol groups may inhibit important biochemical events that could lead to toxicity (Hughes, 2002). Binding of As^{III} to GSH has been demonstrated by several

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investigators (Anundi *et al.*, 1982; Delnomdedieu *et al.,* 1994a; Khan H *et al.,* 2010, 2011a and 2011b; Muktiar *et al.,* 2013; Shah *et al.,* 2013 & 2013a; Khan J *et al.,* 2012) Arsenite (As^{III}) reacts and binds to sulfhydryl groups while arsenate (As^{V}) has chemical properties similar to those of phosphate. As^{V} also has affinity for sulfhydryl groups; however, its affinity is approximately 10-fold less than As^{III} (Jacobson-Kram Montalbano, 1985).

The tripeptide Glutathione is naturally occurring low molecular weight nonprotein thiol, which is endogenously synthesized in the body and every cell is responsible for its own supply of glutathione. It possesses very important biochemical and antioxidant properties due to the presence of sulfhydryl (SH) group. In the body it exist both in oxidized (GSSG) and reduced (GSH) forms. In normal situation, 90% of GSH occurs in reduced form while only 10% of glutathione pool occurs in oxidized or disulfide form. The ratio of oxidized to reduced Glutathione (GSSG/GSH) within the body cells is scientifically used as indicator of cellular toxicity (.Pastore *et al,* 2003).

Exposure to arsenite, arsenic trioxide or organo-arsenic compounds has been reported to result in the generation of reactive oxygen species in laboratory animals or in cultured animal and human cells by many investigators (Wang *et al.,* 1996; Chen *et al.,* 1998; Hei *et al.,* 1998; Ercal *et al.,* 2001; Thomas *et al.,* 2001). One hour after exposure to arsenic, the GSH concentration was significantly depleted and lipid peroxidation was increased in both the high and low protein diet groups (Maiti & Chatterjee, 2001). Once arsenic is absorbed it is transported in the blood to organs throughout the body. It is generally bound to sulfhydryl groups of proteins and low-molecular weight compounds such as glutathione (GSH) and cysteine (NRC, 1999).

Materials

L. Glutathione (GSH) (Fluka), DTNB (Sigma), Sodium Hydroxide (Fluka AG), Sodium chloride (Merck), Potassium Dihydrogen Phosphate (Merck), HCl 35% (Kolchlight), Arsenic Trioxide (ATO) (Across, Belgium) Nitro Benzene Arsonic Acid (NBA) (Sigma), Distilled Water (Double Refined), Chloroform (Merck), Ethanol (Merck), Spectrophotometer: UV. Visible, 1601 (Schimadzu, Japan), PH Meter: Model NOV-210 (Nova Scientific Company Ltd. Korea), Analytical Balance AX 200 (Schimadzu, Japan) Centrifuge H-200 (Kokusan Ensink Company Japan), Eppendolf's tubes (Plastic, 101) Oven: Memmert Model U-30,854 (Schwa Bach, Germany), RPMI-1640, Fetal calf serum and Ficol paque plus (Sigma), Magnetic Stirrer, Nylon wool, Syringe, Pastuer pipette.

Preparation of stock solution

To make 0.9%-NaCl solution, 90 mg of pharmaceutical grade NaCl was added to D/W quantity sufficient (q.s.) 100 ml. Arsenic Trioxide (ATO), 2mM solution contained 19.8 mg of ATO in D/W q.s. 50 ml, which was again gradually diluted to get 10 different concentrations (0.2, 0.4, 0.6, 0.8, 1.00, 1.2, 1.4, 1.6, 1.8 and 2.00 mM) of ATO. Nitro Benzene Arsenic acid (NBA), 2mM solution contained 26.304 mg of NBA in D/W q.s. 50 ml, which was again gradually diluted to get 10 different concentrations (0.2, 0.4, 0.6, 0.8, 1.00, 1.2, 1.4, 1.6, 1.8 and 2.00 mM) of NBA. GSH (1mM) solution was obtained through dissolving 15.375 mg of GSH in 50 ml of phosphate buffer (pH 7.6). 19.8 mg of 5-5, Dithiobis 2-nitrobenzoic acid (DTNB) was added to phosphate buffer (pH 7.6) to get 50 ml of 1mM solution of DTNB. Balanced salt solution was prepared by mixing 4-parts of fetal calf serum with 45 parts of RPMI-1640. Ficol paque plus was used without further purification.

Isolation of T. cells and B. cells

B and T lymphocytes were isolated according to the supplier's instructions of Ficoll-Paque PLUS®.

Anticoagulant-treated blood (2ml) collected from a healthy human volunteer was diluted with an equal volume of balanced salt solution (RPMI-1640 plus 10% fetal calf serum) (Flow laboratories) was layered on the Ficoll-Paque PLUS® solution. This two-phase system was centrifuged at 400 × g for 30-40 minutes at 18–20 °C. Differential migration during centrifugation resulted in the formation of layers containing plasma and different cell types. The bottom layer contained erythrocytes which sedimented completely through the Ficoll-Paque PLUS. The layer immediately above the erythrocyte layer contains mostly granulocytes having density great enough to migrate through the Ficoll-Paque PLUS® layer. Because of their lower density, the lymphocytes accumulated at the interface between the plasma and the Ficoll-Paque PLUS with other slowly sedimenting particles (platelets and monocytes). The lymphocytes are then recovered from the interface and subjected to short washing steps with RPMI-1640 to remove any Ficoll-Paque PLUS® and plasma. Platelet contamination was finally effectively removed with the 20% sucrose gradient layered over Ficoll-Paque PLUS®. The platelets remained at the top of the sucrose gradient and the lymphocytes sedimented through the sucrose gradient to the top of the Ficoll-Paque PLUS® layer.

T. cells and B. cells Controls

T.cells control was prepared through mixing isolated T.cells fraction and 0.9%-NaCl solution in 1:1 ratio without treating with metal solution.

B.cells control was prepared through mixing isolated B.cells fraction and 0.9%-NaCl solution in 1:1 ratio without treating with metal solution.

Experimental design

Through centrifugation, plasma and cytosolic fraction were collected in separate sample tubes. The concentration dependent effect of ATO and NBA (0.2,

0.4, 0.6, 0.8, 1.00, 1.2, 1.4, 1.6, 1.8 and 2.00 mM) on GSH level was studied in T.cells and B.cells fractions each. The final concentrations of ATO and NBA (in each 10 samples of T.cells and 10 samples of B.cells fractions) were 100,200, 300, 400, 500, 600, 700, 800, 900 and 1000 µM. Data for effect of ATO and NBA on GSH level at different time fixations (00, 30, 60 and 90 minutes) was also collected. The effect of ATO and NBA on GSH level in T.cells and B.cells was also studied at different pH (6.5, 7.6 and 8.5). The effect of different temperatures (25°C, 37°C and 45°C) The time, pH and temprature studies were carried for change in GSH level in T.cells and B.cells by 10 different concentrations of ATO and NBA viz 100 to 1000 µM.

Experimental parameters

Assay of T. cells and B. cells fractions incubated with different ATO and NBA concentrations, at different time fixations, pH and temperature were made for estimation of effective toxicity marker compound, glutathione, in reduced form (GSH).

Determination of GSH concentration

The reduced glutathione level was determined by the method of Ellman (Ellman's, 1959).

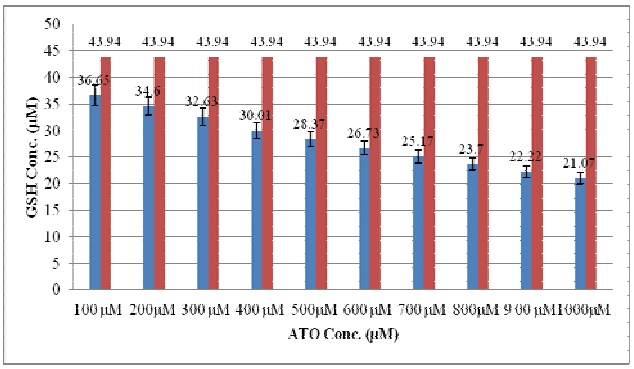
Samples of the isolated T.cells and B.cells fractions (having different ATO and NBA concentrations) were mixed with each of the10 different concentrations of ATO and NBA in equal volumes. 0.2 ml from each of these mixtures was added to 2.3 ml of phosphate buffer (pH 7.6). Then 0.5 ml of reagent (DTNB) was added. The absorbance was determined at 412 nm against respective T.cells and B.cells control, where ATO and NBA had not been added. The concentration of GSH was expressed as µM.

RESULTS

Change in isolated T. cells-GSH level by Arsenic Trioxide (ATO)

GSH level was determined in isolated T. cells, after the addition of different aqueous solutions of ATO, having the final concentrations of ATO (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000µM). The spectrophotometric analysis showed significant decrease in T. cells-GSH level (36.65 µM) with the initial ATO concentration i.e. 100µM compared to the T. cells-control (43.94 µM). The T. cells-GSH level was continuously decreasing in other samples which contained increasing ATO concentrations. The maximum used ATO concentration (1000 µM) brought drop in T. cells -GSH level up to (21.07 µM) compared to the T. cells-control (43.94 µM) as shown in table 1 and fig. 1.

Two aqueous solutions of ATO having final concentrations of ATO (100 and 1000µM) were incubated upto different time intervals (0-min, 30-min, 60-min and 90-min) with isolated T. cells. By extending the time of incubation, the T. cells-GSH level was further decreasing, the maximum decrease in T. cells-GSH level being with the maximum provided time as shown in table 1 and fig. 2.



Fig, 1: Effect of ATO Concentration on T. cells-GSH Content

■ T. cells Control (1ml 0.9% NaCl+1ml of T. cells)

■ATO (100-1000 µM)

Results are the mean ± SE of 3 experiments

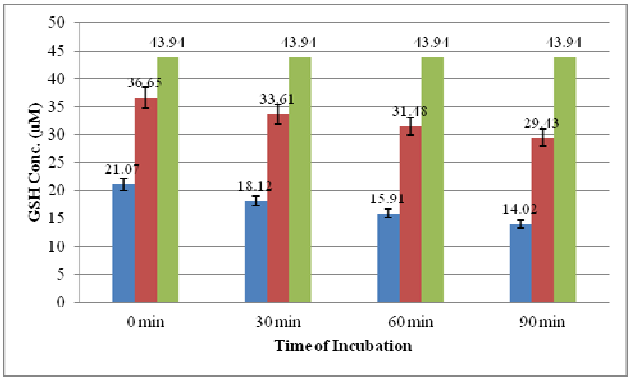


Fig. 2: Effect of ATO Concentration on the T. cells –GSH content with time incubation period (0-90 min)

■T. cells Control (1ml 0.9% NaCl+1ml of T. cells)

■ATO (100 µM)

■ ATO (1000 µM)

Results are the mean ± SE of 3 experiments

Change in isolated T. cells-GSH level by Nitro Benzene arsonic acid (NBA)

GSH level was determined in isolated T. cells, after the addition of different aqueous solutions of NBA, having the final concentrations of NBA (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µM). The spectrophotometric analysis showed significant decrease in T. cells-GSH level (38.86 µM) with the initial NBA concentration i.e. 100µM compared to the T. cells-control (35.75 µM). The T. cells-GSH level was continuously decreasing in other samples which contained increasing NBA concentrations. The maximum used NBA concentration (1000 µM) brought drop in T. cells-GSH level up to (13.04 µM) compared to the T. cells-control (35.75 µM) as shown in table 2 and fig. 3.

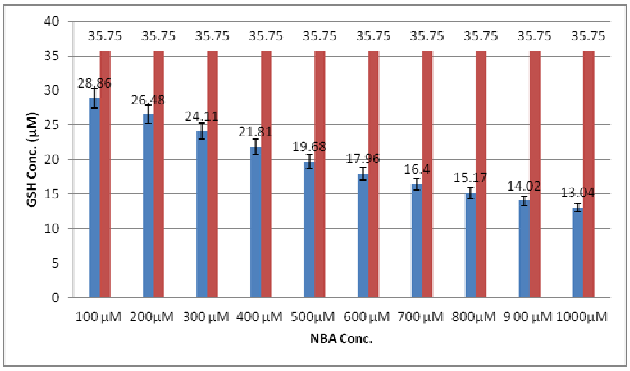


Fig. 3: Effect of NBA Concentration on T.cells-GSH Content

■ T. cells Control (1ml 0.9% NaCl+1ml of T.cells)
■ NBA (100-1000 µM)
Results are the mean ± SE of 3 experiments

Two aqueous solutions of NBA having final concentrations of NBA (100 and 1000µM) were incubated upto different time intervals (0-min, 30-min, 60-min and 90-min) with isolated T. cells. By extending the time of incubation, the T. cells-GSH level was further decreasing, the maximum decrease in T. cells-GSH level being with the maximum provided time as shown in table 2 and fig. 4.

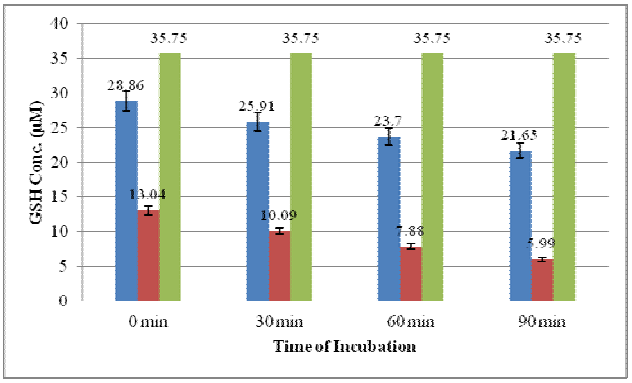


Fig. 4: Effect of NBA Concentration on the T. cells -GSH content with time incubation period (0-90 min)

■T. cells Control (1ml 0.9% NaCl+1ml of T. cells)
■ PAA (100 µM)
■ PAA (1000 µM)
Results are the mean ± SE of 3 experiments

Change in isolated B. cells-GSH level by Arsenic Trioxide (ATO)

GSH level was determined in isolated B. cells, after the addition of different aqueous solutions of ATO, having the final concentrations of ATO (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000µM). The spectrophotometric analysis showed significant decrease in B. cells-GSH level (39.27 µM) with the initial ATO concentration i.e. 100µM compared to the B. cells-control (48.37 µM). The B. cells-GSH level was continuously decreasing in other samples which contained increasing ATO concentrations. The maximum used ATO concentration (1000 µM) brought drop in B. cells-GSH level up to (19.68 µM) compared to the B. cells-control (48.37 µM) as shown in table 3 and fig. 5.

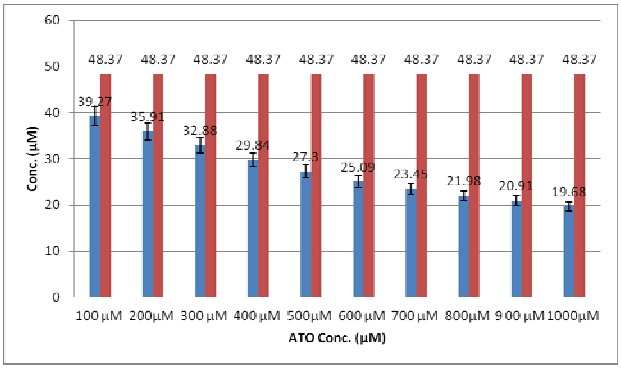


Fig. 5: Effect of ATO Concentration on B. cells-GSH Content

■ B. cells Control (1ml 0.9% NaCl + 1ml of B. cells)
■ ATO (100-1000 µM)
Results are the mean ± SE of 3 experiments

Two aqueous solutions of ATO having final concentrations of ATO (100 and 1000µM) were incubated upto different time intervals (0-min, 30-min, 60-min and 90-min) with isolated B. cells. By extending the time of incubation, the B. cells-GSH level was further decreasing, the maximum decrease in B. cells-GSH level being with the maximum provided time as shown in table 3 and fig. 6.

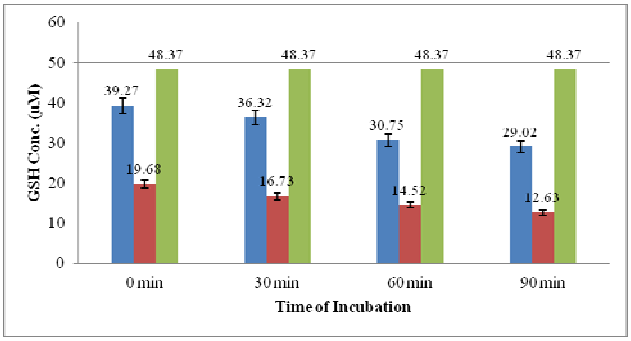


Fig. 6: Effect of ATO Concentration on the B. cells -GSH content with time incubation period (0-90 min)

■B. cells Control (1ml 0.9% NaCl+1ml of B. cells)
■ATO (100 µM)
■ATO (1000 µM)
Results are the mean ± SE of 3 experiments

Change in isolated B. cells-GSH level by Nitro Benzene arsonic acid (NBA)

GSH level was determined in isolated B. cells, after the addition of different aqueous solutions of NBA, having the final concentrations of NBA (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000µM). The spectrophotometric analysis showed significant decrease in B. cells-GSH level (39.84 µM) with the initial NBA concentration i.e. 100µM compared to the B. cells-control (47.22 µM). The B. cells-GSH level was continuously decreasing in other

samples which contained increasing NBA concentrations. The maximum used NBA concentration (1000 µM) brought drop in B. cells-GSH level up to (17.63 µM) compared to the B. cells-control (47.22 µM) as shown in table 4 and fig. 7.

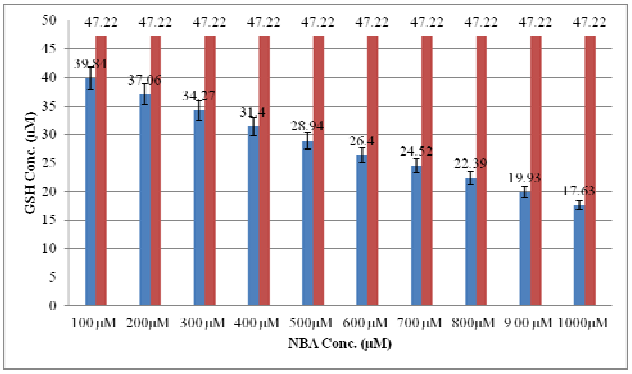


Fig. 7: Effect of NBA Concentration on B. cells-GSH Content

■ B. cells Control (1ml 0.9% NaCl + 1ml of B. cells)
■ NBA (100-1000 µM)
Results are the mean ± SE of 3 experiments

Two aqueous solutions of NBA having final concentrations of NBA (100 and 1000µM) were incubated upto different time intervals (0-min, 30-min, 60-min and 90-min) with isolated B. cells. By extending the time of incubation, the B. cells-GSH level was further decreasing, the maximum decrease in B. cells-GSH level being with the maximum provided time as shown in table 4 and fig. 8.

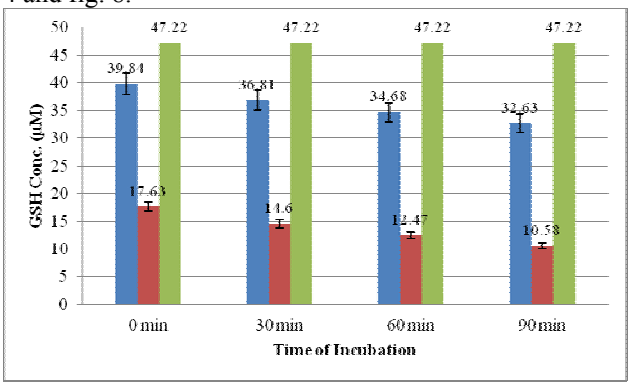


Fig. 8: Effect of NBA Concentration on the B. cells – GSH content with time incubation period (0-90 min)

■ B. cells Control (1ml 0.9% NaCl + 1ml of B. cells)
■ NBA (100 µM)
■ NBA (1000 µM)
Results are the mean ± SE of 3 experiments

DISCUSSION

We have studied the effect of inorganic and organic forms [Arsenic trioxide (ATO) and Nitro Benzene arsonic acid (NBA)] of Arsenic (As^{+III}) on GSH level in T. cells and B. cells before and after isolation of whole blood spectrophotometrically. The concentrations of ATO and NBA used in the present study ranged from 100uM - 1000uM. The effect of As^{III} on the chemical and metabolic status of GSH content of T. cells and B. cells was studied in terms of determination of absorbance of GSH at λ max 412nm. This λ max (412nm) is being used for the determination of GSH concentration in samples according to a well known Elman's method (Elman's, 1959).

Thus the interaction of As^{+III} with reduced glutathione content of T. cells and B. cells *in vitro* as a model of *in vivo reaction* will establish further scientific data and will strengthen our knowledge about the toxicological profile of As^{+III} and Pb^{+III} as well as the role of GSH in the protection of our body from their harmful effects. According to our findings the As^{+III} in the form of ATO and NBA induced the depletion of GSH content of T. cells and B. cells in a dose dependent manner. The results also show positive correlation between the exposure of glutathione content of T. cells and B. cells to the above given concentrations of ATO and NBA and the depletion of GSH content of T. cells and B. cells as the time passed from 0 to 90 minutes.

The fact that inorganic and organic compounds of As^{+III} have considerable toxicological and pharmacological effects prompted us to examine their interactions with glutathione (GSH) of T. cells and B. cells, the most important intracellular antioxidant in T. cells and B. cells. Furthermore little information is available on the mechanism of action at the molecular level. Our experimental work proposes the molecular mechanism of action of ATO and NBA. Our research hypothesis was that ATO and NBA cause modulation in the status of GSH of T. cells and B. cells either to glutathione disulfide (GSSG) or formation of Arsenic-glutathione (As-SG) complex.

The exact mechanism of action of these compounds on GSH metabolic status in this study is not known. However the proposed hypothetical mechanism of action of ATO and NBA on GSH metabolic status is the formation of As-$(SG)_3$ complex.

The proposed reactions involve the coordination of As^{+III} in ATO and NBA with the S-H group of GSH with the formation of As-$(SG)_3$ complex as under:

$$As_2O_3 \longrightarrow 2As^{+3} + 3O^{-2}$$

$$2As^{+3} + 6 \longrightarrow \text{GSH } 2\text{As-(SG)}$$

CONCLUSION

Our findings show that changes in GSH status produced by Arsenic could be due to adduct (As-$(SG)_3$) formation. This change in GSH metabolic status provides information regarding mechanism of toxicity of inorganic and organic arsenicals. These findings are important for the rational design of antidote for the prevention of arsenic induced toxicity.

Table 1: Effect of Arsenic Trioxide (ATO) on GSH-level in Isolated T.cells

	Time	100 µM			200 µM	300 µM	400 µM	500 µM	600 µM	700 µM	800 µM	900 µM	1000 µM	Control
1	Avg Conc. at 0 Min.	Conc		36.65	34.60	32.63	30.01	28.37	26.73	25.17	23.70	22.22	21.07	43.94
		PH	6.5	38.04	35.58	33.78	30.91	29.60	27.88	26.57	24.76	23.53	22.06	43.94
			7.6	33.61	31.65	29.68	27.06	25.34	23.78	22.14	20.66	19.19	18.12	43.94
			8.5	31.48	29.43	27.47	24.84	23.20	21.57	20.01	18.53	17.06	15.91	43.94
		Temp	25C	36.65	34.60	32.63	30.01	28.37	26.73	25.17	23.70	22.22	21.07	43.94
			37C	35.42	33.12	31.40	28.45	27.39	25.34	24.43	22.39	21.16	19.60	43.94
			45C	37.71	36.07	33.86	31.48	29.84	27.55	26.73	24.68	23.53	22.30	43.94
2	Avg Conc. at 30 Min.	33.61			31.65	29.68	27.06	25.34	23.78	22.14	20.66	19.19	18.12	43.94
3	Avg Conc. at 60 Min.	31.48			29.43	27.47	24.84	23.20	21.57	20.01	18.53	17.06	15.91	43.94
4	Avg Conc. at 90 Min.	29.43			27.71	25.50	23.04	21.32	19.52	18.29	16.57	15.25	14.02	43.94

Table 2: Effect of Nitro Benzene arsonic acid (NBA) on GSH-level in Isolated T.cells

	Time	100 µM			200 µM	300 µM	400 µM	600 µM	500 µM	700 µM	800 µM	900 µM	1000 µM	Control
1	Avg Conc. at 0 Min.	Conc		28.86	26.48	24.11	21.81	19.68	17.96	16.40	15.17	14.02	13.04	35.75
		PH	6.5	30.25	27.47	25.25	22.71	20.91	19.11	17.80	16.24	15.34	14.02	35.75
			7.6	25.91	23.45	21.07	18.78	16.65	14.93	13.45	12.14	10.99	10.09	35.75
			8.5	23.70	21.32	18.94	16.65	14.52	12.80	11.24	10.01	8.86	7.88	35.75
		Temp	25C	28.86	26.48	24.11	21.81	19.68	17.96	16.40	15.17	14.02	13.04	35.75
			37C	27.47	25.75	22.80	20.75	18.20	16.73	14.84	14.19	12.63	12.30	35.75
			45C	29.93	27.96	25.34	23.29	21.16	18.78	17.96	16.16	15.34	14.27	35.75
2	Avg Conc. at 30 Min.	25.91			23.45	21.07	18.78	16.65	14.93	13.45	12.14	10.99	10.09	35.75
3	Avg Conc. at 60 Min.	23.70			21.32	18.94	16.65	14.52	12.80	11.24	10.01	8.86	7.88	35.75
4	Avg Conc. at 90 Min.	21.65			19.60	16.98	14.84	12.63	10.75	9.52	8.04	7.06	5.99	35.75

Table 3: Effect of Arsenic Trioxide (ATO) on GSH-level in Isolated B. cells

	Time	100 µM			200 µM	300 µM	400 µM	500 µM	600 µM	700 µM	800 µM	900 µM	1000 µM	Control
1	Avg Conc. at 0 Min.	Conc		39.27	35.91	32.88	29.84	27.30	25.09	23.45	21.98	20.91	19.68	48.37
		PH	6.5	40.66	36.89	34.02	30.75	28.53	26.24	24.84	23.04	22.22	20.66	48.37
			7.6	36.32	32.88	29.84	26.89	24.35	22.06	20.50	19.02	17.88	16.73	48.37
			8.5	66.64	59.84	53.77	47.79	42.70	38.20	35.00	32.05	29.84	27.46	92.95
		Temp	25C	69.59	62.87	56.80	50.74	45.66	41.23	37.95	35.00	32.87	30.41	92.95
			37C	38.53	34.60	31.81	28.37	26.07	23.61	22.71	21.24	19.60	18.61	48.37
			45C	40.34	37.39	34.11	31.32	28.78	25.91	25.01	22.96	22.22	20.91	48.37
2	Avg Conc. at 30 Min.	36.32			32.88	29.84	26.89	24.35	22.06	20.50	19.02	17.88	16.73	48.37
3	Avg Conc. at 60 Min.	34.11			30.75	27.71	24.68	22.14	19.93	18.29	16.81	15.75	14.52	48.37
4	Avg Conc. at 90 Min.	32.06			29.02	25.75	22.88	20.25	17.88	16.57	14.84	13.94	12.63	48.37

Table 4: Effect of Nitro Benzene arsonic acid (NBA) on GSH-level in Isolated B. cells

	Time	100 µM			200 µM	300 µM	400 µM	500 µM	600 µM	700 µM	800 µM	900 µM	1000 µM	Control
1	Avg Conc. at 0 Min.	Conc		39.84	37.06	34.27	31.40	28.94	26.40	24.52	22.39	19.93	17.63	47.22
		PH	6.5	41.24	38.04	35.42	32.30	30.17	27.55	25.91	23.45	21.24	18.61	47.22
			7.6	36.81	34.11	31.24	28.37	25.99	23.45	21.48	19.43	16.89	14.60	47.22
			8.5	34.68	31.89	29.11	26.24	23.78	21.24	19.35	17.22	14.76	12.47	47.22
		Temp	25C	39.84	37.06	34.27	31.40	28.94	26.40	24.52	22.39	19.93	17.63	47.22
			37C	38.61	35.58	33.04	29.84	27.96	25.01	23.78	21.07	18.86	16.16	47.22
			45C	40.91	38.53	35.50	32.88	30.42	27.22	26.07	23.37	21.24	18.86	47.22
2	Avg Conc. at 30 Min.	36.81			34.11	31.24	28.37	25.99	23.45	21.48	19.43	16.89	14.60	47.22
3	Avg Conc. at 60 Min.	34.68			31.89	29.11	26.24	23.78	21.24	19.35	17.22	14.76	12.47	47.22
4	Avg Conc. at 90 Min.	32.63			30.17	27.14	24.43	21.89	19.19	17.63	15.25	12.96	10.58	47.22

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