academicJournals

Vol. 7(19), pp. 1194-1204, 22 May, 2013
DOI 10.5897/AJPP12.545
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Metabolic changes of glutathione in human T and B lymphocytes induced by organo-aluminum complex

Haroon Khan[1]*, Muhammad Farid Khan[1], Barkat Ali Khan[2], Naseem Ullah[1], Muhammad Mukhtiar[1] and Arshad Farid[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University D.I. Khan Pakistan.
[2]Department 0f Pharmaceutics, Faculty of Pharmacy, Gomal University D.I. Khan Pakistan.
[3]Gomal Center of Biochemistry and Biotechnology, Gomal university D.I. khan Pakistan

Accepted 15 April, 2013

Even though aluminium is not considered to be a heavy metal like lead, silver, arsenic and cadmium, it can be toxic when taken in excessive amounts and even in small amounts if deposited in the brain. Glutathione, a major antioxidant in the cells, so its depletion weakens the tissue resistance to oxidant. Glutathione is the sulfhydryl (-SH) antioxidant, antitoxin and enzyme cofactor which plays an important role in aluminum detoxification. The present study was designed to investigate the extent of changes in glutathione level by inorganic and organic alumni metal. Biocordination of aluminum acetylacetonate and aluminum sulphate with glutathione in T-cells and b-cells of lymphocytes have been described using Ellman's method. The decline of glutathione level is due to increased aluminum concentration and time of incubation. The decline of glutathione level was consistent with increasing pH, while at physiological temperature, the drop was more significant. Our study indicates that changes in glutathione level produced by aluminium could be due to conjugate (Al-$(SG)_3$) formation. This change in glutathione level endowed with information regarding mechanism of toxicity of aluminium inorganic and organic complexes. This study is important for the design of rational antidote for the prevention of aluminium toxicity.

Key words: Glutathione (GSH), aluminium sulphate $Al_2(SO_4)_3$, aluminium acetylacetonate ($Al(acac)_3$) , T-cells, B-cells.

INTRODUCTION

Motivations for controlling heavy metal concentrations in gas streams are diverse. Some of them are dangerous to health or to the environment (for example, mercury, cadmium, lead, chromium), some may cause corrosion (for example, zinc, lead), some are harmful in other ways (for example, arsenic may pollute catalysts) (Michael, 2011). Some of these elements are actually necessary for humans in minute amounts (cobalt, copper, chromium, manganese, nickel) while others are carcinogenic or toxic, affecting among others, the central nervous system (manganese, mercury, lead, arsenic), the kidneys or liver (mercury, lead, cadmium, copper) or skin, bones, or teeth (nickel, cadmium, copper, chromium) and medical usage, heavy metals are loosely defined (Ron and Kilpinen, 2001) and include all toxic metals irrespective of their atomic weight: "heavy metal poisoning"

*Corresponding author. E-mail: haroonscholar@gmail.com. Tel: 0092-322-9830144

can possibly include excessive amounts of iron, manganese, aluminium, mercury, cadmium. All metals can cause disease through excess exposure. In addition, essential metals can affect the human body in the case of deficiency or imbalance. Until now, no biological function has been attributed to aluminium metal, and more importantly, aluminum accumulation in tissues and organs, results in their dysfunction and toxicity (Proudfoot, 2009; Verstraeten et al., 2008). No known physiologic need exists for aluminum; however, because of its atomic size and electric charge (0.051 nm and 3^+, respectively), it is sometimes a competitive inhibitor of several essential elements of similar characteristics, such as magnesium (0.066 nm, 2^+), calcium (0.099 nm, 2^+), and iron (0.064 nm, 3^+).

At physiological pH, aluminum forms a barely soluble compound $Al(OH)_3$ that can be easily dissolved by minor changes in the acidity of the media (Verstraeten et al., 2008). Aluminum causes an oxidative stress within brain tissue (Drago et al., 2008) since the elimination half-life of aluminum from the human brain is 7 years. This can result in cumulative damage via the element's interference with neurofilament axonal transport and neurofilament assembly. Some experts believe it plays a role in leading to the formation of Alzheimer-like neurofibrillary tangles.

Blaylock and Strunecka (2009) suggest that the heterogeneous symptoms of autism spectrum disorders have a connection with dysregulation of glutamatergic neurotransmission in the brain, along with enhancement of excitatory receptor function by proinflammatory immune cytokines as the underlying pathophysiological process. Animal studies in rats and case reports have implicated the use of oral aluminum-containing antacids during pregnancy as a possible cause for abnormal fetal neurologic development (Exley et al., 2009; Shuchang et al., 2008).

Glutathione (GSH) is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Shuchang et al., 2003; Pastore et al., 2003). These free radicals are produce due to chronic exposer of aluminum metal (Priya and Bimla, 2006).

Many studies have been carried out in order to study the molecular mechanism responsible for the toxic effects of this metal ion on both humans and animals (Xiao et al., 2008). The results indicate that aluminum has been found inside cells in many illnesses (Vina et al., 2004; Xiao et al., 2008). Therefore, studying the binding of metal ion and its complexes by peptides and proteins is important for understanding many biological systems. It has been proposed that, in some cases, aluminum binds to certain natural carriers (Haroon et al., 2011). The interaction of aluminum with amino acids, peptides and proteins is a subject of current interest (Yves, 2000).

MATERIALS AND METHODS

L-Glutathione (GSH) was purchase from fluka chemical Co. All glutathione were prepared freshly daily with double distilled water, and all samples were flushed with high purity nitrogen or purified argon. Aluminium sulphate and aluminum acetyl acetonate were prepared by dissolving highly purified metallic aluminum powder of specific quantity in distilled water. More dilution were prepared by diluting these solutions with double distilled water. Roswell Park Memorial Institute (RPMI)-1640, fetal calf serum and ficol paque plus (Sigma) Ellman's reagent (DTNB) was obtained from sigma chemical Co. Other chemical reagents were of analytical grade. All measurements were taken on Schimadzu ultra violet (UV)/visible spectrophotometer. All glass wares were soaked in 10% HNO_3 for at least 24 h and then washed with double distilled water.

Preparation of stock solution

To make 0.9%-NaCl solution, 90 mg of pharmaceutical grade NaCl was added to distilled water quantity sufficient (q.s.) 100 ml. A 100 ml of 1 mM aluminum sulphate (molar weight (MW) = 342) solution was prepared by dissolving 34.2 mg of aluminium sulphate in 100 ml of distilled water, which was again gradually diluted to get 10 different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.00 mM) of aluminium sulphate. Aluminium acetyl acetonate [Al(acac)3], 1 mM solution contained 32.4 mg of [$Al(acac)_3$] in D/W q.s. 100 ml, which was again gradually diluted to get 10 different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.00 mM) of [$Al(acac)_3$]. GSH (1 mM) solution was obtained through dissolving 15.375 mg of GSH in 50 ml of phosphate buffer (pH 7.6). A 19.8 mg of 5-5,Dithiobis 2-nitrobenzoic acid (DTNB) was added to phosphate buffer (pH 7.6) to get 50 ml of 1 mM 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 (2 ml) 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) which was layered on the Ficoll-paque plus solution. This two-phase system was centrifuged at 400 × g for 30 to 40 min at 18 to 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 aluminium sulphate and [Al(acac)$_3$] (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.00 mM) on GSH level was studied in T-cells and B-cells fractions each. The final concentrations of aluminium sulphate and [Al(acac)$_3$] (in each 10 samples of T-cells and 10 samples of B-cells fractions) were 50,100, 150, 200, 250, 300, 350, 400, 450 and 500 µM. Data for effect of aluminium sulphate and [Al(acac)$_3$] on GSH level at different time fixations (00, 30, 60 and 90 min) was also collected. The effect of aluminium sulphate and [Al(acac)$_3$] 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, 37 and 45°C), the time, and pH studies were carried out for change in GSH level in T-cells and B-cells by 10 different concentrations of aluminium sulphate and [Al(acac)$_3$] from 50 to 500 µM.

Experimental parameters

Assay of T-cells and B-cells fractions incubated with different aluminium sulphate and [Al(acac)$_3$] concentrations, at different time fixations, pH and temperature were made for estimation of effective toxicity marker compound, and glutathione in reduced form of GSH.

Determination of GSH concentration

The reduced glutathione level was determined by the method of (Ellman, 1959). Samples of the isolated T-cells and B-cells fractions (having different aluminium sulphate and [Al(acac)$_3$] concentrations) were mixed with each of the10 different concentrations of aluminium sulphate and [Al(acac)$_3$] 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 T-cells and B-cells control, respectively, where aluminium sulphate and [Al(acac)$_3$] had not been added. The concentration of GSH was expressed as µM.

Effect of aluminum sulphate on glutathione level adding into T-cell after separation

GSH level was determined in isolated T-cells after the addition of different aqueous solutions of aluminium sulphate, having the final concentrations of aluminium sulphate (50,100, 150, 200, 250, 300, 350, 400, 450 and 500 µM). The spectrophotometric analysis showed significant decrease in T-cells-GSH level (36.65 µM), with the initial aluminium sulphate concentration that is, 50 µM compared to the T-cells-control (0.445). The T-cells-GSH level was continuously decreasing in other samples which contained increasing aluminium sulphate concentrations. The maximum used aluminium sulphate concentration (500 µM) brought a drop in T-cells-GSH level up to (0.223), compared to the T-cells-control (0.445) as shown in Table 1 and Figure 1. Two aqueous solutions of 0.445 having final concentrations of 0.445 (50 and 500 µM) were incubated up to different time intervals (0, 30, 60 and 90 min) with isolated T-cells. By extending the time of incubation, the T-cells-GSH level was further decreased, with the maximum decrease in T-cells-GSH level being with the maximum provided time as shown in Table 1 and Figure 2.

Effect of aluminium acetylacetonate on glutathione level adding into T-cell after separation

GSH level was determined in isolated T-cells after the addition of different aqueous solutions of [Al(acac)$_3$], having the final concentrations of aluminium acetylacetonate (50, 100, 150, 200, 250, 300, 350, 400, 450 and 50 µM). The spectrophotometric analysis showed significant decrease in T-cells-GSH level (38.86 µM) with the initial [Al(acac)$_3$] concentration that is, 50 µM compared to the T-cells-control (0.458). The T-cells-GSH level was continuously decreasing in other samples which contained increasing [Al(acac)$_3$] concentrations. The maximum used [Al(acac)$_3$] concentration (500 µM) brought a drop in T-cells-GSH level up to (0.173) compared to the T-cells-control (0.458) as shown in Table 2 and Figure 3. Two aqueous solutions of [Al(acac)$_3$] having final concentrations of [Al(acac)$_3$] (50 and 500 µM) were incubated up to different time intervals (0, 30, 60 and 90 min) with isolated T-cells. By extending the time of incubation, the T-cells-GSH level was further decreasing, with the maximum decrease in T-cells-GSH level being with the maximum provided time as shown in Table 2 and Figure 4.

Effect of aluminum sulphate on glutathione level adding into B-cell after separation

GSH level was determined in isolated B-cells after the addition of different aqueous solutions of aluminium sulphate, having the final concentrations of aluminium sulphate (50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µM). The spectrophotometric analysis showed significant decrease in B-cells-GSH level (39.27 µM) with the initial aluminium sulphate concentration that is, 50 µM compared to the B-cells-control (0.391). The B-cells-GSH level was continuously decreasing in other samples which contained increasing aluminium sulphate concentrations.

Table 1. Effect of aluminum sulphate on glutathione level adding into T-cell after separation.

No.	Time		50 µM		100 µM	150 µM	200 µM	250 µM	300 µM	350 µM	400 µM	450 µM	500 µM	Control
			0.345		0.329	0.319	0.299	0.285	0.273	0.259	0.240	0.233	0.223	0.445
			6.5	0.340	0.323	0.311	0.293	0.285	0.270	0.261	0.243	0.234	0.224	0.445
		pH	7.6	0.264	0.251	0.237	0.222	0.210	0.196	0.184	0.170	0.158	0.152	0.445
1	Average ABS at 0 min		8.5	0.288	0.275	0.262	0.246	0.235	0.221	0.208	0.194	0.182	0.176	0.445
			25	0.345	0.329	0.319	0.299	0.285	0.273	0.259	0.240	0.233	0.223	0.445
		Temperature (°C)	37	0.323	0.311	0.297	0.282	0.270	0.256	0.244	0.230	0.218	0.212	0.445
			45	0.336	0.321	0.313	0.294	0.284	0.273	0.257	0.239	0.231	0.222	0.445
2	Average ABS at 30 min		0.287		0.274	0.261	0.245	0.233	0.220	0.207	0.193	0.181	0.175	0.445
3	Average ABS At 60 min		0.260		0.248	0.234	0.219	0.207	0.193	0.181	0.167	0.155	0.149	0.445
4	Average ABS at 90 min		0.240		0.227	0.214	0.199	0.187	0.173	0.160	0.147	0.135	0.128	0.445

Table 2. Effect of aluminium acetylacetonate on glutathione level adding into T-cell after separation.

No.	Time		100 µM		200 µM	300 µM	400 µM	500 µM	600 µM	700 µM	800 µM	900 µM	1000 µM	Control
			0.360		0.333	0.316	0.293	0.273	0.257	0.236	0.213	0.199	0.173	0.458
			6.5	0.355	0.327	0.308	0.287	0.273	0.254	0.237	0.216	0.168	0.153	0.458
		pH	7.6	0.278	0.255	0.234	0.216	0.198	0.180	0.160	0.143	0.123	0.101	0.458
1	Average ABS at 0 min		8.5	0.303	0.280	0.259	0.241	0.223	0.204	0.185	0.168	0.148	0.126	0.458
			25	0.360	0.333	0.316	0.293	0.273	0.257	0.236	0.213	0.199	0.173	0.458
		Temperature (°C)	37	0.338	0.315	0.294	0.276	0.258	0.240	0.220	0.203	0.183	0.161	0.458
			45	0.351	0.325	0.309	0.288	0.272	0.256	0.234	0.212	0.196	0.171	0.458
2	Average ABS at 30 min		0.355		0.327	0.308	0.287	0.273	0.254	0.237	0.216	0.199	0.155	0.458
3	Average ABS at 60 min		0.275		0.252	0.231	0.213	0.195	0.177	0.157	0.140	0.120	0.098	0.458
4	Average ABS at 90 min		0.255		0.232	0.211	0.193	0.175	0.157	0.137	0.120	0.100	0.078	0.458

concentrations. The maximum used aluminium sulphate concentration (500 µM) brought a drop in B-cells-GSH level up to (0.183) compared to the B-cells-control (0.391) as shown in Table 3 and Figure 5. Two aqueous solutions of aluminium sulphate having final concentrations of aluminium sulphate (50 and 500 µM) were incubated up to different time intervals (0, 30, 60 and 90 min) with isolated B-cells. By extending the time of incubation, the B-cells-GSH level was further decreased with the maximum decrease in B-cells-GSH level being with the maximum provided time as shown in Table 3 and Figure 6.

Effect of aluminum acetylacetonate on glutathione level adding into B-cell after separation

GSH level was determined in isolated B-cells after the addition of different aqueous solutions of $[Al(acac)_3]$, having the final concentrations of $[Al(acac)_3]$ (50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µM). The spectrophotometric analysis showed significant decrease in B-cells-GSH level (39.84 µM) with the initial $[Al(acac)_3]$ concentration that is, 50 µM compared to the B-cells-control (0.398). The B-cells-GSH level was continuously decreasing in other samples which contained increasing $[Al(acac)_3]$ concentrations.

Table 3. Effect of aluminum sulphate on glutathione level adding into B-cell after separation.

No.	Time		100 µM		200 µM	300 µM	400 µM	500 µM	600 µM	700 µM	800 µM	900 µM	1000 µM	Control
			0.269		0.256	0.252	0.237	0.226	0.220	0.209	0.194	0.191	0.183	0.391
			6.5	0.265	0.250	0.244	0.231	0.226	0.217	0.210	0.197	0.191	0.184	0.391
		pH	7.6	0.188	0.178	0.170	0.160	0.151	0.143	0.134	0.124	0.115	0.112	0.391
1	Average ABS at 0 min		8.5	0.213	0.203	0.195	0.185	0.176	0.168	0.158	0.149	0.140	0.136	0.391
			25	0.269	0.256	0.252	0.237	0.226	0.220	0.209	0.194	0.191	0.183	0.391
		Temperature (°C)	37	0.248	0.238	0.230	0.220	0.211	0.203	0.193	0.184	0.175	0.172	0.391
			45	0.260	0.248	0.245	0.232	0.225	0.219	0.207	0.193	0.188	0.182	0.391
2	Average ABS at 30 min		0.267		0.256	0.244	0.232	0.223	0.212	0.201	0.189	0.176	0.164	0.391
3	Average ABS at 60 min		0.240		0.229	0.218	0.206	0.197	0.185	0.175	0.162	0.150	0.138	0.391
4	Average ABS at 90 min		0.220		0.209	0.198	0.186	0.177	0.165	0.155	0.142	0.130	0.118	0.391

Table 4. Effect of aluminum acetylacetonate on glutathione level adding into B-cell after separation.

No.	Time		100 µM		200 µM	300 µM	400 µM	500 µM	600 µM	700 µM	800 µM	900 µM	1000 µM	Control
			0.314		0.291	0.276	0.250	0.229	0.215	0.197	0.175	0.167	0.155	0.398
			6.5	0.310	0.285	0.268	0.244	0.229	0.212	0.199	0.179	0.137	0.145	0.398
		pH	7.6	0.233	0.213	0.194	0.173	0.154	0.138	0.122	0.106	0.091	0.083	0.398
1	Average ABS at 0 min		8.5	0.258	0.238	0.219	0.197	0.178	0.163	0.147	0.130	0.116	0.108	0.398
			25	0.314	0.291	0.276	0.250	0.229	0.215	0.197	0.175	0.167	0.155	0.398
		Temperature (°C)	37	0.293	0.273	0.254	0.233	0.214	0.198	0.182	0.166	0.151	0.143	0.398
			45	0.306	0.284	0.269	0.245	0.228	0.215	0.196	0.175	0.165	0.153	0.398
2	Average ABS at 30 min		0.256		0.237	0.217	0.196	0.177	0.162	0.145	0.129	0.115	0.106	0.398
3	Average ABS at 60 min		0.230		0.210	0.191	0.170	0.151	0.135	0.119	0.103	0.088	0.080	0.398
4	Average ABS at 90 min		0.210		0.190	0.171	0.150	0.131	0.115	0.099	0.082	0.068	0.060	0.398

The maximum used [Al(acac)$_3$] concentration (500 µM) brought a drop in B-cells-GSH level up to (0.155) compared to the B-cells-control (0.398) as shown in Table 4 and Figure 7. Two aqueous solutions of [Al(acac)$_3$] having final concentrations of [Al(acac)$_3$] (50 and 500 µM) were incubated up to different time intervals (0, 30, 60 and 90 min) with isolated B-cells. By extending the time of incubation, the B-cells-GSH level was further decreased with the maximum decrease in B-cells-GSH level being with the maximum provided time as shown in Table 4 and Figure 8

DISCUSSION

In the present studiey, an attempt has been made to determine the effect of aluminum sulphate and aluminum acetyl acetonate on the glutathione level in T-cell and B-cell of human blood by the use of influential parameters like concentration and time of incubation. We have found. that there was a depletion of glutathione level in T-cell and B-cell by increasing the concentration and with the passage of time. The study was conducted in terms of determination of absorbance of glutathionespectrophotometicallyinT-cellandB-cell

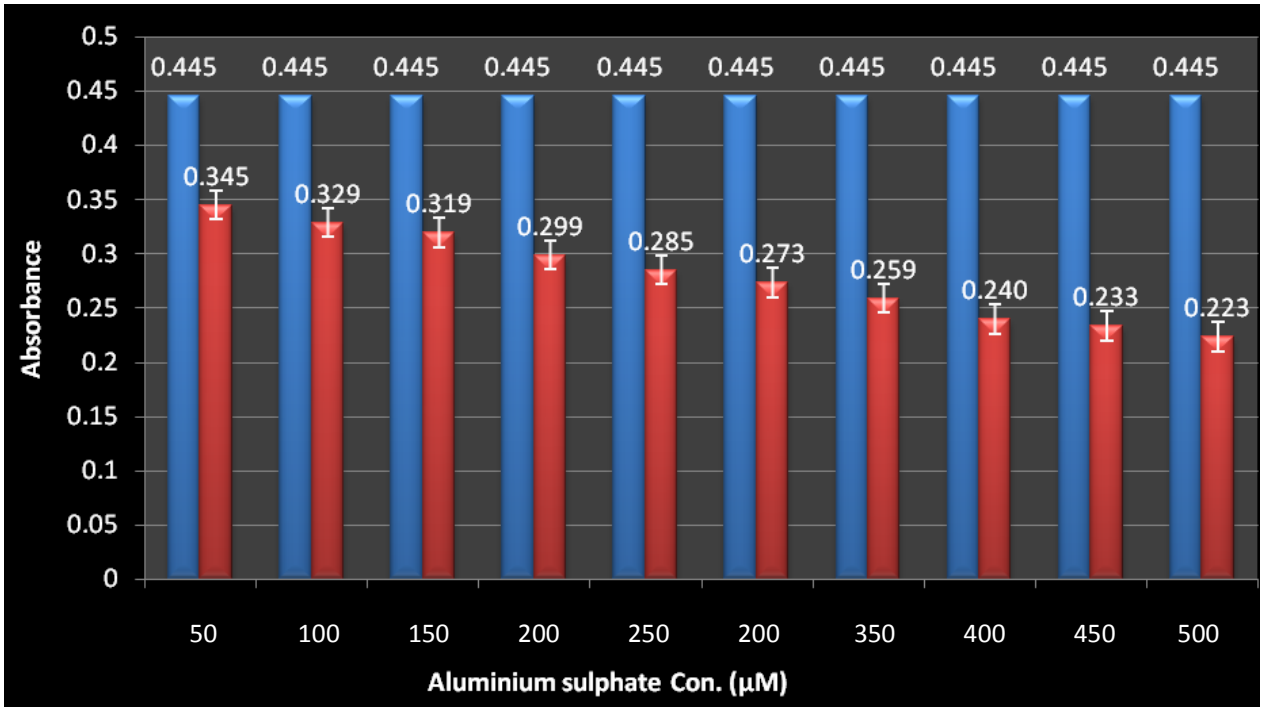

Figure 1. Effect of aluminium sulphate concentration on T-cells-GSH content. ■ T-cells Control (1 ml 0.9% NaCl + 1 ml of T-cells). ■ Aluminium sulphate (50 to 500 µM). Results are the mean ±SE of 3 experiments. Con = concentration.

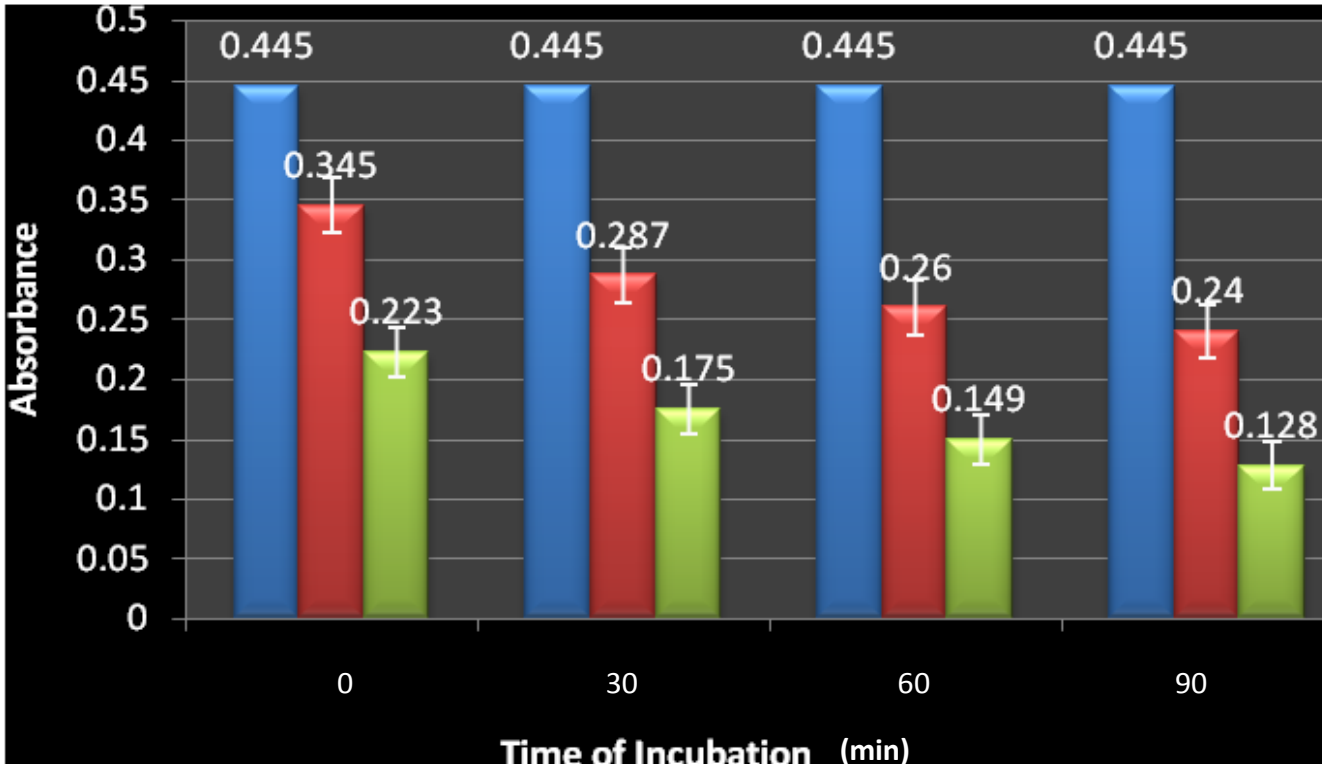

Figure 2. Effect of aluminium sulphate concentration on the T-cells-GSH content with time incubation period (0 to 90 min). ■ T-cells control (1 ml 0.9% NaCl + 1 ml of T-cells). ■ Aluminium sulphate (50 µM). ■ Aluminium sulphate (500 µM). Results are the mean ± SE of 3 experiments.

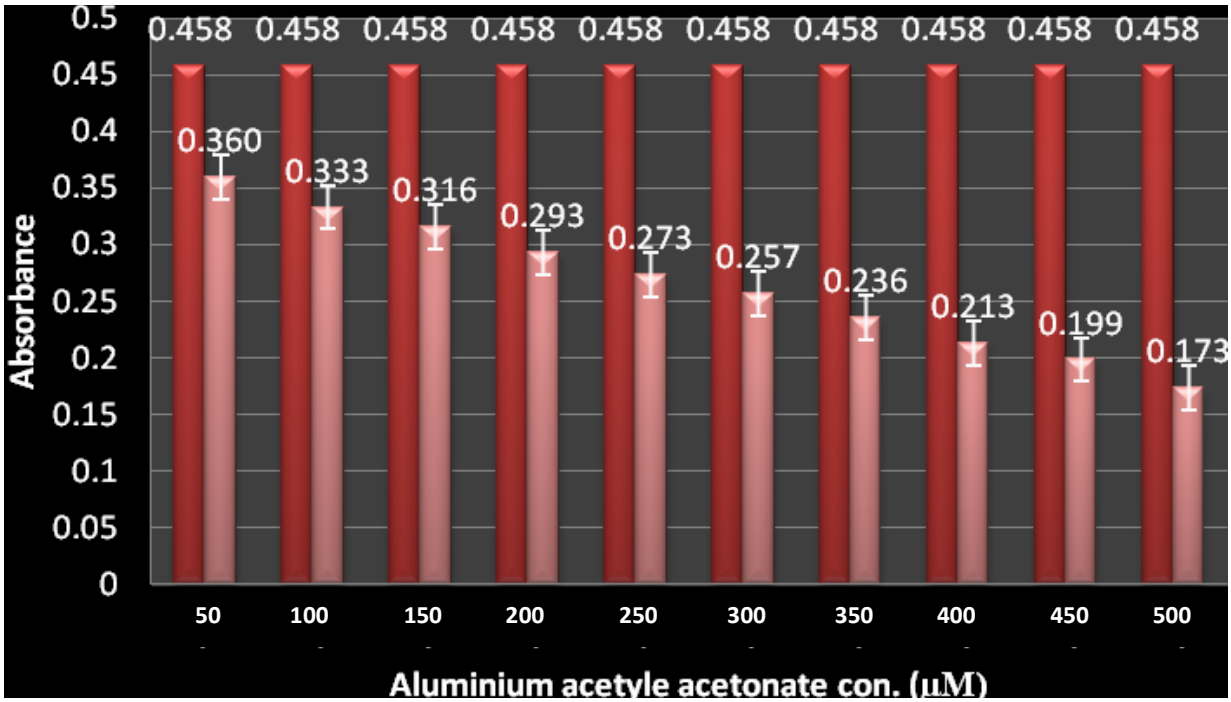

Figure 3. Effect of Aluminium acetylacetonate concentration on T.cells-GSH content. ■ T.cells control (1 ml 0.9% NaCl +1 ml of T-cells). ■ Aluminium acetylacetonate (50 to 500 µM). Results are the mean ± SE of 3 experiments. Con = concentration.

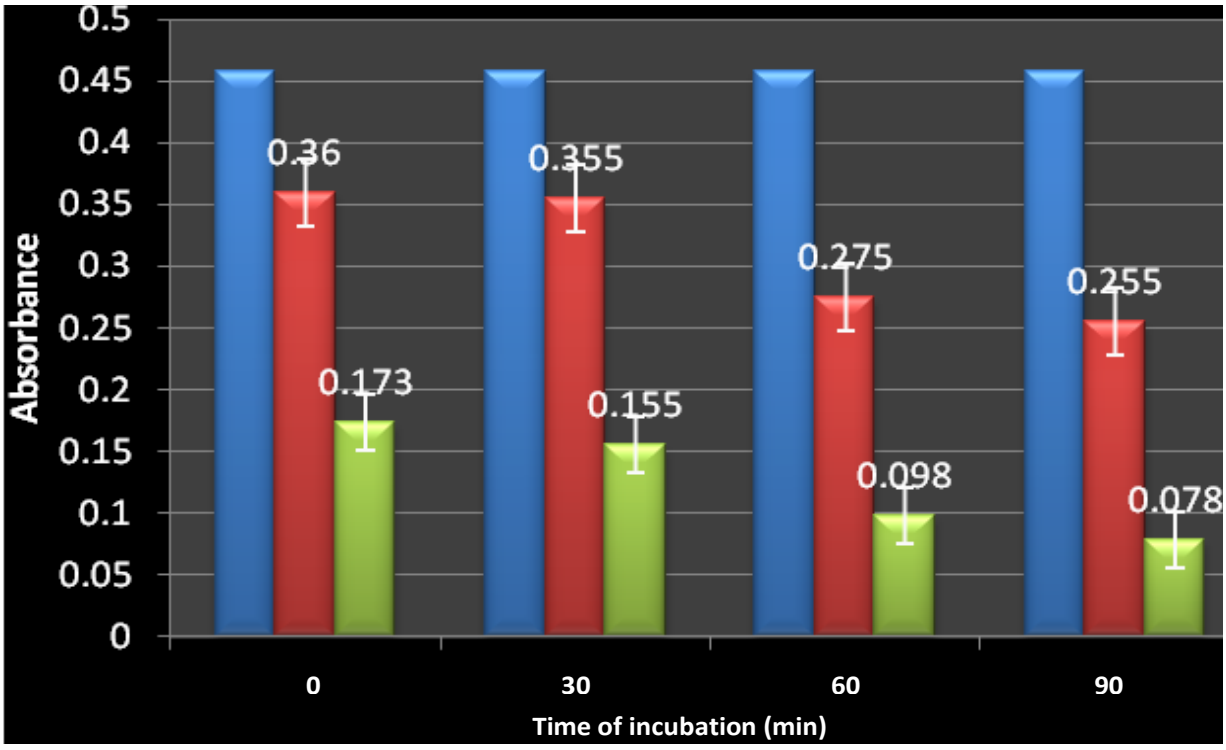

Figure 4. Effect of Aluminium acetylacetonate Concentration on the T.cells-GSH content with time incubation period (0 to 90 min). ■T-cells control (1 ml 0.9% NaCl +1 ml of T-cells). ■ Aluminium acetylacetonate (50 µM). ■ Aluminium acetylacetonate (500 µM). Results are the mean ± SE of 3 experiments.

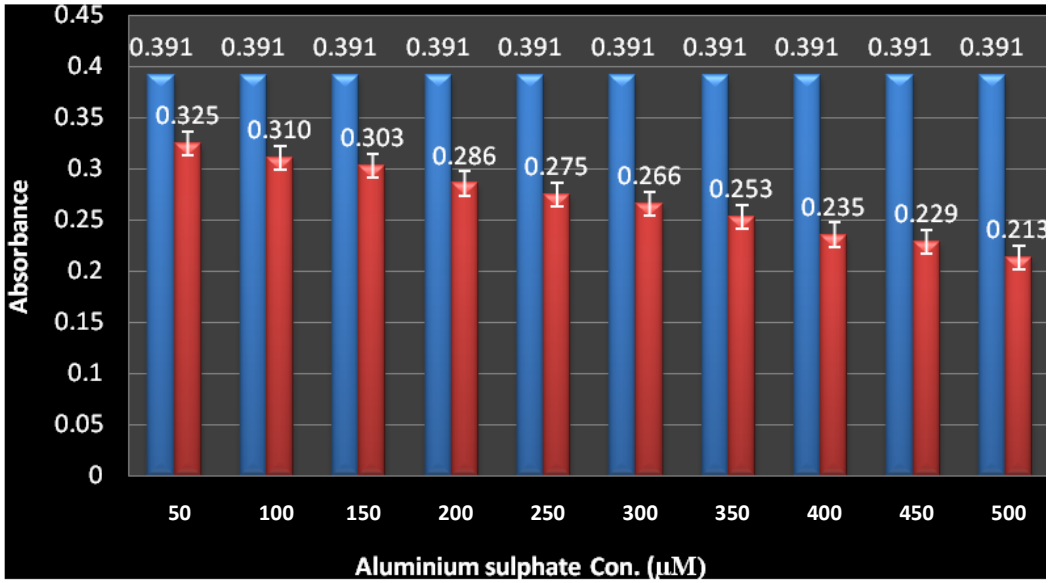

Figure 5. Effect of aluminium sulphate concentration on B.cells-GSH content. ■ B-cells control (1 ml 0.9% NaCl + 1 ml of B-cells). ■ Aluminium sulphate (50 to 500 µM). Results are the mean ± SE of 3 experiments. Con = concentration.

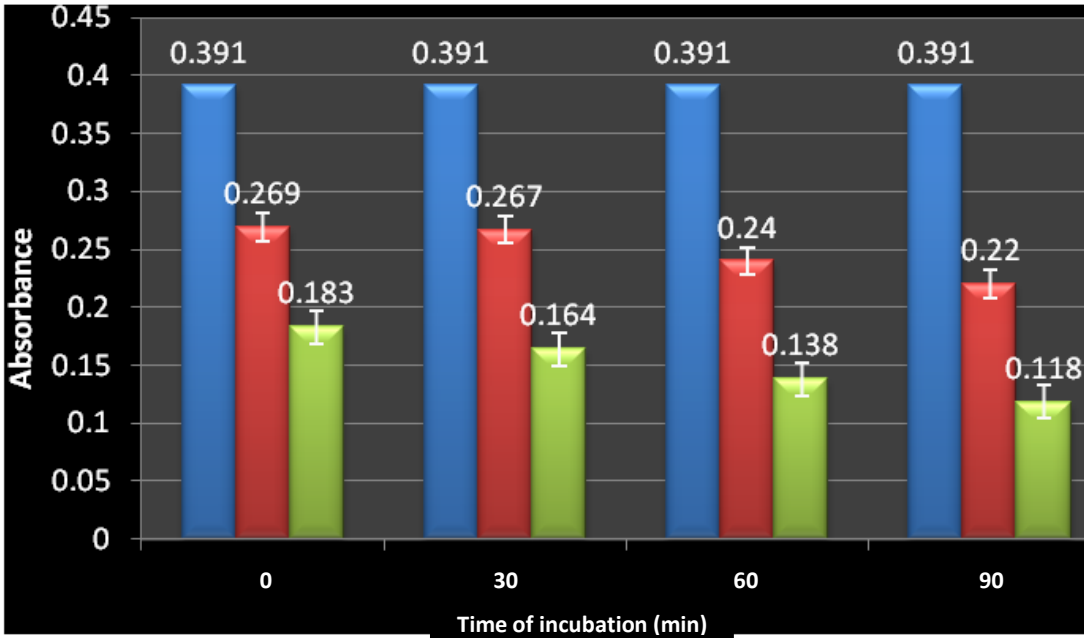

Figure 6. Effect of ATO Concentration on the B.cells -GSH content with time incubation period (0-90 min). ■ B-cells control (1 ml 0.9% NaCl + 1 ml of B-cells). ■ Aluminium sulphate (50 µM). ■ Aluminium sulphate (500 µM). Results are the mean ± SE of 3 experiments.

at ʎ max 412 nm by well-known Ellmans'method (Ellman, 1959). Our results confirm the finding report that aluminum has strong affinity with glutathione which in turn decrease the concentration of glutathione (Priya and Bimla, 2006).

Glutathione coordinates with Al (III) with an average efficiency, like simple monodentate amino acids. The carboxylate groups are effective binding sites for Al (III).

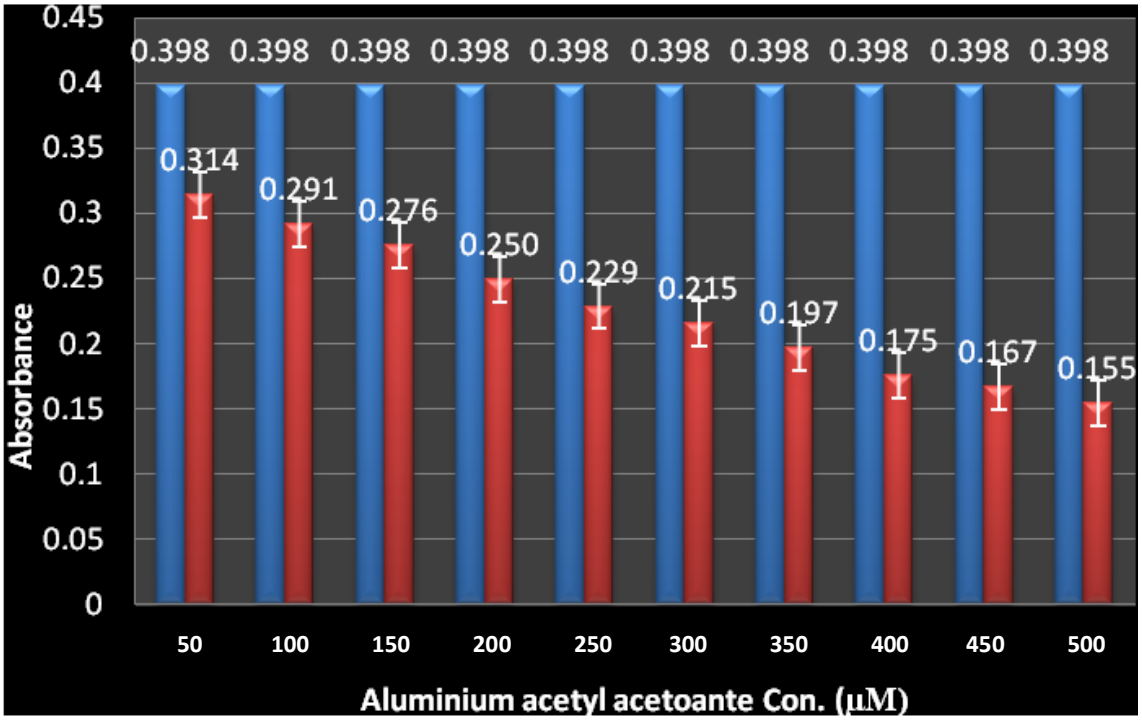

Figure 7. Effect of aluminium acetylacetonate concentration on B-cells-GSH content. ■ B-cells control (1 ml 0.9% NaCl + 1 ml of B-cells). ■ Aluminium acetylacetonate (50 to 500 µM). Results are the mean ± SE of 3 experiments. Con = concentration.

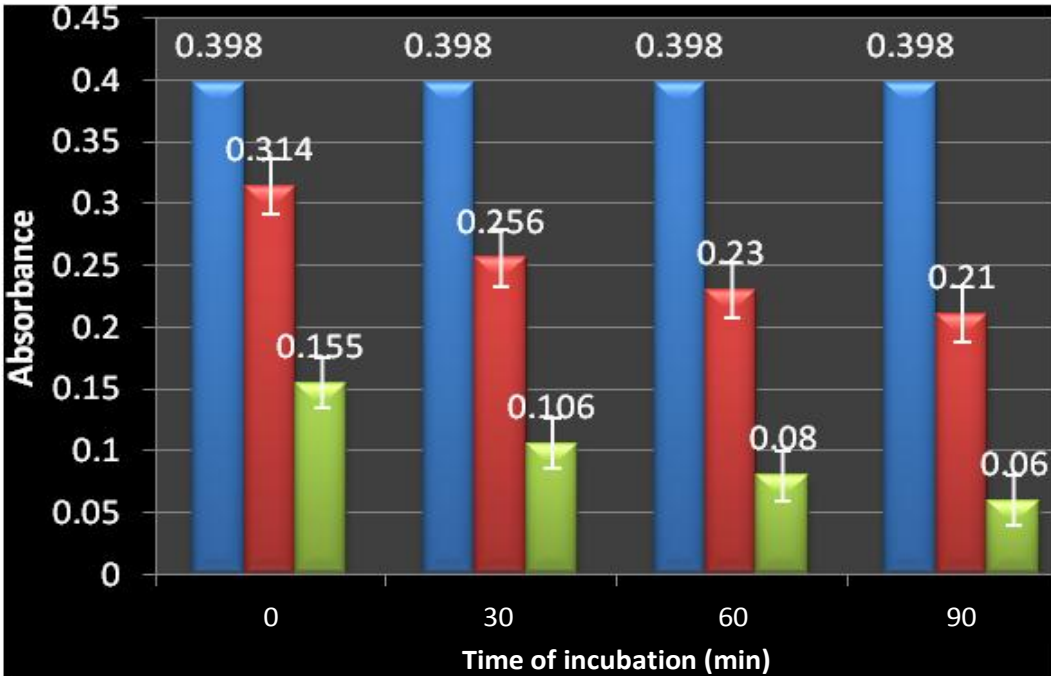

Figure 8. Effect of Aluminium acetylacetonate Concentration on the B.cells-GSH content with time incubation period (0 to 90 min). ■ B-cells control (1 ml 0.9% NaCl + 1 ml of B-cells). ■ Aluminium acetylacetonate (50 µM). ■ Aluminium acetylacetonate (500 µM). Results are the mean ± SE of 3 experiments.

The possible binding sites are the negatively charged C-terminal Gly-COO- and Glu-COO groups. Unfortunately, this small peptide glutathione disulfide (GSSG) cannot prevent the precipitation of Al (III) complexes around the physiological pH range; it only keeps Al (III) ions in aqueous solution in an acidic pH range (Xiao et al., 2008). Our studies also confirm the findings report of (Haroon et al., 2011) that aluminium metal depleted the glutathione level in aqueous medium by increasing the influential parameters like concentration, time of incubation, pH and temperature. We also compare the studies with other findings and found that metals like silver, aluminum, lead, arsenic, mercury has high binding affinity for glutathione in aqueous as well as in blood components. The decline of glutathione level in aqueous and blood components is due to interaction of sulfhydryl group of glutathione and these metals (Haroon et al., 2011, 2012; Naseem et al., 2011).

Thus, the interaction of aluminium 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 aluminium as well as the role of GSH in the protection of our body from their harmful effects. This also confirm the findings reported that adult rats are exposed to the aluminium dose 100 mg/kg bwt for 60 days, and registered a highly significant decrease in the level of glutathione as compared to control animal. According to our findings, the aluminium in the form of aluminum sulphate and aluminum acetyl acetonate 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 aluminum sulphate and aluminum acetyl acetonate and the depletion of GSH content of T-cells and B-cells as the time passed from 0 to 90 min.

Studies by haroon et al. (2011) indicated that increase in the concentration of aluminum depleted the glutathione level in blood components. It also indicated that the decrease is more pronounced when time elapsed from 0 to 90 min. Furthermore, little information is available on the mechanism of action at the molecular level. Our experimental work proposes the molecular mechanism of action of aluminum sulphate and aluminum acetyl acetonate. Our research hypothesis was that aluminum sulphate and aluminum acetyl acetonate caused modulation in the status of GSH of T-cells and B-cells either to glutathione disulfide (GSSG) or formation of aluminum-glutathione (Al-SG) complex.

The exact mechanism of action of these compounds on GSH level in this study is not known. However, the proposed hypothetical mechanism of action of aluminum sulphate and aluminum acetyl acetonate on GSH level is the formation of Al-$(SG)_3$ complex.

The proposed reactions involve the coordination of Al^{+III} in aluminum sulphate and aluminum acetyl acetonate with the S-H group of GSH with the formation of Al-$(SG)_3$ complex as shown below

$$Al_2(So_4)_3 \longrightarrow 2Al^{+3} + 3SO^{-2}$$

$$2Al^{+3} + 6GSH \longrightarrow 2Al\text{-}(SG)_3$$

The importance of interaction of metalloelements including aluminium metal with GSH as a biomarker of detoxification may guide biochemical scientist to take account of metal salt/complexes and metal/ drug complexes for implementation into clinical settings

Conclusion

From these findings we concluded that when human B and T lymphates were exposes to aluminum sulphate and aluminum acetyl acetonate there was significant decrees in glutathione level present in T-cells and B-cells of lymphocytes of human blood. So the people exposed to aluminum may be more prone to Parkinsonism and alzimer as aluminum causes reduction in glutathione level which is natural antioxidant. It is further suppose that a targeted study may be conducted in future.

ABBREVATIONS

D/W; distilled water, **q.s;** quantity sufficient.

REFERENCES

Blaylock RL, Strunecka A (2009). Immune-glutamatergic dysfunction as a central mechanism of the autism spectrum disorders. Curr. Med. Chem.16(2):157-170.

Drago D, Cavaliere A, Mascetra N (2008). Aluminum modulates effects of beta amyloid (1-42) on neuronal calcium homeostasis and mitochondria functioning and is altered in a triple transgenic mouse model of Alzheimer's disease. Rejuvenation Res.11(5):861-171.

Exley C, Swarbrick L, Gherardi RK, Authier FJ (2009). A role for the body burden of aluminium in vaccine-associated macrophagic myofasciitis and chronic fatigue syndrome. Med. Hypotheses. 72(2):135-139.

Ellman GL (1959). Determination of sulfhydryl group.Arch. Biochem. Biophys. 82:70-74.

Haroon K, Muhammad FK, Syed UJ, Naseem U (2012)."Effect of Aluminium metal on Glutathione (GSH) Level in Plasma and Cytosolic Fraction of Human Blood". Pak. J. Pharm. Sci. 24(1):13-18.

Haroon K, Mohammad FK, Naseem U, Muhammad M, Naheed H, Barkat AK, Abdul W, Arshad F, Kamal S (2011). Effect of Aluminum Acetyl Acetonate on the Chemical Status of Glutathione by Influential Parameters in Aqueous Medium. IJBMSP 1(1):23-26

Naseem U, Muhammad FK, Haroon K, Muhammad M (2011). Metabolic Modulation of Glutathione in Whole Blood Components against Lead Induced Toxicity A. J. Biotechnol. 10(77):17853-17858

Michael H (2011). *Heavy metal*. Encyclopedia of Earth. National Council for Science and the Environment. eds. E. Monosson & C. Cleveland. Washington, D.C.

Pastore A, Piemonte F, Locatelli M, Russo AL, Gaeta LM,Tozzi G, Federici, Giorgio (2003). "Determination of blood total, reduced, and

oxidized glutathione in pediatric subjects". Clin. Chem. 47(8):1467–1469.
Priya A, Bimla N (2006). Alteration in glutathione system in adult and pups rate brains following chronic aluminum exposer. Ind, J. Ocup. Environ. Med. 10(3):128-132.
Proudfoot AT (2009). Aluminium and zinc phosphide poisoning. Clin. Toxicol. 47(2):89-100.
Ron Z, Kilpinen P (2001). Control of Pollutants in Flue Gases and Fuel Gases.
Shuchang H, Qiao N, Piye N (2008). Protective effects of gastrodia elata on aluminium-chloride-induced learning impairments and alterations of amino acid neurotransmitter release in adult rats. Restor. Neurol. Neurosci. 26(6):467-473.
Verstraeten SV, Aimo L, Oteiza PI (2008). Aluminium and lead: molecular mechanisms of brain toxicity. Arch Toxicol .82(11):789-802.
Vina J, Lloret, A, and Orti D (2004). Molecular bases of the treatment of Alzheimer's disease with antioxidants. Mol. Aspect Med, 25, 117.
Yves C (2000). Oxidative stress and Alzheimer disease. Am. J. Clin. Nutr. 71*(2)*:621S-629S.
Xiao DY, Qian QZ, Rong FC, Ren FS (2006). Speciation of aluminum complexes with oxidized glutathione in aqueous solutions. Jpn. Sci. Anal. Chem. 24(1):1005-1012.