Research Article

CONCENTRATION AND TIME DEPENDENT EFFECT OF ALUMINIUM METAL ON GLUTATHIONE LEVEL IN PLASMA OF HUMAN BLOOD

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ABSTRACT

Aluminium is an important metalloelement and has many medicinal uses like use of aluminum as an antacid. Thus it is interesting to study the effect of Aluminium on the glutathione (GSH) *in* v*ivo* conditions. The concentration and time dependent effect of Aluminium on glutathione level in plasma was studied by using Ellman's method. A drastic effect on decreasing the concentration of glutathione level in plasma was found by increasing the concentration of Aluminium sulphate over the time. The reason could probably be due to oxidation of GSH to corresponding disulphide (GSSG). In this paper the effect of Aluminium metal on thiol/GSH level was discussed *in vitro*, which in principal may present a model of *in vivo* reaction.

***Keyword*:** Aluminium (Al), Glutathione (GSH), Ellman's method. 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB)

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INTRODUCTION:

GSH (g-glutamylcysteinylglycine) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is found in animals, plants, and microorganisms, and due to its water solubility is mainly found in the cell cytosol and other aqueous phases of the living system (Kosower and Kosower, 1978; Kidd, 1991; Lomaestro and Malone, 1995; Meister, 1976). Glutathione exists in two forms: the antioxidant "reduced glutathione" tripeptide is conventionally called glutathione and abbreviated GSH; the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress.

GSH has potent electron-donating capacity, as indicated by the high negative redox potential of the GSH/GSSH "redox couple" (E'0 =-0.33v) (Lewin, 1976). Its high redox potential renders GSH both a potent antioxidant and a convenient cofactor for enzymatic reactions that require readily available electron pairs (Kehrer and lund, 1994). The reducing power of GSH is a measure of its free radical scavenging, electron-donating, and sulfhydryl-donating capacity.

The reduced glutathione molecule consists of three amino acids - glutamic acid, cysteine, and glycine - covalently joined end-to-end. The sulfhydryl (-SH) group, which gives the molecule its electron-donating character, comes from the cysteine residue.Glutathione is present inside cells mainly in its reduced (electron-rich, antioxidant) GSH form. In the healthy cell GSSG, the oxidized (electron-poor) form, rarely exceeds 10 percent of total cell glutathione (Kosower and Kosower, 1978). Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Duke *et al*., 1996; Slater *et al*., 1995). Aluminium compounds are used in many diverse and important industrial applications such as alums in water-treatment and alumina in abrasives and furnace linings. They are found in consumer products such as antacids, astringents, buffered aspirin, food additives, and antiperspirants. Powdered Aluminium metal is often used in explosives and fireworks (Sidney, 2007). The use of Aluminium cookware, popular because of its corrosion resistance and good heat conduction, has not been shown to lead to Aluminium toxicity in general. Excessive consumption of antacids containing Aluminium compounds and excessive use of Aluminium-containing antiperspirants are more likely causes of toxicity. In research published in the Journal of Applied Toxicology, Dr. Philippa D. Darby of the University of Reading has shown that Aluminium salts increase estrogen-related gene expression in human breast cancer cells grown in the laboratory. These salts estrogen-like effects have lead to their classification as a metalloestrogen. It has been suggested that Aluminium is a cause of Alzheimer's disease, as some brain plaques have been found to contain the metal. Aluminium metal has affinity for the glutathione present in aqueous phases of blood. This affinity is mainly formed between Aluminium metal and sulfhydryl groups of glutathione (Quig, 1998). This affinity can cause a depletion of the reduced form glutathione in the blood, but with the depletion of the glutathione, GSH synthesizing systems start making more GSH from cysteine via the γ-glutamyl cycle but if GSH is usually not effectively supplied, however, if GSH depletion continues because of chronic metal exposure (Quig, 1998; Hultberg *et al.*, 2001 Stohs and Bagchi,1993) then the pharmacological benefits of the Aluminium metal being used for the help of body defenses can be harmful in nature to the body defense system . The following study makes a design to see the effects of Aluminium Sulphate, in respect of concentration and time, on glutathione level in Plasma.

MATERIALS AND METHODS

Materials

Sodium Hydroxide (Fluka AG), L.Glutathione (GSH)(Fluka), DTNB (Sigma), Potassium Dihydrogen Phosphate (Merck), HCl 35% (Kolchlight) Aluminium Sulphate (Merck,Germany), Sodium Chloride (Merck), Disodium Edetate (Riedel Dehean AG Sleeze Hannover) Chloroform (Merck), Ethanol(Merck). Distilled Water (Double Distilled). U.V 1601 spectrophotometer (Shimadzu). Centrifuge (H-200). PH Meter: Model NOV-210, Nova Scientific Company Ltd.

Korea, Oven: Memmert Model U-30,854 Schwabach (Germany). Magnetic Stirrer, hot plate 400(England). Micropipettes 200 µl, 500 µl, 1000 µl were used of Socorex Swiss (Finaland), Sortorius Balance, Disposable Rubber Gloves, were used in this research work.

METHODS (Ellman's, 1959)

Isolation of Plasma

Sample of 5 ml of human venous blood treated with heparin to prevent clotting was collected. The blood was centrifuge on H-200 centrifuge at 10,000 rpm for 2 minutes. The plasma was removed with Pasteur pipette. One ml of plasma was incubated for different concentration and time interval with I ml of metal, and analyzed for GSH level.

Determination of GSH in Plasma

The assay of GSH with DTNB was performed followed a standard Ellman's method for plasma of blood. 2.3 ml of potassium phosphate (0.2 M, pH 7.6) buffer was taken in the cell and/or cuvete followed addition of 0.2ml aqueous solution or plasma of blood. To it 0.5 ml DTNB (0.001M) in a buffer was added. An absorbance of reaction product in cuvette was read after 5 minutes at 412 nm using shamadzo 1601 UV/Visible double bean spectrophotometer and GSH level was determined, from standard curve of reduced GSH obtained with 0.2, 0.4, 0.6, 0.8 and 1mM GSH concentration.

Standard Curve for Glutathione

200µl of 0.2, 0.4, 0.6, 0.8 and 1mM solutions of glutathione was added to 2.3ml of phosphate buffer pH 7.6, followed by the addition of 0.5ml of 1mM DTNB Stock solution. The mixtures were shaken thoroughly and incubated for 5 minutes at 30^0C. Absorbances were taken after 5 minutes at fixed wavelength of 412nm.

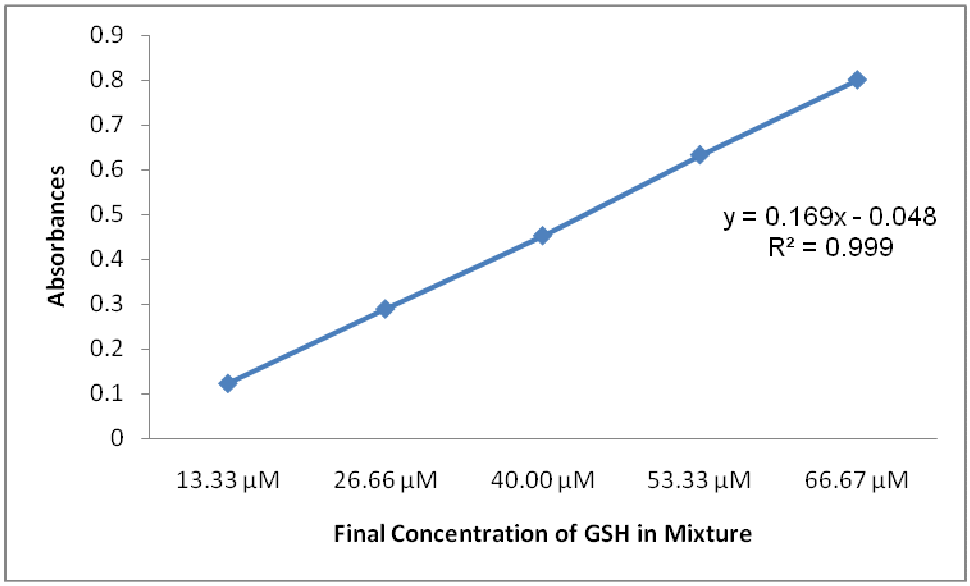

Figure 1. Standard Curve of Glutathione

Blank was prepared in which GSH was omitted. Standard curve was constructed by plotting the change of absorbance versus final concentration of GSH in the mixture. Straight line was drawn by using linear regression analysis. The correlation coefficient of plot was 0.999. Standard curve was obtained as shown in the figure 1.

Effect of Different Concentrations of Aluminium Sulphate on Glutathione (GSH) level in Plasma of Human Blood

To 1ml (1000µl) of plasma taken in five separate test tubes, 1ml (1000µl) of different concentrations of 0.2, 0.4, 0.6, 0.8 and 1mM solution of Aluminium Sulphate were added separately and shacked. Five separate test tubes was prepared with 0.2ml (200µl) Aluminium Sulphate plus plasma mixture from each previously made five tubes diluted with 2.3ml (2300µl) of phosphate Buffer pH 7.6 and added 0.5ml (500µl) of 1mM DTNB stock solution. A control for plasma was also prepared by taking 1ml (1000µl) of plasma in a test tube and diluted with 1ml (1000µl) of phosphate buffer pH 7.6.The effect of Aluminium sulphate on the chemical status of glutathione in plasma was studied in terms of determination of concentration of GSH in mixtures by a well known Ellman's method, as mentioned in standard curve for GSH. The concentrations of GSH were determined from the GSH standard curve.

Table 1-Effect of different concentrations of Aluminium Sulphate on Glutathione (GSH) level in Plasma.

Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank Solution Was 0.060 ABS at 412nm

S No	Conc. Used of $Al_2(So_4)_3$	Final Conc. of$Al_2(So_4)_3$ in Mixture	1st ABS	2nd ABS	3rd ABS	Average of 3 Readings	Real absorbance*	Real Absorbance for Plasma Blank
1	0.2mM	6.67µM	0.540	0.525	0.544	0.536	0.473	0.484
2	0.4mM	13.33µM	0.520	0.522	0.533	0.525	0.462	0.474
3	0.6mM	20.00µM	0.480	0.513	0.509	0.501	0.438	0.463
4	0.8mM	26.67µµM	0.460	0.498	0.491	0.483	0.420	0.463
5	1mM	33.33µM	0.445	0.495	0.482	0.474	0.411	0.472

***** *Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution.*

Effect of Aluminium Sulphate on Glutathione (GSH) level in Plasma with Time

To 1ml (1000µl) of Plasma taken in a test tube, 1ml (1000µl) of 1mM solution of Aluminium Sulphate was added and shacked. The final concentration of Aluminium sulphate was 0.5mM (500µM). A test tube with 0.2ml (200µl) Aluminium sulphate plus plasma mixture was prepared from previously made test tube diluted with 2.3ml (2300µl) of phosphate buffer pH 7.6 and added 0.5ml (500µl) of 1mM DTNB stock solution. The final concentration of Aluminium sulphate was 0.03333mM (33.33µM).A control for plasma was also prepared by taking 1ml (1000µl) of plasma in a test tube and diluted with 1ml (1000µl) of phosphate buffer pH 7.6.The effect of Aluminium sulphate glutathione level in plasma was studied in terms of determination

of concentration of GSH in mixtures by a well known Ellman's method, as mentioned in standard curve for GSH. The absorbances were read at 0, 30, 60, 90, 120, 150 minutes after preparing mixture (1ml of plasma plus 1ml of Aluminium sulphate).The concentrations of GSH in plasma were determined from the glutathione standard curve.

Table 2- Effect of Aluminium Sulphate on Glutathione (GSH) level in Plasma with time.

Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank solution was 0.060 ABS at 412nm

Final Concentration of of Aluminium Sulphate was 33.33µM in Final Mixture

S #	Time Interval	1st ABS	2nd ABS	3rd ABS	Average of 3 Readings	Real absorbance*	GSH Blank ABS	Real Absorbance for GSH BLank
1	0 min	0.439	0.451	0.433	0.441	0.383	0.550	0.492
2	30 min	0.429	0.445	0.425	0.433	0.375	0.540	0.482
3	60 min	0.419	0.431	0.413	0.421	0.363	0.545	0.487
4	90 min	0.406	0.425	0.404	0.412	0.354	0.538	0.480
5	120 min	0.396	0.401	0.387	0.395	0.337	0.543	0.485
6	150 min	0.379	0.389	0.372	0.380	0.322	0.530	0.472

***** *Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution*

RESULTS

Effect of Aluminium Sulphate on Glutathione (GSH) level in Plasma

Effect of Aluminium metal on the chemical status of glutathione present in plasma was studied in term of determination of concentration of glutathione. Aluminium metal caused a decrease in the concentration of glutathione present in plasma. Different concentrations of Aluminium cause a gradual decrease in the concentration of glutathione in plasma as the concentration of metal increased as shown figure 2 and table 3.

Effect of Aluminium on the chemical status of glutathione was also studied for the time dependency and noted that the concentration of glutathione was gradually decreased as the time passes from 0minute interval of time to 150 minutes as shown figure 3 and table 4.

Statistical Analysis

Statistical Analysis for Effect of Aluminium Sulphate Glutathione (GSH) Level in Plasma

Statistical approach for the effect of Aluminium sulphate on the chemical status of GSH was also conducted for the concentration and time dependent effects. The paired comparison T-test (Table 5) of concentration dependent effect of Aluminium sulphate and GSH blank gave the decision that there is an effect of Aluminium on GSH level in plasma with increase in concentration of Aluminium sulphate, as compared to GSH blank solution treatment.

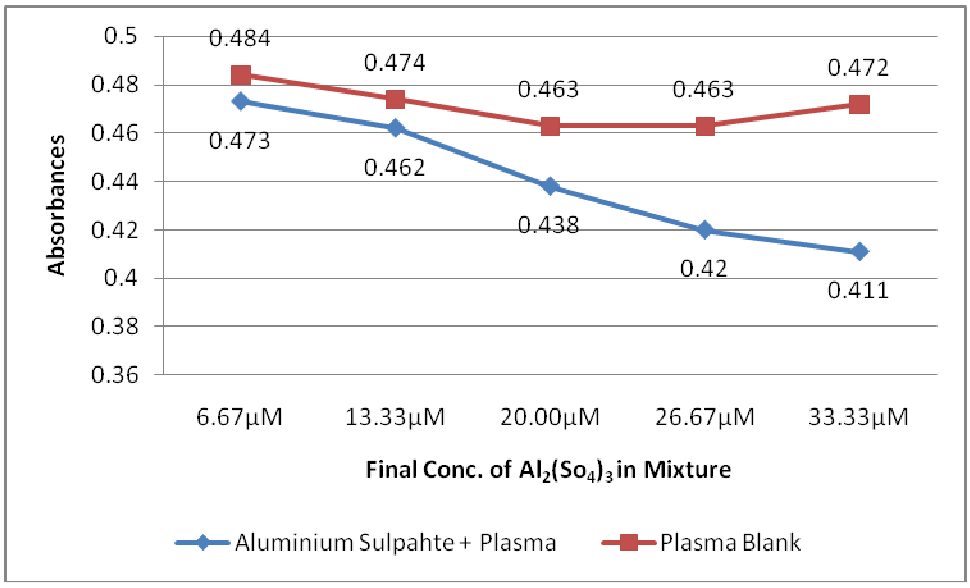

Figure 2- ***Curves for Plasma Thiol Control Level &*** $Al_2(So_4)_3$ ***Effected Plasma Thiol Level***

Table 3- Calculation for Concentration of GSH after reaction with Aluminium Sulphate by Ellman's Method		
S/No.	Real Absorbance(ABS)	Concentration of GSH (µM) Remained
1	0.473	40.664
2	0.462	39.762
3	0.438	37.795
4	0.420	36.320
	0.411	35.582

Table 4- Calculation for Concentration of GSH after reaction with Aluminium Sulphate by Ellman's Method		
S/No.	Real Absorbance(ABS)	Concentration of GSH (µM) Remained in plasma.
1	0.383	33.287
2	0.375	32.631
3	0.363	31.648
4	0.354	30.910
5	0.337	29.516
6	0.322	28.287

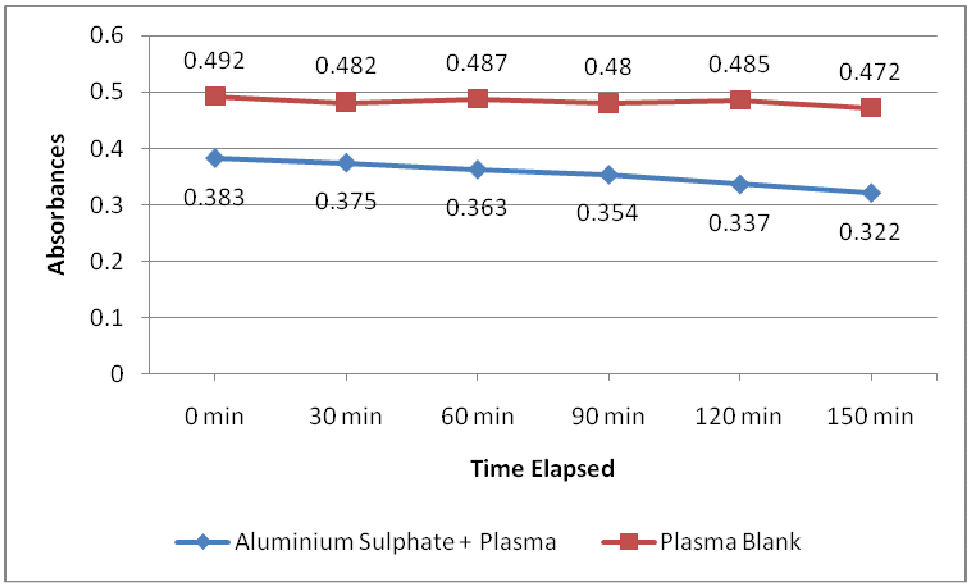

Figure-3 ***Time Dependent Curves for Plasma Thiol Control Level &*** $Al_2(So_4)_3$ ***Effected Plasma Thiol Level***

Table 5 -Paired comparison t-test for concentration dependent effect of $Al_2(SO_4)_3$		
	Affect of concentrations Of Aluminium on Plasma Glutathione	***GSH control solution (Blank)***
Mean	0.441	0.4712
Variance	0.0008	7.67E-05
Observations	5	5
Pearson Correlation	0.690	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-3.171	
P(T<=t) one-tail	0.017	
t Critical one-tail	2.139	
P(T<=t) two-tail	0.0339	
t Critical two-tail	2.777	

Similarly the paired comparison T-test (Table 6) of time dependent effect of Aluminium sulphate and GSH blank gave the decision that there is an effect of Aluminium sulphate on the chemical status of GSH in plasma as the passage of time is increased with a specific concentration of Aluminium sulphate as compared to GSH blank solution treatment.

Table 6- t-Test: Paired comparison t-test for time dependent effect of $Al_2(SO_4)_3$

	Aluminium Affect Plasma Glutathione with time	***GSH control solution (Blank)***
Mean	0.356	0.483
Variance	0.0005	4.64E-05
Observations	6	6
Pearson Correlation	0.757	
Hypothesized Mean Difference	0	
Df	5	
t Stat	-16.899	
P(T<=t) one-tail	6.636E-06	
t Critical one-tail	2.016	
P(T<=t) two-tail	1.328E-05	
t Critical two-tail	2.571	

DISCUSSION

There is increasing interest in glutathione due to its varied physiological and pharmacological properties including detoxification through participation in the redox system, activation of SH-Enzymes, co-enzymatic action and conjugation. Aluminium has been found to play a role in apoptosis (gene-directed cell death), a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases. Cells in the salivary gland, prostate, immune system and intestine can secrete Aluminium. Thus it was of interest to study the interaction of this metal in vivo to establish further scientific data. This scientific data about the interaction and the effect of Aluminium sulphate on the chemical modulation of GSH will enable us to understand further the role of, Aluminium sulphate and GSH and strengthen our knowledge about their therapeutic uses in many diseases. Different concentrations of Aluminium caused decrease of concentration of glutathione and play important role in the conversion of GSH to either GS Al or GS-Al of reduced form SG in plasma. The effect of Aluminium Sulphate was studied for the concentration and time dependent effects on the glutathione level and was found that the concentration of reduced glutathione was decreased with increasing concentration of Aluminium metal in solution and with the passage of time respectively. The following sequences of reactions are suggested to be happened in the experiment.

Equation

$$GSH + Aluminium\ (Al) \rightarrow Al\ (GS)_3$$

The results also suggested that there was a possibility of formation of intermediate or conjugate between Aluminium and GSH. However it was not possible to estimate or determined those conjugates under those conditions .Since both GSH and Aluminium, is biological active compounds. It was of interest to study the possible interaction of this metal in *vitro* as a model of in *vivo* interaction.

CONCLUSION

The tripeptide thiole glutathione has facile electron-donating capacity, linked to it sulfhydryl (_SH) group. Glutathione is important water phase antioxidant and essential cofactor for antioxidant enzyme. It provides protection also for the mitochondria against endogenous radicals. Its high electron donating capacity combined with its high molecular concentration endows (GSH) with great reducing power, which is used to regulate a complex thiole- exchange system**.**

Different concentration of Aluminium metal caused a gradual decreased in the concentration of glutathione in plasma. Effect of Aluminium on the chemical status of glutathione was also studied for the time dependency and noted that the concentration of glutathione gradually decreased as the time passes from 0 minute interval of time to 150minutes in plasma.

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