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CYTOSOLIC FRACTION OF GLUTHTION LEVEL AFTER ADDITION OF ALUMINUM TO HUMAN BLOOD

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ABSTRACT
Aluminium is being used in the medicines in the form of antacids. The Aluminium metal can be leached from our utensils and can harm the body for its side effects, if become available to the systemic circulation. Thus it is interesting to study the Effect of Aluminium on the Glutathione (GSH) in *Vivo* Conditions. The effect of Aluminium on the chemical status of the Glutathione (GSH) in cytosolic fraction has been studied using U.V Spectrophotometer by using Ellman's method. The effect of Aluminium on the chemical status of glutathione (GSH) was checked in cytosolic fraction for concentration and time dependent effects. There was found a profound effect on decreasing the concentration of glutathione (GSH) in cytosolic fraction as the concentration is increased and time has passed. The decrease in the glutathione level was concentration and time of interaction dependent, probably 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.
KEYWORDS: Aluminium, Glutathione (GSH), Red Blood Cell (Cytosolic Fraction), Ellman's method, 5, 5-Dithiobis, 2-Nitrobenzoic Acid (DTNB)

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INTRODUCTION

GSH (g-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is found in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system[1-4]. 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)[5]. Its high redox potential renders GSH both a potent antioxidant and a convenient cofactor for enzymatic reactions that require readily available electron pairs[6]. 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 (GSH)[1]. 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[7-8].

Aluminium Sulphate has affinity for the Glutathione (GSH) present in aqueous phases of blood. This affinity is mainly formed between Aluminium Sulphate and sulfhydryl groups of glutathione[9].This affinity can cause a depletion of the reduced form Glutathione in the blood ,but with the depletion of the Glutathione (GSH),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[10-12] 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 cytosolic fraction.

MATERIALS AND METHODS

Materials

L.glutathione (GSH) was purchase from (Fluka), DTNB was from (Sigma) chemical Co, Aluminium Sulphate was obtained from (Merck, Germany). All other reagent were of the highest purity commercially available. U.V 1601 spectrophotometer (Shimadzu). PH Meter: Model NOV-210, Nova Scientific Company Ltd. Korea, Oven: Memmert Model U-30,854 Schwabach (Germany).Centrifuge (H-200).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 also used in this research work .

Isolation of Cytosolic Fraction

Sample of 5 ml of human venous blood of patient/or treated with heparin to prevent clotting was collected. The blood was centrifuge on h-200 centrifuge at 10,000rmp for 2 minutes. The plasma was removed with Pasteur pipette. One ml of red cell were incubated for different time interval with I ml of Aluminium metal. after incubation, these red cell fraction washed twice with isotonic saline(0.9% NaCl)solution and lysed with an equal volume(1:1)of distill water for one hour at 4C.o.8 ml of a mixture of chloroform and ethanol(3:5v/v) at o C was added to 2ml of lysed cells Hemoglobin was precipitated followed by addition of 0.3ml of distill water. Clear supernatant (pale yellow), a cytosolic fraction of red cells was removed by pastuer pipette, after centrifugation as before, and analyzed for GSH level.

DETERMINATION OF GSH IN CYTOSOLIC FRACTION

The assay of GSH with DTNB was performed followed a standard Ellman's method[19] for cytosolic fraction of blood.2.3ml of potassium phosphate (0.2M,PH 7.6)buffer was taken in the cell and/or cuvete followed addition of 0.2ml aqueous solution or cytosolic fraction of blood .To it 0.5ml 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.

METHODS

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[13]. 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 0.9984. Standard curve was obtained as shown in **figure 1**.

Effect of different concentrations of Aluminium Sulphate on Glutathione Level in Cytosolic Fraction

To 1ml of cytosolic fraction taken in five separate test tubes, 1ml of different concentrations of 0.2, 0.4, 0.6, 0.8 and 1mM solution of Aluminium Sulphate were added separately and shaked. Five separate tubes were prepared with 0.2ml Aluminium Sulphate plus cytosolic fraction mixture from each previously made five tubes diluted with 2.3ml of phosphate Buffer pH 7.6 and added 0.5ml of 1mM DTNB stock solution. A control for cytosolic fraction was also prepared by taking 1ml of cytosolic fraction in a test tube and diluted with 1ml of phosphate buffer pH 7.6.

The effect of Aluminium Sulphate on the chemical status of GSH in cytosolic fraction 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.

Effect of Aluminium Sulphate on Glutathione (GSH) Level in Cytosolic Fraction with time

To 1ml of cytosolic fraction taken in a test tube, 1ml of 0.1mM solution of Aluminium was added and shaked. The final concentration of Aluminium Sulphate was 0.5mM .A test tube with 0.2ml Aluminium plus cytosolic fraction mixture was prepared from previously made test tube diluted with 2.3ml of phosphate Buffer pH 7.6 and added 0.5ml of 1mM DTNB stock solution. The final concentration of Aluminium Sulphate 0.03333mM .A control for cytosolic fraction was also prepared by taking 1ml of cytosolic fraction in a test tube and diluted with 1ml of phosphate buffer pH 7.6. The effect of Aluminium Sulphate on the chemical status of GSH in cytosolic fraction 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 cytosolic fraction plus 1ml of Aluminium Sulphate. The concentrations of GSH in cytosolic fraction were determined from the GSH standard curve.

RESULTS

Effect of Aluminium Sulphate on the Chemical Status of Glutathione (GSH) in Cytosolic Fraction

Effect of Aluminium Sulphate on the chemical status of glutathione present in cytosolic fraction was studied in term of determination of concentration of GSH.

Aluminium Sulphate caused a decrease in the concentration of GSH present in cytosolic fraction. Different concentrations of Aluminium Sulphate cause a gradual decrease in the concentration of GSH in Cytosolic Fraction as the concentration of metal increased as shown **figure 2** and **table 3**.

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

Statistical Analysis

Statistical Analysis for Effect of Aluminium Sulphate on Glutathione (GSH) level in Cytosolic Fraction

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 Sulphate Aluminium Sulphate on the chemical status of GSH in cytosolic fraction with increase in concentration of Aluminium Sulphate, as compared to GSH Blank solution treatment.

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 GSH level in cytosolic fraction as the passage of time is increased with a specific concentration of Aluminium Sulphate as compared to GSH Blank solution treatment.

DISCUSSION

There is increasing interest in GSH 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 Sulphate 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 Sulphate. Thus it was of interest to study the interaction of this metal in vitro 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.

The study conducted showed that the concentration of GSH present in cytosolic fraction was shown to be low as compared to the level of GSH present in cytosolic fraction. The effect was same as viewed in the case of performance of effect of metal on GSH in aqueous medium and in cytosolic fraction. Different concentrations of Aluminium Sulphate caused decrease of concentration of glutathione and play important role in the conversion of GSH to either GSAl or GS-Al-of reduced form SG in cytosolic fraction. In the same manner the effect of Aluminium Sulphate was also time dependent on the chemical status of GSH and the concentration of reduced GSH present in cytosolic fraction was decreased with the passage of time. The following sequences of reactions are suggested to be happened in the experiment.

Equation

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

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 Sulphate, 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 glutatine (GSH) 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 Sulphate caused a gradual decreased in the concentration of GSH in cytosolic fraction. Effect of Aluminium Sulphate 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 cytosolic fraction.

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Table 1- Effect of different concentrations of Aluminium Sulphate on the chemical Status of Glutathione (GSH) in Cytosolic Fraction.

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

S#	Conc. Used of Al_2SO_4	Final Conc. of Al_2SO_4 in Mixture	1st ABS	2nd ABS	3rd ABS	Average of 3 Readings	Real absorbance*	Real Absorbance for Cytosolic Fraction Blank
1	**0.2mM**	**6.67mM**	0.142	0.139	0.150	0.144	**0.086**	**0.098**
2	**0.4mM**	**13.33mM**	0.115	0.113	0.123	0.117	**0.059**	**0.100**
3	**0.6mM**	**20.00mM**	0.106	0.104	0.114	0.108	**0.050**	**0.089**
4	**0.8mM**	**26.67mM**	0.100	0.097	0.108	0.102	**0.044**	**0.101**
5	**1mM**	**33.33mM**	0.090	0.093	0.101	0.095	**0.037**	**0.094**

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

Table 2- Effect of Aluminium Sulphate on the Chemical Status of Glutathione (GSH) in Cytosolic Fraction 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**	0.155	0.143	0.136	0.145	**0.089**	0.179	**0.121**
2	**30 min**	0.153	0.141	0.134	0.143	**0.087**	0.176	**0.118**
3	**60 min**	0.152	0.140	0.133	0.142	**0.086**	0.174	**0.116**
4	**90 min**	0.139	0.127	0.120	0.129	**0.073**	0.173	**0.115**
5	**120 min**	0.129	0.117	0.110	0.119	**0.063**	0.170	**0.112**
6	**150 min**	0.103	0.091	0.084	0.093	**0.037**	0.167	**0.109**

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

Table 3- Calculation for Concentration of GSH after reaction with Aluminium Sulphate by Ellman's Method

S/No.	Real Absorbance(ABS)	Concetration of GSH (µM) Remained
1	**0.086**	8.943
2	**0.059**	6.730
3	**0.050**	5.992
4	**0.044**	5.500
5	**0.037**	4.926

Table 4- Calculation for Concentration of GSH after reaction with Aluminium Sulphate by Ellman's Method

S/No.	Real Absorbance(ABS)	Concetration of GSH (µM) Remained in Cytosolic Fraction.
1	**0.089**	9.189
2	**0.087**	9.025
3	**0.086**	8.943
4	**0.073**	7.877
5	**0.063**	7.057
6	**0.037**	4.926

Table5- Statistical analysis of effect of zinc chloride on GSH chemical status in cytosolic fraction (C.F) of blood.

1- Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair	Zinc +C.F	0.0552	5	0.018708	0.004
	BLANK C.F	0.0964	5	0.00493	0.001

2- Paired Samples Correlations

		N	Pearson Correlation	P-value
Pair	Zinc +C.F *BLANK C.F*	5	0.245	<0.05

3- Paired Samples Test

		Paired Differences					t	df	t-Critical (1-Tail)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	Zinc +C.F *BLANK C.F*	0.0552	0.0188	0.000	0.030	0.061	-5.00	5	2.132

Table 6- Paired comparison t-test for time dependent effect of $Al_2(so_4)_3$

	Affect of concentrations Of Aluminium Sulphate **on cytosolic Fraction of Glutathione with time**	**GSH(Blank)**
Mean	0.0725	0.1151667
Variance	0.0004031	1.817E-05
Observations	6	6
Pearson Correlation	0.929015812	
Hypothesized Mean Difference	0	
Df	5	
t Stat	-6.453462516	
P(T<=t) one-tail	0.000664668	
t Critical one-tail	2.015048372	
P(T<=t) two-tail	0.001329336	
t Critical two-tail	2.570581835	

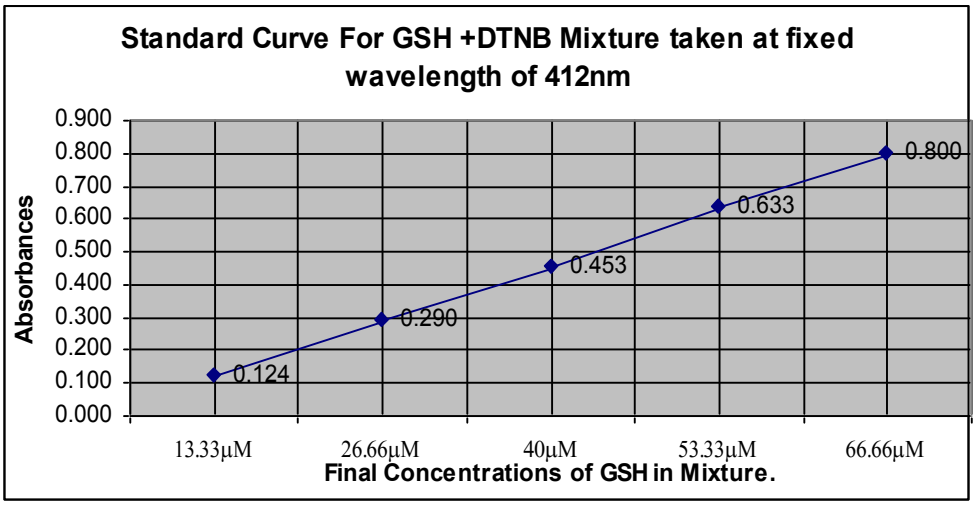

Figure 1- Standard Curve for Glutathione (GSH) + DTNB Mixture taken at fixed wavelength of 412nm

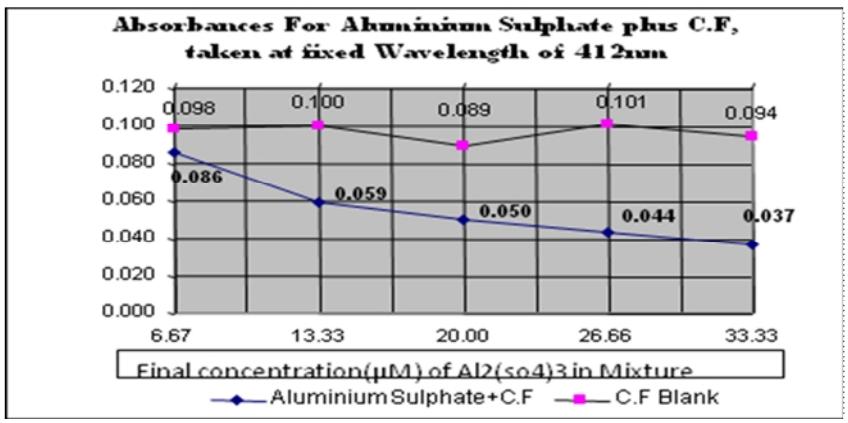

Figure 2- Curves For Control Thiol Level of Cytosolic Fraction & $Al_2(so_4)_3$ effected Thiol Level of Cytosolic Fraction

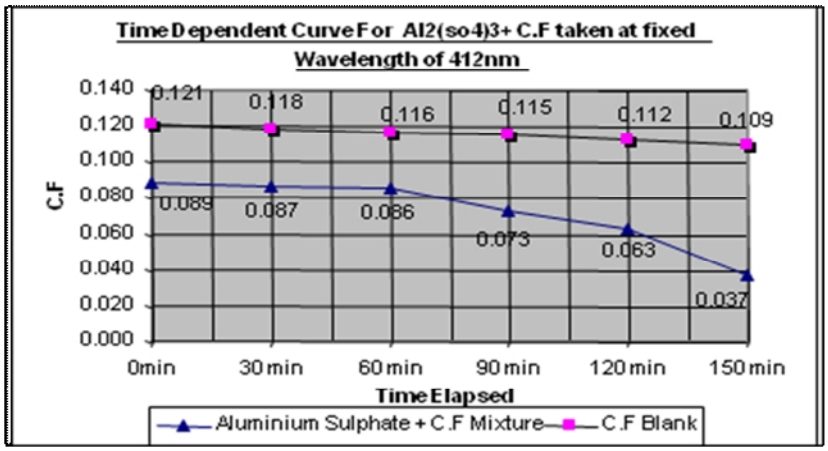

Figure 3- Time Dependent Curves For Control Thiol Level Of Cytosolic Fraction & $Al_2(so_4)_3$ effected Thiol Level of Cytosolic Fraction

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