STUDY OF THE CHEMICAL AND METABOLIC CHANGES IN PLASMA GLUTATHIONE (GSH) OF HUMAN BLOOD AFTER LITHIUM INTRODUCTION

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ABSTRACT

Lithium remains a mainstay in the acute and prophylactic treatment of bipolar affective disorder. It is used in the augmentation of antidepressant treatment and, less frequently, in the augmentation of antipsychotic treatment of schizophrenia. It is reported to have specific anti-suicidal effects. Systematic reviews by the Cochrane collaboration and others have examined the evidence base or its use in these contexts. Thus it is interesting to study the effect of Lithium on the Glutathione. The effect of Lithium on the chemical status of the glutathione in plasma has been studied using Ellman's method. The effect of Lithium on the chemical status of glutathione was determined in plasma for concentration and time dependent effects. There was found a drastic effect on decreasing the concentration of glutathione in plasma 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 Lithium metal on thiol /GSH level was discussed *in vitro*, which in principal may present a model of *in vivo* reaction.

Key words: Lithium, Glutathione (GSH), Blood, Plasma, Ellman's method

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INTRODUCTION:

Glutathione (g-glutamylcysteinylglycine, GSH) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is ubiquitous in animals, plants, and microorganisms, and being water soluble is found mainly 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. Glutathione synthesis occurs within cells in two closely linked, enzymatically controlled reactions that utilize ATP and draw on nonessential amino acids as substrates. First, cysteine and glutamate are combined (by the enzyme gamma-glutamyl cysteinyl synthetase, with availability of cysteine usually being the rate-limiting factor. Cysteine is generated from the essential amino acid methionine, from the degradation of dietary protein, or from turnover of endogenous proteins. The buildup of GSH acts to feedback-inhibit this enzyme, thereby helping to ensure homeostatic control over GSH synthesis.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). Lithium in pharmacology refers to the lithium ion, Li^+, used as a drug (Hecht *at el*., 2000). Lithium is administered in a number of chemical salts of lithium, which are used primarily in the treatment of Bipolar disorder as mood stabilizing drugs. In bipolar disorder they have a role in the treatment of depression and mania acutely and in the long term. As a mood stabiliser, lithium is probably more effective in preventing mania than depression, and may reduce the risk of suicide. In depression alone (unipolar disorder) lithium can be used to augment other antidepressants. Lithium carbonate (Li_2CO_3), sold as is the most commonly prescribed, whilst the citrate salt lithium citrate ($Li_3C_6H_5O_7$), the sulfate salt lithium sulfate (Li_2SO_4), aspartate and the orotate salt lithium orotate are alternatives (www.nlm.nih.gov). Lithium has affinity for the glutathione present in aqueous phases of blood. This affinity is mainly formed between metal and sulfhydryl groups of glutathione (Quig, 1998).This affinity can cause a depletion of the reduced form of 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 Blichi,1993) then the pharmacological benefits of the 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 Lithium, in respect of concentration and time, on glutathione level in plasma.

MATERIALS AND METHODS

L.Glutathione (GSH) (Fluka), Lithium Carbonate (Across, Belgium), Di,thiobis, dinitrobenzoic acid (DTNB), U.V 1601 spectrophotometer (Shimadzu). PH Meter: Model NOV-210, Nova Scientific Company Ltd

ISOLATION OF PLASMA

Sample of 5 ml of human venous blood was 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 plasma were 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.2.3ml 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 plasma 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 shimadzo 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.

Effect of different concentrations of Lithium Carbonate on the chemical Status of Glutathione (GSH) in plasma of Human Blood

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 the figure 1

To 1ml (1000µl) of plasma taken in five separate test tubes, 1ml (1000µl) of different concentrations of 0.4, 0.8, 1.2, 1.6 and 2mM solution of Lithium carbonate were added separately and shacked. Five separate test tubes was prepared with 0.2ml (200µl) Lithium carbonate 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 Lithium carbonate 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.

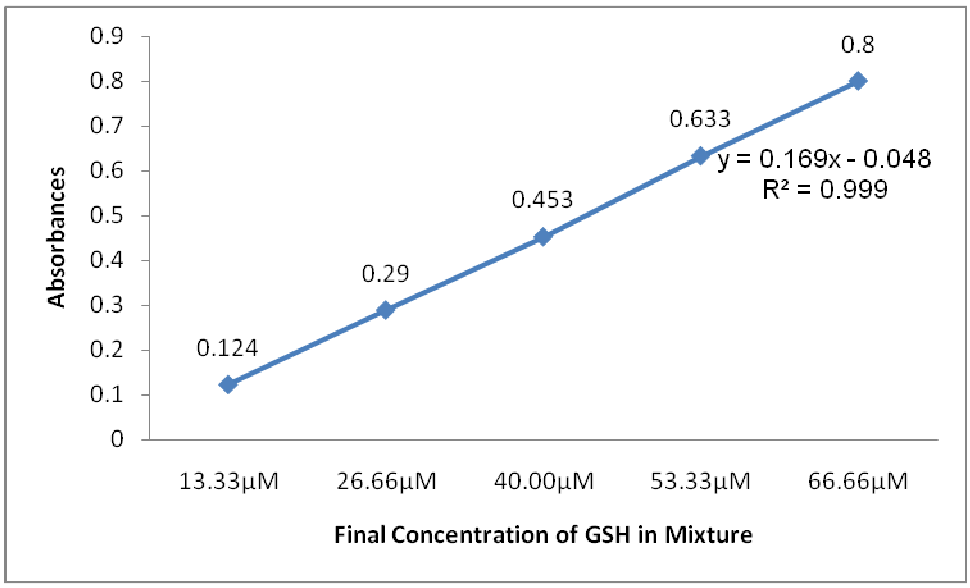

Figure 1- Standard Curve for Glutathione (GSH) + DTNB Mixture taken at fixed wavelength of 412nm

Effect of Lithium Carbonate on the Chemical Status of Glutathione (GSH) in Plasma with time

To 1ml (1000µl) of Plasma taken in a test tube, 1ml (1000µl) of 2mM solution of Lithium carbonate was added and shaked. The final concentration of Lithium carbonate was 1mM (500µM). A test tube with 0.2ml (200µl) Lithium carbonate 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 Lithium carbonate 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 Lithium carbonate 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.

RESULTS

Effect of Lithium on the Chemical Status of Glutathione (GSH) in Plasma

Effect of Lithium metal on the chemical status of glutathione present in plasma was studied in term of determination of concentration of glutathione.

Lithium metal caused a decrease in the concentration of glutathione present in plasma. GSH in plasma as the concentration of metal increased. Different concentrations of Lithium cause a gradual decrease in the concentration of glutathione.

Table 1. Effect of different concentrations of Lithium Carbonate on the chemical Status of Glutathione (GSH) in Plasma

Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank Solution was0.060 at 412nm								
S.NO	**Conc. Used of Li_2CO_3**	**Final Conc. of Li_2CO_3 in Mixture**	1st ABS	2nd ABS	3rd ABS	**Average of 3 Readings**	**Real absorbance***	**Real Absorbance for Plasma Blank**
1	**0.4mM**	**6.67µM**	0.530	0.517	0.511	0.519	**0.459**	**0.484**
2	**0.8mM**	**13.33µM**	0.512	0.499	0.493	0.501	**0.441**	**0.474**
3	**1.2mM**	**20.00µM**	0.496	0.483	0.477	0.485	**0.425**	**0.463**
4	**1.6mM**	**26.67µM**	0.476	0.463	0.457	0.465	**0.405**	**0.463**
5	**2mM**	**33.33µM**	0.450	0.437	0.431	0.439	**0.379**	**0.472**
***** Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution.***								

Effect of Lithium 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

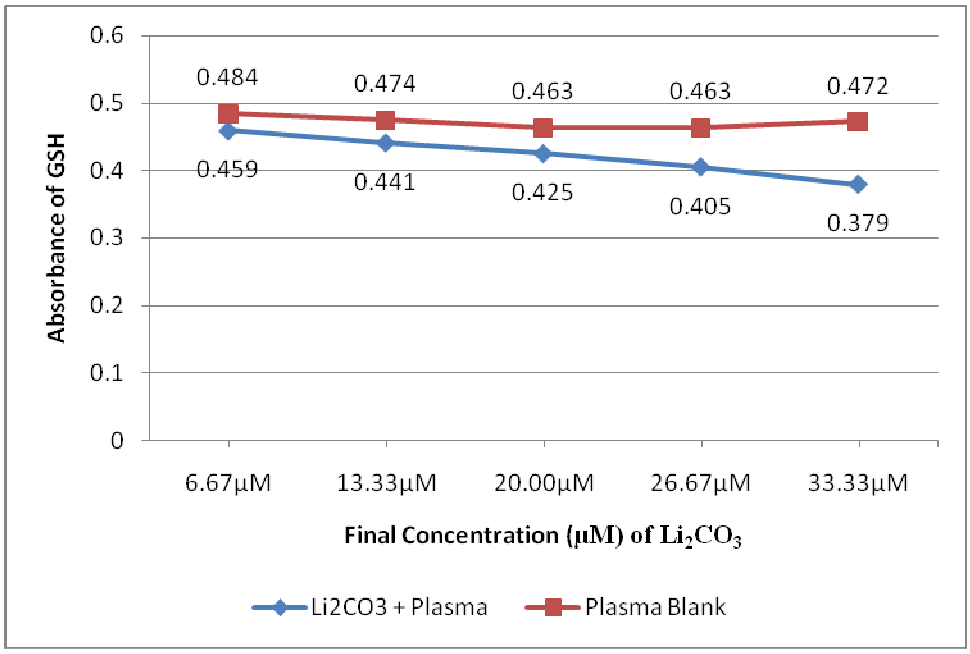

Figure 2-The effect of lithium metal (2mM) on GSH level.GSH level was determined before and after adding lithium metal.Upon addition of lithium metal in the plasma of blood,decrease in GSH level in plasma of blood was observed and found to be concentration dependent

Table 2. Calculation for Concentration of GSH after reaction with Lithium Carbonate by Ellman's Method

S/No.	Real Absorbance(ABS)	Concentration of GSH (µM) Remained in Plasma
1	**0.459**	39.52
2	**0.441**	38.04
3	**0.425**	36.73
4	**0.405**	35.09
5	**0.379**	32.96

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Table 3. Effect of Lithium Carbonate on the Chemical Status of Glutathione (GSH) in Plasma with time

Absorbance of 5,5-Dithiobis,2-Nitrobenzoic Acid (DTNB) blank Solution Was 0.060 ABS at 412nm								
Final Concentration of Lithium Carbonate was 33.33µM in Final Mixture								
S.NO	**Time Interval**	**1st ABS**	**2nd ABS**	**3rd ABS**	**Average of 3 Readings**	**Real absorbance***	**GSH Blank ABS**	**Real Absorbance for Plasma Blank**
1	0 min	0.450	0.440	0.457	0.449	0.391	0.550	0.492
2	30 min	0.439	0.427	0.445	0.437	0.379	0.540	0.482
3	60 min	0.426	0.414	0.432	0.424	0.366	0.545	0.487
4	90 min	0.420	0.408	0.426	0.418	0.360	0.538	0.480
5	120 min	0.417	0.405	0.423	0.415	0.357	0.543	0.485
6	150 min	0.403	0.391	0.409	0.401	0.343	0.530	0.472
***** *Real Absorbance = Absorbance of Mixture - Absorbance of DTNB blank Solution*								

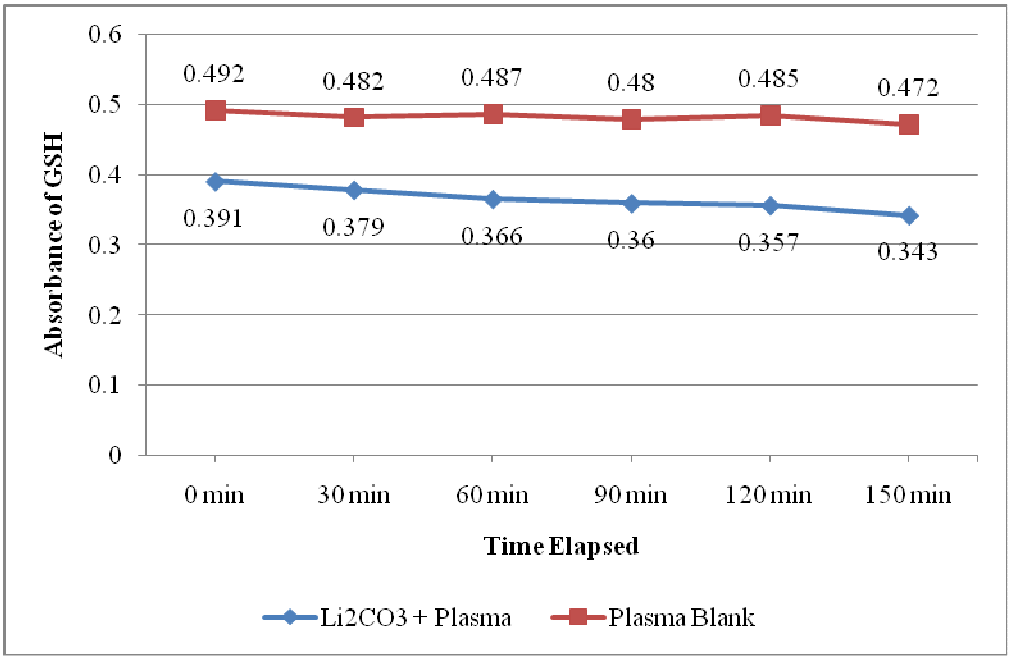

Figure-3 The effect of lithium metal (2mM) on GSH level.GSH level was determined before and after adding lithium metal.Upon addition of lithium metal in the plasma of blood,decrease in GSH level in plasma of blood was observed and found to be time dependent

Table 4. Calculation for Concentration of GSH after reaction with Lithium Carbonate by Ellman's Method

S/No.	Real Absorbance(ABS)	Concentration of GSH (μM) Remained in Plasma
1	0.391	33.94
2	0.379	32.96
3	0.366	31.89
4	0.360	31.40
5	0.357	31.16
6	0.343	30.01

Statistical Analysis for Effect of Lithium on the Chemical Status of Glutathione (GSH) in Plasma

Statistical approach for the effect of Lithium 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 Lithium and GSH blank gave the decision that there is an effect of Lithium on the chemical status of GSH in plasma with increase in concentration of Lithium, as compared to GSH blank solution treatment.

Table 5- Paired comparison t-test for concentration dependent effect of Li_2Co_3

	Effect of concentrations of Lithium on plasma Glutathione	GSH control solution(Blank)
Mean	0.4218	0.4712
Variance	0.0009692	7.67E-05
Observations	5	5
Pearson Correlation	0.55675918	
Hypothesized Mean Difference	0	
df	4	
t Stat	-4.054359181	
P(T<=t) one-tail	0.007710101	
t Critical one-tail	2.131846782	
P(T<=t) two-tail	0.015420201	
t Critical two-tail	2.776445105	

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

Table 6- Paired comparison t-test for time dependent effect of Li_2Co_3

	Effect of concentrations of Lithium on plasma Glutathione with time	**GSH control solution (Blank)**
Mean	0.366	0.483
Variance	0.000288	4.64E-05
Observations	6	6
Pearson Correlation	0.804503086	
Hypothesized Mean Difference	0	
df	5	
t Stat	-23.52580736	
P(T<=t) one-tail	1.29176E-06	
t Critical one-tail	2.015048372	
P(T<=t) two-tail	2.58351E-06	
t Critical two-tail	2.570581835	

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. Lithium 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 Lithium. 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 Lithium on the chemical modulation of GSH will enable us to understand further the role of, Lithium and GSH and strengthen our knowledge about their therapeutic uses in many diseases. The effect of Lithium was studied for the concentration and time dependent effects on the chemical status of glutathione and was found that the concentration of reduced glutathione was decreased with increasing concentration of Lithium metal in solution and with the passage of time, respectively. The following sequences of reactions are suggested to be happened in the experiment.

Equation

$$GSH + Lithium\ (Li) \rightarrow GS\text{-}Li$$

The results also suggested that there was a possibility of formation of intermediate or conjugate between Lithium and GSH. However it was not possible to estimate or determined those

conjugates under those conditions .Since both GSH and Lithium, 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 thiol 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 Liainst 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 thiol-exchange system**..**Different concentration of Lithium metal caused a gradual decreased in the concentration of Glutathione (GSH) in plasma. Effect of Lithium 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 150 minutes in plasma.

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