Effect of organo and inorganic lithium salt on human blood plasma glutathione- A comparative study

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Abstract: Investigation of toxicological effect of various metals is the field of interest for toxicological scientists since four to five decades and especially the toxicological effect of those drugs containing metals and there use is common because there is no other choice except to use these metal containing drugs. Inorganic as well as organic salts of lithium are commonly used in prophylaxis and treatments of many psychiatric disorders. The aim of the present study was to see the difference between the effect of organic and inorganic salt of lithium commonly used in psychiatric disorders on the GSH of human blood plasma. It is the scientific fact that ionic dissociation of organic and inorganic salts of any metal is always quite different hence to prove this fact, the effect of lithium citrate (organic salt of lithium) and lithium carbonate (inorganic salt of lithium) was investigated on human blood plasma GSH to find the difference between the effect of two. Ellman's method was used for the quantification of glutathione contents in plasma. It was found that lithium citrate decrease plasma GSH contents less than lithium carbonate indicating that organic salts of lithium are safe than inorganic salts of lithium when are used in psychiatric disorders. Further to analyze the effect of organic and inorganic salt of lithium on blood plasma GSH with the increase in incubation time was also evaluated and was found that both concentration and time dependent effect of organic salt of lithium shows that this salt has decreased plasma GSH contents of human blood less than inorganic salt of lithium either by promoting oxidation of GSH into GSSG or by lithium glutathione complex formation. These results suggest the physicians that the use of organic lithium salts is much safer than inorganic salts of lithium in terms of depletion of blood plasma GSH contents.

Keywords: Lithium citrate, toxicology, depletion, GSH (reduced glutathione), toxicity, time.

INTRODUCTION

Ample evidences are present in literature that among many causes, metals especially transition metals are the major cause of generation of free radicals or reactive oxygen species as hydrogen peroxide, super oxide and hydroxyl radicals (Halliwell and Gutteridge, 1986). This condition is called oxidative stress and oxidative stress leads to oxidative deterioration in the complex biological system resulting in depletion of GSH, DNA damage, lipid per oxidation (LPO), depletion of proteins sulfhydryl. When transition metals like cadmium, arsenic, aluminum mercury and other metals enter inside the human body through any source, the reduced glutathione promote a per oxidant effect by a metal reducing action (Khan H *et al.,* 2010, 2011a, 2011b and 2012; Muktiar *et al., 2012* and 2013; Shah *et al.,* 2013 & 2013a; Khan J *et al.,* 2012; Naseem *et al., 2015;* Hashmat *et al., 2015*). Depletion of glutathione results in neurological damage (Packer *et al*., 2001). GSH depletion may disturb all the physiological functions which are attributed to glutathione for example a). Scavenging of hydrogen peroxide, b). Other peroxides and free radicals, c). Preservation of SH group in a reduced form in proteins, enzymes and in all other molecules, d). Detoxification of foreign compounds and metals by their conjugation with reduced form of glutathione, e). Acting as a co-enzyme for some enzymes for example glyoxalase, f). Translocation of amino acids across cell membranes, g). Catalysis of disulfide exchange reaction (Meister, 1985), h) increased activity of nitric oxide synthetase (Heales *et al*., 1996), i) ROS i-e reactive oxygen species formation and per oxidation (Garcia *et al*., 1995). The concentration of reduced form of glutathione in cells reflects a steady-state balance between the synthesis of glutathione and its loss.

Depletion of GSH due to metals or conversion of GSH due to metals into GSSG impairs the GSH/2GSSG balance so increases contents of GSSG are considered as oxidative stress marker and thus 2GSH / GSSG redox couple can serve as an available index of oxidation redaction state (Slivka *et al*., 1987). Moreover "redox state" does not only represent GSSG/2GSH (redox pair) of the cells but it expresses more generally the redox environment of the cells (Schafer and Buettner, 2001). The 2GSH/GSSG couple is typical cellular redox buffer which represents the redox environment of the cell (Droge, 2002).

Recent studies have consistently reported increased production of ROS, lipid per oxidation, alterations in levels of GSH and in major antioxidant enzymes in people with bipolar disorders in which lithium

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compounds are use as drug of choice (Kuloglu *et al*., 2002; Ranjekar *et al*., 2003; Ozcan *et al*., 2004).

Lithium citrate (organo-lithium) was selected because it is used for the treatment and prophylaxis of mania, bipolar affective disorder, recurrent depression and aggression or self multilating behavior (Lang and Davis, 2002) and because of the reason that lithium is also used as anti-suicide agent as well.

It has been reported that lithium produces a variety of adverse effect even at therapeutic levels of lithium. These effect include hypotension, bradycardia i.e. cardio-vascular effect (Nciri *et al*., 2008) because there is a narrow margin between therapeutic dose and toxic level of lithium. Symptoms of lithium toxicity at the start may be drowsiness, vomiting, nausea, muscular weakness and pain, impaired coordination while with high doses the symptoms of toxicity are tinnitus, ataxia and twitching of facial muscles and limbs. Due to the toxic effects of lithium, death may occur because of seizures, arrhythmias and delirium. Therefore when aforementioned symptoms appear, immediately the patient should consult his/ her physician. Although lithium is used in the treatment of various acute and chronic diseases and abnormalities of human and is also used for its neuroprotective effects but daily use of lithium as interperitoneal injection for at least one month leads to transitory and increased serum lithium concentration able to generate an oxidative stress and a renal insufficiency (Nciri *et al*., 2009). Lithium is used in the form of lithium carbonate and lithium citrate as well as lithium acetate (Hal and Angelica, 2008), that is why we have decided to investigate the comparative effect of lithium carbonate and lithium citrate on the chemical status/ modulation of reduced form of glutathione of blood plasma.

MATERIALS AND METHODS

Materials

Disodium Edetate (Merck), NaOH (Sodium Hydrooxide, Merck), NaCl (Sodium Chloride, Fluka), Ethanol (Sigma), Chloroform (sigma), Lithium citrate (sigma) potassium dihydrogen phosphate (sigma), HCl (Kolchlight), Ellman's reagent, 5,5 Dithiobis, 2 nitrobenzoic acid (sigma) reduced form of glutathione (GSH) (Fluka), Distilled water (D/W), Water for injection. Beakers, flasks, graduated cylinders, and funnels of different sizes volumes made up of Pyrex glass were used in this piece of research work. Pyrex glass test tubes sterile, (Germany) and micropipettes made up of scorex company Finland were also used. UV-visible spectrophotometer of shimadzu model-1601, Japan, Pyrex eppendolf's tubes, centrifuge model H200 Kokuson Ensik company of Japan, pH meter (Nov-210, Korean Nov scientific company), disposable rubber gloves, sterile syringes of surg pharmaceuticals. Chemicals were used as were purchased without further any purification.

Methods

Required solutions preparation

From the available 37% HCl solution, its 0.9ml was dissolved in quantity sufficient of double refined distilled water to prepare 0.1N stock solution of HCl. 75.0ml of 1.0mM glutathione (reduced) solution was prepared by adding 23.055mg of GSH in 0.1N HCl solution. To prepare 0.9% sodium chloride solution, 90mg of NaCl was carefully weighed and dissolved in 100ml of double refined distilled water. 5:3 chloroform plus ethanol stock solution was prepared by adding 50ml of $CHCl_3$ to 30ml of C_2H_5OH. 0.2M phosphate buffer stock solution of pH 7.6 was prepared by mixing 42.4ml of 0.2M NaOH solution with 50ml of 0.2M potassium dihydrogen phosphate and final volume was made 200ml with quantity sufficient of water. 19.8mg Ellman's reagent i.e. 5,5-dithio-bis-2-nitro benzoic acid was dissolved in 50ml of 0.2M phosphate buffer to prepared 1.0mM DTNB stock solution. The pH of 0.2M phosphate buffer was adjusted to 7.6 with 0.2M NaOH and 0.2M KH_2PO_4 and pH meter.

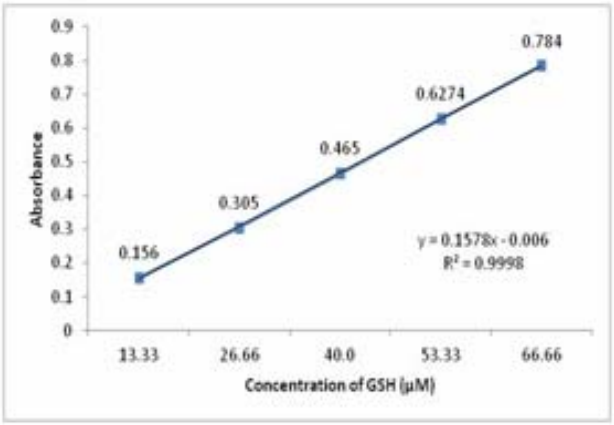

Fig. 1: Standard curve of GSH

Preparation and isolation of blood components

Isolation of human blood plasma

Fresh venous blood of a healthy human volunteer exactly 12ml was collected and immediately was treated with 500µl disodium edetate (0.5M) in order to avoid any clotting, as six different concentrations of lithium citrate/lithium carbonate were prepared, to each 1000µl concentration of lithium citrate/lithium carbonate, 1000µl of this blood was mixed and in this way 6 reaction mixtures -1 were prepared which were kept for 10 minutes to allow the different dilutions of lithium citrate/lithium carbonate and blood to incubate. In these reactions mixture-1 used concentration of lithium citrate/lithium carbonate was left from 0.00005µM to 1000µM. To separate plasma portion from whole blood, from these six reaction mixture-1 containing 2ml (1ml blood + 1ml of various metal concentrations), each of the reaction mixture-1 was centrifuged at 10000 rpm for 5 minutes. After the end of centrifugation, with the help of Pasteur pipette, 0.8ml plasma was removed from

supernatant fluid and each was transformed to sample tubes, which were kept on ice until analyzed. The lower layer underneath the supernatant fluid was layer of packed cells. This layer of packed cells was further processed for collection of cytosolic fraction.

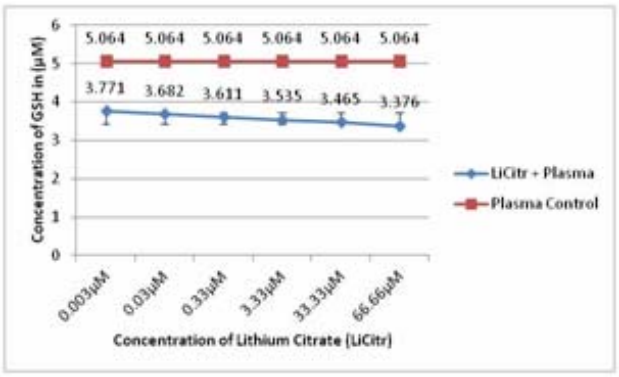

Fig. 2: Effect of different concentrations of lithium citrate (LiCitr) on the chemical status of plasma-GSH level (concentration effect). Results are the mean ±SE of 3 experiments of plasma fraction.

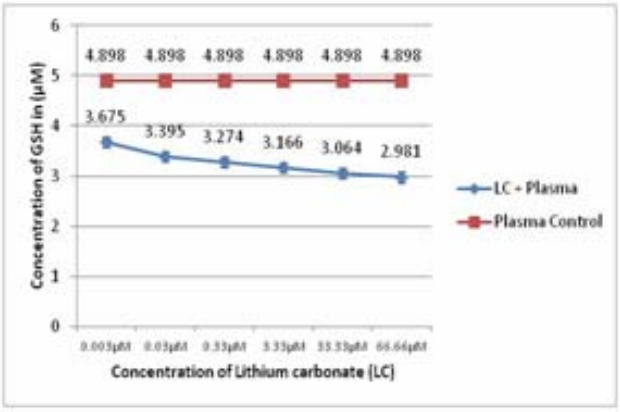

Fig. 3: Effect of different concentrations (0.0001mM, 0.001mM, 0.01mM, 0.1mM, 1.0mM & 2.0mM) of lithium carbonate (LC) on the chemical status of Plasma GSH. Results are the mean ±SE of 3 experiments.

Plasma control

1:1 mixture of fresh venous blood and 0.9% NaCl solution was prepared and centrifuged for the purpose of getting sample of plasma control.

Determination of glutathione contents of plasma fraction of human blood.

Ellman's method (Ellman's 1959) was used to estimate glutathione contents in plasma fraction of human blood. To 2300µl phosphate buffer 200 µl of sample (plasma fraction of blood) was mixed followed by the addition of 500µl of 1.0mM Ellman's reagent (DTNB). This reaction mixture was transformed to spectrophotometer cell (cuvette). Reference cell was containing phosphate buffer. Absorbance of each sample was recorded at fixed wave length λ_{max}: 412nm under U.V-visible spectrophotometer.

DTNB blank

DTNB blank solution was prepared by taking 2500µl of phosphate buffer to which 500µl of DTNB (Ellman's reagent was added). Its absorbance was recorded at λmax: 412nm under U.V-visible spectrophotometer against reference cell containing phosphate buffer.

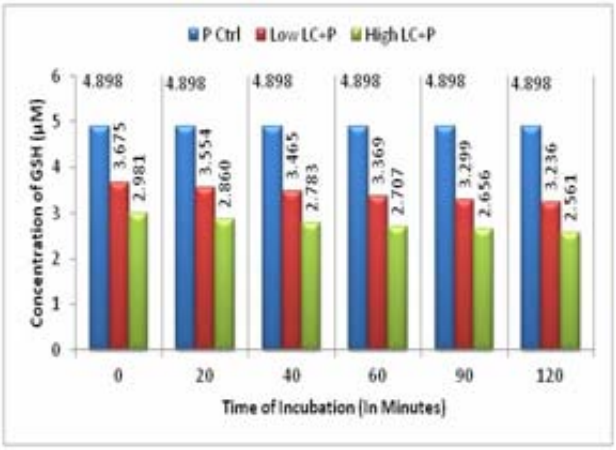

Fig. 4: Comparison between the decrease in concentration of plasma GSH by lowest used and highest use concentration of lithium carboanate (LC) at 0,20,40,60,90 and 120 minutes.

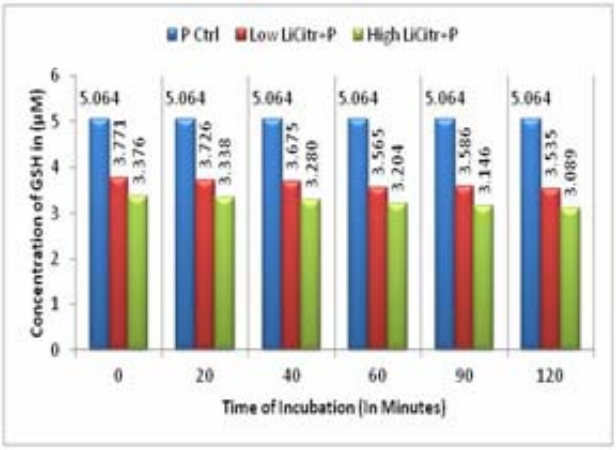

Fig. 5: Comparison between the decrease in concentration of plasma-GSH by lowest used and highest use concentration of lithium citrate (LiCitr) at 0,20,40,60,90 and 120 minutes.

Ethical consideration

Four healthy male volunteers age group 25-32 years were selected for this piece of research work. They were examined by physician to confirm that they are healthy and have no serious disease or medical history of long sickness due to any serious disease. The volunteers were provided with volunteer protocol before the start of study. The protocol contained terms and conditions of testing and this protocol was signed by each volunteer individually while the whole process was completed between temperature range 15°C to 40°C with relative humidity conditions during this temperature range.

Standard curve

Ellman's method was followed to prepare standard curve of GSH for which we have used 13.33µM-66.66µM of GSH (reduced form of glutathione).

Table 1: T-Test: Paired two samples for means in case of effect of LC on plasma GSH

t-Test: Paired Two Sample for Means		
	LC+Plasma	GSH Blank
Mean	3.259167	4.898
Variance	0.063147	0
Observations	6	6
Pearson Correlation	0	
Hypothesized mean Difference	0	
Df	5	
T-stat	-15.9748	
P(T<==t) one-tail	8.75E-06	
T Critical one-tail	2.015048	
P (< =t) two-tail	1.75E-05	
T Critical two-tail	2.570582	

Table 2: T-Test: Paired two samples for means in case of effect of LiCitr on plasma GSH

t-Test: Paired Two Sample for Means		
	LiCitr +Plasma	GSH Blank
Mean	3.573333	5.064
Variance	0.020889	0
Observations	6	6
Pearson Correlation	0	
Hypothesized mean Difference	0	
Df	5	
T-stat	-25.2637	
P(T<==t) one-tail	9.07E-07	
T Critical one-tail	2.015048	
P (< =t) two-tail	1.81E-06	
T Critical two-tail	2.570582	

RESULTS

Effect of lithium citrate on the plasma-GSH

It was found that there is statistically significant ($p<0.001$) decrease in plasma GSH level after the interaction of lithium citrate with plasma GSH. The remaining GSH level was up to 3.771µM (74.47%) after the interaction of lowest used concentration (0.003µM) of lithium citrate (LiCitr) while the remaining GSH level after the interaction of other used concentrations of lithium citrate (LiCitr) with plasma GSH level was 3.682µM (72.71%), 3.611µM (71.31%), 3.353µM (69.81%), 3.465 µM (68.42%) and 3.376µM (66.67%) respectively (fig. 2). With the increase in time of incubation between lithium citrate and plasma-GSH, it was found that there is further decrease in plasma-GSH level as the time passes from 0 minute to 120 minutes. The drop by various concentrations of lithium citrate (LiCitr) from 0 to 120 minutes was up to 3.771µM (74.47)%, 3.726µM (73.58)%, 3.675µM (72.57%), 3.665 µM (72.37 %), 3.586µM (70.81%) and 3.535µM (69.81%) respectively (figs. 5 and 7).

Effect of lithium carbonate on blood plasma GSH

It was noted that isolated plasma GSH contents were decreased significantly ($p<0.001$) by all the used concentration (0.0001-2.0mM) of lithium carbonate (LC) and after the interaction of various concentrations, the remaining GSH level was 75.03%, 69.31%, 66.84%, 64.64%, 62.56% and 60.86% respectively from lowest to highest used concentration of lithium carbonate (LC) (fig. 3) It was further found that with the increase in time of incubation between various concentration of lithium carbonate (LC) and plasma GSH contents results in significant ($p<0.001$) decrease in plasma level of GSH. The lowest used concentration (0.0001mM) of lithium carbonate (LC) has decreased GSH level of plasma 3.675 µM (75.03%) to 3.236µM (66.07%) from 0 to 120 minutes while the highest used concentration (2.0mM) of lithium carbonate (LC) has dropped plasma GSH level 2.981 µM (60.86%) to 2.561µM (52.29%) from 0 to 120 minutes (fig. 4,6).

STATISTICAL ANALYSIS

Statistical analysis was conducted to investigate the effect of lithium citrate/lithium carbonate on the chemical modulation of plasma GSH contents and it was found that using the 95% confidence interval (CI) level. Results are mean of ± SE. The statistical significance and difference from plasma GSH control and plasma GSH plus salt test values obtained from t-test paired two samples for means gave the decision that there is an effect of lithium citrate/lithium carbonate on plasma GSH as shown in tables 1 and 2.

DISCUSSION

Glutathione is a low molecular weight, three peptide molecule which is present inside as well as outside the cells of all organisms but inside the cells it reaches relatively to high concentration i.e. 0.1-10mM (Deneke and Fanburg, 1989). Being 90% intracellular non protein thiol makes it important intracellular reducing agent. It is found both in free and bound form in cytosol, nucleus and mitochondria (Sen *et al*., 1992).

The depletion or decrease in its concentration regardless in intracellular or extra cellular compartments may cause serious metabolic changes in these compartments because disturbance in GSH/ GSSG ratio leads to imbalance redox status of the cell. Our study shows that lithium carbonate although is common in use for the treatment of many

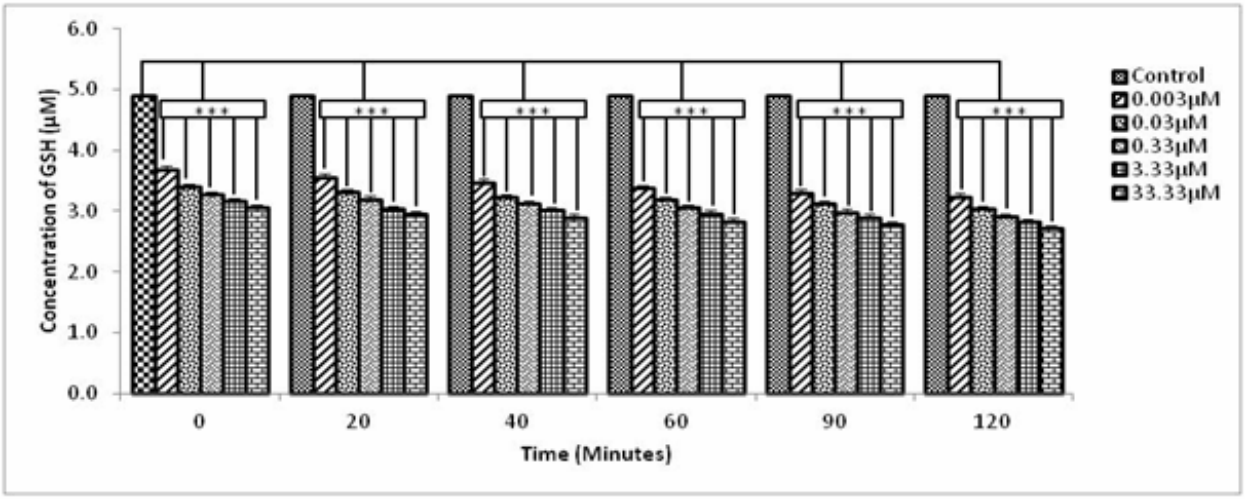

Fig. 6: Result of effect of different concentration of lithium carbonate (LC) on plasma GSH with time (i.e. 0 min, 20min, 40min, 60min, 90min, 120min) Results are the mean ± SE of 3 experiments

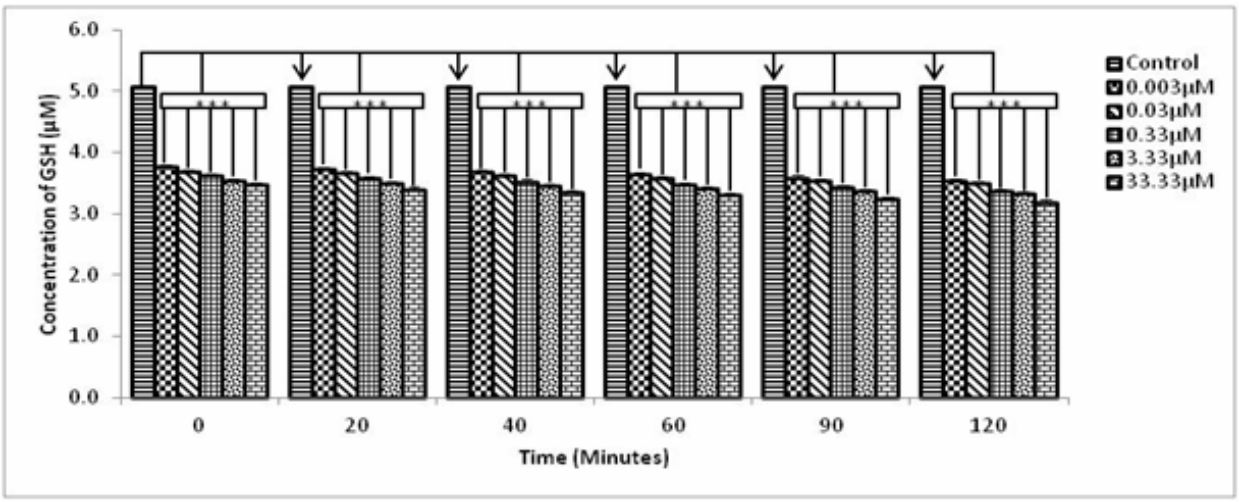

Fig. 7: Result of effect of different concentration of lithium citrate (LiCitr) on plasma GSH with time (i.e. 0 min , 20min, 40min, 60min, 90min, 120min) Results are the mean ± SE of 3 experiments

psychiatric disorders and as an anti suicide agent but at the same time it is depleting GSH in the above mentioned compartment more than organic salt of lithium therefore suggesting that there should be a check and balance in its use as there is a narrow margin between the therapeutic dose and toxic effect of lithium. We also suggest that green vegetables and fruits should be taken during lithium therapy especially when inorganic lithium salt is used as published data shows that glutathione contents depends on the type of nutrition (Tylor *et al*., 1996). Several enzymes are involved in the metabolism of glutathione so cellular contents of GSH are not constant and depend on the rates of synthesis, conjugation with metals like lithium, oxidation of glutathione etc (Viarengo *et al*., 1991; Porte *et al*., 2000). Lithium citrate/lithium carbonate has depleted plasma glutathione indicating the possibility of either lithium glutathione complex formation or oxidation of GSH into GSSG by the following possible reactions.

$$C_6H_5Li_3O_7 + 3GSH \longrightarrow 3Li\text{-}SG + C_3H_5(COO)_3^{3-} + 3H^+$$

$$2GSH \longrightarrow 2H^+ + 2GS^{-1}$$

$$GS^{-1} + GS^{-1} \longrightarrow GSSG$$

There is possibility of other intermediates however under the giving conditions in which the experiment was performed, it was not possible to determine these intermediates. In the presence of xenobiotics or stress, reduced form of glutathione is converted into its oxidized form and an elevated GSSG concentration is considered as oxidative stress marker as in the case of lithium citrate ($C_6H_5Li_3O_7$) in general and in lithium carbonate in particular while the GSH/GSSG oxidation reduction pair can serve as an available index of oxidation reduction state (Slivka *et al*., 1987). Glutathione performs a large number of physiological functions including scavenging of hydrogen peroxide, other peroxides and free radicals like metals which come inside into human body either as pollutant or along with drugs as a part of drugs as lithium salts (in the form of lithium carbonate, lithium citrate,

lithium orate) and cisplatin etc in the treatment of psychiatric disorders and cancer respectively. GSH involved in catalysis of disulfide exchange reaction (Meister, 1985). As the time passes, there is further, more and more depletion in GSH contents of blood plasma, it is probably due to the property of lithium that it has greater affinity to interact with glutathione as lithium is mano-valent and can easily lose its one electron present in its outermost shell and becomes Li^+ ion in this environment. Thus lithium easily interact with GSH and chemically becomes attached with it by forming possibly the Li-SG adduct. In this situation there is great possibility of conversion of GSH into GS^{-1} (thiyl ion) in the presence of lithium thus forming GSSG form of glutathione. So depletion of GSH either by its conversion into GSSG or metal-adduct formation leads to cell death and has been documented in many degenerative conditions. In short glutathione plays a vital and key role in cellular defiance and serves as a store/ reservoir to restore the cellular defense system in intact state.

Hence glutathione as career of lithium binds with lithium, forms a complex that prevent lithium from binding to cellular proteins and causing damage to both enzymes as well as tissues and it seems that glutathione lithium complex reduces intracellular damage by preventing lithium from entering tissues cells and becoming an intracellular toxin. Our study suggests that pre-causations and causations should be exercised during the treatment of diseases (psychiatric disorders) for which the lithium compounds are considered as drug of choice because depletion of GSH either by GS-Li complex formation or by GSSG formation may give birth to many other serious physiological abnormalities resulting in cells apoptosis. Results of our present study endorse our previous findings (Khan *et al*., 2010) that lithium carbonate depletes plasma GSH contents and new finding is that comparatively plasma GSH contents are decreased more by inorganic salts of lithium than organic salts of lithium suggesting that physicians should prefer organic lithium salts then inorganic lithium salts while prescribing lithium salts in the treatment of some psychiatric disorders.

CONCLUSION

Organic salt of lithium depletes plasma GSH less than inorganic salt of lithium indicating that inorganic form of lithium is more harmful to antioxidant system of human body than organic form of lithium. Our results suggest physicians that they should prefer organic salts of lithium while prescribing lithium therapy for the treatment of different psychiatric disorders of their patients.

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