



## GLUTATHIONE;

### IS IT DEPLETED IN T-CELLS AND B-CELLS WHEN LITHIUM COMPOUNDS ARE USED AS DRUG OF CHOICE IN MANY PSYCHIATRIC DISORDERS?

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**ABSTRACT...Background:** Compounds of lithium are used as drug of choice in many psychiatric disorders including bipolar disorder, depression, schizophrenia etc. **Objective:** The aim of this study was to analyze the effect of lithium on lymphocyte's GSH levels for which terasaki technique was used to separate T-cells and B-cells of human volunteer's venous blood. **Study Design:** Experimental Study. **Setting:** Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University, Dera Ismail Khan. **Period:** 1<sup>st</sup> December 2012 to 26 February 2013. **Statistical Analysis:** One-way ANOVA followed by Dunnet's HSD test. **Results:** Thiol quantification was done by using Ellman's method and was found statistically significant ( $p < 0.001$ ) decrease in T-cells/B-cells GSH level which was dose and time dependent. T-cells/B-cells dose dependent drop in GSH level was  $2.752\mu\text{M}$  (9.41%) and  $2.554\mu\text{M}$  (16.12%) by lowest used concentration ( $0.003\mu\text{M}$ ) of lithium citrate. **Conclusion:** We have noted that there is significant drop in T-cells and B-cells GSH due to which immunological alterations happen which are linked with GSH contents of lymphocytes and hence inhibition in lymphocytes activity is co-related with depletion in GSH level of these cells which ultimately with the increase in  $\text{Li}^{+1}$  concentration cause further decrease in GSH level leading to cells death.

**Keywords:** Lithium citrate, Terasaki technique, T-cells, B-cells, Depletion.

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## INTRODUCTION

When there is talk of psychiatric disorders, lithium comes first because lithium compounds are used as time tested medication in psychiatric disorders including bipolar disorder, depression, schizophrenia etc. Lithium is even used to treat the epileptic patients where lithium has shown drop in seizure and improved patient behavior. About 70 to 90 percent patients on lithium therapy show symptoms of toxicity at some stage during their course of treatment<sup>1</sup>. Serum level of Li from 0 to 1.2-6 m mole/ L may be a serious level of lithium side effects<sup>2,3,4</sup>. Various toxic states may appear at serum level 1.5mM per litter while kidney damage or death may occur when its serum level increases from 2.5mM per litter<sup>5</sup>.

Toxicity of lithium can modulate T and B-cells functions for example cell growth and production of cytokine and many other vital cellular processes which leads to apoptotic cell death<sup>7,8</sup>. Lithium toxicity is very complex which depends on dose

and age of patient at exposure. In humans as lithium is used for the treatment of many psychiatric disorders and even for prophylaxes of many other such conditions and as lithium has narrow index between its therapeutic and toxic dose so there is always chance of lithium toxicity which can be control by constant monitoring of the patient's health profile. Lithium induced toxicity, regarding the formation and role of reactive oxygen species in case of T-cells and B-cells is not well known. Lithium mediated generation of free radicals results in various modifications of DNA, increase lipid peroxidation, alters  $\text{Ca}^{+2}$  and sulfhydryl homeostasis thus lipid peroxides produced by the action of free radicals on poly-unsaturated fatty acids residues of phospholipids will further react with redox metals which finally produced mutagenic and carcinogenic malondialdehydes exocyclic DNA adducts like etheno and propano adducts etc. Bonding to sulfhydryl group of glutathione and proteins is the primary route of lithium toxicity which results in depletion of

glutathione. Lithium causes oxidative stress via production of reactive oxygen species, reducing antioxidant defense system of all cells including T-cells and B-cells GSH, interfering some essential metals inhibiting sulfhydryl reliant enzymes and antioxidant enzymes activities thus increasing the sensitivity of cells to oxidative attack by altering membrane integrity and fatty acid composition<sup>9,10</sup>.

The aim of present study was quantification of thiol contents in T-cells and B-cells by using Ellman's method of thiol quantification to analyze that up to what extent thiol contents of these lymphocytes are depleted when they were treated with various concentrations of lithium citrate for which concentration and time dependent effect of lithium citrate on T and B-cells was evaluated.

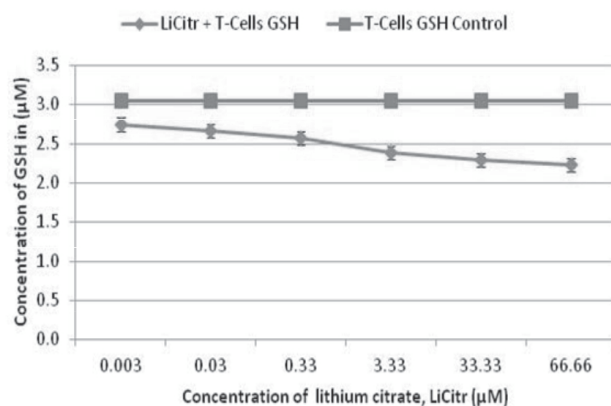
**MATERIALS**

Potassium dihydrogen phosphate (Merck), Reduced glutathione (Fluka), RPMI-1640, Ellman's reagent (DTNB), Fetal calf serum (>98%; agarose gel electrophoresis lyophilized), Ficol paque plus were purchased from Sigma Aldrich. Sodium dihydrogen phosphate (Merk), were purchased from (fluka), Hydro-chloric acid, HCl 35% (Kolchlight), (10M Perchloric Acid 70% (fluka), Sodium- chloride ,NaCl (Merck), Chloroform, CHCl<sub>3</sub> (Merck), Ethanol (Merck), pH-Tablets (pH; 4 & 7), NaCl (Fluka), Sodium edetate (Riedel Dehean AG Sneeze Hannover),Dextrose were purchased from (Merck). Distilled water (Double refined), Rubber gloves (Disposable). Magnetic Stirrer, U.V-visible spectro-photometer (Schimadzu, 1601 Japan),pH meter (NOV-210, Nova Scientific Co. Ltd, Korea), Hot plate-400(England), Oven: Memmert Model U-30,854 Schwabach (Germany), Micropipettes of 100 µl, 200 µl, 500 µl, 1000 µl (Socorex Swiss, Finland), Eppendorf's tubes (Plastic, 10l),Centrifuge (H-200, Kokusan Ensink company Japan), Potter-eveljhem homogenizer (japan), Sterile pyrogen free disposable syringes (Surge Pharmaceuticals), Analytical weighing balance AX 200 (Schimadzu, Japan), Siliconized glass test tubes, Pyrex (Germany) glassware, which were carefully washed with detergent, chromic mixture, distilled water (double refined) and organic solvents etc.

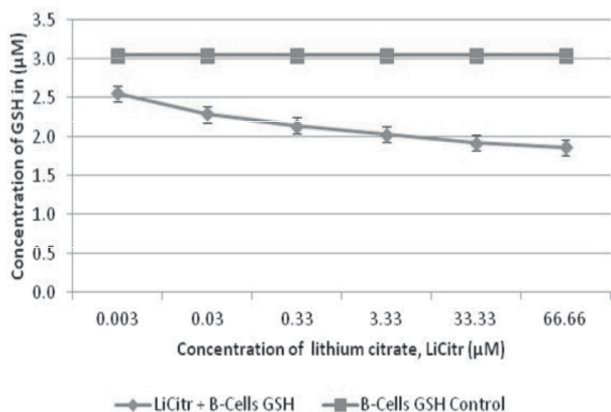
All apparatus were dried at 110°C for two hours in oven. Chromatographic column, Glass pastuer pippets (disposable), Pipette tips of different sizes (100 µl, 200 µl, 500 µl 1000 µl) were used.

**METHODS**

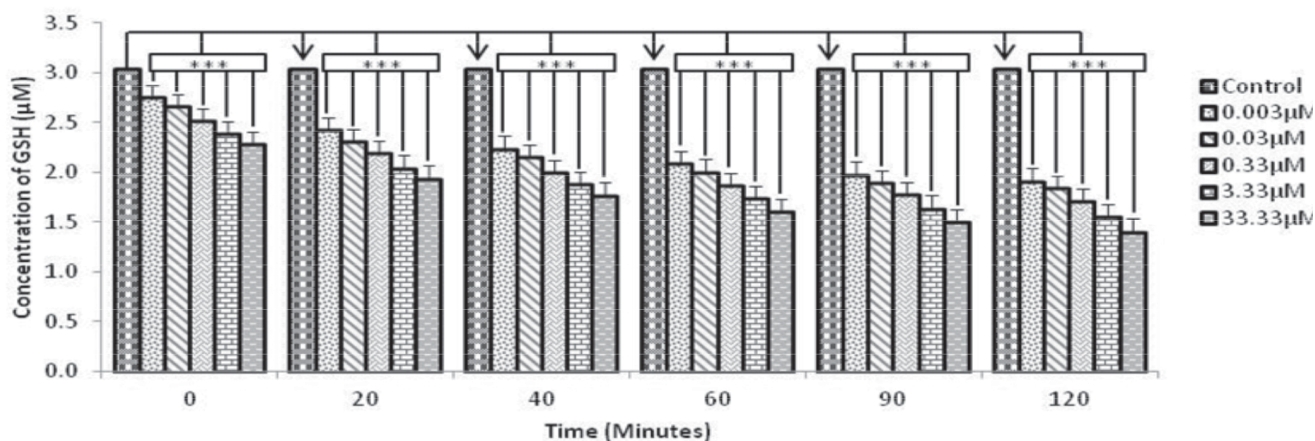
Effect of organo-lithium (lithium citrate) was investigated on isolated T-cells/ B-cells GSH, for which various concen-trations (0.0001-2.0mM) of lithium citrate were used. Absorbance of each sample mixture was recorded under UV-visible spectrophotometer at fixed wave length λmax: 412 nm and then each absorbance were converted to concentration of T-cells/ B-cells GSH. This concentration of unknown T-cells/B-cells GSH left after the interaction of various concentrations of lithium citrate with T-cells/B-cells GSH was calculated by using standard curve for known



**Fig-1 Concentration dependent effect of lithium citrate on T-cells GSH**



**Fig-1 Concentration dependent effect of lithium citrate on B-cells GSH**



**Fig-3. Time dependent effect of lithium citrate on T-cells GSH**  
One-way ANOVA followed by Dunnet's HSD test was applied. \*\*\*  $p < 0.001$ .

concentration of GSH.

was calculated by using standard curve for known concentration of GSH.

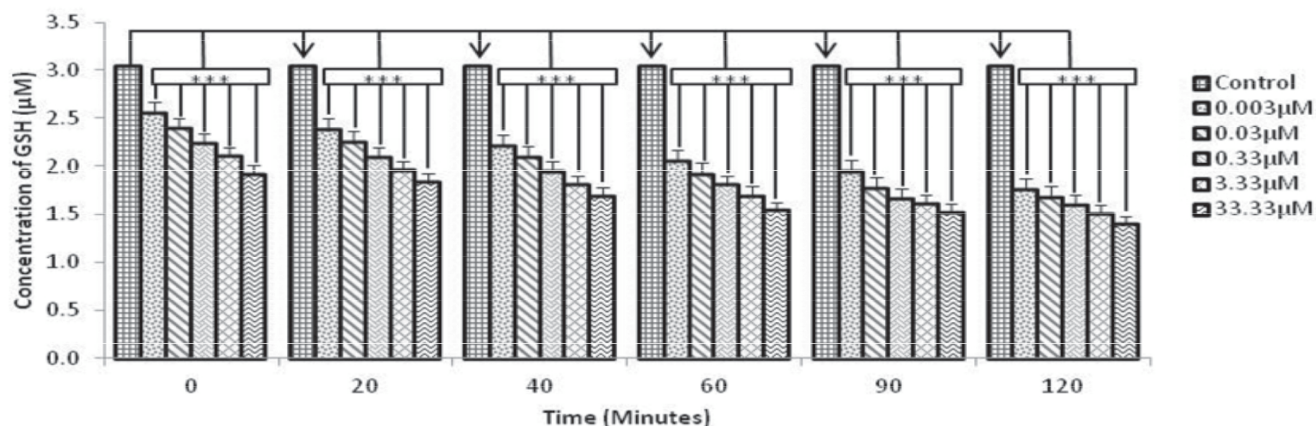
## RESULTS

Isolated T-cells/ B-cells GSH was exposed to these selected concentrations of lithium citrate and it was found that there is statistically significant ( $p < 0.001$ ) decrease in T-cells GSH level as well as in B-cells GSH level. In case of T-cells (Fig-1), the drop in GSH level was  $2.752\mu\text{M}$  (9.41%) by lowest used concentration ( $0.003\mu\text{M}$ ) of lithium citrate while the drop by other used concentrations of lithium citrate in case of T-cells GSH level was  $2.662\mu\text{M}$  (12.38%),  $2.580\mu\text{M}$  (15.08%),  $2.389\mu\text{M}$  (21.36%),  $2.299\mu\text{M}$  (24.33%) and  $2.229\mu\text{M}$  (28.17%) respectively. The B-cells GSH level was decreased significantly ( $p < 0.001$ ) which was  $2.554\mu\text{M}$  (16.12%) by lowest used concentration of lithium citrate while the drop in B-cells GSH by other used concentrations of lithium citrate was  $2.287\mu\text{M}$  (24.89%),  $2.240\mu\text{M}$  (26.44%),  $2.032\mu\text{M}$  (33.27%),  $1.924\mu\text{M}$  (36.81%) and  $1.866\mu\text{M}$  (38.72%) respectively (Fig-2). The decrease in B-cells GSH level is greater than decrease in T-cells GSH level showing that lithium citrate has the penetrating capability into the semipermeable membrane of B-cells of human blood.

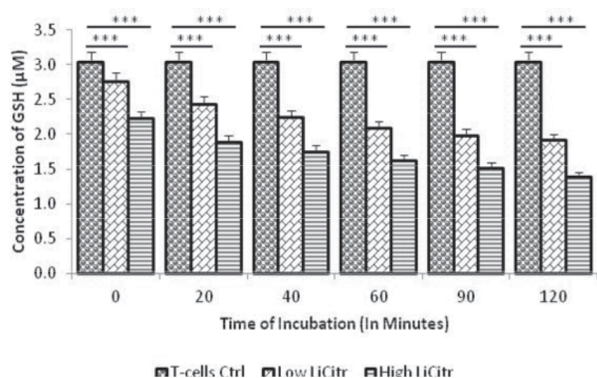
T-cells/B-cells GSH contents were also exposed to various concentrations ( $0.0001$ - $2.0\text{mM}$ ) of lithium citrate for different time of incubation which were 0,20,40,60,90 and 120 minutes

(intervals). It was found that there is further significant ( $p < 0.001$ ) decrease in T-cells/B-cells GSH level as the incubation time between various concentrations of lithium citrate and T-cells/ B-cells GSH increases. In case of T-cells GSH level the drop by various concentrations of lithium citrate from 0 to 120 minutes (Fig-3) was up to  $1.911\mu\text{M}$  (37.10%),  $1.841\mu\text{M}$  (39.40%),  $1.771\mu\text{M}$  (41.70%),  $1.561\mu\text{M}$  (48.62%),  $1.490\mu\text{M}$  (50.95%) and  $1.433\mu\text{M}$  (52.83%) in time interval 0-120 minutes respectively with respect to T-cells control ( $3.038\mu\text{M}$ ) while in case of B-cells GSH level the drop by these used concentrations of lithium citrate from 0 to 120 minutes (Fig- 4) was  $1.764\mu\text{M}$  (43.93%),  $1.682\mu\text{M}$  (46.54%),  $1.599\mu\text{M}$  (49.17%),  $1.510\mu\text{M}$  (52.00%),  $1.433\mu\text{M}$  (54.45%) and  $1.350\mu\text{M}$  (57.09%) respectively with respect to B-cells control ( $3.146\mu\text{M}$ ).

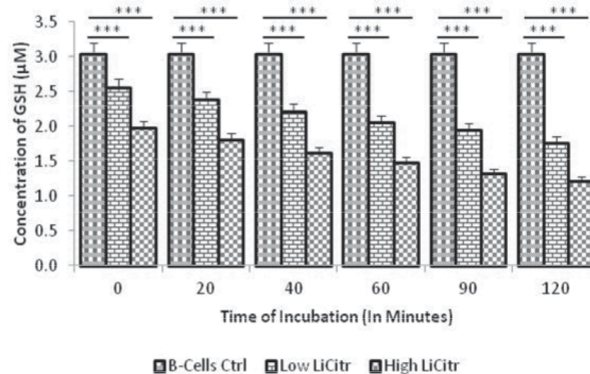
Fig-5,6 Shows a comparison between the effect of lowest and highest used concentrations of lithium citrate on T-lymphocytes-GSH and B-lymphocytes respectively with time of incubation from 0 min: to 120 minutes. Blue bars show T/B-lymphocytes-GSH control. Red bars indicate the effect of lowest used concentration of lithium citrate and green bars show highest used concentration of lithium citrate. In all experiments, results are the mean  $\pm$  SEM of three experiments of T/B-lymphocytes-GSH. The significance of differences in various groups was evaluated by One-way ANOVA followed by Dunnet's HSD test (\*\*\*)  $p < 0.001$ .



**Fig-4. Time dependent effect of lithium citrate on B-cells GSH**  
 One-way ANOVA followed by Dunnet's HSD test was applied. \*\*\* p < 0.001.



**Fig-5. Effect of lowest and highest used concentration of lithium citrate on T-cell-GSH**  
 One-way ANOVA followed by Dunnet's HSD test. \*\*\*p < 0.001



**Fig-6. Effect of lowest and highest used concentration of lithium citrate on B-cell-GSH**  
 One-way ANOVA followed by Dunnet's HSD test. \*\*\*p < 0.001

**DISCUSSION**

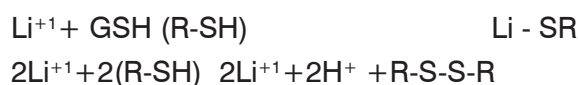
A system inside the human body that protects it from attack of various diseases and foreign particles including heavy metals etc is called as immune system which is a system of biological structures and processes. Depletion of antioxidant glutathione results in weak immune system which is the disorder of the immune system causing auto immune disease, inflammatory disease and cancer<sup>11</sup>. Lymphocytes are the special types of leukocytes which are parts of adaptive immune system and have two types, T-lymphocytes and B-lymphocytes. These are derived from hematopoietic stem cells in bone marrow<sup>12</sup>. T-lymphocytes and B-lymphocytes are involved in cell-mediated immune response and humoral immune response respectively. Reduced glutathione is necessary for various functions of both innate and adaptive immune

systems including T-lymphocytes proliferation<sup>13,14</sup>, pathocytic activity of polymorphonuclear neutrophils<sup>15</sup>.

In our study, the findings shows that the organic compound of lithium have depleted GSH in T-lymphocytes and B-lymphocytes which was dose and time dependent. These findings also indicate that GSH acts as first line defense against lithium metal toxicity and thus protects human body from the toxic effects of this metal either by forming metal-SG adducts by making single covalent bond with this metal or converting it in to GSSG form after giving an electron to free radicals thus stopping free radicals from chain reactions. Glutathione becomes attached with xenobiotics and their metabolits by conjugation<sup>16</sup> while its hydrophilicity greatly increase the aqueous solubility of lipophilic moieties with which it

becomes attached through its -SH (sulfhydryl) group. However there are two proposed possibilities of conversion of T-lymphocytes and B-lymphocytes GSH by lithium to a) conversion of GSH to oxidized form b) conversion of GSH to Li-SG complex<sup>17</sup>.

Proposed reactions can be written in the form of equation as



Cellular GSH concentration is closely linked with cellular smash up that can results from oxidative stress due to metals like lithium<sup>18</sup>.

## CONCLUSIONS

Conjugation reactions of thiol have great importance in bio-transformation and excretion of toxic metabolites of chemicals, drugs and their metal salts hence thiol conjugation with  $\text{Li}^+$  will indicate importance of GSH as protectant against metal toxicity and chelating properties of GSH as well. Moreover 'YES' lithium therapy should be given with great care in psychiatric disorders as our study has proved experimentally that lithium causes depletion of GSH in T and B-lymphocytes which ultimately will results in compromised immune system of psychiatric patients.

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