

IMPACT OF DEPLETION OF ERYTHROCYTE CYTOSOLIC-FRACTION GLUTATHIONE BY ORGANIC LITHIUM COMPOUND ON IMMUNE SYSTEM

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ABSTRACT

Background: Lithium organic compounds are considered safe in use as compare to inorganic compounds of lithium for the treatment of various psychiatric disorders. The aim of present study was to check the effect of lithium citrate on tripeptide master antioxidant glutathione of cytosolic fraction of human blood.

Material & Methods: It was an experimental study which was conducted in PhD research laboratory in Faculty of Pharmacy, Gomal University, D.I.Khan, Pakistan, from January 2013 to March 2014. Blood components were separated by centrifugation at 10000 – 12000 rpm for 10 minutes method. The supernatant pale yellow layer was cytosolic fraction or lysate. 50µl of 0.1N HCl solution was added to keep GSH in reduced form.

Results: The cytosolic fraction GSH level was decreased significantly ($p < 0.001$) which was $3.739 \mu\text{M}$ (72.66%) by lowest used concentration of lithium citrate while the drop in GSH by other used concentrations of lithium citrate was $3.624 \mu\text{M}$ (70.42%), $3.567 \mu\text{M}$ (69.32%), $3.484 \mu\text{M}$ (67.70%), $3.420 \mu\text{M}$ (66.46) and $3.338 \mu\text{M}$ (64.87%) respectively.

Conclusion: Although organic compounds of lithium are said to be safer in use as compare to inorganic compounds of lithium but our study suggests that lithium citrate can considerably depletes GSH in cytosolic fraction of human blood.

KEYWORDS: Lithium citrate; Tripeptide; Human blood; Organic compounds.

This article may be cited as: Ullah H, Khan MF, Bakhsh S, Khan H, Hashmat F, Shaikh U. Impact of depletion of erythrocyte cytosolic-fraction glutathione by organic lithium compound on immune system. Gomal J Med Sci 2016; 14:75-8.

INTRODUCTION

The concentration of reduced glutathione (GSH) is in millimolar in many tissues¹ making it a potent and in abundant intracellular tripeptide. Cytosolic fraction of cells contains 80 to 90% while its remaining concentration is found in many cell organelles. In plasma its concentration is 2 to 20 $\mu\text{mol/L}$.² It is stored mainly in liver and it is released to blood stream to protect WBCs and maintain the integrity of RBCs.³ Depletion of GSH in intracellular compartment is thought to be associated with a va-

riety of diseases including alcoholic liver disease⁴, liver disorders, cancer, and diabetes.⁵ Glutathione is a nucleophilic scavenger of many chemical compounds and there metabolites through enzymatic and chemical mechanisms thus gives protection against oxidative damage which is caused due to reactive oxygen species.⁶

Lithium salts have been used therapeutically for almost 150 years, beginning with its use for the treatment of gout (or uric acid diathesis) in the 1850s.⁷ Although gout was believed to include symptoms of mania and depression, it wasn't until 1880s that John Aulde and Carl Lange observed that lithium could be used to treat symptoms associated with depression, independent of gout.⁷ However the use of lithium became problematic and was discarded due to the serious toxicity associated with the widespread use of lithium in tonics, elixirs and as salt substitute.⁷

Lithium toxicity occurs when the level of lithium in the blood becomes too high and the signs

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Date Submitted: 22-3-2016

Date Revised: 13-6-2016

Date Accepted: 16-6-2016

of lithium toxicity are Shaking and trembling, confusion, slurred speech, nausea, vomiting, Diarrhea, Abdominal pain, Unsteadiness on the feet, coma and seizures while the patients who have lithium toxicity usually look sick, pale, gray and weak. Normally, lithium is not present in significant amounts in body fluid (<0.2 mEq/L), however lithium salts have been used therapeutically for almost 150 years, beginning with its use for the treatment of gout (or uric acid diathesis) in the 1850s.⁷ Although gout was believed to include symptoms of mania and depression, it wasn't until 1880s that John Aulde and Carl Lange observed that lithium could be used to treat symptoms associated with depression, independent of gout.⁷ However the use of lithium became problematic and was discarded due to the serious toxicity associated with the widespread use of lithium in tonics, elixirs and as salt substitute.⁷ The concentration of lithium within the cerebrospinal fluid is only 40% of serum levels due to its transport out of the cerebrospinal fluid by brain capillary endothelium and/ or arachnoid membranes.⁸ Lithium has a variable half life within plasma. Factor altering its half-life include patient age, duration of lithium therapy and level of renal function.⁸ Lithium has an elimination half-life of 12 to 27 hours after a single dose but its elimination half-life can increase to as long as 58 hours in elderly individual or patient taking lithium chronically.⁸ Thus, one must measure lithium levels several times after a toxic ingestion, because its rate of illumination is variable and cannot be predicted in any given patient. The pharmacokinetic disposition of lithium is generally described as an open, two compartment model, and clinical comparisons by convention are made using serum samples acquired during the terminal phase (at least 10 hours after an oral dose).⁹

The aim of present study was to check the effect of lithium citrate on tripeptide master antioxidant glutathione of cytosolic fraction of human blood.

MATERIAL AND METHODS

The centrifugation method for the separation of cytosolic fraction of red blood cells was used and isolated cytosolic fraction was treated with different concentrations of lithium citrate. For centrifugation process Centrifuge, H-200, Kokusan Ensink company Japan was used which is considered time tested for the separation of blood components. The other chemicals which were used in this piece of work were RPMI-1640, Glutathione (Fluka), Ellman's reagent.

RESULTS

Effect of organo-lithium (lithium citrate) was investigated on isolated cytosolic fraction GSH, for which various concentrations (0.0001-2.0mM) of lithium citrate (LiCit) were used. Isolated cytosolic fraction GSH was exposed to these selected concentrations of LiCit and it was found that there is statistically significant ($p < 0.001$) decrease cytosolic

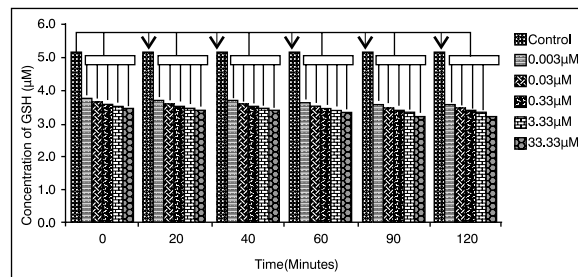


Figure 1: Result of effect of different concentration of lithium citrate on cytosolic fraction GSH with time.

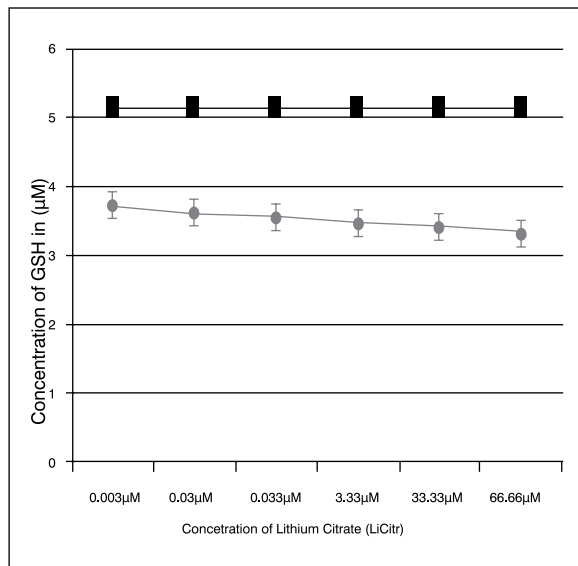


Figure 2: Effect of different concentrations of Lithium citrate.

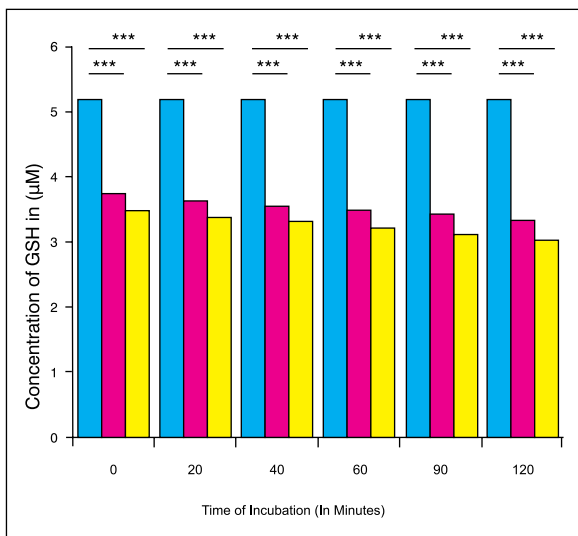


Figure 3: Comparison between the decrease in concentration of cytosolic fraction-GSH by lowest used and highest use concentration of Lithium citrate.

fraction GSH level. The cytosolic fraction GSH level was decreased significantly ($p < 0.001$) which was $3.739 \mu\text{M}$ (72.66%) by lowest used concentration of LiCitr while the drop in cytosolic fraction GSH by other used concentrations of lithium citrate was $3.624 \mu\text{M}$ (70.42%), $3.567 \mu\text{M}$ (69.32%), $3.484 \mu\text{M}$ (67.70%), $3.420 \mu\text{M}$ (66.46) and $3.338 \mu\text{M}$ (64.87%) respectively (Fig. 2). Cytosolic fraction GSH contents were also exposed to various concentrations (0.0001–2.0 mM) of LiCitr for different time of incubation which were 0, 20, 40, 60, 90 and 120 minutes (intervals) while the drop in cytosolic fraction GSH level by these used concentrations of LiCitr from 0 to 120 minutes (Fig. 1) was $3.739 \mu\text{M}$ (72.66 %), $3.675 \mu\text{M}$ (71.41%), $3.643 \mu\text{M}$ (70.79 %), $3.599 \mu\text{M}$ (69.94 %), $3.554 \mu\text{M}$ (69.06%) and $3.503 \mu\text{M}$ (68.07%) respectively.

DISCUSSION

The functions of erythrocytes along with other factors, mainly depend on the concentration of GSH as they have a large amount of reduced glutathione (GSH) which is almost 2 to 3 mM.^{9,10} In each and every cell the amount of GSH is different and depends on the types of metabolic reactions in different cells and tissues¹¹ and depletion in GSH leads to oxidative stress and it is well known that GSH is a crucial cellular multivalent bioprotector playing a major role in a number of processes as the regulation of the level of reactive species, maintenance of redox potential and transport of amino acids.¹²⁻¹⁴ Glutathione redox ratio (GSSG.GSH-1) is an important cellular oxidative stress marker, which stays below 0.1 in normal physiological conditions.¹⁵ A particular concentration of glutathione is necessary for the normal physiological function of cell. GSH is tripeptide which is non-enzymatic antioxidant consisting of three amino acids namely glutamic acid, cysteine and glycine while it is the cysteine residue of GSH which has –SH group serving as proton donor and biological activity of glutathione is only due to –SH group such as detoxification of xenobiotics, protection of cells from oxidative stress, acting as a storage and a transport form of cysteine and affecting cellular thiol redox status, metals like Li have strong affinity for –SH group and they become attached with –SH group thus these metals deplete GSH level everywhere in the cells specially in blood plasma and erythrocytes. Depletion of GSH in plasma and erythrocytes is thought to compromise cell function which promotes tissue damage and increases morbidity under various disease conditions, including inflammatory bowel disease, HIV infection, any critical illness, and acetaminophen-induced GSH depletion which may result in hepatic and renal failure and ultimately in death.¹⁶⁻¹⁸ The ultimate mechanism which is responsible for altered GSH homeostasis in human subjects under various conditions have not been fully determined, but this knowledge is important for the design of safe and effective clinical strategies aimed

at maintaining or enhancing GSH status in defined physiological conditions. The table no.1 shows that paired comparison t-test of concentration dependent effect of lithium citrate and cytosolic fraction GSH blank gave the decision that there is a significant ($p < 0.001$) effect of lithium citrate on the chemical status of cytosolic fraction GSH in sample mixture as the concentration of lithium citrate increased as compare to cytosolic fraction GSH blank solution treatment.

CONCLUSION

GSH has very important role in immune system, if depleted due to any reason, it should be minimized at any cost. The decrease in GSH in present study due to lithium citrate suggests that organic compounds of lithium should be used with great care because immunological alterations will occur which are associated with GSH of cytosolic fraction of red blood cells resulting in weak immune system of the body of patients.

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| <p style="text-align: center;">CONFLICT OF INTEREST Authors declare no conflict of interest. GRANT SUPPORT AND FINANCIAL DISCLOSURE None declared.</p> |
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| <p style="text-align: center;">AUTHORS' CONTRIBUTION</p> |
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|---|-------------------------|
| Conception and Design: | HU, MFK, SB, FH, US |
| Data collection, analysis & interpretation: | HU, MFK, SB, FH, US |
| Manuscript writing: | HU, MFK, SB, HK, FH, US |