

## Full Length Research Paper

# Glutathione role in gallium induced toxicity

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**Metallo-elements have strong affinity for sulfhydryl group (-SH) of glutathione (GSH) present in tissues. It is very important and interesting to study the reaction of gallium nitrate and glutathione as biomarker of glutathione role in detoxification and conjugation in whole blood components (plasma and cytosolic fraction). The effect of gallium nitrate different concentrations was examined on GSH present in whole blood components. Decrease in GSH level was dependant on gallium nitrate concentration. The decrease in GSH level of whole blood components was more prominent with the time of incubation of gallium nitrate. Decrease in the concentration of reduced glutathione may be due to the interaction of reduce glutathione and gallium nitrate to form oxidized glutathione (GSSG) or gallium-glutathione complex. This change in GSH metabolic status provides information regarding the role of GSH in detoxification of gallium nitrate. The effect of gallium metal on glutathione in blood components was discussed in this study in *in vitro* condition as a model for *in vivo* condition.**

**Key words:** Gallium nitrate, reduced glutathione (GSH), whole blood, plasma, cytosolic fraction (CF), oxidized glutathione (GSSG), Di-thiobis-dinitro-benzoic acid (DTNB).

## INTRODUCTION

Gallium can be effectively used for suppression of bone. Its role in the treatment of hypercalcemia of malignancy and Paget's disease of bone (Bockman and Bosco, 1994; Bockman et al., 1995) is due to its antiresorptive activity (Warrell and Bockman, 1989). Clinical efficacy of gallium in suppressing osteolysis and bone pain associated with multiple myeloma and bone metastases (Warrell et al., 1993) is also recorded, and also considered to have role in the treatment of osteoporosis (Warrell, 1995). Besides its therapeutic uses, it can cause metal toxicity in the human body.

Glutathione (GSH) is the main non protein thiol-compound in mammalian-cells and this tri-peptide has

bio-reducing activity (Lomaestro and Malone, 1995). Inside cells, GSH is present in two forms: Reduced state (GSH) and oxidized state (GSSG). Inside cells, glutathione is present in reduced state (GSH) and is more than 95% of the total (GSH+GSSG) content. Intra-cellular oxidized glutathione (GSSG) is present in small quantity but may increase with oxidative-stress or pathological-conditions and reduced glutathione depletion occurs at the same time (Cereser et al., 2001). GSH and GSSG act as a thiol redox-couple and have role in gene regulation and intra-cellular signal transduction (Dalton et al. 1999). Glutathione in reduced state (GSH) has important roles in cell division, immune-response, signal processes control, programmed cell-death, some xenobiotics and heavy metals detoxification (Ogawa, 2005; Meister and Anderson, 1983). GSH has very high redox potential due to the fact that it has anti-oxidant activity and a cofactor for enzymatic reactions that need readily available electron pairs (Khan et al., 2010). GSH is very reactive from physico-chemical point of view and conjugate to other molecules including the heavy metal ions because

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**Abbreviations:** GSH, Glutathione; -SH, sulfhydryl group; GSSG, oxidized glutathione.

of its sulfhydryl moiety (Balendiran et al., 2004; Vitecek et al., 2006). GSH/GSSG ratio gives an early indication of oxidative stress or risk of disease (Cereser et al., 2001). Metals toxicity includes geno-toxicity, carcinogenicity or neuro-toxicity (Flora et al., 2006). Glutathione concentration is decreased when it reacts with GaAs, increased the concentration of oxidized-glutathione (GSSG) (Flora et al. 2002). Blood components having high glutathione level have been selected to examine the effect of gallium nitrate on GSH in these components. This study shows the influence of gallium as gallium nitrate on glutathione level in whole blood constituents, particularly, plasma and cytosolic fraction of whole blood *in vitro* which may accounts for a model for *in vivo* interaction.

## MATERIALS AND METHODS

Gallium nitrate (Sigma), NaCl (Fluka), NaOH (Sigma), potassium dihydrogen phosphate (Sigma), disodium-edetate (Merck). L- glutathione (GSH), 5-5 di thiobis dinitro-benzoic acid (DTNB), HCl, chloroform (Sigma) and ethanol (Sigma) were used. All the chemicals were used for research work without any further purification.

Disposable-rubber gloves, siliconised glass test-tube, sterile syringes (Surge; Pharmaceuticals), Eppendolfs-tubes (Pyrex, Germany), UV-Visible Spectrophotometer Model-1601(Shimadzu), Centrifuge (H-200, Kokusan, Ensink-Company Japan) and PH-Meter (Denver, USA) were used.

### Preparation of solutions

GSH stock solution (1 mM) was made by dissolving 30.7 mg of GSH in 100 ml of 0.1 N HCl. 90 mg of NaCl was dissolved in 100 ml of distilled water and resulted in 0.9% NaCl isotonic solution. 18.96 mg of gallium nitrate (1 mM) was dissolved in 100 ml of distilled water. Phosphate buffer (PH 7.6) resulted from mixing of 42.2 ml (0.2 M) of NaOH, 50 ml (0.2 M) of monobasic potassium phosphate and distilled water was added to make final volume of 200 ml. pH was adjusted with pH-meter (Accumet-Meter, Denver Instrument Company USA). Di, thiobis, dinitro-benzoic acid (DTNB) (1 mM) was obtained by dissolving 39.6 mg of DTNB in 100 ml of buffer solution.

### Preparation and isolation of blood components

#### Separation of plasma from whole blood

Twelve milliliters blood was taken from healthy human volunteer and treated with 0.5 mM sodium-EDTA (500 µl) for the prevention of clotting and 1 ml of this venous blood was mixed with 1 ml of gallium nitrate (200 to 2000 µM) in separate test tubes and incubated for 10 min. Gallium nitrate final concentration (100 to 1000 µM) was obtained in each 2 ml mixture of venous blood and gallium nitrate. This 2 ml mixture sample was centrifuged at 1000 rpm for 5 min. With Pasteur pipette, 0.8 ml supernatant fluid portion (plasma) was taken and kept in ice till further use and the cellular fraction was processed for cytosolic fraction. Control plasma was isolated from centrifugation at 1000 rpm for 5 min of mixture of 1 ml venous blood and 1 ml of 0.9% NaCl solution.

#### Separation of cytosolic fraction from whole blood

The cellular portion was washed two times with isotonic (0.9% NaCl) solution and the blood cells were lysed by addition of 1 ml of distilled water at 4°C for 1 h. To the lysed blood cells, was added 0.8 ml of chloroform and ethanol cold mixture (3:5 v/v) and hemoglobin was precipitated at 0°C and 0.3ml of distilled water was added. The mixture obtained was centrifuged and pale yellowish supernatant was separated with Pasteur pipette and stored on ice till further use. Control cytosolic fraction was obtained in the same way from venous blood by taking 1 ml of venous blood and 1 ml of 0.9% NaCl solution. Plasma and cytosolic fraction of venous blood were also obtained without the addition of gallium nitrate to venous blood for separate experiments.

#### Determination of biological inorganic parameters

1. Plasma GSH (extra-cellular)
2. Cytosolic fraction GSH (intra-cellular)

GSH level was determined by the well known Ellman's method (Ellman, 1959) as mentioned below. 0.2 ml of sample (plasma or fraction cytosolic) was added to 2.3 ml of phosphate buffer (PH7.6) followed by the addition of 0.5 ml of DTNB. Absorbances were taken for each sample after 5 min at 412 nm against phosphate buffer in reference cell. Blank DTNB absorbencies reading was also taken for the sample containing 2.5 ml phosphate buffer and 0.5 ml DTNB against reference cell containing 3 ml phosphate buffer solution.

#### GSH standard curve

Ellman's method was used for the construction of GSH standard curve as given in the Figure 1.

#### Statistical analysis

Statistical analysis applied to the data shows that gallium nitrate caused reduction in the GSH concentration of plasma and cytosolic fraction of blood significantly ( $p < 0.05$ ) as shown in the Table 1.

## RESULTS

### Effect of gallium nitrate on the plasma GSH concentration of whole blood

Concentration of GSH was determined in each test tube in isolated plasma fraction having different concentrations of gallium nitrate (100 to 1000 µM). Results show decreased concentration of GSH as the concentration of gallium nitrate increased from 100 to 1000 µM when compared with the control plasma GSH concentration as shown in Figure 2. Decrease GSH concentration in plasma was found to be  $\text{GaNO}_3$  concentration dependent.

GSH concentration was also found to be decreased with time interval, taken in two separate test tubes containing plasma and 100 and 1000 µM of gallium nitrate solution after time interval of 0 to 180 min as

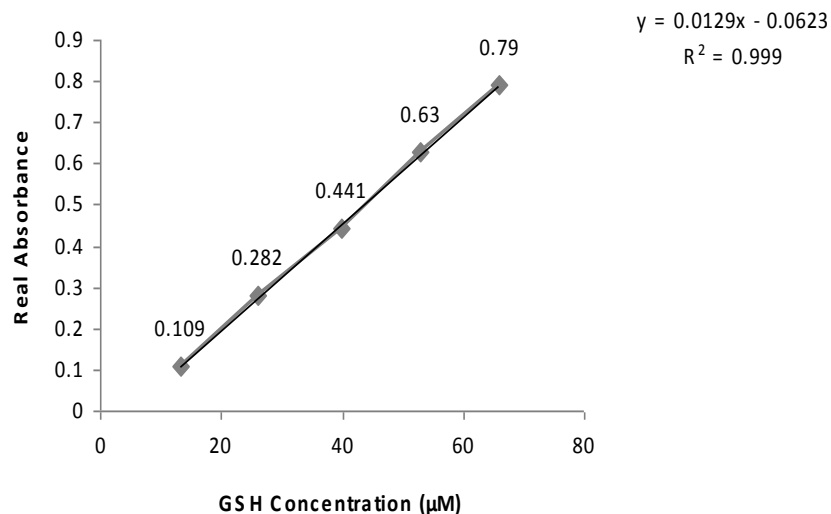


Figure 1. Standard curve of GSH.

**Table 1.** Statistical analysis applied to the concentration and time dependent effect of gallium nitrate on the GSH level in plasma and cytosolic fraction of whole blood.

<b>Concentration dependent effect of gallium nitrate on GSH level in plasma and cytosolic fraction</b>			
	<b>GSH level (Gallium nitrate + plasma)</b>	<b>GSH level (Gallium nitrate + cytosolic fraction)</b>	<b>Control GSH level</b>
Mean±SD	20.947±7.679	23.27±7.663	48.58 ± 7.489
	P < 0.05		
<b>Time dependent (0-180 minutes) effect of gallium nitrate on GSH level in plasma and cytosolic fraction</b>			
	<b>GSH level (Gallium nitrate + plasma)</b>	<b>GSH level (Gallium nitrate + cytosolic fraction)</b>	<b>Control GSH level</b>
Mean±SD	29.332±6.677	25.725±7.320	48.58±7.674
	p < 0.05		

shown in Figure 3. Decrease in GSH concentration was significant ( $p < 0.05$ ).

#### Gallium nitrate effect on GSH content of intracellular cytosolic fraction of blood

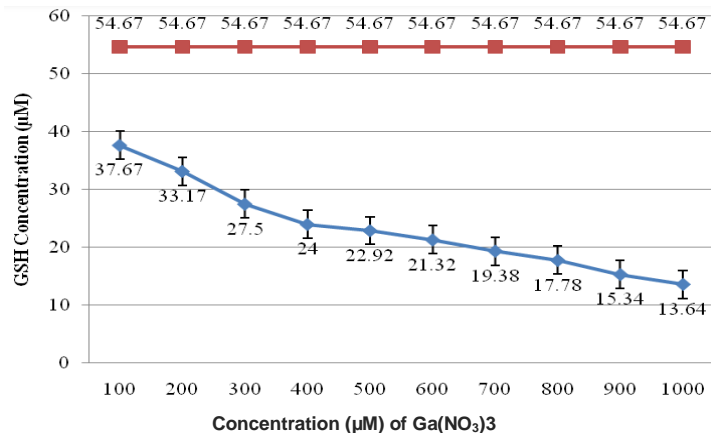
GSH concentration in each of the 10 test tube of cytosolic fraction having different concentrations of gallium nitrate (100 to 1000 µM) was determined by well known Ellmans method. GSH concentration was decreased with increase in concentration of gallium nitrate as given in Figure 4.

GSH concentration was also determined with time interval of 0 to 180 min in two separate tubes having pure cytosolic fraction of venous blood and gallium nitrate solution as given in Figure 5. Final concentration of gallium nitrate in two separate tubes was 100 and 1000 µM. The effect of gallium nitrate on GSH concentration reduction with time was significant ( $p < 0.05$ ). The results

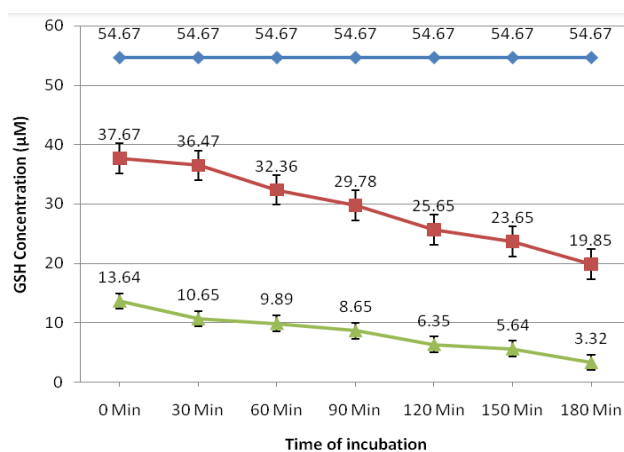
show that decrease in GSH concentration was both concentration and time dependent.

#### DISCUSSION

The main aim of this study was to determine the effect of gallium metal as gallium nitrate on the chemical status of GSH present in both plasma (extracellular component) and cytosolic fraction (intracellular component) of the human venous blood sample. The main focus was to explore the protective role of GSH in the metals induced toxicity. GSH has variety of role as antitoxin, free radical scavenger and participating in the conjugation. Gallium as gallium nitrate caused depletion of GSH concentration in plasma and cytosolic fraction of human venous blood. Depletion of GSH concentration in plasma and cytosolic fraction could be due to the interaction of reduced glutathione and gallium nitrate either from oxidized



**Figure 2.** Effect of different concentrations of gallium nitrate on the extra-cellular plasma GSH concentration. ■ Control plasma GSH; ♦ gallium nitrate (100 to 1000 μM). Results are the mean ±SE of 3 experiments of plasma GSH.



**Figure 3.** Effect of gallium nitrate on the extra-cellular plasma GSH concentration with time interval (0 to 180 min). ♦ Control plasma GSH; ■ gallium nitrate (100 μM); ▲ gallium nitrate (1000 μM). Results are the mean ±SE of 3 experiments of plasma GSH.

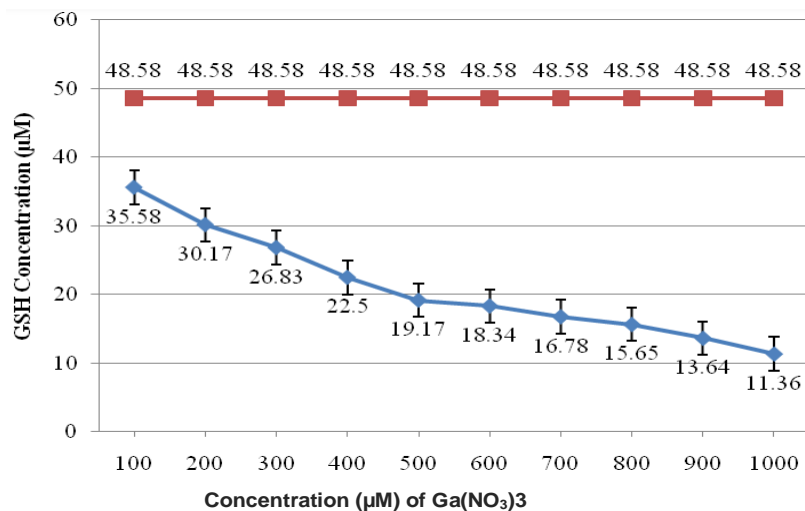
glutathione (GSSG) or Ga-SG complex.



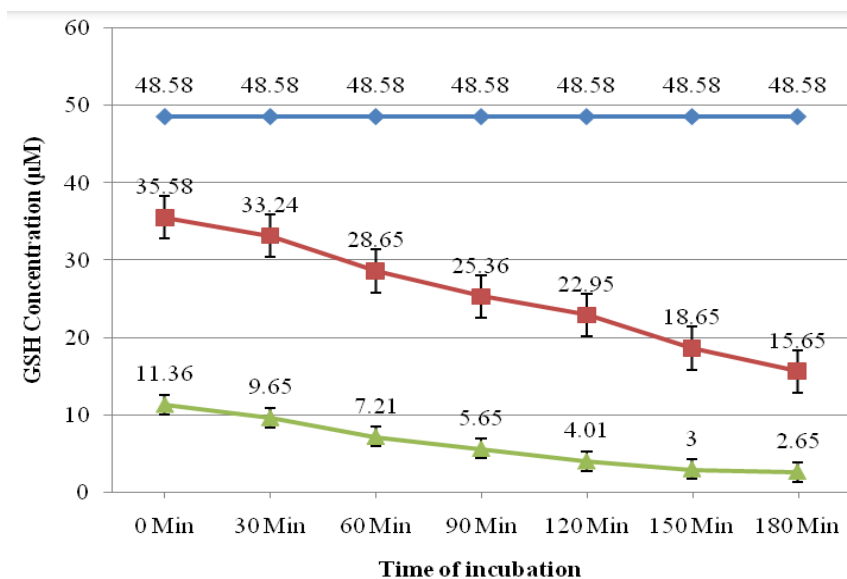
This study confirms the finding of other authors that concentration of reduced GSH present in whole blood of human volunteer is decreased by metal due to interaction of reduced GSH and metal to form the oxidized glutathione (GSSG) or metals-SG complex (Khan et al., 2010).

This study shows that GSH present in whole blood may provide protection by conjugating with metals and very

likely may form metal-SG complexes. In this way, GSH provides a first line of defense mechanism and causes detoxification of heavy metals by a process of enzymatic and non enzymatic conjugation. Evolvement of GSH in detoxification process is a source of protection against metals toxicity and thus prevents heavy metals and all such toxic substances from damaging the cells of the body. If at all Ga-SG complex is formed, it is very likely that Ga-SG complex may be excreted from the body and hence cells are protected from the severe damaging effects of heavy metals. This *in vitro* study of the effect of gallium nitrate and the likely formation of Ga-SG complex is proposed as a model of *in vivo* reaction which may have great chemical implications.



**Figure 4.** Effect of different concentrations of gallium nitrate on the intra-cellular cytosolic fraction of GSH concentration. ■ Control cytosolic fraction GSH; ♦ gallium nitrate (100-1000  $\mu\text{M}$ ). Results are the mean  $\pm$ SE of 3 experiments of cytosolic fraction GSH.



**Figure 5.** Effect of gallium nitrate on the intra-cellular cytosolic fraction of GSH content with time incubation period (0 to 180 min). ♦ Control cytosolic fraction GSH; ■ gallium nitrate (100  $\mu\text{M}$ ); ▲ gallium nitrate (1000  $\mu\text{M}$ ). Results are the mean  $\pm$ SE of 3 experiments of cytosolic fraction of GSH.

## Conclusion

Since SH group of GSH is the most reactive functional group of biochemical processes, hence many pharmacophores interact with and modify SH group of GSH *in-vitro* and *in-vivo*. The expected mechanism of metalloelements, organometallic complexes and drug action with thiols such as GSH is expected to contribute

to further development of rationally designed effective drugs which may account for the welfare of human and treatment of diseases with the expected rationally developed drugs.

## REFERENCES

Balendiran GK, Dabur R, Fraser D (2004). The role of glutathione in cancer. *Cell Biochem Funct.* 22: 343-352

- Bockman RS, Bosco B (1994). Treatment of patients with advanced Paget's disease of bone with two cycles of gallium nitrate. *Semin Arthritis Rheum* 23: 268-269
- Bockman RS, Wilhelm F, Siris E, Singer F, Chausmer A, Bitton R, Kotler J, Bosco BJ, Eyre DR, Levenson D (1995). A multicenter trial of low dose gallium nitrate in patients with advanced Paget's disease of bone. *J Clin Endocrinol Metab.* 80: 595-602.
- Cereser C, Guichard J, Drai J, Bannier E, Garcia I, Boget S, Parvaz P, Revol A (2001). Quantitation of reduced and total glutathione at the femtomole level by high-performance liquid chromatography with fluorescence detection: application to red blood cells and cultured fibroblasts. *J. Chromatogr. B*, 752: 123-132.
- Dalton TP, Shertzer HG, Puga A (1999). Regulation of gene expression by reactive oxygen. *Annu. Rev. Pharmacol. Toxicol.* 39: 67-101.
- Ellman, G.L (1959), Determination of sulfhydryl groups. *Arch. Biochem. Biophys.* 82: 70-74.
- Flora SJ, Dubey R, Kannan GM, Chauhan RS, Pant BP, Jaiswal DK (2002). Meso 2,3-dimercaptosuccinic acid (DMSA) and monoisoamyl DMSA effect on gallium arsenide induced pathological liver injury in rats. *Toxicol Lett*, 132(1): 9-17.
- Flora SJS, Flora G, Saxena G (2006). Environmental occurrence, health effects and management of lead poisoning" In: Cascas SB, Sordo J, editors. *Lead chemistry, analytical aspects, environmental impacts and health effects*. Netherlands: Elsevier Publication; pp. 158-228.
- Khan H, Jan SJ, Hashmatullah, Khan MF, Khan KA, Asim ur rehman, Wahab A (2010). Effect of Lithium Metal on the Chemical Status of Glutathione (GSH) Present in Whole Blood (Especially in Plasma and Cytosolic Fraction in Human Blood). *Pak. J. Pharm. Sci.* 23(2): 188-193.
- Lomaestro BM, Malone M (1995). Glutathione in health and disease: pharmacotherapeutic issues. *Ann. Pharmacother.* 29: 1263-1273.
- Meister A, Anderson ME (1983). Glutathione. *Annu. Rev Biochem.* 52: 711-760.
- Ogawa K (2005). Glutathione-associated regulation of plant growth and stress responses. *Antiox Redox Signaling*, 7: 973-981.
- Vitecek J, Petrova J, Petrek J, Adam V, Potesil D, Havel L, Mikelova R, Trnkova L, Kizek R (2006). Electrochemical study of S-nitrosoglutathione and nitric oxide by carbon fibre NO sensor and cyclic voltammetry—possible way of monitoring of nitric oxide. *Electrochim Acta.* 51: 5087-5094.
- Warrell RP, Bockman RS (1989). Gallium in the treatment of hypercalcemia and bone metastasis, in *Important Advances in Oncology 1989* (DeVita VT, Hellman S and Rosenberg SA eds) pp. 205-220, J.B. Lippincott, Philadelphia.
- Warrell RP Jr, Lovett D, Dilmanian FA, Schneider R, Heelan RT (1993). Low-dose gallium nitrate for prevention of osteolysis in myeloma: Results of a pilot randomized study. *J Clin Oncol.* 11: 2443-2450.
- Warrell RP Jr (1995). Gallium for treatment of bone disease, in *Handbook of Metal- Ligand Interactions in Biological Fluids, Bioinorganic Medicine* (Berthon G ed) vol 2, pp 1253–1265, Marcel Dekker, New York.