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Full Length Research Paper

Metabolic modulation of glutathione in whole blood components against lead-induced toxicity

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Lead has been found to have the ability to interfere in the metabolism and biological activities of many proteins. It has also been found that metalloelements have strong affinity for sulfhydryl (-SH) groups in biological molecules including glutathione (GSH) in tissues. Because of these facts, it was of interest to investigate further the interaction of lead acetate [Pb $(CH_3COO)_2$] with GSH as a biomarker of toxicity and the role of GSH in the detoxification and conjugation processes in whole blood components. Effects of different concentrations of $Pb(CH_3COO)_2$ on the level of reduced GSH in whole blood components (plasma and cytosolic fraction) were examined. GSH depletion in whole blood components was lead acetate concentration dependent. Depleted GSH level was however more pronounced with time. These findings show that changes in the GSH status produced by $Pb(CH_3COO)_2$ could be due to either by adduct (Pb-SG) formation or by increased production of oxidized glutathione (2GSH $\xrightarrow{Pb^{+2}}$ GSSG). This change in GSH metabolic status provides information regarding the mechanism of toxicity of $Pb(CH_3COO)_2$. These findings are therefore important for the rational design of antidote against the prevention of lead induced toxicity.

Key words: Lead acetate, glutathione (GSH), dithiobisdinitrobenzoic acid (DTNB), plasma and cytosolic fraction (CF).

INTRODUCTION

Interest in heavy metal pollution in environmental and occupational health studies has been increased recently because of its environmental pervasiveness (Harrison and Laxen, 1981; Finkel et al., 1983). Metal intoxication is known in neurotoxicity, genotoxicity, or carcinogenicity (Flora et al., 2006). Lead is considered to be the most toxic metalloelement among the toxic substances and has been noted for its serious toxic repercussion in bones, brain, blood, kidneys and thyroid glands (Roberts, 1989). Lead has also been found to cause physiological, biochemical, and behavioral dysfunctions in laboratory animals and humans (Flora et al., 2006). This metal is known to posses the ability to produce reactive radicals, which are known for serious toxicity including oxidative stress and carcinogenicity. It strongly reacts with thiol (-SH) groups found in enzymes and proteins and thiol-containing biological molecules including GSH that are required for normal defense mechanism (Flora et al., 2006). More also, lead has been found to reduce GSH level and cause lipid peroxidation in biological systems (Nakagawa, 1991; Donaldson and Knowles, 1993; Sugawara et al., 1991). High affinity of heavy metals for sulfhydryl groups can result in the formation of covalent attachments mainly between heavy metal and sulphydryl groups (Meister, 1988).

Glutathione, a tripeptide, is found in all forms of life and plays a protective role in the health of organisms. In humans, animals and plants, glutathione is the predominant non-protein sulfhydryl (-SH) containing biological molecule and acts as an antioxidant (Hermes et al., 1991; Nakagawa, 1991; Donaldson and Knowles, 1993). Glutathione has been known for its protection against heavy metal such as cadmium and lead (Daggett et al., 1998; Hunaiti et al., 1995). Blood or blood constituents rich in GSH have been chosen to examine the effect

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of lead toxicity in these constituents. The present study examines the effect of lead as lead acetate on redox status of glutathione in whole blood components including plasma and cytosolic fraction *in vitro* as a model of *in vivo* reaction.

MATERIALS AND METHODS

The followings were used; lead acetate (Sigma), disodium-edetate (Merck), L-glutathione (GSH), di,thiobisdinitrobenzoic acid (DTNB), Eppendolfs tubes (Pyrex, Germany), UV-Spectrophotometer Model 1601(Shimadzu), Centrifuge (H 200, Kokusan, Ensink-Company Japan) and pH-meter (Accumet meter, Denver instrument company USA). Chemicals were used for research work without any further purification.

Preparation of solutions

0.9% Sodium chloride solution was prepared by dissolving 9 mg of NaCl in 100 ml of water. 50 ml of lead acetate stock solution was prepared by dissolving 18.96 mg of lead acetate in distilled water. Glutathione standard solution was prepared by dissolving 30.74 mg of GSH in 100 ml of 0.1 N HCl. Di,thiobis, dinitrobenzoic acid (DTNB) was prepared by dissolving 39.6 mg of DTNB in 100 ml of buffer solution. Phosphate buffer (pH 7.6) was prepared by mixing 42.2 ml of NaOH (0.2 M) and 50 ml mono-basic potassium phosphate solution (0.2 M) and making the volume up to 200 ml with distilled water. pH was measured and adjusted by using pH-meter.

Preparation of blood samples

12 ml fresh venous blood treated with 0.5 mM Na-EDTA (500 µL) to prevent clotting, was collected from healthy human volunteers. 1 ml venous blood was then taken and mixed with 1 ml of each concentration of lead acetate (200 to 2000 µM) solution and incubated for 10 min. Final concentration of lead acetate in each tube was from (100 to 1000 µM). Each of this 2 ml sample containing blood and lead solution in 1:1 ratio was then centrifuged at 10000 rpm for 5 min. The supernatant fluid (plasma), 0.8 ml, was removed with Pasteur pipette, transferred to sample tubes and kept on ice till use and packed cells were further processed for cytosolic fraction. Control containing 1 ml of venous blood and 1 ml of 0.9% NaCl solution was also centrifuged for isolation of plasma.

The packed cells were washed twice with isotonic saline (0.9% NaCl) solution and the blood cells were lysed at 4°C with an equal volume (1:1) of distilled water for 1 h. After 1 h lysis at 4°C, 0.8 ml of cold mixture of chloroform- ethanol (3:5 V/V) at 0°C was added to 2 ml of lysed cells to precipitate the hemoglobin, followed by 0.3 ml of distilled water. The resulting mixture was centrifuged as before and the pale yellow clear supernatant (cytosolic fraction) was removed by Pasteur pipette, transferred to sample tubes and stored on ice till use. Control containing 1 ml each of venous blood and of 0.9% NaCl solution was centrifuged as before. After separation of plasma fraction, the packed cells were processed for the collection of cytosolic fraction as aforementioned.

Biological organic parameters

Biological parameters determined were; plasma glutathione (extracellular) and Lysate glutathione (Intracellular). All glutathione estimation were carried out following the modified standard of Ellman's method (Ellman, 1959) indicated as follows; 2.3 ml buffer was added to 0.2 ml of the sample (plasma or cytosolic fraction of blood), followed by the addition of 0.5 ml of DTNB. This mixture was transferred to a spectrophotometer cell. The reference cell contained buffer. DTNB blank consisting of 2.5 ml buffer and 0.5 ml DTNB was measured against a reference cell containing 3 ml buffer. All measurements were taken after 5 min at 412 nm. The glutathione contents were calculated using the standard curve. Absorbance readings were taken using a UV visible spectro-photometer.

RESULTS

Results indicate that the GSH content of plasma fraction of blood decreased with increasing concentration of $Pb(CH_3COO)_2$ addition as shown in Figure 1. A significant change in the content of plasma GSH from control was observed at $p<0.05$. Plasma GSH content at time intervals (0 to 90 min) was also measured when two different concentration of $Pb(CH_3COO)_2$ (100 and 1000µM) were added. The GSH content of extracellular plasma also decreased at time intervals (Figure 2). These results show that decrease in plasma GSH content were both $Pb(CH_3COO)_2$ concentration and time dependent.

Upon addition of $Pb(CH_3COO)_2$ to venous blood, measurement of intracellular cytosolic GSH content showed gradual decrease in GSH content as shown in Figure 3. Intracellular cytosolic GSH content was also measured at 0 to 90 min after the addition of $Pb(CH_3COO)_2$ to venous blood. A significant change in the content of cytosolic fraction GSH from control was observed at $p<0.05$. Results show the time dependent decrease in GSH content as shown in Figure 4. Results are the mean ± SE of 3 experiments of cytosolic fraction GSH. In addition, investigations with GSH percentage decrease in plasma and cytosolic fraction revealed the same behavior of GSH content decrease following the addition of $Pb(CH_3COO)_2$ to venous blood as shown in Figure 5. Figure 5 also shows that $Pb(CH_3COO)_2$ exerts more oxidative stress on the extracellular plasma GSH.

DISCUSSION

The aim and objective of this study was to investigate the possible interaction of $Pb(CH_3COO)_2$ with the extracellular and intracellular GSH content upon the addition of $Pb(CH_3COO)_2$ to the venous of healthy human volunteers. Moreover, the effect of heavy metals on the chemical modulation of biologically active low molecular weight molecules such as GSH, in biological fluids is an important and active area of research. The importance of interaction of heavy metals including $Pb(CH_3COO)_2$ with GSH in biological fluids as a biomarker of toxicity and detoxification is receiving increasing clinical interest. Blood components are rich in GSH content. The determination of content of GSH in biological fluids after or before incubation with heavy metals have been of value in further understanding of mechanism of action of heavy metal induced toxicity. Results derived from

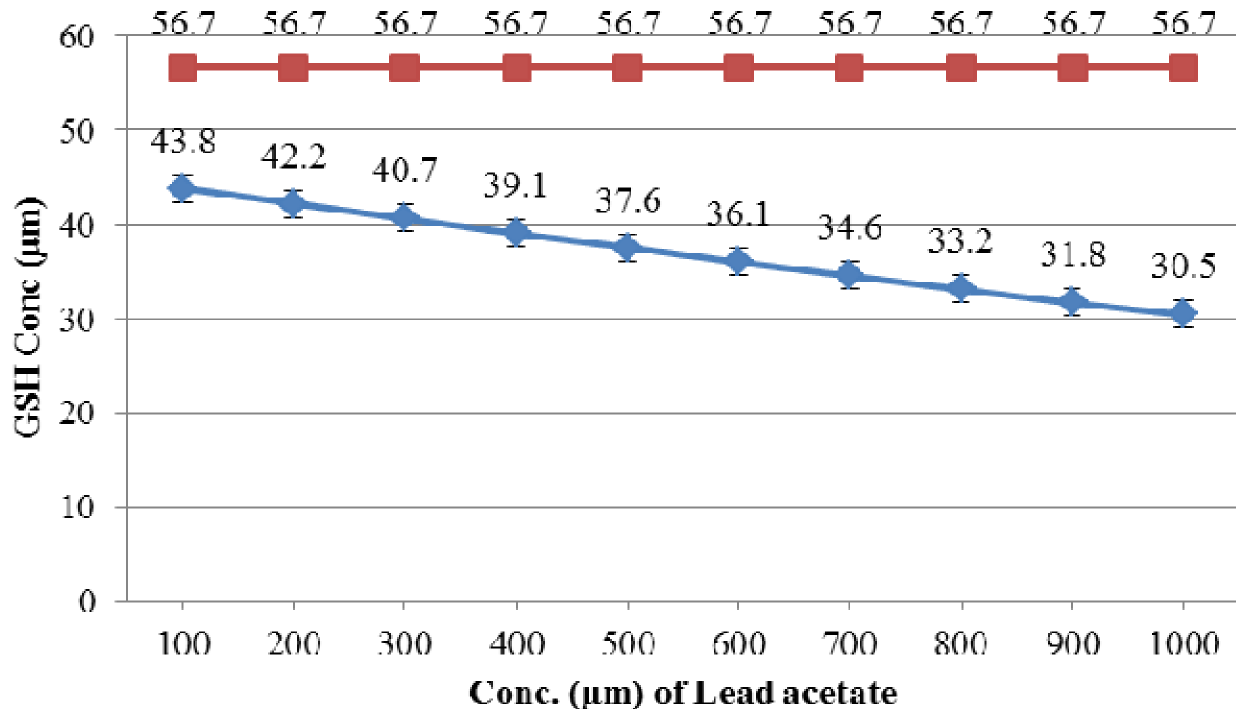

Figure 1. Effect of $Pb(CH_3COO)_2$ concentration on extracellular plasma GSH content. ■ Control (1ml 0.9% NaCl / 1ml of blood); ♦ $Pb(CH_3COO)_2$ (100 to 1000 µM). Results are the mean ± SE of 3 experiments of plasma GSH.

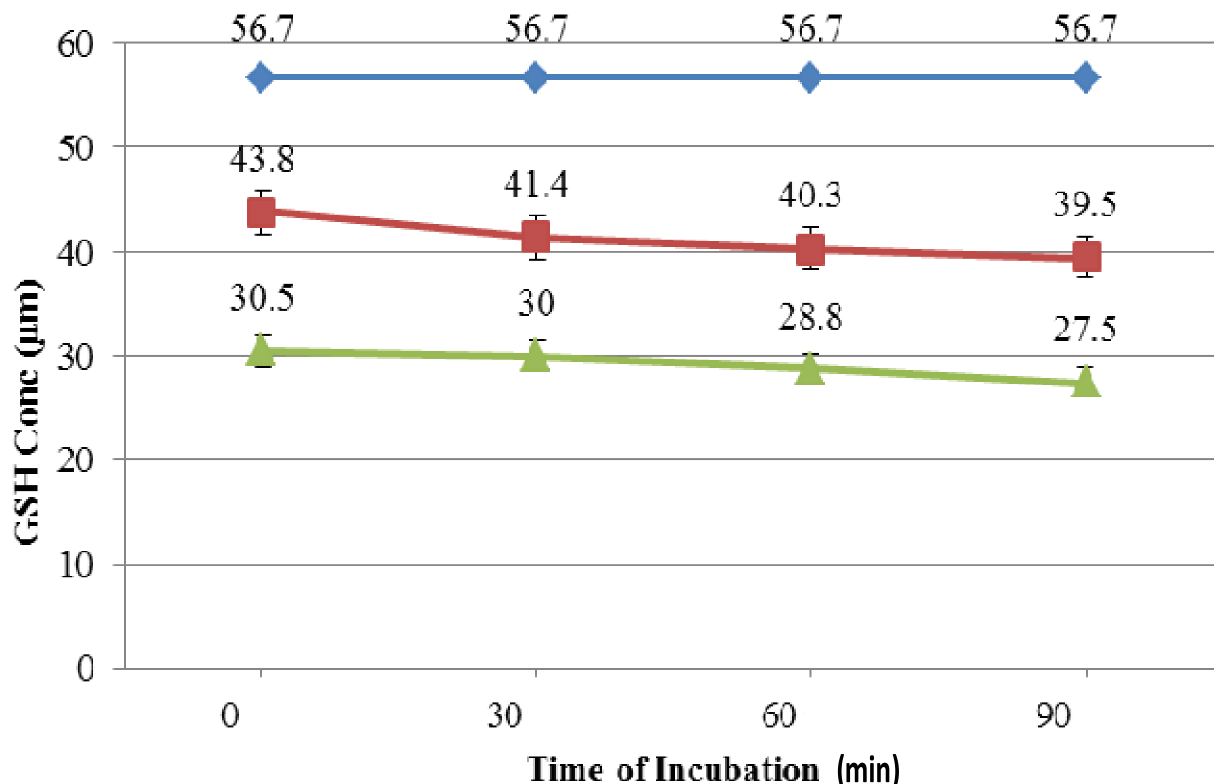

Figure 2. Effect of $Pb(CH_3COO)_2$ concentration on the extracellular plasma GSH content with time of incubation period (0 to 90 min). ♦Control (1 ml 0.9% NaCl / 1 ml of blood); ■ $Pb(CH_3COO)_2$ (100 µM); ▲$Pb(CH_3COO)_2$ (1000 µM). Results are the mean ±SE of 3 experiments of plasma GSH.

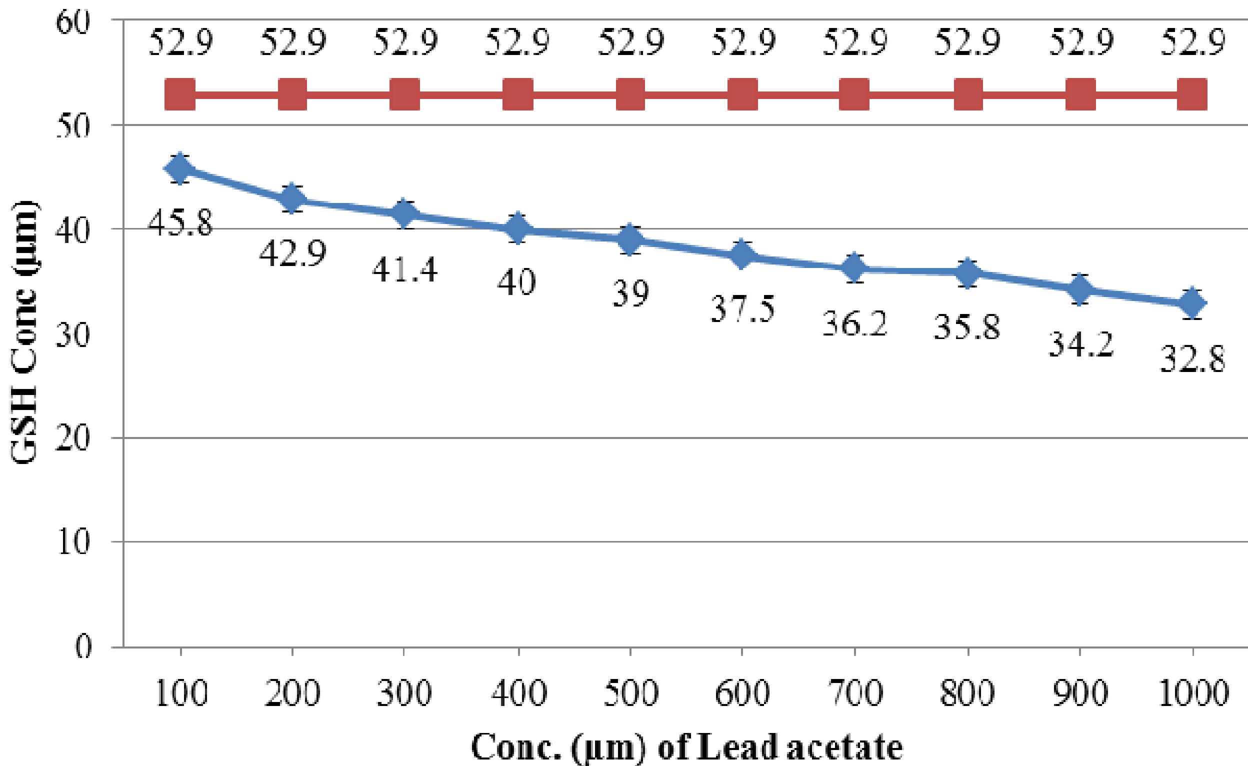

Figure 3. Effect of $Pb(CH_3COO)_2$ intracellular cytosolic fraction GSH content. ■ Control (1 ml 0.9% NaCl / 1 ml of blood) ♦ $Pb(CH_3COO)_2$ (100 to 1000 µM); Results are the mean ± SE of 3 experiments of cytosolic fraction GSH.

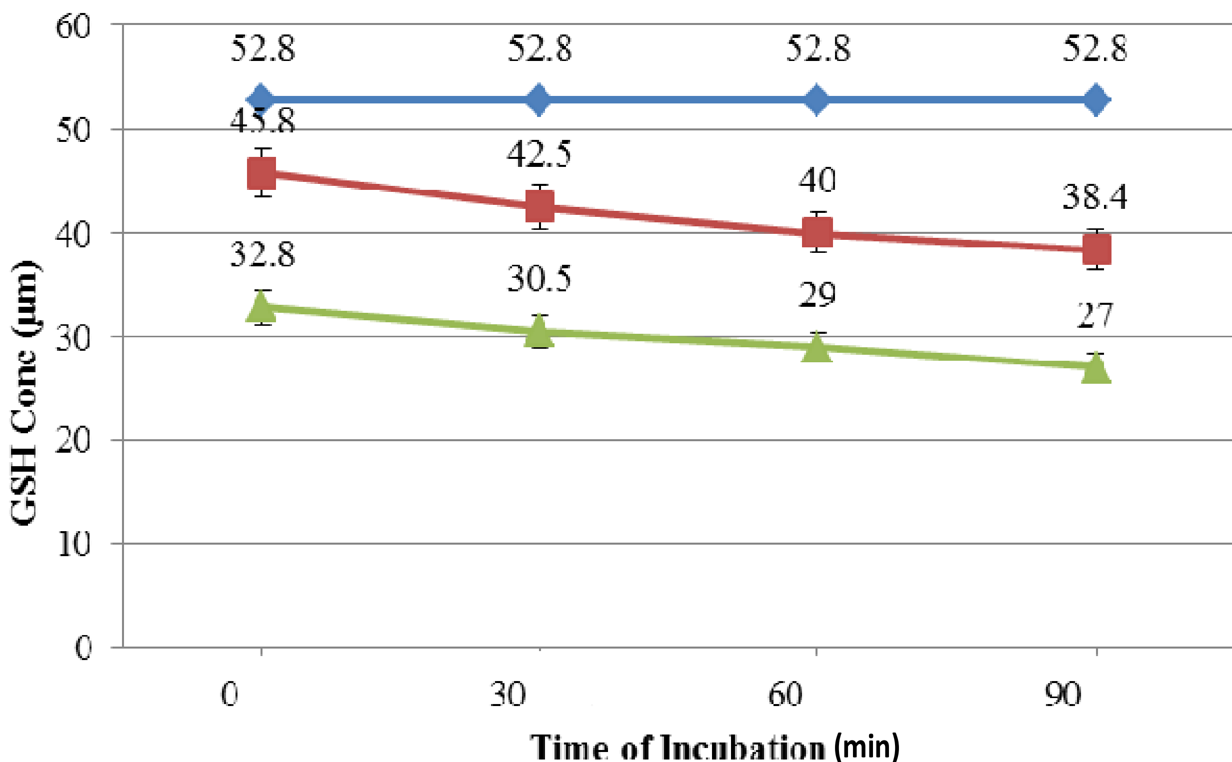

Figure 4. Effect of $Pb(CH_3COO)_2$ on intracellular cytosolic fraction GSH content with time of incubation period (0 to 90 min).♦Control (1 ml 0.9% NaCl / 1 ml of blood); ■ $Pb(CH_3COO)_2$ (100 µM); ▲$Pb(CH_3COO)_2$ (1000 µM).

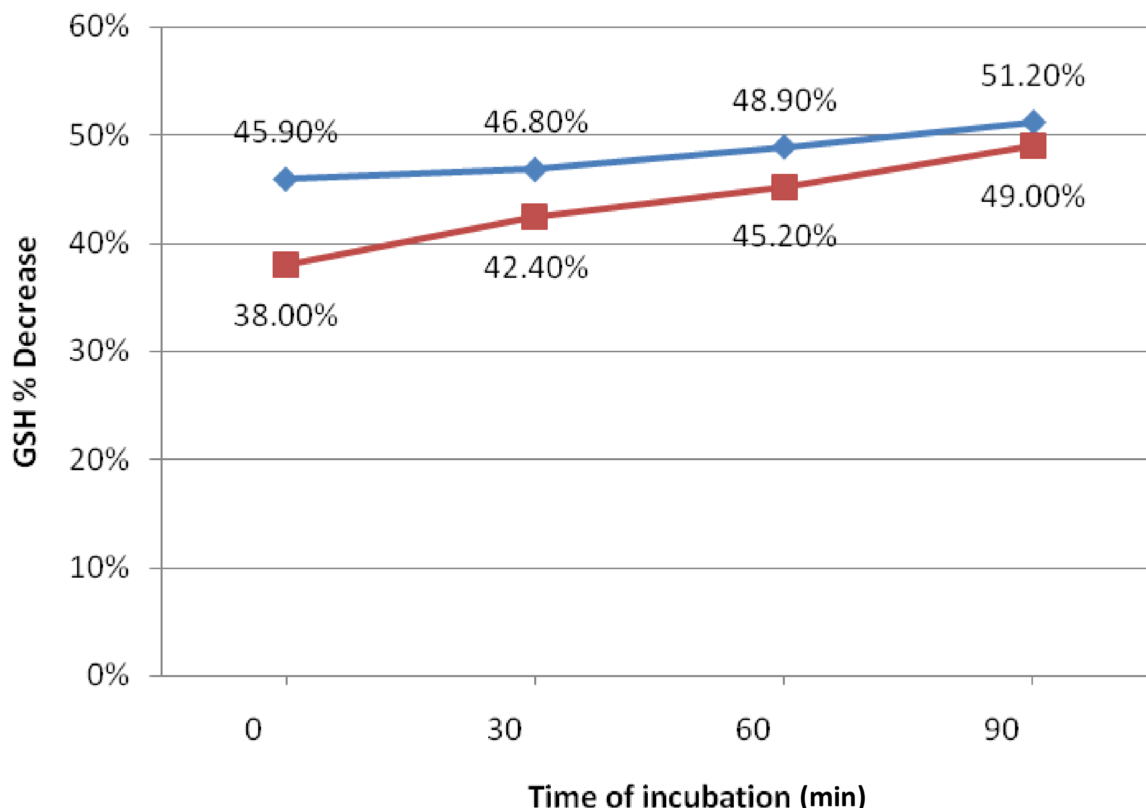

Figure 5. Percentage decrease of extracellular and intracellular plasma and cytosolic GSH. ♦ Plasma GSH content; ■ Cytosolic GSH content.

chemical modulation and conjugation of GSH in plasma and cytosolic fraction caused by heavy metals like lead can be used for safety evaluation.

Treatment of venous blood with $Pb(CH_3COO)_2$ and measurement of GSH content in extra and intracellular compartment may possibly indicate the protective role of GSH against Pb^{+2}. This observation is consistent with previous observations that indicate that Pb^{+2} forms complexes with GSH (Flora et al., 2006; Meister, 1988). Our findings may offer further evidence that GSH plays a role of protection against Pb^{+2}. Our findings also show the effect of Pb^{+2}-induced blood toxicity in which blood GSH content in extracellular and intracellular compartments were decreased by addition of $Pb(CH_3COO)_2$ to venous blood. These results can be consistent with a role of GSH protective mechanism. However, the mechanism by which Pb^{+2} induces toxicity in our research work is not yet known. From our findings, it appears that the oxidative stress or toxic effect of Pb^{+2} is more exerted at extracellular than intracellular level. This evidence further suggests that extracellular compartment is more oxidized than intracellular compartment. Presumably, GSH provides protection by complexing Pb^{+2}, thus, decreasing the availability of Pb^{+2} for toxic effect. This suggests that GSH provides a first line of defense against Pb^{+2}. The protective role of GSH and the known affinity of GSH for other metal ions may have therapeutic implications. More also, the percentage decrease of GSH in extra and intracellular level indicated that $Pb(CH_3COO)_2$ acted more effectively at extracellular then intracellular level. This could be due to non-lipid solubility nature of $Pb(CH_3COO)_2$ rather quite soluble in aqueous media.

Therefore, it could be concluded that the addition of $Pb(CH_3COO)_2$ to venous blood caused the decrease in GSH content in plasma and cytosolic fraction of blood as measured by Ellman's method (Ellman, 1959). This may be due to oxidation GSH to GSSG or by the binding of Pb^{+2} to GSH to form the corresponding Pb-SG complex.

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