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Effects of Tranexamic acid and its derivatives on the metabolism of Glutathione/Thiols in freshly isolated Leukocytes

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Abstarct

Objective: The current study was to examines the effects of tranexamic acid and its derivatives (N-Acetyl-Tranexamic acid and N-Phthaloyl-Tranexamic acid) on the metabolism and chemical modulation of glutathione (GSH)/ thiol in freshly isolated leukocytes from human blood.

Method: Blood from human volunteers was taken and leukocytes were isolated, by avoiding oxidation and coagulation of the blood. The pH of phosphate buffer solution on was adjusted to pH 7.62 and absorbances were measured at 412 nm using U.V/ Visible spectroscopy.

Results: The effect of the different concentrations of N-Acetyl tranexamic acid on the metabolism of thiol in leukocytes was found to exhibit greater changes as compared to the effects of tranexamic acid and phthaloyl tranexamic acid. Furthermore, these changes in thiol concentration were found to be time dependent.

Conclusions: It appears that N-Acetyl tranexamic acid causes the depletion of GSH markedly on thiols in leukocytes solutions due to the oxidation while N-Phthaloyl tranexamic acid shows slight change.

Key words:

Tranexamic Acid, Glutathione (GSH), N-Phthaloyl-Tranexamic acid, N-Acetyl-Tranexamic acid, Leukocytes.

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Introduction

Living organisms possess a range of enzymes including glutathione for protecting it from a range of deleterious effects and prevent the accumulation of free radicals in the cells thus reducing oxidative stress [1-2]. Glutathione is present in nearly all cells and is a major intracellular enzyme it also regulates the activity of other antioxidants such as vitamin C and E within the body. Glutathione is a tripeptide thiol synthesiaed with in the cells from glutamate, cystcine, glycine [3-5]. Glutathione possess various activities such as antioxidant defense, detoxification of electrophilic xenobiotics, and modulation of redox regulated, signal transduction, regulation of cell proliferation, synthesis of deoxyribonucleotide synthesis, regulation of immune responses, and regulation of leukotriene and prostaglandin metabolism [1, 6]. Cells that exhibit a lack of glutathione typically show severe oxidative damage associated with the degeneration of mitochondria [7].

The concentrations of GSH in tissues vary according to the number and kind of the metabolic processes requiring glutathione in the particular tissues. The concentration of GSH in certain tissues such as lens and liver are 15 mM and 5-10 mM, respectively. The liver is chief source of the glutathione synthesis; it also provides glutathione for cellular protection against reactive intermediates and pollutant xenobiotics. Blood is also an important site of GSH presence, Leukocytes contains 14-21 mM, which is seven folds of the contents of the erythrocytes 2-3 mM. The peripheries of the lens, particularly cortex, anterior epithelium of the cornea, optic fasiculus and retina also exhibit appreciable quantities of GSH [8-11].

Reduction in the levels of glutathione are associated with aging and many diseases such as; Alzheimer's disease, Parkinson's, atherosclerosis, neuro-degenerative diseases and viral hepatitis A, B, and C, it also leads to reduced chances of survival in case of a HIV infection. Glutathione reduces the chances of complications associated with diabetes, lung diseases, raising

glutathione levels restores normal liver function tranexamic acid (Trans-4-aminomethylcyclohexane Carboxylic acid) a derivative lysine, inhibits the proteolytic activity of plasmin and the conversion of plasminogen to plasmin with the aid of plasminogen activators. Plasmin is also known to cleave fibrinogen and a series of other proteins involved in coagulation [12-15]. Tranexamic acid is indicated in various symptoms as described in the literature [12-13].

Materials and Methods

Chemicals and instruments

Chemical such as: Acid citrate dextrose (ACD), 0.20M Sodium citrate Dihydrate (Merck), 0.14M Citric acid (pecking chemical company), 0.22M glucose [D (+) glucose] (Merck), 6% Dextran saline (Sigma chemical company), Sodium chloride (Merck), L-glutathione (GSH) (sigma chemical company), 5, 5-Dithiobis (2-nitrobenzoic acid). Cupric chloride dihydrate ($CuCl_2.2H_2O$) (Pecking Chemical Company), Disodium hydrogen phosphate ($Na_2HPO_4.H_2O$) (Riedel Dehaen AG Sleeze Hannover) ethylene diamine tetra acetate disodium EDTA–2 Na (Riedel Dehean AG sleeze Hannover) were used as purchased without further purification. The derivative of tranexamic acid, acetyl tranexamic acid and phthaloyl tranexamic acid were prepared according to a published method [13].

Centrifuge (H-200, Kokusan, Ensink Company, Japan), Eppendolf,s tubes (plastic, 10 ml), Siliconisid glass test tubes, sterile pyrogen free syringes (Disposable, plastic, MFD Sam woo Corporation) were used in this research work.

Stock solutions

Preparation of anticoagulant (acid-citrate-dextrose,ACD): 10 ml of anticoagulant solution was prepared by dissolving 0.294 gm citric acid (M.W.210.14),0.714 gm sodium citrate dihydrate (MW. 294.10) and 0.438 gm glucose (MW. 180.76) in 10 ml of distilled water. The final concentration of the citric acid, sodium citrate dihydrate and glucose were 0.14 M, 0.20 M and 0.22 M respectively.

Preparation of normal saline: (0.9% w/v (0.154 M) solution of sodium chloride (normal saline) was prepared by dissolving 0.90 gm or 90 mg sodium chloride in 10ml of distilled water.

Preparation of 6% dextran: 10 ml of 6% dextran solutions was prepared by dissolving 0.6gm dextran in 10ml of normal saline (0.9%w/v or 0.154 M). Preparation of 0.5M EDTA (M.W. 292.2) solution: 10ml of 0.5M EDTA (M.W 292.2) solution was prepared by dissolving 1.861 gm EDTA-2 Na in 10 ml; of distilled water .5 mm EDTA solution was prepared by diluting 100 µl of 0.5 M EDTA solution to 10ml with distilled water.

Preparation of glutathione (GSH) DTNB Buffer Solutions: 20 ml of 1mM GSH (M.W. 307.4) solution was prepared by dissolving 0.0062 grams GSH in 0.1N hydrochloric acid (HCl). 20 ml of 10^{-2} M DTNB (M.W 396.4) solution was prepared by dissolving 0.07928 grams in 0.1 M phosphate buffer (pH 7.6) 1000 ml of 0.1 M phosphate buffer (pH 7.6) was prepared by dissolving 17.799 g in 1000 ml of doubly distilled water and pH was adjusted to 7.6 with 0.1N HCl [12]. Stock solutions of 1mM tranexamic acid, 1mM acetyl tranexamic acid, and 1mM phthaloyl tranexamic acid were also prepared.

Isolation of leukocytes

Five milliliters of venous blood was withdrawn from human volunteers (male, female) using sterile pyrogen free syringe bathed in ACD solution to prevent coagulation of blood and oxidation of blood constituents including thiols and especially glutathione. The blood was then mixed with 2.5 ml of 6% Dextran 70(in 10ml of saline) in a wide mouth glass tube, followed by mixing with 1 ml of ACD solutions. The mixture was allowed to stand vertically for for about half an hour at room temperature. The straw colored leukocytes (supernatant) were collected with the help of micropipette in two separate glass test tubes (1 ml of leukocytes in each test tube).

Standard curve of GSH

In five separate test tubes 100, 200, 300, 400, and 500 µL, of 1mM GSH solution was taken and are diluted with phosphate buffer (pH 7.62), to yield five solutions with the concentrations 0.1, 0.2, 0.3, 0.4, & 0.5 mM, respectively. A standard curve for GSH was according to the method reported in the literature [16].

From each of these five test tubes 200 µl GSH solution was taken and added to 2.3 ml of phosphate buffer (PH 7.62), followed by the addition of 500µl of 10^{-2} M DTNB. A DTNB blank was also obtained by taking 2.5 ml of phosphate buffer (PH 7.62) followed by the addition of 500µl of DTNB. The mixture was shaken thoroughly & incubated for five minutes. Absorbance was taken after five minutes at 412 nm.

The final absorbance was obtained by subtracting the absorbance for DTNB from the absorbance for GSH plus DTNB mixture. A standard curve for GSH was obtained as shown in figure 1.

Effect of tranexamic acid on the metabolism of glutathione in leukocytes

To 1 ml of leukocytes in a test tube, 1 ml of 1 mM tranexamic acid solution was added, and shaken for a few seconds, and the final concentration of tranexamic acid was made 500 µM. A control was also prepared by adding 1 ml of leukocytes to 1ml phosphate buffer (pH 7.6) to give a final volume of 2 ml.

The effect of tranexamic acid on the metabolism of glutathione in leukocytes was studied in terms of determination of concentration of glutathione according to the method described in the literature [16]. The absorbances were read at 0, 30, 60, 90 minutes after mixing and the concentration of glutathione was determined from the GSH standard curve as shown in the figure 2.

Effects of different concentrations of tranexamic acid on the metabolism of glutathione in leukocytes

To 1 ml leukocytes in three separate test tubes, 1000 µl, 800 µl and 600 µl of 1 mM tranexamic acid solutions were added, and further diluted with phosphate buffer (PH 7.6) to a volume of 2 ml. The tubes were shacked for 5 minutes with, till the final concentrations of GSH in each test tube were 500 µM, 400 µM and 300 µM respectively. A control was also prepared, to 1 ml leukocytes; 1 ml phosphate buffer (pH 7.6) was added to make a total volume of 2 ml.

The effect of different concentrations of tranexamic acid on the metabolism of glutathione in leukocytes was

studied for the determination of the concentration of GSH in the mixture according to the method described in the literature [16]. The absorbances were read at 0, 30, 60, 90 minutes after mixing and the concentration of GSH was determined from the GSH standard curve as shown in figure 3.

Effect of acetyl tranexamic acid on the metabolism of glutathione in leukocytes

To 1 ml of leukocytes in a test tube, 1 ml of 1mM acetyl tranexamic acid solution was added, the resulting solution was shaken for a few seconds, and the final concentration of acetyl tranexamic acid was made up to 500 μM. A control was also prepared, by adding 1 ml leukocytes to 1 ml phosphate buffer (pH 7.6) to make a total volume of 2 ml.

The effect of acetyl tranexamic acid on the metabolism of glutathione in leukocytes was studied in terms of the determination of the concentration of glutathione as described in the literature [13, 16]. The absorbances were taken at 0, 30, 60, 90 minutes after mixing, and the concentrations of GSH were determined from the standard curve as shown in figure 4.

Effect of different concentrations of acetyl tranexamic acid on the metabolism of Glutathione in leukocytes

To 1 ml leukocytes in three separate test tubes, 1000 μl, 800μl, and 600μ of 1mM acetyl tranexamic acid solutions were added and further diluted with phosphate buffer (pH 7.6) to make a final volume of 2 ml. The tubes were shaken for 5 minutes till the final concentration of GSH in each test tube was 500 μM and that of acetyl tranexamic acid in each tube was 500 μM, 400 μM, 300 μM, respectively. A control was also prepared, by adding 1 ml leukocytes to 1 ml phosphate buffer (pH 7.6) to make a total volume of 2 ml.

The effect of different concentrations of acetyl tranexamic acid on the metabolism of glutathione in leukocytes were studied for the determination of concentration of GSH in the mixture, according to the method described in the literature [13, 16]. The absorbances were read at 0, 30, 60and 90 minutes after mixing. GSH concentrations were determined from the GSH stander curve as shown in figure 5.

Effect of different concentrations of phthaloyl tranexamic acid on the metabolism of glutathione in leukocytes

To 1 ml of leukocytes in a test tube, 1 ml of 1mM phthaloyl tranexamic acid solution was added, and shaken for a few seconds. The final concentration of phthaloyl tranexamic acid was 500 μM. A control was also prepared, by adding 1 ml leukocytes to 1 ml phosphate buffer (pH 7.6) to make a total volume of 2 ml.

The effect of phthaloyl tranexamic acid on the metabolism of glutathione in leukocytes was studied by determining the concentration of glutathione, according to the method described in the literature [13, 16]. The absorbances were read at 0, 30, 60, 60, 90 minutes after mixing, and the concentration of GSH was determined from the standard curve as shown in figure 6.

Later, to 1 ml leukocytes in three separate test tubes 1000 μl, 800μl, and 600μ of 1mM phthaloyl tranexamic acid solutions were added and further diluted with phosphate buffer (pH 7.6) to 2 ml. The tubes were shaken for 5 minutes and the final concentration of GSH in each test tube was 500 μM and that of phthaloyl tranexamic acid in each tube was 500 μM, 400 μM, 300 μM respectively. A control was also prepared by adding 1 ml of leukocytes to 1ml phosphate buffer (pH 7.6).

The effect of different concentration of phthaloyl tranexamic acid on the metabolism of glutathione in leukocytes were studied for the determination of concentration of GSH in the mixture according to the method described in the literature [16]. The absorbances were read at 0, 30, 60and 90 minutes after mixing. GSH concentrations were determined from the GSH stander curve as shown in figure 7.

Results

The effect of tranexamic acid on metabolism of glutathione in leukocytes was determined by measuring the concentration. With the addition of tranexamic acid (to a final concentration of 500μM) to leukocytes, it was observed that the concentration of GSH first decreased at 0 minute, and then increased after 30 minutes, and slight increase was observed at 60 minutes and 90 minutes as shown in figure 2.

The effects of the different concentration of tranexamic acid on the metabolism of GSH in leukocytes were also studied. With the addition of Tranexamic acid to a final concentration of 400 μ M, 300 μ M, it was observed that GSH concentration decreased slightly with the passage of times as compared to 500 μM, which increased during 30-60 minutes. It was further observed that this decrease in GSH concentration in leukocytes was tranexamic acid concentration dependant (Figure 3)

Effect of acetyl tranexamic acid on GSH concentration of leukocytes was studied. With the addition of acetyl tranexamic acid (to a final concentration of 500 μM) to leukocytes, a decrease in the concentration of GSH was observed with the passage of time (Figure 4).

The effect of the different concentrations of acetyl tranexamic acid on the concentration of GSH in leukocytes was also studied. With addition of acetyl tranexamic acid, to a final concentration of 500 μM, 400 μM and 300 μM, it was observed that GSH concentration increases at 0 minute and then decreases slowly with the passage of time (Figure 5).

The effect of phthaloyl tranexamic acid on GSH concentration of leukocytes was also studied. With the addition of phthaloyl tranexamic acid (to a final concentration of 500μM) to leukocytes, an increase in the concentration of GSH was observed at 0 minutes which decreased slightly with the passage of time as shown in figure 6. However, between 30-60 minutes it remained stable and between 60-90 minutes it showed a decline.

The Effect of different concentration of phthaloyl tranexamic acid on the GSH concentration of leukocytes was also studied. With the addition of phthaloyl tranexamic acid to a final concentration of 500 μM, 400 μM and 300 μM, it was observed that the concentration GSH increases at 0 minutes and 30 minutes but later on shows a decrease at 90 minutes (Figure 7).

Discussion

To study effect of chemicals and/or drugs and their metabolites on the chemical modulation and metabolism of biologically active low molecular weight molecules such as glutathione, in biological fluids is an important and active area of research. The importance of interaction of chemical and drugs and their metabolites with GSH in biological fluids as a biomarker of toxicity and detoxification is receiving increasing clinical interest [2, 7].

The biological fluids include components of blood such as leukocytes, plasma and erythrocytes, these biological fluids are rich in GSH and/or thiols. The determination of concentration of GSH and/or thiols in biological fluids after or before interaction with chemical and/or drugs and their metabolites have been of valve in further understanding of mechanism of action of chemicals and /or thiols with regards to chemical and/or drugs induced toxicity [8, 17].

Results derived from chemical modulation, conjugation and metabolism of GSH and/or thiols in leukocytes and plasma caused by tranexamic acid, phthaloyl tranexamic acid and acetyl tranexamic acid can be used for safety evaluations. Availability of safe and quality chemicals and drugs and their complexes worthy of study to monitor, in vitro, thiols and/or GSH chemical and/or drugs their complexes reactions as an excellent model in vivo reaction.

The complexes of tranexamic acid may be considered to produce its varied pharmacological effects. All of these effects may account for biochemical activity and lipophilic character of complexes [6, 18].

Addition of phthaloyl tranexamic acid and acetyl tranexamic acid to leukocytes caused a reduction or lowering in concentration of GSH. This reduction in GSH concentration was not prominent but decreased slightly with the passage of time. The rate of the reduction of the concentration of GSH in leukocytes was time dependent as shown in (figures 3 and 5).

The oxidation of thiols to disulfides is well known [18]. Data obtained in these studies suggested that the addition of Phthaloyl tranexamic acid and acetyl tranexamic acid to plasma produced Phthaloyl tranexamic acid thiol and acetyl tranexamic acid thiol complexes, and these complexes are unstable in the presence of air, thus the complex thiol after oxidation may produce disulfide complexes [19].

Conclusion

It appears that N-Acetyl tranexamic acid causes the depletion of GSH markedly on thiols in leukocytes solutions due to the oxidation while N-Phthaloyl tranexamic acid shows slight change.

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Standard Curve

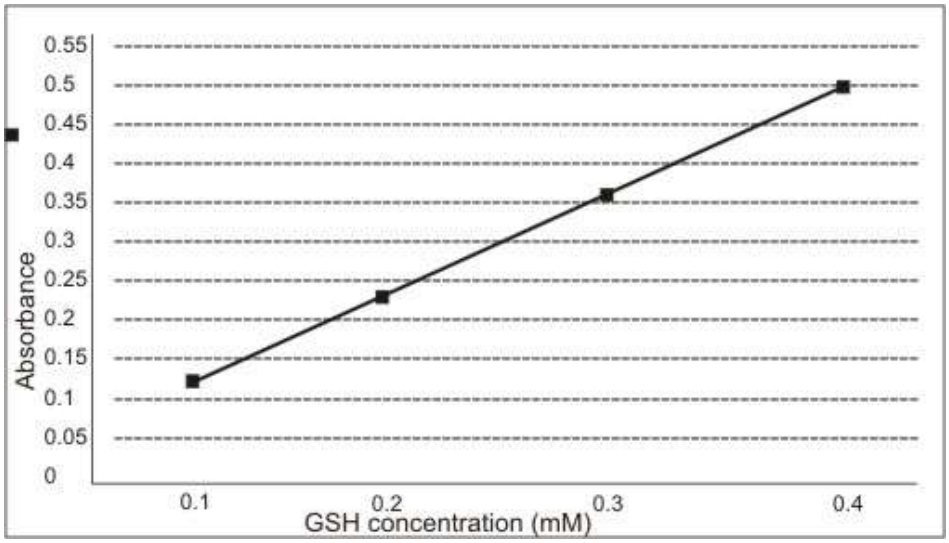

Figure 1: Standard Curve for GSH.

Tranexamic Acid Leukocytes (500 µM)

Control = Black Color

500µM = Red Color

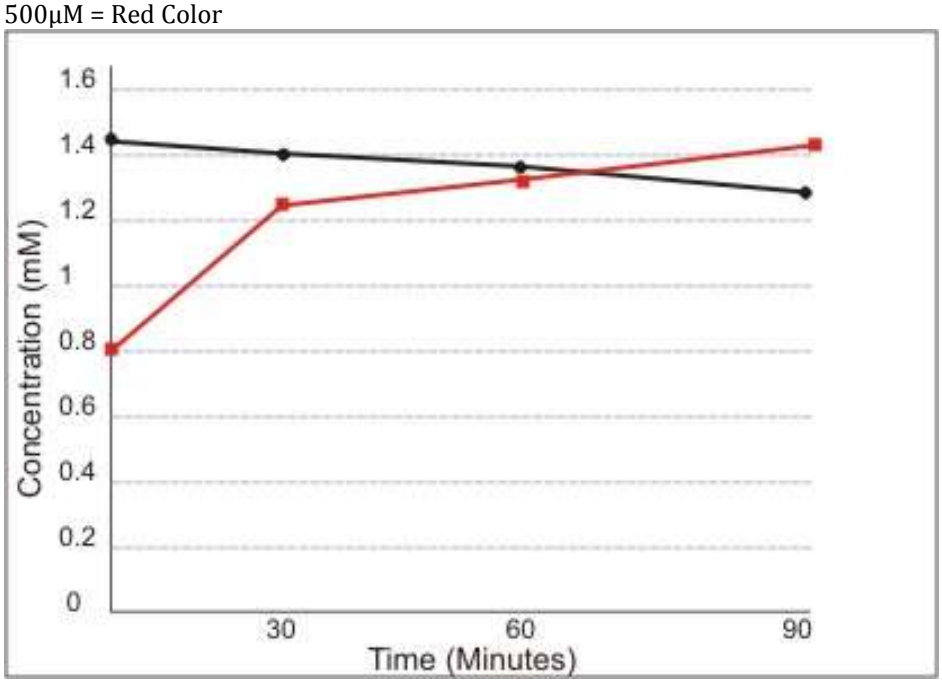

Figure 2: Effect of Tranexamic Acid on the concentration of GSH in Leukocytes. The final concentration of Leukocytes and Tranexamic Acid mixture was 500 µM. each point represent the mean of three components.

Tranexamic Acid Leukocytes (400 µM 300µM)
Control = Black Color
400µM = Blue Color
300µM = Green Color

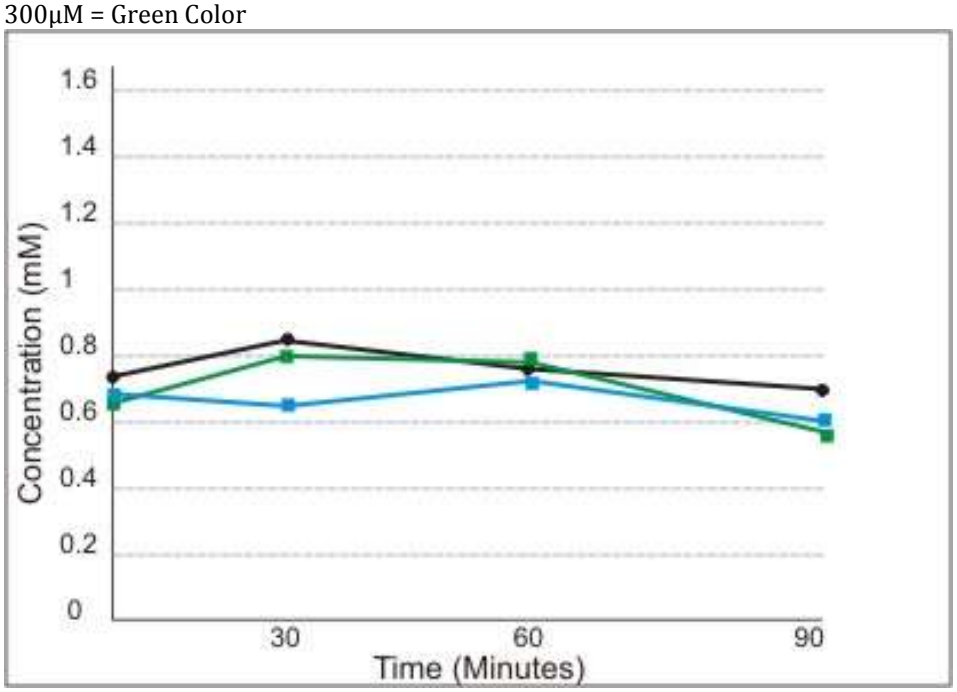

Figure 3: Effect of different concentration of Tranexamic Acid on the concentration of GSH in Leukocytes. The final concentration of mixture containing Leukocytes and Tranexamic Acid were 400µM and 300 µM.

Acetyl Tranexamic Acid (500 µM) + Leukocytes
Control = Black Color
500µM = Red Color

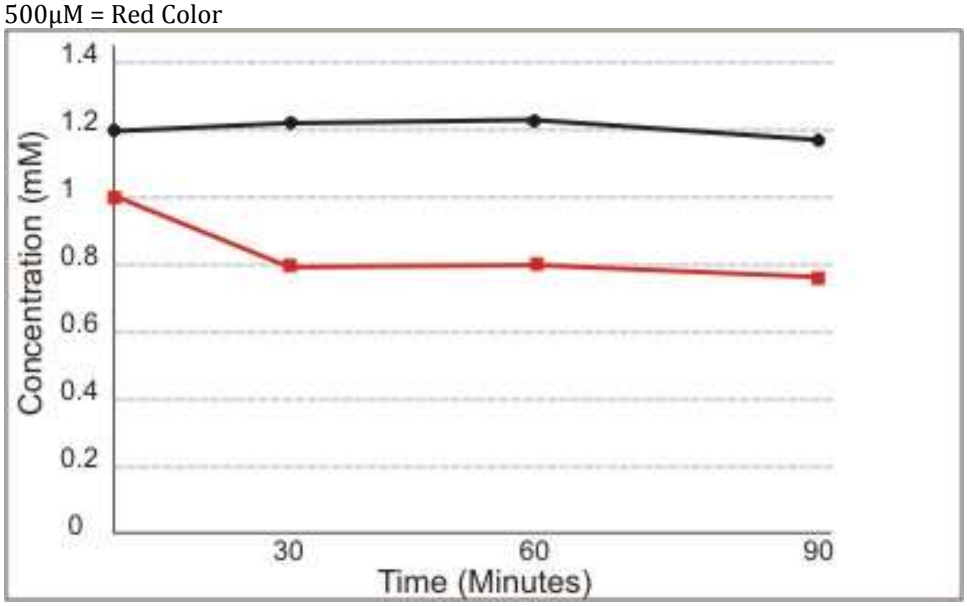

Figure 4: Effect of Acetyl Tranexamic Acid on the concentration of GSH in Leukocytes. The Final concentration of Leukocytes and Tranexamic Acid was 500µM. Each point represent the mean of three experiments.

Acetyl Tranexamic Acid + Leukocytes
Control = Black Color
500µM = Red Color
400µM = Blue Color
300 µM = Green Color

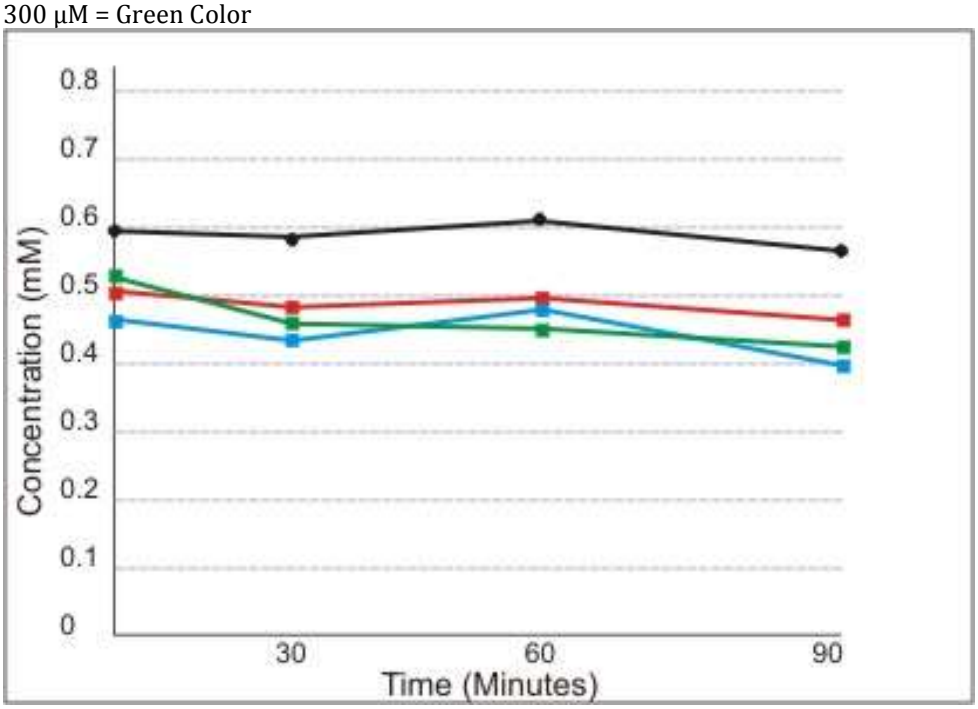

Figure 5: Effect of different concentration of Acetyl Tranexamic Acid on the concentration of GSH in Leukocytes. The final concentration of mixture containing Leukocytes and Tranexamic Acid were 500µM, 400µM and 300 µM.

Phthaloyl Tranexamic Acid (500µM) + Leukocytes
Control = Black Color
500µM = Red Color

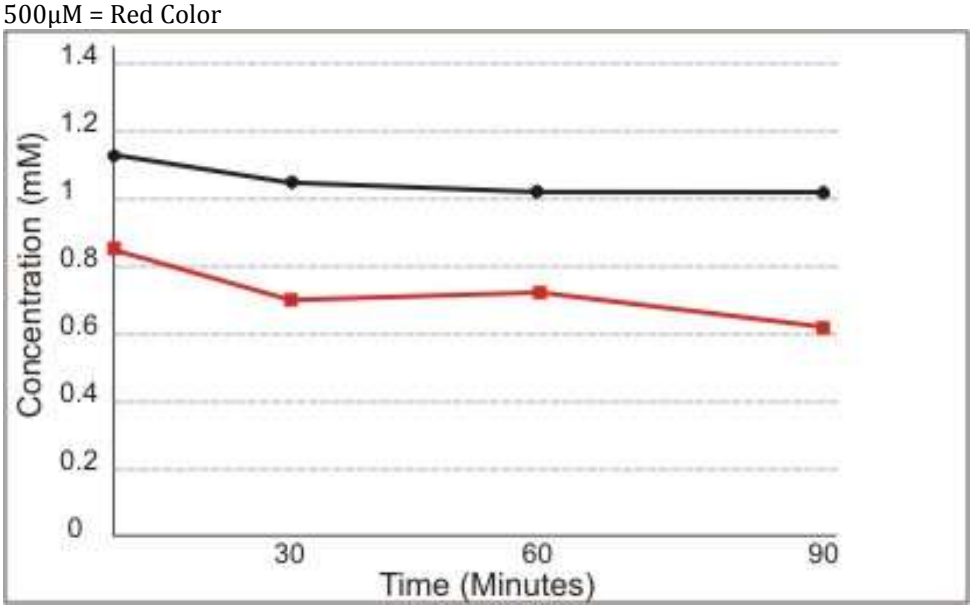

Fig. 6: Effect of Phthaloyl Tranexamic Acid on the concentration of GSH. The final concentration of Phthaloyl Tranexamic Acid was 500 µM. Each point represent the mean of three experiments.

Phthaloyl Tranexamic Acid + Leukocytes
Control = Black Color
500µM = Red Color
400µM = Blue Color
300 µM = Green Color

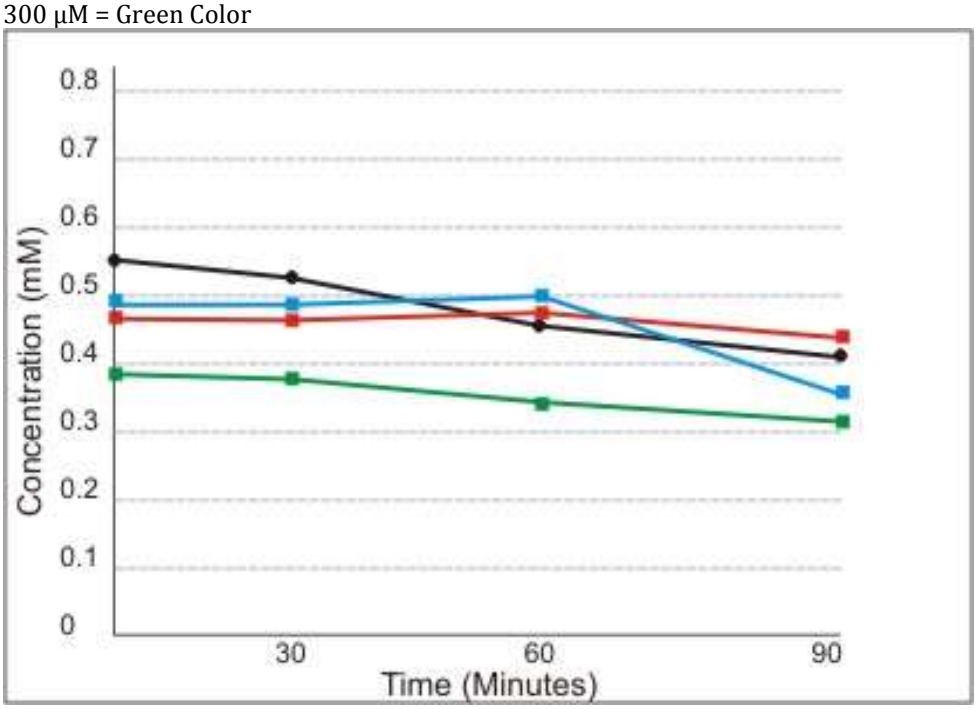

Figure 7: Effects of Phthaloyl Tranexamic Acid on the concentration of GSH. The final concentration (Phthaloyl Tranexamic Acid + GSH) were 500µM, 400µM, and 300µM. Each point represent the mean of three experiment.