IN-VITRO, CONCENTRATION AND TIME DEPENDENT EFFECT OF PHENYL MERCURIC ACETATE ON THE CHEMICAL STATUS OF GLUTATHIONE (GSH)

Hashmat Ullah[1], Muhammad Farid Khan[1]& Farwa Hashmat [2]

[1]*Department of pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University Dera Ismail Khan.*

[2] *Department of Chemistry, Gomal University, Dera Ismail Khan.*

ABSTRACT

Mercury toxicity is known since long and is a significant clinical entity as mercury is ubiquitous in the whole environment and poses serious risk to human health. In humans mercury toxicity encompasses direct damage to tissues and enzyme functions. It is the strong oxidant among heavy metal and is a neurotoxin. It was of interest to investiage the concentration and time dependent effect of phenyl mercuric acetate on the chemical status of reduced glutathione (GSH) in vitro conditions. Spectrophotometric analysis shows that with the increase in concentration of PMA (Phenyl Mercuric Acetate) and increase in incubation times cause greater depletion in reduced form of glutathione perhaps due to the conversion of GSH into GSSH or GS-HG complex formation.

Key words: Phenyl Mercuric Acetate (PMA), DTNB, Spectrophotometer, concentration.

INTRODUCTION

Reduced glutathione has a number of important roles in almost all cellular processes especially in the protection of cells from many toxic chemical compounds, radiation and oxidative damage (Meister, 1983; Meister, 1983). GSH has great affinity to form complexes with heavy metals including cadmium mercury etc (Li, 1955; Perrin 1971) and in this way might function in protection cells against metal toxicity. The reduced glutathione intracellular levels are regulated by a complex mechanism involving control of synthesis, transport and utilization of GSH. Glutathione is an essential cellular component (Antioxidant) and prolonged failure to maintains adequate level of GSH in the cells is detrimental to the cells (Will, 1999).

Mercury salt is use in many cosmetic products, laxatives, teething powders, diuretics and antiseptics. (Ozuah, 2000) PMA is used in eye drops and paints to preserve them, as a disinfectant, and as a catalyst in polyurethane systems (Li, 1955; Perrin, 1971). Inorganic mercury is also formed by the elemental mercury vapor or methyl mercury metabolism in the body (Clerkson, 2002). Mercury cannot reach the placenta or cannot cross the blood brain barrier but it can reach in the neonatal brain because of complete absence of blood brain barrier (National Research Council, 2000). Exposure to mercury salts effects the renal cortex

(Kojima, 1989) and the result will be renal failure like dysuria, proteinuria, Hematuria, Oligruia and uremia. Human exposure to mercury salts may also cause gastrointestinal problems like colitis, gingivitis, stomatitis and increase salivation (Kojima, 1989) while acrodynia and irritability may occur occasionally (Ozuah, 2000)

MATERIALS AND METHODS

Reduced glutathione i.e. GSH (Fluka), DTNB (Sigma), phenyl mercuric acetate (Aldrech), potassium dihydrogen phosphate (Merck) Double refined distilled water, hydrochloric acid Hcl 35% (Kolchlight), NaoH (Sodium Hydroxide) (Fluka) Schimadzu spectrophotometer (UV-1601, Japan) pH meter: model Nov: 2012 (Scientific Company Nova Ltd, Korea), oven: Memmert model U-30854 (Schwabach, Germany), Analytical balance model Ax 200 (Schemadzu, Japan), Magnetic stirrer, Beaker: 100ml, 50ml (pyrex Iwaki Glass, Japan), Digital micro pipette 200µl, 500 µl, 1000 µl (Scorex Swiss Finland) Were used.

PREPARATION OF STOCK SOLUTIONS

i. Phosphate Buffer (pH 7.6).

For preparing 0.2m Phosphate buffer, 42.4ml of 0.2m Sodium Hydroxide solution was taken into 250ml volume metric flask and to this 50ml of 0.2M potassium dihydrogen phosphate solution was added, to this solution which is now 92.2ml quantity sufficient of distill water was added to make total volume of this solution upto 200ml.

ii. Glutathione Stock Solution.

15.375 mg of reduced glutathione (GSH) was dissolve into 50ml of 0.1 NHcl (Hydrochloric Acid) solution to prepare 1mM glutathione solution.

iii. DTNB stock solution

19.8mg of Ellman's reagent (DTNB) was dissolved in 50ml of 0.2m phosphate buffer pH 7.6 to prepare 1mM DTNB stock solution.

iv. Phenyl Mercuric Acetate Stock Solution.

0.396 mg of phenyl Mercuric Acetate was dissolved in 50ml of distill water to prepare 2mM stock solution of PMA (Phenyl Mercuric Acetate).

STANDER CURVE

Five different solutions were prepared from 1mM stock solution of reduced

glutathione and from each dilution 0.2ml was taken into five different test tubes in which already 2.3ml of phosphate buffer pH 7.6 was added. To all these five test tubes each containing 2.5ml solution in total 0.5ml of 1mM DTNB stock solution was added. These test tubes were well shaken and incubated for 5 minutes. After 5 minutes of incubation time, absorbance of each mixture was recorded at fixed wavelength λ max 412nM. 0.5ml of DTNB stock solution was added to 2.5ml of phosphate buffer to prepare DTNB blank, absorbance of DTNB blank was also recorded at λ max 412nM. Real absorbance of each mixture was obtained by subtracting absorbance of DTNB blank from the absorbance of each of the mixture.

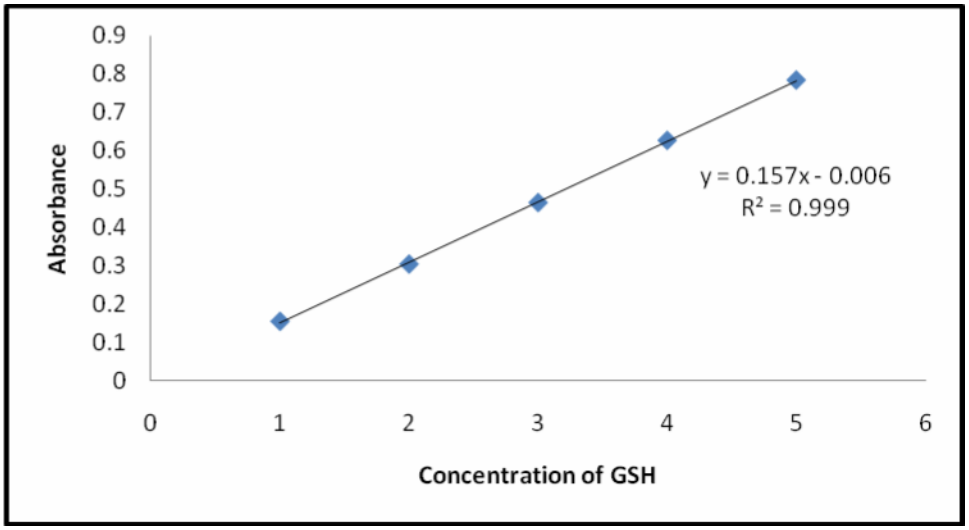

Fig 1: Standard Curve for Glutathione (GSH)

AFFECT OF DIFFERENT CONCENTRATIONS OF PHENYL MERCURIC ACETATE (PMA) ON GLUTATHIONE IN-VITRO CONDITIONS.

From 0.0001mM to 2mM, 6 different concentrations of PMA (Phenyl Mercuric Acetate) were prepared then into 6 test tubes 2ml of 1mM reduced glutathione solution was added. To each test tube containing 2ml of 1ml GSH, 2ml from different concentration were added and

these test tubes were incubated for 10 minutes, called as reaction mixture-1.

To six other separate test tubes 2.3ml of phosphate buffer pH 7.6 was added and to each these test tube 0.2ml from each mixture 1 was added then mixture-1 was added then 0.5ml of 1mM DTNB stock solution was added to each these second group of 6 test tubes, called as mixture-2. The mixture-2 were incubated for 5 minutes and absorbance of each mixture-2 was recorded after 5 minutes at fixed wavelength λ max 412nm. In each mixture-1 glutathione concentration is 0.5mM while PMA (Phenyl mercuric acetate) concentration is (from lower to higher concentration) 0.00005mM to 1mM respectively. The recorded absorbance of each mixture-2 was converted into GSH concentration by using Ellman's modified method (Ellman, 1959) as described in stander curve. Table 1 fig: 2 shows affect of different concentrations of PMA (Phenyl Mercuric Acetate) on reduced glutathione (GSH).

Table #1

Effect of different concentrations of Phenyl Mercuric Acetate (PMA) i.e. from 0.0001 mM to 2.0mM on the chemical status of glutathione in aqueous medium

Concentration of Glutathione (GSH) in final mixture 33.33µM DTNB blank
Absorbance of 5,5-dithiobis, 2-nitrobenzoic acid (DTNB) blank solution was 0.061

S No	Used conc: of PMA	Final conc: of PMA	1st ABS	2nd ABS	3rd ABS	Mean of reading	Real abs* / conc: of GSH after reaction with PMA		Real abs*/ conc: of GSH Blank	
							ABS	Conc. Of GSH (µM)	ABS	Conc. Of GSH (µM)
1	0.0001 mM	0.003 µM	0.553	0.548	0.544	0.548	0.488	3.15	0.785	5.04
2	0.001 mM	0.03 µM	0.491	0.487	0.483	0.487	0.427	2.76	0.785	5.04
3	0.01 mM	0.33 µM	0.399	0.396	0.394	0.396	0.336	2.18	0.785	5.04
4	0.1 mM	3.33 µM	0.295	0.289	0.283	0.289	0.229	1.50	0.785	5.04
5	1.0 mM	33.33 µM	0.099	0.095	0.079	0.089	0.031	0.24	0.785	5.04
6	2.0 mM	66.66 µM	0.065	0.063	0.060	0.063	0.003	0.06	0.785	5.04

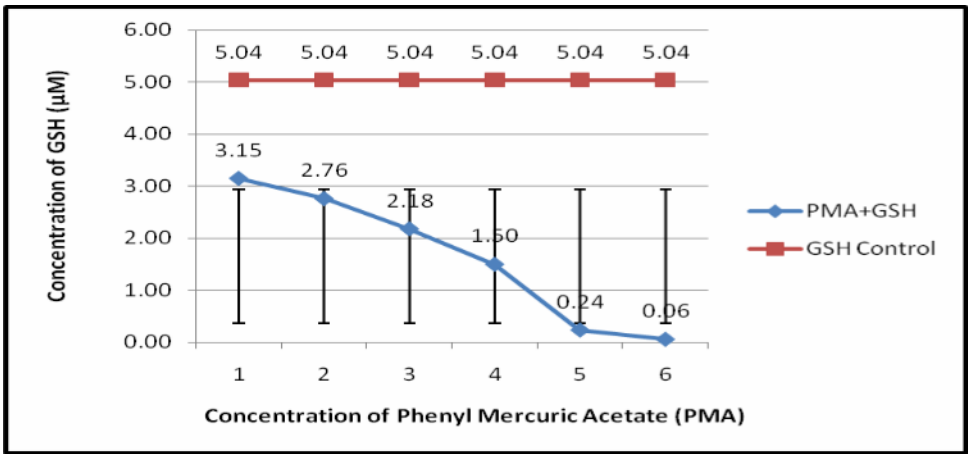

Figure 2.◆Effect of different concentrations (0.0001mM, 0.001mM, 0.01mM, 0.1mM, 1.0mM & 2.0 mM) of PMA on the chemical status of glutathione (GSH)in aqueous medium. GSH control. Results are the mean ±SE of 3 experiments.

AFFECT OF PHENYL MERCURIC ACETATE ON GLUTATHIONE (GSH) WITH TIME IN-VITRO CONDITIONS

The procedure used for the determination of affect of different concentrations of PMA (Phenyl Mercuric Acetate) on glutathione was also repeated to check the intention of different concentration of PMA on glutathione by giving different time of incubation to mixture-2. The incubation periods were from 0 minutes to 90 minutes as shown in table 2 fig: 3.

Table #2

Effect of different concentrations of Phenyl Mercuric Acetate (PMA) on chemical status of Glutathione (GSH) at different time intervals (incubation time)

Concentration of Glutathione (GSH) in final mixture 33.33µM DTNB blank
Absorbance of 5,5-dithiobis, 2-nitrobenzoic acid (DTNB) blank solution was 0.061

S.NO	Conc: of PMA Used (µ M)	Final Conc: of PMA (µM)	At 0 mint		At 20 mint		At 40 Mint		At 60 Mint		At 90 Mint		At 120 Mint	
			ABS	Conc	ABS	Conc	ABS	Conc	ABS	Conc	ABS	Conc	ABS	Conc
1	0.0001 µ M	0.003 µ M	0.471	3.04	0.431	2.78	0.394	2.55	0.356	2.31	0.317	2.06	0.287	1.87
2	0.001 µ M	0.03 µ M	0.415	2.68	0.379	2.45	0.343	2.22	0.308	2.00	0.299	1.94	0.244	1.59
3	0.01 µ M	0.33 µ M	0.325	2.11	0.293	1.90	0.259	1.69	0.225	1.47	0.195	1.28	0.165	1.09
4	0.1 µ M	3.33 µ M	0.220	1.44	0.203	1.33	0.174	1.15	0.142	0.94	0.112	0.75	0.082	0.56
5	1.0 µ M	33.33 µ M	0.020	0.70	0.000	0.04	0.000	0.04	0.000	0.04	0.000	0.04	0.000	0.04
6	2.0 µ M	66.66 µ M	0.000	0.04	0.000	0.04	0.000	0.04	0.000	0.04	0.000	0.04	0.000	0.04
Real abs*/ conc: of GSH Blank			0.765	4.91	0.765	4.91	0.765	4.91	0.765	4.91	0.765	4.91	0.765	4.91

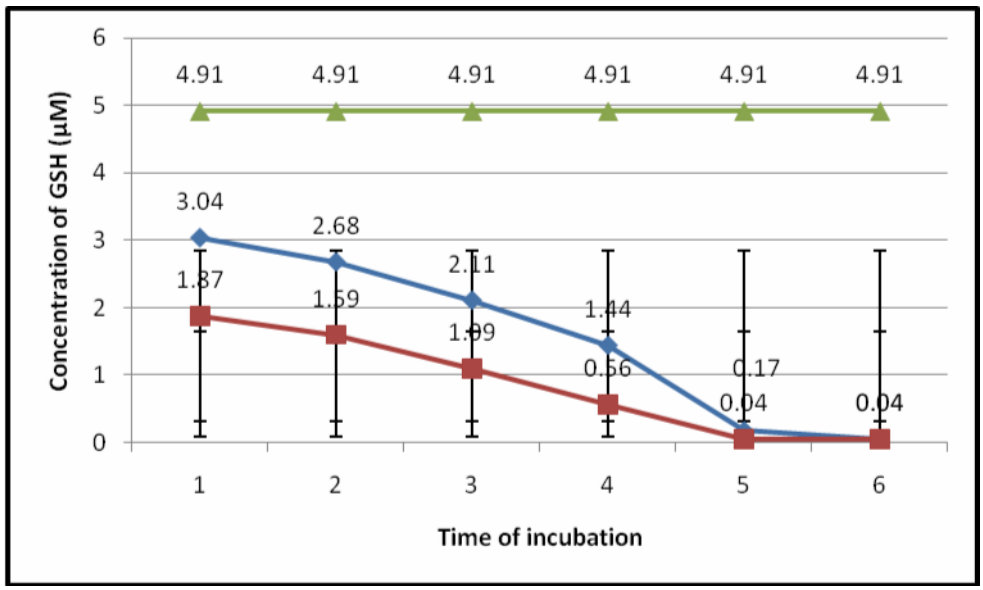

Figure 3. Effect of different concentration of PMA on glutathione (GSH) in aqueous medium with time (i.e. 0 min: ,20 min:,40 min:,60 min:,90 min:,120 min:) GSH▲control Effec◆f lowest used PMA concentration (0.001 µM) Effec■f highest used PMA (66.66 µM). Results are the mean ± SE of 3 experiments.

RESULTS

AFFECT OF DIFFERENT CONCENTRATIONS OF PHENYL MERCURIC ACETATE (PMA) ON GLUTATHIONE IN VITRO CONDITIONS.

In vitro conditions, at 0 minutes, the fact is that all the used concentrations of PMA (Phenyl Mercuric Acetate) deplete reduced glutathione very much with the fact that lower used concentration of PMA deplete reduced glutathione less than the higher used concentration of PMA (Phenyl Mercuric Acetate) indicating that products or chemicals (Antifungal, Preservatives) containing high concentration of mercury will damage the human antioxidant defense system to the extent of serious risk. When the human are exposed to these mercury products.

AFFECT OF DIFFERENT CONCENTRATION OF PHENYL MERCURIC ACETATE (PMA) ON GLUTATHIONE IN VITRO CONDITIONS

PMA (Phenyl Mercuric Acetate) concentrations which were six in number, each shows a gradual and simultaneous reduction in concentration of reduced glutathione (GSH) different times of incubation with the passage of time. Our investigation shows that there are more

and more chances are interaction between mercury and reduced glutathione resulting in more depletion of GSH because of either its conversion into GS-Hg-Sg complex or GSSG (Oxidized form of glutathione) formation.

DISCUSSIONS

Mercury (Hg) is a harsh metal and potent cellular poison whose mood of action and target system in humans are mainly depend on chemical form of mercury involved specially the PMA (Phenyl Mercuric Acetate) is the mercurial of greatest environmental concerned and hence our study/results are positive in indicating the fact that humans are at the greatest risk when are exposed to mercurials. If they (Mercurials) are constantly used in different products of human use/ interest whether in pharmaceutical, cosmetics, agriculture or in any other filed in which the humans are exposed to mercurials.

Stability constants (the energy necessary to form and break bonds) are very high for mercury and glutathione (reduced form) so mercury binds to reduced glutathione freely which is in the highest concentrations in cells (Divine KK, 1999). The reaction between mercury and reduced glutathione is rapid and instantaneous (Clarkson TW, 2002). The endogenous thiol- containing molecule-glutathione (reduced Glutathione) binds to mercuric ions and determine the biological fate of mercury compounds in the body (Zalups, 2000)

In our investigation, it is clear that with the increase in concentration in mercury and incubation time, there is greater depletion of reduced glutathione (GSH).

PROPOSED REACTIONS

$GSH + C_8H_8HgO_2 \longrightarrow$ GS-Hg-SG+C_6H_6+CH_3COOH

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