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BLOOD;
A SPECIFIC TARGET DURING ORGANIC MERCURIAL TOXICITY

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ABSTRACT… All forms of mercury are global pollutants having no environmental limits. Human exposure to mercury occurs basically through food chain due to accumulation of organic forms of mercury in fish. **Objectives:** The purpose of the present study was to analyze the effect of phenyl mercuric acetate on plasma and cytosolic fraction GSH. **Study Design:** Experimental Study**. Setting:** Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University, Dera Ismail Khan. **Period:** 29 January 2011 to 11 march 2012 .**Statistical Analysis:** One-way ANOVA followed by Dunnet's HSD test. **Results:** For the estimation of thiols Ellman's method was used and was found statistically significant ($p < 0.001$) decrease in plasma and cytosolic fraction GSH which was dose and time dependent. The plasma GSH contents drop in 0 to 120 minutes by various concentrations of phenyl mercuric acetate were 64.45%, 59.33%, 50.89%, 41.56%, 33.63% and 32.99% while drop in cytosolic fraction GSH level from 0 to 120 minutes by different concentrations of phenyl mercuric acetate (PMA) was 53.86%, 48.60%, 45.41%, 36.11%, 29.38% and 27.06%.**Conclusions:** It is clear that during organic mercury toxicity the blood components are also affected which is proved from our results. With the increase of time ,the mercury toxicity would be more harmful so detoxification of organic mercury should be done on emergency bases at the earliest with the help of suitable chelating agents along with antioxidant therapy.

Key words: Phenyl mercuric acetate, Detoxification, Estimation, Experimental, Pollutants.

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INTRODUCTION

Mercury infectivity occurs from different sources including coal and bio-fuel combustion, agrochemicals, mining, urban discharges, battery and fluorescent lamp production, cement production and industrial waste discharges[1,2]. In countries like Russia, America, Brazil, China, Africa, Columbia, Mongolia, Peru, Spain, etc, mercury is commonly used in extraction of gold in both large and small scale gold mining[1]. It has already been estimated that for the upturn of 1 kg of gold, about 1.32 kilograms of mercury is lost to the environment and about 40% of it is lost in liquid form which is release to rivers, lakes, streams or sediments where as 60% of mercury in the form of vapors is release directly to the atmosphere[3,4]. About 70 to 80% mercury is directly release to the atmosphere[5]. The inorganic mercury released to environment undergoes to several reactions to form organic compounds of mercury such as methyl and phenyl mercurial which accumulates in the food chain[6,7,8]. Methyl mercury is documented as extremely toxic fraction of mercury and both occupational and non-occupational people are exposed to it through the utilization of mercury polluted food. The bigger fraction of mercury enters directly to the air as vapors of inorganic mercury and professional people frequently expose to it through inhalation and direct skin contact. It means that consumption of contaminated water and food items[9], inhalation, ingestion of soil and dermal contact are the major human exposure routes[10]. Compounds of Hg react with glutathione particularly with active sulfhydryl group of this compound forming complexes. The high chemical affinity of Hg for -SH group is accountable for the toxicological behavior of mercury compounds[11,12]. When mercury compounds are identified in biological media, it has been found that mercury has formed complexes with-SH containing ligands. Mercury complexes with glutathione and cysteine have been identified in blood[13,14], brain[15], liver[16], and bile[17]. The aim of this study was to analyze the

effect of mercury on plasma and cytosolic fraction GSH.

MATERIALS

Ethanol (Sigma), chloroform (sigma), Phenyl mercuric acetate(sigma), Disodium edetate (Merk), HCl (Kolchlight), NaOH (Sodium Hydrooxide, Merk), NaCl (Fluka), Potassium dihydrogen phosphate (sigma), Ellman's reagent,(sigma) reduced form of glutathione (Fluka), Distilled water and water for injection. Flask, graduated cylinders, beakers and funnels of different volumes made of Pyrex glass were used in this piece of work. Pyrex glass test tubes sterile (Germany) and micropipettes made up of scorex company Finland were also used. UV-visible spectrophotometer of shimadzu model-1601, Japan, Pyrex eppendolf's tubes, centrifuge model H 200 Kokuson Ensik company of Japan, pH meter (Nov-210, Korean Nov scientific company), disposable rubber gloves, sterile syringes of surg pharmaceuticals. No further purification of chemicals was made and were used as were purchased

METHODS

For the purpose of isolation of blood plasma, 200ml of fresh venous blood from healthy human volunteer was collected in a hepranized bag. The blood was processed for centrifugation for which it was centrifuged at 3000rpm for 10 minutes at 4°C. The supernatant yellow layer was plasma layer which was pipette of without disturbing the white buffy layer. Then plasma was deproteinated by the addition of 5.1% tri-acetic acid solution. After deproteination, the mixture was allowed to stand for 5 minutes at room temperature. It was again centrifuged at 3000rpm for 5 minutes, after centrifugation, the supernatant was carefully collected without disturbing the precipitate. In order to maintain the plasma GSH in reduced form, 50μl 0.1N HCl was added to the plasma and was stored below 20°C to refrigerator till use.

After the isolation of plasma the remaining packed cells (red blood cells fraction) were further processed for isolation of cytosolic fraction. The red blood cells fraction was gently taken and washed two times with normal saline solution (0.9% NaCl solution). After washing red blood cells fraction was then softly centrifuged for 5 minutes. At the end of centrifugation, supernatant layer was discarded, 0.5ml red blood cells fraction was taken and mixed with 0.5ml of double refined distilled water to lyse the RBCs. This was placed in refrigerator for 1 hour, 0.6ml chloroform: ethanol (3:5) mixture was added to the above mixture in order to precipitate hemoglobin. It was mixed softly with the further addition of 0.1ml of distilled water. The above mixture was centrifuged hard at 10000 – 12000rpm for 10 minutes. The supernatant pale yellow layer was cytosolic fraction or lysate, 50μl of 0.1N HCl solution was added to keep GSH in reduced form. The obtained lysate was kept in refrigerator till use.

RESULTS

The level of GSH contents was depleted significantly ($p<0.001$) in both the fraction. In case of plasma(Fig-1), the drop in plasma GSH contents by all used concentrations of phenyl mercuric acetate (PMA) with respect to plasma GSH control was 27.50% (3.611 μM), 32.36% (3.369 μM), 36.32% (3.172 μM), 46.68% (2.656 μM),52.82% (2.350 μM) and 60.49% (1.968 μM) respectively. In case of cytosolic fraction(Fig-2), the drop in cytoslic fraction GSH contents with respect to cytosolic fraction GSH control by all used concentrations of phenyl mercuric acetate (PMA) was 35.49% (3.357 μM), 36.72% (3.292 μM), 46.02% (2.809 μM), 53.25% (2.433 μM), 57.78% (2.197 μM) and 64.26% (1.860 μM). The decrease in cytosolic fraction GSH level is greater than decrease in plasma GSH level which is showing that phenyl mercuric acetate has the penetrating capability into the semi permeable membrane of red blood cells of human blood.

Isolated plasma and cytosolic fraction were separately exposed to different concentrations of phenyl mercuric acetate (PMA) for various times of incubation in order to evaluate extent of interaction between isolated plasma/cytosolic fraction GSH and various concentrations of PMA with the elapse of time. The results of effect of phenyl mercuric acetate on GSH of plasma

and cytosolic fraction with time is shown is Fig-3,4. Ellman's method was used to measure the absorbance at λmax: 412nm of each sample test tube and absorbance of each sample was converted into concentration of plasma/ cytosolic fraction GSH, this was the unknown concentration of plasma/cytosolic fraction GSH left after interaction with various concentrations of phenyl mercuric acetate. In case of plasma (Fig-5) the plasma GSH contents were dropped from 0 to 120 minutes by various concentrations of PMA were 64.45%, 59.33%, 50.89%, 41.56%, 33.63% and 32.99% while in case of cytosolic fraction (Fig-6), the drop in GSH level from 0 to 120 minutes by different concentrations of phenyl mercuric acetate (PMA) was 53.86%, 48.60%, 45.41%, 36.11%, 29.38% and 27.06%.

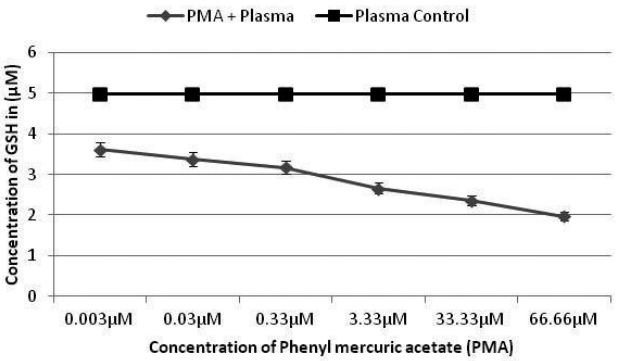


Fig-1. Concentration dependent effect of phenyle mercuric acetate on Plasma GSH

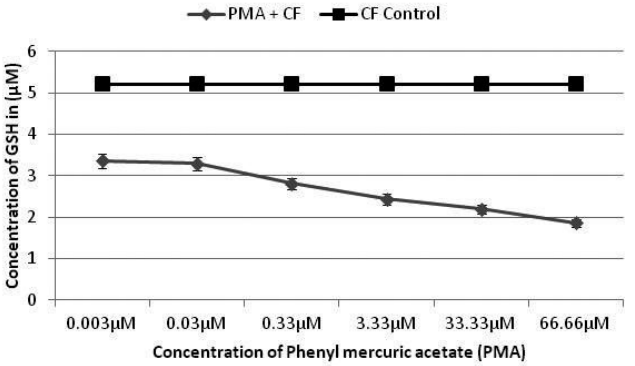


Fig-2. Concentration dependent effect of phenyle mercuric acetate on Cytosolic Fraction GSH

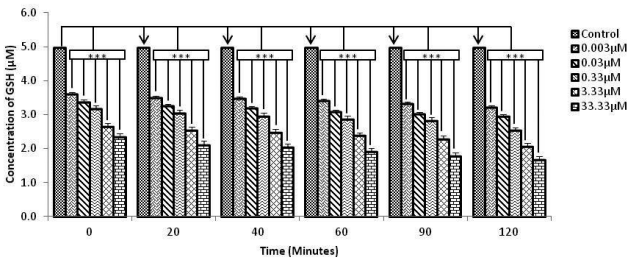


Fig-3. Time dependent effect of phenyl mercuric acetate on plasma GSH One-way ANOVA followed by Dunnet's HSD test was applied.* p<0.001**

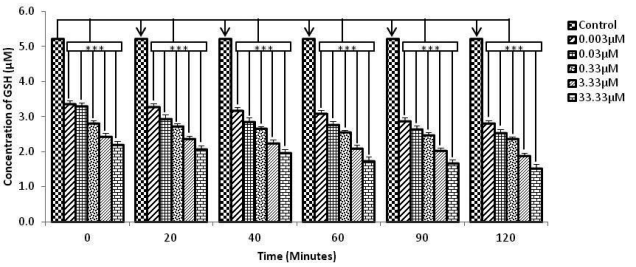
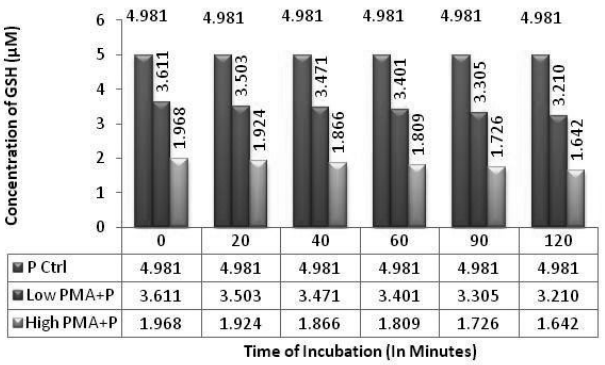
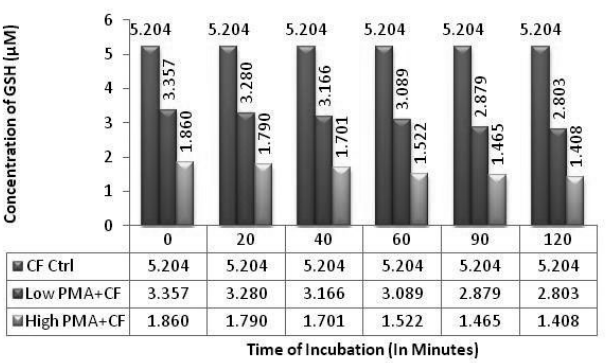


Fig-4. Time dependent effect of phenyl mercuric acetate on cytosolic fraction GSH One-way ANOVA followed by Dunnet's HSD test was applied.* p<0.001**



	0	20	40	60	90	120
P Ctrl	4.981	4.981	4.981	4.981	4.981	4.981
Low PMA+P	3.611	3.503	3.471	3.401	3.305	3.210
High PMA+P	1.968	1.924	1.866	1.809	1.726	1.642

Fig-5. Effect of phenyl mercuric acetate on plasma GSH with time of incubation from 0 min: to 120 min, One-way ANOVA followed by Dunnet's HSD test was applied. *** p<0.001



	0	20	40	60	90	120
CF Ctrl	5.204	5.204	5.204	5.204	5.204	5.204
Low PMA+CF	3.357	3.280	3.166	3.089	2.879	2.803
High PMA+CF	1.860	1.790	1.701	1.522	1.465	1.408

Fig-6. Effect of phenyl mercuric acetate on cytosolic fraction GSH with time of incubation from 0 min: to 120 min, One-way ANOVA followed by Dunnet's HSD test was applied. *** p<0.001

DISCUSSION

Mercury is found everywhere in its different forms like organic, inorganic and in its elemental form as pollutant of the environment. No human being is safe from its toxicity as it comes simultaneously into the food chain and then ultimately into the human body especially through fish foods etc. Glutathione is found in intracellular as well as in extracellular compartments in human body and constitutes the most important antioxidant system of the body. It has great chemical affinity for those oxidants which have unpaired electrons including the heavy metals ions like Hg^{1+} $Hg^{2+,}$ Cd^{2+} etc. As soon as mercury enters in human body in any form, glutathione scavenges it as free radical. Our aim of this study was to evaluate the effect of phenyl mercuric acetate on the GSH contents of blood plasma and cytosolic fraction GSH for which thiol quantification was done by using Ellman's method. Our results shows that there is significant drop in GSH level of both the compartments and comparatively there was more drop in GSH contents in cytosolic fraction GSH which indicates that organic mercury can easily penetrates into the semi permeable membranes of red blood cells of human blood. The drop in plasma and cytosolic GSH was dose and time dependant as with the passage of time there was further decrease in GSH levels of these components of blood. These results reveal that with the passage of time organic mercury toxicity is more and more harmful for human so at the earliest measures for detoxification of mercury should be taken. The high concentration of organic mercury was detected in blood samples of those peoples who on average ingested two to three times fish in a week as compared to those who ingested two to three times in a month [18]. Organic mercury may also open the pore by oxidizing GSH and/or NADH resulting in the oxidation of the sensitive vicinal dithiol present in the S site and P-site of the permeability transition pore, respectively, or by cross-linking the dithiol in the S-site[19]. It is well documented in literature that exposure to organic mercurial partly inactivates cytochrome *i* oxidase[20]. It is remarkable that cytochrome c oxidase has seven cysteine residues as sulfhydryl groups which are subject to alteration by hydrophobic mercurial, such as phenyl and methyl mercury. It is also worth mentioning that apoptosis and the stress response are highly interconnected and altered expression of heat shock proteins exerts great influence on the development of apoptosis [21].

CONCLUSIONS

During organic mercury toxicity the blood components are also affected and this affect is dose and time dependent, as a result with the increase of time, the organic mercury toxicity would be more harmful so detoxification of mercury should be done on emergency bases at the earliest with the help of suitable chelating agents along with antioxidant therapy.

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PREVIOUS RELATED STUDY

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Hashmat Ullah[1],	Conception, design, statistical expertise and drafting of the article	
2	Muhammad Farid Khan[2]	Critical revision of the article for important intellectual content