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The interplay of antioxidant activities in rats exposed to sub chronic low level inorganic mercury is gender specific

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Abstract: *To investigate sex differences in the effects of inorganic mercury on antioxidant enzymes in different compartments, male and female albino rats were exposed to mercury (0.5, 1.0 and 1.5mg/kg) for 12 weeks. Post exposure, total mercury in liver, kidney and whole blood was determined using Inductively-Coupled Plasma Spectrometry (ICP-MS) while superoxide dismutase (SOD) and catalase (CAT) activities in the liver, kidney, plasma and erythrocytes were also determined. Inhibition of CAT and SOD in plasma and erythrocyte characterised the effects of inorganic mercury in female animals with the same trend in SOD of the male, but vice versa in CAT respectively. Inorganic mercury exposure inhibited CAT by 23% (liver) and 84% (kidney); SOD by 30% (liver) and 16% (kidney) respectively in female animals. In the male animals, a 26% inhibition of SOD was observed in the liver, whereas CAT was inhibited (66%) in the kidney. Correlation analysis shows a negative relationship between tissue mercury levels and the two antioxidant enzymes specifically in females. The findings from this support sex differences in the effects of mercury on the depletion of antioxidant enzymes.*

Keywords: *Inorganic mercury, Antioxidant enzymes, superoxide dismutase (SOD), Catalase (CAT).*

I. Introduction

Mercury is the second-most prevalent heavy metal toxicant. It is of ubiquitous environmental and epidemiological importance, posing severe health risk to millions of people worldwide (Patrick 2002). Mercury toxicity has intense neurological, renal, cellular, cardiovascular, pulmonary, haematological, respiratory, immune, dermatological, reproductive and developmental disorders implications (Rice et al. 2014; Risher and Amler 2005). The toxicity of mercury depends on its chemical form. Mercury is found in the environment in three major forms, viz: elemental mercury, organic mercury and inorganic mercury compounds or mercury salts example eg. mercuric chloride ($HgCl_2$) (Magos and Clarkson 2006).

Mercuric chloride ($HgCl_2$) is an inorganic compound with extensive applications in various field such a: catalyst in polyvinylchloride production, in sample preservation, photography etc. It is equally a widespread environmental and industrial pollutant, which induces severe alterations in tissues and enzyme functions of both animals and men (Mahboob et al. 2001). Exposure to inorganic mercury compounds ($HgCl_2$) had increased through its extensive usage in a range of medical and cosmetic products such as antiseptics, laxatives, diuretics, teething powders, skin-lighting creams (Syversen and Kaur 2012). Once absorbed, $HgCl_2$ is distributed in all tissues, and low fractions have been shown to easily cross the brain-blood barrier and the placenta. Critical target organs of mercury chloride exposure include kidney, liver, blood, intestinal epithelium and lungs (Berlin 1978; Warfvinge et al. 1992). The kidney is however considered as the primary target organ, in which $HgCl_2$ is intensively accumulated following chronic exposure (WHO 1991). Mercury and its compounds mainly inhibit the activities of the free radical quenching enzymes; catalase, superoxide dismutase and glutathione peroxidase (Benov et al. 1990)

Different mechanisms have been highlighted to account for the biological toxicity of mercury chloride (Sener et al. 2007). Beside the reported affinity of mercury chloride for thiol containing enzymes, a common factor in these mechanisms is the excessive generation of reactive Oxygen species, manifesting as increased lipid peroxidation in the cells (Durak et al. 2010b). Significant reductions in glutathione levels, GSH-Px, GST and catalase activities of the liver have been reported to accompany increased oxidative stress and lipid peroxidation (Temmar et al. 2013)

There is a dearth of literature on sex differences in mercury chloride induced oxidative stress, though there is increased evidence that the effects of metal induced oxidative stress on health are manifested differently in males and females (Vahter et al. 2007). Higher resting plasma antioxidant levels have been observed in women compared with men (Goldfarb et al. 2007). Females have also been shown to have higher levels of mitochondrial reduced glutathione, SOD and glutathione peroxidase than their male counterparts. Similarly, mitochondrial from female rats has higher expression of antioxidants enzymes and lower production of reactive oxygen species compared to mitochondria of male rats (Borras et al. 2003).

Several studies have reported that the adaptation to changes in antioxidant capacity is affected by gender difference. However, studies on the impact of gender differences in mercury chloride toxicity in the rat are scanty and inadequate. This research aimed at providing information on the influence of gender in antioxidant enzymes activities in rats exposed to mercury chloride toxicity.

II. Materials and methods

2.1 Chemicals

All chemicals and kits used in this study were obtained from British Drug House (BDH) Chemicals Limited, Poole, England, except Mercuric chloride (Sigma-Aldrich, Missouri, USA).

2.2 Animals and treatment

Experimental protocols were conducted in accord with guidelines of the Institutional Animal Care and Use Committee and were approved by the Ethical Committee of the Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria. Sixty-four male and female albino Sprague-Dawley rats (bred in the College of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria) with a mean body weight of 150g were used for the experiment. The rats were housed in an animal room with average temperature (22±2˚C), and a regular 12h light-dark cycle (06:00-18: 00h). They were allowed 14 days to acclimatize before the commencement of mercury exposure. The animals maintained on a standard pellet diet.

Animals were divided into eight groups of eight animals each. While two groups served as control (male and female) and received distilled water, the remaining groups (three groups each for male and female) were exposed to mercury chloride (0.5, 1.0 and 1.5mg/kg body weight) respectively for 12 weeks. At the end of mercury exposure, blood was collected by cardiac puncture into heparinized tubes under light anesthesia after an overnight fast. Liver and kidney were also removed from the animals, weighed and stored at −4 °C until needed. An aliquot of the blood samples was used for mercury determination while the remaining blood samples were centrifuged (5000rpm) on a model 90.1 centrifuge (England). The resulting red blood cells were washed twice with physiological saline and used for antioxidant assays. All samples were stored at -20˚C until needed.

2.3 Mercury determination

A portion of the frozen organs (≈200mg) and whole blood (200µL) were digested in nitric and sulphuric acid mixture. Total mercury was determined using Inductively-coupled plasma spectrometry (ICP-MS) in the central analytical laboratory; Stellenbosch University, South Africa Results are expressed as µg Hg/ml for blood and µg Hg/g wet weight for the organs.

2.4 Determination of Catalase activity in plasma, erythrocyte, liver and kidney

The activity of the catalase in the plasma, erythrocyte and homogenates of liver and kidney were estimated spectrophotometrically and expressed in units/ml for the plasma and erythrocyte, while that of the kidney and liver were expressed in units/g tissue as described (Claiborne 1985). The used reaction mixture (1 ml) contained 100 mM phosphate buffer (pH 7.4), 50 mM of H_2O_2 and plasma or erythrocyte or tissues homogenate. The reaction was started by adding H_2O_2, and its decomposition was observed by following the decrease in absorbance at 240 nm for 1 min.

2.5 Determination of Superoxide dismutase activity in plasma, erythrocyte, liver and kidney

The activity of the superoxide dismutase in the plasma, erythrocyte and homogenate of liver, brain and kidney was estimated spectrophotometrically and express in units/ml for the plasma and erythrocyte while that of the kidney, brain and liver were expressed in units/g tissue as described (Sun and Zigman ,1978). The reaction was carried out in 0.05M sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005NHCl. One unit of SOD is the amount of the enzyme required for 50% inhibition of the auto-oxidation of epinephrine to adenochrome per minute.

2.6 Statistical analysis

Results are expressed as mean ± sem. One-way analysis of variance (ANOVA) followed by Tukey's test was used to analyze the results with $p<0.05$ considered significant. Associations among the parameters and their magnitudes were tested for by using Multiple Linear Regression analysis.

III. Results

Table 1: Mercury concentrations in the tissues of the animals

Mercury dose(mg/kg body weight)	Sex	Liver Mercury µg/100g	Kidney Mercury µg/100g	Whole mercury µg/100ml
Control	Male	8 ± 0.000^{a}	14.3 ± 0.009^{a}	0.4 ± 0.000^{a}
	Female	5.5 ± 0.003^{a}*	32.2 ± 0.029^{a}*	0.5 ± 0.001^{a}

0.5	Male	0.173±0.010[b]	29.207±0.881[b]	0.035±0.010[c]
	Female	27.3±0.049[b]	22582±1.754[b]	2±0.004[b]
1.0	Male	42.9±0.031[c]	1747±0.214[c]	2.2±0.005[b]
	Female	68.9±0.055[c]*	4383.4±4.913[c]*	3.7±0.010[c]
1.5	Male	136.5±0.186[d]	2378.2±1.587[c]	4.1±0.007[c]
	Female	88.6±0.064[d]*	7789.3±7.708[d]*	6.7±0.016[d]

Each value represents the mean ± S.E.M. of 8 rats. Values within a row with different alphabets are significantly different from each other. *p < 0.05, compared to male.

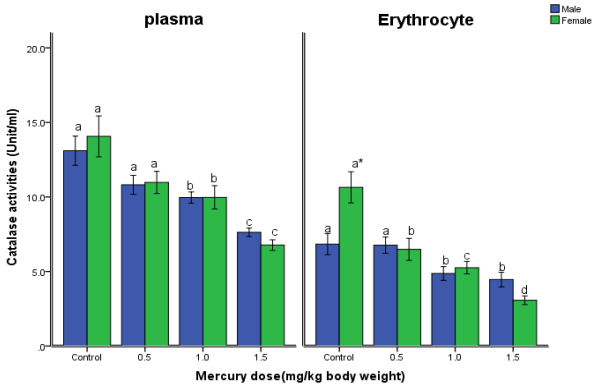

Figure 1: Effects of inorganic mercury on plasma and erythrocyte catalase activities in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

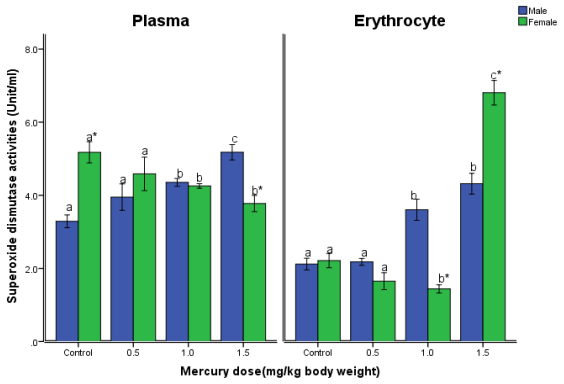

Figure 2: Effects of inorganic mercury on plasma and erythrocyte superoxide dismutase activities in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

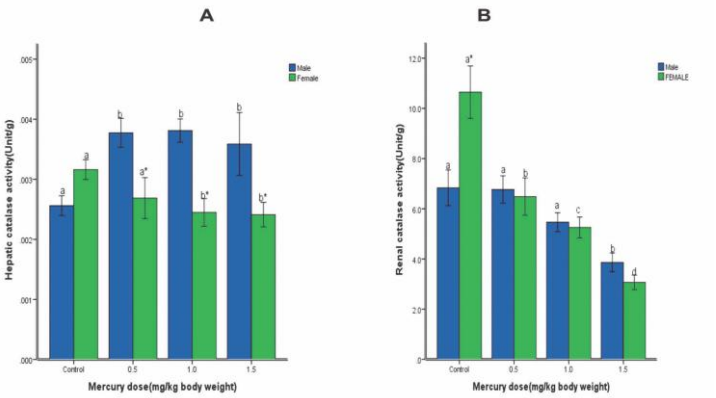

Figure 3: Effects of inorganic mercury on liver (A) and kidney (B) catalase activity in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

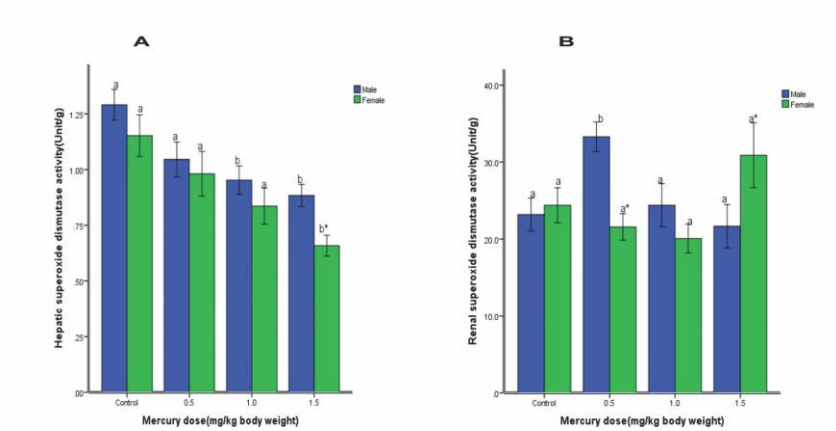

Figure 4: Effects of inorganic mercury on liver (A) and kidney (B) superoxide dismutase activity in the animals. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

Table 2: Associations between tissue mercury levels and Catalase activity in various compartments of male and female animals

	Tissue mercury		Blood mercury	Hepatic mercury	Renal mercury
Plasma superoxide dismutase activity (unit/ml)	Male	Cor. Coef. (r)	0.372*	0.698**	0.486**
		P val.	0.036	0.000	0.005
	Female	Cor. Coef. (r)	-0.273	-0.517**	-0.555**
		P val.	0.130	0.002	0.001
Erythrocyte superoxide dismutase activity (unit/ml)	Male	Cor. Coef. (r)	-0.036	0.641**	0.262
		P val.	0.334	0.000	0.148
	Female	Cor. Coef. (r)	0.634**	0.582**	0.660**
		P val.	0.000	0.000	0.000
Hepatic superoxide dismutase activity (unit/g)	Male	Cor. Coef. (r)	-0.368*	-0.375*	-0.511**
		P val.	0.038	0.034	0.003
	Female	Cor. Coef. (r)	-0.483**	-0.531**	-0.623**
		P val.	0.005	0.002	0.000
Renal superoxide dismutase activity (unit/g)	Male	Cor. Coef. (r)	0.262	-0.199	0.339
		P val.	0.147	0.276	0.058
	Female	Cor. Coef. (r)	0.077	0.117	0.209
		P val.	0.674	0.524	0.252

Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level

Table 3: Associations between tissue mercury levels and superoxide dismutase activity in various compartments of male and female animals

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Erythrocyte superoxide dismutase activity (unit/ml)	Male	Cor. Coef. (r)	-0.036	0.641**	0.262
		P val.	0.334	0.000	0.148
	Female	Cor. Coef. (r)	0.634**	0.582**	0.660**
		P val.	0.000	0.000	0.000
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		P val.	0.674	0.524	0.252

Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level

IV. Discussion

Exposure of the animals to inorganic mercury resulted in accumulation of mercury in blood, liver and kidney of both sexes compared with the control. In all the tissues of female animals was the building up found to be dose-dependent (Table 1). In male animals, exposure to inorganic mercury at the highest dose of 1.5mg/kg

resulted in a mercury content of blood (4.1µg Hg/100ml) and of the kidney (2378.2µg Hg/100g). Whereas in the female, at same dose the mercury content of blood and kidney was 6.0µg Hg/100ml and 7789.3µg Hg/100g respectively.

As depicted in fig 1.0, $HgCl_2$ at highest dose resulted in decreased level in both plasma and erythrocyte catalase activities in both sexes mostly in female (51.8%) than male (42.0%), 70.75% and 33.82 respectively. The same trend was also observed in the kidney of both sexes with the highest level of inhibition, 70.75% in female (fig. 3B). Meanwhile, $HgCl_2$ resulted to decrease in female hepatic catalase whereas the increase was observed in male animals as shown in fig.3A.

As illustrated in fig 2.0. as compared with the control group, HgCI2 at the highest dose, caused 26.96% reduction in plasma SOD activity in female. On the contrary, SOD activity in plasma was significantly increased in male rats with a percentage increase of 57.57%. Also, increased in the erythrocyte of both sexes with 104.76% and 209.09% respectively.

Oxidative stress can occur either as a result of increased reactive oxygen species (ROS) generation, depressed antioxidant system or both. Various mechanisms have been proposed to explain the biological toxicity of $HgCl_2$, including oxidative stress. Accumulation and toxicity of HgCl in target tissues (El-Shenawy and Hassan 2008) increases the production of many endogenous oxidants, such as hydrogen peroxide, depletes protective antioxidants, such as glutathione (GSH), and reduces free radical scavenging systems, such as superoxide dismutase (SOD) and GSH peroxidase (GPx) (Mahboob et al. 2001). These clinical manifestations support the involvement of oxidative stress in the toxicity of mercury chloride.

The accumulation of mercury in the liver, kidney and whole blood of the male and female rats used in this study were observed to be significantly different, which were higher in female than male. This result corroborates another study on mercury chloride toxicity (Oriquat 2012). Increasing concentration of mercury in the blood with increasing mercury chloride exposure suggests increased distribution of mercury into vital organs. However, distribution of mercury into specific tissue is affected by its chemical form. Inorganic mercury has a non-uniform distribution after absorption, been accumulated mainly in the liver, and the kidney. (Bharathi et al. 2012; Nielsen and Andersen 1989). Uptake, accumulation and toxicity of inorganic mercury in the kidney is related to its binding to endogenous thiol-containing molecules, while its accumulation in the liver is related to the role of the liver in toxicant metabolism.

The expected sex differences as a result of sub chronic $HgCI_2$ were observed more in superoxide dismutase activities in all the compartments studied, with inhibition being higher in females than male. Meanwhile, the sex differences observed in catalase activity was only obsserved in the liver. The result from this study is similar to other reports on mercury chloride toxicity. Treatment with mercury chloride has been reported to cause a decrease in catalase activity in erythrocytes (Durak et al. 2010a), plasma (Pinheiro et al. 2008) and liver tissue (Jagadeesan and Sankarsami Pillai 2007). The decreased activity of these enzymatic antioxidants may be due to the accumulation of free radicals which in turn causes the inhibition of these enzymes. High level of peroxides may cause the inhibition of catalase activity in liver tissue (Mary and Reddy 1999). These data suggest that chronic mercury intoxication may deplete antioxidant enzymatic activity.

Various mechanisms have been proposed to explain the biological toxicity of $HgCl_2$, including oxidative stress. Experimental models suggest that oxidative stress plays an important role in the toxicodynamics of heavy metals, including mercury (Soares et al. 2003). The capability of mercury to initiate oxidative stress lies in its affinity and ability to bind to reduced sulphur especially in thiol containing molecules like albumin, cysteine, metallothionein (MT) and glutathione (GSH). Glutathione helps in removing toxic peroxides by serving as a cofactor of glutathione peroxidise, GPx (Bharathi et al. 2012). Glutathione peroxidase is the most important peroxidase for the detoxification of hydrogen peroxide. A single Mercury (Hg) ion can bind to and cause irreversible excretion of up to two GSH molecules (Quig 1998). Depletion of GSH, the cofactor of GPX leads to a reduction in the antioxidant ability of GPX against H_2O_2, an increase in the H_2O_2 burden, and a final inhibition of catalase activity by H_2O_2.

In most studies on mercury chloride induced toxicity in rats, oxidative stress has been suggested as a mechanism of toxicity and a decrease in SOD activity in tissues and erythrocyte has been mostly reported (Durak et al. 2010a; Miller 1991). Data obtained in this present study for SOD activity in the kidney, plasma, and erythrocytes of male rats deviated from the trend previously reported.. While a significant increase in kidney, plasma, and erythrocytes SOD activity was observed in male rats, a decrease (not significant) was observed in female rats. A significant decrease in SOD activity was however observed in the hepatic tissue of both male and female rats corroborating an earlier study. (Surapaneni and Vishnu Priya ,2009)

V. Conclusion

The present study suggests that the peroxide-removing antioxidant system of male rats was less affected on exposure to different levels of inorganic mercury compared to female in all the compartments studied except the liver. Whereas female rats had stronger superoxide-removing antioxidative system, as the

levels were significantly elevated except in the liver. Conclusively, these results suggests that female kidney are particularly vulnerable to oxidative stress on exposure to mercury..

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