Research Article

Sodium Selenite Pretreatment Ameliorates Aspects of the Nephropathy Induced by Mercuric Chloride in the Ratte *Albinos Wistar*

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ABSTRACT

The study was designed to investigate the possible protective role of sodium selenite in mercuric chloride induced renal stress, by using biochemical approaches. Female rats *Albinos Wistar* were randomly divided into four groups. The first group was served as the control, the second group was given sodium selenite (1 mg/kg b.w), while the third group was given mercuric chloride (1 mg/kg), finally, the fourth group was given combined treatment of sodium selenite and mercuric chloride for 10 days. The effects of sodium selenite on mercuric chloride induced oxidative and renal stress were evaluated by serum creatinine, urea and uric acid, kidney tissue lipid peroxidation, GSH levels, GSH-Px and GST activities. Administration of mercuric chloride induced significant increase in serum: creatinine, urea and uric acid concentration showing renal stress. Mercuric chloride also induced oxidative stress, as indicate by decreased kidney tissue of GSH level, GSH-Px and GST activities along with increase the level of lipid peroxidation. Furthermore, treatment with mercuric chloride caused a marked elevation of kidney weight and decreased body weight. Sodium selenite treatment markedly reduced elevated serum: creatinine, urea and uric acid levels, and counteracted the deterious effects of mercuric chloride on oxidative stress markers and attenuated histopathological changes caused by $HgCl_2$ in kidney. Our results indicate that sodium selenite could have a beneficial role against mercuric chloride induced nephrotoxicity and oxidative stress in rat.

Keywords: Mercury, sodium selenite, lipid peroxidation, reduced glutathione, antioxidant enzymes.

INTRODUCTION

Mercury is a well-known human and animal induces extensive kidney damage nephrotoxicant. acute oral or parenteral exposure induces extensive kidney damage[1]. Studies in vivo and in vitro have demonstrated that mercury induced lipid peroxidation, suggesting the involvement of oxidative stress in its cytotoxicity. Lund et al. (1993)[2] reported that mercury enhances renal mitochondrial hydrogen peroxide formation *in vivo* and *in vitro*. However, cansative correlation between mercury induced lipid peroxidation and cellular toxicity remains controversial. Some authors reported that lipid peroxidation plays a critical role in cell injury induced by mercury[2] in renal cells, whereas other investigators showed that lipid peroxidation is not directly responsible for mercury induced cell injury in hepatocytes and renal cells[3]. It is believed that antioxidants should one of the important components of effective treatment for mercury poisoning. Indeed, $HgCl_2$ induced injury can be ameliorated by superoxide dismutase[4] and non-enzymatic antioxidants like vitamin C, Vitamin E, cystein and selenium[6] have proven helpful to overcome oxidative damage.

The biological important of selenium is at least 3-fold. First, it forms the prosthetic group of some critical selenocysteine containing enzymes, such as glutathione peroxidase, iodothyronine 5'-deiodinase, and thioredoxin reductase[7]. Second, sodium selenite is protective against a number of toxicants. Third, selenium excessive intake cause toxic potential[8]. The purpose of this study was to evaluate the protective role of selenium on mercury chloride induced oxidative and renal stress in rats.

MATERIALS AND METHODS

All chemicals used in this work were purchased from sigma chemical company. Laboratory animals, *Albino Wistar* male rats, were brought from the Algiers Pasteur institute at the age of 8 weeks, with an average live weight of 200g. They were located in a room with an ambient temperature of 21±1°C and up to 12h of light daily. The rats were divided into four experimental groups; each consists of six rats. The first group was served as the control. The second group was given sodium selenite at a dose of 1 mg/kg body weight, while the third group ($HgCl_2$) was intraperitoneally given mercuric chloride at a dose of 1 mg/kg body weight. Finally, the fourth group was given combined treatment with sodium selenite and mercuric chloride .The treatment of all groups was lasted for 10 days.

Twenty four hours after the last administration, blood was collected by retro-orbital sinus punction, After centrifugation at 3000 rpm for 10min, the serum was separated immediately and stored at – 20°c until determination of urea, creatinine, and uric acid levels. Subsequently, rats were decapitated and kidneys were removed.

Tissue preparation

About 500mg of kidney was homogenized in 4mlof buffer solution of phosphate buffered saline (w/v: 500mg tissue with 4ml PBS, PH 7.4) homogenates were centrifuged at 10.000xg for 15min at 4°c. And the resultant supernatant was used for determination of: reduced glutathione (GSH) according the method of Weeckbekeretcory (1988)[9], Thiobarbituric acid- reactive substance (TBARS) level by method of Buege and Aust (1978)[10], and glutathione peroxidase (GSH-PX) and glutathione –S-transferase (GST) activities were measured by the method Flohe and Gunzler(1984)[11] and Habig et al (1974)[12] respectively. However, protein content was measured by the method of Bradford (1976)[13].

Histopathological examination

Kidney from autopsied animals were excised out and fixed in formalin (10%). five micron think section were prepared by using microtome and these section were stained with hematoxyline and eosin. For histological alterations these slides were observed under light microscope.

Determination of Biochemical parameters

Serum urea, creatinine and uric acid levels were determined using commercial kits (Spinreact).

Statistical analysis

The data were subjected to student *t* test for comparison between groups. The values are expressed as mean ± SEM. Significance level was set at P<0.05, P<0.01, P<0.001.

RESULTS

Effects of treatments on body, absolute and relative kidney weights

Table 1 shows the effect of mercuric chloride, sodium selenite and combined treatment with mercuric chloride and sodium selenite. The marked decreased in rats body weight was observed in mercuric chloride treated rats and mercuric chloride + sodium selenite group, but the result was not significant as compared to control. Along sodium selenite showed increased body weight but result was not significant. The kidneys of rats treated with mercuric chloride were enlarged. Mercuric chloride treated rats showed a highly significant increased kidney weight and relative kidney weight (P≤0.001) as compared to control. Combined treatment with sodium selenite showed significant increased relative kidney weight, while alone sodium selenite treatment had showed no significant effect.

Effects of treatment on serum biochemical parameters

A highly significant (P≤0.001) elevation in serum urea, creatinine and uric acid levels was observed in mercuric chloride intoxicated rats. Only sodium selenite treatment did not show any significant alteration. However, the combined treatment of sodium selenite with mercuric chloride show a highly significant decline in serum urea, creatinine and uric acid was noticed respect to mercuric chloride treated animals (table 2).

Table 1: Changes in body and absolute and relative kidney weights of control and rats treated with selenium (Se), mercuric chloride, and combined treatment of mercuric chloride with selenium after 10 days of treatment.

Parameters	Treatment groups			
	Control	Se	$HgCl_2$	Se + $HgCl_2$
Initial body weight (g)	226.66±26.56	222.5±12.56	221±35.36	224.66±25.5
Final body weight (g)	227±18.4	225.83±18.9	193.16±31.0	218.81±15.05
Absolute kidney weight (g)	1.04±0.24	1.11±0.53	2.23±0.82**	1.76±0.18**
Relative kidney weight (g/100g b.w)	0.45±0.008	0.49±0.006	1.15±0.02**	0.8±0.01***

Values are given as mean ± SEM for group of 6 animals each. *P≤0.05, compared to controls. **P≤0.01, compared to controls. ***P≤0.001, compared to controls.

Table 2: Changes in biochemical parameters of control and rats treated with selenium (Se), mercuric chloride, and combined treatment of mercuric chloride with selenium after 10 days of treatment.

Parameters	Treatment groups			
	Control	Se	$HgCl_2$	Se + $HgCl_2$
Urea (g/l)	0.25±0.02	0.23±0.01	0.37±0.03	0.33±0.05**
Creatinine (mg/l)	2.89±0.71	2.92±0.55	3.94±0.98	3.17±0.54
Uric acid (mg/l)	15.55±4.50	16.55±2.43	24.81±6.87*	23.04±4.35**

Values are given as mean ± SEM for group of 6 animals each. *P≤0.05, compared to controls. **P≤0.01, compared to controls. ***P≤0.001, compared to controls.

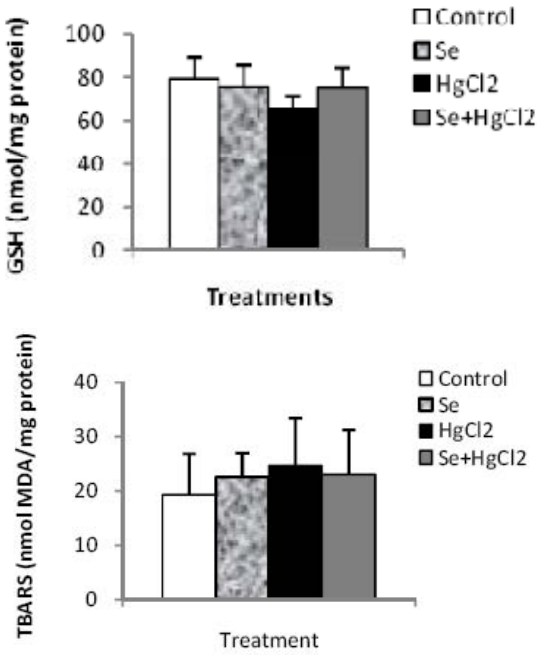

Figure 1: Reduced glutathione (nmol/ mg protein) and TBARS (nmol MDA /mg protein) levels in kidney of control and rats treated with selenium, mercuric chloride, and combined treatment of mercuric chloride with selenium after 10 days of treatment. Values are given as mean ± SEM for group of 6 animals each significant difference: * compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).

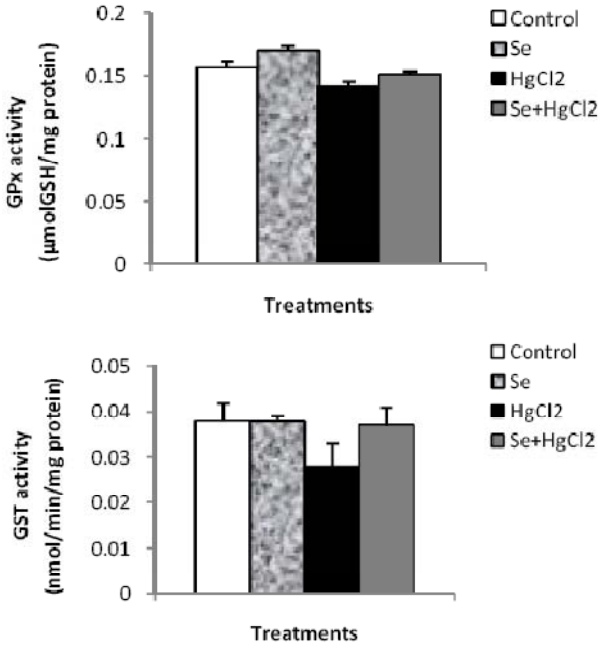

Figure 2: Enzyme activities of GPx (µmol GSH/ mg protein) and GST (nmol /min/mg protein) in kidney of control and rats treated with selenium, mercuric chloride, and combined treatment of mercuric chloride with selenium after 10 days of treatment. Values are given as mean ± SEM for group of 6 animals each significant difference: * compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).

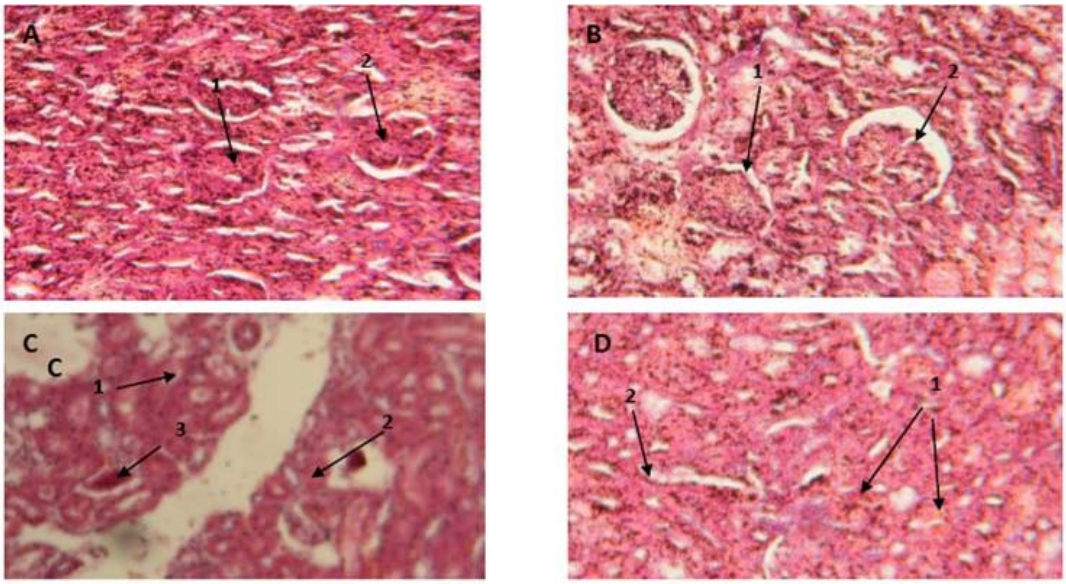

Figure 3: T.S. of kidney of Female rat treated with mercuric chloride (Hg) alone, and in combination with sodium selenite. (A) control (H&E100X): showing well develop glomerulus (1), with normal tubular cells; (B) sodium selenite alone treatment (H&E 100X): showing normal glomerulus (1), and normal tubular cells; (C) mercury treatment (H&E100X): showing degeneration of tubular cells (1), loss of nucleus (2), degeneration of glomerulus (3); (D) combined treatment of mercuric chloride with sodium selenite (H&E100X): showing normal glomerulus (1), normal tubular cells (2).

Effects of treatments on renal oxidative stress parameters

Mercuric chloride exposure a significant depleted in reduced glutathione level, GSH-Px and GST activities. And a significant increase in kidney lipid peroxidation level in mercury intoxicated rats was noticed. Sodium selenite alone treatment did not show any significant decline. In combined treatment of mercuric chloride with sodium selenite highly significant increase in reduced glutathione level, GSH-Px and GST activities. And significant depletion in lipid peroxidation level was recorded with respect to mercury intoxicated rats (Fig.1 and 2).

Histological studies

The histological changes in Kidney are presented in Fig.3. Mercuric chloride induced various pathological alterations in kidney of rats. These alterations were characterized by renal tubular damage, indicating by tubular necrosis (Fig. 3C). In combination group were sodium selenite was administration with mercuric chloride showed reparative changes. Kidney showed prominent recovery in the form of normal renal tubular and very less tubular necrosis (Fig. 3D). kidney of the control group had a regular histological structure (Fig.3A). Furthermore, no histological alterations were observed in the kidney of sodium selenite treated group (Fig. 3B).

DISCUSSION

In the present study, oxidative stress induced by $HgCl_2$ was evidenced in kidney of rats by increase in lipid peroxidation level and the stimulation of GSH-Px, and GST activities. Accordingly, oxidative stress induced by $HgCl_2$ has been previously reported[2]. As a consequence of lipid peroxidation biological membranes are affected causing cellular damage. Renal damage observed in rats exposed to $HgCl_2$ was also evidenced by increase in the plasmatic levels of urea, creatinine and uric acid, which are renal markers of damage. In the present study, serum urea, creatinine and uric acid levels were significantly increased after 10 days mercuric chloride (1mg/kg), showing insufficiency of renal function. Studies in animals have established that tubular injury plays a central role in the reduction of glomerular filtration rate in acute tubular necrosis. Two major tubular abnormalities could be involved in the decrease in glomerular function in mercuric chloride treated rats: obstruction and backleak of glomerular filtrate[5]. The alterations in glomerular function in mercuric chloride treated rats may also be secondary to ROS (reactive oxygen species), which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rat[14]. The activity of GSH-Px and GST that can clear to protect the cells from being injured represents the competence of clearing free radicals from the organism. MDA content manifests the level of lipid peroxidation, and then indirectly represents the level of damage of the cell of renal mitochondria. Evaluating from GSH, MDA levels and GSH-Px and GST catalase activities in kidney of rats. Hg alone significantly decreased GSH level, GSH-Px and GST activities and increased MDA content along with histological damage in kidney.

Co-administration of Hg and Se significantly increased GSH level, and activities of GSH-Px and GST, catalase and decreased MDA content. The effect of Hg and Se interaction depended on the molar ratio of these elements administrated to animals. The maximal effect of Se on Hg induced nephrotoxicity was observed ween Se was given the same mol as Hg. Hg can give rise to free radicals that induce lipid, protein, and DNA oxidation. Hg has a great affinity for SH groups of proteins and enzymes that are crucial in cell metabolism. Endogenous antioxidant enzymes such as GSH-Px and GST are involved in the protection against oxidative stress and lipid peroxidation in kidney[15]. Induction of these antioxidant enzymes indicates an adaptive onset of the redox defence system, whereas inhibition is thought to contribute to oxidative stress in mouse brain following mercury intoxication[16,17]. Se can enhance antioxidant ability by enhancing activities of antioxidant enzymes and by increasing contents of the antioxidants. Xia et al. (2003)[18] reported that Se is crucial in several enzymes with physiological antioxidant properties, including GSH-Px and thioredoxin. Besides, the ability of Se to reduce Hg toxicity has been extensively investigated. It have been demonstrated that $HgCl_2$ lower the activity of the selenoenzyme GSH-Px in the renal mitochondria after prolonged treatment. Their direct inhibitory action, preseumably via covalent reaction with the selenol group of the selenocysteine residue[19] is one mechanism whereby they impair the activity of GSH-Px and possible other selenoenzymes, following prolonged exposure. Selenite treatment prevented $HgCl_2$ induced decline in GSH-Px activity in the renal mitochondria of rats[20]. The protective effect of Se against Hg induced nephrotoxicity may be related to the formation of a Se-Hg complex. This conclusion is based on previous studies demonstrating that pre-treatment with sodium selenite increased whole retention of Hg, conceivably due to the formation of inert Se-Hg complexes[21] and the complexes reduced the availability of Hg[20]. Yoneda and Suzuki (1997)[22] also demonstrated that Se forms an equimolar complex with Hg in the plasma which subsequently binds to selenoprotein P. simultaneous administration equimolar doses of sodium selenite prevented not only methyl mercury induced increased of oxidized glutathione, inhibition of GSH-Px in kidney[23], but also histological and functional damage in kidney as well.[24] Although the exact mechanism of mercuric chloride induced nephrotoxicity is not well understood, several studies suggested the involvement of free radicals. Oxidative stress develops when the disturbances between reactive oxygen forms are produced in excess and the factors preventing their harmful effect occur. It has been show in various studies that mercuric chloride administrations are associated with increased formation of free radicals, and with heavy oxidative stress. This will lead to oxidative damage cell components e.g proteins, lipids and nucleic acids[25]. $HgCl_2$ inhibits activities of antioxidant enzymes (GSH-Px and GST) and there is depletion of cellular thiols[26] in rat kidney and testes suggesting that $HgCl_2$ toxicity results from generation of reactive oxygen species. Selenite metabolite are similar to thiols, and therefore compounds that react with thiols are expected to react also with selenols[27] may be the one mechanism to restore the activity of antioxidant. Mercuric chloride induced nephrotoxicity is associated with increased level of MDA. MDA and 4-HNE(4-hydroxy-2-nonenal) are the end products produced by the decomposition of W_3 and W_6 polyunsaturated fatty acids[28,29] due to $HgCl_2$

administration, platinum sulphydryl group complexes formed are taken up by renal cells and stabilized by intracellular GSH for several hours, in case of intracellular GSH depletion the complexes undergo the rapid transformation to receive metabolites, this depletion seems to be the prime factor that permits lipid peroxidation and impair antioxidant enzymes. Nephroprotectant by the exogenous selenite might be directly related to its antioxidant activity.

CONCLUSION

Taking into account the results of this study, it is concluded that $HgCl_2$ administration produces severe nephrotoxicity in rats, increase in the serum: urea, creatinine and uric acid levels, and activity of antioxidant enzymes and GSH level were decreased in kidney along with increase the lipid peroxidation level. Co-administration with sodium selenite, show significant modification in the activity of antioxidant enzymes and histological damage caused by $HgCl_2$ might be achieved by the use of sodium selenite. We suggest that the use of sodium selenite may offer a beneficial strategy against $HgCl_2$ toxicity.

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