

# Role of Selenium in Mercury Intoxication in Mice

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**Abstract:** Studies were conducted to examine the effect of pre and post-treatment of selenium in mercury intoxication (20  $\mu\text{mole/kg}$  b.w. each given intraperitoneally) in mice in terms of lipid peroxidation (LPO), glutathione (GSH) content, activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and mercury concentration in liver, kidney and brain. No significant alteration was observed in all the organs examined after mercury or selenium treatment in LPO and GSH but administration of selenium (pre and post) resulted in an increase in the level of LPO and GSH. The activity of SOD was depleted in liver and kidney while that of GPx was lowered in liver of mercury exposed animals. Selenium administration resulted in restoration of the depletion of these enzymatic activities. The activity of CAT in liver and brain was enhanced both in mercury and selenium treated animals. Administration of selenium significantly arrested enhanced CAT activity. Kidney showed the highest mercury concentration among the organs examined. Administration of selenium resulted in further enhancement of mercury concentration in the tissues. An increase in selenium level in liver was observed after mercury treatment, which was also restored by mercury selenium co-administration. Our results indicate that the prooxidant effect of selenium was greater by its pretreatment.

**Key words:** Mercury, Selenium, Lipid peroxidation, Glutathione, Superoxide dismutase, Glutathione peroxidase, Catalase

## Introduction

Mainly after the environmental disaster at Minamata and several poisoning accidents due to the use of mercury pesticides in agriculture, mercury has been considered as an environmental pollutant<sup>1</sup>. Inorganic mercury is widely used in certain types of batteries (usually mercuric oxide) and continues to be an essential component of fluorescent light bulbs and thermometers<sup>2</sup>. The effects of mercury poisoning are determined by the amount and rate of absorption as well as the physical and chemical properties of the mercury compound. However, each form of mercury exhibits a different pattern of toxicity, and variation exists between species. The kidney, liver, gastrointestinal system, and central nervous system are the main target sites of mercury toxicity<sup>3</sup>.

The primary target organ for inorganic salts of mercury

is the kidney hence it is known as nephrotoxic agent<sup>2,4</sup>. Because of the high bonding affinity between mercury and sulfur, mercury binds to metallothioneins and small molecular weight thiols such as cysteine<sup>5</sup> and glutathione<sup>6</sup>. Mercury exposure has also been demonstrated to induce lipid peroxidation detected by increased thiobarbituric acid reactive substances (TBARS) in liver, kidney, brain and other tissues<sup>7-9</sup>. Mercury is known to increase the intracellular levels of reactive oxygen species such as superoxide<sup>10</sup> and hydrogen peroxides<sup>11</sup>, which induce oxidative stress, resulting in tissue damaging effects<sup>12</sup>. Alteration in renal glutathione (GSH) contents is an important feature in the expression of mercury nephrotoxicity<sup>13</sup>.

Selenium is a structural component of several enzymes with physiological antioxidant properties, including glutathione peroxidases and thioredoxin reductase<sup>14,15</sup>. Although, selenium is an essential dietary nutrient for all mammalian species, its compounds are also toxic *in vivo*<sup>16</sup>. Toxicity of selenium is now thought to occur due to its

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prooxidant ability to catalyze the oxidation of thiols and simultaneously generate superoxides<sup>17</sup> and as a result of increased thiol oxidation, redox cycling and superoxide generation, in a dose dependant manner<sup>17, 18</sup>). It has been well documented that mercury and selenium interact in the body of mammals, and the co-administration of both reduces the toxicity of each other<sup>19, 20</sup>). Besides, the ability of selenium to reduce mercury toxicity has been extensively investigated<sup>20, 21</sup>). Most studies have evaluated the effects of the inorganic selenium compound (sodium selenite) on mercury toxicity<sup>9, 11, 22-25</sup>). In the presence of selenium, the accumulation of mercury in the target organ kidney is reported to be reduced, but body retention of mercury on the whole is increased, especially in the liver<sup>20</sup>). Selenium also affects mercury elimination, by reducing its urinary and fecal excretion<sup>26, 27</sup>), the main pathway for the elimination of inorganic mercury from the body. Thus Hg-Se interactions have been experimentally reported to counteract mercury toxicity<sup>8, 9, 28</sup>). Simultaneous administration of sodium selenite with mercuric chloride usually protects against mercury toxicity *in vivo*<sup>9, 29, 30</sup>). The present study was undertaken to investigate the effect of mercuric chloride on hepatic, renal and cerebral oxidative damage alongwith antioxidant enzymatic activities, and to examine the antioxidant or prooxidant role of sodium selenite both in pre and post treatment in mercury intoxication.

## Materials and Methods

### *Chemicals and reagents*

1,1,3,3-Tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), glutathione (GSH), Folin Ciocalteu's phenol reagent, butanol, tris (hydroxymethyl) aminomethane, bovine serum albumin (BSA), sodium dodecylsulphate, tetrasodium pyrophosphate, nitro blue tetrazolium salt, phenazene methosulphate,  $\beta$ -nicotinamide adenine dinucleotide reduced disolidium salt were obtained from Sigma Chemical Co. Hydrogen peroxide, sodium hydroxide, copper sulphate, sodium potassium tartrate and hydroxylammonium chloride were procured from Merck, India. Potassium dichromate, potassium chloride, trichloroacetic acid, ethylenediamine tetraacetic acid, acetic acid, sodium dihydrogen orthophosphate, sodium phosphate dibasic, sodium azide, mercuric chloride, sodium selenite, hydrochloric acid and nitric acid were obtained from Qualigens, India. Sodium carbonate of S. D. Fine-chemicals Limited and perchloric acid from Ranbaxy, India were used.

### *Animals and treatment*

Thirty adult Swiss albino male mice ( $30 \pm 5$  g) from ITRC, Lucknow breeding colony maintained under controlled temperature ( $22-25^{\circ}\text{C}$ ) and 12 h alternate light and dark conditions, with free access to water and pellet food (Lipton India Ltd, Mumbai) were used for the study. Mercuric chloride and sodium selenite were dissolved in physiological saline solution for intraperitoneal (ip) administration ( $20 \mu\text{mole/kg b.w.}$ ). Animals were randomly divided into five groups (06 animals each) and named as control (I), mercury (II), selenium (III), selenium + mercury (selenium pre treatment) (IV) and mercury + selenium (selenium post treatment) (V). Group I, received intraperitoneal injection of physiological saline to serve as control. Group II and III, received one ip injection of mercury or selenium respectively. Group IV was first treated with selenium and then mercury with the time difference of 2 h. Group V was injected with mercury and after 2 h treated with selenium.

Mice were anesthetized with chloroform and sacrificed 24 h after the last treatment. Liver, kidney and brain were quickly removed, trimmed of extraneous tissue, washed with ice-cold physiological saline solution, blotted dry, placed on ice chilled dish and weighed. Tissues were divided into two parts and one half were homogenized with ice-cold 0.15 M KCl solution (10% w/v) for LPO, GSH, CAT and mercury concentration while another half were homogenized with 0.1 M phosphate buffer solution for SOD, GPx and selenium concentration.

### *Lipid peroxidation (LPO) assay*

Levels of MDA (TBARS), an end product of polyunsaturated fatty acid peroxidation (lipid peroxidation), were measured in tissue homogenates on the basis of the reaction with thiobarbituric acid (TBA) to form a pink coloured complex, MDA produced was determined with the absorbance coefficient of the MDA-TBA complex at 532 nm on GBC Cintra 20 Spectrophotometer using 1,1,3,3-tetraethoxypropane as standard<sup>31</sup>).

### *Determination of glutathione level*

Glutathione level was determined using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) for colour development and reading taken at 412 nm on GBC Cintra 20 Spectrophotometer after 15 min. A standard curve using reduced glutathione was used for calibration<sup>32</sup>).

### *Superoxide dismutase activity*

Activity of superoxide dismutase was determined in the tissue homogenates by modified method of NADH-

phenazinemethosulphate-nitroblue tetrazolium formazan inhibition reaction spectrophotometrically measured at 560 nm on Spectrophotometer Genesys 10 UV<sup>33</sup>).

#### *Glutathione peroxidase activity*

Activity of glutathione peroxidase was determined by the method of Flohe and Gunzler<sup>34</sup>) and expressed in terms of  $\mu$ mole GSH consumed/min/mg protein measured at 420 nm on Spectrophotometer Genesys 10 UV.

#### *Catalase assay*

The activity of catalase was determined by the method in which catalase preparation is allowed to decompose H<sub>2</sub>O<sub>2</sub> for a fixed period of time<sup>35</sup>). The reaction was then stopped by addition of dichromate- acetic acid reagent followed by heating for 15 min in boiling water bath. The remaining H<sub>2</sub>O<sub>2</sub> was determined by measuring chromic acetate generated at 570 nm on GBC Cintra 20 Spectrophotometer.

#### *Determination of protein*

Protein was assayed using Bovine serum albumin (BSA) as standard and O.D. read at 690 nm on GBC Cintra 20 Spectrophotometer<sup>36</sup>).

#### *Determination of mercury and selenium concentration*

Tissue homogenates were used for analysis of mercury<sup>37</sup>) and selenium concentration<sup>38</sup>). They were analyzed by Atomic Absorption Spectrophotometer equipped with vapor generation assembly (GBC Avanta- $\Sigma$ ). For mercury determination 1 ml tissue homogenates (10% w/v) were digested with 7 ml of nitric acid and perchloric acid (6:1) mixture by heating on a water bath maintained at 85°C for 18–20 h. When the digestion was complete, clear homogeneous digest was obtained, allowed to cool at room temperature and 2 ml of 20% hydroxyl ammonium chloride solution was added. Finally the volume was made up to 20 ml by the addition of distilled water, while for selenium determination 1 ml tissue homogenates (50%w/v) were digested with 2 ml of nitric acid by heating on a water bath maintained at 90°C for 2 h, cooled and then added 5.5 ml hydrochloric acid, shaken well and again heated on water bath maintained at 70°C for 1 h. When the digestion was complete, clear homogeneous digest was obtained, it was allowed to cool at room temperature and raised the volume up to 15 ml by the addition of distilled water. All samples were analyzed on AAS against standards in linear range of concentration.

#### *Statistical analysis*

Statistical significance of mean value of different biochemical parameters (LPO, GSH, SOD, GPx, CAT) and concentration of mercury and selenium in different tissues (liver, kidney and brain) for different treatments (control, mercury, selenium, selenium pre and selenium post treatments) was tested using one way analysis of variance (ANOVA). The analysis was repeated for each parameter and for each tissue separately. Prior to this analysis, the homogeneity of variance between treatment groups was ascertained using Bartlett's test<sup>39</sup>). Post hoc analysis was carried out to compare the mean values between the pair of treatments using Scheffe's multiple comparison procedure<sup>40</sup>). A probability of less than or equal to 0.05 was considered to be a significant.

## **Results**

#### *Lipid peroxidation measurement*

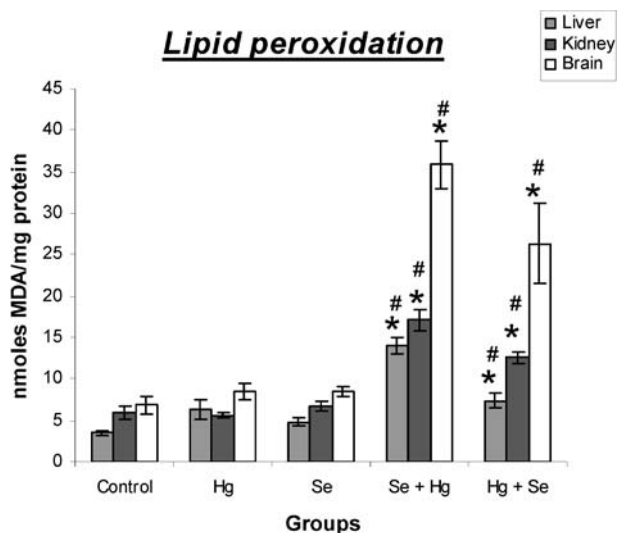
No significant alteration in LPO as measured by in Malondialdehyde (MDA) levels was observed in mercury or selenium treated animals. MDA level however, significantly increased in all the tissues examined i.e. liver, kidney and brain, in selenium pre and post treated mercury-exposed animals when compared to control group (Fig. 1). The increase was more marked in selenium-pretreated group when compared to mercury treated animals.

#### *Glutathione levels*

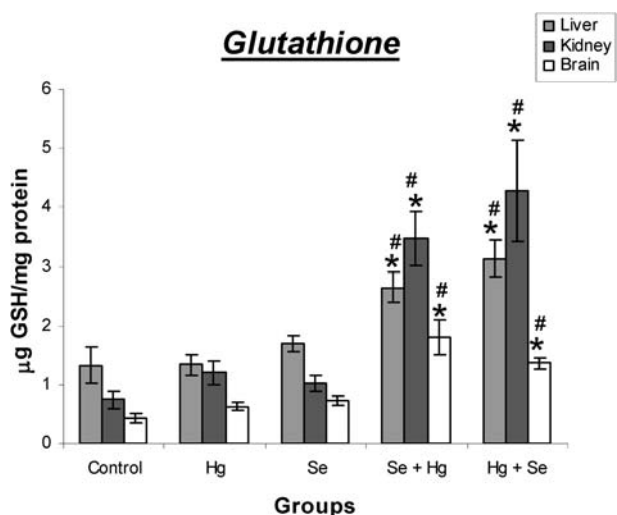
The content of GSH did not alter by either mercury or selenium treatment in the tissues examined (Fig. 2). But the administration of the two metals together resulted in a significance increase in GSH level in both pre and post treatment of selenium when compared to control or mercury treated animals.

#### *Superoxide dismutase activity*

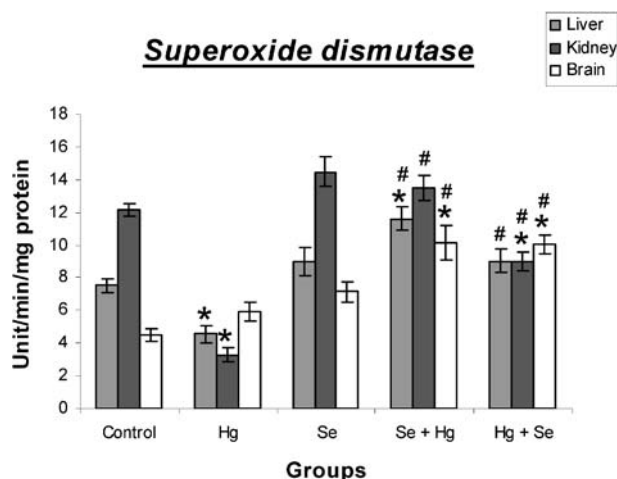
Treatment with mercury resulted in reduction in SOD activity in liver and kidney of mercury treated animals (Fig. 3). Selenium pretreatment resulted in enhanced SOD activity in all the three tissues examined. Significant restoration was observed in kidney of the selenium pretreated group while in liver and brain, further enhancement was also observed when compared to control group. In case of selenium post treatment the recovery was almost to the normal control level in liver, while in kidney although there was significant restoration but the level remained low compared to normal control. In case of brain, the level was significantly enhanced when compared to both control as well as mercury treated animals.



**Fig. 1. MDA levels in mouse tissues after single ip administration of mercuric chloride and/or sodium selenite.**  
MDA levels are expressed as nmoles MDA/ mg protein. The results are presented as mean ± SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.



**Fig. 2. GSH Contents in mouse tissues after single ip administration of mercuric chloride and/or sodium selenite.**  
GSH Contents are expressed as µg GSH / mg protein. The results are presented as mean ± SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.



**Fig. 3. SOD activity in mouse tissues after single ip administration of mercuric chloride and/or sodium selenite.**  
SOD activity is expressed as Unit/ min/ mg protein. The results are presented as mean ± SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.

*Glutathione peroxidase activity*

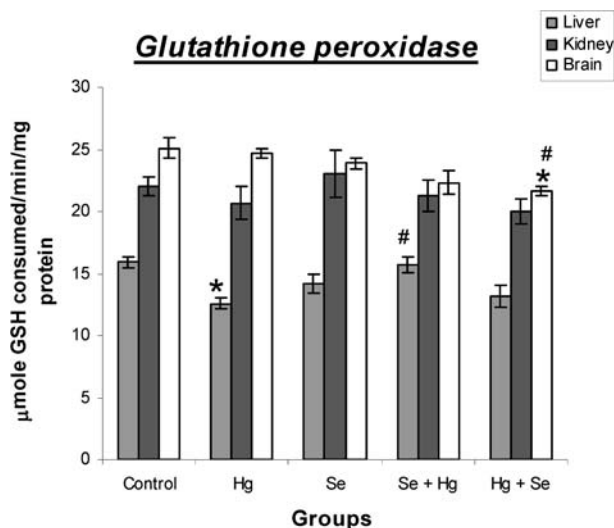
The activity of glutathione peroxidase was decreased in mercury treated animals in liver only which was recovered both in selenium pre or post treatment (Fig. 4). The effect was observed significantly marked in selenium pretreated group. However, significant depletion was observed in brain of selenium post treated group when compared to control as well as mercury treated animals.

*Catalase activity*

The activity of catalase in liver was significantly increased both by the treatment of mercury and selenium individually (Fig. 5). This enhancement was arrested significantly by the pre treatment of selenium in mercury exposed animals while the same effect was not observed in selenium post treatment. However, no change occurred in kidney in groups treated with both the metals given alone or in combination when compared to control as well as mercury treated animals. In brain tissue, enhancement in catalase activity in either mercury or selenium treated animals was restored in both the pre and post treatment of selenium which was more marked in selenium post treated animals.

*Mercury concentration*

The highest accumulation of mercury was observed in kidney followed by liver and brain (Fig. 6). Administration of selenium to the mercury treated animals further enhanced



**Fig. 4.** GPx activity in mouse tissues after single ip administration of mercuric chloride and/or sodium selenite.

GPx activity is expressed as  $\mu\text{moles GSH consumed/min/mg protein}$ . The results are presented as mean  $\pm$  SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.

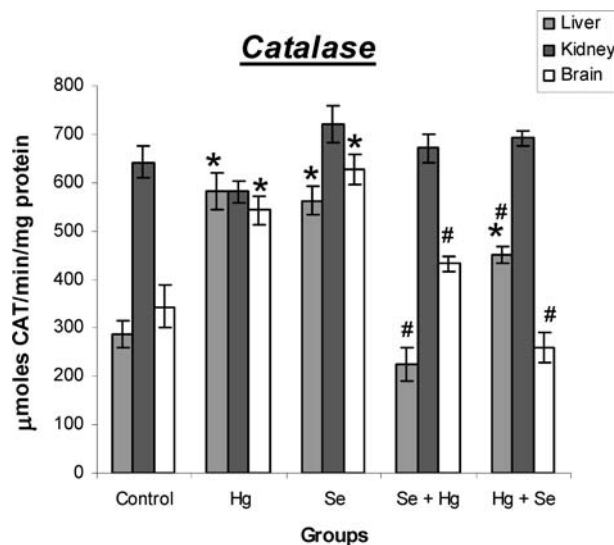
the accumulation pattern of mercury in both selenium pre and post treated groups. A significant enhancement in liver, kidney and brain tissues was observed in mercury accumulation pattern of selenium pretreated mercury exposed animals, while the selenium post treatment showed enhancement in kidney and brain only. Selenium pre treated group showed marked elevation in liver and kidney tissues while the same was observed more prominently in brain tissue of selenium post treated animals when compared to mercury treated group.

#### Selenium concentration

An increase in selenium level in liver was observed after mercury treatment, which however was restored to normal level by mercury selenium co-administration (Fig. 7).

## Discussion

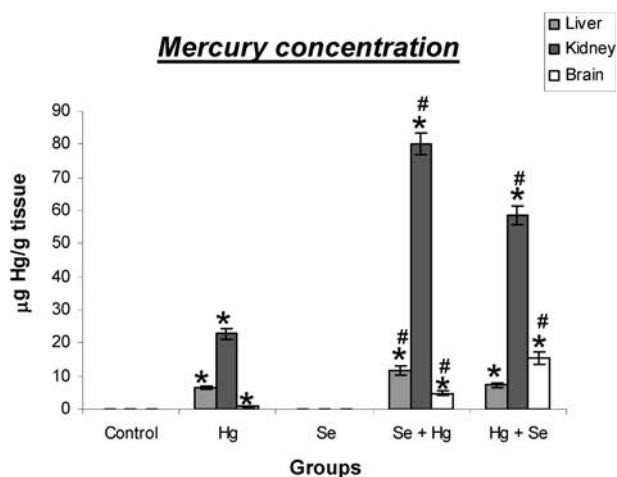
The interaction between mercury and selenium in the body of mammals has been studied for over three decades. It is also reported that the toxicity of inorganic mercury decreased by simultaneous injection of selenite<sup>41</sup>. Many studies have been carried out to examine the role of selenium in the detoxification of mercury<sup>20</sup>. Although the complete mechanism of mercury-selenium interaction is still



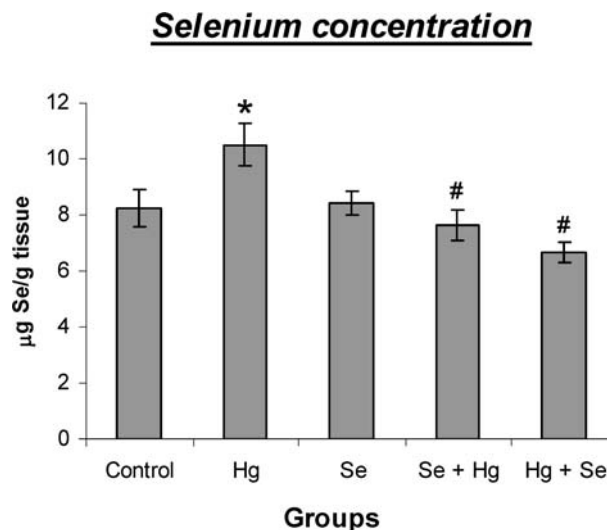
**Fig. 5.** CAT activity in mouse tissues after single ip administration of mercuric chloride and/or sodium selenite.

Catalase activity is expressed as  $\mu\text{moles CAT/min/mg protein}$ . The results are presented as mean  $\pm$  SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.

unresolved, there is evidence that selenium in plasma forms a complex with mercury, which then binds to selenoprotein P<sup>21, 24</sup>. Formation of mercury-selenium complex with selenoprotein P causes redistribution of mercury in the organism. Nevertheless, there is some controversy concerning the protection afforded by pre treatment of sodium selenite in mercury intoxication. Some authors have reported an absence of protection<sup>29</sup> and even an increase in mercury toxicity when selenite was given 1–2 h before mercuric chloride<sup>42</sup>. This indicates its prooxidant activity to induce the oxidative stress and produce their effect on the TBARS generation, GSH contents and activity of antioxidant enzymes viz SOD, GPx and CAT. In our results, among mercury treated animals, although there was accumulation of mercury in all the tissues examined but no significant changes were observed in MDA level in any of the organs. A decrease in the activity of SOD in liver and kidney but the elevation of CAT activity in liver and brain of mercury exposed animals indicates that there might be generation of reactive oxygen species ( $\text{O}_2^-$  or  $\text{H}_2\text{O}_2$ ), which however seems insufficient to result in significant enhancement in MDA levels or alteration in GSH level. It seems that there is an induction of selenium level to combat mercury toxicity at the metabolic level in liver. However, when selenium supplementation pre or post is given, it appears that exogenous selenium takes care of



**Fig. 6. Mercury concentration in mouse tissues after single ip administration of mercuric chloride and/or sodium selenite.** Mercury concentration is expressed as µg Hg/ g tissue. The results are presented as mean ± SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.



**Fig. 7. Selenium concentration in mouse liver tissue after single ip administration of mercuric chloride and/or sodium selenite.** Selenium concentration is expressed as µg Se/g tissue. The results are presented as mean ± SE (n=6). Difference between groups were considered to be significant when  $p < 0.05$ . \*Values significantly different from control group. #Values significantly different from mercury treated group.

mercury induced selenium requirement. In case of selenium administration in mercury exposed animals the enhanced accumulation of mercury in all the three tissues shows that there is some mechanism which facilitates the accumulation of mercury which may be through its binding with glutathione in view of enhanced GSH level in tissues and also may be due to the formation of mercury selenium complex with plasma protein<sup>21,24</sup> which increases the tendency of mercury to accumulate rather than eliminating it from the body<sup>43</sup>. High content of mercury in the tissues by administration of both mercury and selenium might also cause high level of superoxides as indicated by the enhanced activity of SOD and almost no significant alteration in the activity of GPx and CAT. This may cause the enhanced level of hydrogen peroxide and the hydroxyl radical subsequently and lead to enhanced LPO as indicated by enhancement in MDA levels in all the tissues examined both in pre and post treatment of selenium in mercury exposed animals. Also significant positive correlation was observed between mercury concentration and lipid peroxidation in all the tissues examined ( $r=0.776, p < 0.001$ ;  $r=0.86, p < 0.001$ ;  $r=0.83, p < 0.001$  for liver, kidney and brain, respectively). The increased level of selenium in liver of mercury exposed animals further suggests alterations in selenium homeostasis due to mercury but the increase in endogenous selenium at hepatic level seems insufficient or not responsible for

elevation in MDA level. Our results thus indicate that there is a prooxidant activity observed on the part of selenium in mercury treated animals. We have selected the dose of mercuric chloride on the basis of study by Fukino who used 15 µmoles mercury in rats<sup>44</sup>, which was slightly lower than our dose and the dose of selenium was taken in a way to provide 1:1 molar ratio with the dose of mercury. This may have provided higher selenium dose to produce selenium toxicity in combination with mercury due to the action on thiols to generate superoxides and reactive oxygen species, similar results were reported by Spallholz<sup>45</sup>. Sodium selenite is known to result in characteristic sign of selenosis (sub chronic disease due to selenium toxicity), which causes oxidative stress as observed by increase in lipid peroxidation and other biochemical parameters among cow calves<sup>46</sup>. Toxicity of selenium is also well established and selenosis is well reported in humans as well as in animals<sup>47</sup>. Results further show that the prooxidant effect of selenium was more marked in selenium pretreatment rather than its post treatment as indicated by the concentration of mercury in liver and kidney tissues, which was high in selenium pretreated group rather than its post treatment. However, in brain post treatment of selenium showed more mercury concentration. This also gets support from the fact that selenium blocks the effects of chelating agents in mercury intoxication and the process may be holding good for elimination of mercury

from the body in natural course leading to higher accumulation of mercury in the body organs<sup>43</sup>. Our results show high mercury accumulation in kidney than in liver, which is also supported by the other studies<sup>2, 4, 12, 48</sup>. Enhancement of GSH level may be towards the defense mechanism against the effect of ROS in the cell, which was found to be more in selenium post treated animals in liver and kidney tissues rather than its pre treatment.

Thus administration of selenium at the dose, which was used in our experiment, showed its synergistic effect on mercury toxicity. Although, selenium is an essential trace element it can elicit toxic effects at modest intake as demonstrated in our experiment. It may be that the window of beneficial functionality of selenium is narrow. Further studies are in progress to examine the effects of selenium at lower dose in mercury intoxication.

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