

# Investigating the Role of Peroxidative Index in Mercury-Induced Kidney Toxicity

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## ABSTRACT

Oxidative stress is an important molecular mechanism for kidney injury in mercury (Hg) poisoning. Usually, oxidative stress was measured by measuring the levels reactive oxygen species (ROS) and antioxidant enzymatic activity. In this present study, we proposed a new marker for oxidative stress in kidney toxicity induced by Hg. The new marker is the peroxidative index (PI). In this experiment, a kidney sample was taken from male rats (*Rattus norvegicus*). Samples then homogenized and divided into five groups with; T1 served as control which contains kidney homogenate only; T2 which contains kidney homogenate+0.1 mg/l of mercury chloride (HgCl<sub>2</sub>); T3 which contains kidney homogenate+1 mg/l of HgCl<sub>2</sub>; T4 which contains kidney homogenate+10 mg/l of HgCl<sub>2</sub>; and T5 which contains kidney homogenate+100 mg/l of HgCl<sub>2</sub>. After treatment, kidney catalase (CAT) and peroxidases (Pox) activity, PI, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and PC level were estimated. The results revealed that Hg level is strong negatively correlated with both CAT and Pox activities, and strongly positively correlated with PI. Also, the results revealed that PI is strongly positively correlated with PI with the presence of Hg in different concentration of Hg in kidney cells. In conclusion, PI might be a useful marker for oxidative stress in kidney damage induced by Hg. For our knowledge, the proposed mechanism according to our results is Hg inhibited antioxidant enzymatic activity and increase ROS in the kidney. Thus, induced oxidative stress which promotes a further reaction to damage protein and resulted in kidney damage.

**Keywords:** Kidney, Mercury, Oxidative Stress, Peroxidative Index.

## INTRODUCTION

Mercury (Hg) is ubiquitously distributed in the environment and is non-essential and toxic to the human body<sup>1</sup>. Hg is considered as one of the highest priority pollutants to humans<sup>2</sup>. Hg is widely used in industry, agriculture, and medicine, and circulates in ecosystems, but is never destroyed<sup>1</sup>. Hg exists in two forms; as inorganic Hg or organometallic Hg compounds.<sup>2</sup> The fate and behavior of mercury in the environment depend on these chemical form<sup>3</sup>. Although organic Hg is the most toxic form, inorganic Hg is the most common form of mercury released into the aquatic environment by industries, having a more significant effect on fish tissue<sup>4</sup>. High exposures to inorganic Hg may result in damage to the several of human organs, mainly kidney<sup>3,5</sup>. Inorganic Hg salts are taken up and accumulated in the proximal tubules of the kidneys. Clinical findings are polyuria and proteinuria (especially low molecular proteinuria) which are the main indicators of tubular damage in kidneys<sup>6</sup>. Hg may also cause nephrotic syndrome, either because of membranous nephropathy or minimal change disease<sup>7</sup>. It is widely accepted that Hg exerts toxic health effects by different mechanisms including, disturbing or inhibition of enzymes and inducing oxidative stress<sup>6</sup>. Recent reports suggest that mercury toxicity involves the generation of ROS with marked alterations in the antioxidant defense systems and oxidative damage induction such as lipid peroxidation leading to cell death<sup>4</sup>. Begam and Sagupta<sup>8</sup> result study shows that Hg exposure could decrease the catalase (CAT) activity in intestinal macrophages of fish. Agarwal and Behari<sup>9</sup> also show that Hg exposure could decrease both glutathione peroxidase and CAT activity. Recently, oxidative stress state not only evaluated by analyzing the

level of oxidants and antioxidants, but also analyze through a proportion or ratio. Several proportion ratio is known, such as oxidative stress index and peroxidative index (PI)<sup>10-11</sup>. Kania et al. study<sup>10</sup> suggested that PI is the ratio between lipid peroxidation to hydrogen peroxide levels. We use a different approach in this present study. In this present study, PI is measured by the ratio between hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) level and the sum of peroxidase (Pox) and CAT activity, as which have been done in Setiawan research<sup>12</sup>.

Among a wide range of oxidative stress state modifications, biomolecule carbonylation is known to be a major hallmark of oxidative stress<sup>13</sup>. Among biomolecule carbonylation, protein carbonylation (PC) is a common parameter that measured to assess biomolecule carbonylation. PC can be formed via the  $\alpha$ -amidation pathway, oxidative cleavage of glutamyl residues, formation of protein-protein cross-linked derivatives, and cell membrane damage by lipid oxidation products give rise to reactive aldehydes and ketones<sup>14-16</sup>. PC content in blood and tissues is a reliable indicator of protein oxidation<sup>17</sup>.

As mentioned above, in this present study we try to evaluate the kidney toxicity induced by Hg through the measurement of PI. Since the PI is the ratio between H<sub>2</sub>O<sub>2</sub> level and the sum of Pox and CAT activity, each of these parameters is measured one by one. To investigate the mechanism, we correlated Pox and CAT activities, and PI, with different concentrations of Hg and we, also correlated the PI with PC with the presence of Hg in different concentrations.

## MATERIAL AND METHODS

**Animals and Homogenate Preparation** Male rats (*Rattus novergicus*) weighing 200–250 gram with 2-3 months old were obtained from the Abadi Jaya farm at Yogyakarta, Indonesia, in healthy condition. The experiment was approved by the Ethical Committee of the Lambung Mangkurat University, South Kalimantan, Indonesia. Animals were fed under standard conditions and acclimatized with a 12 hours light/dark cycle. The animals were sacrificed by surgical procedure and the kidney was removed. Then, the organs homogenized in phosphate buffer saline (pH 7.0) and were ready to use for in vitro experimental models.

### Experimental Models

Kidney cells will be exposed to different concentration of HgCl. The HgCl concentrations were 0 mg/l, 0.1 mg/l, 1 mg/l, 10 mg/l, and 100 mg/l. Each solution then incubated at 37°C for 3 hours. After incubation, the CAT and Pox activity, the PI, and the PC and H<sub>2</sub>O<sub>2</sub> level were estimated.

### CAT activity analysis

CAT activity estimated by using Aebi's method<sup>18</sup>. The first step was to prepare the stock solution by using 2 ml and 1 ml from phosphate buffer at pH 7 and H<sub>2</sub>O<sub>2</sub> (30 mM) respectively. Then, 50 µl of the lysate was added to the stock solution. The ability of catalase to work a reducing factor was measured by determining the changes in absorbance at 240 nm. **Peroxidase activity analysis**

Determination of Pox activity was measured by the method of Pruitt et al.<sup>19</sup> The assay was performed by mixing 1.0 ml phosphate buffer (pH 7.0), 1.0 ml guaiacol solution and 1.0 ml of a sample. The reaction was started by adding 1.0 ml of H<sub>2</sub>O<sub>2</sub> stock solution. Absorbance at 470 nm (A) and time (T) data were monitored.

### H<sub>2</sub>O<sub>2</sub> level analysis

The H<sub>2</sub>O<sub>2</sub> level was calculated by the FOX2 method with slight modification<sup>14</sup>. Solutions measured spectrophotometrically at  $\lambda = 505$  nm. Standard and test solutions consisted of 1 M H<sub>2</sub>O<sub>2</sub> 200 µL and 200 µL serum, respectively, with the addition of 160 µL PBS pH 7.4, 160 µL FeCl<sub>3</sub> (251.5 mg FeCl<sub>3</sub> dissolved in 250 ml distilled water) and 160 µL o-phenanthroline (120 mg ophenanthroline dissolved in 100 ml distilled water) for both solutions. The composition of the blank solution was identical to that of the test solution, except for the absence of FeCl<sub>3</sub> in the blank. Subsequent to preparation, all solutions were incubated for 30 minutes at room temperature, then centrifuged at 12,000 rpm for 10 minutes, and the absorbance of the standard (As), test (Au) and blank (Ab) solutions measured at  $\lambda=505$  nm, using the supernatant of each solution<sup>22</sup>.

### Peroxidative Index (PI) analysis

PI was a ratio between H<sub>2</sub>O<sub>2</sub> level and CAT plus Pox activity. PI was calculated following to equation:<sup>12</sup>

$$PI = \frac{(H_2O_2) \text{ level}}{(CAT + Pox) \text{ activity}}$$

### PC level analysis

PC was calculated by measuring the total protein carbonyl content. The total protein carbonyl content was determined by colorimetric method. The liver homogenate (0.5ml) was

pipetted into 1.5 ml centrifuge tube and 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine in 2 M HCl was added and allowed to stand at room temperature for 1 hour, with vortexing every 10-15 minutes. Then, 0.5ml of 20% Trichloroacetic acid was added followed by centrifugation. The supernatant was discarded and the pellets were washed 3 times with 1 ml ethanol-ethyl acetate (1:1) to remove free reagent. The obtained precipitated protein was redissolved in 0.6 ml guanidine solution. Carbonyl content was calculated from maximum absorbance (390nm).

### Statistical analysis

The results were expressed as mean±SE for six replicates. The data was analyzed between each parameter level and cyanide concentration. For analyzing the data, Microsoft excel 2010 was used and was examined by simple correlation regression.

## RESULTS AND DISCUSSION

The present study is aimed to investigate the kidney damage induced by Hg through the measurement of PI. First, we measured the activity of CAT and Pox with the presence of Hg in different concentrations in kidney homogenate and correlated. The results show in figure 1 and 2. From figure 1 and 2, we can see that Hg level is strong negatively correlated with both CAT and Pox activities. It means, with the presence of Hg, both CAT Pox activities decrease. Similarly, Ibrahim<sup>20</sup> found that CAT and glutathione peroxidase (GPx) activities were decreased in the kidney of *Clarias gariepinus* exposed to Hg, and Ogunrinola et al.<sup>21</sup> also found the depletion of CAT activity in the kidney of rats exposed to Hg.

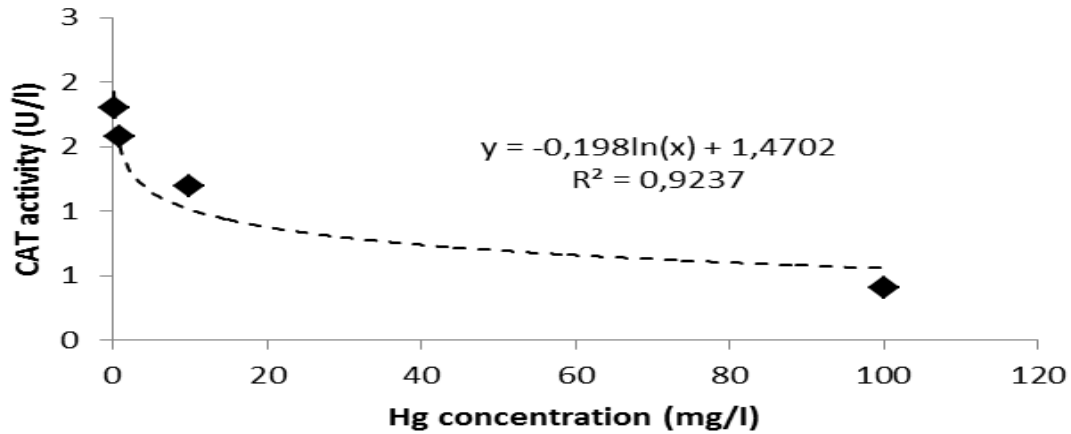


Figure 1: The correlation between Hg level and CAT activity in kidney cells homogenate. Hg: mercury; CAT: catalase.

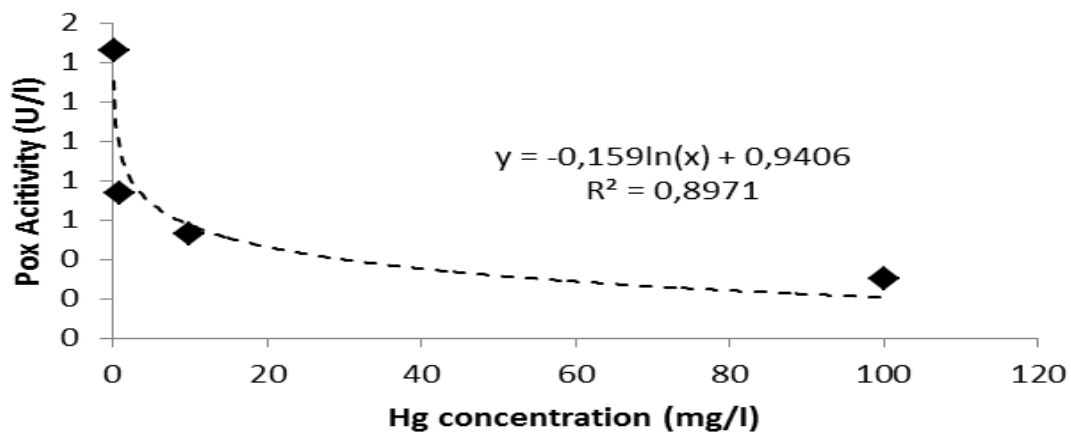


Figure 2: The correlation between Hg level and Pox activity in kidney cells homogenate. Hg: mercury; Pox: peroxidase.

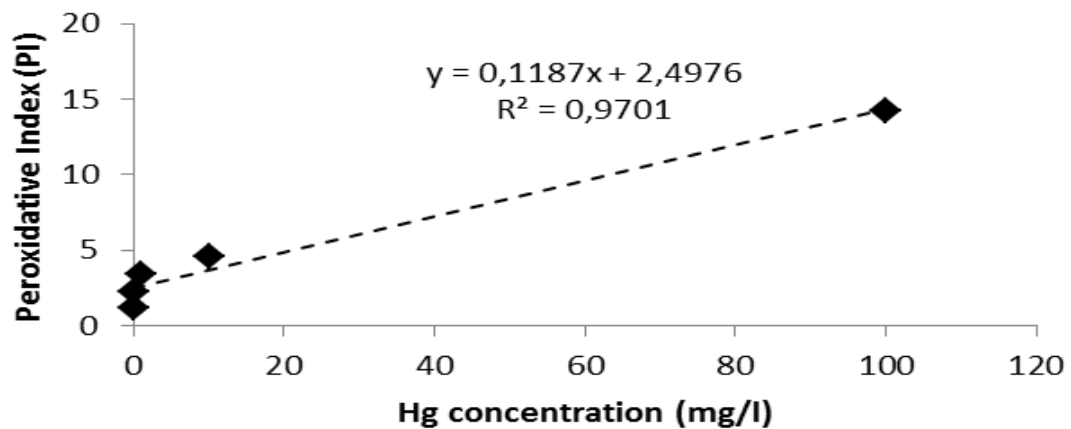


Figure 3: The correlation between Hg level and PI in kidney cells homogenate. Hg: mercury; PI: peroxidative index.

It is widely accepted that Hg exposure could lower the defense system of antioxidants. These effects is caused two basic mechanisms according to Karantika et al.<sup>22</sup>, i.e (1) heavy metals including Hg can make a bond formation with the sulfhydryl groups (-SH) of cysteine, thus inhibited the activity of enzymes; and (2) Hg can replace a metal ion in the body's of several enzymes, leading to inactivation of the enzymes. Furthermore, the inactivation of CAT and Pox can cause the accumulation

of ROS, such as H<sub>2</sub>O<sub>2</sub>, which in turn causes oxidative damage in the cells<sup>23-25</sup>.

To prove the oxidative damage in kidney cells by Hg, in this present study, we measure the correlation between Hg exposure and PI. To best of our knowledge, PI is a new parameter that we try in this study to assess oxidative damage from Hg exposure. The result shows in figure 3. From figure 3 we can see that the PI has increased with the increasing of Hg concentrations in kidney cells

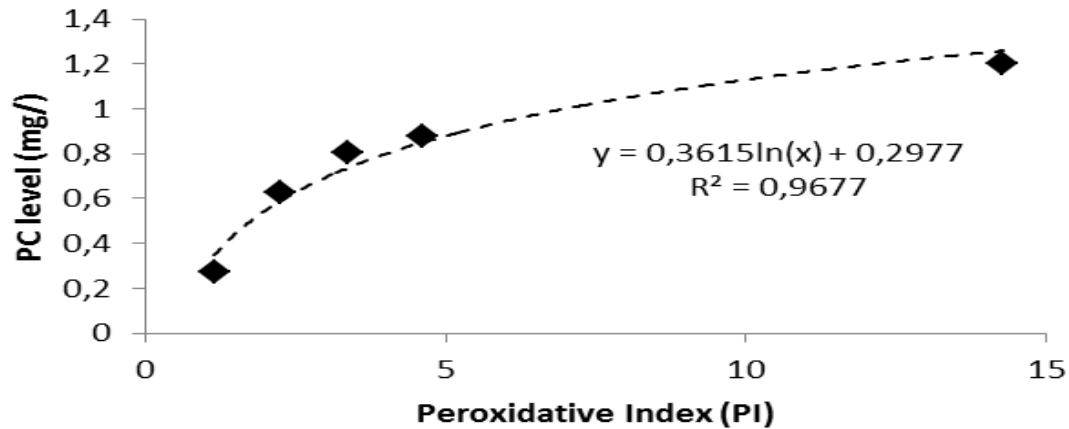


Figure 4: The correlation between PI and PC level in kidney cells homogenate. PI: peroxidative index; PC: protein carbonylation.

homogenate. However, as far as we know, there have been no investigations of the association between Hg exposure and PI in kidney cells. This is the first study to examine the effects of Hg exposure on PI in kidney cells. These results reinforce previous results in figure 1 and 2 that Hg exposure can cause oxidative stress. The exact mechanism is still unknown. Several studies indicated that Hg reacts with thiol groups (-SH), thus depleting intracellular thiol, especially glutathione and causing cellular oxidative stress or predisposing cells to it and forming ROS<sup>26</sup>. In other hand, results of this study indicated that Hg could interact with antioxidant enzymatic, thus also increase the level ROS resulted in oxidative stress.

It is widely accepted that oxidative stress situations can cause damage of biomolecules<sup>27</sup>. Among biomolecules, proteins are the principal target of ROS because they are present in high concentrations in biological systems and remove 50-75% of the generated ROS<sup>28</sup>. The ROS could oxidize the protein and change the structure of proteins. This process is known as PC. PC has attracted much attention in the last four decades and recognized as a universal marker of oxidative stress induced protein modification<sup>13</sup>.

To evaluate the link between oxidative stress and PC during Hg exposure, we correlated the PI and PC level with the presence of Hg in different concentrations in kidney cells homogenate. The results show in figure 4. From figure 4, we can see that PI is strong positively correlated with PC level. It means, if the PI is increased, the PC level in kidney cells also increase with the presence of Hg. Several study and review indicated that PC appear through side-chain oxidation of proline, arginine, and lysine. They can also result from backbone cleavage through the  $\alpha$ -amidation pathway or  $\beta$ -scission. Alternatively, they can be introduced into proteins through Michael addition of unsaturated aldehydes produced by peroxidation of lipids<sup>29</sup>.

In conclusion, the present study indicated that the Hg exposure induced kidney toxicity through the oxidative stress condition, which indicated by the strong correlation between Hg level and PI. PI might be a useful marker for oxidative stress-induced kidney damage by Hg exposure.

However, further longitudinal studies are needed to validate PI as a marker of oxidative stress induced kidney damage in Hg exposure. In addition, this results studies also suggested that oxidative stress by Hg might be through the depletion of antioxidant enzymatic such as CAT and Pox, which promote a further reaction to promote PC.

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