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Kinetic Parameters Analysis of Liver and Kidney Catalase Under the Influence of Cadmium (Cd) and Mercury (Hg) *In Vitro*

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ABSTRACT

Cadmium (Cd) and mercury (Hg) are toxic metals that affect human organs function, including liver and kidney. This toxic activity is because the heavy metal could induce oxidative stress and interfere antioxidant activities, including catalase (CAT). The present study was aims to evaluate the effect of Cd and Hg to liver and kidney CAT kinetic parameters *in vitro*. In this experiment, liver and kidney were taken from male rats (*Rattus novergicus*). Sample the homogenized and divided into three groups with; T0 served as control which contains liver or kidney homogenate + H_2O_2, T1 which contains liver or kidney homogenate + H_2O_2 + 0.03 mg/L $CdSO_4$; and T2 which contains liver or kidney homogenate + H_2O_2 + 1 mg/L Hg. Solutions then incubated at 37°C for 1 hour and then was prepared for CAT activity measurement. The CAT activity was measured using spectrophotometer at 240 nm. For measuring the kinetic parameters, different concentration of H_2O_2 were used. The kinetics parameters (Km and Vmax) were calculated using Lineweaver-Burk plot. The results shows that Cd and Hg could decrease the affinity of CAT-H_2O_2 complex which expressed by the higher Km and Vmax values. Also from the results, Cd has better activity to decreased the affinity of CAT-H_2O_2 complex than Hg. From this results, it can be concluded that Cd and Hg treatments could inhibit CAT activity in liver and kidney *in vitro*.

Keywords: *Cadmium (Cd), catalase, kinetic parameters, mercury (Hg)*

INTRODUCTION

Heavy metal such as Cd and Hg are toxic metals that are widespread in the environment [1, 2]. These metals can occur as a results from natural, anthropogenic, and human sources [2, 3]. These heavy metals are not biodegradable, so as consequences these can accumulate in living organisms [4]. Because of the nature of heavy metals tend to accumulate in living organisms, these metals can enter the food chain to humans [5].

Human exposure can cause many various effects to human organs, especially kidney and liver [6, 7]. The previous study had shown that chronic exposure to Cd could induce kidney damaged, manifested by proteinuria, glucosuria, aminoaciduria, and phosphaturia [8]. The Cd also lead to a liver damaged [9], both in acute and chronic exposure. Many studies has well documented that Cd exposure could cause the swelling, necrosis, and degeneration of hepatocytes [10]. Besides Cd, Hg also well documented to induced liver and kidney damaged. The previous study had shown that Hg exposure will lead to kidney and liver cells necrosis [11, 12].

The basic mechanism of Cd and Hg toxicity is an activity to induce oxidative stress [13]. Cd and Hg also impair antioxidant defenses. These antioxidant defenses involving thiol-containing antioxidants and enzymes, including CAT [14, 15].

CAT is a heme-containing enzyme and found in almost all aerobically respiring organisms. CAT is an intracellular enzyme that can be found in all tissues and organs. The highest CAT concentrations are found in erythrocytes, liver, and kidney [16]. The main function of CAT catalyzes the conversion of hydrogen peroxide

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to molecular oxygen and water [17].

Although many researches in the interaction between these heavy metals (Cd and Hg) and the activity of CAT has well documented, but still the kinetics of CAT by the presence of both Cd and Hg has not been explored fully. Since CAT has a great function to maintain the cell function, especially in liver and kidney, investigation of the effect of Cd and Hg in CAT kinetic parameters seems to be importance. Therefore, in this study, we try to investigate the kinetics parameters of CAT with the presence of Cd and Hg in liver and kidney *in vitro*.

MATERIALS 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 from the University of Lambung Mangkurat. Animals were fed under standard conditions and acclimatized with a 12 hours light/dark cycle. The animals were sacrificed by surgical procedure. The livers and kidneys were removed. Then, the both organs homogenized in phosphate buffer saline (pH 7.0). Then, each of homogenate was used for in vitro experimental models.

Experimental models

Samples was divided into 3 groups (1 control group and 2 treatment groups). Control: Homogenate + H_2O_2, T1: Homogenate + H_2O_2 + 0.03 mg/L $CdSO_4$; and T2: Homogenate + H_2O_2 + 1 mg/L Hg. Each solution then incubated at 37°C for 1 hour.

Catalase activity measurements

The CAT activity was measured by the method of Aebi [18], using spectrophotometer at 240 nm. The activity defined as mmol of H_2O_2 consumed per minute, in 50 mM phosphate buffer, pH 7.0.

Catalase kinetic parameter measurements

Kinetic parameters were determined by using five different concentrations of the substrate, H2O2. The substrate concentrations are 6.25, 12.5, 25, and 50 mM of H_2O_2. The kinetic parameters, Vmax, and Km, were determined using the Lineweaver-Burk version of the Michaelis-Menten equation [19], as follows:

$$\frac{1}{V} = \frac{km}{Vmax} \times \frac{1}{[S]} + \frac{1}{Vmax} \qquad (1)$$

V : reaction velocity
Vmax : maximum reaction velocity
Km : Michaelis constant (the substrate concentration at half-maximal reaction velocity)
[S] : substrate concentration

RESULTS AND DISCUSSION

In this present study, the kinetic parameters of liver and kidney CAT with the presence of Cd and Hg were investigated. The result shows in figure 1 for liver CAT, figure 2 for kidney CAT, and table 1 for Km and Vmax of both liver and kidney CAT in the group of treatments.

Figure 1 and 2 represent the Lineweaver-Burk plot of liver and kidney CAT respectively. From that plot, kinetic parameters Km and Vmax for liver and kidney CAT was calculated and presented in Table 1. Results revealed Km and Vmax values seems to be higher in T1 and T2 groups, in comparison with T0 group. Results of this present study also show that the highest Km and Vmax values are in T1 group (Table 1).

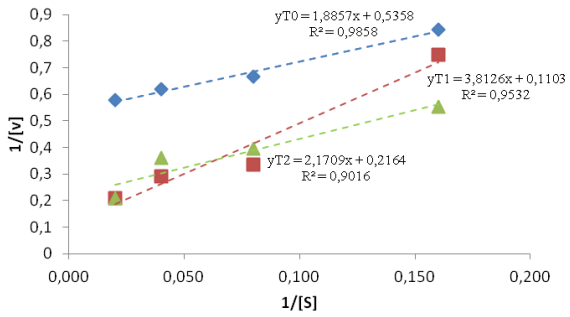

Figure 1. The Lineweaver-Burk plot for liver catalase. ◆ : Control (T0); ■ Cd group (T1); ▲ : Hg group (T2)

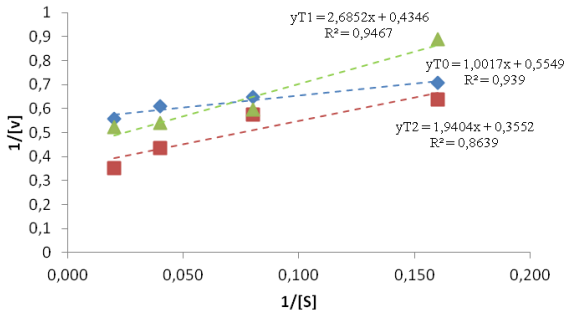

Figure 2. The Lineweaver-Burk plot for kidney catalase. ◆ : Control (T0); ■ : Cd group (T1); ▲ : Hg group (T2)

Table 1. Kinetic parameters and coefficient correlation for liver and kidney catalase in different group of treatments

Groups	*Organs*					
	Liver			*Kidney*		
	Km	*Vmax*	*r*	*Km*	*Vmax*	*r*
T0	3.530	1868	0.993	1.804	1.802	0.969
T1	34.579	9.066	0.996	5.470	2.817	0.973
T2	10.041	4624	0.950	6.186	2.302	929

Vmax and Km values reflect the catalytic and substrate binding ability of an enzyme, respectively. The smaller Km value represents, the greater affinity of enzyme-substrate complex. This means the enzyme can to catalyze the reaction to formed the product [20-21]. From the results suggest, with the presence of Cd and Hg, Km and Vmax values are increase in both of organs. This suggests that Cd and Hg decrease the affinity between enzyme substrate. This means the presence of Cd and Hg decrease the affinity between CAT-H_2O_2 complex. The enzyme in not able to catalyze the reaction. Cd and Hg inhibit the CAT activity both in liver and kidney homogenate.

Results of this present study also show that Cd has a greater proportion to increase the Km and Vmax of liver CAT than Hg. Interestingly, the opposite results showed in the Km of kidney CAT. In kidney CAT, Km values for the presence of Hg seems higher than with the presence of Cd. This suggests, Cd has more inhibition activity in liver CAT, and Hg shows more in kidney CAT.

The presence of these both heavy metal, Cd, and Hg, will be able to inhibits the CAT activity in liver and kidney. The inhibition of CAT by Cd and Hg is based on these two basic mechanisms, as follow:

1. Heavy metals including Cd and Hg can make a bond formation with the sulfhydryl groups (-SH) of cysteine. The presence of metals can replace the hydrogen atoms –SH groups, thus inhibited the activity of enzymes [22-23].
2. Hg and Cd can replace a metal ion in the body's metalloenzyme [24]. It is well known that CAT is metalloenzyme, that contain four subunits and each subunit contains 1 Fe-protoheme IX [25]. The presence of Cd and Hg can replace Fe in CAT subunit leading to inactivation of CAT [24].

CONCLUSION

From this present study, it can be concluded that Cd and Hg could increase the Km and Vmax values of liver and kidney CAT. It seems Cd and Hg inhibit the activity of CAT by decreased the affinity of CAT-H_2O_2 complex.

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