STUDY OF INTERACTION BETWEEN LEAD AND GASTRIC MUCOSAL PROTEIN OF RATS WITH FORENSIC TOXICOLOGY APPROACH

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***Abstract**: **Recently, forensic toxicology has been an interesting concern, especially in exposing the phenomena associated with the law.** Using the forensic toxicology approach, several cases of lead (Pb) poisoning have been widely revealed. In this present study will be investigate the interaction between Pb and amino acid gastric mucosal constituent proteins, especially cysteine and tyrosine groups. This research is a pure experimental research with posttest control group design, which is divided into 4 groups with 6 rats (Rattus novergicus) in each group. Treatment in each group as follows; P0 was control group were given 2 ml of distilled water; P1 = administration of Pb 0.1 g/L; P2 = Pb administration of 1 mg/L; and P3 = Pb administration of 10 g/L for 4 weeks repectively. According to the results, it can be concluded that Pb-Protein interaction tends to binding of Pb-Cysteine rather than Pb-Tyrosine*

***Keywords:** Gastric Mucosa, Lead, Protein*

INTRODUCTION

Recently, forensic toxicology has been an interesting concern, especially in exposing the phenomena which was associated with law. Forensic toxicology is the use of toxicology and other disciplines such as chemical analysis, pharmacology and clinical chemistry for the purpose of legal or medical investigations of cases of death, poisoning, and drug use.[1] The main concern of forensic toxicology is not a legal result of toxicology investigation or use of technology, but obtains and interprets the results. Toxicological analysis can be done for various types of samples, such as body fluids (blood, urine, and saliva) and organs.[2]

However, some several cases were revelaed with the forensic toxicology approach. In 2010 it was revealed that the deaths of 400 children in Nigeria were caused by lead (Pb) poisoning and 30,000 Pb-contaminated children. This is because the water has been polluted by Pb from gold mining in these country.[3] In addition, 24 children aged 9 months until 16 years in Xinhua China have been hospitalized due to Pb poisoning.[4]

Generally, Pb could enter into the body through the gastrointestinal system. About 5-10% of Pb will be absorbed through the gastrointestinal mucosa.[5,6] Furthermore, 75-80% of Pb is excreted through the urine, and 15% through the feces, bile, sweat, nails and hair (7-8). The average daily intake of Pb are about 0.3 mg/day, and when the Pb intake reaches 0.6 mg/day, it can cause a positive poisoning symptoms. However, due to slow Pb deposits, these dose will not show a poisoning symptoms, but can continue to accumulate in the body until the symptoms of poisoning is appear.[9,10]

The basic known mechanism how Pb can cause poisoning was the interaction between Pb and the amino acids, especially thiol groups in cysteine.[11,12] It has been reported that Pb forms mercaptide compounds with thiol (-SH) cysteine groups and decreases this complex stability with other amino acids. This leads to changes in the components of amino acids in the mucosal protein, which can lead to Pb poisoning.[13,14] The mechanism of the interaction of Pb with proteins has been disclosed by Patrick[15], which states that in general, the toxicity begins with a Pb reaction with proteins containing cysteine residues. Furthermore, the cysteine residue will react with Pb which results in damage to the mucosal constituent protein.

Although it has been known that Pb could interacted with proteins in vitro, but there is a few research that examines the interaction of Pb with gastric mucosal protein in vivo. Therefore, this present study aim to investigate the interaction of Pb with the gastric mucosal protein in Pb-exposed mice.

RESEARCH METHODS

The present study was a true experimental study design to examine the interaction between Pb and gastric mucosal protein of rats (Rattus novergicus). Male rats, Sprague-Dawley furrow, healthy and have normal activity with 8-10 weeks of age and weighing 300±10 grams were obtained from the Abadi Jaya farm at Yogyakarta, Indonesia, in healthy condition.

All rats were caged separately for acclimation period for one week. During the acclimation period, the rats were fed the same drinking water and foods, ie C-05 pellets and PDAM water as drinking water. Before being treated, rats were fasted for 1-2 hours would be to ensure that the rat stomach empty. In addition, all experiment was approved by the Ethical Committee of the Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia.

The study involved 4 groups of 24 male rats with 6 rats each group. Group one (P0) was the control group, while the other was the case group with exposure of Pb. The control

group rats were given 2 ml of distilled water, while in the treatment group rats were exposed Pb in the different concentration. P1 = administration of Pb 0.1 g/L, P2 = Pb administration of 1 mg/L, and P3 = Pb administration of 10 g/L. All treatment period were lasted for 4 weeks.

After the treatment period a surgery were performed and the gastric mucosa were taken and immediately fixed in phosphate buffer solution pH 7. Then the gastric mucosa was cut into small pieces and ground to form a liquid. Subsequently, 5 ml of the solution was taken and centrifuged at 3500 rpm for 10 minutes. The top layer of 200 µL were taken to be examined.

Lead and Gastric Mucosa Protein Interaction Analysis

The top layer of homogenate was taken and the absorbance was measured at 220-300 nm using UV-Vis spectrophotometry.

RESULTS AND DISCUSSION

Based on the observations, the increasing of Pb concentration causes an increase in absorbance. This suggests that there is an interaction between Pb and gastric mucosal proteins, as shown in figure 1.

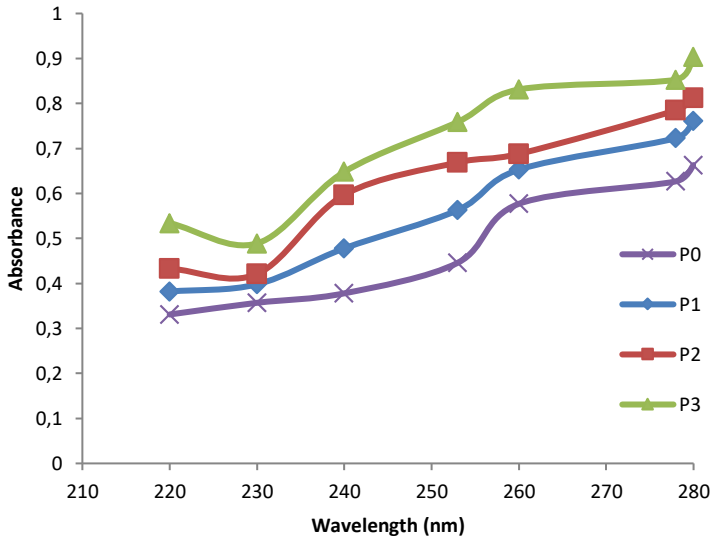

Figure 1. Graph of wavelength relationship with absorbance in group of treatments

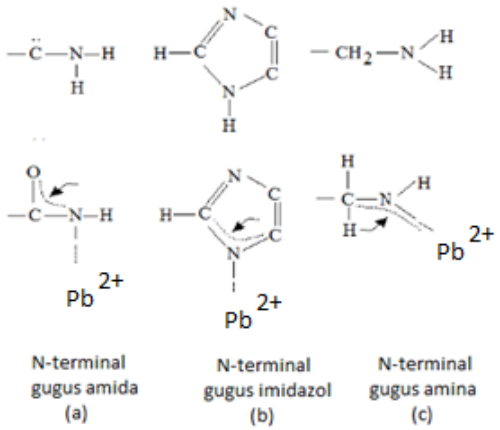

Figure 2. Interaction between Pb and protein by covalent binding with (a) N-terminal amide group; (b) N-terminal of imidazole group; and (c) N-terminal amine group (16)

Figure 1 shows that the increasing of Pb concentration can causes the changing of the absorbance band at 220-280 nm wavelength. The change is allegedly due to the covalent binding of Pb with the N-terminal amide group, the N-terminal of the imidazole group, and the N-terminal of the amine group which promote a further reaction to changes the structure of protein molecules resulted in changes in absorbance bands (figure 2).

The interaction between the metal (L) with protein (P) is based on the reaction equation[17]:

Metals + Proteins ⇆ Metals : Protein

So the bonding constant (K) is formulated:

$$K = \frac{[\text{Metal:Protein}]}{[\text{Metal}][\text{Protein}]} \qquad (1)$$

If it is assumed [Metals: Protein] = $C_{b,}$ then

$$K = \frac{[\text{Cb}]}{[\text{Cl}-\text{Cb}][\text{Cp}-\text{Cb}]} \qquad (2)$$

Under Lambert-Beer's law, then

$$Cl = \frac{\text{Ao}}{\varepsilon \text{l}.\,\text{b}}$$

$$Cp = \frac{\text{Ao}}{\varepsilon \text{p}.\,\text{b}}$$

By entering a value of C_l and C_p in equation (2) then obtained equation (3):

$$\frac{Ao}{A-Ao} = \frac{\varepsilon \text{l}}{\varepsilon \text{b}} + \frac{\varepsilon \text{l}}{\varepsilon \text{b}.\,\text{K}} \cdot \frac{1}{Cl}$$

If n = εb/εl, then the equation (4) is as follows:

$$\frac{1}{A-Ao} = \frac{1}{\text{n}} + \frac{1}{\text{n}.\,\text{K}} \cdot \frac{1}{Cl}$$

By using graph linear curve between the $\frac{1}{A-Ao}$ and $1/C_L$ will be obtained the values of n, the number of the active site binding proteins and metal on metal-protein binding constants (K). In this research, cysteine and tyrosine absorbance data (253 nm for cysteine and 278 nm for tyrosine) at various concentrations of Pb were used, to obtain Pb-cysteine and Pb-tyrosine binding constant (figure 3).

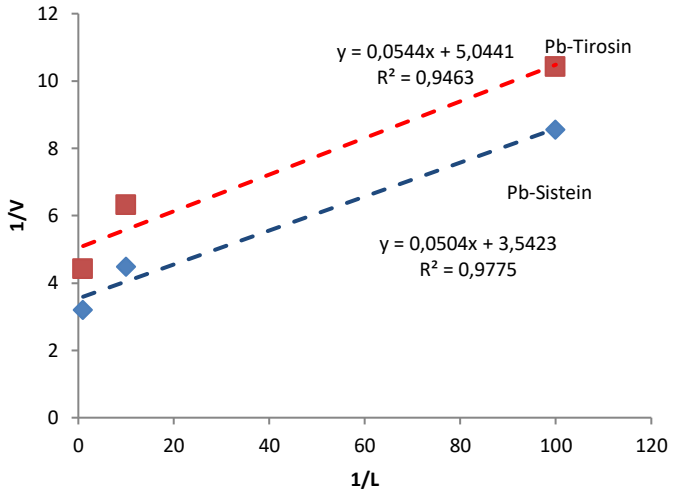

Figure 3. The graph between 1 / V with 1 / L to determine the value of K.

Based on the figure 3, the value of K for Pb-cysteine is 5.65 while for Pb-Tirosin is 3.67. This means that the cysteine in the gastric mucosa has a high affinity to Pb that tends to bind Pb more strongly than tyrosine. The results of this study is in line with the results of Gajawat (13), which states that Pb poisoning will change the components of amino acids protein in the mucosa.

CONCLUSION

In conclusion, the value of K for Pb-cysteine of 5.65 while for Pb-tyrosine for 3.67. This means that the cysteine in the mucosa has a high affinity to Pb that tends to bind Pb more strongly than tyrosine.

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