Lung peroxidative index in mouse models drowning in fresh water

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Lung Peroxidative Index in Mouse Models Drowning in Fresh Water

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Abstract. Drowning is a form of suffocation in the form of victims immersed in liquid so that there is a decrease in oxygen supply resulting hypoxia. Hypoxia can cause oxidative stress which can be determined by measuring the peroxidative index. This research used 24 *Rattus novergicus* which were divided into a control group and five treatment groups. In control groups (P_0), the rats were not drowned; (P1) = post-drowned rats and left for one minute in freshwater; (P2) post-drowned rats and left for two minutes in the freshwater; (P3) = post-drowned rats and left for 15 minutes in the freshwater; (P4) = post-drowned rats and left for 30 minutes in the freshwater; (P5) = post-drowned rats and left for one hour in the freshwater. Peroxide levels were determined by the colorimetric method, while catalase activity was determined by the Aebi method. The results of *Kruskal Wallis* test and *Mann Whitney* test in H2O2 level and catalase enzyme activity shows significant differences toward the control group (p>0,05). Lung peroxidative index correlation with drowning time is used Spearman test. Based on these statistic test, it can be concluded that the length of post drowning duration, the peroxidative index in the lung will increase significantly. This case causes the increasing of oxidative damage in the lungs.

Keywords: drowning, hypoxia, hydrogen peroxide, catalase.

INTRODUCTION

Drowning is a form of suffocation which is the victim immersed in a liquid which is aspirated into the respiratory tract up to the alveoli. Many of drowning incidence occurred. According to the World Health Organization (WHO) in 2012 around the world, there were 372,000 people dying from drowning, with 91% of deaths occurring in developing countries, the case of men 4 times higher than drowning women [1]. This result is supported by statistical data from the Centers for Disease Control and Prevention (CDC) that there is an increase in the incidence of drowning in adulthood and 80% of it were men. This happens because men are less careful and interaction with alcohol is higher [2].

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Drowning begins with the aspiration of water into the lungs resulting in asphyxia caused by obstruction of the respiratory tract resulting in reduced oxygen supply to the tissue until the occurrence of hypoxia [3]. Hypoxia is a condition that causes the oxygen concentration in cells becomes very low so that there is an increase in the production of Reactive Oxygen Species (ROS). The imbalance between the production of ROS and antioxidants in the body causes oxidative stress. The presence of oxidative stress can be expressed by the Peroxidative Index parameter, which is the ratio between peroxide and catalase activity (an enzyme that catalyzes peroxide) [4].

Peroxide (H_2O_2) is a strong oxidant of oxygen derivative compound and reacts slowly with organic substrates. That molecule is toxic and produced in mitochondria during the process of cellular respiration. The peroxide compound produced will then be converted to H_2O and O_2 by the enzyme catalase [5].

Catalase enzyme is one of an intracellular endogenous antioxidant enzyme to neutralize and accelerate the degradation of peroxide compound. In human, catalase can be found in erythrocytes, renal, lymph, pancreas, and brain. In the animal and plant, catalase has hemoprotein which contains four heme groups [4,6].

Researches on the peroxidative index have been done a lot. The research of Lestarisa et al [7] stated that peroxidative index can be as a sign of oxidative stress in kidney damage caused by mercury. In addition, the research of Kania et all [8] concluded that coal dust exposure that caused oxidative stress is marked by the significantly increasing of peroxidative stress. Research on the peroxidative index of drowning in freshwater has not been done much. Therefore, this research needs to be done.

MATERIALS AND METHOD

This research used 24 male *Rattus novergicus* with weight 250gr and was adapted for 24 hours by feeding and drinking. After the adapting process, then terminated by dividing into six groups: control group (P0), the undrowned group; (P1): post-drowned rats and left for one minute in freshwater; (P2): post-drowned rats and left for two minutes in the freshwater; (P3): post-drowned rats and left for 15 minutes in the freshwater; (P4): post-drowned rats and left for 30 minutes in the freshwater; (P5) = post-drowned rats and left for one hour in the freshwater.

One by one *Rattus novergicus* were put into a mouse trap cage; then they were put into the bucket with 30-liter fresh water, according to each group. The drowning duration counted since the rat was declared dead, by confirmed of the rats response when it was shaken. When the rats have been declared dead, the duration can be calculated according to the treatment, which is left in fresh water for 1 minute, 2 minutes, 15 minutes, 30 minutes, and 1 hour. After the treatment, the rats were removed from the cage immediately. Next, the rats were opened up removed for their lungs, then pulmonary homogenates were executed and measured the levels of hydrogen peroxide (H_2O_2) and enzyme catalase activity.

Lung homogenate preparation

The lung of *Rattus novergicus* was removed by surgery and the lung volume was measured, and then crushed with mortar by adding 1 ml buffer phosphate pH 7,4. Homogenate was removed to microtube to be centrifuged in rate 8000 rpm for 20 minutes. After that process, the supernatant was taken to be examined for its H_2O_2 and catalase activity in each group [9].

Lung H_2O_2 analysis

Peroxide level was measured by the colorimetric method. The technique was to put 5 ml buffer phosphate into a tube and add 1 ml lung homogenate solution and homogenize it slowly. Take 1 ml of the result, and add it into 2 ml dichromate / glacial acetate. Heat the tube for 10 minutes in boiling water to remove the blue precipitate and formed a green solution. Measure the absorbance in a 570 nm wavelength [10].

Lung Catalase Activity analysis

Catalase activity was determined by Aebi method [7]. As much as 0,1 ml supernatant was added to kuvet contained 1,9 ml, 50 mm buffer phosphate (pH 7,0). The reaction will be started by adding 1,0 ml H_2O_2 30 mm. The reaction would occur after the addition. Read the first absorbance (A1) after 15 seconds (t1) and the second absorbance (A2) was after 30 seconds (t2). The absorbance was read on 240 nm of wavelength. Catalase activity in unit/mg protein. One unit is defined as a constant rate per seconds.

Peroxidative index calculation

The peroxidative index is calculated by formula [7]

$$\text{Peroxidative index} = \frac{H_2O_2 \text{ Level}}{\text{Catalase activity}}$$

Data analysis

The data were analyzed by *the Kruskal-Wallis* test and Mann-Whitney test. This research was done in Biochemistry-Biomolecular Laboratory of Faculty of Medicine, Universitas Lambung Mangkurat, Banjarbaru.

RESULTS AND DISCUSSION

The research results from the data of peroxide level and catalase activity. The data is in table 1.

TABLE 1. H_2O_2 level and catalase activity in lung *Rattus novergicus* in drowning freshwater

Duration (minutes)	Parameter	
	H_2O_2 (µM)	Catalase Activity (unit/mg protein)
undrawning	3.272	17.320
1	8.690*	11.667*
2	9.873*	3.029*
15	11.272*	1.820*
30	11.608*	1.074*
60	12.292*	0.636*

*) = significantly different from controls ($p<0.05$)

Therefore, the calculation of the peroxidative index is in figure 1.

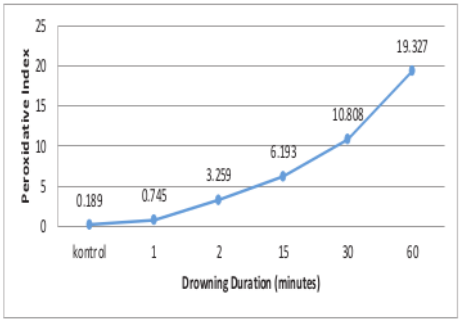

FIGURE 1. Peroxidative index in *Rattus novergicus* lung drowning in freshwater.

By using Spearman test, obtained Rs=1 (p=0,02; p<0,05), that means the increasing of drowning duration cause the significantly increasing of oxidative stress. The increasing of the peroxidative index in death drowning can be caused by the increasing of H_2O_2 level as the transition of duration. The increasing of H2O2 level in *Rattus novergicus* lung is caused by hypoxia. Hypoxia can damage the body tissue by stress oxidative mechanism that causes oxidative damage. As long as the body get hypoxia, the produced peroxide will increase. The increasing of peroxide will cause the body compensates by increasing the production of SOD enzyme, peroxide glutathione, and catalase to form and reduce excess peroxide concentration in the body [11-13].

Peroxidative index in research can be caused by the reducing of catalase activity. Catalase has Km (Konstanta Michaelis-Menten) for H_2O_2 that is bigger than Glutathione Peroxidase so that catalase has an important role in catalyzing H_2O_2 with high concentration.[5] The catalyzing of catalase to peroxide contains two phases. The first reaction phase explains one molecule of H_2O_2 oxidize heme group *(ferricatalase)* in the catalase in form of *resting-state* (Enz(Por-Fe^{III})) becomes *oxyferryl* form. For oxidation is equivalent to Iron (Fe) removal and one of Porphyrin (Por) ring so that resulting I compound, that is radical cationic porphyrin (Por^{+}-Fe^{IV}=O). In the second reaction phase, hydrogen peroxide molecule is used as the reducing agent of I compound to regenerate of the resting-state enzyme (Enz(Por-FeIII)), water (H_2O), and oxygen (O_2) [5,13].

CONCLUSION

From the research, it can be concluded that the longer of post drowning duration, the peroxidative index in the lung will increase significantly. This case causes the increasing of oxidative damage in the lungs.

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REFERENCES

1. Tayler MD, Nielsen CR, Saghafi O, Morse EA, Carey R et al. The epidemiology of drowning in low-and-middle-income countries: a systematic review. BMC Public Health. 2017;413(17): 1-7.
2. Centers for Disease Control and Prevention. Unintentional drowning: get the facts. CDC; 2016 [cited 2016 April 28]. Available from: https://www.cdc.gov/homeandrecreationalsafety/water-safety/waterinjuries-factsheet.html
3. Wilianto W. The diatom examination of the victim allegedly drowned. Indonesian Forensic Medicine Journal. 2012;14(3): 39-45.

4. Zainuri M, Wanadi SI. Spesific activity of manganese superoxide dismutase (MnSOD) and catalase in the rat liver induced systemic hypoxia: relationship with oxidative damage. Media Health Research and Development. 2012;22(2): 87-92.
5. Suhartono E. Reactive oxygen and antioxidant toxicity in the field of medicine and health. Banjarbaru: Gosyen Publishing; 2016.
6. Salomez F, Vincent JL. Drowning: a review of epidemiology pathophysiology, treatment, and prevention. Resuscitation. 2004;63: 261-8.
7. Lestarisa T, Alexandra FD, Jelita H, Suhartono E. Peroxidative index: a new marker in kidney toxicity induced by mercury. International Journal of Toxicological and Pharmacological Research. 2016;8(6): 440-444.
8. Kania N, Setiawan B, Widjajanto E, Nurdiana N, Widodo MA, Kusuma HMSC. Peroxidative index as novel marker of hydrogen peroxide involvement in lipid peroxidation from coal dust exposure. Oxidants and Antioxidants in Medical Science. 2012;1(3): 209-15.
9. Oktaviyanti IK, Thalib I, Suhartono E. The Protective Efficacy of Quercetin on Mefenamic Acid-Induced Gastric Mucosal Damage International Journal of Pharmaceutical and Clinical Research. 2016; 8(10): 1390-5.
10. Hartoyo E, Thalib I, Suhartono E, Yunanto A. Oxidative and chlorinative stress in children with dengue hemorrhagic fever. International Journal of Pharmaceutical and Clinical Research. 2016;8(8): 1186-1191.
11. Simpson K. Forensic Medicine. London: The English Language Book Society; 1982.
12. Idries AM. Forensic Medical Science Guidelines. Ed I. Jakarta: Binarupa Aksara; 1997.
13. Marks DB, Marks AD, Smite CM. Medical biochemistry is the basis of a clinical approach. Jakarta: Pendit, EGC; 2000.

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