Efektivitas Paparan Hipoksia Berdasarkan Aktivitas Antioksidan Katalase dan Kadar Malondialdehyde (MDA) Sebagai Indikator Pada Jaringan Jantung Tikus

The Effectiveness of Hypoxia Exposure Based on Catalase Antioxidant Activity and Malondialdehyde (MDA) Levels as Indicators in Tissue Rat Heart

Muhammad Farikh[1)], Nabila Sulaeman[1)], Balkis Alfatimah Azzahra[1)], Siska Alicia Farma[1)],Rinti Mutiara Sari [1)], Atiqah Nabila Febril[1)], Shalmita Sakinah[1)]

[1)]*Program Studi Biologi, Fakultas Matematika dan Ilmu Pengetahuan Universitas Negeri Padang*
Jalan Prof. Dr. Hamka, Air Barat, Kec. Padang Utara, Kota Padang, Sumatera Barat 25171
Email: Bukittinggi.farikh12@gmail.com

ABSTRACT

Free radicals can cause oxidative stress, due to an imbalance between oxidants and antioxidants that have the potential to cause cell damage. Free radicals can increase lipid peroxidation, which breaks down into malondialdehyde (MDA) in the blood. MDA is a marker of cellular defects caused by free radicals. In this study, rats were treated with hypoxia in a hypoxia chamber with a concentration of 10% oxygen and 90% nitrogen. Based on the results of statistical tests, the levels of MDA in the liver tissue of rats with catalase activity were Accept H0 i.e. there was no difference in the average duration of exposure to O2 gas. This event can occur because an organism will try to maintain its life when environmental conditions are not favorable. In this study, the effect of continued hypoxia on catalase as an endogenous antioxidant that produces malondialdehyde (MDA) was not found. The results of this study indicate that exposure to hypoxia can reduce the value of pH, pCO2 and pO2 of rat bloodplasma.

Key words: ***Hypoxia, Malondialdehyde*** **(MDA),** ***Antioxidant catalase, Peroxidation***

PRELIMINARY

Oxygen has a very important role in all body processes functionally as well as the need for oxygen which is the most important and vital requirement for the whole body. Oxygen is known to exist in the air (atmosphere) and in waters. Normal levels of O in the atmosphere are about 21% or 160 mmHg (Sherwood, 2010). In a state of lack of oxygen can cause metabolism to run unstable, the presence of a lack of O2 can be characterized by a state known as hypoxia. Hypoxia is a condition of lack of oxygen supply in the body. In this condition, damage to nerves, brain, liver and other organs can occur and can cause death in humans. These symptoms can be characterized by an above-average heart rate, very low oxygen saturation, fast and short rhythms and volumes. (Seprani, 2017).

Hypoxia is characterized by a condition of low oxygen levels in cells/ tissues to below the physiological level (Hadiarto, 2010). Oxygen levels in cells/ tissues can be said to be hypoxic if the partial pressure of oxygen gas (Pgas) in arterial blood is < 100 mmHg because there are < 20% O gas in the atmospheric air (Law, 1999).

The lack of sufficient oxygen in the tissues will have an impact on the stability of aerobic respiration in cells, more precisely at the electron transport stage. At this stage, living cells need oxygen molecules as the final electron acceptor in cell metabolism. Asa result of insufficient oxygen demand, it will release free electrons that are radical or unstable, known as free radicals. These unstable electrons will act as Reactive Oxygen Species (ROS). One of the ROS that is naturally produced by the body is hydrogen peroxide (H2O2). Hydrogen peroxide is a highly reactive chemical composed of hydrogen and oxygen components.

Naturally, the body can make efforts to protect against the negative effects of ROS. The body's way to fight back by overcoming the number of free radicals is to produce antioxidants, one of which is catalase. Catalase is an enzyme class antioxidant that can convert $H_2O_2$to water (H_2O) and O_2. If free radicals do not bind to antioxidants, the oxidation reaction will continue or form a cascade that causes cell damage (Figure 1).

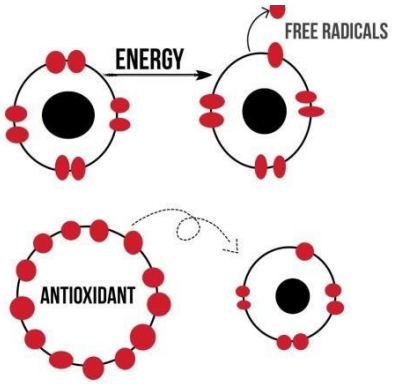

Image 1. Formation o ree radicals and their role antioxidants stabilize free radicals

In addition, oxidative stress will trigger ROS to carry out fatty peroxide reactions that will damage cells/tissues. $H_2O_2$2is a type of ROS that is highly reactive to cell membranes. One of the end products of the lipid peroxide reaction is the formation of malondialdehyde (MDA) compounds, so that the level of cell damage can be measured based on the levels of MDA contained in the cells/tissues. The liver is the main site for the presence of catalase (Kyo Ah Kang et al., 2006). Enhancement this ROS also results in an increase in MDA due to the lipid peroxidation process, so it can be interpreted that MDA is used as a marker to determine oxidative stress in cells (Shofia et al., 2013).

This is in accordance with the main task of the liver as a neutralizer of toxins such as

free radicals in the body. Some of the functions of the liver include processing major nutrient metabolites (carbohydrates, fats, proteins), detoxification or degradation of waste substances, synthesis of various plasma proteins and glycogen storage. In the liver parenchyma cells, an oxygen gradient is formed due to the oneway flow of blood from the portal and periportal veins to the central (perivenus) vein. This oxygen gradient increases because of a metabolic process that consumes oxygen in the parenchyma cells and makes the oxygen pressure decrease from 60-65 mmHg in the periportal area to 30-35mmHg in the central vein. The oxygen gradient in the liver parenchyma plays an important role in the regulation of genes encoding enzymes for carbohydrate metabolism. For example, glycolytic enzymes such as pyruvate kinase are expressed more strongly in a less aerobic area, namely the perivenous zone, whereas gluconeogenesis enzymes are more dominant in the more aerobic periportal zone. Given the important role of oxygen for cells in enzyme activity in the liver. In this study, the specific activity of malondialdehyde (MDA) levels as an information indicator in rat liver tissue and catalase in rat liver induced by systemic hypoxia was analyzed and its relationship to oxidative damage.

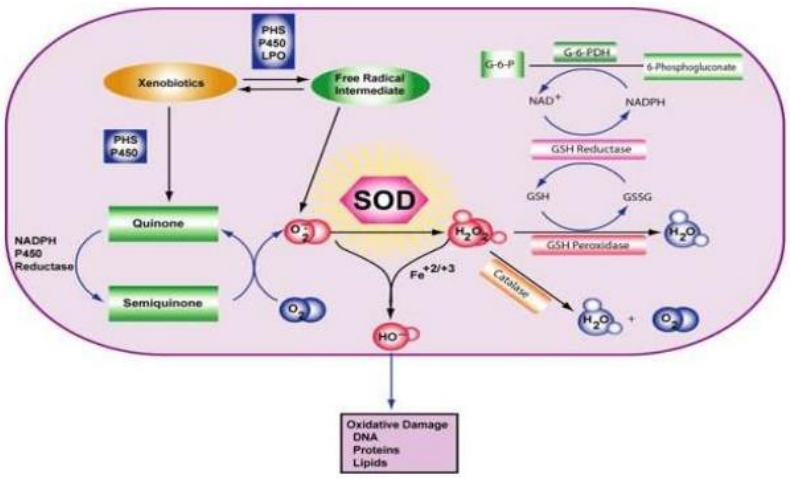

Image 2. MDA formation mechanism

RESEARCH METHODS

1. Sample or research

Male wistar rats, as many as 24 rats using the Frederer's formula. The sample was found from the Veterinary Research Agency (BALITVET), Bogor with a susceptible age ranging from 2.5 - 3 months and a weight of ± 100 gr.

2. Adaptation of Rats to the Environment

A cage for rats was prepared containing enough oven wood coir, feed for rats at a dose of 15 g/head/day, equipped with a wire covering measuring about 20 x 40 cm, and drinking water. Wood fiber is replaced every 4 days. Trays are changed once a week. All rats were adjusted to the environment for 2 weeks in the available cage space at UNP

before carrying out treatment actions

3. Sections in Hypoxia Exposure

A set of hypoxic chambers/cages is prepared and then connected directly using a plastic hose to a gas cylinder that already contains 10% Oxygen accompanied by 90% Nitrogen. Wood fibers can be directly inserted into the cage. A total of 60g of rat feed was added along with the water. 4 male wistar rats from the adaptation process can be taken, weighed, then the weight of the rats is recorded and marked. The 4 rats can be directly put into a tightly closed cage. Oxygen gas of 10% and 90% N was flowed at a speed of 1L/min into the chamber. The flow of gas into the chamber corresponds to the exposure time (length exposure) namely 1,3,7,14, and 21 days. Maintenance is carried out by replacing the wood coir oven and drinking water 2x a day (morning and evening), wiping the steam on the chamber walls, feed rats at a dose of 60 gr/day.

4. Control Group

Prepared a set of cages for each rat. The weight of the rats was weighed and given a sign or label. The maintenance of rats in the control room lasted for 21 days. Perform maintenance actions by replacing oven coir once a week, feeding rats 15 g/day, and drinking water ad libitum.

5. Rat Sugery

Each rat was removed from the cage when it had reached the day of exposure to hypoxia. Rats can be killed in various ways, one of which is neck dislocation. After that the west of the body of the tusk can be measured. Perform surgery on rats, followed by taking blood through the ventricles of the heart using a 2.5 cc syringe containing heparin. The blood will be measured for blood gases using Lab services. IKAFKUI/RSCM Intensive Care Unit. Parameters needed to describe hypoxic conditions are pH values, pCO_2, and pO_2. Liver stored in freezer-2 ◦ C.

6. Making Liver Homogenates

For each rat tissue in the treatment and control rat groups, 1gr each can be weighed. Liver tissue in rats was washed with 150 µl of PBS + PMSF to clear from blood. The liver tissue was crushed with pestle and then PBS + PMSF was added with a ratio of 1:1. The homogenate solution was centrifuged at 12,000 rpm 4 OC with a duration of 10 minutes and then separated between pellets and supernatant. This supernatant will be used as a protein homogenate which is then stored in a freezer at -20 ◦ C until the test stage for catalase activity and MDA levels.

7. Examination of the specific activity of the enzyme catalase

The centrifuged substance which had a lower specific gravity was diluted using PBS+PMSF with a ratio of 1:500. The 10mM H2O2 was diluted using phosphate buffer in a ratio of 1: 100. Cuvettes for UV spectrophotometer were prepared. In the blank cuvette, 50 µ of PBS and H2O2 of 950 µ of the test cuvette, 50 µ of supernatant was added and 950

µ of H2O2 was added and mixed manually. Using a wavelength of 240 nm for each cuvette, absorbance was measured in the first minute (t_1) and the second minute (t_x). Catalase activity (U/mL) was determined based on the following formula:

$$\frac{\Delta \text{Absorbance Test} - \Delta \text{Absorbance Blank}}{\text{Molarity } H_2O_2 \text{ X Volume}} \times \text{Diluent Sample Factor}$$

8. Examination of MDA levels

Performing MDA measurements using a modified spectrophotometer thiobarbituric acid (TBA) test method. 16 A total of 400 µl of sample was mixed with 200 µl of *trichloroacetic acid* (TCA) 20% for deproteinization. Then it was vortexed and centrifuged at 5000 rpm for 10 minutes. The supernatant formed was taken and added 400 L of 0.67% TBA. Furthermore, the sample was vortexed and incubated in a water heater at a temperature of 960 C, 10 minutes later removed and immediately cooled at room temperature. After that read the absorption with a wavelength of 530 nm.

9. Data Analysis

Main data in the form of antioxidant activity of catalase, MDA levels of rat liver tissue were spectrophotometrically tested for normality (Kolmogorov-Smirnov) and 1- way ANOVA ($p \leq 0.01$) using SPSS 17.

RESEARCH RESULT

1. Body weight O experimental animal rats

Rats in the treatment group based on Figure 3 decreased according to the length of time for exposure to 10% O2 gasin the cage. This treatment group at 3 days had a minimum value with an average of 4.25 g. While the minimum weight loss value is at 21 days of maintenance, which is as high as 14.25 gr. The control group rats experienced an increase in body weight as high as 49 g. Changes in body weight of rats can be observed in Figure 3.

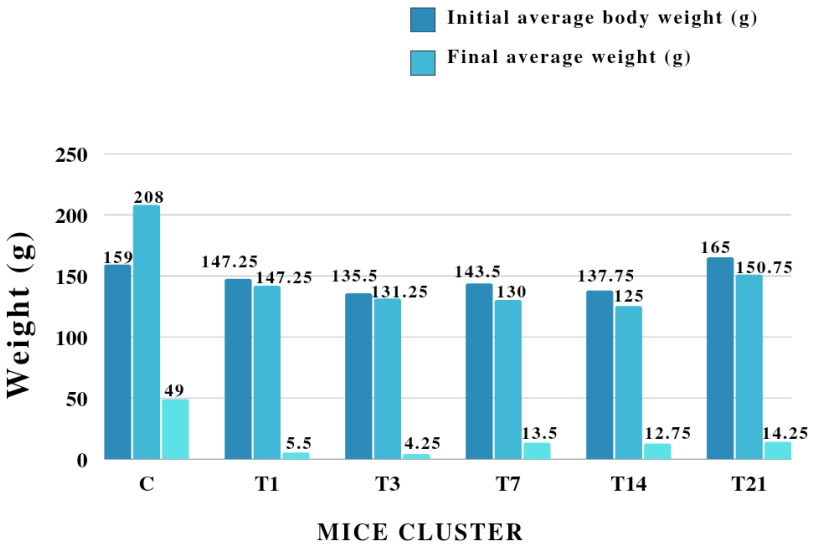

Figure 3. Changes in Rat Body Weight; C = Control Group Rats; T = Rats in the treatment group with O_2 gas exposure: 10%; 1, 3, 7, 14, 21 = Length of exposure to O_2 gas: 10%/day.

2. Blood gas profile of experimental rats

This test during the study was to obtain a profile of pH, pO2 , and pCO2 . Normal mice have blood with a pH value of 7.25-7.35;32-43mmHg 50-100mmHg, pO2 (Heeijen, 2002). Based on the review of Table 1, the results of the measurement of rat blood gases were obtained between the treatment group and the control group. The blood pH value in the rats in the treatment group decreased with the duration of 10% O2 gas induction. In the control group the results of measurements of pH showed a value of 7.1. This means that the pH value in the control group shows a lower value when comparedto the standard pH of normal rats. When compared with the pH value of the blood of the rats in the treatment group, the pH value of the rats in the control group was still higher. The pCO2 value of pCO2 the treated group rats had a higher value than. the control group However, on exposure to 10% O2 gas14 levels of pCO2 rats in the treatment group showed a very low value when compared to the control group. The results of the measurement of blood pCO2 58 mmHg. The blood pCO2 level of the control group rats showed a higher value when compared to the Pco2 of normal rats. The increase in the pCO2 value of the rat blood in the treatment group according to the longer exposure time to O2 gas can be seen from the results of the rat blood gas measurement during the study. 10%. The results of the measurement of blood pO2 levels of rats in the control group were 25.5 mmHg. pO2 levels of control group rats showed a lower value when compared to the standard pO2 of normal rats. The value of pO levels of rats in the treatment group did not differ much from the pO2 of. the control group.

Tabel 1. Results of Measurement of Mice Blood Gas Treatment Group; K = Control Group Rats; P = Rats in the treatment group with 10% O gas exposure; 1, 3, 7, 14, 21 = Duration of exposure to O gas 10% (days)

Parameter			
Label	pH	pCO_2(mmHg)	pO_2(mmHg)
Standar *	7,25-7,35	32-43	50-100
K	7,1	58	25.5
1 H	7,1	75,25	33,5
3 H	7	82,52	13,32
7 H	7,023	43,825	24,725
14 H	7,02	95,725	24,475
21 H	6,7	94,95	27,97

Keterangan *: Standard rat blood gas analysis is normal

3. Catalase Activity

Catalase activity values were obtained in the rats in the treatment group and the control group. The treatment group showed a higher value of catalase activity but fluctuated during the study. On exposure within 3 days, the antioxidant activity of catalase showed the highest number, which was as high as 126.75 Unit/ml. The value of catalase activity decreases at 7 and 14 days of exposure and will increase at 21 days, which is as high as 92.25 Unit/ml. The value of catalase antioxidant activity in the treatment group rats was higher than the control group rats.

Based on the results of the normality test (Kolmogorov-Smirnov) the catalase activity data were normally distributed. The results of the 1-way ANOVA statistical test ($p \leq 0.01$) showed that the F table was 1.343 while the calculated F was 0.701. Because F count < F table, it can be interpreted that there is no average difference between the antioxidant activity of catalase in rat liver tissue for each duration of 10% O2 exposure1,3,7,14 , and 21 days). The value of catalase antioxidant activity in the liver tissue of the treated rats can be seen in Figure 4.

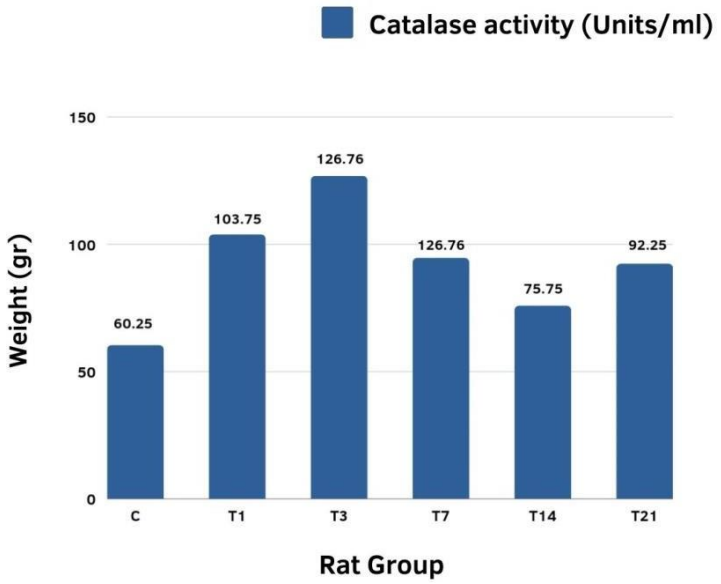

Figure 4. Catalase Activity (Units/ml) Rat liver tissue, C = Rat Control Group, T = Rat Treatment Group with Gas Exposure O_2 10%; 1, 3, 7, 14, 21 = Gas Exposure Duration O_2 10 % (days).

4. Profile of malondialdehyde (MDA) levels in the liver tissue of experimental mice during the study

The highest MDA level value based on Figure 5 shows that the experimental group rats were higher than the control group mice. However, the value of MDA levels in the treatment group fluctuated during the research stage up to 21 days. In the treatment group, the value of MDA levels showed a lower value at 3-day exposure, which was as high as 0.836 nmol/ml while the MDA value at 14-day exposure was as high as 0.856 nmol/ml. The highest value of MDA levels in the treatment group occurred at 7 days of exposure, which was as high as 1,417 nmol/ml. MDA levels in rat liver tissue can be seen in Figure 5.

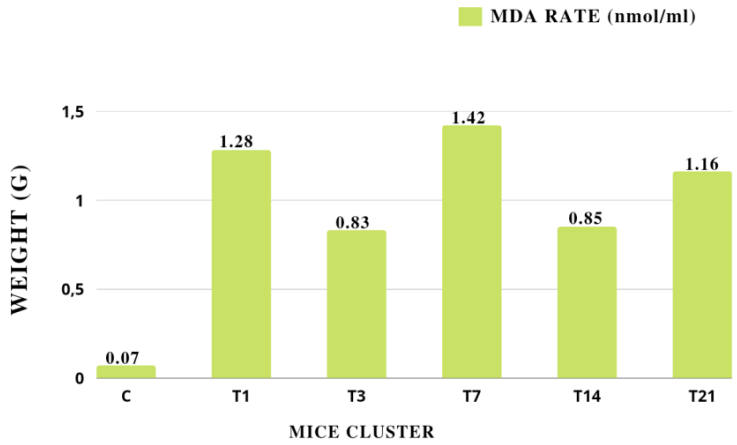

Figure 3. Malondialdehyde levels (nmol/ml) Rat liver tissue. C= Control Group Rats; T= Treatment group mice with 10% O_2 gas exposure; 1, 3, 7, 14, 21 = Length of exposure to gas O_2 10% (days).

So, based on a review of the results of the normality test (Kolmogorov-Smirnov) the data on MDA levels were normally distributed. The results of the 1-way ANOVA statistical test ($p \leq 0.01$) showed the F table of 4.25 while the calculated F was 1.343. Because F count < F table, it can be said that there is no difference in the average MDA levels of rat liver tissue on the duration of exposure to O210 % (1,3,7,14, and 21 days).

DISCUSSION

1. Mice Weight Change Profile

The group of treated rats experienced a decrease in body weight along with the duration of induction. The treatment group resulted in weight loss with the lowest value occurring in maintenance for 3 days with an average of 4.25 g and the highest result in maintenance for 21 days of 14.25 g. Weight loss in the treatment group could have occurred due to a lack of oxygen supply.

Based on research conducted by Chen (2007), that hypoxic conditions will cause weight loss. Supporting theory in this regard is the effect of high levels of reactive oxygen species (ROS) and the events of cell death programmes. ROS are reactive to cell constituents such as DNA and cell membranes. ROS can damage cell membranes by taking electrons in the cell membrane so that the stability of the cell membrane will decrease (Catala, 2011). This event is known as lipid peroxidation and leaves the final compound in the form of an aldehyde or malondialdehyde (MDA). Weight loss due to hypoxic conditions is a matter that has been studied. Several factors that cause this to happen are increased metabolic rate, decreased energy consumption, and decreasednutrient intake due

to reduced appetite (Liippl et al., 2009). In addition, weight loss during hypoxic conditions is caused by the use of adipose tissue as an energy source, dueto decreased food intake (Ge et al., 2001).

2. Mice Blood Gas Profile

Based on the results of blood gas analysis in the treatment group rats, according tothe duration of exposure to 10% O2, the pH value decreased, with an average of 6.7 at 21 days. The high and low pH values in blood plasma depend on the number of HCO molecules dissolved in the blood (Sherwood, 2010). The end result of metabolism is the formation of CO2. This molecule must be immediately removed from the body through the bloodstream to the lungs. When flowing in the bloodstream, CO2 will bind to water to form HCO ions. High levels of HCO ions will lower the pH value.

The pCO2 valuetreated rats showed a value greater than the standard pCO2of normal rats. pCO2 14 days of exposure, which was 95.7mmHg. The lowest pCO2 value7 days of exposure, it is possible on that day the rats were able to do homeostasis to CO2. The pCO2 valuesecond result of higher anaerobic respiration will make the rats adapt by hyperventilating. When the supply of O2is not met, the body will adapt by doinganaerobic respiration. Anaerobic respiration aims to meet the needs of ATP. The end result of anaerobic respiration is the formation of lactic acid and H+ ions. The increase in H+ ions is related to the production of CO2 in the tissues. The significant reasons for the increase in pCO2 are currently still being debated, considering that CO2 can be formed either from aerobic respiration or anaerobic respiration (Guiterrez, 2004). Apart from anaerobic respiration, supporting theories explaining the increase in pCO2are the dissociation curve and blood flow in tissues. Hypoxic conditions cause the plasma pH to become acidic thereby increasing the affinity of hemoglobin to bind to CO2, thereby increasing the pCO2 (Vallet et al., 2000).

The pO2 valuecontrol of the treatment group's blood showed different results from the pO2 value of the. groupThe low pO2 control valueis possible that the atmospheric pO2is indeed low. While the low pO2 valueby the percentage of O2 gas10%, so that it would further reduce the pO2. in the blood circulationWhen it reaches the bloodstream, the pO2 value 160 mmHg) will decrease by about 37.5% to 100mmHg (Sherwood, 2010). Gas O2 that is given into the chamber is only 10%, then the pO2 valuein the blood circulation is only about 47.5 mmHg. However, based on the results ofrat blood gas measurements, the average pO2 value. of the treatment group was around 24mmHgrats trying to get as much O24 as possible, so that the O2 intakethey get is decreasing.seen that the exposure to 10% O2 gas3 days was the lowest pO2 value7 , 14, and 21 days. This event indicates that the mice are trying to survive by hyperventilating to meet the needs of O2.

3. Antioxidant Activity Profile of Rat Liver Tissue Catalase

In the treatment group rats, the highest catalase antioxidant activity occurred during the third day and decreased further on exposure for 7 and 14 days but increased again to 75.75 Unit/ml at 21 days of exposure. The catalytic function of the antioxidant catalase is to maintain homeostasis from increasing levels of ROS. This catalytic function can be measured by the ability of antioxidants to convert the substrate into a product. If the concentration of the solution in the antioxidant is large, the ability to accelerate the work of the antioxidant will also be large which will be directly proportional to the result of a product. In addition, the number of substrate molecules available in a solution will determine the frequency of the substrate reaction to the enzyme so that it will determine the amount of product that will be formed.

The low activity of catalase in the group of treated rats was caused by a low concentration of the antioxidant catalase which will be formed by liver tissue on that day. When compared the antioxidant activity of catalase between the control group and the treatment group, the treatment group obtained greater catalase antioxidant activity but tended to decrease at 7 and 14 days. However, the antioxidant activity of catalase again increased when exposed to 21 days. Based on these conditions, at 21 days of hypoxic exposure, rats made adaptations to maintain life. In addition, catalase antioxidants are also reactive to H2O2, probably because the catalase measured by spectrophotometer is catalase that has not been damaged from environmental influences.

4. Mice Liver Network MDA Profile

Based on data on MDA levels in liver tissue, it can be said that under normal or hypoxic conditions it will still produce MDA. The treatment group produced higher levels of MDA but fluctuated with each length of exposure to hypoxia. The value of MDA levels decreased on exposure for 3 days by 0.836 nmol/ml and 14 days by 0.856 nmol/ml. The highest levels of MDA occurred at the duration of exposure for 7 days at 1,417 nmol/ml. The resultant MDA is an indicator of the occurrence of damage to the tissue caused by the presence of ROS. This hypoxic condition can later increase the levels of free electrons or known as ROS. One ROS like one of them is hydrogen peroxide. Hydrogen peroxide with H2O2 has lipid-soluble properties and causes damageto the outer cell membrane. Damage to cell membranes occurs through the process of fat peroxidation. At the end of fat peroxidation, an aldehyde compound in the form of malondialdehyde (MDA) will be formed (Catala, 2011).

Based on the data on MDA levels in the treatment group, MDA levels fluctuated at each time of 10% oxygen exposure. It can be seen that at 7 days of exposure the highest MDA levels occurred, so it can be indicated that on that day the highest ROS were formed. This is also supported by the antioxidant activity of catalase which decreased on exposure

for 7 days. On exposure for 14 days, MDA levels decreased, this indicated that the antioxidant catalase was able to suppress the presence of ROS. According to research that has been done by Chen et al. (2007), one of the various types of ROS that is abundant under hypoxic conditions is H2O2. H2O2reactiveis. to cell membranes Catalase antioxidants are known as endogenous antioxidants that have a balancing role when the body's physiological ability decreases when fighting free radicals. The antioxidant catalase serves to prevent the chain reaction of lipid peroxidation by converting H2O2 into O2 and H2O.

5. Relationship between Catalase Activity and MDA Levels

Catalase is an antioxidant enzyme produced by the body itself in the form of enzymes and reacts with H2O2 molecules oxidation will reactionoccur when both form enzyme substrates so that H2O2H2OO2. be broken down intoand. The will MDA levels, is quite related because H2O2 or peroxidewith is one type of ROS that is abundant during hypoxia. At 3 days of exposure to hypoxia, catalase activity increased by 0.83gr when compared to 1-day exposure of 1.28gr. When observed at the MDA levels of exposure for 3 days, it was seen that the MDA levels on that day decreased. This event illustrates that the role of catalase in suppressing ROS that is reactive to cell membranes occurs. The increase in MDA levels occurred at 7 days of exposure and catalase activity on that day decreased. This event may be caused by the mouse body not being able to produce antioxidants optimally so that the reaction of ROS with cell membranes cannot beavoided. At 14 days of exposure, catalase activity decreased, with the lower than 7 days of exposure and the MDA levels of 14 days of exposure showed a decrease when compared to 7 days of exposure. This could be due to the presence of antioxidants other than catalase which can suppress ROS so that cell damage does not occur. At 21 days of exposure, both catalase activity and MDA levels increased. This event shows that with exposure to O2 gas21 days, ROS increased, but the body's defenses also increased, so that the rats in the chamber/cage would still be able to survive.

6. Analysis of Statistical Test Result

Based on the results of statistical tests, the levels of MDA in the liver tissue of rats with catalase activity were Accept H0 i.e. there was no difference in the average duration of gas exposure O_2. This event can occur because an organism will try to maintain its life when environmental conditions are not favorable. However, these results did not occur in previous studies, which said that O. gas exposure$_2$10% for 1, 3,7, 14, and 21 days showed a significant difference. This may have happened because the resistance of the mice used in this study was higher than the resistance of the mice used in previous studies. So it needs a lot of improvement or further research on the activityof catalase and MDA by

reducing oxygen levels in exposure at the same time can be reviewed in other body organs.

CONCLUSION

In this study, it was not found the effect of hypoxia on catalase as an endogenous antioxidant that produces malondialdehyde (MDA) which is the end product of the lipid peroxide reaction in rat liver tissue. The research results that can be reviewed are related to rat body weight. The results of this study also showed that exposure to hypoxia can reduce the value of pH, pCO2and pO2So of rat blood plasma.

REFERENCE

Sepriani, R. 2017. Pengaruh Pemberian Minuman Beroksigen Terhadap Kemampuan Volume Oksigen Maksimal (VO2 Maks). *Jurnal Menssana*. 2(2). 89-98.

Catala,A. (2011*). Lipid Peroxidation*. (INIFTA-CCT La Plata-CONICET), Facultad de Ciencias Exactas, UNLP.

Chen, X.Q., Dong, J., Niu.C.Y., Fan. J. M., Du. J.Z. (2007). Effects of Hypoxia on Glucose, Insulin, Glucagon, and Modulation by Corticotropin-Releasing Factor Receptor Type 1 in the Rat. *Endocrine Journal*; 148 (7): 3271.

Ge., Wang.,Yand., Lui. (2001). *Progressin Studying Weight Loss at High Altitude and Its Potential Application In Patients With Obesity*. Qinghai: Research center for high altitude medicine.

Law, Rob dan Bukwirwa. (1999). *The Physiology of Oxygen Delivery*. Issue 10 (3): 1-2. Diunduh dari http://www.nda.ox.ac.uk/wfsa/html/u10/u1003_01.html padatanggal 14 Desember 2011.

Uyun, H. F., & Indriawati, R. (2013). Pengaruh Lama Hipoksia terhadap Angka Eritrosit dan Kadar Hemoglobin *Rattus norvegicus*. *Mutiara Medika: Jurnal Kedokteran dan Kesehatan*, *13*(1), 49-54.

Sherwood, L. (2010). *Human Physioloy From Cells to Systems 7th Edition*. Canada: Brocks/ Cole**.**

Law,Rob dan Bukwirwa.(1999). *The Physiology of Oxygen Delivery*. Issue10(3):1-2. Diunduhdari http://www.nda.ox.ac.uk/wfsa/html/u10/u1003_01.html pada tanggal 14 Desember 2011.

Andarina, R., & Djauhari, T. (2017). Antioksidan dalam Dermatologi. *Jurnal Kedokteran dan Kesehatan: Publikasi Ilmiah Fakultas Kedokteran Universitas Sriwijaya*, *4*(1), 39-48.

Yuniarti, Y., Achadiyani, A., & Murniati, N. (2016). Penggunaan Pemutih Gigi Mengandung Hidrogen Peroksida 40% Dibanding Dengan Strawberry (Fragaria x ananassa) Terhadap Ketebalan Email, Kadar Kalsium, Dan Kekuatan Tekan Gigi. *Global Medical and Health Communication*, *4*(1), 7-15.

Puspitaningrum, R., Lestari, A. P., & Murtiati, T. (2014). Pengaruh Paparan Hipoksia Terhadap Aktivitas Antioksidan Katalase Dan Kadar Malondialdehid (MDA) Pada Jaringan Hati Tikus. *Bioma*, *10*(2), 27-34.

Shofia, V., Aulanni'am, Mahdi, C., 2013, Studi Pemberian Ekstrak Rumput Laut Coklat (Sargassum Prismaticum) terhadap Kadar Malondialdehid dan Gambaran Histologi Jaringan Ginjal pada Tikus (*Rattus norvegicus*) Diabetes Melitus Tipe 1, *Kimia. Studentjournal*, 1, pp. 119-125.

Sunaryo, H., RAHMANIA, R. A., DWITIYANTI, D., & SISKA, S. (2017). Aktivitas Antioksidan Kombinasi Ekstrak Jahe Gajah (*Zingiber officinale* Rosc.) dan Zink Hiperkolesterolemia dan Hiperglikemia dengan Penginduksi Streptozotosin. *Jurnal Ilmu Kefarmasian Indonesia*, 13(2), 187-193.

Halliwell B, Gutteridge JMC. 2007. *Cellular Respones to Oxidative Stress: Adaptation, Damage, Repair, Senescence and Death. In Free radicals in biology and medicine. 4th ed*. London: Oxford. University Press, 187-267.

Vallet, B., Teboul, J.L., Cain, S., Curtis, S. (2000). Venoarterial CO difference during regional ischemic or hypoxic hypoxia. *Journal Application of Physiology*. Vol. 89: 1317-1321.

Zaetun, S., Dewi, L. B. K., Wiadnya, I. B. R., & Gede, L. S. (2019). Profil Kadar Mda (Malondialdehide) Sebagai Penanda Kerusakan Seluler Akibat Radikal Bebas Pada Tikus Yang Diberikan Air Beroksigen. *Jurnal Analis Medika Biosains* (*JAMBS*), 4(2), 63-68.