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Research Article

The Effect of Hyperbaric Oxygen Therapy (HBOT) on the Histopathological Lung Cells of Male White Wistar Rats Which are Intoxicated with Methanol

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ABSTRACT

Methanol is a liquid that has the characteristics of being light, very easy to evaporate, colorless, flammable, toxic, and has a very distinctive odor like ethanol but lighter. The World Health Organization (WHO) globally states that 320,000 people die every year due to methanol poisoning in the age range of around 15 years to 29 years. Usually, symptoms of poisoning caused by methanol appear within 30 minutes to 2 hours. Based on the phenomenon that occurred, the author wanted to find out more about the effect of HBOT administration on the histopathological appearance of lung cells in mice intoxicated with methanol. This study used 30 male white wistar rat lung samples aged 2 to 3 months weighing around 150 grams to 250 grams, divided into three groups, namely the control group, treatment group I, and treatment group II. The control group was not given methanol treatment and hyperbaric oxygen therapy (HBOT). Treatment group I was given methanol for 14 days without hyperbaric oxygen therapy. Meanwhile, the treatment group was given methanol for 14 days and then given hyperbaric therapy for 10 days. The results show $p<0.0006$, which means the data is significant and there are differences between groups. The results of this study can be concluded that administering hyperbaric oxygen therapy to rats intoxicated with methanol had a statistically significant improvement effect on the alveolar membrane, alveolar lumen, and connections between the alveoli.

Key words: Hyperbaric Oxygen Therapy, Methanol, Histopathological Lung Cells.

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INTRODUCTION

Drinks containing alcohol known as liquor are a type of drink that contains ethyl alcohol or ethanol which is made from distilled or non-distilled fermentation. Liquor that is commonly consumed in Indonesia contains ethyl alcohol or ethanol with levels of 5-40%, but it is not uncommon to find Indonesian people consuming liquor mixed with other ingredients. This liquor can be said to be mixed liquor, the ingredient most often used is methanol. Methanol is an ingredient that is often used as a paint cleaner or paint solvent. Methanol is very toxic to the human body even if it is not mixed with other ingredients, and can even cause death.[1,2]

The World Health Organization (WHO) globally states that 320,000 people die every year due to methanol poisoning in the age range of around 15 years to 29 years. Usually, symptoms of poisoning caused by methanol appear within 30 minutes to 2 hours after consuming alcohol contaminated with methanol. The first symptoms of poisoning that appear include nausea, vomiting, drowsiness, dizziness, gastritis, diarrhea, back pain, and stiffness in the legs. After a latent period of 6 hours to 30 hours, mild to severe metabolic acidosis can also be experienced by patients. Patients can also experience problems with their vision (up to blindness), even convulsions leading to coma, and acute kidney failure with myoglobinuria, until death can occur.[3]

Most of the disorders are caused by the effects of formic acid which is the main metabolite. This formic acid can irritate the lining of the airways and disrupt the normal defense mechanisms of the airways. Formic acid is metabolized in the body which takes a long time and can accumulate in the body which will result in metabolic acidosis, it can cause a slowdown in cellular respiration and lactic acidosis.[4]

HBOT is a type of therapy that has not been studied in depth, and its benefits in various diseases can be said to be small, especially in clinical testing. Scientifically, its use is effective in infectious diseases, decreased oxygenation levels (hyperfusion), infarction, or lack of blood supply to the tissue (ischemia). HBOT increases plasma oxygen tension and increases the amount of oxygen delivered to cells, which increases the production of reactive oxygen and nitrogen to stimulate angiogenesis.[5,6]

METHODS

This study used 30 male white wistar rat lung samples aged 2 to 3 months weighing around 150 grams to 250 grams, divided into three groups, namely the control group, treatment group I, and treatment group II. The control group was not given methanol treatment and hyperbaric oxygen therapy (HBOT). Treatment group I was given methanol for 14 days without hyperbaric oxygen therapy. Meanwhile, the treatment group II was given methanol for 14 days and then given hyperbaric therapy for 10 days. The dose of methanol used is 6 cc/kilogram of body weight.

On the 14th day (control group and treatment I) and 24th (treatment group II) 9 animals were taken for euthanasia each. Next, rat lungs were processed to

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Table 1. Lung damage assessment criteria.

Histopathology Features	Score		
	1	2	3
Alveolar Membrane	The alveolar membrane is intact, nucleated, and complete with >75% endothelium cells	alveolar membrane intact, nucleated, and complete with 25-75% endothelium cells	alveolar membrane intact, nucleated, and complete with <25% endothelium cells
Alveolar Lumen	Rounded proportional size >75%	Rounded proportional size 25-75%	Rounded proportional size <25%
Connections Between Alveolar	Tight >75%	Tight 25-75%	Tight <25%

Source: Kartika Murti et al, 2016.[7]

Table 2. Normality test results using Shapiro-Wilk.

	Group	Significance
Grade of Lung Tissue Damage	Treatment Group II	0.008

Table 3. Results of the Kruskal-Wallis non-parametric statistical test.

	Significance
Grade of Lung Tissue Damage	0.0006

make preparations using the paraffin method. In addition, rat lungs were processed using the paraffin method to prepare preparations, while hematoxylin-eosin staining was used in the staining procedure. Histopathological images were viewed at 100x magnification followed by 400x magnification. The scoring criteria used to evaluate lung histopathological features are the histopathological scoring system presented below.

Then the data that has been obtained will enter the analysis stage. Then a normality test will be carried out using Shapiro-Wilk to assess the distribution of the data. The data in the table below shows the results of the normality test using Shapiro-Wilk. Then a non-parametric test will be carried out, namely using the Kruskall-Wallis test to see whether there are differences between the three experimental groups.

RESULTS

After the process of making lung histology preparations using the paraffin method, observations will be carried out using 400x magnification.

Based on the research that has been carried out, the results show that administration of HBOT affects the histopathological picture of the lungs of rats. The histopathological changes in Figure 1 that can be seen from microscopic observation are the alveolar membrane, alveolar lumen, and connections between alveolar. In the control group, there were: The alveolar membrane is intact, nucleated, and complete with >75% endothelium cells; the alveolar lumen rounded proportional size >75%; and the connection between alveolar is tight >75%. In treatment group I, there were: The alveolar membrane is intact, nucleated, and complete with <25% endothelium cells; the alveolar lumen rounded proportional size <25%; and the connection between alveolar is tight <25%. The last is treatment group II was found: The alveolar membrane is intact, nucleated, and complete with >75% endothelium cells; the alveolar lumen rounded proportional size >75%; and the connection between alveolar is tight >75%.

The data in the graph below shows the degree of damage to lung tissue. With data from the control group, 8 samples of rats showed grade 1, with 2 samples of rats that died before treatment was carried out due to room temperature adjustments. Then in treatment group I, it showed that 9 samples of rats showed grade 3, with 1 sample of rats that died before treatment was carried out due to room temperature adjustments, the same as 2 samples of rats in the control group. Finally, treatment group II, namely 2 rats samples showing grade 3, 3 rats samples showing grade 2, and 5 rats samples showing grade 1.

Then a normality test will be carried out using Shapiro-Wilk to assess the distribution of the data. The data in the table below shows the results of the normality test using Shapiro-Wilk.

From the data above, the results show $p<0.05$, which means the data is not normally distributed, therefore a non-parametric test will be carried out, namely using the Kruskall-Wallis test to see whether there are differences between the three experimental groups.

From the data above, the results show $p<0.0006$, which means the data is significant and there are differences between groups.

DISCUSSION

In the control group, according to what is shown in Figure 1, it shows that all of them are included in grade 1. Then in treatment group I all samples show results of grade 3. Finally, in treatment group II the results show that 5 samples are classified as grade 1, 3 samples are classified as grade 1. into grade 2, and 2 samples are classified into grade 3. This is in accordance with the results of statistical analysis of lung tissue damage data. For a score of 1, the alveolus is composed of complete and nucleated endothelial cells, the shape of the intact alveolus is rounded, and the structure of the alveolus is tight. At a score of 2, the alveolar membrane is still relatively intact with the endothelium around it, the shape of the alveolus is still relatively intact, and rounded, and the alveolus is relatively tight. Meanwhile, with a score of 3, the alveolar membrane cells do not have nuclei and the surrounding endothelium cells are not visible, the alveoli are widened, and the connections between the alveoli are stretched.

This shows that the administration of hyperbaric oxygen therapy showed good results, as seen in the difference between treatment group I which was intoxicated with methanol without being given HBOT, and treatment group II which was intoxicated with methanol and given HBOT showed improvement. However, there were 2 samples that remained at grade 3 and 3 samples that experienced a slight improvement to grade 2, this was possibly caused by giving therapy that was not given for a long time.

Formic acid produced by methanol metabolites can irritate mucosal membranes, which can reduce normal airway defenses. Changes in the structure of lung tissue due to exposure to formic acid are a clinical manifestation of acute respiratory distress syndrome (ARDS) which is characterized by inflammatory cell infiltration, pulmonary edema, and injury to the lung and alveolar walls. Accumulation of formic acid also causes metabolic acidosis which is characterized by a decrease in pH

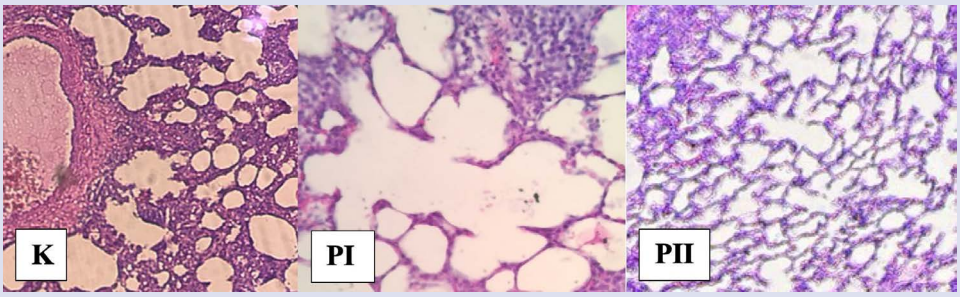

Figure 1. Lung histopathological images obtained using paraffin method and using 400x magnification: K as control (not given methanol treatment and HBOT), PI as treatment group I (was given methanol for 14 days without HBOT), PII as treatment group II (was given methanol for 14 days and then given HBOT for 10 days).

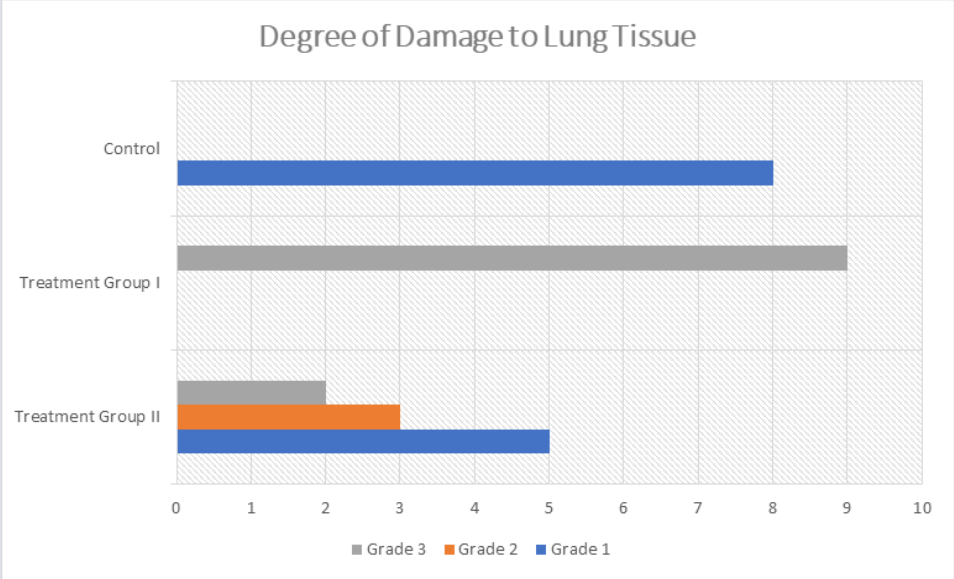

Figure 2. Graph Degree of Damage to Lung Tissue.

and an increase in CO2 in the blood, causing rapid and deep breathing. Respiratory failure can also occur due to exposure to formic acid.[8]

HBOT improves wound healing by increasing the oxygen gradient along the edges of ischemic wounds and facilitating collagen matrix formation for angiogenesis. This also increases the solubility of oxygen so that it can reach the cells. When increased to 3 ATA, the oxygen diffusion capacity increases 4 times compared to the pressure of 1 ATA[9,10] This can save cells or tissue from hypoxia so that they can survive. When breathing normal air, the arterial oxygen pressure is approximately 100 mmHg, and the tissue oxygen pressure is approximately 55 mmHg. However, 100% oxygen at 3 ATA can increase arterial oxygen pressure up to 2,000 mmHg and tissue oxygen pressure up to 500 mmHg, allowing an oxygen intake of 60 mL/L of blood (compared to 3 ml/L at atmospheric pressure), sufficient to meet oxygen requirements. tissue without hemoglobin contribution. Thanks to dissolved oxygen, it can reach dense areas unreachable by red blood cells allowing for tissue oxygenation.[11]

By increasing the tissue oxygen diffusion gradient, the respiratory acidosis caused by formic acid can improve, in addition to being caused by the formation of new blood vessel tissue or neovascularization. When lung tissue perfusion improves, existing damage will begin to be repaired.[12] Apart from neovascularization, HBOT will also reduce tissue edema.[13,14] Just like research conducted by Mortensen (2008), the same beneficial effects of hyperbaric oxygenation that have been observed in the myocardium are also seen in lung tissue. Bone tissue has previously demonstrated the effectiveness of hyperbaric oxygen in bone infections and as an adjunct in cardiothoracic surgery with sternotomy, as well as in osteonecrosis secondary to trauma due to surgery or ingestion of bisphosphonates.[15,16]

CONCLUSION

The results of this study can be concluded that administering hyperbaric oxygen therapy to rats intoxicated with methanol had a statistically significant improvement effect on the alveolar membrane, alveolar lumen, and connections between the alveoli. It is possible that providing longer therapy can improve the condition of damaged tissue for better results. Suggestions for further research are to increase the duration of HBOT administration because in this study it was only 10 days.

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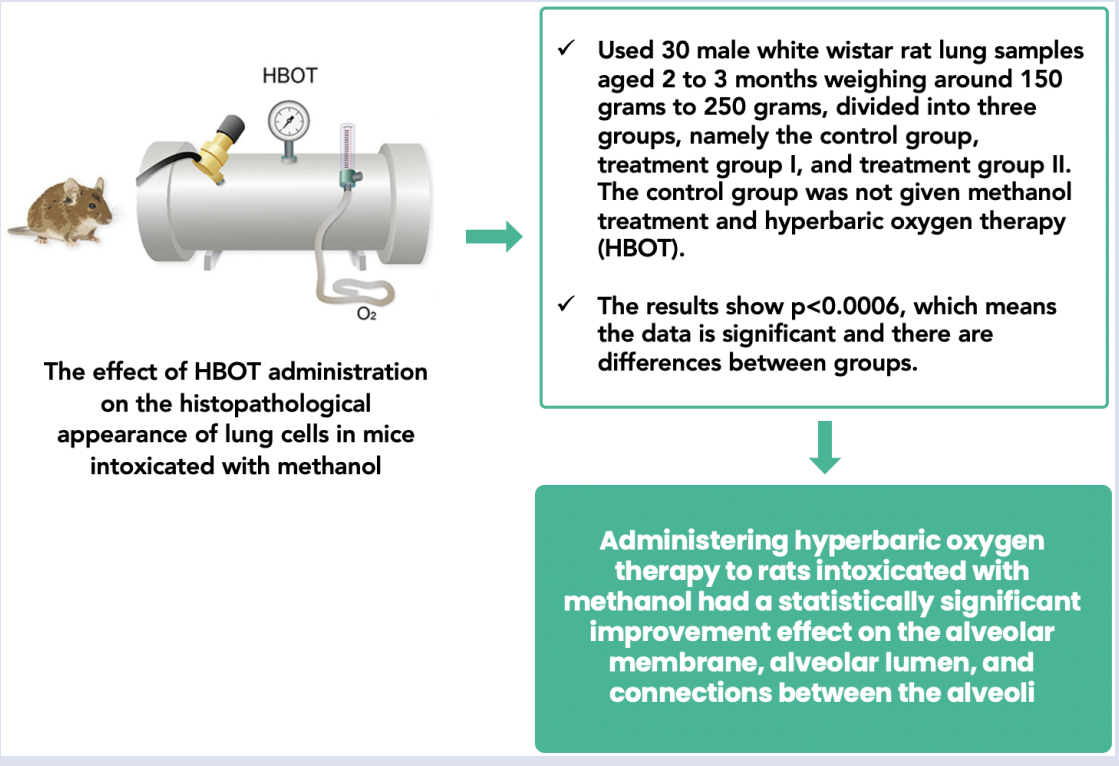
GRAPHICAL ABSTRACT



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