RESEARCH
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HYPERBARIC OXYGEN TREATMENT REDUCES STRESS OXIDATIVE AND INFLAMMATION THROUGH SUPEROXIDE DISMUTASE AND INTERLEUKIN 1 B IN ANIMAL MODELS EXPOSED TO MOTOR VEHICLE SMOKE

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Abstract

The development of human civilization and the increasing variety and quantity of vehicles cause air pollution which is one of the biggest environmental risks for health. Exposure to air pollution is estimated to cause 7 million premature deaths each year. This study examined hyperbaric oxygen therapy (HBOT) strategies to reduce oxidative stress in experimental animals exposed to motor vehicle smoke through analysis of superoxide dismutase (SOD), interleukine 1b (IL-1b) and hemoglobin (Hb). Material and Methods: The research design used is true experimental research. The research design was a post-test only randomized control group design. 21 female rats (Rattus Norvegicus) aged 6-8 weeks weighing 180-200 grams were divided into 3 groups, the normal group (G0), the group exposed to vehicle smoke which were not given Hyperbaric oxygen therapy (HBOT) (G1) and the group exposed to vehicle smoke which were given HBOT (G2). HBOT is inhaling 100% O2 with a pressure of 1.7 ATA for 3x30 minutes with 5-minute intervals for 10 consecutive days. Oxidative stress was assessed based on the activity levels of the enzymes SOD, IL-1β and hemoglobin (Hb)as measured by the enzyme-linked sorbent assay (ELISA).There was a significant increase ($p < 0.05$) in SOD and Hb levels, there was no significant decrease ($p > 0.05$) in IL-1β levels in the animal model group exposed to vehicle smoke that were given HBOT (G2) compared to with experimental animal models exposed to vehicle smoke that were not given HBOT (G1). Results: There was a significant increase ($p < 0.05$) in SOD and Hb levels, there was no significant decrease ($p > 0.05$) in IL-1β levels in the animal model group exposed to vehicle smoke that were given HBOT (K2) compared to with experimental animal models exposed to vehicle smoke that were not given HBOT (K1). Conclusion: HBOT has an effect on reducing oxidative stress through increasing SOD enzyme activity and Hb levels and decreasing levels of the pro-inflammatory cytokine IL-1β in animal models exposed to vehicle smoke.
Keywords: HBOT; exposure to vehicle smoke; SOD; IL-1β; Hb

INTRODUCTION

Vehicle fumes cause impaired lung function resulting in premature death due to oxidative stress [1-3]. Oxidative stress is an imbalance between antioxidants and oxidants which causes disruption of signaling, redox control and molecular damage [4]. Oxidative stress is associated with inflammation and hypoxia which can be caused by two pathological processes, namely narrowing of the small airways and destruction of the lung parenchyma [5]. Superoxide dismutases (SODs) a very important antioxidant defense against oxidative stress in the body. This enzim acts as a good therapeutic agent against diseases mediated by oxidants or reactive [6]. Interleukine (IL)-1 is a pro-inflammatory cytokine that is mainly produced by macrophage and respiratory epithelium, and its release, accompanied by IL-6, IL-8 and TNF-α, can cause neutrophilia, macrophage activation and also a response from T cells [7]. Hypoxia in chronic lung disease often includes anemia or low hemoglobin (Hb) levels as a cause of comorbidity [8].

Hyperbaric oxygen therapy (HBOT) is a therapy that provides '100%' oxygen (O2) or a higher level than normal air in a high-pressure air chamber of more than 1 atmosphere absolute (ATA). The effect of HBOT on lung function has been investigated. HBOT increases pulse oxygen saturation (SpO2), overcomes hypoxia and reduces inflammation so it is hoped that it can be a new breakthrough in preventing damage and impaired lung function [9]. HBOT increases maximum respiratory flow or peak expiratory flow (PEF) and forced vital capacity (FVC) [10].

The research I conducted further examined therapeutic strategies in reducing oxidative stress in experimental animals exposed to motor vehicle fumes using animal models [11]. HBOT dosage includes oxygen levels, pressure, duration, interval and number of sessions. In this

study, experimental animals were given HBOT inhaling '100%' O2 3x30 minutes alternated with 2x5 minutes of breathing normal air at a pressure of 1.7 ATA for 10 consecutive days after being exposed to motor vehicle gas emissions and then blood and lung tissue analysis was carried out.

MATERIALS AND METHODS:

Ethical implications for Wistar rats as experimental animals following animal ethics. The research was carried out after obtaining ethical suitability certificate No. 06/EC/LKS/VII/2022 from the Naval Health Institute Research Ethics Commission. The location and time of the research was carried out at the Lakesla Hyperbaric Animal Research Laboratory, Drs. Med. R. Rijadi S., Phys Surabaya in January – September 2023.

This research design was true experimental with a post test only randomized control group design. The sampling technique was simple random sampling, namely a simple random sampling technique from the population so that each member of the population has the same opportunity to be selected as a research sample. The sample size (n = 7) in the study was obtained based on the results of analysis from preliminary research. There were 3 groups in this study, namely the negative control group (G0), positive control (G1), and treatment group (G2). The G0 group was not exposed to motor vehicle fumes and was not given HBOT. Group G1 was exposed to motor vehicle fumes, not given HBOT. Group G2 was exposed to motor vehicle fumes and given HBOT. In these three groups, blood serum samples were taken to analyze the levels of SOD enzyme activity, IL-1β and Hb levels.

The samples used were Wistar rats, female, 6-8 weeks old with a body weight (BW) of 200-300 grams which met the inclusion and exclusion criteria. Inclusion criteria included healthy mice (normal anatomical structure, clear eyes, thick fur), active (lively movements), after being exposed to vehicle fumes, mice coughing experienced in groups G1 and G2. Exclusion criteria include experimental animals with anatomical defects (broken legs, missing ears, etc.), experiencing illness during adaptation (excessive hair loss, loose stools, etc.), suffering from other diseases not caused by treatment. Drop out criteria include experimental animals that died during treatment.

Twenty-one Wistar rats (Rattus Norvegicus) were adapted for 7 days. The smoke exposure technique using a Honda PM 2.5 motor vehicle was 5 days a week, 2 days of rest, then 5 days a week with an interval of 1 hour of exposure, followed by 30 minutes of rest, 4 times every hour 08.00-09.00 WIB, 10.00-11.00 WIB, 14.00- 15.00 WIB, 16.00-17.00 WIB using a Honda 2.5 PM motor vehicle for 2x5 days (5 consecutive days interspersed with 2 days of rest) for 30 days [11].

The treatment was carried out by taking experimental animals with models of chronic obstructive pulmonary disease (COPD) from the treatment group (G3) and placing them in a special animal chamber for experimental animals. Providing oxygen at a higher level than ordinary air in a high-pressure chamber made of steel (hyperbaric chamber) at a room temperature of 28oC and air humidity of 50%. In this case, it was sucking 100% O2 1.7 ATA for 3 x 30 minutes, 2 x 5-minute intervals, breathing normal air 10 times, namely for 10 consecutive days in a research animal chamber specifically for experimental animals. After each HBOT exposure, the mice were returned to their original cages.

On days 1, 8, 39, 49, an external physical examination of the mice was carried out, namely body weight was measured using scales and an internal examination by observing blood.

On the 49th day, anesthesia was performed using Ketamine 10% intraperitoneally, tracheotomy, and intubation and placed supine [11].

On the 49th day, mice blood samples were taken after anesthesia. Blood from mice was taken directly from the heart in the ventricles slowly to prevent collapse of the heart using the cardiac puncture technique with a 3-cc syringe and using a 23 G needle [12].

RESULT

All data was analyzed statistically using SPSS. The significance level for analyzing statistics ($\alpha = 0.05$), namely a p value < 0.05, was considered significant. Before carrying out a difference test, a test was carried out on the normality and homogeneity of the data to be studied.

The mean and standard deviation of SOD levels originating from blood serum of experimental animals in groups G0, G1, and G2 can be seen in table 1.

Table 1: Mean values and standard deviation of SOD levels in groups G0, G1 and G2

Group	Mean ± SD	Min.	Max.	p-value*
G0	0.01486 ± 0.001676	0.012	0.017	0.007
G1	0.01229 ± 0.001496	0.010	0.014	
G2	0.01529 ± 0.000756	0.014	0.016	0.002

* Mean difference was compared to group 1 (G1), significant if p<0.05.

The Shapiro Wilk normality test had a significance of

p > 0.05 in each group, so the results of the normality test showed that the three groups of data were normally distributed. The results of Levene's homogeneity test had a significance of p = 0.186 (p > 0.05) so that no variants or homogeneous data were found in the data. The One-Way Anova test used 24 samples divided into 3 groups with 1 measurement at the end of the study (post test only) had significance between groups showing a figure of p = 0.001 (p <0.05) so there was a significant difference in the mean SOD levels between the negative control group (G0), positive control group (G1) and treatment group (G2) were significant. Tukey type post hoc test (Honestly Significant Difference) to determine significant differences between each group. Comparison of the negative control group (G0) with the control group (G1) showed a significance of p = 0.007 (p < 0.05), so there was difference in mean SOD levels in the negative control group (G1) and the normal group (G0).

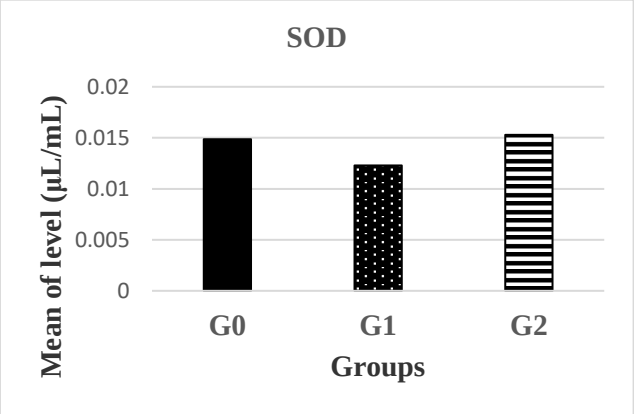

Figure 1: Diagram of average SOD levels in groups G0, G1 and G2. (Note: Each bar showed the mean ± SD of SOD level)

Comparison of the negative control group (G0) with the treatment group (G2) showed a significance of p = 0.829 (p > 0.05), so there was no difference in mean SOD levels in the negative control group (G0) and the treatment group (G2). A comparison between the positive control group (G1) and the treatment group (G2) showed a significance of p = 0.002 (p < 0.05), so there was a difference in the mean SOD levels in the positive control group (G1) and the treatment group (G2). In the G1 group, the mean SOD value was 0.01229, which was lower than in the G2 group which had a mean of 0.01529, so there was a significant increase in SOD levels in the group of experimental animal models exposed to motor vehicle fumes given HBOT compared to the experimental animals models exposed to motor vehicle fumes that were not given HBOT. The distribution of SOD level measurement results in groups G0, G1 and G2 can be seen in table 1 and figure 1.

The mean and standard deviation of IL-1β levels originating from the blood serum of experimental animals in groups G0, G1, and K2 can be seen in table 2.

Table 2: Mean Value and Standard Deviation of IL-1β levels in blood serum of experimental animals in groups G0, G1, and G2

Group	Mean ± SD	Min.	Max.	*p*-value*
G0	0.15386 ± 0.029002	0.125	0.198	0.002
G1	10.20586 ± 7.451402	2.921	21.689	
G2	6.58043 ± 3.715129	2.213	11.774	0.338

*Mean difference was compared to group 1 (G1), significant if p<0.05.

The normality test using the Shapiro-Wilk test has a significance of p > 0.05 in each group, so the results of the normality test showed that the three groups of data were normally distributed. Levene's Test of homogeneity showed a figure of p = 0.000 (p < 0.05), so the data was found to be variant or the data was not homogeneous. The non-parametric test, namely the Kruskal Wallis test, showed that there was a statistically significant difference of p = 0.001 (p < 0.05) in IL-1β levels from the three groups, both G0, G1, and G2.

The Mann-Whitney U test showed that there was a statistically significant difference p = 0.002 (p < 0.05) in IL-1β levels between G0 and G1, and there was no significant difference p = 0.338 (p > 0.05) in levels IL-1β was statistically between G1 and G2.

In the G1 group, the IL-1β mean value was 10.20586, which was higher than in the G2 group which had a mean of 6.58043, so there was no significant decrease in IL-1β levels in the experimental animal model group exposed to motor vehicle fumes given HBOT was compared with experimental animal models exposed to motor vehicle exhaust that were not given HBOT. The distribution of IL-1β level measurement results in groups G0, G1, and G2 can be seen in table 2 and figure 2.

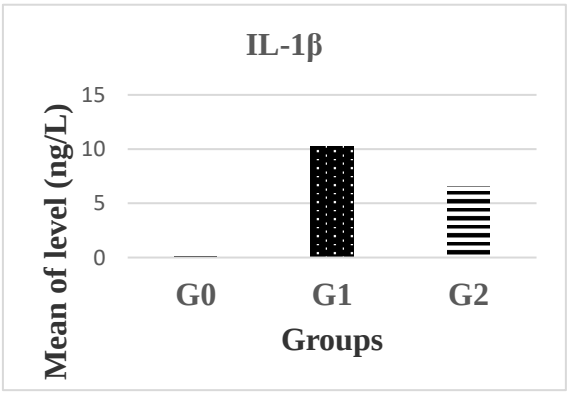

Figure 2: Diagram of mean IL-1β levels in groups G0, G1 and G2. (Note: Each bar showed the mean ± SD of IL-1β level)

The mean and standard deviation of Hb levels

originating from blood serum of experimental animals in groups G0, G1 and G2 can be seen in table 3.

Table 3: Mean values and standard deviation of blood serum Hb levels of experimental animals in groups G0, G1, and G2

Group	Mean ± SD	Min.	Max.	p-value*
G0	13.743 ± 1.4593	11.7	16.3	0.004
G1	10.629 ± 1.1686	9.1	12.8	
G2	11.657 ± 0.8696	11.0	13.4	0.040

*Mean difference was compared to group 1 (G1), significant if p<0.05.

The normality test using the Shapiro-Wilk test has a significance of p < 0.28 in each group, so the results of the normality test show that the three groups of data are not normally distributed. The Kruskal Wallis non-parametric test showed that there was a statistically significant difference of p = 0.002 (p < 0.05) in the Hb levels of the three groups, both G0, G1 and G2. Non-parametric tests using the Mann-Whitney U test showed that there was a statistically significant difference of p = 0.004 (p < 0.05) in Hb levels between G0 and G1, there was a significant difference of p = 0.006 (p < 0.05) in levels Hb is statistically between G0 and G2 and there is a statistically significant difference p = 0.040 (p < 0.05) of Hb levels between G1 and G2. In the G1 group, the mean Hb value was 10.629, which was lower than in the G2 group which had a mean of 11.657, so there was a significant increase in Hb levels in the group of animal models exposed to motor vehicle fumes that were given HBOT compared to the animal models exposed to vehicle fumes. motorbikes that are not given HBOT. The distribution of Hb level measurement results in groups G0, G1, and G2 can be seen in table 3 and figure 3.

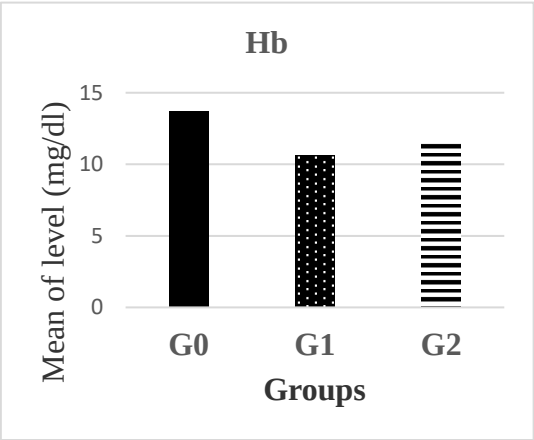

Figure 3: Diagram of average Hb levels in groups G0, G1, and G2. (Note: Each bar showed the mean ± SD of Hb level)

The concept of HBOT dosage comes from the definition of HBOT as a drug. HBOT dosage includes O2 levels, pressure depth, duration, interval, and frequency. This study used 100% oxygen at a pressure of 1.7 ATA with 3x30 minute intervals, and 2x5 minute breaks by breathing normal air in a research animal room specifically for experimental animals every day for 10 consecutive days

This pressure dose of 1.7 ATA is a lower dose than the standard dose, namely the 2.4 ATA dose which is often used by the Naval Health Institute Surabaya Indonesia to treat several clinical diseases. Lung damage due to exposure to vehicle fumes will cause inflammation so the elasticity of lung tissue will decrease [13]. If high pressure is applied, it can cause a risk of side effects of HBOT called pulmonary barotrauma [14].

Barotrauma is physical damage to body tissue caused by pressure differences between gas spaces within, or in contact with the body, and the surrounding fluid. This situation usually occurs when the organism is exposed to significant changes in ambient pressure, such as when a scuba diver, freediver, or airplane passenger ascends or descends, or during uncontrolled decompression of a pressure vessel, but it can also occur due to shock waves. Barotrauma should be considered a complication of the use of positive pressure in tissues, where normal air movement is largely passive. It is defined as the presence of extra alveolar air in a location not normally found in patients receiving mechanical ventilation. The application of positive pressure ventilation has an impact on inflamed lungs, risking pulmonary barotrauma [15,16]. Therefore, in this study, a lower pressure dose of HBOT was used compared to doses for other clinical diseases.

The O2 levels used are higher than ordinary air. It is hoped that with higher pO2 pressure, the HIF-1α transcription factor will decrease even though there is a risk of higher reactive oxygen species (ROS) formation as well, as stated by Thom SR who stated that high pO2 gives rise to ROS [17]. Even high ROS can stimulate oxidative stress which induces inflammation. These two seemingly contradictory things are one of the things that make researchers interested in research. The higher pressure used in this study, as stated by Eggleton et al, namely not exceeding 3 ATA (equivalent to a depth of 20 meters in water-seawater) and the duration of treatment for elective therapy generally does not exceed 2 hours in one session is still considered safe [18].

DISCUSSION:

Effect of HBOT on SOD levels

In this study, a significant increase in SOD levels was also

found in the experimental animal treatment group exposed to vehicle fumes treated with HBOT (G2) compared to the positive control group without HBOT therapy (G1). This is because exposure to HBOT causes significant oxidative stress at the start of therapy, but with continued exposure to the induced oxidative stress, adaptive processes and signaling from antioxidants such as SOD will occur [19].

Superoxide dismutase (SOD) is a very important antioxidant defense against oxidative stress in the body. SOD is formed from the transcriptional regulation of genes controlled in part through Antioxidant Response Elements (AREs) transcription factors Nuclear Factor NF-E2-related Factor 2 (Nrf2) plays an important role in the ARE-mediated basal and inducible expression of more than 200 genes that can be grouped into several categories including antioxidant genes and phase II detoxification enzymes [20]. SOD enzyme acts as a good therapeutic agent against ROS-mediated diseases. SOD has several therapeutic effects such as on physiological and pathological conditions such as cancer, inflammatory diseases, cystic fibrosis, and ischemia. ROS including O2- and its reaction product peroxynitrite have an important role in endothelial and tissue injury associated with ischemia and reperfusion. Overexpression of SOD reduces ischemic damage due to ischemia or reperfusion. Removal of O2- and peroxynitrite by SOD mimetics helps in the prevention of cellular energetic failure and tissue damage associated with ischemia and perfusion and has a beneficial effect in these situations [6].

This study, to repair lung tissue damage in experimental models of animals exposed to vehicle fumes caused by ROS. HBOT therapy was carried out to increase SOD levels. Previous studies conducted on transgenic mice overexpressing extracellular SOD and SOD mimetics have shown that O2- inhibition can prevent neutrophil infiltration at the site of damage. Neutrophil apoptosis may also be an important step in the resolution of inflammation. SOD can function as an inhibitory agent of neutrophil-mediated inflammation and may stand for a new therapeutic approach for ROS-dependent tissue damage caused by neutrophils through several mechanisms [6,19].

This shows that administering HBOT 1.7 ATA therapy with a duration of 3 x 30 minutes, with 5-minute intervals of breathing normal air for 10 consecutive days has been proven to have a positive effect on inflammation and symptoms in female Wistar mice model exposed to vehicle fumes through decreasing ROS with an increase in antioxidants, namely SOD with a significance of $p = 0.000$ ($p < 0.05$) which can reduce tissue damage due to ROS in the blood serum of Wistar rats with a COPD model. Hence, it can be concluded that the Wistar strain model of mice exposed to vehicle fumes experienced an increase in SOD and improved inflammatory symptoms after undergoing HBOT 1.7 ATA therapy with a duration of 3x30 minutes, with 5-minute intervals breathing normal air for 10 consecutive days. Improvements in terms of inflammation can improve clinical conditions and help reduce the symptoms felt.

Effect of HBOT on IL-1β levels

In this study, there was also a significant increase in levels of the pro-inflammatory cytokine IL-1β in the treatment group of COPD animal models that were not treated with HBOT (G1) with a significance of 0.001 ($p < 0.05$) compared to the control group without treatment or intervention. (G0), where this increased pro-inflammatory cytokine occurs due to increased transcription of TNF-α and interleukin which is regulated by NF-κB during inflammation, where the main cytokine that plays a role in COPD is IL-1β [5,11].

IL-1β is a pro-inflammatory cytokine originating from the IL-1 family which is the main regulator of inflammation by controlling variations in the innate immune system. IL-1 has a wide range of biological functions, including leukocytic pyrogen, a mediator of fever, and an endogenous leukocytic mediator, as well as inducing various components of the acute phase response and lymphocyte-activating factor (LAF). There is an apoptosis-associated speck-like protein containing a CARD (ASC) adapter protein which is an important component of the NRLP3 inflammasome, which recruits pro-caspase-1 to the protein complex and is increased in the lungs of COPD patients. ASC accumulation is associated with the formation of extracellular specks, which continually form IL-1β outside the cell [5,21].

In this study, to reduce the inflammatory reaction that occurred in animal models of COPD, HBOT therapy was carried out. HBOT therapy had several effects, the decreased CRP levels caused a decrease in the infiltration of inflammatory cells such as monocytes on HIF-1α signaling. OHB therapy is also involved in reducing the release of inflammatory cytokines from monocyte cells which is mediated by heat shock proteins 70 (HSP 70) and heme oxygenase-1 (HO-1). OHB therapy can increase oxygen and tissue saturation which can then increase the release of ROS and reactive nitrogen species (RNS). During hypoxia with inflammation, it will be followed by an increase in ROS and RNS which can induce HIF-1α

(Hypoxia-Inducible factor - 1α). Activated HIF-1α signaling will lead to higher expression of Catalase (CAT) and Glutathione peroxidase 1 (GPx1) mRNA thereby reducing oxidative stress. HIF-1α reduces oxidative tissue injury by reducing adhesion of neutrophils, monocytes, and lymphocytes as well as infiltration into inflamed tissue [22].

This shows that administering HBOT 1.3 ATA therapy with a duration of 3 x 30 minutes, with 5-minute intervals of breathing normal air for 10 consecutive days has been proven to have a positive effect on inflammation and symptoms in Wistar strain mice model of COPD through reducing ROS which can inhibit the regulation of cytokines in the blood serum of Wistar strain mice with a model of COPD, and also increase levels of anti-inflammatory cytokines such as IL-10. So, it can be concluded that Wistar mice in the COPD model experienced a decrease in ROS and improvement in inflammatory symptoms after undergoing HBOT 1.3 ATA therapy with a duration of 3 x 30 minutes, with 5-minute intervals breathing normal air for 10 consecutive days. Improvements in terms of inflammation can improve the patient's clinical condition and help reduce the symptoms felt by the patient.

Effect of HBOT on Hb levels

There was a significant difference in the increase in Hb levels in the treatment group (G2) of model animals exposed to vehicle fumes that were given HBOT compared to the positive control group of model animals exposed to vehicle fumes that were not given HBOT (G1). The condition of anemia resulting from low Hb levels is common in patients with severe COPD. Showing that a decrease in Hb levels can affect the rate of oxygen uptake across the alveolocapillary bed and reduce the diffusion capacity of the lung [23]. The high activity of lipid peroxidase (LPO) due to increased ROS can reduce erythrocyte antioxidants which causes damage to the erythrocyte membrane, erythrocytes become easily lysed, and the number of erythrocytes decreases, the amount of Hb decreases [24]. Increased ROS can also cause damage to the spinal cord so that erythrocyte and Hb production is reduced [25,26].

The basis of HBOT therapy is three main factors: (1) By breathing 100% O2, positive gradient, thus favoring diffusion of hyperoxygenated lung to hypoxic tissue; (2) due to high pressure, the concentration of O2 in the blood arises according to Henry's Law (the amount of gas dissolved in a liquid is directly proportional to the partial pressure), and (3) reduces the size of gas bubbles in the blood by Boyle-Mariotte's Law and Henry's Law. In other words, the creation of a hyperbaric environment with pure oxygen allows a significant increase in the oxygen supply to the blood (hypoxemia) and to the tissues (hyperoxia) resulting in overcoming tissue hypoxia and causing ROS levels to decrease [26].

When hemoglobin drops to critical levels and reduces oxygen delivery, HBOT can be used as a therapy to supply oxygen on an emergency basis. Oxygen administered hyperbarically allows dissolved oxygen in increased concentrations in low red blood cell plasma or crystalloid/colloid diluted intravascular fluid in resuscitated patients. In addition, in subacute and chronic anemia patients, pulsed, normobaric, or hyperbaric oxygen administered intermittently induces an increase in red blood cells/hemoglobin mass [27]. So, in this study, a significant increase in hemoglobin was found in the treatment group of model animals exposed to vehicle fumes that were treated with HBOT (G2) compared to the positive control group of model animals exposed to vehicle fumes that were not treated with HBOT (G1).

CONCLUSION

HBOT has an effect on reducing oxidative stress through increasing SOD enzyme activity and Hb levels and decreasing levels of the pro-inflammatory cytokine IL-1β in animal models exposed to vehicle smoke. HBOT can be an adjuvant therapy for chronic obstructive pulmonary disease due to exposure to vehicle smoke.

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Conflict of Interest

Authors declare that no conflict of interest.

Authorship Contributions

Idea/Concept: Dony Irawan Suwardhono; **Design:** Dony Irawan Suwardhono, Arifa Mustika; **Control/Supervision:** Arifa Mustika , Titut Harnanik; **Data Collection and/or**

Processing: Dony Irawan Suwardhono; **Analysis and/or Interpretation:** Dony Irawan Suwardhono, Arifa Mustika, Titut Harnanik; **Literature Review:** Dony Irawan Suwardhono; **Writing the Article:** Dony Irawan Suwardhono; **Critical Review:** Arifa Mustika , Titut Harnanik; **References and Fundings:** Dony Irawan Suwardhono, Arifa Mustika, Titut Harnanik; **Materials:** Dony Irawan Suwardhono.

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