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ARTIKEL PENELITIAN

NEUROPROTECTIVE EFFECTS OF INTERMITTENT HYPOBARIC HYPOXIA IN RATS: CASPASE-3, NT-3, SOD, AND CARBONYL PROFILES

EFEK NEUROPROTEKTIF HIPOKSIA HIPOBARIK INTERMITEN PADA TIKUS: PROFIL CASPASE-3, NT-3, SOD, DAN KARBONIL

Defli Yuandika Ruso[1], **Ninik Mudjihartini**[2,*], **Wawan Mulyawan**[3], **Atikah Chalida Barasila**[4,5], **Yulhasri**[2]

[1] Master's Program in Biomedical Sciences, Faculty of Medicine, University of Indonesia, Jl. Salemba Raya no. 6, Jakarta, Indonesia
[2] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Indonesia, Jl. Salemba Raya No. 6, Jakarta, Indonesia
[3] Department of Aerophysiology, Lakespra dr. Saryanto, Indonesian National Armed Forces Air Force, Jl. MT Haryono No. 41, Jakarta, Indonesia
[4] Department of Histology, Faculty of Medicine, University of Indonesia, Jl. Salemba Raya No. 6, Jakarta, Indonesia
[5] Medical Acupuncture Study Program, Faculty of Medicine, University of Indonesia, Jl. Salemba Raya No. 6, Jakarta, Indonesia
*** Correspondence:** ninik.mujihartini@ui.ac.id

ABSTRAK
Pendahuluan*: Otak adalah organ yang mengonsumsi energi terbesar dalam tubuh dan sangat rentan terhadap kerusakan dalam kondisi hipoksia. Namun, periode hipoksia tertentu dapat ditoleransi dan memberikan efek perlindungan. Penelitian ini bertujuan untuk menganalisis efek perlindungan dari hipoksia hipobarik intermiten (IHH) terhadap ekspresi caspase-3, neurotropin-3, karbonil, dan superoksida dismutase (SOD) pada jaringan otak tikus.*
Metode*: Sebanyak 25 ekor tikus Wistar jantan dibagi menjadi lima kelompok: satu kelompok kontrol (kondisi normoksia) dan empat kelompok perlakuan yang dipaparkan dengan kondisi hipoksia di dalam ruangan simulasi ketinggian 25.000 kaki selama 5 menit dengan interval 7 hari untuk setiap paparan. Kelompok 1 terpapar satu kali kondisi hipoksia (IHH 1), kelompok 2 terpapar dua kali (IHH 2), kelompok 3 terpapar tiga kali (IHH 3), dan kelompok 4 terpapar empat kali (IHH 4).*
Hasil*: Kadar caspase-3 menurun secara signifikan pada kelompok perlakuan dibandingkan dengan kelompok kontrol (p<0,05), menunjukkan respons positif terhadap IHH. Di sisi lain, kadar neurotropin-3 dan karbonil tidak menunjukkan perubahan signifikan antar kelompok. Selain itu, peningkatan signifikan kadar SOD terjadi antara kelompok 1 dan kelompok 3 (p<0,05), menunjukkan peningkatan pertahanan antioksidan dengan paparan berulang terhadap IHH.*
Kesimpulan*: Hipoksia hipobarik intermiten dapat memberikan efek perlindungan terhadap kerusakan akibat hipoksia dengan menekan ekspresi caspase-3. Paparan IHH berulang meningkatkan pertahanan antioksidan dengan meningkatkan kadar SOD. Sebaliknya, kadar neurotropin-3 dan karbonil tetap tidak berubah, menunjukkan bahwa penanda ini tidak responsif terhadap IHH jangka pendek.*
Kata Kunci: *caspase-3, hipoksia hipobarik intermitten, karbonil, NT-3, SOD*

ABSTRACT
Introduction: The brain is the largest energy-consuming organ in the body and is highly vulnerable to damage under hypoxic conditions. However, certain periods of hypoxia can be tolerated and provide protective effects. This study aimed to investigate the protective effects of intermittent hypobaric hypoxia (IHH) on the expression of caspase-3, neurotrophin-3, carbonyl, and superoxide dismutase (SOD) levels in rat brain tissues.
Methods: A total of 25 male Wistar rats were divided into five groups: a control group (normoxic conditions) and four treatment groups exposed to hypobaric hypoxia in a chamber simulating 25,000 feet altitude for 5 minutes at intervals of 7 days for each exposure. Group 1 was exposed to a one-time hypoxia condition (IHH 1), group 2 was exposed two times (IHH 2), group 3 was exposed three times (IHH 3), and group four were exposed four times (IHH 4).

Results: Caspase-3 levels significantly decreased in the treatment groups compared to the control group ($p<0.05$), indicating a positive response to IHH. In contrast, neurotrophin-3 and carbonyl levels showed no significant changes across the groups, maintaining a stable trend. Additionally, a significant increase in SOD levels was observed between group 1 and group 3 ($p<0.05$), suggesting enhanced antioxidant defense with repeated IHH exposure.
Conclusion: Intermittent hypobaric hypoxia can suggest a protective effect against hypoxic damage by suppressing caspase-3 expression. Repeated IHH exposure enhances antioxidant defense by elevating SOD levels. In contrast, neurotrophin-3 and carbonyl levels remain unchanged, suggesting these markers are unresponsive to short-term IHH.
Key Words: carbonyl, caspase-3, intermittent hypobaric hypoxia, NT-3, SOD

INTRODUCTION

Human adult nerve cells have limited proliferative capacity, making damage to nerve cells in the brain more critical compared to other organs, like the skin or liver, which exhibit higher regenerative potential.[1,2] Although the brain has a high metabolic demand, it remains particularly vulnerable to conditions that reduce oxygen supply, such as hypoxia and ischemia. Reduced oxygen availability can induce oxidative stress, leading to brain cell injury, impairs blood brain barrier and apoptosis.[3] Additionally, hypoxia can disrupt brain metabolic activity, impairing normal neurological functions. While the Central Nervous System (CNS) can tolerate a wide range of oxygen levels, a critical threshold of hypoxia can cause significant functional disruption.[4] Hypoxia characterized by insufficient oxygen supply to the body or specific regions, initially triggers compensatory responses in the cardiovascular and respiratory systems, followed by adaptive responses in other organs, including the brain.[5]

Under certain conditions, hypoxia can be tolerated and may exert protective effects, a phenomenon known as hypoxia preconditioning. During hypoxia preconditioning, changes in gene expression and cellular signalling pathways occur, enhancing cell survival capacity.[6] Intermittent hypoxia, characterized by repeated exposures to sublethal hypoxic conditions at low barometric pressures, serves as an example of hypoxia preconditioning.[7] Studies have shown that intermittent hypoxia exerts neuroprotective effects on both the brain and heart.[8,9]

Hypoxia triggers necrotic and apoptotic pathways primarily through reactive oxygen species (ROS) formation. Elevated ROS levels stimulate mitochondrial release of cytochrome c, activating initiator caspase (caspase-9) and effector caspase (caspase-3), which promote apoptosis. Evidence suggests that repeated and intermittent hypoxic exposure can reduce caspase-3 expression, thereby potentially mitigating apoptotic processes.[10]

Neurotrophin-3 (NT-3) is part of the neurotrophic factor (NTF) family expressed by neurons and astrocytes within the nervous system. Neurotrophin-3 plays a critical role in nerve protection, regulating cell proliferation, promoting axonal and dendritic growth, and inducing myelination by activating Schwann cells.[11] Neurotrophin-3 interacts with several receptors, including TrkC, TrkA, and TrkB, facilitating the survival and differentiation of neuronal pathways throughout both the central and peripheral nervous systems.

Furthermore, neurotrophin including NT-3, have been shown to support neuronal proliferation and maturation during brain development and to exert neuroprotective effects in the adult brain.[12]

In response to hypoxic conditions, neurons upregulate the expression of superoxide dismutase (SOD) as a protective mechanism against oxidative stress. However, sustained or severe hypoxia may exceed the antioxidant defense capacity, resulting in cellular damage and neurodegeneration. Astrocytes and microglia also increase SOD expression in response to hypoxia, enhancing the antioxidant defense within the brain's microenvironment.[13]

Hypoxia often leads to increased production of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide. These ROS can react with proteins, leading to carbonylation. The presence of carbonylated proteins and other carbonyl compounds serves as a marker for oxidative damage in the brain under hypoxia. Increased levels of carbonylated proteins have been observed in various models of brain ischemia and neurodegenerative diseases associated with hypoxic conditions.[14]

Hypoxia preconditioning induces adaptive responses that enhance cell survival through mechanisms involving the regulation of oxidative stress levels, apoptotic rate, and cell proliferation. This study was conducted to examine the expression of caspase-3 mediated apoptosis, neurotrophin-3 mediated cell survival, and antioxidant defense mechanisms, as indicated by SOD and carbonyl levels, in the brain during intermittent hypobaric hypoxia exposure.

METHODS

The samples for this experiment were obtained from male rats previously treated in a related study. The brains of these rats were used as biological specimens in the present study. A total of twenty-five male rats were randomly divided into five groups: one control group and four treatment groups, with each group comprising five rats. This study has been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia (Protocol number: KET-1359/UN2. F1/ETIK/PPM.00.02/2020).

The treatment groups in this study were exposed to hypobaric hypoxia. Rats were placed in a hypobaric chamber, simulating an altitude of 25,000 feet, for 5 minutes at intervals of 7 days per exposure (Figure 1). The first treatment group was exposed to hypoxia once (IHH 1), the second group twice (IHH 2), the third group three times (IHH 3), and the fourth group four times (IHH 4). Following the final exposure, the rats were sacrificed right after the treatment, and their brains were collected and stored at -80°C. The brain samples were homogenized in Phosphate-Buffered Saline (PBS) at pH 7.2, centrifuged at 5,000 g for 5 minutes, and the supernatant was collected for analysis.

Brain tissue homogenates were treated with lysis buffer for protein extraction. SDS-PAGE was conducted at 120 volts for 85 minutes, followed by wet transfer at 300 mA for 75 minutes.

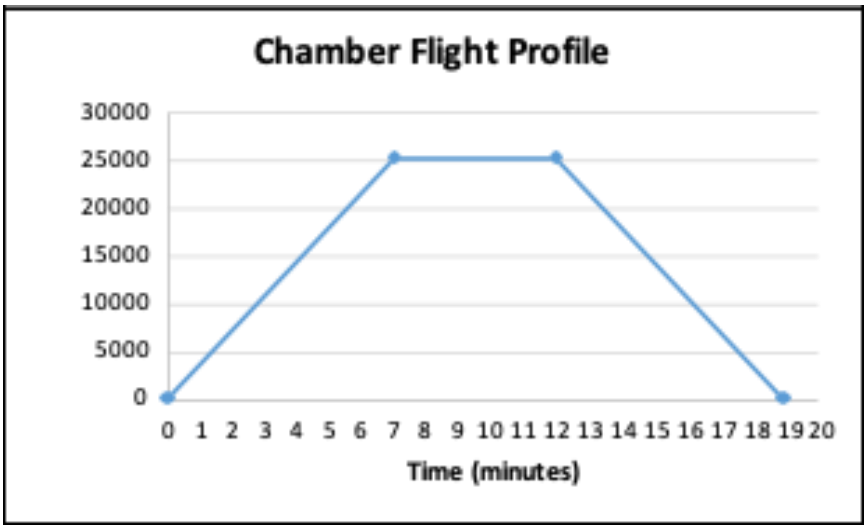

Figure 1. Hypobaric Chamber Flight Profile
The hypobaric chamber flight protocol, commonly used in human experiments, was adapted for use with experimental animals. The animals were exposed to an altitude of 25,000 feet for 5 minutes before being returned to ground level (0 feet)

The membrane was then blocked with blocking buffer (5% skim milk in PBST). Incubation with the primary antibody was performed in 5% BSA in PBST, and the secondary antibody was applied in TBST at room temperature.

Caspase-3 protein levels were measured using the Rat CASP3 ELISA kit (E-EL-R0160, Elabscience®), and NT-3 levels were measured with the Neurotrophin-3 ELISA kit (ab213905, Abcam®). The procedures were conducted according to the manufacturer's protocols.

Protein carbonyl levels were measured using the DNPH assay. Protein samples were incubated with 10 mM DNPH in 2 N HCl for 1 hour in the dark. Following precipitation with 20% TCA, samples were washed three times with ethanol-ethyl acetate (1:1, v/v) and dissolved in 6 M guanidine hydrochloride. Absorbance was measured at 390 nm, and carbonyl content was calculated using an extinction coefficient of 22,000 M^{-1} cm^{-1}.[15]

The SOD total activity was measured using the Ransond kit W125 (Randox Company®) and performed according to manufacturing protocols.

Data were presented as means with standard deviations (SD). Data normality was assessed using the Kolmogorov-Smirnov test. For data meeting the criteria of normal distribution and homogeneity of variances, a one-way unpaired analysis of variance (ANOVA) was conducted to evaluate group differences. Post hoc comparisons were then performed using Tukey's test to determine specific group differences. Data that did not meet these criteria will be analyzed using non-parametric tests. All statistical analyses were conducted using GraphPad Prism ver 9.

RESULT

Caspase-3 levels, measured using the ELISA method, yielding the following values: IHH 1 group, 4.90 ± 0.64 pg/mg protein; IHH 2 group, 4.69 ± 0.38 pg/mg protein; IHH 3 group,

4.23 ± 0.50 pg/mg protein; IHH 4 group, 3.03 ± 1.21 pg/mg protein; and normoxia/control group, 2.98 ± 0.15 pg/mg protein. A comparison of caspase-3 levels between the treatment and the control (normoxia) group is presented in Figure 2.

The results of statistical tests revealed significant differences between intermittent hypoxia-hypoxia (IHH) treatment groups and the normoxia/control group in rat brain tissue, as well as distinct differences between certain IHH groups. Specifically, the IHH 1 group exhibited significantly elevated Caspase-3 levels compared to both the IHH 4 group ($p=0.003$) and the control group ($p=0.002$), while the IHH 2 group also demonstrated a significant increase in Caspase-3 compared to the control group ($p=0.006$) and the IHH 4 group ($p=0.008$). These findings suggest that intermittent hypoxia induction can significantly modulate Caspase-3 expression in a group-dependent manner.

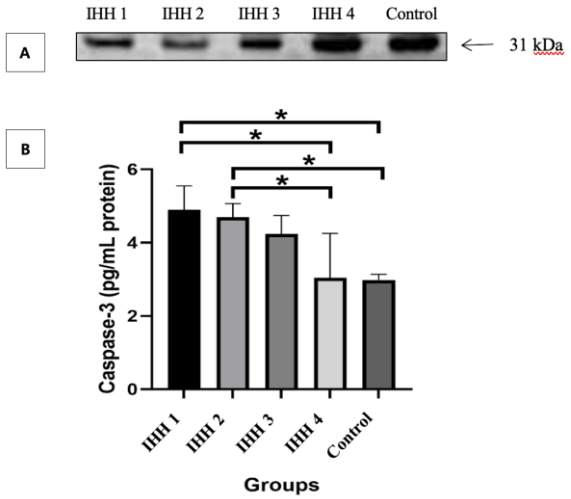

Figure 2. Caspase-3 Level in Rat Brains
(A) Western blot of caspase-3. IHH 1, IHH 2, IHH 3, IHH 4 and Control, with molecular weight of 31Kda.
(B) Measurement of Protein Caspase-3 levels ELISA. (*p <0.05; ANOVA, post hoc Tukey's test).

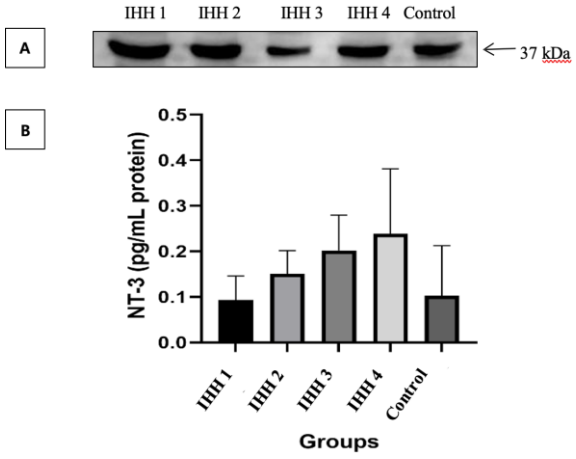

Figure 3. NT-3 Level in Rat Brains
(A) Western blot of NT-3 with molecular weight of 37 KDa
(B) Measurement of Protein NT-3 levels after induction using ELISA (*p<0.05; ANOVA).

Although statistical tests did not reveal a significant difference between the treatment and control groups, a discernible tendency for increased NT-3 levels was observed in the IHH 2, IHH 3, and IHH 4 groups compared to the IHH 1 and control groups.

Superoxide Dismutase levels in rat brain tissue showed no significant difference between treatment group and control group. A significant difference in SOD levels was observed between the groups, particularly highlighting a noteworthy increase in the IHH 4 group compared to the IHH 2 group ($p<0.005$) as shown in Figure 4.

Carbonyl levels in rat brain tissue showed no significant difference between IHH groups and the control group as shown in Figure 5.

Neurotrophin-3 levels in rat brain tissue were measured using the ELISA method, with the following results: IHH 1 group, 0.093 ± 0.053 pg/mg protein; IHH 2 group, 0.151 ± 0.051 pg/mg protein; IHH 3 group, 0.202 ± 0.077 pg/mg protein; IHH 4 group, 0.238 ± 0.143 pg/mg protein; and Control group, 0.102 ± 0.11 pg/mg protein. Comparison of NT-3 levels among the treated groups and the control group is presented in Figure 3.

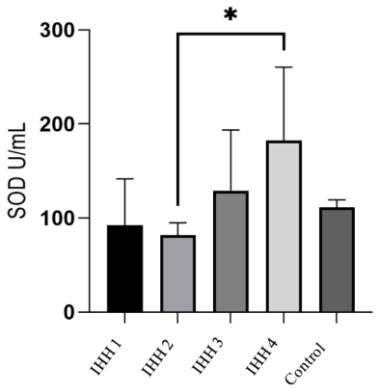

Figure 4. Total SOD Activity in Rats Brain
Statistical test results (*p<0.05; ANOVA, post hoc Tukey's test).

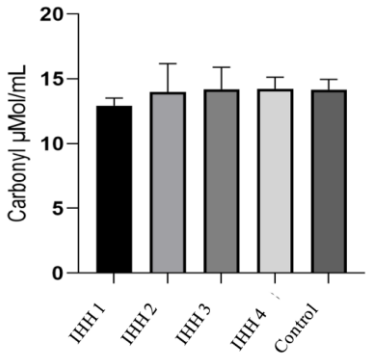

Figure 5. Carbonyl Levels in Rat Brain.
Statistical test results (*p<0.05; ANOVA).

DISCUSSION

In the altitude chamber protocol, Wistar rats were maintained at an altitude of 25,000 feet for 5 minutes before a gradual decrease in altitude. This exposure period corresponds to the Effective Performance Time (EPT) at 25,000 feet, which ranges from 3 to 5 minutes, representing the maximum time a pilot can remain conscious and capable of decision-making under hypoxic conditions at this altitude.[16] A previous study by Triveda, *et al*. demonstrated that at altitudes between 20,000 and 25,000 feet, Wistar rats exposed to hypoxia exhibited reduced activity, with some animals becoming somnolent.[17] Blood oxygen saturation levels at 25,000 feet ranged between 40–60%, categorizing the exposure as moderate hypoxia per established criteria, while in the normoxia group, blood oxygen saturation was recorded at 96%.[18]

This study demonstrated a decrease in caspase-3 levels in the treatment groups (IHH 1 and IHH 2) compared to the control group (normoxia). Reduction in caspase-3 levels was observed consistently with repeated exposure to hypobaric conditions, suggesting that IHH treatment may provide preconditioning against acute hypoxic stress in rats. However, IHH 3 treatment did not result in a significant caspase-3 reduction compared to the control, while IHH 4 showed caspase-3 levels comparable to the control group. These findings align with previous research by Hidayat, *et al*., which reported a decrease in caspase-3 expression in mice subjected to repeated hypoxia at 35,000 feet, indicating that frequent hypoxic exposure can attenuate caspase-3 protein expression.[19]

The intensity of intermittent hypoxic induction proved influential in eliciting significant differrences when compared with acute hypobaric hypoxia treatments. In this study, caspase-3 levels in the IHH 3 and IHH 4 groups were not significantly different from the control group, with only minimal increases observed in the IHH 4 group, resulting in caspase-3 levels close to those of the control group. This finding suggests that the hypobaric IHH 4 treatment provided a protective effect against apoptosis in rat brain tissue.

Similarly, Imbriani, *et al*. found that low levels of caspase-3 can activate molecular mechanisms involved in synaptic plasticity.[20] At these low concentrations, caspase-3 does not induce apoptosis but instead contributes to synaptic plasticity. Additional studies support this role of caspase-3: Mudjihartini, *et al*. reported a significant increase (*$p<0.05$) in caspase-3 levels in aging mice treated with *Centella asiatica* extract compared to an untreated aged group, while studies by Hollville, *et al*. and Nakajima, *et al*. showed that caspase-3 in neuronal cells exerts non-apoptotic functions, facilitating synaptic plasticity, dendritic growth, cell proliferation and differentiation, and cytoskeletal rearrangements.[21-23]

The levels of NT-3 protein were not significantly different between the treatment groups and the control group, likely due to the seven-day interval between each hypobaric hypoxia exposure. This interval may have allowed sufficient time for the rats' physiological and psychological states to return to

baseline.[19] No statistically significant differences in NT-3 levels were detected. However, as shown in Figure 3, mean NT-3 levels were visually higher in the IHH 2, IHH 3, and IHH 4 groups relative to the IHH 1 and control groups. This may indicate a potential relationship between exposure intensity and NT-3 levels that was not confirmed statistically in this study.

The measurements of superoxide dismutase (SOD) activity indicate that the levels of SOD in rats exposed to intermittent hypobaric hypoxia twice (IHH 2) are significantly different from those in the group exposed four times (IHH 4). This suggests that the frequency of hypoxic exposure influences SOD activity, potentially reflecting adaptive responses to oxidative stress. The increased SOD levels in the two-time exposure group may signify an initial adaptive response to hypoxia, whereas the IHH 4 group may experience a plateau or diminished response due to cellular exhaustion or altered regulation of antioxidant mechanisms. Supporting this, Dewi, *et al*. demonstrated that exposure to acute intermittent hypobaric hypoxia increases the specific activity of SOD in the rat brain, with significant enhancement noted in groups subjected to the most frequent hypoxia exposure, indicating an adaptive response to oxidative stress.[24] The study also concluded that the specific activity of SOD increases significantly under intermittent hypobaric hypoxia, underscoring the importance of SOD in mitigating oxidative damage during hypoxic conditions. Other study showed that together with GSH, SOD, and GPx, it tries to prevent lipid peroxidation (MDA) which continues to increase as the amount of IHH exposures increases.[25]

Carbonyl levels in rat brain homogenates revealed no significant changes in all groups. Previous research by Dewi, *et al*. indicated that inhibitory gene expression and MnSOD activity exhibit distinct patterns across tissues such as the heart, brain, and blood cells in chronic systemic hypoxia, with MnSOD activity varying by organ and influenced by factors like hypoxic exposure.[26] The relative MnSOD levels also depend on tissue type and species.

CONCLUSION

Collectively, these findings provide compelling evidence for IHH as a multifaceted neuroprotective strategy. The significant decrease in caspase-3 confirms IHH's role in inhibiting apoptotic pathways. This is complemented by two key adaptive responses: a positive trend in neurotrophic support (NT-3) and a frequency-dependent modulation of the antioxidant enzyme SOD, indicating an enhanced ability to manage oxidative stress. The stability in carbonyl levels, while requiring further study, does not detract from the clear protective signals observed. These findings position IHH as a promising therapeutic strategy for conditions involving hypoxic and oxidative damage.

REFERENCES

1. Tibbetts P. Fundamental neuroscience. Third edition. Edited by Larry R Squire, Darwin Berg, Floyd E Bloom, Sascha du Lac, Anirvan Ghosh, and Nicholas C Spitzer. Q Rev Biol. 2009;84(1).

2. Semenov M. Proliferative capacity of adult mouse brain. Int J Mol Sci. 2021;22(7):3449.
3. Shi Q, Li S, Lyu Q, Zhang S, Bai Y, Ma J. Hypoxia inhibits cell cycle progression and cell proliferation in brain microvascular endothelial cells via the miR-212-3p/MCM2 axis. Int J Mol Sci. 2023;24(3):2788.
4. Dunn JF, Isaacs AM. The impact of hypoxia on blood-brain, blood-CSF, and CSF-brain barriers. J Appl Physiol (1985). 2021;131(3):977-85.
5. Kane AD, Kothmann E, Giussani DA. Detection and response to acute systemic hypoxia. BJA Educ. 2020;20(2):58-64.
6. Li G, Guan Y, Gu Y, Guo M, Ma W, Shao Q, et al. Intermittent hypoxic conditioning restores neurological dysfunction of mice induced by long-term hypoxia. CNS Neurosci Ther. 2023;29(1):202-15.
7. Coimbra-Costa D, Garzón F, Alva N, Pinto TCC, Aguado F, Torrella JR, et al. Intermittent hypobaric hypoxic preconditioning provides neuroprotection by increasing antioxidant activity, erythropoietin expression and preventing apoptosis and astrogliosis in the brain of adult rats exposed to acute severe hypoxia. Int J Mol Sci. 2021;22(10):5272.
8. Costa DC, Alva N, Trigueros L, Gámez A, Carbonell T, Rama R. Intermittent hypobaric hypoxia induces neuroprotection in kainate-induced oxidative stress in rats. J Mol Neurosci. 2013;50(3):402-10.
9. Wang H, Shi X, Schenck H, Hall JR, Ross SE, Kline GP, et al. Intermittent hypoxia training for treating mild cognitive impairment: A pilot study. Am J Alzheimers Dis Other Demen. 2020;35: 1533317519896725.
10. Coimbra-Costa D, Alva N, Duran M, Carbonell T, Rama R. Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. Redox Biol. 2017;12:216-25.
11. Liu DB, Yang JS, Lu QB, Zhu ZF, Fang Q. Effect of NT-3 on infection-induced memory impairment of neonatal rats. Eur Rev Med Pharmacol Sci. 2019 Mar;23(5):2182-7.
12. Barh D, García-Solano ME, Tiwari S, Bhattacharya A, Jain N, Torres-Moreno D, et al. BARHL1 is downregulated in Alzheimer's disease and may regulate cognitive functions through ESR1 and multiple pathways. Genes (Basel). 2017;8(10):245.
13. Lee KH, Cha M, Lee BH. Neuroprotective effect of antioxidants in the brain. Int J Mol Sci. 2020; 21(19):7152.
14. Martínez-Orgado J, Martínez-Vega M, Silva L, Romero A, de Hoz-Rivera M, Villa M, et al. Protein carbonylation as a biomarker of oxidative stress and a therapeutic target in neonatal brain damage. Antioxidants (Basel). 2023;12(10):1839.
15. Mudjihartini N, Paramita R, Siregar AMK, Filzadiyanti E, Sarsanti PAN, Purwaningsih E. Comparing the effect of *Centella asiatica* L. and *Acalypha indica* L. treatment to carbonyl and glutathione level in the brains of old rats. Acta Biochim Indones. 2022;5(1):79.
16. Reinhart RO. Basic flight physiology. 3rd ed. New York: McGraw-Hill Professional; 2007.
17. Triweda RH. Perubahan vaskularisasi otot jantung secara kuantitatif pada tikus Wistar yang mengalami hipoksia hipobarik intermiten = Quantitative vascularization changes of cardiac muscle in Wistar rats with intermittent hypobaric hypoxia [tugas akhir]. Jakarta: Fakultas Kedokteran Universitas Indonesia; 2018.
18. El-Khoury R, Bradford A, O'Halloran KD. Chronic hypobaric hypoxia increases isolated rat fast-twitch and slow-twitch limb muscle force and fatigue. Physiol Res. 2012;61(2):195-201.
19. Hidayat A, Wiradisastra K, Hernowo BS, Achmad TH. Effects of IHH exposures towards the heart by measuring LDH, caspase-3 expressions and lactic acid level. Maranatha J Med Health. 2010;10:61-8.
20. Imbriani P, Tassone A, Meringolo M, Ponterio G, Madeo G, Pisani A, et al. Loss of non-apoptotic role of caspase-3 in the PINK1 mouse model of Parkinson's disease. Int J Mol Sci. 2019;20(14):3407.
21. Mudjihartini N, Paramita R, Prijanti AR, Sarsanti PAN, Fadilah F, Purwaningsih EH. The effects of *Centella asiatica* and *Acalypha indica* L. extracts on aging process. Res J Pharm Technol. 2020;13(2):787-90.
22. Hollville E, Deshmukh M. Physiological functions of non-apoptotic caspase activity in the nervous system. Semin Cell Dev Biol. 2018;82:127-36.
23. Nakajima YI, Kuranaga E. Caspase-dependent non-apoptotic processes in development. Cell Death Differ. 2017;24(8):1422-30.

24. Dewi S, Mulyawan W, Wanandi SI, Sadikin M. The effect of intermittent hypobaric hypoxia on oxidative stress status and antioxidant enzymes activity in rat brain. Acta Biochim Indones. 2018;1(2):46-51.
25. Wardaya W, Sukmawati D, Ibrahim N, Ferdinal F, Mudjihartini N, Sadikin M, et al. Intermittent exposure to hypobaric hypoxia increases VEGF, HIF-1α, and Nrf-2 expressions in brain tissue. Indones Biomed J. 2025;17(2):197-206.
26. Dewi S, Wanandi SI, Jusman SW. Manganese superoxide dismutase gene expression in rats' heart, brain, and blood induced by systemic hypoxia [thesis]. Jakarta: Fakultas Kedokteran Universitas Indonesia; 2008.