Biomedical Journal of Indonesia
Journal Homepage: https://bji-fk.ejournal.unsri.ac.id

Effect of Penta Herbs Forte on Antioxidant Capacity and Cardiac Protein Carbonyl Levels in a Hypoxia-Induced Rat Model

Sentanu Widiarto[1], Shirly Gunawan[2*], David Limanan[3], Frans Ferdinal[4], Eny Yulianti[5]

[1]Bachelor of Medicine Study Programme, Faculty of Medicine, Universitas Tarumanagara, West Jakarta, Indonesia
[2]Department of Pharmacology, Faculty of Medicine, Universitas Tarumanagara, West Jakarta, Indonesia
[3,4,5]Department of Biochemistry and Biology Molecular, Faculty of Medicine, Universitas Tarumanagara, West Jakarta, Indonesia

ARTICLE INFO

Keywords:
Antioxidant
Hypoxia
Penta herbs forte
Protein carbonyl

Corresponding author:
Shirly Gunawan
E-mail address:
shirlyg@fk.untar.ac.id

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/BJI.v12i2.304

Received 4 December 2025;
Accepted 11 February 2026

ABSTRACT

Introduction. Hypoxia, a state of inadequate oxygen supply in tissues, can induce the generation of reactive oxygen species (ROS), leading to oxidative stress and damage to biomolecules such as proteins. This damage can be quantified by measuring protein carbonyl levels. Penta Herbs Forte (PHF), a polyherbal formulation composed of Andrographis paniculata, Blumea balsamifera, Phyllanthus urinaria, Zingiber officinale, and Curcuma xanthorrhiza, is rich in bioactive compounds with known antioxidant properties. This study aimed to evaluate the antioxidant activity of PHF extract and its effects on cardiac protein carbonyl levels in hypoxia-induced rats. **Methods.** This experimental study included 32 male Sprague-Dawley rats, divided into eight groups: control and treatment groups under normoxia, 1-day hypoxia, 7-day hypoxia, and 14-day hypoxia, either with or without PHF extract administration. Antioxidant capacity was determined using the ABTS and DPPH assays, and cardiac protein carbonyl levels were measured using a spectrophotometer. **Results.** PHF extract showed an antioxidant activity yielding LC_{50} values of 22.135 µg/mL (ABTS) and 105.04 µg/mL (DPPH), indicating strong antioxidant potential. PHF-treated groups exhibited lower cardiac protein carbonyl levels than their respective controls across all hypoxic durations. **Conclusion.** Administration of PHF extract reduced cardiac protein carbonyl levels via its antioxidant activity, suggesting its potential as an exogenous antioxidant source to protect against hypoxia-induced oxidative damage.

1. Introduction

Hypoxia is a condition where oxygen supply to cells or tissues is insufficient, which can disrupt the body's metabolism. Hypoxia can trigger an increase in Reactive Oxygen Species (ROS), causing oxidative stress.[1] This occurs when the balance between ROS and cellular antioxidants is disrupted, resulting in an increase in oxidation potential that threatens the stability of cellular structures.[2–5] Increased oxidative stress can have negative consequences for biological systems, including molecular damage to components such as nucleic acids, lipids, and proteins, which can lead to cell death (apoptosis or necrosis) and have a detrimental impact on health. Oxidative stress is associated with more than 100 diseases, including cardiovascular disease, cancer, hypertension, diabetes, neurodegenerative diseases, aging, and other diseases.[6]

The effect of hypoxia on the heart organ causes several changes within the organ, for example, alterations in gene expression patterns that depend on oxygen availability, which HIF-1α regulates through gene transcription. Another change is the occurrence of energy metabolism disorders to maintain heart function and viability. In addition, it also causes various disorders in the structure and function of the heart muscle, such as ventricular dysfunction and muscle hypertrophy, which are the result of cardiac compensation in dealing with hypoxic conditions.[7]

Previous research has demonstrated the role of antioxidants derived from natural ingredients in mitigating health issues associated with oxidative stress. Antioxidants are beneficial for health because they help prevent the aging process and the development of degenerative diseases.[8] Antioxidants have a role in protecting the heart muscle, as well as improving cardiovascular structure and function, including hypertrophy, inflammation, and fibrosis. Antioxidants can prevent atherosclerosis by lowering total cholesterol, triglycerides, and insulin levels.[9] This mechanism occurs through enzymatic and non-enzymatic pathways to overcome the harmful effects of ROS, such as the work of the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase

(MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GPX) and peroxidase (POX), as well as non-enzymatic compounds such as ascorbate (AsA), glutathione (GSH), protein carbonyls, carotenoids, and tocopherols.[10]

Indonesia, with its tropical forests and abundant biodiversity, is a significant source of medicinal plants. Several plants have been proven to be potential sources of antioxidants. It is known that plants such as sambiloto, temulawak, ginger, meniran, and sembung have been used traditionally as medicines for various diseases. Sambiloto (Andrographis paniculata) has been used to treat liver disease, heart disease, snake bites, leprosy, jaundice, and bronchitis, as well as to treat worms.[11–13]

Research by Dev et al. shows that sambiloto contains flavonoids, which are compounds often associated with antioxidant activity.[14] Kusumawati and Yogeswara's research shows that sembung (Blumea balsamifera) has high levels of phenolics, tannins, and oxidant capacity.[15] Liu and Li's research shows that meniran (Phyllanthus urinaria) has a strong ability to fight free radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), has good reducing power, and has a total antioxidant capacity.[16] Mustafa and Chin's research shows that ginger (Zingiber officinale) has high flavonoid content, Ferric Reducing Ability of Plasma (FRAP), ABTS, and high antioxidant activity.[17] Rosidi et al reported that temulawak (Curcuma xanthorrhiza) has a high antioxidant content.[18]

Extensive research has established the antioxidant effects of single-herbal extracts under oxidative stress conditions. However, evidence regarding the biological impact of polyherbal formulations, particularly across distinct temporal phases of hypoxia, is still limited. To bridge this gap, our study explicitly underscores the novelty of examining polyherbal approaches, revealing that such combinations confer greater protective benefits than individual extracts.

Based on previous studies, this research investigates the antioxidant effects of Penta Herbs Forte (PHF), a polyherbal extract composed of five medicinal plants—*Andrographis paniculata*, *Blumea balsamifera*, *Phyllanthus urinaria*, *Zingiber officinale*, and *Curcuma xanthorrhiza.* This study aims to evaluate the antioxidant capacity of PHF and to determine whether its protective effects against protein oxidation are time-dependent across acute and sub-chronic hypoxia models. In addition, it investigates potential non-linear oxidative responses, particularly the absence of significant effects at intermediate hypoxia durations, which may indicate endogenous antioxidant adaptation. This research examines the antioxidant effects of PHF in a hypoxia-induced rat model and evaluates its impact on cardiac protein carbonyl levels**.**

2. Methods

This study employed an in vitro and in vivo experimental design. In vitro analyses included the antioxidant capacity assays using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods. In vivo experiments were conducted in male Sprague-Dawley rats to assess the effect of Penta Herbs Forte (PHF) extract on cardiac protein carbonyl levels under hypoxic conditions.

A total of 32 male Sprague-Dawley rats (10–12 weeks old, 200–250 g) were randomly divided into eight groups (n = 4 each). The estimated sample size in this study was calculated using Federer's formula.

PHF-treated and untreated groups, each exposed to normoxia, 1-day, 7-day, and 14-day hypoxia. The study was conducted from December 2024 to April 2025 at the Biochemistry and Molecular Biology Laboratory, Faculty of Medicine, Universitas Tarumanagara.

The PHF extract consisted of five medicinal plants: Andrographis paniculata, Blumea balsamifera, Phyllanthus urinaria, Zingiber officinale, and Curcuma xanthorrhiza. Each plant was cleaned, chopped, air-dried at room temperature, and ground into fine powder (simplicia). About 75 grams of each powder were macerated in ethanol for 24 hours, stirred twice daily, then filtered and evaporated using a rotary evaporator to obtain a thick paste. Each extract (0.2 g) was combined, mixed with a drop of Tween-80, vortexed, and diluted with 10 mL of distilled water to prepare the PHF solution. PHF extract was administered orally at a dose of 100 mg/kg body weight per day, adjusted to a final volume of 2 mL/day for 14 days.

Hypoxia was induced using a sealed chamber filled with 10% oxygen and 90% nitrogen. After treatment, blood and cardiac tissue samples were collected for analysis. Tissues were homogenized in phosphate-buffered saline and centrifuged to obtain supernatants. Protein carbonyl levels were measured using the Solarbio Protein Carbonyl Assay Kit with spectrophotometry at 370 nm.

Antioxidant activities were evaluated via standard ABTS and DPPH assays. All experimental protocols involving animals were reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia (Ethical Clearance No. 003/KER/FK/09/2024).

Statistical tests in this study were performed using GraphPad Prism 6.05. The analyzed variables were statistically significant if $P < 0.05$. The obtained data were averaged and then displayed in tables and graphs. The Mann–Whitney test and linear regression were used to compare data from the control and treatment groups. The Pearson test was used to compare the two variables.

3. Results

3.1. Antioxidant Activity Test of PHF Extract Using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Method

Wavelength and Absorbance of the ABTS Test

The control absorbance at the optimal wavelength of 520 nm was 0.411, which was used as a blank for the PHF extract test.

ABTS Test of Reference Solution (Trolox)

Trolox solutions were prepared using absorbance concentrations of 5, 10, 15, 20, and 25 µg/mL, which were then read using a spectrophotometer. A standard Trolox curve was constructed showing the absorbance and percentage inhibition values with a linear equation of Y = 1.542x + 29.54 and R^2 = 0.9987 (Figure 1). The LC_{50} value was 13.266 µg/mL (Table 1).

ABTS Test of PHF Extract

The absorbance value of PHF extract with concentrations of 10, 15, 20, 25, 30 µg/mL was then read using a spectrophotometer, and a linear equation of Y = 3.294x – 22.92 and R^2 = 0.9826 was obtained (Figure 2). The LC_{50} value was 22.135 µg/mL. (Table 2).

3.2. Antioxidant activity test of PHF extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method

DPPH test of reference solution (Trolox)

Trolox solutions were prepared using absorbance concentrations of 10, 20, 30, 40, and 50 µg/mL, which were then read using a spectrophotometer. A standard Trolox curve was constructed, showing the absorbance and percentage inhibition values with a linear equation of Y = 1.317x + 21.87 and R^2 = 0.9976 (Figure 3). The LC_{50} value was 21.353 µg/mL (Table 3).

DPPH Test of PHF Extract

The absorbance values of PHF extracts at concentrations of 30, 60, 90, 120, and 150 µg/mL were then read using a spectrophotometer, yielding a linear equation of Y = 0.5377x – 6.478 and R^2 = 0.9825 (Figure 4). The LC_{50} value was 80.940 µg/mL (Table 4).

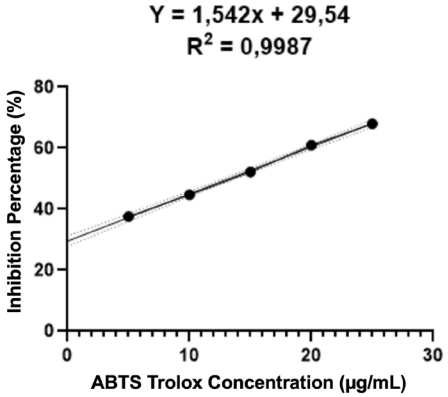

Figure 1. Trolox ABTS standard comparative test result curve

Table 1. ABTS Trolox standard comparison

Concentration (µg/mL)	Inhibition Percentage (%)	LC_{50} (µg/mL)
5	37.546	13.266
10	44.689	
15	52.198	
20	60.989	
25	67.949	

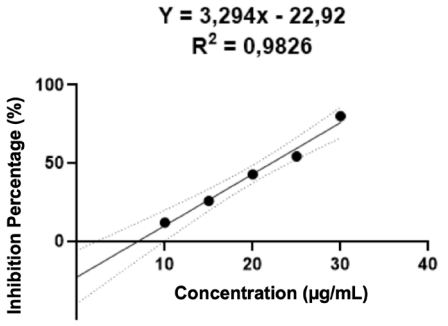

Figure 2. ABTS test result of the PHF extract

Table 2. ABTS test results of the PHF extract

Concentration (µg/mL)	Inhibition Percentage (%)	LC_{50} (µg/mL)
10	11.922	22.135
15	25.791	
20	42.822	
25	54.258	
30	80.049	

LC_{50} Value (µg/mL) >200 = Very weak ; 151-200 = Weak ; 101-150 = Moderate ; 50-100 = Strong ; <50 = Very strong [22]

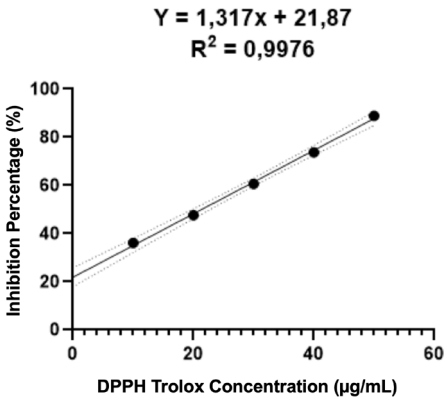

Figure 3. Trolox DPPH standard comparative test result curve

Table 3. Trolox DPPH comparison standards

Concentration (µg/mL)	Inhibition Percentage (%)	LC_{50} (µg/mL)
10	36.087	21.353
20	47.609	
30	60.652	
40	73.696	
50	88.913	

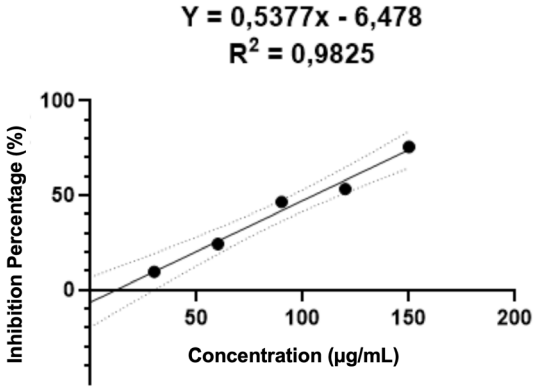

Figure 4. DPPH test result of the PHF extract

Table 4. DPPH test results of PHF extract

Concentration (µg/mL)	Inhibition Percentage (%)	LC_{50} (µg/mL)
30	9.565	105.04
60	24.348	
90	46.522	
120	53.478	
150	75.652	

LC_{50} Value (µg/mL) >200 = Very weak ; 151-200 = Weak ; 101-150 = Moderate ; 50-100 = Strong ; <50 = Very strong [22]

3.3. Animal Testing

Control Cardiac Carbonyl Protein Levels

The Y-axis shows the carbonyl protein levels (µmol/gram tissue), and the X-axis shows the hypoxia treatment groups (Table 5) (Figure 5). Increasing the duration of hypoxia treatment increases carbonyl protein levels in the hearts of control animals. Comparisons between the normoxia treatment group

and the 1- and 7-day hypoxia treatment groups, and between the hypoxia treatment groups, showed no significant difference. There was a significant difference between the normoxia and 14-day hypoxia treatment groups (p = 0.0329). This is due to the adjusted p-value (p = <0.05).

Test Cardiac Carbonyl Protein Levels

Figure 6 shows a graph of protein carbonyl levels in the test group. The Y-axis shows the value of protein carbonyl levels (µmol/gram tissue), and the X-axis shows the hypoxia treatment group. Increasing the duration of hypoxia treatment increases protein carbonyl levels in the test group's heart (Table 6). The results show a significant difference between the normoxia and 14-day hypoxia groups (p = 0.0028). Meanwhile, the cardiac protein carbonyl levels between the normoxia group and the 1- and 7-day hypoxia treatment groups were not significantly different, and the same was true between the hypoxia treatment groups.

Comparison of Cardiac Carbonyl Protein Levels In Control and Test Groups

Figure 7 shows that the administration of PHF extract significantly reduced carbonylated protein levels in the normoxia test group (p = 0.0435), the 1-day hypoxia (p = 0.0181), and the 14-day hypoxia (p = 0.0181), compared to the control group. Meanwhile, the 7-day hypoxia treatment group did not show a significant difference in carbonyl protein levels.

Table 5. Cardiac carbonyl protein levels of rats not given PHF extract

Hypoxia Treatment	Carbonyl Protein Level (µmol/gram tissue)
Normoxia	0.0475
1 Day of Hypoxia	0.068125
7 Days of Hypoxia	0.07375
14 Days of Hypoxia	0.09625

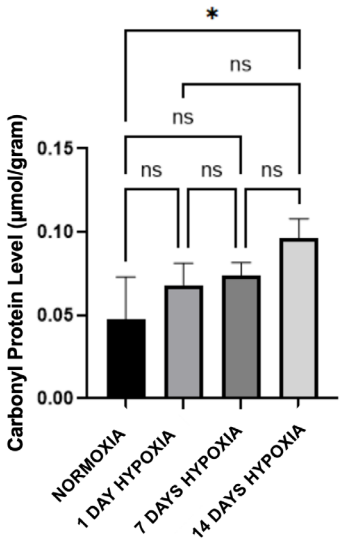

Fig. 5. Cardiac carbonyl protein level in the control group.* All values are presented as the mean ± SD; n = 4; *p < 0.05 vs normoxia; ns = not significant; Statistical analysis was performed using one-way ANOVA followed by multiple comparisons

Table 6. Carbonyl protein levels in the hearts of rats given PHF extract

Hypoxia Treatment	Carbonyl Protein Level (µmol/gram tissue)
Normoxia	0.014
1 Day of Hypoxia	0.031
7 Days of Hypoxia	0.057
14 Days of Hypoxia	0.059

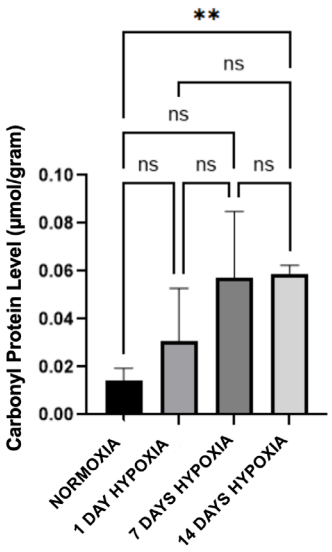

Fig. 6. Cardiac carbonyl protein level in the treatment group. * All values are presented as the mean ± SD; n=4; **p < 0.01 vs normoxia; ns = not significant; Statistical analysis was performed using one-way ANOVA followed by multiple comparisons

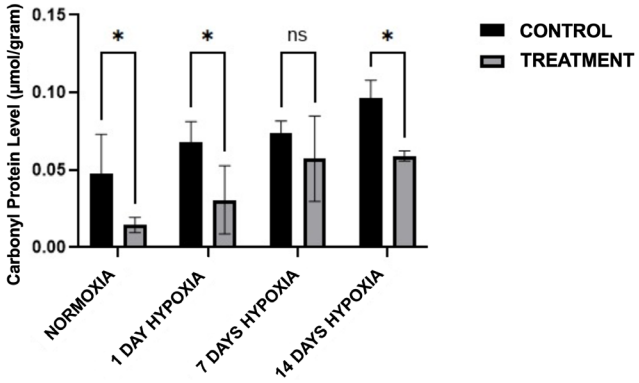

Figure 7. Comparison of cardiac carbonyl protein levels in control and treatment groups. * All values are presented as the mean ± SD; n = 4; *p < 0.05 vs control; ns = not significant; Statistical analysis was performed using two-way ANOVA followed by multiple comparisons

4. Discussion

The rationale for combining five medicinal plants in PHF is based on the diversity of their phytochemical profiles and antioxidant mechanisms. The administered dose of PHF (100 mg/kg body weight) was selected to ensure biological activity while remaining within a safe range based on preliminary toxicity screening. However, as this study employed a single-dose design, dose–response relationships and pharmacokinetic profiles could not be assessed and should be addressed in future studies. Andrographis paniculata and Curcuma xanthorrhiza are rich in diterpenoids and curcuminoids that act as direct ROS scavengers, whereas Phyllanthus urinaria and Blumea balsamifera are known to modulate endogenous antioxidant enzymes. Zingiber officinale contributes phenolic compounds with metal-chelating and anti-inflammatory properties.[12-13, 15-18]

Such diversity suggests potential functional complementarity, although synergistic or antagonistic interactions cannot be excluded. We assessed antioxidant capacity using the ABTS and DPPH assays, as both methods are classified as mixed-mode (HAT/SET) assays that represent multiple approaches to antioxidant capacity evaluation based on their underlying mechanisms. These assays combine hydrogen atom transfer (HAT), which measures the ability of antioxidants to neutralize free radicals, generally peroxyl radicals considered more biologically relevant, through hydrogen atom donation, and single electron transfer (SET), which reflects the ability of antioxidants to transfer a single electron to reduce metal ions, carbonyl compounds, and radicals, resulting in the reduction of oxidants accompanied by a measurable color change. The discrepancy between ABTS and DPPH results may reflect differences in radical solubility, steric accessibility, and competitive interactions among phytochemicals within the extract.

4.1. Antioxidant Capacity Test using ABTS Method

In the antioxidant capacity test using the ABTS (2,2' - azino - bis (3 - ethylbenzothiazoline - 6 -sulfonic acid)) method, the PHF extract was found to have an antioxidant capacity of 22.135 µg/mL. This result indicates a higher level than that of Ali et al., in which the pure ginger extract showed antioxidant capacities of 5.35 ± 0.33 and 7.05 ± 0.23 µg/mL.[19]

Other studies also showed lower antioxidant capacity in single extract tests. Liwanda et al. reported that the antioxidant capacity of sambiloto extract obtained with a combination of ethanol and acetone solvents was 4.026 ± 0.02 µg/mL.[20] Rusmana et al. found the antioxidant capacity of meniran extract was 1.53 µg/mL.[21] Based on the antioxidant activity criteria of Blois et al., the ABTS test for the PHF extract was considered to have very strong antioxidant activity.[22] The type of solvent can influence the ABTS test results in research conducted by other researchers; the solvent that is more effective or produces a higher value is a solvent that has radical scavenging properties.[20]

4.2. Antioxidant Capacity Test using DPPH Method

The antioxidant capacity test using the DPPH method for the PHF extract yielded an LC_{50} value of 105.04 µg/mL, and based on the antioxidant activity criteria of Blois et al., the PHF extract has moderate antioxidant activity (Table 7).[22] For Andrographis paniculata extract, a study conducted by Singh et al. found DPPH levels in Andrographis paniculata extract at a concentration of 100 µg/mL of 69.52 ± 0.52 µg/mL, and the results increased with the concentration of the extract tested.[23] The DPPH test results conducted by Singh et al. concluded that Andrographis paniculata extract has strong antioxidant activity (Table 7). In the study by Rusmana et al. The DPPH content in the meniran extract was 4.24 µg/mL.[21] Interactions between plants, which can increase or decrease antioxidant activity in DPPH testing, can cause lower and higher results from the constituent plants.[24]

4.3. Cardiac Carbonyl Protein Levels

Test results showed an increase in cardiac carbonyl protein levels in the control and test groups during normoxia, 1-day hypoxia, 7-day hypoxia, and 14-day hypoxia. A significant increase in carbonyl protein levels was observed in the control group between normoxia and 14-day hypoxia ($p = <0.05$) (Figure 5). Furthermore, in the test group, there was a very significant increase in carbonyl protein levels between normoxia and 14-day hypoxia ($p = <0.01$) (Figure 6). This is because the longer the exposure to hypoxia, the greater the oxidative-stress-induced protein damage caused by oxidative stress, which increases carbonyl levels in the heart. This increase in carbonyl levels is also consistent with the findings of Marques et al. As the mice aged to 12 months, protein carbonyl levels increased due to the accumulation of oxidative protein damage in heart tissue.[25]

Interestingly, PHF administration did not significantly reduce protein carbonyl levels in the 7-day hypoxia group ($p = >0.05$) (Figure 7). This finding may reflect a phase of endogenous antioxidant adaptation, where upregulation of intrinsic defense systems temporarily compensates oxidative burden. Such adaptive responses have been described in subacute hypoxia and may represent a form of hormetic regulation, where oxidative remodeling occurs before overt damage manifests. [26]

The protein carbonyl test on the hearts of the mice showed a difference between the control and test groups. The protein carbonyl levels in the test group given PHF extract were lower than those in the control group not given PHF extract. This indicates that administering PHF extract to the heart tissue can better suppress oxidative stress and reduce protein damage.

5. Conclusion

This study demonstrated that the Penta Herbs Forte (PHF) extract contains a high level of phenolic compounds. The extract exhibited potent antioxidant activity. Administration of PHF extract in hypoxia-induced Sprague Dawley rats led to a decrease in cardiac protein carbonyl levels across all treatment groups—normoxia, 1-day, 7-day, and 14-day hypoxia, respectively. Notably, a significant reduction in protein carbonyl levels was observed in the PHF-treated groups compared to their respective controls, particularly under normoxia, 1-day, and 14-day hypoxia conditions. In conclusion, PHF demonstrated antioxidant activity and was associated with reduced cardiac protein oxidation in a hypoxia-induced rat model. While these findings support its potential antioxidative role, further studies incorporating dose–response analysis, mechanistic biomarkers, and standard antioxidant comparators are required before translational implications can be inferred. Future investigations should incorporate a broader oxidative stress panel, including endogenous antioxidant enzymes (SOD, CAT, GPx), inflammatory markers, and apoptotic signaling pathways, to provide a more comprehensive understanding of PHF-mediated cardioprotection.

6. Author Contribution

S.W. experimented. S.W and S.G wrote the manuscript. S.W and D.L collected the data sample. F.F., E.Y., D.L., and S.G. supervised the project. F.F. conceived the original idea.

7. Acknowledgements

We express our gratitude to the faculty of medicine, Universitas Tarumanagara, for providing facilities for this research.

8. References

1. Sies H, Jones DP. Reactive oxygen species (ROS) are pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21(7):363–83.
2. Poljsak B, Šuput D, Milisav I. Achieving the

balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxidative Med Cell Longev. 2013;1–11.
3. Bagchi K, Puri S. Free radicals and antioxidants in health and disease. East Mediterr Health J. 1998;4(2):350–60.
4. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44–84.
5. Waterman KC, Adami RC, Alsante KM, Hong J, Landis MS, Lombardo F, et al. Stabilization of pharmaceuticals against oxidative degradation. Pharm Dev Technol. 2002;7(1):1–32.
6. Liu Y, Chen C, Wang X, Sun Y, Zhang J, Chen J, et al. An epigenetic role of mitochondria in cancer. Cells. 2022;11(16):2518.
7. Giaccia AJ, Simon MC, Johnson R. The biology of hypoxia: the role of oxygen sensing in development, normal function, and disease. Genes Dev. 2004;18(18):2183–94.
8. Werdhasari A. Peran antioksidan bagi kesehatan. J Biotek Medisiana Indon. 2014;3(2):59–68.
9. Młynarska E, Hajdys J, Czarnik W, Fularski P, Leszto K, Majchrowicz G, et al. The role of antioxidants in the therapy of cardiovascular diseases—a literature review. Nutrients. 2024;16(16):1–26.
10. Hassanuzaman M, Hossain MA, da Silva JAT, Fujita M. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Crop stress and its management: perspectives and strategies. Berlin: Springer; 2012. p. 261–315.
11. Dewi IP, Verawaty V, Taslim T. Aktivitas imunomodulator ekstrak etanol daun sambiloto (Andrographis paniculata Ness) pada mencit putih. J Kesehat Perintis. 2023;10(1):1–6.
12. Jayakumar T, Hsieh C, Lee J, Sheu J. Experimental and clinical pharmacology of Andrographis Paniculata and its major bioactive phytoconstituent Andrographolide. Evid Based Complement Alternat Med. 2013;846740.
13. Nagajothi S, Mekala P, Raja A, Raja MJ, Senthilkumar P. Andrographis Paniculata: qualitative and quantitative phytochemical analysis. J Pharmacogn Phytochem. 2018;7(4):1251–3.
14. Dev S. Prime Ayurvedic plant drugs. 2nd ed. London: Springer; 2023.
15. Kusumawati IGAW, Yogeswara IBA. Antioxidant and antibacterial capacity of loloh sembung (Blumea balsamifera) based on the extraction method. Tradit Med J. 2016;21(3):143–8.
16. Liu Y, Li SM. Extraction optimization and antioxidant activity of Phyllanthus urinaria polysaccharides. Food Sci Technol. 2021;41(Suppl. 1):91–7.
17. Mustafa I, Chin NL. Antioxidant properties of dried ginger (Zingiber officinale Roscoe) var. Bentong. Foods. 2023;12(1):1–18.
18. Rosidi A, Khomsan A, Setiawan B, Riyadi H, Briawan D. Antioxidant potential of temulawak (Curcuma xanthorrhiza Roxb). Pak J Nutr. 2016;15(6):556–60.
19. Ali AMA, El-Nour MEM, Yagi SM. Total phenolic and flavonoid contents and antioxidant activity of ginger (Zingiber officinale Rosc.) rhizome, callus, and callus treated with some elicitors. Journal of Genetic Engineering and Biotechnology. 2018;16(2):677–82. doi: 10.1016/j.jgeb.2018.03.003
20. Liwanda N, Zahra A, Sudarjat KSA, Mulyati T, Nurcholis W. Total phenolic content and antioxidant capacity from stems and leaves of Andrographis paniculate in different solvent combinations. Curr Appl Sci Technol. 2024;25(2):e0261033.
21. Rusmana D, Wahyudianingsih R, Elisabeth M, Balqis, Maesaroh, Widowati W. Antioxidant activity of Phyllanthus niruri extract, rutin, and quercetin. The Indonesian Biomedical Journal. 2017;9(2):84–90.
22. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–200.
23. Singh RK, Reddy SM. Andrographis paniculata: a study of its phytochemical makeup and antioxidant properties. Ayden International Journal of Biomedical Research and Technology. 2022;10(4):1–8.
24. Ginting B, Maulana I, Yahya M, Saidi N, Murniana M, Hasballah K, et al. Antioxidant and antiproliferative activities of n-hexane extract and its fractions from *Blumea balsamifera* L. leaves. J Adv Pharm Technol Res. 2022;13(3):216
25. Marques GL, Neto FF, Ribeiro CAO, Liebel S, de Fraga R, Bueno RRL. Oxidative damage in the aging heart: an experimental rat model. Open Cardiovasc Med J. 2015;9:78–82.
26. Sun W, Yin X, Wang Y, Tan Y, Cai L, Wang B, et al. Intermittent hypoxia-induced renal antioxidants and oxidative damage in male mice: hormetic dose response. Dose Response. 2013;11(3):385-400.