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ORIGINAL ARTICLE

Ficus carica Puree Exerts a Higher Antioxidative Profile in Hypoxic Lungs after Intermittently Inducing Hypoxia in Sprague–Dawley Rats

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Background: The body produces more hypoxia-inducible factor and reactive oxygen species in response to hypoxic situations because they cause unbalanced oxygen levels. *Ficus carica* provides numerous benefits because of its high antioxidant and mineral content. Nevertheless, the mechanism by which *F. carica* consumption confers this protective benefit remains incompletely understood. **Aim:** This study aimed to evaluate how *F. carica* puree (FCP) can affect the antioxidant enzyme activity and malondialdehyde (MDA) levels in the lung by mitigating the effects of intermittent hypoxia (IH). **Methods:** Thirty Sprague–Dawley rats were divided into five groups: negative control (NC), untreated; positive control (PC), treated with aquadest; FCP1; FCP2; and FCP3, which received FCP at doses of 1.25, 2.5, and 5 mL/200 g body weight. The treatment was administered for 4 weeks before inducing IH (10% O_2 and 90% N_2) into all groups (except NC) for 4 h for 7 days. Furthermore, hemoglobin (Hb) level, lung MDA level, and lung superoxide dismutase (SOD) enzyme activity were assessed. **Results:** The Hb level did not exhibit a significant increase under IH conditions. Conversely, the PC group exhibited the least activity of lung antioxidant enzymes and the highest lung MDA levels. In addition, the FCP intervention group exhibited lower MDA levels than the PC group and ameliorated relative lung weight loss. **Conclusion:** All FCP intervention groups showed lower MDA levels and higher SOD levels compared to the PC group, suggesting that FCP could mitigate the effects of hypoxia in rat lungs.

Key words: Intermittent hypoxia, *Ficus carica*, antioxidant, malondialdehyde, superoxide dismutase

INTRODUCTION

Oxygen is crucial in maintaining the body's acid-base equilibrium, providing nutrients to body cells, and facilitating other chemical transport activities. The human body uses the lungs to facilitate gas exchange. Hypoxia occurs when the body's oxygen supply is reduced or imbalanced.[1] Under these circumstances, the body will increase the synthesis of hypoxia-inducible factor (HIF-1) and reactive oxygen species (ROS), which can damage different biomolecules, such as proteins, polyunsaturated fatty acids, and DNA. Arachidonic acid, a polyunsaturated fatty acid, functions as a peroxidase, generating malondialdehyde (MDA), 4-hydroxy-2-nonenal, and other compounds through various biochemical pathways. Several studies have shown that MDA can be used as a biomarker for oxidative stress in the lungs. MDA correlates with other biomarkers that indicate lipid breakdown and may be detected in many bodily fluids, such as blood, urine, and exhaled breath condensate.[2]

If the body has adequate antioxidants, then the increased ROS can be controlled. Antioxidants are compounds acting as the body's protective mechanism against harmful free radicals and the resulting oxidative stress,[3] and the body employs enzymatic and nonenzymatic antioxidant systems to protect against ROS

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and the subsequent membrane and macromolecule damage. Antioxidant molecules possess the direct capacity to regulate ROS to prevent oxidative damage. The main endogenous antioxidant enzymes in the body responsible for controlling ROS are catalase, glutathione peroxidase, and superoxide dismutase (SOD);[4] of these antioxidant enzymes, SOD is the primary defensive mechanism, facilitating the conversion of superoxide anion (O^-) into hydrogen peroxide (H_2O_2). Within the body, three distinct variations of SOD were identified: extracellular SOD (ecSOD3), manganese SOD (SOD2), and copper/zinc SOD (SOD1). The lungs is one of the major tissues that strongly express the mRNA and protein of ecSOD.[5,6] The smooth muscle cells generate the ecSOD isoform, which is then released into the extracellular space. Some research[7,8] reported that ecSOD functions as a master regulator of nitric oxide (NO) produced from the endothelium.

Nevertheless, the body's antioxidant enzyme (SOD) activity declines under hypoxic conditions.[9] Antioxidants can originate from two sources: endogenous, made within the body, and exogenous, came from external sources. Fruits include antioxidants that the body cannot synthesize. The fruit commonly referred to as fig or *Ficus carica* is rich in valuable nutrients and antioxidants. Figs are referred to as "plants of life" due to their ability to adapt to many environmental circumstances. Intermittent hypoxia (IH)-induced oxidative stress is yet to be thoroughly understood. Furthermore, this study aimed to evaluate the protective role of *F. carica* puree (FCP) in reducing the impact of IH induction on the antioxidant enzyme activity in the lung.

MATERIALS AND METHODS

Preparation of *Ficus carica* puree

Whole fig fruits (*F. carica* cv. Jordan) grown in Ciwidey, Kabupaten Bandung, West Java, were used in this study. The figs utilized in this study were fully mature and ready to consume. Figs require 4 months from the time, they are planted to start harvesting. The fig fruit utilized is the Jordanian cultivar, which turns blackish purple when fully ripe. It is then crushed into a puree and stored at a temperature of 4°C. The condition of the puree is regularly monitored. The puree is placed in a sealed container with a volume adjusted to the required dosage.

The puree was made by washing, slicing, crushing, and blending fresh whole fig fruits, including the skin, pulp, and seeds. After homogenizing the mixture for 20 min at 8033 rpm using an Armfield L4R homogenizer, the seeds were removed by sifting the mixture through a fine mesh sieve. The resulting FCP (Ficus Carica Puree) was kept in an airtight plastic cup and stored in a refrigerator at 2°C without any light. FCP was made every 3 days to ensure freshness.

Animal and experimental design

The Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (approval number: KE/FK/0532/EC/2022; approval date: April 28, 2022) approved the study protocol, and the institution rules were followed for the care and use of laboratory animals. A total of 30 adult male Sprague–Dawley rats (180–200 g) were used in this study. The rats were kept in a standard cage (40 cm × 30 cm × 20 cm; 3 rats/cage) with a 12–12-h light-dark cycle and free access to water and diet. The environmental temperature and humidity were maintained at 22°C–24°C and 40%–60%, respectively. Before the trial, the rats were given 7 days for acclimatization. During the trial, intensive care was enforced to minimize their suffering to ensure their well-being following the experimental timeline as shown in Figure 1.

After acclimatization, the rats were distributed into five groups (n = 5 or 6): negative control (NC), untreated, (n = 6); positive control (PC), treated with aquadest, (n = 5); FCP1; FCP2; and FCP3 (groups receiving FCP with 1.25, 2.5, and 5 mL/200 g body weight, each with (n = 6). The treatment was administered for 4 weeks before inducing IH (10% O_2 and 90% N_2) in all groups (except NC) for 4 h for 7 days. The treatment was orally administered through force-feeding every morning at 07:00 AM, FCP is given for 4 weeks, and at the time of IH induction (with the FCP administration interval being 2 h before IH induction). Body weight measurements are conducted weekly. Furthermore, hemoglobin (Hb), lung MDA levels, and lung SOD enzyme activity were assessed.

Intermittent hypoxia induction

Rats subjected to IH were placed under exogenous and normobaric hypoxia stimulation (760 mmHg, 10% O_2, and 90% N_2) for 4 h over 7 days. IH was induced using Meliala *et al.*[10] method, involving placing the rats in a hypoxic chamber with dimensions of 25 cm × 15 cm × 15 cm. Temperature and humidity changes are observed every 30 min. Before inducing hypoxia in rats, the oxygen levels of the chamber were determined by comparing the oxygen levels inside and outside the chamber and then adjusted and documented. This was performed to reduce bias in the study. Following calibration, the rats were placed in an airtight chamber equipped with temperature and humidity sensors. The chamber is subsequently associated with a gas mixture of 10% oxygen and 90% nitrogen. This ensures the entry of oxygen and nitrogen gasses from the mixture into the chamber, as observed in the water reintroduced into the gas mixture. An oximeter in the chamber shows an oxygen level ranging from 10% to 13%. The oxygen levels in the chamber were measured every 30 min for 4 h/day over 7 consecutive days in the PC, FCP1, FCP2, and FCP3 groups. The administration of FCP

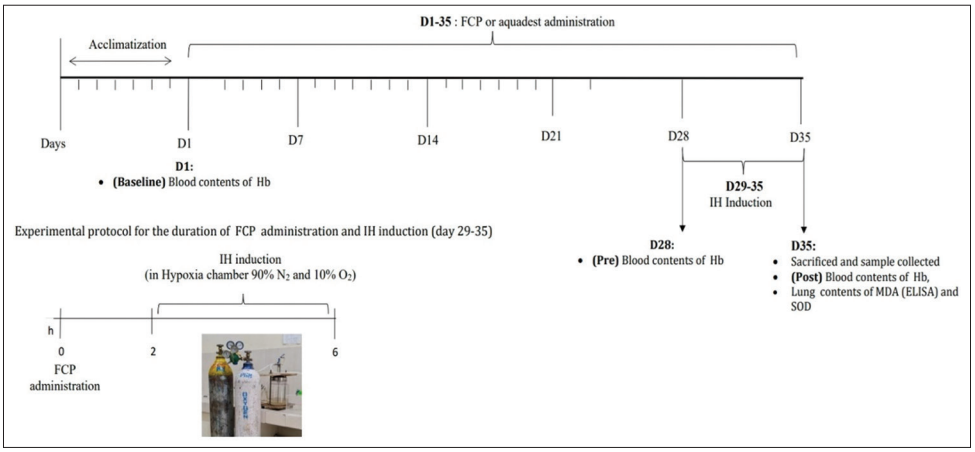

Figure 1: Experimental timeline. FCP = *Ficus carica* puree, IH = Intermittent hypoxia, MDA = Malondialdehyde, Hb = Hemoglobin, SOD = Superoxide dismutase

is used as a preventive measure.[10,11] This administration is done for 4 weeks before hypoxia induction, and it continues for 1 week within hypoxia induction. After 7 days of hypoxic exposure, the rats were collected as postmortem samples for assessment. The lung tissues were blended and examined for MDA and SOD concentrations and Hb levels.

Body weight and lung index

To determine body weight changes, they were measured before the FCP intervention period (basal) and at days 0 and 7 during IH induction. The lung index was calculated as a percentage of lung/body weight. This calculation is based on a study showing that the lung weight increases relative to the body weight in rats exposed to hypoxic conditions.[12]

Collection and examination of lung organs and plasma

Blood sampling was performed in the eye orbital sinus with a volume of ± 2 mL, which was then placed into a 3-mL ethylenediaminetetraacetic acid tube, on day 1 after acclimatization (baseline), day 29 (pretest), and day 35 (posttest). The blood sample is then centrifuged at 4000 rpm for 15 min to separate the serum and blood plasma. Blood plasma was then used to measure Hb at the Gadjah Mada University Integrated Research and Testing Laboratory.

On day 35, the rats were terminated. The lung organs were divided into two groups. Some samples were sent to the Clinical Pathology Laboratory of Gadjah Mada University for MDA examination with an enzyme-linked immunosorbent assay kit (ab118970; Abcam, Cambridge, UK). In contrast, the remaining samples were sent to the Nutrition Laboratory of Gadjah Mada University Central Building (PAU) to analyze the SOD enzyme activity using amsbio SOD activity assay kit (Catalog #K335-100; 100 assays).

Statistical analysis

Fold changes in Hb levels in the plasma were determined as the average of the final/initial values and adjusted to percentage changes (i.e., 1.60-fold, 60% increase; 0.60-fold, 40% reduction). All data (body and lung weights; MDA and SOD levels in lung organs) were normally distributed and reported as means and standard deviations (SDs). To compare the initial and final values, a paired sample *t*-test was performed. To compare the means of fold changes in all groups, a one-way analysis of variance and Fisher's least significant difference *post hoc* test were performed. At $P < 0.05$, differences were considered significant. Pearson's correlation coefficient was used to analyze the correlation between oxidative stress parameters, with a statistical significance level of $P < 0.05$. The SPSS 23 for Windows (IBM Corp., Chicago, USA) was used for statistical analysis.

RESULTS

Impact of *Ficus carica* puree administration and intermittent hypoxia induction on lung and body weights

Body weight under basal conditions (after a 7-day adaption period) and during IH induction on days 0 and 7 [Table 1]. Under normal conditions and during IH exposure, no significant differences were observed in body weight among the groups. However, the absolute lung weight was higher in the IH-exposed group than in the NC group, except for the FCP1 and FCP3 groups. The relative lung weight was significantly higher in the PC group than in other groups, whereas the FCP intervention group had a relative lung weight similar to the NC group.

Protective role of *Ficus carica* puree against hemoglobin levels induced by intermittent hypoxia

The Hb level was calculated by analyzing the results of the three stages: baseline, pre, and post, as indicated in Table 2. At the baseline stage, no significant difference was observed ($P > 0.05$) in Hb levels between the groups. However, after a 4-week treatment intervention involving FCP consumption, FCP3 showed considerably higher Hb levels than FCP1 and FCP2. Based on the results provided, hypoxia induction did not have a significant effect on Hb levels. However, FCP3 exhibited significantly greater Hb levels than NC, PC, and FCP1, although the difference was not significant when compared with FCP2.

Protective role of *Ficus carica* puree on oxidative stress-related parameters in the lung induced by intermittent hypoxia

Figure 2 shows data on indicators associated with oxidative stress. PC had notable disparities in comparison with other groups, whereas FCP1, FCP2, and FCP3 did not display any major distinctions compared with NC. Regarding the antioxidant enzyme activity parameter SOD, FCP2 exhibited significantly higher SOD activity than PC, and FCP1. Regarding the antioxidant enzyme activity parameter SOD, FCP2 exhibited significantly higher SOD activity than PC, and FCP1 [Figure 3]. Nevertheless, the SOD activity in FCP2 is not significantly different from that in FCP3. The MDA levels in the PC group were substantially higher than those in other groups. Pretreatment with FCP efficiently regulated the MDA levels, reducing them to a nonsignificant level compared with the normal group. No significant differences were observed among the groups that received FCP at varying dosages.

Comparative box plots for SOD/MDA ratio among the groups [Figure 4] and the correlation between lung SOD enzyme activity versus lung MDA concentration is shown in Figure 5. The NC group exhibited a greater SOD/MDA ratio than the other groups, whereas the PC group displayed the lowest SOD/MDA ratio. The difference in SOD/MDA levels between the FCP2 and FCP3 groups was not significant.

DISCUSSION

IH is typically defined as the periodic and alternating occurrence of low oxygen levels (hypoxia) and normal oxygen levels (normoxia). This study induced hypoxia by exposing the subject to a chamber with an atmosphere consisting of 10% oxygen and 90% nitrogen. The rats were exposed to this environment for 4 h/day over 7 days. The decision to choose a 4-h length of hypoxia for the study was influenced by a previous study investigating the impact of hypoxia on oxidative stress. In that study, hypoxia was induced for 4 h over a period of 5 days.[13] The IH duration pertains to the findings of the orientation study conducted to determine the highest resistance level exhibited by the research subjects against the air composition in the chamber, namely, 10% oxygen and 90% nitrogen. During that period, the subjects exhibited indications of organ impairment, characterized by both the urine and blood in their excretions.

This study used SD rats categorized into five groups. Among these groups, three received interventions at varying FCP dosages, namely, low, medium, and high. The remaining two groups served as NC and PC groups. The antioxidant content of fig puree was assessed using 1,1-diphenyl-2-pikrylhidrazyl (DPPH), revealing that FCP contains 54% of antioxidants.[10] A percentage of DPPH of >50% is included in the category of high free radical inhibition.[14] The IH induction in this study is derived from previous studies, showing that subjecting individuals to IH for

Table 1: Effect of *Ficus carica* puree and intermittent hypoxia - induced rats on body weight and relative lung weight (lung index)

	NC	PC	FCP1	FCP2	FCP3
Body weight (g)					
Basal	219.00±16.52[a]	225.50±20.51[a]	230.00±20.22[a]	239.25±8.34[a]	235.33±21.50[a]
During IH induction					
Day-0	223.67±13.58[a]	269.00±9.89[a]	256.75±18.23[a]	272.00±21.56[a]	283.33±22.50[a]
Day-7	263.33±20.60[a]	246.50±7.78[a]	239.75±20.85[a]	269.25±14.80[a]	264.33±14.36[a]
Lung					
Absolute	1.81±0.08[a]	2.60±0.08[b]	1.61±0.10[c]	1.81±0.09[a]	1.59±0.14[c]
Relative ($\times 10^3$)	6.89±0.37[a]	10.57±0.02[b]	6.72±0.61[a]	6.72±0.28[a]	6.03±0.81[a]

Different superscripts in the same column show significant differences ($P \leq 0.05$). The values represent the mean±SD. The ANOVA test was used and continued with the Duncan test. FCP1, FCP2, and FCP3 groups which received FCP with doses of 1.25, 2.5, and 5 mL/200 g BW. NC=Negative control, untreated; PC=Positive control, treated with aquadest; SD=Standard deviation; FCP=*Ficus carica* puree; ANOVA=Analysis of variance; IH=Intermittent hypoxia

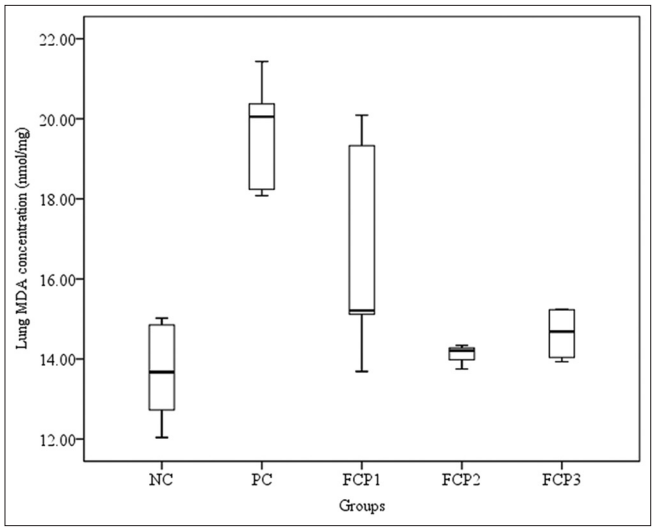

Figure 2: Box plot graph for lung malondialdehyde concentration, representing highest, lowest, and mean values in among group. Negative control, untreated; positive control, treated with aquadest, and *Ficus carica* puree (FCP) 1, FCP2, and FCP3 groups which received FCP with doses of 1.25, 2.5, and 5 mL/200 g BW. Different letters indicate statistical differences among groups. FCP = *Ficus carica* puree, MDA = Malondialdehyde, NC = Negative control, PC = Positive control

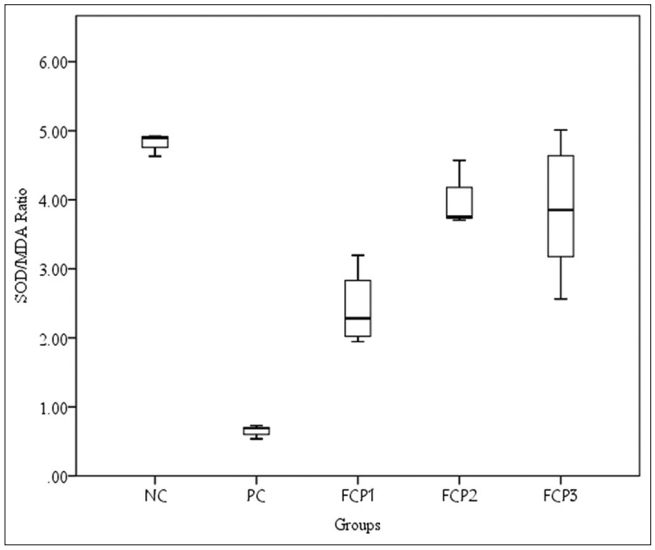

Figure 4: Box plot graph for superoxide dismutase/malondialdehyde ratio, representing highest, lowest, and mean values in among group. Negative control, untreated; positive control, treated with aquadest, and *Ficus carica* puree (FCP) 1, FCP2, and FCP3 groups which received FCP with doses of 1.25, 2.5, and 5 mL/200 g BW. Different letters indicate statistical differences among groups. FCP = *Ficus carica* puree, SOD = Superoxide dismutase, MDA = Malondialdehyde, NC = Negative control, PC = Positive control

4 h/day over 7 days resulted in elevated HIF-1α and HIF-2α levels.[10]

This study examined the preventive effects of FCP consumption and induced hypoxia on lung weight and lung-to-body weight ratio. The PC group exhibited a markedly

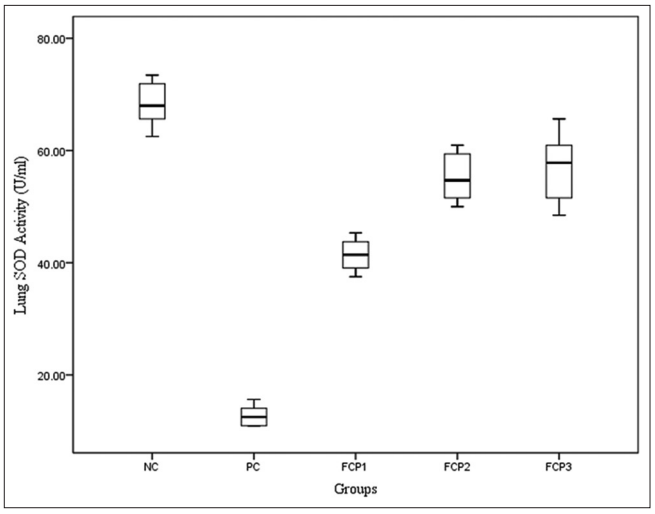

Figure 3: Box plot graph for lung superoxide dismutase activity, representing highest, lowest, and mean values in among group. Negative control, untreated; positive control, treated with aquadest, and *Ficus carica* puree (FCP) 1, FCP2, and FCP3 groups which received FCP with doses of 1.25, 2.5, and 5 mL/200 g BW. Different letters indicate statistical differences among groups. FCP = *Ficus carica* puree, SOD = Superoxide dismutase, NC = Negative control, PC = Positive control

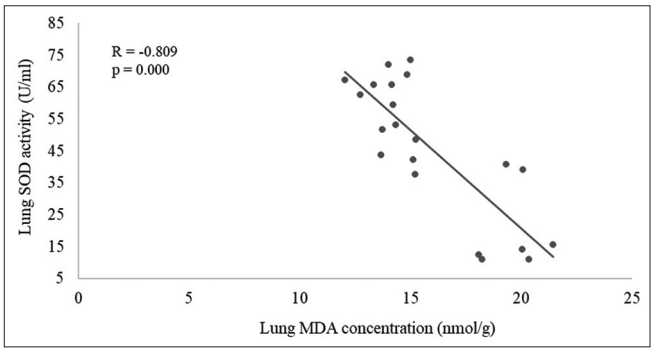

Figure 5: Correlation analysis of lung superoxide dismutase enzyme activity versus lung malondialdehyde concentration (Pearson's correlation). SOD = Superoxide dismutase, MDA = Malondialdehyde

elevated lung-to-body weight ratio compared with other groups. The study findings align with the other research that is indicating that hypoxia in normobaric settings leads to an elevated lung-to-body weight ratio compared with the normal control group. [12]

Figs contain antioxidant chemicals that can enhance and sustain a stable Hb state in rats, even when subjected to IH. The findings align with those of previous studies, indicating that rats administered figs demonstrated elevated hematological parameters, including red blood cells and Hb levels[15] due to the presence of micronutrients, specifically iron complexes, in the fig content. Previous studies reported that the iron (Fe) level in figs is approximately 27.8–40.0 ppm.[16] Based on the average Hb levels of all groups, each sample had normal Hb levels, indicating that previous studies reported varied typical Hb

Table 2: Hemoglobin concentration of normal rats, intermittent hypoxia - induced rats, and aquadest or *Ficus carica* puree - administration rats

Groups	Sampling stages			Fold change (post/pre)
	Baseline	Pre	Post	
NC	13.73±0.60[a]	13.91±0.31[b,c]	13.23±0.82[a]	0.91±0.05[b]
PC	10.12±2.97[a]	14.22±0.61[b,c]	13.46±1.21[a]	0.94±0.05[b]
FCP1	12.52±3.15[a]	13.47±0.47[a,b]	13.42±0.97[a]	0.99±0.08[b]
FCP2	13.62±0.86[a]	12.23±2.83[a]	14.10±0.67[a,b]	1.10±0.11[a]
FCP3	12.72±2.88[a]	15.41±0.53[c]	14.77±0.75[b]	0.96±0.04[b]
P	0.121	0.009*	0.038*	0.003*

*A significant difference between groups in each column is shown by a *P* value ≤ 0.05. Different superscripts in the same colums show significant differences ($P \leq 0.05$). The values represent the mean±SD. The ANOVA test was used and continued with the Duncan test. FCP1, FCP2, and FCP3 groups which received FCP with doses of 1.25, 2.5, and 5 mL/200 g BW. NC=Negative control, untreated; PC=Positive control, treated with aquadest; SD=Standard deviation; FCP=*Ficus carica* puree; ANOVA=Analysis of variance

levels of male Sprague–Dawley rats between 10.4 and 16.5 g/dL;[17] furthermore, IH does not induce anemia, consistent with previous study findings showing no alteration in Hb levels after IH exposure.[18]

The pulmonary MDA levels in the control group exhibited elevated levels in the PC group compared with the other groups. Elevated MDA concentrations in the pulmonary system indicate that IH induces oxidative stress. Consistent with a previous study, IH can stimulate ROS production and trigger an oxidative stress reaction.[19] The IH induction group, which received medium and high FCP doses before the experiment, did not exhibit any significant difference in MDA levels compared with the normal group. However, the group exposed to IH without FCP intervention showed considerably higher MDA levels than the other groups.

SOD is a crucial antioxidant enzyme that helps prevent free radical reactions in the body by neutralizing free radicals and promptly repairing tissue damage. Combating ROS involves the use of both endogenous enzymatic antioxidants and exogenous nonenzymatic antioxidants. Enzymatic antioxidants help convert lipid metabolism products into H_2O_2, whereas nonenzymatic antioxidants prevent and halt free radical reactions[20] The negative correlation between the lung MDA concentration and lung SOD enzyme activity suggests that the reduction in SOD activity could contribute to the lipid peroxidation level. MDA is a byproduct of lipid peroxidation, whereas SOD is a key component of the body's antioxidant defense mechanism that helps eliminate ROS. These markers indicate the oxidative stress level in the body. This study demonstrates a negative association between SOD and MDA in the lung, indicating that IH induction can harm lung tissues. During hypoxia, lung cells trigger pulmonary vasoconstriction to enhance gas exchange through several mechanisms. Multiple studies have confirmed that the mitochondria play a crucial role in enhancement. ROS communication in hypoxic conditions.[21] Consistent with a previous study, hypoxia exposure can lead to an increased ROS from the mitochondria, including NADPH oxidase, xanthine oxidase/reductase, and the NO synthase enzyme, which can initiate an inflammatory response in the lungs. The mitochondria are a place where ROS, particularly superoxide anions, are continuously produced, primarily in complexes I and III of the mitochondrial electron transport chain.[22]

CONCLUSION

Antioxidant compounds in FCP can reduce the IH effects on the lungs by lowering lung MDA levels and increasing SOD levels. The most effective FCP dose for combating oxidative stress in the lungs is 2.5 mL/200 g of body weight. Further evaluation of this antioxidant mechanism of FCP can provide valuable insights into managing IH-induced oxidative stress.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, AM, upon reasonable request.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

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