Antioxidative Parameters Improvements on Nutritional Approach: A Study on Hypoxic Multiple Organs of Sprague-Dawley

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1. Introduction

Recent investigations have shown that hypoxia causes the formation of reactive oxygen species (ROS) in the brain (Chen et al. 2018; Bhattacharjee et al. 2022), kidneys (Farias et al. 2012; Zhang et al. 2021), and colon (Aviello and Knaus 2017; Zhao et al. 2020). The reoxygenation phase that occurs under intermittent hypoxia (IH) conditions is also thought to contribute to the formation of ROS and affect the physiological response of the body (Gonchar and Mankovska 2017; Bartman et al. 2021). The generation of ROS and the antioxidant system have a natural physiological equilibrium, particularly through superoxide dismutase (SOD) (Checa and Aran 2020). An imbalance in the creation and clearance of ROS might result in oxidative stress. Hypoxia alters the activity of the cytochrome chain responsible for mitochondrial oxidative phosphorylation, resulting in a decrease in adenosine triphosphate (ATP) generation, an increase in ROS, and a decrease in the activity of cellular antioxidant mechanisms (Ham and Raju 2017). The kidneys are particularly vulnerable to ROS damage due to the high concentration of long-chain polyunsaturated fatty acids in renal lipids (Ozbek 2012). Because the heart has less antioxidant capacity than other tissues, it is also prone to ROS damage (e.g., the liver), and high amounts of ROS can impair cellular signaling pathways in the cardiovascular system by depleting endogenous antioxidant defenses (Varricchi et al. 2018). Additionally, the colon is a major generator of ROS due to its constant contact with external chemicals and microbial infections. Long-term exposure to ROS triggers a variety of intestinal

ABSTRACT

In various organs, such as the heart, kidneys, and colon, hypoxia enhances the generation of reactive oxygen species (ROS). However, the effects of reoxygenation, as occurs in intermittent hypoxia (IH) to achieve full recovery of hypoxic organs, are not yet clear. The acclimatization response can boost blood oxygen transport capacity, while hypoxia ROS can impact erythrocytes and plasma behavior, resulting in poor peripheral blood flow. This study aimed to study the antioxidant impact of puree Ficus carica (PFC) in rats with IH-induced oxidative stress. Twenty-nine Sprague-Dawley rats were randomly divided into five groups: group N (control, untreated and not exposed to IH, and Group HC was exposed to hypoxia and received distilled water. Group HPF-6.25, HPF-12.5, and HPF-25 (n = 6) received PFC with doses 6.25; 12.5; and 25 ml/kg/d, respectively for 4 weeks before IH exposure. At the end of 4 weeks, all animals except controls were exposed to IH (10% O2 and 90% N2; 4 hours/day for one week). Hematological parameters were measured with several oxidative stress indicators. Hypoxic rats exhibited substantially higher hemoglobin, hematocrit, and mean corpuscular hemoglobin concentrations. All groups exposed to IH showed increased malondialdehyde (MDA) and decreased superoxide dismutase (SOD) activity in the heart, kidneys, and colon. The increasing MDA and decreasing SOD compared to controls and pre-treatment using PFC had a dose-dependent protective effect on the heart, kidneys, and colon.
disorders, including irritable bowel disease (IBD), enteric infections, ischemic intestinal damage, and colon cancer (Wang et al. 2020). Repeated periods of hypoxia and reoxygenation, such as in IH, are common since they often occur in a wide range of clinical diseases. The key contributors to ROS generation and oxidative stress development are assumed to be oxygen depletion during hypoxia and following the reoxygenation (Gonchar and Mankovska 2017).

Oxidative stress can have an impact on the function of blood cells, the coagulation system, and the lipid profile (Danesh et al. 2022). Acclimatization occurs at the cellular, molecular, and systemic levels to maintain critical processes during acute and chronic hypoxia. Increased oxygen transport throughout the circulation is one outstanding physiological response to hypoxia. Acute hypoxia causes a 10% drop in plasma volume (PV) within hours of arrival at high altitude. However, red cell volume (RCV) remains constant (Sawka et al. 2000), decreased oxygen availability promotes the release of erythropoietin (EPO), a glycoprotein controlling red blood cell formation; while a few minutes of hypoxia exposure stabilizes hypoxia-inducible factor-1, activating EPO gene transcription and production; consequently, serum EPO concentrations rise for several hours and result in the formation of reticulocytes, which develop into red blood cells in 5–6 days (Nagel et al. 2020).

Ficus carica (F. carica) is one of the first known medicinal plants to cure internal and exterior illnesses. The fruits, bark, leaves, twigs, young shoots, and latex of F. carica have all been used as traditional therapies for tumors and inflammation-related illnesses (Meziant et al. 2022). According to reports, dried and fresh F. carica are high in amino acids, carbohydrates, sugars, fibers, minerals (copper, manganese, magnesium, potassium, and calcium), vitamins, organic acids, and phenolic compounds. Secondary metabolites generated by plant cells are thought to be responsible for the therapeutic effects of plant extracts and have been extensively examined for various biological and pharmacological activities (Petkova et al. 2019).

The antioxidant activity of tryptophan in vivo is related to the radical scavenging activity of certain tryptophan metabolites or the activation of antioxidative systems in the body following tryptophan supplementation rather than the radical scavenging activity of tryptophan molecules. Some tryptophan hydroxylated metabolites (such as serotonin, 3-hydroxykynurenine, and xanthurenic acid) exhibit radical scavenging activity, but tryptophan does not (Xu et al. 2018). Many studies have determined that tryptophan metabolites, particularly melatonin, have antioxidant effects. Melatonin can eliminate free radicals and enhance antioxidation activity by increasing the production of antioxidant enzymes, such as SOD (Xu et al. 2017) However, there are not many studies related to the role of total phenolic and tryptophan amino acid content in puree F. carica (PFC) as a preventive measure to increase antioxidant defenses in overcoming excessive ROS production during hypoxia followed by reoxygenation in IH. Accordingly, this study aimed to evaluate the effect of PFC on hematology parameters and oxidative stress caused by IH conditions in the heart, kidneys, and large intestines from a biochemical perspective. In this article, we also discuss the possible mechanisms of PFC’s antioxidant preventive effects and their interactions.

2. Materials and Methods

2.1. Preparation of PFC

F. carica cv. Jordan, grown in Bogor, West Java, Indonesia, was used in this research. The fresh F. carica was weighed, sliced, crushed, blended, and homogenized with a homogenizer (Armfield L4R, 8033 rpm, 20 min) into a puree, with a soluble solid content of 10.75±0.070 Brix, then stored at −4°C until usage.

2.2. Active Compound, Antioxidant Activity, and Proximate Analysis

High-Performance Liquid Chromatography (HPLC) was used to analyze the amino acid composition, except for tryptophan. To prepare a standard solution for tryptophan, 25 mg standard L-tryptophan was dissolved in 10 ml aqua bidest. As much as 0.3 ml HCl was added and sonicated for 15 minutes before diluting to 25 ml with aquabidest. 1 ml stock solution was diluted to 10 ml with aqua bidest and properly mixed. From the stock solution, individual working standard solutions of eight concentrations were made (Kusharto et al. 2018). Although free amino acids are often examined without hydrolyzing the material, acid hydrolysis (typically 100-120°C, 6N HCl, and 22–24 h) is required to estimate total amino acids in a protein, since tryptophan is unstable under these circumstances (Çevikkalp et al. 2016).
The Hypoxia Control (HC) group (n = 5) was exposed to hypoxia and received distilled water. Group HPF-6.25 (n = 6), HPF-12.5 (n = 6), and HPF-25 (n = 6) received pre-treatment of PFC with doses 6.25, 12.5; and 25 ml/kg/d, respectively, for 4 weeks before IH exposure. The water and the PFC administration in varied doses was done orally by force-feeding, conducted at 07:30 a.m. daily for four weeks, except for the Controls (Group 1). Body weights were measured weekly.

2.4. Hypoxia Induction
Rats were given IH as per the protocol suggested by Saxena et al. (2020) with some modifications, in which the rats were placed into a chamber having dimensions 25 cm × 15 cm × 15 cm. Briefly, the chamber was manufactured using a laser cutting machine and an acrylic sheet with a thickness of 3 mm, and the rats were under hypoxic condition (O₂ 10% and N₂ 90%) for 4 h/d for 1 week.

2.5. Plasma Collection
Blood samples (~1 ml) were taken from retroorbital veins and collected from rats in EDTA-coated vacutainer tubes (BD Bioscience, Franklin Lakes, NJ, USA). Furthermore, To separate plasma, vacutainer tubes containing blood were centrifuged at 1,200 g for 25 minutes at 4°C (Sigma 3K-30).

2.6. Hematological Analysis
The total number of white blood cells (WBC), total red blood cell (RBC) count, hemoglobin content (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), and platelets (PLT) were all determined using an automated hematology analyzer (Hematology Analyzer Sysmex KX-21) (Corporation, Sysmex 2023).

2.7. SOD Activity in the Heart, Kidneys, and Colon in Response to IH
The colon was the section of the gut studied in this investigation. SOD activity was quantified by SOD assay kit-WST (Sigma) (Kaushik et al. 2018). The reduction rates with oxygen are proportional to the activity of xanthine oxidase (XO), which is suppressed by SOD. Plasma samples were utilized to analyze SOD activity by the WST kit procedure. The plate’s absorbance was measured at 440 nm (SpectraMax). Each sample’s SOD activity was estimated as follows:

$$\text{SOD activity (Inhibition rate %)} = \frac{(\text{Blank 1} - \text{Blank 3}) - (\text{Sample} - \text{Blank 2}) \times 100}{\text{(Blank 1-Blank 2)}}$$
results revealed that the concentration of tryptophan was 402.17 mg/L PFC.

3.2. Effect of PFC Consumption on Hematological Parameters

Figure 2 shows the variables in the study groups and a comparison between them using the ANOVA test. According to the results, the hypoxic exposure group had significantly higher RBC, Hb, HCT, and PLT concentrations than the Normal Control (NC) group. There were no significant differences in the MCV, MCH, and MCHC parameters between groups. In the WBC parameter, the group with a PFC dose of 12.5 mg/kg/day showed significantly higher concentration than the other groups but not significantly higher than those with a PFC dose of 25 mg/kg/day.

3.3. Effect of PFC Consumption on Heart and Gut-Weight/Body Weight Ratio

Body and organ weights measured in rats are shown in Figure 2. The organs were weighed after treatment with PFC for 30 days and IH exposure to obtain comparable data. Exposure to hypoxia affected significantly lower body weight values compared to the NC group, and the presence of pretreatment with PFC affected body weight values that were not significantly different compared to the standard group. However, this study did not show any differences in the relative weight of the heart, kidneys, and colon in all groups.

3.4. Effect of PFC Consumption on Oxidative Stress Parameters

The MDA concentration in the HPF-25 group was significantly lower than the HC group, and the MDA concentrations in the NC, HPF-6.25, and HPF-25 groups showed no significant difference (Table 2). When compared to the NC, there was a significant increase in MDA concentration after HPF treatment.
Figure 1. HPLC chromatogram of standard tryptophan (1A) and PFC (1B)
Figure 2. Effect of IH exposure on hematological parameters. Rats in all groups, except groups N and HC, were pretreated with PFC for 30 days. Values are mean ± SD. WBC (White Blood Cell), RBC (Red Blood Cell), Hb (Hemoglobin), HCT (Hematocrit), PLT (Platelet), MCH (Mean Corpuscular Hemoglobin or mean corpuscular volume), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume). Different letters indicate that there are significant differences according to the ANOVA (P<0.05).
but a significant increase occurred in the HC and HPF-6.25 groups, which were 1.18-fold (18% increase) and 1.29-fold (29% increase), respectively. The decrease in MDA concentration occurred in the HPF-12.5 and HPF-25 groups, which were 18% and 23%, respectively, and did not differ significantly from the NC.

The concentration of MDA and antioxidant enzyme (SOD) in normal and IH-induced rats were found in the tissues used. Significant increases in MDA and a decrease in SOD were found in the heart, kidneys, and colon tissues of IH-induced rats groups as compared to the normal group (Table 3). Treatment of PFC before IH significantly reduced the MDA in all tissues and increased the activities of SOD dose-dependently. In summary, the heart, kidneys, and colon were responsive to IH exposure. PFC caused a significant increase in antioxidant activity with a corresponding decrease in MDA concentration (Table 4).

Figure 3 shows that PFC administration significantly affects the SOD/MDA ratio, which is proportionate to the increase in PFC doses in rats exposed to IH. The IH-exposed rats had a considerably greater value ratio than the NC group.

Table 2. Preventive effects of Ficus carica puree administration on MDA level in plasma rats after hypoxia induction

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA level (nmol/ml)</th>
<th>Fold changeFinal/Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>NC</td>
<td>515.57±70.46</td>
<td>377.42±172.65</td>
</tr>
<tr>
<td>HC</td>
<td>433.44±80.47</td>
<td>506.09±52.64</td>
</tr>
<tr>
<td>HPF-6.25</td>
<td>377.44±62.26</td>
<td>486.43±90.21</td>
</tr>
<tr>
<td>HPF-12.5</td>
<td>459.74±119.09</td>
<td>383.03±103.60</td>
</tr>
<tr>
<td>HPF-25</td>
<td>440.88±55.06</td>
<td>289.78±55.61</td>
</tr>
</tbody>
</table>

Values were expressed in Mean ± SD (n = 5-6/group).

Superscripts (a, b, c) for a particular variable in the same column, mode means with different superscript are significantly (P<0.05) different.

Mode means with same superscripts are not significantly (P>0.05) different.

NC : Normal control
HC : Hypoxic control
HPF-6.25 : Group treated with 6.25 g/kg BW/day PFC before hypoxia exposure
HPF-12.5 : Group treated with 12.5 g/kg BW/day PFC before hypoxia exposure
HPF-25 : Group treated with 25 g/kg BW/day PFC before hypoxia exposure

Table 3. Effect of consuming PFC on antioxidant status and lipid peroxide in healthy and IH-induced rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (nmol/g)</th>
<th>SOD (inhition rate, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Kidney</td>
<td>Colon</td>
</tr>
<tr>
<td>NC</td>
<td>1.99±0.11</td>
<td>1.85±0.12</td>
<td>1.42±0.10</td>
</tr>
<tr>
<td>HC</td>
<td>11.07±0.10</td>
<td>10.83±0.09</td>
<td>9.93±0.37</td>
</tr>
<tr>
<td>HPF-6.25</td>
<td>6.94±0.14</td>
<td>6.81±0.14</td>
<td>6.26±0.17</td>
</tr>
<tr>
<td>HPF-12.5</td>
<td>3.97±0.09</td>
<td>3.83±0.05</td>
<td>3.12±0.12</td>
</tr>
<tr>
<td>HPF-25</td>
<td>3.14±0.08</td>
<td>2.97±0.08</td>
<td>2.44±0.10</td>
</tr>
</tbody>
</table>

Values were expressed in Mean ± SD (n = 5-6/group)

Superscripts (a, b, c) for a particular variable in the same column, mode means with different superscript are significantly (P<0.05) different.

Mode means with same superscripts are not significantly (P>0.05) different.

NC : Normal control
HC : Hypoxic control
HPF-6.25 : Group treated with 6.25 g/kg BW/day PFC before hypoxia exposure
HPF-12.5 : Group treated with 12.5 g/kg BW/day PFC before hypoxia exposure
HPF-25 : Group treated with 25 g/kg BW/day PFC before hypoxia exposure
4. Discussion

The major findings in the present study demonstrated that the puree *Ficus carica* (PFC) contains potent radical scavenging phenolic and flavonoid compounds. In addition, the presence of the amino acid tryptophan and dietary fiber also have the potential as antioxidants and anti-inflammation agents to reduce the hypoxia effect in the tissues important in the treatment of recovery from hypoxia. The percentage of PFC is 54%, where the percent reduction of DPPH radicals >50% is included in the category of high free radical inhibition (Wulansari et al. 2011). Since dietary fiber and the amino acid tryptophan are abundant in the PFC, the potential is significant for these two components to have a health benefit as a preventive effort in overcoming the effects of hypoxia on the heart and intestinal tissues (Ullah et al. 2020).

The kidney has an important role in maintaining a healthy and regulated fluid balance, salt concentration, acid-base balance in the blood, and excretion of waste materials such as urea and waste other nitrogen in the blood. The kidney is sensitive to hypoxia, and regardless of the underlying etiology, renal hypoxia is a frequent final route to end-stage kidney disease. Kidney hypoxia exacerbates oxidative stress, while increased oxidative stress exacerbates renal hypoxia (Honda et al. 2019). Furthermore, recent epidemiological studies have associated a flavonoid-rich diet with a lower prevalence of kidney disease. It was found that the major flavonoids in Ficus are quercetin and luteolin, with a total of 631 and 681 mg/kg extract, respectively (Vaya and Mahmood 2006). Similarly, in the current investigation, the dosage-dependent tests revealed that the optimal dose of quercetin supplementation gave the least kidney impairment at 50 mg/kg BW compared to other tested doses after 12 hours of hypobaric hypoxia exposure (Rathi et al. 2023).

When the ability of antioxidant defense systems to buffer ROS surpasses the ability of ROS formation, oxidative stress occurs, resulting in cellular and molecular problems and, finally, heart failure. Oxidative stress is a major contributor to the development of hypoxia and ischemia-reperfusion-related cardiovascular diseases (Kurian et al. 2016; Ferretti et al. 2020). Flavonoids, in particular, have antiplatelet, antioxidant, and anti-inflammatory properties and the ability to modulate endothelial cells. Furthermore, flavonoids' bioactivity includes vascularization reduction and vasodilation promotion. Finally, flavonoids have been shown to influence important cardiometabolic risk factors such as body weight, lipid profile, blood glucose, blood pressure, and metabolic syndrome (Valaei et al. 2021).

Since the exposures to IH are accompanied by body weight reduction (Gozal et al. 2017), our current
study indicates that the IH exposure in the group without pretreatment with PFC shows a significantly smaller bodyweight value compared to controls. Still, the presence of PFC pretreatment before IH exposure can offset the impact of weight loss caused by IH. Weight loss due to hypoxic conditions has been previously studied. Several factors that cause this to happen are increased metabolic rate, decreased energy use, and decreased nutritional intake due to reduced appetite, in addition to decreased body weight when hypoxic conditions are caused by the use of adipose tissue as an energy source, due to reduced food intake (Matu et al. 2018). In an F. carica study without involving IH exposures, it was found that consuming F. carica extracts at doses of 50 and 100 mg/kg affected weight gain (Ain et al. 2022). In this study, it is believed that there was some effect on weight gain, but the presence of IH exposure makes the body focus on maintaining body condition so that it remains constant during the IH exposure. Repeated exposure to IH in 7 days in rats did not lead to a change in organ weight. However, the results of this study are not in line with the results of another study, which stated that hypoxia makes the size of organs such as the liver, kidneys, and brain smaller compared to not being exposed to hypoxia on postnatal day 2 with an O2 level of 11% for four weeks (Farahani et al. 2008). This may be due to the shorter duration of IH in this study not affecting the organ weight of the rats.

The hematological parameters for the five experimental groups were within the range of the results previously found in the Sprague-Dawley rats (Rosidah et al. 2020). The most remarkable adaptations to acclimatization are those observed in the hematological parameters. The significant increases in RBC, HCT, and Hb concentration in group-induced IH exposure are associated with an enhancement of blood oxygen transport capacity. As a result, IH may stimulate erythropoiesis in the rats to the same level as chronic hypoxia. The iron mineral component in figs also contributed to higher Hb concentration in the PFC-administered group compared to controls. It has been found that the most predominant mineral in figs is iron, and fresh F. carica contains 6.69-10.09 mg/100 g (Khan et al. 2011). Both iron and protein are required for Hb production. Only small quantities of iron are required in the daily diet to restore the tiny amounts lost through urine and feces elimination (Al-Jowari et al. 2011). During the Hb breakdown process, iron is recycled by macrophages in the liver and spleen, and the globin component is broken into amino acids, which are subsequently utilized to create other proteins. The iron is liberated and can be used in the bone marrow to manufacture new Hb molecules. As a result, the PFC’s trace amounts of iron and protein may have contributed to the significant increase in Hb content and PCV.

The presence of phenolic compounds in F. carica that act as immunostimulatory agents may explain the increase in WBC concentration in the hypoxic-induced rat group that had previously been given PFC for 30 days. The immunostimulant activity activates the lymphocytic system and increases WBC production by stimulating the body’s macrophage response (Vigila and Baskaran 2008). The changes in WBC concentration were within the normal range as follows: 6.93-20.48 ×10^3/µL (Rosidah et al. 2020). The rise in leukocyte concentration outside of the normal range, on the other hand, may imply that blood cell synthesis increases to counteract the toxin attack from digestion and foods in the diet since leukocytes are known to be among the body’s defense systems that fight against the pathogenic organism.

Regarding PLT concentration, several research findings reveal inconsistencies in the effect of IH exposure on PLT. Reactivity of PLT is enhanced in rats after 6 hours of acute hypoxia and in mice after three weeks of chronic hypoxia (Tyagi et al. 2014). In the results of this study, overall, the hypoxic exposure group showed significantly higher PLT concentration compared to the controls. This might be because hypoxia has been associated with direct coagulation activation, a higher risk of thrombotic events, and enhanced PLT reactivity in rats (Kiers et al. 2019). Previous research has shown that the effect of hypoxia on PLT parameters is dependent on hypoxia exposure, which is consistent with our findings. Short-term hypoxia has been shown to increase thrombocytosis; however, PLT concentrations quickly fell between the fifth and ninth days of hypoxia, eventually leveling out at half their usual amount (Alvarez-Martins et al. 2016). ROS produced by IH exposure is critical in controlling PLT activity and mitochondrial organelles. PLT activation causes significant changes in platelet redox balance and metabolism; PLT-derived ROS, in turn, increases ROS generation and, as a result, PLT activation, adhesion,
and attenuation in an automatic reinforcement loop. This vicious cycle leads to a procoagulant phenotype and PLT death, which contribute to the elevated thrombotic risk associated with oxidative stress-related illnesses (Masselli et al. 2020). As a result, in addition to systemic inflammation, hypoxia in critically ill patients may contribute to reduced PLT function and coagulopathy, increasing their risk of organ failure.

To maintain ROS homeostasis, the body contains a fundamental antioxidant defense system against ROS imbalance: endogenous enzymatic antioxidants and endogenous non-enzymatic antioxidants. Endogenous enzymatic antioxidants such as SOD, catalase, and others. Glutathione, thioredoxin (Trx), and irisin are endogenous non-enzymatic antioxidants. As exogenous antioxidants, critical nutrients and dietary supplements, such as PFC, play a crucial function in the antioxidant system (Wang et al. 2020). One of the most important antioxidant defense systems identified in both tissues and blood is SOD metabolism. SOD is an antioxidant that safeguards the heart against ischemia and the lungs against inflammation and fibrosis (Corrine R Kliment et al. 2009).

PFC contains tryptophan (Badgujar et al. 2009), which functions as an antioxidant molecule that collaborates with other amino acids, such as histidine and lysine, to boost Glutathione S-transferase activity (GST). It is known to play a key role in detoxifying and reducing ROS and could stimulate antioxidant metabolism (Gaafar et al. 2022). In which tryptophan is the raw material for serotonin synthesis, the antioxidant mechanism might be caused by the presence of phenolic hydroxyl groups from serotonin, which acts as antioxidant compounds in the mechanism of radical scavenging activity with a high content of active antioxidant substances (Nurrahma et al. 2021; Meliala et al. 2022). We report the generation of ROS as an increase in lipid peroxidation product (MDA) and the decrease of antioxidant enzymes (SOD) in the heart, kidneys, and colon. In support of the hypotheses under consideration, 7 days of IH (4h/d) induced an increase in oxidative stress with changes in antioxidant enzyme activities, suggesting that ROS were overproduced during the IH phase of the process. To the best of our knowledge, this is the first in-vivo study of PFC pretreatment in IH (4h/d for 7 days)-induced oxidative stress in rats. An imbalance between the creation of ROS and antioxidant activity causes oxidative stress. As a result, the absence of change in antioxidant enzyme effectiveness suggests that an excess of ROS generation causes the oxidative stress seen after seven days of IH.

It has been demonstrated that animal bodies have an efficient method for preventing free radical-induced tissue cell damage, which is done by endogenous antioxidant enzymes such as SOD (Noeman et al. 2011). SOD is an important oxygen radical scavenger, while MDA is a byproduct of lipid peroxidation (Qaid et al. 2017). The SOD/MDA ratio may reflect the extent to which cells were harmed by free radicals and antioxidant capacity (Zhu et al. 2019). The results showed that the ratio of SOD/MDA was lower in hypoxia condition and increased with pre-treatment of PFC dose-dependently, which suggested that an enhanced antioxidative response appeared in rats while in IH exposure and that there was a protective mechanism underlying the body's ability to reduce subsequent cell damage. The immunostimulant action activates the lymphocytic system and boosts WBC production by stimulating the body's macrophage response (Vigila and Baskaran 2008). The changes in WBC count were within the normal range ($6.93-20.48 \times 10^3/\mu L$). An increase in leukocyte counts outside the normal range, on the other hand, may indicate that blood cell synthesis increases in an attempt to counteract the toxin attack from digestion and food in the diet because leukocytes are known to be among the body's defense systems that fight against non-self or pathogenic organisms.

The results obtained evidence that the puree Ficus carica with dose 25 ml/kg/d can effectively protect the body from reactive oxygen species generated during the reoxygenation phase of intermittent hypoxia (IH) exposure. This immunogenic effect may contribute to the adaptation process in blood hematological conditions to be able to survive the IH conditions, as evidenced by increased concentrations of red blood cells, hemoglobin, platelets, mean corpuscular hemoglobin and hematocrit. Here, we report antioxidant activity in PFC against IH-induced oxidative stress. These findings suggest that consumption of PFS containing bioactive compounds (flavonoids, tryptophan, and dietary fiber) might be beneficial for the preventive strategy for the hypoxia condition.
Acknowledgements

The authors thank the Faculty of Pharmacy and Faculty of Medicine, Public Health and Nursing of Universitas Gadjah Mada for supporting the study by providing the facilities and Mr. Erik C. Hookom BA, TEFL, M.Ed (Senior Consultant) for proofreading assistance.

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