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

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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% O_2 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.

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 (Farías *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

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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; consequentially, 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 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) test was conducted using the prior published procedure (20) with slight modifications. The antioxidant study was conducted on the sample extracts by dissolving 100 L of extracts in 2.9 ml 0.1 mm DPPH. After vibrating it with a vortex to ensure that everything was well-mixed, this solution was allowed to stand for 30 minutes at room temperature. An ultraviolet (UV)/Visible spectrophotometer was used to test the absorbance of the standard against the reagent blank (MeOH 70%) at 517 nm. The percentage inhibition (%) was calculated using the following formula ((A0 (blank) – A1 (sample)) / A0) × 100.

The proximate components of the PFC, which comprised ash, carbohydrates, fat, moisture, protein, and dietary fiber, were analyzed using standard protocols at the Chemistry and Biochemistry Laboratory, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

2.3. Animal Model

This greenhouse experiment was conducted at All animal studies were authorized by the Medical and Health Research Ethics Committee at the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (approval number: KE/FK/0532/EC/2022) and conducted under the institutional rules for the care and use of laboratory animals. Adult male Sprague Dawley rats (220-250 g) were kept in standard cages in a temperature-controlled environment (22-24°C) with a humidity of 40-60%,. A 12-hour dark-12-hour light cycle, food, and drink were freely accessible, and all the rats were housed in standard rat cages with three rats per cage, with the dimension of cage 40 × 30 × 20 cm for keeping the health standard for rats. All animal experiments were performed with utmost care to minimize the suffering of rats.

After a 7-day adaptation period, rats were distributed into five groups: group NC n = 6) was the Normal Control group. The number of rats in each group was the same at the beginning of the study (n = 6) to ensure that they remained within the formula federer range if mortality occurred during the hypoxia induction stage. Still, when hypoxia was induced in group 2 (HC-group), one rat died, but the number of rats in each group was within Federer's sample size range for calculating the number of replications. The number of samples, according to Federer's formula (Rukmana *et al.* 2022), follows the following formula: t(r - 1) > 15, where t = number of treatments, and r = number of replications; 5(r - 1) > 15; r > 4.

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 (O_2 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:

$$\text{SOD activity (Inhibition rate \%)} = \frac{(\text{Blank 1 - Blank 3}) - (\text{Sample - Blank 2}) \times 100}{(\text{Blank 1-Blank 2})}$$

Then, we calculated the ratio of SOD/MDA in the heart, kidneys, and colon, and then the differences between groups were analyzed.

2.8. Measurement of Lipid Peroxidation

The MDA concentration was determined using the Lipid Peroxidation Assay Kit (ab118970; Abcam, Cambridge, UK). The heart, kidneys, and colon were homogenized on ice in 303 L MDA lysis buffer before being centrifuged at 14,000 g for 10 minutes at 4°C. Following that, 200 L of the supernatant was combined with 600 L of the thiobarbituric acid (TBA) solution, incubated at 95°C for 60 minutes, refrigerated on ice for 10 minutes, and 200 L of the mixed solution was placed in a 96-well microplate. The MDA level was calculated using the standard curve at 532 nm and following the manufacturer's specifications (Seenak *et al.* 2020).

2.9. Data Analysis

Fold changes in MDA concentration were determined as the average of the final/initial values and adjusted to percentage changes (i.e., 1.40-fold = 40% increase and 0.60-fold = 40% reduction). All the data had a normal distribution and were reported as mean and standard deviation (SD). A one-way analysis of variance (ANOVA) was used to compare the means of groups. At $p<0.05$, differences were considered significant. Pearson's correlation coefficient was used to analyze the correlation between oxidative stress parameters, with a significance level of <0.05. SPSS 23 for Windows (IBM Corp., Chicago, USA) was used for the statistical analysis.

3. Results

3.1. Bioactive Compounds, Biochemical Compositions, and Antioxidant Capacity of PFC

The DPPH assay method was used to assess the antioxidant capability of PFC. Proximate analysis showed the components of PFC have high moisture content at 87.88 g/100 g, low fat at 0.51 g/100 g, and high dietary fiber at 9.32 g/100 g (Table 1). The DPPH free radical scavenging activity of PFC was investigated in this work to establish its antioxidant activity. These results indicated that dietary fiber and tryptophan compounds were present in the PFC.

The chromatogram of standard tryptophan revealed a prominent peak at a retention time indicated by a similar retention time of the PFC for the assessment of tryptophan content in PFC by HPLC (Figure 1). Our results revealed that the concentration of tryptophan was 402.17 mg/L PFC.

Table 1. The measured bioactive compounds of PFC

	Amount of analytes (mean±SD) (g/100 g of HPF)
Oxidant scavenging (DPPH assay)	54%±0.04
Dietary Fiber	9.32±0.16
Tryptophan (ppm, mg/L)	402.17±0.23
Carbohydrate	9.67±0.115
Fat	0.51±0.059
Moisture	87.88±0.014
Protein (%N × 6.25)	1.20±0.028
Ash	0.75±0.014

3.2. Effect of PFC Consumption on Hematological Parameters

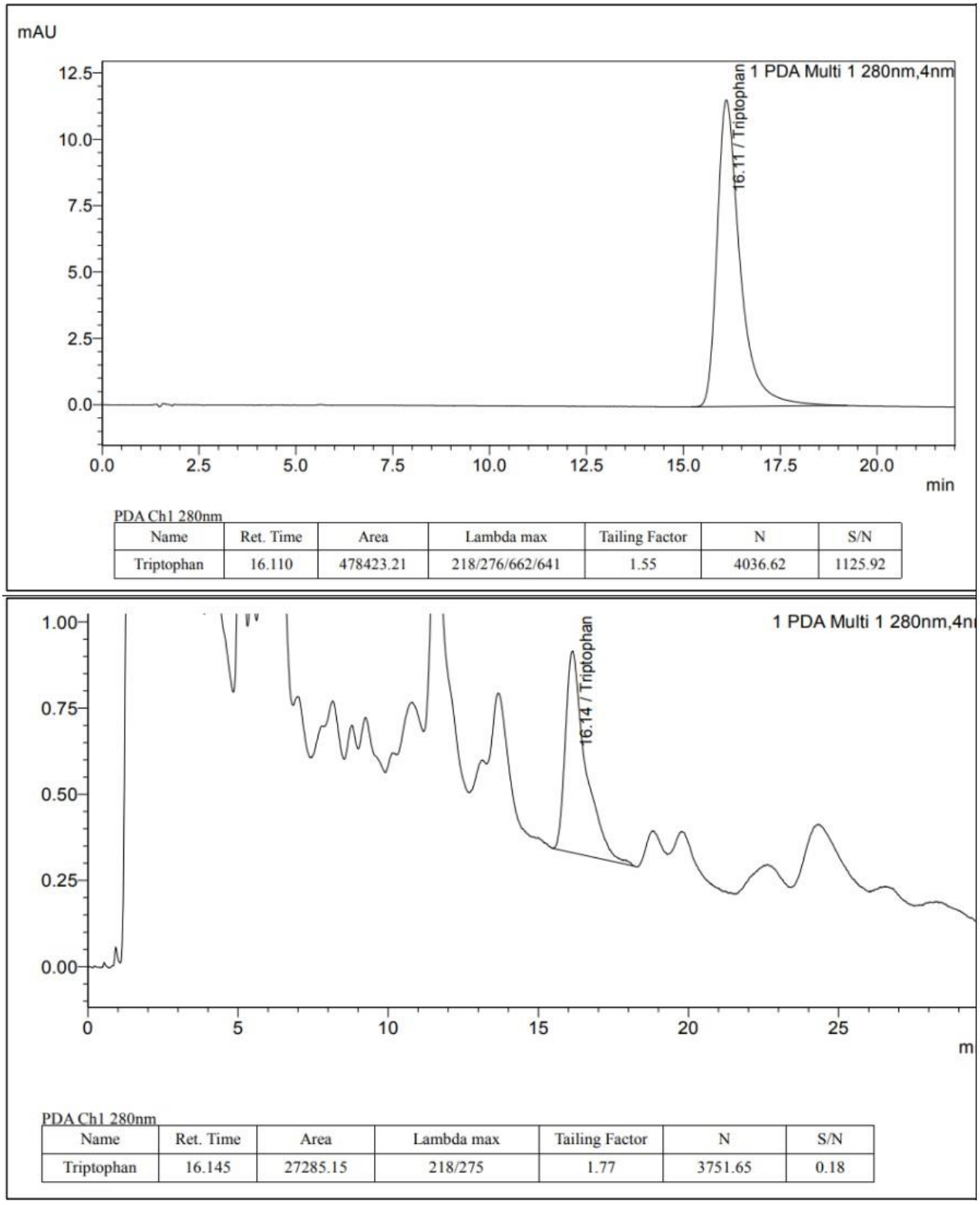
Figure 2 shows the variables in the study groups and a comparison between them using the ANOVA test. According to the results, the hypoxic exposure group had significantly higher RBC, Hb, HCT, and PLT concentrations than the Normal Control (NC) group. There were no significant differences in the MCV, 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



PDA Ch1 280nm

Name	Ret. Time	Area	Lambda max	Tailing Factor	N	S/N
Triptophan	16.110	478423.21	218/276/662/641	1.55	4036.62	1125.92

PDA Ch1 280nm

Name	Ret. Time	Area	Lambda max	Tailing Factor	N	S/N
Triptophan	16.145	27285.15	218/275	1.77	3751.65	0.18

Figure 1. HPLC chromatogram of standard tryptophan (1A) and PFC (1B)

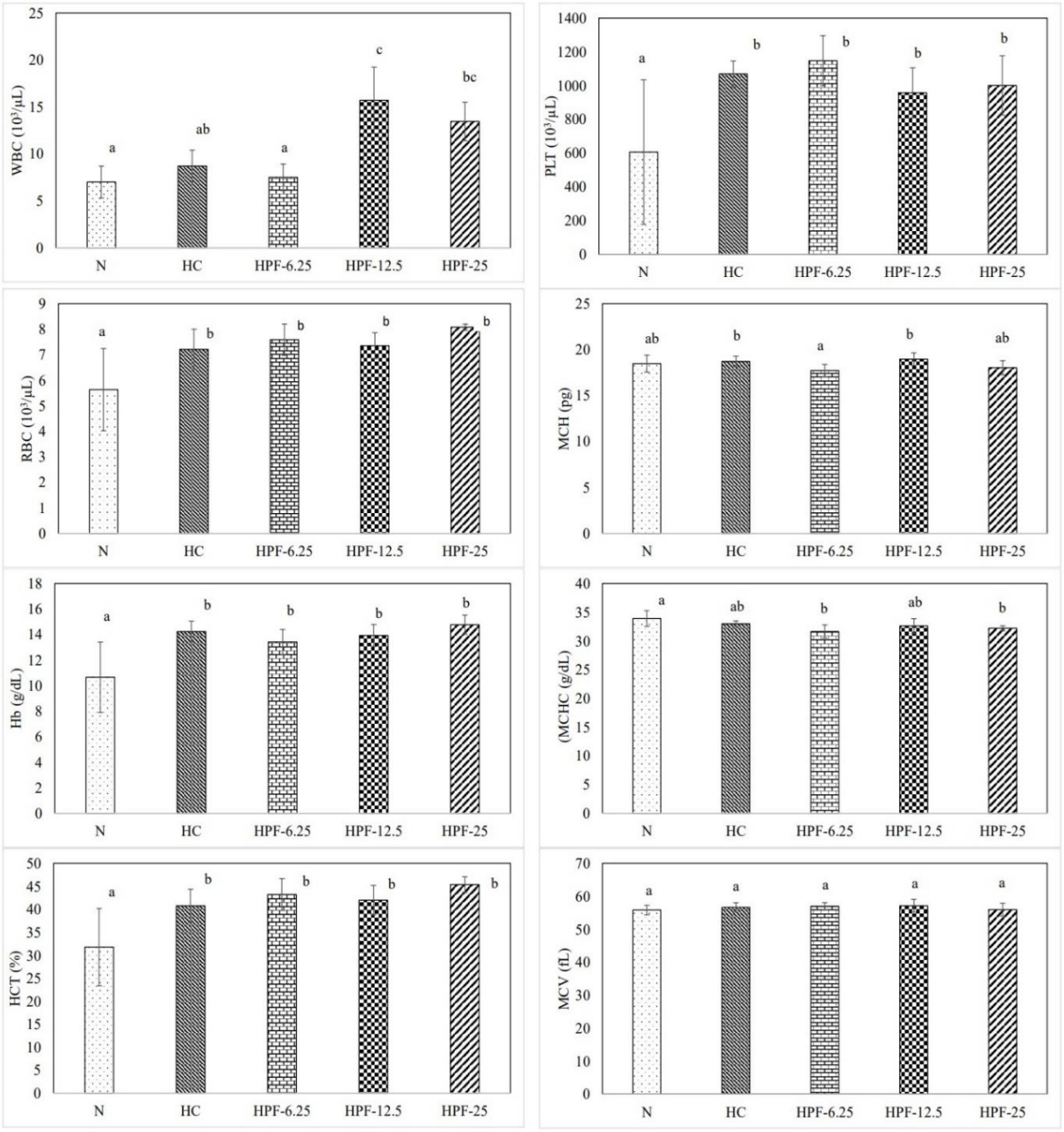


Figure 2. Effect of IH exposure on hematological parameters. Rats in all groups, except groups N and HC, were pretreated with PFC for 30 days. Values are mean ± SD. WBC (White Blood Cell), RBC (Red Blood Cell), Hb (Hemoglobin), HCT (Hematocrit), PLT (Platelet), MCH (Mean Corpuscular Hemoglobin or mean corpuscular volume), MCHC (Mean Corpuscular Hemoglobin Concentration), MCV (Mean Corpuscular Volume). Different letters indicate that there are significative differences according to the ANOVA ($P<0.05$)

(or before exposure to hypoxia), 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 4. Correlation matrix between hematology indices, MDA concentration and SOD activity

	Organ		SOD
MDA Organ	Heart	r	-0.966
		p-value	0.000
	Kidney	r	-0.961
		p-value	0.000
	Colon	r	-0.961
		p-value	0.000

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

Groups	MDA level (nmol/ml)		Fold change$^{Final/Initial}$
	Initial	Final	
NC	515.57±70.46^{a}	377.42±172.65^{ab}	0.72±0.23^{a}
HC	433.44±80.47^{a}	506.09±52.64^{b}	1.18±0.10^{b}
HPF-6.25	377.44±62.26^{a}	486.43±90.21^{a}	1.29±0.04^{b}
HPF-12.5	459.74±119.09^{a}	383.03±103.60^{ab}	0.82±0.21^{a}
HPF-25	440.88±55.06^{a}	289.78±55.61^{b}	0.67±0.17^{a}

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

Groups	Parameters					
	MDA (nmol/g)			SOD (inhition rate, %)		
	Heart	Kidney	Colon	Heart	Kidney	Colon
NC	1.99±0.11^{a}	1.85±0.12^{a}	1.42±0.10^{a}	73.70±5.17^{a}	82.03±2.92^{a}	89.32±4.78^{a}
HC	11.07±0.10^{b}	10.83±0.09^{b}	9.93±0.37^{b}	22.19±5.23^{b}	18.44±3.73^{b}	31.25±2.47^{b}
HPF-6.25	6.94±0.14^{c}	6.81±0.14^{c}	6.26±0.17^{c}	49.74±3.62^{c}	54.43±4.12^{c}	66.67±3.38^{c}
HPF-12.5	3.97±0.09^{d}	3.83±0.05^{d}	3.12±0.12^{d}	63.80±6.66^{d}	71.10±2.92^{d}	75.78±2.92^{d}
HPF-25	3.14±0.08^{e}	2.97±0.08^{e}	2.44±0.10^{e}	69.53±2.92^{e}	74.22±3.88^{e}	80.99±3.88^{e}

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

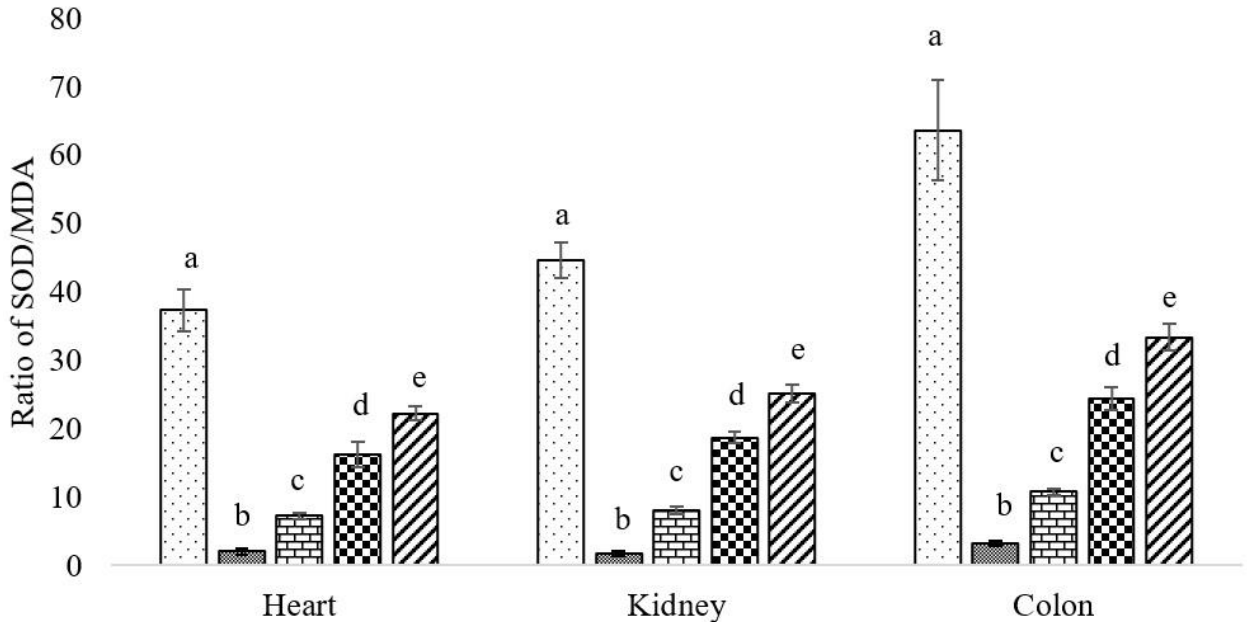


Figure 3. Effect of consuming PFC on the ratio of SOD/MDA

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 O_2 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 $\times 10^3/\mu L$ (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 × 10^3/μL). 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.

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References

Ain, N. ul., Khan, R.A., Mirza, T., Fayyaz, T.B., 2022. The effects of *Ficus carica* on male and female reproductive capabilities in rats. *Evidence-Based Complementary and Alternative Medicine*. 2022, 1–12. https://doi.org/10.1155/2022/1799431

Al-Jowari, S.A., Al-Obaidi, S.A., Yousif, W.H., 2011. Effect of aqueous extract of Fig (*Ficus carica*) fruit on some hematological parameters in female rabbits. *Baghdad Science Journal*. 8, 59–65.

Alvarez-Martins, I., Remédio, L., Matias, I., Diogo, L.N., Monteiro, E.C., Dias, S., 2016. The impact of chronic intermittent hypoxia on hematopoiesis and the bone marrow microenvironment. *Pflugers Archiv European Journal of Physiology*. 468, 919–932. https://doi.org/10.1007/s00424-016-1797-6

Aviello, G., Knaus, U.G., 2017. ROS in gastrointestinal inflammation: rescue or sabotage? *British Journal of Pharmacology*. 174, 1704–1718. https://doi.org/10.1111/BPH.13428

Badgujar, S.B., Patel, V.V., Bandivdekar, A.H., Mahajan, R.T., 2009. Traditional uses, phytochemistry and pharmacology of *Ficus carica*: a review. *Food and Chemical Toxicology*. 52, 1487–1503.

Bartman, C.M., Awari, D.W., Pabelick, C.M., Prakash, Y.S., 2021. Intermittent hypoxia-hyperoxia and oxidative stress in developing human airway smooth muscle. *Antioxidants*. 10, 1–19.

Bhattacharjee, M., Manoharan, S., Deshetty, U. M., Perumal, E., 2022. Acute hypobaric hypoxia exposure causes neurobehavioral impairments in rats: role of brain catecholamines and tetrahydrobiopterin alterations. *Neurochemical Research*. 48, 1–16.

Çevikkalp, S.A., Löker, G.B., Yaman, M., Amoutzopoulos, B., 2016. A simplified HPLC method for determination of tryptophan in some cereals and legumes. *Food Chemistry*. 193, 26–29. https://doi.org/10.1016/j.foodchem.2015.02.108

Checa, J., Aran, J.M., 2020. Reactive oxygen species: drivers of physiological and pathological processes. *J Inflamm Res*. 13, 1057–1073.

Chen, R., Lai, U.H., Zhu, L., Singh, A., Ahmed, M., Forsyth, N.R., 2018. Reactive oxygen species formation in the brain at different oxygen levels: the role of hypoxia inducible factors. *Front. Cell Dev. Biol.* 6, 1–12. https://doi.org/10.3389/fcell.2018.00132

Corporation, Sysmex, S., 2023. Hematology analyzer sysmex KX-21N. Availbale at: https://bioprom.gr/en/product/analytis-sysmex-kx-21/#:~:text=The KX-21N hematology analyzer,use of automatic floating discriminators. [Date accesseed: 6 October 2023]

Corrine R Kliment, Suliman, H.B., Tobolewski, J.M., Reynolds, C.M., Day, B.J., Zhu, X., McTiernan, C.F., McGaffin, K.R., Piantadosi, C.A., Oury, T.D., 2009. Extracellular superoxide dismutase regulates cardiac function and fibrosis. *J. Mol. Cell. Cardiol.* 47, 730–742.

Danesh, H., Ziamajidi, N., Mesbah-Namin, S.A., Nafisi, N., Abbasalipourkabir, R., 2022. Association between oxidative stress parameters and hematological indices in breast cancer patients. *International Journal of Breast Cancer*. 2022, 1-8. https://doi.org/10.1155/2022/1459410

Farahani, R., Kanaan, A., Gavrialov, O., Brunnert, S., Douglas, R. M., Morcillo, P., Haddad, G.G., 2008. Differential effects of chronic intermittent and chronic constant hypoxia on postnatal growth and development. *Pediatric Pulmonology*. 43, 20–28. https://doi.org/10.1002/ppul.20729

Farías, J.G., Zepeda, A.B., Calaf, G.M., 2012. Melatonin protects the heart, lungs and kidneys from oxidative stress under intermittent hypobaric hypoxia in rats. *Biological Research*. 45, 81–85. https://doi.org/10.4067/S0716-97602012000100011

Ferretti, G., D'Oria, R., Schipani, R., Leonardini, A., Natalicchio, A., Perrini, S., Cignarelli, A., Laviola, L., Giorgino, F., 2020. The role of oxidative stress in cardiac disease: from physiological response to injury factor. *Oxidative Medicine and Cellular Longevity*. 2020, 1–29. https://doi.org/10.1155/2020/5732956

Gaafar, R.M., Osman, M.E.A.H., Abo-Shady, A.M., Almohisen, I.A.A., Badawy, G.A., El-Nagar, M.M.F., Ismail, G.A., 2022. Role of antioxidant enzymes and glutathione S-Transferase in bromoxynil herbicide stress tolerance in wheat plants. *Plants*. 11, 2679. https://doi.org/10.3390/plants11202679

Gonchar, O., Mankovska, I., 2017. Hypoxia/reoxygenation modulates oxidative stress level and antioxidative potential in lung mitochondria: possible participation of P53 and NF-KB target proteins. *Archives of Pulmonology and Respiratory Care*. 3, 35–43. https://doi.org/10.17352/aprc.000022

Gozal, D., Khalyfa, A., Qiao, Z., Almendros, I., Farré, R., 2017. Temporal trajectories of novel object recognition performance in mice exposed to intermittent hypoxia. *European Respiratory Journal*. 50, 1701456. https://doi.org/10.1183/13993003.01456-2017

Ham, P.B., Raju, R., 2017. Mitochondrial function in hypoxic ischemic injury and influence of aging. *Prog Neurobiol.* 157, 92–116.

Honda, T., Hirakawa, Y., Nangaku, M., 2019. The role of oxidative stress and hypoxia in renal disease. *Kidney Res. Clin. Pract*. 38, 414–426.

Kaushik, R., Dige, M., Dass, G., Ramachandran, N., Rout, P.K., 2018. Superoxide dismutase activity in response to heat stress in Jamunapari goats. *Indian Journal of Biochemistry and Biophysics*. 55, 39–43.

Khan, M., Sarwar, A., Adeel, M., Wahab, M., 2011. Nutritional evaluation of *Ficus carica* indigenous to Pakistan. *African Journal of Food, Agriculture, Nutrition and Development*. 11, 5187–5202. https://doi.org/10.4314/ajfand.v11i5.70445

Kiers, D., Tunjungputri, R.N., Borkus, R., Scheffer, G.J., de groot, P.G., Urbanus, R.T., van der ven, A.J., Pickkers, P., de Mast, Q., Kox, M., 2019. The influence of hypoxia on platelet function and plasmatic coagulation during systemic inflammation in humans *in vivo*. *Platelets*. 30, 927–930. https://doi.org/10.1080/09537104.2018.1557617

Kurian, G.A., Rajagopal, R., Vedantham, S., Rajesh, M., 2016. The role of oxidative stress in myocardial ischemia and reperfusion injury and remodeling: revisited. *Oxid Med Cell Longev*. 2016, 1–14.

Kusharto, C.M., Rosmiati, R., Marta, E.O., Palupi, E., 2018. Amino acid composition and protein quality of instant liquid food based on Catfish (*Clarias gariepinus*) and Kelor (*Moringa oleifera*) flour. *IOP Conference Series: Earth and Environmental Science*. 196, 012033. https://doi.org/10.1088/1755-1315/196/1/012033

Masselli, E., Pozzi, G., Vaccarezza, M., Mirandola, P., Galli, D., Vitale, M., Carubbi, C., Gobbi, G., 2020. ROS in platelet biology: functional aspects and methodological insights. *International Journal of Molecular Sciences*, 21, 1–35. https://doi.org/10.3390/ijms21144866

Matu, J., Gonzalez, J.T., Ispoglou, T., Duckworth, L., Deighton, K., 2018. The effects of hypoxia on hunger perceptions, appetite-related hormone concentrations and energy intake: a systematic review and meta-analysis. *Appetite*. 125, 98–108. https://doi.org/10.1016/j.appet.2018.01.015

Meliala, A., Narwidina, P., Nurrahma, H.A., Supriyanto, I., 2022. Banana (*Musa balbisiana* Colla) peels flour modulates HTR2B receptor expression in the liver diabetic rats. HAYATI J. Biosci. 29, 279–287. https://doi.org/10.4308/hjb.29.3.279-287

Meziant, L., Bachir-bey, M., Boutiche, M., Gali, L., Ikhlef, A., Louaileche, H., 2022. Assessment of flavonoid-rich extracts from dark peels of *Ficus carica* L. fruits for cosmeceutical and antimicrobial applications. *Traditional Medicine Research*. 7, 52. https://doi.org/10.53388/TMR20220313002.Executive

Nagel, M.J., Jarrard, C.P., Lalande, S., 2020. Effect of a single session of intermittent hypoxia on erythropoietin and oxygen-carrying capacity. *International Journal of Environmental Research and Public Health*. 17, 1–10. https://doi.org/10.3390/ijerph17197257

Noeman, S.A., Hamooda, H.E., Baalash, A.A., 2011. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. *Diabetology and Metabolic Syndrome*. 3, 1–8. https://doi.org/10.1186/1758-5996-3-17

Nurrahma, H.A., Meliala, A., Narwidina, P., Herwiyanti, S., 2021. Hepatoprotective effect of banana peel flour on histological and liver function in diabetic rats. *Indonesian Journal of Pharmacy*. 32, 529–538. https://doi.org/10.22146/ijp.1917

Ozbek, E., 2012. Induction of oxidative stress in kidney. *International Journal of Nephrology*. 2012, 1–9.

Petkova, N., Ivanov, Denev, I., Denev, P., 2019. Changes in phytochemical compounds and antioxidant potential of fresh, frozen, and processed figs (*Ficus carica* L.). *International Food Research Journal*. 126, 881–1888.

Qaid, E.Y.A., Zakaria, R., Sulaiman, S.F., Yusof, N.M., Shafin, N., Othman, Z., Ahmad, A.H., Aziz, C.A., 2017. Insight into potential mechanisms of hypobaric hypoxia–induced learning and memory deficit–lessons from rat studies. *Human and Experimental Toxicology*. 36, 1315–1325. https://doi.org/10.1177/0960327116689714

Rathi, V., Tiwari, I., Kulshreshtha, R., Sagi, S.S.K., 2023. Hypobaric hypoxia induced renal injury in rats: Prophylactic amelioration by quercetin supplementation. *PLoS ONE*. 18, 1–23. https://doi.org/10.1371/journal.pone.0279304

Rosidah, I., Ningsih, S., Renggani, T.N., Efendi, J., Agustini, K., 2020. Profil hematologi tikus (*Rattus norvegicus*) galur sprague-dawley jantan umur 7 dan 10 minggu. *JBBI*, 7, 136–145. https://doi.org/10.29122/jbbi.v7i1.3568

Rukmana, A., Supardi, L.A., Sjatha, F., Nurfadilah, M., 2022. Responses of humoral and cellular immune mediators in BALB/c Mice to LipX (PE11) as seed tuberculosis vaccine candidates. *Genes*. 13, 1-10. https://doi.org/10.3390/genes13111954

Sawka, M.N., Convertino, Eichner, E.R., Schnieder, S.M., Young, A.J., 2000. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Med Sci Sports Exerc*. 32, 332–348.

Saxena, S., Agrawal, I., Singh, P., Jha, S., 2020. Portable, low-cost hypoxia chamber for simulating hypoxic environments: development, characterization and applications. *Medical Devices and Sensors*. 3, 1–8. https://doi.org/10.1002/mds3.10064

Seenak, P., Kumphune, S., Malakul, W., Chotima, R., Nernpermpisooth, N., 2020. Pineapple consumption reduced cardiac oxidative stress and inflammation in high cholesterol diet-fed rats. *Nutrition and Metabolism*. 18, 1-11. https://doi.org/10.1186/s12986-021-00566-z

Tyagi, T., Ahmad, S., Gupta, N., Sahu, A., Ahmad, Y., Nair, V., Chatterjee, T., Bajaj, N., Sengupta, S., Ganju, L., Singh, S.B., Ashraf, M.Z., 2014. Altered expression of platelet proteins and calpain activity mediate hypoxia-induced prothrombotic phenotype. *Blood*. 123, 1250–1260.

Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.H., Jaremko, M., 2020. Important flavonoids and their role as a therapeutic agent. *Molecules*. 25, 1–39.

Valaei, K., Taherkhani, S., Arazi, H., Suzuki, K., 2021. Cardiac oxidative stress and the therapeutic approaches to the intake of antioxidant supplements and physical activity. *Nutrients*. 13, 3483. https://doi.org/10.3390/nu13103483

Varricchi, G., Ameri, P., Cadeddu, C., Ghigo, A., Madonna, R., Marone, G., Mercurio, V., Monte, I., Novo, G., Parrella, P., Pirozzi, F., Pecoraro, A., Spallarossa, P., Zito, C., Mercuro, G., Pagliaro, P., Tocchetti, C.G., 2018. Antineoplastic drug-induced cardiotoxicity: a redox perspective. *Frontiers in Physiology*. 9, 1–18. https://doi.org/10.3389/fphys.2018.00167

Vaya, J., Mahmood, S., 2006. Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). *Biofactors*. 28, 169–175.

Vigila, A.G., Baskaran, X., 2008. Immunomodulatory effect of coconut protein on cyclophosphamide induced immune suppressed swiss albino mice. *Ethnobotanical Leaflets*. 12, 1206–1218.

Wang, Y., Chen, Y., Zhang, X., Lu, Y., Chen, H., 2020. New insights in intestinal oxidative stress damage and the health intervention effects of nutrients: a review. *Journal of Functional Foods*. 75, 104248. https://doi.org/10.1016/j.jff.2020.104248

Wulansari, D., Chairul, D., Botani, B., Penelitian, P., Abstrak, B.L., 2011. Antioxidant screening activity of several indonesian medicinal plants using 2,2-difenil 1-1 picrylhidrazyl (DPPH). *Majalah Obat Tradisional*. 16, 2011.

Xu, K., Liu, H., Bai, M., Gao, J., Wu, X., Yin, Y., 2017. Redox properties of tryptophan metabolism and the concept of tryptophan Use in pregnancy. *Int J Mol Sci*. 18, 1–28.

Xu, K., Liu, G., Fu, C., 2018. The tryptophan pathway targeting antioxidant capacity in the placenta. *Oxidative Medicine and Cellular Longevity*. 2018, 1-8. https://doi.org/10.1155/2018/1054797

Zhang, H., Renfeng, X., Zhengchao, W., 2021. Contribution of oxidative stress to HIF-1-mediated profibrotic changes during the kidney damage. *Oxidative Stress and Trauma Repair*. 2021, 1–8.

Zhao, L.L., Sun, J.L., Liang, J., Liu, Q., Luo, J., Li, Z.Q., Yan, T.M., Zhou, J., Yang, S., 2020. Enhancing lipid metabolism and inducing antioxidant and immune responses to adapt to acute hypoxic stress in *Schizothorax prenanti*. *Aquaculture*, 519, 734933. https://doi.org/10.1016/j.aquaculture.2020.734933

Zhu, M., Xu, M., Zhang, K., Li, J., Ma, H., Xia, G., Li, X., Zhang, B., Shi, H., 2019. Effect of acute exposure to hypobaric hypoxia on learning and memory in adult Sprague-Dawley rats. *Behavioural Brain Research*. 367, 82–90. https://doi.org/10.1016/j.bbr.2019.03.047