Jurnal Biodjati 7(2):234–246, November 2022
e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Hemotoxicity of Hairy Fig (*Ficus hispida* L.f.) Fruits on Male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769)

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Received: 19 July, 2022
Revise from: 23 July, 2022
Accepted: 06 September, 2022

DOI: 10.15575/biodjati.v7i2.19241

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Abstract. *Hairy fig (*Ficus hispida*) fruits (HFF) are widely consumed as food and traditional medicine in several West Asian countries, both the unripe fruit (UHFF) and ripe fruit (RHFF). However, they are not commonly utilized in Indonesia. Acute oral toxicity studies reported No Observed adverse effect level (NOAEL). Further, a reproductive toxicity study found that UHFF boosted spermatogenesis and increased the quality and quantity of spermatozoa. Meanwhile, RHFF exhibited the opposite effects. To provide comprehensive information from the previous study, this research was conducted to evaluate the hemotoxicity of UHFF and RHFF about their impact on the male reproductive system. Nine Wistar rats were assigned into three groups: the first group received UHFF juice, the second group received RHFF juice, and the third group received distilled water as control. The volume of each treatment was 2 mL/individual/day for 77 days. On days 0, 28, and 77, blood samples were collected for routine hematological profile examination using a hematology analyzer (Sysmex®XP-100). Data were analyzed using a one-way ANOVA test and Duncan's test (α=0.05) to discover significant differences between groups and times. Results showed that consuming hairy fig fruit, especially the UHFF, had an unfavorable effect on erythrocytes resulting in hypochromic microcytic anemia. Still, there was no adverse effect on leukocytes and platelets. Anemia may have occurred due to the presence of hemotoxic compounds that interfere with the synthesis and binding of hemoglobin or because the hairy fig fruit filtrates were oxidized, thereby increasing the level of oxidative stress within the body, of which is a drop in hemoglobin levels.*

Keywords: *anemia, Ficus hispida, hairy fig, hematological profile, hemotoxicity, reproductive toxicity study*

Citation
Fitria, L., Sari, A. L., Handayani, L. & Widiyanto, S. (2022). Hemotoxicity of Hairy Fig (*Ficus hispida* L.f.) Fruits on Male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769). *Jurnal Biodjati*, 7(2), 234–246.

INTRODUCTION

Plant metabolism produces a variety of phytochemical compounds, such as secondary metabolites. Some secondary metabolites have medicinal properties, while others are potentially toxic (Mudimba et al., 2019). Plants synthesize secondary metabolites to protect themselves from other organisms (pathogens, pests, and herbivores), respond to environmental stresses, mediate organismal interactions, and competition with other

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plants (Wink, 2006; Pang et al., 2021). Some plants contain phytoconstituents that are cytotoxic, hemotoxic, nephrotoxic, hepatotoxic, carcinogenic, and other noxious properties. The parts which may accommodate toxic substances are roots, tubers, stems, leaves, buds, flowers, and fruits (Saidu et al., 2007; Friday, 2019). Natural toxins are also found in some fresh edible fruits that harm human and animal health. They are produced by plants to defend themselves against fungi, insects, and predators, and provide a protection mechanism against environmental stress (The Government of the Hong Kong Special Administrative Region, 2005; Friday, 2019).

Ficus hispida or hairy fig (Familia *Moraceae*) is a medium-sized tree that grows wild in tropic regions, is easy to reproduce, and bears heavy fruit throughout the year (Ali & Chaudhary, 2011; Lee et al., 2013; Cheng et al., 2020). This species in Indonesia, known as *luwingan*, can be found in various locations, especially as shade trees (Fitria et al., 2019; Fitria et al., 2020, Fitria et al., 2021; Fitria et al., 2022). Although the fruit of *F. hispida* (HFF) is edible (Mon et al., 2020; Rusmadi et al., 2020) like common figs fruit (*F. carica*), however, it is rarely consumed because it could cause headache, dizziness, nausea, vomiting, and indigestion, all of which are signs of toxicity or food poisoning (Berg et al., 2005; Kehati, 2009; Slik, 2009; Mon et al., 2020). However, people in some countries in West Asia and Southeast Asia utilize HFF as traditional medicine and consume it as a daily food menu (Kunwar & Bussmann, 2006; Ali & Chaudhary, 2011; Shahreen et al., 2012; Aziz et al., 2014; Mon et al., 2020; Rusmadi et al., 2020). Phytochemical studies on HFF reported a variety of secondary metabolites including sesquiterpenoids, terpenoids, flavonoids, coumarins, phenylpropionic acids, benzoic acid derivatives, alkaloids, phenols, sterols, glycosides, and alkanes. Terpenoids, flavonoids, and alkaloids are the major phytoconstituents in HFF (Zhang et al., 2018; Cheng et al., 2020).

The single-dose (14 days) oral toxicity study revealed that HFF could be consumed, but there is a tendency to increase the number of lymphocytes (Fitria et al., 2019). The repeated-dose (28 days) oral toxicity study on female rats demonstrated that HFF could be consumed but there is a tendency to cause anemia and impaired renal function (Fitria et al., 2020). A similar study conducted on male rats, on the other hand, did not show these findings (Fitria et al., 2021). This indicates that male and female individuals have different physiological responses when exposed to the same substance (Clayton, 2016; Miller et al., 2017). The reproductive toxicity study on male rats (77 days) showed that HFF is safe for reproductive health and even can improve spermatogenesis efficiency and sperm quality (Fitria et al., 2022). Nevertheless, the information on the safety and adverse effects of consuming HFF for 77 days on the general physiological condition are not yet provided.

Hematological parameters are essential in toxicity studies because they provide information about the direct effect of a substance on blood cells (hemotoxicity) or indirect effect which describes the physiological conditions as a response to the administration of toxic substances (Budinsky, 2000; Keohane et al., 2016; Mudimba et al., 2019). This research is a continuation of the series of toxicity studies on HFF which aimed to investigate the potential hemotoxic properties of HFF as an adverse effect of its benefit as a promising profertility agent. Studies on hematological profile of Wistar rats after oral administration of HFF have been carried out, however on female rats (Fitria et al., 2019; Fitria et al., 2020). Male rats have also been the subject of

the study, however, this was a short-term observation of 28 days (Fitria et al., 2021). The duration in this research was longer (77 days) based on the length of the spermatogenesis cycle in rats (Fitria et al., 2022).

MATERIALS AND METHODS

Animal Samples

Nine male Wistar rats aged 8 weeks with an initial body weight of 124-147 g (138±7 g) were obtained from The Faculty of Pharmacy, Universitas Gadjah Mada. Animal care and experimental procedures were conducted at the animal facility of Faculty of Biology Gadjah Mada University referred to the standard guideline for laboratory rats provided by NRC (2011) including housing, feeding, drinking water, health monitoring, environmental control, husbandry, and sanitation. All procedures related to the handling and treatment of animals in this study have been approved by The Research Ethics Committee of Integrated Research and Testing Laboratory (LPPT) Universitas Gadjah Mada (Certificate of Ethical Clearance No: 00100/04/LPPT/VIII/2018).

Plant Samples

Unripe and ripe hairy fig fruits were picked directly from a tree growing in the area of Faculty of Biology Universitas Gadjah Mada (Figure 1A). Species identification was based on morphological characters according to Backer & van den Brink (1965) and confirmed as *Ficus hispida* (Certificate of Identification from Laboratroy of Plant Systematics, Faculty of Biology Universitas Gadjah Mada No: 0141073/S.Tb./IX/2021).

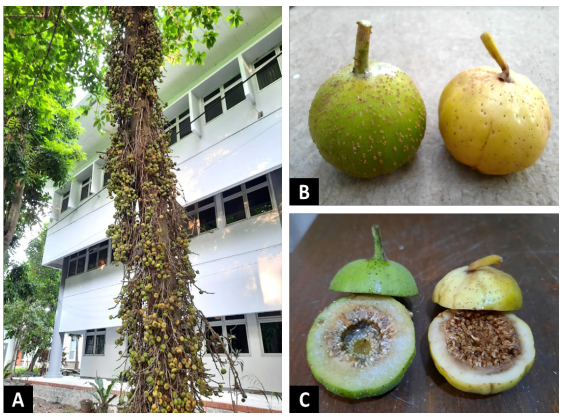

Figure 1. Hairy figs (*Ficus hispida* L.f.) (Fitria et al., 2022). A. Hairy fig tree bearing a lot of fruits, B. Unripe fruit (left) and ripe fruit (right), C. Opened fruit showing tiny flowers.

We only used good quality fruits with the following criteria: unripe fruits (UHFF) are green with a hard texture, whereas ripe fruits (RHFF) are yellow with a soft and watery texture, with no defects or wounds due to insect infection (Figure 1B). Besides color and texture, there are various secondary metabolites inside UHFF and RHFF (Fitria et al., 2015). That was the reason of our study to use both fruits to compare their effect on physiological aspects through hematological parameters. UHFF and RHFF were rinsed with running water and sliced to clean the inside (Figure 1C), then crushed using a blender and filtered to produce pure juice (100 %). Each juice was administered via oral gavage every

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day at 2 PM after rats fasted for 6 hours, immediately to anticipate oxidation as indicated by color changing of the juice (Fitria et al., 2019; Fitria et al., 2020; Fitria et al., 2021; Fitria et al., 2022).

Experimental Design

Nine rats were assigned into three groups: the first group received UHFF filtrate, the second group received RHFF filtrate, and the third group received distilled water as control (CTRL). UHFF, RHFF, or distilled water was administered orally (gavage feeding) as much as 2 mL/individual/day for consecutive 77 days, which is 1.5× of the cycle of spermatogenesis in rats, following the method by Fitria et al. (2022).

Data Sampling

Body weight and rectal temperature were measured weekly from the beginning to the end of the experiment. On days 0, 28, and 77, one milliliter blood was collected from the orbital sinus after animals were anesthetized under Ketamine-Xylazine cocktail (10:1) with volume 0.1 mL/100 g bb IM (IACUC UIOWA, 2020). EDTA-blood was examined for the routine hematological profile using a fully automated hematology analyzer (Sysmex®XP-100). Blood samples were also processed for regular thin blood smear preparation following Vu et al. (2021) for morphological observation and confirmation with results generated by the machine.

Data Analysis

Data were recapitulated in Microsoft®-Excel®2019 and statistically analyzed based on one-way ANOVA test followed by Duncan's test (α=0.05) using IBM®SPSS®v.23 to discover significant differences between groups and times. Reference intervals were obtained based on the lowest and highest values of each variable from the animal population in this study on Day 0 or "the baseline" (Poitout-Belissent & McCartney, 2010). Percent increase (+) or decrease (-) in the number and size of blood cells were calculated using the formula by Kuriya *et al.* (1985) as follows: [(final value - initial value)/initial value] x 100. The table shows all values as descriptive statistic values (mean±standard deviation).

RESULTS AND DISCUSSION

The hematological profile, or examination of cellular components of blood, is one of the important physiological parameters in preclinical studies because blood can be used to evaluate the body's responses to various diseases and treatments (Fitria & Sarto, 2014; Delwatta et al., 2018), including effects on exposure to toxicants (Etim et al., 2014). Table 1 shows that the hematological profile of male Wistar rats (Baseline) has slightly different values when compared to the database by Giknis & Clifford (2008). Erythrocyte and platelet profiles in our animal were relatively lower than the reference even though the readings were within the reference intervals. On the other hand, the leukocyte profile tends to be greater than the reference although the value is still within the reference intervals (Giknis & Clifford (2008); Fitria et al. (2021)). Our baseline values are close to Fitria et al. (2021) because our animal comes from the same animal facility, with the relatively same management and care methods. In the meantime, Giknis & Clifford (2008) provides hematological profile of rats which raised in foreign country (Charles River, USA).

Table 1. Values of selected hematological profile of wistar rats on this study compared to references

Parameters	This study (Baseline)	Giknis & Clifford (2008)	Fitria et al. (2021)
Erythrocyte profile			
RBC ($\times 10^6/\mu L$)	6.33-8.22	7.27-9.65	4.87-8.22
HCT (%)	36.4-47.3	39.6-52.5	36.40-52.17
HGB (g/dL)	12.2-15.2	13.7-17.6	10.09-15.33
MCV (fL)	54.9-58.6	48.9-57.9	52.49-63.47
MCH (pg)	17.4-19.3	17.1-20.4	17.40-18.65
MCHC (g/dL)	31.7-33.5	32.9-37.5	27.72-36.50
Leukocyte profile			
WBC ($\times 10^3/\mu L$)	8.2-14.1	1.96-8.25	7.16-14.10
#NEU ($\times 10^3/\mu L$)	1.0-1.7	0.22-1.57	1.00-3.37
#LYM ($\times 10^3/\mu L$)	6.6-12.4	1.41-7.11	4.60-12.40
%NEU (%)	10.1-19.3	6.2-26.7	13.9-37.44
%LYM (%)	80.7-89.9	66.6-90.3	61.44-87.94
N/L	0.11-0.24	0.12-0.26	0.11-0.61
Platelet profile			
PLT ($\times 10^3/\mu L$)	524-1417	638-1177	147-1417
PCT (%)	0.32-0.86	0.39-1.10	n/a
MPV (fL)	5.8-6.4	6.2-9.4	n/a

Notes: RBC= number of red blood cells, HCT= hematocrit, HGB= hemoglobin, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration, WBC= total number of white blood cells, #NEU= number of neutrophils, #LYM= number of lymphocytes, %NEU= percentage of neutrophils, %LYM= percentage of lymphocytes, N/L= neutrophil to lymphocyte ratio, PLT= number of platelets, PCT= plateletcrit, MPV= mean platelet volume

Delwatta et al. (2018) also experienced the same thing, they found discrepancies in the baseline value of the hematological profile of their animals when compared to the reference. This is because the hematological profile of animals, particularly laboratory rats, varies depending on several conditions such as age, sex, nutrition, health status, environmental factors, care management or husbandry, and genetic factors (breed and genotype). Although the species and even the strain (breed, strain, or stock) are the same, blood collection methods, sampling frequency, number of samples, blood volume, and methods of analysis in each laboratory or animal facility may differ (Etim et al., 2014; Keohane et al., 2016; Delwatta et al., 2018). As a result, rather than using general reference values for control in the experiment, it is recommended to use local reference values from where the animals were obtained (Fitria & Sarto, 2014) or based on the baseline (Poitout-Belissent & McCartney 2010).

Erythrocyte Profile

Erythrocytes or red blood cells play an essential role in the body system as they provide oxygen through hemoglobin to supply energy for various metabolic processes. Erythrocytes are also associated with activity and stress (Fitria & Sarto, 2014). Therefore, alterations in erythrocyte profile, such as anemia, will seriously impact the physiological condition and health status of individuals. Th erythrocyte profile of male wistar rats with oral administration of HFF for 77 days is shown in table 2.

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Table 2. The erythrocyte profile of male Wistar rats in the study of hemotoxicity of hairy figs fruits filtrates for 77 days

Parameters	Group	Day			PD (%)
		0	28	77	
RBC (×10⁶/µL)	CTRL	7.48±0.08[a]	7.36±0.20[a]	8.12±0.16[b]	8.56
	UHFF	6.83±0.37[a]	7.27±0.17[b]	8.09±0.29[c]	18.45
	RHFF	8.05±0.12[a]	7.32±0.60[b]	9.07±0.99[c]	12.67
HCT (%)	CTRL	42.50±0.78[a]	41.23±2.01[a]	43.00±0.22[a]	1.18
	UHFF	39.50±2.45[a]	41.80±1.18[b]	43.53±2.15[b]	10.20
	RHFF	45.30±1.47[a]	40.50±3.00[b]	47.27±5.69[a]	4.35
HGB (g/dL)	CTRL	13.80±0.43[a]	13.90±1.02[a]	14.30±0.54[a]	3.62
	UHFF	12.97±0.70[a]	14.17±0.37[b]	11.13±5.22[a]	-14.19
	RHFF	14.47±0.54[ab]	13.63±0.62[a]	15.10±2.24[b]	4.35
MCV (fL)	CTRL	56.83±1.24[a]	56.03±1.33[a]	53.00±1.31[a]	-6.74
	UHFF	57.80±0.57[a]	57.50±0.80[a]	53.83±0.87[a]	-6.87
	RHFF	56.23±1.06[a]	55.37±0.49[a]	52.07±0.59[a]	-7.40
MCH (pg)	CTRL	18.47±0.68[a]	18.83±0.90[a]	17.63±1.02[a]	-4.55
	UHFF	19.00±0.36[a]	19.47±0.12[a]	13.67±6.27[b]	-28.05
	RHFF	17.97±0.45[ab]	18.70±0.70[b]	16.60±0.86[a]	-7.62
MCHC (g/dL)	CTRL	32.43±0,54[a]	33.67±0.84[a]	33.27±1.07[a]	2.59
	UHFF	32.83±0.53[a]	33.87±0.31[a]	25.2±1.31[b]	-23.24
	RHFF	31.90±0.16[a]	33.77±0.98[a]	31.83±1.51[a]	-0.22

Notes: RBC= number of red blood cells, HCT= hematocrit, HGB= hemoglobin, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration, CTRL= control, administered orally with distilled water 1 mL/individual/day, UHFF= administered orally with unripe hairy fig fruit filtrate 1 mL/individual/day, RHFF= administered orally with ripe hairy fig fruit filtrate 1 mL/individual/day. Different letters at the end of each value in the same row indicates a significant difference ($p<0.05$). PD= percentage of difference, positive value indicates an increase, negative value indicates a decrease.

Table 2 shows that erythrocyte numbers in CTRL and RHFF fluctuated with an increasing trend, with RHFF having a higher value than the baseline (significant). UHFF showed a steady and significant rise, with the largest percentage of difference among the three groups, but it remained within the baseline. The hematocrit followed the same pattern, but with all values were maintained within the baseline. In the case of hemoglobin, UHFF decreased over time until it fell below the baseline (significant), whereas CTRL improved continuously but still within the baseline. Meanwhile, RHFF also fell but it had a tendency to rise, and the value was still within the baseline. UHFF had the lowest hemoglobin level but the highest erythrocyte numbers. This could be a compensatory mechanism to anticipate anemia, whereby erythrocyte production is increased in order to bind more hemoglobin (Keohane et al., 2016).

To determine whether this condition is safe or should be considered, we carried out an evaluation based on the calculation of erythrocyte indices (MCV, MCH, MCHC). These three variables are used to confirm the possibility of anemia and identify the type of anemia (Keohane et al., 2016). Results showed that MCV in all groups fell below the baseline, indicating a reduction in the size or

volume of erythrocytes (microcytic). Microcytic anemia generally occurs due to impaired hemoglobin synthesis (Doig & Zhang, 2017). However, this was not solely a toxic effect of HFF because the decrease was insignificant and also occurred in control animals (CTRL). The difference in erythrocyte size could not be seen clearly in blood smear preparation because the changes were very small, only 6-7 % of the total volume. MCH and MCHC in UHFF dan RHFF also fell below the baseline. The most significant drop was in UHFF (>20%). This, combined with MCV calculation, indicated hypochromic microcytic anemia (Doig & Zhang, 2017). HFF is thought to contain toxic compounds that reduce hemoglobin levels. Ladokun et al. (2015) stated that herbal medicine is not completely safe; it can possess various adverse effects, one of which is anemia. Therefore, in addition, to exploring HFF as a potential medicinal property, we also conducted this study to investigate its adverse effects.

Fitria et al. (2022) reported that UHFF can improve male reproductive function, however, we discovered that it induced hypochromic microcytic anemia. Another possibility for the toxic effect generated by HFF is that the filtrates had already oxidized when they were administered to the animals, as evidenced by the dark brown color. Browning is the enzymatic reaction that occurs when fruits are exposed to air, particularly after being cut, peeled, chopped, or subjected to other processing. The fruits darken rapidly due to the oxidation of polyphenol oxidase (PPO) to form melanin (Jiang et al., 2016). According to Idu et al. (2022), reactive oxygen species (ROS) are involved in the pathogenesis of anemia. Consumption of damaged food due to excessive oxidation increases oxidative stress within the body, interfering with the synthesis of erythrocytes and hemoglobin. Natural oxidation or autooxidation can occur during food processing. Excessive oxidation is characterized by the browning of fruits and vegetables (Penicaud et al. 2011; Moon et al, 2020; Poljsak et al. 2021). In this case, excessive oxidation occurred during the making of HFF juice and filtering the product for oral administration.

Budinsky (2000) stated that hemotoxicity or hematotoxicity in erythrocytes is particularly the incidence of anemia due to a decrease in cell counts or low hemoglobin levels. Anemia is mainly caused by the inhibition of oxygen binding by hemoglobin and damage to the structure of erythrocytes. Observations of the blood smear preparations and complete blood count (CBC) did not show a decrease in erythrocytes resulting from a structural injury that causes cell damage or lysis. However, HFF can potentially interfere with erythrocyte function since it reduces hemoglobin levels. Therefore, we stated that HFF, especially UHFF, is hemotoxic to erythrocytes. Furthermore, proper processing methods can reduce the toxicity of HFF by preventing it from being oxidized quickly before consumption.

Leukocyte Profile

Leukocytes or white blood cells are responsible for the body's defense system. There are five types of leukocytes found in the peripheral blood, namely neutrophils, monocytes, eosinophils, basophils, and lymphocytes. Neutrophils are predominant in innate immune responses, which are phagocytic in nature. Lymphocytes play an important role in adaptive immune response, serving as regulators or effectors depending on the subset. Our machine, as well as manual observation with blood smear preparations, failed to detect monocytes, eosinophils, and basophils. These three types of leukocytes are extremely rare in normal conditions, but their numbers

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will increase as a result of pathological conditions (Fitria & Sarto, 2014; Chmielewski & Strzelec, 2017; Rosales, 2018). Therefore, in this study, we focused solely on the discussion of neutrophils and lymphocytes.

Leukocyte profile is influenced by age, sex, hormonal conditions, genetic factors, stress, diet, and lifestyle (Chmielewski & Strzelec, 2017). Food can cause inflammation, altering the value of leukocyte profile. Consumption of fruits and vegetables can help to reduce inflammation and improve health (Menni et al., 2021). However, some fresh vegetables and fruits naturally contain toxins that are harmful to health. For instance, beans contain phytohaemagglutinin (PHA) causing blood cells to clump together. Cassava, sorghum, stone fruits, bamboo roots, and almonds contain cyanogenic glycosides which generate mild symptoms of intoxication to fatal death (The Government of the Hong Kong Special Administrative Region, 2005; WHO, 2018). Therefore, a toxicity study should be carried out to determine the long-term safety of consuming such substances. The leukocyte profile of male Wistar rats with oral administration of HFF for 77 days is shown in Table 3.

Table 3. The leukocyte profile of male Wistar rats in the study of hemotoxicity of hairy figs fruits filtrates for 77 days

Parameters	Group	Day			PD (%)
		0	28	77	
WBC (×10³/µL)	CTRL	10.83±2.45[a]	14.57±4.77[a]	13.03±1.68[a]	20.31
	UHFF	10.77±0.95[a]	14.53±1.43[a]	10.33±5.17[a]	-4.09
	RHFF	9.37±1.58[a]	10.70±1.53[a]	12.97±2.39[a]	38.42
#NEU (×10³/µL)	CTRL	1.30±0.29[a]	4.47±2.44[c]	3.80±0.94[b]	192.31
	UHFF	1.63±0.09[a]	3.30±0.83[b]	2.23±1.96[a]	36.81
	RHFF	1.40±0.16[a]	2.63±0.90[a]	4.17±1.65[b]	197.86
#LYM (×10³/µL)	CTRL	9.53±2.22[a]	10.10±2.33[a]	9.23±0.74[a]	-3.15
	UHFF	9.13±1.03[a]	11.23±0,66[a]	8.10±3.65[a]	-11.28
	RHFF	7.97±1.59[a]	8.07±1.07[a]	8.80±0.94[a]	10.41
%NEU (%)	CTRL	12.27±1.80[a]	28.70±6.00[b]	29.00±3.26[b]	136.35
	UHFF	15.33±1.98[a]	22.27±3.47[b]	19.47±8.70[a]	27.01
	RHFF	15.40±2.94[a]	24.30±6.00[b]	30.93±8.00[b]	57.79
%LYM (|%)	CTRL	87.73±1.80[b]	71.30±6.00[a]	71.00±3.26[a]	-19.07
	UHFF	84.67±1.98[b]	77.73±3.47[a]	80.53±8.70[ab]	-90.49
	RHFF	84.60±2.94[b]	75.70±6.00[ab]	69.07±8.00[a]	-18.36
N/L	CTRL	0.14±0.02[a]	0.41±0.13[b]	0.41±0.07[b]	199.16
	UHFF	0.18±0.03[a]	0.29±0.06[b]	0.26±0.14[b]	44.48
	RHFF	0.18±0.04[a]	0.33±0.10[b]	0.47±0.17[b]	160.66

Notes: WBC= total number of white blood cells, #NEU= number of neutrophils, #LYM= number of lymphocytes, %NEU= percentage of neutrophils, %LYM= percentage of lymphocytes, N/L= neutrophil to lymphocyte ratio, CTRL= control, administered orally with distilled water 1 mL/individual/day, UHFF= administered orally with unripe hairy fig fruit filtrate 1 mL/individual/day, RHFF= administered orally with ripe hairy fig fruit filtrate 1 mL/individual/day. Different letters at the end of each value in the same row indicates a significant difference ($p<0.05$). PD= percentage of difference, positive value indicates an increase, negative value indicates a decrease.

The total number of leukocytes and lymphocytes in all groups fluctuated but still maintained within the baseline. The number of neutrophils in all groups also fluctuated and was higher than the baseline (significant). This increase altered the percentage of neutrophils and lymphocytes as well as the ratio of neutrophils to lymphocytes or N/L (Table 3). Based on the calculation of the percentage difference of leukocytes before and after the experiment, UHFF has the lowest value. This indicated that the immune system is tolerant of UHFF consumption. This contrast with the erythrocyte profile, where UHFF had the largest differences (Table 2). We suspect that UHFF contains bioactive compounds that are toxic to erythrocytes but not to leukocytes. Meanwhile, RHFF was found to be relatively safe for the profile of erythrocytes (Table 2) and leukocytes (Table 3).

As previously stated, fresh fruit can contain natural toxins that are harmful to health (The Government of the Hong Kong Special Administrative Region, 2005). Some *Ficus* fruits are safe to eat, but others are not. Fruit of *F. carica* is known to be safe to consume, and even beneficial to health (Salma et al., 2020). Fruit of *F. variegata* has never been consumed by humans and frugivorous animals, indicating that it contains natural toxins that should be avoided (Lee, 2020). Fruit of *F. hispida* (HFF) is edible (Kunwar & Bussmann, 2006; Ali & Chaudhary, 2011; Shahreen et al., 2012) but can cause intoxication and indigestion (Berg et al., 2005; Kehati, 2009; Slik, 2009). However, an oral toxicity study of HFF by Fitria et al. (2019), Fitria et al. (2020), and Fitria et al. (2021) using Wistar rats found no signs of toxicity. When HFF is consumed continuously for an extended period of time, toxic effects may develop as a cumulative effect. The accumulation of toxic substances within the body will slow down the detoxification process, increase systemic inflammation, disrupt immune and hormonal systems, and increase the risk of various diseases (The Institute for Functional Medicine, 2020). According to Chmielewski & Strzelec (2017), food or substances containing toxins can rise the number of leukocytes significantly as they are considered foreign components (antigens), thus evoking the immune response. A significant number of neutrophils are released to phagocytose foreign particles. The high number of neutrophils also indicates inflammation and tissue damage due to oxidative stress (Chmielewski & Strzelec, 2017; Rosales, 2018). Because the increase of neutrophils was observed in all groups including the control, it can be concluded that this is not a toxic effect of HFF.

Platelet Profile

Platelets are the major component of blood coagulation which functions to maintain blood volume and pressure or hemostasis (Fitria & Sarto, 2014). Platelets are also involved in inflammation (Pogorzelska et al., 2020). A low platelet count (thrombocytopenia) raises the risk of bleeding. However, not all cases of thrombocytopenia result in bleeding (Vinholt et al., 2014). Platelet profile is greatly influenced by the blood collection technique, and the result is usually lower than the true value (thrombocytopenia). This is due to the fact that a large number of platelets contributed to the damaged tissue during phlebotomy. When blood sampling is not smooth or bleeding occurs during the process, the number of platelets in the blood sample will also drop. Bleeding activates platelets to aggregate, and some of them are stuck to injured blood vessels during coagulation and clotting reactions of the hemostasis mechanism (Tien, 1995).

Platelet count alone is not sufficient to

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study hemotoxicity. Platelet profile indices, particularly MPV (mean platelet volume) and PCT (plateletcrit), are essential for predicting and prognosing acute and chronic health status. MPV stands for platelet production and activation. High MPV levels are linked to poor health, whereas low MPV levels are associated with inflammation. PCT shows the proportion of platelets in a unit of blood volume. A low platelet count or PCT followed by a low MPV value triggers spontaneous bleeding (Vinholt et al., 2014; Pogorzelska et al., 2020). The platelet profile of male Wistar rats in the study of hemotoxicity of hairy figs fruits filtrates for 77 days is shown in Table 4.

Table 4. The platelet profile of male Wistar rats in the study of hemotoxicity of hairy figs fruits filtrates for 77 days

Parameters	Group	Day			PD (%)
		0	28	77	
PLT ($\times 10^3/\mu L$)	CTRL	1071.33±303.57[a]	1129.67±152.97[a]	1129.67±177.91[a]	5.45
	UHFF	829.33±282.96[a]	843.33±188.24[a]	900.00±128.41[a]	8.52
	RHFF	1163.33±59.02[a]	1022.00±101.04[a]	1099.00±33.24[a]	-5.53
PCT (%)	CTRL	0.64±0.18[a]	0.69±0.13[a]	0.78±0.08[a]	21.88
	UHFF	0.50±0.18[a]	0.55±0.11[a]	0.63±0.02[a]	26.00
	RHFF	0.72±0.03[a]	0.63±0.04[a]	0.75±0.11[a]	4.17
MPV (fL)	CTRL	5.97±0.12[a]	6.13±0.29[a]	6.93±0.68[a]	16.08
	UHFF	6.00±0.14[a]	6.53±0.39[a]	7.10±0.79[a]	18.33
	RHFF	6.23±0.17[a]	6.17±0.24[a]	6.83±0.83[a]	9.63

Notes: PLT= number of platelets, PCT= plateletcrit, MPV= mean platelet volume, CTRL= control, administered orally with distilled water 1 mL/individual/day, UHFF= administered orally with unripe hairy fig fruit filtrate 1 mL/individual/day, RHFF= administered orally with ripe hairy fig fruit filtrate 1 mL/individual/day. Different letters at the end of each value in the same row indicates a significant difference ($p<0.05$). PD= percentage of difference, positive value indicates an increase, negative value indicates a decrease.

Table 4 shows that almost all values are within the baseline, with the exception of MPV, where some values are lower than the baseline but increase over time and return to the baseline. In all parameters observed, CTRL and UHFF exhibited the same trend, which was increasing, whereas RHFF fluctuated but was not significant. It is possible to conclude that HFF has no toxic effect on platelets, and there is no intervention of technical factors that cause a decrease in platelet count. Studies on the effects of traditional herbal medicines, health supplements, and food and drink ingredients on platelet profiles are still limited. For example, quinine is one of phytoconstituents linked to thrombocytopenia (Royer et al., 2010), whereas alkaloids act as antithrombocytopenia (Patil et al., 2013). HFF contains fourteen alkaloids as the main bioactive ingredients (Ali and Chaudhary, 2011; Lee et al., 2013; Cheng et al. 2020), therefore, rather than being hemotoxic to platelets, HFF maintains platelets' normal structure and function.

CONCLUSION

Results showed that consuming hairy fig fruits, especially the unripe fruit, had an unfavorable effect on erythrocytes resulting in the hypochromic microcytic anemia. Still, there were no adverse effects on leukocytes and platelets. Anemia may have occurred due to the presence of hemotoxic compounds that interfere with the synthesis and binding

of hemoglobin, or because the hairy fig fruit filtrates we administered to animals had undergone excessive oxidation, thereby increasing the level of oxidative stress within the body, one of which is a drop in hemoglobin levels. Further research, therefore, is needed to elucidate these findings. We put forward to conduct phytochemical screening of bioactive substances in hairy fig fruits which potential to cause anemia and application of several methods to prevent enzymatic browning in the fruit, for instance, treatment with antioxidant agents, blanching, and lyophilization.

AUTHOR CONTRIBUTION

L.H. and L.F. conceived and designed the study, A.L.S. and LH conducted the experiment and collected data under supervision by L.F., A.L.S. and S.W. performed data analysis and interpretation. L.F., A.L.S. and S.W. wrote the manuscript. All authors have read and approved the final manuscript.

ACKNOWLEDGMENTS

We are grateful to Head of Laboratory of Animal Physiology, Gadjah Mada University for giving us permission and facilitating the “Ficus Project” research. We'd also like to thank Dr. Bambang Retnoaji, M.Sc. for his valuable input on this research. Last but not least, we would like to express our gratitude to Lina Noor Na’ilah for her assistance during the experiment.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

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