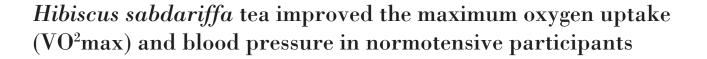
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ABSTRACT

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Key words: Normotensive participants, cardiovascular fitness, reactive oxygen species, VO,max cell damage. Previous studies announced that treatment with antioxidants might decrease oxidative stress caused by physical practice. Studies on the ethanol and water extracts of *Hibiscus sabdariffa* calyces had proven their antioxidant activity. Our work is the first report on the effect of 30 days of daily intake of *H. sabdariffa* tea on the maximum oxygen uptake (VO₂max) and blood pressure (BP) in healthy male and female normotensive participants. The VO₂max, measured to obtain the participants' cardiovascular fitness status, was performed by following the Young Men's Christian Association step test. Our study revealed that *H. sabdariffa* tea intake decreased the BP of the participants in week 4 and increased the number of participants with average VO₂max status, starting from week 2 to 4, compared to baseline. Taking these results together, *H. sabdariffa* tea may be beneficial to improve the maximum oxygen uptake status in normotensive participants.

The heavy physical practice may escalate the production of reactive oxygen species, which eventually results in

INTRODUCTION

Hibiscus sabdariffa L. has been widely explored in both healthy participants and patients with metabolic disorders. *Hibiscus sabdariffa* has indicated a good effect on hypertensive patients (Herrera-Arellano *et al.*, 2007; McKay *et al.*, 2010), as proven by a randomized double-blind clinical study of *H. sabdariffa* L. (containing 250 mg of total anthocyanins/dose) administered for 4 weeks. This study resulted in a decrease in blood pressure (BP) (Herrera-Arellano *et al.*, 2007). Another randomized double-blind clinical study on 65 participants with pre- and mild hypertension treated with brewed *H. sabdariffa* L. tea reported a significantly lower systolic BP, but not significantly lower diastolic BP (McKay *et al.*, 2010). A study on type II Diabetes mellitus patients with mild hypertension consuming *H. sabdariffa* L. infusion demonstrated

Jutti Levita, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, Indonesia. E-mail: jutti.levita @ unpad.ac.id positive effects on their BP (Mozaffari-Khosravi *et al.*, 2009). In a randomized control trial, patients with nonalcoholic fatty liver disease treated with a 450 mg capsule of *H. sabdariffa* or a placebo capsule daily for 8 weeks experienced a decrease in serum triglyceride, alanine aminotransferase, and aspartate aminotransferase levels compared with the placebo. A significant decrease in systolic BP and diastolic BP and a significant elevation in total antioxidant capacity had also been described (Izadi *et al.*, 2021). Moreover, a rise in high-density lipoprotein cholesterol and a significant decrement in BP of healthy participants treated with *H. sabdariffa* tea for 30 days were also reported (Diantini *et al.*, 2021).

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The ethanol and water extracts of *H. sabdariffa* calyces have been proclaimed for their antioxidant activity (Adusei, 2020; Anokwuru *et al.*, 2011; D'Heureux–Calix and Badrie, 2004; Hamrita *et al.*, 2022; Pozos *et al.*, 2020; Purbowati and Maksum, 2019) and various other pharmacology activities tested in animal models, for example, antidyslipidemia (Ajay *et al.*, 2007; Ekor *et al.*, 2010; Hirunpanich *et al.*, 2006). These activities were predicted due to the plant's total phenol compound (Anokwuru *et al.*, 2011; D'Heureux–Calix and Badrie, 2004; Purbowati and

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Maksum, 2019) and anthocyanins (D'Heureux–Calix and Badrie, 2004; Guardiola and Mach, 2014; Purbowati and Maksum, 2019). Anthocyanins could remarkably reduce the oxidation of low-density lipoprotein, inhibit adipogenesis, and alter the expression of a particular gene (Guardiola and Mach, 2014). An *in vitro* study on cultured hematopoietic stem cells demonstrated the effect of *H. sabdariffa* on rising the glutathione level and superoxide dismutase activity but the amount of reactive oxygen species (ROS) was unchanged (Lin *et al.*, 2014).

The physical practice is believed to be the foundation of good health; however, this activity may escalate the production of ROS, which eventually results in cell damage. Moreover, physical practice results in increasing the levels of malondialdehyde in the blood which works as an indirect indicator of lipid peroxidation (Clarkson and Thompson, 2000). The physical practice is believed to be able to stimulate inflammation and muscle fatigue (Simioni et al., 2018). Also, during heavy physical practice, the oxygen flux to active skeletal muscles is significantly elevated (Atalay et al., 2015; Radak et al., 2013). The demand for O₂ in skeletal muscle during physical workups leads to a remarkable change in blood flow (Kawamura and Muraoka, 2018). Previous studies announced that treatment with antioxidants might decrease oxidative stress caused by heavy physical practice (Clarkson and Thompson, 2000). Participants supplemented with vitamin E had shown a decrease in creatine kinase and lactic dehydrogenase levels which confirmed the protective effect of vitamin E against oxidative stress (Itoh et al., 2000).

Considering this, it is interesting to explore the effect of *H. sabdariffa*, which has been reported for its ability to increase the activity of antioxidant enzymes, on normotensive participants' post-physical practice in terms of cardiovascular fitness. Thus, our work assessed the effect of 30 days of daily intake of *H. sabdariffa* tea on the VO₂max and BP in healthy participants. VO₂max equals the maximal O₂ consumption during exercise to approximately the depletion of physical strength. It is one of the best methods of predicting cardiorespiratory endurance and is related to body weight (Blair *et al.*, 2001).

MATERIALS AND METHODS

Study design

This study was a quasi-experiment with a pre-post design for 32 adult males (59.4%) and females (40.6%), aged 21–55 years. The participants were affirmed to be healthy by a physician. Participants diagnosed with hypertension, peptic ulcers, knee arthritis, and/or musculoskeletal disorders were excluded. The protocol of this study was approved by the Research Ethics Committee of Universitas Padjadjaran (approval no. 35/UN6.C.10/PN/2018). The participants had agreed to sign the informed consent in compliance with the Ethical Principles for Medical Research Involving Human Subjects of the WMA Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/) with the knowledge that they could withdraw at any time.

The general characteristics of the participants based on their age and body mass index (BMI) are shown in Tables 1 and 2, respectively. The participants were given 200 ml of *H. sabdariffa* tea, twice daily for 30 successive days. The *H. sabdariffa* tea was prepared by boiling 100 g of dried calyces and 500 g of sugar in

Table 1. Characteristics of the participants based on their ages.

Age (years)	N (%)
21–30	8 (25.0%)
31-40	7 (21.9%)
41–50	13 (40.6%)
>50	4 (12.25%)
Total	32 (100%)

Table 2. Characteristics of the participants based on their BMI.

Category	N (%)
Obese	2 (6.2%)
Overweight	12 (37.5%)
Normal	18 (56.3%)
Total	32 (100%)

10 l of potable water for 15 minutes; thus, the daily dose of the *H.* sabdariffa tea $(2 \times 200 \text{ ml})$ contains approximately 4 g of dried calyces and 20 g of sugar.

Measurement of VO₂max and BP before and after treatment with *H. sabdariffa* tea

Before the intervention and every Friday during the treatment, the participants were required to undertake cardiovascular fitness by precisely obeying the Young Men's Christian Association (YMCA) step test (Beutner *et al.*, 2015; Diantini *et al.*, 2021; Morrow *et al.*, 2005; Teren *et al.*, 2016). The participants were prepared to avoid taking meals, cigarettes, and caffeine for at least 2 hours before the test. The test was conducted as follows.

The BP of participants was measured before the step test. The participants were told to sit for 5 minutes on an upright chair in a position with their feet flat on the floor. BP was measured using a sphygmomanometer and stethoscope (Muntner *et al.*, 2019) with three replicates per reading.

Afterward, the participants were directed to stand facing a bench (about 30 cm in height/Harvard bench). A sensor plate connected to a computer was equipped on their chest to record the heart rate. The operator set the metronome to 96 beats per minute (24 steps per minute). The participants started stepping on and off (right foot up, left foot up, right foot down, and left foot down) the bench with a regular rhythm for 3 minutes. After 3 minutes, the participants stopped and sat on the bench for 1 minute. The VO₂max was determined by adopting the table described by the American Heart Association Cardiorespiratory Fitness Chart published in 1972. The classification is presented in Table 3 (Herdy and Caixeta, 2016; Morrow *et al.*, 2005).

Statistical analysis

Data were analyzed using the Wilcoxon test in IBM Statistical Package for the Social Sciences Statistics version 20.0 for Windows with a p-value <0.05 indicating a statistical significance.

RESULTS

Figure 1 presents the cardiovascular fitness of the participants based on BP and VO₂max. A decrease in systolic and

 Table 3. VO₂max (ml/kg/minute) based on the American Heart

 Association cardiorespiratory fitness chart.

Age group	Very poor	Poor	Average	Good	Excellent
		Men			
20-29	<25	25-33	34–42	43-52	≥53
30–39	<23	23-30	31-38	39–48	≥49
40-49	<20	20-26	27-35	36–44	≥45
50-59	<18	18-24	25-33	34–42	≥43
60–69	<16	16–22	23-30	31-40	≥41
		Wome	n		
20–29	<24	24-30	31-37	38–48	≥49
30–39	<20	20-27	28-33	34–44	≥45
40-49	<17	17-23	24-30	31-41	≥42
50-59	<15	15-20	21-27	28-37	≥38
60–69	<13	13-17	18–23	24–34	≥35

diastolic BP (p = 0.036 and p = 0.03, respectively) compared to baseline was observed in week 4 (Fig. 1a). A decline in the number of participants with very poor VO₂max (from 23 participants before intervention to 6 participants at week 4 of intervention) was also observed. However, six participants with very poor VO₂max status have not changed. Inversely, an increase in the number of participants with average VO₂max (from 4 participants before intervention to 14 participants at week 3 of intervention) was confirmed. Detailed information on the VO₂max of all participants from the baseline (before intervention with *H. sabdariffa* tea) to week 4 is presented in Table 4.

Statistical analysis revealed a significant difference in the number of participants with very poor and average VO₂max after intervention with *H. sabdariffa* tea (p < 0.05) compared to baseline (Fig. 1b). Therefore, the intervention with *H. sabdariffa* tea might influence cardiovascular fitness, in terms of the BP and VO₂max parameters.

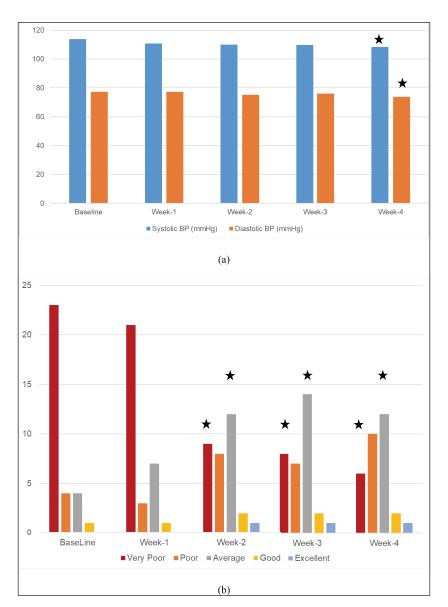


Figure 1. BP (a) and VO₂max (b) of the participants before and after intervention with *H. sabdariffa*. Stars indicate a significant difference compared to the baseline (p < 0.05).

Participants	Baseline	Week 1	Week 2	Week 3	Week 4
А	Poor	Very poor	Poor	Average	Average
В	Poor	Very poor	Average	Poor	Poor
С	Very poor	Very poor	Poor	Poor	Poor
D	Very poor	Very poor	Poor	Very poor	Poor
Е	Poor	Poor	Average	Average	Average
F	Poor	Average	Average	Average	Average
G	Very poor	Very poor	Average	Average	Average
Н	Very poor	Very poor	Poor	Very poor	Poor
Ι	Very poor				
J	Very poor	Very poor	Poor	Average	Average
K	Average	Average	Average	Average	Average
L	Very poor	Very poor	Poor	Average	Poor
М	Very poor	Very poor	Poor	Poor	Poor
Ν	Very poor	Very poor	Poor	Average	Average
0	Very poor				
Р	Very poor				
Q	Very poor	Average	Good	Average	Good
R	Very poor				
S	Very poor	Very poor	Very poor	Good	Good
Т	Very poor	Very poor	Average	Poor	Poor
U	Very poor				
V	Very poor	Average	Average	Average	Average
W	Very poor				
Х	Very poor	Very poor	Very poor	Poor	Poor
Y	Average	Average	Average	Average	Average
Z	Very poor	Average	Average	Average	Average
AA	Very poor	Very poor	Very poor	Poor	Poor
BB	Average	Poor	Average	Average	Average
CC	Average	Average	Good	Good	Average
DD	Very poor	Very poor	Poor	Poor	Poor
EE	Good	Good	Excellent	Excellent	Excellent

Table 4. VO₂max status of the participants (measured every week).

DISCUSSION

In our study, *H. sabdariffa* tea was administered twice daily to 32 healthy normotensive Indonesian participants and its effect on cardiovascular fitness was observed in terms of BP and VO₂max every week until 4 weeks. Both the systolic and diastolic BP of the participants reduced significantly. Our study is in accordance with previous studies reported elsewhere (Al-Shafei and El-Gendy, 2013; Diantini *et al.*, 2021; Herrera-Arellano *et al.*, 2007; Izadi *et al.*, 2021; McKay *et al.*, 2010; Mozaffari-Khosravi *et al.*, 2009). Moreover, a non-randomized quasi-experimental study in Egypt reported a significant decrement in pulse pressure and BP of the normotensive and hypertensive participants compared to their baseline values by the end of week 4 (Al-Shafei and El-Gendy, 2013).

However, apparently, no human studies on the *H. sabdariffa* effect on cardiovascular fitness were reported. Our study is the first report on the effect of *H. sabdariffa* tea on the VO,max of normotensive participants. The intake of *H. sabdariffa*

tea twice daily for 30 days could significantly reduce the number of participants with very poor cardiovascular fitness and increase the number of participants with average cardiovascular fitness. Based on these data, *H. sabdariffa* tea could improve the VO₂max and BP in normotensive participants.

The calyces of *H. sabdariffa* have been proven to contain abundant anthocyanins, which is indicated by the dark red color. We predict that these anthocyanins may play important role in improving the cardiovascular fitness of the participants. In fact, several epidemiological studies have described the correlation between anthocyanins and cardiovascular diseases. An earlier cohort study reported that the intake of 0.2 mg/day of anthocyanins (strawberries and blueberries) was associated with a decreased risk of cardiovascular diseases in elderly females (Mink *et al.*, 2007). Anthocyanins intake was also reported to have the ability to prevent chronic inflammatory diseases in men and women (aged 40–74 years) (Karlsen *et al.*, 2007). Generally, VO₂max is being used as the marker of cardiovascular health status. It is considered an important physiological measure in healthy adults (Bennett *et al.*, 2016). A previous study on Korean women (aged 30–59 years), with more than 30% body fat, reported that systolic and diastolic BP was significantly correlated with VO₂max (Shin and Ha, 2016). Moreover, in a study on 24 Sweden athletes and 5 healthy non-athletes, no significant correlation between VO₂max and the systolic BP/VO₂ slope was found, but a significant correlation between VO₂max and systolic BP in males was observed (Eklund, 2021).

CONCLUSION

Our study assessed the effect of 30 days of *H. sabdariffa* tea consumption on the VO₂max and BP in healthy male and female normotensive participants. We declared that this is the first study on normotensive humans assessing the effect of *H. sabdariffa* tea on their cardiovascular fitness. *Hibiscus sabdariffa* tea intake could significantly reduce the systolic and diastolic BP of the participants. Moreover, *H. sabdariffa* tea significantly reduced the number of participants with very poor VO₂max status and significantly increased the number of participants with average VO₂max status. Although this study was applied to a limited number of participants (n = 32), we concluded that *H. sabdariffa* tea could improve the VO₂max and BP in normotensive participants.

AUTHORS' CONTRIBUTIONS

Ajeng Diantini contributed to conceptualization, methodology, funding acquisition, and supervision. Leonardo Lubis contributed to methodology and data curation. Sri Adi Sumiwi contributed to supervision. Syamsul Rahmat contributed to participant recruitment and clinical investigation. Annisa Alpiani contributed to participant recruitment and clinical investigation. Jutti Levita contributed to writing the original draft, reviewing, editing, and supervision. All the authors have read and approved the final article.

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CONFLICTS OF INTEREST

All the authors declared no potential conflicts of interest.

ETHICAL APPROVALS

The protocol of this study has been approved by The Ethical Committee of Padjadjaran University (document no. 35/UN6.C.10/PN/2018). Data availability can be accessed from the first author upon request.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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