The Acute Treatment Effect Of *Phyllanthus Amarus* on Heart Rate Variability Responses to Moderate- and High-Intensity Exercise in Sedentary Healthy Men

Thapanee Roengrit[1,4], Panakaporn Wannanon[1], Piyapong Prasertsri[1,4], Yupaporn Kanpetta[2,4] Bung-orn Sripanidkulchai[3], Naruemon Leelayuwat[1,4*]
[1]Department of Physiology, Faculty of Medicine, Khon Kaen University
[2]Exercise and Sport Sciences Program, Graduate School, Khon Kaen University
[3] The Center for Research and Development of Herbal Health Products, Khon Kaen University
[4]The Exercise and Sport Sciences Research and Development Group, Khon Kaen University

Background and Objective: Heart rate variability (HRV) is used to assess cardiac autonomic nervous function. Oxidative stress was shown to be associated with the heart function. *Phyllanthus amarus* (PA) is an herbal plant which acts as an antioxidant. Thus, this study aimed to evaluate the acute treatment effects of PA on HRV after a single bout of moderate- and high-intensity exercise.
Method: Twelve men participated in two 3-day phases with a week washout in a randomized double-blinded, cross-over design. On the first day, the subjects ingested two capsules of either *P.amarus* (PA) (600 mg/day) or placebo (PLA) 20 min before cycling at moderate- (M) or high intensity (H). They then ingested the same kind of 4 capsules (2 capsules each meal after lunch and dinner) on the same day of the exercise and 6 capsules/day for the next 2 days.
Results: At 48h after moderate-intensity exercise, RMSSD in the PLA group was higher than in the PA group (M+PLA; 155.1$\pm$30.3 ms, M+PA; 76.1$\pm$31.7 ms;$p<0.05$). However, all frequency domain variables were not significantly different between PLA and PA groups.
Conclusion: This study demonstrates that PA supplementation did not improve HRV induced by a single bout of moderate- and high-intensity exercise.
Keywords: antioxidant, exercise, cardiac autonomic nervous function

ศรีนครินทร์เวชสาร 2556;28 (supple) Srinagarind Med J 2013;28 (supple)

Proceeding

Introduction

Heart rate variability (HRV) is a non-invasive method used to assess cardiac autonomic nervous function (CAN)[1]. Previously, oxidative stress is inversely associated with significant alterations in the functional of heart[2]. Moderate-and high-intensity exercise were encouraged to people to decrease mortality[3]. It is known that increased aerobic metabolism during the exercise is a potential source of oxidative stress[4]. Any intervention improves exercise-induced oxidative stress is likely to positively influence the cardiac autonomic activity. Thus, the antioxidant supplementation during the exercise may increase health fitness by improving the cardiac autonomic activity.

Phyllanthus amarus Schumach. et Thonn. (PA) is an herbal plant which is widely spread throughout the tropical and subtropical areas especially in Thailand. Previous phytochemical studies have shown that it has alkaloids, lignins, flavonoids and polyphenols compounds[5,6]. These substances are known to have beneficial effects on haematological and immunological properties[7], chemoprotective agent against cyclophosphamide induced toxicity in mice6, anti-carcinogenic and anti-inflammatory properties[6-9] and antinociceptive[10]. According to the antioxidant property and availability of PA in Thailand, we are interested in investigating its antioxidant effect on improving HRV after exercise. Interestingly, until now, no scientific study has been reported on the effect of PA on the HRV during recovery process of moderate exercise.

Objective

The aim of this study was to examine the acute treatment effect of PA on the HRV after either a single bout of moderate- or high-intensity exercise. We hypothesized that acute PA supplementation improves HRV during recovery from the moderate- or high-intensity exercise.

Methods

1. Study design and subjects

This study was a randomized crossover (double blind) design. Twelve sedentary males participated in the study. They did not take vigorous exercise regularly, had no medical disorders, and had not taken other antioxidant supplements or medications. This study was approved by the Khon Kaen University Ethical Committee and conformed to the standard set by the Declaration of Helsinki in 2010 (HE531029).

2. Supplement preparation

The supplements consisted of PA and placebo (PLA). One PA capsule contained 100 mg of dried powder without any binders, fillers or additives of PA, while one placebo capsule contained Avicel PH101 (cellulose microcrystalline), Aerosil (dessicant) and artificial colors. And it *weighed* the *same* as PA capsule (100 mg/ capsule). Both supplements were manufactured by the Center for Research and Development of Herbal Health Products, Khon Kaen University. The dosage of the PA was based on the recommendations of the product in Thailand (Khaolaor Laboratories Co. Ltd, G 357/42). Aerial part of PA was collected from Khon Kaen Province. After washing with distilled water and drying at 50^{0}Celcius, the plant was ground and tested for the microbial contamination and insecticide contamination. Then the plant powder was put in the capsule containing recipients.

3. Experimental protocol

Each subject started the experiment by performing an incremental exercise test to determine maximal oxygen consumption ($\dot{V}O_{2,peak}$) on the cycle ergometer (Corival, Lode, The Netherlands) by using a gas analysis system (AD instrument, ML206, Australia). The $\dot{V}O_{2,peak}$ of the subject was determined when any of the following was achieved; the subject's $\dot{V}O_2$ reached a plateau with an increased workload, or a respiratory gas exchange ratio greater than 1.15, or heart rate (HR) reached maximum HR (calculated by the equation: 220 – age), or maximal symptoms of dyspnea and fatigue by using the *ratings of perceived* dyspnea (RPD) and the rating of perceived exertion (RPE) scales or were unable to maintain cycling at a speed of 60 rpm. HR and ECG were continuously monitored during the test. This test was done at least one week before the initial exercise session. A relationship between workload and $\dot{V}O_2$ from the test was used to obtain workload for the subsequent exercise session.

Then, subjects randomly participated in two 3-day phases with one week apart to prevent carryover effect. At the first day of both phases they randomly ingested two capsules of either PA or PLA 20 min before performing 20-min moderate-intensity exercise with 3-min warming-up on the cycle ergometer. After that, they exercised for 20 min at either a moderate or high-intensity (65% or 85% of their $\dot{V}O_{2,peak}$). They then ingested four capsules of the same supplementation (two capsules each meal after lunch and dinner) on the same day of the exercise and six capsules/day for the next 2 days.

4. HRV analysis

Five-min 3-lead ECG was recorded at rest before the supplementation and throughout the subsequent exercise. The 3-min ECG were also recorded at 24 and 48 h of recovery from the exercise. It was analyzed using a Labchart 6 (PowerLab 8/30 AD instruments, Australia) by both time and frequency domains. The time domain consisted of standard deviation of all NN intervals (SDNN), square root of the mean of the sum of the squares of differences between adjacent NN interval (RMSSD). In addition, frequency domain consisted of the total power (TP), the low frequency (LF) (0.04 - 0.15Hz), the high frequency (HF) (0.15 - 0.4 Hz), very low frequency (VLF) (0.003 - 0.04 Hz), LF in normalized unit, HF in normalized unit and the ratio LF/HF. LF and HF were measured in normalized units [(n.u.=(LF or HF)x100/(total power - VLF)].

5. Statistical analysis

Descriptive data were presented by mean $\pm$ SD. One-way ANOVA with repeated measures was used to test the effect of time and supplementation. All statistical significance was set at $p < 0.05$. Statistical analysis was performed using SPSS statistical software, version 18 (SPSS, Chicago, IL).

Results

1. Subject characteristics

The subjects were 12 sedentary men, aged 22ฑ2.90 years. Their mean $\dot{V}O_{2,peak}$ was 36.9$\pm$5.21 ml/kgBM/min. Anthropometric and physiological characteristics were summarized in Table 1.

2. HRV index

2.1 HR

In both supplement groups and exercise intensities, HR was significantly increased during exercise from pre-exercise ($p< 0.05$). In contrast, it was significantly decreased at 24 h and 48 h after the exercise when compare with during and immediately after the exercise in both supplement groups ($p< 0.05$). At 24 and 48 hafter exercise, there were significantly decrease from immediately after the moderate-intensity exercise in both supplement ($p< 0.05$), and only PA group in high -intensity exercise ($p< 0.05$) (Figure 1).

2.2 Time domain

At the moderate-intensity exercise, SDNN and RMSSD were significantly increased at 48 h after exercise when compared with during exercise in PLA group ($p< 0.05$). There were significantly lower during high- intensity exercise than during moderate-intensity exercise in only PA group ($p< 0.05$). Interestingly, RMSSD in the PLA group was higher than the PA group at 48h after moderate-intensity exercise ($p<0.05$). At high-intensity exercise, SDNN was significantly increase at 24 h after exercise from immediately after exercise (post-exercise) in PLA group ($p< 0.05$) (Figure 2A-B).

2.3 Frequency domain

Total power, TP; low frequency, LF; high frequency, HF, very low frequency; VLF, LF nu., HF nu., and the ratio LF/HF *were not significant different between* PLA and PA groups after a single bout of moderate- and high-intensity exercise. However, at high-intensity exercise, TP was significantly lower than the moderate-intensity exercise in PLA group after post-exercise ($p< 0.05$) (Table 2). At high-intensity exercise, LF (nu.) during exercise was significantly lower than pre-exercise in PLA group ($p< 0.05$) (Figure 3A). Both exercise intensities, HF (nu.) was significantly decrease at post- exercise from pre-exercise in PA group ($p< 0.05$). At moderate-intensity exercise, there was significantly increase at 24 and 48 h after exercise from during exercise in PLA group ($p< 0.05$). At high-intensity exercise in PA group, HF (nu.) was significantly decreased at post-exercise compared with pre-exercise. However, at 48 h after the high-intensity exercise in the PA group, HF (nu.) was significantly higher than during and immediately post-exercise. Moreover, there was significantly increase when compare with moderate–intensity exercise group at the same supplement and time (Figure 3B). At 48 h after high-intensity exercise, VLF was significantly lower

Table 1 Anthropometric and physiological characteristics of subjects

Variables	Mean±SD (n=12)
Age (yr)	22±2.90
Height (m)	1.69±0.06
BM (kg)	63.6±10.7
BMI (kg/m^2)	22.8±3.27
%BF	19.5±9.00
FM (kg)	12.5±7.30
FFM (kg)	48.4±6.62
Waist circumference (cm)	76.7±7.46
Hip circumference (cm)	94.8±6.08
Waist: hip ratio	0.81±0.04
$VO_{2,peak}$ (ml/kgBM/min)	36.9±5.21
Work load$_{max}$ (watts)	136.07±32.35
65% $VO_{2,peak}$ (ml/kgBM/min)	21.4±3.77
85% $VO_{2,peak}$ (ml/kgBM/min)	28.0±4.93
measured %$VO_{2,peak}$ during moderate-intensity exercise	68.4±18.91
measured %$VO_{2,peak}$ during high-intensity exercise	82.4±7.0

Values are presented as mean±SD; n=12.BM, body mass; BMI, body mass index; %BF, percentage of body fat; FM, fat mass; FFM, fat free mass; $VO_{2,peak}$, peak oxygen consumption

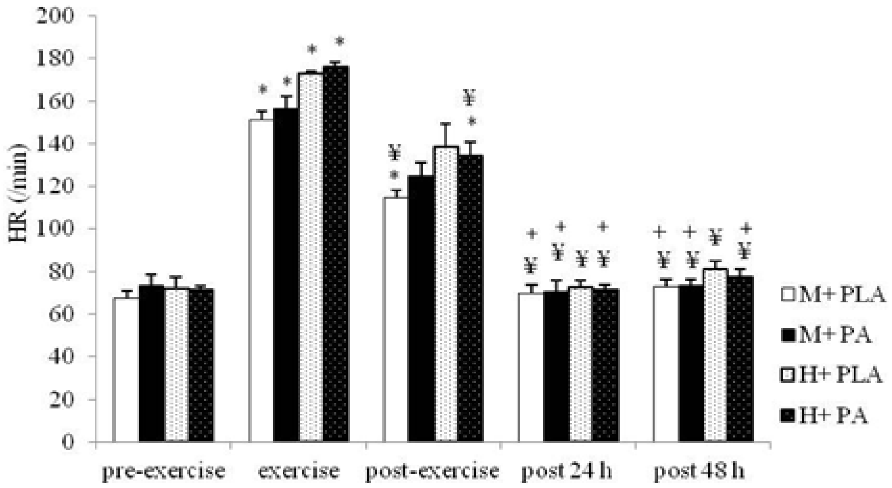

Figure 1 HR before the exercise (pre-exercise), immediately (post-exercise), 24 h (post 24 h) and 48 h (post 48 h) after moderate- and high-intensity exercise

Values are presented as mean ± SE; n=12.

M, moderate intensity; H, high intensity; PA, *P. amarus*; PLA, placebo.

* Significantly different from pre-exercise (p<0.05),

¥ significantly different from during exercise (p<0.05),

+ significantly different from immediately after the exercise (post-exercise) (p<0.05).

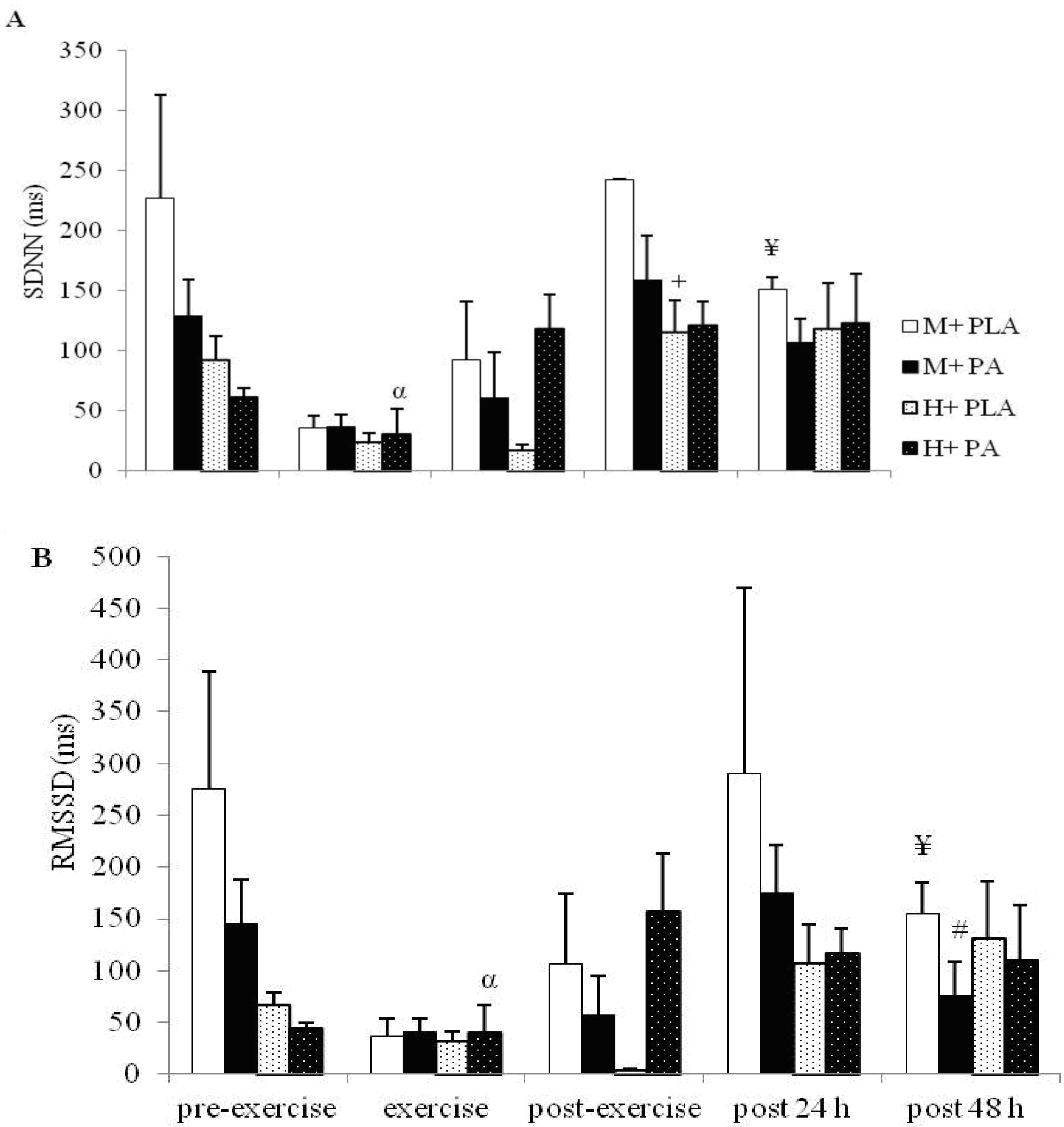

Figure 2 SDNN (A) and RMSSD (B) before the exercise (pre-exercise), immediately (post-exercise), 24 h (post 24 h) and 48 h (post 48 h) after moderate- and high–intensity exercise

Values are presented as mean ± SE; n=12.

SDNN, standard deviation of all NN intervals; RMSSD, square root of the mean of the sum of the squares of differences between adjacent NN interval; M, moderate intensity; H, high intensity; PA, *P. amarus*; PLA, placebo

¥ significantly different from during exercise ($p<0.05$),

+ significantly different from immediately after the exercise (post-exercise) ($p<0.05$),

significantly different from the PLA group at the same time point ($p<0.05$).

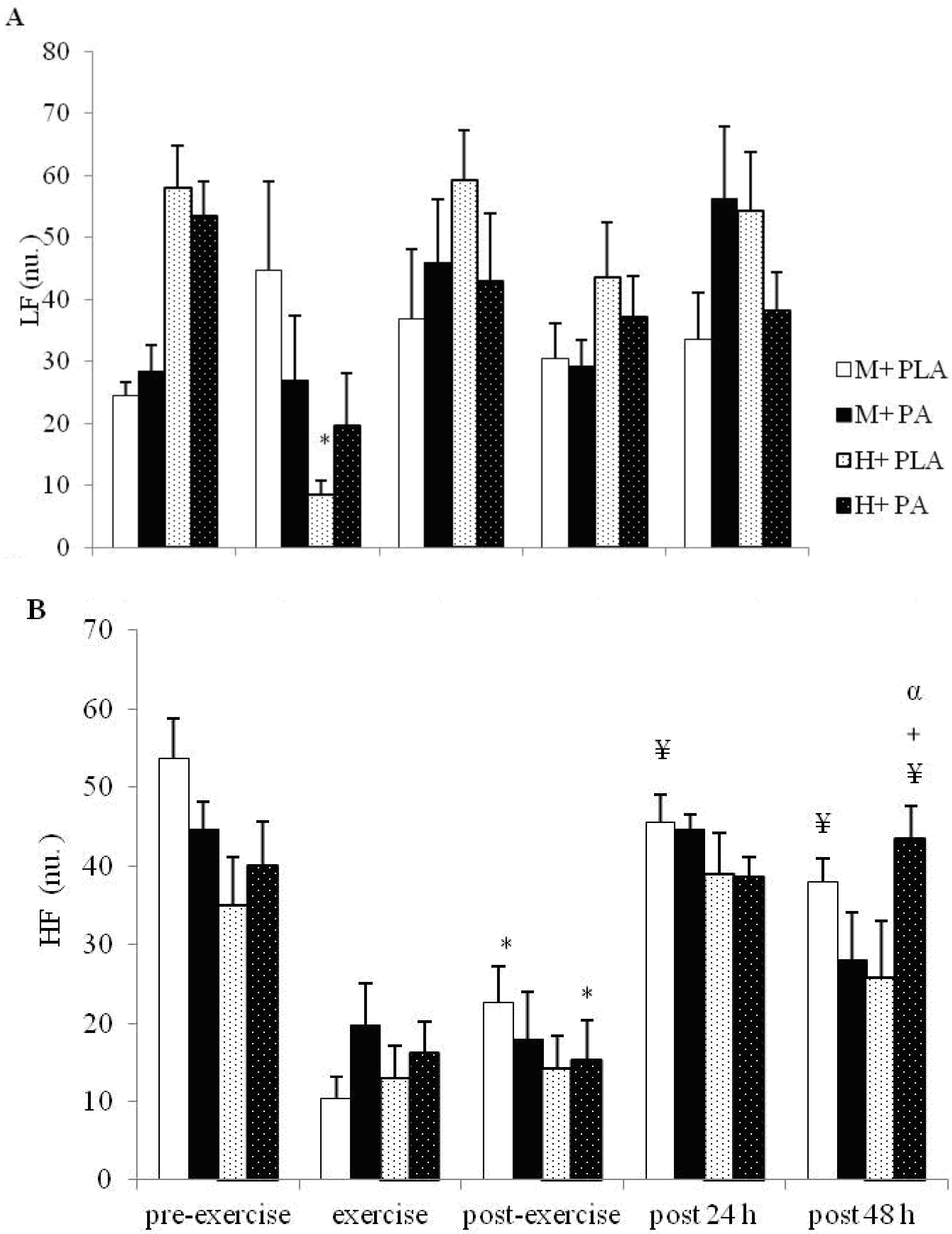

Figure 3 LFnu. (A), and HFnu. (B) before the exercise (pre-exercise), immediately (post-exercise), 24 h (post 24 h) and 48 h (post 48 h) after moderate- and high–intensity exercise

Values are presented as mean + SE; n=12.

LF, low frequency; HF, high frequency; nu., normalize unit; M, moderate intensity; H, high intensity; PA, *P. amarus*; PLA, placebo

* Significantly different from pre-exercise (p<0.05),

¥ significantly different from during exercise (p<0.05),

+ significantly different from immediately after the exercise (post-exercise) (p<0.05),

α significantly different from the moderate intensity exercise at the same time point (p<0.05).

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Table 2 Frequency domain of HRV before the exercise (pre-exercise), immediately (post-exercise), 24 h (post 24 h) and 48 h (post 48 h) after the moderate- and high–intensity exercise

			Time-points				
Parameter	Exercise intensity	Supplement	pre-exercise	during exercise	post-exercise	post-24 h	post-48 h
TP (ms^2)	Moderate	PLA	64901.13±36519	1946.42±1038	16613.04±13427	85284.13±73710	18511.31±4511
		PA	20006.98±9644	2010.82±1242	9376.43±8956	23092.23±7190	11147.93±3785
	High	PLA	9157±3224	927.77±432	206.34±64 แ	12839.21±7737	23774±13346
		PA	3900±830	3844.38±3599	20264.89±7041	14090.74±4218	28044±22338
HF (ms^2)	Moderate	PLA	25629.39±13081	326.71±204	4031.40±3535	33032.73±28436	5171.87±2341
		PA	7037.90±3669	514.93±323	1583.95±1478	7857.53±3034	2122.26±1505
	High	PLA	1528.53±417	160.69±99.32	7.40±5.57	5645.29±4530	8133.34±6436
		PA	826.07±168	773.78±719	2185.02±945	3973.43±1445	10814.17±9078
LF (ms^2)	Moderate	PLA	11371.92±6317	247.94±155	2133.45±1808	19120.96±17346	2967.84±926
		PA	3193.93±1400	281.55±184	1991.11±1907	5560.22±2417	1783.99±648
	High	PLA	3259.63±1432	111.69±72.25	26.21±14.05	4055.17±2623	7459.15±4217
		PA	1413.27±451	269.05±247	3521.49±1728	2803.64±1221	4566.96±3431
VLF (ms^2)	Moderate	PLA	17320.36±13947	227.52±66.78	2053.98±1094	17654.22±15950	5940.94±1274
		PA	5401.37±2428	209.57±107	1664.37±1541	6580.33±2607	5403.04±2673
	High	PLA	3992.74±1776	49.60±23.06	168.40±60.93	2301.22±503	2684.91±1276 แ
		PA	1541.85±344	322.97±297	1301.50±808	4174.07±1017	8875.20±7007
LF/HF	Moderate	PLA	0.49±0.07	12.73±6.13	2.36±1.07	0.70±0.15	0.97±0.3
		PA	0.67±0.12	4.97±2.91	4.19±1.34	0.65±0.08	3.01±1.14
	High	PLA	2.07±0.48	0.97±0.33	6.50±2.43	1.33±0.41	3.97±1.6
		PA	1.66±0.34	3.86±2.68	4.16±1.26	1.00±0.21	1.18±0.45

Values are presented as mean±SE; n=12.

TP, total power; HF, high frequency; LF, low frequency; VLF, very low frequency; nu., normalize unit

* Significantly different from pre-exercise ($p<0.05$),

ฅ significantly different from during exercise ($p<0.05$),

+ significantly different from immediately after the exercise (post-exercise) ($p<0.05$),

แ significantly different from the moderate intensity exercise at the same time point ($p<0.05$),

significantly different from the PLA group at the same time point ($p<0.05$)

than the moderate-intensity exercise in PA group ($p< 0.05$). However, LF/HF ratio was not significant different in both supplement groups and exercise intensities at any time points (Table 2).

Conclusion

In conclusion, the present study did not confirm the possible effects of PA supplementation on HRV after a single bout of moderate- and high-intensity exercise. A limitation of this study may be due to inadequate dose of the PA which is the dose for resting condition. Therefore, the investigation of higher dose of the whole PA powder or extract of the PA supplementation on HRV after the moderate- and high-intensity exercise are needed.

Acknowledgments

We would like to thank the volunteers for their enthusiastic participation in this study. This study was supported by grants from Graduate Research Fund Academic Year 2010 (53212102) Khon Kaen University, National Research University (MIH-2554-Ph.D-10), and Invitation research (I 55145). It was partially supported by the Exercise and Sport Sciences Research and Development Group and the Joint Research group, Khon

Kaen University. Finally, we thank the Center for Research and Development of Herbal Health Products, Khon Kaen University, for the supplement manufacture.

Reference

1.Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology Circulation. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation 1996; 93:1043–65.

2. Rhoden CR, Wellenius GA, Ghelfi E, Lawrence J, Gonzแlez-Flecha B. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. Biochim Biophys Acta 2005;3:305-13.

3. World health organization. Global recommendations on physical activity for health. 2010.

4. Leeuwenburgh C, Heinecke JW. Oxidative stress and antioxidants in exercise. Curr Med Chem. 2001;8: 829-38.

5. Kumaran A, and Karunakaran RJ. In vitro antioxidant activities *of methanol extracts of five Phyllanthus species from India.* LWT- Food Science and Technology 2007; 40: 344-52.

6. Kassuya CA, Silvestre AA, Rehder VL, Calixto JB. Anti-allodynic and anti-oedematogenic properties of the extract and lignans from Phyllanthus amarus in models of persistent inflammatory and neuropathic pain. *European* Journal of *Pharmacology* 2003;478:145-53.

7.Taiwo IA, Bola O. Oboh, Francis-Garuba PN. Haematological Properties of Aqueous Extracts of Phyllantus amarus (Schum and Thonn.) and Xylopia aethiopica (Dunal) A. Rich in Albino Rats. Ethno-Med 2009; 3: 99-110.

8.Kumar KB, and Kuttan R.Chemoprotective activity of an extract of Phyllanthus amarus against cyclophosphamide induced toxicity in mice. Phytomedicine 2005; 12: 494-500.

9.Kiemer AK, Hartung T, Huber C, Vollmar AM. Phyllanthus amarus has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-kappaB pathway.J Hepatology 2003; 38: 289-97.

10.Santos AR, De Campos RO, Miguel OG, Filho VC, Siani AC, Yunes RA, Calixto JB. Antinociceptive properties of extracts of new species of plants of the genus Phyllanthus (Euphorbiaceae)," Journal of Ethnopharmacology 2000; 72: 229-38.