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Research Article
Moringa oleifera Leaves Extract Alters Exercise-Induced Cardiac Hypertrophy Adaptation

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Abstract

Background and Objective: Cardiomyocyte adaptation to exercise might require ROS as a central regulator. There is a limited study regarding the importance of ROS for inducing exercise-induced adaptation and its correlations with changes in histological scoring of cardiac muscles. The study aimed to explore the importance of physiological ROS induced by exercise and its correlation with Cardiomyocyte' histological appearance that is altered by *Moringa oleifera* leaves extract in Wistar rats. **Materials and Methods:** This was an animal experimental study, which use 4 groups of 24 Wistar rats divided into Control (Co), *Moringa* leaves extract (Mo), Exercise (Ex) and a combination of *Moringa* leaves extract and Exercise (MoEx). The *Moringa* leaves extract were given orally, 5 days a week, for 4 consecutive weeks. The exercise was given in moderate intensity, 5 days a week, also for 4 consecutive weeks. **Results:** This study found significant differences in heart weight and heart weight/body weight ratio in Ex group compared to the control. As for histology scoring, found that MoEx group has 16.7% cardiac hypertrophy and myofiber disarray compared to 83.3% mild hypertrophy and 50% mild disarray in Ex group. **Conclusion:** In summary, the study showed that the potential central role of exercise-induced physiological ROS for cardiac hypertrophy adaptation is altered by *Moringa oleifera* leaves extract treatment.

Key words: Cardiac hypertrophy, *Moringa oleifera*, ROS, cardiomyocyte, cardiac hypertrophy, myofiber disarray

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acute aerobic exercise induces oxidative stress in blood and other organs such as the skeletal muscle and the heart[1-3]. Regulation response to oxidative stress needs to be controlled balance to maintain physiological stability[4]. Increased oxidative stress by exercise provides benefits until it reaches maximal levels, while further increase would cause damage in tissue[3]. This exercise-induced oxidative stress is determined by many factors including exercise intensities and duration, nutritional and training status and other factors[2]. Intense and long duration of exercise might lead to prolonged inflammation, while moderate-intensity or high intensity with resting interval might deliver optimal benefit on body health[5].

Animal and human studies showed that antioxidants supplementation prevented exercise-induced oxidative stress and as a result, it might blunt the adaptation process to exercise in skeletal muscle[3,6-8]. As for cardiac muscles, Graham *et al.*[9] also proved that mitochondria-targeted antioxidant attenuates cardiac hypertrophy in spontaneously hypertensive rats. Some studies showed that significant cardiac hypertrophy could be detected starting from 4 weeks of exercise training and become plateau after 6-8 weeks of exercise duration[10-12]. Molecular aspects of cardiac hypertrophy as a result of exercise-induced adaptation are a complex process involving many pathways and modulators including growth factors, hormones and ROS[13,14]. Signaling pathway in physiological cardiac hypertrophy is involving IGF-PI3KCA-AKT-mTOR pathway[14]. The activation of AKT-mTOR has an important role in activating HIF, which is regulated by ROS[15].

Indonesian people have long used medicinal plants as an alternative in treating many diseases. These medicinal plants have many potential benefits such as anticancer, antiviral, anti-inflammatory, analgesic, Cardioprotective and other benefits for health[16]. Using medicinal plants should be conducted while considering the potential risks, including herb-drug interactions, overdosage, toxicity and contamination of medicinal plants[16-18]. *Moringa oleifera* is a plant that is predominantly found in Asia and Africa[19]. In Indonesia, *Moringa oleifera* has been known as a local medicinal plant that could be found in Java and the lesser Sunda islands[20]. This plant is well known for its antioxidant, anti-inflammation, protective role in the cardiac, liver, nervous system and many other activities that bring health benefits[21-23]. Of all the parts of *Moringa oleifera* plant, it is the leaves that are most often used for the prevention and treatment of chronic disease. The leaves have many bioactive compounds such as polyphenols, flavonoids, tannins, phenolic acids and saponins, which are associated with their antioxidant and cardioprotective properties[19,24].

This study hypothesized that uncontrolled antioxidants supplements consumption under normal conditions might alter the cardiac hypertrophy process after exercise that is regulated by ROS. So, this study aimed to investigate the effect of *Moringa oleifera* leaves extract on cardiac hypertrophy induced by exercise.

MATERIALS AND METHODS

Study area: The animal study was carried out in the Animal Physiology Laboratory, Physiology Division, Faculty of Medicine, Universitas Padjadjaran, while the histological study was carried out in Maranatha Biomedical Research Laboratory and Pathology Anatomy Department, Maranatha Christian University. This study was conducted from July, 2020 until July, 2021.

Animals: Twenty-four male Wistar rats aged 8 weeks, weighed 200±20 g, were purchased from Biofarma and divided into 4 groups: Co (Control), Mo (*Moringa*), Ex (Exercise) and MoEx (*Moringa* and Exercise), each group consisted of 6 rats (n = 6). *Moringa* leaves extract were given orally 5 days per week, while exercise was given by running on a treadmill for 30 min a day, 5 days/week. All treatments were given for 4 consecutive weeks. During the experiments, all the rats were given a standard chow diet and water ad lib, housed in groups of three at a temperature between 22 and 24°C, with 12 hrs cycles of light and dark each day. Implementation of all procedures was based on the use and care of laboratory guidelines[25] and has received approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Kristen Maranatha-Rumah Sakit Immanuel Bandung No117/KEP/IX/2020.

Treadmill training protocol: At first, all the rats were given 2 weeks of environmental habituation. For the rats in Ex and MoEx groups, 1 week of treadmill habituation was carried out preceding treadmill exercises of 20 m min^{-1} in 30 min a day, 5 days/week, for 4 weeks. This speed was determined based on an intensity that stimulates lactate threshold, according to a study conducted by Ronny Lesmana *et al.*[26]. Sedentary control and *Moringa* served as non-exercise groups. At the end of the study, all the rats were terminated, the body weight and heart weight were obtained and

cardiac tissues were collected then formalin-fixed for hematoxylin-eosin staining.

Preparation of *Moringa oleifera* leaves extract: *Moringa oleifera* leaves extract powder was collected from West Java, Indonesia. The dose used in this study was 5.7 mg kg^{-1} b.wt., for each rat, given orally 5 times a week, for 4 weeks. At the end of the study, the rats were terminated and cardiac tissues were collected for making histological slides.

Hematoxylin and eosin (H and E staining): Cardiac tissues were obtained, formalin-fixed, dehydrated, embedded in paraffin wax, 2 µm thick sections were excised, placed on slides, then stained with hematoxylin-eosin. Blinded histology visualization was conducted by an expert pathologist using a microscope (LEICA ICC50) with the magnification of 100 and 400x. The scoring system for evaluation of the slides was taken from previous study[27], after some modification from a study conducted by McLeod *et al.*[28]. Level of cardiomyocyte hypertrophy and myofiber disarray was evaluated. As for cardiomyocyte hypertrophy, used this scoring:

0 = No cardiomyocyte hypertrophy
1 = Mild cardiomyocyte hypertrophy (cardiomyocyte diameters were 3-4 RBCs)
2 = Moderate cardiomyocyte hypertrophy (cardiomyocyte diameters were 4-5 RBCs)
3 = Severe cardiomyocyte hypertrophy (cardiomyocyte diameters were >5 RBCs)

And as for the level of myofiber disarray, used this scoring:

0 = No myofiber disarray
1 = Mild myofiber disarray (1-25% of the myocardial area)
2 = Moderate myofiber disarray (26-50% of the myocardial area)
3 = Severe myofiber disarray (>50% of the myocardial area)

Statistics: Results were presented as Mean±Standard error of the mean. Data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey HSD test using Statistical Package for Social Science (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). The results of the scoring system for cardio myocyte slides were analyzed using Kruskal-Wallis test. Values of $p<0.05$ were considered as statistically significant.

RESULTS

Effects on bodyweight, heart weight and heart weight/body weight ratio: In this study, there was no significant difference in body weight (Co 289.17±4.8, Mo 283±5.05, Ex 283.17±4.21, MoEx 290.83±9.68) in all treatment groups ($p = 0.747$) compared to the control as shown in Fig. 1a. This study also found significant differences in heart weight

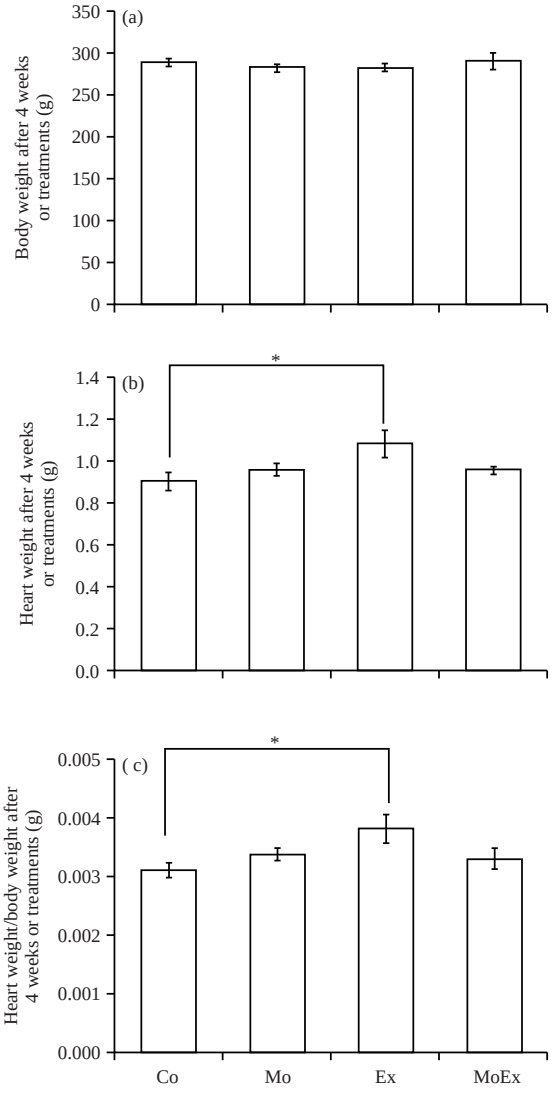

Fig. 1(a-c): Effect of *Moringa oleifera* extracts supplementation and exercise on body weight, heart weight and heart weight/body weight ratio (a) Body weight of Wistar rats, (b) Heart weight of Wistar rats and (c) Heart weight/body weight ratio of Wistar rats

Co: Control, Mo: *Moringa* leaves extract, Ex: Exercise, MoEx: Combination of *Moringa* leaves extract and exercise, *: $p<0.05$ (Ex compared to Co)

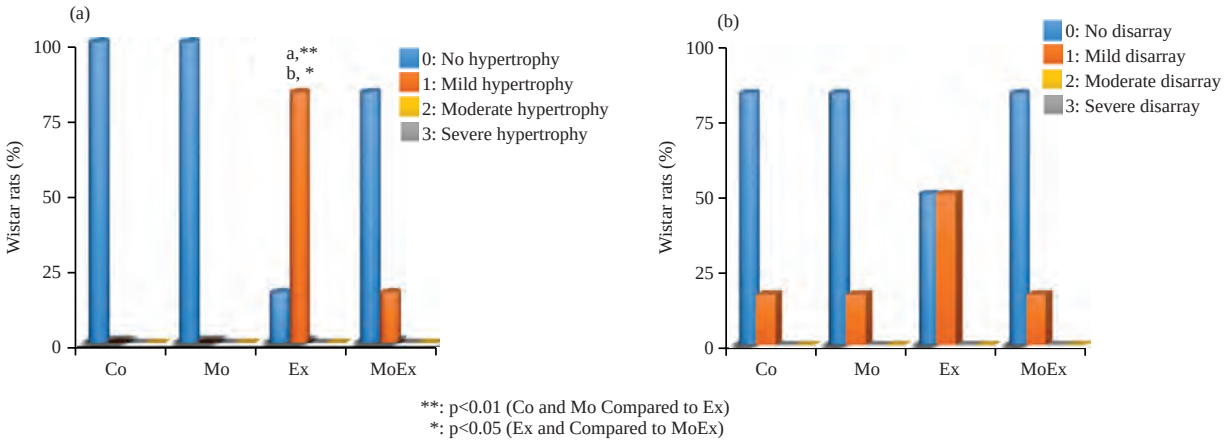

Fig. 2(a-b): Effect of *Moringa oleifera* extracts supplementation and exercise on histology scoring, (a) cardiomyocyte hypertrophy and (b) Myofiber disarray

Co: Control, Mo: *Moringa* leaves extract, Ex: Exercise, MoEx: Combination of *Moringa* leaves extract and exercise

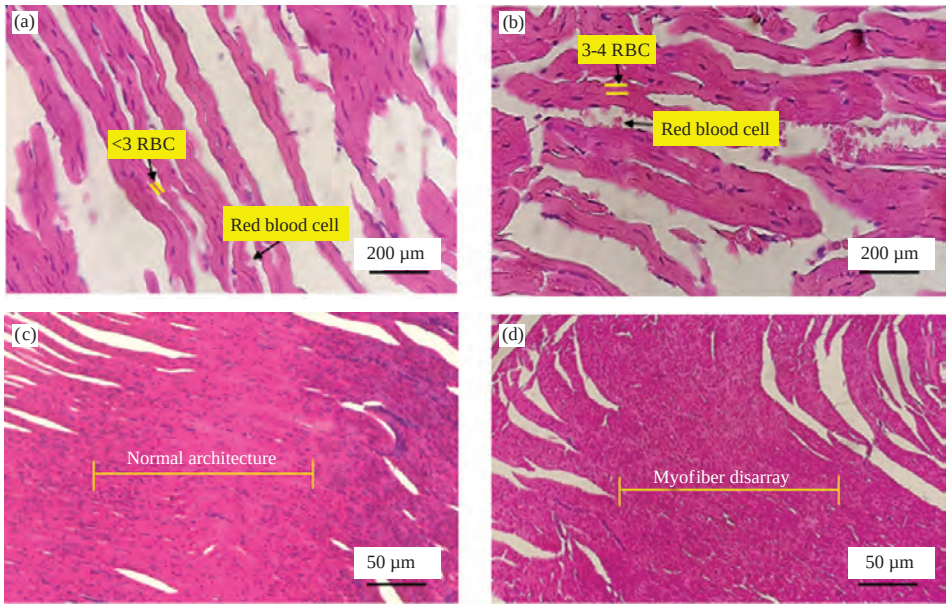

Fig. 3(a-d): Effect of *Moringa oleifera* extracts supplementation and exercise on histology appearances, (a) Level 0 cardiomyocyte hypertrophy (400x), (b) Level 1 cardiomyocyte hypertrophy (400x), (c) Level 0 myofiber disarray (100x) and (d) Level 1 myofiber disarray (100x)

Co: Control, Mo: *Moringa* leaves extract, Ex: Exercise, MoEx: Combination of *Moringa* leaves extract and exercise

(Co 0.90±0.043, Mo 0.96±0.029, Ex 1.08±0.064, MoEx 0.96±0.018) with p = 0.033 as shown in Fig. 1b and heart weight/body weight ratio (Co 0.0031±0.00013, Mo 0.0034±0.00010, Ex 0.0038±0.00025, MoEx 0.0033±0.00017) between the Co and Ex groups with p = 0.041 as shown in Fig. 1c.

Effects on histology scoring of wistar cardiac rats muscles: *Moringa oleifera* leaves extract and treadmill exercise-induced different histology characteristics in Wistar cardiac rat muscles, based on cardiomyocyte hypertrophy in Fig. 2a and myofiber disarray in Fig. 2b. For cardiomyocyte hypertrophy, found no hypertrophy in Co and Mo groups (100%) but found 83.3% mild hypertrophy and 16.7% no hypertrophy in the Ex group but interestingly, found only 16.7% mild hypertrophy and 83.3% no hypertrophy in MoEx group. And as for myofiber disarray, found 83.3% no disarray and 16.7% mild disarray in Co, Mo and MoEx groups, while in Ex groups there were 50%

no disarray and 50% mild disarray. Statistical analysis showed a very significant difference in cardiac hypertrophy between the Co and Mo compared to Ex groups ($p = 0.05$) and a significant difference in cardiac hypertrophy between the Ex and MoEx groups ($p = 0.027$) as shown in Fig. 2a. This study did not find a significant difference between myofiber disarray between all groups as presented in Fig. 2b ($p = 0.575$). The effect of *Moringa oleifera* extract and treadmill exercise on general histology appearance of level 0 cardiomyocyte hypertrophy was presented in Fig. 3a and level 1 cardiomyocyte hypertrophy in Fig. 3b. Level 0 cardiomyocyte hypertrophy was defined as no cardiomyocyte hypertrophy, whereas level 1 cardiomyocyte hypertrophy was defined as mild cardiomyocyte hypertrophy (cardiomyocyte diameters were 3-4 RBCs), with 400x magnification. The effect of *Moringa oleifera* extract and treadmill exercise on general histology appearance level 0 myofiber disarray in Fig. 3c and level 1 myofiber disarray in level 3D. Level 0 myofiber disarray was defined as no myofiber disarray, whereas level 1 myofiber disarray was defined as mild myofiber disarray (1-25% of the myocardial area), with 100x magnification.

DISCUSSION

Moringa oleifera has been known as the herbal plant that contains phytochemicals that could exert its effect as an anti-inflammatory, antioxidants, anti-obesity and cardioprotective[29,30]. Exercise was proven to be an effective way of preventing chronic diseases and promoting cardiovascular health[31]. But recently, a concept of exercise-induced hormesis has been postulated. Hormesis is a term that is used to explain a curve showing biphasic dose-response, where low levels of oxidative stress deliver benefit effect on tissue but high levels induce damage[3,32]. It is proved that exercise-induced oxidative stress delivers optimal benefit when the exercise was done in low to moderate intensity or high intensity with intervals of resting periods[5,33].

This study used 4 weeks of moderate-intensity of treadmill exercise to induce cardiac hypertrophy. This study found a significant increase in heart weight and heart weight/body weight between the exercise groups compared to the control in Fig. 1. As for histological examination, this study found 83.3% of rats with mild cardiac hypertrophy and 50% with myofiber disarray (Fig. 2 and 3). These characteristics might be induced by the adaptation process after exercise, which results in cardiac remodelling that leads to physiological cardiac hypertrophy. This result is in line with other studies that found cardiac hypertrophy after four weeks of treadmill exercise[10,11]. Many signalling pathways and substances might involve in the detailed mechanism of cardiac hypertrophy, including ROS[13,14].

Cellular and redox signalling modification play an important role in cardiac hypertrophy induced by exercise[34]. The key process of redox signalling is protein post-translational modification as a result of oxygen and ROS effects[35]. As the highest O_2 consumption organ, O_2 metabolism alteration in cardiac-exercise adaptation might activate the redox signalling pathway correlated with cardiac hypertrophy[35,36]. The major signalling pathway involved in physiological cardiac hypertrophy is IGF-PI3Kα-AKT pathway, whereas the activation of AKT would increase mTOR which eventually increases angiogenesis induced by HIF[13,14]. Another signalling pathway is cGMP/PKG that inhibits pathological cardiac hypertrophy through inhibition of NFAT[14,37]. Some studies have confirmed ROS involvement in these pathways (AKT-PKB and PKG)[15,38] but the sources of ROS and the extent of its role in cardiac hypertrophy still need to be further elucidated[14].

Sources of ROS in the cellular state are mitochondria, endoplasmic reticulum and NADPH oxidases (NOX)[14,39-41]. In cardiovascular systems, NOX 1, 2, 4 and 5 produce low levels of ROS and were correlated with the proliferation and differentiation of cardiac cells[40,42]. In cardiac hypertrophy, NOX2 and NOX4 are both upregulated but have different effects, while NOX2 is a source of ROS activating CaMKII-HDAC in pathological cardiac hypertrophy[43], NOX4 is a source of ROS activating cytoprotective genes (HIF-VEGF, Nrf2) and promoting autophagy[44-46]. Although NOX4 is confirmed to be protective, a very high level of ROS production might have a detrimental effect that overwhelms its protective effect but the exact mechanism for this effect is still unknown[14,47].

Another source of ROS is endothelial (eNOS) and neuronal synthases (NOS) in cardiomyocytes[14]. NOS coupled with NO has a beneficial effect through the cGMP-PKG pathway[38] but uncoupled NOS leads to hyper oxidation and PKA activation that results in maladaptive cardiac hypertrophy[14]. The vascular wall stress-induced in exercise activates the PI3Kα-AKT pathway that increases the production of eNOS and NO leads to cardioprotection against apoptosis[13,48].

In the control and *Moringa* groups, this study found no cardiac hypertrophy and 16.7% mild myofiber disarray, while in the exercise group found 83.3% mild hypertrophy and 50% mild disarray. Interestingly, this study found only 16.7% of rats with mild cardiac hypertrophy and myofiber disarray in the group given *Moringa oleifera* leaves extract and exercise. This finding might suggest that *Moringa* leaves extract alters cardiac hypertrophy induced by exercise. Antioxidants have been used in pathological cardiac hypertrophy because of

their effect on attenuating cardiac hypertrophy by decreasing the accumulation of ROS that served as suppression of the pro-hypertrophic signalling pathway[49]. Considering multiple pathways of redox signalling in cardiac hypertrophy, this study postulated that *Moringa* leaves extract might alter either one or more pathways. Biomarker modulations correlated with the effects of *Moringa* are oxidative stress decrease (ROS, NO, iNOS), signal transduction modification (Nrf2, PI3Kα-AKT-mTOR and NOX4)[30,50-52]. Those modulations might attenuate the effect of cardiac hypertrophy induced by moderate exercise. Nevertheless, there is still limited data regarding the molecular mechanism of *Moringa* leaves extract on cardiac hypertrophy induced by exercise, therefore a future study needs to be elucidated.

CONCLUSION

Regular treadmill exercise induces cardiac hypertrophy as a part of cardiac adaptation to exercise, in which physiological ROS might play a role. *Moringa oleifera* with its antioxidant potential could affect the process of cardiac hypertrophy induced by exercise. In this study, a significant increase in heart weight and heart weight/body weight were found in the exercise group after 4 weeks of treadmill exercise with moderate intensity, accompanied by histological findings that showed 83.3% of rats with mild cardiac hypertrophy and 50% with myofiber disarray. On the contrary, histological findings showed 16.7% of mild cardiac hypertrophy and myofiber disarray in the group given *Moringa oleifera* leaves extract and exercise. This finding might suggest that *Moringa* leaves extract alters cardiac hypertrophy induced by the moderate intensity of exercise.

SIGNIFICANCE STATEMENT

This study discovered that *Moringa oleifera* alters cardiac hypertrophy induced by moderate-intensity of treadmill exercise and the reason for this effect might be correlated with its antioxidant potential. *Moringa oleifera* was found to be beneficial in exercise-induced cardiac adaptation because it might reduce the effects of exercise in increasing ROS production thus attenuating cardiac hypertrophy. This study will help the researchers to uncover the critical ideas of the exercise and antioxidants combination to suppress the production of ROS that might be induced by cardiac adaptation to exercise, that many researchers were not able to explore. Thus, a new theory on the potential of *Moringa oleifera* may have arrived at its role in altering cardiac hypertrophy in response to moderate intensity of treadmill training.

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