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Original Article

EFFECT OF *HIBISCUS SABDARIFFA LINN* ON IL-6 AND TNF- α LEVELS IN OVERTRAINED RAT HEART

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ABSTRACT

Objective: This study aims to determine the effect of *Hibiscus sabdariffa Linn*. (HSL) administration on the interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) levels in rat heart. Overtraining was proven to increase the IL-6 and TNF-α levels in the blood, and HSL had anti-inflammatory and anti-oxidant properties. However, no studies have been conducted on the effect of methanolic extract of HSL administration on the IL-6 and TNF-α levels in overtrained rat heart.

Methods: This study used 25 male adult Wistar rats aged 8–10 w and weighing 200–250 g. The rats were randomly divided into five groups: control (C), control *H. sabdariffa Linn* (C+HSL), overtraining (OT), overtraining *H. sabdariffa Linn* (OT+HSL), and aerobic (A). Treatment was given 5 times a week for 11 w. At the end of the study, the IL-6 and TNF-α levels were measured using a standard ELISA kit.

Results: IL-6 and TNF-α levels in the heart were the highest in the overtraining group. The group that received HSL administration showed the lowest TNF-α and IL-6 levels.

Conclusion: HSL could be a used to protect the heart from an inflammatory state, particularly in an overtraining condition.

Keywords: Overtraining, Interleukin-6, Tumor Necrosis Factor-alpha, *Hibiscus sabdariffa Linn*

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INTRODUCTION

Competitive athletes train to improve their performance by increasing training load combined with an adequate recovery period. However, if the excessive training load is not accompanied by an adequate recovery period, an abnormal training response may occur and a state of overreaching and overtraining may develop. Overreaching is the accumulation of training and/or non-training stress that results in a short-term decrement in performance capacity with or without related the physiological and psychological signs and symptoms of maladaptation; the restoration of performance capacity may take from several days to several weeks. Conversely, overtraining (OTS) is the accumulation of training and/or non-training stress that results in a long-term decrement in performance capacity with or without the related physiological and psychological signs and symptoms of maladaptation; the restoration of performance capacity may take several weeks or months [1, 2].

OTS is an extreme condition of maladapted physiology. The symptoms of OTS are varied, and they include fatigue, depression, bradycardia, loss of motivation, insomnia, irritability, agitation, tachycardia, hypertension, restlessness, anorexia, weight loss, lack of mental concentration, stiff muscles, anxiety, and awakening unrefreshed [3]. In mice, OTS led to left ventricular hypertrophy and increased gene expression related to pathological hypertrophy, such as mRNA for the ANP, mRNA for β-myosin heavy chain, and mRNA for skeletal muscle actin [4].

The exact etiology and pathogenesis of OTS are actively being investigated. Numerous hypotheses on the pathophysiology of OTS have been proposed. OTS has been assumed to result from the decrement of muscle glycogen level (glycogen hypothesis), increase in serotonin level in the brain that causes central fatigue (central fatigued hypothesis), decrement in plasma glutamine level (glutamine hypothesis), oxidative stress, autonomic nervous system imbalance, alterations in the hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes, and local inflammation response accompanied with an increased cytokine level (cytokine hypothesis) [3].

In the cytokine hypothesis, training causes degrees of micro trauma to the muscle, connective tissue, and/or bones and joints. This trauma produces a mild inflammatory response with the final purpose of adaptive healing. However, with a continuous high load of training without an adequate resting period, the local acute inflammation becomes chronic, the cytokine released in this process activates the circulating monocyte, and a systemic inflammation with OTS symptoms occurs. The cytokines central to the OTS are the proinflammatory interleukin-1 β (IL-1β) and tumor necrosis factor α (TNF-α). Aside from IL-1β and TNF-α, IL-6 also plays an important role in OTS. IL-6 has both pro inflammatory and anti-inflammatory effects [5].

IL-6 is a pleiotropic cytokine that bridges the innate and adaptive immune systems [6]. In the heart, the IL-6 family signaling on the cardiac myocyte is cardioprotective during the acute response. However, when this cytokine remains elevated chronically, it can induce maladaptive hypertrophy and decreases contractile function. Increased IL-6 production is associated with depressed cardiac function. Acutely, the IL-6 family cytokine protects myocytes against oxidative stress, and its signaling induces an anti-apoptotic program. However, the IL-6 family signaling also depresses the basal contractility of myocytes and the beta adrenergic responsiveness of the cells, leading to a decreased function. The IL-6 family signaling also induces gene expression in the myocytes associated with pathological hypertrophy [7].

Cardiac cells secrete TNF-α as an inflammatory response to stress. TNF-α triggers intra-cellular signaling cascades that modulate host defense against injury, facilitate growth and survival, and promote apoptosis and matrix metalloproteinase expression [8]. Circulating and cardiac TNF-α levels are elevated in heart diseases, such as dilated cardiomyopathy, myocardial infarction, and left ventricular (LV) pressure overload. Consequently, TNF-α has been implicated in the pathogenesis of ventricular remodeling in infarcted heart and in cardiac dysfunction. TNF-α contributes to adverse left ventricular remodeling during pressure overload through the regulation of cardiac repair and remodeling, leading to ventricular dysfunction [9].

Hibiscus sabdariffa Linn (HSL) is a medicinal and food plant rich in phytochemical compounds, which are the source of its biological properties. HSL has cardioprotective and anti-inflammatory activities in the heart, ear, and hippocampus [10-12]. HSL also has antioxidant, anti-nociceptive, anti-diarrheal, antibacterial, antifungal, anti-parasitic, antipyretic, hepatoprotective, nephron-protective, cancer-preventive, and anti-diabetic activities [12, 13]. HSL contains several phytochemical compounds, including organic acids, phenolic acids, anthocyanin, flavonoids, trace elements, and vitamins. Flavonoid quercetin inhibits the TNF-α gene expression by modulating the NF-κβ. However, there is no evidence yet suggesting the effect of HSL on IL-6 and TNF-α levels in rat heart in the overtraining condition. In this study, we investigated the effect of HSL extract on the IL-6 and TNF-α levels in overtrained rat heart.

MATERIALS AND METHODS

Experimental animals

This experimental *in vivo* study used 25 male adult Wistar rats (*Rattus norvegicus)* aged 8–10 w old and weighed 200–250 g. All the rats were from PT Bio Farma (Persero), Bandung, Indonesia. The rats were maintained in individual cages in the FKUI laboratory under a controlled temperature (22±2 °C) on a 12:12 h light–dark inverted cycle. Food and water were provided ad libitum. The experimental procedures were approved by the ethics committee of the Medical Research-Faculty of the Medicine Universitas Indonesia/Cipto Mangunkusumo Hospital (FMUI/RSCM) No: 0955/UN2. F1/ETIK/2018. The rats were randomly divided into five groups: control (C, sedentary), control *H. sabdariffa* (C+HSL, sedentary, given *HSL* methanolic calyx. extract), aerobic (A, mild aerobic protocol), overtraining (OT, overtraining protocol), and overtraining *HSL* (OT+HSL, overtraining protocol, given *H. sabdariffa* calyx methanolic) group.

Training protocols

Before entering the physical exercise program, the exercising groups (A, OT, and OT+HSL) followed an adaptation procedure with low speed and duration of running until the rats were accustomed to run on the treadmill.

The protocol used for overtraining was adapted from Hohl [14]. The overtraining program consists of 11 experimental weeks, with consecutive days of training sessions followed by 2 d of recovery, and is divided into the adaptive training phase 1 (AT1), adaptive training phase 2 (AT2), and incremental training phase (T2X, T3X, and T4X). AT1 was conducted for 4 w with a progressive increase in speed and duration. Then, in AT2, the running speed and the duration that were reached at the end of AT1 were maintained for 4 w to reach adaptation at a stable training load. AT1 and AT2 were performed during the daytime between 13:00 and 17:00. A 24-h recovery time was given between training sessions. In the last 3 w of training, the frequency of daily exercise sessions was increased to two (T2X), three (T3X), and four (T4X) times with the AT2 training load. During this period, recovery time was also reduced between training sessions (4, 3, and 2 h, respectively) to cause an imbalance between overload and recovery. T3X and T4X were performed during an extended daytime period between 10:00 and 17:00. The amount of training in minutes was individually quantified in each training session [14].

In the aerobic experimental group, the rats were subjected to 11 w of training session, twice a week, at the same duration and velocity of running (12 m/mins for 10 min).

HSL extract

The methanolic calyx extract of HSL was obtained from the Central Laboratory of Medicinal Studies Institut Pertanian Bogor (Bogor, Indonesia) with 86.34% purity. The extract was administered orally at a dose of 500 mg/kg BW at 5 times a week for 11 w. Its administration to the overtraining and mild aerobic groups was conducted 3 h before physical exercises.

Heart extraction and biochemical assay

After 11 w of treatment, the rats were sacrificed by decapitation. Thoracotomy was performed, and the heart was removed and then directly stored at −80 °C. The heart tissue was then homogenized according to the instruction of the IL-6 and TNF-α ELISA kit manufacturer. The total protein of the tissue homogenate was measured using the Bradford method. The measurement of IL-6 and TNF-α levels was performed using a commercial kit from Elabscience (Hubei, China) with catalog no: E-EL-R0015 and E-EL-R0019 for IL-6 and TNF-α, respectively. The concentrations of IL-6 and TNF-α were presented in pg per μg of the total protein.

Statistical analysis

The results are expressed as the mean±standard error of the mean. The data were normally distributed. One-way analysis of variance (ANOVA) with a significance level of 0.05 was used for statistical analysis, and Tukey's test was used for pairwise comparisons.

RESULTS

Overtraining obtained the highest level of IL-6 in rat heart

No significant difference in the IL-6 level among the groups was found. Nevertheless, the overtraining group (67.2±14.42 pg/mg) obtained the highest IL-6 level in comparison with the aerobic (57.37±8.338 pg/mg) and the two control groups (C = 32.34±8.67 and C+HSL = 32.97±4.097 pg/mg, respectively), as shown in fig. 1.

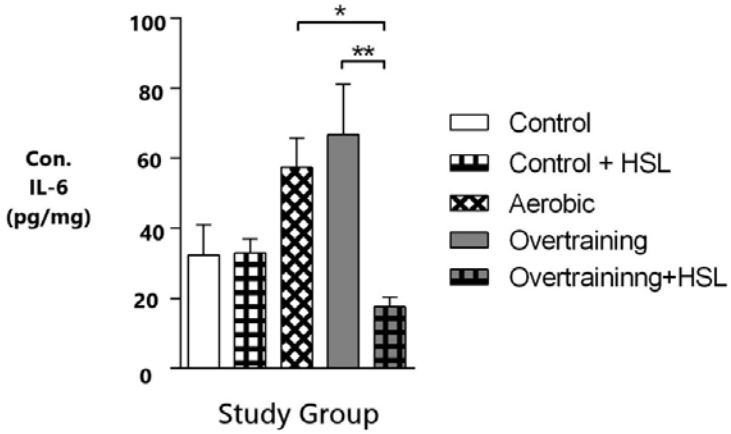

Fig. 1: IL-6 level in rat heart

Overtraining and aerobic training obtained the highest level of TNF-α in rat heart

The overtraining group (206.7±40.96 pg/mg) exhibited a significantly higher ($p<0.05$) TNF-α level compared with the C group (93.03±20.23 pg/mg) and the C+HSL group (93.19±10.72 pg/mg). The level of TNF-α in the aerobic group (162.2±17.31 pg/mg) was not significantly different compared with that in the C and C+HSL groups.

Overtraining with the administration of HSL Linn produced lower TNF-α and IL-6 levels than overtraining only

The level of IL-6 in the OT+HSL (17.62±2.612 pg/mg) group was lower than that in the OT group (6.72±14.42 pg/mg). This level of IL-6 was more than threefold lower than that in the OT group and was statistically significant (p<0.006). Interestingly, the OT+HSL group (17.62±2.612 pg/mg) also had a lower IL-6 level than the aerobic group (57.37±8.338 pg/mg, p<0.03). A similar observation was found in the TNF-α level. The OT+HSL group (44.95±6.252 pg/mg) had a lower TNF-α level of up to twofold than the OT group (206.7±40.96 pg/mg) (p<0.001, fig. 2).

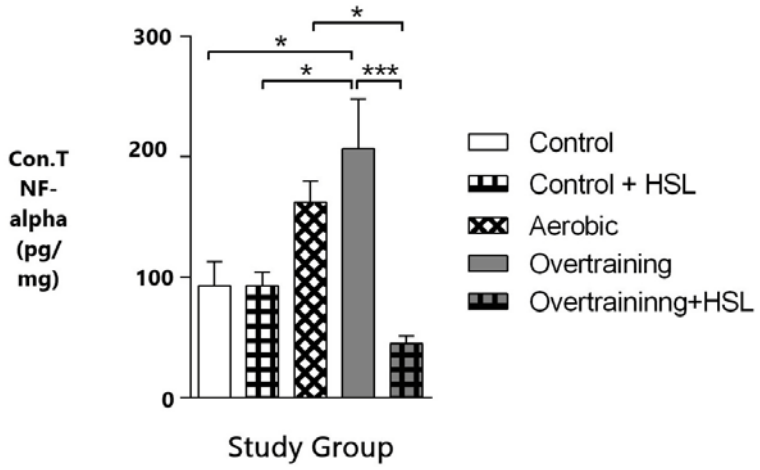

Fig. 2: TNF-α level in rat heart

DISCUSSION

This study showed that overtraining increased the TNF-α level and tended to increase the IL-6 level in the heart, consistent with other studies [4, 15, 16]. Overtraining has been proved to induce acute local inflammation, which may develop into chronic inflammation, thus producing systemic inflammation. The cytokines involved in this process are IL-1β, TNF-α, and IL-6 [17].

The expression of TNF-α, which is a cytokine that is produced in cardiac myocytes, smooth muscle cells, and endothelial cells in response to endotoxin independent of inflammatory cells in ex vivo and *in vitro* cardiac studies [18], was up-regulated in isolated cardiac myocytes and fibroblast following experimental ischemia and myocardial infarction. It was also up-regulated in response to pressure overload and stretch in isolated cardiac myocytes [19]. The cardiac-restricted over-expression of the secreted and trans-membrane form of TNF-α was shown to induce cardiac myocyte hypertrophy and the re-expression of the fetal gene program. TNF-α also reduced myocardial contractility. TNF-α induced apoptosis in cardiac myocytes, which contribute to the progressive LV wall thinning and adverse cardiac remodeling [20]. Thus, we assumed that the marked increase in TNF-α level in overtraining could cause a pathologic phenomenon, such as the morphological and functional alteration of the heart.

Conversely, IL-6 is a pleiotropic cytokine that is produced not only by immune cells and immune accessory cells, including monocytes and macrophages but also by cardiovascular components, such as endothelial cells, vascular smooth muscle cells, and ischemic myocytes. Increasing the IL-6 and IL-6R levels contributes to the signaling pathway, leading to cardiac hypertrophy [21]. IL-6 has been shown to depress papillary muscle contraction and is negatively inotropic in cardiomyocyte cultures. High IL-6 concentrations usually precede the development of multi-organ failure in patients with cardiogenic shock due to acute myocardial infarction, dilated cardiomyopathy, and valvular heart disease [22]. In our study, overtraining only tended to increase the IL-6 level in rat heart. Nevertheless, TNF-α, which is the other inflammation cytokine, increased robustly and should be enough to cause a negative effect on the heart.

Thus, we administrated HSL extract for the overtraining condition and found that this extract prevented the increase in the IL-6 and TNF-α levels in rat heart. This result supports those of previous studies elucidating the anti-inflammatory effect of HSL [10, 23]. The suppression of IL-6 and TNF-α production could be related to the quercetin and anthocyanin compounds in HSL. Quercetin has been proved to have an inhibitory effect on IL-6 production through its effect on regulating the p38-mitogen-activated protein kinase (p38-MAPK) [24]. Quercetin also suppresses the TNF-α gene and expression. The possible mechanism of this suppression may be mediated by downregulating the gene expression of NF-κβ and decreasing the phosphorylation of Iκβα and Iκββ by quercetin, thus suggesting that quercetin decreases the activation of NF-κβ [25]. Anthocyanins, the other active compound of HSL, have antioxidant, anti-inflammation, and anti-cancer properties [26]. Anthocyanins inhibit the production of nitric oxide, prostaglandine E2, TNF-α, and IL-6 in a dose-dependent manner without a cytotoxic effect. Kim *et al.* showed that anthocyanins decreased the reactive oxygen species production, thereby reducing the MAPK activation and inflammatory cytokine production [27]. Anthocyanins inhibit the translocation of NF-κβ from the cytosol to the nucleus and prevent the phosphorylation of Iκβ, thus decreasing the production of proinflammatory mediators such as IL-6 and TNF-α [28].

In conclusion, this study demonstrates that overtraining along with the administration of HSL extract produces lower TNF-α and IL-6 levels in rat heart than overtraining only. Therefore, HSL extract can be a promising natural compound candidate to protect the heart from a chronic inflammation state particularly caused by overtraining.

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ETHICS AND HUMAN AND ANIMAL EXPERIMENTATION

The experimental procedures were approved by the Health Research Ethical Committee FKUI No: 0955/UN2. F1/ETIK/2018.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declare no potential conflict of interest

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