

Basic Medical Research

Effects of *Hibiscus sabdariffa* Linn. on insulin-like growth factor binding protein 3 (IGFBP-3) to prevent overtraining syndrome

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Abstrak

Latar belakang: Latihan fisik berat (overtraining) dapat meningkatkan produksi reactive oxygen species (ROS). Salah satu indikator sindrom overtraining adalah penurunan kadar insulin-like growth factor binding protein 3 (IGFBP-3) dalam darah. *Hibiscus sabdariffa* Linn., suatu antioksidan kuat, diharapkan mampu mencegah sindrom overtraining. Penelitian ini bertujuan mengetahui pengaruh pemberian *H. sabdariffa* pada tikus yang diberi "latihan fisik overtraining".

Metode: Penelitian eksperimental ini menggunakan 30 tikus *Rattus norvegicus* galur Wistar jantan (200-250 gram), dibagi menjadi 5 kelompok: 1) tikus kontrol (C); 2) tikus kontrol diberi *H. sabdariffa* (C-Hib); 3) tikus dengan latihan fisik aerobik ringan (A-Ex), 4) tikus dengan latihan fisik overtraining (OT); 5) tikus dengan latihan fisik overtraining dan diberi *H. sabdariffa* (OT-Hib). *H. sabdariffa* diberikan secara oral dengan dosis 400 mg/kgBB/hari selama 11 minggu. IGFBP-3 diukur dengan teknik ELISA dan dianalisis dengan uji ANOVA.

Hasil: Kadar IGFBP-3 pada kelompok C adalah $17,4 \pm 10$ mIU/L, paling rendah pada kelompok OT ($10,7 \pm 9,9$ mIU/L) dan paling tinggi pada kelompok OT-Hib ($31,5 \pm 6,2$ mIU/L), $p < 0,05$. Terdapat perbedaan bermakna kadar IGFBP-3 pada kelompok OT dengan A-Ex ($10,7 \pm 9,9$ vs $23,5 \pm 9,7$ mIU/L; $p < 0,05$). Perbedaan kadar IGFBP 3 pada kelompok C dan OT-Hib adalah ($17,4 \pm 10$ vs $31,5 \pm 6,2$; $p < 0,05$).

Kesimpulan: Pemberian *H. sabdariffa* dapat mencegah penurunan kadar IGFBP-3 pada tikus dengan overtraining, yang menunjukkan *H. sabdariffa* dapat mencegah terjadinya sindrom overtraining.

Abstract

Background: Excessive physical exercises (overtraining) can increase the production of reactive oxygen species (ROS). One of the indicators of overtraining syndrome is a decrease in insulin-like growth factor binding protein 3 (IGFBP-3). Administration of *Hibiscus sabdariffa* Linn., a powerful antioxidant, is expected to boost endogenous antioxidants, and thus prevents overtraining. The aim of this study is to determine the effect of *H. sabdariffa* on IGFBP-3 levels in rats under "overtraining physical exercise".

Methods: This experimental study was conducted on 30 male rats (*Rattus norvegicus* 200-250 grams), randomly allocated into 5 groups: 1) control group (C); 2) control with *H. sabdariffa* (C-Hib); 3) mild aerobic exercise (A-Ex); 4) overtraining exercise (OT); 5) overtraining exercise with *H. Sabdariffa* (OT-Hib). *H. sabdariffa* (400 mg/kg/d, 11 weeks) were administered orally via syringe cannula. IGFBP-3 was measured by using ELISA (Cusa bio kit) and data were analyzed with ANOVA test.

Results: Plasma level of IGFBP-3 in the C and OT groups were 17.4 ± 10 mIU/L, the lowest in OT groups (10.7 ± 9.9 mIU/L) and the OT-Hib group had the highest level (31.5 ± 6.2 mIU/L). There was significant difference of the level IGFBP-3 in OT groups with A-Ex groups (10.7 ± 9.9 vs 23.5 ± 9.7 mIU/L; $p < 0,05$). The significant difference was also observed in the level of IGFBP 3 between C groups and the OT-Hib groups (17.4 ± 10 vs 31.5 ± 6.2 ; $p < 0.05$).

Conclusion: Administration of *H. sabdariffa* can prevent the decrease of IGFBP-3 levels in overtraining rats, indicating its role in preventing overtraining syndrome.

Keywords: *Hibiscus sabdariffa* Linn., IGFBP-3, overtraining

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Overtraining syndrome is a result of excessive volume of exercise which, in the long run, will cause performance declining. This decrease in performance is particularly associated with physical, immunological and emotional disturbances.¹⁻⁴ Overtraining syndrome frequently occurs due to long-term trainings before a competition with the exercise volume exceeding the capabilities of an athlete. It is also caused by imbalance between training and resting days, and between competitions and recovery.^{2,4} The incidence of overtraining syndrome in elite athletes is 7-20% and occurs more commonly in predominantly aerobic sports such as swimming, running, and cycling.^{1,5,6} However, this high incidence of overtraining syndrome is related to various and less known factors that precede or follow the overtraining syndrome.

Some theories try to explain the pathophysiology and indicators of overtraining syndrome. However, the existing theories are still unable to completely explain the pathophysiology of overtraining, because each theory has its advantages and disadvantages. One of the theories states that overtraining symptoms is associated with the disruption in the hypothalamic regulation of hormones. Other studies indicate a decrease in the hormone insulin-like growth factor binding protein 3 (IGFBP-3) in athletes who suffer overtraining.^{7,8} The secretion of IGFBP-3, a carrier protein for insulin-like growth factor 1 (IGF-1), is stimulated by growth hormone (GH). The decrease in IGFBP-3 is proposed to be used as the indicator of overtraining in athletes.

Overtraining syndrome often occurs in athletes following aerobic type exercises with high oxygen consumption. Some studies suggest a link between physical exercise, increased oxygen consumption and reactive oxygen species (ROS) production. Therefore, the excessive ROS production during overtraining may result in an imbalance between the endogenous antioxidant system and pro-oxidants, which is referred to as oxidative stress.

Indonesia is rich of herbs which contain antioxidants such as *Hibiscus sabdariffa* Linn. This plant belongs to the family of *Malvaceae* and is often used as antihypertensive, antioxidant, anticancer, antipyretic, antibacterial, anti-inflammatory, and anti-hyperlipidemic phytotherapy.^{9,10} Calyx is the major part of *H. sabdariffa* used in phytomedicine with anthocyanin being the main active component.¹¹ Under certain conditions, anthocyanin shows higher

antioxidant activity compared to vitamin E, ascorbic acid, and β -carotene.^{10,12}

The aim of this study was to evaluate the effect of *H. sabdariffa* on overtraining by measuring the IGFBP-3.

METHODS

The protocol of this study has been approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia/Cipto Mangunkusumo Hospital (No. 289/H2.F1/ETIK/2013).

Thirty male Wistar rats (*Rattus norvegicus*) weighing 200-250 g were divided randomly into five groups: 1) control group (C), 2) control group given *H. sabdariffa* (C-Hib), 3) the mild aerobic exercise group (A-Ex), 4) the overtraining exercise group (OT), and 5) the overtraining exercise group given *H. sabdariffa* (OT-Hib). Before and during intervention, the rats were maintained properly according to the ethics of experimental animal usage. Rats were given standardized food and water *ad libitum*. The cages were arranged 12 hours of light (18:00 pm to 6:00 am) and 12 hours of dark (6:00 am to 18:00 pm), thus a treatment of overtraining exercise could be done during the day. Room temperature was set at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The extract of *H. sabdariffa* was administered orally via a syringe cannula to the rats in C-Hib and OT-Hib groups at a dose of 400 mg/kgBW/day, five days a week (except Saturdays and Sundays) for 11 consecutive weeks.

H. sabdariffa was extracted by the following process: one kg of calyx *H. sabdariffa* was mixed with 5 liters of water for 3 hours. The mixture was kept for 24 hours at room temperature, then filtered and dried in a Rotavapor (Büchi, Switzerland).

Before starting the physical exercise program, the rats in the exercise groups A-Ex, OT, and OT-Hib followed an adaptation procedure until the rats were accustomed to running on the treadmill. The adaptation process on the treadmill started at low speed and short duration, then the speed was gradually increased until the rats could run at a speed of 10 m/min for 10 minutes. Group A-Ex group was running at a speed of 12 m/min for 10 minutes twice a week, representing mild aerobic exercise. The rats in the OT and OT-Hib group, obtained an overtraining program with gradually increased speed, duration, and frequency for 11 weeks (Table 1).¹³

Table 1. Program of aerobic exercise overtraining

Time of experiment	Phase of training	Speed (m/min)	Duration (min)	Frequency/day	Resting time between sessions (hours)
week-1	A1	15	20	1	24
week-2	A1	20	30	1	24
week-3	A1	22.5	45	1	24
week-4	A1	25	60	1	24
week-5-8	A2	25	60	1	24
week-9	T2x	25	60	2	4
week-10	T3x	25	60	3	3
week-11	T4x	25	60	4	2

A1: adaptation phase 1; A2: adaptation phase 2 with increased speed and duration; T2x: treadmill exercise twice/day; T3x: treadmill exercise 3 times/day; T4x: treadmill exercise 4 times/day. Modified from Margonis, et al¹³

Blood sample were drawn on day-3 after treatment, to avoid the acute effects on overtraining. The rats were anesthetized with ether, the chest was opened and blood was collected by cardiac puncture. The blood was centrifuged at 1000 x g for 15 minutes to obtain plasma for the IGFBP-3 measurement. The level of free IGFBP-3 (i.e. not bound to IGF-1) was measured by ELISA (CusaBio kit).

Data analysis

All data in the experiments were analyzed using one way Anova. Multiple comparisons were done with post-hoc LSD method. The $p < 0.05$ was taken as the limit of statistical significance.

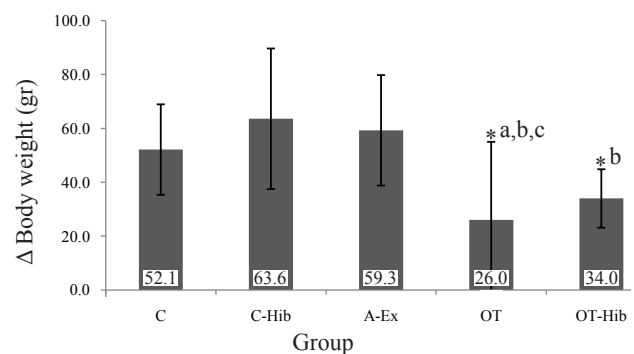
RESULTS

Body weight

Figure 1 shows the differences in the average body weights between pre-test and post-test measurement of all groups. The rats in all groups gained weight in the course of the training programs; however, the OT group had the lowest post-test body weight compared to other groups. There were significant differences in Δ body weight between OT and C group, between groups OT and C-Hib, and between OT and A-Ex group ($p < 0.05$). There was no significant difference in the Δ body weight between OT and OT-Hib group ($p > 0.05$). Nevertheless, there was a tendency to higher Δ body weight in OT than in OT-Hib group.

Serum levels of IGFBP-3

The levels of IGFBP-3 in the five group are presented in figure 2. The level in A-Ex group (23.5 ± 0.7



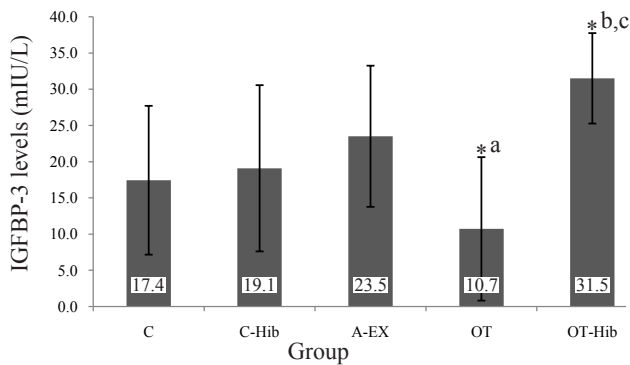
* $p < 0.05$; ^acompared to C; ^bcompared to C-Hib; ^ccompared to A-Ex

Figure 1. Body weights before and after the intervention in the control (C), control-*H. sabdariffa* (C-Hib), mild aerobic exercise (A-Ex), overtraining exercise (OT), and overtraining exercise-*H. sabdariffa* (OT-Hib).

mIU/L) was higher than in groups C (17.4 ± 10.2 mIU/L) and C-Hib (19.1 ± 11.4 mIU/L). The level of IGFBP-3 in C-Hib group was slightly higher than group C; the difference was statistically not significant. The lowest IGFBP-3 level was obtained in the OT group (10.7 ± 9.9 mIU/L), which differed significantly from A-Ex and OT-Hib groups (31.4 ± 6.2 mIU/L) ($p < 0.05$) which was the highest level of all groups.

DISCUSSION

The results of this study showed that Δ body weight in the OT group was lower than in controls. This difference is assumed to be due to the increased body metabolism and/or the skeletal muscle catabolism in OT compared to the control group. The OT group has a longer catabolic phase due to higher energy demand that is obtained from fat and protein. Xiao, et al¹⁴ reported that the body weight of rats in



*p < 0.05; ^acompared to A-Ex; ^bcompared to C; ^ccompared to OT

Figure 2. The IGFBP 3 level in the control (C), control-*H. sabdariffa* (C-Hib), mild aerobic exercise (A-Ex), overtraining exercise (OT), and overtraining exercise-*H. sabdariffa* groups

overtraining group was 23.6% lower than control group. In addition, the decreased body weight in this study is also due to decreased appetite in OT rats, one of the markers of the overtraining syndrome. The loss of body weight in the OT group indicates that this group was experiencing the overtraining syndrome. Other supporting parameters of the overtraining condition in this study were signs of fatigue and decreased performance when the rats were put on treadmill training. According to Margonis, et al¹³ one of the indicators of the overtraining syndrome is a decrease in the physical exercise capacity which is related to chronic fatigue of rats.

The OT-Hib group did not experience fatigue and decreased performance when running on a treadmill. The results showed that the performance in the OT-Hib group was better than in the OT group. In addition, the OT-Hib group had higher Δ body weight than the OT group. This finding suggests that the OT-Hib group did not experience the overtraining syndrome, since loss of body weight or decreased weight gain is one of the signs of overtraining syndrome.

The IGFBP-3 levels of the A-Ex group were higher than in group C and C-Hib. This suggests that physical exercise can increase IGFBP-3 levels. Exercise can increase the levels of GH, IGF-1 and IGFBP-3 because it is a powerful stimulus of growth hormone releasing hormone (GHRH) and GH release.¹⁵ The increase of GH secretion from the pituitary gland will stimulate the liver and other tissues to produce IGF-1 and IGFBP-3.¹⁶⁻¹⁸ However, in the condition of overtraining syndrome the level of IGFBP-3 will

decrease.^{7,8} Therefore, the decreased IGFBP-3 level can be used as a specific marker of the overtraining syndrome, although the mechanism of such decrease is still unclear. The decrease of IGFBP-3 is allegedly due to disturbance in the GH /IGF-1 axis or disruption in IGFBP-3 synthesis in liver cells by oxidative stress. Heavy physical exercises can increase the ROS production resulting in oxidative stress.

It is well known that oxidative stress is an imbalance between free radicals production and antioxidant defends mechanism in the body.⁶ Production of ROS and damage of lipids, proteins and genetic materials (DNA, RNA) through ROS are believed to be the main mechanisms of the biochemical and physiological changes that occur during physical exercise.¹⁹ Further studies are needed to disclose the mechanisms of the decreased IGFBP-3 levels in the overtraining syndrome associated with oxidative stress.

In the present study, the IGFBP-3 level in the OT group is lower than in the A-Ex group. This finding suggests that exercise program given to the rats in this group leads to the overtraining syndrome. However, when the overtraining exercise program is accompanied by the provision of *H. sabdariffa* extract the rats do not experience the overtraining syndrome. The IGFBP-3 levels in the OT-Hib group are even higher than in A-Ex and OT groups. It seems that *H. sabdariffa* works more effectively in overtraining animals compared to animals in normal condition. The higher level of IGFBP-3 in the OT-Hib group are likely caused by combination of physical exercise with the antioxidant role of *H. sabdariffa*.

Antioxidants are compounds that may prevent or inhibit the oxidation of proteins, DNA, and lipids in cell membranes. *H. sabdariffa* contains anthocyanins, flavonoids, protocatechuic acid, ascorbic acid, and other compounds.²⁰ As anthocyanins are potent antioxidants, *H. sabdariffa* can reduce free radicals by inhibiting the activity of xanthine oxidase and by increasing the activity of glutathione peroxidase (GPx).²¹ Therefore, we suggest *H. sabdariffa* may prevent overtraining in heavy physical exercise. The capacity of *H. sabdariffa* to reduce ROS and increase GPx activity may alleviate ROS damage at the levels of molecules, cells, organs, and systems. This study found a tendency of *H. sabdariffa* to increase IGFBP-3 level in the C-Hib group, although

the differences were not statistically significant compared to control group.

It is suspected the high levels of IGFBP-3 in the OT-Hib group are caused by the combined effects of physical exercise and *H. sabdariffa*. Physical exercise can increase IGFBP-3 levels through the increase of GH secretion. There is a positive correlation between the intensity of the exercise with the magnitude of GH secretion.¹⁵ Increased GH secretion will intensify liver stimulation to produce IGFBP-3. On the other hand, the greater exercise intensity will result in higher ROS production. Therefore, the highest level of IGFBP-3 found in OT-Hib may be caused by exercise in high intensity and the concomitant provision of *H. sabdariffa*.

From the above results, we conclude that *H. sabdariffa* can maintain the IGFBP-3 level and is expected to prevent overtraining syndrome in excessive physical exercise.

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Conflict of interest

The authors affirm no conflict of interest in this study.

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