

HIBISCUS INCREASES FUNCTIONAL CAPACITY AND THE ANTI-OBESITY EFFECT IN TRAINED OBESE RATS

O HIBISCUS AUMENTA A CAPACIDADE FUNCIONAL E O EFEITO ANTI-OBESIDADE EM RATOS OBESOS TREINADOS

EL HIBISCUS AUMENTA LA CAPACIDAD FUNCIONAL Y EL EFECTO ANTI-OBESIDAD EN RATAS OBESAS ENTRENADAS

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ABSTRACT

Introduction: Hibiscus sabdariffa (Hs) has been widely used for weight loss and in the fight against obesity-associated comorbidities. **Objective:** To evaluate the effects of Hs and physical training on the functional capacity of normal-weight and obese rats. **Methods:** Wistar rats were distributed into eight experimental groups: control (C, n = 8), Hibiscus Sabdariffa (Hs, n = 8), high-intensity interval training (IT, n = 8), high-intensity interval training + Hibiscus Sabdariffa (ITHs, n = 8), obese (O, n = 8), obese + continuous aerobic training (OAT, n = 8), obese + Hibiscus Sabdariffa (OHS, n = 8), and obese + continuous aerobic training + Hibiscus Sabdariffa (OATHs, n = 8). Hibiscus Sabdariffa extract was administered for 60 days in a dose of 150 mg/kg of body weight. The maximum progressive effort test (MPET) was performed on a treadmill at the beginning and end of the study. The variables analyzed were maximum speed V_{max} , time, and distance covered. Lactate was measured immediately after the MPET. Functional capacity was evaluated by the distance/adiposity index. The ANOVA with Bonferroni post hoc and Pearson's correlation tests were used at a 5% significance level. **Results:** After both types of training, moderate-intensity continuous and high-intensity interval performed on the treadmill, final body weight, weight gain, and the adiposity index decreased, and V_{max} , time, and distance covered in the MPET increased, in addition to an improvement in functional capacity. Hs supplementation reduced the adiposity index in normal-weight and obese rats. Hs associated with aerobic training reduced final body weight and increased functional capacity. **Conclusion:** Hs supplementation promoted a reduction in the adiposity index in normal-weight and obese rats and an increase in the functional capacity of trained obese rats. **Level of Evidence III; Therapeutic Studies - Outcome Investigation. Case study – control.**

Keywords: Hibiscus; High-intensity interval training; Endurance training; Obesity; Exercise test.

RESUMO

Introdução: O Hibiscus Sabdariffa (Hs) tem sido amplamente utilizado para emagrecimento e no combate às comorbidades associadas à obesidade. **Objetivos:** Avaliar os efeitos do Hs e do treinamento físico sobre a capacidade funcional de ratos eutróficos e obesos. **Métodos:** Ratos Wistar foram distribuídos em oito grupos experimentais: Controle (C, n = 8), Hibiscus Sabdariffa (Hs, n = 8), treinamento intervalado de alta intensidade (TI, n = 8), treinamento intervalado de alta intensidade + Hibiscus Sabdariffa (TIHs, n = 8), obeso (O, n = 8), obeso + treinamento contínuo aeróbico (OTA, n = 8), obeso + Hibiscus Sabdariffa (OHS, n = 8), obeso + treinamento contínuo aeróbico + Hibiscus Sabdariffa (OATHs, n = 8). O extrato de Hibiscus Sabdariffa foi administrado por 60 dias na dose de 150 mg/kg de peso corporal. Foi realizado teste de esforço progressivo máximo (TEPM) em esteira no início e no final do estudo. As variáveis analisadas foram: velocidade máxima (V_{max}), tempo e distância percorrida. O lactato foi mensurado imediatamente depois do TEPM. A capacidade funcional foi avaliada pela distância/índice de adiposidade. Empregou-se ANOVA com post hoc de Bonferroni e correlação de Pearson, adotando-se o nível de significância de 5%. **Resultados:** Depois de ambos os tipos de treinamento, o contínuo em moderada intensidade e o intervalado de alta intensidade realizados em esteira, reduziram o peso corporal final, o ganho de peso e o índice de adiposidade, bem como aumentaram a V_{max} , tempo e distância percorrida no TEPM, além de melhora da capacidade funcional. A suplementação de Hs diminuiu o índice de adiposidade nos ratos eutróficos e obesos. O Hs associado ao treinamento aeróbico reduziu o peso corporal final e aumentou a capacidade funcional. **Conclusão:** A suplementação de Hs promoveu redução no índice de adiposidade em ratos eutróficos e obesos e aumentou a capacidade funcional de ratos obesos treinados.

Nível de evidência III; Estudos Terapêuticos - Investigação de Resultados. Estudo caso controle.

Descritores: Hibiscus; Treinamento intervalado de alta intensidade; Treino aeróbico; Obesidade; Teste de esforço.

RESUMEN

Introducción: El Hibiscus Sabdariffa (Hs) ha sido ampliamente utilizado para promover la pérdida de peso y tratar las comorbilidades asociadas a la obesidad. **Objetivos:** Evaluar los efectos del Hs y el entrenamiento físico sobre la capacidad funcional de ratos eutróficos y obesos. **Métodos:** Se distribuyeron ratos Wistar en ocho grupos experimentales: Control (C, n=8), Hibiscus Sabdariffa (Hs, n=8), , entrenamiento de intervalos de alta intensidad (EI,



n=8)), *entrenamiento de intervalos de alta intensidad + Hibiscus Sabdariffa (EIHs, n=8)*, *obeso (O, n=8)*, *obeso + entrenamiento continuo aeróbico (OEA, n=8)*, *obeso + Hibiscus Sabdariffa (OHs, n=8)*, *obeso + entrenamiento continuo aeróbico + Hibiscus Sabdariffa (OEAHs, n=8)*. Se administró extracto de *Hibiscus Sabdariffa* durante 60 días a una dosis de 150 mg/kg de peso corporal. Se realizó una prueba de esfuerzo progresivo máximo (PEPM) en una cinta rodante al principio y al final del estudio. Las variables analizadas fueron: velocidad máxima $V_{m\acute{a}x}$, tiempo y distancia recorrida. El nivel de lactato se midió inmediatamente después de la PEPM. La capacidad funcional se evaluó mediante el índice de distancia / adiposidad. Se empleó el método ANOVA con post hoc de Bonferroni y las pruebas de correlación de Pearson, a un nivel de significancia del 5%. Resultados: Después de ambos tipos de entrenamiento, el entrenamiento continuo a intensidad moderada y el entrenamiento de intervalos de alta intensidad realizados en cinta rodante, las ratas presentaron disminución del peso corporal final, aumento de peso e índice de adiposidad, así como aumentaron la $V_{m\acute{a}x}$, el tiempo y la distancia recorrida en la PEPM, y mejoraron la capacidad funcional. La suplementación con Hs disminuyó el índice de adiposidad en ratas eutróficas y obesas. El *Hibiscus* asociado al entrenamiento aeróbico redujo el peso corporal final y aumentó la capacidad funcional. Conclusión: La suplementación con Hs redujo el índice de adiposidad en ratas eutróficas y obesas y aumentó la capacidad funcional de ratas obesas entrenadas. **Nivel de Evidencia III; Estudios Terapéuticos - Investigación de Resultados. Estudio de caso – control.**

Descriptores: *Hibiscus*; Entrenamiento de intervalos de alta intensidad; Entrenamiento aeróbico; Obesidad; Prueba de esfuerzo.

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INTRODUCTION

Currently, obesity is considered one of the most important public health problems, being associated with increased risk of mortality, reduced life expectancy, and numerous comorbidities such as cancer and metabolic and cardiovascular diseases.¹

Obesity is caused by a sedentary lifestyle, poor diet, and psychological and genetic factors.² Several approaches, including drug treatments, lifestyle changes such as regular exercise, and dietary approaches, are aimed at reducing body weight and fat accumulation. However, they are considered temporary and difficult to maintain for long periods, causing the so-called “yo-yo effect”.³ For long-term solutions, many researchers focused on using bioactive compounds as a new therapeutic approach to treat obesity. Recently, natural bioactive compounds such as flavonoids and phenols have shown efficacy in obesity treatment. Bioactive compounds from edible plants, such as epigallocatechin gallate, nobiletin, curcumin, resveratrol and pterostilbene, and anthocyanins from green tea, citrus peel, turmeric, grapes and berries, and *Hibiscus sabdariffa* (Hs), respectively, have demonstrated potential anti-obesity activity *in vivo* and *in vitro*.^{4,5}

Hs is a botanical species belonging to the family *Malvaceae*, originally from Malaysia, and cultivated in tropical and subtropical regions, including Brazil. Hs production has increased over the years for the preparation of culinary and medicinal beverages. It is rich in polyphenols, flavonoids, and anthocyanins. Its anti-obesity, hypocholesterolemic, hypoglycemic, anti-atherosclerotic, anti-inflammatory, antioxidant, diuretic, antihypertensive, and hepatoprotective therapeutic effects have been reported.^{4,7}

Regular physical activity is considered the primary key in obesity prevention and treatment. Systematic reviews⁸ confirmed the anti-obesity effects of exercise training (ET) in reducing body weight and fat, increasing physical conditioning, cardiorespiratory capacity, lean mass, and functional capacity.

Body weight can be reduced with different forms of ET. Traditional training, i.e., continuous moderate intensity exercise, being efficient in reducing body weight, is the most investigated. Recently, high-intensity interval training has been extensively investigated, presenting a time-efficiency component in reducing body weight. A meta-analysis showed that both forms of exercise were effective in reducing body weight.⁹

There are no reports on the effects of Hs on the functional capacity. Since obesity leads to fat accumulation and reduces functional capacity, and both Hs and ET have anti-obesity effects, this study hypothesizes that

Hs supplementation combined with exercise training will reduce body fat and increase the functional capacity of normal-weight and obese rats. To this end, we evaluated the effects of Hs and exercise training on the functional capacity of normal-weight and obese rats.

MATERIALS AND METHODS

Animals and experimental model

The study included 30-day-old Wistar rats (n=64) from the Central Vivarium of the Federal University of Cuiabá, UFMT, Brazil. After 30 d of acclimatization, rats were distributed into eight experimental groups (eight rats each), control (C), Hs, high-intensity interval training (IT), high-intensity interval training+Hs (ITHs), obese (O), obese+Hs (OHs), obese+moderate aerobic training (OAT), and obese+moderate aerobic training+Hs (OATHs).

Body weight was measured weekly to monitor the development of the rats, which were kept in collective cages under controlled temperature (24±2 °C) and humidity (55±5%) in an alternate light-dark cycle (12 h-12 h). The study protocol was approved by the UFMT Ethics Committee (no. 23108,722977/2017-35) and followed the recommendations of the Guide for the Care and Use of Experimental Animals and of the Ethical Principles in Animal Experimentation of the Brazilian College of Animal Experimentation.¹⁰

Diet and supplementation model

The groups C, Hs, IT, and ITHs received standard rodent feed (NUVILAB CR-1, Colombo, Paraná, Brazil) and water *ad libitum*. The rats in groups O, OHs, OAT, and OATHs received a high-calorie diet (NUVILAB CR-1 feed powder, casein, lard, condensed milk, cornstarch biscuit, vitamin, and mineral mixture) and water with sucrose (300 g/ 500 mL). (Table 1)

Hibiscus sabdariffa extract preparation and treatment

Hs was purchased from *Chá e Cia* store (www.chaecia.com.br). The hibiscus powder was diluted in absolute ethyl alcohol in a 10 g:100 mL ratio. This solution was stored in the refrigerator for seven days, and stirred for 2 h daily. After this period, the solution was filtered, followed by alcohol evaporation in a rotary evaporator. Extract was then dried in an oven for two days at 37 °C, and subsequently stored and diluted in distilled water. The rats received Hs extract daily (150 mg/kg of body weight) for 60 days.

Maximum progressive exercise test

The rats were subjected to maximum progressive exercise test (*Extest*) on a treadmill to evaluate their pre/post-training physical performance

Table 1. Chows nutritional composition.

Components	Chow	
	Control	High fat *
Carbohydrate	65.6	45.2
Protein (casein > 99%)	22	20.9
Fat	4	24.5
Fibers	4	4
Vitamin mixture +	1	Adicionada
Mineral mixture +	3.5	Adicionada
Total (%)	100	100
Energy (Kcal/100g)	380	485 [#]
Energy (Kj/100g)	1,591	2,031 [#]

Notes: Commercial diet NUVILABVR Cr-1 contains fat from soy oil and carbohydrates as a sum of starch and saccharose. *High-fat diet designed by our group contained a powdered commercial chow diet, casein, maize biscuit, condensed milk, lard (main lipid source), vitamins and minerals (4.5%). +Based on the chow diet vitamin/mineral amounts, for each 100 g of the high-fat diet, the following was added: iron: 25.2 mg; potassium: 104.8 mg; selenium: 73.1 mg; molybdenum sulphate: 150.0 mg; vitamin B12: 34.5 mg; vitamin B6: 6.6 mg; biotin: 0.12 mg; vitamin E: 48.9 IU; vitamin D: 2,447.0 IU; and vitamin A: 15,291.2. #Calories from sugar in the drinking water (1.2 kcal/mL) are not included

and to determine the training load.¹¹ The variables analyzed during the test were maximum speed ($Speed_{max}$), time, and distance covered. The initial test speed was 10 m/min, being progressively increased (two m/min) every two min until exhaustion. The exhaustion criterion for the test was the inability to maintain the required speed for cinco seconds. The *Extest* was repeated 30 days after the beginning of the training to adjust the training load. At the end of the experimental period, all rats underwent *Extest* again.

High-intensity interval and continuous aerobic training protocols

The animals in the IT and ITHs groups underwent a high-intensity IT program on a treadmill (eight and two min at a speed corresponding to 80% and 20% of $Speed_{max}$, respectively) for 60 days, with daily training time of 60 min. The rats in the OAT and OATH groups underwent a continuous moderate intensity aerobic training (AT) on a treadmill (60 min at a speed corresponding to 50% of $Speed_{max}$, five day/ week) for 60 days.

Adiposity index characterization

The body adiposity index (BAI) is an obesity indicator that analyzes the amount of body fat in animals. After euthanasia, epididymal, visceral, and retroperitoneal fat deposits of the rats were dissected and weighed. The sum of the fat weight of the deposits normalized by the body weight [(epididymal+retroperitoneal+visceral)/body weight×100] is considered the BAI.¹²

Blood lactate determination and functional capacity evaluation

Blood lactate levels were determined immediately after *Extest* to evaluate the intensity of the exercise using a portable analyzer (Accutrend Plus, Roche, Germany) that quantifies lactate levels in small amounts of blood. A drop of blood was collected from the tip of the tail for each test.

Functional capacity was measured by the distance covered in the *Extest* divided by the total fat.

Statistical analyses

The data are expressed as mean and standard deviation of the mean or standard error of the mean. The normality of all variables was evaluated by the Shapiro-Wilk test. Two-way ANOVA was used to compare the groups (physical training, *Hs*), being complemented by the Bonferroni test.¹³ The Pearson's correlation test was used to analyze the relationship between distance covered and total body fat. A five % significance level was considered for all variables. The GraphPad Prism 8 software for Windows (Graphpad Software, San Diego, CA, USA) was used to analyze and create the graphs.

RESULTS

In normal-weight rats, non-supplemented IT significantly reduced the final body weight (FBW), weight gain, and BAI by 10%, 26%, and 32%, respectively. Supplemented IT reduced weight gain by 20% and BAI by 43% (Table 2). In obese rats, the non-supplemented AT significantly reduced FBW, weight gain, caloric consumption, and BAI by 25%, 79%, 21%, and 21%, respectively. Supplemented aerobic training reduced weight gain by 32% and BAI by 32% (Table 3). *Hs* supplementation had no effect on the FBW and weight gain of normal-weight rats. However, BAI decreased significantly by 24% and 12% with and without IT, respectively (Table two). In obese rats, *Hs* supplementation significantly reduced the FBW, weight gain, and BAI in sedentary rats by 22%, 45%, and six%, respectively. In obese rats exercising, *Hs* increased the FBW by six%, weight gain by 78%, and reduced BAI by 18%. There was an association between the effect of *Hs* and AT in context of FBW and weight gain of obese rats. (Table 2 and 3)

Performance evaluation using *Extest* showed that IT increased the distance covered, total time, $Speed_{max}$, and blood lactate level by 327%, 147%, 99%, and 14% in normal-weight rats, and by 311%, 106%, 73%, and 157%, respectively, when combined with *Hs* supplementation. *Hs* supplementation alone showed no impact on *Extest* parameters in normal-weight or obese rats. (Table 4). In obese rats, AT increased the distance covered, total time, and $Speed_{max}$ by 187%, 97%, and 61%, and by 191%, 101%, and 66%, respectively, when supplemented with *Hs*. (Table 4 and 5)

In the evaluation of the association between body fat and performance, the Pearson's correlation test showed a significant association between

Table 2. General characteristics of normal-weight rats submitted to the IT protocol.

	C (n=9)	Hs (n=8)	IT (n=9)	ITHs (n=9)
IBW (g)	358 ± 26	349 ± 22	343 ± 25	345 ± 10
FBW (g)	438 ± 29	419 ± 26	396 ± 20*	401 ± 20
WG (g)	80 ± 17	70 ± 14	59 ± 13*	56 ± 18*
BAI (%)	4,76 ± 1,16	4,03 ± 1,26*	3,33 ± 0,62*	2,91 ± 0,55*

Notes: Values expressed as mean and standard deviation of the mean; C: Control; Hs: *Hibiscus Sabdariffa*; IT: high-intensity interval training; ITHs: high-intensity interval training+Hs; n: number of animals; IBW: initial body weight; FBW: final body weight; WG: weight gain; IBA: body adiposity index. ANOVA, Bonferroni, p < 0,05; * vs C; + vs Hs; # vs IT.

Table 3. General characteristics of obese rats submitted to the AT protocol.

	C (n=8)	O (n=8)	OHS (n=8)	OAT (n=6)	OATHs (n=7)
IBW (g)	435 ± 62	517 ± 55	463 ± 49	478 ± 41	485 ± 52
FBW (g)	454 ± 44	712 ± 62	553 ± 99*	531 ± 66*	559 ± 83*
WG (g)	30 ± 0,8	205 ± 28	111 ± 18*	42 ± 15*	75 ± 19**
CC (Kcal/d)	105 ± 15	133 ± 21	114 ± 16	105 ± 15*	112 ± 11
BAI (%)	4,72 ± 1,77	11,9 ± 1,92	11,7 ± 3,59*	9,93 ± 0,83*	7,95 ± 2,02**

Notes: Values expressed as mean and standard deviation of the mean; C: Control; O: obese; OHS: Obese+ *Hibiscus Sabdariffa*; OAT: obese+continuous moderate aerobic training; OATHs: obese+continuous moderate aerobic training+Hs; n: number of animals; IBW: initial body weight; FBW: final body weight; WG: weight gain; CC: (Kcal/dia/rato): ingestão calórica inclui a energia da dieta rica em gordura e o açúcar da água potável; IBA: body adiposity index. ANOVA, Bonferroni, p < 0,05; * vs O; + vs OHS; # vs OAT.

Table 4. Data regarding the distance covered, time and the maximum speed in MPET and blood lactate in normal-weight rats.

	C (n=9)	Hs (n=8)	IT (n=9)	ITHs (n=9)
Distance covered (m)	354 ± 61	334 ± 93	1511 ± 307*	1376 ± 319*
Time (min)	18,7 ± 2,2	21,1 ± 3,1	46,3 ± 5,4*	43,4 ± 6,2+
Speedmax (m/min)	27,6 ± 2,2	30,2 ± 3,1	55,0 ± 5,6*	52,4 ± 6,1+
Lactate	10,7 ± 4,1	6,3 ± 5,1	12,2 ± 3,4*	16,2 ± 3,2+##

Notes: Values expressed as mean and standard deviation of the mean; C: Control; Hs: *Hibiscus Sabdariffa*; IT: high-intensity interval training; ITHs: high-intensity interval training+Hs; n: number of animals; Distance: distance covered by meters; Time: time spent in minutes; Speedmax: maximum speed traveled in meters per minute; Lactate: lactic acid measured in millimol per liter. ANOVA, Bonferroni, p < 0,05; * vs C; + vs Hs; # vs IT.

distance and BAI in normal-weight and obese rats. The association between distance and BAI was inversely proportional in normal-weight and obese rats, demonstrating that the lower the BAI, the greater the distance covered in the *Extest*. (Figure 1)

The evaluation of functional capacity (distance/BAI) showed that ET increased functional capacity in normal-weight and obese animals. (Figure 2A and 2B). *Hs* supplementation showed no effect on the functional capacity of normal-weight rats but increased the functional capacity of obese rats. There was also an effect of the interaction between *Hs* and AT on the functional capacity of obese rats. (Figure 2B)

DISCUSSION

The objective of this study was to evaluate the effects of *Hs* supplementation and ET on the functional capacity of normal-weight and obese rats subjected to different exercise training protocols. The main finding was that combining ET and *Hs* administration decreased body fat and increased functional capacity in obese rats. However, although *Hs* supplementation decreases BAI, it showed no effect on the functional capacity of normal-weight rats.

Table 5. Data regarding the distance covered, time and the maximum speed in MPET and blood lactate in obese rats.

	C (n=8)	O (n=8)	OHS (n=8)	OAT (n=6)	OATHs (n=7)
Distance covered (m)	505 ± 85	234 ± 66	265 ± 130	673 ± 224*	773 ± 116*
Time (min)	24,8 ± 2,8	14,3 ± 2,7	15,5 ± 5,1	28,3 ± 6,2*	31,2 ± 2,9*
Speedmax (m/min)	34,3 ± 3,4	23,3 ± 2,6	24,3 ± 5,4	37,7 ± 6,4*	40,3 ± 2,7*
Lactate	9,6 ± 2,1	12,1 ± 4,7	11,3 ± 3,6	12,5 ± 5,4	11,2 ± 2,6

Notes: Values expressed as mean and standard deviation of the mean; C: Control; O: obese; OHS: Obese+ Hibiscus Sabdariffa; OAT: obese+continuous moderate aerobic training; OATHs: obese+continuous moderate aerobic training+Hs; n: number of animals; Distance: distance covered by meters; Time: time spent in minutes; Speedmax: maximum speed traveled in meters per minute; Lactate: lactic acid measured in millimol per liter. ANOVA, Bonferroni, p <0,05; *vs. O; +vs. Hs; #vs. OAT.

The ET protocol was established for each group according to their clinical conditions.¹⁵ In a pilot study, obese rats could not perform interval training due to excess weight. Thus, moderate intensity AT was used for them (50% of Speed_{max} in the MPET). Normal-weight rats underwent standardized high-intensity IT in the laboratory, considering the best physical conditioning results.¹¹ High-calorie-diet-induced obesity resulted in increased weight and body fat corroborating other studies conducted in the same laboratory.¹⁵

The ET protocols used in this study were effective in reducing body weight and fat. Body weight decreased 10% and 25% in normal-weight and obese rats after 60 days of ET, respectively. Body fat was 295% higher in obese than in normal-weight rats. The analysis of BAI showed that IT decreased body fat by 32% in normal-weight rats and AT by 21% in obese rats, indicating that body fat does not directly reflect body weight. These results confirm that regardless of intensity, regular physical exercise controls body weight, and is a powerful tool to treat obesity.¹⁶ These data corroborate meta-analyses¹⁷ reporting that both IT and AT (continuous and moderate intensity) promote body fat reduction. Silva¹⁸ reported that a continuous moderate intensity exercise training protocol five day/ week for 12 weeks reduced body fat in obese rats under a high-calorie diet, showing the efficiency of ET in combating obesity.

The high-intensity IT and the continuous moderate intensity AT training protocols increased the performance *Extest* parameters (maximum speed, total test time, and distance covered) in normal-weight and obese rats in this study. These data corroborate studies showing that physical training significantly improves the maximum volume of oxygen (VO_{2max}) consumed regardless of the training protocol used.¹⁹ steady-state exercise (SSE; n=15 Kapravelou et al.²⁰ reported that obese rats had lower performance in a physical exercise test compared to normal-weight rats. Although our study did not compare the different training models, these results indicate that the training model and clinical condition resulted in decreased *Extest* performance.

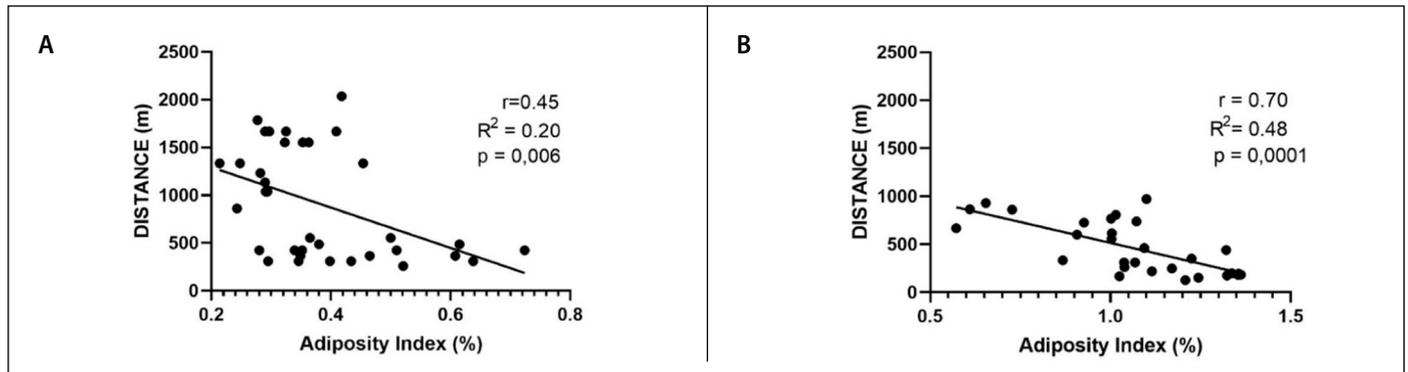


Figure 1. Correlation between the distance and the adiposity index of normal-weight rats. (A) and obese rats (B).

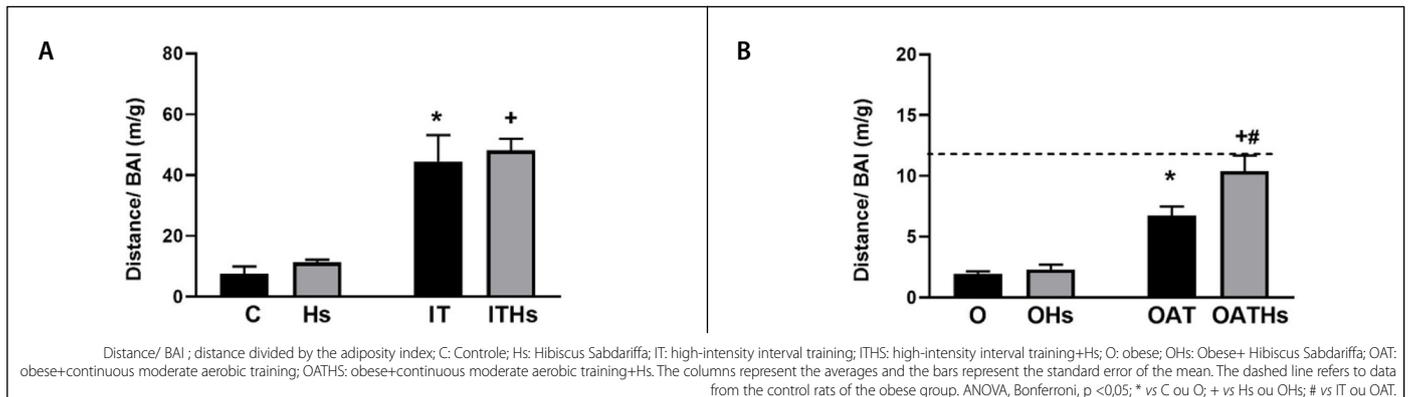


Figure 2. Functional capacity assessment. Distance/BAI; distance divided by the adiposity index; C: Controle; Hs: Hibiscus Sabdariffa; IT: high-intensity interval training; IT+Hs: high-intensity interval training+Hs; O: obese; OHS: Obese+ Hibiscus Sabdariffa; OAT: obese+continuous moderate aerobic training; OATHs: obese+continuous moderate aerobic training+Hs. The columns represent the averages and the bars represent the standard error of the mean. The dashed line refers to data from the control rats of the obese group. ANOVA, Bonferroni, p <0,05; * vs C ou O; + vs Hs ou OHS; # vs IT ou OAT.

Hs supplementation reduced body fat in normal-weight and obese rats corroborating with previous findings.¹⁵ Interleukin (IL) The effect of *Hs* on body fat in obese people is demonstrated in the meta-analysis by Zhang et al.,²¹ which showed that the use of *Hs* as a herbal treatment reduced cholesterol levels in patients with metabolic syndrome. A study by Diez-Echave et al.²² showed that *Hs* treatment with doses one, 10, and 25 mg/kg/d for six weeks decreased fat content in obesity induced by a hypercaloric diet in obese rats. A study by Morales-Luna et al.²³ showed that *Hs* treatment using 500 and 750 mg/100 mL of water at will for 16 weeks had an anti-obesogenic effect in rats with obesity induced by a high-calorie diet. According to the authors, both *Hs* concentration promoted lower triglyceride storage in adipocytes. A clinical trial with obese patients with hepatic steatosis showed that *Hs* treatment (six capsules of 450 mg/d for 12 weeks) reduced body weight, body fat, and the waist-hip ratio, in addition to reducing free fatty acids, showing a beneficial effect on metabolic regulation.²⁴ Fat reduction effects of *Hs* may be due to several mechanisms. The bioactive compounds (polyphenolic and flavonoids) contained in *Hs* can reduce oxysterols (a cholesterol derivative) in the bile acid metabolism and block lipid accumulation in the liver, also modulating fat absorption by increasing palmitic acid excretion in the feces, associated with decreased triglyceride and cholesterol levels. *Hs* polyphenols are efficient in suppressing adipogenesis, being considered its main active components and responsible for its anti-obesogenic effect. Literature reports that the natural bioactive compounds found in *Hs*, such as polyphenols, it a potential treatment agent to inhibit adipogenesis and, therefore, combat obesity,^{4,25} which is a crucial factor in the development of obesity, hepatitis, and hyperlipidemia. In this study, we investigated the anti-obesity effect of Hibiscus sabdariffa extract (HSE)

Hs supplementation reduced body weight gain and increased the functional capacity of obese rats, but not of normal-weight rats. Studies on the effect of the association between *Hs* supplementation and ET

to improve physical performance are still scarce. A study by Anel et al.²⁶ reported improved VO_{2max} and speed test results in young athletes under *Hs* supplementation before and after training with a dose of 2.5 g/240 mL of water. Most studies associating *Hs* with performance improvement show its antioxidant effect. Hsieh et al.²⁷ conducted a research on rodents and showed that hibiscus protocatechuic acid extracted from dry *Hs* leaves, which have antioxidant properties, inhibited the level of oxidative stress caused by exhaustive exercise. In addition, a study by Ilyas et al.²⁸ demonstrated the potential antioxidant effect of *Hs* as it contains anthocyanins, causing decreased free radicals, which may prevent overtraining syndrome. Our study suggests that *Hs* positively influences functional capacity, thereby accelerating body fat reduction. Several studies show the negative influence of body fat on functional capacity. The longitudinal study by Pribis et al.²⁹ conducted for over 13 years with more than 5,000 students, showed a negative correlation between increased body composition and decreased VO_{2max} , since the participants with increased BMI over the years showed decreased aerobic fitness. A study by Capel et al.³⁰ body mass index (BMI) evaluated 197 girls with a mean age of 13 years and showed that the participants who had higher body fat percentage and body mass index had lower aerobic capacity (VO_{2max}) compared with normal-weight girls.

CONCLUSION

To conclude, this study showed that *Hs* supplementation can reduce caloric consumption, body weight, and body fat, thereby decreasing the body weight and increasing the functional capacity of obese rats when associated with moderate intensity AT. New studies are warranted to establish the beneficial effects of combining *Hs* supplementation and ET.

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