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Original article

Anti-exercise-fatigue and promotion of sexual interest activity of total flavonoids from wasps drone-pupae in male mice



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ARTICLE INFO	A B S T R A C T
<i>Keywords:</i> Drone pupae total flavonoids Anti-fatigue Promotion of sexual interest Response surface methodology	The aim of this research was to evaluate the anti-exercise-fatigue and promotion of sexual interest of total flavonoids from drone pupae of wasps. DPTF was prepared by ethanol extracting and its extraction conditions were optimized by response surface methodology. Then, anti-exercise-fatigue and promotion of sexual interest of DPTF were evaluated. The optimums extraction conditions by RSM were ethanol concentration 65%, extraction time 3 h and solid-to-liquid 20:1(mL/g). No mortality and general symptoms of toxicity were observed in the DPTF treated mice(1 g/kg,3 g/kg,5 g/kg body weight) the body weight and food consumption were not significantly changed compared with the normal control group. The relative weights of main organ did not markedly change. DPTF can significantly extend the duration of the swimming time to exhaustion and the times of capture the female in mice, decrease BUN, LAC and Cr levels, increase LG, GG and T activities in the DPTF treated mice. The dose of 5 g/kg body weight is the optimal dose for anti-exercise-fatigue activity and promotion of sexual interest in male mice. In conclusion, DPTF is promising traditional natural-based therapeutic remedy

for relieving exercise-fatigue with high safety.

1. Introduction

Exercise fatigue is a physiological process after strenuous exercise, which involves a significant decline in ability for work, vitality, and activities [1,2]. With the improvement of living standards, people's awareness of sports and health is becoming more and more strong [3]. Nevertheless, strenuous exercise could cause physical and energy consumption in the body. It was occurred from widespread inflammation, neuropathology, and immune system dysfunction [4,5]. Meanwhile, the recovery time of exercise fatigue is relatively prolong [6], which also disadvantage of people's normal life to a certain extent [7]. In addition, fatigue is also a significant sign of disorders, such as immunologic, hematological, rheumatological, cardiac, renal, and endocrinological diseases, etc. The forced swimming test (FST) has been used to investigate whether certain agents have anti-fatigue [8].

Sexual intimacy is an important aspect of normal and healthy living. However, sexual desire and arousal disorders are among the most common problems presented. Sexuality is dynamic and alterable with time and may be addressed through different scientific angles: through the physiological and psychological aspects. Sexuality is directly influenced by sociocultural aspects, being addressed by the social sciences, human sciences (biological and genetic domains), and political sciences [9]. Especially, the process of fatigue recovery is rather long after strenuous exercise. It affected various functions of the human body, refers to the lack of interest in sexual activity, and there is no initiative requirement [10,11]. This is an urgent problem that needs to be solved. On the other hand, testosterone levels have been linked with sexual function, specifically erection quality, libido, and ejaculatory function in a variety of studies [12]. It provided experimental evidence for the promotion of sexual interest in medicine and food. Thus, the aim to find a quick and effective food to improve sexual performance is great significance [13,14]. Although a lack of sexual interest is rarely a threat to physical health, it can lead to psychological conditions such as depression and weary feelings of inadequacy [15]. Studies have shown that there is a certain relationship between the reduction of sexual function and physical fatigue [16,17]. Thus, this study was proposed to study the aphrodisiac potential of total flavonoids extract of drone

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Abbreviations: DPTF, total flavonoids extract from wasps drone pupae; BUN, blood urea nitrogen; LA, lactic acid; LG, liver glycogen; Cr, creatinine; GG, gastrocnemius glycogen; T, testosterone; FCT, first capture time; CTS, catching times

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Table 1

Factors and the levels of experiment of Response Surface Analysis.

Factors	Factor levels			
	-1	0	1	
A. Extraction time/h	1	2	3	
B. Concentration of ethanol/%	65	70	80	
C. The ratio of solid-fluid(g/ml)	10	20	30	

pupae.

Drone pupae of wasps is the nutrient body of the abnormal period of the male larva of the bee [18,19]. Under normal circumstances, it appears in the breeding season [20,21]. Drone pupae was ideal nutritional foods that contain high protein [22], low fatty [23], various vitamins [24] and microelements [25]. The nutritional value of drone pupae was not lower than bee honey, especially the content of flavonoids is much higher than bee pollen and honey [26]. It could be used as a food, as well as a nutritional supplement. However, there are few reports about the pharmacological effects of drone pupae total flavonoids(DPTF), which have limited the development of this material. Honey product is considered as health products and aphrodisiacs; however, there is little or no scientific confirmation to support this assertion. On the other hand, drone pupae is one of its by-products, and its research reports are rare. Thus, at present investigation, in order to have a deeper understanding of DPTF, we focused on the Anti-exercise-fatigue and promoting sexual interest activity of DPTF in male mice.

2. Materials and methods

2.1. Materials and chemicals

Wasps drone pupae was prepared by Shandong Huakang Apiculture Ltd. Co. (Rizhao China), Materials were identified by Prof. Jia Li, School of pharmacy, Shandong University of traditional Chinese medicine, Jinan, China. The herbarium samples of rutin standard (Chinese medicine Shanghai Chemical Reagent Company); Gold Day American ginseng infusion was prepared by Gaozhou DongTai nutrition food factory Ltd. Co. (Gaozhou, China).

Determination of biochemical parameters, including GLU (Glycogen), BLA(Blood lactic acid), T (Testosterone), Cr (Creatinine), COR(cortisol)and BUN (Blood urea nitrogen) were conducted using reagent kits that were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China)

Standard total flavonoids including rutin, quercetin, and quercetin glycosides were obtained from Chinese medicine Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Other reagents and solvents were purchased from Sigma Aldrich Chemical Co., Ltd (St. Louis, MO, USA).

Table 2

Table 3The effects of DPTF on the body weight of mice.

Groups	Initial body weight after a week of adjustment(g)	Final weight(g)	Addition of weight (g)	Food intake(g)
Blank group	31.86 ± 1.94	$44.10~\pm~2.02$	12.54 ± 1.83	762.4 ± 33.86
Ginseng granu- le group	29.59 ± 1.40	43.00 ± 3.88	11.41 ± 2.16	752.4 ± 28.23
DPTF-L	29.77 ± 2.47	42.89 ± 3.94	12.52 ± 1.47	758.4 ± 29.43
DPTF-M	30.84 ± 1.50	44.26 ± 4.55	12.62 ± 3.25	772.4 ± 31.63
DPTF-H	30.91 ± 1.90	44.89 ± 4.65	13.38 ± 2.79	782.4 ± 34.93

Values expressed as mean \pm SD, n = 20animals/group.

No statistically significant differences were found.

2.2. Experimental animals

Healthy Kunming mice (aged 4 weeks, half males and half females, weight 22 ± 2 g) were obtained from Jinan Pengyue experimental animal breeding Co., Ltd (Approval No. SYXK (Lu) 20140007, Jinan, China). Mice were housed under controlled temperature and humidity conditions, and allowed free access to food and water. Before experiments, mice were acclimated to housing conditions for at least one week. Animal experiments were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978), and were approved by the Animal Care and Welfare Committee of Jinan.

2.3. Extraction of drone pupae total flavonoids

DPTF was prepared using the method of response surface methodology (RSM) reported by Wang et al. [27] with some modifications. RSM was performed in using the reasonable experimental design method and through the experiment to get some data, the multivariate two regression equation is used to fit the function relationship between the factors and the response values. By the analysis of the regression equation, the optimal process parameters are sought, and a statistical method for solving the multivariable problem is solved [28].

According to Box-Benhnkende's central combinatorial design principle. We were taken ethanol concentration, extraction time, ratio of liquid to solid as a significant influence on the total flavonoids of drone pupae. Three factors and three levels of response surface analysis method to optimize the extraction process of total flavonoids from drone pupae. Table1 shows the factors and design conditions of response surface optimization.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	14.76	9	1.64	8.57	0.0049	significant
A(extraction time)	0.11	1	0.11	0.55	0.4815	
B.(Concentration of ethanol)	4.68	1	4.68	24.45	0.0017	
C The ratio of solid-fluid	0.59	1	0.59	3.10	0.1216	
AB	0.036	1	0.036	0.19	0.6772	
AC	2.500E-003	1	2.500-003	0.013	0.9122	
BC	0.12	1	0.12	0.64	0.4501	
A ²	0.034	1	0.034	0.18	0.6857	
B ²	8.25	1	8.25	43.10	0.0003	
C^2	0.69	1	0.69	3.61	0.0993	
Residual	1.34	7	0.19			
Lack of fit	1.28	3	0.43	26.86	0.0841	Not significant
Pure Error	0.063	4	0.016			-
Cor Total	16.10	16				

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