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Acute toxicity and anti-fatigue activity of polysaccharide-rich extract from corn silk

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ABSTRACT

The aim of this study was to evaluate the safety and potential of PCS as the anti-fatigue functional food. PCS was prepared by water extracting-alcohol precipitating method, and its chemical compositions of monosaccharide were analyzed. Then, acute toxicity and anti-fatigue activity of PCS were evaluated. PCS is composed of Rha, Arab, Xyl, Man, Glu, and Gal, its molar ratio is 0.17: 0.30: 0.26: 0.35: 1.00: 0.57. No mortality and general symptoms of toxicity were observed in the PCS treated mice (7.5, 15, and 20 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, and biochemical indicators also did not markedly change. PCS can significantly prolong the duration of the swimming time to exhaustion in mice, decrease BUN, LA levels, increase LDH activities, and the contents of HG in the PCS treated mice. The dose of 400 mg/kg body weight is the optimal dose for anti-fatigue activity both in male and female mice. In conclusion, PCS is a promising traditional natural-based therapeutic remedy for relieving fatigue with high safety.

1. Introduction

Fatigue is a commonly used term relating to progressive decline in the ability for vitality and activity [1]. Fatigue can be divided into two types, i.e. central and peripheral fatigue. Central fatigue may be caused by the impaired alpha motor cord neuron firing, which subsequently reduces the mean spectral frequency on electromyography (EMG), and the reduced amplitude of EMG signal usually indicates loss of muscles activation. Peripheral fatigue mainly arises from the reduced maximum voluntary contraction (MVC) of muscle during a task. Some mechanisms including impaired calcium release and reuptake, bioenergetic failure, loss of electrical conduction, and impaired interactions between myosin and actin, involve the MVC reduction [2]. In addition to a physiological response to physical stress, fatigue is also a significant sign of disorders, such as immunologic, hematological, rheumatological, cardiac, renal, and endocrinological diseases, etc. Despite a wide range of options to alleviate fatigue, response to patients who suffer from the above-mentioned diseases, medication-based therapy is still necessary [3]. Dietary supplements as well as traditional Chinese medicine are the principal drugs to treat fatigue, such as *L*-carnitine [4], creatine [5], coenzyme Q10 [6], and ginseng [3].

Corn silk (*Maydis stigma*), the stigma and style of corn (*Zea mays* L.), is an abundantly available waste material from corn cultivation and widely distributed in many parts of the world [7,8]. It has been consumed as a traditional natural-based therapeutic remedy and functional food for a long time in China, Turkey, United States and

Abbreviations: PCS, polysaccharide-rich extract from corn silk; BUN, blood urea nitrogen; LA, lactic acid; LDH, lactate dehydrogenase; HG, hepatic glycogen; GLU, glucose; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; TC, total cholesterol; TG, triglycerides; Cr, creatinine; NPP, *p*-nitrophenylphosphate; AMP, 2-amino-2-methyl-1-propanol; CHOD, cholesterol oxidase; GPO, glycerol phosphate oxidase; PAP, peroxidase/4-aminopyrine/4-chlorophenol; Rha, *D*-rhamnose; Arab, *D*-arabinose; Xyl, *D*-xylose; Man, *D*-mannose; Glu, *D*-glucose; Gal, *D*-galactose; Fru, *D*-fructose; GC, gas chromatography.

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France [9,10]. The bioactive components of corn silk consist of flavonoids, carbohydrates, steroids, alkaloids, volatile oils, saponins, tannins, vitamins, and proteins [7,10–12]. These active ingredients contribute to its beneficial potential for the applications of healthcare, such as anti-diabetic capacities [13], antioxidant effects [14], anticoagulant [15], anti-tumor [16], antifungal activities [17], and diuretic [18]. In China, corn silk has been listed in the Chinese Pharmacopeia 1977 edition, and its traditional medicinal application was used for the treatment of chronic diseases, including diabetes, cystitis, prostatitis, hypertension, gout, and edema [19]. Nowadays, the traditional use of corn silk has been expanded to an array of aspects, such as hepatic protection [20], immunoenhancement [21], and gastroprokinetic effects [22], especially, the research and development of anti-fatigue functional food. Hu et al., [23] studied the anti-fatigue activity of flavonoid from corn silk (FCS). Results indicated that FCS is able to prolong ($P < 0.05$) the swimming time significantly in mice at doses of 100 mg/kg and 400 mg/kg, when compared with the control group, and significantly lower ($P < 0.05$) LA, BUN levels, elevate HG concentrations. In addition, corn silk extract (CSE) can enhance the anti-fatigue effects of *Portulaca oleracea* L. extract (POE) at a dose of 500 mg/kg (mass ratio of CSE/POE = 1: 2) in mice [24]. Preparations of corn silk have been proved to significantly extend ($P < 0.05$) the swimming time at a dose of 0.73 g/mL, and significantly prolong ($P < 0.05$) the hypoxia endurance time at a dose of 2.19 g/mL in mice, when compared with the control group [25]. Therefore, the anti-fatigue function of corn silk has been becoming the inheritance and innovation of traditional medicinal use in China.

Polysaccharides are a kind of natural active substances, which have been reported to exhibit anti-fatigue activities [26,27]. Previous researches indicated that corn silk is rich in polysaccharides [9]. However, no attempt has been made to evaluate the anti-fatigue activity of polysaccharide-rich extract from corn silk (PCS). Safety is the crucial consideration for the development of natural-based functional food, even though, natural products are safer and harmless than total or semi-synthetic products. In previous studies, subchronic toxicity of corn silk was conducted to assess its safety [28]. The results suggested that consumption of corn silk has no adverse effects. Nevertheless, there is no report on the acute toxicity of PCS.

In previous work, the antioxidant activity, subchronic toxicity and genotoxicity of the flavonoid-rich extract from corn silk (FCS) have been studied by our group [19,29]. Thus, in present investigation, with the aim to obtain the possibilities of PCS becoming functional food for relieving fatigue, we focused on the acute toxicity and anti-fatigue activity of PCS in mice.

2. Materials and methods

2.1. Materials and chemicals

Corn silk was collected in the suburbs of Jilin City, Jilin Province, China. Materials were identified by Prof. Guang-shu Wang, School of pharmacy, Jilin University, Changchun, China. The herbarium samples (Voucher No. HG-CS-036) were deposited at School of Chemistry and Pharmaceutical Engineering, Jilin Institute of Chemical Technology, Jilin, China. Ginsenoside was provided by Dalian Fusheng Pharmaceutical Co., Ltd (Dalian, China).

Determination of biochemical parameters, including GLU (hexokinase method), AST (substrate method), ALT (substrate method), ALP (NPP substrate-AMP buffer method), TC (CHOD-PAP method), TG (GPO-PAP method), Cr (sarcosine oxidase method), and BUN (urease/glutamate dehydrogenase method) were conducted using AU2700 Beckman coulter chemistry analyzer (Beckman Coulter, Brea, CA, USA).

Reagent kits for the determination of BUN (lot No. 20150925), HG (lot No. 20150919), and LDH (lot No. 20150922) were purchased from Jiancheng Biotechnology Co., Ltd (Nanjing, China).

LA was determined by Lactate Scout$^+$ analyzer (EKF Diagnostics, Cardiff, WAL, England).

Standard sugars including Glu, Rha, Arab, Xyl, Man, Gal, and Fru were obtained from Sino-pharm Chemical Reagent Co., Ltd (Shanghai, China). Other reagents and solvents were purchased from Sigma Aldrich Chemical Co., Ltd (St. Louis, MO, USA).

2.2. Experimental animals

Healthy Kunming mice (aged 4 weeks, half male and half female, weight 20 ± 2 g) were obtained from Liaoning Changsheng Biotechnology Co., Ltd (Approval No. SCXK (Liao) 2015-0001, Liaoning, 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 Jilin Institute of Chemical Technology.

2.3. Polysaccharides preparation

PCS was prepared using the method of ultrasonic-assisted extraction (UAE) reported by Prakash Maran et al., [30] with some modifications. UAE was performed in an ultrasonic extracting device equipped with digital temperature and time controller (Voshin, VS-200UE, Jiangsu, China) working at input power of 250 W, frequency of 20 kHz, and heating power of 500 W. About 500 g of dried ground corn silk was soaked in 1000 ml of water at room temperature overnight, and the extract was prepared by UAE at 60 °C for 30 min. After filtration, the filter cake was subjected to the above method for twice. The resulting filtrate was combined, concentrated to the original volume of 20%, and precipitated by the addition of anhydrous ethanol to a final concentration of 75% (v/v). The concentrated filtrate was refrigerated at 4 °C overnight to obtain crude PCS, which was isolated by centrifugation at 4500 rpm for 5 min. The lower solid was freeze-dried, and grounded to a powder, giving 26.9 g of crude PCS. The percentage of PCS extraction yield was 5.38%.

2.4. Preliminary purification

Deproteinization of crude PCS was conducted according to the method reported by Huang et al., [31] with some modifications. 10 g of crude PCS was weighed to prepare 1.25% of PCS solution, which was then adjusted to pH 3 with 10% trichloroacetic acid (TCA) solution, and kept it overnight. The treated sample was centrifuged at 5000 rpm for 10 min. The lower solid was discarded to obtain the deproteinized PCS solution, and small molecular impurities was removed by dialysis (MD10, Viskase, Darien, IL, USA) for three times.

2.5. Total polysaccharide determination

Polysaccharide content of purified PCS was determined according to the phenol-sulfuric acid method using glucose as standard [32,33]. The absorbance was measured at 490 nm using distilled water as blank in an ultraviolet-visible spectrophotometer (752N, Jinke, Shanghai, China).