European Journal of Pharmacology 764 (2015) 100–108

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural pharmacology

# Anti-fatigue effect of Myelophil in a chronic forced exercise mouse model

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ARTICLE INFO

*Article history:*
Received 26 February 2015
Received in revised form
26 June 2015
Accepted 26 June 2015
Available online 2 July 2015

*Keywords:*
Chronic fatigue
Chronic forced exercise
Oxidative stress
Antioxidant
Myelophil

ABSTRACT

This study was performed to evaluate the anti-fatigue effects of Myelophil. ICR male mice (10 weeks old) were forced to run for 1 hour, 5 days/week for 4 weeks. Each running session was followed by administration of distilled water, Myelophil (50 or 100 mg/kg), or ascorbic acid (100 mg/kg) 1 h later. Equal proportions of Astragali Radix and Salviae Miltiorrhizae Radix were extracted using 30% ethanol, and formulated into Myelophil. To evaluate the anti-fatigue effects of Myelophil, exercise tolerance and forced swimming tests were conducted. Underlying mechanisms, including oxidant–antioxidant balance, inflammatory response, and energy metabolism, were investigated by analyzing skeletal muscle tissues and/or sera. Myelophil significantly increased exercise ability and latency times, and decreased the number of electric shocks and immobility time on exercise tolerance and forced swimming tests compared with control group. Myelophil also significantly ameliorated fatigue-induced alterations in oxidative stress biomarkers, antioxidant enzymes and antioxidant capacity, as measured by multiple assays, including enzyme activity assays and western blotting, as well as alterations in pro- and anti-inflammatory cytokines in skeletal muscle. Furthermore, Myelophil normalized alterations in energy metabolic markers in sera. These findings suggest that Myelophil reduces the effects of chronic fatigue, likely by attenuating oxidative and inflammatory responses and normalizing energy metabolism. Consequently, this study provides evidence for the clinical relevance of Myelophil.



## 1. Introduction

Chronic fatigue is a condition defined by persistent tiredness lasting more than 6 months, which is not ameliorated by rest (Kroenke et al., 1988). This condition, which includes medically unexplained chronic fatigue, idiopathic chronic fatigue, and chronic fatigue syndrome, is thought to affect as much as 10% of the general population worldwide (Son, 2012), with significant detrimental effects on quality of life (Swain, 2000), including impaired concentration, memory issues, muscle pain, and sleep problem (Chaudhuri and Behan, 2004; Finsterer, 2012). Despite the prevalence of this condition, there remain no effective therapies to date. Not surprisingly, in the absence of approved treatments, many patients with chronic fatigue and fatigue-related disorders turn to alternative medicine, and other non-traditional practices (Adams et al., 2009).

The pathophysiology of chronic fatigue remains poorly understood, though mounting evidence points to issues associated with the immune and endocrine systems, as well as dysregulation of antioxidant defenses and energy metabolism (Norheim et al., 2011). Among these potential causes, oxidative stress is the most well-characterized factor (Ferreira and Reid, 2008), often resulting in the release of inflammatory cytokines a secondary response (Pedersen and Febbraio, 2008). Previous studies have shown elevated levels of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL)-1β, and IL-6 in affected humans, as well as animal models of chronic fatigue syndrome (Clarkson and Hubal, 2002; Davis et al., 2007).

On the other hand, an alternative theory has focused on the disruption of energy homeostasis in chronic fatigue patients (Hultman et al., 1991). Constant, repetitive muscle contraction during intense exercise provokes the depletion of muscle ATP, which is reflected in levels of glycolysis and glycogenesis associated with muscle fatigue (Allen et al., 2008; Ament and Verkerke, 2009). Accordingly, many groups have begun investigating conditions associated with muscle fatigue as a potential treatment for chronic fatigue disorders (Powers et al., 2011; Yeh et al., 2014).

Myelophil, a 30% ethanolic extract consisting of equal parts *Radix Astragali* and *Radix Salviae Miltiorrhizae*, is a commercially available supplement in Korea, commonly used for the treatment of fatigue-associated disorders. Previous investigations into the

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http://dx.doi.org/10.1016/j.ejphar.2015.06.055
0014-2999/

pharmacological properties of these two herbs have demonstrated potent anti-inflammatory, immunomodulatory, antioxidative, and anti-tumor properties (Kang et al., 2004; Lu and Foo, 2002; Ryu et al., 2008), as well as anti-fatigue effects in a clinical study of chronic fatigue syndrome (Cho et al., 2009).

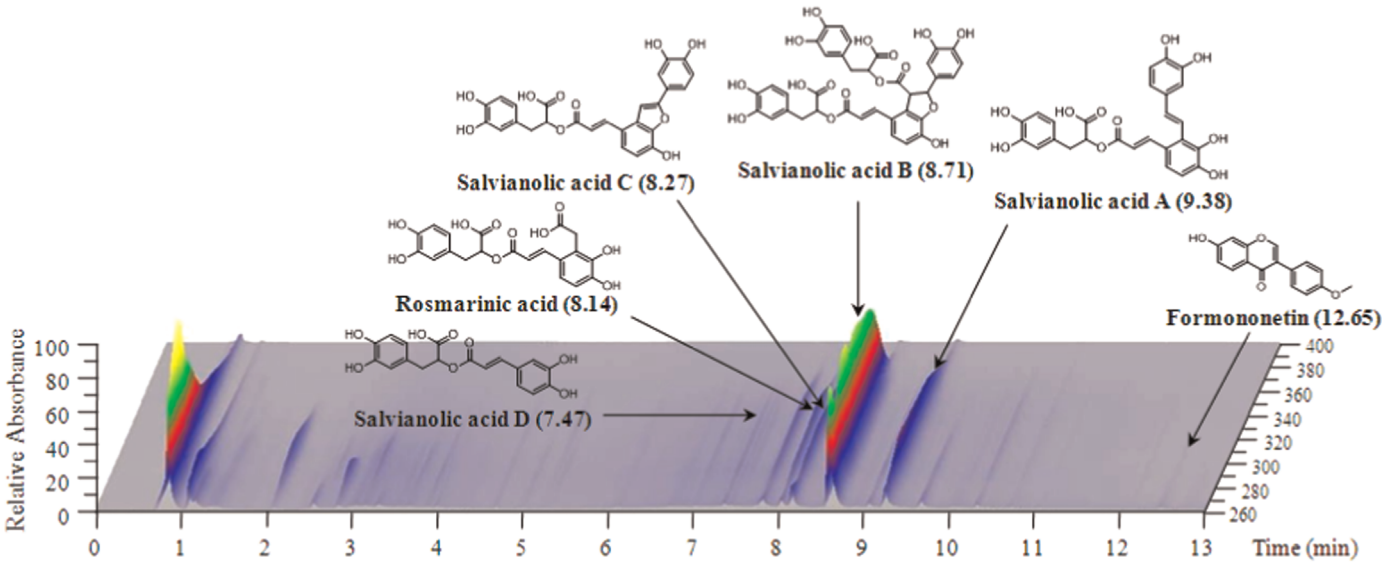
Here, we have expanded on these earlier works, evaluating the anti-fatigue effects of Myelophil in murine model of forced exercise-induced chronic muscle fatigue. The anti-fatigue effects of Myelophil were evaluated by means of behavioral tests, including exercise tolerance and swimming tests, along with molecular analyses examining oxidative stress, inflammatory cytokines, and energy metabolism in skeletal muscle and serum.

## 2. Materials and methods

### 2.1. Preparation and standardization of Myelophil

Astragali Radix (*Astragalus membranaceus Bunge*, cultivated in Jecheon, South Korea Ser. No. 20101106-JC-HG) and Salviae Miltiorrhizae Radix (*Salvia miltiorrhiza Bunge*, cultivated in Hebei, China; Ser. NO. 20110302-CHN-DS) were purchased from an Eastern medicine company (Jeong-Seong Drugstore, Daejeon, Korea). Equal proportions of Astragali Radix and Salviae Miltiorrhizae Radix were extracted using 30% ethanol, and formulated into Myelophil (Kyung-Bang Pharmacy, Incheon, South Korea). It was produced in accordance with the approved good manufacturing practice (GMP) guidelines of the Korean Food and Drug Administration (KFDA). The final Myelophil product [yield: 20.52% (w/w)] was stored for future use. Molecular fingerprinting of Myelophil (Fig. 1A) was conducted by ultra-high-performance liquid chromatography (UHPLC, Thermo Scientific, San Jose, CA, USA) as described previously (Lee et al., 2012). The quantitative analysis (Fig. 1B) of four reference compounds (astragaloside IV and formononetin for Radix Astragali, and salvianolic acid B and Rosmarinic acid for Radix Salviae Miltiorrhizae) and Myelophil was performed using liquid chromatography–mass spectrometry (LC/MS, LTQ Orbitrap XL linear ion-trap MS system, Thermo Scientific Co., San Jose, CA, USA), as described previously (Lee et al., 2014).

A




B

| Compounds | Quantitative Analysis (mg/g of Myelophil) | | |
|---|---|---|---|
| | Astragali Radix | Salviae Radix | Myelophil |
| Astragaloside IV | 1.81 ± 0.01 | Not detected | 0.87 ± 0.01 |
| Formononetin | 0.77 ± 0.01 | Not detected | 0.21 ± 0.01 |
| Salvianolic acid B | Not detected | 86.89 ± 0.19 | 36.21 ± 0.04 |
| Rosmarinic acid | Not detected | 2.53 ± 0.01 | 1.03 ± 0.01 |

C

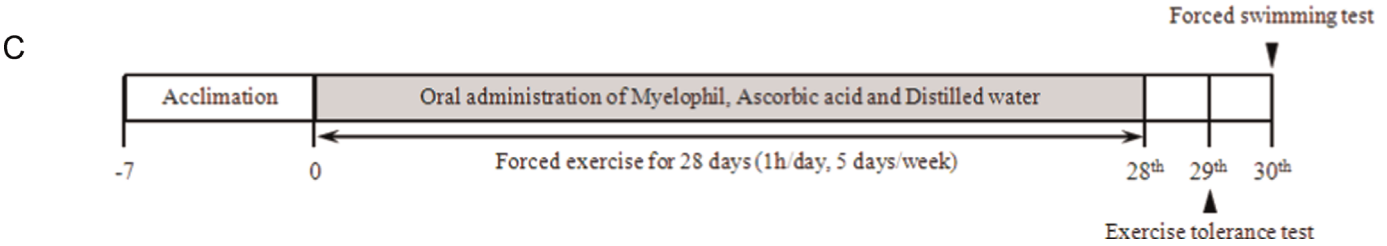



**Fig. 1.** UHPLC and LC/MS chromatogram of Myelophil. Myelophil and reference compounds were subjected to UHPLC analysis (A). Myelophil and four major compounds were analyzed by LC/MS (B). The diagram depicts the experimental design used in this study (C); including exercise tolerance tests (ETT) and forced swimming tests (FST).