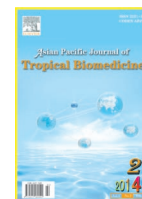




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## Inhalation of Shin-I essential oil enhances lactate clearance in treadmill exercise

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## PEER REVIEW

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## Comments

The mechanisms of some well-known effects of essential oils on physiological and psychological disorders have not been clearly established. This study provides evidence-base data and suggests that Shin-I essential oil inhalation may accelerate recovery after exercise in rats. In addition, lactate clearance is significantly enhanced after Shin-I aromatherapy.

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## ABSTRACT

**Objective:** To evaluate the effect of Shin-I essential oil inhalation on blood lactate changes in rats subjected to treadmill exercise.

**Methods:** Adult male Sprague Dawley rats ( $n=12$ ) were randomly divided into the control or the Shin-I group. Rats were subjected to a treadmill exercise program (15 m/min for 30 min). After exercise, rats were exposed to 200  $\mu$ L of water or Shin-I essential oil, respectively, using a nebulizer for 180 min during the recovery period. Blood samples were collected every 15 min. Blood glucose and lactate concentrations were determined in a CMA 600 analyzer.

**Results:** The basal glucose and lactate levels were no significantly different between two groups. After exercise, glucose levels were slightly increased to about 110%–120% of the basal level in both groups. Lactate levels of both groups reached to 110%–140% of basal levels during exercise. In the recovery period, lactate levels further increased to 180% of the basal level and were maintained at a plateau in the control group. However, lactate levels gradually decreased to 60%–65% of the basal level in the Shin-I group. Lactate clearance was significantly enhanced after Shin-I essential oil inhalation.

**Conclusions:** Our results provide evidence that Shin-I essential oil inhalation may accelerate recovery after exercise in rats.

## KEYWORDS

Blood, Lactate, Shin-I essential oil, Treadmill exercise

### 1. Introduction

Exercise results in a depletion of blood glucose and an accumulation of blood lactate concentrations[1]. Numerous studies have indicated that glucose and lactate concentrations are closely correlated with exercise

intensity[2]. In general, the onset or threshold of blood lactate represents the balance between lactate production and removal and may not suggest the efficiency of aerobic or anaerobic metabolism. However, lactate production is considered to be one of the major causes of muscle fatigue during exercise. Lactate appears to be an important

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source of systemic energy and plays an important role in providing optimal conditions for anaerobic exercise[3]. In addition, lactate accumulation reflects a mismatch between the inefficient rapid ATP production by glycogenolysis and the oxidative processes. The interference with muscle contraction and metabolism during this mismatch may result in the inhibition of oxidative phosphorylation[4]. Lack of oxidative phosphorylation is generally accepted to be one of the critical factors in muscle fatigue. Meanwhile, the clearance of lactate is crucial to allow the muscle to continue working. Previous studies found that lactate clearance was enhanced when performing continuous exercise or massage during recovery periods[5–7]. Among these methods, massage and aromatherapy have been shown to provide an efficient relief from muscle fatigue[6,7].

Aromatherapy has been used for thousands of years as a folk remedy and has become popular in complementary and alternative medicine in recent years[8]. Shin-I (or Flos Magnolia) is a commonly used Chinese medicinal herb, and it is one of the most widely used essential oils. Shin-I has been traditionally used for the treatment of allergic rhinitis, nasal empyema, sinusitis, hypotension and fungal infection, as well as for its skeletal muscle contracting effects[9,10]. Although aromatherapy is widely used, there is little scientific evidence in support of its efficacy as a therapeutic agent.

Our previous studies have used our auto-blood sampling system to explore dynamic changes of energy metabolites during exercise in animal models[1,11]. The aim of the present study was to investigate the effects of the inhalation of Shin-I essential oil on dynamic lactate changes in rats by using the auto-sampling system after treadmill exercise. Blood lactate accumulation and clearance were evaluated.

## 2. Materials and methods

### 2.1. Preparation of Shin-I essential oil

The Shin-I essential oil was extracted by conventional steam distillation from the buds of *Magnolia biondii* Pamp (Nanzhao County, Henan, China). In brief, 200 g of fresh buds of *Magnolia biondii* Pamp were ground and mixed with 3 000 mL of water. The temperature of the steam decomposed the plant fibers sufficiently to expose the essential oil molecules, which were then carried by the water vapor up and out of the container. The steam/oil mixture was then cooled via a Liebig condenser at 4 °C to obtain the essential oil, which was then analyzed by gas

chromatography (GC)–mass spectrometry (MS) analysis.

### 2.2. GC–MS analysis

Essential oil extract (1 µL) was analyzed by GC–MS using an Agilent 6890N GC interfaced with a Hewlett–Packard 5973 MS. A Zebron–5 mass cross-linked fused–silica capillary column (HP–5MS, 0.25 mm×30 m) coated with 5% phenyl polymethylsiloxane (0.25 µm thickness) was used. The oven temperature was maintained at 40 °C, then increased from 40 to 100 °C (2 °C/min), and then increased from 100 to 250 °C (7 °C/min), and finally maintained at 250 °C for 10 min. The pressure of the helium inlet was set at 11.57 pounds per square inch, with a linear velocity of 33 cm/s. The injector temperature was maintained at 250 °C. The percentage composition of the essential oils was computed from GC peak areas. Chromatographic peaks were checked for homogeneity with the aid of mass chromatograms of characteristic fragment ions. The Wiley NIST database was used for automatic identification of the GC peaks.

### 2.3. Animals

Adult male Sprague Dawley rats (300–350 g) were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). All rats were housed in a temperature–(25 °C) and light–controlled room (12:12 h light–dark cycle), with standard rat chow and water *ad libitum*. Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital (La–97524) in accordance with the guidelines for use of laboratory animals. Rats ( $n=12$ ) were randomly separated into the control or the Shin-I group. Each rat was anesthetized by isoflurane (Anaesthesia Unity, Univentor 400; Phymep, Paris, France), and a catheter (TPC/40, EICOM, Kyoto, Japan) was inserted into the jugular vein for repeated blood samplings. To prevent the TPC/40 catheter from injuring the rats, the catheter was tunneled under the rat's skin to the nape of the back of the neck. Blood samples (50 µL) were automatically collected every 15 min via the implanted catheter in the jugular vein by the auto-blood sampling system (DR–II, EICOM, Kyoto, Japan). Blood samples were centrifuged at 3 000 r/min for 10 min at 4 °C. A microdialysis analyzer (CMA/600, Carnegie Medicin, Stockholm, Sweden) was employed for determination of glucose and lactate concentrations. Glucose and lactate are oxidized by glucose and lactate oxidase, respectively. Peroxidase catalyses the reaction between the hydrogen peroxidase formed, phenol, and 4-amino–antipyrine

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