Journal of Ethnopharmacology 138 (2011) 156–161

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Effects of an isopropanolic-aqueous black cohosh extract on central body temperature of ovariectomized rats

Xiaoyan Ma[a], Hui Zhang[a], Ke Wang[b], Liyuan Yang[b], Lihua Qin[b,*], Wenpei Bai[a,*], Youfei Guan[c], Jing Jia[d], Jihong Kang[c], Changman Zhou[b]

[a] Department of Gynecology, The First Hospital of Peking University, Beijing, China
[b] Department of Anatomy and Embryology, Peking University Health Science Center, Beijing, China
[c] Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China
[d] Department of Stomatology, General Hospital of Armed Police, Beijing, China

ARTICLE INFO

Article history:
Received 10 May 2011
Received in revised form 22 August 2011
Accepted 30 August 2011
Available online 7 September 2011

Keywords:
Black cohosh
Cimicifuga racemosa
Abnormal thermoregulation
Central body temperature
Rats

ABSTRACT

Ethnopharmacological relevance: Black cohosh (*Cimicifuga racemosa*) is widely used in menopause symptoms strategy.
Aim of the study: The aim of this study was to examine the effect of isopropanolic black cohosh extract (iCR) on the central body temperature (CBT) of ovariectomized rats (OVX) and elaborate its possible effects in alleviating menopause related hot flushes.
Materials and methods: 64 female Sprague-Dawley rats, weighing 230 ± 10 g and aged 6–8 weeks, were divided into four groups: ovariectomy (OVX), sham, ovariectomy plus estradiol valerate (OVX + E), and ovariectomy plus iCR (OVX + ICR). The sham group underwent a sham surgery without ovariectomies, while the other three groups underwent bilateral ovariectomies under sterile conditions and a temperature implant was embedded in the abdominal cavity of all four groups. After 2-week recovery period, the temperature of all animals was monitored for 6 weeks.
Results: CBT of four groups maintained a normal circadian rhythm, with a low day CBT and a high night CBT. CBTs of the sham group were lower than that of the other three groups. The day CBTs of the (OVX + E) group and (OVX + ICR) group were lower than that of the OVX group from day 2 and day 22 respectively. For the difference between day and night CBT, the sham group was smallest, while (OVX + E) and (OVX + ICR) groups were higher than that of OVX group. The amplitude of day and night CBT, CBT fluctuation frequency at 5 min intervals, were higher for the OVX group than the sham group; the amplitude of day and night CBT of (OVX + E) group and the amplitude of night CBT of (OVX + ICR) group were higher than those of OVX group; while the amplitude of day CBT of (OVX + ICR) group was lower than that of OVX group; CBT fluctuation frequency at 5 min intervals was higher for the (OVX + E) and (OVX + ICR) groups than the OVX group.
Conclusions: Ovariectomized rats had abnormal thermoregulation, demonstrating an increase in day and night CBT, greater difference between day and night CBT, higher amplitude of day and night CBT, and more CBT fluctuation frequency. For the herbal extract iCR, the onset of affecting abnormal thermoregulation took longer than that of estradiol valerate. ICR had a significant effect on day CBT but was only little effective on night CBT of ovariectomized rats.

1. Introduction

The hot flush associated with the menopause is an unique symptom and causes considerable distress and impairment of quality of life. Menopausal hot flush occurs in as many as 74% of menopausal women (Kritz-Silverstein et al., 2000), and for some 40% the symptom is troublesome enough to seek for medical help. The underlying mechanism of hot flushes is still unclear. However, one long-standing assumption has been that hot flashes involve dysregulation of the thermoregulatory system, triggering homeostatic heat loss mechanisms to return the system to normal (Kronenberg, 2010).

It is well known that preoptic anterior hypothalamus (PO/AH), which contains warmth-sensitive/cold-sensitive neurons, receives input from peripheral and deep tissue thermal receptors when

* Corresponding authors. Tel.: +86 10 83573226; fax: +86 10 66551211.
E-mail addresses: qinlihua88@163.com (L. Qin), bwp66@163.com, jane-19831028@163.com (W. Bai).

0378-8741/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2011.08.076

core body temperature (CBT) is low/high. Normal homeostasis operates to keep the core body temperature within a very narrow range, sweating and cooling thresholds, which is called the thermoneutral zone (Cabanac and Massonnet, 1977).

Freedman has demonstrated that women with hot flushes have an increased core temperature and a reduced thermoneutral zone (Freedman, 2001; Freedman and Subramanian, 2005) compared with their asymptomatic counterparts indicated that women with significant peri-menopausal symptoms have abnormal thermoregulation, with a raised cooling threshold, lowered sweating threshold and narrowed thermoneutral zone, which is recognized as the mechanism of hot flushes. Currently, estrogen treatment is still the most effective method of alleviating peri-menopausal hot flushes (Nelson, 2008). However, its use has been seriously challenged since the announcement of the Women's Health Initiative (WHI) results in 2002. Therefore, studies of non-estrogen treatments for peri-menopausal hot flushes have been aroused wide-spread concern. Black cohosh (*Cimicifuga racemosa*) has gained wide clinical attention in alleviating peri-menopausal symptoms, and its efficacy and safety have been confirmed by many randomized controlled trials (Liske et al., 2002; Frei-Kleiner et al., 2005; Bai et al., 2007). There was one study aimed to examine the underlying mechanism of black cohosh, found that black cohosh had no estrogenic effect on the uterine weight of ovariectomized rats, but could specifically bind to the 5-hydroxytryptamine receptors, 5-HT1AR and 5-HT7R (Burdette et al., 2003). The two serotonin receptor subtypes are mainly distributed in the hypothalamus—the central integrator of thermoregulation (Bruck and Zeisberger, 1987). Based on this finding one can assume that black cohosh might alleviate peri-menopausal hot flushes by acting on the thermoregulation center through various neurotransmitter pathways.

In recent years, researches confirmed that ethanolic black cohosh extract can reduce hot flush frequency and subcutaneous temperature in ovariectomized rats (Winterhoff et al., 2003; Kapur et al., 2010). However, there is no report reveals how black cohosh preparations affect the CBT of ovariectomized rats. Because CBT directly determines whether the thermoregulation center issues temperature adjustment signals, the effect of black cohosh preparations on CBT is crucial to explain the underlying mechanism of peri-menopausal hot flushes.

We aimed to study the effect of isopropanolic black cohosh extract on CBT of ovariectomized rats by telemetric measurement system, to further explore possible mechanisms of black cohosh in temperature modulation which might support our understanding of the mechanisms in alleviating hot flushes during the peri-menopausal period.

2. Materials and methods

2.1. Drugs

Isopropanolic-aqueous black cohosh extract (Remifemin® tablets) used in this study was manufactured by Schaper & Brümmer GmbH & Co. KG, Germany (bulk batch number 824821) and available on the Chinese drug market. With reference to the active ingredients in each tablet: 0.018–0.026 mL liquid extract corresponded to, on average, 2.5 mg dry extract and to 20 mg crude drug (extraction agent isopropanol 40%, v/v). Estradiol valerate used in this study was manufactured by the Guandong Branch of Bayer Healthcare Co, Ltd, bulk batch number 169A 11 (Bujiale®, 1 mg/tablet).

2.2. Subjects

Eighty female Sprague-Dawley rats aged 6–8 weeks were used, weighing 230 ± 10 g, purchased from the Department of Laboratory Animal Science, Peking University Health Science Center. The study was approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine of Peking University Health Science Center. The rats were housed in the laboratory and given 1 week to adapt to the surroundings before operation. To eliminate the effect of phytoestrogen on the experimental results, the rats were fed soy-free pelleted feed throughout the experiment. Rat were maintained in barrier rooms under a 12/12-h light/dark cycle, with a temperature of 25 °C and a relative humidity of 50%.

2.3. Ovariectomies and telemetric measurement of rats' CBT

Eighty rats were housed in the laboratory for 1 week, and then their rectal temperature was measured with a digital electronic thermometer (BT-A11CN-2) twice a day at 9:00 am and 16:00 pm for 1 week. Sixty-four rats with mean temperature of 37.2 ± 0.2 °C were selected and divided into four groups randomly: ovariectomy (OVX), sham, ovariectomy plus estradiol valerate treatment (OVX + E), and ovariectomy plus isopropanolic black cohosh extract (OVX + ICR). All except the animals of the sham group underwent bilateral ovariectomies under sterile conditions. The rats were anesthetized with an intraperitoneal injection of 1% sodium pentobarbital (40 mL/kg), and then fixed and laid on its back. The fur on the abdomen was clipped, and the skin disinfected with Betadine® and 70% ethanol. A 2–3 cm long skin incision was made in the midline of the abdomen and extended through the abdominal musculature; milky white fat tissue in the abdominal cavity was revealed, then raised lightly out of the abdominal cavity and dissected to reveal the uterus. The ovaries were revealed at the end of the uterus. One ovary was ligated using a clamp and thread and was resected. The other ovary on the lateral side was resected similarly. The fat tissue and uterus were placed back in the abdominal cavity after the absence of bleeding was confirmed, and a temperature plant (TA10TA-F40, Data Sciences International) was implanted in the abdominal cavity. The abdomen and skin were closed with wound clips and disinfected. The sham group animals had a sham operation—that is, the abdominal cavity was opened to reveal the ovaries, but they were not resected.

Rats were given 7 days to recuperate before monitoring temperature. Three days after the operation, all rats had vaginal smears to observe the effect of the operation. The presence of external basal layer cells for 5 days in succession indicated that the rat had a decreased estrogen level, and that ovaries had been successfully removed. Before body temperature monitoring, a receptor RPC-1 (Data Sciences International) was connected to a PC through a digital–analog converter—data exchange matrix (Data Sciences International). The CBT of each rat was measured over a 20 s period at 1 min intervals, and the data were recorded on computer by Dataquest Acquisition & Analysis System ART 3.0.

After 1 week of continuous CBT monitoring, the rats were given 6 weeks of treatment as follows: the (OVX + E) group received estradiol valerate (0.8 mg/kg body weight (BW), once/day); the (OVX + ICR) group received iCR (crude drug 60 mg/kg BW, once/day), and the OVX and sham groups underwent lavage with physical saline (10 mL/kg BW, once/day). The CBT of the rats was continuously monitored during the treatment period.

2.4. Dose and route of administration

The drugs used in the study were prepared by the following method: tablets of estradiol valerate and isopropanolic black cohosh extract were suspended in saline with sonification and