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Research Note

Effects of Aqueous Extracts of *Cynanchum wilfordii* in Rat Models for Postmenopausal Hot Flush

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ABSTRACT: Menopausal hot flushes (HFs), which manifest as a transient increase in skin temperature, occur most frequently in postmenopausal women, and sometimes negatively influence daily life. We investigated the effect of an aqueous extract of *Cynanchum wilfordii* (CWW) in a rat model of menopausal HFs, where tail skin temperature (TST) is increased after the rapid estrogen decline induced by ovariectomy. Ten-week-old female rats were ovariectomized and treated with CWW for 1 week. We measured TST and rectal temperatures (RT) and investigated serum estradiol. The TST in ovariectomized (OVX) rats was significantly elevated after ovariectomy compared with control rats, whereas the RT in OVX rats was not elevated. Administration of CWW (200 mg/kg/d for 7 days, p.o.) significantly improved the skin temperature increase in OVX rats. The lower level of serum estradiol in OVX rats was significantly increased by supplying E2, but it was not affected by CWW. The present study indicates a need for future research involving treatment with high concentrations of *C. wilfordii* and measurement over 24 h.

Keywords: anti-hot flush, *Cynanchum wilfordii*, tail skin temperature, ovariectomy

INTRODUCTION

Hot flushes (HFs) are the most common postmenopausal symptom and are reported as feelings of intense warmth along with sweating, flushing, shivering, and chills. HFs usually last for 1∼5 min, with some lasting as long as an hour (1). The physiology of HFs on temperature regulation are not known in detail, but probably involves core body temperature, central processing areas in the central nervous system, neuromodulators, peripheral vasculature, and sweat glands (2). A HF occurs as a transient increase in skin temperature, associated with objective signs of cutaneous vasodilation and vasoconstriction when the core temperature drops. Accumulating evidence regarding the etiology of HFs suggests that vasomotor instability associated with a significant withdrawal of estrogen is the base for the pathophysiology (3-5). It is well known that estrogen has anti-inflammatory and vasoprotective effects, modulates vascular physiology and function *in vitro*, *in vivo*, and in human models (6-11). For many years, estrogen-based hormone therapy has been an effective treatment for HFs. However, results obtained from numerous clinical trials indicated increased problems of thromboembolic incidents, heart disease, and breast cancer in women receiving long-term hormone replacement therapy (12). As an alternative to hormone replacement therapy, selective serotonin reuptake inhibitors and venlafaxine have been introduced as primary therapy for HFs (13). Regrettably, selective serotonin reuptake inhibitors can interrupt the metabolism of tamoxifen in patients with breast cancer (14). Accordingly, the development of effective and safe non-hormonal treatments for patients with menopausal syndromes (including HFs) is required.

Cynanchum is a genus of about 200 species that are widely distributed in Asia. Most *Cynanchum* species are used in traditional herbal medicine in Korea for the prevention and treatment of various diseases such as rheumatic arthritis, geriatric diseases, atherosclerotic vascular diseases, and ischemia-induced diseases (15). *Cynanchum wilfordii* Radix is described as the roots of *C. wilfordii* in Korean pharmacopoeia. In a previous human study, it was reported that a combination of EstroG (*C. wilfordii*, *Angelica gigas*, and *Phlomis umbrose*), vitamin, and minerals improved various menopausal associated disorders (16).

In a clinical trial, EstroG-100 appeared to be an effective and safe dietary supplement for use in pre-, peri-, and

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post-menopausal women (15). Unfortunately, there is currently little research on improving HFs with *C. wilfordii in vivo*.

Therefore, the present study was designed to study the anti-HF effect of *C. wilfordii* in an ovariectomized rat model.

MATERIALS AND METHODS

Samples and preparation

C. wilfordii root (2 kg) was extracted with distilled water at 100°C for 4 h, 0.7~0.75 kgf/cm^2. The extract was filtered through Whatman No. 4 filter paper and concentrated in vacuum at 40°C using a rotary evaporator (R-210, BÜCHI Labortechnik AG, Flawil, Switzerland). The extracted compound was lyophilized using a freeze-dryer and was stored at −20°C until needed. The aqueous extract of *C. wilfordii* was defined as CWW, which was used for treatments. Chemicals used were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Animals and treatments

This study was approved by the Animal Ethical Committee of Jeollanamdo Institute for Natural Resources Research (JINR1515). All experimental procedures were undertaken in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA) and the National Animal Welfare Law of the Republic of Korea. Ten-week-old female Sprague-Dawley rats weighing 210~230 g were purchased from Samtako (Osan, Korea). The animals were allowed tap water and standard laboratory food *ad libitum*, and were housed in polycarbonate cages at a temperature of 23±2°C, relative humidity of 55±10% and a 12-h light/dark cycle, with lights on from 07:00 h to 19:00 h daily. The rats were randomly allocated to two groups before the operation. Both groups were anesthetized with Zoletil 50 (Virbac, Carros, France) 50 mg/kg IP. Then, one group received bilateral ovariectomy using the dorsal approach (OVX; n=45) and the other group underwent a sham operation (sham; n=7) as controls. The OVX rats were randomly divided into 4 groups: OVX group (OVX/vehicle; n=9), E2 treatment group (OVX/E2; n=9), and two CWW treatment groups (OVX/CWW100 or 200; n=9). CWW (100 or 200 mg/kg body weight/d), E2 (91 μg/kg body weight/d) or distilled water were orally administered to rats at 0.05 mL/kg body weight once a day for 7 days, starting 1 week post surgery. Distilled water (10 mg/kg) as the control was administered to the sham-operated rats for 7 days following the same schedule.

Experimental procedure

Rats underwent bilateral ovariectomy or sham operations. After 1 week recovery period, CWW or E2 was administered once daily by oral gavage for 7 days. On the measurement day, water, E2, or CWW were administered orally 30 min before tail skin temperature (TST) and rectal temperature (RT) measurements. After the measurement of TST and RT, rats were sacrificed and then blood was collected to measure serum estradiol levels.

Measurement of TST and RT

Rats were restrained in a holder in a conscious state and the TST was measured for 1 h at the dorsal surface of the tail about 2 cm from the fur line with an infrared thermometer (AMIR 7210, Ahlborn Meßtechnik GmbH, Holzkirchen, Germany). Before testing, all animals were settled in the laboratory room for 15 min. The environment temperature was 25°C. Measuring points were identified and marked on the dorsal surface of the tail 2 cm from its base. TST data were measured at 10 min intervals throughout the experimental period. TST during the 1 h measurement period was calculated and data were analyzed as the change in TST for each 10 min measurement compared with the mean TST at 0 min. Changes in TST were assessed using ΔTST.

$$\Delta TST = (TST \text{ in each 10 min block}) - (TST \text{ at 0 min})$$

Values were expressed as the means±standard error of the mean (SEM).

RT was measured with a microprobe thermometer (BAT-12, Physitemp, Clifton, NJ, USA) inserted 5 cm into the rectum. The probe, dipped into glycerol before insertion, was held in the rectum for about 20 s. Measurements were taken at each location twice. Temperature recordings were carried out by the same person that handles the animals prior to the experiment.

$$\Delta RT = (RT \text{ in each 10 min block}) - (RT \text{ at 0 min})$$

Values were expressed as mean±SEM.

Both TST and RT were measured every 10 min for 1 h, and their mean values were calculated for data analysis.

Assay for serum chemistry

For the measurement of individual serum E2 concentrations, blood samples were collected via abdominal aorta puncture and were kept at room temperature for 30 min followed by centrifugation at 3,000 rpm for 10 min. Serum samples were stored at −80°C until assayed. Steroids from serum samples were extracted with diethyl ether twice and concentrated for estradiol determination. The level of estradiol was measured using a competitive ELISA assay Kit (ADI-901-174, Enzo Life Scien-

ces, Farmingdale, NY, USA) according to the manufacturer's instructions. The limit of detection for the assay was 14.0 pg/mL.

Statistical analysis

All values represent as the mean±SEM. The statistical significance was evaluated by a one-way analysis of variance (ANOVA) followed by Dunnett's test or Fisher's *F*-test. The significance level was accepted at $P<0.05$.

RESULTS AND DISCUSSION

The results of the change of TST are presented in Fig. 1. The ΔTST was maximally 50~60 min after measurement and those of the sham/vehicle rats were lower than those of the OVX rats. Either CWW rats (100 or 200 mg/kg) were significantly ($P<0.05$) lower than that of the OVX/vehicle group. E2 treatment (OVX/E2) significantly ($P<0.01$) inhibited the elevation of skin temperature in OVX rats, as did the 200 mg/kg dose of CWW. However, a difference in the dose-response curves seen for ΔTST was not observed among CWW-treated groups. As shown in Fig. 2, there were no differences in ΔRT among any of the groups during the measurement period.

The serum concentrations of estradiol in OVX and sham-operated rats are shown in Fig. 3. The estradiol level in sham rats was significantly higher ($P<0.05$) than in OVX rats. The lower level of estradiol was significantly ($P<0.05$) increased by supplying E2 (91 μg/kg, p.o.), but it was not affected by treatment with CWW.

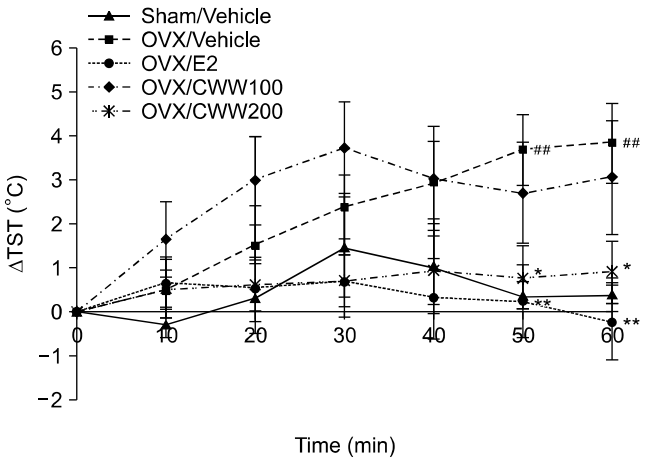

Fig. 1. Changes of the tail skin temperature (TST) in the ovariectomised (OVX) rats. Aqueous extract of *Cynanchum wilfordii* (CWW), E2, and distilled water (vehicle) were orally administered to rats once a day for 7 days starting one week after surgery. Shown are the mean changes in TST compared with the mean calculated at 0 min (ΔTST). Data are presented as the mean±SEM. Significantly different at $^{\#\#}P<0.01$ compared with the sham/vehicle group and $^{*}P<0.05$ and $^{**}P<0.01$ compared with OVX/vehicle group.

HFs in women are generally considered a thermoregulatory event, with the vasomotor symptoms and increased sweating being consistent with a heat dissipation response. The majority of HFs are preceded by an increase in core body temperature and their incidence is higher in a warm situation or following heating or exercise (17). It has been hypothesized that this thermoregulatory response is due to a dramatically narrowed thermoregulatory neutral zone, meaning that even a very small change in core body temperature may cross the temperature threshold for a heat dissipation response (18). This thermoregulatory process of HFs has led to the assumption that they are generated in the thermoregulatory areas of the anterior hypothalamus, as this area contains neurons that monitor and regulate body temperature. However, although the central thermoregulatory regions of the brain may be involved, there is no evidence as to the pathophysiology of HFs. Research into the role of estrogen withdrawal in HFs has generally as-

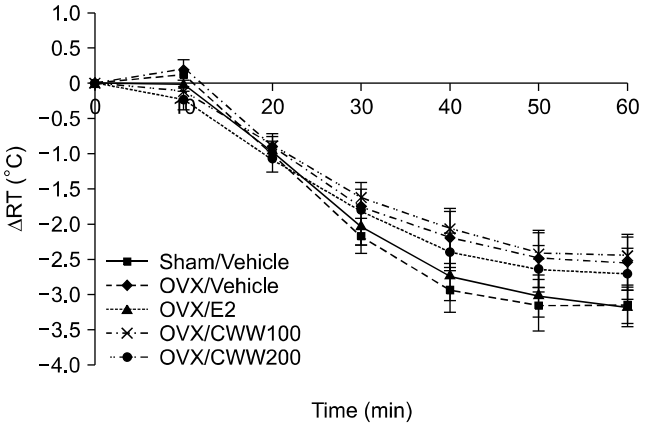

Fig. 2. Changes of the rectal temperature (RT) in the ovariectomised (OVX) rats. Aqueous extract of *Cynanchum wilfordii* (CWW), E2, and distilled water (vehicle) were orally administered to rats once a day for 7 days starting one week after surgery. Shown are the mean changes in RT compared with the mean calculated at 0 min (ΔRT). Data are presented as the mean±SEM.

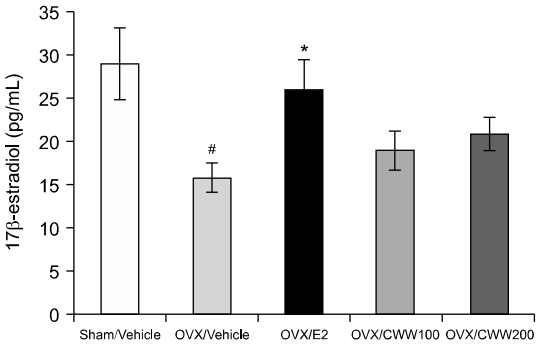

Fig. 3. Changes in serum estradiol levels in the ovariectomised (OVX) rats. Each value is expressed as the mean±SEM. Significantly different at $^{\#}P<0.05$ compared with the sham/vehicle group and $^{*}P<0.05$ compared with OVX/vehicle group. CWW, aqueous extract of *Cynanchum wilfordii*.

sumed it to be a central effect, but there is little direct experimental evidence.

There has been growing attention to the use of phytoestrogens as 'alternative' therapies for HFs. However, limited evidence from small randomized controlled trials provides mixed results suggesting that soy protein and isolated isoflavones do not substantially improve HFs (4).

Flushing of the tail skin in OVX animals is regarded as a good indicator for climacteric HFs, although the spontaneous appearance of flushing is irregular. After ovariectomy, TST increases, and this effect can be reduced by supplying estrogen (19-23). RT has been reported to lower, to increase, or to have no effect. These conflicting results may be caused by differences in environmental factor (restrained or free-moving condition, measurement period, etc.) (24-26). In this paper, *C. wilfordii* inhibited the elevation of skin temperature in OVX rats, but *C. wilfordii* did not restore the lowered serum estrogen in OVX rats. These findings suggest that *C. wilfordii* does not confer estrogenic activity to serum, and it does not potentiate estrogen-production in the extra ovular tissue in OVX rats. This is consistent with human studies, which showed that serum estadiol levels in the EstroG-100 group did not change, implying that EstroG-100 was not an active estrogenic compound (15). Some plants contain substances called "phytoestrogens", such as isoflavones (27) and coumestrol (28,29), that activate estrogen-controlled functions via estrogen receptors. In addition, raloxifene, which is a synthetic selective estrogen receptor modulator (SERM), exhibits antiestrogenicity in the breast and uterus, but acts as an agonistic in the bone and liver (30). If such phytoestrogen-like or SERM-like substances are contained in *C. wilfordii*, the estrogen-like activity regarding thermoregulation may be induced without restoring the decreased serum estrogen level in OVX rats. Accordingly, further experiments are required to determine the anti-HFs effect of *C. wilfordii* at higher doses and to search for other possible mechanisms such as neuromodulators.

In conclusion, *C. wilfordii* inhibited the potentiation of skin temperature elevation in OVX rats. The present study results also suggest that estrogen is useful as hormone replacement therapy for menopausal hot flashes.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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