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Effects of *Alternanthera sessilis* extract on Tail Skin Temperature in Rats; A Preliminary study

Chamawan P[1*], Thisayakorn K[2], Phornchirasilp S[1]

[1]Pharmacology and Biomolecular Sciences Program, Department of Pharmacology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
[2]Pharmaceutical and Natural Product Department (PNPD), Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand

Keywords: Hot flush, tail skin temperature (TST), core body temperature, *Alternanthera sessilis*

Objective: To evaluate the effects of *Alternanthera sessilis* (L.) R.Br. ex DC. on hot flushes simulation in both female and male Wistar rats.
Methods: Cutaneuos vasodilatation and peripheral blood flow increasing are the hallmarks of hot flush. The change of tail skin temperature in the rat model was used to screen the compounds for relieving hot flush. Calcitonin gene-related peptide (CGRP) (10 µg/kg; a potent vasodilator) and leuprorelin acetate (1 mg/kg; a potent GnRH-analogue) were administered in female and male rats via subcutaneously (S.C.) injection, respectively. Temperatures from tail skin (TST) and core body were determined after feeding with conjugated equine estrogen (CEE) 10 mg/kg, *A. sessilis* extract 100,300, and 500 mg/kg.
Results: All doses of *A. sessilis* extract and CEE inhibited the effects of αCGRP-induced increasing of TST in female rats. While, *A. sessilis* extract 500 mg/kg and CEE diminished the effects of leuprorelin acetate-induced elevation of TST in male rats.
Conclution: These results suggest that *A. sessilis* may be useful for alternative treatments in hot flushes patients. In addition, the results obtained from this study would be used to develop an animal model for screening the substances for relieving hot flushes in human.

* Corresponding author: Pharmacology and Biomolecular Sciences Program, Department of Pharmacology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; +66(0)2644-8679, +66(0)2644-8680, +66(0)2644-8681, +66(0)2644-8685; Fax. +66(0)2354-4326
E-mail address: panida.cha@mahidol.ac.th

Introduction

Hot flush is a common symptom that is often seen in postmenopausal women and andropausal men. It relates with sex hormone deficiency and the reflection of a disorder of hypothalamic thermoregulatory mechanisms. This symptom generally begins with a sudden outpouring of sweat, and then increasing in heart rate and peripheral blood flow resulting in skin temperature elevation.[1]

Alternanthera sessilis (L.) R. Br. ex DC. has called many names including sessile joyweed, dwarf copperleaf, and Ponnanganni in Tamil. It is a perennial herb that found widespread and abundant in wet or damp spots throughout tropical and subtropical regions of the world. It belongs to Amaranthaceae family and contains many phytochemical compounds especially stigmasterol, campesterol, β-sitosterol, β-spinasterol, palmitates of sterol, etc.[2] It is classified in vegetable and can be used as a herbal medicine in the southeast asia traditional medicine. Thai pharmacopoeia argues that the medical properties of both tree and roots of *A. sessilis* are mild laxative, treatment of irregular menstruation, fever heat neutralization, antipyretic, treatment of apthous ulcer, diarrhea, dysentery, treatment of urinary tract infections, stimulation the flow of bile, stimulation of blood circulation in the fire postpartum women, treatment of breast gland inflammation, galactogogue in postpartum, and treatment of lesions.[3,4] The benefits of *A. sessilis* could be useful in the treatment of vasomotor symptom in sex hormone deficiency people.

Therefore, the objective of this study is to evaluate the effects of *A. sessilis* extract for relieving hot flushes induced by vasodilator substances in both female and male Wistar rats. The results obtained from this study may be useful to develop a rat model for screening various plant extracts that are proposed to relieve hot flushes in human.

Methods

Animals

Fifteen male Wistar rats weighting 300±20 g, and fifteen female Wistar rats weighting 250±20 g were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom. The rats were maintained on 12 h light/ dark schedule at a temperature of 23±1 °C, with free access to food and water. All procedures and animal care

were approved by the Institutional Animal Care and Use Committee of Thailand Institute of scientific and Technological Research (TISTR).

Drug and reagents

Rat αCGRP and leuprorelin acetate were dissolved in saline and solvent that accompanied with a prefilled syringe, respectively, on the day of use. Conjugated equine estrogen (CEE) was prepared in vehicle (7% w/v of acacia) and used as a standard drug.

Whole parts of fresh *A. sessilis* plants without flowers were extracted with 95% ethanol at room temperature for 2 weeks, and then evaporated to be ethanolic *A. sessilis* extract (% yield = 7).

Effects of A. sessilis extract on αCGRP-induced increasing of tail skin temperature in female rats

Measurements of tail skin and core body temperatures were performed between 08:00-12:00 A.M., and the room temperature was maintained at 23±1 ˚C throughout the recording period.

Female rats were subcutaneously injected with aCGRP at a dose of 10 µg/kg every other day. On the fifth day of experiment, rats were orally administered with 7% w/v of acacia solution (as control), CEE 10 mg/kg, *A. sessilis* extract 100, 300, and 500 mg/kg daily in the morning for 11 consecutive days.

A digital thermometer probe (TL8009, Keyan Technology Co. Ltd., China) was taped to the dorsal surface of the tail about 3 cm from rat's tip. The mean of tail skin temperature (TST) was measured 1 hr after feeding throughout the 15-day experiment. The core body temperature was determined by mercury thermometer (CRW-23A, Jianggsu Yuyue Medical Instruments Co. Ltd., China) at the first and the last day of the experiment. The data were expressed as the changes of tail skin temperature; whereas the average tail skin temperature in each corresponding period after feeding – the basal tail skin temperature in each rat.

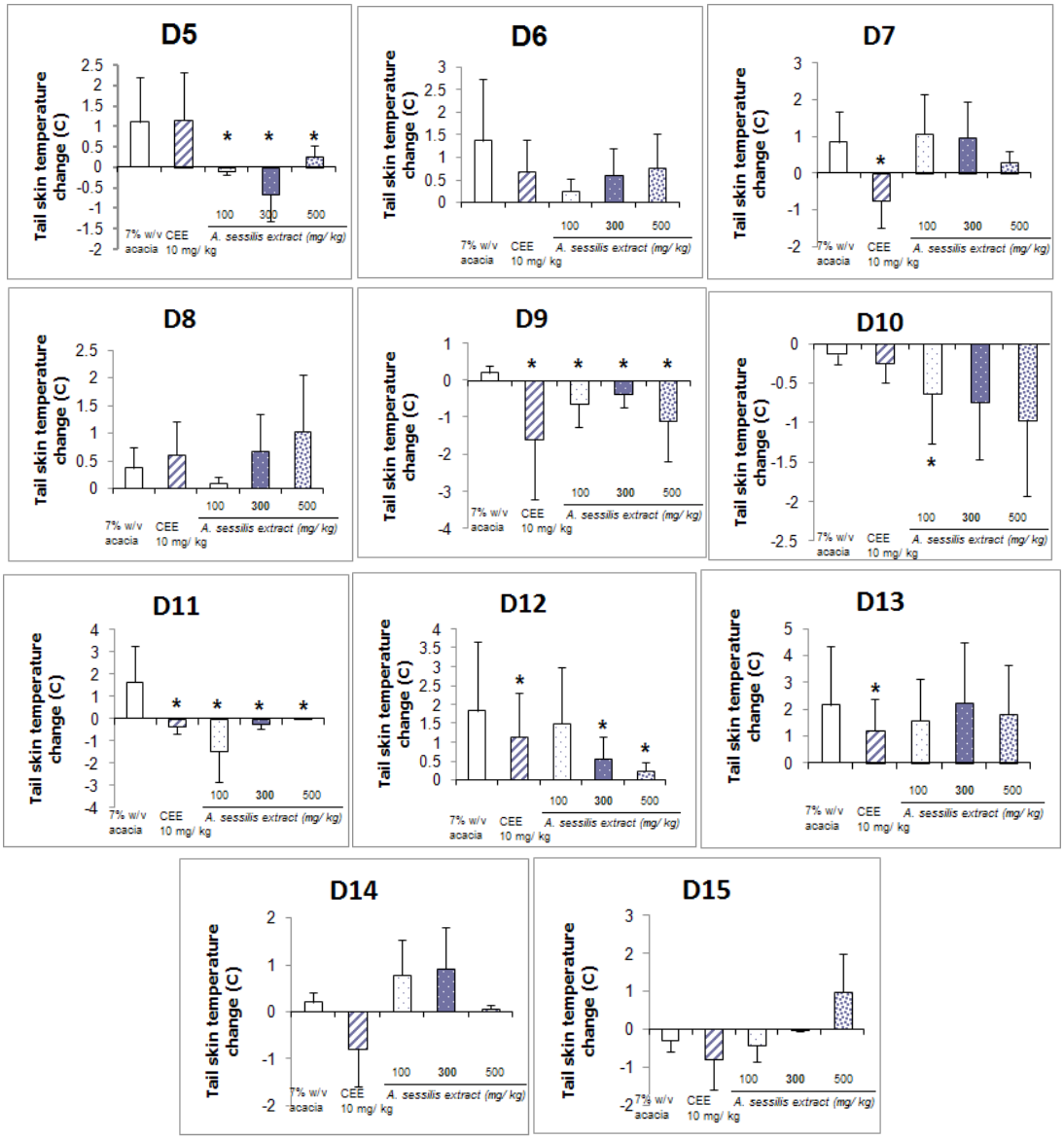

Figure. 1: Effects of *A. sessilis* extract on αCGRP-induced increasing of tail skin temperature in female rats. Each value was expressed as the mean ± S.E.M. (n = 3). Significance with Dunnett's test following a one-way ANOVA, * p< 0.05 vs. 7%w/v of acacia-treated group in female rats.

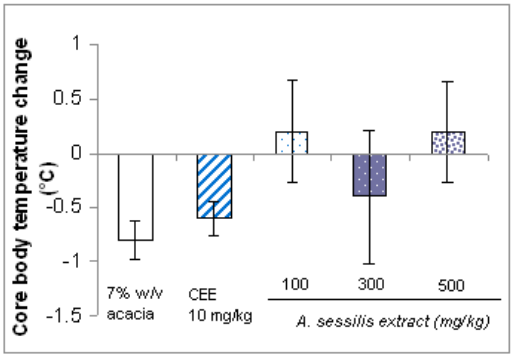

Figure. 2: Effects of *A. sessilis* extract on αCGRP-induced changes of core body temperature in female rats. Each value was expressed as the mean ± S.E.M. (n = 3). Significance with Dunnett's test following a one-way ANOVA, * p< 0.05 vs. 7%w/v of acacia-treated group in female rats.

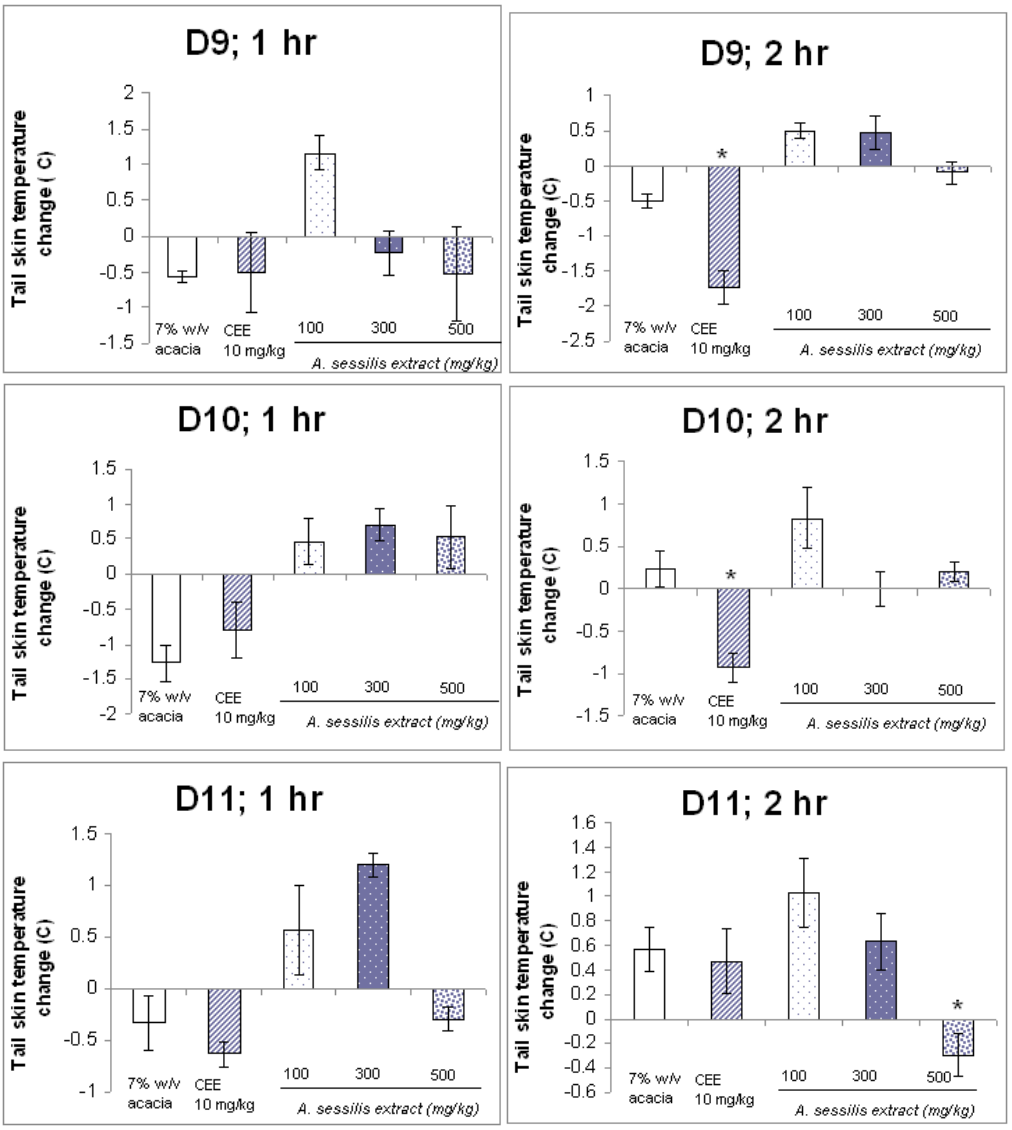

Figure. 3: Effects of *A. sessilis* extract on leuprorelin acetate-induced increasing of tail skin temperature in male rats. Each value was expressed as the mean ± S.E.M. (n = 3). Significance with Dunnett's test following a one-way ANOVA, * p< 0.05 vs. 7%w/v of acacia-treated group in male rats.

Effects of A. sessilis extract on leuprorelin acetate-induced increasing of tail skin temperature in male rats

In another set of experiments, leuprorelin acatate was subcutaneously injected at a dose of 1 mg/kg everyday in male rats. On the ninth day of experiment, rats were orally treated with 7% w/v of acacia solution (as control), CEE 10 mg/kg, *A. sessilis* extract 100, 300, and 500 mg/kg daily in the morning for 3 consecutive days. Procedures of temperature evaluations were similar to rat aCGRP groups throughout the 11-day experiment.

Statistical analysis

All data represent the mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of P<0.05 was considered for statistical significance.

Results

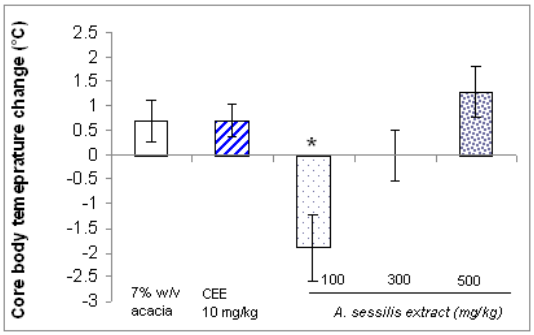

Figure. 4: Effects of *A. sessilis* extract on leuprorelin acetate-induced changes of core body temperature in male rats. Each value was expressed as the mean ± S.E.M. (n = 3). Significance with Dunnett's test following a one-way ANOVA, * p< 0.05 vs. 7%w/v of acacia-treated group in male rats.

In female rats, All doses of *A. sessilis* extract significantly reduced TST when compared with control (p<0.05) and exhibited similar to that with CEE 10 mg/kg (Figure. 1). On the 9th -12th day of experiment, the tail skin temperature in *A. sessilis* extract -treated rats was diminished approximately 0.5 - 1.5 °C in a dose-dependent manner (Figure. 1). While, the change of core body temperature in every group was not statistically significant as showing in Figure. 2.

In male rats, at two hour after feeding, CEE and *A. sessilis* extract 500 mg/kg significantly reduced TST when compared with control (Figure. 3). Only group received *A. sessilis* extract 300 mg/kg significantly changed of core body temperature that exhibited in Figure. 4.

Discussion

In our previous studies, the αCGRP and leuprorelin acetate were used to elevate tail skin temperature in female and male rats, respectively, for mimicking hot flush simulation. The estrogen plays an important role in body's thermoregulation.[5] Conjugated equine estrogens (CEE) consists of ester sulfate of estrogens to diminish hot flush in postmenopausal women. *A. sessilis* extract and CEE significantly reduced TST in αCGRP and leuprorelin acetate-induced tail skin temperature elevation in female and male rats, respectively. *A. sessilis* may activate estrogen-controlled functions for thermoregulation activity similar to CEE does.

The changes of core body temperature in every αCGRP-treated group were not statistically significant, because they were based on the assumption that exogenous αCGRP might be involved in the physiological control of blood flow circulating contributed to peripheral organ.[6] On the other hand, some groups of leuprorelin-treated rats that have significantly changed of core body temperature because these rats are sensitive to drugs than others.

Conclusion

We demonstrated that *A. sessilis* extract inhibited the effects of both αCGRP and leuprorelin acetate in increasing of tail skin temperature in both female and male rats, respectively. In addition, *A. sessilis* might be useful for women and men whom had experiencing in hot flushes. However, an increase in number of rats is still needed to be done in order to confirm these results in the further study, so it may be useful to develop an animal model for screening the substances that are proposed to relieve hot flushes in human.

Acknowledgements

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