Original Article

Potential of *Alternanthera philoxeroides* on Improvement of Anxiety-Like Behavior Induced By Ovariectomized Mice Model

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ABSTRACT

Background: Ovariectomy (OVX) causes estrogen deprivation and oxidative stress leads to the hippocampus cell death and affected on mood disorders such as anxiety. *Alternanthera philoxeroides* was folk used for blood tonic and neuroprotective. **Objectives:** The action of *A. philoxeroides* extract (AP) on anti-anxiety in OVX mice model was evaluated and chromatographic fingerprint of AP was analyzed. **Methods:** Mice were ovariectomized to induce estrogen deprivation which involved anxiety. AP (250 and 500 mg/kg/day) and 17β-estradiol (E_2, 1μg/kg/day) was treated. Six weeks after treatment, anxiety-like behavior was evaluated by the elevated plus maze test (EPM) and light/dark exploration test (LD). Additionally, fingerprint of AP was characterised by high performance liquid chromatography (HPLC) technique. **Results:** E_2 and AP 250 and AP 500 significantly diminished the anxiety behavior. Rutin, quercetin and kaempferol were detected in this plant. **Conclusion:** AP can manifest anti-anxiety in mice via estrogenic activity and it may action by rutin, quercetin and kaempferol.

Keywords: *Alternanthera philoxeroides*, Ovariectomy, Anxiety, Neuroprotective, Behavior, Flavonoids

INTRODUCTION

Estrogens are firmly established as regulator of mood such as anxiety and depression in humans and animals. Women suffer from anxiety disorders at twice the rate of men. As life expand of women also has risen so more women will live longer in an E_2-deprivation state.[1-3] Estrogen replacement therapy (ERT) in menopausal women is consistently reported to improve mood and learning[4]. Similar effects of E_2 on anxiety also have been reported in rodents. OVX indicate behavioral indices of anxiety increase. E_2-treated to OVX mice exhibited anxiolytic actions.[5] For safety concern, ERT come with many undesirable side effects such as thromboembolism. Anti-anxiety, benzodiazepines, have also been found many side effects such as suicidal ideation.[6] As a result, there has been increased interest in the use of herbal medicines. We are also interested in herbal plant that has not yet been evaluated scientifically. *A. philoxeroides* belongs to the family Amaranthaceae. In Ayurvedic and traditional Chinese medicine is used for antiviral, antibacterial in brain, antitumor, anti-inflammatory and postnatal complaints such as anxiety.[7-10] There is no report in antianxiety effect for this potential herb. Our studies aim to evaluate the anxiolytic effect and characterize active compounds from *A. philoxeroides*.

MATERIALS AND METHODS

Plants materials and preparation of A. philoxeroides

A. philoxeroides was collected in Khon Kaen province, Thailand. Dry plant was refluxed with ethanol at 50 °C. The combined extract was concentrated by rotary evaporator at 40 °C and was vaporized by Freeze Dryer.

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Animals

Female ICR mice (5 weeks old) were obtained from Thai National Laboratory Animal Center. The animals were in a light-controlled room with a 12 h dark/light cycle, under controlled temperature 22°C ± 2°C, humidity 45% ± 2%. This study was approved by the Animal Ethics Committee of Khon Kaen University. Ovariectomy was performed as previously described[11]. The animals were divided into five groups: (1) sham, (2) OVX, (3) OVX+1 µg/kg, i.p. 17β-estradiol, (OVX+E_2), (4) OVX+250 mg/kg, p.o. of AP (OVX+AP250), and (5) OVX+500 mg/kg, p.o. of AP (OVX+AP500). Animals were treated once daily for 6 weeks.

Behavioral analysis

Elevated Plus Maze: The testing apparatus was consisted of plus-shaped platform elevated 50 cm from the floor. Two opposite open arms have no wall and two opposite closed arm have 15 cm tall of wall. The activities of mice were recorded for 5 min. The percentage proportion of entries into open and close arms, and the percentage proportion of time spent in open and close arms were calculated[12]. *Light/Dark transition test:* The apparatus was partitioned into two chambers, which were bright and darken chamber. The activity of mice were recorded for 5 min. The time spent in dark chamber was calculated as index of anxiety.[12] *Locomotor activity test:* Y-maze task was used to determine the locomotor activity. Total arm entries were recorded.[12]

Fingerprint analysis: HPLC separation of the extracts was performed on a Hypersil ODS column and detected by UV at wavelength 254, 275 and 370 nm. Mobile phase composed of 1% formic acid containing 10%isopropanol as solvent A and acetonitrile as solvent B. Gradient elution system was operated for the analysis.

Statistical analysis

Data were expressed as the mean ± S.E.M. and were examined one-way ANOVA followed by the Tukey test for multiple comparisons among different groups. Differences of $p \leq 0.05$ were considered to be statistically significant. SigmaStat® ver. 3.5 (SYSTAT Software Inc., Richmond, CA, USA) was used.

RESULTS AND DISCUSSION

Elevated plus maze: Spontaneously rodents avoid the open, bright and elevated arms of the maze and prefer to stay in the more dark or closed arms. A reduction in anxiety is indicated by an animal's tendency to spend more time interacting with their environment through exploration of the maze that leads to their entering and spending more time in the open arms of the maze. In the elevated plus maze, OVX mice treated with E_2, AP250 and AP500 displayed significantly fewer anxiety related behaviors by increasing numbers of entries onto the open arms and reducing numbers of entries into close arms (P<0.01)(Figure 1A). All treatments increased time spent on the open arms (P<0.01) (Figure 1B) compared with OVX groups. *Light/Dark transition test:* Consistent with our results in EPM, OVX mice treated with E_2, AP250 and AP500 showed significantly less anxious behavior in the light/dark apparatus. AP250 and AP500 spent more time in light compartment and fewer spent time in dark chamber (P<0.01) in dose dependent manner (Figure 2). In order to exclude the false positive results from hopeless behavioral tests, locomotor activity test was conducted. It was exhibited that no effect of locomotor activity induced by AP and E_2 (Figure 3). Moreover, result from HPLC revealed that some flavonoids such as rutin, quercetin and kaemp-

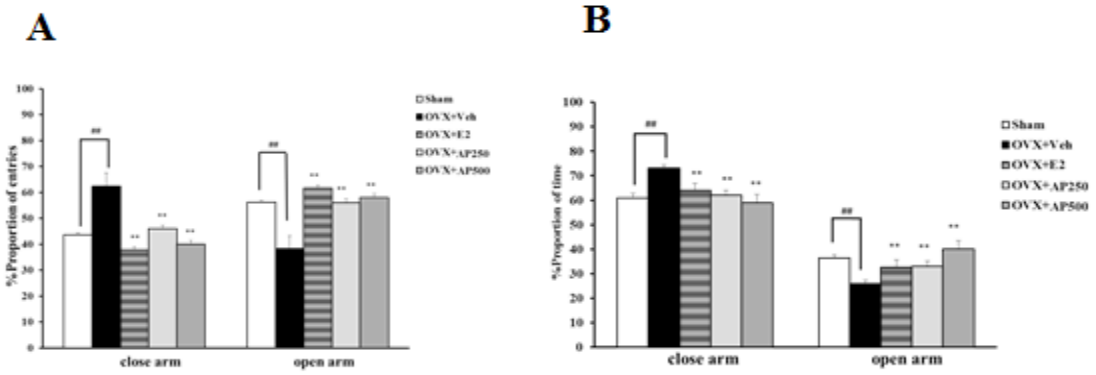

Figure 1: The effect of E_2 and *A. philoxeroides* on anxiety in sham and ovariectomized mice using elevated plus maze test. A) The percentage proportion of entries into close and open arms were displayed. B) The percentage proportion of time spent in close and open arms. Values given were the mean ± S.E.M (n=8-10). Significant ANOVA effect were represented by ##p<0.001 vs. sham, **p< 0.001 vs. ovariectomized mice.

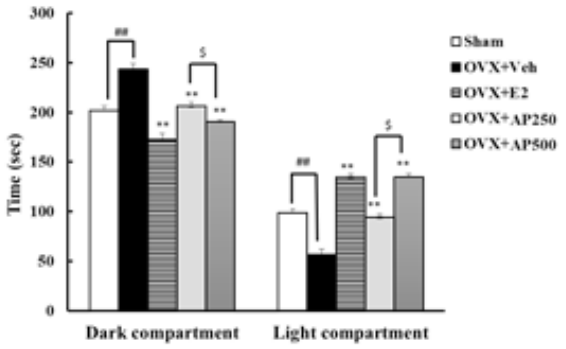

Figure 2: The effect of E_2 and *A. philoxeroides* on anxiety in sham and ovariectomized mice using light/dark transition test. The time spent in dark chamber was evaluated. Values given were the mean ± S.E.M (n=8). Significant ANOVA effect were represented by ##*p*<0.001 vs. sham, ***p*< 0.001 vs. ovariectomized mice and $p< 0.05 vs. *A. philoxeroides.*

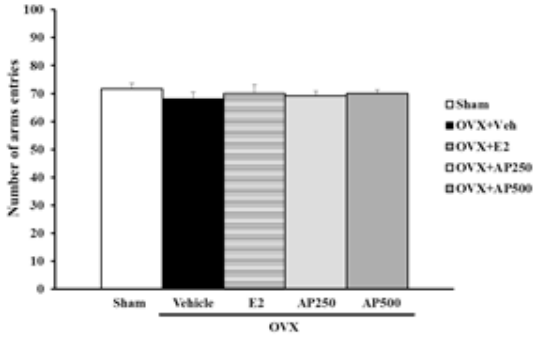

Figure 3: The effect of daily administration of E_2 and *A. philoxeroides* extract on locomotor activity in Y-maze test. Number of arms entries of each animal group was determined. Each data column represents the mean ± S.E.M (n= 8-10 in each animal group).

ferol were detected and the content was approximately 0.4 mg/g extract per each. These fingerprint chromatograms showed the specific characteristic of AP which could be used for controlling the quality of this plant. Some literatures reported the neuroprotective also anti-anxiety action of these found flavonoids.[13-14] However, it will be necessary in subsequent studies the relevant mechanism such as estrogen receptor, γ-aminobutyric acid A receptor, serotonin receptor and hypothalamic-pituitary-adrenal.

CONCLUSION

These finding suggested that *A. philoxeroides* significantly reduced anxiety behavior investigated by EPM test and LD transition test in OVX mice model. The action may affect from flavonoids such as rutin, quercetin and kaempferol that found in this plant. Thus *A. philoxeroides* has potential to develop for antianxiety especially menopausal women.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ABBREVIATION USED

AP: Alternanthera philoxeroides; E_2: 17β-Estradiol; EPM: Elevated plus maze; ERT: Estrogen replacement therapy; LD: Light/Dark transition test; OVX: Ovariectomy.

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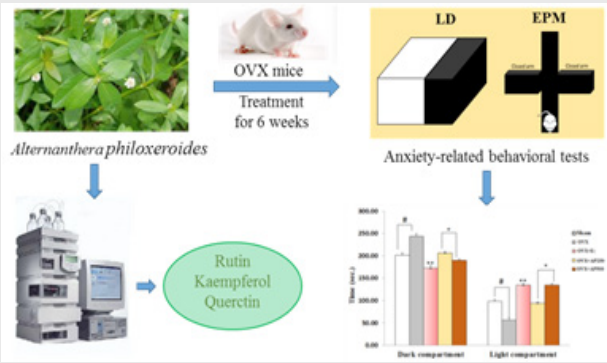
PICTORIAL ABSTRACT



SUMMARY

- Ovariectimy that mimics to estrogen deprivation or menopausal condition can induce anxiety-like behavior.
- *A. philoxeroides* showed its potential on improvement of anxiety-like behavior induced by ovariectomized mice model similar to estrogen replacement therapy by 17β-estradiol.
- Flavonoids, rutin, quercetin and kaempferol were found and they might act as phytoestrogen and/or anti-oxidative stress in brain. Therefore, the mechanism of action needs for future studies.
- From this studies, it is indicating that *A. philoxeroides* is one of herbal medicine that exhibit the potential to develop as anxiolytic drug especially for the menopausal women.

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