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*Original Article*

# Yakae-Prajamduen-Jamod recipe reduced anxiety behavior and brain oxidative damage in ovariectomized mice

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**Abstract**

Yakae-Prajamduen-Jamod (ABP), which was acquired from Chao Phraya Abhaibhubejhr Hospital, is a traditional Thai herbal formula to treat perimenopausal and menopausal symptoms. However, its mechanism remains unknown. The present study aimed to investigate the effects of ABP on ovariectomy-induced anxiety and oxidative stress in mice. Ovariectomized mice exhibited not only anxiety in elevated plus maze and mirror chamber tests, but also brain oxidative stress when compared with control. Ovariectomy-induced behavioral and neurochemical alterations were attenuated by ABP treatment (500 mg/kg/day). We also analyzed antioxidant activities of ABP using DPPH and ABTS assays. ABP showed radical scavenging activity with $IC_{50}$ 58.59 and 44.99 µg/mL, respectively. Total phenolic and flavonoid contents were 110.53±1.50 mg GAE and 152.25±0.5 mg rutin equivalents per gram extract, respectively. These findings suggest that ABP attenuated the ovariectomy-induced anxiety and oxidative brain injury via antioxidative properties. Therefore, ABP is an alternative choice of anxiety disorder in the menopausal transition.

**Keywords:** ABP, menopause, ovariectomy, anxiety, lipid peroxidation

## 1. Introduction

Recently, Thailand has one of the most rapid rates of ageing populations among the developing countries in the world (World Health Organization [WHO], 2017). This report coincides with the increase in the number of menopausal woman in Thailand. The unstable estrogen level during menopausal transition results in an unbalance of many systems in the body such as the central nervous system (CNS), cardiovascular system, endocrine system, immune system, reproductive system, renal system, and musculoskeletal system (Prossnitz & Barton, 2011). Particularly in the central nervous system, estrogen modulates various functions such as learning, memory, emotions, motivation, and sensory perception (Brinton, Yao, Yin, Mack, & Cadenas, 2015).

Normally, free radicals are generated in our body. However, the defensive mechanisms against oxidation help prevent damage from too many free radicals. Oxidative stress

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is defined as the imbalance of free radicals and antioxidant defense mechanisms. The deprivation of estrogen can destroy the balancing of the antioxidant system which leads to an overproduction of free radicals and high oxidative stress (Al-Rahbi, Zakaria, Othman, Hassan, & Ahmad, 2014). Oxidative stress is associated with neurological disease and aging by damage to the DNA, proteins, and lipids. Evidence suggests that disruption of the CNS results in various mental illnesses in menopausal women and an imbalance of the CNS redox state may influence such pathologies. Oxidative stress was reported to involve anxiety-related disorders (Hassan, Silva, Mohammadzai, da Rocha, & Landeira-Fernandez, 2014).

Yakae-Prajamduen-Jamod (ABP) is a traditional Thai herbal formula obtained from Chao Phraya Abhaibhubejhr Hospital. ABP is widely used to treat perimenopausal and menopausal symptoms in Thailand. The ABP capsule is prescribed to patients by a Thai traditional doctor in Chao Phraya Abhaibhubejhr Hospital, which is a central public hospital in Prachinburi, Thailand. ABP consists of 22 medicinal herbs: *Cassia garrettiana* Craib; *Cassia siamea* (Lam.) H.S.Irwin et Barneby; *Derris scandens* (Roxb.) Benth; *Mesua ferrea* L.; *Mammea siamensis* Kosterm; *Coriandrum sativum* L.; *Myristica fragrans* Houtt.; *Amomum testaceum* Ridl.; *Cyperus rotundus* Linn; *Nigella sativa* L.; *Piper ribesoides* Wall.; *Aucklandia lappa* DC.; *Artemisia annua* L.; Radix *Angelica sinensis*; *Dracaena loureiri* Gagnep; *Tarenna hoaensis* Pitard; *Bridelia ovata* Decne.; *Carthamus tinctorius* L.; *Terminalia chebula* Retz. var. chebula.; *Phyllanthus emblica* L.; *Terminalia arjuna* Roxb.; and *Aloe vera* (L.) Burm.f.. The medicinal efficacy of ABP may be due to the estrogenic activity and antioxidant activity of various herbs in the formula. However, the effect of ABP on ovariectomy-induced anxiety and oxidative brain damage has not been reported. In addition, data indicated that ovariectomized rats showed behavioral alterations, including anxiety-like profiles associated with oxidative damage in different CNS areas (Hassan, Silva, Mohammadzai, da Rocha, & Landeira-Fernandez, 2014). Thus, the present study aimed to investigate the antioxidant property of this Thai herbal formula and explore the effects of ABP on ovariectomized mice by behavioral and oxidative parameters in different CNS areas of ovariectomized mice.

## 2. Materials and Methods

### 2.1 Thai medicine formula for menopause

A traditional Thai medicine formula for menopause (ABP) was received from Pho-Ngern-Abhaibhubejhr Osot, Chao Phraya Abhaibhubejhr Hospital, Prachinburi, Thailand, as a commercial product (ABP powder in capsule). It consists of 22 dry medicinal herbs (Table 1).

### 2.2 Preparation of the ethanolic extract

The ABP powder was taken from the capsules and extracted three times by maceration methods with 95% ethanol at room temperature. The ethanol extracts were combined, filtered, and evaporated at 60 ºC. Two hundred grams of ABP powder yielded 25.21 g of ethanolic extract. The extract was kept at −20 °C until the experiment.

### 2.3 Measurement of antioxidant capacity

The Sunrise™ microplate reader (Tecan) was used to measure the absorbance of DPPH radical scavenging capacity and ABTS radical scavenging capacity. The chemical reagent for DPPH was 1,1-diphenyl-2-picrylhydrazyl (Trolox, Sigma-Aldrich, MO, USA). The chemical reagents for ABTS were 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid, diammonium chloride, potassium peroxodisulfate (Trolox, Sigma-Aldrich, MO, USA). All reagents used in this study

Table 1. Components of a traditional Thai medicine formula (ABP) for menopause.

| No. | Botanical name | Thai name | Part used | Ratio in ABP capsule |
|---|---|---|---|---|
| 1 | *Senna garrettiana* (Craib) H.S.Irwin & Barneby | Sa-mae-sarn | heartwood | 1 |
| 2 | *Senna siamea* (Lam.) H.S.Irwin et Barneby | Khi-lek | heartwood | 1 |
| 3 | *Derris scandens* (Roxb.) Benth | Tao-wal-piang | stem | 1 |
| 4 | *Mesua ferrea* L. | Boon-nak | flower | 1 |
| 5 | *Mammea siamensis* Kosterm | Sa-ra-pee | flower | 1 |
| 6 | *Coriandrum sativum* L. | Louk-pak-chee | fruits | 1 |
| 7 | *Myristica fragrans* Houtt. | Louk-chan | fruits | 1 |
| 8 | *Amomum testaceum* Ridl. | Kra-waan | fruits | 1 |
| 9 | *Cyperus rotundus* Linn | Haek-moo | tuber | 1 |
| 10 | *Nigella sativa* L. | Tian-dum | seed | 1 |
| 11 | *Piper ribesoides* Wall. | Sa-karn | stem | 1 |
| 12 | *Saussurea lappa* Clarke | Koth-kra-douk | root | 1 |
| 13 | *Artemisia annua* L. | Koth-chula-lumpa | whole | 1 |
| 14 | *Angelica sinensis* | Koth-chiang | root | 1 |
| 15 | *Dracaena loureiri* Gagnep | Chan-daeng | heartwood | 1.5 |
| 16 | *Tarenna hoaensis* Pitard | Chan-khao | heartwood | 1.5 |
| 17 | *Bridelia ovata* Decne. | Ma-kaa | leaves | 1.5 |
| 18 | *Carthamus tinctorius* L. | Kham-foi | flower | 1.5 |
| 19 | *Terminalia chebula* Retz. var. chebula. | Sa-mor-thai | fruits | 1.5 |
| 20 | *Phyllanthus emblica* L. | Ma-kham-pom | fruits | 1.5 |
| 21 | *Terminalia arjuna* Roxb. | Sa-mor-ted | fruits | 1.5 |
| 22 | *Aloe vera* (L.) Burm.f. | Yaa-dum | resin | 1.5 |

were analytical grade. The DPPH solution in ethanol was prepared and mixed with the ABP ethanolic extract. Absorbance was measured at 520 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration (Kedare & Singh, 2011). The ABTS+• radical was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1:1, v/v) and leaving the mixture for 4–16 h until the reaction was complete and the absorbance was stable. The ABTS+• solution was diluted with ethanol to an absorbance value of 0.7±0.02 at 734 nm for measurements. The photometric assay was conducted on 990 µL ABTS+• solution and 10 µL of ABP tested solution and incubated at room temperature for 15 min. The absorbance was measured immediately at 734 nm. The antioxidative activity of the tested samples was calculated as percentage inhibition of radical cation absorbance. $IC_{50}$ values were reported and compared with Trolox which was the reference antioxidant (Re *et al.*, 1999).

### 2.4. Determination of total phenolic content (TPC)

The total phenolic content was determined by Folin–Ciocalteu's colorimetric assay modified by Todaro *et al.* (Todaro *et al.*, 2017). Briefly, 20 µL of extract solution, 100 µL of 10% Folin–Ciocalteu reagent, and 80 µL of 7% sodium carbonate (Sigma-Aldrich, MO, USA) were mixed and keep at room temperature for 30 min. The absorbance was measured at room temperature at 760 nm using an EnSight Multimode Plate Reader® (PerkinElmer, MA, USA). The total phenolic content was represented as mg of gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g) using a standard curve with 0.1–1000 mg/L gallic acid (Sigma-Aldrich, MO, USA). All the determinations were performed in triplicate.

### 2.5 Determination of total flavonoid content (TFC)

Total flavonoid content of the ABP ethanolic extract was determined by the aluminum chloride colorimetric method with slight modification (Woisky & Salatino, 1998). One mg of ethanolic extract was dissolved with 1 mL of 80% ethanol. An amount of 20 µL of extract solution, 15 µL of 2.5% aluminum chloride, 20 µL of 10% acetate (Sigma-Aldrich, MO, USA), and 145 µL of distilled water were mixed and incubated at room temperature for 15 min. Absorbance was measured at 430 nm using an EnSight Multimode Plate Reader® (PerkinElmer, MA, USA). Flavonoid content was expressed as rutin equivalents in mg per gram of dry extract using a standard curve with 0.1–1000 mg/L rutin (Sigma-Aldrich, MO, USA). All determinations were performed in triplicate.

### 2.6 Experimental animals

Seventy-five 4-week old female ICR mice that weighed from 20 to 30 g were obtained from the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand). The mice were housed on wood chip bedding in cages and given food and water *ad libitum*. The housing conditions were 12-h dark and light cycles (light 06:00–18:00) under temperature controlled conditions (22±2 ºC) and constant humidity (45±2%). All behavioral experiments were performed from 08:00 to 17:00 hours and each animal was used once. The experiment protocol was approved by the Animal Ethics Committee of Khon Kaen University (ACUC-KKU-54/2559, Reference No. 0514.1.75/60).

### 2.7 Surgical operation and treatments

Ovariectomy (OVX) was conducted as previously described (Monthakantirat *et al.*, 2014). The ovary and oviduct were removed from the mice in the OVX group. The sham control group was ovary-intact mice that underwent operations. Three days after the operations, the ovariectomized mice were divided into four groups of 10 to 15 animals each. The treatments were: 1) vehicle (0.5% sodium carboxymethyl cellulose [SCMC]); 2) 1 µg/kg 17β-estradiol; 3) ABP 100 mg/kg; and 4) ABP 500 mg/kg. The sham control group received the vehicle. The 17β-estradiol (Sigma-Aldrich Co. LLC.) was suspended in corn oil. ABP was suspended in 0.5% SCMC as the vehicle. The vehicle, 17β-estradiol, and ABP were administered orally once daily between 07:00 and 09:00 hours for eight weeks. The behavioral tests were conducted at one hour after treatment.

### 2.8 Behavioral tests

#### 2.8.1 Elevated plus maze test (EPM)

The elevated plus maze (EPM) test is used to evaluate anxiety-related behavior in rodent models with CNS disorders. The EPM apparatus consists of two open arms and two closed arms (30x5 cm) elevated to 35 cm from the floor. The two closed arms with 15 cm-high black walls are located at the opposite side (Figure 1). All treatments were performed one hour prior to the mice being placed at the intersection between arms (5x5 cm). A mouse was placed to face an opened arm and allowed free exploration for 5 min. The number of entries and the time spent in both the closed and open arms were recorded (Pellow, Chopin, File, & Briley, 1985). The %proportion of the number of entries and time spend in each closed and open arm were calculated using this equation:

$$\%\text{proportion of time spent in opened arm} = \text{ x } 100.$$

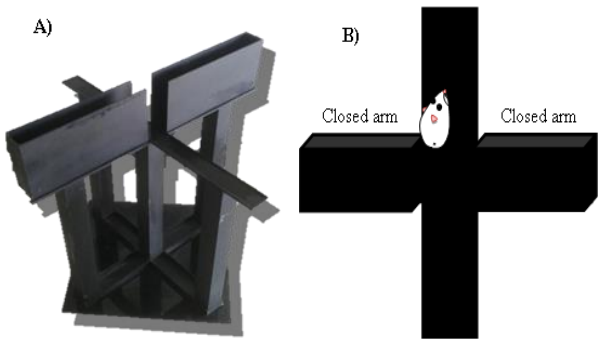


Figure 1. Elevated plus maze (EPM) apparatus: A) Side view; B) Top view.

### 2.8.2 Mirror chamber test (MC)

The mirror chamber (MC) test demonstrates approach-avoidance conflict behavior when faced with a mirror image (Toubas, Abla, Cao, Logan, & Seale, 1990). The mirror chamber apparatus consisted of two open-top connected boxes (35x35x30 cm). The larger box contained walls with mirrors. The smaller box had a black wall without a mirror (Figure 2). The mice were treated one hour prior to being placed in the corner of the larger box with the mirror and were allowed free exploration for 5 min. The average time spent on at the dark sides was recorded (Lamberty, 1998).

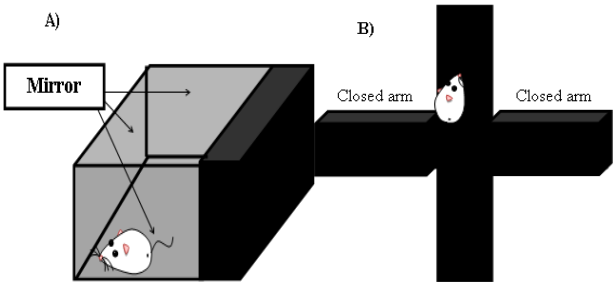


Figure 2. Mirror chamber (MC) apparatus: (A) Side view; (B) Top view.

### 2.9 The dissection of brain tissue

The animals were sacrificed by decapitation. Their hippocampus and frontal cortex were resected and kept at −80 °C until use.

### 2.10 Determination of brain lipid peroxidation (TBARs assay)

Lipid peroxidation in the brain (hippocampus and frontal cortex) was demonstrated according to Matsumoto *et al*. (Matsumoto *et al.,* 1999). The hippocampus and frontal cortex were weighed and homogenized in 10 volumes of phosphate buffer (5 mM, pH 7.4). The homogenized brain was mixed with trichloroacetic acid and centrifuged at 8000*g* for 10 min at 4 °C. The supernatant was collected and incubated with 0.8% (w/v) 2-thiobarbituric acid at 100 °C for 15 min. The intensity of pink pigment formed from MDA-TBA condensation indicated the extent of lipid peroxidation. The complexes were determined by UV-visible spectrophotometer at 532 nm. The level of malondialdehyde (MDA) was used as the standard. The protein contents in the hippocampus homogenates were measured by the Bradford method. (Chatuphonprasert *et al.,* 2013) The results are represented as nmol of MDA/mg protein. (Grotto *et al.,* 2009)

### 2.11 Statistical analysis

All data are expressed as mean±SEM. Multiple comparisons among the different groups were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. A significant difference was considered at $P<0.05$. SigmaStat® version 3.5 (SYSTAT Software Inc., Richmond, CA, USA) was used for the analysis.

## 3. Results

### 3.1 Antioxidant capacity of ABP

The radical scavenging capacity of ABP was evaluated by the DPPH and ABTS assay methods. The value expressed as $IC_{50}$ was defined as the concentration of the test compound that inhibited 50% of the DPPH and ABTS radicals. Trolox was used as the standard antioxidant in this assay. The $IC_{50}$ values for radical scavenging capacity are given in Tables 2 and 3. The ethanolic extract of ABP in this study had $IC_{50}$ values for radical scavenging capacity from the DPPH and ABTS assays of 58.59 and 44.99 µg/mL, respectively.

Table 2. Radical scavenging capacity of ABP evaluated by DPPH assay.

| Compound | Concentration | %Inhibition (n=3) (mean±SEM) | $IC_{50}$ |
|---|---|---|---|
| Trolox | 10 µM | 11.93±4.43 | |
| | 20 µM | 30.22±13.87 | |
| | 30 µM | 55.01±2.78 | 27.72 µM or |
| | 40 µM | 80.42±1.71 | 6.94 µg/mL |
| | 50 µM | 97.73±0.72 | |
| ABP | 30 µg/mL | 15.34±2.11 | |
| | 45 µg/mL | 25.10±2.35 | |
| | 60 µg/mL | 41.06±1.76 | 58.59 µg/mL |
| | 75 µg/mL | 55.17±2.68 | |
| | 90 µg/mL | 64.36±2.06 | |

Table 3. Radical scavenging capacity of ABP evaluated by the ABTS assay.

| Compound | Concentration | %Inhibition (n=4) (mean±SEM) | $IC_{50}$ |
|---|---|---|---|
| Trolox | 10 µM | 9.71±0.42 | |
| | 20 µM | 24.56±1.59 | |
| | 30 µM | 35.05±0.44 | 40.78 µM or |
| | 40 µM | 49.35±0.42 | 10.21 µg/mL |
| | 50 µM | 61.82±0.08 | |
| ABP | 15 µg/mL | 23.18±1.72 | |
| | 30 µg/mL | 37.34±0.68 | |
| | 45 µg/mL | 52.10±1.63 | 44.99 µg/mL |
| | 60 µg/mL | 63.97±1.72 | |
| | 75 µg/mL | 73.46±2.03 | |

### 3.2 Total phenolic and total flavonoid contents

Total phenolic content and total flavonoid content were determined from the calibration curves of gallic acid (($y = 2.5424x + 0.0977$, $R^2 = 0.9967$)), and rutin ($y = 0.9378x + 0.0042$, $R^2 = 0.9999$), respectively. TPC was found to be 110.53±1.50 mg GAE/g extract. It was also observed that the ABP extract was rich in flavonoids. The total flavonoid content was 152.25±0.5 mg rutin equivalents/g extract.

### 3.3 Effects of ABP on anxiety-like behavior in ovariectomized mice model

The EPM and MC tests were performed to evaluate the anxiolytic effect of ABP. The EPM has closed arms which represent safety zones for an anxious mouse. The results are shown in Figures 3 and 4. The EPM test also revealed OVX-induced anxiety-like behavior in the mice. The %proportion of entries in the open arm of the sham control group was significantly greater (P<0.05), while the vehicle-treated ovariectomized mice had a smaller %proportion of entries into the open arm which indicated OVX-induced anxiety behavior. On the other hand, the ovariectomized animals that received the 17β-estradiol (1 μg/kg) and supplementation of ABP (500 mg/kg) for eight weeks exhibited significant improvement in the performance. There were statistically significant effects of estrogen deprivation in the ovariectomized mice in the anxiety model (P<0.001) demonstrated by the increased %proportion of time spent in the closed arm. The 17β-estradiol and ABP (500 mg/kg)-treated ovariectomized groups showed a significant reduction of time spent in the dark compartment (P<0.001) compared to the vehicle-treated ovariectomized group.

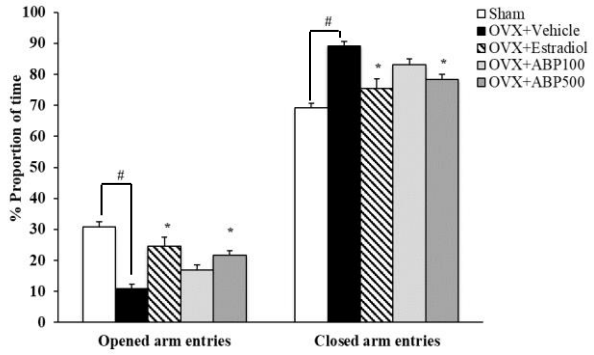


Figure 3. Effects of ABP on OVX-induced anxiety-like behavior in the elevated plus maze test as the %proportion of time. The value given in each column represents the mean±SEM (n=10–12). A significant ANOVA effect is represent by * P<0.05 vs. the ovariectomized groups. # P<0.001 vs. the sham control group.

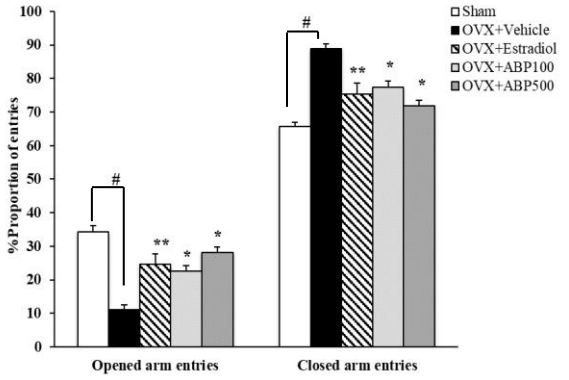


Figure 4. Effects of ABP on OVX-induced anxiety-like behavior in the elevated plus maze test as the %proportion of entries. The value given in each column represents the mean±SEM (n=10–12). Significant ANOVA effects are represent by * P<0.05 and ** P<0.001 vs. the ovariectomized groups. # P<0.001 vs. the sham control group.

The anxiolytic effect of ABP was also conducted in the MC test to confirm the anti-stress activity of the ABP. The results of experiment are shown in Figure 5. The sham control group spent significantly more time in the dark compartment while the vehicle treated showed less time in the dark compartment which indicated OVX-induced anxiety behaviors. The 17β-estradiol and ABP (100 and 500 mg/kg)-treated groups showed a significant decrease in time spent in the dark compartment (P<0.001) compared to the vehicle-treated ovariectomized group.

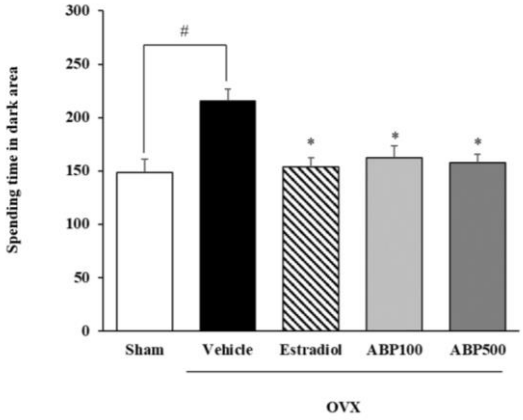


Figure 5. Effects of ABP on OVX-induced anxiety-like behavior in the mirror chamber test. The values given the columns represent the mean±SEM (n=10–12). Significant ANOVA effects are represented by * P<0.05 and ** P<0.001 vs. the ovariectomized groups. # P<0.001 vs. the sham control group.

### 3.4 Brain lipid peroxidation (TBARs assay)

Lipid peroxidation is a source of free radicals which cause damage to lipids in the brain (Lefevre *et al.,* 1998). Malondialdehyde (MDA) is a secondary product of lipid peroxidation. MDA was estimated by measuring the thiobarbituric acid-reactive substance (TBARS) levels and the results are shown in Figures 6A and 6B. There was a significant effect of estrogen deprivation in the ovariectomized mice on lipid peroxidation in the brain (P<0.001) indicated by increased MDA levels in the hippocampus and frontal cortex compared with the sham control mice. The ovariectimized mice treated with 17β-estradiol- and ABP (500 mg/kg) showed a significant decrease in the MDA levels in both brain areas compared with the ovariectomized mice.

## 4. Discussion

The present study aimed to investigate the effects of ABP on stress related-behaviors and oxidative stress in ovariectomized mice model of menopause and clarify the possible mechanism. The amounts of active constituents in ABP were also determined by the HPLC method. Our results demonstrated that ovariectomy induced anxiety-like behavior. Moreover, ovariectomized rats showed increased TBARS levels in the frontal cortex and hippocampal areas. ABP treatment ameliorated anxiety behaviors and brain oxidative damage caused by OVX.

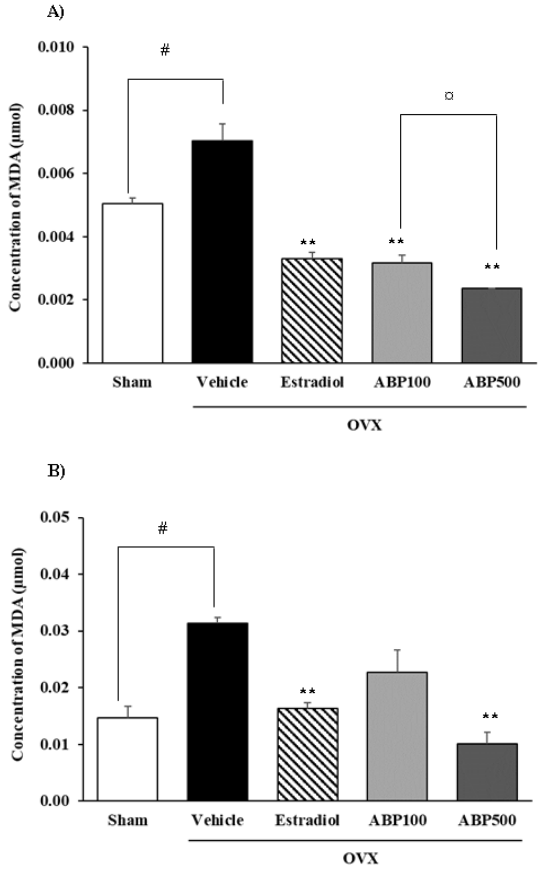


Figure 6. Effects of ABP on lipid peroxidation in the (A) hippocampus and (B) frontal cortex. Each column represents the mean±SEM (n=3–5). # P<0.01 vs. the sham control group. ** P<0.001 vs. the ovariectomized groups. ¤ P<0.05 vs. dose dependent.

It is well known that the OVX model mimics many alterations observed in menopausal women, such as atrophy of the uterus, cognitive dysfunctions, anxiety, oxidative stress, and strongly suggests that estrogen deprivation is the important factor that causes menopause-related dysfunctions (Monthakantirat *et al.,* 2014), (Chaves *et al.,* 2009). In particular, depression and anxiety are among the main psychological symptoms related to sexual hormone deprivation in postmenopausal women (Chaves *et al.,* 2009). These disorders emerge together due to a pro-oxidant status caused by estrogen deficiency, whereas estrogen itself can act as an antioxidant. The important part of estrogen for antioxidant effect is the C-3 hydroxyl on the phenolic A-ring (Prokai *et al.,* 2003). Physiological circulating levels of estrogen in the blood are associated with improved antioxidant status and also a lower incidence of mood disorders and oxidative stress-associated neurodegenerative disorders (Chakrabarti *et al.,* 2014).

In the present work, the anxiety-like behavior was observed in ovariectomized mice by approach-avoidance behavior when confronted with a mirror in MC test and increased proportion of time or entries in the closed arm of the EPM test. The EPM is a pharmacologically validated assay of anxiety-like behaviors in rodents and it is based on the natural preference of rodents for enclosed versus exposed spaces. A high percentage of time spent in the closed arms indicates the primary measure of anxiety-like behavior and is statistically decreased by administration of clinically effective anxiolytic compounds such as benzodiazepines (Rodgers & Dalvi, 1997). The MC paradigm is based on the principle that many species show approach–avoidance conflict behavior when mirrors are placed in their environment. A distortion in the appearance of a readily accessible environment via a compartment constructed of mirrored glass produces an anxiogenic state that was quantitatively/qualitatively. These are non-invasive measures of anxiety-like behavior (Toubas *et al.,* 1990). Treatment with ABP (500 mg/kg) showed significant attenuated anxiety-like behavior in both tests as well as estrogen replacement therapy (P<0.001).

This study also examined the effect of OVX-induced oxidative brain damage. As a result, oxidative stress can alter neuronal function, neurotransmission, neurogenesis, and overall brain activity which have been implicated in anxiety disorders, depression, and high anxiety levels (Bouayed *et al.,* 2009). The findings establish a link between oxidative stress and pathological anxiety. Many researchers have correlated oxidative stress and anxiety-like behavior. For example, OVX causes oxidative stress in different CNS structures owing to depletion of antioxidant content leading to an anxiogenic profile (Behr *et al.,* 2012). Da Silva Morrone and coworker observed elevated carbonylated protein levels in the hippocampus and striatum, increased TBARS levels in the striatum and frontal cortex, and also a decrease in thiol content in striatal non-protein fractions which indicated a pro-oxidant effect in different brain structures after estrogen deprivation and these alterations were related to anxiety behaviors. ABP-treated ovariectomized mice exhibited a reduction in TBARS levels in the frontal cortex and hippocampus which suggested a neuroprotective effect of ABP. Moreover, ABP extract also showed potent radical scavenging activities *in vitro* demonstrated by the ABTS and DPPH assays.

In addition, it was also observed that the ABP extracts are rich in phenolic and flavonoid compounds. Much evidence reported that these chemical compounds possess strong antioxidant activity. The phenolic compounds are very important constituents in medicinal plants because of their scavenging ability due to their hydroxyl groups. The planar structure of flavonoids, number and position of their hydroxyl groups, as well as the presence of the C2−C3 double bond, are important for metal chelation, radical scavenging activity, and the inhibition of free radical producing enzymes (Badhani, Sharma, & Kakkar, 2015), (Azevedo *et al.,* 2013), (Akinboro, Mohamed, Asmawi, Sulaiman, & Sofiman, 2011) and anxiolytic activity. On the other hand, phenolic compounds and flavonoids also exhibit anxiolytic activity. Treatment of 10 mg/kg of gallic acid for 10 days significantly showed antianxiety-like activity in stressed mice (Dhingra, Chhillar, & Gupta, 2012), and 300 mg/kg of rutin for 9 days exhibited a significant anxiolytic effect in rat (Hernandez-Leon, Gonzalez-Trujano, & Fernandez-Guasti, 2017).

Taken together, our findings suggest that ovariectimized mice treated with ABP for 8 weeks showed decreased anxiety-like behavior and attenuation of oxidative stress in the hippocampus and frontal cortex. The plausible mechanisms may be related to the active constituents in ABP

such as gallic acid, and rutin which are strong antioxidants and anxiolytic agents. This is the first scientific-evidence that showed the benefits of ABP oral administration against anxiety-like behavior and brain oxidative damage induced by hormone deprivation. Our results support the potential use of ABP as a natural alternative to hormone replacement therapy.

## 5. Conclusions

In conclusion, a traditional Thai herbal formula for menopause, ABP, attenuated stress related anxiety disorder comparable to estrogen in ovariectomized mice. This effect is possibly associated with improvement of antioxidant activity against brain oxidative stress which was observed in the hippocampus and frontal cortex. Therefore, ABP may offer an alternative therapeutics choice of stress-related anxiety disorder observed in menopausal transition.

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