Effect of Inhaling Bergamot Oil on Depression-Related Behaviors in Chronic Stressed Rats

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Background: *Bergamot essential oil (BEO) possesses sedation and anxiolytic properties similar to diazepam. After long period of exposure to stressors, including restrained stress, depressive-like behavior can be produced. BEO has been suggested to reduce depression. However, there is no scientific evidence supporting this property.*
Objective: *To investigate the effect of BEO in chronic stressed rats on: 1) behavior related depressive disorder, 2) hypothalamic pituitary adrenal (HPA) axis response, and iii) brain-derived neurotrophic factor (BDNF) protein levels in hippocampus.*
Material and Method: *Male Wistar rats, weighing 200 to 250 g, were induced chronic restrained stress 15 minutes daily for two weeks. For the next two weeks, these rats were divided into four groups, control-i.p., fluoxetine-i.p., control-inhale, and BEO-inhale. Fluoxetine (10 mg/kg i.p.) or saline was intraperitoneally administered daily while 2.5% BEO or saline was inhaled daily. At the end of the treatment, rats were assessed for depressive-like behavior using the forced swimming test (FST). After the behavioral test, the animals were immediately decapitated and trunk blood samples were collected for the measurement of corticosterone and adrenocorticotropic hormone (ACTH) levels and hippocampus was dissected and stored in a freezer at -80 °C until assay for BDNF protein.*
Results: *BEO and fluoxetine significantly decreased the immobility time in the FST ($p<0.05$). Fluoxetine tended to decrease serum corticosterone and significantly ($p<0.05$) decreased serum ACTH while BEO had no effect on these two stress hormones. For BDNF protein determination, neither BEO nor fluoxetine had any effect on BDNF protein levels in hippocampus compared to their controls.*
Conclusion: *The inhalation of BEO decrease behavior related depressive disorder similar to fluoxetine but has no effect on HPA axis response and BDNF protein levels in chronic restrained stress.*

Keywords: *Depression, Bergamot oil, Force swimming test, HPA axis, BDNF*

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Depression is a chronic disease that causes a major public health problem. The World Health Organization (WHO) ranked depression as the fourth greatest disease burden worldwide, resulting in disability and premature death among people aged 18 to 44 years. It is expected to be among the two main causes of disability, together with heart disease by the year 2020[1]. Depression is the most important risk factor for suicide[2]. Patients affected by chronic illness have a higher risk for depression while depressed patients have significantly higher rates of other chronic medical diseases, including cardiac diseases, diabetes, and cancer[3].

Although synthetic antidepressants have been widely used to treat depressive disorders, the poor tolerability and side effects of antidepressants limit their real efficacy and acceptance especially by many patients who often discontinue their therapy and seek alternative antidepressants[4]. Aromatherapy, using essential oil for the treatment, has a long history of use in supporting health and many diseases including depression[5]. For example, *Lavandula angustifilia* decreased mean depression score, the common index of depression[6]. An animal study has showed that clove oil reduced immobility in the force swimming test (FST), suggesting its antidepressant-like activity[7].

Bergamot, *Citrus bergamia*, Risso, has been used in aromatherapy to reduce stress, anxiety, and depressive disorders[8]. However, there are only a few systematic investigations on these biological properties. A previous human study showed that a mixture of lavender and bergamot demonstrated a

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relaxing effect, leading to the treatment of depression and anxiety[9]. In the cellular study, BEO has been shown to minimize neuronal damage caused by excitotoxic stimuli[10]. Moreover, the animal study showed that inhalation of BEO attenuated the activity of hypothalamic pituitary adrenal (HPA) axis by reducing the corticosterone (one of major hormones in the HPA axis of rats) response to the stress, exposure to the elevated plus-maze[11].

Several studies have demonstrated that chronic stress, one of risk factors for depression, induces significant changes in the HPA axis and brain-derived neurotrophic factor (BDNF) protein[12,13]. Hyperactivity of HPA axis resulted from chronic stress is associated with depression. The elevation of plasma adrenocorticotropic hormone (ACTH) and corticosterone, indicators of HPA axis activity, was observed following chronic stress. Expression of BDNF, playing a major role in the regulation of the growth, survival, and differentiation of neurons, has decreased in the chronic stress, while antidepressant treatment increases expression of BDNF[14]. Fluoxetine, a selective serotonin reuptake inhibitor, is one of the most commonly prescribed antidepressant for the treatment of depression. Therefore, many studies have selected this drug to investigate the efficiency of novel antidepressants compared with fluoxetine. Several animal studies have demonstrated that fluoxetine ameliorated depressive-like behavior in stress-induced depression may be related to the up-regulation of BDNF levels in the hippocampus and normalizing hyperactivity in the HPA axis[12,13].

Thus, the main objective of the present study was to compare behavioral, hormonal and molecular effect induced by chronic administration of BEO and fluoxetine to rats with chronic restrained stress. The behavioral effects of BEO and fluoxetine were evaluated in the FST a valid behavior model widely used for screening new antidepressants. Additionally, ACTH and corticosterone serum levels were assessed to explore the chronic effect of BEO on the HPA axis. In order to investigate the mechanism underlying the antidepressant-like action of BEO, BDNF protein level was also measured from hippocampus of rats.

Material and Method

Animals

Male Wistar rats weighing 200 to 250 g (National Laboratory Animal Centre, Mahidol University, Bangkok, Thailand) were used for all experiments. The rats were housed, four per cage, at constant temperature (22±0.5°C) and humidity under a 24-hour light/dark cycle with free access to food pellets (National Laboratory Animal Center, Thailand) and tap water for the duration of the study. The rats, which were used after an adaptation period of one week, were inexperienced to the FST and naive to exposure to essential oil or drugs.

All procedures were carried out in accordance with the Animal Ethics Committee of Srinakharinwirot University (under license No. 8/2552), which is in compliance with the International Guiding Principles for Biomedical Research Involving Animals provided by the National Research Council of Thailand.

Chemicals

Fluoxetine was purchased from Sigma Chemical (USA) and diluted with 0.9% saline solution. BEO, extracted from Kaffir Lime peels and without bergapten, was purchased from Make Scent Limited (Bangkok, Thailand). It was extracted from the washed, chopped, dried, and soaked rind of bergamot fruit and rootlets by steam distillation and yields about 0.5%. The BEO samples were analyzed using Gas Chromatography (GC) and confirmed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify the major compounds (10.86% linalool, 18.57% linalyl acetate and 26.70% limonene). A voucher sample has been deposited at Thailand Institute of Scientific and Technological Research. Emulsions (water/oil) of BEO at 2.5% (w/w) were freshly prepared just before the start of the experiments. Control inhalations were performed using 0.9% saline solution.

Experimental design

The rats were induced chronic restrained stress 15 minutes daily for two weeks. For the next two weeks, these rats were divided into four groups, control-i.p., fluoxetine-i.p., control-inhale, and BEO-inhale. Fluoxetine (10 mg/kg, i.p.) or saline was intraperitoneally administered daily. While 2.5% BEO or saline was inhaled (INH) daily. The procedures of restrain stress and inhalation were performed between 09:00 and 12:00 daily. At the end of the treatment, rats were assessed for depressive-like behavior using the FST. After the behavioral test, the animals were immediately decapitated and trunk blood samples were taken and put into tubes and then centrifuged (1,500 xg for five minutes at 4°C). Serum was collected and subsequently stored at -20°C until required for the corticosterone and ACTH assay. Hippocampus was dissected and stored in a freezer at -80°C until assay for BDNF protein

(Fig. 1).

Inhalation of essential oils

For the experiments involving inhalation of essential oils, the inhalation apparatus consisted of a square transparent Plexiglas chamber (36x30x29 cm) adapted from de Almeida et al[15]. The floor of the apparatus was made of a stainless steel grid. The apparatus was made of acrylic fiber used to made walls, front and back walls with four holes into which cotton soaked with 0.9% saline or BEO was inserted. The top wall, containing 30 small holes for ventilation, could be opened to allow the rat in the chamber. Rats were placed individually into the chamber seven minutes daily for two weeks.

Restrained stress

The restraint was performed in a transparent Plexiglas tube adjustable to animal size (15.5±2.5 cm long and 6.3 cm diameter) with ample holes for ventilation. Rats were exposed to 15 minutes stress session daily for two weeks. This procedure is considered to be psychological stressor because it does not produce pain or direct physical insult[16]. At the end of each daily stress, the animals were returned to their cages.

Forced swimming test (FST)

The procedure used for the FST was performed as previously described[17]. The forced swimming tank consisted of a clear, cylindrical tank (40 cm height x 18 cm diameter) filled with a water (25°C) depth of 30 cm. The FST was comprised of a two-day protocol. On the first day of the test, the rats were individually placed into forced swimming tank for 15 minutes. On the second day, each rat was left in the swimming tank for six minutes. After the first two minutes, the total duration of immobility (floating and movements to keep the head out of the water only) was measured during a 4-minute test. The duration of immobility was regarded to be an index of the depressive-like state of the animals. This behavior was recorded with a videotaped for the subsequent analysis.

Determination of serum corticosterone and ACTH levels

The levels of serum corticosterone and ACTH were determined in rat serum under competitive enzyme immunoassay (EIA) principle by using Corticosterone EIA and ACTH EIA kits (Phoenix Pharmaceuticals, Inc; USA), respectively. The assays were performed according to the manufacturer's instructions. Levels of both hormones were quantified on a microplate reader (BioTek Instruments, Inc; USA). A standard curve of known concentrations of each protein was established and the unknown concentrations of the protein were determined by extrapolation to the appropriate protein standard curve. All samples from this study were

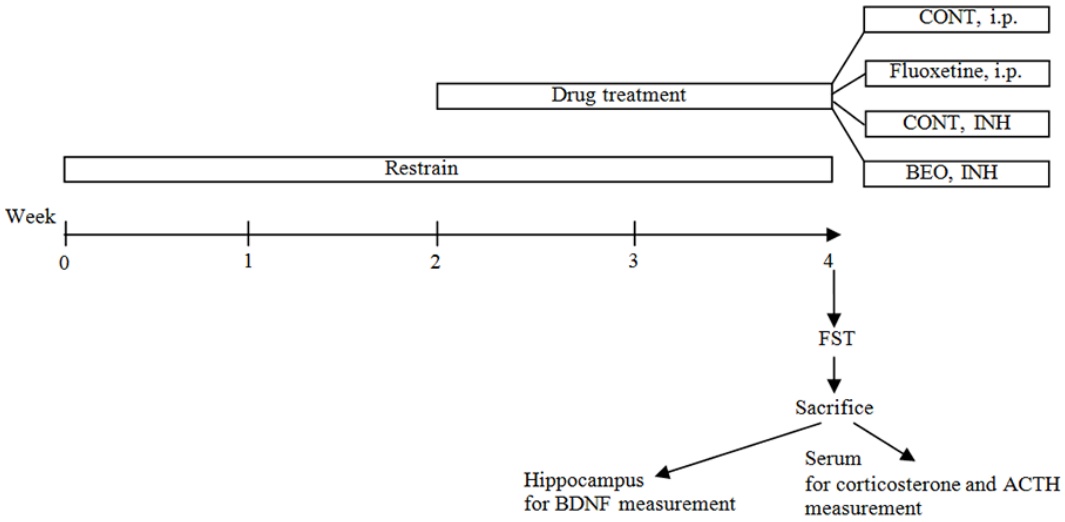

Fig. 1 Experimental timeline. All rats were induced chronic restrained stress 15 minute daily for 2 weeks. For the next 2 weeks, these rats were divided into 4 groups; control-i.p., fluoxetine-i.p., control-inhale, and BEO-inhale. At the end of the treatment, rats were assessed for depressive-like behavior using FST. After the behavioral test, the animals were immediately decapitated and trunk blood samples were collected for the measurement of corticosterone and ACTH level. Hippocampus was dissected for the determination of BDNF protein.

assayed in duplicate.

Measurement of BDNF protein levels

BDNF protein levels in hippocampus were measured by the Promega BDNF Emax ImmunoAssay System (Promaga, USA). Briefly, hippocampus samples were weighed and homogenized in ice-cold lysis buffer. The homogenate was then centrifuged at 14,000xg for 20 minutes at 4°C and supernatants were used for BDNF assays. A 96-well Nunc ELISA plates were coated with 100 µl anti-BDNF monoclonal antibody (mAb) diluted 1:1,000 in carbonate coating buffer (pH 9.7) and incubated overnight at 4°C. The plates were then blocked with Block & Sample 1X buffer for one hour. The samples, diluted to within the range of the standard curve, were added and incubated for two hours at room temperature. Wells were washed with TBST and incubated with an anti-human BDNF polyclonal antibody (pAb) for two hours. After washing, anti-IgY HRP was added for one hour at room temperature. Samples were washed and then incubated with TMB One solution for 10 minutes to develop color. Finally, the reaction was stopped with 1N hydrochloric acid and the optical density (OD) was measured using an ELISA-plate reader at a plate reader at a wavelength of 450 nm. All samples were assayed in triplicate and total protein concentrations were determined from the regression line for BDNF standards.

Statistical analysis

All data are expressed as the mean ± SEM. The data were analyzed by one-way ANOVA with a post hoc Dunnett's test on GraphPad Prism. The differences were considered statistically significant when $p<0.05$.

Results

Effect of BEO on depressive behavior in the force-swimming test

The FST showed that stressed rats inhaled with 2.5 % BEO for two weeks had a reduced immobility time compared to their controls ($p<0.05$). Similarly, immobility time of stressed rats treated with fluoxetine (10 mg/kg, i.p. for two weeks) was significantly less than that of their controls ($p<0.05$) (Fig. 2).

Effect of BEO on HPA axis

In the pilot study, the serum corticosterone and ACTH levels in restrained rats were higher than those of control rats. After exposure to the FST by stressed rats, there was no significant difference in the serum corticosterone levels between either BEO-treated rats (2.5% BEO, INH. for two weeks) or fluoxetine-treated rats (10 mg/kg, i.p. for two weeks) and their controls (Fig. 3A). The serum ACTH following fluoxetine administration was significantly lower than that of control rats ($p<0.05$) (Fig. 3B). However, the serum ACTH of BEO-treated rats were not significantly different with that of control group.

Effect of BEO on BDNF protein levels in hippocampus

There was no significant difference in the BDNF expression in hippocampus between BEO-treated rats (2.5% BEO, INH. for two weeks) and their vehicle treatment groups. Chronic administration of fluoxetine (10 mg/kg, i.p. for two weeks) failed to alter BDNF protein levels in hippocampus of stressed rats (Fig. 4).

Discussion

The main finding of the current study was that BEO and fluoxetine significantly decreased the immobility time in the FST, indicating the antidepressant effects. Fluoxetine has a tendency to decrease serum corticosterone and significantly decreased serum ACTH while BEO had no effect on these two stress hormones. Neither BEO nor fluoxetine changed BDNF protein in hippocampus compared to their own controls.

FST technique, previously described in detail

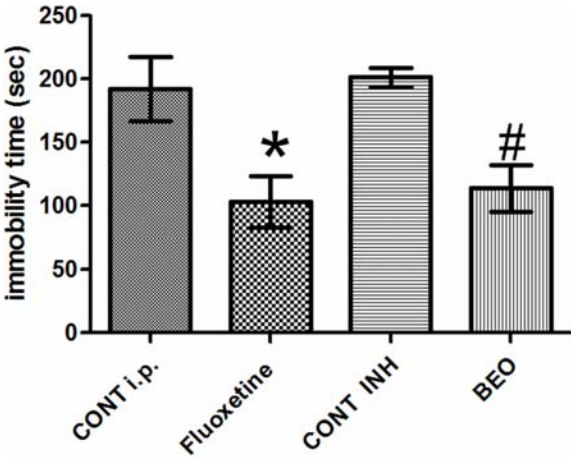

Fig. 2 Effect of bergamot essential oil (BEO) on the FST. BEO inhalation (2.5% w/w, INH) and fluoxetine (10 mg/kg, i.p.) injection were compared to controls (0.9% saline (CONT) either i.p. or INH) on immobility time. Data are expressed as mean ± SEM (n = 10). * $p<0.05$ compared to CONT (i.p.) and # $p<0.05$ compared to CONT (INH).

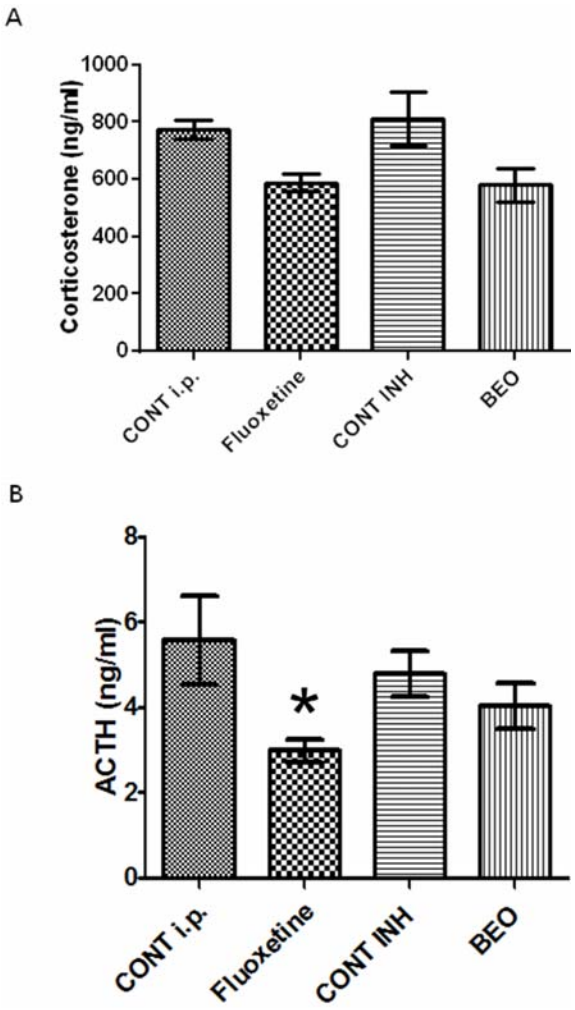

Fig. 3 Effect of bergamot essential oil (BEO) on serum levels of ACTH and corticosterone. Effect of BEO on serum levels of corticosterone (A) and ACTH (B). BEO inhalation (2.5% w/w, INH) and fluoxetine (10 mg/kg, imps) were compared to controls [0.9% saline (CONT) either i.p. or INH]. Data are expressed as mean ± SEM (n = 10). * $p<0.05$ compared to CONT (i.p.).

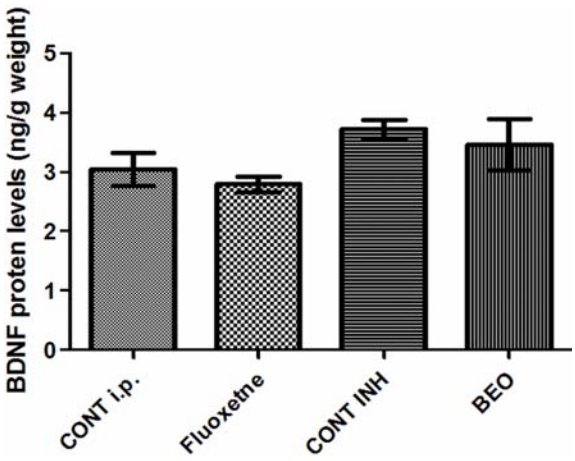

Fig. 4 Effect of bergamot essential oil (BEO) on BDNF protein levels in hippocampus. BEO inhalation (2.5% w/w, INH) and fluoxetine (10 mg/kg, i.p.) compared to controls [0.9% saline (CONT) either i.p. or INH] on BDNF protein levels in hippocampus. Data are expressed as mean ± SEM (n = 10).

by Porsolt et al, has been widely used to evaluate depressive behavior, increased immobility time during swimming in the apparatus, in animals including rats[17]. Administration of fluoxetine, one of the popular antidepressants, to prenatal stressed rats reversed the decrease in immobility in the FST[18]. Others have shown that the neonatal treatment with fluoxetine reduced depressive behavior induced by the FST in adult rats[19]. These is consistent with the present result showing that not only fluoxetine but also BEO significantly decreased the immobility time of stressed rats in the FST, suggesting their antidepressant properties.

There is a close relation between the incidence of depressive disorder and dysregulation of HPA axis. The current study showed that fluoxetine tended to decrease serum corticosterone and significantly decreased serum ACTH. This is in agreement with the previous study that fluoxetine significantly reduced stress-induced increases in plasma ACTH and corticosterone levels[20]. Interestingly, this HPA axis response produced by fluoxetine treatment may be related to the reduction in the immobility time in the FST. However, chronic inhalation of BEO had no effect either on serum corticosterone or ACTH, indicating no effect on HPA axis response. Our study was similar to the previous result demonstrating that chronic administration of fluoxetine in drinking water for two weeks did not change corticosterone level in restrained mice[21]. Essential oil inhalation can be absorbed by nasal mucosa and then carried to the lungs and the blood stream respectively; therefore, the active compounds can reach the central nervous system, including hypothalamus. For example, it was found that rose essential oil inhalation significantly inhibited the increase in plasma corticosterone and reduced the increase in the number of c-Fos-positive cells in paraventricular nucleus (PVN) of hypothalamus in rats subjected to acute restrained stress[22]. Besides,

essential oil inhalation stimulates olfactory pathway and then transmits the impulses to the limbic system, which can regulate the activity of HPA axis[23].

BDNF, one of neurotropic factors in human brain, plays a major role in the regulation of developing brain and synaptic plasticity and neuronal survival in adult brain. Chronic stress decreases BDNF expression whereas antidepressants increase BDNF expression[14]. The current study demonstrated that chronic administration of either BEO or fluoxetine for two weeks failed to alter BDNF protein level in hippocampus. The results agreed with the recent finding that chronic treatment with fluoxetine did not alter BDNF protein in frontal cortex and hippocampuss of mice receiving social defeat stress[24]. Moreover, previous studies suggested that fluoxetine-induced change in BDNF in rat brain depends on the length of treatment, which could contribute to the explanation of the slow onset of antidepressant activity. After 2-week treatment, there was a tendency for the number of BDNF-immuno-reactive cells to increase in hippocampus in treated rats compared with vehicle-treated controls[25]. Therefore, the shorter time course of either BEO or fluoxetine treatment in the present study may have led to the lack of effects on BDNF protein level in hippocampus.

Conclusion

The results of the present study indicate that the inhalation of BEO decreased behavior related depressive disorder similar to fluoxetine but had no effect on HPA axis response and BDNF expression in chronic restrained stress. Future work should be performed by increasing the period of BEO treatment, which may produce adaptive changes in cellular level, like other standard antidepressants.

What is already known on this topic ?

BEO possesses sedation and anxiolytic properties similar to diazepam. After prolonged exposure to stressors, depressive-like behavior can be produced. Although BEO has been traditionally used to relive depression for many years, there are no scientific data supporting this effect.

What is this study adds ?

The inhalation of BEO to rats exhibited antidepressant-like effects on FST similar to fluoxetine however; this essential oil did not change HPA axis response and BDNF expression in chronic restrained stress.

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Potential conflicts of interest

None.

References

1. Ustun TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. Br J Psychiatry 2004; 184: 386-92.
2. World Health Organization. World suicide prevention day. Geneva: WHO; 2012.
3. Cassano P, Fava M. Depression and public health: an overview. J Psychosom Res 2002; 53: 849-57.
4. Fava GA, Offidani E. The mechanisms of tolerance in antidepressant action. Prog Neuro-psychopharmacol Biol Psychiatry 2011; 35: 1593-602.
5. Tang SK, Tse MY. Aromatherapy: does it help to relieve pain, depression, anxiety, and stress in community-dwelling older persons? Biomed Res Int 2014; 430195.
6. Nikfarjam M, Parvin N, Assarzadegan N, Asghari S. The Effects of Lavandula Angustifolia Mill Infusion on Depression in Patients Using Citalopram: A comparison Study. Iran Red Crescent Med J 2013; 15: 734-9.
7. Mehta AK, Halder S, Khanna N, Tandon OP, Sharma KK. The effect of the essential oil of Eugenia caryophyllata in animal models of depression and locomotor activity. Nutr Neurosci 2013; 16: 233-8.
8. Bagetta G, Morrone LA, Rombola L, Amantea D, Russo R, Berliocchi L, et al. Neuropharmacology of the essential oil of bergamot. Fitoterapia 2010; 81: 453-61.
9. Hongratanaworakit T. Aroma-therapeutic effects of massage blended essential oils on humans. Nat Prod Commun 2011; 6: 1199-204.
10. Corasaniti MT, Maiuolo J, Maida S, Fratto V, Navarra M, Russo R, et al. Cell signaling pathways in the mechanisms of neuroprotection afforded by

bergamot essential oil against NMDA-induced cell death in vitro. Br J Pharmacol 2007; 151: 518-29.
11. Saiyudthong S, Marsden CA. Acute effects of bergamot oil on anxiety-related behaviour and corticosterone level in rats. Phytother Res 2011; 25: 858-62.
12. Tyler CR, Solomon BR, Ulibarri AL, Allan AM. Fluoxetine treatment ameliorates depression induced by perinatal arsenic exposure via a neurogenic mechanism. Neurotoxicology 2014; 44: 98-109.
13. Song L, Che W, Min-Wei W, Murakami Y, Matsumoto K. Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. Pharmacol Biochem Behav 2006; 83: 186-93.
14. Zhang Y, Gu F, Chen J, Dong W. Chronic antidepressant administration alleviates frontal and hippocampal BDNF deficits in CUMS rat. Brain Res 2010; 1366: 141-8.
15. de Almeida RN, Motta SC, de Brito FC, Catallani B, Leite JR. Anxiolytic-like effects of rose oil inhalation on the elevated plus-maze test in rats. Pharmacol Biochem Behav 2004; 77: 361-4.
16. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 1997; 20: 78-84.
17. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978; 47: 379-91.
18. Rayen I, van den Hove DL, Prickaerts J, Steinbusch HW, Pawluski JL. Fluoxetine during development reverses the effects of prenatal stress on depressive-like behavior and hippocampal neurogenesis in adolescence. PLoS One 2011; 6: e24003.
19. Mendes-da-Silva C, de Souza SL, Barreto-Medeiros JM, Freitas-Silva SR, Antunes DE, Cunha AD, et al. Neonatal treatment with fluoxetine reduces depressive behavior induced by forced swim in adult rats. Arq Neuropsiquiatr 2002; 60: 928-31.
20. Grundmann O, Lv Y, Kelber O, Butterweck V. Mechanism of St. John's wort extract (STW3-VI) during chronic restraint stress is mediated by the interrelationship of the immune, oxidative defense, and neuroendocrine system. Neuropharmacology 2010; 58: 767-73.
21. Ihne JL, Fitzgerald PJ, Hefner KR, Holmes A. Pharmacological modulation of stress-induced behavioral changes in the light/dark exploration test in male C57BL/6J mice. Neuropharmacology 2012; 62: 464-73.
22. Shaw D, Norwood K, Leslie JC. Chlordiazepoxide and lavender oil alter unconditioned anxiety-induced c-fos expression in the rat brain. Behav Brain Res 2011; 224: 1-7.
23. Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Ann N Y Acad Sci 2008; 1148: 64-73.
24. Brown RW, Noel DM, Smith JJ, Smith ML, Huggins KN, Szebeni K, et al. Eszopiclone facilitation of the antidepressant efficacy of fluoxetine using a social defeat stress model. Pharmacol Biochem Behav 2011; 99: 648-58.
25. De Foubert G, Carney SL, Robinson CS, Destexhe EJ, Tomlinson R, Hicks CA, et al. Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. Neuroscience 2004; 128: 597-604.

ผลของการสูดดมน้ำมันหอมระเหยมะกรูดต่อพฤติกรรมที่สัมพันธ์กับภาวะซึมเศร้าในหนูขาวที่อยู่ในภาวะเครียดเรื้อรัง

สมฤดี สายหยุดทอง, จันทนา เมฆสีประหลาด

ภูมิหลัง: การศึกษาที่ผ่านมาพบว่าน้ำมันหอมระเหยมะกรูด (bergamot essential oil, BEO) มีฤทธิ์ทำให้นอนหลับดีและลดความเครียดได้คล้ายกับฤทธิ์ของ diazepam ตัวกระตุ้นที่ทำให้การเกิดความเครียดหลายชนิด เช่น การทำให้สัตว์ทดลองอยู่ในภาวะเครียดโดยการกักขัง ส่งผลให้มีภาวะซึมเศร้าเกิดขึ้นได้มีการแนะนำให้ใช้ BEO ในการลดภาวะซึมเศร้าทั้งๆ ที่ยังไม่มีหลักทางวิทยาศาสตร์ยืนยันคุณสมบัตินี้

วัตถุประสงค์: ศึกษาถึงผลของ BEO ในหนูขาวที่มีภาวะเครียดเรื้อรังต่อ 1) พฤติกรรมที่แสดงถึงภาวะซึมเศร้า 2) การตอบสนองของ HPA axis และ 3) ระดับโปรตีน BDNF ในสมองส่วน hippocampus

วัสดุและวิธีการ: การศึกษาครั้งนี้ใช้หนูขาว (Wistar rat น้ำหนัก 200-250 กรัม) โดยทำให้หนูขาวอยู่ในภาวะเครียดโดยการจำกัดความเคลื่อนไหววันละ 15 นาที เป็นเวลา 2 สัปดาห์ ต่อมาในสัปดาห์ที่ 3-4 แบ่งหนูขาวออกเป็น 4 กลุ่ม ได้แก่ กลุ่มที่ 1 ได้รับการฉีด saline solution เข้าทางช่องท้อง, กลุ่มที่ 2 ได้รับการฉีด fluoxetine 10 mg/kg เข้าทางช่องท้อง, กลุ่มที่ 3 ให้สูดดม saline solution และกลุ่มที่ 4 ให้สูดดมน้ำมันหอมระเหยมะกรูด 2.5% (w/w = water/oil)) ต่อมานำหนูขาวมาทดสอบบน force swimming test (FST) แล้ว decapitate เพื่อเก็บตัวอย่างเลือดเพื่อใช้ตรวจหาระดับฮอร์โมน corticosterone กับ ACTH ในซีรัมและเก็บสมองส่วน hippocampus เพื่อใช้ตรวจหาระดับโปรตีน BDNF โดยวิธี ELISA

ผลการศึกษา: พบว่า BEO และ fluoxetine ทำให้ระยะเวลาที่หนูขาวหยุดเคลื่อนไหว (immobility time) ใน FST ลดลงอย่างมีนัยสำคัญ ($p<0.05$) หนูขาวที่ได้รับการฉีด fluoxetine มีระดับของฮอร์โมน corticosterone ในซีรั่มมีแนวโน้มลดลงแต่มีระดับของฮอร์โมน ACTH ในซีรัมลดลงอย่างมีนัยสำคัญ ($p<0.05$) ในขณะที่ BEO ไม่ทำให้ระดับของฮอร์โมนความเครียดทั้งสองชนิดนี้แตกต่างจากหนูขาวกลุ่มควบคุม สำหรับการตรวจหา BDNF protein นั้นพบว่าหนูขาวที่สูดดม BEO หรือหนูขาวที่ได้รับการฉีด fluoxetine มีระดับของ BDNF protein ไม่แตกต่างจากหนูขาวกลุ่มควบคุม

สรุป: การสูดดม BEO ลดพฤติกรรมที่แสดงออกถึงภาวะซึมเศร้าได้เช่นเดียวกับ fluoxetine แต่ไม่มีผลต่อการสนองตอบของ HPA axis และระดับ BDNF protein ในภาวะเครียดเรื้อรัง