

ฤทธิ์ปกป้องเซลล์ประสาทของน้ำมันแฟกหอมต่อการตายของเซลล์จากฮอริโมนคอร์ติโคสเตอโรน ในเซลล์ประสาท neuroblastoma SH-SY5Y cells ผ่านกลไกการต้านอนุมูลอิสระ

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บทคัดย่อ

น้ำมันแฟกหอมมีคุณสมบัติในการลดความเครียดและภาวะซึมเศร้าได้ พยาธิสรีรวิทยาของภาวะซึมเศร้าเกิดจากความเสียหายของระบบประสาทที่มีสาเหตุหลายประการ โดยเฉพาะอย่างยิ่งจากอนุมูลอิสระที่เพิ่มขึ้น ดังนั้น การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อตรวจสอบฤทธิ์ต้านอนุมูลอิสระของน้ำมันแฟกหอมต่อการตายของ neuroblastoma SH-SY5Y จากพิษของคอร์ติโคสเตอโรน โดยเลี้ยงเซลล์ด้วยคอร์ติโคสเตอโรน 650 μM ร่วมกับน้ำมันแฟกหอม (0.025, 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2.5, 5 $\mu\text{g/ml}$) หรือ fluoxetine 10 μM เป็นเวลา 24 ชั่วโมง แล้วศึกษาความอยู่รอดของเซลล์โดยการวิเคราะห์ด้วย MTT และ lactate dehydrogenase (LDH) โดยเทคนิค spectrometry และฤทธิ์ต้านอนุมูลอิสระโดยการวัด caspase-3 และ reactive oxygen species (ROS) โดยเทคนิค ELISA จากการศึกษาพบว่าน้ำมันแฟกหอม (0.1 $\mu\text{g/ml}$) ทำให้เซลล์รอดชีวิตมากกว่าการเลี้ยงเซลล์ด้วย corticosterone เพียงอย่างเดียว เมื่อเลี้ยงเซลล์ด้วย corticosterone จะทำให้การหลั่ง LDH เพิ่มขึ้น ในขณะที่น้ำมันแฟกหอมความเข้มข้น 0.025, 0.05, 0.5, 0.1, 0.25 และ 0.5 $\mu\text{g/ml}$ และ fluoxetine 10 μM สามารถยับยั้งการเพิ่มการหลั่ง LDH เนื่องจากพิษต่อเซลล์จากการได้รับ corticosterone ที่น่าสนใจคือ corticosterone เพิ่ม caspase-3 อย่างมีนัยสำคัญในทางตรงข้ามพบว่าน้ำมันแฟกหอม 0.5 $\mu\text{g/ml}$ ช่วยบรรเทาการเพิ่มของ caspase-3 จากการได้รับ corticosterone ในขณะที่ fluoxetine ไม่มีผลดังกล่าว นอกจากนี้พบว่าน้ำมันแฟกหอม 0.025, 0.05, 0.5, 0.1, 0.25, 0.5 $\mu\text{g/ml}$ และ fluoxetine ยับยั้ง ROS ภายในเซลล์ที่เพิ่มจากการได้รับคอร์ติโคสเตอโรน ดังนั้น การศึกษาครั้งนี้แสดงให้เห็นว่าน้ำมันแฟกหอมมีฤทธิ์ปกป้องเซลล์ประสาทผ่านกลไกในการต้านอนุมูลอิสระ

คำสำคัญ: ภาวะซึมเศร้า น้ำมันแฟกหอม caspase-3 แลคเตทดีไฮโดรจีเนส การมีชีวิตรอดของเซลล์ การต้านอนุมูลอิสระ

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Neuroprotective effects of vetiver oil against corticosterone-induced cell death in human neuroblastoma SH-SY5Y cells through antioxidant activity

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Abstract

Vetiver oil is known to reduce stress and depression. The pathophysiology of depression is associated with neuronal damage, especially from increased free radicals. Therefore, the aim of this study was to investigate the antioxidant activities of vetiver oil on corticosterone-induced apoptosis in neuroblastoma SH-SY5Y cells. The cells were treated with 650 μ M of corticosterone in the absence or the presence of vetiver oil (0.025, 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2.5, 5 μ g/ml) compared with fluoxetine 10 μ M for 24 hours. Cell viability was measured by MTT assay. Lactate dehydrogenase (LDH), caspase-3 and reactive oxygen species (ROS) were investigated by spectrometry and ELISA techniques, respectively. Corticosterone significantly decreased cell viability while vetiver oil (0.1 μ g/ml) significantly reversed the decreased viability induced by corticosterone. Cells treated with corticosterone significantly increased LDH release, but vetiver oil at 0.025, 0.05, 0.5, 0.1, 0.25 and 0.5 μ g/ml and fluoxetine 10 μ M attenuated increased LDH release from cells due to corticosterone-induced cytotoxicity. Interestingly, corticosterone significantly increased caspase-3, while vetiver oil but not fluoxetine attenuated this increase. Vetiver oil (0.025, 0.05, 0.5, 0.1, 0.25, 0.5 μ g/ml) and fluoxetine suppressed intracellular ROS was induced by corticosterone. The findings demonstrated that vetiver oil had a neuroprotective effect. The mechanism by which the neuroprotective effects of vetiver on corticosterone-induced neurotoxicity in SH-SY5Y cells may involve antioxidant activity.

Keywords: depression, vetiver oil, caspase-3, lactate dehydrogenase, cell viability, antioxidants

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Introduction

Chronic stress is known to contribute to the development of psychotic disorders, especially depression. Stress caused by hypothalamus–pituitary–adrenal (HPA) axis dysregulation is mainly associated with depression. Cortisol hypersecretion, resulting from over-activation of the HPA axis is highly associated with the severity of depression¹. There are currently many treatments for depression but patients show different responses. Antidepressants, in particular, often have negative side effects such as insomnia, sweating, nausea, constipation, diarrhea, loss of appetite, weight loss and decreased libido². Therefore, complementary or alternative treatments including aromatherapy have been widely used to relieve stress and depression³⁻⁵.

Essential oils used in aromatherapy demonstrate antidepressant properties both in animals and humans⁴. Lavender and chamomile oil are effective in attenuating symptoms of depression, anxiety and stress in adults⁶⁻⁸, while inhalation of *Litsea cubeba* essential oil significantly reduces salivary cortisol, suggesting its anti-stress properties⁹. Many essential oils and their active compounds possess anti-stress and anti-depressive-like effects in animal models¹⁰⁻¹³. Essential oil extracted from *Santalum album* L. attenuated oxidative stress-induced damages, reactive oxygen species (ROS) generation and apoptosis, indicating its antioxidant activity¹⁴. Many previous studies have shown that depressive mood is highly related with impaired antioxidant defense and

inflammatory status that can be reduced by treatment with antidepressants¹⁵.

Vetiver oil, extracted from the root of vetiver grass is used in aromatherapy and believed to have anti-stress, anti-anxiety and sedative properties¹⁶. Inhalation of vetiver oil in rats demonstrated decreased rearing activity in an open field test, indicating a sedative effect¹⁷. Similarly, oral administration of vetiver oil showed significant anticonvulsant activity in mice¹⁸. Vetiver oil also exhibited an anxiolytic-like profile in the elevated plus maze test, involving altering neuronal activation in the lateral division of the central amygdaloid nucleus¹¹. An *in vitro* study showed that vetiver oil exhibited a strong free radical scavenging activity compared to standard antioxidants¹⁹. Vetiver oil also produced antioxidant activity against oxidative stress with tert-butyl hydroperoxide (t-BHP) and hydrogen peroxide (H₂O₂)²⁰. Excessive free radicals promote one of the major mechanisms underlying depression but no studies have demonstrated the antioxidant activity of vetiver oil in neuronal cells. Therefore, this study investigated the antioxidant activities of vetiver oil on corticosterone-induced apoptosis in SH-SY5Y human neuroblastoma cells.

Materials and methods

Cell culture

SH-SY5Y human neuroblastoma cell line was obtained from the American Type Culture Collection (ATCC) (Manassas, USA). SH-SY5Y cells were cultured in cell culture plates coated with collagen and maintained

in DMEM with phenol red medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), 10% fetal bovine serum and 5% fetal bovine serum at 37 °C in a humidified atmosphere incubator (95% air and 5% CO₂) for 48 hours until cell growth of 70% on cell culture plates before seeding in a 96-well plate. After 24-48 hours, the cells were incubated with vetiver oil and corticosterone 650 µM for 24 hours. To study cell viability, the cells were divided into ten groups as the control (with no drug added), 650 µM of corticosterone and 650 µM of corticosterone mixed with fluoxetine 10 µM or vetiver oil (0.1, 0.25, 0.5, 1, 1.5, 2.5, 5 µg/ml). Lactate dehydrogenase (LDH) release, ROS production and caspase-3 activity were measured at low concentration of vetiver oil (0.025, 0.05, 0.5, 0.1, 0.25, 0.5 µg/ml). Experiments were carried out 24 hours after the cells were seeded.

Chemicals

Vetiver oil was supplied by The Royal Project Shop, Thailand. The essential oil extracted from vetiver root containing 0.27% (w/w) fresh vetiver was identified and chemically analyzed using gas chromatography-mass spectrometry (GC-MS). Fluoxetine was purchased from Sigma-Aldrich (St Louis, MO, USA). Dimethyl sulfoxide (DMSO) was obtained from Millipore (Billerica, MA, USA) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from MP Biomedical (Illkirch-Graffenstaden, France). 2', 7'-Dichlorodihydrofluorescein diacetate

(H₂DCFDA) was purchased from Invitrogen (Waltham, MA, USA).

Research method

Cell viability assay

Cell viability was determined by the MTT assay. In brief, SH-SY5Y cells were seeded in 96-well plates at a density of 2×10⁴ cells/well in serum-free DMEM and co-treated with various concentrations of vetiver oil (0.1, 0.25, 0.5, 1, 1.5, 2.5, 5 µg/ml) and corticosterone 650 µM before further incubating at 37 °C in a 5% CO₂ incubator for 24 hours. After treatment, MTT reagent (final concentration, 0.5 mg/ml) was added and the cells were further incubated in a CO₂ incubator at 37 °C for 4 hours. The medium was then aspirated off and 100 µl DMSO was replaced to dissolve formazan. The absorbance was measured at 570 nm by a spectrophotometer (Epoch™ Microplate Spectrophotometer; Biotek, Winooski, VT, USA). Cell viability was calculated as a percentage of the control.

Activity of lactate dehydrogenase (LDH)

The presence of stable LDH in cell culture medium can be detected by cell damage and used as an indicator of cellular toxicity. After treatment with various concentrations of vetiver oil (0.025, 0.05, 0.5, 0.1, 0.25, 0.5 µg/ml), the remaining cells on the plates were denatured using buffer lysis pH 7.5 and performed following the instruction manual of the LDH activity assay kit before measurement of LDH activity using a spectrophotometer (Epoch™ Microplate

Spectrophotometer; Biotek, Winooski, VT, USA).

Activity analysis of caspase-3

To determine cell apoptosis caused by oxidative stress, caspase-3 fluorometric assay was used to detect caspase-3 activity in cell damage. After incubating SH-SY5Y cells in a 96-well plate for 24 hours, the cells were centrifuged to collect the pellets and lysed by adding a lysis buffer. Then the samples were added with reaction buffer and 5 μ l of DEVD-pNA substrate and subsequently incubated at 37 °C for 2 hours. Finally, the lysates were measured by a fluorescence microplate reader (Epoch™ Microplate Spectrophotometer; Biotek, Winooski, VT, USA) at 400 nm and 505 nm. Caspase-3 activity was expressed as fold difference compared to the control.

Reactive oxygen species (ROS) measurement

A fluorescence probe, H₂DCFDA, was used to measure the ROS production in SH-SY5Y cells activated by corticosterone. In brief, the cells were plated at a density of 6×10⁴ cells in 96-well cell culture plates. After 24 hours, the cells were treated with H₂DCFDA at a final concentration of 100 μ M in DMEM for 30 min at 37 °C and then washed twice with PBS. The cells were further co-incubated with corticosterone 650 μ M and fluoxetine 10 μ M or various concentrations of vetiver oil (0.025, 0.05, 0.5, 0.1, 0.25, 0.5 μ g/ml) for 1 hour. After treatment, ROS generation was monitored using a fluorescent multiwell

plate reader (Infinite 200PRO; Tecan Austria GmbH, Grodig, Austria) with excitation at 485 nm and emission at 530 nm. All treatments were performed in triplicate, with data presented as a percentage of the control.

Statistical analysis

Results were presented as mean $\pm$ standard error of mean (SEM). All statistical analyses were performed by one-way ANOVA followed by the Newman-Keuls multiple comparison test using GraphPad Prism 9 software. Differences were accepted as statistically significant at $p \leq 0.05$. Each experimental group consisted of six cell samples.

Results

Effect of vetiver oil on viability of SH-SY5Y cells after inactivation by corticosterone

As shown in Figure 1, when SH-SY5Y cells were treated with corticosterone (650 μ M) alone for 24 hours, cell viability significantly decreased compared to the control group ($p < 0.05$). Co-treatment of vetiver oil at concentrations of 0.1 μ g/ml with corticosterone significantly increased cell viability compared to the corticosterone group ($p < 0.05$). By contrast, higher doses of vetiver oil (0.5, 1, 1.5, 2.5, 5 μ g/ml) potentiated decreased cell viability produced by corticosterone ($p < 0.001$ and $p < 0.01$). Therefore, vetiver oil at low dose concentration was chosen for further investigation.

Effect of vetiver oil on LDH secretion from SH-SY5Y cells after destruction by corticosterone

As indicated in Figure 2, LDH secretion significantly increased in corticosterone-treated SH-SY5Y cells. Co-incubated cells with fluoxetine 10 μ M or vetiver oil at 0.025, 0.05, 0.5, 0.1, 0.25, 0.5 μ g/ml and corticosterone significantly attenuated LDH secretion ($p<0.001$ and $p<0.01$).

Effect of vetiver oil on caspase-3 levels in SH-SY5Y cells after destruction by corticosterone

Treatment of SH-SY5Y cells with corticosterone (650 μ M) significantly increased caspase-3 levels compared to the control

($p<0.01$). However, fluoxetine and vetiver oil (0.025, 0.05, 0.5, 0.1, 0.25 μ g/ml) did not alleviate increase of caspase-3 activity in cells induced by corticosterone treatment. By contrast, vetiver oil (0.5 μ g/ml) attenuated the increase in caspase-3 activity in cells induced by corticosterone treatment (Figure 3).

Effect of vetiver oil on ROS levels from SH-SY5Y cells after destruction by corticosterone

Treatment of SH-SY5Y cells with corticosterone (650 μ M) significantly increased ROS levels compared to the control ($p<0.05$). Fluoxetine and vetiver oil (0.025, 0.05, 0.5, 0.1, 0.25, 0.5 μ g/ml) attenuated increased ROS in cells induced by corticosterone treatment ($p<0.05$, $p<0.01$ and $p<0.001$) (Figure 4).

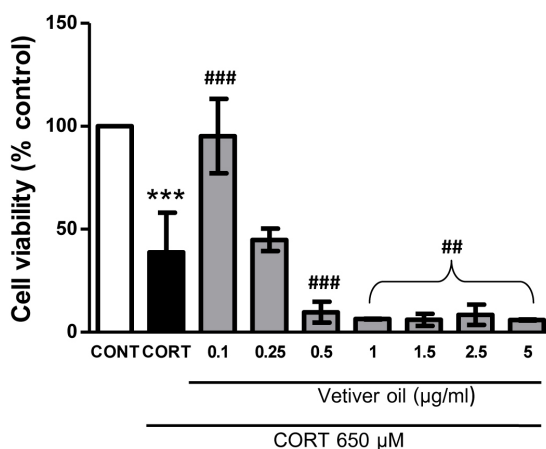


Figure 1 Effects of vetiver oil on corticosterone (CORT)-induced cytotoxicity of SH-SY5Y cells measured by MTT assay (mean $\pm$ SD, n= 4-6) *** $p<0.001$ compared with the control (CONT) group; ## $p<0.01$ and ### $p<0.001$ compared with the CORT group.

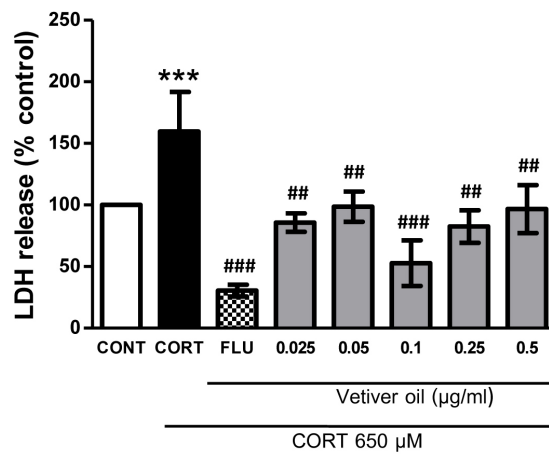


Figure 2 Effects of vetiver oil on LDH release in corticosterone (CORT)-induced SH-SY5Y cells (mean $\pm$ SD, n= 4-6) *** p <0.001 compared with the control (CONT) group; # p <0.05, ## p <0.01 and ### p <0.001 compared with the CORT group.

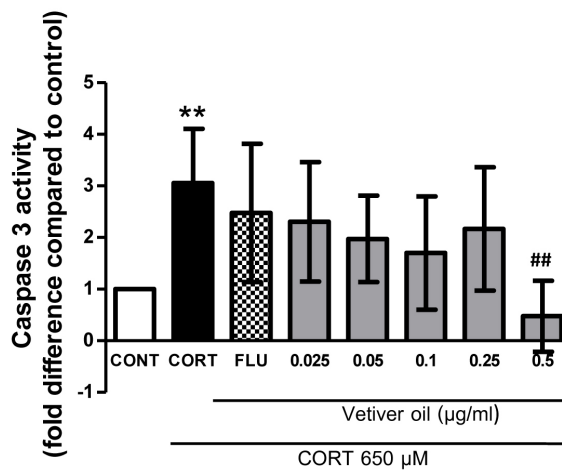


Figure 3 Effects of vetiver oil on caspase-3 activity in corticosterone (CORT)-induced SH-SY5Y cells (mean $\pm$ SD, n= 4-6) ** p <0.01 compared with the control (CONT) group; ## p <0.001 compared with the CORT group.

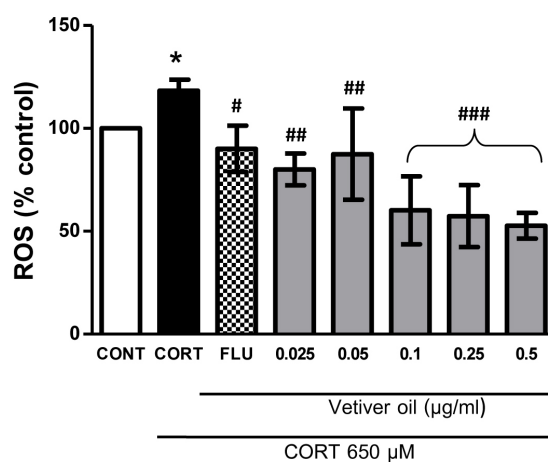


Figure 4 Effects of vetiver oil on ROS levels on corticosterone (CORT)-induced SH-SY5Y cells (mean $\pm$ SD, n= 4-6) * p <0.05 compared with the control (CONT) group; # p <0.05, ## p <0.01 and ### p <0.001 compared with the CORT group.

Discussion

Chronic stress causes hyperactivation of the HPA axis, which then results in the development of depression¹. SH-SY5Y cells share some physiological functions with normal neurons and have been used to study depressive disorders at the cellular level²¹. High corticosterone causes significant damage to SH-SY5Y cells, including decreased cell viability and lower activity of BDNF/ERK/AKT/CREB/mTOR pathways. However, LPM580153, which has an antidepressant effect, significantly attenuated corticosterone-induced damage in SH-SY5Y cells²². Similarly, vetiver oil and fluoxetine, an antidepressant, showed greater SH-SY5Y cell survival than cells incubated with high concentrations of corticosterone alone. Therefore, vetiver oil showed neuroprotective activity against the cytotoxic activity of

corticosterone, indicating potential use as an antidepressant.

The LDH enzyme is present in the cytoplasm of all mammalian cells. Normally, the plasma membrane does not allow secretion of LDH but LDH is released into extracellular fluid following cell damage. Thus, LDH has been used as an indicator of cell damage or injury²³. The previous study showed that VMY-2-95, a potential pharmacological solution for depression, reduced SH-SY5Y cell damage by attenuating decreased cell viability from corticosterone²⁴. Consistently, this study found that both fluoxetine and vetiver oil attenuated increased LDH secretion from SH-SY5Y cells produced by corticosterone. Therefore, vetiver oil prevented SH-SY5Y cell damage from corticosterone exposure, indicating anti-apoptosis activity.

Moreover, corticosterone contributes to oxidative damage which results in neuronal apoptosis and damage. Excessive production of reactive oxygen species (ROS) stimulated the activity of caspase-3, with cells consequently undergoing apoptosis²⁴. A previous study found that VMY-2-95 behaved as an antidepressant and exhibited protective effects on cell viability, cell oxidation and cell apoptosis²⁴. Exposure of SH-SY5Y cells to corticosterone led to the production of reactive oxygen species (ROS) and apoptotic cell death via activation of caspase-9 and caspase-3. In agreement with previous results, our study demonstrated that both vetiver oil and fluoxetine attenuated increase in ROS in SH-SY5Y cells produced by corticosterone. Surprisingly, vetiver oil but not fluoxetine reduced the increased caspase-3 produced by corticosterone. Hwang and Kim (2020) also reported that fluoxetine induced caspase-3 generation and neuronal death²⁶. A previous study also found that the active antioxidant components of vetiver oil were β -vetivenene, β -vetivone and α -vetivone, which were also found in the oil in this study²⁷. Taken together, vetiver oil showed potential cytoprotection associated with antioxidant activity superior to fluoxetine following corticosterone exposure. Further studies are required to examine other mechanisms of vetiver oil that reduce apoptosis of corticosterone-impaired SH-SY5Y cells.

Conclusion

Findings demonstrated that vetiver oil has the neuroprotective effect. The mechanism by which vetiver oil attenuated corticosterone-induced SH-SY5Y cell apoptosis may involve antioxidant activity profile.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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