THE EFFECT OF 10% AND 30% LAVENDER ESSENTIAL OIL BALM ON SERUM CORTISOL LEVELS IN RATS GIVEN STRESSOR

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ABSTRACT

Introduction: Indonesian Ministry of Health published Basic Health Research stated that the incidence of stress in Indonesia increased between 2013 and 2018. Untreated stress is a risk factor for suicide and can cause the onset of depression. Stress associated with cortisol, this hormone has many functions in our body, such as increasing blood sugar levels, reducing inflammation, and suppressing the immune system. One of the essential oils commonly used is English Lavender (Lavandula angustifolia). The lavender essential oil has many benefits, such as reduce anxiety, relieve pain, improve sleep quality, bactericidal, and repellent

Purpose: This study aims to research the effectiveness of 10% and 30% lavender essential oil balm on serum cortisol levels in rats given stressor.

Method: This study used 37 male rats randomly divided into four groups: negative control, positive control, 10% lavender balm, and 30% lavender balm. The forced swim test was given as the stressor every day for ten days, 20 days, and 30 days. The lavender oil balm was applied to the back after the forced swim test. ELISA Kit measured the serum cortisol levels.

Results: The results showed that 10% lavender essential oil balm significantly (p=0.007 dan p=0.041) decreased serum cortisol levels compared to negative control and positive control group. However, there was no statistically significant difference in serum cortisol levels in the 30% lavender essential oil group. Furthermore, there was no significant difference in serum cortisol levels between 10 days, 20 days, and 30 days of the 10% and 30% lavender essential oil balm.

Conclusion: The effectiveness of lavender essential oil balm to decrease the serum cortisol levels depends on the concentration and not depending on the duration of administration. 10% lavender essential oil balm lowers the serum cortisol levels more than 30% lavender essential oil balm.

Keywords: Lavender, forced swim test, cortisol, stressor

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INTRODUCTION

The incidence of stress is still high in various groups and professions in the world. More than 300 million people in the world suffer from stress which is the leading cause of morbidity and disability in the world. In 2013, the Indonesian Ministry of Health published Basic Health Research showed that 6% of the total population in Indonesia experienced emotional mental disorder. This figure had increased to 9.8% in 2018.

Stress is the individual perception of threat that results in anxiety discomfort, emotional tension, and difficulty in adjustment. In neuroendocrinology, stress is any stimulus that provokes adrenocorticotropic hormone (ACTH) and adrenal glucocorticoid. Untreated stress can lead to many problems such as the onset of depression and the risk of completed suicide.

Cortisol is a glucocorticoid hormone produced by adrenal glands, regulated by ACTH, and synthesized from cholesterol. This hormone has many functions in our body, such as increasing blood sugar levels, reducing inflammation, and suppressing the immune system. The body’s response to a stressor is divided into acute responses and chronic responses. The acute response involves the sympathetic adrenal medullary axis (SAM Axis), which produces epinephrine and norepinephrine hormones. In contrast, the chronic responses involve the hypothalamic-pituitary-adrenal axis (HPA Axis), which produces the cortisol hormone. Therefore, serum cortisol levels are often used as an indicator of stress conditions.

Essential oils are aromatic and volatile liquids obtained from the plant material and often used for cosmetics, perfume, and aromatherapy. One of the essential oils commonly used is English Lavender (Lavandula angustifolia). The lavender essential oil has many benefits, such as reduce anxiety, relieve pain, improve sleep quality, bactericidal, and repellent.

The lavender essential oil can be administered orally, topically, and by inhalation. Previous study showed that oral administration of lavender essential oil useful in the treatment for anxiety disorder. Furthermore, inhalation of lavender essential oil can reduce the cortisol levels in serum and saliva. Lavender essential oil has two major components. There are linalool and linalyl acetate. The research by Jager et al. concluded that linalool and linalyl acetate are rapidly absorbed through the skin and peak after 19 minutes.

This study aims to research the usage of natural topical medication lavender essential oil balm effectiveness to treat...
stress conditions. Nowadays, treatment for stress conditions always oriented to synthetic drugs like sedative, psychotropic drugs that are susceptible to misuse. The use of natural ingredients are expected to be an alternative in stress condition therapy.

**METHODS**

**Materials**

The materials used in this research are lavender essential oil (Lavandula angustifolia), formulated into the balm. Lavender essential oil balm consists of 5 ml (4.62 gram) virgin coconut oil, 1 gram beeswax, 0.625 gram lavender essential oil (10% lavender essential oil balm), dan 2.409 gram lavender essential oil (30% lavender essential oil balm).

**Animals**

Male Wistar rats (100-200 grams, 2-3 months) were habituated for seven days before the experiment. Foods were given 60 grams, and water was available ad libitum. Animals were individually housed in the cage.

**Experimental Procedures**

This study used 37 male Wistar rats randomly divided into 4 groups [negative control (C-) = no stressor and no balm; positive control (C+) = stressor only and no balm; lavender 10% (L10) = stressor and 10% lavender essential oil balm; lavender 30% (L30)= stressor and 30% lavender essential oil balm]. The forced swim test was given as the stressor. The animal was placed in a water-filled cylinder for 10 seconds every day for ten days, 20 days, and 30 days. Lavender essential oil balm was given to the treatment group (L10 and L30) within 30 minutes after the forced swim test.

**Measurement of Serum Cortisol Levels**

For the measurement of serum cortisol, the blood sample was taken in the morning after treatment (days 10, 20, and 30). the intracardiac puncture obtained a 1 ml blood sample. Then the blood was centrifuged to collect the plasma. The plasma was stored in a refrigerator under freezing conditions at -20°C until processing. The blood concentration of cortisol (ng/ml) was determined with Enzyme-Linked Immunosorbent Assay (ELISA).

**Data Analysis**

The design was an experimental study with a post-test only control group design. All the results are presented as means ± standard deviation. Statistical significance was analyzed using the Kruskal-Wallis test with the Mann-Whitney U as posthoc analysis. P<0.05 was considered to indicate a statistically significant difference.
RESULT

Characterization of Lavender Essential Oil

Observation of lavender essential oil was done before processing it into the form of a balm. The purpose of this observation is to ensure its quality, purity, and effectiveness. These observations include organoleptic test (odor, shape, and color), pH, density, refractive index, solubility, and clarity. The results of the characterization test are similar to the reference result (Table 1).

Table 1. Characterization of Lavender Essential Oil

| Parameter          | Result             | Reference Result |
|--------------------|--------------------|------------------|
| Odor               | Aromatic           | Aromatic         |
| Shape              | Liquid             | Liquid           |
| Color              | Colorless          | Colorless or yellow |
| Density (g/ml)     | 0.888              | 0.875–0.888      |
| Refractive Index   | 1.4610 ± 0.0082    | 1.459 – 1.470    |
| Solubility         | Soluble in ethanol 96% | Slightly soluble in water, Soluble in ethanol 96% |
| Clarity            | Clear              | Clear            |

Characterization of Lavender Essential Oil Balm

The ingredient of the balm was lavender essential oil, beeswax, and virgin coconut oil. The balm that has been made was observed to ensure the quality of the balm. The results was compared with the reference result (Table 2).

Table 2. Characterization of Lavender Essential Oil Balm

| Parameter          | Result             | Reference Result |
|--------------------|--------------------|------------------|
| Odor               | Aromatic           | Aromatic         |
| Shape              | Semi solid         | Semi solid       |
| Color              | White              | White            |
| pH                 | 7                  |                  |

Effect of Forced Swim Test for 10 Second Daily against Serum Cortisol Levels

The stressor given in this study was a forced swim test for 10 seconds every morning for ten days, 20 days, and 30 days. The result of serum cortisol levels after exposure to the forced swim test was 728.13 ± 48.125 ng/ml (Table 3). compared to the negative control group, the result showed no significant difference (Table 4).

Figure 1: Mean Serum Cortisol Levels in All Groups

Table 3. Mean Serum Cortisol Levels in All Groups

| Groups | n  | Cortisol (ng/ml) |
|--------|----|-----------------|
| C-     | 9  | 712.95 ± 129.589 |
| C+     | 9  | 728.13 ± 48.125  |
| L10    | 10 | 684.19 ± 54.081  |
| L30    | 9  | 731.47 ± 37.944  |

n : number of sample
C- : no stressor and no balm
C+ : stressor only and no balm
L10 : stressor and 10% lavender essential oil balm
L30 : stressor and 30% lavender essential oil balm
Table 4. Negative Control Group (C-) and Positive Control Group (C+)

| Parameter                  | C-                  | C+                  | Sig (p<0.05) |
|----------------------------|---------------------|---------------------|--------------|
| Cortisol Serum (mean ± SD) | 712.95 ± 129.589    | 728.13 ± 48.125     | 0.2          |

C- : no stressor and no balm  
C+ : stressor only and no balm

Effect of 10% and 30% Lavender Essential Oil Balm against Serum Cortisol Levels

Kruskal Wallis test showed a significant difference in serum cortisol levels in all groups (p=0.01) (Table 5). The serum cortisol levels in 10% lavender essential oil balm were 684.19 ± 54.081 ng/ml. However, the serum cortisol levels in 30% lavender essential oil balm were 731.47 ± 37.944 ng/ml (Table 3).

The 10% lavender essential oil balm significantly decreased the serum cortisol levels in male Wistar rats compared to the negative control group (p=0.007) and the positive control group (p=0.041) (Table 6).

As shown in (Table 6), comparison in serum cortisol levels between 30% lavender essential oil balm and negative control group was not statistically significant in male Wistar rats (p=0.145) (Table 6). Furthermore, there was no significant difference in serum cortisol levels between 30% lavender essential oil balm and positive control group (p=0.27) (Table 6). However, there was a significant difference in serum cortisol levels between 10% and 30% lavender essential oil balm (p=0.009) (Table 6).

Table 5. Analysis of Serum Cortisol Levels based on Lavender Essential Oil Balm Concentration

| Parameter                  | Kelompok | Sig (P<0.05) |
|----------------------------|----------|--------------|
| Serum Cortisol (mean ± SD) | C-       | C+    | L10 | L30 | 0.01* |

* : statistically significant  
C- : no stressor and no balm  
C+ : stressor only and no balm  
L10 : stressor and 10% lavender essential oil balm  
L30 : stressor and 30% lavender essential oil balm

Table 6. Comparison of Serum Cortisol Levels

| Group (Serum Cortisol) | Sig (p<0.05) |
|------------------------|--------------|
| C- (712.95 ± 129.589)  | 0.007*       |
| C+ (728.13 ± 48.125)   | 0.041*       |
| C- (712.95 ± 129.589)  | 0.145        |
| C+ (728.13 ± 48.125)   | 0.27         |
| L10 (684.19 ± 54.081)  | 0.009*       |

C- : no stressor and no balm  
C+ : stressor only and no balm  
L10 : stressor and 10% lavender essential oil balm  
L30 : stressor and 30% lavender essential oil balm  
* : statistically significant

Differences in Serum Cortisol Levels Based on the 10th Day, 20th Day, and 30th Day Measurement

In this experiment, we measured the serum cortisol levels three times: day 10, day 20, and day 30. Kruskal Wallis test showed that there was no significant difference in serum cortisol levels based on the 10th day, 20th day, and 30th-day measurement (p=0.057, p=0.147, and p=0.264) (Table 7).
Table 7. Serum Cortisol Levels Based on the 10th Day, 20th Day, and 30th Day Measurement

| Day Measurement | Group | Sig (P<0.05) |
|-----------------|-------|-------------|
| Day 10          | C- C+ | L10 L30     |
| Day 20          | C- C+ | L10 L30     |
| Day 30          | C- C+ | L10 L30     |

C-: no stressor and no balm  
C+: stressor only and no balm  
L10: stressor and 10% lavender essential oil balm  
L30: stressor and 30% lavender essential oil balm

Effectiveness of 10% and 30% Lavender Essential Oil Balm to Serum Cortisol Levels

The Friedman test was used to analyze the effective duration of lavender essential oil balm. The Friedman test indicated that no significant difference in serum cortisol levels was found (Table 8). The serum cortisol levels were decreased in the 20th day and increased in the 30th day on 10% lavender essential oil balm group (Figure 2) and 30% lavender essential oil balm group (Figure 3).

Table 8. Effectiveness of 10% and 30% Lavender Essential Oil Balm to Serum Cortisol Levels

| Group (Day of Measurement) | Sig (P<0.05) |
|----------------------------|--------------|
| L10(10) L10(20) L10(30)   | 0.097        |
| L30(10) L30(20) L30(30)   | 0.264        |

L10: stressor and 10% lavender essential oil balm  
L30: stressor and 30% lavender essential oil balm  
(10): measurement of 10th day  
(20): measurement of 20th day  
(30): measurement of 30th day

DISCUSSION

The present study was planned to research the effect of 10% and 30% lavender essential oil balm against serum cortisol levels in male Wistar rats given stressor, the difference in serum cortisol levels based on different times in measurement, and the effectiveness of 10% and 30% lavender essential oil against serum cortisol levels.

The stressor given in this study was forced to swim test 10 seconds daily for ten days, 20 days, and 30 days. The forced swim test is a behavioral test often used to
test an antidepressant effect, assuming that animals will always try to escape from any unfavorable situations. As shown in (Table 4), there was no significant difference in serum cortisol levels after 10 seconds forced swim test daily has been given—the result contradicted earlier findings by Jameel et al., in 2014. We hypothesized there was habituation of cortisol response to repeated stressor. Habituation is a reduction in individual physiological response as a result of repeated exposure.

Lavender (Lavandula angustifolia) is a native Mediterranean plant. This plant can flourish on a plateau with a height of 600-1350 meters above sea level. Currently, lavender plants have been cultivated all over the world. The composition of the lavender essential oil consist of 47,56% linalyl acetate, 28,06% linalool, 4,34% lavandulyl acetate, 3,75% α-terpineol, dan 1,14% 1,8-cineole. From the data above, we conclude that the major component of lavender essential oil are linalyl acetate and linalool.

As shown in (Table 6), the 10% lavender essential oil balm decrease serum cortisol level significantly than the negative control group and positive control group. This result similar to previous research by Kim et al. that reported the administration of lavender essential oil through inhalation can reduce the saliva cortisol level. Furthermore, a study by Hosseini et al. showed that serum cortisol levels of open-heart surgery patients were decreased after inhalation of lavender essential oil.

This effect occurs due to the modulation of gamma-aminobutyric acid A (GABAA) receptor in the brain by linalool and linalyl acetate. As a result, the concentration of GABA will be increase. GABA is the primary inhibitory neurotransmitter in the body. GABA will suppress the HPA Axis by inhibiting the paraventricular nucleus in the hypothalamus, so the secretion of the corticotropin-releasing hormone will be decreased.

As can be seen from (Table 6), there was no significant difference in serum cortisol levels between the 30% lavender essential oil balm and the control group. There was also an increase in cortisol levels when compared to a control group. This increase can be caused by toxic effects of 30% lavender essential oil balm. The phenomenon of elevated cortisol levels is similar to the previous research by Strac et al. that the administration of diazepam 1 mg/kg can lower cortisol levels. However, if the dose increased to 10 mg/kg, the serum cortisol levels would be increased. We suspected other neuroendocrine mechanisms could
increase cortisol levels, such as cAMP modulation in the brain.  

From the data in (Table 7), there was no significant difference in serum cortisol level on the 10th day, 20th day, and 30th-day measurement between 10% and 30% lavender essential oil balm group. We suggest that therapeutic effectiveness is not affected by the length of administration but rather based on dosing.

It can be seen from the data in [Table 8] that there was no significant difference in serum cortisol level in 10% and 30% lavender essential oil balm either for ten days, 20 days, and 30 days. However, the serum cortisol levels were decreased on day 20 and increased on day 30 (Figure 2) (Figure 3). this happened because of the negative feedback mechanism as compensation for a decline in serum cortisol levels.

CONCLUSION

Based on the research that has been held, we conclude that lavender essential oil balm can affect serum cortisol levels in male Wistar rats that are given stressor.

The 10% lavender essential oil balm can lower the serum cortisol levels better than 30% lavender essential oil balm. However, the duration of the administration of lavender essential oil balm does not affect the serum cortisol levels.

REFERENCES

1. World Health Organization. Depression and Other Common Mental Disorders. Cc By-Nc-Sa 30 Igo. 2017;(1):1–22.
2. Kementerian Kesehatan. Riset Kesehatan Dasar (Riskesdas) 2013. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan Republik Indonesia; 2013.
3. Kementerian Kesehatan. Riset Kesehatan Dasar (Riskesdas) 2018. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan Republik Indonesia; 2018.
4. Fink G. Stress: Definition and history. Encycl Neurosci. 2010;549–55.
5. Fink G. Stress, Definitions, Mechanisms, and Effects Outlined Lessons from Anxiety. Stress Concepts and Cognition, Emotion, and Behavior. Elsevier Inc.; 2016. 3–11 p.
6. Wada K, Sairenchi T, Haruyama Y, Taneichi H, Ishikawa Y. Relationship between the Onset of Depression and Stress Response Measured by the Brief Job Stress Questionnaire among Japanese Employees: A Cohort Study. 2013;8(2).
7. Gradus JL, Qin P, Lincoln AK, Miller M, Lawler E, Sorensen HT, et al. Acute stress reaction and completed suicide. Int J Epidemiol. 2010;39(6):1478–84.
8. Miller DB, O’Callaghan JP. Neuroendocrine aspects of the response to stress. Metabolism. 2002;51(6 SUPPL. 1):5–10.
9. Guyton AC, Hall JE. Textbook of Medical Physiology 13th Edition. Philadelphia, PA: Elsevier; 2016.
10. Mostl E, Palme R. Hormones as indicators of stress. Domest Anim Endocrinol. 2002;23(1–2):67–74.
11. Cavanagh HMA, Wilkinson JM. Biological activities of Lavender essential oil. Phyther Res. 2002 Jun;16(4):301–8.
12. Dornic N, Ficheux AS, Roudot AC, Saboureau D, Ezzedine K. Usage patterns of aromatherapy among the French general population: A descriptive study focusing on dermal exposure. Regul Toxicol Pharmacol. 2016;76:87–93.
13. Kasper S, Gastpar M, Müller WE, Volz HP, Möller HJ, Dienel A, et al. Silexan, an orally administered Lavandula oil preparation, is effective in the treatment of “subsyndromal” anxiety disorder: A randomized, double-blind, placebo controlled trial. Int Clin Psychopharmacol. 2010;25(5):277–87.
14. Rajai N, Sajadi SA, Teymouri F, Zareiyan A, Siavoshi S, Malmir M. The Effect of Aromatherapy with Lavender Essential Oil on Anxiety and Stress in Patients Undergoing Coronary Artery Bypass Graft Surgery. Jundishapur J Chronic Dis Care. 2016 Sep 13;5(4).
15. Hosseini S, Heydari A, Vakili M, Moghadam S, Tazyky S. Effect of lavender essence inhalation on the level of anxiety and blood cortisol in candidates for open-heart surgery. Iran J Nurs Midwifery Res. 2016;21(4):397.
16. Umezuz, Nagano K, Ito H, Kosakai K, Sakaniwa M, Morita M. Anticonflict effects of lavender oil and identification of its active constituents. Pharmacol Biochem Behav. 2006;85(4):713–21.
17. Jager W, Buchbauer G, Jirovetz L, Fritzer M. Percutaneous absorption of lavender oil from a massage oil. J Soc Cosmet Chem. 1992;43(1):49–54.
18. Neil MJ., Smith A, Heekelman, P.E. Obenchain, J.R. Gallipeau JAR, Arecea MAD, Budavari S. The Merck Index: An Encyclopedia of Chemicals Drugs and Biologicals. Thirteenth. New Jersey: Merck & Co. Inc.; 2001.
19. Kementerian Kesehatan. Farmakope Indonesia Edisi V. Jakarta: Kementrian Kesehatan RI; 2013.
20. Belovicova K, Bogi E, Csatoslova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40–3.
21. Khaleel Jameel M, Rajiv Joshi A, Dawane J, Padwal M, Joshi AR, Pandit VA, et al. Effect of various physical stress models on serum cortisol level in Wistar rats. J Clin Diagnostic Res. 2014;8(3):181–3.
22. Wüst S, Federenko IS, Van Rossum EFC, Koper JW, Hellhammer DH. Habituation of cortisol responses to repeated psychosocial stress - Further characterization and impact of genetic factors. Psychoneuroendocrinology. 2005;30(2):199–211.
23. Grissom N, Bhatnagar S. Habituation to repeated stress: Get used to it. Neurobiol Learn Mem [Internet]. 2009;92(2):215–24. Available from: http://dx.doi.org/10.1016/j.nlm.2008.07.001
24. Basch E, Foppa I, Liebowitz R, Nelson J, Smith M, Sollars D, et al. Lavender (Lavandula angustifolia Miller). J Herb Pharmacother. 2004 Sep 7;4(2):63–78.
25. Platt ES. Lavender How to Grow and Use the Fragrant Herb Second Edition. Pennsylvania: Stackpole Books; 2009.
26. Verma RS, Rahman LU, Chanotiya CS, Verma RK, Chauhan A, Yadav A, et al. Esential oil composition of Lavandula angustifolia Mill. cultivated in the mid hills of Uttarakhand, India. J Serbian Chem Soc. 2010;75(3):343–8.
27. Kim I-H, Kim C, Seong K, Hur M-
H, Lim HM, Lee MS. Essential Oil Inhalation on Blood Pressure and Salivary Cortisol Levels in Prehypertensive and Hypertensive Subjects. Evidence-Based Complement Altern Med. 2012;2012:1–9.

28. Milanos S, Elsharif SA, Janzen D, Villmann C, Buettner A. Metabolic Products of Linalool and Modulation of GABAA Receptors. Front Chem. 2017;5(June):1–9.

29. Harada H, Kashiwadani H, Kanmura Y, Kuwaki T. Linalool Odor-Induced Anxiolytic Effects in Mice. Front Behav Neurosci. 2018;12(October):1–8.

30. Cullinan WE, Ziegler DR, Herman JP. Functional role of local GABAergic influences on the HPA axis. Brain Struct Funct. 2008;213(1–2):63–72.

31. Švob Štrac, Dubravka; Muck Šeler, Dorotea; Pivac N. The effects of GABA and GABAergic drugs on the HPA axis activity. Biosynthesis, Med Uses Heal Eff. 2014;71–92.

32. Vargas ML, Abella C, Hernandez J. Diazepam increases the hypothalamic-pituitary-adrenocortical (HPA) axis activity by a cyclic AMP-dependent mechanism. Br J Pharmacol. 2001;133(8):1355–61.

33. Gjerstad JK, Lightman SL, Spiga F. Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. Stress [Internet]. 2018;21(5):403–16. Available from: https://doi.org/10.1080/10253890.2018.1470238