

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 ¹. In 2013, the Indonesian Ministry of Health published Basic Health Research showed that 6% of the total population in Indonesia experienced emotional mental disorder ². This figure had increased to 9.8% in 2018 ³.

Stress is the individual perception of threat that results in anxiety discomfort, emotional tension, and difficulty in adjustment ⁴. In neuroendocrinology, stress is any stimulus that provokes adrenocorticotrophic hormone (ACTH) and adrenal glucocorticoid ⁵. Untreated stress can lead to many problems such as the onset of depression and the risk of completed suicide ⁷.

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 ⁸. 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 ⁹. Therefore, serum cortisol levels are often used as an indicator of stress conditions ¹⁰.

Essential oils are aromatic and volatile liquids obtained from the plant material and often used for cosmetics, perfume, and aromatherapy ¹¹. 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 ¹¹.

The lavender essential oil can be administered orally, topically, and by inhalation ¹². previous study showed that oral administration of lavender essential oil useful in the treatment for anxiety disorder ¹³. Furthermore, inhalation of lavender essential oil can reduce the cortisol levels in serum and saliva ^{14 15}. lavender essential oil has two major components. There are linalool and linalyl acetate ¹⁶. The research by Jager et al. concluded that linalool and linalyl acetate are rapidly absorbed through the skin and peak after 19 minutes ¹⁷.

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 \pm 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 ¹⁸
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 ¹⁹
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

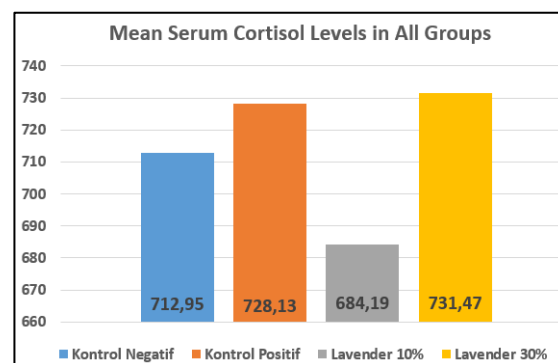


Table 3. Mean Serum Cortisol Levels in All Groups

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)	L10 (684.19 ± 54.081)	0.007*
C+ (728.13 ± 48.125)	L10 (684.19 ± 54.081)	0.041*
C- (712.95 ± 129.589)	L30 (731.47 ± 37.944)	0.145
C+ (728.13 ± 48.125)	L30 (731.47 ± 37.944)	0.27
L10 (684.19 ± 54.081)	L30 (731.47 ± 37.944)	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)
	C-	C+	L10	L30	
Day 10	C-	C+	L10	L30	0.057
Day 20	C-	C+	L10	L30	0.147
Day 30	C-	C+	L10	L30	0.264

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)	
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

Figure 2: Comparison Chart of Serum Cortisol Levels Based on Measurement Time in 10% Lavender Essential Oil Balm Group

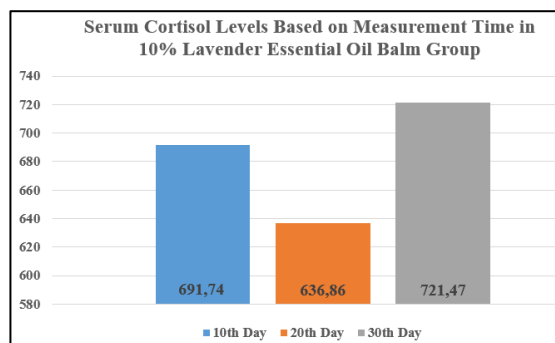
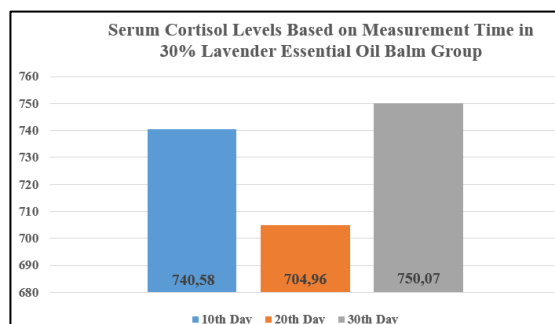


Figure 3: Comparison Chart of Serum Cortisol Levels Based on Measurement Time in 30% Lavender Essential Oil Balm Group



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 situations²⁰. 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 2014²¹. We hypothesized there was habituation of cortisol response to repeated stressor²². Habituation is a reduction in individual physiological response as a result of repeated exposure²³.

Lavender (*Lavandula angustifolia*) is a native Mediterranean plant²⁴. 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 world²⁵. 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-cineole²⁶. 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 level²⁷. Furthermore, a study by Hosseini et al. showed that serum cortisol levels of open-heart surgery patients were decreased after inhalation of lavender essential oil¹⁵.

This effect occurs due to the modulation of gamma-aminobutyric acid A (GABAA) receptor in the brain by linalool and linalyl acetate^{28 29}. As a result, the concentration of GABA will be increase. GABA is the primary inhibitory neurotransmitter in the body⁹. GABA will suppress the HPA Axis by inhibiting the paraventricular nucleus in the hypothalamus, so the secretion of the corticotropin-releasing hormone will be decreased³⁰.

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 increased³¹. We suspected other neuroendocrine mechanisms could

increase cortisol levels, such as cAMP modulation in the brain³².

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 levels³³.

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.

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