

EFFECT OF VETIVER ESSENTIAL OIL IN 10% AND 30% CONCENTRATION TO THE CORTISOL HORMONES OF STRESSOR-INDUCED RATS

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ABSTRACT

Background: The number of people with mental health problems is still high in the world, especially depression. This incident is triggered by the stress of each individual on an ongoing basis and failure to adapt. Stress regulation is a preventive measure to prevent depression in individuals. Currently, there are many developing uses of essential oils as anti-stress relaxation therapy. Vetiver essential oil is believed to reduce stress levels.

Purpose: This study aimed to determine the effect of using vetiver essential oil with different concentrations and administration periods on changes in cortisol levels.

Methods: This study used *Rattus norvegicus* experimental animals with the Forced Swim Test (FST) as a stressor. Vetiver essential oil concentrations of 10% and 30% were administered with the essential oil preparation given after giving stressor. Stress level in experimental animals was measured from the hormone cortisol in the blood. The method of measuring cortisol in experimental animals was carried out using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

Results: This study obtained significant results from the Kruskal Wallis statistical test on decreasing cortisol levels in experimental animals by administering vetiver essential oil with 10% concentration. Changes in the cortisol hormone in experimental animals and cortisol levels were not affected by the length of time of administration.

Keyword: Vertiver Essential Oil, anti-stress effect, *Forced Swim Test* (FST), cortisol serum, *Enzyme-Linked Immuno Sorbent Assay* (ELISA).

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INTRODUCTION

Depression is one of the significant mental health problems that occur almost everywhere globally, including in Indonesia.¹ This affective disorder has an impact on the productivity of an individual's life. Over an extended period, many people with depression decide to commit suicide. One of the causes of depression is untreated stress. Stress is a condition of mental and emotional disorders or confusion because of a problem, and depression is one of the complications that can occur.²

Prevention is needed to avoid depression. This can be assisted by providing additives that can affect cortisol levels as an indicator of stress. Vetiver essential oil is one of the additives that can be used for relaxation.³ Vetiver essential oil has medical benefits and is widely used for its relaxing effect. Vetiver zizanioides is one of the essential oil-producing plants included in the Poaceae family.⁴ Indonesia is the leading producing country of vetiver essential oil in the world, with its cultivation location in Samarang Garut, West Java.^{5,6}

Vetiver essential oil is obtained by distillation from its root extract.⁵ The uses of this essential oil can be in the form of perfume, cosmetic ingredients, anti-inflammatory, anti-bacterial, anti-fungal, insect repellent, and aromatherapy.⁷ To

date, research on vetiver oil has proven to help reduce symptoms of depression, anxiety, insomnia, and improve sleep quality in humans.^{8,9,10}

Based on the explanation above, researchers are interested in examining vetiver essential oil to reduce stress. The use of natural essential oils can be an alternative to empirical treatment in dealing with stress. This study aims to compare the effectiveness of the use of *Vetiveria zizanioides* essential oil on levels of the hormone cortisol in the blood as a marker of stress conditions.

METHOD

Materials and Method

The materials used in this study were 10% vetiver essential oil and 30% vetiver essential oil, each containing 5ml of virgin coconut oil (VCO), one gram of beeswax, 0.626 grams of essential oil (10%), and 2,213 grams of essential oil. vetiver (30%). Vetiver oil was rubbed on the rats' backs after applying stressor.

Preparation of Experimental Animals

The experimental animal used was white male rat Wistar (*Rattus norvegicus*). The experimental animals were 2-3 months old and weighing 100-200 grams, as many as 36 rats. The experimental animals kept, were given an adaptation time of one week and placed in a cage containing one rat.

The male Wistar white rats were randomly divided into four groups, the first control group (K1) was the rats that were not given stressors and balsam, the second control group (K2) was the stressor group, but not balsam, the first treatment group (P1) the group that was given stressors and given vetiver balsam with a concentration of 10%, and the second treatment group (P2) was the group that was given stressors and given 30% vetiver balsam.

Experiment Procedure

In this study, rats were given a stressor in the form of a Forced Swim Test (FST) in groups K2, P1, and P2 every day for 30 days. The treatment in the form of FST was given by placing the rats in a cylindrical tube with a 20 cm diameter, a height of 30 cm, and containing 20 cm of water.

The rats forcibly swam for 10 seconds, dried with a tissue, and let stand for 30 minutes at room temperature. After that, the rats were smeared with 10% and 30% vetiver oil according to the groups.

Measurement of Serum Cortisol Levels

Blood sampling was carried out on all experimental animal groups. Blood was drawn intracardially in the morning as much as ± 1 ml, then the blood was collected in an Ethylenediaminetetraacetic Acid (EDTA) tube and stored in a refrigerator at -20°C . This process was

carried out on days 10, 20, and 30 to test the cortisol concentration with Enzyme-Linked Immuno Sorbent Assay (ELISA).

Data Analysis

The data from this study's results were tested for normality using the Kolmogorov-Smirnov test and homogeneity with Levene's test. The normality test results were abnormal, so a non-parametric statistical test was used, namely the Kruskal-Wallis test and followed by the Mann-Whitney test (post-hoc test).

RESULTS

The Effect of Giving Vetiver Balsam with Different Concentrations on Cortisol Levels

The effect of giving vetiver essential oil on cortisol levels is described in Figure 1. In this study, a hypothesis test was carried out using Kruskal-Wallis followed by a post hoc test with Mann-Whitney U. Statistical test with Kruskal-Wallis comparing all groups (K1: K2 : P1: P2) showed a significant result, which means that there were significant differences in cortisol levels between groups. In the P1 group, the lowest cortisol levels were found when compared to other groups [Table 1].

Post-hoc test with Mann-Whitney U showed significant results when comparing between the P1 group and the

K2 group. The results showed that the cortisol levels in the P1 group were 692.16 ng/ml and 728.38 in the K2 group [Table 3].

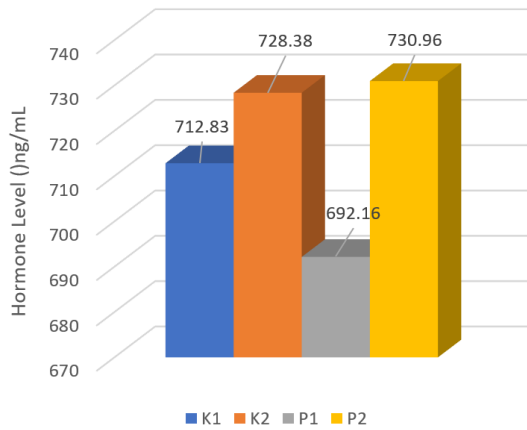


Figure 1 Comparison Cortisol Hormone Test Results

The results showed insignificant values in the vetiver balsam group 30% (P2) compared to the stressor-only group (K2), with cortisol levels of 736.03 ng/ml in the P2 group and 728.34 ng/ml in the K2 group [Table 4].

Table 1 Cortisol Hormone Levels

Group	N	Cortisol Levels (ng/ml)
K1	9	712.83
K2	9	728.38
P1	9	692.16
P2	9	730.96

N : sample number
 K1 : not stressor-induced and essential oil
 K2 : stressor-induced in a form of FST
 P1 : FST and vetiver essential oil (10%)
 P2 : FST and vetiver essential oil (30%)

Table 2 Hypothesis Testing Based on Cortisol Hormone Concentrations

Group				Sig (P<0.05)
K1	K2	P1	P2	0.027*
K1	K2			0.085**
	P1			0.038*
	P2			0.047*
K2	K1			0.085**
	P1			0.047*
	P2			0.354**

P1	K1	0,038*
	K2	0,047*
	P2	0.024*
P2	K1	0.145**
	K2	0.354**
	P1	0.024*

* : statistically significant
 ** : statistically insignificant
 K1 : not stressor-induced and essential oil
 K2 : stressor-induced in a form of FST
 P1 : FST and vetiver essential oil (10%)
 P2 : FST and vetiver essential oil (30%)

Table 3 Comparison of K2 and P1

Parameter	K2	P1	Sig (p<0.05)
Cortisol Serum (mean ± SD)	728.38 ± 48.13	692.16 ± 32.38	0.047*

K1 : not stressor-induced and essential oil
 P1 : FST and vetiver essential oil (10%)

Table 4 K2 and P2 Comparison

Parameter	K2	P2	Sig (p<0.05)
Cortisol Serum (mean ± SD)	728.38 ± 48.13	730.96 ± 26.06	0.354**

K2 : stressor-induced in a form of FST
 P2 : FST and vetiver essential oil (30%)

Hypothesis Testing Based on Cortisol Hormone Concentrations

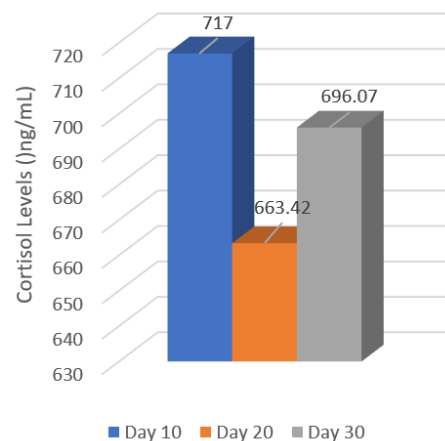


Figure 2 Levels of the essential oil group cortisol with a concentration of 10% on day 10, 20, and 30

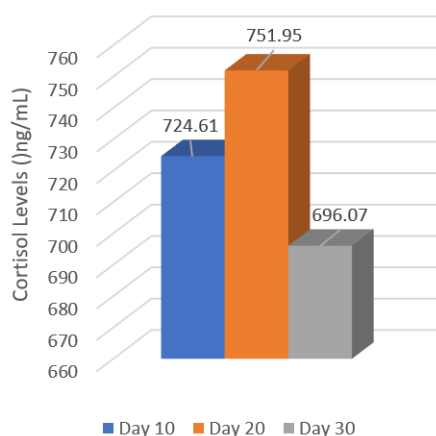


Figure 3 Levels of the essential oil group cortisol with a concentration of 30% on day 10, 20, and 30

This study conducted a hypothesis test to see the effectiveness of therapy against the time of administering balsam for 10, 20, and 30 days. The hypothesis test used was Kruskal-Wallis by comparing groups K1, K2, P1, and P2 on days 10, 20, and 30. The results obtained were not significant [Table 5], which means no differences in cortisol levels between groups. The difference is seen in the mean number of the results [Figure 2 and Figure 3].

Table 5 Hypothesis Testing Based on Cortisol Hormone Concentrations

Day n	Groups				Sig (P<0.05)
Day 10	K1	K2	P1	P2	0.118**
Day 20	K1	K2	P1	P2	0.168**
Day 30	K1	K2	P1	P2	0.108**

** : statistically insignificant
 K1 : not stressor-induced and essential oil
 K2 : stressor-induced in a form of FST
 P1 : FST and vetiver essential oil (10%)
 P2 : FST and vetiver essential oil (30%)

Differences in the Effectiveness of Giving Vetiver Essential with Different Concentrations and Periods Against Cortisol Levels.

The hypothesis test results to see the difference in the effectiveness of the time of giving vetiver balsam using the Friedmann test was carried out by all groups showing insignificant results, which indicated no difference in cortisol levels.

Group P1 obtained insignificant results [Table 6] based on statistical tests. This finding also occurs in the P2 group who got insignificant results [Table 6].

Table 6 Hypothesis Test Based on the Effectiveness of Giving Essential Oil

Groups			Sig (P<0.05)
P1(10)	P1(20)	P1(30)	0.05**
P2(10)	P2(20)	P2(30)	0.05**

** : statistically insignificant
 P1 : FST and vetiver essential oil (10%)
 P2 : FST and vetiver essential oil (30%)

DISCUSSION

This research was conducted by measuring cortisol levels in rats given different vetiver essential oil concentrations, with varying administration duration. Some studies suggest that vetiver oil has a stress-reducing effect, but the mechanism of action of this essential oil is not fully understood. The way these essential oil works is thought to be related to the regulation of γ -aminobutyric acid (GABA), which functions as an inhibitory system in the brain, thereby reducing stress levels.⁹

The results of this study indicated that 10% of vetiver balsam had the effect of

reducing stress by reducing cortisol levels in stress-treated rats. This can be seen in the P1 group, which had significantly lower cortisol levels than the K2 group. This low cortisol level is thought to be an effect of vetiver essential oil.

Vetiveria zizanioides or vetiver has important compounds that give it the distinctive smell of vetiver in the form of khusimene, δ -selinene, β -vetivenene, cyclocopacamphan-12-ol (epimers A and B), vetiselinenol, khusimol, isovalencenol, khusimone, α -vetivone, and β -vetivone. This essential oil component is thought to have a therapeutic effect on reducing stress.¹¹

The mechanism by which vetiver balsam produces a relaxing effect is not fully elucidated. Vetiver oil is thought to increase GABA receptors' sensitivity and increase the number of GABA neurotransmitters in the central nervous system, which facilitates the inhibition system.¹² GABA neurotransmitters will attach to the receptors and open Cl⁻ ion channels making depolarization difficult at the cellular level.¹³ This system will inhibit activation at the cellular level in the hypothalamus, particularly the paraventricular nucleus, which controls the endocrine system.¹⁴

Giving vetiver oil 30% (P2) showed no significant difference in cortisol levels than the control group that was given

stress without being given the essential oil (K2). What is suspected is the occurrence of a rebound effect on changes in the hormone cortisol.¹⁵ This mechanism occurs because the body tries to achieve a balance so that cortisol is not too low due to the administration of vetiver essential oil. The impact of reaching the point of homeostasis resulted in a drastic increase in the rat's hormone cortisol. The previous theory gave physical stressors that benefit athletes by providing a treadmill test for 30 minutes. After this test was carried out, there was a surge in the hormone cortisol, and it decreased rapidly the day after.¹⁵ This also occurred in soccer players, that was found to have a decrease in the hormone cortisol about 48 hours after a match. The reduction in cortisol levels is thought to be the body's mechanism to reduce cortisol levels if they are too high. This is thought to also occur the other way around in the decrease in the hormone cortisol, which is too low so that the body adapts to increase the cortisol levels in the blood again to achieve balance.^{15,16}

Another factor that occurred is the local toxic effect on the skin. The previous theory regarding vetiver essential oil states that vetiver essential oil is relatively safe and has no toxic properties on the body, but it may cause phototoxic effects and local irritation to the skin at high enough concentrations.¹⁷ Vetiver essential oil at

30% concentration makes it possible to provide the impact of new lesions or stressors.

The results of the research and statistical tests showed that there was no significant difference in the effectiveness of the day of giving vetiver essential oil balsam, both for 10% and 30% concentrations. This occurred in the groups of mice that were operated on day 10, 20, and 30. This statement confirms that the effectiveness of therapy was not affected by the essential oil's timing.

There was no significant difference in the effectiveness of the length of time given vetiver essential oil balsam with 10% concentration. This shows that this concentration does not have a statistically distinct difference in the effectiveness of therapy on administration duration, from day 10 to day 30. Still, there is a difference in the average change in cortisol levels with the duration of administration.

CONCLUSION

Based on the research carried out, it is concluded that there are differences in the effectiveness of giving vetiver oil balsam with a concentration of 10% and 30% against the cortisol hormone of male Wistar rats given stressors.

Giving vetiver oil with a concentration of 10% can reduce hormone cortisol levels. However, based on the duration of

administration of the essential oil, it was found that there was no difference in the effectiveness of giving vetiver oil balsam with the duration of administration of = 10, 20, and 30 days to the experimental animal's cortisol hormone.

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