Effect of Essential Oil of Cedarwood (*Cedrus Atlantica*) against Serum Cortisol Levels in Rats Which Were Given Stressor

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Abstract: Stress is a response of the body to any demand that can affect the body's endocrine system such as the release of cortisol into the bloodstream. Essential oils have been widely used for stress treatment because they have a calming effect. One of them is cedarwood essential oil. This study was conducted to determine the effect of cedarwood balm against serum cortisol levels in rats which were given stressor. In this study wistar male rats were randomly selected. This animal was exposed to forced swim test as stressor and then they were given cedarwood balm. We used 3 groups, the first group were given a daily forced swim test and applied cedarwood balm on the shaved back, the second group were given a daily forced swim test only, the third group were given nothing. Their serum cortisol levels were measured by ELISA test after 30 days. Result were analysed by Kruskal Wallis test for all group and man whitney test as a post test ($p < 0,05$). Serum cortisol level was significantly lower in cedarwood balm group then the other groups. When the all group were compared, serum cortisol level was significantly different ($p = 0,018$). In conclusion this indicates that cedarwood balm affect the endocrine regulatory mechanism to modulate stress responses.

1 INTRODUCTION

Stress is the body's response to mental, emotional and / or physical needs that exceed the body's regulatory capacity (Cohen et al., 2013; Fink, 2016). Stress is well known to change serum cortisol in animal models (Hall, 2016). There are a variety of stress delivery techniques, one of which is the forced swim test. Forced swim test is a stressor technique that is often used for cases of depression in experimental animals, the forced swim test (FST), which is one of the most commonly used assays for the study of depressive-like behavior in rodents). The use of FST as a stressor to increase cortisol hormone levels has been demonstrated by various researchers (Khaleel Jameel et al., 2014).

The central regulation of stress and cortisol release occurs in the hypothalamus, which contains multiple responses from the brain. This biological response is the activation of three systems namely the sensory system in the brain, the Sympathetic Adrenal Medullary (SAM), and the hypothalamic-pituitary-adrenal (HPA) axis. Stressors that are stimulated by the sensory system in the brain will activate two hormonal systems to help individuals cope with this condition, the first of which is mediated by a sympathetic nerve called a "fight-or-flight" response which rapidly results in the release of epinephrine and norepinephrine6. Second is a slower and longer hormonal response, mediated by the hypothalamic-pituitary-adrenal (HPA) axis. This response is mediated by a group of neurons in the hypothalamic paraventricular nucleus (PVN), which secretes corticotrophin-releasing hormone (CRH) to give pituitary signals so that the pituitary releases adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal glands to synthesize and secrete Cortisol (Hall, 2016).

Cortisol is an indication of a stress condition. Almost all types of stress that are physical or mental cause an increase in ACTH secretion which increases cortisol secretion within a period of several minutes. Stimulation resulting from physical stress or tissue damage will be delivered to the eminence of the hypothalamic median through the brain stem which causes the activation of the HPA axis so that there is an increase in cortisol secretion into the blood (Cohen et al., 2013; Fink, 2016). In rats given stressor by

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forced swim test method has been shown to increase levels of the hormone cortisol, but the signaling of cortisol hormone levels is higher in acute exposure to FST in mice, it increases stress hormone more than chronic FST exposure (Khaleel Jameel et al., 2014).

Cedrus atlantica plant is one species that comes from the family Pinaceae. Oil from the cedarwood plant is extracted from the Cedrus atlantica tree by distilling it from shavings and splinters from the Cedrus atlantica tree. especially in α-atlantone which can help calm the mind (Rhind, 2012). The high sesquiterpene alcohol content in cedarwood oil has a sedative effect that can overcome tension, fear, anxiety, and depression (Fradelos and Komini, 2015; K G Stiles, 2017). In previous studies the use of cedarwood oil to reduce stress is still unclear, because the method is not clearly explained and there are still many cases of stress management with the use of synthetic drugs such as psychotropic drugs. The use of drugs with this class is still often misused, therefore researchers are interested in further research on the use of natural ingredients such as cedarwood oil to see the effectiveness of cedarwood oil in dealing with stress conditions, with the hormone cortisol as a marker of stress conditions.

2 METHODS AND MATERIALS

2.1 Animal

Rats in this study were male Wistar strain rats weighing 120-150gr, aged 2-3 months, and healthy. The experiments were performed after the animal had been habituated to the experimental environment for 1 week.. foods were given 60 gram and water were given 45 ml every day. These animals were individually housed in cage. The male rat were divided into 3 groups, the first group is negative control group who were not given balms and stressors (C^-), the second group is positive control group who were given forced swim test but were not given balms (C^+), and the treatment groups who were given cedarwood oil balms and given stressors (T^1). Rats will be shaved feathers on the back to apply cedarwood oil balm to the group to be given balm. Mice will be given a forced swim test for 30 days. Experiments were conducted between 1 to 4pm every day.

2.2 Materials

10 % Cedarwood balm consist of 5 ml virgin coconut oil, 1-gram beeswax, and 0,625 gram cedarwood essential oil (*Cedrus atlantica*). Cedarwood balm will be applied at the back of the rats after getting forced swim test.

2.3 Forced Swim Test and Cedarwood Balm

Forced swim tests were given to control group two and treatment group for 30 days, every day on cylinder tubes with a diameter of 20 cm and a height of 30 cm. This cylinder tube will be filled with water as high as 20 cm then the mouse will be placed in a cylindrical tube filled with water and will be quenched for 10 seconds then the rat will be removed and dried by wiping with a towel and allowed to stand for 30 minutes at room temperature. After that the treatment group will be applied cedarwood oil balm with a concentration of 10%.

2.4 Measurement of Serum Cortisol Level

Blood was collected from all the study group animals after 30 days at 4 pm, all blood samples were taken at the same time to get the same result. 3ml of blood was collected by intra cardiac and then the serum was separated by centrifugation at 3000rpm for 5 minutes and stored at -20c. The serum sample was analyzed with ELISA KIT to determine the cortisol level. This assay has high sensitivity and specificity for estimation of cortisol levels in wistar rats.

2.5 Statistic

Data were analyzed by statistical test and the Kruskal Wallis followed Whitney test man as a post hoc test with SPSS 25.0 software to indicate a statistically significant difference between the control group and the treatment group with a significant value of $p < 0.05$.

3 RESULTS

The effect of cedarwood balm against serum cortisol levels in the treatment group shown in Figure 1. Comparison of serum cortisol level between

treatment group (T^1), positive control group (C^+), and negative control group (C^-) was done in study group animals. Statistical tests using Kruskal Wallis show that the data is significant and has significant differences. It was observed that serum cortisol level was decrease more after given cedarwood balm (Table 3).

Post hoc test (Mann-Whitney U test) showed that treatment control group significantly decreasing serum cortisol level in wistar rats compared to positive control group and negative control group. While the serum cortisol level was 706.85 ng/ml in treatment group, 728.34 ng/ml in positive control group, and 713.04 in negative control group (Table 4 and Table 5).

Result showed no significant different in serum cortisol level between negative control group and positive control group. Mean ± SD of serum cortisol level in negative control group were 713.04 ± 129.533 and positive group were 728.34 ± 48.12 (Table 2).

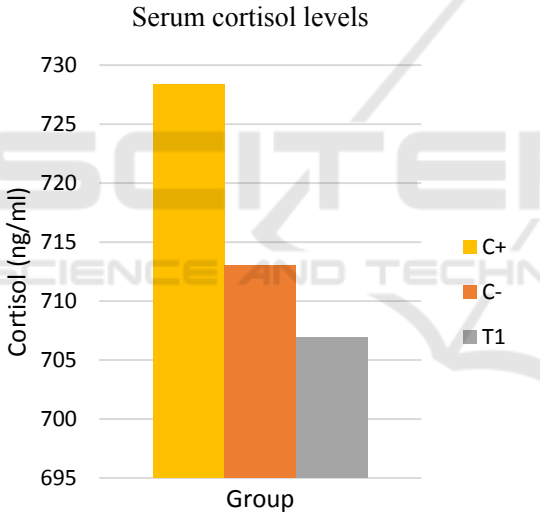

Figure 1: Comparison of serum cortisol level

Table 1: Serum cortisol levels.

Groups	N	Serum cortisol
C^+	9	728.34
C^-	9	713.04
T^1	9	706.95
N: number of samples C^+ : forced swim test only C^- : no stressor and no treatment were given T^1 : forced swim test and 10% cedarwood essential oil balm were given		

Table 2: Comparison of serum cortisol levels between groups.

Groups	Groups	Sig (P<0.05)
C^+	C-	0.200**
	T1	0.047*
C^-	C+	0.200**
	T1	0.007*
T^1	C+	0,047*
	C-	0,007*
*: statistically significance **: statistically not significance C^+ : forced swim test only C^- : no stressor and no treatment were given T^1 : forced swim test and 10% cedarwood essential oil balm were given		

Table 3: Comparison of negative control group and treatment group.

Parameter	C^-	C^+	T^1	Sig (p<0.05)
Serum Cotisol (mean ± SD)	713.04 ± 129.533	728.34 ± 48.12	706.95 ± 37.78	0.018*
C^- : no stressor and no treatment were given T^1 : forced swim test and 10% cedarwood essential oil balm were given				

Table 4: Comparison of negative control group and treatment group.

Parameter	C^-	T^1	Sig (p<0.05)
Serum Cortisol (mean ± SD)	713.04 ± 129.533	706.95 ± 37.78	0.007*
C^- : no stressor and no treatment were given T^1 : forced swim test and 10% cedarwood essential oil balm were given			

Table 5: Comparison of negative control group and treatment group.

Parameter	C^+	T^1	Sig (p<0.05)
Serum Cortisol (mean ± SD)	728.34 ± 48.12	706.95 ± 37.78	0.047*
C^+ : forced swim test only T^1 : forced swim test and 10% cedarwood essential oil balm were given			

4 DISCUSSION

In the present study we investigated the effects of cedarwood balm on serum cortisol level in male wistar rats. Several studies have said that cedarwood can reduce stress with the hormone cortisol as an

indication. However, the mechanisms is not clearly explained and the species used is different (Worwood, 2016).

Our result showed that cedarwood balm has an effect to reduce stress by reducing the level of the hormone cortisol in male wistar rats. This happen when we compare treatment group and positive control group. There was significant different between serum cortisol level, which is a decrease in the level of the serum cortisol, which is thought to be caused by the content of cedarwood oil.

There are four major constituent in cedarwood essential oil such as cedrol, α-atlantone, α-pinene, and himachalol (Aberchane and Fechtal, 2004; Tisserand and Young, 2013). The composition of essential oils differ according to the part of the plant used, according to the region or origin of the plant, the stage of germination and its extraction methods (Ainane et al., 2018; Fidah et al., 2016; Satrani et al., 2015). All of this major constituent has an effect on stress. The high content of sesquiterpene alcohol in cedarwood oil has a sedative effect that can overcome tension, fear, anxiety, and depression10,13.

Cedrol works by activating GABA so that it causes a sedative effect that can cope with stress conditions8,30,31. In research conducted by Ryuji et al. (2016) cedrol is given inhaled in male wistar rats and provides a sedation and relaxation effect that is thought to be caused by the mechanism of GABA inhibition (Kagawa et al., 2003). In another study cedrol improve sleep in young women by heightening parasympathetic activity (Takeda et al., 2017). Another constituent that can cause sedation and relaxation effect is α-pinene that produces cinnamon scent which can help calm the mind and works on GABAergic transmission like cedrol (Aoshima and Hamamoto, 1999; Rhind, 2012).

The mechanism of action of cedarwood essential oils to produce sedative and relaxing effects remains to be determined. These effect may occur through inhibition of the activity of am-aminobutyric acid (GABA) transaminases which are enzymes for GABA metabolism in synapses. This inhibitory activity causes an increase in GABA levels and a decrease in glutamate levels which will cause a sedative effect (Franz and Novak, 2015). Previous studies have suggested that there is a barrier to the activity of the HPA axis after injecting GABA-A agonists by inhibiting the production of CRH in the parvocellular paraventricular nucleus (Herman et al., 2004). As a result of HPA axis suppression and the cessation of CRH production, the serum cortisol level will be reduced.

5 CONCLUSIONS

Our study showed that 10% cedarwood balm has the effect of reducing stress by reducing the level of the hormone cortisol in male wistar rats given the stressor. Cedarwood balm showed potential to be used as alternative treatment to relief stress condition but further studies will be needed to have more conclusive evidence on this aromatherapy.

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