

# Blood Cortisol Level and Blood Serotonin Level in Depression Mice with Basil Leaf Essential Oil Treatment

Machnizar Sentari<sup>1\*</sup>, Urip Harahap<sup>2</sup>, Tuti Wahmurti A. Sapiie<sup>3</sup>, Kiking Ritarwan<sup>4</sup>

<sup>1</sup>Department of Psychiatry, Faculty of Medicine, University of Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, 20155, Indonesia; <sup>2</sup>Department of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, 20155, Indonesia; <sup>3</sup>Department of Psychiatry, Faculty of Medicine, University of Padjajaran, Jatinangor, Sumedang, Bandung, Jawa Barat, 45363, Indonesia; <sup>4</sup>Department of Neurology, Faculty of Medicine, University of Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, 20155, Indonesia

## Abstract

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**\*Correspondence:** Machnizar Sentari. Department of Psychiatry, Faculty of Medicine, University of Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, 20155, Indonesia. E-mail: [nyzarsntari96@gmail.com](mailto:nyzarsntari96@gmail.com)

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**BACKGROUND:** Depression is a serious public health problem. The impact of depression is enormous, ranging from decreasing work productivity, interpersonal disorders, sleep and eating disorders, susceptible to disease to an increase in suicides. In a state of depression, there was an increase in cortisol and changes in the neurotransmitter of the brain monoamine, norepinephrine and dopamine and specifically serotonin level. Treatment of depression using synthetic drugs such as the Selective Serotonin Reuptake Inhibitor (SSRI) drug which is said to be safe turns out to still have side effects, such as stomach disorders, erectile disorders, weight gain and sometimes sleep disorders. So, the usage of traditional medicines can be an alternative. One of the traditional medicines that have been studied in Indonesia was the essential oil of basil leaves, which is known to function as an antidepressant at a dose of  $2.5 \times 10^{-2}$  mL/kg b.w.

**AIM:** To determine the comparison of cortisol and serotonin level between depressed mice which get basil leaf essential oil as intervention, depression mice, and normal mice.

**METHODS:** This research was an experimental type purely using experimental animal models, with an experimental research design Post Test Only Context Group Design in mice (*Mus musculus*), by dividing 3 groups of mice, namely depressed mice which received essential oil of basil leaves dose of  $2.5 \times 10^{-2}$  mL/kg BW, depression mice, and normal mice, to compare the level of blood cortisol and serotonin while to determine effect of administration basil leaves essential oils in depression mice.

**RESULTS:** Cortisol levels between intervened depression mice, depression mice, and normal mice groups were significantly comparable with  $p < 0.001$ , while serotonin level was significantly comparable between intervened depression mice, depression mice, and normal mice group with  $p < 0.001$ .

**CONCLUSION:** Evidenced by differences in cortisol levels, serotonin levels between normal mice groups, depressed mice and mice that received essential oils of basil leaves. The comparisons showed that the cortisol level of the intervened depression group was lower than the depression mice group; however the level didn't reach the level of cortisol in the normal group. While the serotonin level of intervened depression mice group was higher than the depression mice group or normal mice group.

## Introduction

Depression is a serious public health problem. The impact of depression is great, starting from the decline in work productivity, disruption in one's interpersonal relationships, sleep and eating disorders, susceptibility to various diseases, and an increase in suicides [1]. From the Rihmer clinical study, it was found that 78-89% of patients with the major depressive disorder had a desire and attempted suicide [2]. Depression is a syndrome characterised

by clinical symptoms whose manifestations can vary by an individual [3].

Based on psychopathology, it is known that there are three symptoms of depression, namely decreased mood, anhedonia and reduced energy or fatigue. Other symptoms such as weight loss, sleep disorders, loss of concentration to increased suicidal desire [4]. In 2017, the WHO stated the number of depressed patients had increased sharply over the past 10 years, between 2005-2015, where sufferers of depression were estimated to have reached more than 300 million people [5]. While in Indonesia, the

prevalence of depressive disorders in the Basic Health Research study reached 6% or around 14 million people [6].

Genetic factors, neuroendocrine disorders, neurophysiological changes are neurobiological factors [7]. The changes in neurotransmitters that occur are associated with brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine. It's just that a decrease in serotonin levels is thought to have a greater role in the occurrence of depression [8]. The initial sign of depression is an increase in cortisol caused by dysregulation of the hypothalamic Pituitary Adrenal (HPA) axis triggered by recurrent chronic stress [9]. On the other hand, the treatment of depression using synthetic drugs has experienced rapid development since the introduction of fluoxetine in 1988, a drug class of Selective Serotonin Reuptake Inhibitors (SSRIs). However, SSRI drugs that are said to be safe still have side effects, such as stomach disorders, erectile disorders, weight gain and sometimes sleep disorders. And this is what encourages the interest in using herbal or traditional medicines to increase in the last 10 years [10].

Traditional medicines derived from natural ingredients have been widely used in Indonesia. The government supports this by issuing a decree of the Minister of Health of the Republic of Indonesia Number: 381/MENKES/SK/III/2007 concerning National Traditional Medicine Policy, which states that the use of traditional medicines needs to be improved and developed and tested scientifically. This is where the role of research and good assessment of the efficacy, side effects, legality, and marketing continue [11]. One of the traditional medicines that have been studied in Indonesia is Basil essential oil. The results obtained from the previous study found that the essential oils of basil leave that function as antidepressants were found in doses of  $2.5 \times 10^{-2}$  mL/kg b.w. [12]. In this research, the researcher will examine the blood cortisol level and blood serotonin level to prove the antidepressant activity.

## Material and Methods

### Tools

The tools used in this study were plastic cages 40 cm × 60 cm that was given woven fibre for 10 mice, food containers, mice drinks, 1 ml syringe, alcohol cotton, stopwatch, syringe, test tube. Elisa Reader to measure the blood cortisol level and blood serotonin level. Standardised electric scales for weighing the body weight.

### Materials

The materials used in this study were basil essential oil (*Ocimum basilicum*) is obtained by steam distillation of basil leaves, obtained from the Bogor Research Institute for Medicinal Plants and Aromatic Plants which has been certified by Ministry of Agriculture.

### Research Methods

The study was conducted in August 2018 to November 2018 in the Laboratory of Biochemistry at the Faculty of Medicine, University of Brawijaya. This type of research was preclinical research using experimental animal models and experimental research designs Posttest Only Control Group Design. The researcher divided the three groups of mice (*Mus musculus*), namely the treatment group (depressed mice that treated with essential oils of basil leaves at a dose of  $2.5 \times 10^{-2}$  ml/kg b.w.), negative control (depression mice without treatment), and normal control (mice that). This study included the preparation stage of experimental animals, intervened experimental animals depression and administering essential oils (doses of  $2.5 \times 10^{-2}$  ml/kg b.w.). Testing the characteristics of the experimental animals was carried out at the start and the end of the study, the examination of blood cortisol level and blood serotonin level in each group of mice, was only done once.

Furthermore, the mean and median data were analysed. Normality of data was analysed using the Shapiro Wilk test. In normal distribution data, the mean difference was tested by one-way analysis of variance test while in the opposite condition, the differences were analysed using Mann Whitney between each intervention group. This analysis will be significant if the value of  $p < 0.05$ .

### Experimental Animal

The experimental animal was maintained and injected by corticosterone to induced depression. The study sample was randomly selected using the inclusion and exclusion criteria. Inclusion criteria were male mice (*Mus musculus*) with weight 18 g-30 g and in good health. Exclusion criteria were mice whose hair falls and move slowly. This research was approved by the health research ethics committee of the Faculty of Medicine, the University of North Sumatra with No: 641/TGL/KEPK FK USU-RSUO HAM/2018. The experimental animal was 27 healthy male mice were randomly selected and fulfilled the inclusion and exclusion criteria. Conducted in the Laboratory of Biochemistry at the Faculty of Medicine, University of Brawijaya, where mice were kept in plastic cages with woven fibre to feel like outside environment.

## Depression Induction

Male mice were placed in a 12-hour light cycle and 12 hours dark. The lights were turned off at 6:00 p.m. with a temperature of approximately 25°C. In experimental animals (n = 27) who were given independent access to food and drink, while 18 experimental animals were given an intraperitoneal injection of subcutaneous corticosterone at a dose of 20 mL/kg b.w. Once a day. Duration of administration was 3 weeks, then 9 mice from depression group were weighed and observed with a swimming test (FST) to get immobility time. Immobility time is obtained with 6 minutes of swimming mice reduces with the duration of mice for swimming and determination of blood cortisol and serotonin level. While the other 9 mice, after one day of depression induction was treated with basil leaf essential oil (dose of  $2.5 \times 10^{-2}$  mg/kg b.w.) orally every day. Then at the 4<sup>th</sup> week, all the animal has measured the weight gain, blood cortisol level and blood serotonin level.

## Results

Experiment animal was measured of their body weight. In this case, the results were measured at the beginning and the ending of research.

**Table 1: Bodyweight results**

Group	Average Body Weight		
	Early	Final	Difference
Normal	25.52	29.00	3.48
Negative	27.46	19.78	7.69
Treated	26.80	24.32	2.48

Normal control group was a mouse without treatment of depression and intervention with early average body weight of 25.52 g and final average body weight of 29.00 g, there is an additional weight of mice by 3.48 g while mice in the negative control group were depressed mice group without intervention, the early average body weight was 27.46 g and the final average was 19.78 g, resulting in a weight loss of 7.69 g. This value shows that the treatment of depression without intervention causes the body weight of mice to drop and this shows that the presence of depressed conditions in mice causes a decrease in weight. While the group given the treatment of depression and given the intervention of essential oils of basil leaves with a dose of  $2.5 \times 10^{-2}$  had an average body weight of 26.8 g and an average body weight of 24.32 g, a decrease was 2.48 g. This decrease in body weight is not as much as a decrease in positive control weight but not as good as normal mice that have increased body weight.

Examination of blood cortisol level and blood serotonin level was carried out in each group of mice that were intervened by administering essential oils of basil leaves dose of  $2.5 \times 10^{-2}$  mL/kg b.w., depressed

mice and normal mice. The intervention is indicated by the administration of essential oils of basil leaves with dose doses of  $2.5 \times 10^{-2}$  mL/kg b.w. indicating that there are differences in blood cortisol level and blood serotonin level.

**Table 2: Cortisol level**

Group	Cortisol Level		P-Value
	Mean	Standard Deviation	
Normal	349.052	18.032	< 0.001*
Negative	490.001	18.004	
Treated	400.363	30.875	

\* One Way Analysis of Variance Test.

The average cortisol level of normal mice was 349.052 ng/mL; negative control mice were 490.001 ng/mL. The cortisol levels between the normal group and negative control group were significantly different (140.949 ng/mL with p-value < 0.001). The cortisol level decreased after the treatment with basil leaf essential oil. The between groups of treated mice and negative control mice were significantly different (89.638 ng/mL with p-value < 0.001).

**Table 3: Serotonin level**

Group	Cortisol Level		P-Value
	Median	Range	
Normal	544.902	525.098 - 582.098	< 0.001*
Negative	367.110	348.751 - 473.899	
Treated	741.577	693.403 - 779.803	

\* Shapiro Wilk Test.

The median of serotonin level of normal mice was 544.902 ng/mL; negative control mice were 367.110 ng/mL. The cortisol levels between the normal group and negative control group were significantly different (177,792 ng/mL with p-value < 0.001). The cortisol level decreased after the treatment with basil leaf essential oil. The between groups of treated mice and negative control mice were significantly different (374.467 ng/mL with p-value < 0.001).

Stress alone is not enough to induce depression. Serotonin reduction is needed to cause this disorder. Therefore, a tryptophan diet study in mice as a source of serotonin is reduced slowly and added with repeated acoustic stress which is considered as chronic stress, to get a state of depressed mice in which the condition of cortisol increases and serotonin decreases [13]. Pathophysiology turns out that all of that, both biology and psychology are interrelated, the mechanism of neurotransmitter changes, dysregulation of the HPA axis due to chronic stress, inflammation, reduce neuroplasticity and network disfunction as integral parts that are connected directly or not [14].

One of the initial parameters that show normal mice treated with depression is physical changes and psychomotor movements, where the bodyweight of the mice decreases, the psychomotor movement becomes weaker. This is in line with the theory of depression which describes a state of anhedonia

where someone who is depressed tends to be lazy for activity, lacks enthusiasm and motivation, even the absence of appetite which causes a person to be lazy to move and lose weight. The decrease in body weight that occurs is actually caused by many factors, but in this study, we see from increasing cortisol levels and decreasing serotonin due to repeated injections of corticosterone which causes depression in mice. The increase in cortisol in the blood known as hypercortisolism and continues to cause chronic glucocorticoid receptors that have metabolic, catabolic, immunosuppressive and anti-inflammatory effects that are very complex both in organ tissues and at the cellular level and if this condition is left in the brain tissue it will cause damage to the hippocampus finally disrupting the stability of the neurotransmitter [15]. Increased cortisol causes lipolysis, which is lysis of fat and stimulates the breakdown of many proteins in the tissues. This can be explained simply because of the result of hypercortisolism that causes insulin resistance (IR) which ultimately results in high peripheral glucose levels and hyperinsulinemia because of the reduced ability of insulin to reduce blood glucose levels by taking to muscle tissue, for example, type 2 Diabetes Mellitus [16].

For cortisol levels, a significant increase occurred in mice treated with depression. This shows that an increase in cortisol is one sign of depression in mice. After treated with essential oils of basil leaves (dose  $2.5 \times 10^{-2}$  mL/kg b.w.), a significant decrease in cortisol and a significant increase in serotonin. Several studies that support a decrease in cortisol levels caused by herbal medicines have been carried out several times. The decrease in cortisol levels in mice has been studied for different herbs. Mangosteen peel juice for 4 weeks and the result that cortisol levels were significantly decreased compared to negative controls [17] or mangosteen peel extract reduce cortisol levels in mice [18]. The effects of *Ocimum basilicum* (basil leaves) on depressed mice by inhalation can reduce cortisol level [19].

It was proven that there were differences in blood cortisol and serotonin levels between depressed mice groups that received basil leaf essential oil, depressed mice and normal mice. The comparison showed that the cortisol level of the treated mice in depression state mice was lower than negative control mice but higher than normal control mice, while the serotonin level of treated mice in a depression state mice was higher than negative control mice but also higher than normal control mice.

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