

## The Effect of Long Exposure Reed Diffuser Essential Oil *Plumeria alba* on Cortisol Levels of Male Wistar Rats

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### Abstract

Stress can occur due to a person's inability to respond to a stressor, resulting in bodily or mental disorders. Anxiety can be characterized by increased levels of cortisol, which is regulated by the Hypothalamus-Pituitary-Adrenaline (HPA-axis). Aromatherapy is a therapy using essential oils that give a distinctive aroma to plant parts such as flowers, roots, leaves, and stems. Aromatherapy can be done through a reed diffuser. The frangipani (*Plumeria alba*) is one of the plants in Indonesia that has a particular scent in its flowers. This study aims to determine the effect of prolonged exposure to *P. alba* essential oil reed diffuser on cortisol levels in rats. Twenty-four rats were divided into four groups: the control group without exposure to a reed diffuser for 5 and 10 days and the other group with exposure for 5 and 10 days. Each blood was drawn through the retro-orbital, centrifuged to obtain serum, and tested using LC-MS to determine cortisol levels. The group with ten days of essential oil exposure showed lower cortisol levels. It can be concluded that the duration of aromatherapy exposure is connected to cortisol levels and that aromatherapy can be utilized as a stress-reduction therapy.

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## INTRODUCTION

Stress levels affect memory function. Stress is a condition where the demands that must be met exceed their capabilities, the cause of stress is called a stressor<sup>1</sup>. Stress can occur due to a person's inability to respond to a stressor. It can result in bodily or mental disorders. Stress can be characterized by the increased hormone cortisol, regulated by the Hypothalamus-Pituitary-Adrenaline (HPA-axis), causing cortisol levels to rise<sup>2,3</sup>. Some therapies to prevent dementia and decreased memory function include using drugs, physical exercise, and aromatherapy<sup>4</sup>.

Aromatherapy is a therapy using essential oils that give a distinctive aroma to plant parts such as flowers, roots, leaves, and stems. Aromatherapy can be done through a diffuser either electrically or using a reed<sup>4</sup>. Several studies have shown that aromatherapy using rosemary and lemon essential oils spread through a diffuser in the morning and lavender and orange at night can improve cognitive function in dementia patients<sup>5</sup>. Aromatherapy alleviated stress and improved sleep quality in intensive care unit patients after two days of the experimental treatment by exposure to lavender aroma oil<sup>6</sup>. Essential oil (or volatile or flying oil) is a compound of natural ingredients with a distinctive aroma. It can be found in the roots, stems, twigs, flowers, fruit, seeds, or rhizomes of plants with an aroma<sup>7</sup>. Essential oils are obtained through several extraction methods, such as distillation, certain solvents, or pressing the scented plant parts<sup>8,9</sup>. Essential oils are used as additives in

medicine, cosmetics, perfumes, aroma enhancers in food and beverages, cleaning agents, and others<sup>10</sup>. Essential oils have aromatherapy benefits and pharmacological effects such as antibacterial, antioxidant, anti-inflammatory, antifungal, and antiviral<sup>11</sup>.

One of Indonesia's plants with a distinctive aroma in its flowers is the *Plumeria alba*. *Plumeria alba*, also known as frangipani, is widely planted as decoration in the house's yard because the flowers are attractive and have an aroma. *Plumeria alba* also grows wild on roadsides and in burial areas. Several studies have shown that aromatherapy can improve memory function and reduce stress. Therefore, this study aims to determine the effect of *P. alba* essential oil as aromatherapy on male white rats' memory function and stress levels.

## MATERIALS AND METHODS

### Materials

*Plumeria alba* was harvested from a Botanical Garden Balai Penelitian Tanaman Rempah dan Obat (BALITRO), Bogor, West Java, Indonesia, and identified at Herbarium Bogoriense of Indonesia Institute of Sciences with specimen number B-830/V.

### Methods

#### Essential oil extraction

As much as 1 kg of *P. alba* flowers was distilled in a steam container at a temperature of  $\pm 100^{\circ}\text{C}$  for two hours. The collected steam was then separated using a separatory funnel to obtain the essential oil. *Plumeria alba* essential oil compound was detected using GC-MS and dissolved in an alcohol solvent, and placed in a reed diffuser bottle.

#### Animal treatment

Twenty-four male rats weighing 150-200 g were provided by the Center of Laboratory Animal Breeding, Kemuning Karanganyar, Indonesia. During the experiment, the animals were housed under standard environmental ( $25\pm 3^{\circ}\text{C}$  temperature and 12:12 hours light and dark) and nutritional (standard pellet diet and water ad libitum) conditions. The Medical and Health Research Ethics Committee Universitas Muhammadiyah Prof. Dr. HAMKA (KEPKK-UHAMKA) approval was obtained, and all procedures performed in the study complied with the desired ethical rules, with ethical approval number 02/22.07/01740.

#### Experimental design

The rats were divided into four groups ( $n=6$ ): the group without exposure to the reed diffuser for 5 and 10 days and exposure to the reed diffuser for 5 and 10 days. Rats were placed in standard cages containing three rats. The cages were placed in a 2 x 1 m box and given a reed diffuser. The box still had air vents but was not too big so that the intensity of the aroma inhaled from the reed diffuser was more and limited the use of the scent to the outside air. The group was exposed to the reed diffuser for five days, then on the 6<sup>th</sup> day, their blood was drawn and their cortisol levels checked. In the group that was exposed to the reed diffuser for ten days, on the 11<sup>th</sup> day, their blood will be drawn and their cortisol levels checked. Data were analyzed using a t-test to determine the significance.

#### Blood sampling

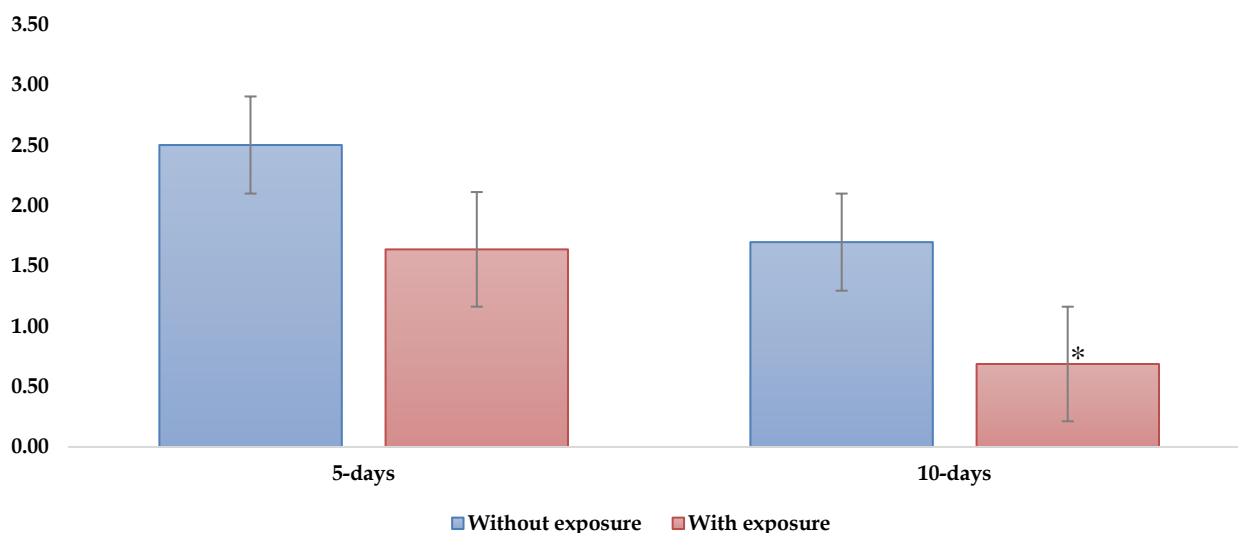
The blood sample was drawn retro-orbitally for each rat and centrifuged at 4000 rpm for 10 minutes to obtain serum on the fifth and tenth days. The serum was then tested for cortisol levels using the LC-MS method. The results of cortisol levels were tested using the ANOVA test to see the significance.

#### Data analysis

The experimental data were expressed as the mean $\pm$ SEM. Data were analyzed by t-test. The significance of the difference between means was determined, where a p-value  $<0.05$  was considered significant.

## RESULTS AND DISCUSSION

Cortisol test levels (**Figure 1**) between the control (no exposure) and treatment (with exposure) groups significantly differed and decreased. Exposure to essential oils for ten days reduced cortisol levels more than exposure to essential oils for five days. The group exposure within ten days has a significant difference from others.



**Figure 1.** Cortisol levels (ug/mL) on the blood serum of male white rats (\*p <0.05).

The body reacts to stress by releasing two chemical messengers: hormones in the blood and neurotransmitters in the nervous system<sup>12</sup>. Stress can be defined as a condition where the demands that must be met exceed the existing capabilities of the object. Stress can also be interpreted as a condition that shows changes due to responding to a stressor<sup>2</sup>.

Each type of body response to physical and psychological stress can increase ACTH secretion, increasing cortisol levels. The release of stress hormones begins with corticotrophin-releasing factor (CRF) secretion<sup>13</sup>. The CRF is first released from the hypothalamus in the brain into the bloodstream, reaching the pituitary gland, which is located just below the hypothalamus<sup>14</sup>. In this place, CRF stimulates the release of adrenocorticotrophin hormone (ACTH) by the pituitary, which stimulates the adrenal glands to release various hormones. One of them is cortisol. Cortisol circulates in the body and plays a role in coping mechanisms. When the stressor received by the hypothalamus is robust, the CRF secreted will increase so that the stimulation obtained by the pituitary also increases, and the secretion of cortisol by the adrenal gland also increases. If the emotional state has stabilized, the coping mechanism becomes positive, then signals in the brain will inhibit the release of CRF, and the stress-hormone cycle will repeat<sup>14,15</sup>. In conditions of restlessness, anxiety, and depression, cortisol secretion can increase up to 20 times due to stress<sup>16</sup>.

Essential oils are compounds of natural ingredients with a distinctive aroma due to the nature of their blends that contain fragrance and are volatile, so essential oils are also known as volatile oils<sup>7</sup>. In clinical applications, trans nasal inhalation of essential oils can be used nasal inhaler, vapor diffuser, spraying into the air, vapor balms, or direct inhalation by evaporation using tissue or cotton round<sup>17</sup>. Notably, inhalation of essential oils or aromatic plant volatile oils can send signals directly to the olfactory system and trigger the brain to produce neurotransmitters, e.g., serotonin (5-hydroxytryptamine, 5-HT) and dopamine, influence the neuroendocrinological system, neurophysiological brain activity, sympathetic and parasympathetic nervous system, biomarkers changes, psychological and behavioral effects, and modulate mental disorders further. This indicates that the effects of aromatherapy on mental diseases are attributable to the pharmacological action brought on by systemic absorption or act on the neurological system rather than just the psychological sense of the scent<sup>5</sup>.

The research data showed increased cortisol levels in the normal group with the group exposed to aromatherapy (**Figure 1**). This is in line with previous studies that stated conditions of restlessness, anxiety, and depression, and cortisol secretion can increase up to 20 times due to stress<sup>16</sup>. The rats exposed to aromatherapy for ten days had lower blood serum cortisol

levels than rats treated for only five days. It means the stress level also decreases. These results align with previous research on the impact of aromatherapy on stress and sleep quality in ICU patients. The experimental group received aromatherapy treatment, and the control group significantly changed subjective and objective stress indexes<sup>6</sup>. According to previous research, *P. alba* oil contains linalool and linalyl acetate<sup>18,19</sup>. Linalool contains sedative and narcotic properties, whereas linalyl acetate possesses narcotic properties. In addition, it can also activate the parasympathetic nervous system [20]. These results showed a relationship between the duration of exposure to essential oils in cortisol levels.

## CONCLUSION

This research shows that the length of exposure to *P. alba* aromatherapy is related to cortisol levels. The group with exposure for ten days has the lowest cortisol levels and can be used as a therapy to reduce stress.

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## AUTHORS' CONTRIBUTION

SS, TB, and SP revised the initial manuscript before submission to the journal website. SS and TB managed the experiment in the laboratory during the research. All authors read, reviewed, and approved the manuscript and English language.

## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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