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Protective effect of lavender essential oils on depression and multi-organ stress

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ABSTRACT

Introduction: Lavender essential oils (LEO) have been known to have relaxing effects, improve mood, and treat anxiety, but the effect on multiple organ stress concurrently is unknown. This multiorgan stress is related to depression can be caused by chronic psychological stress due to excessive cortisol levels and can lead to organ damage. This study analyzed LEO in preventing depression and multiorgan failure using intraperitoneal injection of corticosteroids in animal-model.

Methods: Rats (*Rattus norvegicus*) Wistar strain, male, aged 7-8 weeks were involved in this study. Depression in animal-model is defined by immobilization using tail suspension test and anhedonia using sucrose preference test. LEO 5% was diluted in virgin coconut oils as vehicle. Serum cortisol was analyzed using enzyme-linked immunosorbent assay (ELISA). Organs were extracted and processed using hematoxylin eosin staining.

Results: The results of this study indicate that LEO was able to prevent damage to the glial cells, myocardiac cells, and gastrointestinal mast cells infiltration, but not to the hepatocytes and renal cells damage. LEO also induced behavioral activation as improvement of depression, but anhedonia was still remained.

Conclusion: The effect of LEO is to prevent the increase in blood cortisol levels, thus reduce the reactivity of depressed individuals to stress, although the individual still has anhedonia as a residual symptoms.

Keywords: Lavender essential oils, depression, cortisol, multi-organ stress.
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INTRODUCTION

Psychological stress appears in the form of somatic complaints is still unexplained, such as heart and gastrointestinal organs.[1] Chronic stress leads to impaired brain function as well as immune system disorders and gastrointestinal-brain disorders.[2] The effect of stress on the body has been explained by the failure of the cortisol feedback loop in the of the hypothalamus-pituitary-adrenal axis (HPA axis).[3]

The corticosteroid injection is the most widely used to measure HPA axis activity in psychiatric patients using dexamethasone suppression test. Dexamethasone acts on the anterior pituitary to reduce the secretion of adrenocorticotropic hormone (ACTH), thereby decreasing the synthesis and release of cortisol by the adrenal cortex. Failure to suppress plasma cortisol concentrations after dexamethasone administration suggests impaired HPA axis feedback regulation.[4] This principle is used to create a depression model rat using corticosteroid injection.

Aromatherapy as a non-pharmacological method has been used by many studies because of its harmless and convenient use.[5] Aromatherapy is a popular form of complementary alternative medicine for the prevention and treatment of various health conditions.[6] Lavender essential oils (LEO) are known to have a relaxing effect, so they are good for symptoms of insomnia, anxiety, and depression.[7]

Lavender has active ingredients that can inhibit the release of acetylcholine and provide a relaxing effect. Lavender blooms (*Lavandula angustifolia*) contain 0.5% to 3% essential oil, the main ingredients of which are linalyl acetate (30-60%) and linalool (20-50%).[8] Linalool and linalyl acetate found in lavender can stimulate the parasympathetic system which affects mood states, resulting in feeling better and more refreshed, as well as being more active and relaxed.[9] It is known that contraindications, side effects, or drug interactions in LEO are minimal with inhalation, in contrast to topicals which can cause contact allergic reactions.[10]

This study aims to analyze the effect of LEO administration on improving serum cortisol levels as a marker of multi-organ stress, and to reduce the impact of multiorgan failure due to exposure to high serum cortisol in Wistar strain rats (*Rattus norvegicus*), especially in the brain, cardiac, gastrointestinal, and liver.

METHODS

Sample Preparation

Rats (*Rattus norvegicus*) Wistar strain, male, aged 7-8 weeks with average weight is 150 grams, were acclimatized of housing

and food bottles for 7 days. Rats in this age were considered as similar model of adult human, marked with mature motile sperms.[11] This acclimatization was to avoid stress bias in rats after relocation of cages, including introducing the rats to a standard feed (pellet) and a 5% sucrose solution.

Rats were randomized into 4 (four) groups, each 6 (six) rats per group based on "E" value for animal research experiment.[12] The corticosteroids injection was using a generic injectable dexamethasone product to induce acute depression in two weeks.[13] The injection dose was reduced than it should be (20 milligram-per-kilogram body-weight) and then equalized in all groups of rats with 0.5 milligram dose, because in the preliminary test most of the rats died with gangrene wounds at the injection site. Intraperitoneal (ip) injection was done so that absorption can occur directly in the blood vessels of internal organs. Corticosteroid injections were given around 07.00-09.00, then the rats were returned to their cages.

The concentration of LEO used was 5%.[14] The solvent for LEO was virgin coconut oil (VCO), which was colorless and odorless organic oil. Five cotton balls (saturated with VCO or LEOs) were placed in the rat container. The intervention was carried out every day for 14 consecutive days according to the following arrangement:[6]

1. The negative control group [K1] received intraperitoneal (ip) injection of propylene glycol and an hour of exposure to VCO cotton balls.
2. Corticosteroid group [K2] received daily intraperitoneal (ip) injection of 0.5mg of dexamethasone and an hour exposure to VCO cotton balls.
3. The LEO group [K3] received an intraperitoneal (ip) injection of propylene glycol and an hour exposure to 5% LEO cotton balls.
4. The LEO + corticosteroid injection group [K4] received an intraperitoneal (ip) injection of 0.5mg of corticosterone and an hour exposure to 5% LEO cotton balls.

Aromatherapy Preparation

The LEO preparation for this research was obtained from a patent product at Rumah Atsiri, Tawangmangu, Karanganyar, Central Java, Indonesia. This product is extracted using the principle of hydrodistillation. The lavender flower sample (40 g) was hydrodistilled for 5 hours with 500 cc of deionized water. After extraction, the essential oil was dehydrated with anhydrous sodium sulfate and weighed. All steps are repeated three times. This step can produce 100% LEO which can later be diluted according to research needs.[15,16] A cotton ball with diameter of three centimeter saturated with the 1 cc 5% LEO preparation. The following is a container design for aromatherapy treatment in rats.

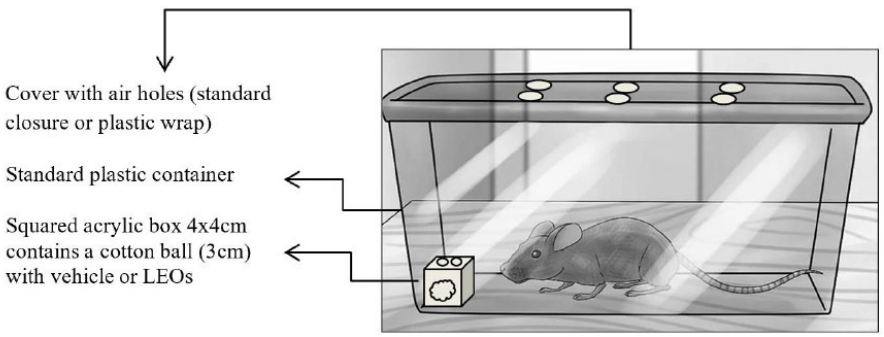

Figure 1. Design of containers for aromatherapy treatment.

The container was a standard container for laboratory rats, according to availability in the laboratory can be made of plastic or acrylic basins. Containers were designed for one rat per container. The container is modified by providing a lid made of clear plastic/acrylic, which can be tightly closed on the sides of the container. At the top of the lid given holes about 6-8 air holes which were also placed for the sucrose solution. Five cotton balls (VCOs or LEOs) were placed in a small plastic/acrylic box measuring 4x4 centimeter in one corner of the container. This box has a hole at the top so that the aromatherapy vapors can still be smelled by rats. The cotton is placed all day until it is replaced the next day.

Behavioral Examination

Depression in rat model is defined by immobilization and low preference on sucrose.[17,18] Immobilization indicates psychomotor retardation, while low preference on sucrose indicates anhedonia. Immobility was counted in seconds during tail suspension test (TST). Rats were hung by tail using support poles (wooden or iron stick) at a distance of 50 centimeter above the floor in six minutes, as two minutes earlier were habituation, and four minutes later were actual counting using digital stopwatch. The longer the time rat immobile the more depressed the rats were, the lower the time the more active or agitated rats. Preference on sucrose was examined using sucrose preference test (SPT). Sucrose 5% diluted in 100 milliliter regular tap water, placed inside a bottle as daily intake for rats. Preference on sucrose was measured using average residual volume every next morning for 14 days. More residual volume indicates anhedonia on rats.[19]

Biological Material Examination

Corticosteroid examination using enzyme-linked immunosorbent assay (ELISA) method using blood samples. Rats were knocked-out using chloroform. The surgical area should be disinfected with 70% alcohol and incised as wide as possible following a vertical Y line from the abdominal area to the upper extremities, and an inverted Y from the abdominal area to the lower extremities. Blood was taken by dissecting experimental animals until the heart was visible and blood was taken using a 5 ml syringe from the left ventricle. Blood samples taken as much as 3-5 cc through cardiac puncture. Blood was left to clot and serum is separated by centrifugation 5000 rpm for 5 minutes and stored at -80 ^{0}C until use. Total serum corticosterone levels were measured with an ELISA kit (CORT ELISA Kit; CSB-E7014r). After blood collection, rats were sacrificed.

The rats that had been sacrificed were then extracted from the brain, heart, stomach, liver, and kidneys. Organs were placed in 10% formalin normal *buffer* for 24 hours. The brain was cut on sagittal

from 1/3 dorsal-anterior with a thickness of 3-5 millimeter to obtain the cortex, parietal lobe, temporal lobe, and hippocampus. The heart was cut on the walls of the atria and ventricles, the liver and kidney were in the middle (sagittal section), the stomach is in the fundus area (coronal cut). Histopathological analysis was using hematoxylin eosin (HE) staining. Cells were counted average in ten fields. Normal cells contained single cell nucleus, clear, normal size, clear cytoplasm, fine chromatin, and regular membrane. Abnormal cells contained parenchymatous degeneration, hydropic degeneration and necrosis (pyknosis, cariorexis, or karyolysis).

Statistical analysis

Data were analyzed statistically by using SPSS with Kruskal Wallis test and Mann Whitney test, also the results were presented as mean ± standard deviation (SD). The analysis was carried out using the ANOVA test followed by post-hoc. When *p*-value less than 0.05 was indicated any significant differences.

RESULTS

Effect on Depression Model

Normality and homogeneity test showed that the distribution of the research sample was normal and homogeneous (p>0.05). The cortisol level of [K2] group was significantly the highest and the [K4] group was the lowest among the others. Post hoc test concluded that a model of depression had been established (Table 1).

Table 1 shows that mice in [K2] and [K4] were more active than the other groups, with [K4] being the most active. This finding in [K2] reinforces that in acute depression the individual experiences an agitation rather than a psychomotor retardation.

Effect on Multi-organ Stress

Results of multi-organ stress were various. Normality and homogeneity test were differ in each organ, thus analysis was conducted separately. Observation analysis of organ cells using a microscope with a 400x objective lens magnification. Normal and abnormal glial cells were counted based on the average per 10 visual fields.

Table 2 shows normal glial cells [K4] group were more abundant than other groups, and abnormal glial cells [K4] were the least compared to other groups.

Table 3 shows that LEO could prevent myocardial cells damage, where abnormal cells on [K4] were less than [K2] even there was no significant difference within groups. Depression is a known risk factor for cardiovascular disease including myocardial infarction and coronary artery disease. Excessive activation of corticotropin releasing hormone (CRH) due to depression causes autonomic disorders, including cardiovascular disease. Variability in heart rate in model of depression indicate a cardiovascular disorder also occurs in depressed patients.[20] Histological features of the heart of depressed mice showed changes such as cytoplasmic enlargement (Figure 3), vacuolar degeneration, infiltration of mononuclear cells in the myocardium, necrotic cells in the myocardium, and cardiac muscle cells had a dark color and a shrinking nucleus and eosinophilic cytoplasm.[21]

Table 4 shows the least normal hepatocyte cells [K4] compared to other groups, and the most abnormal cells [K4] compared to other groups. This means that aromatherapy has not been able to prevent liver cell damage. High cortisol levels can increase the burden of liver performance and trigger cell necrosis [31] (Figure 4).

Table 5 depicts more normal mast cells [K4] than [K2] and [K3], and the least abnormal mast cells [K4] compared to other groups. This means that aromatherapy can help prevent gastrointestinal disease related to depression.

The finding in Table 6 also suggests that LEO could not affect proximal tubules activity. It can be assumed that fluids and sodium are substantial elements that the body needs in dealing with stress and depression.

Table 1. Depression model and mean difference between groups.

No	Group	n	Mean ± SD		
			Cortisol level (ml)*	Immobility (s)**	Residual volume (ml)***
1.	[K1] control	6	12.219 ± 1.034	158.278 ± 32.355	27.743 ± 8.079
2.	[K2] CORT only	6	15.270 ± 0.907*	140.833 ± 25.972	28.474 ± 4.589
3.	[K3] LEO only	6	13.075 ± 1.688*	158.222 ± 19.343	26.917 ± 3.542
4.	[K4] CORT + LEO	6	11.187 ± 1.364	130.444 ± 15.701	31.117 ± 6.628

* Post hoc test was significantly different compared to [K4] (p < 0.05; with ANOVA test p < 0.05).
** Immobility (silent time) was counted in second during tail suspension test (ANOVA test p > 0.05).
*** Residual volume was counted in milliliter during sucrose preference test (ANOVA test p > 0.05).

Table 2. Effect of Aromatherapy on Glia Cells.

No	Group	n	Mean ± SD (Normal Cell)	*p* post hoc* (compared with [K4])	Mean ± SD (Abnormal Cells)	*p* Mann-whitney** (compared with [K4])
1.	[K1] control	6	6.67 ± 2.16	0.138	0.00 ± 0.000	0.138
2.	[K2] CORT only	6	8.33 ± 1.862	0.282	1.33 ± 1.633	0.282
3.	[K3] LEO only	6	9.33 ± 0816	1,000	0.33 ± 0.516	1,000
4.	[K4] CORT + LEO	6	9.50 ± 2.429	-	0.33 ± 0.516	-

* Post hoc Tukey test was not significantly different (p > 0.05; with ANOVA test p > 0.05)
** Mann-Whitney test was not significantly different (p > 0.05; with Kruskal-Wallis test p > 0.05)

DISCUSSION

Based on this study, the cortisol level of [K2] group was significantly the highest and the [K4] group was the lowest among the others. Post hoc test concluded that a model of depression had been established (Table 1). High levels of cortisol in the blood indicate a disturbance in the HPA-axis feedback. The failure of HPA axis feedback is a marker of serious mental disorders such as attempted suicide in individuals with major depression.[4] LEO was able to lower cortisol levels in K4 compared to other groups.

This study shows that mice in [K2] and [K4] were more active than the other groups, with [K4] being the most active. This finding in [K2] reinforces that in acute depression the individual experiences an agitation rather than a psychomotor retardation. The high level of cortisol in depressed individuals is related to their sensitivity to external stressors.[22] Reducing sensitivity requires an adaptive approach to help individuals deal with stress, such as using aromatherapy as adjuvant. As previously known, LEO is supposed to have a relaxing effect on agitated states, but in [K4] shows the behavior of mice that are increasingly active.[23] According to a Thailand study, subjects inhaled LEO were reported have more behavioral activation in terms of mood compared to control group, even though they were more relaxed, confirmed with increasing of theta and alpha waves significantly using electroencephalography.[24] Thus, behavioral activation should be distinguished from agitation.

Residual volume of SPT on [K4] was higher than other group indicated that rats were still in anhedonia state. Taken together, although cortisol level was lower and rats were being active, anhedonia was still remaining problem of individual with improved depressive symptoms. Anhedonia is an inability to respond to rewards and pleasures, and can be a predictor of poor treatment outcome of depression.[25] This finding confirms that anhedonia could persist as a residual symptom of depression.

This study shows normal glial cells [K4] group were more abundant than other groups, and abnormal glial cells [K4] were the least compared to other groups. Glial cells are relatively smaller than neurons and appear to have a darker nucleus because they contain a lot of chromatin.[26] Damaged glial cells are characterized by swollen cytoplasm and cell nuclei that begin to pyknosis.[27] Depressive symptoms are associated with high apoptotic brain cells and decreased BDNF.[28] Continuous corticosteroid injection caused massive

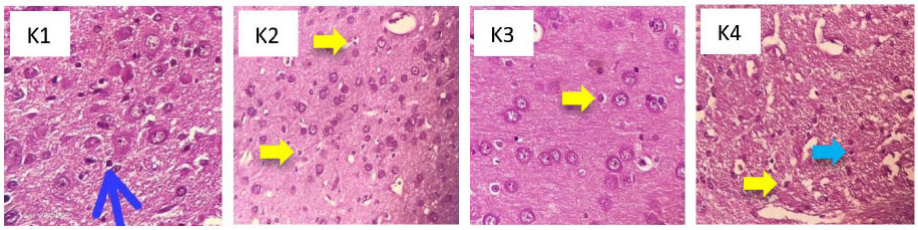

Figure 2. Abnormal glial cells, swollen cytoplasm or become pyknosis (yellow arrow) and normal glial cells (blue arrow) (HE staining, objective lens 400x magnification).

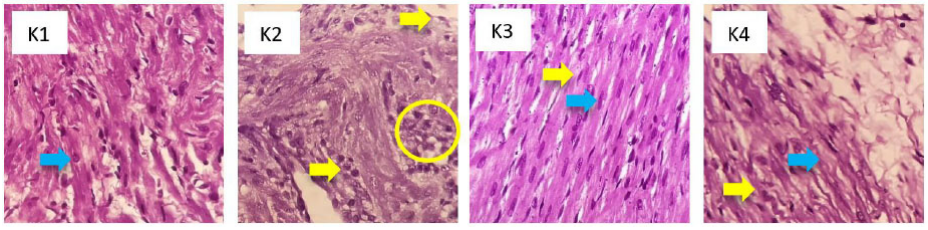

Figure 3. Abnormal myocardial cells showed cytoplasmic enlargement and eosinophilic or shrinking nucleus (yellow arrow and circle). Normal myocardial cells are in blue arrow (HE staining, objective lens 400x magnification).

Table 3. Effect of Aromatherapy on Myocardial Cells.

No	Group	n	Mean ± SD (Abnormal Cells)	*p* Mann-Whitney*			
				[K1]	[K2]	[K3]	[K4]
1.	[K1] control	6	1,000 ± 0.000	-	0.021*	1,000	0.138
2.	[K2] CORT only	6	1.830 ± 0.753		-	0.021*	0.212
3.	[K3] LEO only	6	1,000 ± 0.000			-	0.138
4.	[K4] CORT + LEO	6	1.330 ± 0.516				-

* Mann-Whitney test was significantly different ($p < 0.05$; with Kruskal-Wallis test $p < 0.05$)

Table 4. Effect of Aromatherapy on Hepatocyte Cells.

No	Group	n	Mean ± SD (Normal Cell)	*p* post hoc* (compared with [K4])	Mean ± SD (Abnormal Cells)	*p* post hoc** (compared with [K4])
1.	[K1] control	6	81.67 ± 6.831	0.000*	3.00 ± 3.950	0.006**
2.	[K2] CORT only	6	82.50 ± 5.244	0.000*	9.63 ± 2.739	0.601
3.	[K3] LEO only	6	76.14 ± 6.646	0.001*	13.33 ± 5.164	0.191
4.	[K4] CORT + LEO	6	56.67 ± 4.082	-	19.17 ± 8.010	-

* Post hoc Tukey test was significantly different ($p < 0.05$; with ANOVA test $p < 0.05$)
** Post hoc Tukey test was significantly different ($p < 0.05$; with ANOVA test $p < 0.05$)

stress in the brain and induced glial cells to apoptosis and cell death.[29] This research confirmed that LEO prevents glial cell from damage as mentioned by Sanchez-Vidana et al, 2019.[30] The high number of normal glial cells and the relatively small number of abnormal cells in [K4] compared to other groups indicate that LEO plays a role in the process of neurogenesis and neuroplasticity.

This study shows the least normal hepatocyte cells [K4] compared to other groups, and the most abnormal cells [K4] compared to other groups. This means that aromatherapy has not been able to prevent liver cell damage. High cortisol levels can increase the burden of liver performance and trigger cell necrosis.[31] The linalool compound in LEO has the strongest odorous effect. Most of the linalool will be metabolized by the liver into water-soluble polar compounds by cytochrome P450 enzymes. The metabolic process of linalool by liver enzymes basically increases the work of the liver and has the potential to cause stress on the endoplasmic reticulum and dysfunction of hepatocyte mitochondria.[32]

This study shows depicts more normal mast cells [K4] than [K2] and [K3], and the least abnormal mast cells [K4] compared to other groups. This means that aromatherapy can help prevent gastrointestinal disease related to depression. Mast cell infiltration indicates inflammatory response during psychological stress, as known in functional gastrointestinal disorders (FGIDs). It is a disease that is prevalent with depression in the general population.[33,34] The prevalence of depression in FGIDs is 61%.[35] Gastrointestinal symptoms are also common in patients with major depression.[36] FGIDs are chronic recurrent gastrointestinal disorders without structural or biochemical abnormalities, with a variety of pathophysiological causes including changes in motility, visceral hyperalgesia, including the effects of psychological stress and gut-brain disturbances.[37]

Depression affects the kidneys through the vasopressin pathway, where vasopressin directly affects CRH activity. Individuals with depression are known to have higher mean plasma vasopressin levels than controls, causing individuals to experience more massive sodium and fluid retention than controls. This fluid and sodium retention is associated with a decreased appetite condition, so the kidneys automatically maintain the required amount of fluid and sodium in the body.[38] The closure of the proximal tubules of this research indicates that an effort was being made to maintain the required amount of fluid and sodium in the body. This condition is dangerous for the individual because it can trigger

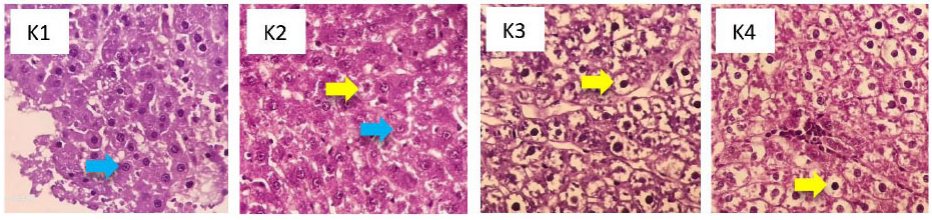

Figure 4. Abnormal hepatocyte cells showed cytoplasmic enlargement and eosinophilic or shrinking nucleus (yellow arrow), normal hepatocytes in blue arrow. (HE staining, lens 400x magnification).

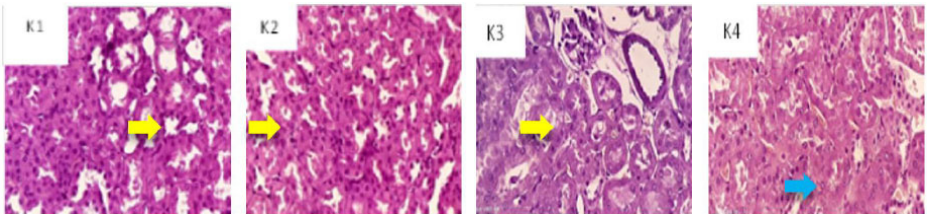

Figure 5. Opened (yellow arrow) and closed (blue arrow) proximal tubule (HE staining, objective lens 400x magnification).

Table 5. Effect of Aromatherapy on Gastrointestinal Mast Cells.

No	Group	n	Mean ± SD (Normal Cell)	*p* Mann-Whitney* (compared with [K4])	Mean ± SD (Abnormal Cells)	*p* Mann-whitney** (compared with [K4])
1.	[K1]	6	304.17 ± 189.273	(1,000)	7.50±8.803	(0.493)
2.	[K2]	6	250.00 ± 126.807	(0.108)	6.67±2.582	(0.032)
3.	[K3]	6	215.00 ± 71.484	(0.124)	4.50±3.937	(0.359)
4.	[K4]	6	275.00 ± 31,464	-	2.59±3.920	-

* Mann-Whitney test was not significantly different ($p > 0.05$; with Kruskal-Wallis test $p > 0.05$)
** Mann-Whitney test was not significantly different ($p > 0.05$; with Kruskal-Wallis test $p > 0.05$)

Table 6. Effect of Aromatherapy on Renal Cells.

No	Group	n	Mean ± SD (Σ impaired proximal tubules)	*p* post hoc*			
				[K1]	[K2]	[K3]	[K4]
1.	[K1] control	6	4.833 ± 0.983	-	0.000*	0.000*	0.000*
2.	[K2] CORT only	6	2.500 ± 0.548		-	1.000	0.007*
3.	[K3] LEO only	6	2.500 ± 0.548			-	0.007*
4.	[K4] CORT + LEO	6	1.000 ± 0.632				-

* post hoc Tukey test was significantly different ($p < 0.05$; with ANOVA test $p < 0.05$)

nephrotoxicity.[39]

CONCLUSION

This study confirms a biological depression model of rats using continuous corticosteroid intraperitoneal injection. The effect of LEO is to prevent the increase in blood cortisol levels, thus reduce the reactivity of depressed individuals to stress, although the individual still has anhedonia as a residual symptoms. Behavioral activation as symptom marker for mood improvement still needs to be distinguished from agitation. LEO prevents damage to glial cells and supports neuroplasticity in depression. LEO also prevents damage to myocardial cells and gastrointestinal mast cells, but not to hepatocytes and renal cells. These two organs need to be considered in future experiments using aromatherapy because their function is to neutralize foreign substances that enter the body, including aromatherapy. These two organs were also the most affected by oxidative stress due to corticosteroid injection. This study observation is still limited to the damage of anatomical structure, so it requires organ function analysis for the future research.

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AUTHOR CONTRIBUTION

All authors contributed to this study's conception and design, data analysis and interpretation, article drafting, critical revision of the article, final approval of the article, and data collection.

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CONFLICT OF INTEREST

There is no conflict of interest for this manuscript.

ETHICAL CONSIDERATION

This research has been registered with ethical certificate from FK UNUSA with the number 076/EC/KEPK/UNUSA/2021.

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